

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS1-01

Landscape of the breast tumour microenvironment at single-cell resolution

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Breast cancers are a complex 'ecosystem' of diverse cell types, whose heterotypic interactions play central roles in defining the aetiology of disease and its response to therapy. The next generation of therapies will likely be based upon an integrated understanding of the malignant, microenvironmental and immune states that define the tumour and inform treatment response. However, our poor understanding of the tumour microenvironment (TME) of breast cancers has limited the development and implementation of new drugs that target stromal and immune cells.

Single cell genomics (SCG) is a remarkable new platform to examine the compositional, gene expression and other parameters of thousands of cells, rapidly and at scale. We have used a multi-dimensional SCG approach to characterise the TME in a unique cohort of early and metastatic breast cancers with rich clinico-pathological annotation. We have conducted single cell RNA-Sequencing on more than 125,000 cells collected from 22 patients.

Malignant cells showed remarkable intra-tumoural heterogeneity for canonical breast cancer features, such as intrinsic subtype, hormone receptor expression and activity, drug targets, drug resistance signatures and transcriptional drivers.

Cancer Associated Fibroblasts (CAFs), which are classically studied as a single cell type, were heterogeneous across primary and metastatic sites. Interestingly we identified a myofibroblast-like subset and an inflammatory-mediator subset and propose multi-faceted roles in regulating malignancy and tumour immunity. Distinct transcription factor networks regulated these polarised states.

We applied a new method known as CITE-Seq to measure cell surface immune markers and checkpoint proteins simultaneous to RNA-Sequencing. We resolve the tumour-immune milieu with high precision and generate new transcriptional signatures of breast tumour-infiltrating leukocytes.

To track lymphocyte clonal dynamics through space and time, we developed a novel method known as RAGE-Seq to permit simultaneous full length lymphocyte receptor- and RNA-sequencing at single cell resolution. We observe clonal expansion and trafficking of CD4+ and CD8+ T lymphocytes between the lymph nodes, blood and tumor of patients. In comparison, B cells were polyclonal, suggesting an absence of antigen-dependent clonal expansion.

This data provides by far the most extensive insights into the cellular landscape of breast cancer and will reveal new biomarkers and opportunities for stromal- and immune-based therapy.

## 2018 San Antonio Breast Cancer Symposium ®

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Towards a human breast cell atlas

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The human breast tissue consists of lobules connected to a complex network of ducts that are evolutionarily designed to produce and transport milk to nourish offspring. Histopathology has identified 10 major cell types based on morphological features but have provided limited information on cell states - the transcriptional programs of cell types that reflect different biological functions. In this study, we have generated an unbiased 'cell atlas' of the normal human breast to define the cell types and cell states using single cell RNA sequencing methods. We performed 3' microdroplet based single cell RNA sequencing of 31,442 stromal cells from 11 women with pathologically normal breast tissues that were collected from mastectomies. Unbiased expression analysis identified three major cell types: epithelial cells (luminal and basal), fibroblasts and endothelial cells, in addition to several minor cell types: macrophages, T-cells, natural killer cells, pericytes and smooth muscle cells. Analysis of cell states of these cell types revealed different transcriptional programs in luminal epithelial cells (hormone receptor positive and secretory), basal epithelial cells (myoepithelial or basement-like), endothelial cells (lymphatic or vascular), macrophages (M1 or M2) and fibroblasts (three subgroups) and provided insight into progenitors of each cell types. These data provide a valuable reference for the research community and will provide new insights into how normal cell types are transformed in the tumor microenvironment to promote or inhibit the progression of breast cancer.

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Crosstalk between osteoblasts and breast cancer cells alters breast cancer proliferation through multiple mechanisms

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BrCa preferentially metastasizes to bone, where the 5-year relative survival rate is <10%. While the mechanism for preferred metastasis is unknown, bone likely provides a hospitable environment that attracts BrCa cells (BCCs) and allows them to colonize and grow.

In a cancer-free environment in the adult, the skeleton continuously undergoes remodeling. Osteoclasts excavate erosion cavities and osteoblasts (OB) synthesize bone matrix, with no net bone gain or loss. However, when metastatic BCC invade bone, this balance is disrupted to favor bone loss. Bisphosphonate treatments are not curative. OBs do not deposit new bone. This result suggests that OBs may be altered or experience a loss-of-function in the tumor niche.

We have new, late-breaking evidence to suggest that communication between OBs and BCCs 'educates' OBs to produce factors that suppress BCC proliferation in bone. We have in-vitro and in-vivo mouse-model evidence that 'educated' osteoblasts (EOs) have a unique secretory protein profile compared to 'uneducated' OBs. We also identified EOs as being present in the bone tissue samples of human patients with bone metastatic BrCa via multi-plex immunofluorescence. When we treated BCCs with EO conditioned media (CM), BCC proliferation was reduced in both triple negative and ER+ metastatic BCCs, while CM from 'uneducated' OBs did not affect BCC proliferation. This effect was mediated through alterations in EO production of decorin and NOV. We identified EO CM as a rich source of exosomes (exo) and confirmed the presence of an exo population via iodixanol density gradient and western blotting for specific exo protein markers. We found that EO-derived exo, but not 'uneducated' OB-derived exo, decreased proliferation of ER+ and triple negative BCC. Also, treatment with EO-derived exosomes increased the number of Ki67 *negative* metastatic BCC. Moreover, we labeled EO exo with RFP-conjugated CD63 to visually confirm exo transfer from EO cells to BCCs using confocal microscopy. And, co-culture with EOs increased triple negative and ER+ metastatic BrCa expression of p21 compared to co-cultures with 'uneducated' OBs. Our data suggest that EOs use multiple mechanisms of cellular communication to regulate BCC proliferation in bone.

**Impact:** Our late-breaking data suggest that OBs produce factors that suppress metastatic BCC growth. Much less attention has been given to OB interactions with tumor cells at sites of bone metastasis due to observations that OB populations are reduced at sites of advanced osteolysis. However, we propose that OBs may be valuable endogenous targets to aid in restoration of bone deposition and suppression of metastatic BrCa growth in the niche in concert with therapeutic drugs to kill the cancer cells. Our data suggest there is a population of OBs that demonstrate a functional role in retarding metastatic BCC growth; a property capable of exploitation. Moreover, restoration of the OBs' ability to deposit new bone would lead to better quality of life and increased time of survival for bone metastatic BrCa patients where bone loss is found. For these reasons, OBs and EOs are suitable candidates for therapeutic targeting and will open new avenues for retarding the growth of BrCa bone metastases.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS1-04

IMpassion130: Efficacy in immune biomarker subgroups from the global, randomized, double-blind, placebo-controlled, phase III study of atezolizumab + *nab*-paclitaxel in patients with treatment-naïve, locally advanced or metastatic triple-negative breast cancer

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**Background:** The Phase III IMpassion130 study (NCT02425891) evaluated atezolizumab (anti-PD-L1) + *nab*-paclitaxel (*nabPx*) vs placebo + *nabPx* as first-line treatment for pts with metastatic triple-negative breast cancer (TNBC). The study met its co-primary PFS endpoint in ITT pts and in pts with PD-L1 ≥1% on tumor-infiltrating immune cells (IC+). Clinically meaningful OS benefit was seen at interim OS analysis, notably in pts with PD-L1 IC+ tumors (Table). Here we report exploratory efficacy data in immunologically and clinically relevant, biomarker-defined subgroups.

**Methods:** Pts had histologically documented metastatic or unresectable locally advanced TNBC (evaluated locally per ASCO-CAP). Pts were randomized 1:1 to *nabPx* 100 mg/m<sup>2</sup> IV (d1, 8 and 15 of a 28-d cycle) + atezolizumab 840 mg IV q2w or placebo (A-*nabPx* or P-*nabPx*) until progression or toxicity. Exploratory biomarkers were centrally analyzed: PD-L1 on tumor cells (TC) by VENTANA SP142 IHC assay, intratumoral CD8 by IHC, stromal tumor-infiltrating lymphocytes (sTILs), *BRCA1/2* mutational status and ER/PR/HER2 status.

**Results:** PD-L1 IC was highly predictive of A-*nabPx* efficacy (Table). The majority of PD-L1 TC+ tumors were also PD-L1 IC+. Intratumoral CD8, but not sTILs, were well correlated with PD-L1 IC. Consequently, CD8 was predictive of A-*nabPx* efficacy for PFS/OS, while sTILs only predicted PFS benefit. Local vs central TNBC assessment was concordant in most pts. Local vs central lab-defined TNBC populations derived similar benefit from A-*nabPx*. Efficacy by *BRCA* status will be presented to evaluate the benefits of immunotherapy for this subgroup.

**Conclusions:** Exploratory efficacy analyses from IMpassion130 suggest consistency between local and central ER/PR/HER2 testing and that PD-L1 IC is the most robust predictive biomarker for selecting untreated mTNBC pts who benefit from A-*nabPx*.

Population	A- <i>nabPx</i>	P- <i>nabPx</i>
<b>Primary data, stratified</b>		
<b>ITT, n</b>	451	451
mPFS (95% CI), mo	7.2 (5.6-7.5)	5.5 (5.3-5.6)
PFS HR (95% CI)	0.80 (0.69-0.92); P=0.0025	
mOS (95% CI), mo	21.3 (17.3-23.4)	17.6 (15.9-20.0)
OS HR (95% CI)	0.84 (0.69-1.02); P=0.0840	
<b>PD-L1 IC+, n (%)</b>	185 (41%)	184 (41%)
mPFS (95% CI), mo	7.5 (6.7-9.2)	5.0 (3.8-5.6)
PFS HR (95% CI)	0.62 (0.49-0.78); P<0.0001	
mOS (95% CI) mo	25.0 (22.6-NE)	15.5 (13.1-19.4)
OS HR (95% CI)	0.62 (0.45-0.86) <sup>a</sup>	

Exploratory/biomarker data, unstratified		
<b>PD-L1 TC evaluable, n</b>	449	451
<b>PD-L1 TC+, n (%)</b>	38 (8%)	40 (9%)
PFS HR (95% CI)	0.51 (0.31-0.84)	
OS HR (95% CI)	0.63 (0.33-1.21)	
<b>CD8 evaluable, n</b>	371	349
<b>CD8 ≥0.5%, n (%)</b>	261 (70%)	239 (68%)
PFS HR (95% CI)	0.74 (0.61-0.91)	
OS HR (95% CI)	0.66 (0.50-0.88)	
<b>sTIL evaluable, n</b>	448	444
<b>sTIL ≥10%, n (%)</b>	147 (33%)	137 (31%)
PFS HR (95% CI)	0.66 (0.50-0.86)	
OS HR (95% CI)	0.75 (0.51-1.10)	
<b>cTNBC evaluable, n</b>	420	412
<b>cTNBC ITT, n (%)</b>	307 (73%)	317 (77%)
PFS HR (95% CI)	0.81 (0.68-0.98)	
OS HR (95% CI)	0.85 (0.67-1.08)	
<b>cTNBC PD-L1 IC+, n (%)</b>	133 (43%)	134 (42%)
PFS HR (95% CI)	0.67 (0.51-0.88)	
OS HR (95% CI)	0.69 (0.47-1.00)	

Data cutoff: 17 April 2018 (12.9-mo median follow up).

cTNBC, centrally confirmed TNBC

TC/IC+, PD-L1 ≥1% (VENTANA SP142 assay)

a Not formally tested per hierarchical study design.

## 2018 San Antonio Breast Cancer Symposium ®

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Apobec3 induced mutagenesis sensitizes murine models of triple negative breast cancer to immunotherapy by activating B-cells and CD4+ T-cells

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Immune checkpoint inhibitor (ICI) therapies have led to remarkable clinical responses in cancers such as melanoma and non-small cell lung cancer. In breast cancer, current immunotherapy trials have placed an emphasis on triple negative breast cancers (TNBC), where early results suggest response rates of 10-20%. Thus, it is critical to identify predictive biomarkers to enhance patient selection for immunotherapy. With this goal in mind, we simulated a clinical trial employing anti-PD1 and anti-CTLA therapies in immune-intact genetically engineered mouse models (GEMMs) of TNBC. Testing of ICI therapies on 8 different GEMMs demonstrated that each model was resistant. Whole exome sequencing showed that each model also harbored a low mutation burden. Given that mutation load is predictive of immunotherapy response in other cancer types, and that Apobec3B activity is associated with higher tumor mutation burden (TMB) in breast cancer, we created two different tumor lines with overexpression of murine Apobec3.

In contrast to the parental lines, the Apobec3 overexpressing lines showed an elevated tumor mutation burden and new mutations were consistent with the Apobec mutation signature. These TNBC lines with new mutations resulting from Apobec3 activity were exquisitely sensitive to anti-PD1/anti-CTLA4 combination therapy; as assessed by reduction in tumor volume and extended overall survival. To identify features that predict response, we examined resistant and sensitive tumors at pretreatment, at 1 week of treatment, and at end stage by flow cytometry and mRNA-seq. Gene expression profiling identified multiple immune signatures as predictive of response to ICI therapy; specifically CD8+ T-effector memory cells, CD4+ T-cells, and activated B-Cells. Similarly, gene expression analysis showed that these cell types increased at 1 week of therapy in sensitive models but not in resistant models. Flow cytometry confirmed these predictions.

Next, we used an antibody based approach to separately deplete CD4+ T-Cells, CD8+ T-cells, or B-cells in Apobec3 mutagenized murine tumors receiving aPD1/aCTLA4 combination therapy. In each case, depletion of these populations significantly reduced the therapeutic response. However, mice receiving combination immunotherapy and depleted for CD8+ T-cells still exhibited a significant extension in overall survival compared to non-treated controls. In contrast, the CD4+ T-cell depleted mice and B-cell depleted mice exhibited no ICI therapeutic benefit.

Together, these data point to key immune biomarkers of response to anti-PD1/anti-CTLA4 therapy; we have further developed a genomic predictor of ICI response using our murine models and will test this on a human TNBC data set. Lastly, this GEMM system provides a rich RNA-seq resource, and new immune-activated models for TNBC, which uncovered a key role for B-cells and CD4+ T-cells in response to ICI therapies.

# 2018 San Antonio Breast Cancer Symposium ®

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Unraveling lobular breast cancer progression and endocrine resistance mechanisms through genomic and immune characterization of matched primary and metastatic samples

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## Background:

Invasive lobular breast cancer (ILC) represents the second most common histology of breast cancer, accounts for 10-15% of all invasive cases and generally expresses the estrogen receptor (ER, coded by the *ESR1* gene). Little is known about the genomic alterations associated with tumor progression and endocrine resistance in ILC. Here, we therefore molecularly characterized a unique series of matched primary and metastatic ILC.

## Patients and methods:

We retrospectively identified 129 metastatic ER-positive ILC patients from 6 institutions. Following central pathology review and available DNA from the primary tumor (P), the metastasi(e)s (M), as well as from normal tissue, 80 patients (279 samples) were eligible for this study. All but 6 patients (7.5%) received endocrine treatment before metastatic sampling. Low pass whole genome and targeted gene screen (N=20 genes) sequencing was conducted to detect copy number aberrations (CNAs) and mutations associated with ILC metastatic progression respectively. *ESR1* mutations were further assessed using droplet digital PCR (ddPCR). Publicly available data from IJB (n=413 ILC Ps), TCGA (n=172 ILC Ps), and MSKCC-IMPACT (n=116 ILC Ms) were used to compare and validate the frequencies of the detected alterations in ILC. Stromal tumor infiltrating lymphocytes (TILs) were assessed by two experienced pathologists.

## Results:

The overall matched CNA comparison revealed a significant positive association between relapse-free survival and the P/M genomic distance defined by the number of CNAs private to P or M ( $r^2 = 0.52$ ,  $p < .001$ ), suggesting that the longer the disease evolution, the more M differs from P. Regarding CNA changes in cancer genes, we observed the acquisition in M of *MYC*, *CCND1*, *FGFR1/ZNF703*, and *ZNF217* amplifications in 17, 9, 6 and 6% of the patients, as well as *RB1*, *PTEN*, and *TP53* deletions in 9, 11, and 6% of the patients, respectively. The matched P/M comparison of the mutations highlighted the acquisition in M of mutations in 11% of the patients for *CDH1*, in 10% for *ESR1*, in 8% for *ARID1A*, in 5% for *ERBB2*, *GATA3*, *IGF1R*, *MAP3K1*, and *PIK3CA*, and in 3% for *AKT1* and *TBX3*. Of interest, when comparing the overall CNA frequencies of our Ms to publicly available data from ILC Ps, we observed a higher frequency of *CCND1* and *MYC* amplification, of *PTEN*, *RB1* and *TP53* deletions, as well of *AKT1*, *ERBB2*, *ESR1* and *IGF1R* mutations in our ILC Ms. Of note, we did not detect any significant difference in CNA or mutational frequency when comparing our ILC Ms to those from MSKCC-IMPACT.

Regarding the immune infiltration, higher TILs in Ps were significantly associated with younger age at diagnosis, high grade tumors, and with mixed non-classic and trabecular histology. A paired analysis revealed no significant difference in TIL levels between P and M. TIL levels in the P or M were not associated with survival.

## Conclusion:

This is to our knowledge the largest metastatic ILC series in which matched P and M samples were interrogated, revealing several genomic alterations, some of which potentially targetable, driving disease progression and endocrine resistance.

# 2018 San Antonio Breast Cancer Symposium ®

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The genomic landscape of 501 metastatic breast cancer patients

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**Background:** In depth sequencing of primary breast cancer (BC) has identified a heterogeneous repertoire of disease drivers, evidence of clonal evolution and underlying mutational processes. As cancer evolves over time and under treatment pressure, whole genome sequencing (WGS) of metastatic BC (MBC) tissue is crucial to gain further insight into its genetic make-up and driving forces, thereby allowing improved patient management.

**Methods:** Metastatic tissue and matched germline DNA of patients with MBC (N=501) were prospectively recruited under the biopsy protocol of the Center of Personalized Cancer Treatment (CPCT-02; NCT01855477) and analyzed by WGS. Sequence reads were mapped to the reference genome to call somatic single nucleotide variants (SNV), small insertions and deletions (InDels) and copy number variations from which mutational signatures and tumor mutational burden (TMB; the number of SNV and InDels relative to the genome) were derived. The incidence of called aberrations in our cohort was compared to previously reported WGS data of 560 primary BC (BASIS cohort, Nik-Zainal et al. Nature 2016) (Table 1).

**Results:** According to routine diagnostics 303 patients (60.5%) were ER+/HER2-, 70 (14%) triple negative (TNBC), 95 (19%) HER2+ and the remaining 33 (6.6%) had as of yet an unknown subtype. Top 5 recurrently affected genes were *TP53* (47%), *ATM* (33%), *MAP2K4* (32%), *NCOR1* (31%), *ERBB2* (30%). In the metastatic lesions, median TMB was 2.9 per million base pairs (IQR: 1.7-5.3). Interestingly, 53 (11%) patients had a high TMB ( $\geq 10$ ).

Compared to primary BC (BASIS cohort), we found (subtype-specific) enrichment of alterations in multiple genes such as *ATM* (0.4% to 33%), *GPS2* (1.3% to 29%), *MAP2K4* (6.4% to 32%), *CBFB* (2.7% to 25%), and, as previously reported, *ESR1* (1.3% to 20%). APOBEC signature mutations appeared to be enriched in MBC while HRD signature mutations seemed less prevalent.

Analyses to reveal additional genomic features is ongoing as well as the association of genomic alterations with uniquely collected information on prior treatments and response to treatment received directly after biopsy (i.e. endocrine therapy alone or combined with CDK4/6 inhibitors and chemotherapy). Also, to exclude methodological bias the raw data of the BASIS cohort will be processed through our pipeline.

Table 1: Comparison of ER+/HER2- and TNBC subtypes: BASIS versus CPCT-02 MBC

	BASIS		CPCT-02 MBC	
	ER+/HER2-(%)	TNBC(%)	ER+/HER2-(%)	TNBC(%)
Number of samples	326	155	303	70
Median TMB	0.96	2.63	2.73	2.91
SNV burden/Mbp	0.89	2.5	2.41	2.64
InDel burden/Mbp	0.06	0.12	0.21	0.3
Top 5 affected genes	<i>PIK3CA</i> (37)	<i>TP53</i> (83)	<i>TP53</i> (43)	<i>TP53</i> (61)
	<i>TP53</i> (20)	<i>PTEN</i> (37)	<i>ATM</i> (43)	<i>MYC</i> (37)



	<i>CCND1</i> (20)	<i>MYC</i> (26)	<i>MAP2K4</i> (37)	<i>CDKN2A</i> (33)
	<i>GATA3</i> (15)	<i>RB1</i> (24)	<i>NCOR1</i> (35)	<i>CDKN2B</i> (33)
	<i>MYC</i> (14)	<i>PIK3CA</i> (17)	<i>CDH1</i> (34)	<i>RB1</i> (33)
Mutational signatures (median relative contribution)				
Age	23.2	7.2	13.7	11.8
APOBEC	6.9	7.8	14.6	8.3
Homologous-recombinant deficiency	4.4	39.9	4.9	19.8
DNA mismatch Repair Deficiency	0.6	0	0.3	0

**Conclusion:** WGS of this unique cohort of patients with MBC shows a genetic make-up roughly similar to primary BC, but does show subtype-specific enrichment of selected driver mutations in metastatic disease. This study provides better insight into the tumor biology of MBC potentially improving management of these patients.

# 2018 San Antonio Breast Cancer Symposium ®

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## Genomic characterisation of metastatic breast cancer

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**Rationale:** while large efforts have been done to characterize early breast cancer, little is known about the genomic landscape of metastatic breast cancer. In the present study, we performed whole exome sequencing of 800 metastatic breast cancers, in order to identify new candidate targets and better stratify patients eligible for innovative therapies.

**Patients and Methods:** Patients were selected to present a metastatic breast cancer and to have received a biopsy in the context of precision medicine trials (SAFIR01, SAFIR02, PERMED, MOSCATO, SHIVA). Samples with >30% cancer cells, and normal DNA, were sequenced using Hiseq and Novaseq. Drivers were identified using MutSigCV. Actionability of somatic genetic alterations was determined based on OncoKB. Decomposition of mutational signatures was performed using deconstructSigs. Prognostic value was assessed using a cox model. TCGA database was used as comparator to identify gene alterations enriched in metastatic samples.

**Results:** results presented in the current abstract are based on the first 629 patients analyzed. Sequencing was performed in 387 patients with HR+/Her2- breast cancer, 186 triple negative breast cancers, and 32 Her2-overexpressing breast cancers. only 9 patients received a pretreatment with a CDK4 inhibitor. 24 driver genes were significantly mutated. In patients with HR+/Her2- breast cancer, 11 genes were found more frequently mutated in the metastatic setting as compared to early stage breast cancer. This includes TP53 (29%), KMT2C (13%), NCOR1 (8%), NF1 (7%), RB1 (4%), C16orf3 (2%), FRG1 (6%), ESR1 (21%), RIC8A (4%), AKT1 (7%), PLSCR5 (2%). In addition, in the whole population, KRAS was found mutated in 3% of samples (G12A/C/R/V) while its frequency of mutation in early breast cancer is <1%. No gene alteration was found enriched in metastatic Her2+++ and TNBC. Copy number alterations were compared between metastatic and early breast cancer. 18 amplicons were found more frequently in HR+/Her2- metastatic breast cancers as compared to eBC. Among the genes enriched in metastatic samples, mutations in RB1 or NF1 were associated with a poor outcome ( median OS 9 and 13 months respectively, p=0.0038 and 0.01 respectively). 73% of patients presenting HR+/Her2- mBC had an actionable alteration, as compared to 55% of patients presenting HR+/Her2- eBC (p<0.01). Patients with HR+/Her2- mBC presented an enrichment of gene alterations in the MAPK/ERK pathway (37% vs 22%) and in the HRD pathway (22% vs 10%). When the analysis focuses on mTNBC; the proportion of patient presenting an actionable alteration was comparable to the eTNBC. 11 (6%) and 16 (9%) patients presented a somatic mutation or homozygous gene deletion on BRCA1 and PTEN respectively.

We further assessed the mutational signatures in order to better understand which mutational processes could drive cancer progression. Metastatic HR+/Her2- mBC presented an increase in APOBEC, S3 (HRD), S10 (POLE-associated signature), S17 signatures as compared to early HR+/Her2- BC.

**Conclusion:** the present study, based on 629 patients, identifies 11 driver gene alterations and four mutational processes enriched in HR+/Her2- metastatic breast cancers. Final results on 800 patients will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS2-01

Age-related breast cancer risk estimates for the general population based on sequencing of cancer predisposition genes in 19,228 breast cancer patients and 20,211 matched unaffected controls from US based cohorts in the CARRIERS study

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**Background:** Clinical germline genetic testing of cancer predisposition gene panels is used to identify women at increased risk for breast cancer. The identification of pathogenic mutations in established high and moderate predisposition genes may result in improved risk management of breast cancer for tested patients and their family members through tailored screening, prophylactic surgeries, or chemoprevention. However, the risks of breast cancer associated with mutations in these genes have likely been overestimated for many women in the general population because previous studies have focused on individuals with a family history of breast and/or ovarian cancer, early onset disease, or triple negative breast cancer. The goal of the “CAnceR Risk Estimates Related to Susceptibility” (CARRIERS) study is to estimate breast cancer risks associated with mutations in hereditary cancer panel genes in the general population.

**Methods:** Germline DNA samples from blood or saliva were obtained from 39,439 breast cancer patients and matched unaffected controls from six US-based cohorts (BWHS, CPSII, CTS, MEC, NHS1, NHS2, WHI). DNA was subjected to dual bar-coded QIAseq multiplex PCR-based amplification of 1733 target regions covering all coding regions of 37 cancer predisposition genes and sequenced. Mutation calling was conducted with Haplotype Caller and Vardict.

**Results:** High quality sequence data was obtained for 38,990 of 39,439 samples (98.9%) and for 99.3% of target regions. Pathogenic mutations in 12 known breast cancer predisposition genes were identified 4.5% of all breast cancer cases and 2.1% of controls; and in 6.7% of African American breast cancer cases and 1.8% of controls. Differences in mutation frequencies were observed by age with mutations in 7.8% of cases diagnosed £50 years of age and 4.0% of cases diagnosed over age 50. Mutations in *ATM*, *BRCA1*, *BRCA2*, and *PALB2* were enriched 2 to 3-fold in cases diagnosed under age 50 relative to older cases. No change in frequency of *CHEK2* mutations by age was observed. In case-control analyses mutations in *BRCA1*, *BRCA2* and *PALB2* were significantly associated with a high risk of breast cancer (odds ratio (OR)>4.0). Of these, *BRCA1* and *BRCA2* displayed ORs of 13.5 and 16.6 in the £50 age group, but only 5.7 and 3.2 in the >50 age group. Only minor age-specific effects were observed for *PALB2*. Mutations in *ATM* and *CHEK2* were associated with moderate risks of breast cancer (OR=2.0 to 4.0) in the younger age group, but not in the older age group.

**Conclusions:** Results from the CARRIERS cohort-based study establish that mutations in known breast cancer predisposition genes are associated with only moderate risks of breast cancer in the general population. However, risks are substantially increased for *BRCA1* and *BRCA2* but not *ATM*, *CHEK2* or *PALB2* mutations in those £50 years of age. The age-related estimates of breast cancer risk for each of the hereditary cancer panel genes in this study may inform selection of individuals in the general population who may benefit from genetic testing and associated risk management strategies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS2-02

Efficacy and utilization trends of adjuvant chemotherapy for stage I, II, and III breast cancer in the elderly population: A National Cancer Database (NCDB) analysis

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**Background:** The role of adjuvant chemotherapy in early stage breast cancer is well established with survival benefit seen in long term follow up studies, but only a small minority of patients in these studies were >65 years old. Dose and schedule can be tailored according to the special requirements of an elderly patient, as stated by the International Society of Geriatric Oncology (SIOG). However the magnitude of the benefit and trends in utilization of adjuvant chemotherapy has not been well studied in this population.

**Methods:** Female patients above 65 years age with stage I to III breast cancer were identified from the NCDB database from 2004-2015. Factors predicting utility of chemotherapy were assessed with multivariate analysis. Kaplan Meier curves were constructed for calculation of overall survival (OS) with hazard ratio (HR) estimated from cox model. Log rank test and pearson chi square was used for comparison between groups. Groups were compared for OS benefit at 5 and 10 years.

**Results:** Of a total of 2,445,730 patients analyzed, 160,676 met our inclusion criteria. Of them, 21,743 were >80 years old. Factors predicting use of adjuvant chemotherapy were shown in table 1.

Table 1-Factors predicting utilization of adjuvant chemotherapy

		No chemotherapy	With chemotherapy	p-value
Histology				<0.0001
	All others	9980 (16.8)	12502 (13.9)	
	Ductal, infiltrating	43453 (73)	69631 (77.2)	
	Lobular	6107 (10.3)	8073 (8.9)	
Grade				<0.0001
	Well differentiated	15268 (25.6)	8182 (9.1)	
	Moderately differentiated	28813 (48.4)	36028 (39.9)	
	Poorly differentiated	15459 (26.0)	45996 (51.0)	
Age				<0.0001
	>80	15766 (26.5)	4501 (5.0)	
	65-80	43774 (73.5)	85705 (95.0)	
Race				<0.0001
	Black	5150 (8.6)	9882 (11.0)	
	White	52420 (88.0)	76956 (85.3)	
	Others	1970 (3.3)	3368 (3.7)	
CDCC				<0.0001
	0	43902 (73.7)	70256 (77.9)	
	1	11883 (20.0)	16219 (18.0)	
	2	2840 (4.8)	3053 (3.4)	
	>3	915 (1.5)	678 (0.8)	
Radiation Therapy				<0.0001
	With Radiation	28978 (48.8)	57507 (64.1)	
	Without Radiation	30395 (51.2)	32184 (35.9)	

Type of Surgery				<0.0001
	Local/partial mastectomy	35823 (60.2)	43026 (47.7)	
	Mastectomy	1234 (2.1)	1005 (1.1)	
	None	22467 (37.7)	46112 (51.2)	
Stage				<0.0001
	1	33574 (56.4)	27829 (30.9)	
	2	20544 (34.5)	41087 (45.5)	
	3	5422 (9.1)	21290 (23.6)	
ER/PR Status				<0.0001
	+/+	42842 (73.1)	46000 (52.0)	
	+/-	7049 (12.0)	13033 (14.7)	
	-/+	451 (0.8)	1315 (1.5)	
	-/-	8279 (14.1)	28155 (31.8)	

OS benefit was seen in patients who received adjuvant chemotherapy regardless of their age, ER, PR, HER-2 status or stage. Patients with TNBC had an HR of 0.547. More benefit was seen in the higher stages. HR for stages I, II, and III were 0.801, 0.608, and 0.666 respectively.

Conclusions: Adjuvant chemotherapy is considered standard of care for patients with early stage breast cancer. Elderly patients are more likely to get adjuvant chemotherapy based on histology, age<80, grade, stage, and hormone receptor status. In this study, we also learn that the OS benefit with adjuvant chemotherapy is significant in all subgroups analyzed for the elderly population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS2-07

PHARE randomized trial final results comparing 6 to 12 months of trastuzumab in adjuvant early breast cancer

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Since 2005, 12 months of trastuzumab added to chemotherapy alone is the standard of care in patients with HER2-positive breast cancer. PHARE ('Protocol for Herceptin® as Adjuvant therapy with Reduced Exposure') is the first trial comparing a reduction of adjuvant trastuzumab versus the standard 12 months. In 2012, the first analysis failed to prove that 6-months was non-inferior to 12-months of adjuvant trastuzumab (NCT00381901). The current presentation reports the final analysis. Methods: The trial was sponsored by the French National Cancer Institute (INCa) ([www.e-cancer.fr](http://www.e-cancer.fr)), and approved by central Ethical Committee on May 15<sup>th</sup> 2006. Patients with HER2-positive early breast cancer were randomly assigned between 12 and 6 months of adjuvant trastuzumab duration. The randomization was stratified by concomitant or sequential trastuzumab administration with chemotherapy, estrogen receptor (ER) status and center. The primary objective was non-inferiority of 6- versus 12-months arms in the intent to treat population, in terms of disease-free survival (DFS) with a pre-specified hazard margin of 1.15. Overall Survival (OS) and metastasis free survival (MFS) were secondary endpoints.

Results: A total of 3380 patients were randomized, their median age was 54 years (21-86). Patients and disease characteristics were well balanced between the two arms. No involved axillary node was observed in 54.5% of cases, 41.7% of tumors were ER negative. At a median follow-up of 7.5 years, 704 events counting for DFS were observed. Between the 12- and 6-months arms, the adjusted Hazard Ratio (HR) for DFS rates was 1.08 (95%CI: 0.93-1.25; p=0.39) favoring the longer exposure. The 1.15 margin of non-inferiority was included in the 95%CI. No heterogeneity in terms of treatment effect was observed, no significant difference for trastuzumab duration effects was found in any subgroups. For OS and MFS, the adjusted HR were 1.13 (95%CI 0.92-1.39) and 1.15 (95%CI 0.96-1.37), respectively.

Conclusion: The choice of the non-inferiority margin will remain inherently a subject of controversy especially in the context of oncology trials where the primary outcome is survival and the least additional death could be considered unacceptable questioning the very feasibility of such trials. Nevertheless, PHARE failed to show that 6 months of adjuvant trastuzumab was non-inferior to 12 months. The standard of care should remain 12 months of adjuvant trastuzumab.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD4-06

A clinical model for assessing the individual breast cancer risk in mammography screening

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**Background.** Mammography screening reduces breast cancer mortality, but is suboptimal for the breast cancers that are not detected by the screening. These women are identified as symptomatic interval cancers with more aggressive tumors and worse prognosis. To efficiently screen for breast cancer the individual breast cancer risk should be determined. We describe a model that is suited for bi-annual screening programs and estimates the 2-year risk of breast cancer. The risk model could be used at most mammography screening units without adding substantial cost.

**Methods.** The study was based on the population based prospective KARMA cohort including 70,877 participants. Mammograms were collected up to five years following baseline mammogram. A prediction model was developed using mammographic features (density, microcalcifications and masses), use of hormone replacement therapy (HRT), family history of breast cancer, menopausal status, and body mass index. Relative risks were calculated using conditional logistic regression and 2-year absolute risks were calculated

**Results.** Comparing women at highest and lowest mammographic density yielded a 5-fold higher risk of breast cancer for women at highest density. When adding microcalcifications and masses to the model, high-risk women had a nearly 9-fold higher risk of breast cancer compared to those at lowest risk. In the full model, taking HRT use, family history of breast cancer and menopausal status into consideration, area under the curve (AUC) reached 0.73.

We calculated the absolute 2-year risk of breast cancer based on national incidence and mortality rates. We also stratified women into risk groups using the NICE guidelines adapted to 2-year risks. The 20% women with moderate or high breast cancer risk were 7.6 times more likely to develop breast cancer compared to the general risk. Also 18% of the women showed 4 times reduced risk compared to the average population.

**Conclusions.** This risk model can improve mammography screening by identifying women that are in need of additional examination procedures. There is also a substantial proportion of women with low breast cancer risk who will have little benefit from screening.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS3-02

**PALLET:** A neoadjuvant study to compare the clinical and antiproliferative effects of letrozole with and without palbociclib

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**Background:** CDK4/6 inhibitors, such as palbociclib, are used to treat ER+ metastatic breast cancer in combination with endocrine therapy with trials ongoing in patients with primary disease. No biomarkers exist to identify those who do/do not benefit from added CDK4/6 inhibition. PALLET is an investigator-initiated/led phase II randomized trial collaboration between UK and NSABP investigators evaluating the biological and clinical effects of palbociclib with letrozole combination as neoadjuvant therapy.

**Methods:** Postmenopausal women with ER+ primary breast cancer and tumors >2.0cm (ultrasound) were randomized to one of 4 treatment groups (3:2:2:2 ratio): Group A: letrozole (2.5mg/d) for 14 weeks; Group B: letrozole for 2 weeks followed by letrozole + palbociclib to 14 weeks; Group C: palbociclib for 2 weeks followed by letrozole + palbociclib to 14 weeks; Group D: letrozole + palbociclib for 14 weeks. Palbociclib was given 125mg/d PO on a 21 days on, 7 days off schedule. Post-14 week treatment was at the discretion of the treating clinician including letrozole until surgery. Core-cut biopsies were taken at baseline, 2 weeks and 14 weeks. Co-primary endpoints for letrozole alone vs palbociclib groups (Group A vs Groups B+C+D) were: (i) change in Ki67 (IHC) between baseline and 14 weeks (log-fold change, Mann-Whitney test); (ii) clinical response (ultrasound) after 14 weeks (4 group, ordinal, Mann-Whitney test). Complete cell-cycle arrest (CCCA) (Ki67≤2.7%) was analyzed using a logistic regression model adjusting for recruitment region. Pre-specified exploratory biomarkers included c-PARP (apoptosis).

**Results:** 307 patients were recruited between 27 Feb 2015 and 08 Mar 2018; 103 were randomized to letrozole alone and 204 to letrozole + palbociclib. 279 (90.9%) patients were evaluable for 14 week clinical response. Clinical response was not significantly different between letrozole vs letrozole + palbociclib groups [(p=0.20; CR+PR 49.5% (46/93) vs 54.3% (101/186) and PD 5.4% (5/93) vs 3.2% (6/186)] nor was the small proportion of patients with pathological CR (1/87, 1.1% vs 6/180, 3.3%; p=0.43). 190 (61.9%) patients were evaluable for 14 week change in Ki67. The median log-fold change in Ki67 was greater with letrozole + palbociclib vs letrozole alone (-4.1 vs -2.2; p<0.001) corresponding to a geometric mean change of -97.4% vs -88.5%. Similarly, a greater proportion of patients who received letrozole + palbociclib achieved CCCA (90% vs 59%, p<0.001). 146 (47.6%) patients were evaluable for c-PARP and the log-fold change (suppression) was greater with letrozole + palbociclib vs letrozole alone (-0.80 vs -0.42; p=0.003) corresponding to a geometric mean change of -56.8% vs -31.4%. Other biomarkers of response / resistance are being evaluated. A higher proportion of patients had a grade ≥3 toxicity on letrozole + palbociclib than letrozole alone (49.8% vs 17.0%; p<0.001) mainly due to asymptomatic neutropenia.

**Conclusion:** Adding palbociclib to letrozole markedly enhanced the suppression of malignant cell proliferation as assessed by Ki67 but did not substantially increase the clinical response of primary ER+ breast cancer over a 14-week period. Concurrent reductions in cell death may have reduced the speed of tumor shrinkage.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS3-03

Effects of prolonging adjuvant aromatase inhibitor therapy beyond five years on recurrence and cause-specific mortality: An EBCTCG meta-analysis of individual patient data from 12 randomised trials including 24,912 women

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Effects of prolonging adjuvant aromatase inhibitor therapy beyond five years on recurrence and cause-specific mortality: an EBCTCG meta-analysis of individual patient data from 12 randomised trials including 24,912 women

**Background:** Five years of endocrine therapy with tamoxifen and/or an aromatase inhibitor is highly effective in reducing the risk of recurrence but a substantial risk remains after treatment discontinuation. Continuing treatment with an aromatase inhibitor may mitigate this risk.

**Methods:** We sought individual patient data for meta-analysis from 12 randomised trials that compared 3-5 years of aromatase inhibitor versus no further treatment after five or more years of endocrine therapy. Primary outcomes were recurrence, and breast cancer mortality. Predefined subgroup comparisons were or prior endocrine therapy (tamoxifen alone, tamoxifen then AI, AI alone), site of recurrence (distant, local, contralateral), age, nodal status, tumour size, grade, and period of follow-up (yrs 0-1, 2-5, 5-9, 10+). Five trials randomised 2-3 years prior to treatment divergence and the primary analyses included only women who were recurrence and second primary cancer free and still alive at the point of treatment divergence.

**Results:** Data have so far been received on 7,488 women (100% of those randomised) in trials of extended AI following tamoxifen alone, 10,796 women (82% of 13,192 randomised) following prior tamoxifen then AI, and 959 (23% of 4,229 randomised) following AI alone. Preliminary analyses including 1,617 breast cancer recurrences and 854 breast cancer deaths confirm a 35% reduction in recurrence with extended AI following tamoxifen alone but suggest a more moderate reduction after prior AI therapy. Data from two trials (NSABP B-42 & N-SAS BC 05) contributing ~5666 women should be available before SABCS to allow definitive analyses.

**Conclusion:** This meta-analysis will provide the most reliable possible summary of the available evidence to inform clinicians on the efficacy of extending AI therapy compared to stopping AI after about 5 years of endocrine therapy in preventing disease recurrence and death from breast cancer, both overall and in different categories of women.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS3-04

A prospective randomized multi-center open-label phase III trial of extending aromatase-inhibitor adjuvant therapy to 10 years - Results from 1697 postmenopausal women in the N-SAS BC 05 trial: Arimidex extended adjuvant randomized study (AERAS)

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**Background:** Treatment with an aromatase inhibitor for 5 years as up-front monotherapy or after tamoxifen therapy for 2-3 years is the treatment of choice for hormone-receptor-positive breast cancer in postmenopausal women. Extending treatment with an aromatase inhibitor to 10 years may reduce the risk of breast cancer recurrence.

**Methods:** We conducted a prospective randomized multi-center open-label phase III trial to assess the effect of the extended use of anastrozole for an additional 5 years. Postmenopausal patients with stage-III, hormone-receptor-positive breast cancer, disease-free after 5 years of either anastrozole alone or tamoxifen 2-3 years followed by anastrozole 3-2 years were randomized to continual group with anastrozole for an additional 5 years or stop group without an additional anastrozole. Our primary end point was disease-free survival.

**Results:** We enrolled 1697 women. After a median follow up of 4.9 years, there were 149 events involving disease recurrence or the occurrence of contralateral breast cancer (51 in continual group and 98 in stop group) and 7 deaths (3 in continual group and 4 in stop group). The 5-year disease-free survival rate was 91.9% (95% confidence interval [CI], 89.4 to 93.8) in continual group and 84.4% (95% CI: 80.0 to 88.0) in stop group (hazard ratio for disease-free survival, 0.548 ;P=0.0004, by a two-sided log-rank test stratified according to nodal status, prior adjuvant chemotherapy, institution, and choice of anastrozole or tamoxifen). The rate of 5-year overall survival was 99.5% in continual group and 99.6% in stop group. (hazard ratio, 1.389 ;P=0.665). The rate of 5-year distant disease-free survival was 97.2% in continual group and 94.3% in stop group (hazard ratio, 0.514 ;P=0.0077).

Bone-related adverse events were observed more frequently among patients in continual group than among patients in stop group, including a higher incidence of bone pain, stiff joints, bone fractures, and new-onset osteoporosis.

**Conclusion:** The extension of treatment with an adjuvant aromatase inhibitor (anastrozole) to 10 years resulted in significantly higher rates of disease-free survival and distant disease-free survival than those with no additional anastrozole, but the rate of overall survival was not different between two groups. Our study shows that it is safe and beneficial for postmenopausal patients with hormone-receptor-positive breast cancer to take an anastrozole as adjuvant therapy for an additional 5 years after initial treatment. (UMIN:000000818)

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS3-05

Prospective optimization of estrogen receptor degradation yields ER ligands with variable capacities for ER transcriptional suppression

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ER+ breast cancers can depend on ER signaling throughout disease progression, including after acquired resistance to existing endocrine agents, providing a rationale for further optimization and development of ER-targeting agents. Fulvestrant is unique amongst currently approved ER ligand therapeutics due to classification as a full ER antagonist, which is thought to be achieved through degradation of ER protein. However, the full clinical potential of fulvestrant is believed to be limited by poor bioavailability, spurring attempts to generate ligands capable of driving ER degradation but with improved drug-like properties.

Here, we evaluate three ER ligand clinical candidates that recently emerged from prospective optimization of ER degradation – GDC-0810, AZD9496 and GDC-0927 - and show that they display distinct mechanistic features. GDC-0810 and AZD9496 are more limited in their ER degradation capacity relative to GDC-0927 and fulvestrant, display evidence of weak transcriptional activation of ER in breast cancer cells (i.e. partial agonist activity), and do not achieve the same degree of *in vitro* anti-proliferative activity as GDC-0927 and fulvestrant. In the HCl-013 (ER.Y537S) and HCl-011 (ER.WT) ER+ patient-derived xenograft models, GDC-0927 drives greater transcriptional suppression of ER, and greater anti-tumor activity relative to GDC-0810.

We found that despite their full antagonist phenotype, GDC-0927 and fulvestrant promote association of ER with DNA, including at canonical ERE motifs, prior to ER degradation. Interestingly however, integration of ER ChIP-Seq and ATAC-Seq data revealed that ER complexed with fulvestrant or GDC-0927 fails to increase chromatin accessibility at DNA binding sites, in contrast to partial agonists which result in increased chromatin accessibility at ER binding sites. Thus, although ER contacts DNA when engaged with fulvestrant and GDC-0927, it is functionally inert. To further explore mechanistic features that might account for the differential activity of full antagonists and partial agonists that occurs prior to ER degradation, we used cell-based fluorescence recovery after photobleaching (FRAP) to measure the kinetics of ER diffusion within the nucleus. We demonstrate that while ER is generally highly mobile, including after engagement with GDC-0810 and AZD9496, GDC-0927 and fulvestrant immobilize intra-nuclear ER. A site saturating mutagenesis screen revealed a series of novel ER mutations that prevent ER immobilization by fulvestrant and GDC-0927. This class of “always mobile” ER variants promotes an antagonist-to-agonist transcriptional switch for fulvestrant and GDC-0927, and simultaneously prevents ER degradation by these molecules, implying that ER immobilization is a key functional determinant of robust transcriptional suppression.

We thus propose that ER degradation is not a driver of full ER antagonism, but rather a downstream consequence of ER immobilization, occurring after a suppressive phenotype has been established at chromatin. We additionally argue that evaluating the transcriptional output of candidate ER therapeutics, both pre-clinically and clinically, will be critical for the identification of ER ligands with best-in-class potential.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS3-06

Dynamics of breast cancer relapse reveal molecularly defined late recurring ER-positive subgroups: Results from the METABRIC study

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**Background:** Recent studies have demonstrated that women with early stage ER-positive (ER+) and HER2-negative (HER2-) breast cancer have a persistent risk of recurrence and cancer related death up to 20 years post diagnosis, highlighting the chronic nature of ER+ breast cancer and critical need to identify tumor characteristics that are more predictive of risk of recurrence than standard clinical covariates. However, progress in delineating the dynamics of breast cancer relapse and biomarkers of late recurrence has been hindered by the lack of large cohorts with long-term clinical follow-up and molecular information.

**Methods:** We report the results of a cohort of 3,240 breast cancer patients from the United Kingdom and Canada with 20 years of follow-up (median 9.75 years), including 1,980 with accompanying molecular data from the primary breast tumor. Information for each patient on loco-regional recurrence (LR), distant recurrence (DR), and site(s) of metastases was collected. We developed a non-homogenous Markov chain model that accounted for different clinical endpoints and timescales, as well as competing risks of mortality and the distinct baseline hazards that characterize different molecular subgroups. This approach enabled robust analysis of the spatio-temporal dynamics of breast cancer recurrence across the clinical subgroups, PAM50 subgroups and the integrative clusters, while also enabling individual risk of relapse predictions.

**Results:** We employed our multistate model to compute the probability of experiencing a LR or DR, as well as the baseline transition probabilities from surgery, LR or DR at various time intervals for average individuals in each of the clinical/molecular subgroups. These analyses reveal four late-recurring ER+ (predominantly HER2-) subgroups, together accounting for 26% of all ER+ tumors, with high (median 42-55%) risk of recurrence up to 20 years post-diagnosis. Each of these four subgroups maps to one of the Integrative Clusters, defined based on genomic copy number alterations and gene expression, and is enriched for a characteristic copy number amplification events: 11q13 (*CCND1*, *RSF1*), 8p12 (*FGFR1*, *ZNF703*), 17q23 (*RPS6KB1*) and 8q24 (*MYC*). These four molecular subgroups are superior in predicting late DR than standard clinical variables.

**Conclusions:** A detailed understanding of the rates and routes of metastasis and their variability across the distinct molecular subtypes is essential for devising personalized approaches to breast cancer care. We describe a molecularly characterized breast cancer cohort with long-term clinical follow-up and a statistical modeling framework, enabling delineation of the dynamics of breast cancer recurrence at unprecedented resolution. These analyses reveal four late recurring ER+ subgroups and accompanying biomarkers that collectively define the quarter of ER+ cases at highest risk of recurrence. Our findings highlight opportunities for improved patient stratification and biomarker-driven clinical trials directed at the subset of breast cancer patients with persistent risk of recurrence.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS4-02

Regional lymph node irradiation in early stage breast cancer: An EBCTCG meta-analysis of 13,000 women in 14 trials

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**Background** There is uncertainty as to which lymph node regions should be irradiated following breast cancer surgery. Systematic review of radiation dosimetry indicates that in randomised trials of nodal radiation therapy (RT) versus not, radiation delivery was qualitatively better in modern trials compared to older trials.

**Methods** We undertook an individual patient data meta-analysis of randomised trials assessing the benefits and risks of RT to different lymph node regions including the axilla, supraclavicular fossa (SCF) and internal mammary chain (IMC). Eligible studies started before 2009, and included a randomisation, or pseudo-randomisation (by left-versus-right sided tumours), in which the only difference between treatment groups was the use, or extent, of nodal irradiation. Surgery/RT to the breast was the same in both arms. Analyses used standard log-rank methods, and were stratified by study, age, nodal status and year of follow-up. – Studies were categorised according to estimated mean heart dose in the nodal RT arm and whether regimens were likely to have delivered ≥85% of prescribed dose to target nodal regions.

**Results** Information was available on 13,132 women in 14 comparisons of nodal RT versus not. There were 3260 recurrences, 2545 deaths from breast cancer and 4147 deaths overall. Eight trials starting 1961–1978, with median follow-up 9.2 (interquartile [IQR] range 3.4–17.5) years, had estimated >8 Gy mean heart dose and likely nodal dose <85% in the nodal RT arm. In these older trials, including 2178 women, nodal RT had no effect on breast cancer recurrence [Rate ratio (RR)=0.98, 95% CI 0.85–1.13, p=0.83] or breast cancer mortality (RR=1.05, 0.91–1.21, p=0.54), but increased non-breast cancer mortality (RR=1.44, 1.20–1.73, p<0.0001), leading to a net increase in any death (RR=1.18, 1.06–1.32, p=0.004).

Six studies starting 1989–2003, with a mean follow-up 9.1 [IQR 7.0–11.0] years, had likely nodal dose ≥85%, and estimated mean heart dose <8 Gy in the nodal RT arm. In these more recent studies, including 10,954 women, nodal RT reduced breast cancer recurrence (RR=0.86, 95% CI 0.79–0.94, p=0.0006), breast cancer mortality (RR=0.81, 0.74–0.90, p<0.0001) and overall mortality (RR=0.86, 0.80–0.93, p=0.0002). No excess of non-breast cancer mortality was apparent (RR=0.96, 0.79–1.18, p=0.71). Recurrence rate ratios did not vary significantly according to nodal region(s) irradiated (axilla/SCF/IMC), or the use of adjuvant chemotherapy.

**Conclusions** RT to regional lymph nodes in older (1961–78) studies increased the overall risk of death, probably explained by radiation exposure of the lungs and heart. Nodal RT in more recent (1989–2003) studies reduced breast cancer recurrence, breast cancer mortality and overall mortality without increasing non-breast cancer mortality. The proportional benefits from today's RT may be larger. Absolute benefits for individual women will depend on their absolute recurrence and breast cancer mortality risks.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS4-03

**RAPID:** A randomized trial of accelerated partial breast irradiation using 3-dimensional conformal radiotherapy (3D-CRT)

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## Background

Whole breast irradiation (WBI) after lumpectomy reduces the risk of local recurrence, thereby avoiding subsequent mastectomy. It is a key component of breast conserving therapy. WBI is usually given in daily fractions over 3-6 weeks. With accelerated partial breast irradiation (APBI), radiation is delivered over a week or less to the surgical cavity with a margin of normal tissue. It was introduced to provide treatment in a shorter more convenient form. 3D-CRT is an attractive approach as it is non-invasive and uses standard techniques for external beam RT that are widely available. The objective of the RAPID trial was to determine if APBI using 3D-CRT was not inferior to WBI following breast conserving surgery (BCS).

## Methods

Women  $\geq 40$  years of age with axillary node-negative invasive ductal carcinoma, or ductal carcinoma in situ (DCIS)  $\leq 3$ cm treated by BCS with clear margins of excision were eligible. Randomization was stratified for age ( $<$  or  $\geq 50$ y), histology (DCIS alone or invasive breast cancer), tumor size ( $<$  or  $\geq 1.5$ cm), ER status (+/-) if invasive disease, and treatment center. Patients were allocated to APBI using 3D-CRT (38.5Gy in 10 fractions delivered twice daily) or WBI (42.5Gy in 16 daily fractions or 50Gy in 25 daily fractions; boost radiation was permitted). The primary outcome was ipsilateral breast tumor recurrence (IBTR). Important secondary outcomes included radiation toxicity and nurse assessed adverse cosmesis (fair or poor on global assessment). The trial was designed to show that the 5-year IBTR rate in the APBI arm was not inferior to the WBI arm by more than 1.5% (hazard ratio [HR]  $\leq 2.02$ ) with 85% power and a one-sided alpha of 5%.

## Results

From February 2006 to July 2011, 2135 patients from sites in Canada, Australia, and New Zealand were randomly assigned: 1070 to APBI and 1065 to WBI. The median follow-up was 8.6 years. The mean age of the study population was 61 years; 82% of patients had invasive breast cancer and 18% had DCIS only. For invasive cancers: 60% were  $< 1.5$ cm and 90% were ER positive. For DCIS tumors: 68% were  $< 1.5$ cm. A total of 65 IBTRs were observed. For the APBI patients, the 5-year and 8-year cumulative rates of IBTR were 2.3% and 3.0%, respectively. The corresponding data for the WBI patients were 1.7% and 2.8%. The HR for APBI versus WBI was 1.27, 90% confidence interval, 0.84 to 1.91. Acute radiation toxicity (occurring within 3 months of treatment start) e.g. radiation dermatitis and breast swelling was less in patients treated with APBI compared with WBI ( $\geq$  Grade 2, 28% vs 45%,  $p < 0.001$ ). Late radiation toxicity (beyond 3 months) e.g. breast induration and telangiectasia was greater in patients treated with APBI ( $\geq$  Grade 2, 32% vs 13%,  $p < 0.001$  and Grade 3, 4.5% vs 1.0%,  $p < 0.001$ ). Adverse cosmesis was higher in patients treated with APBI compared with WBI at 3 years (29% vs 17%,  $p < 0.001$ ) and at 5 years (32% vs 16%,  $p < 0.001$ ).

## Conclusions

The APBI regimen used in our trial was non-inferior to WBI in preventing local recurrence. Although it was associated with less acute toxicity, an increase in late normal tissue toxicity and adverse cosmesis was observed with APBI.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS4-05

Dose escalated simultaneous integrated boost radiotherapy for women treated by breast conservation surgery for early breast cancer: 3-year adverse effects in the IMPORT HIGH trial (CRUK/06/003)

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## Background

IMPORT HIGH is a randomised, multi-centre phase III trial testing dose escalated simultaneous integrated boost (SIB) against sequential boost each delivered by intensity modulated radiotherapy (IMRT) for early stage breast cancer with higher risk of local relapse. The primary endpoint was initially breast induration at 3 years, requiring 840 patients; accrual was extended (target 2568) with the new primary endpoint of local relapse. We report adverse effects (AE) at 3 years.

## Methods

Women age  $\geq 18$  after breast conservation surgery for pT1-3 pN0-pN3a M0 invasive carcinoma were eligible. Randomisation was 1:1:1 between 40Gy/15F to whole breast (WB) + 16Gy/8F sequential photon boost to tumour bed (40+16Gy), 36Gy/15F to WB, 40Gy to partial breast + 48Gy (48Gy) or + 53Gy (53Gy) in 15F SIB to tumour bed. AEs were assessed annually by clinicians in all patients and in a planned sub-set (840) of patients by photographs at 3 years and by patients at 6 months, 1 and 3 years. AE scores were dichotomised as none/mild vs marked for photographs and none/mild vs moderate/marked for patients and clinicians. Fisher's exact tests compared groups; principal comparison (protocol-specified) between 53Gy and 48Gy ( $p < 0.01$  defined as statistical significance).

## Results

2617 women consented between 03/2009 and 09/2015 from 39 UK radiotherapy centres. Median follow-up was 49.1 (IQR 36.8-63.2) months. Median age was 49 (IQR 44-56); 9%, 38% & 53% were tumour grade 1, 2 & 3 respectively; 30% were node positive. 66% received chemotherapy and 73% endocrine therapy. 3-year AE data were available for 2017 clinician assessments, 641 photographs and 842 patient assessments. Proportions of patients with marked AEs were low overall. Rates of moderate/marked AEs at 3 years were broadly similar between the randomised groups; with a suggestion of a slightly increased risk for breast induration in 53Gy compared with control (borderline significance)

AE at 3 years

	40+16Gy n(%)	48Gy n(%)	53Gy n(%)
<b>Clinician</b>			
Breast induration;N	656	668	654
None	451 (69)	483 (72)	445 (68)
Mild	167 (25)	141 (21)	146 (22)
Moderate	32 (5)	42 (6)	56 (9)

	Marked	6 (1)	2 (1)	7 (1)
P-value			0.570 <sup>1</sup>	0.010 <sup>2</sup>
			0.044 <sup>3</sup>	
Breast shrinkage;N		655	669	654
	None	442 (68)	472 (71)	448 (69)
	Mild	167 (26)	161 (24)	166 (25)
	Moderate	40 (6)	33 (5)	35 (5)
	Marked	6 (1)	3 (1)	5 (1)
P-value			0.254 <sup>1</sup>	0.577 <sup>2</sup>
			0.637 <sup>3</sup>	
Breast distortion;N		656	669	654
	None	451 (69)	464 (69)	442 (68)
	Mild	169 (26)	170 (25)	170 (26)
	Moderate	33 (5)	32 (5)	38 (6)
	Marked	3 (1)	3 (1)	4 (1)
P-value			0.903 <sup>1</sup>	0.486 <sup>2</sup>
			0.411 <sup>3</sup>	

### Patient

Change in breast appearance;N		287	264	285
	None	38 (13)	50 (19)	58 (20)
	Mild	164 (57)	151 (57)	142 (50)
	Moderate	57 (20)	45 (17)	54 (19)
	Marked	28 (10)	18 (7)	31 (11)
P-value			0.149 <sup>1</sup>	0.999 <sup>2</sup>
			0.124 <sup>3</sup>	

### Photograph

Change in breast appearance;N		218	210	213
	None	183 (84)	185 (88)	177 (83)
	Mild	25 (11)	23 (11)	32 (15)
	Marked	10 (5)	2 (1)	4 (2)
P-value			0.036 <sup>1</sup>	0.173 <sup>2</sup>
			0.685 <sup>3</sup>	

<sup>1</sup>48Gy v 40+16Gy; <sup>2</sup>53Gy v 40+16Gy; <sup>3</sup>53Gy v 48Gy

### Conclusions

These results represent the largest and most mature reported AE outcomes of breast SIB within a clinical trial. At 3 years, rates of moderate/marked AEs were similar between SIB IMRT and WB + sequential boost IMRT delivered over 3 and 4.5 weeks respectively.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** GS5-01

A randomized community-based trial of an angiotensin converting enzyme inhibitor, lisinopril or a beta blocker, carvedilol for the prevention of cardiotoxicity in patients with early stage HER2-positive breast cancer receiving adjuvant trastuzumab

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## **Background:**

Exposure to trastuzumab for one year is an integral part of therapy for patients with early stage HER2-positive breast cancer. Yet, cardiac side effects, particularly in patients who also receive anthracyclines require frequent monitoring and result in dose interruptions and discontinuation of trastuzumab. Prophylactic use of angiotensin converting enzyme (ACE) inhibitors or beta blockers (BB) may prevent cardiotoxicity associated with chemotherapy and trastuzumab.

## **Methods**

A large community-based prospective double-blind, placebo-controlled trial, evaluated the rates of pre-specified cardiotoxicity in patients with early stage breast cancer treated with one year of trastuzumab. Cardiac events were followed for two years. Patients were randomized to simultaneously receive either the ACE inhibitor, lisinopril, or the BB, carvedilol, or placebo and were further stratified by anthracycline use to determine whether ACE inhibitors or BB can prevent trastuzumab-induced decrease in left ventricular ejection fraction (LVEF) and trastuzumab interruptions.

## **Results:**

The study included 468 eligible patients (median age:51, BMI:27 kg/m<sup>2</sup>, baseline systolic BP: 126mmHg and LVEF :63 ± 6.29%) from 127 community-based practices, 189 patients received an anthracycline. For the entire study population and the non-anthracycline group, no difference in number of trastuzumab interruptions were seen. For patients receiving an anthracycline, cardiac event rates were higher in the placebo group (47%), and reduced in both the lisinopril (37%), and the carvedilol (31%) groups. Interruptions of trastuzumab were required in 23% patients on lisinopril and 20% on carvedilol compared to 40% on placebo (p=0.007). Changes in LVEF from baseline (least square means, SE) were significantly reduced with both carvedilol (-4.5 (0.8), p=0.008, and lisinopril (-4.0 (0.8), p=0.002) than placebo, (-7.7 (0.8). Cardiotoxicity-free survival was longer on both carvedilol (hazard ratio 0.49, 95% confidence intervals 0.27, 0.89, p=0.009) or lisinopril (HR 0.53, CI 0.30, 0.94, p=0.015).

## **Conclusions**

In patients with HER2-positive breast cancer receiving trastuzumab and an anthracycline, both lisinopril and carvedilol during treatment reduced cardiotoxicity in patients, but not in those with non-anthracycline containing regimens. The use of lisinopril or carvedilol may allow the use of an anthracycline without compromising trastuzumab treatment in those who might benefit from an anthracycline.

# 2018 San Antonio Breast Cancer Symposium ®

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Resistance to neoadjuvant chemotherapy in triple negative breast cancer mediated by a reversible drug-tolerant state

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Approximately 50% of patients with localized triple negative breast cancer (TNBC) have substantial residual cancer burden following treatment with neoadjuvant chemotherapy (NACT), resulting in distant metastasis and death for most of these patients. While genomic and phenotypic intra-tumor heterogeneity are pervasive features of TNBCs at the time of diagnosis, the functional contributions of heterogeneous tumor cell populations to chemoresistance have not been elucidated.

To investigate tumor evolution accompanying NACT, we employed orthotopic patient-derived xenograft (PDX) models of treatment-naïve TNBC, which retain intra-tumor heterogeneity characteristic of human TNBC. We discovered that some PDX models initially exhibited partial sensitivity to standard front-line NACT (Adriamycin plus Cyclophosphamide, AC). Following AC, residual tumors were resistant to chemotherapy but repopulated tumors with chemo-sensitive cells if left untreated, indicating that tumor cells possessed inherent plasticity. To identify the tumor cell subpopulation(s) conferring chemoresistance, we conducted barcode-mediated clonal tracking in three independent PDX models by introducing a high-complexity pooled lentiviral barcode library into PDX tumor cells which were then orthotopically engrafted into recipient mice. Strikingly, residual tumors maintained the same heterogeneous clonal architecture as naïve tumors. Concordantly, whole-exome sequencing revealed conservation of genomic subclonal architecture throughout treatment. These results were corroborated by genomic sequencing of serial biopsies pre- and post-AC obtained directly from TNBC patients enrolled on an ongoing clinical trial at MD Anderson (ARTEMIS; NCT02276443). Together, these studies revealed that genomically distinct pre-treatment subclones were equally capable of surviving AC to reconstitute tumors after treatment.

To identify functional addictions of residual tumor cells, we conducted histologic and transcriptomic profiling. Residual tumors following AC-treatment exhibited extensive fibrotic desmoplasia and tumor cell pleomorphism in both PDX models and in serial biopsies obtained from TNBC patients enrolled on the ARTEMIS trial. Strikingly, these AC-induced features were reverted upon regrowth of residual tumors in PDXs and in patients' tumors. Similarly, residual tumors exhibited unique transcriptomic features, many of which are also de-regulated in cohorts of human TNBCs undergoing chemotherapy treatment. These features were nearly completely reverted after tumors regrew, suggesting that the residual tumor state may be a unique and transient therapeutic window. Gene set enrichment analyses revealed that residual tumors had increased activation of oxidative phosphorylation and decreased glycolytic signaling. Pharmacologic targeting of oxidative phosphorylation with a small-molecule inhibitor of mitochondrial electron transport chain complex I (IACS-010759) significantly delayed the regrowth of AC-treated residual tumors in three independent PDX models. Collectively, these studies reveal that a reversible phenotypic state can confer chemoresistance in the absence of genomic selection and that the residual tumor state is a novel therapeutic window for chemo-refractory TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

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No survival benefit of chemotherapy escalation in patients with pCR and “high-immune” triple-negative early breast cancer in the neoadjuvant WSG-ADAPT-TN trial

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**Background:** Immune markers such as tumor infiltrating lymphocytes (TILs), CD8, PDL1, PD1 and other protein or mRNA-based genomic markers have been identified as prognostic / predictive in TNBC regarding survival / chemotherapy (CTx) efficacy. In the adjuvant WSG-PlanB trial, patients with high TILs and/or CD8 by mRNA had excellent outcome, irrespective of anthracycline use; in the neoadjuvant ADAPT-TN trial, high PDL1, PD1 and CD8 and/or TILs were predictive for pCR. Still, optimal markers for potential treatment de-escalation have yet to be determined. Here, we analyse for the first time impact of immune mRNA-based markers and TIL's as prognostic and predictive survival markers.

**Methods:** TNBC patients (ER/PR<1%, HER2-,) were randomized to neoadjuvant 4x nab-paclitaxel 125 mg/m<sup>2</sup>/gemcitabine 1000 mg/m<sup>2</sup> d1/8 q3w (gem arm) or 4x nab-paclitaxel 125 mg/m<sup>2</sup>/carboplatin AUC2 day 1/8 3-weekly (q3w) (carbo arm). Primary endpoint of WSG-ADAPT-TN was pCR (ypT0/is/ypN0); secondary endpoints included translational analyses, e.g., TILs or expression of 119 genes by nCounter platform. Standard adjuvant chemotherapy (4xEC) was optional (not randomized) in patients achieving pCR after 12 weeks. According to protocol, 1<sup>st</sup> safety survival analysis was performed after 3y median follow-up.

**Results:** Present translational analysis included 306 of 336 TNBC patients (36 months median FU). pCR was associated with significantly better survival (3y EFS: 92% vs. 71%, p<.001), but despite substantially higher pCR in the carbo arm (46% vs. 29%), no significant EFS advantage was seen (p=.6) (gem: 78%; carbo: 80%; 3y-EFS).

Bivariate Spearman correlations among CD8, PD1, and PDL1 were strongly positive; their correlations with TILs were moderately positive.

Preliminary Cox analysis of EFS was performed with clinical variables (cN, cT, menopausal status); neoadjuvant study arm; pCR; TILs; proliferation markers (baseline Ki67 by IHC, scores derived from PAM50); baseline immune markers; risk scores; and individual gene expression scores previously identified as prognostic for pCR in one or both neoadjuvant arms. Independent prognostic factors included pCR, cN, Ki67, PD1, and CD8; these were entered into (prognostic) interaction analysis. The resulting model contained cN, high Ki67 and low TILs as (unfavorable) main effects and the interaction of (higher) PD1\*pCR (favorable). Among pCR patients, the groups with/without additional adjuvant CTx were similar with respect to explanatory factors. Baseline TILs, Ki67, cN, and PD1 were entered into exploratory predictive analysis; the model retained only the interaction [adjuvant CTx \* (fractionally ranked) PD1]. In patients with pCR, those with low PD1 benefited from standard anthracycline-containing adjuvant CTx, whereas patients high PD1 did not with an 98% 3y-EFS.

**Conclusions:** Our exploratory results suggest independent prognostic impact of mRNA markers and TIL's in early TNBC. Patients with both pCR (after 12 weeks) and “high-immune” signature (defined here by PD1) had excellent 3y-EFS and may be candidates for treatment de-escalation (e.g. omission of anthracyclines), whereas “low-immune” pCR patients may benefit from standard adjuvant poly-chemotherapy.

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International pooled analysis of the prognostic impact of disseminated tumor cells from the bone marrow in early breast cancer: Results from the PADDY study

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## Introduction

As early breast cancer might relapse even after complete removal of breast and lymphnodes, the disease must persist in secondary sites. The detection of disseminated tumor cells (DTC) in the bone marrow (BM) has been described as a surrogate of residual disease. Various trials showed an impaired prognosis of DTC positive early breast cancer (EBC) patients. The PADDY (Pooled Analysis of DTC Detection in Early Breast Cancer) study is a large international pooled analysis that aimed to assess the prognostic impact of DTC detection in patients with EBC.

## Methods

A pre-specified protocol was followed, and centers known to practice BM sampling for DTC detection were contacted for individual patient data. Patients with EBC, with available follow-up data and BM sampling before any anti-cancer treatment were eligible. BM aspirates were collected at the time of primary surgery. DTC were identified by antibody (A45-B/B3, AE1/AE3, 2E11 and E29) staining against cytokeratin. The DTC status was compared to other prognostic factors using the chi-squared test. Univariate log-rank test and multivariate cox regression were used to compare survival of DTC positive versus DTC negative patients.

## Results

Individual data from 10,320 patients (11 centers from Europe and USA) were included with a median follow-up of 91 months. Of all patients, 2,823 (27.4 %) were DTC positive. DTC detection was associated with higher tumor grade, higher T stage, nodal positivity, ER and PR negativity, and HER2 positivity (all  $p < 0.001$ ). In univariate analyses, overall, breast cancer specific, disease-free and distant disease-free survival (OS, BCSS, DFS, DDFS) were significantly shorter in DTC positive patients with  $p$ -values of  $< 0.001$ . Multivariate analyses showed the DTC status to be an independent prognostic marker for OS, BCSS, DFS and DDFS with hazard ratios (HR) and 95%-confidence intervals (CI) of 1.23 (95%-CI: 1.06-1.42,  $p = 0.007$ ), 1.38 (95%-CI: 1.11-1.72,  $p = 0.004$ ), 1.29 (95%-CI: 1.10-1.50,  $p = 0.001$ ) and 1.32 (95%-CI: 1.10-1.58,  $p = 0.003$ ), respectively.

## Conclusions

Detection of DTC in the bone marrow is an independent prognostic marker in patients with non-metastatic breast cancer. Further studies should investigate the impact of DTC on metastatic cancer progression and their role for clinical decision making.

# 2018 San Antonio Breast Cancer Symposium ®

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## Surgical treatment after neoadjuvant systemic therapy in young women with breast cancer: Results from a prospective cohort study

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### Background

Young women are more likely than older women to present with higher stage breast cancer (BC) and may benefit to a greater extent from downstaging with neoadjuvant systemic treatment (NST). Young age is also associated with greater likelihood of pathologic complete response (pCR). Using a large prospective cohort of young women with BC, we investigated response to neoadjuvant therapy, eligibility for breast conserving surgery (BCS) pre- and post-NST, and surgical treatment.

### Methods

The Young Women's Breast Cancer Study (YWS) is a multi-center cohort of women diagnosed with BC at age  $\leq 40$ , that enrolled 1302 patients from 2006 to 2016. Disease characteristics and treatment information were obtained through medical record and central pathology review. Surgical recommendation before and after NST, conversion from BCS borderline/ineligible to BCS eligible, surgery, documented reasons for choosing mastectomy (MTX) among BCS eligible women, and final pathologic response were independently reviewed.

### Results

Among 1302 women enrolled in YWS, 801 (62%) presented with unilateral stage I-III breast cancer and 317(40%) received NST. Median age was 36 years old (22-40). Pre-NST, 85/317 (27%) were BCS eligible, 49 (15%) were borderline, and 169 (53%) were not eligible (16 inflammatory breast cancer (IBC), 88 large tumor size /cosmetic, 48 diffuse calcifications, and 83 multicentricity). Among the 218 patients who were BCS ineligible/borderline pre-NST, 82 (38%) became eligible for BCS after NST. 4 patients who were BCS eligible pre-NST became ineligible. Of all patients eligible for BCS post-NST (n=163), 80 (49%) attempted BCS, 74 (93%) of whom were successful, and 83 (51%) chose MTX. Reasons for choosing MTX included: patient preference (38/83 (46%)), BRCA or TP53 mutation (31 (37%)), family history (3 (4%)), unknown (11 (13%)). On final pathology, 75 (24%) patients had pCR. Among patients who achieved a pCR, 48 (64%) underwent MTX, fewer than half (21/48 (44%)) were for anatomic indications (IBC, large tumor at diagnosis, diffuse calcifications, multicentric disease).

### Conclusion

While NST doubled the proportion of young women eligible for BCS, nearly half chose MTX regardless of response to NST, mostly for personal preference or high-risk preventative reasons. These data highlight that surgical decision making among young women with breast cancer is often driven by factors beyond extent of disease and clinical response to therapy.

Table 1. Clinical-pathologic characteristics

Characteristics	Number	%
Pre NST surgical recommendation		
BCS eligible	85	26.8
Borderline	49	15.5
BCS ineligible	169	53.3
Unknown	14	4.4
Clinical Response		

Complete	202	63.7
Partial	92	29.0
Stable	3	0.9
Progressing	7	2.2
Unknown	13	4.1
Pathologic Response		
pCR (No invasive or DCIS)	75	24
No pCR	242	76
Post NST Surgical recommendation		
BCS eligible	163	51.4
BCS ineligible	144	45.4
Unknown	10	3.2
Attempted surgery		
BCS	80	25.2
MTX	236	74.1
Unknown	2	0.6
Final Surgery		
BCS	74	23.3
MTX	241	76
unknown	2	0.6

# 2018 San Antonio Breast Cancer Symposium ®

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Symptoms and health-related quality of life on endocrine therapy alone (E) versus chemoendocrine therapy (C+E): TAILORx patient-reported outcomes results

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**Background:** TAILORx patient-reported outcomes (PRO) quantify symptoms and health-related quality of life (HRQL) from C+E beyond E alone from the patient's perspective, thus can inform decision-making for women in the intermediate risk group for whom chemotherapy may still be considered.

**Methods:** TAILORx participants with OncoType DX Recurrence Scores 11-25 were randomly assigned to E or C+E. All TAILORx participants enrolled 1/2010-10/2010 (N=612) completed PROs measuring fatigue, endocrine symptoms, cognitive impairments (PCI), and fear of recurrence at baseline, 3, 6, 12, 24 and 36 months. HRQL was assessed at baseline, 12, and 36 months. Linear regression (LR) examined PRO scores among the per-protocol sample.

**Results:** Overall, participants reported significantly more fatigue, endocrine symptoms and PCI at 3, 6, 12, 24 and 36 months compared to baseline and those randomized to C+E reported a greater magnitude of change baseline-3 months compared to those randomized to E alone (Table 1). Overall, by 12 months symptoms were comparable between groups. Pre-menopausal women had comparable symptoms at 24 and 36 months. Post-menopausal women randomized to C+E had greater endocrine symptoms at 24 and 36 months and greater fatigue at 6 and 24 months. Fear of recurrence was comparable between arms during treatment and follow-up. Multiple linear regression identified increased fatigue (LR slope  $\beta=0.67$ ), endocrine symptoms ( $\beta=0.14$ ), and PCI ( $\beta=0.11$ ) as significant predictors of decreased HRQL across arms ( $p<0.001$ ). HRQL was comparable between E and C+E at 12- and 36-months.

Mean PRO change scores from baseline by treatment arm and menopausal status in per protocol population

	Months				
	3	6	12	24	36
N=Overall	454	469	458	384	343
n=Pre-menopausal	153	151	150	118	103
n=Post-menopausal	301	318	308	266	240
FACIT-Fatigue					
Overall sample					
C+E	-8.77	-4.37	-4.01	-4.27	-3.67
E	-2.48	-1.97	-2.14	-1.49	-1.83
LMED	-5.32***	-1.55	-1.01	-1.76	-0.90
Pre-M					
C+E	-8.01	-3.26	-2.99	-2.45	-1.60
E	-3.87	-1.66	-1.32	-2.52	-2.11
LMED	-3.11	-0.82	-1.12	1.02	1.46
Post-M					

C+E	-9.22	-4.97	-4.55	-5.14	-4.67
E	-1.87	-2.10	-2.52	-1.09	-1.71
LMED	-6.42***	-1.99*	-1.16	-3.02*	-2.01
FACT-Endocrine Symptoms					
Overall sample					
C+E	-5.56	-5.63	-6.96	-6.81	-7.14
E	-3.61	-4.24	-5.62	-5.31	-5.17
LMED	-1.62*	-0.97	-1.08	-1.05	-1.69
Pre-M					
C+E	-7.62	-8.34	-7.94	-8.29	-8.96
E	-5.96	-6.19	-8.95	-10.39	-10.84
LMED	-1.44	-1.63	1.06	2.27	2.18
Post-M					
C+E	-4.39	-4.19	-6.45	-6.10	-6.28
E	-2.55	-3.41	-4.10	-3.23	-2.87
LMED	-1.49	-0.45	-2.04	-2.39*	-3.17**

Significance between mean change scores \*p<0.05;\*\*p<0.01;\*\*\*p<0.001. LMED=estimated tx difference using linear model regressing score on baseline value and tx

Conclusions: TAILORx is the first trial to examine patient-reported fatigue, endocrine symptoms, PCI and HRQL among breast cancer patients randomized to endocrine therapy alone vs chemoendocrine therapy, thus allowing us to quantify acute and long-term symptoms uniquely attributable to chemotherapy. As expected, chemotherapy is associated with greater fatigue, endocrine symptoms and PCI acutely during treatment, and for post-menopausal women with greater long-term endocrine symptoms. Increased symptoms were associated with poorer HRQL. Long-term HRQL was comparable between groups.



# 2018 San Antonio Breast Cancer Symposium ®

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Development and validation of a chemotherapy toxicity (Chemo Tox) risk score for older patients (Pts) with breast cancer (BC) receiving adjuvant/neoadjuvant treatment (Adjuvant Tx): A R01 and BCRF funded prospective multicenter study

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**Background:** Older pts with BC receiving adjuvant tx are at increased risk of chemo tox; however, no BC-specific tool exists to quantify this risk. The Cancer and Aging Research Group (CARG) developed/validated a chemo tox score for older pts with all stages of solid tumor. The goals of this study were to: 1) build upon the CARG score by developing/validating CARG-BC (a BC specific adjuvant chemo tox score for older pts) and 2) evaluate its association with dose modifications, reduced relative dose intensity (RDI) and hospitalizations.

**Methods:** 501 pts age ≥65 with stage I-III BC from 16 sites were accrued (300 development; 201 validation cohort). A pre-chemo assessment captured: CARG chemo tox score, BC tumor/tx variables, and additional geriatric assessment (GA) items. Grade 3-5 chemo tox by NCI CTCAE v 4.0 was captured. Univariate analysis identified chemo tox risk factors (p<0.10) in addition to the CARG score which were used to develop a predictive model by best subset method; each risk factor was assigned a score, and summed to yield a total score (CARG-BC). Model performance was assessed by area under the ROC curves (AUC) of the development cohort, 10-fold internal validation, external validation, and goodness of fit.

**Results:** Among 501 pts, 28 received non-standard regimens and were excluded, leaving 473 evaluable pts: 283 development and 190 validation cohort. The development cohort (median age 70; range 65-85) had Stage I (39%), II (41%), & III (20%) BC with 65% hormone positive, 24% triple negative, 27% Her2 positive; and 37% received an anthracycline. Grade 3-5 tox occurred in 46% (36% grade 3, 10% grade 4, 0.4% grade 5). The CARG score was significantly associated with grade 3-5 tox (p<0.001; AUC 0.64). The addition of BC tumor/tx & GA variables (CARG-BC: see table) improved the AUC to 0.76 (95% CI, 0.70-0.82; goodness of fit p=0.28). The score ranged from 0-19, (low risk 0-5, mid risk 6-9, high risk 10+) and was significantly associated with grade 3-5 tox (p<0.001) while KPS was not (p=0.20). The 10-fold internal validation AUC was 0.78. The external validation AUC (0.69) was not statistically different (p=0.15) from the development AUC. A higher CARG-BC score was associated with dose delay/reduction, chemo discontinuation, hospitalization, and RDI<85% (all p-value <0.001).

**Conclusions:** We developed and validated a risk score (CARG-BC) which identifies an older pt's risk for adjuvant BC chemo tox and is associated with dose reduction, delay, reduced RDI, and hospitalization. This tool could be considered as a part of adjuvant tx decision-making.

## Chemo Tox Risk Score for BC (CARG-BC)

	Grade 3-5 Tox (%)	Score
<b>CARG-Score:</b> age, # of chemo drugs, dose, hemoglobin, creatinine clearance, hearing, falls, ability to walk 1 block and take meds, decreased social activities		
Low	36	0
Middle	57	3
High	61	3
<b>Stage</b>		

I	33	0	
II/III	55	2	
Planned Tx Duration			
≤ 3 mo.	33	0	
> 3 mo.	58	4	
Anthracycline			
No	38	0	
Yes	59	1	
Liver Function			
Normal	45	0	
Abnormal	62	3	
Ability to Walk a Mile			
Not limited	37	0	
Limited	61	3	
Someone to Provide Advice			
Most of Time	44	0	
None to Some of Time	61	3	
CARG-BC Risk Score			
Low	21	0-5	
Middle	45	6-9	
High	79	10+	

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: GS6-05

The impact of breast cancer surgery on quality of life: Long term results from E5103

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**Background:** Breast cancer (BC) treatment, including surgery, can impact not only short-term health outcomes but may also affect longer term health-related and psychosocial quality of life (QOL). We sought to describe the impact of BC surgery on QOL among breast cancer survivors followed in a large randomized trial.

**Methods:** The ECOG-ACRIN protocol E5103 was a phase III trial that randomized BC patients (pts) who had undergone definitive BC surgery to receive adjuvant doxorubicin, cyclophosphamide, and paclitaxel with either bevacizumab (bev) or placebo. Telephone based surveys were administered to all pts enrolled between 01/Jan/10 and 08/Jun/10 as part of a Decision-Making/QOL component until 18 mos post enrollment. Functional/psychosocial QOL domains were assessed by the EQ-5D-3L and the FACT B+G. Fisher's exact test compared categorical and Wilcoxon rank sum test compared continuous variables between subgroups. Multivariable regression was used to evaluate factors in addition to primary surgery at enrollment (age, race, ER/PgR status, tumor size, nodal status) associated with overall FACT score at 18 mos.

**Results:** Patient reported outcomes at 18 mos were available from 89.6% (465/519) pts. At enrollment, 57% (266/465) had a mastectomy; 43% (199/465) breast conserving surgery (BCS). Median age at enrollment was 52 (range: 25-76) years. There were no differences in QOL between bev vs placebo treatment arms (EQ-5D-3L Index Score p=0.65; FACT B+G Score p=0.23) at 18 mos so groups were combined. Using EQ-5D-3L, over half of the pts (58%) reported at least some pain/discomfort; 38% symptoms of anxiety/depression. A higher proportion of mastectomy pts reported problems with usual activities compared to BCS pts (Table). Compared to BCS pts, mastectomy pts had lower average EQ5D-3L scores 0.80 vs. 0.84, p=0.04 and FACT B+G scores 109 vs. 114, p=0.01, indicating worse QOL. In univariate analyses, non-white race (p=0.03), ER/PgR+ status (p=0.04) and mastectomy as primary surgery (p=0.01) were significantly associated with worse QOL (lower FACT B+G scores). In multivariable analyses, non-white race (p=0.02) and ER/PgR+ status (p=0.05) remained associated with worse QOL; mastectomy was borderline significant (p=0.06).

**Conclusions:** Among women participating in a contemporary adjuvant BC chemotherapy trial, a substantial proportion of survivors experience symptoms that may be amenable to intervention, including referral to physical rehabilitation, especially among pts undergoing more extensive surgery. Attention to psychosocial health is also essential both during and after completion of active treatment to optimize QOL outcomes.

5 Dimensions	N(%) reporting problems*		Overall	p**
	BCS	Mastectomy		
Mobility	44(23)	59(23)	103(23)	1.00
Self-care	11(6)	23(9)	34(7)	0.21
Usual activities	49(25)	90(34)	139(30)	0.04
Pain/discomfort	104(53)	161(61)	265(58)	0.08
Anxiety/depression	70(36)	105(40)	175(38)	0.44

\*3L: 3 possible answers: 1) no problems 2) some/moderate problems 3) problems; responses then collapsed into no problems vs. any problems' (=some/moderate problems and problems). \*\* Fisher's exact test p-value.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-01-01

Systemic imaging fails to detect metastasis in early stage breast cancer

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**Background:** Current NCCN guidelines for early stage breast cancer (Stage I and II) do not recommend routine systemic imaging in the absence of symptoms or abnormal labs suggestive of distant metastasis. This study aims to determine the frequency and appropriateness of these imaging studies performed, its impact on staging and the factors that influence physicians in ordering these imaging studies.

**Methods:** Patients with stage I and II breast cancer at initial presentation were retrospectively identified between years 2011-2015 from the tumor registry. Charts were reviewed to determine patients who got systemic imaging (CT scan, non-breast MRI, bone scan or PET scan) within 6 months of diagnosis. Provider notes and laboratory data were analyzed to establish the appropriateness of ordered imaging studies and if the imaging altered the stage. For each patient in the study, age at diagnosis, the grade of the breast tumor, hormonal receptor status and HER-2 status was documented. Statistical analysis was done using appropriate tests.

**Results:** A total of 1067 patient charts were screened, of which 882 were identified for inclusion in the study (544 stage I, 338 stage II). Amongst the cohort, 18.57% (101) of patients with stage I and 50.89% (172) of patients with stage II cancer received imaging studies within the first 6 months of diagnosis. Only 12.68% (69) of stage I patients and 18.24% (62) of stage II patients were judged appropriate for imaging based on symptoms and lab results suggesting metastasis. In the imaged cohort of Stage I patients, only 4.35% (3) of the appropriately imaged group and 13.33% (4) of the inappropriately imaged group had a change in stage. Similarly, in the Stage II cohort, only 4.84% (3) of the appropriately imaged group and 8.18% (9) of the inappropriately imaged group saw a change in state. The difference in stage change in the appropriately and inappropriately imaged groups was not statistically significant. ( $p = 0.11$  for Stage I,  $p=0.41$  for Stage II). Only 5.9% of Stage I and 2.9% of Stage II imaged patients changed to stage IV. Grade 1 patients were less likely to receive systemic imaging than grade 2 and 3 patients ( $p < 0.001$ ). Similarly, the difference in imaging rates ordered in patients with ER and/or PR negative status versus ER and PR positive status was significant ( $p=0.0004$ ). Triple negative ( $p < 0.001$ ) status and age  $\leq 50$  years were statistically significant predictors of patients receiving imaging ( $p = 0.014$ ). HER-2 status alone was not a significant predictor of getting imaged ( $p= 0.527$ ).

**Conclusions:** We performed the first ever study to investigate a correlation between the appropriateness of ordered imaging studies in early stage breast cancer and its ability to detect a change in stage. Distant metastasis identification among stage I & II patients was extremely rare among both appropriately and inappropriately imaged groups. Our findings suggest a wide prevalence of inappropriately ordered imaging studies in Stage I and II breast cancer as well as limited utility for even appropriately ordered ones. Further, other factors such as grade of the tumor, ER/PR/HER2 status and age were found to be statistically significant predictors of whether patients received imaging studies.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-01-02

Quantitative breast MRI to predict response to neoadjuvant therapy in community imaging centers: Preliminary results

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**Introduction:** Early response assessment to neoadjuvant therapy (NAT) for locally advanced breast cancer would allow for more accurate prognosis and provide the opportunity to replace an ineffective treatment with an alternative regimen. This could potentially increase systemic treatment efficacy and avoid unnecessary side effects from ineffective therapies. Quantitative MRI has been shown to be beneficial in predicting breast tumor response to treatment early during the course of NAT within many academic environments. Importantly, integrating quantitative imaging techniques into the community-based setting has the potential to reach a large percentage of breast cancer patients, as most patients receive their care at private practice or community hospitals. This study evaluated the ability to implement quantitative dynamic contrast enhanced MRI (DCE-MRI) and diffusion-weighted MRI (DW-MRI) data in the community setting to predict the eventual response of breast tumors to NAT.

**Experimental Design:** Women undergoing NAT for breast cancer (N=16) were scanned with DCE-MRI and DW-MRI at baseline (prior to beginning therapy,  $t_1$ ) and three longitudinal time points during the course of NAT to evaluate early response to therapy ( $t_2$ ,  $t_3$ ,  $t_4$ ). MRI was performed at two community imaging centers using a 3T Siemens Skyra scanners equipped with 8- or 16-channel breast coils. DW-MRI was acquired with a spin echo sequence with  $TR/TE = 3000/52$  ms,  $b$ -values of 200, 800 s/mm<sup>2</sup> and used to compute the apparent diffusion coefficient (ADC) values for every voxel. DCE-MRI data was collected (following a pre-contrast  $T_1$  map) with  $TR/TE/\alpha = 7.02$  ms/4.60 ms/6°, and a temporal resolution of 7.27 sec for eight minutes. A catheter placed within an antecubital vein delivered gadolinium-based contrast agent (0.1 mmol/kg of Multihance or 10 mL of Gadovist) at 2 mL/sec via a power injector after the acquisition of the first minute of dynamic scans (baseline). Quantitative measures of ADC (evaluating cellularity from DW-MRI) and  $K^{trans}$  (evaluating vascular perfusion and permeability from DCE-MRI) were calculated for the segmented tumor volume. Imaging was compared to pathology reports at the conclusion of NAT.

**Results:** The patients (n = 6) achieving a pathological complete response (pCR) revealed a  $12.9\% \pm 19.1\%$  increase in the mean ADC values of the tumor from  $t_1$  and  $t_2$ . Conversely, patients (n = 10) that had residual disease burden after NAT (i.e., a non-pCR) had a decreased ADC, revealing a  $-8.0\% \pm 19.2\%$  change between  $t_1$  and  $t_2$  ( $p = 0.06$ ). The mean  $K^{trans}$  values of the tumor decreased showing a change of  $-61.4\% \pm 18.2\%$  from  $t_1$  to  $t_2$  in the pCR patients. Conversely, non-pCR patients had a  $10.2\% \pm 80.4\%$  increase in  $K^{trans}$  between  $t_1$  and  $t_2$  ( $p = 0.14$ ).

**Conclusion:** Preliminary evidence reveals that quantitative DCE-MRI and DW-MRI can be implemented in community-based imaging settings to predict the response of breast tumors to NAT. Thus, our results provide evidence that quantitative DW-MRI and DCE-MRI can be disseminated across community imaging facilities, thereby dramatically increasing the patient population for which these techniques can serve.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-02-01

Improvement of breast cancer screening access and quality in an underserved population through system interventions

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**Background:** Differences in access to and quality of screening and treatment are proposed to contribute to racial disparities in breast cancer outcomes. Interventions designed to improve mammography access and quality encompass strategies at the individual patient, healthcare provider, and system levels. In 2016, an urban safety net healthcare system based in Chicago implemented several changes in response to collected data showing variations in quality at institutions performing mammography. These changes included the installation of digital machines at one of four sites, centralizing reading of images from all four sites to a single site with radiologists specialized in mammography and increasing care coordination including enhanced patient outreach efforts. We examined the impact of these systems-based interventions on the access to and quality of mammography services.

**Methods:** Data was obtained on 15,918 screening mammograms performed across four mammography centers within the Cook County Health & Hospitals System from the six months prior to and one year after implementation of changes. Manual chart abstraction was performed for each study that was assessed as BIRADS 0 (Breast Imaging and Reporting Data System), meaning an incomplete study requiring additional imaging evaluation, or mammograms that appeared suspicious or highly suspicious and categorized as BIRADS 4 or 5. Screening mammogram volume at each site was recorded and compared. Quality of screening mammograms was assessed using eleven metrics reflecting radiologist performance and efficiency of facility care processes. These metrics included the rate of recall, cancer detection rate, proportion of cancers that were early stage or minimal in size, proportion of women with timely follow-up imaging and biopsy, and rates of loss to follow-up.

**Results:** The volume of screening mammograms completed at each of the four sites increased from the six months prior to intervention to the six months after (range of 61-322% increase). At one-year post intervention, there were smaller, but sustained increases in volume (range of 12-70% increase). Improvements were seen in at least one quality metric at each site in the post-intervention period (range 1-8). The proportion of women with timely follow-up after abnormal mammogram also improved across all four sites: from 38% getting follow-up imaging within 30 days pre-intervention to 68% after, and from 62% to 75% of women with biopsy completion within 60 days. Rate of cancer detection improved at two of the sites with the lowest pre-intervention values, from 1.7 to 3.1 and 2.8 to 5.7 per 1,000 mammograms (quality benchmark: 3-10/1,000 screening tests). **Conclusion:** Improvements in access to and quality of screening mammography demonstrate the value of implementing system level changes in enhancing breast cancer care and may translate to better outcomes for all women.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-02-02

Is ultrasound screening justified for non-palpable breast cancer in asymptomatic Chinese women: A real-world study based on long-term survival of consecutive cohort (2001-2017)

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**Background:** Ultrasound (US) is an effective initial screening test for breast cancer both in Caucasian and Chinese women [PMID: 26712110, 26715161, and 25668012]. The real-world modality of breast cancer screening in the China is hospital-based screening among asymptomatic self-referred women. In our previous study, we showed that US and mammography (MG) detected non-palpable breast cancer (NPBC) had similar long-term survival and that US detected more invasive NPBC with positive lymph node [2016 SABCS P5-02-05, PMID: 27689334]. This study was to investigate whether these findings would be still true with more NPBC cases included and longer follow-up in the consecutive hospital cohort.

**Methods:** From 2001 to 2017, 5,264 asymptomatic women with positive (BI-RADS 4 and 5) initial screening US underwent biopsies in PUMC Hospital, and 914 US-NPBC in 883 women were diagnosed. Meanwhile, women without dense breasts (defined as BI-RADS category C and D) also received screening MG after physical examination and US. There were 1,159 patients with positive (BI-RADS 4 and 5) MG and normal US (BI-RADS 1, 2 and 3) underwent MG-guided biopsies and 216 MG-NPBC were diagnosed in 214 women. The clinicopathological characteristics and 10-year disease-free survival (DFS) and overall survival (OS) were reviewed and compared between the US-NPBC and MG-NPBC. Prognostic factors of NPBC were identified by univariate and multivariate Cox analysis.

**Result:** Compared to MG, US could detect more invasive (81.2% vs 48.6%,  $p<0.001$ ), lymph node positive (18.3% vs 10.2%,  $p<0.001$ ), stage II+III (21.7% vs 12.5%,  $p<0.001$ ) and low grade cancer ( $p=0.001$ ). Between invasive US-NPBC and MG-NPBC, no significant difference was identified for lymph node status, TNM stage or subtype. US-NPBC received more breast conserving surgery (32.6% vs 24.1%,  $p<0.001$ ) and chemotherapy (37.5% vs 23.6%,  $p<0.001$ ). There was no significant difference in DFS or OS between US- vs MG-NPBC among ductal carcinoma in situ (DCIS), invasive and all NPBC.

Table 1. Kaplan-Meier estimates of DFS and OS between US-NPBC and MG-NPBC§

Patients (No.)		10-year DFS (%)	P value	10-year OS (%)	P value
All	US-NPBC (914)	92.4	0.570	98.2	0.143
	MG-NPBC (216)	94.7		100.0	
DCIS	US-NPBC (172)	97.7	0.170	100.0	-
	MG-NPBC (111)	95.3		100.0	
Invasive	US-NPBC (742)	91.2	0.458	97.9	0.251
	MG-NPBC (105)	94.4		100.0	

§ Kaplan-Meier survival curves between each two subgroups would be displayed in the poster.

For the US-NPBC, the DFS factors included TNM stage and Hormone receptor status whereas OS-predictors were pN and subtype.

**Conclusion:** Overall, US could detect more invasive NPBC patients with positive lymph node and advanced stage compared to MG, and screen invasive NPBC at similar TNM stage and subtype distribution as MG. US-NPBC patients received more breast conserving surgery and chemotherapy, and could achieve comparable 10-year DFS and OS as MG-detected NPBC. Hence US is justified in the real-world as the initial imaging modality in hospital-based screening Chinese women.

# 2018 San Antonio Breast Cancer Symposium ®

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Defining a breast screening “bundle”: Provider reimbursement and patient cost-share

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**Introduction:** Alternative payment models encourage “bundling” of imaging services, where a bundle aggregates all services used by a population of patients for a clinical indication and estimating the per patient cost. We assess provider cost and patient cost-share for screening mammography as a bundle, or episode of care.

**Methods:** We used patient-level analytic files between 2004 and 2014 from Optum™ Clinformatics™ Data Mart including women 40-64 years without a history of breast cancer or mastectomy and at least one year of continuous enrollment in a given plan, examining standardized costs as a proxy for provider cost and patient cost-share, summing copayments, coinsurance and deductibles. In the episode of care, we included screening mammography (SM) and all downstream diagnostic tests up to but not including biopsy. Definition of the diagnostic pathway or episode of care aggregated any subsequent testing after the initial SM independent of test order. Bureau of Labor Statistics Medical Consumer Price Index (CPI) adjusted costs to 2014 dollars.

**Results:** We identified an average of 530,844 commercially insured women ages 40-64 years with at least 12 months of continuous enrollment in a given plan per year. We identified 8 order-independent diagnostic pathways that women can experience during the episode: 1) SM only; 2) SM+ diagnostic mammography (DM); 3) SM + DM + MRI 4) SM + DM + ultrasound (US); 5) SM+DM+US+MRI; 6) SM+MRI; 7) SM+US; 8) SM+US+MRI. Across the whole population, patient cost-share fell from \$9.83 (CI of \$9.68-9.98) in 2004 to \$7.31 (CI of \$7.18-7.45) in 2014, largely due to cost-share elimination for screening mammography after the ACA. However, evaluating the individual diagnostic pathways, patient cost-share increased over time for all pathways except for those who had a negative screening mammogram and required no further diagnostic work-up prior. Among those requiring diagnostic work-up, rate of cost-share increase was steeper for any diagnostic pathway that included breast MRI. In contrast, provider cost held steady or declined, particularly for those pathways that included MRI. Across the whole population, provider cost for the breast screening episode fell from \$176.54 (CI \$175.92-177.16) in 2004 to \$141.16 (CI \$140.83-141.49) in 2014.

**Implications for Practice:** Although provider cost and patient cost-share for the breast cancer screening bundle declined over time, patients experience an uneven burden of cost-sharing dependent on the diagnostic pathway. Patient cost-share bundles need to be created in conjunction with provider cost bundles for the breast cancer screening episode of care.

**Key Words:** Affordable Care Act (ACA), alternative payment models, radiology, mammography, bundled payments, cancer screening, breast cancer



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-02-04

Is asymptomatic surveillance after standard treatment beneficial? : A 10yr-survival analysis of recurrent breast cancer patients by detection method of recurrence

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Surveillance of recurrence after standard treatment of breast cancer (BC) for early detection and its impact on overall survival are known to differ depending on recurrent site. Current guideline recommends asymptomatic surveillance to only detect loco-regional recurrences. As the evidences depend on historical randomized clinical trials we aimed to address questions whether earlier detection might have impact on survival, now that plenty of new treatment strategies can be offered. Also to give answers to heterogeneous surveillance strategy in real-world practice, we performed a retrospective 10yr-survival analysis of a large cohort of recurrent BC patients according to their detection method.

From 4188 operable breast cancer patients who completed standard treatment Asan Medical Center from 2006 to 2008, 469 patients with recurrent BC were analyzed. Median disease free interval was 35.3 months (range 2.8-97.6) and overall survival (OS) was analyzed as time from initial diagnosis/surgery to death. Among 469 patients who developed recurrence, 23.7% were local (ipsilateral breast, skin, chest wall), 22.6% were regional (ipsilateral axillary, internal mammary lymph nodes) and 53.7% developed distant metastasis. 10yr-overall survival was analyzed according to recurrent site and its detection method.

Detection of recurrence were categorized as 'asymptomatic surveillance (N=162, 34.5%)' and 'symptom-guided (N=307, 65.5%)'. Asymptomatic screening method included mammography, breast-ultrasound, serum tumor marker (CA15-3) and systemic images (eg. chest X-ray, bone scan, PET scans). Symptom-guided detection rate for local, regional and distant metastasis was 14.9%, 5.5% and 15.1% respectively. Overall, asymptomatic vs symptomatic 10yr-OS did not differ (81.3 vs 78.8 months, p=0.778).

Among patients with distant metastasis, 10ys-OS was not significantly different (70.3 vs 66.7 months, p=0.846) and was similar according to stage/subtype. Among patients with local recurrence only, 10yr-OS was 95.1 months ('symptomatic' vs 'asymptomatic', 94.4 vs 94.5, p=0.809), which may be insufficient number of events to show significant difference. Among regional recurrent BCs, longer OS was observed in asymptotically detected patients than symptom-guided group (86.1 vs 63.4, p=0.004). In Cox regression analyses, asymptomatic detection showed significant better survival (HR=3.9, 95%CI:1.6-9.5) and this observation was more evident in patients with hormone receptor(HR) negative primary BCs (69.9 vs 47.9, p=0.029). Intriguingly, only 8.6% (7/80) of regional recurrence were diagnosed by mammography.

We observed survival benefit with asymptomatic screening in detecting regional recurrence especially in HR-negative primary BC patients. And role of systemic radiology even in advanced high risk breast cancer patients were limited. Although with limitation that surveillance method varied widely, we emphasize the role of asymptomatic surveillance of regional nodal evaluation including breast-ultrasound. These findings are to be validated from a prospective clinical study along with using cutting edge modalities other than radiology which enable detection of micro-metastasis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-02-05

Who drops out of breast cancer screening? Results from the EDIFICE 6 survey

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**Background** Breast cancer (BC) screening has been part of a nationally organized program in France since 2004. Women aged 50-74 years are invited for a mammography every two years. After stabilization of up-take figures over the period 2008-2014, the latest data from the French health authorities confirm a declining trend which began in 2015-2016. This fall has been observed in all age groups, with the exception of women aged 70-74 years. It therefore appeared important to gain clearer insight into the characteristics of women who have had at least one screening examination but have not returned after the recommended two-year interval for a repeat mammography.

**Methodology** The French nationwide observational survey EDIFICE 6 was conducted online from 26 June to 28 July 2017 on 12 046 individuals (age, 18-69 years). Representativeness was ensured by quota sampling on age, gender, profession, and stratification by geographical area and type of urban district. Multivariate stepwise logistic regression analysis was conducted to identify factors likely to explain the non-uptake of subsequent BC screening. The present analysis included 1954 women (50-69 years) with no history of cancer.

**Results** Of those who were in the target age range for BC screening, 26% (N=380) did not return for the repeat examination within the recommended 2 years. Compared to those who were compliant with the recommendations, the population of non-compliant women was characterized by higher proportions of unmarried women (23% vs 19%,  $P<0.05$ ), socially vulnerable individuals (53% vs 38%,  $P<0.05$ ), and smokers (33% vs 20%,  $P<0.05$ ). No differences were observed between compliant and non-compliant women in terms of mean age (59.3 SD 5.8, years) or socioprofessional categories. In multivariate analysis, the items associated with non-compliance included: current smoking (OR=1.81 [CI=1.40 – 2.34]), individuals who would not encourage someone close to enroll in a clinical trial (OR=1.55 [1.17-2.04]), considering that protection provided by a prevention program is ineffective (OR=1.48 [1.11-1.97]), and social vulnerability (OR=1.38 [1.09-1.74]). The most frequently cited reasons for non-uptake of subsequent screening were "I don't feel concerned" (45%), "individual negligence/not a priority" (29%), fear of the examination/results (25%), "I have not received a screening invitation" (18%), and self-examination (15%).

**Conclusion** Indicators of non-uptake of repeat BC screening show various patterns: behavioral (currently smoking), social (vulnerability), and those related to information/education. In our analysis, this latter appeared concurrently with medical skepticism. The two main underlying reasons for not pursuing with breast cancer screening were "not feeling concerned" and "individual negligence". Our findings highlight the need for novel awareness campaigns that specifically target this population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-02-06

Breast cancer screening in the Republic of Belarus: Advantages and learning perspective

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## **Background:**

Breast cancer (BC) is the most common cancer among women in the Republic of Belarus. To reduce BC-related mortality, the implementation of organized pilot BC screening programmes using digital mammography has been supported by the European Union as part of the BELMED project. During recent years Belarus has developed its own digital mammographs and software that allows storing and processing images in the DICOM format. To assure digital mammographic performance in the local context, the first external clinical image quality assessment (QA) of Belarus mammograms was performed by a leading UK radiologist.

## **Methods:**

The reviewed mammograms from 88 (out of 2,267) Belarus women (mean age: 59.5 years; SD:5.7) attending the a breast screening pilot in Minsk were examined as 81 with positive and 7 with negative findings, according to Belarus readers. This included all 10 histologically confirmed BCs. Within the framework of BELMED project, a total of 400 mammographic images were selected for evaluation by a leading expert radiologist from the UK Public Health England Breast Screening Programme. Evaluated mammographic images were classified according to the Belarus (non-BIRADS) and BIRADS (the UK radiologist) categories, associated with a follow-up plan. Gradings were compared across categories (the Cohen's Kappa) and according to the proportion of cases referred for further tests (the Wilcoxon signed rank test).

## **Results:**

The reviewer concluded that approximately 50% of the positive mammograms didn't require further assessment in screening according to the UK radiologist. All histologically confirmed BCs were not among those downgraded. Substantial agreement was found in the gradings for BC (Kappa: 0.66,  $p < 0.001$ ). The study has resulted in adopting the BIRADS classification in the country and launching additional training of radiologists (e.d. in biopsy) and radiographers (in positioning).

## **Conclusions:**

To minimize additional tests on women, comprehensive QA (audit and regular check of mammographic technique, the opinion of physicists, etc.) is essential. Applying the implementation research may result in further improvement of the national BC programme. Improved practice can be promoted by continuous image review and training for individual staff (i.e., through BELMED, in collaboration with UK training centres) to further improve the skills of Belarus specialists and increase the quality of BC screening programme in Belarus.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-02-07

Adherence to breast cancer screening recommendations among underserved participants in an urban safety net mammography clinic

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**Background:** The Georgetown Lombardi Comprehensive Cancer Center's Capital Breast Care Center (CBCC) is a safety net mammography screening center that uses a community-based patient navigation program to provide underserved minority women guidelines-concordant mammography screening. Given that screening navigation is designed to eliminate some established barriers such as, access, transportation, and cost, we retrospectively examined patient adherence rates to regular (annual/biennial) mammography screening. We also investigated whether patient demographics are associated with adherence to breast cancer screening.

**Methods:** Data were derived from medical records of patients that received a baseline mammogram at CBCC in 2011 (n = 1,637) and were followed up for 4 years. Within the study time period of 2011-2015, patients were of age 40-74 and had not received a prior breast cancer diagnosis. 10 definitive cases of breast cancer were newly diagnosed in this population during the follow-up period and were excluded from the analysis. Adherence was then calculated based on the American College of Radiology (annual screening starting at 40) and the United States Preventive Services Task Force guidelines (biennial screening starting at 50).

**Results:** In 2011, the mean age of women screened at CBCC was 51.25 years with 45% being 40-50 years of age. CBCC has a predominantly minority population with 48% of the women identifying as Black/African American (AA) and 41% identifying as Hispanic in 2011. Over the 4 year follow up period, 41.11% of the patients screened in 2011 did not return for another screen. The adherence rate for annual screening in the 40-74 age group was 3.3% (3.0% in Black/AA; 4.2% in Hispanic) over the 4-year follow-up. The adherence rate for biennial screening among the 50-74-year-old age group was 21% overall (20% among Black/AA; 26% among Hispanics). Approximately 40% of the participants with baseline screenings in 2011 received at least one additional screening over the 4 year follow up but their mammography schedules were not guidelines concordant and they were labeled as "partially adherent" for this analysis.

**Conclusion:** While the number of partially adherent patients was consistent with previous population-based adherence studies, the proportion of non-adherent patients was higher compared with other population-based studies in primarily Non-Hispanic White populations. Our analysis highlights the importance of focusing on adherence to guidelines and not just mammography initiation in underserved minority populations through educational interventions targeted to patients and providers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-02-09

CDA screening technology for multi-ethnic group, early stage breast cancer screening

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**Background:** Breast cancer is the second leading cause of death from cancer in American women. Current breast cancer screening technologies have issues with poor sensitivity for early stage breast cancer, high false positives, radiation side effects, etc. Cancer Differentiation Analysis (CDA) technology is a blood-sample based, multi-level, multi-parameter diagnostic method which detects signals from both proteins, cells, and to some extent, molecular level, in which multiple aspects of information are collected to improve diagnostic accuracy. CDA technology has been investigated as a viable clinical utility in breast cancer screening, particularly for early stage breast screening with clear advantages (both whole blood and serum can be used, ability to detect early, easy, simple, no side effects, and high degree of sensitivity and specificity).

**Methods:** In this study, the human subjects involved are Caucasians, with serum samples of 44 pathologically confirmed breast cancer patients and 34 healthy individuals from 3 blood bank centers in the USA, of which 40 cases were stagelbreast cancer, 2 cases were stagell, and the other 2 cases were stage III breast cancer. CDA data of 44 breast cancer patients and 34 healthy individuals were collected in US lab and analyzed using SPSS, and the results were shown in the table below. Results from the above study was compared with a clinical study on Asian group with data collected in lab in China using CDA technology.

**Results:** The average CDA value of all breast cancer and stagelbreast cancer samples, and controls were 45.99, 45.76 and 42.36 (rel. units) respectively (see Table 1). Both breast cancer and stagelbreast cancer could be significantly distinguished from the control group ( $p < 0.001$ ) (Table 2). For stagelbreast cancer vs. control group, Area under ROC curve was 0.727, sensitivity and specificity were 62.5% and 82.4% respectively, which is higher than a typical mammogram. To compare with different ethnic groups, data collected on an Asian group is also shown in Table 2, which showed that overall, AUC, sensitivity and specificity are comparable (some difference may be attributed to sample type difference (whole blood vs. serum)) for early stage breast cancer patients for those two ethnic groups, demonstrating that CDA technology can be extended to multiple ethnic groups.

**Conclusions:** CDA screening can be extended to different ethnic group including Caucasian and Asian with good sensitivity and specificity for stagelbreast cancer.

We thank Ugur Basmaci, Sunsil Pandit and Sharon Vorse-Yu for their support.

Table 1 Summary of CDA Test Results

Group	Sample Size	Age Range	Age Mean	Age Median	CDA Mean (rel. units)	CDA Median (rel. units)	CDA STDEV
Control	34	36 -79	57	57	42.36	42.65	2.75
Breast Cancer	44	36 – 77	60	61	45.99	46.50	4.22
Stage I Breast Cancer	40	36 – 77	60	61	45.76	45.55	4.26
Stage II Breast Cancer	2	51 – 64	58	58	47.05	47.05	4.88
Stage III Breast Cancer	2	62 – 75	69	69	49.50	49.50	2.55

Table 2 AUC, Sensitivity and Specificity of Control vs. Stage I Breast Cancer

Stage I Breast Cancer vs. Control	Area Under the Curve	Sensitivity	Specificity
Caucasian (Stage I)	0.727	62.5%	82.4%
Asian# (Stage I)	0.876	80.0%	80.0%

# Whole blood samples. 10 stage I breast cancer samples and 25 control samples

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-02-10

Early stage breast cancer screening using an emerging novel liquid biopsy screening technology

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**Background:** An emerging novel liquid biopsy technology called Cancer Differentiation Analysis (CDA) has been evaluated as a viable early stage breast cancer screening tool. CDA technology is a blood-sample based, multi-level, multi-parameter diagnostic method which detects signals from both protein, cellular, and to some extent, molecular levels, in which multiple aspects of information can be collected to improve diagnostic accuracy, even for early stage of cancer. Improving capability to screen breast cancer is an important on-going research effort, as breast cancer represents a leading cancer with high incidence rate.

**Methods:** In this single-blind study, 22 breast cancer patients and 25 healthy individuals were recruited at Changhai Hospital of Shanghai. Histopathological examination results of breast cancer patients were collected, 22 cases were diagnosed as infiltrating ductal carcinoma of breast, of which 10 patients were stage I breast cancer. 25 individuals were confirmed healthy after physical examinations. Peripheral blood was drawn in EDTA tubes For CDA tests. CDA data of 22 breast cancer patients and 25 healthy individuals were conducted using SPSS, and the results were shown in the table below.

**Results:** The average CDA of breast cancer, stagelbreast cancer, and controls were 43.20, 44.17 and 36.17 (rel. units) respectively as shown in Table 1. Both breast cancer and stage I breast cancer could be significantly distinguished from the control ( $p = 0.000$ ,  $p = 0.001$ , respectively). For stage I breast cancer vs. control group, Area under ROC curve was 0.876, sensitivity and specificity were both 80.0% (Table 2). In contrast to traditional breast cancer screening methodologies which have relatively low sensitivity and high false positives for stage I detection, often with radiation side effects and high costs, advantages of CDA technology include ability to detect early stage cancer with relatively high sensitivity and specificity, and it is also highly cost effective without side effects.

**Conclusions:** Initial results showed that CDA technology could effectively distinguish stagelbreast cancer from healthy individuals, CDA could be a potential candidate for breast cancer screening.

Table 1 Summary of CDA test results

Group	Sample Size	Age Range	Age Mean	Age Median	CDA Mean (rel. units)	CDA Median (rel. units)	CDA STDEV
Control	25	23 - 67	41	37	35.63	36.17	6.98
Breast Cancer	22	39 - 78	54	53	43.20	42.30	4.18
Stage I Breast Cancer	10	43 - 78	59	59	44.17	43.25	4.29
Stage II Breast Cancer	8	39 - 55	47	49	41.28	40.30	3.06
Stage III Breast Cancer	2	55	55	55	42.20	42.20	2.12
Stage IV Breast Cancer	2	51 - 64	58	58	47.00	47.00	7.78

Table 2 AUC, Sensitivity and Specificity of Control vs. Stage I breast cancer

Stage I Breast Cancer vs. Control	Area Under the Curve	Sensitivity	Specificity
	0.876	80.0%	80.0%



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-03-01

Differential distant disease-free intervals for mammography detected vs. clinical presentation invasive breast cancer: Early detection or lead time bias?

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**Background:** Distant recurrence metastatic breast cancer (rMBC) incidence has declined with coincident improved breast cancer (BC) survival over time. Lead time is time from screening diagnosis to diagnosis that would have been made without screening. The purpose of screening is to detect disease at an early more treatable stage. Lead time bias would indicate early detection led only to a perceived increase in survival time without affecting the course of BC progression. Our objective was to evaluate detection method and treatment changes to distant recurrence and survival over time.

**Methods:** In a longitudinal institutional cohort 1990-2011, we reviewed primary invasive stage I-III BC patients (n = 7991) for initial BC detection method by mammography (MamD) or patient/physician (ClinP), distant recurrence (rMBC), time from initial diagnosis to distant recurrence (DDFI) and distant disease specific survival (DDSS), follow up through 2016 updated annually. 856 patients with distant recurrence were identified and confirmed by imaging, biopsy or both. Diagnosis year time periods were set to coincide with significant treatment changes over time (1990-98, 1999-2004, 2005-2011). Chi square, mean ANOVA, DDSS and Cox proportional hazards analysis were conducted.

**Results:** 48% of the cohort were ClinP and 52% MamD with MamD BC increasing over time [1990-98 45%, 2005-2011 54%,  $p < .001$ ]. 72% of rMBC patients were ClinP BC. ClinP BC had shorter distant recurrence time, 4.99 years vs. 6.05 MamD BC ( $p = .001$ ). Mean time from rMBC diagnosis to death was 2.88 years with no significant difference by detection method. In a Cox proportional hazards model adjusted for race, reduced risk of rMBC was observed for stage I/II, recent diagnostic years, hormone receptor positive, MamD, and low histologic grade [TNM I HzR = .11, 95% CI = .09, .14; TNM II HzR = .36, 95% CI = .31, .43; 1990-2004 HzR = .63, 95% CI = .53, .75; 2005-2011 HzR = .49, 95% CI = .41, .58; HR+ HzR = .70, 95% CI = .59, .82; MamD HzR = .68, 95% CI = .58, .81; low histologic grade HzR = .80, 95% CI = .67, .96; age >40 HzR = .79, 95% CI = .65, .96]. No difference was observed for DDSS by detection method [5-year DDSS: ClinP = 22%, MamD = 21%; log rank test = .019,  $p = .892$ ].

**Conclusion:** At initial diagnosis, the majority of rMBC cases are symptomatic, have shorter DDFI but DDSS equal to MamD BC. MamD BC cases have lower rMBC incidence and longer DDFI possibly due to more responsive earlier diagnosed initial disease, slower progression disease or as yet unidentified biologic/genomic differences. Lead time bias may partially explain lower rMBC incidence given the benefits owed to treatment of MamD invasive BC which preclude disease progression to clinically evident BC. Mammography detected/non-symptomatic and clinically detected/symptomatic BC have differential incidence of and time to distant recurrence but no difference in DDSS. Mammography detection appears to confer a distant disease risk reduction advantage independent of other known prognostic factors but the disease has the same virulence once it becomes metastatic. Our results indicate treatment changes by diagnosis year proxy and mammography detection are both associated with decreased rMBC risk.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-03-02

ASCO/CAP human epidermal growth factor receptor-2 (HER2) in situ hybridization (ISH) categories evaluated by quantitative HER2 protein diagnostic methodologies: A comparative analysis

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**Background:** In 2013, the ASCO/CAP consensus panel published updated guidelines for HER2 testing in breast cancer that modified the definition of HER2 amplification by in situ hybridization (ISH), creating five new prognostic categories (group 1: classic amplified, group 2: monosomy, group 3: co-amplified (polysomy), group 4: equivocal, and group 5: classic non-amplified). Patients determined to be ISH amplified, were considered eligible for HER2-directed therapy. Concern over whether patients from non-classic groups 2-4 would benefit from treatment has led to the recent publication of the 2018 HER2 focused update. This update has modified the criteria for interpreting these ISH categories, recommending that the final diagnosis take into consideration a combination of HER2 immunohistochemistry (IHC) and ISH results. With increased emphasis on the HER2 protein assessment, it has prompted us to quantitatively examine HER2 protein expression in the ISH categories, using two different novel technologies. **Materials & Methods:** A cohort of 170 cases (URMC) and 102 cases (PSHMC) of invasive breast cancers, which had previously undergone HER2 IHC and ISH testing, were selected for this study. Cases were sorted and categorized into the HER2 ISH categories defined by ASCO/CAP. HER2 protein expression was quantitatively measured in the URMC and PSHMC cohorts using a novel immunodetection methodology (streptavidin-coated Phosphor-Integrated Dot (PID) fluorescent nanoparticles), and a novel dual-antibody, proximity-binding immunoassay (HERmark® Breast Cancer Assay, Monogram Biosciences, South San Francisco, California), respectively. HER2 protein expression was compared to the HER2 FISH and IHC results by ASCO/CAP category. **Results:** Cases in group 1 had a significantly ( $p < 0.01$ ) higher average PID/cell and HERmark compared to cases in groups 2-5 (Table 1). Cases in groups 2-4 showed lower quantitative levels of HER2 protein expression, similar to the classic non-amplified cases (group 5). Group 1 was further divided into three subgroups (Table 2): Group A - ISH high-level amplified (ratio  $\geq 2$ , HER2  $\geq 6$ , CEP17  $< 2.7$ ), Group B - amplified with elevated CEP17 (ratio  $\geq 2$ , CEP17  $> 2.7$ ), and Group C - low-level amplified (ratio  $\geq 2$ , HER2  $> 4$  and  $< 6$ ). Group A and B had a significantly ( $p < 0.01$ ) higher average PID/cell and HERmark compared to Group C. Group C was more comparable to cases in groups 2-5 (Table 1). **Conclusion:** Our results suggest that quantitative assessment of HER2 protein expression may help to further classify cases for HER2 status for targeted therapy, supporting the 2018 ASCO/CAP recommendation that non-classic ISH results might be resolved by evaluating protein expression. Follow up studies with a larger patient cohort and dual quantitative assessment are warranted.

Average PID/cell and HERmark in ASCO category groups

ASCO category group	N (URMC)	PID/cell (URMC)*	N (PSHMC)	HERmark (PSHMC)*
1	88	88.0	77	61.5
2	10	11.2	0	N/A
3	20	16.0	2	13.8
4	23	8.5	3	15.9
5	29	6.3	20	8.3

\*average

Table 2: Average PID/cell and HERmark in subgroups of Group 1

Subgroup	N (URMC)	PID/cell (URMC)*	N (PSHMC)	HERmark (PSHMC)*
A	24	157.6	64	65.7
B	34	101.6	10	44.1
C	30	16.9	3	29.8

\*average

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-03-03

Comparing staging assignments based on the 7<sup>th</sup> and 8<sup>th</sup> editions of the American Joint Committee on Cancer staging system for breast cancer

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**Background:** The 8<sup>th</sup> edition of the American Joint Committee on Cancer (AJCC) staging system for breast cancer has redefined breast cancer staging to better represent the heterogeneity of the disease. The previously established TNM system reflected only tumor size (T), nodal status (N), and distant metastasis (M). The updated staging system introduces new clinical and pathological prognostic staging systems by incorporating tumor grade, tumor markers, and genomic assays. Prospective comparisons of staging assignment between AJCC 7<sup>th</sup> and 8<sup>th</sup> edition staging systems have not been performed in the United States. The objective of this study was to compare subjects' assigned stage between the AJCC 7<sup>th</sup> and 8<sup>th</sup> edition staging systems.

**Methods:** Data were collected from patients with non-metastatic breast carcinoma who presented to the University of Chicago between January 1, 2018 and May 31, 2018. Upstaging or downstaging was defined as an increase or decrease by one or more letter or number stages (i.e. from IIA to IIB or IB). Descriptive data were generated for all collected data. Analysis was performed in Stata 14.0.

**Results:** Clinical anatomic and prognostic stages were assigned to 246 patients while pathologic anatomic and prognostic stages were assigned to 142 patients. Forty-seven patients underwent neoadjuvant therapy and were not assigned a pathologic prognostic stage, while 57 patients had not undergone surgery at our institution by the end of the study period and were not assigned a pathologic stage. Two patients had lobular carcinoma *in situ* and were no longer considered to have a malignancy by the 8<sup>th</sup> edition staging system; anatomic staging was otherwise unchanged from 7<sup>th</sup> edition to 8<sup>th</sup> edition staging in this cohort. Of the 246 patients assigned clinical anatomic and prognostic stages, 48 patients were upstaged (19.5%), 37 were downstaged (15.0%), and 161 were not affected (65.5%) by prognostic staging. Of the 142 patients assigned pathologic anatomic and prognostic staging, nine patients were upstaged (6.3%), 32 were downstaged (22.5%), and 101 were not affected (71.1%) by prognostic staging.

## Stage Changes with the AJCC 8th Edition

	Upstaged % (n)	Downstaged % (n)	No Change in Stage % (n)
Clinical Stage	19.5 (48)	15.0 (37)	65.5 (161)
Pathologic Stage	6.3 (9)	22.5 (32)	71.1 (101)

Four patients were eligible to be downstaged in pathologic prognostic staging by genomic assays such as Oncotype (consisting of Stage 1B or higher patients with T1N0M0 HER2 - ER + PR - Grade 3 disease or T2N0M0 HER2 - ER + disease). Of these patients, three had genomic assays performed by the end of the study period. Results from genomic assays did not affect the staging of these three patients.

**Conclusions:** Approximately one third of breast cancer patients were assigned new clinical or pathologic stages with the AJCC 8<sup>th</sup> edition staging system. These findings confirm that implementation of the new criteria for staging will be important to many patients in discussions of treatment and prognosis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-03-04

Molecular subtyping of androgen receptor-positive patients using gene expression profiles

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Breast cancer is a heterogeneous disease, and unsupervised clustering approaches using gene expression data have identified 3-6 distinct subtypes of triple negative breast cancer (TNBC). A genomically and clinically distinct subtype of TNBC is referred to as LAR (Luminal Androgen Receptor). Tumors with this subtype typically express high levels of the AR and exhibit alterations within genes involved in the PI3K pathway (e.g. PIK3CA mutations). Prospective studies are underway using drugs that target the AR alone or in combination with PI3K and CDK 4/6 inhibitors. Given the importance of accurately identifying this subtype, we sought to develop an online tool that uses submitted gene expression data to confidently characterize LAR samples by corroborating the classification with previously published clustering approaches.

**Methods:** We have investigated TNBC RNA-Seq data from The Cancer Genome Atlas (TCGA) breast cancer study (N=123 samples) by cluster analysis. Analysis of the average silhouette width in both biased and unbiased K-means clustering approaches demonstrated LAR and basal as two distinct and significant clusters. A shrunken centroid model of 426 differentially expressed genes, named as CABAL (Clustering Among Basal and Luminal androgen receptor), was constructed by comparing LAR and basal subtypes.

**Results:** We applied the CABAL model to classify the four TNBC microarray datasets that were previously used in clustering experiments as well as an independent RNA-Seq data cohort. Non-negative matrix factorization (NMF) and fuzzy clustering were applied to the samples (N=1046). Clustering similarity among the methods was assessed with the adjusted rand index, and CABAL demonstrated significant similarity with both fuzzy and NMF clustering methods. Similarly, hierarchical clustering analysis performed on the pooled cohort of 1046 samples recapitulated the CABAL classification with an area under the receiver operating curve of 0.91.

**Conclusions:** Confident and robust identification of samples with the LAR phenotype is paramount in the assessment of clinical associations and therapeutic efficacy. To facilitate LAR identification, we have provided a web-based prediction tool of the CABAL classification, integrated with the NMF and fuzzy clustering results to identify candidate LAR samples. The end user is provided with the pair-wise adjusted rand indexes, thus reinforcing in the clustering characterizations. Further, our online LAR depiction tool provides a set of graphical and tabular summaries, which will be illustrated, while providing additional molecular characterizations of the PAM50 and Metabric classifications. The availability of this tool could advance the genomic research and treatment of TNBC patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-03-05

Long term survival and tumor biology of screen-detected small non-palpable breast cancer in Chinese women: The smaller, the better?

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**Background:** Tumor biology would reflect the prognosis and potentially the lead time and over-diagnosis rate of screen-detected small breast cancer [PMID: 28591529, 21452022 and 24888816]. Chinese women had earlier peak age of breast cancer incidence and used ultrasound as the primary screening imaging test on a hospital-basis [2016 SABCS P5-02-05, PMID: 27689334]. In our previous work, we showed that US detected non-palpable breast cancer (NPBC) had higher percentage of invasive and lymph node positive cancer, yet still could be regarded as low-risk cancer [PMID:27689334, 28412736]. This study was performed to investigate the prognostic impact of immunohistochemical subtypes and tumor size: the smaller the NPBC, the better the tumor biology and prognosis?

**Methods:** From January 2001 to December 2017, 6,423 consecutive asymptomatic women underwent mammography (MG) or ultrasound (US) guided biopsy in Peking Union Medical College Hospital. Among them, 159 T1a, 239 T1b, 377 T1c and 72 T2 NPBC were diagnosed and treated. The clinicopathological features, treatment choice, 10-year disease-free survival (DFS) and overall survival (OS) of the small NPBC (defined as  $\leq 1.0$ cm, T1a+b) were reviewed and compared with T1c and T2 NPBC. Prognostic factors of these subgroups of invasive NPBC were identified.

**Results:** Compared to big NPBC, the T1a+b small NPBC showed more lymph node negative ( $p < 0.001$ ) and low Ki67 ( $< 14\%$ ,  $p < 0.001$ ) cancers with earlier TNM stage ( $p < 0.001$ ), more luminal A subtype ( $p = 0.003$ ) and significantly improved 10-year DFS and OS ( $p = 0.004$ ). T1c+T2 NPBC had more triple-negative subtype and received more chemotherapy ( $p < 0.001$ ) and targeted therapy ( $p = 0.008$ ). Breast conserving rate and the use of radiation and endocrine therapy showed no significant difference.

Table 1. Comparison of clinicopathological factors and long term survival of small vs big screen-detected NPBC

Screen-detected NPBC(2001-2017				
Clinical&prognostic factors		T1a+T1b(n=398)	T1c+T2(n=449)	P value
Screening method	US-NPBC(n,%)	336(84.4)	406(90.4)	0.008
	MG-NPBC(n,%)	62(15.6)	43(9.6)	
Lymph node status	Negative(n,%)	343(86.2)	315(70.2)	<0.001
	Positive(n,%)	55(13.8)	134(29.8)	
TNM stage	I(n,%)	344(86.4)	277(61.7)	<0.001
	II(n,%)	37(9.3)	134(29.8)	
	III(n,%)	17(4.3)	38(8.5)	
Ki67	<14%(n,%)	208(52.2)	168(37.4)	<0.001
	$\geq 14\%$ (n,%)	183(46.0)	274(61.0)	
Subtype	Luminal A(n,%)	164(41.3)	135(30.1)	0.003
	Luminal B(n,%)	155(38.9)	218(48.6)	
	Her2(n,%)	28(7.0)	27(6.0)	
	TNBC(n,%)	31(7.8)	52(11.6)	
	Unknown(n,%)	20(5.0)	17(3.7)	
10-year survival	DFS(%)	94.6	88.8	0.004
	OS(%)	100.0	96.4	

**Conclusion:** Small asymptomatic NPBCs were detected when small because they were good in terms of low Ki67 index, favorable subtype, tumor biology and long term prognosis. On the contrary, T1c and T2 NPBCs were screened when already big or even with positive nodes without clinical symptoms indicating that they might have larger chance of becoming interval cancers.

# 2018 San Antonio Breast Cancer Symposium ®

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Risk stratification by ultrasound for screen-detected non-palpable breast cancer in Chinese women: Regular low risk versus ultra-low risk?

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**Background:** Mammography (MG) screen-detected breast cancer has been established as low-risk in the western world. However, ultrasound (US) is currently the 'real-world' initial imaging test for breast cancer in China. In our previous work, we firstly showed with a multi-center randomized controlled trial that US could detect breast cancer with improved sensitivity and accuracy in high risk Chinese women [PMID: 25668012]. Then we demonstrated on a hospital-screening basis that US and MG detected non-palpable breast cancer (NPBC) had similar survival [2016 SABCS P5-02-05, PMID: 27689334]. This study was performed to test the hypothesis [Hypothesis would be published in the journal of Medical Hypothesis, 118 (2018):9-12] whether MG+/US- NPBC could be taken as ultra-low risk cancer which had more favorable clinical characteristics and survival than the regular low-risk NPBC.

**Methods:** From 2015-2017, 1,478 consecutive patients received biopsy with initial positive screening US (BI-RADS 4 and 5) at Peking Union Medical College Hospital. Among them, 206 US+/MG- and 135 US+/MG+ NPBC were diagnosed. Meanwhile, 371 patients who had negative initial screening US (BI-RADS 1, 2 and 3) and positive additional MG (BI-RADS 4 and 5) underwent MG-guided biopsies, and 88 MG+/US- NPBC were diagnosed. Clinical characteristics, treatment and 3-year disease free survival (DFS) and overall survival (OS) were analyzed and compared. Prognostic factors were identified.

**Results:** There was no significant difference in age, lymph node status, hormone receptor status, endocrine therapy, chemotherapy, targeted-therapy among the three subgroups of NPBC. MG detected significantly more ductal carcinoma in situ (DCIS, 59.1% vs 22.8% and 28.1%,  $p<0.001$ ) whereas ultrasound diagnosed more invasive cancers (77.2% and 71.9% vs 40.9%,  $p<0.001$ ), multifocal cancer ( $p=0.020$ ) and patients who received breast-conserving surgery ( $p<0.001$ ) and needed radiotherapy ( $P=0.001$ ). No significant difference was found for 3-year DFS and 3-year OS were all 100%, although MG+/US- NPBC showed a trend of better DFS.

Table 1. Comparison of positive predictive value (PPV), pathology and prognosis of US+/MG-, US+/MG+ and MG+/US- NPBC

PathologyRadiology (2015-2017)	US-detected NPBL (N=1,478)	US-detected NPBL(N=1,478)	MG-detected NPBL (N=371)
MG & US positivity	US+/MG- (N=1,108)	US+/MG+ (N=370)	MG+/US- (N=371)
Imaging presentation	Nodule	Nodule + micro-calcifications	Micro-calcifications
Breast cancer (PPV %)	206 (18.6%)	135 (36.5%)	88 (23.7%)
Pathology ( $p<0.001$ )			
DCIS (%)	47 (22.8)	38 (28.1)	52 (59.1)
Invasive (%)	159 (77.2)	97 (71.9)	36 (40.9)
3-Year survival			
DFS (%)	92.3	91.1	96.5
OS (%)	100.0	100.0	100.0

**Conclusion:** MG+/US- NPBC had satisfactory prognosis, higher percentage of DCIS and might be taken as 'ultra-low risk' cancer. Hence US had the potential of stratifying the screen-detected NPBC into regular low risk (US+/MG+ and US+/MG-) and ultra-low risk (MG+/US-).



# 2018 San Antonio Breast Cancer Symposium ®

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Breast cancer diagnosis is associated with relative left ventricular hypertrophy

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**Background:** Cardiac dysfunction is a major concern for patients with breast cancer (BC) receiving adjuvant therapy. Retrospective, cross-sectional echocardiographic data suggests that patients with cancer have reduced myocardial strain prior to cancer therapy exposure. Cardiac magnetic resonance (CMR) is the gold standard imaging modality for cardiac structure and function and can also evaluate myocardial micro-architecture with T1 mapping. We hypothesized that treatment naïve patients with early-stage BC (ESBC) have abnormal myocardial tissue characteristics on CMR.

**Methods:** Women with newly diagnosed ESBC were prospectively recruited for CMR prior to cancer drug treatment. Those with hypertension, diabetes mellitus or prior cancer treatments were excluded. Age and sex matched healthy controls were identified from a prior prospective study. All participants underwent a non-contrast CMR scan on a 1.5T magnet. Image acquisition included cines for cardiac structure and function as well as T1 mapping using saturation recovery single-shot acquisitions. Global longitudinal strain (GLS) was derived from cine images. Demographics and imaging metrics for healthy controls and patients were compared using two-sample t-test.

**Results:** 106 patients with ESBC, mean age 51±9, were included along with 55 matched healthy controls. Body mass index and systolic blood pressure were similar between groups, however resting heart rate was elevated in patients compared to controls, 77±11 vs 67±11 /min respectively, p<0.001 (Table 1). On CMR there was no difference in left ventricular volume or ejection fraction however global longitudinal strain was higher in patients compared to controls, -20.9±2.3 vs -19.9±3.7%, p=0.04 (Table 2). Left ventricular mass was higher compared to controls, 52±6 and 47±6 g/m<sup>2</sup> respectively, p<0.001. However myocardial T1 was similar between groups, T1=1198±27ms for controls vs 1206±46ms for patients, p=0.42.

**Conclusions:** The cardiac phenotype of patients with ESBC is characterized by relative left ventricular hypertrophy with normal myocardial tissue. Further understanding of the mechanisms involved may provide insight into the cardiovascular risk associated with BC diagnosis.

Table 1. Demographics

	Healthy Controls (n=55)	Breast Cancer (n=106)	P value
Age, years (SD)	52(14)	51(9)	0.49
Body mass index, kg/m <sup>2</sup> (SD)	26(5)	27(6)	0.38
Hypertension, number	0	0	NA
Diabetes Mellitus, number	0	0	NA
Receptor status, number (%)			NA
ER/PR	NA	92(87%)	
HER2	NA	74(70%)	
Triple negative	NA	2(2%)	
Stage, number (%)			NA
1	NA	43(42%)	
2	NA	41(38%)	
3	NA	23(20%)	
Systolic blood pressure, mmHg (SD)	127(15)	124(13)	0.19

Heart rate, /min (SD)	67(11)	77(11)	<0.001
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SD=standard deviation, NA=not applicable

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Table 2. Cardiac Magnetic Resonance

	Healthy Controls (n=55)	Breast Cancer (n=106)	P value
LVEF, % (SD)	62(4)	62(5)	0.91
Indexed LVEDV, ml/m2 (SD)	69(9)	72(14)	0.18
Indexed LV mass, g/m2 (SD)	47(6)	52(6)	<0.001
LV mass/LVEDV (SD)	0.69(0.08)	0.74(0.13)	0.002
Indexed left atrial volume, ml/m2 (SD)	40(9)	37(10)	0.21
Global longitudinal strain, % (SD)	-19.9(3.7)	-20.9(2.3)	0.04
Myocardial T1, ms (SD)	1198(27)	1206(46)	0.42

SD=standard deviation, LVEF=left ventricular ejection fraction, LVEDV=left ventricular end-diastolic volume, LV=left ventricular

# 2018 San Antonio Breast Cancer Symposium ®

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New diagnostic tools for bone health assessment: Perspectives in medical oncology

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## Background

Pituitary down regulators, aromatase inhibitors tamoxifen and chemotherapeutic drugs, all have a negative impact on bone health in breast cancer patients.

Although trabecular bone accounts for only 20% of skeleton mass, bone resistance depends also on the its micro-architecture, or quality, of, in addition to bone density. The BESTEST® is an innovative and inexpensive diagnostic method that gives an indication of the quality of the bone structure: it measures the weight-bearing capacity of the bone structure, evaluated from simulated application of loads on bone structure images acquired by radiograms in the proximal epiphysis of the hand. Results are expressed as BSI\_T-score and BSI\_Z-score (which refers the results to the average value for the same age) and provide precious add-on information to densitometry.

We discuss the preliminary results obtained in female patients undergoing breast cancer treatment.

## Material and methods

100 Caucasian women, took BESTEST® as follow-up while undergoing oncological treatment. Femoral neck DXA T-score available in a subgroup of 60. 10 patients self-reported an osteoporotic fracture, DXA T-score available for 8.

Control population: 200 women, accessing BESTEST® and DXA for screening purposes, 30 self-reporting an osteoporotic fracture.

## Results

**Statistics: mean (min, max).**

	N	Age	BSI_T-score	BSI_Z-score	DXA_T-score
Population	100	61 (33, 88)	-1.7 (-3.4, -0.0)	-1.3 (-2.6, 0.6)	NA
DXA subgroup (of oncological population)	60	62 (35, 88)	-1.8 (-3.4, -0.1)	-1.3 (-2.6, 0.6)	-1.6 (-3.2, 0.5)
Fractured subgroup	10	67 (56, 82)	-2.4 (-2.9, -1.3)	-1.8 (-2.6 -0.1)	NA
Fractured subgroup with DXA	8	68 (60, 82)	-2.4 (-2.9, -1.3)	-1.8 (-2.6 -0.1)	-1.5 (-2.9, 0.1)
Control	200	63 (32, 89)	-1.1 (-3.6, 2.9)	-0.6 (-3.0, 2.9)	-1.9 (-3.7, 1.0)

The fractured subgroup exhibits significantly lower BSI T-score than the population (T-test  $p < 0.0100$ ) and results are similar after BSI Z-score correction for age (T-test  $p = 0.0300$ ). The BSI T-score of the DXA subgroup is representative of the oncological population undergoing treatment (T-test  $p = 0.8668$ ).

As expected, BSI T-score and DXA T-score are not correlated:  $R^2 = 0.0917$  in the control and  $R^2 = 0.0294$  in the population. The DXA subgroup exhibit significantly lower BSI T-score than the control (T-test  $p = 0.0002$ ) and similar results are obtained after BSI Z-score correction for age. A lower significance (T-test  $p = 0.0281$ ) is found for DXA T-score.

The 8 fractured oncological patients exhibit significantly lower values of BSI T-score that the oncological population (T-test  $p = 0.038$ ) and all patients have a BSI T-score indicative of a compromised trabecular structure. DXA T-score values cannot be considered statistically different (T-test  $p = 0.6744$ ) and results span all possible diagnostic results from high risk to normal.

## Conclusions

Statistical analyses show that bone micro-architecture is indeed affected by oncological treatment and that bone alterations due to oncological treatment are easily detected with BESTEST, especially when associated with fractures.

This preliminary study clearly provides a rational background for further, deeper investigations into the use of a new, rapid and

safe technique for monitoring the effect of breast and prostate cancers therapies on bone micro-architecture modifications.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-03-09

The breast cancer care continuum in Tanzania: From accessing care for breast concerns to cancer diagnosis and treatment

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**Background:** Tanzania, like many low to middle income countries, has developed their first ever national guidelines focusing on early diagnosis of breast cancer to combat the high fatality rates. Thus, one component of the “Time to A.C.T.” study sought to provide critical information on women presenting to a health facility who might need clinical breast examination per the new guidelines and ultimately how many of these women were diagnosed and treated for breast cancer.

**Methods:** In this retrospective cohort study, data were abstracted from 777 medical charts from three health facilities spanning the health system hierarchy (district n=103, regional n=64, and zonal n=610 hospitals) in Mwanza, Tanzania. Charts were identified from the departments of oncology, surgery, gynecology, obstetrics and emergency medicine, and focused on women aged 30 and older who were seeking or receiving care related to any “breast concern” from January 2015 to April 2018.

**Results:** The median age of women seeking care for breast concerns was 43 years (IQR: 34-54). Women most commonly presented with complaints of swelling (43%), a palpable lump (27%), or pain (24%) of the breast or nipple. Ultimately, 26% of these care-seeking women were diagnosed with breast cancer (including DCIS=2, invasive ductal or lobular cancer=121, and metastatic cancer =15), of whom 73% were pathologically confirmed. Treatment data were available for only 79 women with breast cancer: 24 had surgery only, 33 had chemotherapy only, 21 had surgery and chemotherapy, and 1 woman had surgery, chemotherapy and radiation.

**Conclusions:** This study increases our understanding of the health services needs associated with Tanzania's commitment to down stage breast cancer through early detection and treatment. Almost a third of women who presented with a breast concern were diagnosed with breast cancer. Although this likely reflects the fact the majority of medical charts were identified at a zonal hospital that has the most capacity for cancer diagnosis and treatment, it also highlights the importance of getting women into care, proper referrals and ultimately diagnosis and treatment. This information on who seeks care, why, and where will help appropriately allocate and scale up capacity for breast cancer care.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-03-10

The implementation of a patient referral service in a Brazilian cancer center

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**BACKGROUND:** Healthcare systems face problems of cost increases and poor delivery organization. Integrated delivery may reduce costs and improve quality and health outcomes.

**OBJECTIVE:** To describe how the A.C. Camargo Cancer Center, aiming at greater integration, implemented a referral service for breast cancer patients.

**METHODS AND RESULTS:** The process was divided in three phases: 1) AS IS ANALYSIS, 2) BENCHMARKING, 3) PILOT & IMPLEMENTATION.

## 1) AS IS ANALYSIS

A.C. Camargo was responsible for treating 16% of all breast cancer cases that arose from 2000 to 2012 in São Paulo State. Prior to implementation of the program, there was no special patient classification at the initial appointments.

## 2) BENCHMARKING

As proposed by MD Anderson Cancer Center, we used the patient's classification at the time of an appointment request (regular screenings, undiagnosed or breast cancer patient).

As proposed by Memorial Sloan Kettering Cancer Center, we used a "Physician Referral Service" staffed by "Referral Specialists" and "Trained Oncology Nurses" to collect patient information prior to the first appointment.

As suggested by Princess Margaret Cancer Center, we collected patient care data (e.g., abnormal imaging, palpable lump).

## 3) PILOT & IMPLEMENTATION

Phase I: Feb. 13, 2017 to Dec. 28, 2017. We reached 7% of new patients in the Breast Surgery Department (BSD): 48% were in the Cancer Group, 45% in the Abnormal Imaging Group (Undiagnosed) and 7% in the Palpable Lump Group.

New patients were classified by the Call Center. Electable cases were referred to the Nurse Navigator, who proceeded with appointments according to protocol.

Root cause analysis of non-captured patients led to the following improvements: extension of participant Call Center cells, script review, and implementation of a training program.

The Phase I results led to the following improvements: 1) reclassification of a subgroup with highly suspicious images as the "Cancer Group"; 2) Transfer of the new referral and scheduling functions to the Call Center; and 3) Implementation of new "first appointment items", personalized for each patient group. This information was displayed to the physician and operations staff in advance of the consultation.

Phase II: Dec. 29, 2017 to June 28, 2018. We reached 100% of new patients in the BSD: 17% were in the Cancer Group, 23% in the Abnormal Imaging Group, 8% in the Palpable Lump Group, and 53% in the Regular Screening Group.

Phase II results led to the following improvement: 1.) Distribution of the cancer groups' first appointment items equally among surgeons.

Phase III: From June 29, 2018 to the operation. Implemented new appointment items for new clinical patients and for pre-treatment returns.

**CONCLUSION:** With implementation of the Referral Service, the BSD at A.C. Camargo is now able to identify the reason for each appointment before the first consultation. This practice promotes operational predictability and more effectively organizes the personalized journey of each patient, including care on the part of Nurse Navigators for the most critical cases.

**REFERENCE:** Institute of Medicine (US) Committee on Quality of Health Care in America. Crossing the Quality Chasm: A New Health System for the 21st Century. Washington (DC): National Academies Press (US); 2001

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-03-11

Harnessing the distinctive properties of tumor-adjacent tissues to develop ethnicity-dependent biomarkers of breast cancer

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Recent data demonstrating a correlation between lymph node positivity at the time of detection, and the probability of disease recurrence even decades post detection only solidifies the principle that the detection of breast cancer prior to lymph node metastasis can appreciably better clinical outcomes. Although radiologic methods have greatly improved early detection and remain the mainstay for detection, molecular assays to complement existing strategies will reduce number of false positives as well as enhance detection in cases that preclude conclusive diagnosis with radiologic techniques. Normal breast biology is routinely studied using tissues from reduction mammoplasty or normal tissues adjacent to tumor (NATs). However, studies have shown histologic abnormalities in reduction mammoplasty samples and DNA methylation and gene expression abnormalities in NATs due to "field" effects of the tumor. To interrogate the differences between normal breast and NATs as potential early detection markers, we created a tissue microarray (TMA) comprising breast tissues of 100 age-matched healthy women from the Komen Tissue Bank (KTB) and tumor-NAT pairs from 100 women (a total of 300 samples). Approximately 50% of women in each set were of African American (AA) ancestry and the remaining was of European decent. The TMAs was curated as such, because of our recent findings on ethnicity-dependent differences in breast stem-luminal progenitor-mature cell hierarchy. TMA was analyzed for ZEB1, an oncogenic transcription factor that is central to cell fate and stemness, and estrogen receptor alpha (ER $\alpha$ ) and FOXA1, which are expressed predominantly in hormone-responsive mature luminal cells. ZEB1 expressing cells were localized to surrounding ductal structures of the normal breast, whereas ER $\alpha$ + and FOXA1+ cells were located within the ductal compartment. KTB-normal of AA women contained significantly higher levels of ZEB1+ cells compared to KTB-normal of Caucasian women (CA). We observed only marginal increases in ZEB1+ cells in NATs or tumors of AA women. By contrast, in CA women, both NATs and tumors compared to KTB-normal contained higher levels of ZEB1+ cells. The unique localization pattern external to the ductal structures, as well as intrinsically higher expression in AA women suggest that ZEB1+ cells serve not only as stem cells from which cancers may originate but could also contribute to the microenvironment conducive for ductal tumor progression leading to aggressive and early onset of breast cancer as observed in AA women. Conversely, KTB-normal of AA showed modestly higher FOXA1 expression compared to CA women, and further, FOXA1 levels were declined in NATs of AA but not CA women. ER $\alpha$  levels did not change in any of our analyses, pointing to the specificity of ethnicity-dependent changes in this TMA. We also noted ethnicity-dependent variations in the levels of CD8+ T cells, PD-1+ immune cells and PD-L1+ cells but not CD68+ macrophages in NATs, suggesting distinctive immune environment in NATs. This comprehensive approach will not only serve as a platform to develop tumor-adjacent "normal" tissues as molecular markers for early detection but also provides a molecular basis for aggressive breast tumor in AA women.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-04-01

Estrogen receptor  $\beta$  agonists inhibits syngeneic mammary tumor growth through cell-cycle arrest by modulating cell-cycle regulators

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Breast Cancer is the main cause of cancer-associated mortality in women worldwide. The estrogen receptors (ER's) play an important role in normal mammary gland development, as well as in breast cancer. Estrogen Receptor  $\alpha$  is expressed in 70% of breast cancers, where it contributes to increased cell proliferation and decreased cell death. Endocrine therapies such as anti-estrogens and aromatase inhibitors target ER $\alpha$  signaling and improve outcomes of these patients. Syngeneic, immunocompetent mouse models are essential for elucidating the mechanisms and for evaluating novel strategies for the treatment of breast cancer. In contrast to the tumor-inducing role of ER $\alpha$ , ER $\beta$  has been shown to have tumor suppressive activities in various cancer, including the breast cancer. Compounds that selectively activate ER $\beta$  hold promise because they could potentially avoid the unwanted effects of ER $\alpha$  activation, while exploiting the tumor-suppressive function of ER $\beta$ . In the present study, we assessed the antitumor effects of ER $\beta$  agonists using three different syngeneic mouse models; D2A1 (BALB/c) and MM51 (FVB) syngeneic models and *ex-vivo* culture of highly metastatic cell line E0771 (C57/B6). Effect on *in vitro* cancer cell growth was evaluated by cell proliferation and clonogenic assays. Cell cycle distribution was analysed by flow cytometry. Our results demonstrate that ER $\beta$  agonists LY500307 and S-Equol not only inhibited the growth of all three mouse mammary tumor cell lines, but also reduced the colony formation ability. ER $\beta$  agonists also induced the cell-cycle arrest in time and dose-dependent manner. In mechanistic studies, ER $\beta$  agonists LY500307 and S-Equol, modulated the protein levels of cyclin-dependent kinases (CDKs) (4, 6, and 2), cyclins (D1 and E), in a differential manner in these three cell lines. Our *in vivo* studies of D2A1 and MM51 cells demonstrates that ER $\beta$  agonist LY500307 inhibited the tumor growth and the effect was more pronounce in combination with aromatase inhibitor letrozole. Ex-vivo model of E0771 cells showed that LY500307 has potential to dramatically reduce the proliferation of mouse mammary tumor growth. Together, these results identify potential molecular targets and anticancer effects of ER $\beta$  agonists in mouse mammary tumors.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-04-02

Consequences of FOXP1-mediated transcriptional repression of the mitotic checkpoint gene MAD2 in breast cancer

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## **Introduction:**

The mitotic checkpoint, or spindle assembly checkpoint (SAC), is a safeguard mechanism that prevents chromosome missegregation during mitosis. It is well established that overexpression of the mitotic checkpoint component MAD2 leads to chromosome instability (CIN) and tumorigenesis. MAD2 expression is controlled by transcriptional activator and repressor E2Fs, as well as by cell cycle dependent element/cell cycle homology region (CDE/CHR) sequences in its promoter. However, precisely how MAD2 transcription is regulated has remained elusive. Focusing on breast cancer, we therefore aimed to identify novel MAD2 transcriptional regulators and to characterize how these might contribute to CIN and tumor formation.

## **Materials and Methods:**

Potential MAD2 transcriptional regulators were identified using a DNA-protein pull-down assay and mass spectrometry. In-depth characterization of protein-DNA interactions and transcriptional effects were studied using chromatin immunoprecipitation, promoter mutagenesis and reporter assays. Cell lines in which identified repressors were stably knocked-down by shRNAs were used to investigate changes in MAD2 protein levels, cell cycle progression and chromosome segregation fidelity. *In silico* analyses and immunohistochemistry on tissue microarrays were used to study associations between expression of the regulators and MAD2 at the mRNA and protein levels in breast cancer. Repressor expression levels were also assessed in the context of breast cancer patient prognosis and survival.

## **Results and discussion:**

We identified the forkhead box transcription factor FOXP1 as a main transcriptional repressor of MAD2 expression. FOXP1 reduces MAD2 promoter activity via direct binding to CDE/CHR elements. Furthermore, knockdown of FOXP1 expression decreases the proportion of cells in G2/M phase. In addition, low FOXP1 expression strongly correlates with high MAD2 expression at the mRNA and protein levels, and with poor patient prognosis in breast cancer, especially triple-negative breast cancer.

## **Conclusion:**

We identified FOXP1 as a novel transcriptional repressor of MAD2 expression. Our results suggest that reduced FOXP1 levels in breast cancer affect both MAD2 expression and cell cycle progression. This may promote CIN and tumor formation via upregulation of MAD2. Our results may have important implications for breast cancer diagnostics and, potentially, therapeutic targeting.

# 2018 San Antonio Breast Cancer Symposium ®

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Breast cancer cell-derived IL-1B drives metastasis and colonisation of the bone microenvironment

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**Background:** Breast cancer bone metastases are incurable and new therapeutic targets needed. After homing and colonising bone, cancer cells remain dormant, until signals from the microenvironment stimulate their proliferation to form overt metastases. We have recently identified interleukin-1B (IL-1B) as a potential marker for predicting breast cancer patients at increased risk of skeletal relapse and established a role for IL-1 signalling in tumour dormancy in bone. Here we present novel data to support that tumour cell-derived IL-1B plays major roles in breast cancer metastasis and growth in bone.

**Methods:** Tumour/stromal IL-1B and IL-1R1 expression was assessed in samples from the AZURE study following immunohistochemical staining. A humanised mouse model of MDA-MB-231 bone metastasis was used to assess effects of the IL-1R antagonist, Anakinra, on tumour growth and spontaneous metastasis. Effects of tumour cell-derived IL-1B on parameters associated with epithelial to mesenchymal transition and metastasis were measured by ELISA, QPCR, Western blot, transwell and scratch assays in MDA-MB-231, MCF7 and T47D cells transfected with IL-1B/control. Homing of breast cancer cells to bone was monitored in BALB/c nude mice intra venously injected with IL-1B overexpressing/control cells. To assess anti-tumour effects of IL-1 inhibition combined with standard of care, BALB/c or C57BL/6 mice were injected with 4T1 or E0771 cells (intra cardiac or intra ductal) 7-days prior to administration of Anakinra, doxorubicin, zoledronic acid (ZA) or placebo, alone or in combination for 14 days.

**Results:** In tissue samples from >1300 patients with stage II/III breast cancer, active IL-1B in tumour cells correlated with relapse in bone (hazard ratio 1.85; 95% CI 1.05-3.26; P=0.02) and other sites (hazard ratio 2.09; 95% CI 1.26-3.48; P=0.0016). In a model of spontaneous human breast cancer metastasis to human bone, Anakinra significantly reduced metastasis to bone (from 80% in control animals to, 20% in Anakinra treated mice) and reduced the number of tumour cells shed into the circulation. Genetic manipulation of breast cancer cells to overexpress IL-1B demonstrated that exogenous production of IL-1B promoted EMT (decreased E-Cadherin, N-Cadherin and G-Catenin), invasion, migration and organ-specific homing in ER-ve (MDA-MB-231) and ER+ve (T47D and MCF7) cells *in vitro* and *in vivo*.

Contact between tumour cells and osteoblasts or bone marrow cells increased IL-1B secretion from all three cell types. Exposure of tumour cells to IL-1B in the absence of bone cells did not stimulate tumour cell proliferation. Instead, elevated concentrations of IL-1B caused expansion of the bone metastatic niche (increased osteoblasts and blood vessels) that in turn stimulated tumour proliferation.

Adding Anakinra to chemotherapy and ZA to mice injected with E0771 cells completely prevented bone metastasis formation and reduced extra-skeletal metastasis by 33%. In mice injected with 4T1 cells, the triple combination therapy reduced bone metastasis by 50% and extra-skeletal metastases by 65%.

**Conclusion:** Our novel data demonstrate that IL-1B/IL-1R1 signalling plays an important role in breast cancer metastasis to bone. Pharmacological inhibition of IL-1B has potential as a novel treatment.

# 2018 San Antonio Breast Cancer Symposium ®

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Novel mechanisms of Rac1 activation by the Cullin-3/KCTD10 ubiquitin E3 complex in HER2-positive breast cancer

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Overexpression of HER2 in breast cancer is correlated with poor prognosis. HER2-targeted drugs, such as trastuzumab and lapatinib, have been successful to treat HER2-positive breast cancers, however, the acquisition of the drug resistance of the cells is recognized. Here we suggest the novel molecular targets to cure HER2-positive breast cancers.

The oncogenic roles of Rac1, a Rho family small GTPase, in a variety of cancers have been demonstrated. For example, the elevated expression or hyperactivation of Rac1 is frequently observed in human cancers, correlating with their aggressiveness and poor prognosis. In breast cancers, upregulation of Rac1 GEF (GTP exchange factor) and downregulation of Rac1 GAP (GTPase activating protein) have been reported. Moreover, activation of Rac1 contributed to trastuzumab resistance, which poses a serious problem during chemotherapy.

In the present study, we first investigated in detail in which subtypes of breast cancers mRNA expression of Rac1 is correlated with their poor prognosis. Using the METABRIC database, we found that high mRNA expression of Rac1 significantly correlated with the poor prognosis of HER2-positive breast cancer ( $p=0.0012$ , High:  $n=49$ , Low:  $n=171$ ). On the other hands, other three types (basal, claudin-low, or luminal-B type) did not show significant correlation between the expression levels of Rac1 mRNA and their prognosis ( $p=0.15$ , High:  $n=97$ , Low:  $n=102$ ;  $p=0.052$ , High:  $n=110$ , Low:  $n=89$ ;  $p=0.17$ , High:  $n=70$ , Low:  $n=391$ ; respectively). In luminal-A type breast cancer, low mRNA expression of Rac1 significantly correlated with poor prognosis ( $p=0.0046$ , High:  $n=492$ , Low:  $n=187$ ).

We next investigated the molecular mechanism underlying Rac1 activation in HER2-positive breast cancer cells, SKBR-3 cells. We found that Cullin-3 (CUL3, a subunit of a RING ubiquitin E3 ligase complex) and its adaptor protein KCTD10 are essential for Rac1 activation. Mechanistically, CUL3/KCTD10 ubiquitinate RhoB, a Rho family small GTPase that suppresses the activation of Rac1, leading to the degradation RhoB. We also found that HER2 signaling is essential for the activation of Rac1.

**Conclusions:** This study reveals that the novel molecular axis CUL3/KCTD10/RhoB regulates the Rac1 activation in HER2-positive breast cancer cells. The interference of CUL3/KCTD10 complex formation may be a new strategy to the treatment of HER2- and Rac1-positive breast cancers.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-05-03

Isoform specific targeting of insulin receptor

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The insulin receptor (InsR) exists in both an A and B isoform. InsR-B differs from InsR-A by the inclusion of exon 11, which encodes 12 amino acid residues at the C-terminus of the InsR alpha-subunit. Increased InsR-A expression is associated with mitogenic signaling pathways while InsR-B is linked to insulin-mediated metabolic functions. Predominant InsR-A expression may therefore be important in growth and fetal development of embryos, whereas predominant InsR-B expression has a role in metabolic insulin action in adult life. Increased InsR-A expression is seen in breast cancer. In endocrine resistant breast cancer, InsR-A is expressed at high levels (Gradishar, et al. Clin Cancer Res 22:301 2016 PMID: 26324738). Thus, developing InsR-A specific inhibitors could be a useful therapy for breast cancer. We have previously published InsR specific binders using a T7 phage gene 2 protein (Gp2), a small protein scaffold (Chan, et al. Mol Cancer Ther 16:1324 2017 PMID: 28468775), with the long-term goal of creating effective InsR inhibitors and diagnostics. Using yeast display and directed evolution, we identified three Gp2 variants (Gp2 #1, #5, and #10) with low nanomolar affinity and specific binding to cell surface InsR. We have shown that these Gp2 variants inhibited insulin-mediated monolayer proliferation in both endocrine-sensitive and resistant breast cancer, but did not downregulate InsR expression. To further characterize the specificity of Gp2 variants, we used two techniques. HEK293T cells were infected with lentiviral vectors expressing either InsR-A tagged with mCherry or InsR-B tagged with eGFP. Using these cells, we performed “mock panning” and showed the Gp2 #5 variant bound both InsR-A and InsR-B, but had higher affinity for InsR-B. We also incorporated Gp2 #5 into the capsid of a tropism-null adeno-associated virus (AAV). Using this Gp2-AAV, we infected HEK293T-InsR-A or InsR-B cells at a number of different multiplicities of infection. These data were consistent with panning data and showed specific Gp2-AAV infection of cells expressing high levels of InsR-B, but not InsR-A. Thus, our data show that Gp2 variants we created have a higher affinity for InsR-B than InsR-A. Despite this preferred affinity, these Gp2 binders have sufficient binding to InsR-A to disrupt the biological effects of insulin in breast cancer cells. Thus, even relatively low affinity binding to InsR-A can disrupt its function. Further development of InsR-A Gp2 binders may be developed and provide more specific targeting of the breast cancer specific isoform of InsR.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-05-04

Leptin modulates exosome biogenesis in breast cancer cells through an enhanced Hsp90/Tsg101 interaction

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Exosomes, small membrane vesicles secreted by both normal and malignant cells upon fusion of endosomal multivesicular bodies (MVBs) with the plasma membrane, play an important role in cell-to-cell communication. Several reports have highlighted the involvement of exosomes in many aspects of breast cancer (BC) development and progression, thus mounting interest in the potential exploitation of these vesicles and their cargoes as cancer biomarkers, drug delivery systems, and for the development of novel therapies. It has been extensively demonstrated the involvement of the obesity hormone leptin in all steps of breast tumorigenesis, but up to now its role in modulating breast cancer exosome generation has not been investigated. Here, we studied the effect of leptin on exosome biogenesis and secretion in estrogen receptor  $\alpha$  (ER $\alpha$ )-positive MCF-7 and triple negative MDA-MB-231 BC cells. First, we revealed by Transmission Electron Microscopy (TEM) that the number of MVBs in the cytoplasm of leptin-treated MCF-7 and MDA-MB-231 BC cells was significantly increased compared to control untreated cells. Next, we characterized size distribution, particle number and protein cargo of exosomes, isolated by ultracentrifugation method, from conditioned media (CM) of cells treated or not with leptin. Nanoparticle Tracking Analysis revealed that the concentration of exosomes in the leptin treated MCF-7- and MDA-MB-231-CM was significantly higher compared to untreated samples. Furthermore, exosomes quantification by Acetylcholinesterase activity showed that the full leptin receptor antagonist, peptide LDFI, abrogated leptin-induced exosome secretion. Exosomes from leptin treated cells showed an increased expression of the leptin target gene Heat shock protein 90 (Hsp90) and its client protein HER2, along with activated leptin signaling effectors (pJAK2, pSTAT3, and pMAPK<sup>42/44</sup>) compared to exosomes from untreated cells. Mechanistically, our results demonstrated that, among proteins involved in exosome biogenesis, leptin significantly increased protein expression of the well-known exosomal luminal marker Tumor susceptibility gene 101 (Tsg101), without affecting its mRNA levels. Thus, we aimed to analyse specific Tsg101 protein-protein interaction as a possible mechanism able to modulate its stability or half-life within the cells. To answer to this question, we performed, in MCF-7 BC cells, co-immunoprecipitation studies combined with mass spectrometry. Analysis of the spectra identified Hsp90 as a specific Tsg101 interacting protein, and concomitantly immunoblotting assays revealed a specific interaction between HSP90 and Tsg101 in basal condition that was further increased upon leptin exposure. Accordingly, leptin-induced Tsg101 protein levels were completely abrogated in the presence of specific Hsp90 inhibitor, 17-allylamino-17-demethoxygeldanamycin.

In conclusion, our results demonstrate for the first time that leptin was able to increase exosomes release in BC cells, through an up-regulation of Tsg101 expression at posttranslational level. These findings, providing additional insights into the molecular mechanism governing exosome generation in BC cells, might open new avenues for therapeutic intervention in BC.

## 2018 San Antonio Breast Cancer Symposium ®

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eEF1A2 facilitates PTEN-GSK3 $\beta$  mediated Aurora-A protein degradation during S-G2 phase inactivated in PTEN-deficient breast cancer

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The *AURKA* gene, encoding Aurora kinase-A (Aurora-A), is frequently amplified and overexpressed across multiple cancer types correlating with poor prognosis. Although the *AURKA* gene is frequently amplified in human cancers, underlying mechanism(s) for Aurora-A protein stability through different phases of cell cycle are not well elucidated. Inhibiting the kinase activity and promoting protein degradation are two well-validated conceptual strategies for targeting protein kinases in cancers. Here, we demonstrate that Eukaryotic Elongation Factor 1 Alpha 2 (eEF1A2) facilitates PTEN-GSK3 $\beta$  mediated Aurora-A protein degradation through the SCF complex (SKP1-Cul1-FBXW7) during the S/G2 phase of proliferating cells. In contrast, this mechanism is inactivated in cancer cells accompanying PTEN-GSK3 $\beta$  pathway deficiency. Mechanistically, eEF1A2 interacts with Aurora-A, GSK3 $\beta$ , FBXW7 and Cul1-E3 ligase, as the SCF complex, to facilitate Aurora-A polyubiquitination for 26S proteasomal degradation. eEF1A2 promotes PTEN phosphorylation at T366 and stability, inactivates AKT and activates GSK3 $\beta$  which in turn phosphorylates Aurora-A at S283, S284 and S342. The phosphorylation of Aurora-A at S342 is detected during S/G2 phase of cell mitosis in parallel with eEF1A2-SCF complex formation with active form of GSK3 $\beta$  and neddylated Cul1. Conversely, genetic ablation of *EEF1A2* and *PTEN*, activation of AKT, inhibition of GSK3 $\beta$ , expression of Aurora-A phosphodeficient-mutant attenuates the Aurora-A protein degradation which is corroborated in Aurora-A overexpressing mouse mammary carcinomas and human breast carcinomas. This study identifies a novel mechanism of Aurora-A protein degradation mediated eEF1A2-PTEN-GSK3 $\beta$  pathway and provides a framework for the discovery of Aurora-A therapeutic targets in breast cancer that harbors deficiency of PTEN tumor suppressor pathway.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-05-06

Endothelial nitric oxide synthase gene intron4 VNTR polymorphism in patients with breast cancer

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**Background:** Endothelial nitric oxide synthase (eNOS), also known as NOS3 is an enzyme that in humans is encoded by the NOS3 gene located in the 7q35-7q36 region of chromosome 7. Nitric oxide (NO) is synthesized from L-arginine by eNOS in the vascular endothelium and plays crucial roles in cellular proliferation. In the present study, it is hypothesized that polymorphisms of the Intron4b/a variable number of tandem repeat (VNTR) polymorphism Intron4b/a in the eNOS gene may be associated with an increased risk in the developing breast cancer.

**Methods:** We included 38 (38 F/Female) patients with breast cancer and 70 (44M/26F) healthy controls. The VNTR polymorphism in intron4 (intron4b/a) was analyzed by PCR. The results were statistically analyzed by calculating the odds ratios (OR) and 95% confidence intervals using the  $\chi^2$  test.

**Results:** The distributions of genotype and allele frequency was compared among the groups. The bb, ab and aa genotypes were observed in 29 [76.3%], 9 [23.7%], 0 [0%] patients with breast cancer and in 33 [47.1%], 15 [21.4%], 22[31.5%] healthy controls respectively. The b and a alleles were observed in 67 [88.2%], 9 [11.8%] patients with breast cancer and in 81 [57.8%], 59 [42.2%] healthy controls respectively. The susceptibility to patients with breast cancer had significantly higher frequencies in bb genotype ( $p=0.004$  OR=3.613). The patients with breastcancer had significantly lower frequencies in aa genotype ( $p=0.001$ ,OR=1.458). The frequency of the a allele was significantly lower in the patients with breast cancer ( $p=0.001$ , OR=4.070)

Comparison of eNOS / VNTR polymorphism between patients with Breast and healthy control subjects.

eNOS VNTR	Breast	Healthy Controls	OR*	%95 CI*	p
Genotypes	n=38 (%)	n=70 (%)			
BB	29 (76.3)	33 (47.1)	3.613 a	1.494-8.735 a	0.004 a
			3.065 b	1.440-6.520 b	0.004 b
AB	9 (23.7)	15 (21.4)	0.879 a	0.343-2.252 a	0.812 a
			0.902	0.388-2.096 b	0.832 b
AA	0 (0)	22 (31.5)	1.458 a	1.244-1.709 a	0.001 a
			12.375 b	2.764-55.396 b	0.001 b
Allele					
B	67 (88.2)	81 (57.8)	5.422 a	2.505-11.740 a	0.001 a
A	9 (11.8)	59 (42.2)	4.070 b	2.199-7.533 b	0.001 b

\* Fisher's Exact Test a, comparison of genotype and allele frequencies between breast and healthy control groups

**Conclusion:** We conclude that there was sensible correlation between eNOS gene intron 4b/a VNTR polymorphism and the risk of breast cancer and bb genotype frequency was significantly higher in these patients than healthy controls.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-06-01

### Regulation of BRCA1 by SIRT2

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We are constantly exposed to a variety of both external and internal DNA damaging agents, such as UV light from the sun and reactive oxygen species created as by-products of aerobic respiration. As a result, our DNA accumulates thousands of instances of damage per cell per day. DNA damage response (DDR) pathways, which include DNA repair and cell-cycle checkpoints, are responsible for the repair of DNA damage and are critical for protecting against mutagenesis and maintaining genome integrity. DNA double-stranded breaks (DSBs) are the most deleterious type of DNA damage and are repaired by one of two pathways: Non-homologous end-joining (NHEJ), an error-prone mechanism of repair active throughout the entire cell cycle, or homologous recombination (HR), considered to be an 'error-free' method for DSB repair that occurs in the S and G2 phases of the cell cycle. Deficiencies in NHEJ or HR can result in genomic instability via genomic incorporation of chromosomal aberrations, which can ultimately lead to an increased risk of cancer. However, in many cases, the mechanisms by which defects in these pathways lead to an increased risk of developing cancer is unknown, making preventative care and treatment of resulting cancers more difficult. Breast Cancer 1 (BRCA1), an established tumor suppressor, is a protein necessary for the proper repair of DNA DSBs through the HR pathway. Defects in BRCA1, whether genetically inherited or spontaneously developed, have been linked to different types of cancer in both men and women, including breast, ovarian, and pancreatic cancer. Yet, the regulation of BRCA1 in HR is not well understood and thus highlights a major gap in our understanding of how deficiencies in HR contribute to the development of cancer. Our lab has discovered that SIRT2, a class III NAD<sup>+</sup> dependent histone deacetylase and putative human tumor suppressor, plays a crucial role in the DDR and repair of DNA DSBs. We have shown that depletion of SIRT2 impairs HR and increases cell sensitivity to ionizing radiation in a deacetylase-dependent manner. A mass spectrometry analysis showed SIRT2 interacts with several proteins involved in DDR, including BRCA1. We validated the interaction between SIRT2 and BRCA1 and found SIRT2 deacetylates BRCA1 both *in vitro* and in cells. Depletion of SIRT2 and subsequent deacetylation of BRCA1 decreases BRCA1 protein levels in cells, impairing HR. Our results show SIRT2 is a novel regulator of BRCA1 and is critical for the repair of DNA DSBs through HR. These findings provide invaluable insights into how to exploit the interplay between SIRT2 and BRCA1 as a novel therapeutic approach for the prevention and treatment of cancer.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-06-02

Mismatch repair protein loss in breast cancer: Clinicopathological associations in a large British Columbia cohort

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**Background:** Alterations to mismatched repair (MMR) pathways are a known cause of cancer (particularly colorectal and endometrial). Recently, the FDA approved pembrolizumab for use in MMR-deficient (MMRD) cancers of any type, and the diagnosis can be made by immunohistochemistry (IHC) or genomic methods. In breast cancer, mutational process analyses indicate MMRD occurs in about 2% of breast cancer (Cancer Res; 77; 4755-62, 2017) and recent functional studies have shown associations with resistance to endocrine therapy and sensitivity to CDK4/6 inhibitors (Cancer Discov; 7; 1168-83, 2017). To date, insufficient cases have been assembled to power meaningful associative or survival studies. Herein, the strong correlation between IHC-determined loss of MLH1, PMS2, MSH2 or MSH6 and genomic evidence allowed the assessment of MMRD on a large tissue microarray (TMA) series linked to detailed biomarkers and long-term outcome data.

**Methods:** IHC markers MLH1, PMS2, MSH2 and MSH6 were optimized on the Ventana automated stainer for application to breast cancer TMAs. The patient cohort consists of females from British Columbia diagnosed with primary invasive breast carcinoma in 1986-1992, referred to the British Columbia Cancer Agency for treatment and follow-up. TMA blocks were sectioned and stained. Slides were scored by a pathologist and only nuclear positivity was evaluated positive. Loss of nuclear positivity for any one of the four tested marker defined MMRD. Clinicopathological associations were tested by Chi-square, and survival by Kaplan-Meier plot with log rank test.

**Result:** 1635 cases were interpretable for all MMR markers. 31 cases (1.9%) met criteria for MMRD. 6 cases had paired losses (4 MLH1-PMS2 loss, 2 MSH2-MSH6 loss) and the remaining 25 cases had singular MMR loss (11 PMS2 loss, 10 MLH1 loss, 3 MSH6 loss, 1 MSH2 loss). Deficiency of the the MutL complex (MLH1/PMS2) predominated over the MutS complex (MSH2/MSH6).

Among the demographic and pathological variables assessed – age, grade, tumour size, lymphovascular invasion, nodal and menstrual status – high grade is associated with MMRD ( $p=0.014$ ). In terms of biomarker, MMRD is significantly associated with PR negativity ( $p=0.003$ ) and PD-L1 expression ( $p=0.049$ ), but not with ER, Her2, Ki67, or basal breast cancer IHC markers, nor does MMRD significantly correlate with any of the established major intrinsic subtypes of breast cancer. Tumor infiltrating lymphocyte (TIL) counts are higher in MMRD cases ( $p=0.009$ ). Although statistically not significant (small numbers), Kaplan-Meier plots of survival analysis demonstrated a trend for MMR loss to be associated with decreased breast cancer disease-specific and overall survival.

**Conclusion:** This large series assessed by IHC corroborates findings from smaller genomic series that MMRD is present in about 2% of breast cancers. MMRD tumors are more likely to be high grade, low PR and immunologically active (higher PD-L1 expression and TIL counts). MMR deficiency is present across all major molecular subtypes (luminal, HER2, basal). Given the efficacy of PD1/PDL1 targeting agents in MMR deficient tumors of other types, evidence for the activity of these agents in MMR deficient breast cancers should be actively sought.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-06-03

A selective Cdk12/13 non-covalent inhibitor with potent anti-breast cancer activity

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Cyclin-dependent kinases 12 and 13 (CDK12 and CDK13) are Ser/Thr protein kinase members of the CDK family. CDKs are regulated by cyclins and members of the family are key regulators of the cell cycle. Other members of this family—CDK7, CDK8, CDK9, CDK12 and CDK13—have been described as transcription-regulating kinases. CDK12 and CDK13 phosphorylate the C-terminal domain (CTD) of rpb1 the largest subunit of RNA polymerase II and interact with RNA processing factors, playing pivotal roles controlling transcription initiation and elongation, as well as posttranscriptional RNA processing. Specifically, CDK12 acts as the limiting factor for the transcription of a small subset of genes that are involved in the DNA damage response pathway and CDK13 affects expression of genes involved in growth signaling pathways. Importantly, like their cell cycle family members CDK12 and CDK13 have also been shown to be promising therapeutic targets for several cancer types. We have generated a novel, selective, and orally bioavailable, small molecule inhibitor of CDK12 and CDK13. Our lead molecule downregulates key DNA damage repair proteins including ATM, ATR, Brca1 and Rad51 leading to induction of apoptotic cell death in all triple negative breast cancer (TNBC) cell lines tested. We have confirmed the selectivity of our compound by kinase profiling and by comparing the gene expression profile by RNA-seq of cells treated with compound or infected with CRISPR sgRNA targeting Cdk12 and/or Cdk13. Importantly, our compound acts in synergy with the chemotherapeutic agents Cisplatin, Irinotecan and Olaparib. Finally, in preclinical efficacy studies, our lead compound blocked tumor progression in two different PDX models of TNBC and demonstrated tumor regression and enhanced efficacy over single treatment when dosed in combination with selected chemotherapeutic agents.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-06-04

Small-molecule screening nominates diverse combination therapies that sensitize BRCA mutant and wild-type triple negative breast cancer to PARP inhibition

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**Background:** Triple negative breast cancer (TNBC) remains a heterogeneous clinical phenotype with few, known therapeutic targets. PARP inhibitors (PARPi) are the first approved, targeted therapy in TNBC, limited to germline *BRCA* mutant (*BRCAm*) cancers that lack homologous recombination repair capacity. Even in this context, resistance quickly emerges via secondary mutations that restore DNA repair ability. While DNA damage repair is an intriguing target in *BRCA* wild type (*BRCAwt*) TNBC due to inherent, genomic instability, PARPi alone have been ineffective in unselected populations. Systematic approaches to define novel drugs that sensitize *BRCAwt* and *BRCAm* TNBC to PARPi would greatly improve therapeutic efficacy and durability. **Methods:** *BRCAwt* (HCC1806) and *BRCAm* (SUM149PT) cell lines were screened in duplicate using a 2,100-compound small molecule library. Cell lines were plated in media containing DMSO or sub-lethal doses of the PARPi, olaparib, onto Selleck Bioactive drug plates. Cell viability was assessed after 72 hours, then normalized to vehicle control. Hit cut-offs were predefined as log2 drug/DMSO of  $\leq -0.7$  with a viability difference greater than 20% -where stringent scoring thresholds were chosen to exceed the full range of scores observed in 816 empty control wells. Hits were sorted by target and pathway to provide mechanistic insight into the synergy of combinations. Drug combinations with the highest potential for near term translation were validated using GI50 viability assays in 9 *BRCAwt* and *BRCAm* TNBC cell lines. The most promising combination was further validated via immunoblotting, colony formation, and apoptosis assays.

**Results:** Several drug classes affecting well-known oncogenic signaling pathways conferred sensitivity to PARPi, with more hits in the *BRCAm* cell line. Relevant druggable targets sensitizing cells to olaparib in *BRCAm* TNBC that met the predefined cut-point were inhibitors of PI3K (pan-PI3K, PI3K $\alpha$  and PI3K $\beta$  specific), VEGFR, MEK, EGFR, NF-kB, aurora kinase and several DNA damaging agents. Aurora kinase, EGFR, and NF-kB inhibition sensitized cells to olaparib, yet upon further validation, synergy was mild. The screen identified ATM inhibitors, KU-55933 and KU-60019, as sensitizers of *BRCAm* cells to olaparib. The potent ATM inhibitor, AZD0156, and olaparib were a highly synergistic combination validated in all 9 *BRCAm* and *BRCAwt* TNBC cell lines via cell viability, annexin V, and colony formation assays. Immunoblotting of relevant DNA damage repair proteins showed that olaparib caused upregulation of p-ATM in *BRCAm* and *BRCAwt* cells. p-ATM expression decreased in response to combination ATM and PARP inhibition. Attenuated levels of p-ATM resulted in increased levels of p- and T- $\gamma$ H2AX, indicating an accumulation of double stranded DNA breaks.

**Conclusion:** *In vitro*, inhibition of several relevant, oncogenic pathways yielded sensitivity to PARPi in TNBC. We identified the ATM inhibitor, AZD0156, and olaparib as a potent combination regardless of *BRCA* status, a finding currently being evaluated in patient-derived *in vivo* models. Combination ATM plus PARP inhibitor therapy is a promising and feasible approach for near term translation in metastatic TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

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Mre11 mediates a p53-independent quiescence program in response to mammary oncogene activation

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**Background:** Oncogenic mutations drive uncontrolled proliferation, imposing significant replication stress and activating the DNA damage response (DDR) in early tumorigenesis. The accumulation of double strand breaks (DSBs) is recognized by the Mre11-Nbs-Rad50 (MRN) Complex, a critical sensor that mediates damage signaling and cell cycle checkpoint responses. The mechanism by which the DDR acts as an anti-tumor barrier is in part due to activation of p53 tumor suppressor. However, the role of the DDR in a p53 null or deficient setting has been poorly investigated. In basal-like breast cancer (BLBC) where p53 loss is ubiquitous, the functional consequence of the DDR is in need of further delineation. Previously, we have observed that Mre11 suppresses proliferation in hyperplastic mammary epithelium through a p53-independent mechanism. Here, we evaluate how Mre11 affects cell cycle regulation in p53 deficient primary mammary epithelium induced to overexpress the oncogene c-Myc, a known driver of BLBC.

**Methods:** We performed single-cell live imaging on primary murine mammary epithelial cells (mMECs) induced to overexpress c-Myc and be p53 deficient, with or without additional mutations in Mre11. Cells were further infected with a fluorescent cell cycle reporter: Proliferating Cell Nuclear Antigen (PCNA)-mCherry. By tracking PCNA foci changes via time-lapse imaging modalities, we were able to calculate distinct cell cycle phase lengths, identify disruption of cell cycle, visualize mitotic aberrancies, and characterize the cell cycle profile of p53 deficient, c-Myc activated primary mammary epithelial cells with or without intact DDR.

**Results:** Our results indicate that the presence of Mre11 mediates a quiescence phenotype even in the absence of p53 tumor suppressor with up to 75% of the cell fraction becoming quiescent. This fraction is significantly reduced ( $p < 0.0001$ ) when Mre11 is targeted using gene editing giving rise to highly aberrant multinucleated mMECs capable of unrestrained replication and entry into mitosis. Cells with intact Mre11 undergo cell cycle arrest post abnormal mitoses and are characterized by quiescent cells with classic micronuclei formation. In contrast, mMECs without Mre11 are likely to be multinucleated with reduced micronuclei formation and rarely undergo cell cycle arrest. The loss of Mre11 enables these cells to continue cell cycle progression despite tremendous mitotic aberrancies. **Impact:** Mre11 is an important mediator of a p53 independent quiescence program in c-Myc expressing cells. It regulates the proliferative activity of multinucleated daughter cells and their ability to become quiescent. Furthering our understanding of how Mre11 regulates this molecular quiescence program may provide new insights into drivers of genomic instability in BLBC and novel therapeutic targets.

# 2018 San Antonio Breast Cancer Symposium ®

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Direct ex vivo observation of homologous recombination defect reversal after DNA damaging chemotherapy in metastatic breast cancer patients

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## Introduction

Better predictive biomarkers for response to Poly ADP-Ribose inhibitors (PARPi) are required, since on the one hand evidence is emerging that PARPi are also effective beyond germline *BRCA* mutated (*gBRCAm*) cancers and on the other hand *gBRCAm* cancers can become resistant to PARPi. Therefore, we previously developed a functional homologous recombination (HR) assay exploiting the formation of RAD51 foci in proliferating cells after *ex vivo* irradiation of fresh primary breast cancer (BrC) tissue (n=148): the REpair CAPacity (RECAP) test. The aim of the current study is to molecularly characterize real-time HR deficient (HRD) tumors and explore the utility of RECAP as a predictive biomarker for PARPi treatment in metastatic BrCs.

## Material and method

Patients with advanced or recurrent BrC with easily accessible metastases were eligible. Fresh tissue biopsies from metastatic BrC lesions were collected in customized medium, irradiated with 5 Gy and cultured for 2 hours. Molecular characterization of functional HRD biopsies as well as platinum/PARP resistant biopsies was performed by targeted sequencing (*BRCA1/2*, *TP53*, *CHEK2*, *PALB2*), *BRCA1* promoter methylation analysis and multiplex ligation-dependent probe amplification (MLPA) analysis of *BRCA1* and *BRCA2* to identify large rearrangements.

## Results

41 biopsies were derived from 38 patients with recurrent or metastatic BrC. The RECAP test had a high success rate (93%) when performed on core needle or punch biopsies, and test results were available within 1 week. HRD was detected in 13 out of 41 biopsies (32%), among which 5 were *gBRCAm*, indicating that the RECAP test identifies more patients who may benefit from PARPi treatment than *gBRCA* analysis only. Among the 8 non-*gBRCAm* HRD tumors was one tumor with a germline *PALB2* mutation, one with *BRCA1* promoter hypermethylation and two with somatic variants of unknown significance (VUSes) in *BRCA2*. In three *gBRCAm* patients *BRCA* reversion was detected, as the HRD tumors became HR proficient (HRP) after showing *in vivo* progressive disease (PD) on cisplatin/PARPi treatment. One of these patients obtained a secondary *BRCA1* mutation that restored the open reading frame and led to production of full-length *BRCA1* protein, while the causative molecular event in the other patients is still elusive.

## Conclusion

The RECAP test is a robust and reproducible HRD test which identifies approximately 60% more potential candidates for PARPi treatment, as 40% of HRD tumors were caused by *gBRCAm*. Due to its functional character, the RECAP test reflects the real-time HR status regardless of *BRCA* mutational status and therefore detects HR reversal upon therapy resistance. Thus, RECAP shows great potential as a predictive biomarker for PARPi treatment of metastatic BrC.

# 2018 San Antonio Breast Cancer Symposium ®

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Characterization of chromosomal instability in the TNT trial: A randomized phase III trial of carboplatin compared with docetaxel for patients with metastatic or recurrent locally advanced triple negative or BRCA1/2 breast cancer (CRUK/07/012)

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## Background

A distinctive trait of triple negative breast cancer (TNBC) is the acquisition of genome wide highly aberrant copy number states, which is more evident in metastatic settings. The level of copy number alterations can be characterized by quantitative estimates of chromosomal instability, such as allelic imbalanced copy number aberrations, telomeric allelic imbalance (NtAI), homologous recombination deficiency (HRD) score, referred here as genomic scars. Several of these scars are reported as being indicative of BRCAness and potential predictive and/or prognostic biomarkers of chemotherapy response, currently mostly demonstrated in neoadjuvant settings in TNBC.

## Aims

Using several genomic scar measures, we aim to capture chromosomal instability and test their predictive and prognostic value in metastatic or recurrent locally advanced triple negative or *BRCA1/2* mutated breast cancer in the TNT trial.

## Methods

Patients recruited to TNT (n=376) had ER-/PR-/Her2- breast cancer or were germline BRCA mutation carriers. Genome-wide copy numbers (CN) were derived from FFPE samples including primary tumours and positive lymph nodes (n=183, docetaxel=93, carboplatin=90; BRCA1 mut=25). Genomic scars were generated using ASCAT (Van loo et al., PNAS 2010) CN profiles. HRD scores were established by Myriad Genetics, Inc. assay (n=272). BRCA1-like classifier was applied as described in Schouten et al., Mol Onc 2015. Shannon diversity index was calculated using ASCAT raw CN profiles. Association of genomic scars with PAM50 subtypes and BRCA1 deficiency status were evaluated using Kruskal-Wallis test; p-values were adjusted for multiple comparisons (Dunn's test). Statistical significance was defined as p<0.05. Association of genomic scars with objective tumour response rate (ORR) and Progression Free Survival (PFS) was assessed using logistic regression and restricted mean survival analysis, respectively.

## Results

HRD and NtAI scores were higher in basal like samples compared to non-basal like (median diff. HRD=11.5, p=0.005; NtAI=3, p=0.04). HRD (p=2e-14) and NtAI (p=0.003) scores were also higher in BRCA1 deficient (BRCA1 germline/somatic mutant or BRCA1 methylated) samples compared to non-deficient. Using the BRCA1-like classifier, 42 out of 50 BRCA1 deficient samples and 93 out of 133 BRCA1 non-deficient/undetermined samples were identified as BRCA1-like. The Shannon diversity index, measuring CN heterogeneity, clustered samples into 3 groups. Analysis of ORR showed non-significant trends to preferential response rates with docetaxel in cluster 1 and 3. Membership of cluster 2 predicted higher ORR to carboplatin over docetaxel (interaction p=0.017). PFS indicated a treatment effect in cluster 2, but not in cluster 1 or 3; there was no evidence of interaction between subgroups and treatment (p=0.15).

## Conclusions

Our results suggest that the overall heterogeneity of the copy number landscape is a promising area for seeking predictive/prognostic biomarkers in metastatic TNBC, and combined with other modalities of high-dimensional omics data could provide essential treatment response information.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-06-08

A p53-independent DNA damage response that regulates breast cancer phenotypes

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Defects in the DNA damage repair system result in increased genomic instability and have recently been implicated as being drivers of tumorigenesis in both familial and sporadic breast cancers. To maintain genomic integrity, cells have a DNA damage response (DDR) mechanism that functions to repair damaged DNA efficiently and commits cells to death if damage is irreparable. Failure of this mechanism results in genomic instability and cancer predisposition. Widespread chromosomal instability is a characteristic feature of Triple-Negative Breast Cancer (TNBC), making it difficult to decipher between genes that drive cancer development from those that play a bystander role. Little is known about what gives rise to the extensive genomic instability of TNBC, and presents a major deficit in our scientific and clinical knowledge.

Oncogene induced hyper-proliferation results in replication-associated double strand breaks (DSBs) that engage an Mre11-Rad50-Nbs1 complex-dependent DDR. Classically, the oncogene induced DDR is believed to suppress tumorigenesis due to downstream activation of p53. Using genetically engineered primary mammary epithelial cell models, we demonstrate p53-independent effects of the Mre11-dependent DDR in suppressing proliferation and DNA damage induced by diverse oncogenic drivers. Single cell whole genome sequencing in *Her2/Neu* expressing primary mammary epithelial cells reveals a landscape of stochastic copy number aberrations induced by oncogenic stress that becomes enriched for a genomic scar pattern of larger-size deletions in cells with Mre11 hypomorphism. We identify Mre11 pathway hypomorphism in a subset of basal-like breast cancers (BLBC), which confers vulnerability to specific DNA damaging agents and DDR inhibitors in murine models of p53-deficient BLBC. Thus, assessing the functional status of the Mre11-dependent DDR pathway in p53-mutant breast cancers may provide an opportunity for therapeutic exploitation.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-06-10

Combined tandem affinity purification mass-spectrometry technique with genome-editing CRISPR-Cas9 knockout screening to identify potential subunits in the BRCA1 complex

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**Background:** Genomic instability is one of tumor characteristics. Enhancing the DNA damage load or impairing the ability of DNA damage repair has been therapeutics of cancer. Breast cancer type 1 susceptibility gene (BRCA1) has been studied a lot about the DNA damage and repair in breast cancer and ovarian cancer. BRCA1 mutation increases the risk of developing breast cancer by an eighth to tenth. The C terminal domain of BRCA1 can associates with three proteins Abraxas, Bach1 and CtIP in a phosphorylation-dependent manner forming mutually exclusive complexes, namely BRCA1-A, B, and C complexes. The BRCA1-A complex is necessary in DNA damage repair, and study implicates the complex play roles in chemotherapy resistance.

**Methods:** We explored tandem affinity purification mass-spectrometry (TAP-MAS) technique to identify potential subunits associated with NBA1(one component of BRCA1-A complex). Then we made the genome-editing CRISPR-Cas9 sgRNA library into lentivirus to infect U2OS cells. And 5 Gy dose of Ionizing radiation (IR) was used to induce DNA damage on the cell. After 14 days cultivation, we extracted the DNA from the cells, performed polymerase chain reaction (PCR) and analyzed the correlation between potential genes and DNA damage-repair passage by bioinformatics methods. We generated 200 breast cancer patient tissue samples in our cancer center and performed immunostaining assay.

**Results:** By the TAP-MAS technique of NBA1 tagged with HA and Flag, we found 93 potential subunits except those known subunits in BRCA1-A complex. Combined with CRISPR-Cas9 sgRNA library screening, we scored the relativity of DNA damage and repair passageway of identified 93 potential subunits. We found that nucleoside-triphosphatase, cancer-related (NTPCR) as a new potential subunit of BRCA1-A complex ( $p$  value=0.0034) had the highest score. Endogenous and exogenous co-IP validated NTPCR physically associates with subunits within BRCA1-A complex. Besides, we found that NTPCR associated with the same domain of NBA1 as the other subunits in BRCA1-A complex. Immunohistochemistry of patient tissue samples indicated that high levels of NTPCR expression was correlated with poor prognosis in multivariate analysis (HR: 4.990; 95%CI: 1.433-17.378;  $p$  value: 0.012).

**Conclusion:** NTPCR is a new subunit in BRCA1-A complex. And high expression of NTPCR is a negative prognostic factor in breast cancer patients.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-06-11

Microsatellite instability in triple negative breast cancers

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**Background:** Microsatellite instability (MSI) is a phenotype resulting from defect in mismatch repair genes. The Food and Drug Administration approved anti-programmed death 1 (PD-1) immune checkpoint inhibitor for any solid tumor with MSI-high (MSI-H). Some tumors had good response to PD-1 blockade and it is a promising treatment for a part of refractory breast cancers. Our goal was to determine the frequency of MSI in triple negative breast cancer (TNBC), one of the most clinically aggressive subtypes.

**Patients and Methods:** This study included 228 patients with primary TNBC underwent resection without neoadjuvant chemotherapy between January 2004 and December 2014. Genomic DNA was extracted from formalin-fixed and paraffin-embedded tissue. Tumor and control DNA were amplified by polymerase chain reaction at the following 5 microsatellite markers: NR-21, BAT-26, BAT-25, NR-24, MONO-27. We classified the tumors as microsatellite stable (MSS), MSI-low or MSI-H.

**Results:** The mean age of patients was 59 years (range: 30-89) and all were women. T1 tumors were 57.9% and N0 were 67.5%. Meanwhile, the tumors with nuclear grade 3 were 66.2% and high Ki-67 (> 30%) were 66.7%. Among the 228 tumors, 222 tumors (97.4%) revealed MSS, of which 6 (2.6%) revealed MSI and 2 (0.9%) were MSI-H. Among the MSI tumors, T and N factor were showed as follows: T1: 2 tumors, T2: 3 tumors, T3: 1 tumor, N0: 5 tumors and N1: 1 tumor. Of two MSI-H tumors, one showed T1N0 and another showed T2N0. The both of them showed nuclear grade 3, high Ki-67 (> 30%) and had common following instable markers: NR-21, BAT-26 and BAT-25.

**Conclusions:** Our results demonstrated that the frequency of MSI-H was 0.9% (2/228). MSI might not be useful as a biomarker for immune check point inhibitors. MSI should be combined with another biomarker such as tumor mutational burden in TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-07-01

Novel role of the tumor suppressor ductal epithelium associated ring chromosome 1 (DEAR1) in regulation of breast stem/progenitor cell properties

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**Introduction:** Breast cancer is the most commonly diagnosed cancer and third-leading cause of cancer-related deaths in women in America. A quarter of lesions diagnosed annually are ductal carcinoma in situ (DCIS), one of the earliest pre-invasive forms of invasive ductal carcinoma (IDC). Without therapeutic intervention, 30-50% of DCIS cases can progress to IDC. Understanding the mechanisms regulating progression from DCIS to IDC would help identify biomarkers to stratify DCIS patients at higher risk of progression or recurrence. Cumulative literature suggests the earliest phase of dissemination from the primary tumor is driven by the epithelial-mesenchymal transition (EMT) program. DEAR1 is a tumor suppressor gene which is mutated, undergoes loss of heterozygosity in breast cancer, and is downregulated in DCIS lesions. DEAR1 regulates acinar morphogenesis and cell polarity, and is a negative regulator of TGF-beta-driven EMT by binding to and ubiquitinating SMAD3, thereby limiting the amount of SMAD3 available to activate an EMT signature. Overexpression of EMT master regulators, or exposure to TGF-beta in immortalized human mammary epithelial cells (HMECs), results in mammosphere formation and breast stem cell markers, thus linking the EMT process to acquisition of stem cell characteristics.

**Methods:** Stable lentiviral shRNA knockdown, in vitro mammosphere assay, cytopsin, immunofluorescence, immunoprecipitation, western blot analysis, ubiquitination and real-time quantitative PCR were performed.

**Results:** DEAR1 knockdown in immortal HMECs resulted in a significant enhancement of primary mammosphere formation and growth compared to controls, suggesting that DEAR1 may regulate stem/progenitor cell properties; this effect was greater when cells were exposed to TGF-beta. To determine if DEAR1 regulates stem cell properties through regulation of SMAD3 levels, DEAR1-SMAD3 double knockdown clones were examined for mammosphere formation. Results indicated fewer mammospheres in double knockdown clones, but only in the presence of TGF-beta, suggesting that the mammosphere phenotype is partially dependent on the TGF-beta-SMAD3 pathway. The observation that loss of DEAR1 alone results in a stem/progenitor cell phenotype indicates that DEAR1 may regulate mammosphere formation independent of its role in regulating the TGF-beta pathway. HMEC mammospheres express higher levels of stem cell marker aldehyde dehydrogenase (ALDH1) and co-express luminal and basal cytokeratins, suggesting bipotential capacity. We also observed upregulation of SNAI2 and ZEB1, master EMT and stem cell regulators, in DEAR1 knockdown HMECs. DEAR1 interacted with SNAI2 by co-immunoprecipitation analysis and also promoted the polyubiquitination of SNAI2.

**Conclusions:** Loss of the tumor suppressor/polarity regulator DEAR1 promotes stem cell properties in part through the DEAR1-TGF-beta-SMAD3 axis. We demonstrate a DEAR1-SNAI2 axis that may partially regulate stem/progenitor cell properties in HMECs through the polyubiquitination of SNAI2, a master regulator of EMT and stemness. These mechanisms governing acquisition of the stem cell properties may contribute to understanding how DCIS progress to IDC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-07-02

**BCL6:** A novel target of triple negative breast cancer stem cells

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**Introduction:** Despite improvements in breast cancer treatment in recent years, triple negative breast cancer (TNBC) is still characterized by a poor prognosis and a lack of specific actionable alterations. Thus, the identification of new molecular targets represents a clinical priority. BCL6 is a well-known transcriptional modulator that acts as a repressor of DNA-damage sensing and cell cycle progression pathways in the context of B-cell lymphomas, while its implication in controlling breast cancer cell growth, survival, and invasiveness has been recently described. This study investigated the role of BCL6 expression in TNBC initiation and stemness (TNBCSCs).

**Methods:** The correlation between BCL6 expression and the overall survival (OS) of TNBC patients, as well as the association with stemness-related pathways were assessed *in silico* using the METABRIC dataset. Following BCL6 silencing or pharmacological inhibition by the small molecule FX1, mammosphere-forming efficiency (MFE) and ALDH and CD44 CSC markers expression were evaluated in SUM149 and SUM159 TNBC cell lines.

**Results:** *In silico* analysis of the METABRIC dataset has showed that BCL6 overexpression is associated with poor overall survival and enrichment in stemness signatures in TNBC patients. *In vitro*, BCL6 silencing resulted in a significantly reduced number of mammospheres compared to internal control, as well as a simultaneous decreased percentage of ALDH+ and CD44+ cells in all the TNBC cell lines tested. Moreover, blocking the transcriptional repressor activity of BCL6, using its selective inhibitor FX1, confirmed the impairment of the MFE. Of note, following BCL6 silencing, we observed a global reduction in the repressive marker H3K27me3, suggesting a cooperation between BCL6 and the breast EMT- and stemness-related enzyme EZH2 in inducing specific gene transcription repression in TNBC.

**Conclusion:** Downregulation and pharmacological inhibition of BCL6 significantly impaired TNBC stem-like phenotype, suggesting a critical role of BCL6 in regulating TNBCSC self-renewal. This still unknown BCL6 biological feature could be mediated by BCL6-dependent epigenetic silencing of stemness-related genes, even though further ongoing studies will be necessary to precisely elucidate such mechanism. Overall, we identified BCL6 as a novel target to counteract TNBCSCs paving the way to a novel “tailored” cure for TNBC.

## 2018 San Antonio Breast Cancer Symposium ®

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Associations of epidemiologic and clinical features with intensity of immune infiltrates in postmenopausal breast cancer cases from the cancer prevention study-II cohort

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Stromal tumor-infiltrating lymphocytes (sTILs) are often prominent in HER2-enriched and triple negative breast cancers and predict a favorable prognosis for these subtypes. Clinical and epidemiological determinants of sTILs have not been defined in large cohorts. Accordingly, we analyzed these associations for 702 eligible, postmenopausal invasive breast cancer cases from the Cancer Prevention Study (CPS)-II Nutrition cohort. CPS-II included 97,786 women who completed baseline and follow-up surveys since 1992. Women with self-reported breast cancer diagnoses were consented for medical record and tumor tissue retrieval. We analyzed pre-diagnostic data from the survey prior to diagnosis on personal history of benign breast disease (BBD), menopausal hormone use, alcohol intake, cigarette smoking status, waist circumference, body mass index, adult weight gain, nonsteroidal anti-inflammatory drugs, and physical activity. One pathologist (TG) evaluated sTILs using whole slide images of H&E stained sections according to recommendations by the International TILs Working Group 2014. sTIL levels were dichotomized: none/minimal (0-10%) and moderate/high (>10%). We compared the sTIL levels by clinical and epidemiologic risk factors using chi-square statistics. Odds ratio (ORs) and 95% confidence intervals (CIs) for the associations of clinical and epidemiologic risk factors with sTILs were estimated with multivariable logistic regression.

Mean age at diagnosis was 71.9 years; 44% of cancers were moderate grade, 74% were localized staged and 88% were luminal-like. Cancers with high/moderate sTIL levels (n=614), compared to none/minimal (n=88), were more likely to be high grade (54% vs. 20%; p-value <0.001), node-positive (41% vs. 22%; p-value <0.001), and non-luminal (36% vs. 8%; p-value <0.001). In a model mutually-adjusted for age at diagnosis and clinical factors, all clinical factors were associated with moderate/high sTIL levels: node-positive (OR=2.15, 95% CI 1.28 – 3.58), high grade (OR=3.98, 95% CI 1.88 – 9.06), and non-luminal subtype (OR=3.65, 95% CI 2.00 – 6.59). Age at diagnosis was not associated with sTIL levels (per year: OR=1.01, 95% CI 0.97 – 1.05).

Women with BBD were less likely to have moderate/high sTIL levels than minimal/none sTIL levels (38% vs. 54%; age-adjusted OR=0.50, 95% CI 0.31 – 0.79). Ever smokers also were less likely to have moderate/high sTIL levels (38% vs. 49%) with an OR=0.61 (95% 0.38 – 0.97). Other risk factors were not significantly associated with sTIL levels.

In this large epidemiologic cohort, non-luminal cancers had more sTILs than luminal cancers. Women with a personal history of BBD and ever cigarette smokers had significant lower levels of sTILs. Our study is among the first to examine pre-diagnostic risk factors in relation to sTILs and provides the impetus for larger, population-based studies with phenotypic characterization of immune cells.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-03

An early look at incidence and survival by HR and HER2 status among young US women with stage I-III breast cancer: SEER 2010-15

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**Background:** Population-based contemporary incidence and survival patterns for breast cancer by receptor status (hormone receptor [HR]; human epidermal growth factor receptor 2 [HER2]) among young US women have not been described.

**Methods:** We identified pre-, peri-, and early postmenopausal women ages 20-29 (n=1,617), 30-39 (n=12,910), 40-49 (n=47,313), and 50-59 (n=68,870) diagnosed with Stage I-III breast cancer from 2010-15 in the Surveillance, Epidemiology and End Results (SEER) database using SEER\*Stat version 8.3.5. Of these, respective totals for women with reported HR and non-borderline HER2 status were 1533 (94.8%), 12,225 (94.6%), 44,684 (94.4%), and 64,844 (94.2%). Using this analytic sample, we estimated annual percentage change (APC) in incidence. Restricting the sample to women diagnosed from 2010-14, we estimated four-year survival (maximum available follow-up). Analyses were stratified by age group and receptor status.

**Results:** The proportion of HER2<sup>+</sup>/HR<sup>+</sup> cancer was highest among women 20-29, decreasing with increasing age; that for HER2<sup>+</sup>/HR<sup>-</sup> cancer was lower, differing less across age groups (Table 1). For HER2<sup>-</sup> cancer, the proportion of HER2<sup>-</sup>/HR<sup>+</sup> cancer was lowest for women 20-29 and increased with age; the opposite pattern was observed for HER2<sup>-</sup>/HR<sup>-</sup> cancer. Overall, stage I-III breast cancer increased in incidence from 2010-15 for all age groups, with the APC greatest in women 20-29 [2.20](30-39 [1.98], 40-49 [1.91], 50-59 [1.62]). The patterns for the APCs by age and receptor status tended to reflect those for the observed proportions, except those for women with HER2<sup>+</sup>/HR<sup>-</sup> cancer, which tended to increase with increasing age (Table 1). Four-year survival estimates for women 20-29, although imprecise, differed by HER2 status, being higher for those with HER2<sup>+</sup> than HER2<sup>-</sup> cancer, and this difference persisted for HR<sup>+</sup> and HR<sup>-</sup> cancers among these women (Table 2). In the other age groups, survival differed by HR status, being higher for women with HR<sup>+</sup> than HR<sup>-</sup> cancer.

**Conclusions:** Using SEER data representative of over one-quarter of the US population, Stage I-III breast cancer has increased among young women in recent years. In particular, HER2<sup>+</sup>/HR<sup>+</sup> and HER2<sup>-</sup>/HR<sup>-</sup> cancers were over-represented and estimated to have the highest APCs among women 20-39 compared to those 40-59. Receptor status was observed to influence survival differently across age groups. The differences in four-year survival observed among women 20-29 with HR<sup>+</sup> cancers may have implications as we consider anti-estrogen therapy options for these youngest premenopausal women.

Table 1: Breast Cancer Frequency and APC

Receptors	Age			
	20-29	30-39	40-49	50-59
	N (%)			
HER2 <sup>+</sup> /HR <sup>+</sup>	331(21.6)	2225(18.2)	5865(13.1)	7706(11.9)
HER2 <sup>+</sup> /HR <sup>-</sup>	109(7.1)	863(7.0)	2254(5.0)	3664(5.7)
HER2 <sup>-</sup> /HR <sup>+</sup>	733(47.8)	6769(55.2)	30809(69.0)	45693(70.5)
HER2 <sup>-</sup> /HR <sup>-</sup>	360(23.5)	2398(19.6)	5756(12.9)	7781(12.0)
	APC			
HER2 <sup>+</sup> /HR <sup>+</sup>	5.4	4.9	3.9	4.4
HER2 <sup>+</sup> /HR <sup>-</sup>	1.2	2.1	4.4	3.2
HER2 <sup>-</sup> /HR <sup>+</sup>	-1.1	1.5	1.9	1.6
HER2 <sup>-</sup> /HR <sup>-</sup>	6.5	0.5	-1.1	-1.5

Table 2: Four-Year Breast Cancer Survival

Receptors	Age			
	20-29	30-39	40-49	50-59
	%(SE)			
HER2+/HR+	95.3(1.7)	95.5(0.6)	96.0(0.4)	94.4(0.4)
HER2-/HR+	95.0(2.9)	91.9(1.3)	90.8(0.9)	90.8(0.7)
HER2-/HR+	90.6(1.6)	93.1(0.4)	96.2(0.2)	95.6(0.1)
HER2-/HR-	82.2(2.8)	80.3(1.1)	82.8(0.7)	82.5(0.6)

SE: standard error

# 2018 San Antonio Breast Cancer Symposium ®

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Type 2 diabetes and survival outcomes among a multi-ethnic cohort of breast cancer patients

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**BACKGROUND:** With increasing prevalence of type 2 diabetes, it has become one of the common comorbidities among breast cancer patients. Few contemporary studies have examined the effect of preexisting diabetes on survival outcomes in breast cancer patients. Furthermore, both breast cancer mortality and diabetes prevalence are higher in African American women compared to Caucasian women, yet data on whether diabetes can explain racial disparity in breast cancer mortality is scarce.

**OBJECTIVE:** To compare clinopathological characteristics and survival outcomes between breast cancer patients with and without co-existing diabetes, and to explore the contribution of diabetes to breast cancer survival differences between African Americans and Caucasians.

**METHODS:** We analyzed data from the Chicago Multiethnic Epidemiologic Breast Cancer Cohort (ChiMEC) comprising of 3170 histologically confirmed breast cancer patients diagnosed between 2004 and 2017. The cohort consists of 55% Caucasians, 38% African Americans, and 7% other ethnicities. Cox models were used to analyze data on several clinical outcomes [Table 1].

**RESULTS:** 245 patients (8%) in the cohort had co-existing diabetes at time of breast cancer diagnosis, with African Americans having highest prevalence (14%). Patients with diabetes were older (mean 66 vs. 56 years old) and had higher proportion of obesity (67% vs. 34%) and Charlson comorbidity index >2 (27% vs. 10%) than those without diabetes. The two groups were similar in terms of surgery, radiation, and hormonal therapy received, while patients with diabetes had slightly more advanced stage (15% vs. 10%). After adjusting for multiple prognostic factors, patients with diabetes had a 97% higher risk of dying from breast cancer and a 62% fold higher risk dying from other causes than patients without diabetes (Table). The two groups had no significant difference in risk of recurrence. In addition, the hazard ratio (HR) comparing African Americans with Caucasians was 2.35 (95% confidence interval [CI] 1.90-2.91), and it changed to 2.19 (95% CI 1.76-2.33) after the adjustment for diabetes.

**CONCLUSIONS:** Pre-existing diabetes among breast cancer patients was associated with higher risk of breast cancer specific and all-cause mortality. About 11% of racial disparities in breast cancer mortality could be attributed to higher difference in diabetes prevalence in African American patients. Further research investigating how pre-existing diabetes may influence breast cancer treatment and survival are warranted.

Table 1

	Unadjusted HR (95% CI)	p	Adjusted HR (95% CI)*	p
All-cause mortality	2.20 (1.65-2.92)	<0.001	1.79 (1.33-2.43)	<0.001
Breast Cancer-specific mortality	1.61 (1.06-2.44)	0.025	1.97 (1.27-3.05)	0.002
Non-breast cancer mortality	3.14 (2.11-4.69)	<0.001	1.62 (1.06-2.47)	0.025
Time to recurrence	1.18 (0.76-1.85)	0.46	1.41 (0.89-2.25)	0.15
Recurrence-free survival	1.73 (1.32-2.26)	<0.001	1.68 (1.27-2.22)	<0.001

\*Adjusted for age, race, stage, hormone therapy, and chemotherapy

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-06

Changes in breast cancer diagnosis and treatment after Medicaid expansion in Pennsylvania

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**Background:** In January 2015, Pennsylvania expanded Medicaid eligibility to all legally present adults in the Commonwealth of Pennsylvania with household incomes up to 138 percent of the federal poverty level under the Affordable Care Act. Pennsylvania is the most populous state to expand Medicaid that did not previously have a large expansion of the program to adults, making it an ideal setting to study the impacts of the program. By March 2017, an estimated 716,000 people had enrolled in Pennsylvania's expanded Medicaid program. We aim to determine how Pennsylvania's Medicaid expansion has impacted breast cancer diagnosis and treatment in the state.

**Methods:** The Pennsylvania Cancer Registry was queried for all women aged 18 to 64 years newly diagnosed with breast cancer between 2007 and 2015. Demographic, tumor, and treatment characteristics were evaluated for each year during this time span. In order to assess the impact that Medicaid expansion had on these variables, they were compared for the years 2007 to 2014 (before Medicaid expansion) and the year 2015 (after).

**Results:** Between 2007 and 2015, 49,606 cases of invasive breast cancer were diagnosed among women aged 18-64 and residing in the state of Pennsylvania, 43,920 of which were diagnosed and treated between 2007 and 2014, and 5,686 of which were diagnosed and treated in 2015. Initial analysis indicates that more women were diagnosed with localized breast cancer (SEER summary stage 1) following Medicaid expansion in 2015 compared to the period of 2007 to 2014 (63.07% v. 60.62%,  $p = 0.0004$ ). Meanwhile, the proportion of women diagnosed with metastatic breast cancer did not change before and after Medicaid expansion (6.52% v. 6.86%,  $p = 0.3298$ ). Subsequent analysis will assess changes in treatment outcomes including time to treatment and receipt of guideline-concordant care.

**Conclusion:** The expansion of Medicaid in Pennsylvania in 2015 under the Affordable Care Act resulted in an increase in the proportion of localized (SEER summary stage 1) breast cancer diagnoses, though there was no change in the proportion of metastatic cases in the first year following expansion. The results suggest a possible increase in earlier stage diagnosis associated with increased access to health insurance. Additional analysis will examine the impact on treatment-related outcomes.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-07

Risk factors for high-grade and low-grade DCIS in the cancer prevention study-II nutrition cohort

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**Background:** Nuclear grade, believed to be established early in carcinogenesis, is an indicator of ductal carcinoma in situ (DCIS) prognosis. Women with high-grade DCIS have a higher risk of local recurrence compared to women with low-grade DCIS. Risk factors for DCIS overall are well-characterized but risk factors by grade are not. Given the prognostic capabilities of grade for DCIS, it is of interest to identify whether risk factors by DCIS grade differ.

**Methods:** Among 75,630 women enrolled in the Cancer Prevention Study-II Nutrition Cohort in 1992-1993, we identified 422 who were diagnosed with low-moderate grade DCIS (i.e. grades 1 or 2) and 355 who were diagnosed with high-grade DCIS (i.e. grade 3) during follow-up through 2013. Beginning in 1997, biennial questionnaires were administered to update exposure status, including screening mammography in the previous two years. For this analysis, these questionnaires were used to partition follow-up time into approximately two-year intervals. Because screening is strongly linked to diagnosis of DCIS, contribution of person-time within a specific interval was conditional on reporting a screening mammogram in the interval prior. Multivariate joint Cox proportional hazards regression models were used to estimate adjusted hazard ratios (HR) and 95% confidence intervals (CI) for the associations of known breast cancer risk factors with DCIS overall, and with high-grade and low-grade DCIS individually.

**Results:** Parity (HR=0.70; 95% CI: 0.63-0.78, parous vs. nulliparous), smoking status (HR=0.69; 95% CI: 0.58-0.82, current vs. never smoking) and menopausal status (HR=0.79; 95% CI: 0.73-0.87, natural menopause <50 years vs. natural menopause ≥ 50 years) were inversely associated with risk of DCIS overall. Whereas, positive family history of breast cancer (HR=1.46; 95% CI: 1.36-1.57), personal history of benign breast disease (BBD) (HR=1.73; 95% CI: 1.62-1.85), and current use of combination estrogen and progestin hormone replacement therapy (HR=1.15; 95% CI: 1.04-1.28) were associated with higher risk of DCIS. In analyses stratified on DCIS grade, history of BBD was more strongly associated with higher risk of low-grade (HR=2.21; 95% CI: 1.81-2.70) than with high-grade DCIS (HR=1.29; 95% CI: 1.04-1.60) (p for heterogeneity=0.001). Current combination estrogen and progestin hormone replacement therapy use was associated with a higher risk of high-grade DCIS (HR=1.40; 95% CI: 1.03-1.90) but not low-grade DCIS (HR=1.02; 95% CI: 0.75-1.40), but the difference by grade was not statistically significant (p=0.5).

**Conclusions:** In this study, which is the first to comprehensively assess risk factors by DCIS grade, the association between personal history of BBD and risk of DCIS appeared to differ by grade. Due to limited power for some risk factor analyses, future studies using larger prospective cohorts or pooled data should be conducted to better identify these associations.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-08

High risk of recurrence up to 30 years after diagnosis among breast cancer patients aged <45 years

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## Introduction

In the recent years, the incidence of breast cancer (BC) among young women increased in several Western countries. Furthermore, compared to older women with BC, young women present in general more aggressive biological characteristics: higher stage at diagnosis and lower survival rates. Except for cancer arising in high genetic risk individuals, BC occurring at young age represents a poorly comprehensible epidemiological situation.

We had constituted a population-based cohort of BC patients aged less than 45 years derived from the Geneva cancer registry with the aim of investigating patient's, tumour's, treatment's characteristics and long-term health outcomes of these young patients.

## Patients and methods

Between 1970 and 2012, 1'586 women were identified as resident in the canton of Geneva, Switzerland and diagnosed with a primary invasive non-metastatic BC at the age of 45 years or less. From the pathological and medical files, we collected numerous variables including social environment, family history, fertility and pregnancy. Women were followed up for local and distant recurrences, second cancer occurrence or death up to 31/12/2015. Cumulative incidence was estimated for local and distant recurrences in the competing risks framework considering deaths (from BC or other causes) as competing events. Analyses were stratified according to age groups and stage of the disease.

## Results

BC was diagnosed before the age of 35 years for 225 women (14.2%), between 35 and 39 years for 368 women (23.2%) and between 40 and 45 years for 993 women (62.6%). Most of the patients were diagnosed with luminal A or luminal B molecular subtypes (respectively 32.8 and 37.5%), stages I or II tumours (75.2%), positive estrogen (74.8%) and progesterone (67.5%) receptors. Regarding treatment, almost all women underwent surgery (breast-conserving surgery for 62% and mastectomy for 36% of the patients). Radiotherapy, chemotherapy and hormonotherapy were administered respectively to 70%, 55% and 36% of the patients. At a median follow-up of 10.2 years, 16.7% of the women (N=265) developed local recurrences and 25.4% (N=403) developed distant metastases, while 66.3% (N=1051) did not present any recurrences. Regarding mortality, 474 (29.9%) women deceased during the study period, 347 of whom (73.2%) from BC. Competing risks analyses shows that women remained at risk of developing both types of recurrences up to 30 years after diagnosis. The probability of presenting distant metastases was higher for all ages and all stages except for patients with stage I who were more likely to have local recurrences.

## Discussion

This study presents the very long term outcomes of young women diagnosed with BC. While the vast majority of the patients did not develop any recurrences, some of them were still at risk even after 30 years of follow-up. The main hazard was of distant rather than local recurrence. Further analyses on this cohort will allow to assess the determinants of these risks.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-09

Breast cancer-related deaths according to grade in ductal carcinoma in situ: A Dutch population-based study on patients diagnosed between 1999 and 2012

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## Background

The incidence of ductal carcinoma in situ (DCIS) has drastically increased over the past decades. Since DCIS is resected after diagnosis similarly to invasive breast cancer, the natural cause and behaviour of DCIS is not well known. We aimed to determine breast cancer-specific (BCSS) and overall survival (OS) according to grade in DCIS patients after surgical treatment in the Netherlands.

## Patients and methods

All DCIS patients diagnosed between 1999-2012 were selected from the Netherlands Cancer Registry. Cause of death was obtained from 'Statistics Netherlands'. BCSS and OS were estimated using multivariable Cox regression in the entire cohort and stratified for grade.

## Results

In total, 12,256 patients were included, of whom 1,509 (12.3%) presented with grade I, 3,675 (30.0%) with grade II, 6,064 (49.5%) with grade III and 1,008 (8.2%) with an unknown grade. During a median follow-up of 7.8 years, 1,138 (9.3%) deaths were observed, and 179 (1.5%) were breast cancer-related. Of these, 10 patients had grade I, 46 grade II, 95 grade III and 28 an unknown grade. After adjustment for confounding, grade II and III were related to worse BCSS compared to grade I with HRs of 1.92 (95% CI:0.97-3.81) and 2.14 (95% CI:1.11-4.12), respectively. No association between grade and OS was observed.

## Conclusion

BCSS and OS rates in DCIS patients are excellent. Since superior rates were observed for low-grade DCIS, and earlier studies have shown that low-grade DCIS have a very low chance on recurrence or upstage to invasive cancer, it seems justified to investigate whether active surveillance may be a balanced alternative for conventional surgical treatment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-10

Insulin resistance and breast cancer incidence and mortality in postmenopausal women

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**Background:** Obese postmenopausal women are at higher breast cancer risk potentially driven by hyperinsulinemia. However, reports of insulin level associations with breast cancer incidence and survival are inconsistent. Therefore, we examined associations among insulin resistance by homeostasis model assessment-insulin resistance (HOMA-IR) index and incident breast cancer, deaths from breast cancer, and deaths after breast cancer in postmenopausal women participating in the Women's Health Initiative (WHI).

**Patients and Methods:** From the 161,808 postmenopausal women, aged 50-79 years, enrolled at 40 US clinical centers from 1993 through 1998 in WHI clinical trials or the observational study, 22,837 with available fasting serum insulin and glucose by the same methodology and no prior breast cancer represent the current study population. The exposure was insulin resistance (HOMA-IR index) as [fasting insulin (qIU/ml) times fasting glucose (mg/dL) / 22.5. Survival by cause was determined by central medical record or death certificate review, enhanced by National Death Index queries. Breast cancers, initially ascertained by serial survey, were confirmed by medical record review. Associations between HOMA-IR quartiles and breast cancer outcomes were examined using Cox multivariate proportional hazards models with results reported as hazard ratios (HR) and 95% confidence intervals.

**Results:** At entry, women in the highest HOMA-IR quartile were more likely to be Black, have lower education level, have higher body mass index (BMI), higher waist circumference  $\geq 88$  cm and lower physical activity levels, but have lower calculated five-year breast cancer risk. After 18.1 years median follow-up from randomization with 1,148 incident breast cancers, breast cancer incidence was higher in women in the highest, compared to the lowest, HOMA-IR index quartile (HR 1.39 95% CI 1.14 - 1.69,  $P = 0.0012$ ). Of the women with incident breast cancer, 353 (31%) have died, with cause of death available on 334 (95% of cases) where breast cancer was the most common cause of death (33%); followed by cardiovascular disease (24%); and other cancers (13%). With median post-breast cancer diagnosis follow-up of 10.5 years, breast cancer mortality was examined from breast cancer diagnosis. No association was found between death from breast cancer and HOMA-IR. However, women with breast cancer in the highest HOMA-IR quartile, compared to women in the lowest, were significantly more likely to experience death after breast cancer from any cause (HR 1.45 95% CI 1.00 - 2.09,  $P = 0.0488$ ) and were at somewhat higher risk of death from cardiovascular disease (HR 1.51 95% CI 0.67 - 3.41) and other causes (HR 1.93 95% CI 0.87-4.27).

**Conclusion:** In postmenopausal women, higher insulin resistance is associated with higher breast cancer incidence and more deaths after breast cancer, likely due to insulin influence on several causes of death. However, deaths from breast cancer, even in this older postmenopausal population, remains a major factor limiting survival which needs to be addressed. The findings suggest insulin resistance represented a potential intervention target for postmenopausal women with early stage

breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-11

The impact of existing comorbidities on survival disparities among women diagnosed with invasive breast cancer

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Missouri is one of many states in the US burdened by high rates of mortality from female breast cancer (BC) as well as comorbidities such as Type-2 diabetes, cardiovascular disease (CVD), and hypertension. These comorbidity rates are higher among vulnerable populations including individuals in poverty and/or living in rural areas, African Americans, and the elderly. There is evidence that women with comorbidities at the time of BC diagnosis have a worse prognosis. We hypothesize that the co-existence of comorbidities is likely to impact survival and may contribute to survival disparities observed among women diagnosed with BC from these vulnerable populations.

**Objective:** To examine whether the number and/or type of comorbidity at BC diagnosis is associated with higher BC and all-cause mortality among women diagnosed with invasive BC in Missouri between 2004 and 2012.

**Methods:** Women age 18+ diagnosed with BC in Missouri during 2004–2012 were identified from the Missouri Cancer Registry. These data were then merged with hospital discharge data from the Missouri Patient Abstract System. Associations were evaluated in all women and by race, neighborhood poverty level, rural/urban residence, and age at diagnosis. A comorbidity score was constructed to account for the number of comorbidities (Type-2 diabetes, hypertension, and CVD) identified for each individual. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated using Cox proportional hazards regression models, adjusting for age at diagnosis, race, tumor hormone receptor status, stage, BC treatment, and rural/urban residence. Models were further stratified by race, poverty level, rural/urban residence, and age group.

**Results:** A total of 31,133 women with incident invasive BC and with comorbidity data at the time of BC diagnosis were included in the analysis. After a median follow-up time of 79 months, 9,912 deaths occurred, of which 4,900 deaths were due to BC. Increasing number of comorbidities was significantly associated with BC mortality ( $p_{\text{trend}} < 0.001$ ). BC mortality (HR, 1.33; 95% CI 1.19-1.49) and all-cause mortality (HR, 1.51; 90% CI 1.32-1.61) were significantly higher in women with  $\geq 2$  comorbidities. CVD accounted for the largest increase in BC mortality (HR, 1.36; 95% CI 1.19-1.55). In stratified analyses, we did not observe significant differences in associations by race, poverty, rural/urban residence, or age; however, there was a statistically significant interaction with age when modeled as a continuous measure, comorbidity score, and risk of mortality outcomes ( $p < 0.001$ ). White women with all 3 comorbidities had the highest risk of death (BC-specific: HR, 1.95; all-cause: HR, 2.28). Women in rural areas with  $\geq 2$  comorbidities were 1.78 times more likely to die of BC while women living in the metro with all 3 comorbidities were almost 2 times more likely to die of any cause.

**Conclusion:** Our results demonstrate the negative impact that comorbidities such as diabetes, CVD, and hypertension can have on BC and overall mortality in a diverse group of BC patients diagnosed and treated in Missouri. The data produced from this study can be utilized to identify and implement targeted preventive strategies to improve the quality of life and survival of BC patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-12

Trends in incidence of bilateral breast cancer: A Population-based comparative study of the United States and Japan

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**Background:** Previous studies demonstrated that the incidence rate of invasive contralateral breast cancer (CBC) was 5% within the first 10 years after the primary breast cancer (BC). However improving long-term breast cancer survivorship and advancements in diagnostic imaging have resulted in an increased detection of bilateral breast cancer (BBC), and trends of bilateral invasive and in situ breast cancer are not well established. The aim of this study was to assess national trends of BBC incidence of the United States (US) and Japan.

**Methods:** The Surveillance, Epidemiology, and End Results (SEER) database (1973-2014) and the clinical database of Breast Oncology Center of Cancer Institute Hospital in Tokyo, Japan (Ganken) database (1946-2015) were used to identify  $n_{SEER}=11,771$  and  $n_{Ganken}=1,499$  women diagnosed with BBC, respectively. BBC was defined as invasive BC and/or ductal or lobular carcinoma in situ diagnosed in both breasts simultaneously or after primary breast cancer diagnosis. BBC was grouped into synchronous or metachronous BBC by the interval between first BC and contralateral BC; synchronous cases were defined as CBC diagnosed at the same time or within an interval of 1 year from primary BC diagnosis whereas metachronous cases were defined as a diagnosis occurring 1 year following the primary BC. We assessed trends of BBC incidence and characteristics of BBC cases between the two countries. To determine temporal trends in the incidence of BBC and proportion of the characteristics, we compared them using the Cochran-Armitage test for trend.

**Results:** The rates of BBC have significantly increased in both countries (Table 1, 2) [1975: 2.6%; 2014: 7.5% in SEER ( $p<0.001$ ), 1946-1980: 3.3%; 2011-2015: 10.7% in Ganken ( $p<0.001$ )]

[Table 1] Crude rates of BBC in all breast cancer in SEER

	1975	1985	1995	2005	2014
All breast cancer	9418	13618	25876	60164	71505
BBC and rates (%)	249 (2.6%)	790(5.8%)	1421(5.5%)	3336(5.6%)	5381(7.5%)
Rates of synchronous BC	2.1%	2.8%	2.3%	2.6%	3.5%
Rates of metachronous BC	0.5%	3.0%	3.2%	3.0%	4.0%

BBC: Bilateral breast cancer

[Table 2] Crude rates of BBC in all breast cancer in Ganken

	1946-1980	1981-1985	1991-1995	2001-2005	2011-2015
All breast cancer	4777	2162	2806	3791	5241
BBC and rates (%)	157(3.3%)	110(5.1%)	188(6.7%)	298(7.9%)	559(10.7%)
Rates of synchronous BC	1.0%	1.9%	2.0%	2.7%	4.9%
Rates of metachronous BC	2.3%	3.2%	4.7%	5.2%	5.8%

The increase was identified in both synchronous and metachronous BBC. In SEER, 40% of synchronous BBC were found as in situ BC and about 15% of BBC presented as invasive lobular carcinoma. More recently, CBC was more likely to be diagnosed at

early stages (in situ and local disease) than in previous years [1975: 65%; 2014: 85% in SEER ( $p<0.001$ )]. The interval between first BC and contralateral BC have shortened, and CBC were more likely to be operated simultaneously in both countries [1985: 40%; 2014: 51% in SEER, 1946-1980: 24%; 2011-2015: 74% in Ganken].

**Conclusion:** In the modern era, the number of BBC cases have increased and are more likely to be found at an early stage. Further studies are needed to demonstrate the usefulness of early detection of CBC and to define the best means to tailor therapy for patients with bilateral disease.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-13

A prospective evaluation of HLA expression in breast implant associated anaplastic large cell lymphoma to identify disease susceptibility

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**Background:** Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is a rare T-cell lymphoma found to occur in association with breast implants. Proposed pathogenic factors for BIA-ALCL include immunologic reaction to textured implants, bacteria and biofilm, chronic inflammation, autoimmune disease, or genetic predisposition. While HLA alleles have been found to be associated with other forms of lymphoma, the frequency of HLA allele polymorphisms have not been described for BIA-ALCL. The aim of this study is to evaluate the frequency of HLA alleles in patients with BIA-ALCL.

**Methods:** We prospectively evaluated HLA alleles in 13 patients with BIA-ALCL seen at a single institution from 2017 to 2018. HLA testing was carried out using probe based sequence specific testing and sequence based typing. The frequency of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 were evaluated in the study population. Allele frequencies in the Caucasian European general population were obtained from the National Marrow Donor Program for normative controls. We estimated the relative risk of BIA-ALCL with 95% confidence intervals for each HLA allele using a t test. All statistics were performed using SPSS.

**Results:** Thirteen patients with BIA-ALCL and HLA testing were identified with ages ranging from 37 – 76 years. We identified 10, 11, and 9 HLA A, B, and C alleles respectively. There were 8 DRB1 alleles and 5 DQB1 alleles in the BIA-ALCL patients. The following alleles occurred more than two times more frequently in patients with BIA-ALCL as compared with the general population: A\*31, A\*32, A\*68, B\*27, B\*39, B\*49, B\*51, and C\*15. Conversely, the A\*01, A\*24, and B\*35 alleles occurred less frequently in patients with BIA-ALCL. The A\*26 allele was found to occur significantly less often in BIA-ALCL patients (0.2992 vs. 0.07692,  $p < 0.001$ ) versus normative controls.

**Conclusions:** Our results identify differences in the frequency of specific HLA alleles in patients who develop BIA-ALCL compared with the general population. These alleles may signify genetic susceptibility factors for germline genetic variation in HLA in Caucasian patients with BIA-ALCL. Further work is needed to elucidate if these alleles confer susceptibility or resistance and are predictive for the disease in women with textured surface breast implants.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-14

Trend of utilization of Oncotype DX testing among female hormone receptor positive breast cancer patients in 17 SEER registries, 2004-2015

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**Background:** Oncotype DX, a 21-gene Recurrence Score (RS) assay, has been validated as assay that effectively predicts recurrence and chemotherapy (chemo) benefits for hormone receptor positive (HR+) human epidermal growth factor receptor 2 negative (HER2-) breast cancer (BC). Although guidelines recommend the test to lymph node (LN) negative (PN0) BC patients, it has also been used in patients with 1-3 positive LN (PN1) in clinical practice. The study aimed to 1) examine the trend of utilization of Oncotype DX testing for HR+ and PN0 or PN1 BC patients from 2004 to 2015, as well as the trend in patients with different clinical risk; 2) investigate the trend of having reported chemo in patients with low, intermediate, and high RS; and 3) compare cause-specific survival (CSS) and overall survival (OS) between patients who had test and those who did not.

**Methods:** Data from Genomic Health Inc., the sole Oncotype DX testing provider in the U.S., was linked with routinely collected data from 17 SEER registries. Women who received surgery for stage I-III, HR+ and PN0 or PN1 BC diagnosed in 2004-2015 were included. The Cochrane-Armitage trend test was conducted for trend analysis. Using the overall sample as standard population (frequency of each age-racial group in overall sample as weight), age-race standardized percentage of test use was calculated. Since HER2 data was available only after 2010, survival analysis was restricted to HR+/HER2- patients diagnosed in 2010-2014 whose BC was the only primary tumor. Patients who used and those who did not use the test were matched on propensity score, which was calculated based on age, race, marital status, insurance, grade, tumor size, surgery type, radiation, and chemo within each diagnosis year. Stratified Cox proportional hazards model was used to compare survival between two matched groups. Proportional hazard assumption was met for each model.

**Results:** Out of 346,380 PN0 and 103,317 PN1 patients, the percentage of using Oncotype DX test increased from 2.0% to 38.2%, and from 0.5% to 28.0% from 2004 to 2015, respectively (P-for-trend < 0.0001 for each). Age-race standardized percentage of test use was the highest and increased most rapidly for tumors with intermediate clinical risk (moderately differentiated or tumor size 2.1-5.0cm) among PN0 patients, but for tumors with low clinical risk (well differentiated or tumor size ≤2.0cm) among PN1 patients. From 2004 to 2015, the percentage of having reported chemo decreased in patients with low (PN0: 14.7% to 1.8%; PN1: 27.3% to 16.2%) and intermediate RS (PN0: 36.1% to 28.6%; PN1: 60.0% to 44.7%), but increased among patients with high RS (PN0: 59.0% to 75.4%; PN1: 66.7% to 74.2%). Test use was associated with better CSS (PN0: hazard ratio [HR] 2.15, 95% CI 1.73-2.67; PN1: HR 2.83, 95% CI 2.02-3.95) and OS (PN0: HR 2.05, 95% CI 1.81-2.33; PN1: HR 2.65, 95% CI 2.10-3.35).

**Conclusions:** The use of Oncotype DX test has increased steadily among female HR+ BC patients since 2004. It reduced unnecessary chemo among patients with low or intermediates RS, and increased chemo use in patients with high RS. Among HR+/HER2- patients, those who used test had better CSS and OS than those who did not.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-08-15

## Characteristics of long-term survivors with metastatic breast cancer

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**Background:** Survival with metastatic breast cancer (MBC) is highly variable and only ~16% survive 10 years from the primary cancer diagnosis. In addition to treatments, proposed contributors to increased survival include cancer subtype, distribution of metastases, diet, exercise, and use of complementary and alternative medicine (CAM). We aimed to characterize long-term survivors with MBC through a self-administered survey.

**Methods:** Patient advocates and researchers collaborated on developing and administering an ongoing web-based questionnaire ([outliers.cancer.wisc.edu](http://outliers.cancer.wisc.edu)). Participants were recruited to this IRB-approved project through fliers, social media, MBC conferences, and Susan Love's Army of Women, with a recruiting focus on long-term survivors. Women and men are eligible if they are age ≥18 years old and have MBC by self-report. Eligible participants were invited to complete a detailed 28-part survey in English. Preliminary analysis was conducted by descriptive comparison of survivors ≥ 10 years from primary diagnosis versus those < 10 years.

**Results:** Between March 6 and June 17, 2018, a total of 475 women and 1 man self-reported as living with MBC consented and completed the survey. Participants represented 48 U.S. states (n=414), and other countries on 4 continents (n=54). Two thirds of respondents (315) reported using medical records in preparing responses. Age of respondents ranged from 32-83 years (median 55), and participants survived from 4 months to 50 years (median 8.7), from primary breast cancer diagnosis. A total of 206 had lived ≥10 y from primary diagnosis and 270 <10 y (Table).

## Characteristics of long-term survivors and non-long term survivors with MBC

Factor	Survival from primary diagnosis		Factor	Survival from primary diagnosis	
	≥10 y	<10 y		≥10 y	<10 y
Age, mean	60	52	Bone metastasis only*	36%	40%
Stage 4	11%	47%	BMI, mean	27.4	28.5
HR+HER2-	66%	65%	Alcohol >2 drinks/wk	26%	20%
HR-HER2+	6%	9%	Smoking history	36%	33%
HR+HER2+	24%	22%	Sedentary	23%	21%
Triple negative	4%	4%	CAM use	20%	20%
Oligometastatic (1-3)*	53%	50%	Sleep hours/d, mean	7.7	7.6

\*Number/site of tumors at time of metastatic presentation

As expected, longer-term survivors were older and were less likely to have been diagnosed at stage 4. Surprisingly, there were no major differences in prevalence of breast cancer subtypes, though triple-negative breast cancer was rare in both groups. Long-term survivors reported modestly more alcohol intake and lower BMI. The two groups were well matched for oligometastatic and bone-only disease, which have been previously associated with longer survival. Additionally, there were similar rates of prior smoking, CAM use, and sedentary behavior.

**Conclusion:** Interim analysis of this ongoing survey of MBC survivors finds that many disease and behavioral characteristics are similar between long-term versus short term survivors. Additional factors such as treatments, diet, and supplements will be reported. Other possible factors, including tumor genetics, treatments and immunologic factors will be evaluated separately.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-16

The influence of patient fitness on the likelihood of receiving primary surgery in older women with breast cancer: A population based cohort study

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**Introduction:** There is evidence of variation in the patterns of treatment of older women with breast cancer (BC). As women age, there are less similarities in terms of their functional ability, physiology and social wellbeing. This multifaceted relationship between disease and ageing makes the interpretation of age-related differences in BC management and outcomes at a population level complex. Measuring frailty is as an emerging concept and offers a way to standardise how characteristics of ageing are used in BC.

**Objective:** We compared the primary treatment patterns of women aged  $\geq 70$  yrs compared to those aged 50–69 yrs, with early invasive BC (EIBC; stage 1-3A); using a novel measure of frailty in addition to commonly used patient fitness measures.

**Methods:** Women aged  $\geq 50$  yrs, diagnosed with unilateral EIBC in England and Wales between 01/01/2014 and 31/12/2016; were identified by linkage of several national datasets. Patient fitness was measured by the reported WHO performance status (WHO PS), a calculated Charlson comorbidity score (CCS) and a developed frailty measure based on the electronic Frailty Index (eFI). Multilevel logistic regression was used to account for clustering in the data.

**Results:** Among 126,111 women aged  $\geq 50$  yrs with BC, 88,028 had EIBC: 88% in women aged 50-69 yrs and 75% in women aged  $\geq 70$  yrs. Table 1 describes the proportion of women who received surgery by age and measures of fitness. Overall, older women were less likely to undergo primary surgery, regardless of fitness. For each measure of fitness, fewer women in both age groups underwent surgery as their levels of fitness decreased; the magnitude of this change was greater for women aged  $\geq 70$  yrs. Older women were also less likely to receive BCS for tumours  $< 5$  cm, compared to women aged 50-69 yrs.

Receipt of surgery in women aged  $\geq 50$  years with EIBC according to CCS, eFi and WHO PS status, by age at diagnosis

Measure of fitness	50-69 years		70+ years	
	No. of patients	% having surgery	No. of patients	% having surgery
Number of women	54817	96%	23436	87%
Charlson comorbidity score				
0	48616	97%	23436	87%
1	3454	95%	4390	67%
1+	1063	89%	3096	45%
Unknown	1684	62%	2289	14%
eFI				
Fit	52506	97%	5707	89%
Mild frailty	564	89%	2369	47%
Moderate to severe frailty	63	62%	669	21%
Unknown	1684	62%	2289	14%
WHO performance status				
0	15073	97%	5707	88%
1	1372	93%	2264	74%
2	198	85%	911	46%
3-4	116	52%	795	17%

Unknown	38058	96%	23534	76%
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The association between independent factors of ageing and fitness, and 'no surgery' remained after accounting for case-mix differences and clustering within geographical region. Compared to women aged 50–69yrs, there was strong regional variation in the adjusted rate of surgical treatment for EIBC in women aged  $\geq 70$ yrs.

**Discussion:** Older women are less likely to undergo surgery for EIBC. Even a minor decrease in fitness levels significantly impacts the likelihood of receiving surgery in women  $\geq 70$ yrs; such a pattern is not observed in women aged 50–69yrs. Long-term follow up of these women will enable further understanding of the implications of this variability in practice on outcomes. We also acknowledge poor data completion for the WHO PS, and propose that eFI is suitable replacement measure of fitness in older patients with BC.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-17

Pharmacy refill synchronization and oral endocrine therapy adherence

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**Background:** One-third to one-half of patients prescribed adjuvant endocrine therapy with tamoxifen or an aromatase inhibitor either discontinue early or skip a substantial number of pills. Research to improve this has been disappointing. We investigated whether poor pharmacy synchronization of medications is an unrecognized barrier to adherence. **Methods:** A cohort of women aged 66-90 years old with Stage 0-3 hormone receptor-positive breast cancer were identified from the Surveillance, Epidemiology and End Result (SEER)-Medicare claims-linked cancer registry. Women with Medicare pharmacy claims documenting at least one endocrine therapy prescription fill and at least one other medication fill were identified, and the 3-month synchronization of their medication fills was calculated as the quotient of the number of pharmacy visits and the number of filled medications subtracted from one. Logistic regression was used to assess the association between synchronization stratified by the number of medications and adherence to endocrine therapy (defined as medication possession ratio >80%) over the subsequent year.

**Results:** The study cohort included 3,112 women treated with endocrine therapy for breast cancer (Table 1) . Over 31% were age 70 or younger, and 38.0% had stage 2 or 3 disease. During the three months after the first endocrine therapy prescription, the mean number of unique medications was 8.0 (S.D. 6.0) and the mean number of pharmacy visits was 8.6 S.D. 4.7) for a mean synchronization of 0.3 ( S.D. 0.2) . In adjusted model, compared with the highest synchronization, those in the lowest synchronization quantile were less likely to be adherent (MPR  $\geq$ 80%) (Odds Ratio 0.71 (95% CI 0.57, 0.88)) as were those in the second-lowest (0.81 (0.65, 0.99)). Neither age nor race/ethnicity were associated with adherence, and results were unchanged with inclusion of information about duration of fills (30 vs 90 days). **Conclusions:** Prescription fill synchronization is strongly associated with adherence to endocrine therapy. Research into interventions to improve adherence should include prescription synchronization and other health systems barriers.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-08-18

Initiating adjuvant endocrine therapy: Choice of drugs and changes in the patent status of aromatase inhibitors

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**Background:** Aromatase inhibitors (AIs) and tamoxifen are guideline recommended adjuvant endocrine therapies for postmenopausal women diagnosed with hormone-receptor positive (HR+) breast cancer. Before 2010, all AIs were brand-name, while tamoxifen was generic. In June 2010, anastrozole became the first AI with generic versions available. Generic exemestane became available in April 2011, and generic letrozole in June 2011. We study the associations between the generic drug availability, choice of drug initiated and changes in out-of-pocket (OOP) costs for elderly women in the Medicare program newly diagnosed with HR+ breast cancer.

**Methods:** From the SEER-Medicare linked database, we selected women whose first HR+ breast cancer was diagnosed at age 65 or older between 2007 and 2013, and who had Medicare Part D (pharmacy) coverage in the 12 months following the diagnosis (or until death).

**Results:** Prior to the availability of all generic AIs, the average monthly OOP costs of AIs were as high as \$150 with a yearly repeating pattern of highs and lows. Consistent with Part D benefit design, the average monthly OOP costs for patients were the lowest around February and the highest around October. The percent of patients who initiated endocrine therapy was stable (60%). After all AIs had generic versions available, the OOP costs decreased, and fluctuations of the average monthly OOP costs disappeared. The OOP costs for the two AIs with the highest market shares stabilized to around \$10. The average monthly OOP costs of tamoxifen were constantly around \$10. Compared to the dramatic changes in the OOP costs of AIs, the changes in utilization were modest. The percent of patients who initiated any drug increased to 70% in 2012-14. The percent of patients choosing anastrozole, exemestane and tamoxifen increased by 3-5%, while letrozole decreased by 10%, compared to that in 2007-2009. Increases in anastrozole were only substantial before generic letrozole was available (June 2010-May 2011).

**Table 1. First Drug Choice among Patients Who Initiated Endocrine Therapy**

	2007-2009	Jan 2010-May 2010	Jun 2010-Mar 2011	Apr 2011-May 2011	Jun 2011-Dec 2011	2012-2014
Generic Availability	No Generic AI	No Generic AI	1 Generic AI	2 Generic AIs	All AIs Generic	All AIs Generic
Anastrozole	59.3%	58.4%	69.2%	77.1%	68.0%	64.6%
Letrozole	28.3%	28.9%	20.0%	8.4%	12.8%	18.3%
Exemestane	3.4%	2.6%	1.6%	3.5%	6.7%	4.7%
Tamoxifen	9.0%	10.1%	9.2%	11.0%	12.5%	12.4%

**Table 2. Range of the Average Monthly Out-of-Pocket Costs (\$)**

Anastrozole	37-135	45-72	12-99	10-11	11-13	9-13
Letrozole	39-137	42-79	41-150	63-78	23-78	8-18
Exemestane	30-141	49-73	49-135	40-46	46-61	33-56
Tamoxifen	6-14	8-10	8-9	8-8	8-9	8-11

**Conclusions:** With generic entry, dramatic decreases in the prices and increases in initiations would be expected. We saw dramatic decreases in average monthly OOP costs. Changes in choice of AI were modest, especially considering tamoxifen initiation also increased after all generic AIs were available.

# 2018 San Antonio Breast Cancer Symposium ®

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10-year conditional recurrence risks, overall and relative survival for breast cancer patients in the Netherlands: Taking account of event-free years

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## Background

Survival estimates valid at the time of diagnosis are of limited value for (ex-)breast cancer patients who survived several years, as it includes information on already deceased patients. This study analyzed the 10-year conditional risk of recurrent breast cancer in specific prognostic subgroups according to T and N stage and breast cancer subtypes. Secondly, we investigated 10-year conditional overall (OS) and relative survival (RS), adjusted for confounding.

## Patients and methods

We selected all women diagnosed in 2005 with operated T1-2N0-1 breast cancer from the Netherlands Cancer Registry. Patients were classified into T1N0, T1N1, T2N0 and T2N1 stage. Ten-year conditional recurrence rates were calculated for every year from diagnosis for patients without an event (local (LR), regional recurrence (RR), distant metastasis (DM) or death). Ten-year conditional OS was calculated using multivariable Cox regression. RS was estimated by dividing patient survival rates by those of the general Dutch population.

## Results

We included 7,969 patients: 52.3% had T1N0, 15.3% T1N1, 19.9% T2N0 and 12.5% T2N1 stage. For T1N0, 10-year LR rates changed from 4.6% at diagnosis to 0.5% in year 10. RR rates decreased from 2.3% to 0.2% and DM rates decreased from 7.8% to 0.6%. For T2N1 stage, the LR, RR and DM rates decreased from 6.2% to 0.8%, 5.2% to 0.4% and 19.6% to 1.5%, respectively. Of all patients, 1,702 patients (21.4%) had an unknown breast cancer subtype and were consequently excluded from the analyses according to subtype. Of the remaining 6,267 patients, 3,774 (60.2%) had luminal A, 1,465 (23.4%) had luminal B, 314 (5.0%) had HER2 positive and 714 (11.4%) had triple negative disease. For the luminal A subtype, LR, RR and DM rates ranged from 3.9% to 0.4%, 1.7% to 0.5% and 7.3% to 1.1%, while for triple negative these rates ranged between 5.6% to 0.7%, 4.9% to 0.2% and 16.7% to 0%, respectively. Differences between subgroups attenuated over time and all recurrence rates became  $\leq 1.5\%$  in year 10. Ten-year OS and RS, adjusted for confounding, showed diminishing risk differences between subgroups over time.

## Conclusion

Differences in recurrence rates, OS and RS between prognostic subgroups decreased as years passed by. These results highlight the importance of taking into account disease-free years to more accurately predict (ex-)breast cancer patients' prognosis over time.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-20

Healthcare use in the last six months of life in advanced breast cancer: An analysis from the Southeast Netherlands Advanced Breast Cancer (SONABRE) registry

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In advanced breast cancer (ABC), a growing number of treatment options have become available the past 10 years, increasing overall survival. Healthcare use near the end of life has increased, although specific data for ABC are limited. This study describes healthcare use during the last 6 months of life in patients diagnosed with ABC in Maastricht University Medical Center (MUMC+).

**Methods:** From our Southeast Netherlands Advanced Breast Cancer (SONABRE) Registry, we selected all patients from MUMC+ who were diagnosed with ABC and who also had passed away between January 1<sup>st</sup>, 2007 and October 1<sup>st</sup>, 2017. Patient, disease and treatment characteristics and data regarding health care use in the last period of life defined as ongoing chemotherapy  $\leq 14$  days before death, start of a new line of chemotherapy  $\leq 30$  days before death, and radiotherapy, hospital admission, surgery, intensive care unit (ICU) admission, mechanical ventilation, cardiopulmonary resuscitation (CPR)  $\leq 6$  months before death, and cause and place of death were collected by trained registration clerks. Healthcare use was described and univariate analyses were carried out for ongoing chemotherapy  $\leq 14$  days or start chemotherapy line  $\leq 30$  days before death, admission and death in the hospital using chi square and Fisher's exact test. The SONABRE Registry was approved by the Medical Research Ethics Committee of Maastricht University Medical Center.

**Results:** Of 203 included patients, chemotherapy was continued  $\leq 14$  days before death in 21%, and a new line of chemotherapy  $\leq 30$  days before death was started in 9% of patients. In the last 6 months of life, radiotherapy was applied in 21% of patients. Hospital admission occurred in 76% of patients, because of tumor-related symptoms in 60%, and because of toxicity in 12% of these. Surgery (4%), ICU admission (6%), mechanical ventilation (5%), and CPR (2%) occurred infrequently. Of all patients, 25% died in the hospital; 74% due to progressive disease, 12% due to complications of therapy for ABC and 14% non-breast cancer related. Ongoing chemotherapy  $\leq 14$  days before death was associated with age < 65 years ( $p < 0.001$ ) and negative hormone receptor (HR) status ( $p = 0.04$ ); start of a new line of chemotherapy  $\leq 30$  days before death was associated with age < 65 years ( $p < 0.001$ ). Hospital admission was associated with age < 65 years ( $p = 0.008$ ), de novo ABC ( $p = 0.01$ ), negative HR status ( $p = 0.04$ ) and chemotherapy as last line of therapy ( $p = 0.001$ ). Death in the hospital was associated with ongoing chemotherapy  $\leq 14$  days ( $p < 0.001$ ) and start of a new line of chemotherapy  $\leq 30$  days before death ( $p < 0.001$ ).

**Conclusion:** During the last 6 months of life, admission due to tumor-related symptoms occurred frequently, whereas ICU admission, mechanical ventilation and CPR occurred rarely. Death in the hospital occurred in a quarter of patients, and more frequently in those receiving chemotherapy shortly before death, which in turn was associated with younger age. Insight in real-life healthcare use may improve shared decision making and advanced care planning for patients with ABC.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-21

Association of depression and anxiety disorder with the risk of mortality in breast cancer: A national health insurance service study in South Korea

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**PURPOSE:** To examine whether depression, anxiety disorder and their co-occurrence would increase the risk of mortality in patients with breast cancer, and whether antidepressant treatment would reduce the same.

**PATIENTS AND METHODS:** Data were retrieved from the database of the Korean National Health Insurance Service. Of 145,251 patients diagnosed with breast cancer between 2007 and 2014, 20,870 patients diagnosed with depression or anxiety disorder one year before breast cancer diagnosis were excluded. Thus, data of 124,381 patients were included in this study.

**RESULTS:** Anxiety disorder was more prevalent than depression in patients with breast cancer, and similar factors were associated with both depression and anxiety disorder. Overall, female sex, older age, residence in metropolitan areas, lower income, higher comorbidity, carcinoma in situ, and the receipt of any type of cancer therapies were associated with an increased risk of depression or anxiety disorder. Depression and anxiety disorder were associated with an increased risk of mortality (Hazard Ratio (HR) = 1.26, 95% CI=1.18–1.36; HR=1.14, 95% CI=1.08–1.22, respectively) and their co-occurrence further increased the risk (HR=1.38, 95% CI=1.24–1.54). Antidepressant treatment was related to a reduced risk of mortality. Compared to patients with no depression, among those with depression, the risk of mortality was 2.18 times higher (95% CI=1.69–2.81) in patients who did not receive antidepressant treatment and 1.25 times higher (95% CI =1.17–1.32) in those who received antidepressant treatment.

**CONCLUSION:** The current findings suggest that psychiatric comorbidities are markers of increased mortality risk in patients with breast cancer. This underscores the need for screening and treating depression and anxiety disorders to improve survival in breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-22

Adenosquamous carcinoma of the breast, an uncommon diagnosis with poor prognosis – Lessons learned from analysis of the National Cancer Data Base 2004-2015

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## Background

Adenosquamous carcinomas (ASQ) of the breast is rare, with a reported incidence of <1%. This tumor is considered to belong to the metaplastic breast carcinoma category and is commonly low grade. It has a distinct adenocarcinomatous component comprised of infiltrating small glands with varying degrees of squamous differentiation. The significance of ASQ differentiation and its impact on diagnosis, treatment and prognosis is based primarily on results from small retrospective case series reports. The objective of our study is to define prevalence, clinicopathologic characteristics, treatment patterns and prognosis of ASQ and compare these to the most frequent invasive breast carcinoma (BC), ductal (IDC), through analysis of the National Cancer Data Base (NCDB).

## Methods

A retrospective observational study of NCDB patients (pts) compared all ASQ pts to the same number of randomly selected IDC pts (ICD-O-3 diagnosis codes 8560 and 8500, respectively) using SPSS software. Patients' clinicopathologic characteristics, treatment, and overall survival (OS) were analyzed using frequency statistics, t-tests, Mann-Whitney U tests, chi-square, Kaplan-Meier and logistic regression.

## Results

From 1,932,688 female pts with invasive BC, 1,421,250 had IDC (73.5%); 453 had ASQ (0.0002%). ASQ pts were significantly ( $p<0.05$ ) more likely to be: older, have grade 1 tumors, negative lymph nodes, ER, PR, and HER2-negative tumors when compared to IDC. No significant difference was found in tumor size, TNM stage, or treatment. ASQ pts had significantly worse 5-year OS than IDC pts ( $p<0.025$ ; 73% vs 82% at 60 months, respectively).

## Conclusion

Our study is the largest study to date on ASQ revealing that this unique disease is an aggressive carcinoma and carries a significantly worse prognosis than IDC. Since prospective randomized clinical trial(s) are unlikely, further studies on the genomic make-up of ASQ are needed in order to understand biology and personalize treatment of pts with this uncommon BC.

## Clinicopathologic characteristics of pts with ASQ & IDC - NCDB analysis (2004-2015)

Demographics	ASQ	IDC (random sample)	Adjusted Odds Ratio	95% CI Lower	95% CI Upper
	N=453	N=453			
	N%	N%			
Age					
Average	63.50*	60.62			
Tumor Size (mm)					
Median	18.0	18.0			
Interquartile Range	24	19			
Grade					
1	202*	88	Referent		
	44.6	19.4			
2	71*	185	.17	.12	.24

	15.7	40.8			
3	136*	147	.40	.29	.57
	30	32.5			
Unknown	44	33			
	9.7	7.3			
<b># of node(s) positive</b>					
0	296*	267	Referent		
	65.3	58.9			
1 to 3	50*	72	.63	.42	.93
	11.0	15.9			
4 to 9	14*	31	.41	.21	.78
	3.1	6.8			
>10	9	10	.81	.33	2.02
	2.0	2.2			
Unknown	84	73			
	18.5	16.1			
<b>TNM Stage</b>					
I	227	223	Referent		
	50.2	50.2			
II	146	130	1.1	.82	1.49
	32.3	29.3			
III	38	55	.68	.43	1.07
	8.4	12.4			
IV	16	22	.71	.37	1.40
	3.5	5.0			
Unknown	25	14			
	5.5	3.2			
<b>ER</b>					
(+)	135*	359			
	31.4	81			
(-)	295*	84	9.3	6.8	12.8
	68.6	19			
<b>PR</b>					
(+)	61*	313			
	14.3	71.1			
(-)	367*	127	14.8	10.5	20.8
	85.7	28.9			
<b>HER2</b>					
(+)	8*	44			
	3.2	18			
(-)	240*	201	6.6	3.0	14.3
	96.8	82			
<b>Surgery</b>					
Yes	427	424			
	94.7	94.2			
No	24	26	.92	.52	1.62

	5.3	5.8			
<b>Chemotherapy</b>					
No	199	206			
	51.7	51.8			
Yes	186	192	1.00	.76	1.33
	48.3	48.2			
<b>Hormone Therapy</b>					
No	308	131			
	76.8	32.7			
Yes	93	270	.15	.11	.2
	23.2	67.3			
<b>Radiation</b>					
No	210	200			
	46.9	44.3			
Yes	238	251	.90	.7	1.17
	53.1	55.7			
<b>5-year Overall Survival</b>					
Alive	137*	165	.65	.43	.98
	64.9	74			
Dead	74*	58			
	35.1	26			

\*Significant (p<.05)

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-23

Hormone receptor status may impact the survival benefit between atypical medullary carcinoma of the breast and medullary breast carcinoma: A population-based study

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**Purpose:** Atypical Medullary Carcinoma of the Breast (AMCB) is a subtype of breast cancer with a more adverse prognosis, compared with Medullary Carcinoma of the Breast (MBC). Our study was designed to identify the prognostic factors of AMCB, MBC and invasive ductal carcinoma (IDC).

**Methods:** Using the Surveillance, Epidemiology and End Results (SEER) database from 2004 to 2013, 147 eligible AMCB patients were compared for tumor characteristics and overall survival with 1863 MBC and 446708 IDC patients.

**Results:** Kaplan-Meier analysis and multivariate Cox analysis showed that patients with AMCB (5-year OS = 86.4%) had significantly worse overall survival (OS) compared to MBC (5-year OS=93.8%,  $p=0.013$ ) (HR=0.525, 95%CI 0.305-0.905) but similar to IDC (5-year OS =90.4%,  $p=0.477$ ) (HR=1.097, 95%CI 0.612-1.684). In addition, it was found that age (HR=1.259, 95%CI 1.228-1.291), high grade (HR=2.510, 95%CI 2.371-2.657), large tumor size (HR=2.504, 95%CI 1.851-3.386) and hormone receptor status (HR=1.439, 95%CI 1.392-1.486) were significantly associated with poor prognosis. In the hormone receptor (HR)-positive population, the AMCB group and MBC group had a similar survival ( $p=0.656$ ). In the HR-negative population, the MBC group gained an additional survival benefit compared with the AMCB group ( $p=0.006$ ).

**Conclusions:** Patients with AMCB showed worse overall survival than the MBC group. In HR+ patients, there did not appear to be a difference between AMCB and MBC given the similarities in prognosis; however, in the HR- population, AMCB did not gain a survival benefit.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-24

Conditional survival of breast cancer patients: Korean nationwide registry

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**Purpose:** Conditional relative survival (CRS) could provide more relevant information on the current prognosis of cancer survivors than standard 5-year relative survival (RS). This study aimed to estimate the 5-year CRS of Korean breast cancer patients.

**Patients and Methods:** We identified 145,083 breast cancer cases with diagnosis between 2002 and 2013 in the Korea Central Cancer Registry. The CRS was estimated for every year after diagnosis, according to sex, age, histologic types, stage, and year of diagnosis.

**Results:** The 5-year RS at diagnosis was 90.8% and 10-year RS was 85.7%. Five-year CRS was 91.0% and 94.3% at 1-year and 5-year after diagnosis. Women had better 5-year CRS than men after 5 years of survival (94.3% vs. 79.5%), and very young and very old patients had worse 5-year CRS after 5 years of survival than other age groups (92.2% in <40yr, 92.6% in ≥70yr vs. 95.4% in 40-49, 94.3% in 50-59, and 93.7% in 60-69, relatively). In histologic types, CRS of metaplastic carcinoma has improved from 82.0% to 95.2%, compared to CRS of lobular carcinoma (from 93.1% to 92.5%). Hardly any excess mortality (5-year CRS ≥ 95%) was seen since 7 years after diagnosis. There was hardly any excess mortality at 5 years of survival, for the patients with 40-49 years (95.4%), with localized disease (97.8%), and with metaplastic carcinoma (95.2%).

**Conclusion:** This study showed that CRS of breast cancer survivors in Korea has been improved, which varied by sex, age, stage, and histologic types. These CRS analysis could provide a more detailed information for survival to breast cancer survivors and clinicians.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-26

Factors associated with survival in a 20-year historical cohort in Brazil

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In Brazil (population over 200 million), breast cancer is the leading cause of death from cancer among women. Nevertheless, little information is available regarding the characteristics of patients. Given this scenario, a private non-profit hospital located in Porto Alegre – the Brazilian city with the highest incidence of breast cancer (crude rate estimated at 114.25/100,000 for 2018) (1) – established a comprehensive breast cancer center (Núcleo Mama Moinhos, NMM). We investigated variables associated with mortality in 896 women diagnosed and treated at NMM over 20 years. The study was approved by the research ethics committee. Only women with centrally reviewed histological confirmation of breast cancer were included. The primary outcome was survival following diagnosis. The correlation between survival and the following variables was tested: age at diagnosis, disease stage at diagnosis, schooling, smoking, body mass index (BMI), menopausal status, hormone replacement therapy (HRT), family history of breast cancer, pathological grading, hormone receptor status, HER2 status, molecular subtype, and biomarker Ki67. The impact of variables as predictors of mortality was analyzed using Cox and Kaplan-Meier models. Mean age at diagnosis was 53.7 years (SD 13.087). Clinical staging at diagnosis was: 107 patients with stage 0 (12.3%), 440 stage I (50.7%), 244 stage II (28.1%), 67 stage III (7.7%), 10 stage IV (1.2%), and 28 with missing data. At 5 years, 96.4% of the patients were alive, and at 10 years 86.6% were alive. At the end of the observation (20 years), 67% were alive. Obesity (BMI>30) was detected in 136 (15%) women; 574 (64.0%) were menopausal, and 244 (27.2%) received hormone therapy. HER2 status was available for 782 cases of invasive breast cancer (87.3%), and 131 (16.8%) were HER2-positive. Triple negative cases were 14.3%. According to multivariate analysis (table 1), increasing stage and age at diagnosis, Ki67, and distant recurrence were significantly associated with survival. None of the other variables, including smoking status, years in school, BMI, and HER2 status, was associated with mortality.

Table 1. Risk of mortality in a historical cohort of 896 women from South Brazil

Variable	Hazard ratio
Pathological staging	
0	
I	1.504
II	2.406
III	3.198
IV	12.186
Age at diagnosis	1.051
Ki67	
Negative	
Low	3.869
Intermediate/high	5.801
Distant recurrence	
Yes	5.55
No	

Missing information: 26 patients for staging; 138 patients for Ki67. Age: hazard ratio for each additional year.

To the best of our knowledge, this is the largest breast cancer cohort with the longest follow-up time in Brazil. These results suggest that Brazilian patients who receive early treatment at a comprehensive cancer center will achieve outcomes that are similar to those of developed countries. The high breast cancer mortality in Brazil seems dependent on the health care that is



available to women (2).

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# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-08-27

Advanced stage at diagnosis and worse clinicopathologic features in young woman with breast cancer. A sub-analysis of Brazilian population through the AMAZONA III study (GBECAM 0115)

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**BACKGROUND:** Breast cancer (BC) in young women is uncommon and often more aggressive. There are disparities in terms of screening coverage, diagnostic features and access to optimal treatment among young BC patients worldwide. To better understand this scenario through real world data we performed a sub-analysis of AMAZONA III study. **METHODS:** The AMAZONA III study (GBECAM 0115) is a prospective registry that included 2950 women newly diagnosed with invasive BC in Brazil during the period of January 2016 to March 2018 within 22 sites. Of them, 2888 patients had valid data regarding age at diagnosis and complete baseline information. For the purpose of comparisons of epidemiologic and clinicopathologic features at the time of diagnosis of BC, patients were divided in two groups: women aged  $\leq 40$  years (Group 1) and  $>40$  years (Group 2). Quantitative variables were expressed with mean, while categorical variables were described as their count and percentage and compared using the chi-square test. **RESULTS:** Of 2888 women, 486 (17%) were  $\leq 40$  years of age. No differences were found between ethnicity, performance status, body mass index, personal income, health insurance and family history of cancer between the two groups. Young women had higher educational level ( $p < 0.001$ ), were more involved into a labor activity ( $p < 0.001$ ) and were more frequently married ( $p < 0.001$ ). There were also significant differences regarding nulliparity ( $p < 0.001$ ) and previous use of oral contraceptives ( $p < 0.001$ ). Mode of detection of BC was symptomatic in 73.4% of young group versus 64.5% in older group and screen-detected was only 26.6% vs. 35.5% respectively ( $p < 0.001$ ). Table 1 describes clinicopathological characteristics of the two groups. Young women presented more frequently with stage III, T3/T4, Grade 3 tumors and HER-2 positive, Luminal B and triple negative subtypes. Women older than 40 years had more stage I, Luminal A and Grade 1/2 tumors. **CONCLUSION:** Brazilian women under the age of 40 have unfavorable clinicopathological features of BC at diagnosis with more aggressive subtypes and advanced stage compared with older women. No differences in socioeconomic and ethnical aspects were found but a higher percentage of young women had symptomatic detection of BC which could explain the later stage of disease at diagnosis. Young women were economically active and the majority married which highlights the socioeconomic impact of this disease in Brazil.

Breast cancer features by age groups at diagnosis in Brazilian women.

Information	Group 1 ( $\leq 40$ years)	Group 2 ( $> 40$ years)	p-value
N: 2888	486 (16.83%)	2402 (83.17%)	

Stage at diagnosis			p< 0.001
I	76 (19.2%)	541 (27.8%)	
II	156 (39.4%)	816 (41.9%)	
III	146 (36.8%)	489 (25.1%)	
IV	19 (4.6%)	101 (5.2%)	
Tumor size			p< 0.001
T1	114 (27.1%)	749 (36.9%)	
T2	141 (33.6%)	764 (37.6%)	
T3	101 (24.1%)	282 (13.9%)	
T4	64 (15.2%)	235 (11.6%)	
Tumor grade			p < 0.001
Grade 1	46 (10.7%)	381 (17.9%)	
Grade 2	198 (46.2%)	1150 (52.0%)	
Grade 3	185 (43.1%)	641 (30.1%)	
Molecular Subtype			p < 0.001
Luminal A	106 (30.6%)	957 (51.3%)	
Luminal B - HER 2 negative	55 (15.8%)	212 (11.4%)	
Luminal B- HER 2 positive	79 (22.8%)	298 (16.0%)	
HER 2 positive	27 (7.8%)	135 (7.2%)	
Triple negative	80 (23.0%)	264 (14.1%)	

# 2018 San Antonio Breast Cancer Symposium ®

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Impact of surgery and time to surgery on breast cancer survival in the United States, 2004–2014 (N=2,211,245)

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**Background:** Surgery is very common for patients diagnosed with breast cancer. Its impact on survival depends on diagnostic, patient, tumor-related, and other-treatment factors. Moreover, time to surgery from the date of diagnosis is also a critical factor affecting outcome.

**Objective:** In this study we investigated the impact of surgery on survival in breast cancer patients using two methods: (1) multivariate regression; and (2) propensity score matching. For the patients undergoing surgical intervention, we aimed to identify the optimum time from diagnosis to surgery.

**Methods:** The study population was taken from the National Cancer Database over the years 2004 through 2014. Of 2,211,245 patients, 99.1% were female, 0.9% male, 85% non-Hispanic white, 10.5% black, 0.7% Hispanic, and 14.5% other races. Mean age of the patient population was 60.0 ± 13.4 years (range: 18–90). The majority of the patients (92.9%) underwent a surgical procedure.

**Results:** Overall, the patients who did not undergo surgery were 6.7 times more likely to die within the study time period (95% confidence interval [CI]: 6.7–6.8, p<0.001) than those who did. However, after adjusting for patients' demographics, tumor-related factors, cancer stages, and combination of other treatments, the risk for dying of patients without surgery was 2.3 times higher (hazard ratio [HR]: 2.3, 95% CI: 2.3–2.4, p<0.001). In the propensity-matched cohort of 51,630 patients that was divided equally into two groups — those who underwent surgery and those who did not — the risk of mortality remained 2.4 times higher for patients without surgery (HR: 2.4, 95% CI: 2.3–2.4, p<0.001). Regarding time to surgery from the date of diagnosis, patient survival was best for the patients whose time to surgery ranged from 31 to 60 days. The next best timeframe was 61 to 90 days, followed by 30 days or fewer, then 91 to 120 days, and finally 120 and more days (p<0.001).

**Conclusion:** Using two different statistical methods, surgery is clearly an independent predictor of survival for patients with breast cancer. After matching for other factors, patients not having surgery were more than twice as likely to die as their surgical counterparts. Time to surgery from the date of diagnosis confirmed earlier findings that surgery is most beneficial within 2–3 months from the date of diagnosis. These findings can provide clinical guidance to clinicians and patients for planning treatment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-29

Current status of clinical and pathological characteristics of breast cancer patients in Brazil: Results of the AMAZONA III study (GBECAM 0115)

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## BACKGROUND

Breast cancer (BC) is the most common tumor in women in Brazil with about 60 thousand new cases estimated per year. In low and middle-income countries, patients with BC are diagnosed with more advanced stages as compared with high-income countries. In Brazil, disparities in access to new therapies are recognized; previous data suggests worse survival of BC patients treated in the public system. The aim of AMAZONA III study (GBECAM 0115) is to describe the current status of BC care in Brazil. Here we report patients data at baseline.

## METHODS

The AMAZONA III is a prospective BC registry that included women 18 years or older with newly diagnosed stage I to IV BC from 22 sites in Brazil in the period of January 2016 to March 2018. All patients provided written informed consent; data was collected from interview and medical charts, comprising clinical-demographic variables, initial treatment and a planned follow-up for 5 years. BC subtypes were defined by hormone receptor (HR) expression, HER2 status and grade according to von Minckwitz G. et al 2012. Here we present a descriptive analysis of the patients' baseline characteristics. Continuous variables are shown as mean (standard-deviation) and categorical variables by its absolute and relative frequencies. The study is registered in clinicaltrials.gov NCT02663973.

## RESULTS

A total of 2950 patients were included in the study. Median age at diagnosis was 53 years old (8.4% ≤ 35 years, 34.8% 36-50 years, 56.8% > 50 years), 58.6% were white, 34.4% had brown skin-color, 83% had children before BC diagnosis (median of 1 child/patient) and 63.1% had public health insurance. In terms of method of detection 34% were screen-detected whereas 66% were symptomatic, the last was even higher (70%) in patients in younger than 50 years. The distribution of BC stage at diagnosis was I (26.4%), II (41.6%), III (27%) and IV (5%). The most common histologies were ductal (80.9%) and lobular carcinoma (6.9%). The pathological characteristics were HR positive in 78.0%, HER-2 positive in 23.4% and grade 2 in 51%. BC subtypes were as follows: Luminal A 48%, Luminal B 12%, Luminal HER2 positive 17%, Non-luminal HER2 positive 7.3% and Triple negative 15.5%.

## DISCUSSION

Breast cancer is diagnosed at an earlier age among Brazilian patients. The majority of patients were detected through symptomatic BC and therefore a significant proportion is still diagnosed in stages III and IV. Among other factors, these findings could have a significant impact in treatment outcomes. Further analysis of this large cohort of patients will help to identify other important elements and direct future strategies for breast cancer control.

**TRIAL REGISTRY:** NCT02663973

**KEYWORDS:** Breast Cancer; Epidemiology; Treatment; Brazil

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-30

Triple negative breast cancer: Does ethnicity impact survival?

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## **Background:**

Triple negative breast cancer (TNBC) is a heterogeneous disease with poor outcomes relative to hormone positive breast cancer. Observations in the clinical setting lead to the hypothesis that there may be phenotypic differences among ethnic groups. Previous studies have conflicting results; some suggesting no differences, others showing inferior outcomes for black patients (pts). The purpose of this study was to use a population-based approach to examine survival amongst different ethnicities in triple negative breast cancer (TNBC).

## **Methods**

Retrospective population-based study using data from the surveillance epidemiology and end results (SEER) database to identify TNBC cases diagnosed 2010 - 2014. We divided pts into 6 ethnic groups: White, Black, American Indian/Alaskan Native (AIAN), Pacific Islander (PI), Asian and Asian Indian (AI). Primary outcome: overall survival (OS); secondary outcome: breast-cancer specific survival (BCSS). Survival analysis was performed using Cox proportional hazards and Kaplan-Meier models.

## **Results**

31482 pts were included; 22752 (72.3%) were white, 6319 (20.4%) black, 1720 (5.5%) Asian, 262 (0.8%) AI, 179 (0.6%) AIAN, and 150 (0.5%) PI. Asian pts had the best OS (HR 0.82, 95% CI 0.72-0.94), while Black pts had the worst (HR 1.21 95% CI 1.13-1.29) when compared with White pts. Differences between other ethnicities were not statistically significant for OS. Black pts had significantly worse BCSS (HR 1.20, 95% CI 1.12 -1.29); no other ethnicity had statistically significant differences compared to Whites. Comparing Asian with Black pts in multivariate analysis the HR was 0.68 (95% CI 0.59 – 0.79) for OS and 0.71 (95% 0.61 – 0.84) for BCSS. Older age, advanced stage, male sex, and lack of chemotherapy, radiation or surgery were also found to be statistically significant variables in multivariate analysis.

## **Conclusions**

In this large population study, we found Asian pts had significantly better OS and Black pts had significantly worse OS and BCSS. These findings confirm the clinical observation and warrant closer molecular analysis of TNBC phenotypes based on patient's ethnicity. Findings may offer insight into the biology of TNBC and lead to development of innovative treatment options.

# 2018 San Antonio Breast Cancer Symposium ®

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Comparing estimates of survival for breast cancer patients

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## Introduction

Net survival can be considered as the most accurate evaluation of survival from cancer, as it encompasses the survival that would occur if the only possible underlying cause of death was the cancer under study. Both relative survival (RS) and cause-specific survival (CSS) have been developed as approaches to estimate net survival at the population level. Given the debated accuracy of death certificates in identifying the underlying cause of death, RS measures are most commonly used by cancer registries. However, RS calculations can also be biased as it is sometimes unavoidable to match a group of cancer patients with a non-comparable disease-free population group.

In this study, we aim to compare RS and CSS using causes of death obtained from death certificates in a cohort of Belgian breast cancer patients by evaluating them to CSS using causes of death obtained from medical files.

## Methods

A total of 3,205 breast cancer patients diagnosed and treated in University Hospitals Leuven between 2009-2014 were included in this study. RS was calculated with the Ederer II method, dividing the observed survival by the expected survival for females of the same age and region. CSS was calculated using cause of death information either gathered from death certificates or collected from medical files. The estimates for RS and CSS as obtained from death certificates were compared to CSS obtained from medical files. Follow-up was guaranteed until 31th of December 2014.

## Results

From the included cohort of breast cancer patients, 255 were deceased. Cause of death was available for 254 patients from death certificates and for 191 patients from medical files. By considering the available cause of death information, 3,141 patients were included to calculate the survival estimates. The 1-year relative survival estimate was 99.3% (95%CI, [98.7, 99.8]), while 1-year cause-specific survival based on death certificates was 99.0% [98.5, 99.3]. The 1-year survival estimate according to CSS based on medical files was 98.1% [97.5, 98.5]. The 5-year survival estimates were 95.8% [94.0, 97.4] for RS, 93.3% [91.8, 94.4] for CSS based on death certificates and 89.7% [88.0, 91.2] for CSS based on medical files.

## Conclusion

This study used causes of death information as derived directly from medical files to calculate CSS in order to compare it with RS and CSS derived from death certificates. This allowed an evaluation of the RS and CSS estimates commonly used at the population level against the presumably more reliable CSS as obtained from medical files. Both RS and CSS from death certificates were divergent from CSS from medical files, but the CSS from death certificates appeared to be a closer estimate to CSS from medical files. After longer follow-up, both survival estimates seemed to deviate more from the CSS from medical files.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-32

Treatment delivery waiting times for stage I-III breast cancer patients in Switzerland : A pooled analysis of 7 cancer registries over the 2003-2008 period

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**Background:** To examine time intervals between needle/core biopsy, breast cancer diagnosis, surgery and radiotherapy (RT) as quality metrics in the management of stage I-III breast cancer in a representative Swiss population sample.

**Methods:** Based on seven regional cancer registries covering 45% of the Swiss population, we identified 2628 women which underwent surgery for stage I-III breast cancer without receiving (neo)-adjuvant chemotherapy between January 1, 2003 and December 31, 2005.

Four different time intervals were defined: a) time between needle/core biopsy and diagnosis of breast cancer, b) time between diagnosis of breast cancer and surgery, c) time between needle/core biopsy and surgery, d) time between surgery and adjuvant RT.

These four time intervals were analyzed according to age, nationality, health insurance status, public vs. private hospitals and geography. We also investigated whether case discussion at tumor board delayed patient management.

A one-way analysis of variance (ANOVA) and multiple comparison tests were used to assess differences between groups. All tests were performed using STATA v.15.

**Results:** 2628 women were identified, median age was 67 years (IQR: 58-77). Breast-conserving surgery was performed in 1899 cases (72.3%), mastectomy in 539 cases (20.5%), unspecified surgery /missing data in 190 cases (7.2%). Adjuvant RT was delivered in 1546/2628 patients (58.8 %).

Time interval between biopsy and surgery was age-dependent, ranging from 22 days (95% CI: 19.6 - 25.2) for women < 60 years to 39 days (95% CI: 27.0-50.3) for women 80+ years old ( $p<0.001$ ). After biopsy, women waited on average 19 days until surgery in private clinics (95% CI: 16.4-21.6) and 30 days in public hospitals (95% CI: 26.6-33.3) ( $p<0.001$ ). Women with private insurance were operated 24 days after biopsy (95% CI: 17.0-31.1), compared with 30 days (95% CI: 27.1-33.2) ( $p<0.01$ ) for women with basic state insurance. After biopsy, time interval for foreign nationals was significantly longer than for Swiss citizen (30 vs 24 days,  $p<0.01$ ). Tumor board presentation postponed surgery by 10 days (31 vs 22 days,  $p<0.01$ ).

Time between surgery and RT did not correlate with age ( $p=0.83$ ); the interval was 33% longer in tertiary teaching hospitals than in private clinics (61 vs. 46 days,  $p<0.001$ ), and 8 days longer for patients with private insurance than for those without (61 vs 53 days,  $p<0.01$ ). There was a trend for foreign nationals to receive adjuvant RT later than Swiss citizen (58 vs 55 days,  $p=0.09$ ). RT started later in larger metropolitan areas compared to more rural regions (59 vs 53 days,  $p<0.01$ ). Presenting patients at a tumor board after surgery had no impact on RT start ( $p=0.12$ ).

**Conclusions:** Major differences in treatment waiting times were observed between patients with stage I-III breast cancer. Elderly and foreign patients were at risk for delayed surgery after biopsy. Data from patients with longer timelines need to be analyzed to identify further reasons for delays.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-33

Molecular breast cancer subtypes in a Guatemalan population. Classification according to immunohistochemical markers: Clinicopathologic feature and survival analysis

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**Introduction:** Breast cancer is a heterogeneous malignancy and it's possible to identify by simple techniques, such as immunohistochemistry, four subtypes with different clinical and biological behavior. Our aim was to evaluate the influence of these subtypes on the local and distant recurrences rates.

**Method:** data of 954 unilateral breast cancer patients primarily treated with radical or conservative surgery from 2008 to 2014 were obtained from medical records. Breast cancer subtypes were categorized in Luminal A (RE+ and/or RP+, HER2-), Luminal B (RE+ and/or RP+, HER2+ o Ki-67 > 14%); HER2 (RE-, RP-, HER2+) and triple negative tumors (TNT) (RE-, RP-, HER2-). Patterns of recurrence for each subtype and clinicopathological variables and the influence of each variable on the overall survival (OS) by the Kaplan-Meier method. In all cases, we determined histological type, grade nuclear (low, intermediate vrs high), tumor size, lymph node involvement

**Results:** Median age at diagnosis was 52 years (range, 23-95). Of these patients 55% were premenopausal. We found that 522 (56%) of the cases were luminal A breast cancer, 190 (20%) of the cases were triple negative tumors (TNT), luminal B were 73 (8%) and it was HER2-positive in 152 (20%) of cases. Nine patients (3%) had other breast cancer contralateral. In 70% of all the cases were found in locally advanced stages (IIB – IIIC). In 438 (70%) of all the cases were found in locally advanced stages (IIB – IIIC), 773 (81%) patients underwent radical mastectomy and 181 (19%) undergoing conservative surgery. We performed univariate analysis to evaluate recurrence-related factors. Variables included in the analysis clinical stage (I – IIa vs IIb – IIIC; 88% vs 70%;  $p = 0.001$ ), nuclear grade (grade 1, 2 vs 3; 83% vs 62%;  $p = 0.01$ ) and pathologic response (total vs parcial, stable disease; 76% vs 54%;  $p = 0.01$ ), and status Her-2 subtype so borderline (positive vs negative;  $p = 0.079$ ). A univariate survival analysis stratification according to clinicopathologic characteristics. Clinical stage, histologic grade, response to neoadjuvant and tumor size were identified as significant factors of prognosis for the OS. ( $p=0.5$ ) and histological subtypes Luminal A (94%), Luminal B (75%), HER2 (79%) and TNT (68%),  $p = 0.001$ .

**Conclusion:** this study demonstrates that treatment efficacy is similar to that reported in the literature and emphasizes the need for establishing the molecular breast cancer subtypes and efforts should be made to reduce the high frequency of advanced-stage diagnoses.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-08-35

Treatment across the four molecular types of breast cancer: Insight from the national cancer database, 2010–2014 (N=827,888)

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**Background:** For patients diagnosed with breast cancer treatment plans are usually determined by biomarkers such as ER, PR and HER2, the absence or presence of which determines molecular subtype of the tumor (luminal A, luminal B, triple-negative, and HER2-enriched). However, it is unclear to what extent each treatment plan contributes to overall survival across these four molecular types of breast cancer (as determined by the presence or combination of biomarkers).

**Objective:** Assuming inherent heterogeneity among breast cancer patients, we sought to determine the benefit of any one or combination of treatment methods among surgery, chemotherapy, radiation, immunotherapy, and hormonal therapy and whether differences exist among various subgroups for predicting mortality risk.

**Methods:** A total of 827,888 patients diagnosed with breast cancer from the National Cancer Database were analyzed. The patient population was 99.1% female, 0.9% male, 75.5% white, 11.2% black, 0.8% Hispanic, and 12.5% other races. Most of the patients (97.1%) were diagnosed with invasive breast cancer; the remaining 2.9% were diagnosed with in situ and/or carcinoma in situ. For molecular subtypes, the distribution of patients was 72.7% with luminal A, 10.4% luminal B, 4.7% HER2-enriched and 12.2% triple-negative.

**Results:** Overall, patients who did not receive treatment were 6.9 times more likely to die (95% confidence interval [CI]: 6.7–7.0) than those who did. Within molecular subtypes, hazards ratios [HR] were for dying without treatment were 7.0 (95% CI: 6.8–7.1) for luminal A, 7.8 (95% CI: 7.3–8.3) for luminal B, 6.9 (95% CI: 6.6–7.2) for triple-negative, and 8.9 (95% CI: 8.2–9.7) for HER2-enriched tumor. Overall survival was best for luminal A, followed by luminal B, HER2-enriched and triple-negative ( $p < 0.001$ ). Multivariate Cox regression showed that the five most significant factors predicting mortality for luminal A were surgical treatment (HR: 0.4,  $p < 0.001$ ), patient age (HR: 1.1 one year increment,  $p < 0.001$ ), cancer stage (HR: 1.2, 2.1, 4.1, and 7.4 for stage I, II, III, and IV [all vs stage 0], respectively,  $p < 0.001$ ), Charlson/Deyo score (HR: 1.4, 2.1, and 2.9 for score 1, 2, and  $\geq 3$  [all vs score 0], respectively,  $p < 0.001$ ), and tumor grade (HR: 0.8, 0.5, and 0.4 for poorly, moderately, and well-differentiated tumors [all vs undifferentiated], respectively). For luminal B, HER2-enriched molecular types, and for triple negative chemotherapy (HR: 0.5, 0.6, 0.5, respectively) replaced grade differentiation as one of the top five predictors.

**Conclusion:** Despite recent suggestions of over diagnoses and unnecessary treatment for certain types of tumor, this retrospective study suggested that treatment remains highly beneficial for breast cancer patients. Among treatments, surgery remains a strong predictor of survival across the board; however, for luminal B, HER2-enriched, and for triple negative subtype chemotherapy along with surgery provides additional survival benefit. Overall survival was better for luminal A, followed by luminal B, HER2 and triple negative breast cancer. These results can provide guidance for clinicians as well as for patients.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-09-01

CD8+ T-cell gene expression and signatures in breast cancer and adjacent normal breast tissue: Association with body mass index, alcohol intake, and age at diagnosis

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**Background:** Our understanding of mediators of immune infiltration in breast cancer and normal breast tissue remains limited. We hypothesize that patient factors known to be associated with inflammation and immune subsets, including body mass index, alcohol intake, and age at diagnosis, may play an important role in the tumor-immune microenvironment. Analyses of immune gene expression and signatures facilitate interrogation of the immune microenvironment in large patient cohorts.

**Methods:** Participants from the Nurses' Health Study cohorts I and II diagnosed with invasive breast cancer were included. Total RNA extracted and microarray performed for 882 tumor and 695 tumor-adjacent samples, of which 623 tumors have matched tumor-adjacent data. CD8+ T-cell expression metrics were assessed: *CD8A* single gene expression (CD8Agene), a CD8 T-cell signature (CD8sig), and a tumor infiltrating lymphocyte signature derived from the GeparSixto clinical trial (GSAct). Standard clinicopathologic features were evaluated, as well as body mass index (BMI) one year prior to diagnosis, cumulative average alcohol intake, and age at diagnosis.

**Results:** Overall, tumor and adjacent normal tissue demonstrated positive correlation of CD8Agene, CD8sig, and GSAct (n=623 pairs, Pearson's  $r = 0.46, 0.36, 0.31$ , respectively; all  $p < 0.001$ ). Similar correlations were present in TCGA breast cancer, an independent cohort (n=112 pairs, Pearson's  $r = 0.34, 0.17, 0.45$ , respectively; all  $p < 0.001$ ). We evaluated paired tumor and adjacent normal samples within individual immunohistochemical (IHC) subtype or PAM50 subtype by Wilcoxon signed-rank test. There was not a consistent trend for CD8Agene, CD8sig, nor GSAct to be greater in tumor or normal within subtypes. We then evaluated patient features/exposures and tumor immune expression metrics. For tumor-adjacent normal, there was no significant association of alcohol intake, BMI, or age at diagnosis with CD8 gene/expression metrics. For tumor tissue, a multivariate model demonstrated that BMI one year before diagnosis was significantly associated with CD8Agene expression. There was no significant association of alcohol intake or age at diagnosis with CD8 gene/expression metrics. We are currently evaluating the association of these CD8 T-cell gene expression signatures with CD8 T-cell immunohistochemistry in a subset of patients, which will be reported at the time of abstract presentation.

**Conclusion:** In this cohort of over 600 tumor:normal pairs and a separate validation cohort, multiple distinct CD8+ T-cell expression metrics are correlated between breast cancer and tumor-adjacent normal breast tissue. This suggests that the adjacent normal breast may reflect an altered immune microenvironment in the context of breast cancer. While age at diagnosis and alcohol intake are not significantly associated with tumor CD8 expression metrics, BMI was significantly associated with tumor CD8Agene expression in a multivariate model.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-09-02

Prevalence of genetic mutations in patients with second primary breast cancers

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**Background:** Women newly diagnosed with primary breast cancer (PBC) often undergo multi-gene panel testing to determine their contralateral breast cancer (BC) risk and whether a contralateral prophylactic mastectomy is warranted. However, with the exception of *BRCA1/2*, gene-specific associations with contralateral or second PBC (SPBC) have not been established.

**Methods:** The study sample was comprised of 83,278 women with BC referred to a single diagnostic laboratory for multi-gene panel testing. The frequency of pathogenic/likely pathogenic variants in clinically-actionable genes (CAG), including highly penetrant genes (HPG: *BRCA1*, *BRCA2*, *TP53*, *PTEN*) and moderately penetrant genes (MPG: *ATM*, *CHEK2*, *PALB2*, *CDH1*, *NBN*, *NF1*) was compared between women with a PBC and SPBC. Women with a SPBC <1 year from their first diagnosis were excluded. Logistic regression burden tests were used to test for associations between mutated genes and SPBC adjusting for age at diagnosis of first BC, histology, presence of first- or second-degree relative with BC, and race/ethnicity. **Results:** The study included 75,550 women with PBC and 7,728 with SPBC. The median (IQR) time between primaries for the SPBC group was 11 (6,17) years. Women with SPBC were slightly more likely to be Caucasian (67.8% vs. 63.4%;  $p<0.001$ ), older when referred for genetic testing (mean difference 9.7 years,  $p<0.001$ ), slightly younger at first BC diagnosis (mean difference 2.1 years,  $p<0.001$ ) and slightly more likely to have >1 first or second degree relative with BC (62.2% vs. 60.8%;  $p=0.004$ ) than PBC. Among women tested for all CAGs, 4,883 (8.1%) were carriers of pathogenic/likely pathogenic variants (11.1% SPBC vs. 7.8% PBC). *CHEK2* was the most frequently mutated gene (3.4% SPBC vs. 2.3% PBC), followed by *BRCA1* (2.7% SPBC vs. 1.6% PBC), *BRCA2* (2.2% SPBC vs. 1.8% PBC), and *PALB2* (1.4% SPBC vs. 0.9% PBC). In fully adjusted models, women with SPBC were 1.38 times as likely ( $p<0.0001$ ) as women with PBC to test positive for a CAG (OR=1.35 for HPG and 1.34 for MPG). *BRCA1* (OR=1.49;  $p<0.0001$ ), followed by *CHEK2* (OR=1.36;  $p<0.0001$ ) and *PALB2* (OR=1.53;  $p<0.001$ ) were most significantly associated with SPBC. *TP53*, *BARD1*, *ATM* and *BRCA2* were marginally associated with SPBC ( $p=0.01$  to  $0.06$ ). When results were stratified by race/ethnicity, ORs among Caucasians were similar to those observed overall. Among African Americans, women with SPBC were 1.76 times as likely to carry a CAG ( $p<0.001$ ) than their PBC counterparts. *PALB2* (OR=2.69;  $p=0.002$ ), *BRCA2* (OR=1.85;  $p=.004$ ), and *TP53* (OR=3.88;  $p=.009$ ) were most significantly associated with SPBC followed by *BRCA1* (OR=1.63;  $p=.002$ ). Analysis of gene associations for other racial/ethnic groups was limited by small sample size. **Conclusions:** There is a significantly higher prevalence of CAG mutations among women with SPBC, even after adjusting for age at diagnosis and family history. These findings support SPBC as a standalone indication for multigene panel testing. Additional studies aimed to assess cumulative risk of SPBC for CAG beyond *BRCA1/2* are needed to help guide clinical management decisions for mutation carriers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-09-04

Association between a breast cancer polygenic risk score and contralateral breast cancer risk

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## Background

Breast cancer patients are at significant risk of a second contralateral, breast cancer (CBC). Identification of women at high or low CBC risk could improve patient management decisions. Previous research has shown that breast cancer-associated single nucleotide polymorphisms (SNPs) summarized in a polygenic risk score (PRS) predict the risk of a first breast cancer with an odds ratio (OR) per 1 SD of 1.55 (95% confidence interval (95%CI)=1.52-1.58) (77-SNP PRS). The aim of this study was to evaluate the association between a recently developed PRS and CBC risk.

## Methods

We identified 19 studies from the Breast Cancer Association Consortium (BCAC) with follow-up information on participating patients and at least 10 patients diagnosed with CBC. This included 38,228 females of European ancestry diagnosed with first invasive breast cancer since 1990. Genotyping was done using the iCOGS array or OncoArray, with genotypes for SNPs not on the arrays estimated by imputation. We used a 313-SNP PRS, optimized for prediction of overall (first) breast cancer in the BCAC dataset. Metachronous CBC risk by PRS was quantified using univariable and multivariable Cox regression analyses stratified by country and adjusted for multiple patient, tumor, and treatment characteristics. We assessed PRS interaction with age, family history, adjuvant systemic therapy, and ER-status.

## Results

Median time to develop a CBC (N=1,046) after a first breast cancer was 5.8 years (range 0.3-21.9). Higher PRS was associated with increased CBC risk: hazard ratio (HR) per 1 SD=1.31 (95%CI=1.23-1.39). Patients in the highest and lowest 5% of the PRS had 1.95 fold and 0.67 fold risks of CBC, respectively, compared with patients in the middle quintile. Adjustments for age, year of diagnosis, family history, tumor size, nodal status, ER-status, or treatment (chemotherapy, endocrine therapy, radiotherapy) did not substantially alter these results. We found an interaction with age at first breast cancer diagnosis ( $P_{\text{interaction}}=.002$ ); the PRS was associated with an increased CBC risk for patients aged  $\geq 40$  years (HR=1.37, 95%CI=1.28-1.47), but not for patients  $< 40$  years (HR=1.06, 95%CI=0.93-1.21).

## Conclusion

The PRS is predictive for the development of CBC in patients  $\geq 40$  years at first breast cancer diagnosis. For this group, the PRS could be incorporated in CBC risk prediction models to help define high and low risk patients, and hence optimize screening and treatment strategies.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-09-05

Clinical and genomic characteristics of borderline ER-positive breast cancers

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**Background:** Though current guidelines classify breast tumors with  $\geq 1\%$  estrogen receptor (ER) positivity by immunohistochemistry as ER positive, “borderline” tumors expressing  $\geq 1 - <10\%$  positivity have biological similarities to ER negative tumors in prior studies. We sought to describe molecular features of ER-borderline breast cancer in a diverse cohort of incident cases and identify characteristics associated with high-risk borderline tumors.

**Methods:** We used the Carolina Breast Cancer Study, a large population-based study that oversampled young and black women in 44 counties of North Carolina, to study characteristics of ER-borderline cases compared to other ER-positive and ER-negative tumors. Gene expression of *ESR1*, PAM50 subtype, and risk of recurrence score (ROR-PT, calculated from subtype, proliferation level, and tumor size; categorized as low, medium, high) were quantified using RNA counting methods, with finite mixture modeling to establish a cutoff for *ESR1*-high vs. -low tumors. The relative frequency differences of clinical and genomic features of borderline vs. positive/negative tumors were estimated using linear regression, adjusted for age and race. Recurrence risk was evaluated by ER status, with and without receipt of endocrine therapy using Kaplan-Meier curves and Cox proportional hazards models.

**Results:** Of 2,859 eligible patients, 8% (n=217) were ER borderline, 26% (n=757) were ER negative and 66% were ER positive. Compared to other ER positive tumors (which were 5% basal-like, 89% luminal, 8% high ROR-PT, 16% *ESR1*-low), borderlines (40% basal-like, 42% luminal, 26% high ROR-PT, 71% *ESR1*-low) had a higher relative frequency of basal-like subtype (+37.7%, 95% CI 27.1, 48.4), high ROR-PT (+52.4%, 95% CI 36.8, 68.0), and *ESR1*-low status (+26.3%, 95% CI 20.2, 32.5). In log-rank analysis, recurrence risk in borderlines was not significantly different from ER negatives. However, after adjusting for patient and tumor factors, recurrence risk in borderlines treated without endocrine therapy was the highest of all subtypes (HR 2.9, 95% CI 1.6, 5.0) and persisted after further adjusting for ROR-PT. Results are summarized in Table 1. Frequency of high ROR-PT was 22% among white women with borderline tumors but was significantly more common among black women with borderline tumors (+26.0%, 95% CI 7.1, 45.0). Among borderlines, high grade was associated with basal-like subtype (+43.4%, 95% CI 18.7, 68.0; reference = non-basal subtype, 52% high grade).

Table 1: Recurrence-free survival by ER subtype and endocrine therapy receipt

	Hazard Ratio (HR) Model 1	HR Model 2
ER Positive	REF	REF
ER Borderline, no endocrine therapy	2.9 (1.6, 5.0)	2.3 (1.1, 4.9)
ER Borderline, endocrine therapy	1.5 (0.7, 3.2)	1.5 (0.6, 3.7)
ER Negative	1.8 (1.3, 2.6)	1.5 (0.9, 2.4)

Adjusted for (1) age, race, stage, grade, year of diagnosis, (2) variables in (1) and ROR-PT

**Conclusion:** Consistent with prior studies, borderline tumors demonstrated characteristics that were intermediate between other ER positive and negative tumors, with heterogeneity of genomic features. Given the higher recurrence risk among borderlines, identifying aggressive borderline tumors is important. Black women and those with high tumor grade may benefit from further genomic assessment to guide treatment decisions.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-09-06

Gene-set enrichment analysis (GSEA) of breast tissue from healthy women with less than six months history of breastfeeding shows enrichment in Hedgehog signaling, notch signaling and luminal progenitor gene signatures

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**Introduction:** Multiple epidemiological studies have shown that prolonged breastfeeding is associated with a reduced risk of developing triple negative/basal-like breast cancer (TN/BLBC). We have modeled abrupt involution (AI) due to lack of breastfeeding and gradual involution (GI) of the mammary gland that occurs over time upon prolonged breastfeeding in wild-type FVB/N mice and discovered prominent histological and molecular changes in the AI glands over time. Our studies revealed for the first time a clear and persistent expansion of mammary luminal progenitor (LP) epithelial cells in AI glands (AACR abstract#2242, 2018). Here, we corroborate animal studies using normal human breast tissue obtained from a reduction mammoplasty tissue collection study (OSU-2011C0094).

**Methods:** Breast tissue obtained from parous premenopausal women with no history of breast cancer who breastfed for  $\geq 6$  months (GI, n=16) versus those who breastfed for  $< 6$  months (AI, n=16) (OSU-2011C0094) was used for gene expression analysis. RNA isolated from these normal mammary tissues was analyzed using Affymatrix Gene ChIP Human Transcriptome array 2.0; Gene Set Enrichment Analysis (GSEA) was used to analyze the microarray data. Molecular Signatures Database was used in GSEA querying C2 curated gene sets, Hallmark gene sets, and Lim-Mammary-Luminal-Progenitor gene sets. H&E sections of the breast tissue were used to assess lobular type by counting number of ductules per terminal ductal lobular unit (TDLU). False discovery rate (FDR) q-values and p-values were used for multiple comparison adjustment.

**Results:** GSEA revealed that breast tissue obtained from women in the AI cohort exhibited strong positive enrichment for Notch and Hedgehog Signaling (Hhg) pathways (FDR q-value= 0.20 and 0.12, respectively). In GI women, GSEA showed an overall trend towards enrichment in metabolic pathways and immune system functions. Moreover, there was non-significant trend towards positive enrichment of mouse LP gene signature in AI women only (FDR q-value= 0.30). Age and BMI were not statistically different between AI and GI cohorts. Analysis of TDLU, the primary anatomical source of most breast cancers, revealed that breast tissue from AI women had proportionally higher lobular type 1 only epithelium than GI women who exhibited more differentiated lobular epithelium (p-value= 0.049).

**Conclusion:** We report here for the first time that mammary glands from women who breastfed  $< 6$  months were enriched for stem-cell signaling pathways and LP gene signature. This reflects some similarity to BRCA1 mutation carriers, who demonstrate expanded luminal progenitor population. In addition, higher Type 1 TDLU's are seen in breast tissue from parous women who breastfed  $< 6$  months. Together, these data demonstrate features for TN/BLBC precursors enriched in patients who breastfed for  $< 6$  months. Understanding this mechanistic link will help in developing prevention strategies, particularly for African-American women who have lower prevalence of breastfeeding and higher incidence of TN/BLBC.



# 2018 San Antonio Breast Cancer Symposium ®

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Breast cancer characteristics and outcomes in patients with TP53 germline mutation

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## Introduction

Li-Fraumeni syndrome (LFS) resulting from monoallelic germline *TP53* gene mutation is a rare hereditary cancer predisposition. Breast cancer (BC) is the most common cancer among women with *TP53* germline mutation with a risk ranging from 49% to 85% by the age of 60 years old. Most of these cancers are early onset. Few patients' cases have been reported so far in the literature. Our aim was to describe the medical history of a cohort of LFS women diagnosed with BC recruited from a single institution. The characteristics combined were genetic alteration diagnosis, tumor characteristics, treatment, outcome, and LFS associated cancers.

## Methods

We retrospectively identified breast cancer patients with *TP53* germline mutation from the Institut Curie (Paris, France) database and described their cancer characteristics and medical history.

## Results

From 1989 to 2015, 25 patients affected with BC (31 tumors) and *TP53* germline mutation carrier were identified, with a median follow up of 6.5 years. Median age at BC diagnosis was 30.5 years. All patients were women. 33% had a previously identified *TP53* mutation in their family. 70% of them had BC as their first cancer event. 60% of the patients presented with another LFS associated cancer or non-related cancers: osteosarcoma (22%), glioblastoma (18%), pulmonary carcinoma (13%), gastric linitis plastica (9%), malignant hemopathy (9%), soft tissue sarcoma (9%), adrenocortical carcinoma (4%), ovarian cystadenocarcinoma (4%), renal tumor (4%), choroid plexus carcinoma (4%).

92% of the breast tumors were ductal carcinoma (28% DCIS and 64% IDC), 7% were sarcoma (1 phyllodesarcoma, 1 pleiomorphic liposarcoma); there were no lobular carcinoma. Among the IDC, 50% were HER2 positive, 72% were hormone-receptor positive.

Most patients had a mastectomy (64%), and most of them received radiation (55%). However, when *TP53* mutation had been identified prior to the treatment, none of the patients received radiotherapy (5 patients). Most patients received chemotherapy (70%) (37% in neoadjuvant setting, 33% in adjuvant setting, 25% for metastatic setting). 40% of the patients received hormone therapy (37% as adjuvant treatment, 7% for metastatic disease)

Most of the patients did not relapse from BC (75%). Overall, only 17% of the patients had metastatic BC. To date, 12 patients of our series have died (48%), 6 patients (24%) from other LFS-associated cancers and 4 patients from BC (16%).

## Conclusion

To the best of our knowledge, this descriptive series is the largest study of tumor characteristics and medical history of LFS-women with BC, the most frequent cancer among women with *TP53* germline mutation. It confirms the higher HER2 overexpression rate observed in LFS-patients BC, as suggested in the literature and showed a high rate of DCIS at initial presentation. Most of the patients developed other LFS-associated cancers. In depth molecular analysis of these BC will be performed in order to gain insight into their biological specificities and to adapt the therapeutic management of this poor prognosis syndrome.

# 2018 San Antonio Breast Cancer Symposium ®

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Hormonal factors associated with elevation of DNA methylation age in breast tissue of healthy women

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**Background:** Healthy breast tissue appears older than matched peripheral blood, when using a biologic aging measurement based on DNA methylation markers. The underlying cause of this acceleration is not known. We hypothesize that cumulative estrogen exposure is associated with accelerated breast epigenetic aging. In this study, we examined factors associated with breast epigenetic age in a healthy population of women.

**Methods:** We used breast tissue samples from 232 healthy women donors (119 pre-menopausal, 113 post-menopausal) to the Komen Tissue Bank, with data available on variables related to cumulative estrogen exposure, including age at menarche, gravidity, parity, and menopausal status. DNA methylation experiments were performed using the Illumina EPIC 850K array platform. DNA methylation age (DNAm age) was calculated using the epigenetic clock methods developed by Horvath (2013). Total years of estrogen exposure was calculated as the difference between age at menopause (or current age) - number of live births x 9 months – number of miscarriages x 3 months. Nonparametric group testing was used to compare mean levels of the difference between DNAm age and chronologic age for pre- and post-menopausal groups. We examined the outcome “age acceleration”, calculated using the residuals of the regression of DNAm age versus chronologic age, because it is age-adjusted and independent of cell distribution. Multivariate linear regression models were used to examine for associations between age acceleration and each of our covariates.

**Results:** Our sample included women aged 19-90 years (mean age 50.7, SD 11.8), with 114 nulliparous women. We confirmed that DNAm age in breast tissue is strongly correlated with chronologic age ( $p=0.89$ ,  $p<0.0001$ ). The difference between DNAm age and chronologic age is greater at earlier ages, and is significantly greater in premenopausal women (mean 8.9 years, SE 0.04), compared with postmenopausal women (mean 2.7 years, SE 0.05) ( $p<0.0001$ ). Age acceleration was significantly associated with earlier age at menarche ( $\beta=-0.395$  for each year,  $p=0.036$ ). For women with limited total years of exposure to estrogen ( $<19$  years), there was a significant association between age acceleration and total estrogen exposure and  $\beta=0.673$  for each year,  $p=0.028$ ).

**Conclusion:** Acceleration of epigenetic age in breast tissues occurs in healthy women and is most pronounced in the pre-menopausal period. Earlier age at menarche and total years of estrogen exposure are associated with higher degree of acceleration, suggesting that cumulative estrogen exposure drives this process.

# 2018 San Antonio Breast Cancer Symposium ®

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Octogenarian breast cancer was associated with higher infiltration of M2 macrophages and tregs and worse disease free survival

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**Backgrounds:** It is known that elderly patients have worse prognosis of breast cancer and commonly the blame is on their medical comorbidities and access to care. We question this dogma and hypothesized that extreme elderly (octogenarians over 80 years old) have biologically worse cancer that can be defined by mutation load, tumor heterogeneity, and its tumor immune microenvironment.

**Patients and Methods:** Two groups; Control (patients aged 40-65), and octogenarians (age over 80) at the time of breast cancer diagnosis were compared in The Cancer Genomic Atlas (TCGA; n=1093) and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC; n=2506) cohorts. Cytolytic activity score (CYT), CIBERSORT analysis, tumor mutation load, as well as mutant-allele tumor heterogeneity (MATH) score were conducted as previously published.

**Results:** The total number of patients in the control group and octogenarians were 675 and 54 in TCGA, and 1001 and 121 in METABRIC, respectively. Octogenarians had significantly worse disease free survival in addition to overall survival in both cohorts ( $p<0.01$  in both), which suggested that they had worse cancer biology. In terms of subtypes, octogenarians had significantly higher rate of ER positive cancers than control group in both cohorts (75.3% vs 87.0%,  $p<0.01$  in TCGA, 72.9% vs 90.0%,  $p<0.01$  in METABRIC), but there was no significant difference in PgR or Her2 positivity. With regard of PAM50 classification, luminal-A and B subtypes were significantly higher in octogenarians (44.6% vs 34.7%, 31.4% vs 20.5%, respectively,  $p<0.01$ ), whereas basal (7.4% vs 11.2%) and claudin-low (2.5% vs 11.8%) subtypes were significantly lower ( $p<0.05$ ) in octogenarians in METABRIC cohort. Given that octogenarians had subtype with favorable prognosis, we examined whether they had higher mutation load or heterogeneity of the tumor. There were no significant difference in tumor mutation load and MATH score that reflect tumor heterogeneity in both cohorts. On the other hand, breast tumors of octogenarians were significantly associated with immune-suppressive cells, such as M2 type macrophages and regulatory T cells in both cohorts ( $p<0.05$ ), whereas they were negatively associated with immune-eliminating cells, such as activated memory CD4 T-cells and M1 type of macrophages in METABRIC cohort ( $p<0.05$ ). There was no significant difference in CYT in TCGA cohort.

**Conclusion:** Our result demonstrated that octogenarians breast tumors were infiltrated with more immune-suppressive cells that may contribute to their biologically worse behavior.

# 2018 San Antonio Breast Cancer Symposium ®

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Molecular and genetic characterisation of contralateral breast cancers in Northern Ireland: Opportunities for personalised surgery

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Contralateral breast cancer (CBC) incidence is reported as 0.4-0.7% per year, equating to an approximate 10-15% 20 year risk after primary cancer diagnosis. Young age at primary diagnosis and a strong family history are consistently cited as significant risk factors for CBC development. Requests for contralateral prophylactic mastectomy (CPM) are rising, despite a lack of evidence that CPM significantly improves breast cancer specific survival. Therefore, a method to stratify CBC prognosis and risk at an individual level is required to allow us to personalise surgical decision-making for individual patients.

This study aims to:

1. Explore prognostic factors following CBC diagnosis;
2. Investigate metastatic CBC prevalence by determining clonal relationships between primary and CBC tumours;
3. Assess the rate of germline predisposition gene mutations in a large cohort of Northern Irish CBC cases.

A cohort of 403 CBC patients and 1:1 matched unilateral breast cancer controls (matched for age, year of diagnosis, nodal positivity and tumour morphology) were identified from the Northern Ireland Cancer Registry. Archival primary, CBC and normal lymph node tissue from these CBC patients were obtained for somatic and germline sequencing of a custom 94-gene panel, comprising genes most commonly mutated in breast cancer and all known predisposition genes.

CBC patients had a significantly increased risk of breast cancer specific mortality when compared with 1:1 matched unilateral breast cancer controls (HR 6.45, CI 4.27-9.77). Within the CBC cohort, a shorter time interval between primary and CBC diagnosis is associated with increased breast cancer mortality, whereby women with <5 years between diagnoses have the poorest survival (p=0.001). In terms of receptor status, the PR status of ER positive primary and CBC tumours differed significantly (p<0.001). 89.1% of ER positive primary tumours were PR positive, whilst PR positivity was only 54.4% in ER positive CBCs. Indeed, 36.6% of patients with an ER+/PR+ primary BC developed an ER+/PR- CBC, after exposure to anti-oestrogen therapy.

Targeted next generation sequencing of 42 cases to date has provided evidence of shared somatic mutations suggestive of metastatic clonality between primary and CBC in four cases (9.5%). Germline pathogenic predisposition gene mutations were identified in five (11.9%) patients and a further ten (23.8%) patients were noted to have germline predisposition variants of unknown significance.

Breast cancer specific mortality risk was significantly higher in CBC patients compared with matched unilateral cancer controls, in excess of what would be expected from two primary cancer events. This finding suggests that there are as yet unquantified factors contributing to this excess hazard. Poorer outcome with shorter inter-tumour diagnostic intervals, in addition to evidence of primary-CBC clonality in some cases, suggests that metastatic CBC may play a role in excess risk. Additionally, the significant reduction in PR positivity of ER positive CBC tumours after ER+/PR+ primary tumours, could suggest that acquired hormonal resistance following primary anti-oestrogen therapy exposure may contribute to this additional mortality hazard.

# 2018 San Antonio Breast Cancer Symposium ®

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Prognostication of genetic alterations of ESR 1 in estrogen receptor positive metastatic breast cancers using targeted ultra-deep sequencing data analysis

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**Introduction:** Genetic alteration of Estrogen Receptor 1(ESR1) gene have been associated with acquired endocrine resistance and occurred in about 20% of endocrine resistant estrogen receptor(ER)-positive metastatic breast cancer(MBC). Mutations in ligand binding domain of ESR1 lead to constitutive activity of the ER without ligand estrogen and stimulated down stream cell growth signal. Therefore, ESR1 ligand binding domain alteration is known resistant mechanism of aromatase inhibitor. Among these ESR1 mutations, Y537S, one of the ligand binding domain mutations, caused ER antagonist, fulvestrant resistance. Therefore, assessment of ESR1 mutation in ER-positive MBC had significant benefit to further precision medicine for MBCs. In this study, we explored to identify the frequency and type of ESR1 genetic alterations of ER-positive MBC.

**Methods:** We performed targeted ultra-deep sequencing (CancerSCAN™) using BC tissue specimens. This sequencing was covered entire coding area of ESR1 gene and also detected copy number alteration and translocation of ESR1.

**Results:** Targeted ultra-deep sequencing of ESR1 was performed using 990 BC tissues. Of 990 tissue samples, 341(34.5%) were MBCs. Of MBCs, 112(11.3%) were ER-positive and human epidermal growth factor receptor 2(HER2)-negative BCs. In ER-positive HER2-negative MBCs (N=112), 21 ESR1 genetic alterations were identified in 19 BCs (17.0%). Nineteen were single nucleotide variants (SNVs) and three were copy number (CN) amplification. Most commonly detected single nucleotide variant (SNV) was D538G (6 of 19, 31.6%) followed by Y537N, Y537S, V382I (4, 2 and 2 cases, respectively). Three mutations occurred in non-ligand binding domain (G415V, V392I and P79A). Two BC samples harbored two ESR1 mutations, respectively (Y537S and D538G, L536P and Y537N). In terms of treatment, 11 of 12 patients with ER-positive MBC harboring ESR1 mutation received palliative endocrine therapies. Eight patients received aromatase inhibitor and two patients received tamoxifen. One patient received letrozole plus palbociclib. In 2 MBCs with Y537S mutation, progression free survival (PFS) of endocrine therapy was 1.4 and 5.3 months. MBCs with D538G had 12.3months of PFS (range, 5.3-23.7(months)) and BCs harboring another ligand binding domain mutations (Y537N, L536H and L536P) had 15.7months of PFS of endocrine therapy (range, 8.4-17.3(months)). BC with mutation observed in non-ligand binding domain had short PFS (1.8 (V392I) and 2.7 (P79A) months, respectively). In terms of ESR1 CN amplification, patients could not receive endocrine therapy because their BCs rapidly progressed and extensive distant metastases were occurred within 3 months after curative surgery.

**Conclusion:** In this exploratory study, ESR1 genetic alterations were detected in about 20% of ER-positive MBC. The type of genetic alterations varied including SNVs, CNAs. Each locus of ESR1 mutation predicted endocrine resistance. In addition, we might suggest that ESR1 CN amplification is prognostic marker of ER-positive BCs.

# 2018 San Antonio Breast Cancer Symposium ®

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TP53 expression in relation to clinical and etiologic factors in breast cancer subtypes

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*TP53* is a well-known tumor suppressor gene and mutations in *TP53* are the most frequent genomic event in most cancers including breast cancer. Recent studies have shown that the frequency, spectrum, timing, and clinical implications of *TP53* mutations varied in different molecular subtypes of breast cancer. For example, the frequency of *TP53* mutations is the highest in basal-like subtype and lowest in luminal A tumors. However, the evaluation of TP53 protein expression, as a surrogate for *TP53* mutations, in large studies in the context of tumor subtypes is limited. In addition, the etiologic relevance of TP53 expression is yet to be investigated. The goal of this study is to evaluate the association of clinical and breast cancer risk factors with TP53 expression, measured using immunohistochemistry (IHC), in breast cancer molecular subtypes. The analysis included 7,226 women with invasive breast cancer who were diagnosed and treated in a tertiary hospital in Beijing, China. Subtypes were defined as Luminal A: ER+ and PR+, HER2–, and low grade (grades 1 or 2); luminal B/HER2–: ER+ and/or PR+, HER2–, and high grade (grade 3); luminal B/HER2+: ER+ and/or PR+, HER2+ (regardless of grade); HER2-enriched: ER–, PR–, and HER2+; Triple-negative (TN): ER–, PR–, and HER2–. As expected, positive TP53 staining showed the lowest frequency in the luminal A (46%) and highest in the TN (61%) and HER2-enriched (63%) subtypes (P-value <0.001). Overall and particularly in luminal A patients, positive TP53 staining was associated with higher frequencies of aggressive tumor features such as higher grade, larger tumor size, higher proliferative index, and EGFR expression. Compared with TP53– patients, TP53+ patients were more likely to have younger ages at onset and increased parity, but these associations were largely driven by the luminal A subtype [OR (95% CI) vs nulliparity = 2.67 (1.59, 4.51); 2.63 (1.52, 4.55); 3.68 (2.01, 6.72) for 1, 2, and ≥3 children, respectively (P-trend = 0.006)]. Luminal A/TP53+ patients were also more likely to have breastfed [OR (95% CI) ever vs never = 1.38 (1.03, 1.85)] than luminal A/TP53– patients. These findings suggest that TP53 IHC staining might be used to further refine the classification of luminal A breast cancer into subgroups with distinct clinical and etiologic relevance.

# 2018 San Antonio Breast Cancer Symposium ®

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A real world evidence study of *BRCA* mutations and survival in HER2-negative breast cancer

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**Background:** Limited data exist on the natural history (treated with standard of care) of metastatic breast cancer (mBC) characterized by germline breast cancer susceptibility gene mutations (*gBRCAm*). Real-world data examining survival for patients with *gBRCAm* mBC, overall and separated into *gBRCA1m* and *gBRCA2m*, compared to *gBRCA* wild type (wt) mBC, can help to clarify the prognostic outlook associated with the *gBRCA* mutation.

**Methods:** Adults with human epidermal growth factor receptor 2 negative (HER2-) mBC diagnosed from January 2013 – August 2017 were retrospectively selected from the Flatiron Health Oncology electronic medical record database. Patients were classified as having *gBRCA1m*, *gBRCA2m*, or *gBRCAwt* disease. Those who did not receive the genetic testing or who had equivocal results were classified as *gBRCA* unknown. Overall survival (OS) was calculated from first diagnosis of mBC, as well as from the start of first- and second-line therapy for metastatic disease. Lines of therapy included both hormonal and systemic therapies. Kaplan-Meier analyses provided median OS with 95% confidence interval (CI). Unadjusted log-rank tests compared OS between *gBRCA1m* and *gBRCA2m*, and between overall *gBRCAm* and *gBRCAwt*.

**Results:** Of 8,080 patients selected, mean age at first mBC diagnosis was 64 years, 98.7% were female, and 82.0% had evidence of hormone receptor positive disease. *gBRCA* status was known for 1,852 (22.9%) of patients, of whom 89 (4.8%) had *gBRCA1m*, 152 (8.2%) had *gBRCA2m*, and 8 (0.4%) had both *gBRCA* mutations. Patients with known *gBRCA* status were younger, with mean ages of 52 years for *gBRCAm*, 55 years for *gBRCAwt*, and 67 years for *gBRCA* unknown. Hormone receptor positive disease was less common among those with known *gBRCA* status (71.9%, 77.2%, and 83.6% for *gBRCAm*, *gBRCAwt*, and *gBRCA* unknown, respectively). Median (95% CI) OS from mBC diagnosis was 22 (14 - 26) months for *gBRCA1m* and 30 (27 - 37) months for *gBRCA2m* ( $p = 0.01$ ), though numbers were quite small by the median timepoint. Overall *gBRCAm* disease was associated with median survival of 28 (25 - 32) months, compared to 32 (30 - 35) months for *gBRCAwt* ( $p = 0.07$ ); survival was similar between groups for the first 24 months but declined thereafter in the *gBRCAm* group. Similar patterns were observed for OS after the start of first- and second-line therapy, although no comparisons were significant. Further analyses will present adjusted results and comparisons with outcomes for the patients with *gBRCA* unknown.

**Conclusions:** This real-world study of patients receiving care in largely community oncology clinics suggests that survival after diagnosis of mBC is reduced in patients with *gBRCA1m* compared to *gBRCA2m* disease and may be reduced in *gBRCAm* mBC overall. Effective treatments targeted for the *gBRCAm* subtypes of mBC appear to be needed.

# 2018 San Antonio Breast Cancer Symposium ®

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Demographic, clinical/disease characteristics, and treatment of patients with germline mutated metastatic breast cancer: A CancerLinQ study

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**Objective:** To describe the demographics, clinical/disease characteristics and treatment patterns of patients with germline BRCA mutated (gBRCAm) metastatic breast cancer (mBC) as compared to those with gBRCA wild type (wt) and those who are untested for gBRCA mutations.

**Methods:** The CancerlinQ Discovery Database (CLQ), launched by the American Society of Clinical Oncology (ASCO) in 2016, consists of longitudinal, demographic and geographic diverse data aggregated from oncology practice electronic health record (EHR) databases. Natural language processing and technology-enabled curation are utilized to identify records of most interest, followed by manual curation to abstract information from unstructured EHR fields. This cohort consists of 7,889 patients diagnosed with mBC between 1982 and 2018, and is enriched for patients with gBRCA testing through the curation process.

**Results:** Overall most patients were female (99.0%), white (55.3%), and the median age at mBC diagnosis was 63 years (yrs). The majority were not tested for gBRCA mutation (88.4%); 2.0% were gBRCAm, 9.2% were gBRCAwt, 0.4% had an undetermined test result, and 0.1% had a variant of unknown significance. Among those tested for estrogen-receptor (ER) (n=6,700) and progesterone-receptor (PR) (n=6,737) status, 76.6% were ER positive (+) and 62.2% were PR+. Among those tested for human epidermal growth factor receptor 2 (HER2) (n=6,696), 21.7% were HER2+. Among those with known results for ER, PR, and HER2 (n=6,063), 10.1% were hormone receptor (HR)+/HER2+, 10.6% were HR negative (-)/HER2+, 65.0% were HR+/HER2-, and 14.3% were HR-/HER2-. The median age at mBC diagnosis was 50 yrs for gBRCAm, 51 yrs for gBRCAwt and 64 yrs among the gBRCA untested group. A similar proportion of patients were diagnosed with metastatic disease among the gBRCAm and gBRCAwt groups (25.3% and 20.3%, respectively), while the proportion was higher among the gBRCA untested group (38.0 %). The most common site of metastasis for all groups was bone (35.1% for gBRCAm, 41.7% for gBRCAwt, and 36.5 % for gBRCA untested), followed by liver (16.2% for gBRCAm, 13.3% for gBRCAwt, and 8.5% for gBRCA untested). The most common first-line therapies for gBRCAm patients were tamoxifen (7.7%), letrozole (7.0%), cyclophosphamide+doxorubicin (6.3%), and paclitaxel (6.3%); for gBRCAwt patients they were cyclophosphamide+doxorubicin (6.6%), paclitaxel (6.4%), and tamoxifen (6.4%); and for gBRCA untested patients they were letrozole (11.1%), fulvestrant (9.3%), and tamoxifen (4.6%). The mean number of lines of therapy (including lines of chemotherapy and hormone therapy) were 3.5, 3.8, and 3.4 for the gBRCAm, gBRCAwt, and gBRCA untested groups, respectively.

**Conclusions:** Patients with gBRCAm were younger than the gBRCA untested group, and more patients had metastatic disease at diagnosis in the gBRCA untested group. Further analyses accounting for HR and HER2 status will be conducted and presented. This is the first example of research using curated breast cancer data from ASCO's CLQ.



## 2018 San Antonio Breast Cancer Symposium ®

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DNA methylation markers influenced by BMI are associated with breast cancer

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Body mass index (BMI) is a well-established risk factor for breast cancer. However, the underlying molecular mechanism through which BMI acts on breast cancer remain largely unknown. DNA methylation plays a critical role in regulating gene expression. Aberrant DNA methylation has been implicated in breast cancer development. We hypothesize that BMI influences DNA methylation that in turn leads to breast cancer. We therefore conducted a molecular epidemiological study to identify DNA methylation markers that are associated with BMI in healthy women and further determine the association of these DNA methylation changes with breast cancer development and occurrence. We analyzed 270 normal breast tissue from healthy women and 109 tumor tissue from breast cancer patients for genome-wide DNA methylation profiling using the Illumina TruSeq® Methyl Capture EPIC sequencing technology. After data quality control, approximately 3 million CpG sites (sequencing depth  $\geq 10$ ) were retained and included in further statistical analysis. Linear regression was performed to examine the association between BMI and each of CpG sites, adjusting for age and race. Bonferroni correction was used for control for multiple comparisons. We further investigated whether these BMI-associated DNA methylation markers were differentially methylated in normal and breast tissue. We found that 30 CpG sites were significantly associated with BMI ( $P < 1.5 \times 10^{-8}$ ) in normal breast tissue. Many of these CpG sites were also differentially methylated in tumor and normal breast tissue. Top CpG hits are near novel and putative cancer genes mainly involved in lipid metabolism and related reproductive phenotypes that increase breast cancer risk, including KIAA0232, SHZ2, LACTB, PAPP, and DLGAP2. The most significant CpG site associated with BMI is on chr. 4 in KIAA0232 gene. Little is known about the function of this gene, but genetic variant near the gene are associated with platelet count and volume in GWAS. The second most significant hit is on chr.20 in gene TSHZ2. This gene is found downregulated in breast and prostate cancer. LACTB gene is a tumor suppressor that modulates lipid metabolism and cell state. Its mechanism of action involves alteration of mitochondrial lipid metabolism and differentiation of breast cancer cells. PAPP gene plays a role in inflammation and promote invasion of cancer cells. DLGAP2 is an imprinted gene, and SNPs near DLGAP2 are associated with age at menarche in AA women. Our results suggest DNA methylation may intermediate the observed association between BMI and breast cancer. Further research is warranted to validate these findings in larger studies as well as to understand the regulatory network by linking data with gene expression.

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Defining the spectrum of germline variants among African American patients with triple negative breast cancer

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**Background:** African American (AA) women are more likely to have breast cancer at a younger age and be diagnosed with triple negative breast cancer (TNBC), which is as yet unexplained. We examined results of multi-gene panel testing in AA women with TNBC tested at a large commercial laboratory to assess the utility of gene panels and findings in this group.

**Methods:** We assessed individuals who had clinical hereditary cancer testing with a multi-gene panel between September 2013 and May 2018. Women were included for analysis if they had a personal history of TNBC and self-identified as having any AA ancestry (n=3,268) or only Caucasian (CA) ancestry (n=8,953). Clinical data was collected from provider-completed test request forms. Comparisons were performed using descriptive statistics, t-tests (continuous variables), and chi-square tests (categorical variables) adjusting for multiple testing when necessary.

**Results:** In this cohort, AA women were significantly more likely than CA women to meet NCCN guidelines (97.5% vs. 96.6%, p=0.010) and significantly less likely to have an additional personal (16.2% vs. 21.8%, p<0.001) or family (79.3% vs. 86.3%, p<0.001) history of cancer. Overall, 11.5% of AA women were found to carry a pathogenic variant (PV) compared to 13.4% of CA women (p=0.004; Table 1). Compared to CA women, AA women with a PV were significantly younger at diagnosis (46.7 vs. 49.5 years of age; p<0.001). The prevalence of PVs in *BRCA1*, *CHEK2* and the Lynch syndrome genes was higher in CA women, whereas the prevalence of *BRCA2* PVs was higher in AA women. While the prevalence of PVs in individual genes was not significantly different according to ancestry after adjusting for multiple comparisons, AA women were significantly less likely to have a PV in any breast cancer-related gene compared to CA women (p=0.048). AA women were significantly more likely to have a Variant of Uncertain Significance (VUS; 35.6% vs. 20.9%; p<0.001) and to have >1 VUS (8.6% vs. 2.6%, p<0.001). Regardless of ancestry, patients diagnosed before age 40 were more likely to carry a PV (19.7% AA, 22.2% CA). However, the prevalence of PVs among patients diagnosed after age 60 was still striking (8.9% AA, 10.9% CA) and was similar to the PV prevalence among patients diagnosed between 40-60 (10.1% AA, 12.3% CA).

**Conclusions:** In the era of multi-gene panel testing, this large cohort of patients with TNBC supports the use of panel testing in AA women with TNBC regardless of age or additional personal/family history of cancer. While additional research to the rate and pathogenicity of VUS in AA women is needed, genetic counseling is necessary to explain the possibility and meaning of a VUS in this group.

[table1]

	AA Women	CA Women
Gene	N (%)	N (%)
Any Breast Cancer-Related Gene	347 (10.6)	1104 (12.3)
<i>BRCA1</i>	132 (4.0)	496 (5.5)
<i>BRCA2</i>	97 (3.0)	236 (2.6)
<i>ATM</i>	6 (0.2)	25 (0.3)
<i>BARD1</i>	19 (0.6)	67 (0.7)
<i>BRIP1</i>	20 (0.6)	46 (0.5)
<i>CDH1</i>	1 (<0.1)	1 (<0.1)
<i>CHEK2</i>	2 (0.1)	33 (0.4)
<i>NBN</i>	2 (0.1)	10 (0.1)
<i>PALB2</i>	44 (1.3)	138 (1.5)

<i>PTEN</i>	2 (0.1)	4 (<0.1)
<i>RAD51C</i>	20 (0.6)	41 (0.5)
<i>STK11</i>	0	1 (<0.1)
<i>TP53</i>	2 (0.1)	6 (0.1)
Lynch Syndrome Genes	10 (0.3)	46 (0.5)
Other Genes	12 (0.4)	24 (0.3)
Multiple PVs	6 (0.2)	28 (0.3)
Total (Any Gene)	375 (11.5)	1202 (13.4)

**Table 1.** Distribution of PVs in BC-related genes according to ancestry

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Type II diabetes and subtype-specific breast cancer risk in medically underserved black and white women

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**Background:** Type II diabetes (T2D) has been found to modestly (10-20%) increase risk for breast cancer (BC) in mostly white populations. Some studies indicate that this association varies by BC receptor subtype, suggesting that T2D increases risk specifically for the more aggressive estrogen-receptor (ER)-negative and triple negative subtypes. While black women are more likely than white women to develop both T2D and ER-negative BC, no studies to date have explicitly focused on subtype-specific risk by race. Further, no studies have examined this association in women from medically underserved areas, where the prevalence and severity of T2D is greater than in areas with adequate resources.

**Methods:** Participants were women from the Southern Community Cohort Study (2002-2009), which prospectively recruited individuals age 40-79 years, primarily from community health centers in medically underserved areas across the Southeastern US. We identified 39,687 women who were cancer-free at baseline and self-reported black or white race. Baseline T2D status was defined as a self-reported diagnosis of medication-treated T2D at age  $\geq 30$  years. BC incidence and receptor status were ascertained by state cancer registry linkages and pathology reports. Cox proportional hazards regression was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for BC (overall and by subtype) associated with T2D. Models were first adjusted for, and then stratified by race. Exploratory analyses modeled self-reported diabetes medication adherence (taking as directed all the time or most of the time vs. sometimes, rarely or never) as an exposure.

**Results:** Among 39,687 women (29.9% white and 70.1% black), 7501 (18.9%) reported having T2D. Over a median of 10.3 years of follow-up, 929 incident cases of invasive BC occurred; 224 (24.1%) were ER-negative, and among those with complete receptor data (n=808), 138 (17.1%) were triple-negative. Women with T2D were significantly more likely to develop BC (HR: 1.19, 95% CI: 1.02-1.40) compared to non-diabetics in models adjusted for age, race, BMI, mammography screening, menopausal status, alcohol use, and family history of BC. In subtype-specific analyses, the risk associated with T2D was greatest for the ER-negative (HR: 1.45, 95% CI: 1.01-2.08) and triple-negative (HR: 1.53, 95% CI: 1.01-2.31) subtypes. Race-stratified models showed similar elevations in risk among black (HR: 1.16, 95% CI: 0.97-1.39) and white women (HR: 1.13, 95% CI: 0.83-1.53) for BC overall, and for triple-negative BC, with a HR of 1.61 (1.02-2.53) in blacks and 1.94 (0.74-5.07) in whites. Across both races and by subtype, women reporting lack of adherence to diabetes medications had far greater risk for BC compared to non-diabetics, with an overall HR of 2.15 (1.27-3.66).

**Conclusion:** In this study, T2D was associated with a 19% increased risk of BC and 53% increased risk for the triple-negative subtype, with similar results for black and white women. The risk was notably greater in women who reported lack of T2D treatment adherence. Given these findings and the prevalence of T2D in medically underserved areas, interventions aimed at T2D prevention and adherence to medications in all races could help reduce the BC burden in this population.

# 2018 San Antonio Breast Cancer Symposium ®

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Real-world effectiveness outcomes by race in patients with metastatic triple negative breast cancer

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**Background:** Age, race, and tumor environment are major contributors in the variance of treatment outcomes in patients (pts) with breast cancer. Few studies have evaluated outcomes in real-world pts to identify high-risk populations. We aimed to compare differences in effectiveness outcomes by race among pts with metastatic triple negative breast cancer (mTNBC).

**Methods:** This retrospective observational study evaluated effectiveness outcomes of progression-free survival (PFS) and overall survival (OS). Eligible pts were female, age  $\geq 18$  years, and diagnosed with mTNBC between 1/1/2010 and 1/31/2016 from 9 US community oncology practices. The sample was stratified by race (White, African-American [AA], Other). Kaplan-Meier methods were used to describe time-to-event outcomes. Cox regression models were used to examine the effect of race-based groups on PFS and OS.

**Results:** The study included 608 pts (60.2% White, 33.9% AA, 5.9% Other; mean [standard deviation] age 57.5 [13.5] years). Pt characteristics largely did not differ across groups, except AA pts appeared younger (AA: 55.3 years vs. White: 58.6 vs. Other: 58.6,  $p=0.015$ ) and less likely to have brain metastasis (AA: 8.3% vs. White: 15.0% vs. Other: 19.4%  $p=0.034$ ). 505 pts (83.1%) received anti-cancer treatment (treated) following mTNBC diagnosis, while 103 pts (16.9%) did not receive anti-cancer treatment (untreated). The prognosis for untreated pts who did not receive anti-cancer therapy was poor, with a median OS of 4.8 months from mTNBC diagnosis compared to 12.8 months for treated pts ( $p=0.030$ ). The proportion of treated pts did not differ by race (AA: 85.0% vs. White 82.5% vs. Other: 77.8%,  $p=0.518$ ). Treatment class also did not differ by race ( $p=0.861$ ), with Taxane therapy being the most common treatment. Among treated pts, median PFS and median OS from the start of first line treatment were 4.2 months and 12.0 months, respectively (Table 1). AA pts had a poorer prognosis than White pts as they were more likely to have a PFS event and also more likely to have an OS event compared to White pts (Hazard Ratio [HR]: 1.24,  $p=0.042$ ; HR: 1.35,  $p=0.006$ ; respectively).

**Conclusions:** This evaluation of effectiveness outcomes indicates high unmet need as 1 in 6 mTNBC pts did not receive anti-cancer treatment. Furthermore, AA race was associated with poor outcomes. AA pts had shorter PFS and OS than other pts, and were more likely to experience progression or death than White pts.; despite being younger and less likely to have brain metastasis, which are typically associated with better outcomes. Future studies should be conducted to address unmet need and health outcome disparities in high-risk populations.

Table 1. Effectiveness Outcomes by Race among Treated Pts

	White	AA	Other	Overall	p-value*
PFS, # of events/# of pts	267/302	163/175	26/28	456/505	0.193
PFS, Median (95% CI of Median)	4.37 (3.91-5.06)	3.78 (3.12-4.34)	4.21 (2.24-6.12)	4.18 (3.72-4.57)	
OS, # of events/# of pts	235/302	155/175	20/28	410/505	0.004
OS, Median (95% CI of Median)	13.74 (12.03-16.50)	9.34 (7.69-10.95)	10.42 (6.58-19.89)	11.97 (10.26-13.38)	

AA African-American; CI confidence interval; PFS progression-free survival; OS overall survival; \*p-value was derived using log rank  $\chi^2$  to evaluate differences across race groups

# 2018 San Antonio Breast Cancer Symposium ®

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Racial and ethnic differences in weight gain during and after chemotherapy among women with early-stage breast cancer

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## Objective

Clinically significant weight gain of  $\geq 5\%$  from baseline has been commonly reported during chemotherapy treatment for early stage breast cancer and persisting after completion. Based on the known poorer outcomes associated with weight gain after a breast cancer diagnosis, we evaluated differential weight gain by race/ethnicity as a potential explanation for disparities in breast cancer clinical outcomes among racial/ethnic minorities compared to non-Hispanic white women.

## Methods

We conducted a retrospective cohort study among women diagnosed with stage I-III breast cancer between 2007 and 2016, who received chemotherapy at Columbia University Medical Center (CUMC) in New York, NY. We extracted data on demographics, clinical characteristics, chemotherapy regimens, and height/weight from the electronic health records. Our main exposure variable of interest was race/ethnicity. The outcome variable was dichotomized as  $\geq 5\%$  weight gain or stable weight (defined as  $< 5\%$  weight gain or loss/ $\geq 5\%$  weight loss) from baseline at 3, 6, 12, and 18 months after initiating chemotherapy. We used multinomial logistic regression analyses to determine the association between race/ethnicity and weight gain before and after adjusting for confounders.

## Results

Among 789 evaluable women, median age was 55 years (range, 19-92) and the study cohort was racially/ethnically diverse: 39.8% non-Hispanic white, 30.4% Hispanic, 18.0% non-Hispanic black, 10.4% Asian, and 1.4% other. Mean baseline body mass index (BMI) was highest among black women ( $30.7 \text{ kg/m}^2 \pm 7.0$ ), followed by Hispanic ( $29.4 \text{ kg/m}^2 \pm 5.2$ ), non-Hispanic white ( $27.9 \text{ kg/m}^2 \pm 6.9$ ), and Asian ( $25.5 \text{ kg/m}^2 \pm 5.4$ ) women. The proportion of women with  $\geq 5\%$  weight gain increased over time with 13.6% at 3 months, 15.2% at 6 months, 19.0% at 12 months, and 23.6% at 18 months. Compared to non-Hispanic whites, Asian women had a 63% lower odds of  $\geq 5\%$  weight gain (95% Confidence Interval [CI]: 0.15-0.92) at 3 months after initiating chemotherapy. No statistically significant associations were found between other racial/ethnic groups and  $\geq 5\%$  weight gain. Factors associated with weight gain after chemotherapy included younger age at diagnosis, lower baseline BMI, longer duration of chemotherapy, and having Medicaid insurance coverage.

## Conclusions

Race/ethnicity was not significantly associated with weight gain after chemotherapy among women with early stage breast cancer. Socioeconomic status (SES) rather than race/ethnicity may be a contributing factor in disparities in weight gain and breast cancer clinical outcomes. Future weight loss programs should target younger, pre-menopausal women and those with lower SES.

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Analysis of genetic mutation in ethnically diverse population with Breast and ovarian cancer: Single institution experience

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Only 5-10% of all breast and ovarian cancers have been attributed to inherited mutation. In unselected breast cancer patients, prevalence of germline BRCA 1 & 2 is about 2%. In Ashkenazi Jewish population the prevalence is estimated to be 11.7%. The rate of mutations in other populations has not been well characterized. The purpose of this study is to identify and characterize the risk of genetic mutations in an ethnically diverse population referred for genetic testing according to NCCN guidelines.

## **Method:**

In this single institution retrospective study, we analyzed 782 patients referred for genetic testing between 2009-2018. Information regarding reason for referral, ethnicity, cancer history and results of genetic mutation using multigene panels were collected.

## **Results:**

Reasons for referral were:

- Family History of cancer (142; 18.6%),
- Breast cancer diagnosed at early age (207; 26.4%),
- Triple negative breast cancer (12 ;53%
- Ovarian cancer (61; 7.8%)
- Multiple cancer (32; 4.09%)
- Known BRCA 1 or 2 mutation (51;6.5%)
- Known other mutation (9;1.15%)
- Family h/o cancer but not diagnosed with cancer (268)

## **Ethnic distribution:**

- 328 Hispanic (41.94%)
- 177 Caucasian (22.6%),
- 27 Chinese, (3.4%)
- 52 Vietnamese (6.6%)
- 86 other- Asian (11%)
- 32 Middle eastern (4.1%),
- 14 South Asian (1.8%)
- 35 African American (4.5%)
- 8 Ashkenazi Jewish (1%)
- 19 Mixed Hispanic (2.1%)

**Genetic testing was done in 545 /782 patients.**

**Deleterious Mutation detected: 88 patients (16.5% of tested)**

- BRCA 1: 32(5.8%)
- BRCA 2:28 (5.14%)
- MSH2:5(0.9%)
- p53:39 (0.5%)
- PLB2:3 (0.38%)
- MUTYH:3 (0.38%)
- CDH1 :2 (0.26%)
- PMS2: 2 ( 0.26%)
- ATM: 1(0.13%)
- CCKn2A: 1(0.13%)
- CHEK 2: 1(0.13)
- MET:1(0.13%)
- MSH6:1 (0.13%)
- RAD50 :1(0.13%)

- RET1;1(0.13%)

**BRCA1/BRCA2 mutation: 60**

**Mutation in BRCA 1/ 2 distribution by ethnicity:**

- Hispanic (28; 8.54 %),
- Chinese (4;14.8%)
- Vietnamese (4; 7.7%)
- other Asian (12;13.9%)
- African American (3, 8.5, %)
- South Asian (0 %)
- Caucasian (8; 4.5 %)
- All Asian (20/165: 12.1%)

**Mutation in other gene by Ethnicity: total 26**

Hispanic (16, 4.5%), Caucasian: (3, 1.7%) Asian other than Chinese and Vietnamese (4, 4.65%)

**Mutation in Breast cancer:** 333 Breast cancer patients tested, 39 patients (11.17%) were found to have mutations.

10/32 triple negative (31%) 4/49 her2neu positive patients (8.1%) and 15/252 (5.9%) ER+ patients found to have mutations.

17/39 breast cancer patients with mutation had prophylactic BSO.

**Mutation in BRCA 1& 2 by reason for referral:**

Known family h/o of BRCA1 or 2 mutations (18; 39.5%)

Early age of cancer (10, 7.4%)

Cancer with positive family history (17, 8.2%)

Triple negative (2, 16%).

**Variant of Unknown significance (VUS): 140 patients (25 %)**

VUS in BRCA 2 were most common. VUS were found in 17% of Hispanic and African American patients.

No significant difference in PFS and OS was found in patient with mutation and without mutation

**Conclusion:**

We found much higher rates (16.5%) than previously reported mutation detection in this very highly diverse patient population.

Rate of BRCA 1&2 mutation was 8.55% in Hispanic and 12.1% in Asian patients compared with 4.5% in Caucasian patient when selected for high risk factors.

We Also noted very high rate of VUS especially in Hispanic and African American patients.



# 2018 San Antonio Breast Cancer Symposium ®

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High participation of African-American women veterans in high risk breast cancer screening pilot program

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**SPECIFIC AIMS:** To assess breast cancer risk in Women Veterans.

**BACKGROUND/RATIONALE:** Women are the fastest growing segment of patients numbering over 2 million and breast cancer incidence has more than tripled from 1995 to 2012 within the Veterans Health Administration (VHA). Preliminary data suggest that Women Veterans may be at an increased breast cancer risk based on unique service-related exposures (e.g., burn pits, depleted uranium) and Post Traumatic Stress Disorder (PTSD). Historically, breast cancer rates among African Americans (AA) are lower than those seen in the general population (8.2-13.3%).

**METHODS:** A pilot study was conducted at Bronx, NY and Washington, DC Veterans Affairs Medical Centers. Participants were enrolled at their regular visit to Women's Health Clinics or at breast cancer education and awareness events. 5-year and lifetime risks of developing invasive breast cancer were calculated using the Gail Breast Cancer Risk Assessment Tool (BCRAT). Demographics, PTSD status, eligibility for chemoprevention and genetic counseling using the Breast Cancer Genetics Referral Screening Tool (B-RST), were also determined.

**ELIGIBILITY CRITERIA:** Women Veterans age  $\geq 35$  years with no personal history of breast cancer.

**RESULTS:** A total of 99 Women Veterans with an average age of 54 years participated between 2015 and 2018; 60% African American (AA); 13% Hispanic; 14% non-Hispanic White and 13% as other. In total, 35% (35/99) were considered high risk with a 5-year BCRAT of  $>1.66\%$  and of these, 51% were AA; 14% Hispanic and 17 % were other. Prior breast biopsies were performed in only 22% (22/99) of our entire Veteran population; 57% (56/99) having a family history positive for breast cancer. Comparatively, in our high risk AA alone, 33% (6/18) had prior breast biopsies with 94% (17/18) having a positive family history. High risk patients were referred for chemoprevention; 5 (19%) accepted; and 13/35 (37%) patients were referred for genetic counseling. PTSD was present in 29% overall and in 31% of the high risk subgroup.

**IMPLICATIONS:** The VHA, which leads the nation in mammogram compliance, is an untapped potential resource for the study of breast cancer. To our knowledge, this is the only study with 60% AA Women Veterans. High participation rates among AA in this pilot study have uncovered the potential for further inquiry into this population, which is otherwise dramatically under-represented in research. Over half (51%) of AA in this pilot study had a high risk score, likely linked to a high rate of prior breast biopsies and family history. Limitations of this study include the small sample size, exclusively urban population, geography and self-selection for screening due to a higher level of concern for breast cancer. If Women Veterans are at higher risk of breast cancer, it has been assumed that this would be related to service-related exposures, PTSD and other factors. However this pilot study, which was not designed or powered to do more than raise questions, suggests the additional possibility of an innate or genetic risk, especially in AAs. Future directions include the evaluation of genetic and molecular mutations in high risk AA Women Veterans, possibly even a role for PTSD epigenetic changes and tumorigenesis.

# 2018 San Antonio Breast Cancer Symposium ®

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Oral care evaluation to prevent oral mucositis in estrogen receptor positive metastatic breast cancer patients treated with everolimus (Oral Care-BC): A randomized controlled phase III trial

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## Introduction

Oral mucositis is a clinically significant complication of mucotoxic cancer therapy. The incidence of oral mucositis (any grade) as an adverse drug reaction of everolimus is 58%, while an analysis of Asian people has reported its occurrence as 81%. This study hypothesizes that the occurrence of oral mucositis will reduce with professional oral care (POC) administered prior to everolimus treatment.

## Method:

This was a randomized, multi-center, open-label, phase III study, to evaluate the efficacy of POC in preventing mucositis induced by everolimus in postmenopausal, estrogen receptor (ER)-positive, metastatic breast cancer patients. Patients were randomized into POC and control groups (1:1 ratio). All patients received everolimus with exemestane and continued the everolimus until disease progression. In the POC group, patients were subjected to teeth surface cleaning, scaling and tongue cleaning, before initiating everolimus, and continued to receive weekly POC from dentist or oral surgeons throughout the 8 weeks of treatment. In the control group, patients brushed their own teeth and gargled with 0.9% sodium chloride solution or water. The primary end-point was to measure the incidence of all grades of oral mucositis. Target accrual was 200 patients with a 2-sided type I error rate of 5% and 80% power to detect 25% risk reduction. This trial has been registered at ClinicalTrials.gov, number NCT 02069093.

## Result:

Between May 26, 2014 and Dec 28, 2017, we enrolled 174 women from 31 institutions; 168 were evaluable for efficacy but 5 were excluded (had not received the protocol treatment [n=4]; no efficacy data [n=1]). In 8 weeks, the incidence of grade 1 oral mucositis was significantly different between the POC group (76.5%, 62 of 81 patients) and control group (89.7%, 78 of 87 patients) (p=0.035). The incidence of grade 2 (severe) oral mucositis was also significantly different between the POC group (34.6%, 28 of 81 patients) and control group (54%, 47 of 87 patients) (p= 0.015). As a result of oral mucositis, 18 (22.2%) patients in the POC group and 28 (32.2%) in the control group had to undergo everolimus dose reduction.

## Conclusion:

POC reduced the incidence and severity of oral mucositis in patients receiving everolimus and exemestane. This could be a new standard in oral care for patients undergoing this treatment.

## Primary Analysis: Incidence Probability of Oral Mucositis

	POC Group (n=81)			Controll (n=87)		P-valune
Oral Mucositis over Grade1	n	%		n	%	
Yes	62	76.5		78	89.7	0.035

No	19	23.5		9	10.3	
Risk Difference, % (95% CI)	-11.83 (-22.80, -0.85)					

**POC: Professional oral Care**

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-02

Meta-regression and meta-analysis of dexrazoxane for cardioprotection in all breast cancer stages in patients treated with anthracyclines

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**Background:** Anthracyclines continue to be a valuable option in chemotherapy for breast cancer (BC), in spite of their well-documented cardiotoxicity. Anthracycline-induced cardiotoxicity depends on cumulative dose, and it actually begins with the first dose. Clinical studies have suggested that dexrazoxane could reduce this toxicity. Dexrazoxane is frequently used when higher anthracycline cumulative doses are needed, but is often omitted in the adjuvant setting. We aimed to analyse by an updated meta-analysis the cardioprotective effect of dexrazoxane in all BC stages in patients receiving anthracycline-based chemotherapy. In addition, we performed subgroup analyses and meta-regression to assess if the average anthracycline dose and the studies publication date would interfere in the cardiac event outcome.

**Methods:** We performed a systematic review and meta-analysis. The review was registered in PROSPERO database (CRD42017077462). We searched data from 1990 to August 2017 in Cochrane Central Register of Controlled Trials, Google Scholar, MEDLINE/Pubmed, LILACS, Web of Science, articles references and ASCO proceedings. Studies assessing congestive heart failure and cardiac event (cardiac function alterations without cardiac symptoms or hospitalization for cardiac reasons) as primary endpoints were included. Two reviewers independently performed the studies selection, risk of bias assessment and data extraction. Meta-analysis was done using random effect model for estimation of treatment effect. Heterogeneity was assessed by visual inspection of forest plots and by Q test. Subgroup analyses were carried out, according with the chemotherapy regimen (use of anthracycline in previous chemotherapy). In the meta-regression we used the random effects model.

**Results:** Our search resulted in 1603 articles, from which we included 7 studies providing 1545 participants. Meta-analysis showed an overall beneficial effect of dexrazoxane on reducing the risk of cardiac events (OR 0.262, CI 95%:0.169-0.407,  $p < 0.0001$ ). In two of the seven studies which evaluated the cardiac event outcome, the patients were previously exposed to anthracyclines. In this patients' subgroup, we found an odds ratio of 0,244 (CI 95%, 0,102 to 0,584). In the study subgroup that the patients didn't report previous exposure to anthracycline, the odds ratio was 0,266 (CI 95%, 0,149 to 0,478). The Q test to evaluate the difference between the subgroups showed a value of 0,026 with  $p = 0,871$  suggesting there was no difference between the subgroups. The multiple meta-regression was performed, adding to the model the average dose and studies age variables, for a combined analysis. The statistical analysis of the impact of the two combined cofactors didn't show significative association (Q test = 2,36, df = 2 and  $p = 0,30$ ).

**Conclusions:** Dexrazoxane reduced the occurrence of cardiac events when added to anthracycline based chemotherapy regimen. There was no evidence that the benefit of the reduction of cardiac events with the use of dexrazoxane was different according to the use of anthracycline in previous chemotherapy or by the used average dose of anthracycline. These findings may have significant implications for clinical practice.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-03

Impact of scalp cooling device (SCD) in preventing alopecia in women undergoing chemotherapy for breast cancer

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## **Background.**

Alopecia (A) is a common and emotionally traumatic adverse effect for breast cancer (BC) patients (pts) undergoing chemotherapy (CT). Food and Drug Administration (FDA) cleared the DigniCap® SCD, for patients with breast cancer in 2015. This device was designed to reduce hair loss during chemotherapy. However, the impact of SCD in pts undergoing anthracycline and taxane-based sequential regimen is not entirely established. Thus, the aim of this analysis was to prospectively explore the role of SCD in a cohort of pts including also this regimen.

## **Methods.**

From February 2016 to June 2018 patients with early/locally advanced breast cancer treated with neoadjuvant/adjuvant CT including anthracycline, taxane or both in sequential regimen were enrolled. The estimate of hair-loss was evaluated by photographs of the head using the Dean scale during and one month after the end of chemotherapy. Alopecia was graduated according to Dean scale: G0 = no A; G1 < 25% A; G2 = 25–50% A; G3 = 50–75% A; G4 > 75%. A score of 0-2 ( $\leq$  50% hair loss) was defined as treatment success. Tolerability was defined as the percentage of patients who completed all chemotherapy cycles using the SCD. All patients received the Patient Symptoms Survey (self-reported). A database for individual data and information was appropriately fulfilled. Descriptive statistics was adopted.

## **Results.**

Overall 121 pts were enrolled; 118 pts were evaluable for efficacy of Dignicap® SCD. Median age was 44 years (range: 24-74 years). CT regimens included docetaxel/cyclophosphamide (37 pts), epirubicin (90 mg/m<sup>2</sup>) and cyclophosphamide (600 mg/m<sup>2</sup> iv) three weekly followed by 12 courses of paclitaxel (80 mg/m<sup>2</sup> iv weekly) (84 pts). Alopecia all grade was showed in 52.5% (n=62): G1 in 35 pts (29.6%) and G2 in 23 pts (19.5%). No hair loss in 42 pts (35.6 %). Treatment success was seen in 103 pts (87.3%). Toxicity included grade 1/2 headache in 56 pts (47.4%), cervical discomfort in 36 pts (30.5%), pain of skin in one pts (8.5%). Discontinuation of SCD was seen in 28 pts (23.7%) primarily for headache G3 (4 pts – 3.4%), hair loss G3 in 15 pts (12.7%), discomfort in 8 pts (6.8%), use of head cover in one pt (0.8%).

## **Conclusions.**

This prospective observational study suggests that SCD is effective in preventing A in a relevant number of patients (87.3%), undergoing also anthracyclines followed by taxanes regimen in sequential schedule.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-04

Association between body mass index (BMI) and response to duloxetine for aromatase inhibitor (AI)-associated musculoskeletal symptoms (AIMSS)

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**Background:** AIMSS occurs often in women treated with AI therapy for early stage breast cancer and can negatively impact adherence and persistence with therapy. Duloxetine is a serotonin norepinephrine reuptake inhibitor used to treat mood disorders and chronic pain. In SWOG S1202, patients with AIMSS treated with duloxetine reported statistically significant improvement in pain by 12 weeks compared to placebo. Obesity is a predictor of increased likelihood of developing AIMSS, and a prior study of omega 3 fatty acid versus placebo for AIMSS showed a potential differential response to therapy by BMI. In this exploratory analysis of S1202, we investigated the association between baseline BMI and response to therapy.

**Methods:** In S1202, 299 postmenopausal women with stage I-III hormone receptor-positive breast cancer on AI therapy who developed new or worsening average pain of 4-10 on a numerical rating scale were enrolled, randomized 1:1 to duloxetine or placebo with randomization stratified by baseline pain (4-6 vs. 7-10) and prior taxane therapy (yes vs. no). Patients were treated for 12 weeks. Patient-reported outcomes including Brief Pain Inventory (BPI) were obtained at baseline and weeks 2, 6, 12, and 24. Patients were categorized into BMI<30 kg/m<sup>2</sup> (non-obese) or BMI≥30 kg/m<sup>2</sup> (obese). The pre-specified aim of this secondary analysis was to examine whether the effect of intervention on BPI average pain at 12 weeks differed between obese and non-obese patients. Multiple linear regression was used, adjusting for the stratification factors and the baseline score. We tested whether the interaction of BMI status and intervention effect was statistically significant at  $\alpha=.05$ .

**Results:** 289 patients were eligible for the analysis, 54% of whom were obese. The cohorts were well balanced other than by race. The difference by intervention arm in the 12-week mean BPI scores between baseline and follow-up scores was substantially different for the obese versus non-obese cohorts. In the patients with BMI<30, the reduction in observed mean average pain score was similar in the duloxetine- and placebo-treated patients (-2.46 points vs. -2.34 points, p=.75). In contrast, in the patients with BMI≥30 the reduction in pain score was statistically significantly greater for the duloxetine-treated compared to the placebo-treated patients (-2.73 points vs. -1.64 points, p=.003; interaction p-value=.02). Differences in intervention effects between obese and non-obese groups were even stronger at 2-weeks (interaction p-value=.001) and 6-weeks (interaction p-value<.0001). Similar findings were evident for other pain-related patient-reported outcomes.

**Conclusions:** In the placebo-controlled S1202 trial, obese patients with AIMSS obtained more analgesic benefit from duloxetine. Additional studies are warranted to determine the biologic basis for these findings, such as a different mechanism underlying development of AIMSS or pain expression in patients with obesity, or other confounding variables related to analgesic response to duloxetine relative to placebo.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-06

Systematic evaluation of ovarian reserve in young breast cancer patients treated by sequential chemotherapy

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Most women in reproductive age diagnosed with breast cancer receive (neo)adjuvant chemotherapy. Fertility preservation is part of the standard of care for these young women.

## Patients and methods

We report preliminary results of a prospective multicentric cohort evaluating ovarian reserve during and after chemotherapy and fertility preservation in young ( $\leq 38$  years old) in women aged  $\leq 38$  years, treated for a breast cancer with (neo)adjuvant anthracyclins and taxanes based chemotherapy, between July 2011 and December 2016. Fertility preservation was offered in patients (pts) who received adjuvant chemotherapy. The median duration of follow up was 2,6 years (4 months-5,3 years). The aim of this study was to evaluate the ovarian reserve assessed by antimullerian hormone and antral follicular count. The incidence of amenorrhea (defined by absence of menses  $\geq 3$  months), ovarian failure (absence of menses  $\geq 12$  months), chemotherapy induced menopause (absence of menses  $\geq 24$  months) was collected.

## Results

One hundred and thirty-two pts were included in 10 centers. Data are available for 127 pts. For 4 pts, the scheduled chemotherapy was not received. One pt withdrew her consent. Chemotherapy was neoadjuvant for 43 pts and adjuvant for the 84 others. Fifty-eight asked for fertility preservation and received ovarian stimulation (all in adjuvant setting).

Median age was 32 years (23-37). Eighty pts had a previous pregnancy. Three of them remained nulliparous. Among the 77 others, 36 had 1 child, 31 had 2 children and 10 pts 3 or more children respectively. At the time of diagnosis, 90% had regular menses and 75% had a contraception.

The median initial antral follicular count was 21.5 (Min 1- Max 100). The AMH level significantly decreased during chemotherapy with no secondary return to baseline value over the first 9 months after end of treatment: median of 20.9 pmol/l (0.5-223) before chemotherapy, 12,8 (0,5-120) at the second cycle of chemotherapy (C2), 3 (0,5-20) at C4, 0,5 (0,5-4,4) at C6, 0,5 (0,5-25,1) 3 months after the end of chemotherapy (M3), 0,6 (0,5-29) at M6, and 3 (0,5-29,8) at M9.

At last follow-up, 46% of pts experienced amenorrhea, 7% an ovarian failure and 3% a chemo-induced menopause. The highest incidence of amenorrhea (61%) was at M3. At M12, 7% of pts remained amenorrhoeic. The AMH initial level was not significantly lower in pts who experienced amenorrhea compared to those who did not (25.5 versus 35.1,  $p = 0,087$ ). Ovarian stimulation and BRCA status did not impact risk of amenorrhea.

At 2 years, overall survival rate was 96% and progression free survival rate was 90% (6 deaths and 18 progression events).

## Conclusion

(Neo)adjuvant sequential breast cancer chemotherapy is associated with a decrease of AMH level and amenorrhea. Our results suggest a significant risk of premature ovarian failure. Fertility preservation has to be proposed to these young patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-07

Risk analysis for chemotherapy induced nausea and vomiting (CINV) in patients receiving FEC100 treatment

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## BACKGROUND:

Anthracycline-containing regimens are standard treatment options in adjuvant and neoadjuvant chemotherapy in breast cancer. Chemotherapy-induced nausea and vomiting (CINV) is experienced frequently in patients receiving these regimens, but the risk factors for CINV are unknown.

## OBJECTIVE:

The aim of this study was to investigate risk factors for CINV in anthracycline-containing regimens retrospectively.

## METHODS:

Data were collected from the JONIE study, which was conducted in order to estimate the efficacy of zoledronic acid in a neoadjuvant setting from March 2010 to June 2012 (UMIN000003261). A total of 180 patients were recruited, and we used CINV data from the first cycle of FEC100 treatment and patient backgrounds. As the protocol regulation allowed the use of antiemetic drugs, in the first cycle of the FEC100 regimen, patients received various types of antiemetic agents, which we classified into four groups: Dexamethasone (DEX)+5-HT<sub>3</sub> receptor antagonist (5-HT<sub>3</sub>)+neurokinin-1 receptor antagonist (NK<sub>1</sub>) (**DEX+5-HT<sub>3</sub>+NK<sub>1</sub>**) group; Dexamethasone (DEX)+5-HT<sub>3</sub> receptor antagonist (5-HT<sub>3</sub>) (**DEX+5HT<sub>3</sub>**) group; Dexamethasone (DEX)+5-HT<sub>3</sub> receptor antagonist (5-HT<sub>3</sub>)+dopamine receptor antagonist (DRA) (**DEX+5HT<sub>3</sub>+DRA**) group; and Dexamethasone (DEX)+5-HT<sub>3</sub> receptor antagonist (5-HT<sub>3</sub>)+neurokinin-1 receptor antagonist (NK<sub>1</sub>)+dopamine receptor antagonist (DRA) (**DEX+5-HT<sub>3</sub>+NK<sub>1</sub>+DRA**) group. Risk factors were selected from patient backgrounds and the combinations of antiemetic drugs. In patient backgrounds, the body mass index (BMI) was stratified into 3 categories: Less than 18.5 (underweight group); equal to or more than 18.5 but less than 25 (standard BMI group); and equal to or more than 25 (overweight group). The risks for CINV were analyzed by univariate and multivariate analyses. P values of less than 0.05 were defined as significant.

## RESULTS:

In a univariate analysis of nausea, the body mass index (BMI) was the only significant factor (P<0.05). On the other hand, BMI and the combination of antiemetic drugs were significant factors in vomiting. (P<0.05 and 0.005, respectively). In a multivariate analysis of nausea, the P value for BMI was 0.02. The odds ratio for the underweight group was 7.745 (confidence interval: 2.171 to 27.634) compared with the standard BMI group. In a multivariate analysis of vomiting, BMI and the combination of antiemetic drugs were significant risk factors (P=0.025 and 0.023, respectively). The odds ratio for the underweight group was 3.481 (confidence interval: 1.183 to 10.241) compared with the standard BMI group. Furthermore, the odds ratios in the DEX+5-HT<sub>3</sub>+DRA and DEX+5HT<sub>3</sub> groups were 5.005 (confidence interval: 1.543 to 16.239) and 4.178 (confidence interval: 1.428 to 12.222), respectively, compared with the DEX+5-HT<sub>3</sub>+NK<sub>1</sub> group, which was consistent with the CINV guidelines in 2011.

## CONCLUSIONS:

This study revealed that BMI was the most important risk factor for nausea, and that BMI and the combination of antiemetic drugs were risk factors for vomiting. Underweight-patients tend to have CINV in anthracycline-containing regimen. The DEX+5-HT<sub>3</sub>+NK<sub>1</sub>



group was the best antiemetic drug combination. These result show that following the CINV guideline treatment is mandatory in order to prevent CINV.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-08

Assay for personalized prediction of chemotherapy-induced delayed nausea

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**Background:** Chemotherapy-induced nausea and vomiting (CINV) is a distressing side effect according to patients. CINV can negatively affect nutritional habits, ability to work and motivation to follow treatment regimens. Assessment of CINV is an essential component of care for patients receiving chemotherapy. Multiple factors influence the incidence of CINV with the chemotherapy regimen, both type and dosage, being the primary risk factor. It is generally assumed that the strongest patient-related factors are younger age and female sex. However, reports in the literature have demonstrated that using these factors clinicians underestimate the prevalence of CINV, especially delayed nausea.

Thus, there is a need for risk assessment tools to accurately identify patients requiring anti-emetic regimens to improve quality of life of patients and their families.

Most chemotherapeutic agents cause bursts of reactive oxygen species (ROS) resulting in cellular damage and release of substances that can activate receptors in the chemoreceptor trigger zone. Glutathione (GSH), a key antioxidant, is responsible for maintaining redox homeostasis by neutralizing ROS elicited from chemotherapy. Therefore, we hypothesized that a patient's risk of CINV may reflect individual variations in the efficiency to scavenge free radicals after chemotherapy.

**Methods:** In our Institutional Review Board approved study, we have enrolled over 300 patients and completed assessment of 133 patients. These patients were treated with highly or moderately emetogenic chemotherapies for lung, colon, or breast cancer. Blood samples were drawn from chemotherapy naïve patients and used to determine the glutathione recycling capacity. The assay detects the conversion of a bioactive probe, hydroxyethyl disulfide, into mercaptoethanol, which once normalized to red blood cell count, indicates glutathione recycling capacity (ChemoTox). Nausea severity was reported using the Rotterdam Symptom Check-List at each treatment cycle. Self-reported symptoms were compared to notes in medical records and anti-emetic prescription history.

**Results:** We previously published the correlation between low ChemoTox and risk of delayed nausea for patients receiving platinum-based therapy for lung and colon cancer (N=64; correctly classified 88.5%; AUC 0.77). In this second evaluation, using SAS/STAT v.14.1 classification trees, we show the results from testing this prediction tool for breast cancer patients that typically are treated with anthracycline- or taxane-based chemotherapies. An early evaluation of anthracycline-based therapies (N=37) demonstrated a weak association between experienced nausea and ChemoTox (correctly classified 69.2%; AUC 0.64). In contrast, patients treated with taxane-based therapies (N=32) demonstrated a similar correlation between ChemoTox and severity of nausea as previously seen for platinum therapies. Also, a similar accuracy in identifying patients at risk of moderate-severe nausea was identified (correctly classified 77.3%; AUC 0.79).

**Conclusion:** The results from our prospective study suggests that a reduced ability to recycle GSH in the blood may offer an objective prediction of delayed nausea, possibly allowing for optimal anti-emetic regimen to improve the quality-of-life for breast cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-11-09

Sleep disturbance and quality of life among breast cancer survivors

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**Introduction:** Breast cancer survivors are at risk for substantial sleep disturbance, which can negatively affect quality of life. Sleep disturbance can be exacerbated by co-occurring emotional concerns such as depressive symptoms and anxiety. Prior research has largely focused on linkages between sleep disturbance and emotional concerns among individuals with early stage disease. To dive deeper, we examined sleep disturbance and its correlates among breast cancer survivors with and without metastatic disease.

**Methods:** 631 female breast cancer survivors (168 ever experiencing metastatic disease [MBC]; 463 never metastatic [BC]) enrolled in the Cancer Support Community's online Cancer Experience Registry, provided socio-demographic information, and reported cancer-related distress (CancerSupportSource®, a 25-item measure with depression and anxiety risk screening subscales) and levels of pain interference (PI) and sleep disturbance (PROMIS-29 subscales). We examined associations between risk for depression/anxiety, PI, and worse sleep disturbance with multivariate regression, adjusting for metastatic disease, treatment history, and number of comorbidities.

**Results:** Participants were 84% non-Hispanic White; mean age=54.8 years,  $SD=12.2$ ; mean time since diagnosis=4.4 years,  $SD=5.5$ . 72% ever received chemotherapy; 60% radiation therapy; 56% hormone therapy; 91% had surgery for their cancer. 47% reported moderate to very serious concern about sleep problems; concern about sleep did not differ by metastatic history. 20% of participants reported a level of sleep disturbance that was significantly worse ( $>1SD$ ) than the U.S. population average and 18% reported PI that was significantly worse ( $>1SD$ ) than the U.S. population average; these did not differ by metastatic history. Using CancerSupportSource anxiety and depression risk screening subscales, 47% of participants were identified as at risk for clinically significant levels of anxiety, and 35% at risk for clinically significant levels of depression. Participants with MBC were more likely to be at risk for clinically significant levels of anxiety ( $\chi^2=7.98$ ,  $p<.01$ ). Depression risk did not differ between MBC and BC survivors. Greater sleep disturbance was associated with having ever received radiation therapy ( $r=.11$ ,  $p<.01$ ), number of reported comorbidities ( $r=.37$ ,  $p<.001$ ), greater PI ( $r=.46$ ,  $p<.001$ ), and greater risk for clinically significant depression ( $r=.38$ ,  $p<.001$ ) and anxiety ( $r=.35$ ,  $p<.001$ ). In multivariate analysis, risk for clinically significant levels of depression (semipartial  $r=.12$ ,  $p<.005$ ), anxiety (semipartial  $r=.05$ ,  $p<.05$ ), and greater PI (semipartial  $r=.24$ ,  $p<.001$ ) remained associated with greater sleep disturbance after controlling for treatment history, metastatic status, and number of comorbidities, ( $R^2=.28$ ,  $F(4,588)=56.26$ ,  $p<.001$ ).

**Conclusion:** Being at risk for clinically significant levels of depression and anxiety and experiencing greater pain interference are associated with worse sleep disturbance among breast cancer survivors across the illness trajectory. Health care providers are encouraged to discuss with patients how they can address sleep disturbance concerns, including referrals to integrative therapies that address the constellation of affective, pain, and sleep symptoms.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-10

Efficacy of NEPA as antiemetic prophylaxis in breast cancer patients receiving highly or moderately emetogenic chemotherapy – Interim results of a German prospective, non-interventional study

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## Background

The oral fixed dose combination of netupitant and palonosetron NEPA has been approved for the prevention of acute and delayed chemotherapy-induced nausea and vomiting (CINV) in cancer patients receiving cisplatin-based highly emetogenic (HEC) or moderately emetogenic chemotherapy (MEC). The primary objective of the prospective, non-interventional study (NIS) AkyPRO is the evaluation of quality of life in adults receiving MEC or HEC and NEPA for CINV prevention. Secondary endpoints are efficacy and safety of NEPA. Here we present an interim analysis of NEPA efficacy in the subgroup of breast cancer patients, who represent the largest subgroup (66%) of enrolled patients. Since September 2015, 2427 patients have been enrolled, of whom 986 are breast cancer patients.

## Methods

The NIS has been designed to evaluate NEPA in 2,500 cancer patients receiving single day or two day MEC or HEC. QoL is recorded by FLIE questionnaires. Efficacy (complete response (CR, no vomiting, no rescue medication)), additional medication, and adverse events are recorded in patient diaries over three consecutive chemotherapy cycles. Additionally, physicians report their efficacy assessments of NEPA online, using an eCRF.

## Results

At the cut-off date November 11, 2017, 2427 patients had been enrolled in the study. For the interim analysis 986 breast cancer patients were evaluated who had been fully documented in the eCRF at the cut-off date.

95% had an ECOG performance status of 0 or 1. 51% received adjuvant, 44% neoadjuvant, and 5% palliative chemotherapy.

80% of patients received HEC, mostly (79%) anthracycline/cyclophosphamide (AC) combinations. Of the women receiving MEC, the majority were treated with carboplatin-based regimens (9%). 7% of patients received other MEC regimens.

81.4-82.8 % of patients reported CR in cycles 1-3 and more than 93% of patients reported no emesis during the 3 treatment cycles covered in the patient diaries. No significant nausea was reported by 62.7-64.2% of patients.

Physicians rated the efficacy of the antiemetic prophylaxis with NEPA using the 4 categories very good, good, satisfactory, and poor. In cycles 1 and 2, more than 89% of physicians rated the efficacy of NEPA very good or good. In cycle 3, 90.6% rated it very good or good. In addition to reporting CR, nausea and emesis episodes in their patient diaries, patients used the same 4 categories to assess the efficacy of NEPA at the end of each treatment cycle. Efficacy assessments of physicians and patients were very similar, with 87% of patients choosing very good or good in cycle 1 compared to 89% of physicians.

NEPA was well tolerated. Low-grade constipation (14.9%) and insomnia (8.3%) were the most frequent treatment-related adverse event.

## Conclusion

In this real life study, NEPA was effective in the prevention of CINV in the subgroup of breast cancer patients receiving HEC or MEC. The efficacy assessments by patients and physicians were comparable, with approximately 90% good or very good efficacy for 3 consecutive cycles. More than 93% of patients reported no emesis and more than 81% reported CR during the 5 days post-chemotherapy during all 3 cycles. The study is ongoing.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-11-11

Cimicifuga racemosa extract prevents menopausal syndrome in LHRHa treatment of breast cancer: A prospective randomized research

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**BACKGROUND** Menopausal syndrome (MPS) is common in pre/peri-menopausal breast cancer patient receiving luteinizing-hormone releasing hormone analogue (LHRHa). According to the results of TEXT & SOFT study, it could affect patients' life quality seriously and even caused 8%~23% treatment break. Cimicifuga racemosa (Remifemin) is previously proved effective for MPS in nature menopause patients. We aim to design a prospective randomized research for investigating the effect of Remifemin on MPS which caused by LHRHa in breast cancer (NCT03339882).

**METHODS** The pre/peri-menopausal patients diagnosed as early breast cancer planning for LHRHa were identified and randomly divided into two groups after surgery and chemotherapy if necessary: the Remifemin group (Remifemin 20mg twice a day for 12 weeks) and the control group. The primary endpoint is Kupperman menopause index (KMI). The estradiol (E2), follicle-stimulating hormone (FSH), luteinizing hormone (LH) levels in blood were also evaluated.

**RESULTS** From January 2017 to December 2017, totally 85 patients (42 in Remifemin group and 43 in control group) were enrolled in Zhejiang Cancer Hospital and there were no significant differences in baseline characteristics including age, BMI, tumor stage, ER, PR, HER2, KMI and hormone levels between two groups (Table 1). At the time of 4, 8, 12 weeks after treatment, the KMI of Remifemin group were all significantly lower than control group (Table 2,  $P < 0.01$ ) while no significant difference in E2, FSH and LH levels between the two groups (Table 2,  $P > 0.05$ ). There was no significant difference in endometrial thickness, ovarian cyst and Uterine fibroids except cervical cyst ( $P = 0.02$ ).

**CONCLUSION** The use of Remifemin is oncological safe and reliable in preventing MPS which caused by LHRHa in breast cancer.

The clinical characteristics of two groups

		Remifemin group	Control group	P
		n=42	n=43	
		N(%)	N(%)	
Age(years)		37.74±6.60	37.16±6.28	0.68
BMI (body mass index)		21.90±2.83	21.65±3.01	0.69
T stage	0	2(4.76)	2 (4.65)	0.16
	1	13 (30.95)	22 (51.16)	
	2	27 (64.29)	19 (44.19)	
N stage	0	21 (50.00)	24 (55.82)	0.63
	1	10 (23.81)	12 (27.91)	
	2	6 (14.29)	5 (11.63)	
	3	5 (11.90)	2 (4.65)	
ER	Positive	30 (71.43)	35 (81.40)	0.28
	Negative	12 (28.57)	8 (18.60)	
PR	Positive	30 (71.43)	32 (74.42)	0.76
	Negative	12 (28.57)	11 (25.58)	
HER2	Positive	8 (19.05)	10 (23.26)	0.64
	Negative	34 (80.95)	33 (76.74)	

Category of LHRHa	Goserelin	32 (76.19)	28 (65.12)	0.26
	Leuprorelin	10 (23.81)	15 (34.88)	
Category of endocrine drug	None	10 (23.81)	6 (13.95)	0.20
	SERM	5 (11.90)	11 (25.58)	
	AI	27 (64.29)	26 (60.47)	

The endpoints of two groups

		Remifemin group	Control group	P
		n=42	n=43	
KMI*	Base line	2.45±1.45	2.30±1.36	0.62
	4th week	3.83±1.86	8.37±4.19	<0.01
	8th week	4.21±1.80	11.00±4.48	<0.01
	12th week	4.67±2.16	11.98±4.93	<0.01
E2	Base line	134.24±64.14	143.49±84.19	0.57
	4th week	20.26±14.81	25.24±13.31	0.11
	8th week	13.55±8.14	15.65±8.02	0.23
	12th week	10.83±7.35	12.53±6.35	0.26
FSH	Base line	8.88±11.40	10.17±9.91	0.58
	4th week	8.23±9.93	5.40±6.45	0.12
	8th week	4.83±8.49	6.53±11.50	0.44
	12th week	3.65±7.55	7.28±16.01	0.19
LH	Base line	6.77±6.31	9.62±10.59	0.14
	4th week	9.08±4.87	7.93±4.83	0.28
	8th week	11.76±11.82	10.18±12.21	0.55
	12th week	7.27±4.95	6.90±4.38	0.72

\*Wilcoxon test

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-12

Effect of wearable wellness brain sensing mindfulness-based technology in newly diagnosed surgical breast cancer patients: A randomized intervention

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**Introduction:** There is insufficient knowledge about the feasibility and effect of mind-body intervention on QOL and stress at initial diagnosis and shortly after undergoing breast cancer surgical treatment. The pre-operative and post-operative period can be stressful, distressing and anxiety provoking for many women. Mind-body interventions such as meditation, relaxation and mindfulness can offset distress and is an option to cope with the stresses of treatment. We investigated the role of wearable mindfulness based technology in patients newly diagnosed breast cancer patients.

**Methods:** This randomized study compared the Muse EEG mindfulness sensing headset device (n=16) to a stress reduction education control group (n=13). Primary outcomes included quality of life (QOL), stress, and fatigue via validated, standardized scales/subscales: Functional Assessment of Cancer Therapies – General (FACT-G), Multidimensional Fatigue Symptom Inventory – Short Form (MFSI-SI), and the Perceived Stress Scale (PSS). Participants from an academic cancer center were randomized to the wearable device or stress education material prior to surgery. Participants completed questionnaires at initial diagnosis and at specific time points during the breast cancer surgical journey: pre-surgery, post-surgery (2 weeks), and at 3 months. Usage was directly from Muse and via written log. Was It Worth It (WIWI) survey was conducted at 3 months.

**Results:** Mindfulness via the headset device was feasible and worthwhile (71.4% intervention group). The scales of QOL, fatigue, and stress improved in both groups at 3 months compared to baseline (MFSI-E, MFSI-M, MFSI-V, FACT-EWB, and PSS). Participants randomized to the mindfulness headset device had improved fatigue and QOL at 2 weeks post-operatively (MFSI-E, MFSI-V, MFSI-T, FACT-PWB, FACT-EWB) and in total MFSI at 3 months. The results were not significantly different between groups. High mindfulness headset utilizers (>30 sessions) compared to below average utilizers differed at the 3 month visit in fatigue and QOL (MFSI-G, MFSI-P, FACT-PWB, and FACT-Total).

**Conclusion:** Our study demonstrated that the use of wearable mindfulness sensing headset device was feasible, simple to use, low cost, and integrated into the surgical breast cancer journey. Overall, among breast cancer patients undergoing surgery there was improvement in QOL, fatigue, and stress with mind-body interventions around surgery compared to baseline. Participants randomized to the wearable mindfulness headset device trended toward outcomes sooner (2 week follow-up) and with high utilization. Further research is needed to determine significance of these outcomes and long term impact in care of cancer survivors.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-13

Depomedroxyprogesterone therapy for hot flashes in survivors of ER-expressing breast cancer: Impact on recurrence and survival

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## Background

Survivors of ER-expressing operable breast cancer (ER+BC) generally do not receive hormone replacement therapy for menopausal symptoms due to concern about provoking recurrence of disease. Single dose depomedroxyprogesterone acetate (MPA) 400 mg IM has previously been shown (Loprinzi CL, et al. *J Clin Oncol* 2006;24:1409) to be the most effective non-estrogen therapy available for menopausal hot flashes (HF) but long-term evidence of safety in survivors of ER+BC is lacking.

## Methods

Consecutive patients previously diagnosed with ER+BC who received MPA for HF between January 2007 and December 2012 were retrospectively identified in the breast cancer patient database at Mayo Clinic Arizona. Medical records were audited for breast cancer outcomes in these cases and in contemporaneous control patients with ER+BC who did not receive MPA, matched for age, stage of disease, and year of diagnosis. Statistical comparisons of local-regional recurrence and event-free survival were performed.

## Results

92 patients who received MPA were identified and matched 1:1 with contemporaneous controls. Median follow-up duration was 5.7 years in cases and 4.5 years in controls. Estimated local-regional recurrence free survival at 10 years was 85% (95% CI, 72-100%) in cases and 95% (95% CI, 86-100%) in controls. Matched pairs hazard ratio was 1.0 (95% CI, 0.06-16.0) for local-regional recurrence free survival. Estimated event-free survival at 10 years was 81% (95% CI, 69-97%) in cases and 76% (95% CI, 64-92%) in controls. Matched pairs hazard ratio was 0.38 (95% CI, 0.10-1.41) for event-free survival. The majority (77%) of case patients experienced satisfactory relief of hot flashes from MPA injection.

## Conclusion

In this retrospective case-control study we were unable to identify a detrimental effect of MPA therapy for HF in survivors of ER+BC. MPA may be acceptable for management of HF in this population.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-14

Efficacy and quality of life analysis of palonosetron vs ondansetron for high and moderate emetogenic chemotherapy for breast cancer

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**Background:** Nausea and vomiting are common complications on the chemotherapy (CINV) and can affect the quality of life of the patients. There are various antiemetic regimens that varies in both cost and effectiveness. The aim of this trial is to evaluate the efficacy in acute and delayed CINV of ondansetron vs palonosetron.

**Patients and methods:** In this was a randomized, open label trial, we included breast cancer patients' candidate to (AC, TC, TCH regimens); arm A received palonosetron, dexamethasone and fosaprepitant and arm B ondansetron, dexamethasone, fosaprepitant; patients who had received previously any chemotherapy or radiotherapy were excluded. Presence of CINV were investigated, as well the ER visits due for CINV and QoL (EORTC QLQ 30 and EORTC B-23) were analyzed during the first cycle of treatment. Local ethics committee approved the trial.

**Results:** 262 patients were included, 87% received AC and 13% TC; acute control was achieved in 87% and 94% respectively, p=ns and delayed control was achieved in 76% and 86% p=ns, toxicity was similar in both arms. QoL analysis showed no differences in family interaction, social life and financial troubles. ER visits due to severe symptoms were similar p=ns

**Conclusions:** Palonosetron or ondansetron are equally effective in prevention acute and delayed CINV and they also maintain similar quality of life.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-11-15

Chemotherapy-induced peripheral neuropathy in breast cancer survivors: Comparison of objective and subjective measures

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**Background:** Chemotherapy-induced peripheral neuropathy (CIPN) is a common, potentially debilitating, and dose-limiting side effect experienced by breast cancer survivors. CIPN encompasses symptoms such as pain, numbness, and tingling, which can be measured subjectively by patient-reported outcomes (PRO), or objectively by quantitative sensory testing (QST); however, little is known how QST correlates with symptom profiles measured by PRO.

**Methods:** We conducted a cross-sectional analysis using baseline data of two ongoing clinical trials of breast cancer survivors who experienced moderate to severe CIPN defined by pain, numbness, or tingling ratings of four or greater on a numeric rating scale (NRS) after chemotherapy completion for at least three months. PRO measures of CIPN symptoms included Neuropathic Pain Scale (NPS) and Functional Assessment of Cancer Therapy-Gynecologic Oncology Group/Neurotoxicity subscale (FACT/GOG-Ntx). QST included tactile threshold (TT) measured by Von Frey's filaments, and vibration threshold (VT) measured by biothesiometer. We ran a Spearman correlation to assess the relationship between the subjective measures (NPS and FACT/GOG-Ntx) and objective measures (TT and VT QST).

**Results:** We included 52 sets of baseline data on 50 unique patients; two patients were enrolled in both trials at different times. Mean age was 61 years (SD 10) and 66% were white. The mean NRS pain score was 3.9 (SD 2.8), numbness 5.7 (SD 2.2), and tingling 4.3 (SD 2.8) on a 0-10 scale. The mean NPS total score was 39.2 (SD 23.1) on a 0-100 scale, and FACT/GOG-Ntx was 26.2 (SD 6.8) on a 0-44 scale. High scores on NRS and NPS and low scores on FACT/GOG-Ntx signify more severe CIPN symptoms. See Table 1 for a summary of the correlation between two questions on FACT/GOG-Ntx on tingling/numbness in hands and feet, and NPS total score with QST. A moderate correlation was observed between FACT/GOG-Ntx and QST results, suggesting patient-reported hand and foot numbness or tingling is associated with decreased hand and foot tactile and vibration perception. NPS was positively correlated with tactile perception for the hand and foot, but not with vibration perception.

Table 1. Correlation between objective and subjective measures of CIPN

	Tactile QST		Vibration QST	
	Hand	Feet	Hand	Feet
FACT/GOG-Ntx	-0.33 (P=0.018)	-0.28 (P=0.045)	-0.37 (P=0.008)	-0.40 (P=0.0034)
NPS	0.34 (P=0.015)	0.32 (P=0.022)	0.22 (P=0.12)	0.03 (P=0.81)

**Conclusions:** A mild to moderate correlation was observed between subjective and objective measurements of CIPN. As CIPN presents a diverse range of symptoms, better quantifying the subjective and objective measures of CIPN can help incorporate these tools in observational and intervention trials. Understanding the correlation between PRO and QST can help establish QST as a reliable objective measurement of CIPN symptoms, and enable targeted interventions to alleviate CIPN symptoms.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-16

Efficacy and safety of acupuncture for the hot flashes in breast cancer patients taking adjuvant tamoxifen: A multicenter study in Korean women

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**Background :** Tamoxifen is widely used in premenopausal patients with estrogen receptor positive (ER+) breast cancer. Among the side-effects of tamoxifen, hot flashes is a common and challenging one. Previous pilot study suggested the effectiveness of acupuncture for relief of symptoms. This study was performed to evaluate the efficacy and safety of acupuncture for treatment of hot flashes in Korean women breast cancer patients taking tamoxifen as adjuvant endocrine therapy.

**Methods :** Thirty(30) breast cancer patients taking tamoxifen and reported moderate to severe hot flashes were enrolled from two institutes. Patients were randomly assigned into acupuncture group(n=15) and control group(n=15). The acupuncture group received acupuncture 3 times a week for 4 consecutive weeks, at 5 predefined points(GV 20, M-HN-3, HT 8, KI 10 and LV 2) for 20 ± 5minutes at each session. Control group received no treatment. The score of hot flash visual analogue scale(VAS) and total hot flash score, EORTC QLQ-C30 and QLQ-BR 23 questionnaire were recorded before treatment, once every treatment week and 4 weeks after treatment for both groups.

**Results :** Acupuncture group showed significantly reduced severity of hot flashes during treatment assessed with either VAS and total hot flash score (p=0.000 and p=0.008, respectively). Also, acupuncture group showed improvement of global health status and physical functioning score assessed with EORTC QLQ-C30 (p=0.004 and p=0.027, respectively). Four weeks after the treatment, these trends were retained. No adverse events were notified

**Conclusion :** Acupuncture may have feasibility and safety for alleviate hot flashes of Korean women breast cancer patients taking tamoxifen as an adjuvant endocrine therapy. Long-term follow up results and further study with a larger sample size is required.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-17

Effects of depression, anxiety, and sexual functioning on quality of life among young breast cancer patients in Mexico

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**Background:** Despite the disproportionately high-rates of breast cancer (BC) in young women in Mexico, cancer-control efforts have been predominantly aimed at improving oncological treatment, bypassing survivorship issues and supportive care for this group. The “Joven & Fuerte” cohort, the first supportive care and research program for young BC patients in Latin America, aims to describe and assess the burden of BC in young Mexican women. In this study, we focused on evaluating the association between quality of life (QoL) and anxiety, depression, and sexual functioning in young women with BC ( $\leq 40$  years).

**Methods:** This study included non-metastatic and non-recurrent patients belonging to the cohort's pilot phase. QoL was assessed with the EORTC QLQ-C30 global score. Patients were classified in the domains of anxiety and depression with the Hospital Anxiety and Depression Scale (HADS) as either probable case, doubtful case, or not a case. Sexual functioning was assessed with the Female Sexual Function Index (FSFI) and the sexual functioning and enjoyment domains of the EORTC QLQ-BR23. Assessments were performed at baseline, 6 months, 1 year, and 2 years. Pearson chi-square and analysis of variance (ANOVA) were used for analysis. Nominal unadjusted significance is reported with  $p \leq 0.05$ .

**Results:** 73 out of 96 (76%) pilot phase patients met the inclusion criteria and had complete assessments up to 2 years follow-up. Global QoL was significantly worse for cases with anxiety and depression at baseline (means for non-cases, doubtful cases and cases, respectively: for anxiety, 81.09, 69.54, and 61.54,  $p < .001$ ; and for depression, 75.63, 64.17, and 55.00,  $p = 0.01$ ) and depression at 6 months (76.55, 66.67, and 35.42, respectively,  $p < .001$ ). Classification of case level anxiety was associated with FSFI morbidity during the first year (baseline,  $p = 0.03$ ; 6 months,  $p = 0.09$ ; 1 year,  $p = 0.04$ ). There was no significant association between case level depression and FSFI morbidity in the first 2 years. Neither anxiety nor depression was generally associated with significantly different BR23 sexual functioning or sexual enjoyment; however, a sporadic association was observed between anxiety and BR23 sexual functioning at 6 months ( $p = 0.04$ ).

**Conclusion:** This study confirmed an association between anxiety and/or depression and worse QoL at diagnosis of BC and after 6 months. Additionally, worse sexual function was significantly associated with the classification of case level anxiety. These findings support the current recommendation that physicians should regularly assess patients' psychosocial health and sexual functioning and provide prompt referral to corresponding supportive care services. Additional efforts must be conducted in low-resource settings, where sexual health and psychosocial care are not considered routine cancer treatment. Dedicated programs that promote multidisciplinary and supportive care services, such as “Joven & Fuerte”, should be incorporated into institutional health-care protocols to systematically address patients' emerging needs and improve QoL.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-18

Measuring severe anxiety in patients with metastatic breast cancer: Living up to the PROMIS

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## Purpose/Objective(s):

Symptom burden in the advanced cancer patient can be substantial. PROMIS (Patient Reported Outcome Measurement Information System) is a powerful PRO symptom assessment tool that is increasingly being utilized at the point of care through patient-facing electronic health record (EHR) systems. However, the PROMIS threshold (T-score) for detecting severe anxiety has not been previously validated for patients with advanced breast cancer. The sensitivity/specificity of current expert derive threshold T-score of  $\geq 75$  in this specific population is not known. In this study, we compared PROMIS scores with the PRO-CTCAE anxiety instrument—a standard PRO tool used in the setting of clinical trials that assesses anxiety frequency, severity, and interference with usual activities—to examine the best T-score to use for PROMIS to guide management in advanced breast cancer care.

## Materials/Methods:

A total of 84 unique visits of 74 English speaking patients on active cancer treatment for metastatic breast cancer were assessed using electronic PROMIS CAT (Computer Adaptive Test) and PRO-CTCAE at the UCSF Carol Buck Breast Cancer Center. Assessment forms were displayed on a touch screen tablet and completed by the patient at check-in for weekly treatment visits. PRO-CTCAE anxiety questions were graded on a scale of 1 (none) to 5 (disabling). Any score of grade  $\geq 4$  (severe to disabling) for frequency, severity or interference was defined as severe anxiety.

## Results:

There was a strong correlation of PROMIS T-score and maximum PRO-CTCAE grade (highest grade of frequency, severity, or interference); Spearman correlation coefficient = 0.77 ( $P = <0.001$ ). No patients reached the expert derived severe threshold of PROMIS T-score 75. Highest PROMIS T-score was 69 (range, 33-69). 11 patients reported severe or disabling anxiety by PRO-CTCAE at one visit. Area under the ROC analysis identified a cutoff that maximizes Youden's index at 55.05—sensitivity = 100% (95% CI) and specificity = 63% (95% CI).

## Conclusion:

Electronic systems with integrated patient reported outcomes (PROs) along with validated thresholds to identify severe anxiety will allow for systematic identification of symptoms at point of care and the opportunity to respond rapidly to those symptoms most distressing to patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-19

E-DomSanté study on the improvement of care in oncology through digital technology

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**Background:** The effectiveness of remote follow-up of cancer patients through weekly medical questionnaires filled in on their smartphone has been reported in international studies. These studies demonstrated an improvement in quality of life (QoL) and survival of several months. The e-DomSanté study proposes the combined use of innovative technological tools for the improvement of patient care.

**Patients and methods:** This 2-year pilot study was carried out with patients followed-up for metastatic breast cancer who lived far from their treatment center. They were offered, in addition to their usual follow-up, weekly medical questionnaires (10 items) on an interactive tablet and a connected watch that registered their falls, bedtime and their general activity. All this data arrived on a secure portal. In the event of an alert, telemedicine was organized by remote consultation with exchange on a secure platform, in parallel, between the center's carers and the treating physician, nurse, pharmacist or the Territorial Support Unit. The evaluation criteria used included telemedicine and patients' QoL (FACT-B questionnaires) as well as their satisfaction with their care.

**Results:** The average age of the 15 patients included in the study was 65 years (range, 36-82 years) with one third of patients who had never used a computer or tablet. Those patients as well as elderly patients ( $\geq 75$  years old) adapted very well to the technology simply with reinforced education and modification of the ergonomics of tablets and questionnaires. The requests for remote consultation were mainly due to 1) symptoms of deteriorating disease detected in advance, 2) toxicities relating to the treatment making it possible to adapt the therapy quickly, 3) the support oncological care (pain, depression or taken into terminal care at home) and 4) exhausted relatives. The QoL of all patients was stable or even improved despite progression of their metastatic disease in most cases. Practically all patients were satisfied (less fatigue due to less travel, high responsiveness of carers, security and confidence, less stress) with just a few criticisms (stress generated by the technology, no space for comments in the questionnaires and the restrictive nature of wearing the watch).

**Conclusions:** The e-DomSanté study confirms the contribution of digital technology in improving cancer care. This system avoids unnecessary consultations that are tiring for the patient and costly for society (transportation and hospitalization). It also avoids acute admissions through the emergency room. This method leads to an improvement in the QoL of patients and their satisfaction with their care. A second, much larger, multicenter randomized trial assessing patients' survival, QoL of patients and their relatives and medico-economic assessment will commence soon.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-20

Patient reported supportive care needs in a dedicated advanced breast cancer center

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**Background:** As of January 1<sup>st</sup>, 2017 nearly 154,794 women in the US will be living with metastatic breast cancer (MBC) and rates are expected to increase. Additionally, 58.3% of individuals with MBC, and their caregivers, believe that people with MBC have unique informational, emotional and physical needs that are unmet. However, there are still few research studies examining these needs.

**Methods:** In October of 2016, the Koontz Center for Advanced Breast Cancer was established in Kansas City, MO. Supportive services assume a large role in this center to assist in caring for the whole person and improving quality of life. During the initial consult, the patient meets with the medical oncologist, psychologist, social worker, nutritionist, exercise physiologist and chaplain. All patients complete forms assessing multiple domains that may influence the patient's treatment and outcomes. We use the PROMIS (Patient-Reported Outcome Measurement Information System) measures to assess sleep, physical function, fatigue and pain interference, the Daily Spiritual Experience Scale (DSES) to assess spiritual concerns, the DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure-Adult to assess mental health concerns and Koontz Center forms to assess social work and nutritional concerns. The following data is from assessments collected through May of 2018.

**Results:** Eighty-two individuals were included in the analysis. The mean age was 60.1 ( $SD=10.6$ ) and 98% ( $n=80$ ) were female. A majority were white, non-Hispanic (88%;  $n=71$ ). Sleep related impairment was mild for 27 (33.8%) individuals, moderate for 22 (27.5%) and severe for 3 (3.8%). For physical function, 12 (14.8%) individuals had mild, 32 (39.5%) moderate and 19 (23.5%) severe impairment. Twelve (15%) individuals had mild, 25 (31.3%) moderate and 6 (7.5%) severe symptoms of fatigue. For pain interference, 14 (17.3%) individuals had mild, 24 (29.6%) moderate and 9 (11.1%) severe symptoms. The average DSES score was 12.6 ( $SD=6.8$ ) out of 36 with lower scores indicating stronger spiritual satisfaction. The top three mental health concerns were anxiety ( $n=47$ ; 58.8%), depression ( $n=40$ ; 50%) and somatic symptoms ( $n=38$ ; 47.5%). Seventeen percent ( $n=14$ ) reported concern over weight and 30% ( $n=24$ ) reported losing weight over the past two weeks. The top three areas individuals reported needing assistance in were finding financial resources ( $n=16$ ; 20.5%), help at home ( $n=11$ ; 14.1%) and insurance questions ( $n=10$ ; 12.8%). The top four areas individuals reported needing support in were information on support groups ( $n=20$ ; 25.6%), managing stress ( $n=20$ ; 25.6%), coping with cancer diagnosis ( $n=14$ ; 17.9%) and communicating with children about cancer ( $n=14$ ; 17.9%). Also, 38% ( $n=28$ ) endorsed wanting information about an advanced directive. Changes across age and symptom complexes were also noted and will be presented.

**Conclusion:** The data indicate that there are a wide variety of physical, spiritual, nutritional, social and psychological concerns for those coping with a MBC diagnosis. Future treatment of those with MBC should integrate supportive services to address these symptoms.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-21

Analysis of cognitive function in elderly HER2-positive breast cancer patients receiving either trastuzumab monotherapy or trastuzumab plus chemotherapy as a postoperative adjuvant treatment: A cognitive function sub-study of a randomized, open-label, phase 3 clinical trial (RESPECT trial)

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**OBJECTIVE:** The effect of trastuzumab(Tmab) or chemotherapy on cognitive function has not been fully understood, especially in elderly breast cancer patients. The RESPECT trial compared 1-year(yr) Tmab monotherapy with Tmab plus standard chemotherapy as adjuvant therapy in elderly patients with HER2-positive breast cancer. The primary objective was to verify the noninferiority of 1-yr Tmab monotherapy compared to Tmab plus chemotherapy in terms of disease-free survival, and the planned analysis showed that the difference of restricted mean survival time between two groups at 3 yrs was 0.45 months (Sawaki at ASCO2018). The goal of this report was to assess the impact of the treatment groups on longitudinal cognitive function.

**PATIENTS AND METHODS:** The study was performed with patients from 99 hospitals in Japan. Elderly women with HER2-positive, stage I-IIIa invasive breast cancer surgery treated with clear resection margins were randomly assigned to either receive 1-yr Tmab or 1-yr Tmab plus standard chemotherapy. 15 institutions participated in the cognitive sub-study. Cognitive function was assessed using the Mini-Mental State Examination (MMSE) at baseline and at 1 and 3 yrs after treatment. The primary outcome was the amount of change in the MMSE score from the baseline. A linear mixed-effects model was used for comparisons of change in the MMSE score between groups, controlling for time and baseline score. Secondary outcomes were the proportion of both suspected mild dementia (MMSE $\leq$ 27) and dementia (MMSE $\leq$ 23) at each time point.

**RESULTS:** Between October 2009 and October 2014, 275 patients were enrolled in the RESPECT trial, and 57 patients were enrolled in the cognitive function sub-study with 2 patients subsequently excluded. The 55-patient sub-study comprised 29 patients assigned to the Tmab monotherapy group and 26 patients assigned to the Tmab plus chemotherapy group. Primary analysis revealed that change in the MMSE score was not significantly different between the two groups (difference -0.6 at 1 yr and -0.9 at 3 yrs; p=0.136), whereas the baseline score was the only significant factor that had an effect on the amount of change in the MMSE score (p<0.001). The proportions of suspected mild dementia at baseline, and at 1 yr and 3 yrs were 15.4, 32.0, and 41.7% in the Tmab monotherapy group, and 45.8, 17.6, and 28.6% in the Tmab plus chemotherapy group. The proportions of suspected mild dementia at baseline were significantly higher in the Tmab plus chemotherapy group (p=0.04). The proportions of suspected dementia at baseline, and at 1 yr and 3 yrs were 0%, 0%, and 4.2% in the Tmab monotherapy group, and 4.2%, 0%, and 4.8% in the Tmab plus chemotherapy group. There were no significant differences in the proportions of suspected dementia between the treatment groups at each time point.

**CONCLUSION:** Postoperative chemotherapy for elderly breast cancer patients was considered to have little effect on the onset of dementia during the follow-up period of 3 yrs. Further long-term observation is necessary to obtain a significant conclusion.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-22

Aromatase inhibitors are significantly better tolerated by early stage breast cancer patients 75 or older and with significantly lower early discontinuation rate

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## Introduction:

Breast cancer is the most common cancer in women. In postmenopausal women with early stage estrogen receptor positive breast cancer, aromatase inhibitors (AI) are a common treatment option. AI's are reported to lead to a high early discontinuation rate in younger post-menopausal women due to poor tolerance. The most common side effects reported to lead to early discontinuation are arthralgia, hot flashes, fatigue, and night sweats. The reported tolerance to AI therapy in women age 75 or older is not well documented. Our study looks at women, ages 75 and older, diagnosed with early stage breast cancer who were placed on adjuvant AI therapy and focuses on tolerability, incidence of common side effects, rate of treatment changes, and on discontinuation rates.

## Objective:

This study evaluates the tolerability, treatment side effects and the discontinuation rate of AI in women over the age of 75 with early stage breast cancer.

## Methods:

Our study is a retrospective chart review of 58 patients' ages 75 to 95 with early stage breast cancer treated with adjuvant AI. Charts of patients were reviewed and duration of treatment, patient reported side effects, treatment changes, and discontinuation rate were recorded.

## Results:

Data analysis showed that 36/55 (65.5%) of patients did not report significant side effects to AI. 6/55 (10.9%) patients required therapy changes due to side effects. 5/6 required one treatment change and 1/6 required multiple treatment changes. In 5/6 therapy was changed to another AI. Only 2/55 (3.6%) of patients discontinued therapy. In both patients who discontinued AI, therapy was discontinued due to medical complications unrelated to AI therapy. Average time to discontinuation was 11 months. The most common reported side effects were arthralgia 9/55 (16.4%), fatigue 3/55 (5.5%), hot flashes 4/55 (7.3%), rash 3/55 (5.5%) and hair thinning 3/55 (5.5%). The most common reported side effect which led to treatment change was arthralgia 4/6 (66.7%). With a median follow up time of 24 months, breast cancer specific mortality was 1/55 (1.8%).

## Reported Side Effects on AI

n=55 patients	Patients who noted symptoms	Patients who changed therapy due to symptoms
Fatigue	3	0
Arthralgia	9	4
Hot flashes	4	0
Rash	3	1
Vaginal dryness	1	0
Hair thinning	3	1

## Conclusion:

Our study evaluated the tolerance of AI in older women diagnosed with early stage breast cancer. 36/55 (65.5%) of elderly patients reported no significant side effects suggesting that AI's are well tolerated in this population and the known side effects

are significantly less common than previously reported in a younger cohort in whom arthralgia as well as vasomotor symptoms affect as many as 30% of women. This improved tolerance led to a significantly lower early discontinuation rate than previously reported in the younger cohort: discontinuation rate of 3.6% by 24 months in the 75 or older population versus 20% by 24 months in the younger cohort of post-menopausal women treated with AI.

Reference:

1 Wagner, L.I., Zhao, F., Goss, P.E. et al. Breast Cancer Res Treat (2018) 169: 537.

<https://doi-org.proxy.library.stonybrook.edu/10.1007/s10549-018-4713-2>

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-23

Patient care in breast cancer: Unmet and fulfilled needs

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**Background** Comprehensive cancer care uses complementary approaches alongside specific anticancer therapy. Using a dedicated questionnaire, the Calista 2 national survey sought to assess the importance of supportive care and activities among breast cancer (BC) patients, how often these services are made available, the uptake rate, and the proportion of unmet needs.

**Methods** Of the 82 physicians who accepted to take part in the survey, 29 recruited 257 patients with BC of whom 210 answered the patient-reported questionnaire. Patients meeting the inclusion criteria were adult females already on specific therapy for early or advanced BC. The patient-reported questionnaires covered drug management of pain, fatigue, adverse events (AE), sleep disorders, social and psychological support, physical activities, and complementary and alternative medicines. Items were rated on a scale of 0 – 10. Questionnaires were collected between September 2016 and October 2017.

**Results** After exclusion of non-valid patient questionnaires, 197 were analyzed. The mean age of these patients was 56.8 years (SD 12.6); 53% had early stage disease and 41% advanced stage disease. Patients perceived the management of AE and pain, and self-image improvement techniques as the three most important items (8.0, 7.5, 6.7, respectively), followed by physical activity (6.3) and the management of fatigue (6.0), while preservation of fertility (2.3), spiritual support (2.5), counselling with regards to employment (3.2), and art therapy (3.3) were the least important. Most facilities were available at the point of care. Physicians frequently suggested management AE and pain (83% and 73%, respectively), self-image improvement techniques (73%) and psychological support for the patient (70%). Management of fatigue was however far less frequently proposed (30%). Management of AE (75%) and pain (60%), and self-image improvement (50%) were the most widely used support techniques. Only 19% of patients who were offered support in the management of fatigue declared actually using it. The management of fatigue nevertheless represented one of the three main unmet needs (for 27% of patients), together with complementary medicines (37%) and relaxation (29%).

**Conclusion** These key findings highlight the fact that support for the management of AE and pain, together with self-image improvement techniques, are important for patients, are available, suggested and used. Although management of fatigue is available, it is rarely suggested by physicians and is therefore seen by patients as an unmet need. Patients also expressed the need for complementary medicines and relaxation techniques; these are however less frequently available at the point of care and seldom proposed.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-24

Aromatase inhibitors and bone health in women 75 and older treated for early stage breast cancer

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## Introduction:

Breast cancer is the most common cancer in women. In estrogen receptor positive breast cancers aromatase inhibitors (AI) are a common treatment option. AIs are associated with a reduction in bone mineral density, and patients with osteopenia at baseline have a higher risk of developing subsequent osteoporosis while on AI therapy. Women age 75 and older are a fast growing subset of breast cancer patients and commonly have osteopenia or osteoporosis at time of breast cancer diagnosis. Studies of long-term effects of AI on bone density in these older women who are at higher risk of osteoporosis and musculoskeletal events are lacking at this time.

## Objective:

To evaluate the objective change in bone density in women over the age of 75 diagnosed with early stage breast cancer and treated with AI.

## Methods:

A retrospective chart review of 49 patients ages 75 to 95 diagnosed with early stage breast cancer and treated with AI. Pretreatment DEXA scan results were recorded as well as prevalence of bone targeted therapy at the time of breast cancer diagnosis. Incidence of bone targeted therapy initiated subsequent to cancer diagnosis and changes in T score on follow up DEXA scans were collected as well. Incidence of musculoskeletal events and osteonecrosis of the jaw were recorded.

## Results:

40/49 (81.6%) of study women were found to have osteopenia (23/49 [46.9%]) or osteoporosis (17/49 [34.7%]) on pre-treatment DEXA scans. Only 16/49 (32.7%) of patients were on bone-targeted treatment prior to breast cancer diagnosis. Of the patient with baseline osteoporosis, only 4/17 (23.5%) were on bone targeted treatment prior to breast cancer diagnosis. 25/49 (51%) of women were initiated on bone targeted therapy subsequent to breast cancer diagnosis and following review of pretreatment DEXA scan results. 5/49 (10.2%) of women were started on bisphosphonates and 7/49 (14.3%) were started on Denosumab. On the first subsequent DEXA scan at a median follow up of 2 years, 14/21 (66.7%) of women were noted to have stable DEXA findings (defined as change in T score less than 0.5). 7/21 (33.3%) had a worsening T score on repeat DEXA. Of those patients with worsening T score, 3/7 (42.9%) changed categories (either from normal density to osteopenia or from osteopenia to osteoporosis). 3/49 (6%) of patients sustained a fracture while on AI therapy. There were no reported events of osteonecrosis of the jaw.

	Subsequent DEXA showing stability	Subsequent DEXA showing worsening T score	Fracture
<b>Baseline Normal bone density</b>	1 (n=3)	2 (n=3)	1 (n=9)
<b>Baseline Osteopenia</b>	9 (n=12)	3 (n=12)	0 (n=12)
<b>Baseline Osteoporosis</b>	4 (n=6)	2 (n=6)	2 (n=17)

## Conclusion:

Many elderly women are found to have osteopenia or osteoporosis at the time of breast cancer diagnosis and AI initiation. Most elderly patients had stable findings on subsequent bone density testing. Women with known osteoporosis initiated on bone-targeted therapy and AI did not have significant worsening in bone health. With appropriate treatment and monitoring elderly

women with baseline decreased bone density can be treated safely with aromatase inhibitors.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-11-25

The importance of social support and self-esteem in quality of life in patients with breast cancer

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## BACKGROUND:

Among the changes that have occurred in the practice of oncology in the last decade is the recognition that the patient well-being is important for the treatment. Another one was to have a greater focus on Quality of Life (QoL) and its questionnaires to evaluate this well-being.

Self-esteem is a factor that can be promoted through integrative oncology, through support groups and other forms of social support (such as meditation, yoga, massage, recreation, etc.). It is believed that promoting self-esteem is of great value for the improvement of patients' Quality of Life.

Integrative oncology coordinates the delivery of evidence-based complementary therapies with conventional cancer care. Complementary therapies encompass a broad range of mind and body practices, natural products, and lifestyle modifications, and are commonly used by patients with breast cancer and survivors of breast cancer.

## METHODS:

A prospective cohort study conducted at three institutions to compare the patients' responses to the Rosenberg Self-Esteem Scale (RSS - 12) questionnaire, before and after watching two videos of SOCIAL SUPPORT, where women received care for the promotion of increase of SELF-ESTEEM.

The study included 109 patients with breast cancer in different stages of the disease, and it was carried out between December 2017 and June 2018. For all the patients the Free and Informed Consent Term was applied.

## RESULTS:

18% of the patients were between 30-40 years old, 28% between 40-50, 28% between 50-60 and 26% had more than 60 years. 55% were in neoadjuvant or adjuvant treatment, while the rest were in palliative treatment. 94% of the patients reported having family support during treatment and 48% of the patients were married.

Of the 10 questions in the questionnaire, four presented a positive change in more than 35% after applying the questionnaire. In these four, the remaining percentage remained with unchanged answers. The four questions are those of numbers 4, 8, 9 and 10 and refer to: 4) ability to do things with the same quality as the general population; 8) feeling of self-respect; 9) feeling useless and 10) feeling a good person at all. It is important to emphasize that question 8 presented a positive change in 53% of respondents. In the rest of the questions, most of the other questions remained unchanged in more than 30% of the time.

## CONCLUSION:

It can be suggested that projects carried out for the promotion of social support must be carried out continuously so that the well-being is promoted in a continuous and permanent way, thus leading to an increase in the quality of life of patients in general.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-12-03

Prevalence, predictive factors, and clinical outcomes of anthracycline induced cardiac dysfunction among patients with breast cancer in Japan, where the normal body weight ( $BMI \leq 25$ ) is dominant

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**Objective:** Cardiac dysfunction (CD) is a major clinical problem for survivors of breast cancer. ASCO released a guideline for prevention and monitoring CD in survivors of adult cancer in 2017. Exposure to anthracycline (A) and trastuzumab are both risk factor for CD, as well as obesity is a part of multiple risk factors in the guideline. Meta-analysis shown obesity itself increases risk of A induced CD (A-CD). Prevalence of obesity and CD among non-oncology patients vary in countries, as many Western countries have obesity dominant population. Then little is known about clinical characteristics of A-CD in survivors of adult cancer among normal weight dominant countries, especially in Asia. This study was conducted to understand characteristics of A-CD among patients with breast cancer in Japan.

**Method:** This study used electrical charts, breast oncology database, and cardiology database to find prevalence, predictive factors, and clinical outcomes of A-CD in Hyogo Cancer Center. The definition of CD is based on diagnosis by the cardiologist. Major Cardiac Events (MACE) is defined as cardiac death or emergency admission due to CD. Obesity is defined as  $BMI \geq 30$ , normal body weight is defined as  $BMI \leq 25$ , and elderly is defined as age  $\geq 60$  years old, same as in ASCO guideline. Patients gave written informed consent. IRB approved this study.

**Result:** From Apr. 2006, to Mar. 2017, 855 patients received A for the treatment of breast cancer. Median body weight was 55 Kg, median BMI was 23, and 93.4 % of patients are non-obese. Half of patients (46.9 %) are elderly. Almost a quarter (24 %) of patients received trastuzumab. At the median follow up 60 months, 20 patients (2.3 %) experienced CD, one patient (0.11 %) passed away due to CD, and four patients were admitted as emergency, then five patients (0.58 %) experienced MACE. Median time to onset of CD after the last dose of A is seven months. Among patients with CD, 18 patients (90 %) recovered their ejection fraction (EF), and the median time to recover of EF was two months. Predictive factors for CD include usage of trastuzumab (15 patients), elderly (eight patients), high dose anthracycline (four patients), and multiple cardiac risk factors at base line (four patients). Among patients treated without trastuzumab, only five (0.76 %) patients experienced CD, but four of them experienced MACE.

**Conclusion:** Prevalence of A-CD in the normal weight dominant population was lower than reported in obesity dominant population, especially in patients treated without trastuzumab. In this population, clinical outcome such as prevalence of MACE may vary depending on the usage of trastuzumab. Further study is warranted to set an optimal strategy for the prevention and monitoring of A-CD in non-obese dominant population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-12-04

Preliminary evaluation of a mind-body intervention to improve body and/or self-image: A phase II randomized trial

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**Background:** The number of cancer survivors is growing and expected to be 18.9 million by 2024. Addressing survivorship issues, such as sexual health, are a critical part of health promotion. Women diagnosed with breast or gynecologic cancer can experience distressing changes in their self and/or body image, which affects their sexual health. This study evaluates hypnosis compared to progressive muscle relaxation (PMR) to improve self/body image in order to improve sexuality.

**Methods:** Women with a history of breast or gynecologic cancer, who also report negative body image changes, were randomized in a 2:1 fashion to a 6-week intervention of hypnotic relaxation or PMR. Both intervention arms consisted of three 30-minute sessions delivered face-to-face by a trained therapist, one every two weeks, along with home practice using a CD. The primary outcome was impact of treatment on body image using the Impact of Treatment Scale (ITS). Secondary outcomes included mood (Positive/Negative Affect Scale-PANAS), sexual satisfaction (PROMIS satisfaction), and perceived change (Global Impression of Change Scale -GCIS). Outcomes were measured at baseline and 6 weeks except for the GCIS measured only at 6 weeks. A series of independent samples t-tests were used to compare changes in outcome measures between arms. The intention-to-treat principle was applied.

**Results:** The final randomized sample consisted of 87 women. There was no statistically significant difference ( $p=.15$ ) in the change in ITS between groups at 6 weeks, with both groups significantly improving (within group effect size Cohen's  $d$  .49 - .75). There were non-significant differences between groups on secondary outcomes. Change from baseline for positive affect (PANAS) was 1.7 in the hypnosis group and 3.8 in the PMR group while negative affect change was very similar being about 2.8 in both groups. Change from baseline for the PROMIS general sexual satisfaction scale was 1.5 in the hypnosis group and 1.3 in the PMR group. On the GCIS at 6 weeks, 42% of the hypnosis group reported moderate to very much improvement on self/body image (GCIS) while only 32% of the PMR group reported this level of improvement; and 36% of the hypnosis group versus 11% of the PMR group reported moderate to very much improvement in their sexuality.

**Implications:** The stress relieving strategies applied in this study may contribute substantially to decreasing the distress of the cancer experience related to altered self/body image and sexuality. The variable improvement across domains suggests that hypnosis and PMR may work in different ways, mechanistically. Further mechanistic studies of interventions to optimally promote survivorship health are warranted.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-12-05

A qualitative exploration of self-developed and peer-recommended techniques used by women with breast cancer to improve sexual functioning during and after treatment

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**Background:** Coping with sexual dysfunction during and after breast cancer treatment is a persistent challenge for many women, even if clinicians offer standard sexual rehabilitative therapies (e.g. lubricants, counseling). This study sought to explore how women with breast cancer supplement clinician recommendations with self-developed and peer-recommended techniques for improving sexual function, what those techniques are, and how well they work.

**Methods:** We conducted a cross-sectional, online survey of 501 adult women with stage I-IV breast cancer who were members of the Breastcancer.org community. Open-ended survey items asked women to describe any techniques used to improve sexual function during and after breast cancer treatment beyond those recommended by clinicians. Closed-ended items asked women to assess the source and perceived efficacy of their techniques. We used qualitative content analysis to extract themes that described women's techniques and calculated frequencies in StataMP 15 to quantify sources and efficacy levels.

**Results:** Participants were, on average, age 53 (range 30-79) and 10 years from diagnosis. Most were partnered (90%), heterosexual (96%), with stage I/II disease (73%). 174/501 (35%) women reported using a sexual self-management technique they developed themselves or that was recommended by someone other than a clinician. Emergent themes in techniques included: 1) pain reduction: trial-and-error to find an effective lubricant or moisturizer (e.g. coconut oil), changing sex positions, choosing oral sex over intercourse 2) intimacy enhancement: open partner communication, planning sex 3) arousal enhancement: masturbation, erotica, vibrator use 4) emotional coping: adopting an attitude of persistence vs. acceptance of loss of sex life, encouraging partners to use sexual surrogates. 77 women developed the technique themselves, 54 with partners, 37 heard about it from survivors, 36 read about it online. 45% of women rated their techniques as moderately or more effective when used in addition to or instead of standard therapies offered by clinicians.

**Conclusion:** In a survey of an Internet-based community of women treated for breast cancer, women reported a variety of successful techniques for increasing intimacy and arousal, reducing vaginal pain, and coping emotionally with changes in sexual life after breast cancer. More women reported developing these techniques on their own or with partners vs. learning them from others. Given that standard therapies are often insufficient to manage sexual dysfunction during and after breast cancer treatment, clinicians should address sexual function during follow-up care and encourage women's safe experimentation with techniques for improving sexual function. Clinicians can refer patients to platforms like Breastcancer.org for peer-to-peer support and information exchange. Existing self-developed and peer-recommended techniques should be evaluated for safety, quality, and generalizability. Future research can then assess the effectiveness of particularly novel techniques as a complement to standard, clinician-developed therapies for the broader population of women with breast cancer experiencing sexual dysfunction.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-12-06

Endothelial dysfunction in breast cancer survivors on aromatase inhibitors (AIs) over time

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## Endothelial dysfunction in breast cancer survivors on aromatase inhibitors (AIs) over time

**Background:** AIs reduce breast cancer-related mortality however they may increase cardiovascular (CV) risk. Our previously published cross-sectional study suggested women on AIs were more likely to have endothelial dysfunction when measured by EndoPAT ratio as compared to healthy postmenopausal women. Reductions in EndoPAT ratio (<1.67) and small artery elasticity (SAE) and increases in highly sensitive C-reactive protein (CRP) are associated with worsening endothelial dysfunction and increased cardiovascular events. We present data from a longitudinal pilot study looking at endothelial dysfunction over time in women on AIs.

**Methods:** Fourteen women with locally advanced breast cancer prescribed an AI underwent vascular testing at baseline (pre-AI) and at 6 months. Subjects with tobacco use, hypertension or hyperlipidemia were excluded. Consented subjects underwent biomarker analysis and radial artery pulse wave analysis using the HDI/Pulse Wave CR-2000 CV Profiling System and pulse contour analysis using the Endo-PAT2000 system. Biomarkers were obtained using a fasting blood draw to evaluate the following lipids and inflammatory markers: serum ultrasensitive estradiol, serum glucose, total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), and triglycerides (TG), CRP, plasminogen-activator 1 (PA1), and tissue-type plasminogen activator (tPA). Changes between baseline and follow-up using Wilcoxon signed-rank tests were analyzed.

**Results:** Mean baseline age was 59 years and median body mass index was 26.5 kg/m<sup>2</sup>. Median systolic blood pressure and total cholesterol were 120/70 mm/Hg and 228 mg/dL, respectively. Baseline ultrasensitive estradiol levels were 7 pg/mL and hsCRP was 2.45 mg/dL. Prior to AI therapy, endoPAT ratio was 2.18 (1.19, 2.43). After six months, EndoPAT ratio declined to a median 1.12 (0.85, 1.86) (p=0.045). There were no statistically significant changes in serum glucose, TC, LDL, HDL, hsCRP, PA1 and tPA. HsCRP remained elevated at median 2.98 mg/L. At six months, estradiol levels decreased to a median of 2 pg/mL (p=0.052), however, there appeared to be no linear association between changes in EndoPAT and estradiol (p=0.91).

**Conclusion:** Breast cancer survivors on AIs have endothelial dysfunction, a predictor of adverse CV disease. These changes develop while on AIs. Underlying pathophysiology requires further evaluation.

## Cardiovascular markers

Measures	Baseline	Follow-Up at 6 Months	Change	P-value
BMI (kg/m <sup>2</sup> )	26.5 (24.4, 31.6)	27.1 (23.9, 32.9)	0.5 (0.0, 1.3)	0.056
SBP (mmHg)	120 (115, 124)	123 (114, 127)	-0.8 (-7.4, 3.6)	0.91
DBP (mmHg)	70 (61, 73)	69 (62, 71)	0.0 (-3.0, 2.6)	0.88
Total Cholesterol (mg/dL)	228 (202, 244)	213 (210, 229)	-1 (-18, 27)	0.70
HDL (mg/dL)	64 (58, 69)	73 (61, 77)	2 (-3, 14)	0.44
LDL (mg/dL)	143 (121, 159)	129 (120, 142)	6 (-11, 14)	0.65
Estradiol (pg/mL)	7 (4, 15)	2 (2, 3)	-8 (-12, -3)	0.05
hsCRP (mg/dL)	2.45 (1.14, 6.07)	2.98 (0.90, 4.81)	-8 (-12, -3)	0.85
EndoPAT Ratio	2.18 (1.19, 2.43)	1.12 (0.85, 1.86)	-0.16 (-1.45, -0.02)	0.045

1. Summaries shown are median (1st quartile, 3rd quartile).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-12-07

Prospective 36-month follow-up to determine changes in body mass index and weight among Chinese breast cancer survivors

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**Background:** Anticancer treatment for breast cancer has been associated with weight gain but such observation has mainly been reported in western patients. More recent data in Asian patients have inconsistent findings. Factors including socio-demographic, clinical and lifestyle may be associated with post-diagnosis weight gain. In this prospective cohort study of women with breast cancer, the objectives were to determine the body mass index (BMI) and weight changes over 36 months after initial diagnosis and the factors associated with such changes.

**Methods:** Chinese women with newly diagnosed early-stage breast cancer were recruited. Individual woman had her weight measured at breast cancer diagnosis (W0), at study entry (W1) and at 36-month follow-up (W2). Body height was measured at W0. We evaluated change in weight and body mass index (BMI) before and after breast cancer diagnosis. Socio-demographic, clinical and lifestyle factors were assessed to identify potential associated factors with weight changes.

**Results:** A total of 1133 women with breast cancer had detailed weight measurements at the 3 time-points of assessment. The mean age at diagnosis was 52 years. Fifty-four percent were premenopausal at W1. The proportion of patients with stage 0-I, II and III diseases were 35%, 46% and 19%, respectively.

The proportions of patients who were overweight and obese at the three assessment time-points were 21.2% and 28.5% at W0, 19.7% and 26.6% at W1, and 21.7% and 30.9% at W2 assessment, respectively. When compared to W0, the proportions of women who gained weight within 2-5kg at W1 and W2 were 2.4% and 20.6% respectively, that with weight gain of >5kg at W1 and W2 were 0.5% and 10.0% respectively; 6.1% and 19.6% of women had weight loss >2kg at W1 and W2 respectively.

Compared to W0, the median value of weight change was -0.5 kg (range: -11.4, 18.3) at W1 and 0.6 kg (range: -19.6, 20.5) at W2. On multivariate analysis, only BMI at diagnosis were significantly associated with weight change between assessments at diagnosis and W2; the median (range) for weight changes for women who were underweight, normal, overweight and obese were respectively 0.9 (-4.8, 7.6), 0.6 (-13.2, 20.5), 0.5 (-11.5, 13.0) and 0.5 (-19.6, 12.6) kg,  $p < 0.001$ .

**Conclusions:** In this prospective study of Chinese women with a history of breast cancer who were followed-up over a 36-months' period, the proportions of women with overweight and obese statuses were relatively stable; weight gain was uncommon among Hong Kong women with breast cancer during the same period. These findings are in contrast with studies conducted in the West, where weight gains were more commonly reported.

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## 2018 San Antonio Breast Cancer Symposium ®

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Association of CBR3 polymorphisms with an early change in cardiac function as assessed by left ventricular global longitudinal strain in breast cancer patients treated with doxorubicin

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Progress made in early cancer diagnosis and therapy has translated into increased longevity for patients with breast cancer. As survival has increased, the potential cardiotoxicity of cancer chemotherapy regimens has become an important issue for survivorship. Doxorubicin-induced cardiotoxicity has been demonstrated at a cumulative dose of  $\leq 300\text{mg/m}^2$ , with histopathological changes seen in endomyocardial biopsy tissue from patients receiving as little as  $240\text{mg/m}^2$  of doxorubicin. Individual risk stratification and early detection of chemotherapy-induced cardiotoxicity are crucial to prevent irreversible cardiac dysfunction. There is accumulating evidence for the utility of echocardiographic indices such as left ventricular global longitudinal strain (GLS) in the detection of early chemotherapy induced cardiac injury. We have previously highlighted a predictive role of genetic polymorphisms in the carbonyl reductase 3 gene *CBR3* in anthracycline-related cardiomyopathy following childhood cancer. Consistent with our prior work, we hypothesized that breast cancer patients homozygous for the *CBR3* V244M G allele would exhibit worsening GLS following DOX treatment when compared with patients homozygous for the A allele. We recruited 138 patients with breast cancer receiving treatment with DOX (total cumulative dose:  $240\text{mg/m}^2$ ). 72 patients received an echocardiogram analyzing global longitudinal strain by speckle tracking at baseline ( $t_{0\text{ month}}$ ) and at 6 months ( $t_{6\text{ months}}$ ) of follow up after DOX treatment. Patients were genotyped for variants associated with anthracycline-related toxicity. In agreement with our previous findings and hypothesis, our interim analysis suggested that patients homozygous for the *CBR3* V244M G allele (*CBR* V244) exhibited GL changes at 6 months after DOX therapy suggestive of cardiotoxicity in comparison to individuals homozygous for the A allele (*CBR3* M244) ( $-1.2 \pm 3.5$  vs  $1 \pm 1.6$ ; mean $\pm$ SEM,  $p=0.8$  by Mann-Whitney test). Although the differences between *CBR3* genotype groups are not significant at  $p<0.05$ , the direction and magnitude of changes in ventricular GL are well in line with various reports describing early detection of cardiotoxicity in similarly treated breast cancer patients. This study outlines a potential role for screening *CBR3* polymorphisms in patients prior to DOX therapy and highlights a need for further investigation into the pharmacogenomics of chemotherapy-induced cardiotoxicity.

# 2018 San Antonio Breast Cancer Symposium ®

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Proposal for integrating the pathologic assessment of lymphovascular invasion and extranodal tumor extension in breast cancer-related lymphedema clinical management

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Breast cancer related lymphoedema (BCRL) occurs in a substantial proportion of breast cancer survivors and is a major contributor to disability, representing a long-term threat to these patients. Given the extremely high incidence of breast cancer worldwide, and the increasing number of long-term survivors, the reduction of BCRL burden represents an urgent clinical need in women's healthcare. However, there are no validated predictive biomarkers, diagnostic tools, and strong evidence-supported therapeutic strategies for BCRL management. Here, we provide a comprehensive clinicopathological characterization of a large series of women with node-positive breast cancers and identify new bona fide predictors of BCRL occurrence.

332 cases of surgically-treated node-positive breast cancers were retrospectively collected (2-10.2 years of follow-up). Among them, 62 patients developed BCRL. To identify demographic and clinicopathologic features related to BCRL, Fisher's exact test or Chi-squared test were carried out for categorical variables; the Wilcoxon rank-sum was employed for continuous variables.

Factors associated with BCRL occurrence were assessed using a Cox proportional hazards regression model.

En-bloc dissection of the axillary lymph nodes but not the type of breast surgery impacted on BCRL development. Most of BCRL patients had a Luminal A-like neoplasm. The median number of lymph nodes involved by metastatic deposits was significantly higher in BCRL compared to the control group ( $p=0.04$ ). Both peritumoral lymphovascular invasion (LVI) and extranodal extension (ENE) of the metastasis had a negative impact on BCRL-free survival ( $p=0.01$ ). Specifically, patients with LVI and left side localization harbored 4-fold higher risk of developing BCRL, while right axillary nodes metastases with ENE increased the probability of BCRL compared to ENE-negative patients.

Here, we document that LVI and ENE have a strong predictive value for BCRL occurrence. Furthermore, we confirm that the full excision of the axillary nodes is one of the major determinants of BCRL, regardless of the extent of the surgical procedure involving the breast. In conclusion, our results suggest that the pathologic data on LVI and ENE should be integrated with information on the laterality of the tumor and the type of surgical procedure. This new integrative approach could be extremely beneficial to improve BCRL risk stratification.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-12-10

Regimen-specific rates of chemotherapy-related amenorrhea in breast cancer survivors

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**Background:** Chemotherapy can damage the ovaries and cause amenorrhea, a surrogate for infertility. Young women often wish to understand and minimize their risk of chemotherapy-related amenorrhea (CRA). However, the incidence of CRA with regimens that do not include either an anthracycline or cyclophosphamide is poorly studied. For patients with HER-2 positive disease, these anthracycline and cyclophosphamide-sparing regimens (e.g., docetaxel-carboplatin) are common (in combination with Her-2 directed therapy) in both the neoadjuvant and adjuvant settings.

**Methods:** Women diagnosed with breast cancer under age 50 and within the past 10 years were recruited through a Dr. Susan Love Research Foundation Army of Women e-mail blast. Those who provided their contact information were mailed a consent form and medical record authorization form. Participants then received a web-based survey that inquired about receipt of and type of chemotherapy (including date of last dose) and date of last menstrual period (LMP). Patient-reported LMP was compared to date of final chemotherapy dose to determine if the LMP occurred before (defined as "CRA") or after the last chemotherapy dose. When available, medical record data was used in place of survey data regarding type of chemotherapy used. Exclusion criteria included: LMP prior to diagnosis date, receipt of multiple chemotherapy regimens or no chemotherapy regimens, receipt of ovarian suppression medications (which interfere with interpretation of menstrual data), surgical menopause prior to or at the same time as diagnosis, a cancer diagnosis more than 10 years prior, incomplete menstrual data on the survey, report of an unknown chemotherapy regimen, and no date available for the last chemotherapy dose without an LMP within a month prior to survey completion. Fisher Exact test was used to compare CRA rates between regimens. Rates after two anthracycline-sparing regimens (taxane/cyclophosphamide; taxane/carboplatin) were compared to rates after anthracycline/cyclophosphamide/taxane. **Results:** 273 women consented to participate in this study, 258 of whom filled out the web survey. 151 of them were eligible for this analysis with a median age at diagnosis of 41 (range 24-49) and a median time from last chemotherapy dose to survey of 62.5 months (range 2-138). CRA occurred in 51.2% of the 86 participants who received an anthracycline, cyclophosphamide, and a taxane, in 41.9% of the 43 participants who received only a taxane and cyclophosphamide ( $p=0.35$ ), and in 13.3% of the 15 participants who received carboplatin with a taxane ( $p=0.01$ ). When the 11 patients who were <12 months since last chemotherapy were excluded, CRA rates changed minimally. Age did not differ by regimen, but median time since chemotherapy was shorter in the taxane/carboplatin group (35 months vs. 68 months). Trastuzumab with or without pertuzumab was administered in 100% of patients who received carboplatin/taxane, in 23.3% of patients who received taxane and cyclophosphamide, and in 22.1% of patients who received anthracycline/cyclophosphamide/taxane.

**Conclusions:** This study suggests that carboplatin/taxane may be substantially less gonadotoxic than cyclophosphamide-based (neo)adjuvant regimens. Further research is necessary to confirm these findings.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-12-11

Exercise interventions for breast cancer (BC) patients in rural areas: The Northern New England (NNE) experience

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## Background:

Women in rural areas are less likely to engage in physical activity (PA) compared with their urban counterparts. It can be even more challenging for BC patients due to the sequelae of their cancer and treatment. A quality improvement project in our cancer center evaluating leisure time activity among BC patients identified that about half the responders were sedentary or insufficiently active. Only one third were aware of the recommendation to achieve 150 active minutes per week while more than half reported not meeting the goal. Lack of motivation was quoted as the main reason for not meeting the goal by one-third of the responders. Goal setting has been an integral part of cardiac rehabilitation and behavior change techniques. It has shown to increase participation and motivation. We performed a combined analysis of collaborative interventions in NNE to identify if effective goal setting can overcome the barriers to PA among BC survivors.

## Methods:

5 IRB-approved interventions promoting PA among BC patients across 3 states were included. Studies were stratified according to the stages of treatment, the types of intervention and whether goal-setting was used. Pooled effects were calculated using Comprehensive Meta-Analysis software and R package metafor (v.2.0.0) with the maximum-likelihood configuration. Subgroup analyses were conducted based on the goal-setting criteria.

## Results:

The 5 studies examining 4 exercise interventions are shown in the table below.

The studies evaluating exercise interventions in BC patients

Study name	IWEB/ W12051	D12030	D1032	D12110	UVM (STW)
Region	VT/ MA/ NH	VT/ NH	VT/ NH	VT/NH	VT
Urban/ Rural Mixed	Mixed	Rural	Rural	Rural	Urban
Randomized	No	Yes	No	Yes	No
Measurement Type of intervention	Intermittent (Web-based)	Pedometer Phone call	Self-reported	Self-reported	Self-reported
Final goal defined as >150 min of PA weekly	Yes	No	No	No	Yes
Goal-setting	Yes	Yes	Yes	Yes	No
BMI eligibility	Yes	No	No	No	No
PA eligibility	No	Yes	No	No	No
Enrolled	50	28	23	59	407
Completed intervention	34	4	17	24	279
Completed/ Assigned to intervention (%)	100	14	73	41	68.6
Completed + change PA (%)	41.3	100	69	75	80.4

D12030 included patients on chemotherapy with the aim to increase activity by 10MET/week, while the W12051, UVM, D1032 and D12110 studies included patients who were post-chemotherapy. Home-based programs were used in W12051 and D12030.

Goal-setting was used in all the studies except for the UVM study which used a supervised program with self-reporting. Weighing by percentage of patients completing the program, the pooled change in PA was 56.6% (95% CI=24.2-89.1%). Pooled PA for the goal-setting subgroup was nearly twice as high as the without-goal-setting subgroup (74.3% and 39.5%, respectively).

**Conclusion:**

Cancer centers with large catchment areas need to design creative approaches to encourage PA among the BC survivors. With the lack of motivation as the most common barrier to achieving the recommended PA, goal-directed programs can be helpful by creating attainable targets as well as providing emotional and psychological support. In addition, geographical barriers need to be addressed through interventions like internet-based programs, personal tracking devices, and small-group social activities.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-12-12

Bubble packaging of adjuvant endocrine therapy: updated analysis of compliance and survival

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**Introduction:** Adherence to endocrine therapy is a long recognized problem despite efficacy of these drugs with reported compliance rates of 89% in first year and 50% in fourth year. Most of our knowledge of noncompliance is observational and retrospective. This final analysis of the Bubble Study reports the compliance rate of adjuvant endocrine therapy among women with early stage breast cancer using “bubble” packaging. We previously reported adherence rates of 97% with bubble packaging. This updated analysis includes disease free survival (DFS) and overall survival (OS) at 5 years.

**Methods:** The Bubble study is a non-blinded, prospective observational cohort study, which enrolled 86 patients between August 2012 and April 2014. Demographic and clinical data were collected prospectively including age, race, insurance, duration of therapy, stage, treatment, comorbidities, recurrence and survival. Duration of therapy was divided into 3 cohorts: <12 months, 12-36 months, and 37-60 months. All patients received routine prescriptions in a “bubble” pack or daily blister pack. Patients returned all used bubble packs at follow up appointments for review and kept a diary of missed doses for analysis. DFS and OS data were obtained at 78 months. Compliance was defined as >90% adherence. We calculated institutional DFS and OS for breast cancer patients treated within a similar time frame from the tumor registry.

**Results:** 53 patients were included in the analysis. The remaining patients withdrew from the study prior to data collection or were deemed ineligible. The overall compliance rate was 97%; however, only 72% of enrolled patients were continued in the analysis. None of the variables examined (race, age, insurance status and stage) had an impact on compliance. Only duration of endocrine therapy had a marginal effect on compliance (p value = 0.06). The latest cohort (duration of therapy 37-60 months) was least likely to be compliant at 89.53%. Our 5-year DFS is 92% and 5-year OS is 96%. There is no statistically significant difference in DFS and OS between patients with compliance >90% and <90%. For ER+ breast cancer patients treated during similar timeframe at our institution outside the trial, 5-year DFS is 94% and 5-year OS is 90%.

**Conclusion:** There was no difference in OS or DFS based on compliance to oral anti-estrogens. Given the high overall compliance rate in this small patient population, the lack of OS and DFS difference is not surprising. However, the compliance rate of bubble packaging (>90%) is higher than expected based on current literature. Although this may suggest improved compliance with bubble packaging, more studies are necessary to confirm this given small sample size and high trial dropout rate. Trial withdrawal likely altered analysis of adherence rates as it selects for a largely compliant group of patients. This bias may also explain the lack of difference in compliance rate among race, insurance status and/or age, which contradicts our current knowledge of high-risk groups. There was a trend towards lower DFS in the bubble cohort, but overall better survival when comparing to institutional rate. Studies are ongoing to confirm bubble packaging adherence rates and compare this to established strategies to improve adherence.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-12-13

Initial report of a prospective, pilot study of patient-reported upper extremity dysfunction in women undergoing radiation for breast cancer

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**Objectives:** Upper extremity dysfunction (UED) is a known side effect of breast cancer treatment. It is unclear, however, to what degree radiation contributes to this morbidity. We aimed to characterize the level of UED using patient-reported outcomes (PROs) prior to, during, and after treatment with radiation for breast cancer. Our secondary aims were to evaluate the association of UED with pain scores.

**Methods:** This is a single-institution, prospective, longitudinal cohort study of patients treated with radiation for breast cancer. The validated patient-reported outcome measure, Quick Disabilities of the Arm, Shoulder and Hand (QD) was used to capture UED prior to radiation, at the end of radiation, and 1 month following the completion of radiation. Pain scores were also collected at these intervals using the numeric pain reporting scale (NPRS) from 0 (no pain) to 10 (worst pain).

**Results:** Forty-four patients were enrolled on this study and 43 (97.7%) had completed radiation at the time of analysis. Thirteen patients (29.5%) were treated with mastectomy, axillary lymph node dissection and regional nodal irradiation in the supine position. The other 31 (70.5%) patients underwent lumpectomy and sentinel lymph node biopsy. Of these patients, 26 (83.9%) were treated in the prone position and 30 (96.8%) received whole breast irradiation. Median time from surgery to radiation was 69 days (range 35 – 212 days), 76 days for mastectomy and 68 days for lumpectomy. Median time from start to end of radiation was 38 days for mastectomy and 28 days for lumpectomy. Pre-treatment median QD score prior to radiation was 12.5 (11.4 for lumpectomy, 15.9 for mastectomy), 9.1 at the end of radiation (9.1 for lumpectomy, 18.2 for mastectomy), and 2.4 at 1 month after radiation (2.3 for lumpectomy, 2.5 for mastectomy). Median NPRS scores at pre-treatment, post-treatment and 1 month follow-up were 1, 1, and 1 for lumpectomy and 0, 1, 0 for mastectomy patients, respectively.

**Conclusion:** In this initial pilot study with 1 month of follow up, patient-reported UED as demonstrated by QD scores were higher pre-radiation and decreased by one month after. This likely reflects recuperation after surgical procedure. Median average pain scores were low at all time points. Further evaluation of UED over time to characterize the long-term effect of radiation and correlation with quality of life and other clinical factors is planned.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-12-14

Quality of life, fear of recurrence, impact of cancer, and dietary habits in breast cancer patients during the early survivorship period - Initial data from the pilot CaS-PET (Cancer Survivorship Patient Engagement Toolkit) study

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**Introduction** – With heightened public awareness, earlier detection, and better treatment, the number of breast cancer survivors continues to grow. Many of these cancer survivors have physical, psychological, social, spiritual, and financial challenges that require coordinated, comprehensive care. Patient involvement programs that focus on education and health promotion may help breast cancer survivors to manage their symptoms and take better control of their health. At the University of Maryland, a collaborative group developed the Cancer Survivorship Patient Engagement Toolkit (CaS-PET). CaS-PET includes the following components: survivorship care plans, online support program (learning modules and discussion board), and ongoing communication between providers and patients using a patient portal. The main aim of the study is to test the preliminary impact of the CaS-PET using a small sample.

**Methods** – After IRB approval, 30 cancer survivors at the University of Maryland Marlene and Stuart Greenebaum Comprehensive Cancer Center who were within 6 months of completion of active treatment were enrolled in a prospective pilot study that used a single group pre-post design. The outcome measures included changes in quality of life, fear of recurrence, impact of cancer, dietary and exercise behaviors, and selected cancer symptoms. In addition, participants' demographic characteristics, as well as Internet and patient portal experiences were also assessed at baseline. Currently, baseline surveys are completed and the 3-month follow-up data collection is ongoing.

**Results** – Among the total of 30 patients, 15 (50%) were breast cancer survivors. The median age was 57.2±13 years and all were female. Ten survivors were African American (66.7%). Most survivors received combination of surgery, chemotherapy, and radiation treatments. At baseline, the mean scores for the positive impact and negative impact of cancer were 3.69 (range, 1-5, higher means more positive) and 2.57 (1-5, higher means more negative) respectively. Among various physical and mental cancer symptoms, breast cancer patients frequently experience lack of energy, pain, bloating, sadness, and constipation. The mean score for fear of recurrence was 14.16 ±6.4 (range of 6-24, higher means more fear). The average scores for fat and fruit/vegetable consumption were 17.7±6.0 and 7.6±4.1, respectively, which indicated a diet high in fat and calories and low in fruit and vegetables.

**Discussion** – This baseline assessment shows breast cancer survivors experience significant residual symptoms following completion of active therapy. Many patients are fearful of recurrence and struggle with other negative impacts of cancer. However, patients have also found positives in their experiences. Participants have expressed enjoyment and benefit of the CaS-PET study. Full outcomes will be available in early September 2019.

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Quality of life, unmet needs and social support of patients with breast cancer during adjuvant endocrine therapy in Mexico

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**Background:** As survival increases in patients with breast cancer (BC), long-term care is required. Studies have shown that the psychosocial situation of this patients changes according to age and the types of treatment they received. Quality of life (QoL), unmet needs (UN), and social support (SS) have been significantly related to the way they live their survivorship. However, it is still unclear how these variables are affected by adjuvant endocrine therapy (AET) in Mexican patients with BC, to address and provide appropriate interventions. **Objective:** To describe the level of quality of life, unmet needs and social support, and to evaluate their relationship with the type of AET. Method: 200 patients with BC under AET were enrolled in a cross-sectional and correlational design. We administered QoL (EORTC-30 and BR-23), UN (SCNS-SF32), SS (MOS-SSS14), validated and standardized instruments in Mexican population. Premenopausal patients with ovarian function suppression were excluded. Non-parametric analyzes of U Mann Whitney and Spearman's correlation were used. **Results:** Mean age of the 186 patients evaluated was 54.52 (SD=10.65) years. Most of patients had elementary school (32.8%), were married (49.5%), housewives (60.2%), and catholics (86%). 54.8% underwent tamoxifen, 25.8% exemestane, 12.9% letrozol, 6.5% anastrozol. Mean time of AET were 2.59 years and 3.53 years since diagnosis. They reported a good level of global QoL (mean= 80 points, 0-100); however, they experienced insomnia (25.44 points), financial difficulties (24.19), fatigue (23.77), constipation (21.68) and pain (18.54); besides, adequate body image perception (91.6 points). The most UN were on information and the health system 31.13 (0-100 score), sexual (29.23) and psychological (28.21). The most frequent SS type was affection (88.46 points) and emotional-informational (79.56 points). Patients under anastrozole in comparison with exemestane, tamoxifen and letrozol reported significantly more nausea-vomiting ( $p=0.028$ ;  $p=0.041$ ;  $p=0.002$  respectively), insomnia ( $p=0.008$ ;  $p=.014$ ;  $p=0.032$ , respectively), and appetite loss ( $p=0.049$ ;  $p=0.049$ ;  $p=0.04$ , respectively). We found that patients with anastrozol perceived significantly less SS than other types of AET ( $p<0.005$ ). It highlights that patients who received letrozol perceived significantly better future perspective (QoL). Our data also shows that the higher QoL level and the fewer reported symptoms the lower the UN. **Conclusions:** These results are consistent with previous studies in terms of information and psychological UN, and its relationship with QoL. Despite the small percent of patients treated with anastrozole in our study, they had more side effects than other aromatase inhibitors. In addition, it shows that postmenopausal survivors patients with BC still have sexual UN. This study is the first on Mexico exploring differences in perception of QoL, UN and SS according AET type. We concluded that the health care professionals who attend postmenopausal survivors patients with BC in the real clinical practice, should be aware of that long-term intervention includes care to sexual and psychological UN in the same importance as to the adverse events of treatment.

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Risk factors for trastuzumab cardiotoxicity in a cohort of Mexican population

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**BACKGROUND:** Advances in chemotherapy and target therapy have made treatment of breast cancer (BC) more effective; however, survivors of **BC** suffer an increased burden of cardiotoxicity. The aim of this study is to determine the risk factors in a Mexican patients (pt)

**METHODS:**Observational study of a cohort of pt with locally BC and Her2 (+++) treated with trastuzumab (TZ). According to clinical stage, they underwent to surgery or neoadjuvant chemotherapy, scheme was at the decision of the physician. The main schemes: AC-T, EC-T, FAC-T, FEC-T, Paclitaxel, intravenous (IV) or subcutaneous TZ. The doses were according to NCCN guidelines and the route of administration of TZ was decision of the oncologist. Ventriculography was performed basally, at 4,8,12 cycles and at the end of treatment. If the pt presented a decrease in the ejection fraction>10% with/without symptoms, they received cardioprotection (metoprolol+atorvastatin). It was the oncologist decision to suspend chemotherapy as well as TZ.

**RESULTS:** 97 pt were included from Jan 2017 to Jan 2018, 35% presented cardiotoxicity. The only variables that presented a statistically significant for cardiotoxicity risk were normal weight, neoadjuvant treatment and IV TZ (table 1,2). Only 32.4% of the pt tolerated full doses of cardioprotective drugs. 13% of the pt with cardiotoxicity presented symptoms and 52.9% of the pt did not complete trastuzumab for 1 year

Table 1. Demographic characteristics of the patients (n=97)

	Total	With Cardiotoxicity (n=34)	Without Cardiotoxicity (n=63)	p
Age, years. Mean (SD)	50.36 (12.91)	51.09 (12.46)	49.97 (13.22)	0.686*
Body mass index, kg/m2. Median (range)	37.98 (21.23 -63.07)	28.15 (21.23 - 43.84)	41.58 (31.67 - 63.07)	0.0001¥
Hypertension, presence. n (%)	18 (18.6)	8 (23.5)	10 (15.9)	0.416
Diabetes, presence. n (%)	16 (16.5)	8 (23.5)	8 (12.7)	0.251
Smoking, presence. n (%)	14 (14.4)	2 (5.9)	12 (19.0)	0.128
Weight. n (%)				
Normal	3 (3.1)	3 (8.8)	0 (0)	0.0001
Overweight	22 (22.7)	22 (64.7)	0 (0)	
Obesity	72 (74.2)	9 (26.5)	63 (100)	

Abbreviation.AJCC. American Joint Committee on Cancer 2010. / \*T-student, ¥U Mann-Whitney. The rest were analyzed with X2

Table 2. Treatment characteristics (n=97)

Systemic treatment. n (%)	Total	With Cardiotoxicity (n=34)	Without Cardiotoxicity (n=63)	p
Neoadjuvant	35 (36.1)	30 (88.2)	5 (7.9)	<0.0001

Adjuvant	62 (63.9)	4 (11.8)	58 (92.1)	
Chemotherapy scheme. n (%)				0.687
AC-T	50 (51.5)	20 (58.8)	30 (49.2)	
EC -T	40 (41.2)	12 (35.3)	28 (45.9)	
FAC-T	2 (2.1)	1 (2.9)	1 (1.6)	
FEC-T	2 (2.1)	0 (0)	2 (3.2)	
Paclitaxel	3 (3.1)	1 (2.9)	2 (3.2)	
Acumulative dose antraciclone, mg. Median (range)	432 (0 - 1440)	420 (0-760)	432 (0-1440)	0.874*
Trastuzumab administration way. n (%)				0.0001
Subcutaneous	87 (89.7)	24 (70.6)	63 (100)	
Intravenous	10 (10.3)	10 (29.4)	0 (0)	
Trastuzumab cycles. Median (range)	17 (3 - 17)	13 (3 - 17)	17 (6 - 17)	0.0001*
Ejection fraction left ventricle. Median (range)	67.4 (38 - 75)	67.2 (38 - 74)	67.4 (61 - 75)	0.428*

Abreviation: \* U Mann-Whitney. The rest were analized with X2 / AC-T: Adriamicin/Cyclophosphamide - Paclitaxel, EC-T: Epirrubicin/Cyclophosphamide - Paclitaxel, FAC-T: Fluorouracil/Cyclophosphamide-Paclitaxel

DISCUSSION: This study shows that normal weight and IV TZ is significantly associated with the risk of cardiotoxicity. The observation of our pt will continue to observe this phenomenon in detail.

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Exercise and nutrition intervention on breast cancer survivors in Taiwan with BMI 25 or more to decrease BMI and maintain at an appropriate level -A preliminary report

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It has been reported that lifestyle changes after completion of breast cancer treatment may decrease recurrence rate. Lifestyle was formed since childhood, and modified and sustained throughout lifetime. Usually, lifestyle changes are difficult without specific motivation.

## Materials and Methods

Breast cancer survivors, aged from 20 to 80 years old with stage I, II, III, who have received surgery and completed courses of chemotherapy within 4 years, were candidates for the study.

According to the American Athletic Association sports guidelines, aerobic, muscular and extension exercises are taught by a sports instructor to the participants, and the participants were asked to exercise at home, 150 minutes a week, 30 minutes each time. Participants were instructed to calculate the calories they needed (25 calories/kg/day). Food selection and diet plan must meet the calories they have calculated, and a diet log was recorded.

The study of one-year diet and exercise intervention study was designed. Group meeting was started at the beginning and then held every 3 months. Measurements of body weight, height, body fat, BMI, waist circumference, muscle mass, and basal metabolic rate were performed; WHO quality of life and brief pain inventory (BPI) were also collected.

## Results

A total of 32 breast cancer survivors were recruited, and 20 participants had completed one-year follow-up. Two of the participants withdrew from the study due to personal reasons, and the remaining 18 were included in the statistical analysis.

Body weight, BMI, and body fat decreased after interventions in diet and exercise and reached a significant lower level (p-value = 0.001, 0.002 and 0.01, respectively). In the linear regression model, it could be seen that body weight has a negative correlation with time; the body weight and BMI gradually decreased during the one-year period ( $\beta = -0.327$ , p-value=0.005;  $\beta = -0.362$ , p-value=0.002, respectively). There was no statistically significant change in waist circumference, muscle mass and basal metabolic rate.

BPI at baseline, 17 reported among 20 participants (80%) and decreased to 11% (2/18) at after one-year follow-up.

In the quality of life questionnaire, after the score calculation, the single factor repeated measurement analysis (calculated under the statistical power  $\alpha=0.05$ ) was used. There was no significant difference and change between the scores after the interventional measures. In the bivariate regression analysis, it was also seen that QOL had not changed due to weight loss, and there was no correlation between the two.

## Discussion

This study results demonstrated that lifestyle adjustment can be motivated, and lifestyle can be changed by exercise and diet intervention; however, statistically, quality of life did not change with weight loss.

# 2018 San Antonio Breast Cancer Symposium ®

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Mechanical properties of the shoulder and pectoralis major in women undergoing breast conserving therapy with axillary dissection and regional nodal radiotherapy versus sentinel node biopsy and radiotherapy to the breast alone

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**Background:** Breast conserving surgery (BCS) and radiotherapy (RT) reduce the risk of breast cancer recurrence, but can cause various functional deficits in breast cancer survivors. Side effects to the shoulder can include pain, stiffness, and restricted mobility, which are difficult to objectively assess in the clinic.

**Methods:** The mechanical integrity of the shoulder and the pectoralis major (PM) was assessed in patients at least 1 year post-treatment with BCS and RT. Nine patients with node-negative breast cancer were treated with 2 RT fields to the breast alone after BCS and sentinel node biopsy (Group 1). Nine patients with node-positive breast cancer were treated with  $\geq 3$  RT fields to the breast and draining lymphatics after BCS and axillary lymph node dissection (ALND) (Group 2). Nodal RT was delivered to the supraclavicular and infraclavicular (level III axillary) nodes in 9/9 patients, the internal mammary nodes in 6/9 patients, and the full axilla (levels I, II, and III) in 1/9 patient. Nine age-matched healthy controls (mean age 54) with no history of breast cancer or shoulder injury were also examined. The mechanical integrity of the shoulder was objectively quantified using robot-assisted biomechanical measures of shoulder stiffness. The shear elastic modulus, or 'stiffness', of the clavicular and sternocostal fiber regions of PM was assessed using ultrasound shear wave elastography. Participants were examined while they were relaxed or actively contracting force with their shoulder muscles. Linear mixed effect models with Bonferroni-corrected multiple comparisons were used to determine if shoulder stiffness or PM shear elastic modulus differed between the two breast cancer groups and controls.

**Results:** Patients in Groups 1 (mean age 54) and 2 (mean age 57) were an average (SD) 754(111) and 988(163) days since initiating RT ( $p=0.003$ ). Shoulder stiffness did not differ between the 2 groups and healthy controls ( $F_{2,27}=0.76$ ,  $p=0.48$ ). There was a significant group difference in PM shear elastic modulus ( $F_{2,27}=8.33$ ,  $p=0.0015$ ), with Group 2 patients exhibiting an average greater stiffness of 14-21% in the sternocostal and 12-28% in the clavicular regions of the PM versus Group 1 patients ( $p<0.001$ ) and healthy controls ( $p=0.021$ ). There was no difference between patients treated with Group 1 and controls ( $p=0.29$ ). **Conclusions:** Although power is limited due to small sample size, this study provides the first evidence that the mechanical integrity of the shoulder remains intact in patients who receive ALND combined with a supraclavicular field (generally without full axillary radiotherapy). The observation of altered PM function without subsequent changes to shoulder stiffness in patients treated with ALND and  $\geq 3$  RT fields suggests these patients likely develop new neuromuscular strategies to stabilize the shoulder joint to compensate for the PM. Future work is needed to appreciate whether certain muscle strategies are associated with poorer quality of life in breast cancer survivors, and to prospectively monitor the impact of breast cancer treatments on PM mechanical properties.



# 2018 San Antonio Breast Cancer Symposium ®

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Quality of life of patients with pregnancy- associated breast cancer

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**Background:** Breast cancer (BC) is the most common cancer diagnosed during pregnancy and occurs between one in 3000 to one in 10 000 pregnancies. The first international guidelines for the diagnosis and therapy of pregnancy associated BC (PABC) has been developed in 2003. The 5 year survival rate is similar to the non-pregnant women with the same stage of disease. In contrast to the data about the oncological outcome of patients with PABC, the literature about quality of life (QoL) of those patients is lacking. The aim of the study was to investigate the QoL of patients with PABC.

**Materials and methods:** Patients with PABC diagnosed and treated at our department in the period between 2000 and 2016 were included in the study. Clinical and demographic data were collected from the data base. The QOL was assessed using the EORTC QLQ-C30, the EORTC QLQ-BR 45 and the EORTC SHQ-22. The QoL results were compared with reference data (EORTC Reference Values) of BC patients. Statistical analysis was performed using descriptive statistics and t-test to test the significance.

**Results:** In the analysed period, twenty-one patients with PABC were found. In 16 patients BC had been diagnosed during the pregnancy and in 5 patients during the first year after delivery. Mean age at the time of diagnosis was 34.24 (SD±3.3), mean pregnancy week 21.14 (SD±10.28), and 8 months (SD±5.0) for patients with the diagnosis in the first year after the delivery. All patients underwent surgery, as well as chemotherapy. In addition, 10 patients received hormonal therapy and 12 irradiation. 16 patients lived at the time of the QoL assessment and received the QoL questionnaire per mail. After the mean follow up of 5.2 years, there were no statistically significant differences in EORTC QLQ C30 scales between our population and reference data except for constipation which a better score in our population. Clinical relevant differences were found in scales: physical functioning, role functioning and symptom dyspnea with the better score in our population. Our patients reported clinical relevant more financial difficulties. In the disease specific questionnaire EORTC BC 45, patients with PABC showed statistically significantly worse score in the following scales: body image, upset by hair loss, side effects of the systemic therapy and future perspective. 40% of the patients had a decreased libido and 37% of patients reported that therapy negatively impaired sexual activity. 25% of patients reported that they feel less feminine because of the disease.

**Conclusion:** The results revealed that QoL in patients with PABC is more impaired compared to the overall QoL reference data of BC patients. Despite the small number of patients, the results indicate that PABC patients are a vulnerable group, especially regarding outer appearance (body image, upset by hair loss, feel less feminine). The QLQ BR 45 showed more sensitivity to assess the impact on QoL of these patients than EORTC QLQ C30 and underlines the necessity to use disease-specific questionnaires in specific patient populations.

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Final results of the ASG1-3 study, a randomized phase III study comparing a standard dose chemotherapy with epirubicin/cyclophosphamide and paclitaxel with a dose dense regimen with epirubicin and paclitaxel

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## Background

Dose dense chemotherapy (DDT) has shown improvements of disease-free survival (DFS) and overall survival for primary breast cancer patients with a high risk of relapse. There are much less data about the effect of DDT in patients with intermediate risk of recurrence (1-3 positive axillary lymph nodes). Aim of this prospectively randomized trial was to investigate the superiority of a DDT schedule over a standard chemotherapy (ST) in primary breast cancer patients with 1-3 positive axillary lymph nodes.

## Methods

The ASG1-3 study is a prospectively randomized, open label phase III study of the *Adjuvant Study Group* of the NOGGO association. Patients were eligible for the trial, if they had a primary invasive breast cancer (pT1-3) with 1-3 positive axillary lymph nodes and no evidence of distant metastases. Patients were randomized to an adjuvant therapy with either 4 cycles epirubicin (90mg/m<sup>2</sup> body surface area, BSA) and cyclophosphamide (600mg/m<sup>2</sup> BSA) q3w, followed by 4 cycles of paclitaxel (175mg/m<sup>2</sup> BSA) referred to as ST or to a therapy with 4 cycles of epirubicin (120 mg/m<sup>2</sup> BSA) q2w and primary G-CSF support followed by 4 cycles of paclitaxel (175mg/m<sup>2</sup> BSA) q2w and primary G-CSF support referred to as DDT. Trastuzumab was not given in this study. The study was designed to show an increase of 70% DFS (ST) to 80% DFS (DDT) 5 years after randomization. Comparisons were conducted using Kaplan Meier estimates, log rank tests and Cox regression analyses. In an exploratory way, subgroup analyses were performed for HER2, hormone receptor status and grading using Cox regression models with interaction terms.

## Results

A total of 936 patients were eligible for survival analysis, of which 465 were randomized to ST and 471 to DDT from 2001 to 2004. Patient characteristics were mainly well balanced, with patients being 52.5/52.1 years old, 71.9/78.1% being hormone receptor positive, 24.4/24.6% being HER2 positive and 38.6/38.8% having a tumor grade of 3 in the ST arm and DDT arm respectively. 53 events occurred after ST and 46 after DDT. Adjusted hazard ratio (HR) was 0.87 (95%CI: 0.57-1.35; p=0.54). 5 year DFS rates were 85% (ST) vs. 87% (DDT). Hematological toxicities were the most common grade 3 or 4 adverse events. Grade 3/4 neutropenia occurred in 57.2 vs. 28.0%, grade 3/4 anemia in 15.3% vs. 17.1% and grade 3/4 pain symptoms were seen in 13.2 vs. 12.4% of the patients in the ST arm vs. DDT arm respectively. Other grade 3/4 toxicities were less frequent than 10%.

Subgroup analysis showed a significant interaction ( $p < 0.001$ ) between HER2 status and randomization arm with regard to DFS. In HER2 negative patients the HR was 1.53 (95%CI: 0.91-2.59), whereas in HER2 positive patients the HR was 0.22 (95%CI: 0.09-0.55). Patients with HER2 positive disease and DDT had a similar prognosis like HER2 negative patients.

### **Conclusion**

In the overall population a statistically significant improvement of DFS could not be shown for the DDT arm. In patients with HER2 positive breast cancer DDT chemotherapy improved the disease-free survival to a prognosis which was similar to patients with HER2 negative disease.

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Real world outcomes of adjuvant FECD, ddACT and ACT for the treatment of early stage breast cancer - A multicenter retrospective analysis

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**Background:** Adjuvant chemotherapy combining anthracyclines and taxanes for early stage breast cancer (ESBC) have demonstrated disease-free survival (DFS) and overall survival (OS) benefits. Among the 3rd generation regimens, 2 options have been favoured: FEC-Docetaxel (FECD) and AC-Paclitaxel (ACT). ACT may be delivered with dose-dense (ddACT) or weekly taxane scheduling (ddACWT), compared to traditional every 3-weekly (q3ACT) scheduling. Despite literature supporting both FECD and (dd)ACT regimens in the management of ESBC, no direct prospective trial has evaluated their comparative effectiveness.

**Methods:** A retrospective review of the BC Cancer Breast Cancer Outcomes Unit (BCOU) and the Alberta Health Services (AHS) databases was performed to identify patients with HER2 negative, stage 1-3 ESBC, who received adjuvant chemotherapy between 2007-2014. The primary endpoint was OS and the secondary endpoint was RFS, defined as freedom from local (invasive), regional or distant recurrence or breast cancer death. Outcome comparisons were made between FECD, ddACT/ddACWT and q3ACT using the Kaplan Meier method. Treatment arms were compared using a log-rank test for univariate analysis. A multivariate analysis was also conducted for OS comprising age, stage, grade, receptor status and type of chemotherapy received (FECD vs combined ACT group).

**Results:** A total of 4047 patients met inclusion criteria, including 2685 FECD, 1259 ddACT and 103 ACT. Median age was 53 (24-77) in the FECD group vs 52 (26-68) in the ddACT/ddACWT group and 58 (43-78) in the q3ACT group. The majority had stage 2 disease, 51.3%, 53.5% and 50.5% in the FECD, ddACT/ddACWT and q3ACT groups, respectively. Most were HR+, 84.5% in the FECD group vs 66.9% in both the ddACT/ddACWT and q3ACT groups. In the FECD group, 42.8% had a grade 2 tumour and 48.2% a grade 3 tumour vs 35.4% and 56.4% in the ddACT/ddACWT group and 35.0% and 58.3% in the q3ACT group. Lymphovascular invasion (LVI) was present in 40.7% of patients who received FECD vs 39.7% for ddACT/ddACWT and 26.2% for ACT. 5-year OS, for the FECD group was 90.3% (95%CI 89.1,91.4) vs 87.0% (95%CI 84.3,89.2) for the ddACT/ddACWT and 84.9% (95%CI 75.5,90.8) for the q3ACT groups,  $p=0.0907$ . 5-year RFS was 85.5% (95%CI 84.0-86.8) with FECD vs 84.4% (95% 81.9,86.6) for ddACT/ddACWT and 87.7% (95%CI 79.2,92.8) with q3ACT,  $p=0.4200$ . In multivariate analysis: age, stage and grade were significantly associated with OS whereas type of chemotherapy received (FECD vs ACT) was not ( $p=0.165$ ). Finally, OS rates were compared across provinces and no significant differences were identified, 87.0% vs 88.0% ( $p=0.6294$ ). Subgroup analyses by receptor type, comparing HR+ and TNBC are ongoing.

**Conclusions:** The use of FECD as compared to ACT based chemotherapy did not reveal significant differences in OS or RFS in this population-based study. Further, chemotherapy regimen was not associated with differences in overall survival, as compared to other well recognized prognostic factors. While the results were obtained from a retrospective analysis, conclusive prospective data is lacking. These results may therefore reassure physicians and patients alike on a comparable efficacy of these regimens in a real-life setting.

# 2018 San Antonio Breast Cancer Symposium ®

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Grade of leukopenia predicts treatment effect in early breast cancer in patients treated with tailored epirubicin/cyclophosphamide chemotherapy

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**Background:** Body surface based dosing of chemotherapy is unreliable due to marked inter-individual variations in pharmacokinetics/-dynamics. Multiple retrospective studies have demonstrated that hematological toxicity could be a surrogate marker for efficacy of chemotherapy. The SBG 2000-1 trial was the first adjuvant randomized trial designed to compare the same drugs and number of courses of individually dosed chemotherapy based on grade of toxicity vs. standard dosed chemotherapy in early breast cancer. The aim was to study the relations between dose of chemotherapy, leukopenia nadir grade and prognosis.

**Methods:** Women (n=1452) in Sweden and Denmark with early breast cancer aged 18-60 years, received the first cycle at a standard dose of FEC (fluorouracil 600 mg/m<sup>2</sup>, epirubicin 60 mg/m<sup>2</sup>, cyclophosphamide 600 mg/m<sup>2</sup>). Patients (n=1052) with nadir leukopenia grade 0-2 after first cycle were randomized between either 6 tailored FEC with increased doses of epirubicin and cyclophosphamide aimed at achieving grade 3 leukopenia or treatment with 6 standard FEC. Patients with nadir leukopenia grade 3-4 represented a second control group (registered group) treated with 6 standard FEC. Dose escalation did not significantly improve 10 year distant disease-free survival (HR 0.87, p=0.32, Eur J Cancer 13:79-86, 2018). In this report grade of leukopenia at course 3 (after final escalation) was assessed as a prognostic marker in a Cox regression model adjusted for chemotherapy doses.

**Results:** Eight-year distant disease-free survival (DDFS) was 73%, 77%, 78% and 83% for patients with leucocyte nadir grade 0, 1, 2 and 3-4 and overall survival (OS) 77%, 81%, 81% and 86% respectively. Cox regression analysis of leucocyte grade and DDFS showed a statistically significant hazard ratio (HR) of 0.84 (CI 0.74-0.96, p=0.008) per grade of leukopenia, with non-significant trend for OS (HR 0.88, CI 0.76-1.02, p=0.066). The correlations with DDFS and cumulative dose of epirubicin and cyclophosphamide were not significant with hazard ratios of 0.96 (0.91-1.014 p=0.15) and 1.002 (1.00-1.005 p=0.21) per mg cumulative dose per meter squared. Patients with grade 3 tumors had a significantly stronger impact of leukopenia on DDFS (HR 0.76, 95% CI 0.65-0.90 p<0.001) and a test of interaction between the prognostic effect of grade and leukopenia was significant (p=0.026).

**Conclusions:** The grade of leukopenia predicts the individual treatment effect better than chemotherapy doses. The results of this prospective trial are in agreement with previous retrospective studies indicating that chemotherapy induced leukopenia is predictive for outcome in early breast cancer. Dose dependent toxicity should be monitored for optimal adjustment of the dosage of chemotherapy.

## 2018 San Antonio Breast Cancer Symposium ®

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Efficacy of adjuvant 5-Fluorouracil in residual HER2-negative breast cancer following neoadjuvant chemotherapy

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**Background:** Patients with residual disease following neoadjuvant chemotherapy have an increased risk of relapse. Recently the CREATE-X trial demonstrated that adjuvant capecitabine (oral prodrug of 5'deoxy-5-fluorouridine), prolonged disease-free survival and overall survival among patients with HER2-negative breast cancer who had residual invasive disease after neoadjuvant chemotherapy containing anthracycline, taxane, or both. Therefore, we sought to evaluate the antitumor efficacy of 5-fluorouracil (5-FU) in patient-derived xenografts (PDXs) from residual tumors resistant to neoadjuvant chemotherapy.

**Methods:** Antitumor efficacy of 5-FU was assessed *in vivo* in three PDXs varying in hormone receptor status (0, 4% and 11% respectively), generated from residual tumors of primary breast cancer patients treated with neoadjuvant chemotherapy containing anthracycline, and taxanes. In addition, significance of timing of therapy was also assessed, comparing efficacy of initiating treatment upon implantation (immediate start cohort; mimicking treating residual disease with adjuvant therapy), with initiating treatment upon establishment of PDX (standard start cohort).

**Results:** 5-FU was efficacious in established PDX models that are triple negative (0% ER;  $p < 0.0001$ ), low ER positive (4% ER,  $p = 0.0213$ ) and ER-positive (11% ER;  $p = 0.0390$ ), decreasing growth compared to the cohort. However, there was no statistically significant difference between the immediate start cohort and standard start cohort. Western blot analysis of the treatment-naïve derived mouse tumors recognized RB as a predictive biomarker for 5-FU response.

**Conclusion:** 5-FU has anti-tumor activity in residual HER2-negative PDX models resistant to taxanes, and anthracyclines in the neoadjuvant setting.

# 2018 San Antonio Breast Cancer Symposium ®

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Efficacy and safety of eflapegrastim confirmed in reducing severe neutropenia in breast cancer patients treated with myelosuppressive chemotherapy in the second Phase 3 randomized controlled multinational trial compared to pegfilgrastim (RECOVER trial)

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## **Background:**

Eflapegrastim is a novel investigational biologic comprised of recombinant human G-CSF covalently linked to the human immunoglobulin G4FC fragment using proprietary LAPSCOVERY™ technology with potentially unique distribution to areas rich in FcRn receptors. RECOVER is the second Phase 3 study to investigate the non-inferiority (NI) of eflapegrastim to pegfilgrastim in patients receiving chemotherapy for breast cancer. The first Phase 3 study, ADVANCE, has demonstrated the non-inferiority of eflapegrastim comparing to pegfilgrastim in the duration of severe neutropenia (DSN) in breast cancer patients receiving docetaxel and cyclophosphamide (TC) and was previously published at ASCO 2018 meeting.

## **Trial Design:**

Patients with Stage I to Stage IIIA breast cancer from centers in the USA, Canada, Poland, Hungary, South Korea and India were treated on Day 1 of each of four 21-day cycles with adjuvant or neo-adjuvant TC. On Day 2 of each cycle, patients received a single subcutaneous dose of either eflapegrastim 13.2 mg/0.6 mL (equivalent to 3.6 mg G-CSF) or pegfilgrastim (6 mg) in a 1:1 ratio. Patients had CBCs drawn on Day 1 prior to chemotherapy and Days 4-15 daily or until recovery of neutropenia in Cycle 1. CBC was also collected on Days 1, 4, 7, 10 and 15 in Cycles 2-4. The primary endpoint was to demonstrate the non-inferiority of eflapegrastim comparing to pegfilgrastim as measured by the mean DSN in Cycle 1 with NI margin of <0.62 day.

## **Results:**

In a total of 237 intent-to-treat patients (randomized to 118 eflapegrastim; 119 pegfilgrastim), median age was 59 years (range 29 to 88 years); mean (SD) DSN was 0.31 (0.688) days for eflapegrastim and 0.39 (0.949) days for pegfilgrastim, demonstrating the non-inferiority (95% CI of  $\Delta$ DSN: [-0.292, 0.129];  $p < 0.0001$ ). Non-inferiority of eflapegrastim for DSN was maintained across all 4 cycles. There were no statistically significant differences in secondary endpoints: time to ANC recovery, depth of ANC nadir and incidence of FN at Cycle 1. The common Grade 3/4 adverse events observed in  $\geq 5\%$  of patients were similar across both arms and were mainly hematologic including neutropenia, lymphopenia, anemia and leukopenia. Grade 3/4 bone pain and febrile neutropenia rates were similar across both arms and were less than 5%.

## **Conclusions:**

Eflapegrastim, a novel long acting G-CSF demonstrated non-inferiority to pegfilgrastim in the reduction of DSN in breast cancer patients treated with TC and has validated the results from the first Phase 3 ADVANCE study. Eflapegrastim was safe and well-tolerated with a similar safety profile to pegfilgrastim.

# 2018 San Antonio Breast Cancer Symposium ®

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Exploring the real-life incidence of toxicities amongst obese breast cancer patients receiving adjuvant chemotherapy dosed based on absolute body weight

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## **Background:**

Obesity is an established risk factor for developing breast cancer and is also a negative prognostic indicator for higher recurrence risk.<sup>1</sup> Adjuvant chemotherapy reduces the likelihood of metastatic recurrence and improves disease-free and overall survival.<sup>2</sup> However, the practice of optimal dosing of adjuvant breast cancer chemotherapy amongst obese patients remains contentious with concerns regarding excessive toxicity if obese patients are dosed based on actual body weight (ABW).<sup>2-4</sup> A 2014 study reported that obese patients were five times more likely to have dose reductions in cycle 1 than non-obese patients.<sup>5</sup> Inadequate dosing amongst obese breast cancer patients may have deleterious implications on ultimate prognosis.<sup>2-4</sup> This study aimed to investigate whether chemotherapy dosing using ABW in obese breast cancer patients is associated with comparable incidences of toxicity.

## **Methods:**

A retrospective cross-sectional study was conducted amongst 257 patients (aged  $\geq 18$ ) treated in the adjuvant setting with doxorubicin-cyclophosphamide-paclitaxel (AC-P) or docetaxel-cyclophosphamide (TC) with/without trastuzumab between 2014 and 2017. Obesity was classified as a body mass index (BMI) of  $\geq 30$ , with morbid obesity defined as a BMI  $\geq 35$ . Chemotherapy dosing based on body surface area calculated using ABW was considered the standard protocol with any variations from this dosing method for cycle 1 being recorded. Subsequent dose adjustments were also noted. The primary outcome was tolerability of chemotherapy regimens dosed using ABW with outcome measures of toxicity defined as the incidence of febrile neutropaenia, the incidence of grade 3 or 4 non-haematological toxicities and the number of hospitalisations during the treatment course.

## **Results:**

257 patients were eligible (1 male, 256 females). Median age was 55 (range, 31-78). AC-P was the most commonly used regimen (48.6%), followed by TC (40.1%). Obesity and morbid obesity were noted amongst 17.9% and 15.6% of patients respectively; with 63.8% with a BMI  $\geq 25$ . Chemotherapy dosing was largely based on ABW, with only 4.3% of patients dosed based on ABW or had their dosing body surface area capped at 2.0m<sup>2</sup>. In patients with a BMI  $\geq 25$ , 25% had febrile neutropaenia compared to 21.8% in those with normal BMI ( $p=0.58$ ). Incidence of febrile neutropaenia during treatment by BMI – underweight: 33.3% ( $p=0.61$ ); normal: 24.1%; overweight: 24.4% ( $p=0.97$ ); obese: 17.4% ( $p=0.37$ ); morbidly obese: 25% ( $p=0.92$ ). Grade 3-4 non-haematological toxicities had comparable incidences between the normal BMI group as opposed to the overweight/obese group (23.0% vs 18.9% respectively,  $p=0.44$ ). Hospitalisations by BMI - underweight: 50% ( $p=0.85$ ); normal BMI: 46%; overweight: 42.3% ( $p=0.64$ ); obese: 54.3% ( $p=0.36$ ); morbidly obese: 57.5% ( $p=0.23$ ).

## **Conclusion:**

This study demonstrates that obese breast cancer patients do not experience higher toxicities when their adjuvant chemotherapy is dosed based on ABW. This supports current guidelines for dosing amongst obese patients.



## 2018 San Antonio Breast Cancer Symposium ®

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Effect of adjuvant chemotherapy for patients with ER-positive/HER2-negative breast cancer assessed by the propensity score matching: Significance of nuclear grade and nodal status

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**Purpose:** Although a survival benefit with chemotherapy (CT) is obtained over all among patients with estrogen receptor-positive (ER+) breast cancer, the degree of benefit differs among the subtypes. The 21-gene Recurrence Score assay is a validated prognostic/predictive tool for early ER+ breast cancer. However, genomic assays have not been approved in Japan and most other countries, except in some developed countries. Based on the St. Gallen 2015, 1-3 positive nodes are not an absolute indication for CT among patients with ER+/HER2-negative breast cancer. In contrast, it is better for CT to be performed except for low-risk groups, based on genomic tests according to the St. Gallen 2017. In fact, no prospective study has proven the survival benefit of CT specifically for luminal-A breast cancer. Therefore, we analyzed the survival benefit of CT for ER+/HER2-negative breast cancer by propensity score matching (PSM).

**Patients and methods:** Between 2000 and 2015, 895 patients with stage I-III ER+/HER2-negative breast cancer who had undergone surgery in our hospital were examined. Patients with bilateral breast cancer, ER<10%, and preoperative treatment were excluded. The primary end point was the 5-year recurrence-free survival (RFS) and overall survival (OS) in patients matched by propensity score that estimated by a logistic regression model that included factors likely to influence the decision of whether or not to administer CT (tumor size, nuclear grade [NG], progesterone receptor status and nodal status).

**Results:** In the entire cohort, the median age was 59 (28-95) years; 223 patients (24.9%) were node positive and 126 patients (14.1%) had NG3 disease. Overall, all patients received endocrine therapy, and 24.1% received additional CT. After a median follow-up of 68.8 months, the 5-year RFS rate was 94.3% in CT-untreated patients (non-CT group) and 90.1% in CT-treated patients (CT group; hazard ratio [HR] for recurrence, 1.47; 95% confidence interval [CI], 0.90-2.33; p=0.106). The 5-year OS rate was 97.5% in the non-CT group and 95.6% in the CT group (HR for death, 1.80; 95% CI, 0.99-3.21; p=0.047). Using PSM, 236 patients were selected (1:1 matching between non-CT and CT groups). After matching, the 5-year RFS rate was higher in the CT group than in the non-CT group (96.8% vs. 82.7%; HR for recurrence, 0.29; 95% CI, 0.11-0.68; p=0.003), and the 5-year OS rate was higher in the CT group than in the non-CT group (100% vs. 91.9%; HR for death, 0.06; 95% CI, 0.003-0.35; p<0.001). Among PSM patients, with node-negative/NG3 and 1-3 node positive/NG2 disease, the 5-year RFS rate was significantly higher in the CT group than in the non-CT group (p=0.041 and p=0.006, respectively).

**Conclusion:** No significant benefit of CT was observed when considering the entire cohort because of the treatment bias. When clinical and tumor features were matched by propensity score, the addition of CT significantly improved both RFS and OS of ER+/HER2-negative breast cancer patients, especially for patients with node-negative/NG3 and 1-3 node positive/NG2 disease.

# 2018 San Antonio Breast Cancer Symposium ®

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A prospective non-randomized clinical trial of adjuvant carboplatin chemotherapy in triple negative breast cancer

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**Background:** The addition of carboplatin in the neoadjuvant setting has demonstrated improved outcomes in patients with triple negative breast cancer (TNBC). Carboplatin in the adjuvant setting has not been demonstrated to be an accepted regimen to date. This trial addresses this issue.

**Methods:** The primary objectives of this study were to assess the toxicity of an adjuvant carboplatin-containing regimen for early stage TNBC patients, and to correlate outcomes with molecular markers including Spy-1. Secondary objectives included determination of progression free (PFS) and overall survival (OS), and comparing these to our institutional historical controls. Patients received the backbone of a dose dense anthracycline, taxane, cyclophosphamide (DD ACT), with the addition of carboplatin.

**Results:** Ninety patients with stage I to III patients with triple negative breast cancer were accrued to this trial between Jan 2011 and June 2017. We discovered that DD ACT with carboplatin with an AUC of 5 given on the second and last paclitaxel was well tolerated. Chemotherapy delays were minimized when the parameters for administration of the carboplatin was to allow chemotherapy to proceed if the platelet count was  $\geq 70,000 \times 10^9/L$  and dose adjustment of paclitaxel was allowed based on neuropathy. There were no grade 4 adverse events. 4% of patients had grade 3 peripheral neuropathy (PN), 28% had grade 2 PN and 45% had grade 1 PN. Results of molecular profiles will be reported. Univariate analysis are reported, and were found to be very promising. Using log-rank statistic, a trend to improvement in overall survival was found when compared to historical controls ( $p=0.089$ ). Median follow-up is 24 months. Longer follow-up is necessary to determine PFS and overall survival benefit.

**Conclusion:** If considering an adjuvant regimen for triple negative breast cancer patients DDAC/TC with the carboplatin administered with an AUC of 5 on the second and fourth taxol of DDACT is a well-tolerated regimen.

Univariate Analysis Comparing Carboplatin vs Historical Controls that did not receive Carboplatin

Variable	Historical (no carbo)	Carbo containing chemo	p overall
	n = 179	n = 82	
Age	56.3 (13.1)	49.8 (11.0)	< 0.001
Stage			0.188
Stage I	47 (26.3%)	15 (18.3%)	
Stage II	88 (49.2%)	50 (61.0%)	
Stage III	44 (24.6%)	17 (20.7%)	
Grade			0.011
Grade 1	8 (4.5%)	0 (0.0%)	
Grade 2	33 (18.4%)	7 (8.8%)	
Grade 3	138 (77.1%)	73 (91.2%)	
Remission Status			0.009
In Remission	126 (70.4%)	70 (86.4%)	
Relapsed	53 (29.6%)	11 (13.6%)	
Survival			< 0.001
Alive	121 (67.6%)	73 (90.1%)	
Deceased	58 (32.4%)	8 (9.9%)	



# 2018 San Antonio Breast Cancer Symposium ®

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Long-term follow-up of two randomized controlled trials (N-SAS-BC01 trial and CUBC trial) comparing oral tegafur-uracil (UFT) versus classical cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) as adjuvant therapy in early breast cancer

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**Background:** Two randomized controlled trials comparing the efficacy of oral tegafur-uracil (UFT) (2 years) with that of classical cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) (6 courses) were conducted in patients with resected early breast cancer. UFT is an oral fluoropyrimidine that combines tegafur, a prodrug of 5-fluorouracil, with uracil in a 1:4 molar ratio. One study was the N-SAS-BC01 trial (Watanabe et al, J Clin Oncol 2009), which was conducted in patients with high-risk node-negative breast cancer (n=733). The other was the CUBC trial (Park et al, Br J Cancer 2009), which was performed in patients with node-positive breast cancer (n=377). We reported the pooled analysis of these two randomized control trials using individual patient data (Ohashi et al, Breast Cancer Res Treat 2010). This pooled analysis showed that UFT was non-inferior to CMF in terms of inhibiting recurrence of estrogen receptor (ER)-positive early breast cancer. In addition, an exploratory subgroup analysis showed that UFT may be more effective in ER-positive patients who were 50 years or older. The present study was conducted to investigate the long-term efficacy of UFT or CMF in patients with early breast cancer.

**Methods:** Long-term follow-up data for disease recurrence and survival were collected. Hazard ratios (HR) were determined using the Cox model stratified by study and adjusted for clinical characteristics, namely age, tumor size, nodal status, histological type, ER, and progesterone receptor (PgR). Survival curves were estimated by the Kaplan-Meier method. Hochberg multiplicity adjustment was applied in the previous pooled analysis, and non-inferiority of UFT versus CMF was shown in terms of relapse-free survival (RFS) in the ER-positive patient group. We investigated the non-inferiority of UFT in the same patient group with updated data. Restricted mean survival time (RMST) was calculated to consider the relative efficacy of UFT. This study is registered with JapicCTI-163321.

**Results:** In total, 1,057 patients were analyzed (CMF, n=528; UFT n=529). The median follow-up time was 11.1 years (12.1 years in the N-SAS-BC 01 trial and 8.3 years in the CUBC trial). Table 1 shows the 10-year RFS and overall survival (OS) in all patients and ER-positive patients.

	UFT (%)	CMF (%)	UFT to CMF; HR (95% CI)
10-year RFS			
all patients	74.4	77.1	1.02 (0.81–1.30)
ER-positive patients	75.0	75.1	0.91 (0.66–1.27)
10-year OS			
all patients	86.8	86.9	0.98 (0.72–1.34)
ER-positive patients	89.9	87.9	0.86 (0.54–1.37)

The difference in RMST between arms at 20 years in terms of RFS was -2.7 months in all patients and 3.4 months in ER-positive patients. Table 2 shows the HR for RFS according to ER status and age.

	Age <50 years	Age ≥50 years
ER negative	1.76 (0.95–3.26)	0.93 (0.58–1.51)
ER positive	1.29 (0.74–2.23)	0.76 (0.50–1.16)

**Conclusion:** This study shows that UFT was shown to be non-inferior to CMF in terms of RFS in ER-positive early breast cancer. This result is similar to that of the previous pooled analysis.

**Sponsor:** Taiho Pharmaceutical Co., LTD

# 2018 San Antonio Breast Cancer Symposium ®

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Comparison of overall and recurrence-free survival between four and six cycles of adjuvant docetaxel and cyclophosphamide in early stage breast cancer

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**Background:** The ABC trials and the WSG Plan B trial demonstrated that certain early stage breast cancer patients can be appropriately treated with docetaxel and cyclophosphamide (TC) rather than doxorubicin and cyclophosphamide (AC); however both of these trials utilized six cycles of TC as the comparator. TC is given for 4 cycles based on the US Oncology 9735 trial. To our knowledge, there is no available data that has compared adverse events or outcomes of four versus six cycles of TC.

**Methods:** This was a single-center, retrospective study evaluating recurrence-free survival, overall survival, and occurrence of toxicities between four and six cycles of docetaxel and cyclophosphamide adjuvant chemotherapy for early stage breast cancer at West Virginia University Cancer Institute.

**Results:** 112 patients were included; 81 received four cycles and 31 received six. Recurrence free survival rates of patients that received four and six cycles of chemotherapy were 94% and 90%, respectively, at three years (P=0.68). Overall survival at three years was 99% versus 97% (P=0.49), respectively. The incidence of peripheral neuropathy requiring treatment was 42% in patients that received six cycles and 19% in patients that received four (P=0.015).

**Conclusion:** The addition of two cycles of docetaxel and cyclophosphamide adjuvant chemotherapy for early stage breast cancer provides no additional recurrence-free or overall survival benefit but does increase the incidence of peripheral neuropathy requiring treatment.

Table 1. Baseline Characteristics

	4 Cycles (n=81)	6 Cycles (n=31)	P-value
Age, median years (range)	57 (37-75)	48 (32-71)	0.001
Receptor status, n (%)			0.63
HR positive, HER2 negative	61 (75)	25 (81)	
Triple negative	20 (25)	6 (19)	
Cancer stage, n (%)			0.031
I	39 (48)	9 (29)	
II	39 (48)	17 (55)	
III	3 (4)	5 (16)	
Lymph node involvement, n (%)	24 (30)	20 (65)	0.001
Ki67 (%), median (range)	23 (2-99)	23.5 (4-99)	0.99
Tumor grade, n (%)			0.072
1	2 (2)	4 (13)	
2	42 (52)	12 (39)	
3	37 (46)	15 (48)	
Surgery, n (%)			
Breast conservation	35 (43)	14 (45)	0.99
Unilateral mastectomy	32 (40)	9 (29)	0.38
Bilateral mastectomy	14 (17)	8 (26)	0.43

Table 2. Adverse Events

	4 Cycles (n=81)	6 Cycles (n=31)	P-value
Dose reductions, n (%)	17 (21)	8 (26)	0.62
Dose delay, n (%)	3 (4)	1 (3)	0.99
Inpatient admissions, n (%)	22 (27)	8 (26)	0.99
Treatment for peripheral neuropathy, n (%)	15 (19)	13 (42)	0.015

## 2018 San Antonio Breast Cancer Symposium ®

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Optimal omission of anthracycline in adjuvant chemotherapy of HER2 positive breast cancer

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**Background** In adjuvant settings of HER2 positive cancer, trastuzumab has been shown to be effective in combination with anthracycline- based chemotherapy followed by taxane-based chemotherapy. However, the role of anthracyclines for the treatment of breast cancer has remained in controversy, due to the increased risk of cardiotoxicity and secondary carcinogenesis. Recently, additional anthracycline-free treatment regimens have also been reported. In a single-arm multicenter trial of 406 patients treated with paclitaxel plus trastuzumab for <3cm, lymph node negative, HER2 positive breast cancer, Tolane et al reported a 98.7% rate of survival free from invasive disease at 3 years of follow-up (NEJM 2015). NCCN guideline 2018 suggests paclitaxel plus trastuzumab to be considered for patients with low-risk T1, N0, M0, HER2 positive breast cancer. However, there is a lack of definitive evidence for anthracycline-free regimens in adjuvant chemotherapy of HER2 positive cancer. We performed a single-center retrospective cohort study to assess the characteristics of patients we can safely omit anthracyclines in the adjuvant settings for HER2 positive breast cancer. **Patients and methods** 238 women were diagnosed with HER2 positive breast cancer and treated with neoadjuvant and/or adjuvant chemotherapy between January 1, 2008 and December 31, 2015 at Keio University Hospital. They were divided in two cohorts: an “anthracycline” cohort of 112 anthracycline-treated women and a “no anthracycline” cohort of 126 anthracycline-untreated women. **Results** Three years disease free survival (DFS) was 91.3% (95% CI, 84.0%–95.4%) and 93.1% (95% CI, 86.1%–96.7%) in the no anthracycline and anthracycline cohorts, respectively. There were no significant differences between the two cohorts ( $P=0.692$ ). Among the cT1N0 subset, no significant differences were observed between two cohorts in 3 years DFS ( $P=0.612$ ). However, the patient characteristics of the cT2 subset were not balanced enough to compare the two cohorts. Of the 238 patients, 122 received neoadjuvant chemotherapy and 36 (29.5%) of them achieved pathological complete response (pCR). Among the pCR group, only one patient in each cohort had recurrence. **Discussion** We suggest that we can safely omit anthracyclines in cT1N0 HER2 positive breast cancer patients and also in patients who achieved pCR by neoadjuvant chemotherapy. However, we need a larger number of cases to assess the possibility of omission in cT2 HER2 positive breast cancer. Current clinical trials indicate the addition of pertuzumab to trastuzumab for neoadjuvant and adjuvant chemotherapy in HER2 positive breast cancer leads to a good pCR rate and increase the efficacy of anti HER2 block therapy. We expect that the additional use of pertuzumab with trastuzumab will widen the possibility of anthracycline-free adjuvant chemotherapy in HER2 positive cancer.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-14-01

Final results of NorBreast-231, a randomized phase II study evaluating weekly oral vinorelbine versus weekly paclitaxel as first-line chemotherapy in patients with advanced breast cancer

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**Background:** Single-agent chemotherapy (CT) is the standard treatment option in endocrine receptor (ER)-positive advanced breast cancer (ABC) after failure of endocrine therapy (ET) or in patients (pts) without visceral crisis. Both weekly paclitaxel (WP) and oral vinorelbine (OV) are among the recommended treatment options in this setting. The aim of this study was to evaluate the efficacy and safety profiles of OV and WP in a face to face study.

**Material and methods:** Pts with ER-positive, HER2-negative ABC, with an age  $\geq 18$  years and with a documented locally recurrent or metastatic involvement previously untreated by CT were eligible. Pts were randomized to receive, as first-line CT, 3 weekly-cycles of either: ARM A: weekly OV, given as a 80 mg/m<sup>2</sup> dose (following a first cycle at 60 mg/m<sup>2</sup>, dose escalation to 80 in the absence of grade 3 or 4 toxicity at cycle 1); ARM B: WP 80 mg/m<sup>2</sup> per week. Primary endpoint was disease control rate (DCR). Pts were stratified according to prior taxane CT and visceral metastases.

**Results:** 131 pts have been treated (OV: 66, WP: 65). Baseline pts characteristics (Arms OV/WP): median age 58/61 years; median number of prior ET: 2/2; prior (neo) adjuvant CT 74/72%; prior anthracycline 67/62%; prior taxane: 41/42%;  $\geq 3$  metastatic sites 42/48%; visceral metastases 79/79%. Median number of cycles (range): 6(1-55)/7(1-44); dose escalation of OV was performed in 75% of pts. Safety: most common non-hematological related G3/4 adverse events per pt were fatigue 8/2%, peripheral neuropathy 0/5%, nausea 3/0%, diarrhoea 3/2%, vomiting 3/0%, constipation 3/2%, alopecia (G2) 2/34%, no toxic deaths; febrile neutropenia was present in 2/0% of pts. Quality of life: over time, no major differences between both arms have been observed. Efficacy: DCR in the intent-to-treat population was [95%CI] 75.8 [63.6-85.5] /75.4 [63.1-85.2]%; overall response rate 20/40%; median progression-free survival: 5.5/6.4 months. Median overall survival was: OV 27.6/WP 22.3 months.

**Conclusion:** Both OV and WP reached similar DCR rates of 75%. Each regimen presented a specific tolerance profile, with, in particular, a lower incidence of alopecia and peripheral neuropathy with OV. OV and WP are valid first-line CT options for ER-positive/HER2-negative pts with ABC.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-14-02

A phase 2 study of low dose metronomic eribulin in metastatic breast cancer

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**Background:** Eribulin mesylate is a non-taxane microtubule dynamics inhibitor approved by FDA in treatment of metastatic breast cancer (MBC) based on improvements in overall survival in the pivotal EMBRACE trial. Eribulin is approved at 1.4mg/m<sup>2</sup> administered D1,8 of q21d cycle. However, this dose and schedule may have significant myelosuppression and peripheral neuropathy requiring dose reductions and treatment delays for some patients. We hypothesized that a low dose metronomic schedule will allow responding patients to remain on treatment, resulting in longer TTP (time to progression) and decreased incidence of toxicities and treatment-related discontinuations.

**Methods:** A multi-site prospective open-label phase II trial of metronomic dosing of eribulin in patients with MBC has completed accrual of 60 patients, outcomes will be updated at presentation. Patients whose disease had progressed following 1-6 prior regimens with prior exposure to a taxane, ECOG performance status of 0 – 2, measurable disease per RECIST 1.1, with normal marrow and organ function were eligible. Eribulin was administered at 0.9mg/m<sup>2</sup> weekly for 3 out of 4 weeks. For patients with HER2 positive disease, concurrent trastuzumab administration was allowed. Concurrent denosumab or bisphosphonates were allowed for patients with bone disease.

**Results:** 60 patients were enrolled, average age 58 (range 34-83). Majority were postmenopausal Caucasian females, but the study included African American, Hispanic, native American, male patients. The majority of tumors were ER+, infiltrating ductal, but the study included 13 HER2+, and 12 TN tumors, with 5 ILC, and 5 mixed ILC/IDC. Nearly half of the enrolled patients had clinical benefit from the regimen, remaining on therapy for 6 months or longer, with stable disease or response; 50% had progression PD at 3 months 32% had stable disease and 18% had a partial or complete response (1, long term). Overall Survival, OS, for the entire group of heavily pre-treated patients was 1.2 years, with TN and HER2 positive patients faring better than ER+ in this small study. One HER2+ patient remains in long-term remission, off chemotherapy.

The regimen was extremely well tolerated. The majority of the patients experienced grade 0 or 1 toxicity for alopecia (48/60) and peripheral neuropathy (7 with grade 2 neuropathy, 5 pre-existing, 2 with grade 3 neuropathy). There were few dose reductions (n=15), thrombocytopenia (11 grade 1 only), or use of G-CSF (14).

**Conclusions:** Metronomic weekly low dose eribulin appears to be an active and tolerable regimen with less myelosuppression, alopecia and peripheral neuropathy than is seen with approved dose, allowing longer duration of use and disease control, with similar outcomes compared to the standard dose regimen. Outcomes will be updated at presentation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-14-04

Outcomes in patients (pts) with metastatic triple-negative breast cancer (mTNBC) treated in second line (2L) in the US real-world setting

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**Background:** mTNBC represents an area of high unmet need, yet studies assessing real-world outcomes in pts treated in 2L are limited. We describe treatment patterns and outcomes in mTNBC pts receiving 2L treatment primarily in community cancer care clinics in the USA.

**Methods:** Pts aged ≥18 y with a confirmed diagnosis of mTNBC between 1 Jan 2011 and 28 Feb 2017 and who received 2L therapy were identified from the Flatiron Health electronic health record-derived database. Overall survival (OS) and time to next treatment (TTNT) were assessed as primary outcomes using Kaplan–Meier methods in both the overall population and subgroups of pts receiving the most commonly used agents (capecitabine, taxane, gemcitabine).

**Results:** Most of the 623 pts analyzed were White (62.9%) and received care in community clinics (94.9%). The most commonly used previous first-line (1L) regimens in this 2L pt population were: capecitabine (14.0%), cyclophosphamide/doxorubicin (11.6%), carboplatin/gemcitabine (9.3%), paclitaxel (7.1%), and nab-paclitaxel (7.1%); the remaining 51.0% of pts received 95 different 1L regimens. The most common 2L treatments were monotherapy regimens: capecitabine (10.9%), paclitaxel (9.3%), eribulin (9.0%), nab-paclitaxel (8.2%), and gemcitabine (7.7%); the remaining 54.9% of pts received 120 different 2L regimens. Selected demographic, clinical, and treatment characteristics and outcomes after median follow-up of 8.5 (interquartile range [IQR] 4.5–15.7) mo are shown below.

No. of pts (%)	All pts (n=623)	Selected subgroups by agent-containing regimen*		
		Capecitabine (n=101)	Taxane (n=222)	Gemcitabine (n=111)
Median age at initial metastatic diagnosis, y (IQR)	58 (50–68)	60 (53–67)	58 (50–68)	61 (51–70)
Recurrent	458 (73.5)	81 (80.2)	130 (58.6)	93 (83.8)
TFI >12 mo†	104 (47.1)	18 (43.9)	21 (42.9)	23 (57.5)
TFI ≤12 mo†	117 (52.9)	23 (56.1)	28 (57.1)	17 (42.5)
TFI missing	237	40	81	53
De novo	164 (26.3)	20 (19.8)	92 (41.4)	18 (16.2)
Missing	1 (0.2)	0	0	0
Median time from 1L to start of 2L, mo (IQR)	3.7 (2.1–6.4)	4.2 (2.6–7.5)	2.5 (1.8–4.3)	4.1 (2.1–7.1)
ECOG PS ≥2 at start of 2L	57 (9.1)	13 (12.9)	15 (6.8)	16 (14.4)
CNS/brain metastases at start of 2L	80 (12.8)	14 (13.9)	21 (9.5)	16 (14.4)
Median TTNT‡, mo (95% CI)	4.5 (4.1–5.0)	4.1 (3.5–5.0)	5.5 (4.4–6.2)	4.1 (3.1–4.8)
Median OS‡, mo (95% CI)	10.2 (8.9–11.1)	10.0 (8.0–13.5)	12.4 (10.6–15.0)	8.3 (6.9–9.6)
1-y OS‡, % (95% CI)	42.3 (38.2–46.4)	43.6 (33.3–53.8)	51.9 (45.2–58.7)	29.3 (20.1–38.6)

TFI=treatment-free interval. \*Any regimen containing each agent (monotherapy or combination); subgroups not mutually exclusive. †Defined as the interval between last (neo)adjuvant therapy and metastatic diagnosis; percentages calculated using pts with recurrent disease and non-missing TFI as the denominator. ‡From start of 2L

**Conclusions:** There was no clear 2L treatment standard used for mTNBC and median OS was short (10.2 mo overall). Notably, pts receiving 2L gemcitabine- vs taxane-containing therapy experienced shorter median OS and lower 1-y OS rates, although

imbalances in prior therapy, disease biology, and demographic and prognostic factors between these subgroups may have contributed to the observed differences. 2L treatment for mTNBC remains an area of high unmet medical need.

# 2018 San Antonio Breast Cancer Symposium ®

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Phase II study of eribulin in combination with gemcitabine for the treatment of patients with locally advanced or metastatic triple negative breast cancer (ERIGE Trial). Clinical and pharmacogenetic results on behalf of the Gruppo Oncologico Italiano di Ricerca Clinica (GOIRC)

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**Background:** There are no well-established chemotherapy regimens for metastatic triple negative breast cancer. The combination of a microtubule inhibitor (eribulin) with a nucleoside analog (gemcitabine) may synergistically induce tumor cell death, especially in tumors like triple negative breast cancers (TNBC) characterized by high cell proliferation, aggressive tumor behavior, and chemo-resistance.

**Materials and Methods:** This is an open-label, national multicenter phase II study evaluating the combination of eribulin (0.88 mg/m<sup>2</sup>) plus gemcitabine (1000 mg/m<sup>2</sup>) on day 1 and 8, q21 as either first- or second-line treatment of locally advanced or metastatic TNBC. The primary endpoint was the objective response rate (ORR) for evaluable patients (pts). The study was designed according to the Simon's two stage optimal design. We chose the lower activity (p0) of 0.20 and target activity level (p1) of 0.35. A prospective, molecular correlative study has been being carried out on germinal DNA of study population to assess the role of BRCA mutations and single nucleotide polymorphisms (SNPs) in predicting efficacy and toxicity of the combination regimen.

**Results:** From July 2013 to September 2016, 83 evaluable pts (37 in the first stage, 46 in the second one) were enrolled. They received a median number of 6 cycles of treatment (range 1-24). The ORR (CR+PR) was 37.35% (90% CI: 28.47-46.93) and the clinical benefit rate (CR+PR+SD ≥ 24wks) was 48.78% (90% CI: 39.24%-58.39%). The most common grade 3-4 adverse events (> 10% of patients) were neutropenia and liver toxicity. With a median follow-up of 28.8 months, the median progression-free survival (PFS) and overall survival (OS) were 5.1 months (95% CI: 4.2-7.0) and 14.7 months (95% CI: 10.2-20.0), respectively. BRCA1/2 deleterious mutations were observed in 15 (22%) out of 68 genotyped pts. Women with BRCA1/2 mutations were associated with worse ORR, PFS and OS than those with BRCA1/2 wild-type. A panel of SNPs in genes of study drug metabolism pathways was evaluated. Among these, CYP3A4 392A >G and FGD4 2044236G>A SNPs were associated with greater liver toxicity by logistic regression analysis. Furthermore, CDA\*2 79A>C, RRM1 2455 A>G, and CYP2C8 416G>A SNPs were associated with poorer overall survival by Cox proportional hazards model.

**Conclusions:** The combination of eribulin and gemcitabine shows promising activity and a moderate toxicity profile in metastatic TNBC. BRCA status and pharmacogenetics tests may help identify pts with high probability of response with negligible toxicity.

# 2018 San Antonio Breast Cancer Symposium ®

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Long-term treatment patterns and OS in metastatic breast cancer by intrinsic subtypes in a clinical setting in Sweden

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## Background

Breast cancer is the most common female cancer in Sweden, and patients diagnosed with early stage have a good curative prognosis. However, once metastases are detected, the disease is incurable. There is lack of detailed knowledge on the current management of metastatic breast cancer (MBC), especially treatment lines beyond second or third line. The aim was to describe long-term treatment sequences and OS for metastatic breast cancer patients by intrinsic subtypes in a clinical setting in Sweden.

## Method

In this observational cohort study, women with MBC treated between 2009-2017 were identified in the RealQ® clinical database (Uppsala University Hospital, Sweden). Clinical characteristics, complete MBC treatment regimens, and overall survival (OS) were obtained. Patients were classified into intrinsic subtypes as determined by gene expression profiling i.e., luminal A, luminal B, HER2 subtype, and basal-like (also called triple negative breast cancer [TNBC]). OS was defined from date of first metastatic diagnosis until date of death, migration or end of study. Survival rates were estimated using the Kaplan-Meier method. Subgroup analyses among deceased patients were conducted for all treatment lines and combinations. Treatment exposure rates for each treatment category were calculated using the following formula: exposure rate = person-years for the treatment category/total person-years × 100.

## Results

The full analysis set comprised 371 MBC patients (mean age 67 years, 88% with performance status 0 or 1) of which 350 patients could be subtyped: 118 (34%) Luminal A, 119 (34%) Luminal B, 68 (19%) HER2, and 45 (13%) TNBC. Median follow-up was 27 months and median OS was 32.5 months; 5-year survival rates for Luminal A, Luminal B, HER2, and TNBC were 27%, 29%, 36%, and 7%, respectively. In total 245 patients died during the observation period. The deceased patients had been treated with mean 4.5 lines (median 4, range 1-18), mean time from last treatment to death was 5.4 weeks (median 0, range 0-224). Luminal B patients (n=79) were younger (mean age 66.4 vs. 70.7 years), had more often 2+ metastatic sites (64.6% vs. 57.8%) and visceral as primary metastatic location (69.6% vs. 60.2%) compared with Luminal A patients (n=83). Generally, Luminal B patients were treated with chemotherapy more frequently than Luminal A patients, and chemotherapy was more often at later treatment lines. Total exposure rate for all chemotherapy were 33.6% among Luminal B patients vs. 24.1% among Luminal A patients (p=0.03), and for all endocrine therapy 49.4% among Luminal B patients vs. 61.9% among Luminal A patients (p=0.02). There was no difference in mortality for Luminal B and A patients when adjusting for age, number of metastatic sites, visceral metastatic location, and treatment (p>0.05).

## Conclusion

This observational study of MBC patients in clinical practice shows relatively short OS despite an extensive management with numerous treatment lines and combination therapies. Regardless of differences in treatment patterns for Luminal A and B patients the long-term OS was similar for these patient groups, highlighting the importance of awareness and careful consideration in management of MBC beyond second or third treatment line.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-14-07

Randomized phase 3 study of anthracycline-containing regimens versus S-1 as first-line treatment for metastatic breast cancer (SELECT BC-CONFIRM)—A combined analysis of two randomized phase 3 studies (SELECT BC-CONFIRM and SELECT BC)—

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**Background:** Anthracycline-containing regimens and taxane have been standard as the first-line chemotherapy for metastatic breast cancer (MBC). We conducted SELECT BC (randomized phase 3 study of taxane versus S-1 as first-line treatment for MBC) for evaluating the efficacy of S-1 for patients with HER2-negative MBC from 2006 to 2010 in Japan. This study demonstrated non-inferiority of S-1 in overall survival (OS) (median OS was 37.2 months in taxane group and 35.0 months in S-1 group (HR 1.05, 95% CI 0.86–1.27,  $p=0.015$ )), and superiority in health-related quality of life (HRQOL) to taxanes. S-1 was also shown as less toxic than taxane (Lancet Oncol 2016; 17: 90-98). S-1 might provide clinical benefit as first-line treatment for patients with HER2-negative MBC. To confirm this suggestion, we have conducted further study (randomized phase 3 study of anthracycline-containing regimens versus S-1 as first-line treatment for HER2-negative MBC: SELECT BC-CONFIRM) from 2011 to present, and a combined analysis of two randomized studies (SELECT-BC CONFIRM and SELECT-BC).

**Methods:** In SELECT BC-CONFIRM, 230 patients receiving first-line treatment for MBC were randomly assigned to either anthracycline group ( $n=115$ ) or S-1 group ( $n=115$ ). Anthracycline group patients received anthracycline-containing regimens (AC, EC, FAC, FEC, q3w) at the discretion of the treating physician. S-1 group patients received S-1 40–60 mg twice daily based on the patient's body surface area for 28 days on, 14-day off. The primary endpoint was OS, and secondary endpoints were progression-free survival (PFS), time to treatment failure (TTF), adverse events, HRQOL, and cost-effectiveness. The results were combined with SELECT-BC, to confirm the hypothesis that S-1 treatment is not inferior to the standard therapy (taxanes / anthracycline) for HER2-negative MBC.

**Results:** A combined analysis of the two studies showed that HR was 1.06, 95%CI 0.90-1.253, and  $p=0.0071$  between the standard therapy group and S-1 group. In addition, the Bayesian posterior probability for which HR would be less than 1.333 was about 99.6%.

**Conclusions:** A combined analysis of SELECT BC-CONFIRM and SELECT BC clearly demonstrated that OS with S-1 was not inferior to that with the standard therapy in patients receiving first-line treatment for HER2-negative MBC. S-1 could become a standard therapy for this patient population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-03

Association between exercise, pathological complete response, and treatment tolerability in patients receiving neoadjuvant chemotherapy for operable breast cancer: Results from the CANTO study

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**Background:** Randomized trials, although not all, suggest exercise therapy improves treatment completion rates / relative dose intensity in patients with early-stage breast cancer receiving adjuvant chemotherapy (CT). In addition, preclinical studies show that exercise therapy adds to the antitumor activity of standard CT in murine models of breast cancer. We evaluated the association between exercise and pathologic complete response (pCR) rate (i.e., ypT0ypN0) in patients receiving neoadjuvant CT for operable breast cancer.

**Methods:** Using a prospective design, patients with stage I-III breast cancer receiving anthracycline-taxane ( $\pm$  trastuzumab) neoadjuvant CT participating in a multicenter, national cohort study in France (CANTO, NCT01993498) completed questionnaire assessing self-reported exercise behavior (GPAQ 16). Multivariate logistic models were performed to determine the relationship between pre-CT exercise exposure (total MET-h/wk categorized into the proportion of patients meeting WHO exercise guidelines, the equivalent of  $\geq 10$  MET-h/wk), pCR rates, CT  $\pm$  trastuzumab dose reductions, delays, treatment completion or interruptions for the overall cohort and on the basis of clinical subtype.

**Results:** Between March, 2012 to December, 2014, a total of 989 patients participating in CANTO received neoadjuvant CT and completed GPAQ 16. Here we present interim analyses on 608 patients. Fifty-four percent of patients engaged on  $\geq 10$  MET-h/wk prior to CT administration. In multivariable analysis for the overall cohort, exercise exposure was not associated with higher pCR ( $p=0.69$ ). The pCR rate was 27.7% for patients reporting  $<10$  MET h/wk compared with 28.0% for those reporting  $\geq 10$  MET-h/wk (OR, 1.02; 95% CI, 0.71-1.45). Stratification analyses indicated no differences on the basis of clinical subtype for hormone receptor (HR) positive/HER2 negative ( $<10$  MET h/wk: 15.1% vs.  $\geq 10$  MET h/wk: 16.5%; OR, 0.95, 0.41-2.16); HER2 positive ( $<10$  MET h/wk: 38.1% vs.  $\geq 10$  MET h/wk: 32.5%; OR, 0.62, 0.28-1.35); or triple-negative disease ( $<10$  MET h/wk: 33.3% vs.  $\geq 10$  MET h/wk: 36.7%; OR, 1.04, 0.52-2.10). Rates of CT dose reductions ( $<10$  MET h/wk: 16.1% vs.  $\geq 10$  MET h/wk: 18.3%), CT dose delays ( $<10$  MET h/wk: 19.9% vs.  $\geq 10$  MET h/wk: 19.8%), CT completion ( $<10$  MET h/wk: 12.03% vs.  $\geq 10$  MET h/wk: 11.45%) trastuzumab interruptions ( $<10$  MET h/wk: 9.01% vs.  $\geq 10$  MET h/wk: 7.95%) were also not different on the basis of exercise exposure.

**Conclusion:** On the basis of interim analyses, higher pretreatment exercise exposure is not associated with higher clinical response or treatment tolerability in breast cancer patients receiving uniform conventional neoadjuvant CT. Full results will be presented at the meeting.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-04

Breast cancer recurrence and predictors for recurrence despite pathologic complete response following neoadjuvant chemotherapy

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## *Introduction*

Breast cancer patients with a high-risk tumor (for example Triple Negative Breast Cancer) who achieve a pathological complete response (pCR) following neoadjuvant chemotherapy (NACT) have a better outcome compared to patients with residual disease at surgery. This study investigated Breast Cancer Free Survival (BCFS) and predictors for distant relapse despite pCR.

## *Methods*

Monocentric retrospective study of 243 consecutive breast cancer patients who achieved pCR (ypT0/is ypN0/N0(i+)) after treatment with NACT in UZ Leuven between 01/2000 and 08/2017. 58% had stage III breast cancer, 40% Triple Negative Breast Cancer (TNBC) and 47% HER2 pos breast cancer. BCFS was defined as any breast cancer related event (local, contra-lateral, regional, metastatic) that first appeared. Primary endpoints were frequency of BCFS and predictors for metastatic relapse: patient demographics (age, body mass index (BMI)) and tumor characteristics (TNM stage, histological type, hormonal receptor status). Secondary endpoints were breast cancer specific survival (BCSS) and overall survival (OS). Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS, version 25). The Kaplan Meier method was used for survival analysis.

## *Results*

Of 1167 breast cancer patients undergoing neoadjuvant treatment, 243 patients (20,8%) achieved pCR and were included. Median follow up was 57 months (range 9-252 months). 22 (9.1%) developed tumor progression; 20 (8.2%) metastatic and 2 (0.8%) contralateral. First metastatic site was the brain in 11/20 patients (55%) and 14/22 (64%) died of breast cancer. Higher clinical tumor stage at diagnosis predicted metastatic relapse (stage I-II 2.9%; stage III 12.1%). Patients with a BMI  $\leq 25$  kg/m<sup>2</sup> had less metastatic relapse than patients with BMI >25kg/m<sup>2</sup> (3.8% versus 12.0%), better OS (94.6% vs 88.0%) and BCSS (97.7 vs 91.7%). Neither tumor type (TNBC 8.2%; HER2-pos 8.1%; HR-pos/HER2 neg 9.3%) nor younger age < 36yrs (3.3% versus 8.9%) was prognostic for post-pCR relapse. There is a lower OS (mean 174m versus 231m, 95% CI 158-190m, median 208m) and BCSS (mean 191m versus 253m, 95% CI 182-200m) in cN1-3 versus cN0 disease at diagnosis.

## *Conclusion*

Despite NACT-induced pCR, a small proportion (9.1%) will develop a metastatic relapse after a median follow-up of 57 months. We found that a higher stage at diagnosis and a higher BMI were prognostic for worse BCFS while age <36 y and negative hormonal receptor status were not prognostic. cN+ at diagnosis and a BMI >25 predict worse OS and BCSS.

# 2018 San Antonio Breast Cancer Symposium ®

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Is absolute lymphocyte count associated with platinum-sensitivity? A phase II single arm study evaluating the efficacy of neoadjuvant carboplatin and docetaxel in triple negative breast cancer

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**Background:** Platinum-based chemotherapy is still considered investigational for the treatment of sporadic triple negative breast cancer (TNBC). Since patients with TNBC have a high rate of chemotherapy resistance, it is critical to identify platinum-sensitive individuals prior to initiating therapy. Higher absolute lymphocyte count (ALC) is associated with improved clinical response to anthracycline-based chemotherapy, the current standard of care in TNBC. We report the initial results of a phase II single arm study evaluating the efficacy of neoadjuvant carboplatin and docetaxel in TNBC. We also report results of an exploratory analysis assessing whether ALC can be used to predict pathologic complete response (pCR) after treatment with platinum-based chemotherapy.

**Patients and Methods:** 78 patients with clinical stage II or III TNBC have been enrolled in this ongoing study evaluating the efficacy of neoadjuvant carboplatin and docetaxel (NCT201404107). Patients received docetaxel 75 mg/m<sup>2</sup> and carboplatin AUC 6 every three weeks for a total of 6 cycles. Blood samples were collected prior to each cycle, and a posttreatment sample was collected > 3 weeks after completing cycle 6. pCR was defined as no residual invasive disease in the breast, with or without ductal carcinoma in situ, and no tumor deposits in sampled lymph nodes. Baseline characteristics of patients were summarized with descriptive statistics. Univariate and multivariate logistic regression analyses were used to identify factors associated with pCR.

**Results:** Out of the 78 enrolled patients, 60 have completed all 6 treatment cycles and surgery. The preliminary pCR rate is 46.7%. Age, race, clinical stage, and tumor grade determined at time of diagnosis were not significantly different between pCR patients and non-pCR patients. In univariate analyses, patients with higher ALCs at the posttreatment time point were more likely to have pCR than those who had lower ALCs (OR 5.5, 95% CI 1.5-20.7, p=0.011). Additionally, patients who had higher minimum ALCs were also more likely to have pCR (OR 9.1, 95% CI 1.5-54.9, p=0.016). Baseline ALC values were not associated with pCR. The associations of posttreatment and minimum ALCs to pCR remained statistically significant even after controlling for age and clinical stage at time of diagnosis (posttreatment ALC OR 7.6, 95% CI 1.7-34.8, p=0.009; minimum ALC OR 9.0, 95% CI 1.5-55.2, p=0.018).

**Conclusion:** The pCR rate of our cohort is similar to that of other trials evaluating neoadjuvant platinum-based chemotherapy in TNBC. Baseline ALC did not predict which patients would achieve pCR. However, the associations of posttreatment and minimum ALCs with pCR indicate patients who are able to maintain a robust population of circulating lymphocytes throughout treatment with platinum-based chemotherapy are more likely to respond favorably. The link between patient immunity and platinum-based chemotherapy suggests addition of immunotherapy agents to neoadjuvant chemotherapy may improve patient outcomes.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-15-06

## Impact of serial biopsies in triple-negative breast cancer patients receiving neoadjuvant systemic therapy

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**Background:** Serial biopsies (bx) of triple-negative breast cancer (TNBC) in the curative neoadjuvant setting provides critical information on dynamic changes in the tumor in response to neoadjuvant systemic therapy (NAST) and can help inform the development of novel therapeutic strategies. However, neoplastic seeding following image guided breast bx has previously been reported in TNBC, raising concerns that serial bx may worsen clinical outcomes. Thus, we sought to determine if serial bx were associated with poorer clinical outcomes (using rates of pathologic complete response [pCR]) in TNBC pts receiving NAST.

**Methods:** We identified 370 TNBC pts who received NAST at MD Anderson Cancer Center from 2011-2017. 200 pts did not have any research bx done (controls) on the index breast carcinoma and 170 pts had at least one research bx done (cases) on the index breast carcinoma as part of the prospective molecular triaging ARTEMIS trial. Baseline characteristics were compared between cases and controls using the Student t-test, Wilcoxon Rank Sum test or Fisher's exact test as appropriate. Univariable and multivariable logistic regression was used to determine if rates of pCR following NAST were significantly different between cases and controls.

**Results:** Demographic characteristics demonstrate no significant differences (Table). However, cases were more likely to have received an anthracycline (99% vs 96%, p=0.02) and a targeted agent (22% vs 0%, p<0.01) in the neoadjuvant setting as part of the ARTEMIS trial. A total of 211 bx of the index carcinoma were performed in the 200 controls, of whom, 6% (11/200) had a second bx of the index carcinoma done solely for diagnostic purposes. In contrast, a total of 407 bx of the index carcinoma were performed in the 170 cases (mean: 2.4 biopsies). Of the 407 bx done in the 170 cases, 58% (237/407) were done for research purposes. The pCR rate in controls and cases was 48% (96/200, 95% confidence interval [CI]: 41-55%) and 42% (72/170, 95% CI: 35-50%), respectively. The odds of pCR following NAST were not significantly different between controls and cases on both univariable (odds ratio [OR]: 0.80; 95% CI: 0.53-1.20, p=0.28) and multivariable logistic regression (adjusted OR: 0.94; 95% CI: 0.58-1.51; p=0.79).

**Conclusion:** This is the first study examining the impact of serial bx on clinical outcomes in TNBC pts in the curative neoadjuvant setting. Our data suggest that research bx in this setting do not compromise rates of pCR.

### Baseline and Treatment Characteristics

Characteristic	Controls (n=200)	Cases (n=170)	p value
Mean age - years (standard deviation [SD])	52 (12)	53 (12)	0.54
Ethnicity			
White – n (%)	113 (57)	115 (68)	0.09
Black – n (%)	48 (24)	29 (17)	
Other – n (%)	39 (20)	26 (15)	
Mean tumor size – cm (SD)	3.2 (1.4)	3.3 (1.7)	0.62
T stage – n (%)			
T1	34 (17)	35 (21)	0.96
T2	145 (73)	111 (65)	
T3	14 (7)	17 (10)	
T4	7 (4)	7 (4)	

<b>N stage – n (%)</b>			
N0	101 (51)	89 (52)	0.83
N1	70 (35)	55 (32)	
N2	7 (4)	6 (4)	
N3	22 (11)	20 (12)	
<b>Stage – n (%)</b>			
I	19 (10)	21 (12)	0.74
II	140 (70)	113 (66)	
III	41 (21)	36 (21)	
<b>Grade – n (%)</b>			
1	9 (5)	0	0.14
2	27 (14)	22 (13)	
3	164 (82)	148 (87)	
<b>Neoadjuvant therapy – n (%)</b>			
Anthracycline	191 (96)	169 (99)	0.02
Taxane	199 (100)	166 (98)	0.18
Platinum	23 (12)	23 (14)	0.63
Targeted agent	0	37 (22)	<0.01

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-07

Phase II trial of neoadjuvant carboplatin and nab-paclitaxel in patients with locally advanced triple negative breast cancer

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**Background:** Response to neoadjuvant therapy (NT) predicts progression-free and overall survival in triple negative breast cancer (TNBC). Carboplatin has shown efficacy in patients with TNBC. The current phase II prospective neoadjuvant trial was designed to decrease toxicities and improve efficacy.

**Methods:** Patients with TNBC received carboplatin (carb) and nab-paclitaxel (nab). Pre-NT biopsies were procured to evaluate for biological predictors of pathological complete response (pCR). Newly diagnosed stage II-III patients with TNBC were treated with 4 cycles of carb (AUC 6, day 1 of 28 day cycle) and weekly nab 100 mg/m<sup>2</sup> x 16. Targeted accrual goal is 70. RNA extracted from formalin fixed paraffin embedded (FFPE) biopsies pre-NT was tested for MammaPrint/Blueprint and custom Agilent full genome microarrays for gene expression (GE, by Agendia Inc). The raw gMeanSignal was log2 transformed and normalized to the 75th percentile for GE analysis. Association between MammaPrint/ Blueprint results and pCR was tested by Fisher exact test. The linear model from R limma package was applied. Ingenuity Pathway Analysis (IPA) was applied to assess functional pathways associated with pCR. Cellular distribution by CIBERSORT analysis was carried out to estimate the abundance of 22 different cell types in each patient sample, and test whether the distribution of cell types is different between pCR and non-responders.

**Results:** A total of 64 patients were enrolled. Two patients were deemed ineligible (Her2+), and three were too early, resulting in 59 patients evaluable for pathological response. The pCR rate was 47% (RCB0, 28/59). Eight patients had RCB I. RCB0 plus RCB I reached 61%. Sufficient quality RNA and DNA were available from the first 43 of 55 pts with TNBC. 44/59 (75%) required dose modifications (mostly hematologic), 5 patients had grade 3 peripheral neuropathy (PN), 3 had grade 2 PN, and 3 patients had grade 2 LFTs. In the 53 pts with GE assessment, pCR was inversely associated with luminal Blueprint type (p=0.04). With fold change >1.5 and p-value < 0.05, 36 genes were differentially expressed (DE) in TNBC. CIBERSORT analysis suggested that T-cell regulatory cells (T<sub>REGS</sub>) were associated with pCR in TNBC, and 5 cell types (plasma cells, T<sub>REGS</sub>, macrophage, dendritic cells and neutrophils) presented differently between all pCR and non-pCRs with P-value <0.05. TDP analysis to assess correlation with pCR is ongoing.

**Conclusions:** The combination of carboplatin and nab-paclitaxel given in the neoadjuvant setting reached a promising pCR rate of 47%. The MammaPrint non-luminal Blueprint subtype was predictive of pCR in TNBC. Preliminary analysis suggested that a 36-gene signature for TNBC was associated with pCR. CIBERSORT analysis revealed 5 cell types with different abundance between the pCR and non-responders, suggesting the need to target the tumor microenvironment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-08

Pathologic complete response rates following neoadjuvant systemic therapy in 794 patients with early breast cancer: The Royal Marsden experience

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## Background

The presence and extent of residual invasive cancer after neoadjuvant treatment (NACT) is a strong prognostic factor for risk of recurrence, especially in triple-negative (TN) and human epidermal growth factor receptor 2-positive (HER2+) breast cancer (BC). Recent advances in the standard-of-care NACT improved pathological complete response (pCR) rates in published clinical trials. We evaluated the pCR rates, defined as ypT0-is ypN0, in our real-world BC population and in estrogen receptor-positive [ER+] HER2-, HER2+ and TN subgroups and their association with tumour, patients' characteristics and disease-free survival (DFS).

## Methods

We retrospectively identified early BC patients receiving NACT between January 2013 and December 2017. Demographics, patient and disease characteristics, pathological responses, toxicities, dose delays and reductions were recorded. Simple statistics, Fisher's exact test, chi-squared method and Cox regression were used as appropriate.

## Results

794 patients identified had median age of 50 years (range 24-87) and 93.9% (745 patients) ECOG performance status (PS) 0. 3.0% (24) had clinical stage I disease, 68.0% (540) stage II and 29.0% (230) stage III. 71.0% (564) had grade 3 disease and 91.8% (729) ductal histology. 33.7% (257) had ER+/HER2-, 25.8% (205) had TN and 38.0% (301) HER2+ disease. Overall, 6.8% (54) patients received platinum. 36.6% (291) patients had dose reductions and 24.3% (193) dose delays. Along with NACT, 51.6% (147) of the HER2+ patients received Trastuzumab and Pertuzumab and 48.4% (138) Trastuzumab alone. pCR rate was 33.1% in the overall population and significantly different in ER+/HER2-, HER2+ and TN subgroups (12.84% versus 52.0% versus 28.43% respectively,  $p < 0.001$ ). pCR was influenced by grade (1: 0%; 2: 24.3%; 3: 36.1%,  $p = 0.005$ ) and histology (ductal: 34.2%; lobular: 10.0%; mixed 25.0%;  $p = 0.01$ ). In the HER2+ subgroup, there was a trend for improved pCR rates for patients receiving Pertuzumab and Trastuzumab (57.0%) versus Trastuzumab alone (51.0%). No statistically significant differences were seen based on patients' characteristics including age and PS or in case of treatment dose reductions and delays. Early discontinuation of NACT was associated with lower pCR rates (20.5% vs 36.29%,  $p < 0.001$ ).

Of interest, pCR rates remained consistent across the period 2013-2017 in the overall population. We observed a trend for improved pCR in the HER2+ (2013: 47.5%; 2014: 44.4%; 2015: 66.7%; 2016: 51.0%; 2017: 51.4%) and TN cohorts (2013: 23.5%; 2014: 25.0%; 2015: 25.0%; 2016: 33.3%; 2017: 34.1%) but not in the ER+/HER2- group.

Median DFS was 83.8 months (95% CI 62.0-NR) in the overall population. Although not reached in the TN cohort, median DFS was different according to disease subgroups (HER2+: 83.78 months; TN: NR; ER+/HER2-: 62.0 months,  $p < 0.0001$ ).

## Conclusions

In our analysis pCR rates are consistent with data published in literature and higher in HER2+ and TN disease. The impact of new agents had a relatively low impact on pCR rates in our overall population over the last 5 years, although they produced gradual improvements in the HER2+ and TN subgroups.

# 2018 San Antonio Breast Cancer Symposium ®

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Histological patterns of response to neoadjuvant chemotherapy in breast cancer and breast conservation

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## Introduction

Neoadjuvant chemotherapy (NAC) in breast cancer is an in vivo marker of chemosensitivity and pathological complete response (pCR) an independent prognostic factor. When there is response, NAC downstages the tumour and may allow for or facilitate a conservative surgery. There are three histological patterns of response to NAC: a concentric pattern in which tumour regression takes place from the periphery to the center, a scatter pattern, where fibrosis is placed between tumoral cells, and a mixed pattern.

## Objective

To determine which clinical and histological variables define the type of response to neoadjuvant chemotherapy that facilitates and allows for breast conservation in women with breast cancer.

## Material and methods

A retrospective observational study was made including 170 patients with breast cancer who underwent NAC in the Hospital Universitari de Bellvitge between February 2010 and October 2013. Different clinicopathological parameters were recorded: age, menopausal, stage, surrogate molecular subtype, histological pattern of response, and pCR.

Median age was 50 (23-78), Stage I (1.1%) IIA (27.1%) IIB (35%), IIIA (20.9%), IIIB (11.4%), IIIC (4.5%). Molecular subrogated types: Triple negative (30.7%), Luminal B Her 2 negative like (26.2%), Her 2 positive (17.7%), Luminal B Her 2 positive like (16.4%) and Luminal A like tumours (9.0%). NAC included Anthracyclines, Taxanes, and Trastuzumab if Her 2 +++.

## Results:

Histological pattern of response: 25,5% of cases achieved a pCR. When residual tumour was observed, 42% of the cases were as scatter pattern, 21.9% as concentric pattern and 8.9% as mixed pattern.

The predictive factors of pCR were in the univariate analysis: absence of multicentricity, negative estrogen receptor, negative progesterone receptor, histological grade 3, Ki 67 > 20%, and her 2 overexpression. In the multivariate analysis, only negative estrogen receptor and her 2 overexpression were predictive factors. The molecular surrogate type Her 2 positive was predictive of pCR.

The predictive factors of the concentric response were in the univariate analysis: tumour size of < 5 cm, absence of nodal involvement, negative estrogen receptor, negative progesterone receptor, presence of tumour necrosis and inflammatory infiltration. In the multivariate analysis, tumour size < 5 cm, absence of lymph node involvement, Ki 67 > 20% and tumour necrosis were statistically significant. The molecular surrogate type predictive of a concentric response was triple negative. Conservative surgery was more frequent in the concentric pattern group (78.4%) than in the scatter pattern (58.1%) (p=0.032) but the histological pattern of response to NAC is not correlated to survival.

## Conclusions

Tumour size < 5 cm, absence of lymph node involvement, Ki 67 > 20% and tumour necrosis were predictive of concentric pattern of response to NAC. Triple negative tumours were related to concentric histological pattern, meanwhile Her 2 overexpressed was predictive of pCR. The conservative treatment was more frequent in the concentric pattern. Histological pattern of response to NAC is not correlated to outcome. Only pCR was related to survival.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-10

A prospective multicenter real-world study on neoadjuvant treatment and clinical outcome in TNBC patients

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## Introduction

Controversy exists with regards to the optimal regimen for neoadjuvant chemotherapy (NAC) of TNBC. Platinum-based regimens seem to be more active in TNBC improving pCR rates significantly. But adding platinum to an anthracycline/taxane chemotherapy regime comes at the expense of greater toxicity. Its impact on survival and long-term-outcomes remains undetermined. Practical guidelines vary across leading international professional societies. In this real-world multicenter study neoadjuvant regimens, pCR rates and survival were evaluated.

## Material and methods

This study was conducted from 2012-2017 in six certified breast cancer centers in the region of Hanover, Germany, including rural and urban populations by using a personal questionnaire and data from the medical records. All patients with primary TNBC (ER<1%, PR<1%, Her2/neu 0, 1+ or 2+ FISH/CISH negative) and no evidence of distant disease were eligible.

## Results

143/217 patients (66%) received NAC and 74/217 patients (34%) adjuvant chemotherapy. 63/143 (44%) patients achieved pCR. 23/63 (35%) received platinum-based NAC and 40/63 (65%) received chemotherapy without platinum. In 80 patients pCR was not achieved. 12/80 patients received platinum and 68/80 non platinum-based NAC. pCR was significantly higher among patients with platinum-based chemotherapy (chi-square,  $p < 0.003$ ). 20/23 patients who achieved pCR received NAC containing anthracycline/taxane/carboplatin, 3/23 patients received a taxane/carboplatin-based regime. Treatment discontinuation was seen in 12/35 (34%) patients receiving platinum-based NAC vs 15/108 (14%) in non-platinum-based NAC. Mean follow up was 17 months (1-70 months). A significant difference in OS ( $p=0.007$ ) and DFS ( $p=0.001$ ) was seen for patients with a pCR.

## Conclusion

Our trial confirms that platinum based NAC achieves significantly higher rates of pCR in patients with TNBC. pCR was associated with significantly longer DFS and OS. The CALBG protocol (CALBG 40603) was the preferred choice of treatment regimen followed by the GeparSixto protocol. In our study the pCR rate was comparable to that of both trials in which pCR rates of 60% (CALBG 40603) and 53% (GeparSixto) were achieved. However, in our trial 34% of the patients discontinued treatment due to toxicity. Therefore these protocols should be used in carefully selected patients. In a real world setting less toxic chemotherapy regimens achieved a pCR rate of 37%. In terms of toxicity and adherence to chemotherapy, these regimens are reasonable alternative options. In daily care close monitoring of treatment response is essential during NAC. In patients who have a rapid clinical response to platinum-free NAC the benefit of adding platinum is questionable. In contrast, the addition of platinum seems to be appropriate in those patients who show only limited response under NAC.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-11

Predicting pathological complete response (pCR) to neoadjuvant chemotherapy (NACT) based on pre- and post-NACT digital mammography and digital breast tomosynthesis findings

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**Background:** In invasive breast cancer patients being treated with neoadjuvant chemotherapy (NACT), achieving pathological complete response (pCR) is a useful goal of treatment. Monitoring response to NACT and predicting pCR is helpful in planning further therapy and providing robust prognostic information. Digital mammography (DM) and additional digital breast tomosynthesis (DBT) features are important tell-tales of tumor characteristics and behaviour. Following NACT, the mammographic features- both DM and DBT- of responding tumors can vary considerably. In this prospective study, we correlated the DM and DBT features of pre-NACT and post-NACT mammograms to investigate if these can reliably predict pCR to NACT. **Methods:** Following approval by institutional ethics committee, starting January 2016, 200 consecutive invasive breast carcinoma patients (mean age 51.2 years, all palpable breast masses) undergoing diagnostic breast imaging had their DM and DBT reviewed by two radiologists independently, who were blinded of the cyto/histology and the original DM and DBT reporting. Of these, 47 patients who were treated with NACT and had pre- and post-NACT DM and DBT were recruited. After a core-biopsy, radio-opaque marker(s) were placed in tumor core/margin. The pre- and post-NACT DM and DBT findings were compared and correlated with the extent of response of the primary breast tumor to NACT. DM and DBT characteristics predictive of (in-breast) pCR of index breast lesion were identified.

**Results:** Of the 47 patients who underwent NACT, 44 received both anthracycline and taxane, and 3 received only an anthracycline based combination chemotherapy. Twelve patients underwent breast conservative surgery and the remaining underwent mastectomy. pCR was seen in 17 (36.2%) patients based on the surgical specimen histology. On clinical examination, 19 (40.4%) patients had clinical complete response (cCR) of the breast tumor, 11 (64.7%) of whom had pCR as well. Five patients had radiological complete response (rCR, no breast lesion visualised on post-NACT imaging)- 2 patients on DM alone, 2 patients on DBT alone, and one patient on both DM and DBT. Radio-opaque clips had some obscuring effects in 3 of these 5 patients, especially on DBT, in form of reduced visibility of breast lesion on DBT, c.w. corresponding DM images. All 5 patients with rCR had pCR (sensitivity=29.4%, specificity=100%), in contrast to only 11 (57.9%) patients with cCR having pCR. Patients with pCR had benign appearing (forced Bi-RADS 2 and 3) lesions on mammography more commonly on DM ( $p<0.001$ ) than on DBT ( $p=0.042$ ) (41.2% vs 23.5%). Post NACT lesion morphology varied significantly between patients with and without pCR on DM ( $p=0.038$ ) but not on DBT ( $p=0.182$ ). Pre-NACT forced Bi-RADS score, lesion morphology or margin characteristics on DM and DBT did not vary significantly amongst patients with and without pCR.

**Conclusions:** Post-NACT DM and DBT features can predict pCR with high specificity but with low sensitivity. Pre-NACT DM and DBT features did not reliably predict response to NACT, and pCR in this study. DM may be better than DBT for assessing response to NACT in the presence of radio-opaque markers/clips.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-12

Axillary lymph node metastasis and HER2-receptor positivity significantly associate with recurrence and worse survival in breast cancer patients who achieved pathological complete response after neoadjuvant chemotherapy

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## BACKGROUND

Neoadjuvant chemotherapy (NAC) has become a common practice in breast cancer care since it not only expands the opportunity for breast conservation surgery, but also allows in vivo assessment of individual cancer biology. Patients who achieved pathological complete response (pCR) after NAC are known to have significantly improved outcomes than those who did not. To date, there has been no large study of factors that associate with tumor recurrence after patients had pCR following NAC. To identify such factors, we examined a cohort of 394 patients.

## METHODS

Patients diagnosed during 2007-16 with clinical stage I-III breast cancer who achieved pCR following NAC were identified from clinical records at four hospitals in urban Japan. Nearly 70% of patients received standard NAC regimen, which was a combination of anthracycline and taxane, with trastuzumab added as needed. pCR was defined as no pathological evidence of invasive cancer in the breast; residual ductal carcinoma in situ (DCIS) and residual axillary lymph node metastasis were included in this study. The median follow-up time was 63 months (range = 16-161 months). Outcomes were assessed by 5-year disease-free survival (DFS) and 5-year overall survival (OS).

## RESULTS

Among the 394 patients with pCR, the breast cancer subtype was as follows: Luminal – 49 (12.4%), Luminal-HER2 – 97 (24.6%), HER2 – 117 (29.7%), and TNBC – 131 (33.2%). During follow up, 28 (7.1%) of the 394 patients had experienced tumor recurrence. In univariate Cox regression analysis, each of HER2-receptor status, pre-NAC tumor size, and pre-NAC axillary lymph node status were associated with recurrence. The hazard ratios, and their 95% confidence intervals (CI) and P values for these significant factors were as follows. HER2-receptor negative vs. positive: 2.5 (CI = 1.0-5.8; P = 0.036); cT1/2 vs. cT3/4: 2.2 (CI = 1.3-6.1; P = 0.008); cN0 vs. cN1-3: 9.5 (2.2-40.7; P = 0.002). However, age (<50 vs. ≥50 y), residual DCIS, post-NAC axillary lymph node status, type of mastectomy (total vs. partial), and adjuvant radiation therapy were not associated with recurrence. Of the 28 patients with recurrence, site of first event was local for 8, and brain and visceral for 10 each. Seven of the 10 patients with brain metastasis were HER2-receptor positive. Eleven of the 28 patients with recurrence had deceased, with a median post-recurrence survival duration of 40 months (range = 2–94 months). Shorter survival was associated with HER2-receptor positivity (P = 0.003).

## CONCLUSION

Axillary lymph node metastasis before rather than after NAC, and HER2-receptor positivity are associated with tumor recurrence in patients who achieved pCR in breast cancer. HER2-receptor positive patients had higher risk for brain metastasis and shorter survival. Given the extreme rarity of local recurrence after pCR, we cannot help but speculate that omitting surgical removal of pCR tissue may be permissible when pCR has been diagnosed accurately.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-13

Sentinel lymph node biopsy after neoadjuvant chemo therapy in patients with clinically node negative breast cancer: Can sentinel lymph node biopsy be omitted in selected tumor subtypes?

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## Background

Selected patients presenting with clinically node negative breast cancer (cN0) stage II-III can be treated with neoadjuvant chemo therapy (NCT) followed by surgery of the breast and sentinel lymph node biopsy (SLNB). Pathological response rates are known to vary by tumor subtype with complete pathological response up to 60% in both ER-, HER2-positive tumors and triple-negative (TN) tumors. However, hormone receptor-positive, HER2-negative tumors exhibits lower response rates to NCT. About 70-80% of the patients with a clinically negative axilla, also have pathologically negative axilla after NCT. Properly selecting those patients with a low probability of sentinel lymph node (SLN) metastasis after NCT might save them an unnecessary SLNB. The aim of our study was to assess the impact of tumor subtypes on final pathologic node (pN) status in patients with clinically node negative (cN0) breast cancer who underwent NCT.

## Methods

All cN0 patients diagnosed from 2014-2017 in one large teaching hospital in the Netherlands who were treated with NCT and subsequent surgery including SLNB were selected. This retrospective cohort contained a series of 107 patients with 105 tumors treated for stage II-III breast cancer resp. stage 2 (n=107) and stage 3 (n=2), all cN0. Patient age, tumor size, uni/multifocality at presentation did not differs across subtypes. Histological grading and histological type at presentation did differ across subtypes. Approximated subtype was TN in 21 (19.3 %), HER2-positive in 34 (31.2%), and hormone-receptor-positive, HER2-negative in 54 (49.5%) patients.

## Results

In a total of 109 tumors, 88 had a negative post-NCT SLNB (80.7%), 4 had isolated tumor cells (3.7%) 7 had micro metastasis (6.4%), 10 had macro metastasis (9.2%). Rates of pathological nodal negativity were significantly higher in patients with TN (100%) and HER2-positive tumors (97.1%) than in those with hormone-receptor-positive, HER2-negative tumors (63%) ( $p < 0.001$ ). Furthermore, a total of 41 (37.6%) patients had a complete pathologic response (pCR) of the primary breast tumor of which 40 (97.6%) had pathologically confirmed negative SLN and 1 (2.4%) had isolated tumor cells. A total of 67 patients had no pCR of which 48 (71.6%) had pathologically confirmed negative SLN and 19 (28.4%) were SLN positive ( $p < 0.001$ ). Rates of pCR were significantly higher in HER2-positive (70.6%) and TN tumors (47.6%) than in those with hormone-receptor-positive, HER2-negative tumors (13.0%) ( $p < 0.001$ ).

## Conclusion

SLNB after NCT might be considered to be omitted in patients presenting with cN0 with TN and HER2+ tumors, as SLN is rarely positive. Furthermore, SLN was rarely found positive in patients who achieved pCR. However, more data are necessary for multivariate logistic regression and definite conclusions.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-14

Neoadjuvant liposomal doxorubicin and carboplatin is effective and tolerable for the treatment of triple negative breast cancer

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**Background:** The use of neoadjuvant platinum with taxane for triple negative breast cancer (TNBC) has gained increased attention for improving rates of pathologic complete response (pCR). Our prior trial combining carboplatin (CAR) with liposomal doxorubicin (DOX) for metastatic TNBC showed good response rates with minimal side effects while allowing for greater platinum dosing compared to a taxane combination. We hypothesized that the doublet of DOX+CAR is effective and tolerable in the neoadjuvant setting for TNBC and that tumor genomics may aid in determining those patients most likely to benefit.

**Methods:** A phase II single arm trial was conducted for patients (pts) diagnosed with stage II-III TNBC. Patients received 4 cycles of neoadjuvant carboplatin (AUC 5) and liposomal doxorubicin (30mg/m<sup>2</sup>) administered every 28 days, then underwent definitive breast surgery followed by 12 weeks of adjuvant paclitaxel 80 mg/m<sup>2</sup> administered weekly. Primary and secondary clinical endpoints were rate of pCR and two year recurrence free survival (RFS) and overall survival (OS), respectively. Cardiac safety of the combination was assessed. Fresh residual tumor samples were obtained at time of surgery for generation of patient derived xenografts (PDX). Tumor genomic profiling was done to determine the mutational spectrum, association of this spectrum in primary tumors with achieving pCR, and identifying alternative treatment strategies for PDX evaluation for patients with resistant disease.

**Results:** From 2/2015 to 5/2018, 36 pts were enrolled and 32 completed treatment; 4 pts await definitive surgery; 12 (33%) are two years from diagnosis. Median age of the cohort was 53 years. There was high participation by under-represented groups: 23% African American, 20% Asian, 14% Hispanic. Most histologies were invasive ductal but included apocrine, pleomorphic lobular, and metaplastic subtypes. Of the 32 pts who completed surgery, 34% (11) achieved pCR and 64% (23) had clinical response on serial physical exam. At 2 years, there were 2 distant and 1 local recurrence. The most common toxicities during DOX+CAR were grade 1 nausea in 19 pts (53%), grade 3/4 neutropenia occurred in 10 pts (28%); these pts received GCSF support with subsequent cycles; febrile neutropenia occurred in 1 pt (3%) in this group. Grade 3 thrombocytopenia (2 pts), pruritis (1 pt), and mucositis (1 pt) were observed. Only 6 pts (17%) had grade 1 alopecia. There were no delays in treatment due to cardiotoxicity or complications from surgical healing. TP53 (93%), PI3K/PTEN (26.6%), and NOTCH (20%) were the most commonly altered pathways. Structural variants, such as amplifications, rearrangements, and frameshifts were the most frequent alterations detected. Of the 25 pts who had residual disease, PDX was attempted from 14 pts, and 10 (71%) PDX were established, including those for all 3 patients experiencing recurrence.

**Conclusion:** Neoadjuvant DOX+CAR demonstrated good efficacy and tolerability. Post-chemotherapy PDX is feasible and may help identify targeted approaches for patients with resistant disease. These results warrant further evaluation of this combination for early stage TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

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Accuracy of MRI after neoadjuvant therapy for invasive lobular carcinoma of the breast

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**Background:** Invasive lobular carcinoma of the breast (ILC) has higher rates of false negative imaging than invasive ductal carcinoma, and lower rates of neoadjuvant therapy (NAT) use. We evaluated the accuracy of Breast Imaging Reporting And Data System (BIRADS) findings on magnetic resonance imaging (MRI) after either neoadjuvant chemotherapy or endocrine therapy, and determined whether imaging change correlates with disease free survival.

**Methods:** We queried a database of 674 ILC cases treated at UCSF from 1981-2017 and identified all patients treated with NAT. We reviewed MRI reports and recorded BIRADS descriptors of findings, maximal tumor diameter for mass or non-mass enhancement (NME), and subjective radiologist comments on progression or improvement. We used the t-test, chi-squared test, Pearson's correlation, and Kaplan Meier survival estimates to evaluate the accuracy of MRI after NAT compared to true tumor size on pathology, and the relationship between imaging change and disease free interval in Stata 14.2.

**Results:** Of 136 patients with ILC treated with NAT, we included 101 women who had a post-treatment breast MRI report available. Of these, 58.4% received neoadjuvant chemotherapy, and 41.6% neoadjuvant endocrine therapy. After NAT, MRI findings were mass only in 43%, both mass/NME in 33%, NME only in 18%, and neither in 5%. Maximal diameter of mass on post-treatment MRI underestimated true size by a mean of 3.3 cm (range -3.6 to 15.3 cm). NME size on post-treatment MRI underestimated true size by a mean of 1.87 cm (range -7.2 to 9.7 cm). Mass size on MRI underestimated true size by  $\geq 1$  cm in 61.5% of cases; this size discrepancy was associated with increased positive margins (46.4% versus 20%,  $p=0.011$ ). NME size on MRI underestimated true size by at  $\geq 1$  cm in 65.6%. The correlation coefficient between mass size on MRI and true size was 0.34 ( $p=0.0041$ ), which increased to 0.67 ( $p<0.0001$ ) when excluding those with associated NME. The correlation coefficient between NME size on MRI and true size was 0.28 ( $p=0.1239$ ). Subjective progression on post-treatment MRI was associated with increased recurrence rates (80% versus 18.3%,  $p=0.001$ ). In those with subjective improvement on MRI, there was a trend towards longer disease free interval (89% versus 73% disease free at 4 years,  $p=0.13$ ).

Table 1. Patient and tumor characteristics.

	Neoadjuvant chemotherapy (n=59)	Neoadjuvant endocrine therapy (n=42)	P value
Mean age (yrs, 95% CI)	53.6 (50.9-56.3)	61.3 (58.4-64.1)	0.0002
Subtype			0.105
ER+ PR+ HER2-	29 (53.7%)	23 (62.16%)	
ER+ PR- HER2-	14 (25.9%)	13 (35.14%)	
ER- PR- HER2-	1 (1.85%)	0	
HER2+	10 (18.5%)	1 (2.7%)	
Grade			0.076
1	14 (25%)	16 (38.1%)	
2	37 (66.1%)	26 (61.9%)	
3	5 (8.93%)	0	
Surgical stage			<0.001
I	17 (28.81%)	28 (66.67%)	
II	26 (44.07%)	6 (14.29%)	
III	16 (27.12%)	8 (19.1%)	
Mean follow-up time (yrs, 95% CI)	5.6 (4.53-6.75)	5.1 (3.84-6.27)	0.48

**Conclusions:** Maximal tumor diameter on MRI after NAT in ILC vastly underestimates true tumor size. While these findings suggest using caution when using an MRI for surgical planning in patients with ILC, particularly if there is associated NME, the trend towards improved disease free survival in those with a subjective improvement is intriguing and suggests that MRI changes could become an early predictor of outcomes.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-15-16

Pathological complete response rates with the addition of carboplatin to standard neoadjuvant chemotherapy in a cohort of real-world patients with triple negative breast cancer

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**Objectives:** Addition of carboplatin to standard neoadjuvant chemotherapy (NACT) for triple negative breast cancer (TNBC) remains controversial. There are several randomized trials showing that carboplatin increases the likelihood of achieving pathological complete response (pCR) in TNBC. Patients with TNBC who achieve pCR has been shown to have better disease-free and overall survival. The aim of this study was to asses the impact of adding carboplatin to standard NACT in TNBC in terms of pCR rates and toxicity. **Methods:**In this cross-sectional study, 252 consecutive patients with primary TNBC who were submitted to neoadjuvant chemotherapy between 2013 and 2018, in a single center, were selected. Patients with biopsy-confirmed TNBC, previously untreated, with clinical stages I-III were included (n=179). Clinical pathological features, pathological response, treatment protocol, and toxicities were analyzed and considered for statistical analysis. Eighty patients treated from 2013 to 2015 received doxorubicin plus cyclophosphamide once every 3 weeks (AC) for four cycles, followed by 12 weeks (wP) or every 3 weeks (P) paclitaxel(AC-T group). Ninety-nine patients, treated from 2015 to 2018 had four cycles of AC followed by wP plus weekly carboplatin (Cb) area under curve (AUC) 1.5-2.0 (AC-TCb group). Pathologic response was determined locally, and pCR was defined as the absence of residual invasive disease with or without ductal carcinoma in situ in the breast and axilla. **Results:** Data from 179 patients were included in the analysis (AC-T: n=80; AC-TCb: n=99). Patients in AC-TCb group had a median age of 51.7 years vs. 47.4 years in AC-T group,  $p=0.01$ . In AC-TCb group 61.6% of patients were postmenopausal vs 43.7% in AC-T group,  $p=0.03$ . The distribution of clinical stage in groups AC-TCb and AC-T were as follows: stage I 6.0% vs 0%; stage II 42.4% vs 43.7%; stage III 51.6% vs 56.3%, respectively ( $p=0.02$ ). In AC-TCbgroup, 34 patients (35.0%) had pCR in comparison to 20 patients (25.0%) on AC-T group ( $p=0.22$ ). Pathological stage distribution in groups AC-TCb and AC-T were: stage I 24.7% vs 33.7%; stage II 23.7% vs 26.3%; stage III 16.4% vs 15%, respectively ( $p=0.42$ ).

Distribution of patients with TNBC submitted to NACT with AC-T and AC-TCb according clinical-pathological characteristics

Clinical pathological characteristics	AC-T n= 80	AC-TCb n=99	p
Menopausal			0.03
yes	35	61	
no	45	38	
Clinical stage			0.02
I	0	6	
II	35	42	
III	45	51	
Histologic type			0.25
IDC	80	96	
others	0	3	
Histologic grade			0.86
1	0	1	

2	29	32	
3	51	64	
Pathological stage			0.42
O	20	34	
I	27	24	
II	21	23	
III	12	16	
pCR			0.22
yes	20	34	
no	60	63	

More than 85.0% of patients in AC-TCb group received at least 9 weeks of carboplatin and less than 20.0% required dose reduction due to toxicity. **Conclusions:** An improved pathological complete response for TNBC patients submitted to standard NACT plus carboplatin was observed. The results are in accordance with previous studies demonstrating that the addition of carboplatin to NACT improves pCR rate in TNBC with a favorable risk to benefit profile. Therefore carboplatin might be a potential component of NACT and should be considered in this context.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-17

Liposomal-encapsulated doxorubicin (Myocet) as part of primary systemic therapy in HER2-positive operable breast cancer: Efficacy data, cardiotoxicity and long-term follow-up in 81 patients diagnosed from 2005-2016 at a single institution

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## Objectives

To evaluate the efficacy, cardiotoxicity profile and long-term benefits of liposomal doxorubicin (Myocet) as part of neoadjuvant therapy in HER2-positive operable breast cancer patients.

## Methods

The treatment consisted of a sequential regimen of paclitaxel and liposomal doxorubicin (Myocet) plus trastuzumab. Clinical and pathologic response were evaluated and correlated with clinical and biological factors. Cardiotoxicity profile and long-term benefits were analyzed.

## Results

Median age was 48 years and 4%, 69%, and 27% were stage I, II, and III, respectively, while 12% had inflammatory breast cancer at diagnosis. Hormone receptor (HR) status was negative in 43% and 70% were grade III.

## Patients Characteristics

	Total number (%)
Age, years	
Median (Range)	48 (30-79)
Tumor (cT)*	
Tx	1 (1)
T1	4 (5)
T2	53 (66)
T3	13 (16)
T4	10 (12)
Nodal Status	
N0	24 (30)
N1-2	57 (70)
Stage	
I	3 (4)
II	56 (69)
III	22 (27)
Hormone Receptor Status	
HR+	35 (43)
HR-	46 (57)
HER2	
IHC 3+	74 (91)
IHC 2+	7 (9)
FISH/SISH	21 (26)
Grade	
I	1(1)

II	22 (27)
III	57 (71)
Unknown	1 (1)
Ki-67	
<20%	6 (7)
20-35%	32 (40)
>35%	43 (53)
Surgical Indication at Diagnosis	
Mastectomy	58 (72)
Tumorectomy	22 (27)
Any surgery in breast*	1 (1)

\* One patient had an occult carcinoma.

The clinical complete response rate by ultrasound and MRI were 47% and 65%, respectively, and allowed a high rate of conservative surgery (72%).

The pathologic complete response (pCR) rate in breast and axilla was 54%, higher in HR-negative (66%) than in HR-positive (46%), in ki-67>35% (65%) than ki-67 between 20-35% (38%) and similar in grade III (54%) and grade II (55%).

#### pCR by Subgroups

Pathologic Response (n=81)	pCR n (%)	No pCR n (%)
Tumor Size (MRI)		
<=5 cm	26 (49)	27 (51)
> 5 cm	10 (67)	5 (33)
Nodes		
Positive	26 (46)	31 (54)
Negative	18 (75)	6 (25)
Histological Subtype		
Ductal	43 (56)	34 (44)
Lobular	0 (0)	2 (100)
Other	1 (50)	1 (50)
Hormone Receptor		
Negative	23 (66)	12 (34)
Positive	21 (46)	25 (54)
Ki-67		
<20	4 (67)	2 (33)
20-35	12 (38)	20 (62)
>35	28 (65)	15 (35)
Grade		
I	0 (0)	1 (100)
II	12 (55)	10 (45)
III	31 (54)	26 (46)

Patients who achieved pCR had longer DFS and a trend to improve OS.

Four percent of patients showed a decrease in the left ventricular ejection fraction below 50% during treatment. All except one of them recovered after discontinuation of trastuzumab.

**Conclusion:** A sequential regimen of taxanes and liposomal doxorubicin (Myocet) plus trastuzumab was active with high pCR rates and long-term benefit with a very good cardiotoxicity profile.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-18

Nomogram for prediction of axillary and breast pathologic response after neoadjuvant chemotherapy in node positive patients with breast cancer

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**Background:** Many patients with cytology prove node-positive breast cancer receive neoadjuvant chemotherapy (NAC). We developed a nomogram to predict probability of breast and axillary pathologic complete response (pCR) in patients with cytologically proven axillary node positive breast cancer with NAC.

**Materials and Methods:** This study is a registered medical record review based on a prospectively collected cohort. We selected 995 patients who were diagnosed with invasive breast cancer and axillary lymph nodes metastasis and treated with NAC followed by curative surgery at Samsung Medical Center between January 2007 and December 2014. . Baseline patient and tumor characteristics, chemotherapy regimen, and tumor and nodal responses were analyzed. A nomogram was developed using a binary logistic regression model with a cross validation.

**Results:** Axillary pCR was achieved in 47.3% of the patients who underwent axillary surgery after NAC. Breast pCR was achieved in 24.3% of the patients who underwent breast surgery. Axillary and breast pCR was associated with initial clinical tumor status, negative progesterone receptor status, positive human epidermal growth factor receptor 2 (HER2) status, and nodal responses

Multivariate analysis for Pathologic Complete Response

	OR	95% CI	p-value
Clinical tumor stage			0.009
cT1			
cT2	0.303	0.136-0.676	
cT3	0.220	0.090-0.535	
cT4	0.286	0.081-1.012	
Progesterone receptor			0.007
Positive			
Negative	0.340	0.154-0.750	
HER2			<0.001
Positive			
Negative	3.957	2.352-6.658	
Clinical node response			<0.001
Disappeared			
Decreased	0.258	0.154-0.433	
No response or Increased	0.003	0.001-0.026	

. A nomogram was developed based on the clinical and statistically significant predictors. It had good discrimination performance (AUC 0.868, 95% CI, 0.84–0.89) and calibration fit. Cross validation had average AUC 0.853 (0.837-0.869)

Model development of risk point

	Parameter	Points
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Clinical tumor stage	cT1	37
	cT2	25
	cT3	12
	cT4	0
Progesterone Receptor	Positive	0
	Negative	35
HER2	Positive	21
	Negative	0
Clinical node response	Disappeared	100
	Decreased	50
	No response or Increased	0

**Conclusion:** Our nomogram might help predict axillary and breast pCR after NAC in patients with initially node-positive breast cancer. Patients with a high probability of achieving pCR might be more minimal surgery trial.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-19

Carboplatin-addition in neoadjuvant treatment of women with triple negative breast cancer (TNBC): Prognostic value in real-world patients

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## Background

The addition of carboplatin to an anthracycline/taxane-based chemotherapy(CT) in neoadjuvant setting has been suggested to improve pathological complete response(pCR) in TNBC. However, the impact of pCR in prognosis is unknown. We aim to study the value and feasibility of the addition of carboplatin in neoadjuvant setting.

## Methods

Demographic and clinical data of TNBC patients treated with neoadjuvant CT in a comprehensive cancer center between 2010-2018 were retrospectively collected. Two cohorts were defined: one treated with Carboplatin/Paclitaxel followed by dose-dense Doxorubicin/Cyclophosphamide(CP-AC) and other with AC followed by Docetaxel(AC-D). Median follow-up time was 3.1 and 6.9 years, respectively. pCR was defined as absence of residual invasive tumor in breast/axilla. Survival analysis using Kaplan-Meier method and Cox proportional-hazards model were applied. Statistical significance was set at  $p < 0.05$ .

## Results

One-hundred and sixty patients were enrolled: 78 CP-AC and 38 AC-D. Groups were balanced regarding patients and tumor characteristics with exception of pre-menopausal status, more frequent in CP-AC(68% vs 47%; $p = .04$ ). Age at diagnosis was 47(28-76)years, the majority had ECOG 0(92%) ductal carcinomas(82%), clinicalT2/3 stages(76%), grade 3(81%) with lymph node involvement(N+)(57%). 14% had Inflammatory breast cancer(IBC)(CP-AC 14%;AC-D 13%;  $p = .9$ ).

Neutropenia was the most prevalent adverse event(G3/4: CP-AC 61%;AC-D 16%; $p = .02$ ), 12% and 16% of febrile neutropenia( $p = .8$ ). G3/4 thrombocytopenia occurred only in CP-AC(6%). Hypersensitivity reactions were more prevalent in CP-AC(19% vs 2.7%; $p = .02$ ), majority to paclitaxel, all G1/2. Hospital admission occurred in 12%(CP-AC 13%;AC-D 9%;  $p = .8$ ). There were no treatment-related deaths. Treatment schedule was complete in 89%(CP-AC 87%;AC-D 92%; $p = .5$ ), with 20% dose reductions(CP-AC 25%;AC-D 11%; $p = 0.9$ ).

pCR was achieved in 42%(CP-AC 50%;AC-D 28%; $p = .03$ ). 1- and 3-year disease-free survival(DFS) was 94%/85% for CP-AC and 72%/58% for AC-D( $p = .3$ ). Risk of recurrence was higher in IBC(HR 25.1;CI95% 7.7-81.3; $p < .0001$ ), N+ disease(HR 3.6;CI95% 1.2-10.5; $p = .02$ ) and non-pCR(HR 10.9;CI95% 2.3-52.3, $p = .003$ ). N+ disease was associated with higher recurrence only in AC-D(HR 11.7;CI95% 1.3-104; $p = .03$ ).

Cancer-related deaths were 20%(CP-AC 10%;AC-D 40%; $p = .001$ ). 1- and 2-year overall survival (OS) was 99%/95% for CP-AC and 70%/61% for AC-D( $p = .06$ ). N+ disease was associated with higher risk of death in AC-D(HR 6.3;CI95% 1.1-24.6; $p = .04$ ). Risk of death was independently associated with IBC(HR 4.1;CI95% 2.1-18.7;  $p = .001$ ) but not with N+ disease(HR 2.7;CI95% 0.8-9.5; $p = .13$ ) or pCR(HR 4.1;CI95% 0.9-19.7; $p = .08$ ) although pCR was statistically significant in univariate analysis (1- and 2-year OS 97% vs 92% and 94% vs 86% for pCR and non-PCR; $p = .003$ ).

## Conclusions

Carboplatin addition clearly increased pCR with a trend to DFS and OS benefit. This regimen was associated with more, nevertheless manageable, adverse events with most of the patients able to tolerate and complete the full-dose regimen. Though we did not find a subgroup of patients that benefit with carboplatin regimen, we should consider avoiding AC-D at least in N+ disease.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-20

Dietary REstriction as an adjunct to neoadjuvant ChemoTherapy for HER2-negative breast cancer: Final results from the DIRECT trial (BOOG 2013-04)

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## **Background:**

Short term fasting (STF) protects from toxicity, while enhancing the efficacy of chemotherapy in cancer bearing mice and is a promising strategy to enhance the efficacy and tolerability of chemotherapy in humans. A specifically designed low calorie, low amino acid substitution diet ("Fasting Mimicking Diet", FMD) has similar effects *in vivo* during chemotherapy as STF. The DIRECT trial evaluates the impact of FMD on toxicity and efficacy of neoadjuvant chemotherapy in women with HER2-negative early breast cancer.

## **Patients and methods:**

Eligible patients had histologically confirmed, HER2-negative, stage II/III early breast cancer, adequate bone marrow, liver and renal function, BMI > 19kg/m<sup>2</sup> and absence of diabetes mellitus. Women receiving 8 neo-adjuvant AC-T courses (adriamycin/cyclophosphamide - docetaxel) or 6 FEC-T courses (5-fluorouracil, epirubicin and cyclophosphamide - docetaxel); day 1, q 3 weeks, were randomized to receive FMD or regular diet for 3 days prior to and at the day of chemotherapy and 3 days prior to surgery. The FMD group received no dexamethasone during the AC or FEC courses. The primary endpoint of the phase II part was feasibility and grade III/IV toxicity and of the phase III pathological complete response (pCR) rate. Additionally, in a side study increase in DNA damage in lymphocytes before and three hours after chemotherapy was compared between the 2 arms.

## **Results**

From February 2014 to January 2018 131 patients from 11 participating Dutch centers were randomized, whereof 100 received AC-T and 31 received FEC-T. Sixty-six of the patients received FMD. Compliance to the diet was low as 32% fasted at least half of the cycles and 24% of patients fasted during all of cycles. The main reasons of non-compliance were food aversion induced by chemotherapy and the taste of the diet. Intention to treat grade III/IV toxicity was not significantly different between the standard arm (67,2%) and in the FMD arm (79,4%), although the majority of the toxicities in the FMD arm were assessed in patients that did not complete the FMD diet preceding the measurements. The total overall pCR rate was 12,8%, lower than assumed in the sample size calculation and would therefore need minimally a doubling in patient numbers to be able to reach the expected pCR difference between both arms. Due to the poor compliance, slow accrual rate and low overall pCR rate the DIRECT study terminated after completion of the phase II part. Subgroup analysis will be presented at SABCS. In a side study, DNA damage after chemotherapy was significantly less increased in lymphocytes in the FMD group as compared to the control group ( $p=0.043$ ).

## **Conclusion**

The effect of STF on toxicity and efficacy of chemotherapy was not established due to poor compliance, however STF by FMD reduced a transient increase in chemotherapy induced DNA damage. Close monitoring of patients by nutritionists with expertise in low calorie diets as well as diets with a more variable taste are probably needed to successfully examine the impact on adverse effects and tumor biology.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-21

The molecular characterisation of early and advanced breast cancer in a Middle-Eastern breast cancer cohort treated with neo/adjuvant anthracycline+/-taxane-based chemotherapy

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Neoadjuvant therapy for breast cancer enables improved conservative operative approaches for breast cancer with similar survival. In addition, it may be used to define clinical as well as molecular systemic therapy sensitivity, aid molecular subtyping analysis of residual disease as well as incorporating potential mechanisms of resistance. Breast cancer in women in the Middle East is characterized by younger median age, more advanced stage at presentation and a higher proportion of patients with triple-negative disease. Recently, Symmans et al. published the results of neoadjuvant anthracycline+/-taxane-based chemotherapy demonstrating a strong association between residual cancer burden (RCB) and overall survival. In this and other cohorts e.g. TCGA, ICGC molecular data of pts from the Middle-East is under-represented.

## Aim:

The aim of this project was to define the above parameters in a cohort of women treated with systemic therapy from June 2016 to October 2017 in a Middle Eastern breast cancer referral centre treated in the neo/adjuvant setting. In the neoadjuvant setting we are examining the association between primary biopsy and pathological response tissue (RCB criteria) integrating molecular pathology using massive parallel sequencing (MPS) analyses.

## Methodology:

We designed a custom 1000 gene panel using Illumina IDT capture-based assay design and sequenced tumour samples to greater than 500X coverage. Sequencing analysis and variant calling were performed using Broad GATK best practice; BWA, Mutect2, Oncotator pipeline.

## Results:

We present a cohort of 57 pts with median age of 45(26-66), presenting with clinical stage I 2(4%), stage II 30(53%), stage III 25(44%) breast cancer for neoadjuvant (20) or adjuvant (37) anthracycline +/- taxane-based chemotherapy. Standard immunohistochemical (IHC) analysis revealed ER-pos PR-pos HER2-neg 38(67%) ER-pos PR-neg HER2-neg 2(4%) ER-neg PR-neg HER2-pos 3(5%), ER-pos PR-pos HER2-pos 5(9%), TNBC 9(16%). In the neoadjuvant cohort (20) pts, 7 were clinical stage II and 12 stage III at presentation. Anthracycline+/-taxane-based chemotherapy achieved pCR/RCB 0 7(35%), RCB I 3(15%), RCB II 4(20%), RCB III 6(30%). All Her2 positive patients received concurrent taxane-trastuzumab.

## Implications:

Predictive molecular expression algorithms for response to systemic chemotherapy in the neoadjuvant setting have been published (Hatzis JAMA 2011; Masuda Clin Ca Res 2013). Molecular characterisation of RCB after neoadjuvant chemotherapy has looked at DNA mutations (Jiang PLOS Med 2016) and RNA expression (Lehmann J PLOS One 2016; Echavarria Clin Ca Res 2018). Integration of both provides insight into mechanisms of sensitivity and relapse using pathway analysis. We present genomic data on 20 of the neoadjuvant samples with sufficient quality DNA analysed using a custom designed 1000 gene panel using Illumina IDT capture-based assay design to greater than 500X coverage. The most commonly aberrant genes *TP53*, *PIK3CA*, *GATA3*, *KMT2D* were observed, with notable differences in *PAX3*, *BRCA2*, *CHD2*, *FGFR4*. Using integrated comprehensive tumour molecular comparisons pre- and post-treatment in the neoadjuvant patients and circulating tumour DNA analyses of the whole cohort will be presented.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-22

Clinicopathological significance of diversity index for c-MYC copy number variation after primary systemic therapy in breast cancer

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**Background:** Chemotherapy can alter tumor cell populations by exerting selection pressure. This study was performed to evaluate the change in Shannon diversity index for c-MYC copy number variation after primary systemic therapy (PST) and its clinical implications in breast cancer patients treated with PST.

**Materials and Methods:** One hundred and forty-four breast cancer patients who had residual disease after anthracycline- or anthracycline and taxane-based PST were included in the study. Associations between Shannon index for c-MYC copy number variation in pre- and post-PST breast cancer samples and clinicopathologic features of tumors as well as patient survival were analyzed.

**Results:** Among the 119 patients with both pre- and post-PST samples available for comparison, 17 (14.3%) underwent change in c-MYC amplification status and 40 (33.6%) in c-MYC copy number gain status with most cases showing positive to negative conversion. In the whole group, Shannon index for c-MYC copy number variation was decreased in post-PST specimens compared to pre-PST specimens. Especially, the chemo-responsive group showed a more significant decrease in Shannon index after PST. However, there was no difference in diversity indices between pre- and post-PST specimens in the chemo-resistant group. c-MYC copy number gain and high Shannon indices for c-MYC copy number variation in both pre- and post-PST specimens were associated with adverse clinicopathologic features of breast cancer. In survival analyses, high Shannon indices for c-MYC copy number variation in post-PST samples as well as pre-PST samples were revealed as an independent prognostic factor for decreased disease-free survival. Upon subgroup analysis according to hormone receptor status, high Shannon indices before and after PST were associated with adverse clinical outcome in hormone receptor-positive group but not in hormone receptor-negative subgroup.

**Conclusions:** These results suggest that a change in Shannon index for c-MYC copy number variation after PST reflects chemo-responsiveness and that Shannon index after PST can be used as a prognostic factor in breast cancer patients who receive PST.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-23

Higher efficacy of 2-week nab-paclitaxel compared to solvent-based paclitaxel in neoadjuvant chemotherapy of triple negative breast cancers

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**Background:** Weekly nab-paclitaxel (nP) has better efficacy and safety for breast cancer patients compared with solvent-based paclitaxel (sP), but the result seems to be compromised when to 3-week nP though more convenient. However, reports about the 2-week nP in early breast cancer (EBC) are few. The current study aims to investigate whether 2-week nP is more effective than 2-week sP when added to an epirubicin/cyclophosphamide containing Neoadjuvant Chemotherapy (NAC) in Chinese EBCs .

**Methods:** Patients included in current retrospective study must meet at least one of the following criteria: tumor size $\geq$ 3cm; positive axillary lymph node; human epidermal growth factor receptor-2 (HER2) overexpression; triple negative breast cancer (TNBC, estrogen receptors (ER), progesterone receptors (PR), and HER2 negative); patients with willingness to receive breastconserving therapy. Based on expressive levels of ER, PR, HER2 and Ki-67, patients were divided into different subgroups. After dose dense EC (E, epirubicin 90 mg/m<sup>2</sup>; C, cyclophosphamide 600 mg/m<sup>2</sup>), patients were received either nP (260 mg/m<sup>2</sup>) q2w or sP (175 mg/m<sup>2</sup>) q2w, with or without herceptin (first time 8mg/kg and followed by 6mg/kg). The primary endpoint was the pathological complete response (pCR) rates (ypT0N0 and ypT0/isN0) and the second endpoint was the toxicity.

**Results:** Total 151 patients were recruited from August 2013 to October 2017. Of them, 77 patients received nP and 74 received sP. Baseline characteristics are similar that the mean age was 47.68/51.82 years (nP/sP) and tumor size was 41.23/39.77 mm (nP/sP). After treatment, the pCR rate of TNBC patients was significantly higher in the group receiving nP (6/11, 54.4%) compared to sP (2/16, 12.5%,  $p=0.0332$ ). No difference of pCR rates was found between nP and sP groups regarding to the threshold (20%) of Ki-67. Only few or no patients in subgroups of Luminal A, Luminal B HER2+ and Ki-67  $\leq$  20% experienced pCR. Although the pCR was observed in other subgroups, there was no significant difference between patients with nP and sP

Table1. pCR rates.

		nP(n=77)	sP(n=74)	P value
Luminal A	ypT0N0	0/15	0/5	/
	ypT0/isN0	0/15	0/5	/
Luminal B HER2-	ypT0N0	3/20	2/33	0.3685
	ypT0/isN0	4/20	2/33	0.1836
Luminal B HER2+	ypT0N0	0/0	0/3	/
	ypT0/isN0	0/0	1/3	/
HER2+	ypT0N0	2/3	0/2	0.4000
	ypT0/isN0	2/3	0/2	0.4000
TNBC	ypT0N0	6/11	2/16	0.0332
	ypT0/isN0	6/11	2/16	0.0332
Luminal B HER2+ a	ypT0N0	1/15	1/6	0.4805
	ypT0/isN0	2/15	1/6	1.0000
HER2+ a	ypT0N0	3/13	5/9	0.1870
	ypT0/isN0	5/13	6/9	0.3870
Ki-67 $\leq$ 20%	ypT0N0	0/16	0/12	/
	ypT0/isN0	1/16	0/12	/
Ki-67 $>$ 20%	ypT0N0	15/61	10/62	0.2437
	ypT0/isN0	19/61	12/62	0.1320

a, patients received EC-TH, other patients received EC-T.

. The side effects included peripheral neuropathy, allergic reactions, bone marrow suppression, and the incidence of peripheral neuropathy in nP was significantly increased.

**Conclusions:** The results suggested that nP (260 mg/m<sup>2</sup>, q2w) was associated with a better efficiency profile compared to sP (175 mg/m<sup>2</sup>, q2w) in NAC for TNBC. Meanwhile, nP also brings more peripheral neuropathy. As a result, nP (260 mg/m<sup>2</sup>, q2w) is also an NAC option for TNBC in China.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-24

Randomized phase II trial to evaluate chemoradiotherapy vs radiotherapy among non-responders breast cancer patients treated with chemotherapy

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## **Background:**

Among patients with locally advanced breast cancer (LABC), preoperative systemic treatment is the standard of care; approximately 80% of the patients treated with neoadjuvant chemotherapy present partial or complete clinical response, however there are patients who progress during this therapy or at the end of it, the tumors remain inoperable, this confers a worse prognosis, with an increase in the rates of metastasis and decrease overall survival. The benefit of concomitant chemoradiotherapy in local control is controversial. The objective of this trial is to analyze the efficacy of local control and survival in patients with locally advanced breast cancer who received systemic treatment plus chemoradiotherapy or radiotherapy alone preoperatively.

## **Patients and Methods**

Prospective, randomized, open label trial; patients with LABC whom after neoadjuvant chemotherapy based on anthracyclines and taxanes have disease progression or inoperable disease. Arm A (standard) received radiotherapy (RT) 50 Gy in 25 fractions or chemoradiotherapy (CRT)(gemcitabine 100 mg/m<sup>2</sup> plus cisplatin 30 mg/m<sup>2</sup>) weekly during radiation. The primary endpoint was local recurrence. Secondary end points included systemic recurrence, overall survival and -surgical complications. Statistical analysis was done with SPSS v 20.0, groups comparison was done with X<sup>2</sup>, survival was analyzed with Kaplan-meier method and comparison among groups with log-rank. Proportional Cox model associate clinical variables with recurrence and death. Local ethics committee approves the trial.

## **Results:**

78 patients were included, median follow-up was 116 months (110-121) at this time, 37 patients had recurrence (local, systemic or both) of which 18 were treated with CRT vs 19 RT alone (51.4 vs 44.2%, p=0.34). Higher rates of local recurrence were in RT alone 63.1% vs 38.9% with chemoradiotherapy with significance difference (p=0.004). There were no differences in disease free survival (p=0.542) and overall survival was found (p=0.303), 57.1 vs 53.5% respectively. Proportion of surgical complications were similar 71.4% for CRT vs 69.8% for RT (p=0.848). However hematological and gastrointestinal toxicity were more prevalent in patients treated with CRT p<0.005

## **Conclusion:**

Chemoradiotherapy it's a feasible option to reduce the risk of local recurrence, however without any additional benefit to improve the recurrence-free survival or overall survival. Surgical complications are still the same.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-15-25

Prognostic factors associated with pathological complete response in early breast cancer patients undergoing neoadjuvant chemotherapy. The importance of Ki-67 and molecular subtype

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**Background:** Ki-67 immunohistochemical determination is a widely used biomarker of cell proliferation in patients (pts) undergoing endocrine treatment for breast BC. The role of Ki-67 in pts undergoing neoadjuvant chemotherapy (NAC) for early BC remains controversial.

**Methods:** We analyzed retrospectively data on 137 patients undergoing taxane and/or anthracycline, trastuzumab based NAC. Luminal A was documented in 6 pts, Luminal B in 29 pts, Her-2 positive in 30 pts and triple negative breast cancers (TNBC) in 72 pts. Pathological complete response (pCR) was defined as the complete disappearance of the invasive cancer in the breast and absence of tumor in the axillary lymph nodes examined by axillary clearance.

**Results:** The pCR rate of the entire cohort was 41.6%. At 2 years 92% of pts who attained a pCR were disease free compared to 80% of pts who did not attain a pCR (log rank test  $p < 0.0147$ ).

On univariate analysis factors associated with higher pCR included primary tumor size (T1 68% vs. T2 41% vs. T3 or T4 0%,  $\chi^2=20.05$ ,  $p<0.00017$ ), nodal disease (N0 49% vs. N1 39% vs. N2 8%,  $p<0.02948$ ), ER receptor status (negative 59% vs. positive 14%,  $p<0.00000$ ), PR receptor status (negative 53% vs. positive 17%,  $p<0.00002$ ), molecular subtype (TNBC 53.4%, Her2=50% and Luminal A + B was 8.5%,  $p<0.00002$ ), Ki67 ( $>40=55\%$  vs.  $15-39=34\%$  vs.  $<15=0\%$ ,  $p<0.00060$ ) and Stage (I= 85% vs. IIA=49% vs. IIB=36% vs. III=5%,  $p<0.00006$ ). Factors not associated with a higher pCR included age, menopausal status, extranodal spread and lympho-vascular invasion. In a logistic regression model Ki-67 as a continuous variable ( $p<0.01203$ ) and molecular subtype ( $p<0.02228$ ) retained its significance; while tumor size, stage of disease, nodal status, ER and PR loss significance.

**Conclusion:** Ki67 and molecular subtype (Her-2 positive disease and TNBC) are independent prognostic factors of pCR in pts with early BC undergoing NAC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-01

Efficacy of endocrine- versus chemotherapy-based treatments in hormone receptor-positive (HR+ve), HER2-negative (HER2-ve) postmenopausal metastatic breast cancer (mBC): A network meta-analysis (NMA)

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**Background:** The international guidelines support upfront use of endocrine therapies (HT), with or without new targeted agents (TA), in HR+ve/HER2-ve mBC. However, routine administration of regimens containing chemotherapy (CT) is still common, even in the absence of visceral crisis. Unfortunately, robust data from head-to-head direct comparisons among CT and HT-based strategies are still not available; therefore, a NMA is now the only valuable methodological approach to compare the efficacy and activity of HT- versus CT-based regimens, and to drive adequate suggestions for a potential therapeutic algorithm in 1<sup>st</sup> and 2<sup>nd</sup> line HR+ve/HER2-ve mBC.

**Methods:** We performed a systematic literature search and selected all available phase II-III RCT published between January 2000 and December 2017, which evaluated CT or HT ± TA as 1<sup>st</sup> and/or 2<sup>nd</sup> line treatments for postmenopausal women with HR+ve/HER2-ve mBC. Primary endpoint was progression-free survival (PFS)/time to tumor progression (TTP); secondary endpoint was overall response rate (ORR). A Bayesian NMA was generated to compare posterior median hazard ratios (HR) for PFS and odds ratios (OR) for ORR. The aromatase inhibitor (AI) anastrozole (ana) was chosen as the common comparator for the overall analyses, being the most frequent treatment in the dataset.

**Results:** A total of 137 eligible trials (48,653 patients) were included in the NMA. Among the regimens approved for clinical use, palbociclib (palbo) + letrozole (let) [HR:0.42, 95% credible intervals (CI):0.27-0.65], ribociclib (ribo) + let (HR:0.43, 95%CI:0.27-0.74), abemaciclib (abe) + ana/let (HR:0.42, 95%CI:0.25-0.71), palbo + fulvestrant (ful) (HR:0.37, 95%CI:0.25-0.55), abe + ful (HR:0.45, 95%CI:0.30-0.65), everolimus (eve) + exemestane (exe) (HR:0.37, 95%CI:0.25-0.54), and ful alone (HR:0.81, 95%CI:0.66-0.98) were significantly superior to ana in terms of PFS/TTP. Conversely, none of the CT-based regimens (i.e. paclitaxel±bevacizumab, anthracycline-based schemes, capecitabine, eribulin) was significantly superior to ana. Additionally, head-to-head comparisons among the CDK 4/6 inhibitors (CDK4/6i) combined with AI showed no significant difference (palbo + let vs. ribo + let, HR:0.98, 95%CI:0.63-1.54; palbo + let vs. abe + ana/let, HR:1.02, 95%CI:0.63-1.60; ribo + let vs. abe + ana/let, HR:1.03, 95%CI:0.62-1.73). On the other hand, CDK4/6i+AI were significantly superior to many 1<sup>st</sup> line CT, including taxane- and/or anthracycline-based regimens. Interestingly, no CT±TA regimen was significantly superior to ana or to HT+TA, in terms of ORR.

**Conclusions:** Our analysis suggests that CT-based regimens are not significantly superior to HT-based therapies as 1<sup>st</sup>/2<sup>nd</sup> line treatments for postmenopausal HR+ve/HER2-ve mBC. Instead, HT+TA, including CDK 4/6i and eve, were significantly superior to HT alone and to many 1<sup>st</sup>/2<sup>nd</sup> line CT regimens. Moreover, the three CDK4/6i combined with AI did not differ significantly among each other. These data strongly support the combination of a CDK 4/6i along with HT as the preferred first choice of treatment for the 1<sup>st</sup> and 2<sup>nd</sup> line in HR+ve/HER2-ve mBC, as indicated in all the international guidelines.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-02

The impact of locoregional treatment on survival in patients with metastatic breast cancer: A national cancer database analysis

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**Purpose and Objectives:** Although systemic therapy is the standard treatment for metastatic breast cancer, the value of locoregional treatment (LRT) to the primary tumor and its impact on survival is controversial. This study evaluates survival outcomes in patients with metastatic breast cancer after receiving LRT (surgery and/or radiation therapy) to the primary tumor.

**Materials and Methods:** The National Cancer Database (NCDB) identified 16,128 qualifying cases of stage IV breast cancer (M1, 2004-2013) who received systemic therapy with or without local therapy. Treatment modality was divided into surgery, radiation therapy (RT), surgery with RT (Sx+RT), and no LRT. Median survival and three-year actuarial survival rates (OS) were analyzed for each treatment group. On multivariate analyses, adjusted hazard ratios (HR) with 95% confidence interval were computed using Cox regression modeling to adjust for patient characteristics, year of diagnosis, clinical T and N staging, and facility type. Additionally, survival outcomes for each treatment group were analyzed by metastasis groups (bone, visceral, multiple).

**Results:** A temporal trend of each treatment modality used in years 2004 – 2013 illustrated that the relative use of LRT decreased from 47.2% to 36.2% (p for trend = 0.041). Overall, the median follow-up was 28.3 months and median survival for all patients was 37.2 months. With 9,761 deaths reported, the estimated 3-year survival rate for all patients was 51.3%. The Sx+RT group (n = 2,166) had the highest 3-year survival rate of 69.4%, followed by the surgery group (n = 4,293) with 57.6%, no LRT group (n = 8,955) with 44.3%, and RT group (n = 714) with 41.5% (p < 0.0001). On multivariate analysis, a decreased hazard of death (adjusted HR, 95% CI) was noted in radiation patients compared to no LRT group but without statistical significance (0.91, 0.81-1.0, p = 0.057). Patients receiving surgery (0.68, 0.65-0.71, p < 0.0001) and Sx+RT group (0.46, 0.43-0.49, p < 0.0001) reported statistically significant improved survival compared to the no LRT group.

Additionally, later year of diagnosis, low Charlson-Deyo score, high income, private insurance, white race, age 18 - <50, low T and N stage, ductal histology, positive ER/PR/HER2 status, bone only metastasis, and academic facility type were considered favorable factors for OS. When stratified by metastasis type, patients with bone metastasis had the longest 3-year survival rates (74.4% for Sx+RT, 69.4% for surgery, 53.8% for no LRT, 49.3% for RT, p < 0.0001) whereas patients with multiple metastases had the worst outcomes (56.0% for Sx+RT, 43.5% for surgery, 37.9% for no LRT, 34.4% for RT, p = 0.003).

**Conclusion:** Patients with metastatic breast cancer have a large range of survival rates. Locoregional treatment, especially surgery followed by RT, in addition to systemic therapy was associated with improved survival in metastatic breast cancer patients. When survival rates for each treatment modality was stratified by metastasis location, the most favorable survival was observed for the surgery with follow-up radiation group, which is consistent with the overall analysis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-03

Phase II randomized clinical trial (RCT) of metformin (MET) vs placebo (PLAC) in combination with chemotherapy (CXT) in refractory locally advanced (LABC) or metastatic breast cancer (MBC)

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**Background:** MET treatment of diabetes is associated with improved BC outcomes. Hirsch et al (Cancer Res 2009;69:7505-7511) suggested MET may act synergistically with CXT in BC rodent models. We conducted a double-blind Phase II RCT of CXT plus MET vs placebo in LABC/MBC.

**Methods:** Non-diabetic BC patients (pts) about to commence 1<sup>st</sup>-4<sup>th</sup> line CXT (prespecified anthracycline, taxane, vinorelbine, platinum or capecitabine; HER2 Rx permitted) for MBC or refractory LABC (any ER, PgR, HER2) were eligible if (i) age 18-75, (ii) ECOG 0-2, (iii) adequate hepatic, renal, bone marrow, cardiac function and (iv) measurable or evaluable disease. Those with CNS metastases, recent MET use or radiotherapy to target lesions, intake of  $\geq 3$  alcoholic drinks/day, history of lactic acidosis or current/planned pregnancy or lactation were ineligible. Randomization was to MET 850 mg po bid (or identical PLAC bid) with a 2 day ramp up of one tablet/day; dose was reduced/drug discontinued in a pre-specified manner for grade 2-4 toxicity. Disease status and toxicity/HRQOL were assessed at baseline and q9 weeks until progression. Primary outcome was progression-free survival (PFS); secondary outcomes included survival (OS), response and toxicity. With 40 subjects and type one error 0.2 (1-sided), a PFS HR of 0.58 could be detected with 80% power. PFS was analyzed using Cox proportional hazards regression.

**Results:** 40 pts were randomized (22 MET, 18 PLAC). Mean age 55.4 vs 56.9 years; ER/PgR+ in 86.4 vs 83.3%; time from 1<sup>st</sup> metastases to randomization 297 vs 405 days, in MET vs PLAC respectively. MET pts were more likely to have visceral metastases (95.5% vs 72.2% PLAC) and less likely to be HER2+ (9.1% vs 23.5% PLAC). CXT was 1<sup>st</sup> line in 68.2% MET and 66.7% PLAC pts. **Toxicity** - # events: Gr 4: 0 MET vs 1 PLAC, Gr 3: 14 MET vs 14 PLAC; Gr 1 or 2: 193 MET (mainly GI) vs 53 PLAC. **Best response**: PR 18.2% MET vs 22.2% PLAC, SD 31.8% MET vs 11.1% PLAC, PD 45.4% MET vs 50.0% PLAC, P = 0.41. Mean **PFS** 164 days MET vs 192 days PLAC; HR (MET vs PLAC) 1.14 (95% CI 0.59-2.2), 1-sided p=0.65. Mean **OS** 645 MET vs 831 PLAC days; HR (MET vs PLAC) 1.6, 95% CI 0.72-3.54, 1-sided p=0.88.

**Conclusion:** In these BC pts receiving 1<sup>st</sup>-4<sup>th</sup> line CXT, MET (vs PLAC) did not improve response rates, PFS or OS. Gr 1 and 2 toxicity was higher with MET than PLAC. These results do not support use of MET with CXT in refractory LABC/MET BC. MA32, an adjuvant trial of MET vs PLAC in early BC will provide information on MET in the adjuvant setting.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-04

Risk of venous thromboembolism with abemaciclib based regimen versus other CDK 4/6 inhibitor containing regimens in patients with hormone receptor-positive HER2-negative metastatic breast cancer

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**Background:** Approximately 70% of patients with metastatic breast cancer (MBC) are hormone receptor (HR) - positive and the cyclin dependent kinases (CDK) along with their D-type cyclin catalysts, have been shown to play a role in mediating the resistance to endocrine therapy. Several CDK-targeted agents have been recently approved by FDA. Nevertheless, the risk of venous thromboembolism (VTE) with the use of different CDK 4/6 inhibitors has never been reported. We undertook a systematic review and meta-analysis of randomized controlled trials (RCT) to determine the risk of VTE with abemaciclib based regimens versus other CDK 4/6 inhibitor containing regimens in patients with HR-positive HER2-negative MBC.

**Methods:** We systematically conducted a comprehensive literature search using MEDLINE, EMBASE databases and meeting abstracts through February 2018. The randomized controlled trials that mention deep vein thrombosis and pulmonary embolism as adverse effects of CDK 4/6 inhibitor therapy were incorporated in the analysis. The primary meta-analytic approach was a fixed effects model using the Mantel-Haenszel (MH) method. It was used to calculate the estimated pooled risk ratio (RR) and risk difference (RD) with 95% confidence interval (CI).

**Results:** Five phase 3 studies and one phase 2 study with a total of 3,159 patients with HR-positive HER2-negative MBC were eligible for analysis. The study arms used palbociclib-letrozole, palbociclib-fulvestrant, ribociclib-letrozole, abemaciclib-fulvestrant, and abemaciclib-nonsteroidal aromatase inhibitors (either letrozole or anastrozole) while the control arms utilized placebo in combination with letrozole or anastrozole or fulvestrant. The randomization ratio was 2 to 1 in PALOMA-2, PALOMA-3, MONARCH-2 and MONARCH-3 studies and 1 to 1 in PALOMA-1 and MONALEESA-2 trials. CDK 4/6 inhibitors were utilized as first line treatment in PALOMA-1, PALOMA-2, MONALEESA-2 and MONARCH-3. The  $I^2$  statistic for heterogeneity was 0, and the heterogeneity  $X^2$  (Cochran's Q) was 1 ( $P = 0.707$ ), suggesting homogeneity among RCTs. The VTE incidence was 25 (3.255%) in the abemaciclib group vs 2 (0.520%) in the control group. The pooled relative risk for VTE was 6.222 (95% CI: 1.481 – 26.145,  $P = 0.013$ ) and the absolute RD was 0.027 (95% CI: 0.013 – 0.042,  $P < 0.0001$ ). In other CDK 4/6 inhibitor containing regimens, the VTE incidence was reported at 15 (1.243%) vs 2 (0.374%) in the control arm. The pooled RR for VTE was 2.312 (95% CI: 0.852 – 6.272,  $P = 0.100$ ) and the absolute RD was 0.008 (95% CI: - 0.000 – 0.017,  $P = 0.259$ ).

**Conclusion:** VTE is a major cause of morbidity and mortality and is particularly common in patients with breast cancer. Our meta-analysis demonstrated that the addition of abemaciclib to endocrine therapy notably contributed to a higher incidence of VTE with a relative risk of 6.22. However, no significant increase in the risk of VTE was noted in other CDK 4/6 inhibitor-based regimen. More randomized trials are required to determine the actual relation and definitive incidence of VTE among different CDK-targeted agents when added to endocrine therapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-05

Prognosis and survival in metastatic breast cancer – Ten years in review, a population-based analysis

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**Background:** The rapidly evolving landscape of systemic treatment for metastatic breast cancer (MBC) during the 1990s led to meaningful improvements in the overall survival (OS) of MBC patients[1]. Despite ongoing and expanded access to new treatments, it remains unclear if this has translated into further advances in survival. Moreover, the prognosis of MBC patients based on subtype, over time, are also important to differentiate.

**Methods:** The BC Cancer Breast Cancer Outcomes Unit (BCOU) database was utilized to identify patients referred to BC Cancer who were diagnosed with MBC during 3 time cohorts (cohort 1:2003-2005; cohort 2:2007-2009; cohort 3:2011-2013), to reflect changes in MBC treatment over these separate time periods. Baseline clinical and pathological criteria were compiled, in addition to adjuvant treatments received, as well as number of lines of treatment in the metastatic setting. OS was compared across time cohorts for all patients and then between subtypes using Kaplan-Meier survival curves.

**Results:** A total of 3,953 patients met the inclusion criteria, consisting of 2,440 (61.7%) estrogen-receptor positive (ER+), 778 (19.7%) HER2 positive and 542 (13.7%) triple-negative breast cancer (TNBC) patients. One hundred and ninety-three patients (4.9%) were unable to be subtyped and were therefore excluded from the analysis. A total of 2,205 (90.4%) ER+ patients received at least 1 line of systemic therapy, with 80.0% receiving at least 1 line of hormonal therapy. The median time on hormonal treatment was 8.9 months (range 0.03 - 156.7) for first-line and 6.1 months (range 0.1 – 173.3) for second-line. In the HER2+ group, 665 (85.5%) patients received at least 1 line of treatment, with a median of 2 lines of treatment (range 1-16). Median duration of anti-HER2 treatment was 6.7 months (range 0.03 - 163.8) with a median of 1 line of anti-HER2 directed treatment (range 1-5). For TNBC patients, 357 (65.9%) received at least 1 line of treatment, with a median of 2 (range 1-10). No significant differences in OS were observed between the 3 time cohorts, with a median overall survival (mOS) of 1.63 years, 1.37 years and 1.57 years in cohorts 1-3, respectively (p=0.12). When comparing across subtypes, the ER+ group fared best with a mOS of 1.96 years (95% CI 1.8-2.1), consistent across time cohorts (p=0.72). This was followed by the HER2+ group with a mOS of 1.53 years (95% CI 1.3-1.7), also consistent across time cohorts (p=0.31). The TNBC group fared worst, with a mOS of 0.67 years (95% CI 0.6-0.8) over time (p=0.87).

**Conclusions:** Despite advances in systemic therapy since the early 2000s, no meaningful improvements in overall survival were observed over time, regardless of subtype. It remains to be seen if developments since 2013 will lead to gains in overall survival for MBC patients, at a real life, population-based level.

[1]Chia SK, Speers CH, D'yachkova Y, et al. Cancer 2007;110:973-979.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-06

Improved survival of patients with metastatic breast cancer in routine care is restricted to tumors with positive hormone receptor and/or Her2-expression. Survival analysis of 1,321 patients treated between 1995 and 2017 in oncology group practices

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**Introduction:** 18,000 women die due to metastatic breast cancer in Germany per year. Median survival is 20–28 months after diagnosis. The question we wanted to answer was whether survival has improved in routine care?

**Methods:** Retrospective analysis of all patients with metastatic breast cancer who were treated between 06/1995-12/2017 in 5 community-based oncology group practices in Germany.

**Results:** 1,321 patients were analyzed with a median age of 62 (23–100). Localizations of metastases were distributed as follows: 49% visceral, 33% bone, 6% CNS, 12% others. 79% were hormone-receptor-positive, 20% Her2-positive, 9% triple-negative. Median overall survival was 37 months (95% Confidence Interval: 34–40), survival probability after 5 years 32.5%. Survival was significantly correlated with localizations of metastases, number of metastasized organs, disease free survival since initial diagnosis, hormone- and Her2-receptor status and age. Patients with hormone-receptor-positive tumors had a median overall survival of 39 months, Her2-positive patients of 45 months and triple-negative patients of 20 months. 86% of hormone-receptor-positive patients received antihormonal therapy. 81% of Her2-positive patients received anti-Her2 therapy. Overall survival according to treatment period 1995-2000, 2001-2005, 2006-2011, 2012-2017 was 34, 35, 37 and 38 months respectively. OS of patients with hormone-positive tumors according to treatment period was 35, 43, 38, and 42 months respectively. OS of patients with Her2-positive tumors according to treatment period was 39, 29, 51, and 54 months respectively. OS of patients with triple-negative tumors according to treatment period was 7, 11, 16, and 25 months respectively.

**Conclusions:** Improved survival of patients with metastatic breast cancer in routine care is strongly restricted to hormone receptor- and Her2-positive tumors most likely due to improved targeted therapies directed against the estrogen-receptor and Her2.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-07

Progression-free survival is a surrogate of survival in maintenance therapy for metastatic breast cancer: Randomized trial level analysis

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**Background:** The validity of progression-free survival (PFS) as a surrogate end point for overall survival (OS) in maintenance therapy trials of metastatic breast cancer (MBC) is uncertain. We aimed to compare treatment effect sizes and the strength of associations between OS and PFS in trials of maintenance therapy for MBC.

**Methods:** We searched for randomized trials investigating maintenance chemotherapy, endocrine therapy or immunotherapy after first-line chemotherapy in MBC and selected those reporting results for both OS and PFS. Treatment effect size differences between OS and PFS by a ratio of hazard ratios (rHRs) with 95% confidence intervals [CIs] were evaluated using random effects analysis. Surrogacy were analyzed using a weighted linear regression model, correlations were evaluated by squared correlation  $R^2$ .

**Results:** We analyzed data from 16 trials and 3,898 patients that received maintenance chemotherapy, endocrine therapy or immunotherapy for MBC. In the all trial-level analysis, treatment effect sizes were 28% greater for PFS than for OS (combined rHR, 0.72; 95% CI, 0.62 to 0.85,  $P < 0.001$ ), and the correlation coefficient  $R^2$  between PFS and OS was 18% (95% CI, 12% to 26%). Differences were greater with PFS than OS for trials of maintenance chemotherapy compared with observation (rHR, 0.72; 95% CI, 0.59 to 0.80,  $P < 0.001$ ), and the correlation coefficient  $R^2$  between treatment effects on PFS and on OS ranged from 12% (95% CI, 8% to 16%) when all trials were considered to 40% (95% CI, 30% to 54%) after exclusion of one highly influential trial by sensitivity analysis. Differences were also great for trials of maintenance endocrine therapy vs. observation (rHR, 0.54; 95% CI, 0.44 to 0.66), and immunotherapy vs. observation (rHR, 0.85; 95% CI, 0.80 to 0.91).

**Conclusion:** PFS was greater than OS in the treatment effect sizes, which is a valid surrogate end point for OS to assess treatment effect in MBC maintenance therapy trials.

**PROSPERO registry:** No. CRD42017071858; **Support:** ChiCTR-IIR-17014036, SYS-C-201801.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-08

Response to subsequent therapy after dual immune checkpoint blockade in metastatic breast cancer

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**Introduction:** While initial studies have found that combining chemotherapy with immune checkpoint blockade (ICB) can augment responses, additional toxicity has been observed. The optimal sequencing of chemotherapy and ICB has not yet been described. Sequential responses to chemotherapy after ICB have been reported in various tumor types; however, data is limited, and this has not been described in breast cancer to date.

**Methods:** We identified patients (pts) from a small pilot study in HER2-negative metastatic breast cancer (MBC) who received at least 1 cycle of durvalumab (PD-L1 inhibitor) and tremelimumab (CTLA-4 inhibitor). We excluded pts without follow up data or if they did not receive subsequent systemic therapy. Comparison of differences between subgroups was calculated by Fisher's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables. Time to treatment failure (TTF) of subsequent therapy and overall survival (OS) were assessed by the Kaplan-Meier method and differences between breast cancer subtype were compared by log-rank tests.

**Results:** Twenty-three pts received at least 1 cycle of ICB of whom 14 pts were eligible for this analysis. Nine had estrogen receptor positive (ER+) BC and 5 had triple negative (TN) BC. There were no statistically significant differences between the ER+ and TN subgroups in age, race, ethnicity, ECOG performance status (PS) at end of ICB, or sites of metastatic disease except for more lymph node metastases in the TN cohort ( $p=0.003$ ). Overall response rates to ICB in this cohort was higher in TN vs ER+ (40% vs 0%,  $p=0.11$ ). Pts received a median of 4 lines of systemic therapy for MBC prior to ICB. Subsequent therapy after ICB was eribulin in 29%, carboplatin/gemcitabine in 21%, palbociclib + endocrine therapy (ET) in 14%, anthracycline in 14%, ixabepilone +/- capecitabine in 14%, and paclitaxel in 7%. Clinical response was seen in 8 pts (57%), of whom 5 had ER+ BC and 3 had TNBC. The median TTF of subsequent therapy was 3.0 mo (1.9, 5.5), which compared to a median TTF for therapy prior to ICB of 2.5 mo. The median OS was 12.3 mo (2.3-13.3). There were no significant differences between the ER+ and TN cohorts (log-rank test  $p=0.74$  and  $0.90$  for TTF and OS, respectively). Subsequent therapy was discontinued due to progressive disease in 44%, decline in PS in 19%, liver failure in 6%, treatment related adverse event in 6%, and unknown cause in 13%. Two pts remain on subsequent therapy with palbociclib + ET beyond 6 mo without disease progression. There were no statistically significant differences between TTF >3 mo ( $n=5$ ) and TTF  $\leq 3$  mo ( $n=9$ ) subgroups. Pts with TTF >3 mo were numerically more likely to have a PS 0-1 (100 vs 78%), liver metastases (80 vs 56%), and ER+ BC (80 vs 56%). Pts with TTF  $\leq 3$  mo had more lymphopenia (66% vs 20%) and more lines of prior systemic therapy for MBC (median 6 vs 4).

**Conclusions:** While median duration of response on subsequent therapy was short, a subset of pts had significant clinical responses. These findings provide rationale for prospective validation as they provide strategies for sequencing ICB with standard therapies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-09

Low-dose chemotherapy (CT) + bevacizumab (Bev) combined with unchanged endocrine treatment (ET) in patients with recurrent luminal breast cancer progressing during ET – Effect determined by standard imaging and changes in ctDNA and CTC during treatment

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**Background:** Several mechanisms are involved in the development of endocrine resistance, such as mutations in the *ESR1*, *PIK3CA*, and *TP53* genes and/or up-regulation of tyrosine kinase receptors such as the endothelial growth factor (VEGF) receptor. Preclinical data have revealed that sensitivity to endocrine therapy may be restored if these pathways are blocked.

**Aims:** To investigate the progression-free survival (PFS), overall response rate (ORR), and the toxicity of the study treatment. To use circulating tumor cells (CTC) and circulating tumor DNA (ct-DNA) at base-line and during treatment for next generation sequencing (NGS) to investigate whether changes in tumor mutations or in levels of CTC/ct-DNA correlate to treatment efficacy.

**Patients and methods:** Thirty-two patients aged 46-77 years with confirmed advanced breast cancer (ABC) progressing during ET were included. Treatment consisted of unchanged ET with the addition of cyclophosphamide 50mg x 1 and capecitabine 500mg x 3 daily + bevacizumab 15mg/kg iv. every third week (q21). Blood samples for analysis of CTC and ctDNA were collected at base-line, after the 1<sup>st</sup> and 2<sup>nd</sup> course, and at progression. CTC were isolated by use of an immune-magnetic selection (ADNA-test) and sequenced by NGS. ctDNA were analysed by the SiMSen-Seq (Simple Multiplexed PCR-based barcoding of DNA for Sensitive mutation detection using Sequencing) that allows mutant frequencies < 0.1% to be detected.

**Results:** One patient did not start treatment and 2 were not evaluable. Palliative chemotherapy (1-2 lines) had been delivered before inclusion to 28% and > 1 line of palliative ET to 58% of the patients. A total of 72% (n=21) of the patients had visceral disease (of whom 7 had liver metastases), and 28% (n=8) patients bone-only disease. Median PFS was 9.1 months (range 2.1-59.3 months). Best responses were: 1 patient (3%) received complete remission; 7 patients (24%) partial response; 16 patients (55%) SD (of whom 12 had CB, defined as SD > 24 weeks), and 5 (17%) had progressive disease. The ORR (defined as CR, PR, or CB) was 69%. The most common toxicity was hypertension (62%), that resulted in termination in 2 patients, and 1 patient stopped treatment due to thrombocytopenia. Other side-effects were proteinuria grade 1-3 (24%); hand-foot-syndrome grade 1-2 (45%); mucositis grade 1 (14%); nausea grade 1 (14%) and diarrhea grade 1-3 (10%). CTC was isolated in 12 patients (37%). Three out of the 5 patients with PD at 12 weeks had detectable CTCs at base-line. Base-line *ESR1*, *PIK3CA* and *TP53* mutations were found in CTC from 2 patients (17%), 7 patients (58%), and 5 patients (42%), respectively, but did not correlate to response.

**Conclusion:** The treatment was well tolerated with an ORR of 69%, which is considered very good in this setting. CTCs were only isolated in 37% of the patients which is comparable to previously reported results in metastatic luminal breast cancer and thus not a feasible method for monitoring treatment effect. Results on levels of consecutive ctDNA, as well as mutation pattern in relation treatment effect will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-16-10

Treatment patterns and outcomes of different subtypes of metastatic breast cancer patients in a Swedish real world setting with a focus on HER2-/HR+ subtype

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**Introduction:** The most frequent molecular subtype of metastatic breast cancer (MBC) is the HER2-/HR+ subtype. While there are several treatments available for HER2-/HR+ MBC patients, there is limited knowledge about how patients are treated in a real world setting. In this retrospective study, the aim was to describe the duration of four initial treatment lines, treatment patterns and outcomes in MBC subtypes, with a focus on the HER2-/HR+ subtype.

**Methods:** The population is a cohort of 370 MBC patients diagnosed during '09-'16 in Uppsala County, Sweden. Data were collected from a regional breast cancer registry which included medical records. The subtypes were HER2-/HR+ (59%); HER2+/HR+ (12%); HER2+/HR- (7%) and HER2-/HR- (12%) based on immunohistochemistry (IHC) and in situ hybridization (ISH) tests, 11% of records had missing data on subtypes. Kaplan-Meier estimates were used to model duration of treatment line, progression-free survival (PFS) and overall survival (OS). Cox proportional hazard models were used to test the association, expressed in hazard ratios (HR), between the subtypes and PFS and OS.

**Results:** The median PFS and OS of HER2-/HR+ subtype were 10.6 and 36.7 months, respectively. Compared to the HER2-/HR+ patients, a statistically significant difference was found for HER2-/HR- patients in terms of PFS (HR: 2.1; p-value<0.001) and OS (HR: 3.6; p-value<0.01), indicating a worse prognosis. HER2+/HR+ and HER2+/HR- patients had similar PFS and OS results to HER2-/HR+ patients.

A statistically significant association was found between HR+ expression and OS (HR: 0.5; p-value<0.001) and not between HER2+ expression and OS (HR: 1.0; p-value 0.79 ).

The median duration of treatment decreased with increasing treatment lines; HER2-/HR+ patients' first-line treatment lasted 7.2, second-line 5.5, third-line 4.7 and fourth-line 4.4 months. The proportion of chemotherapy increased with the number of treatment lines: 32%, 38%, 46% and 59% for first to fourth line, respectively.

The ten most used drugs of HER2-/HR+ cohort are summarized in Table 1. In total, endocrine therapy was given during 66% of the total treatment duration.

Table 1: Ten most used drugs of HER2-/HR+

N=197	Patient-years (sum of treatment durations)	Relative frequency of patient-years (%). Total=511 years
Letrozole	190	37
Tamoxifen	56	11
Capecitabine	55	11
Exemestane	43	8
Fulvestrant	31	6
FEC	24	5
Paclitaxel	23	5
Docetaxel	14	3
Vinorelbine	11	2
Everolimus	9	2

**Conclusion:** In this retrospective study of MBC patients, the expression of HR showed an individual positive impact of OS with a

50% reduction in hazards. In our cohort only the prognosis of HER2-/HR- patients were significantly worsened both in terms of PFS and OS compared to HER2-/HR+ subtype. In the analysis of HER2-/HR+ subtype, letrozole was the most durable therapy, used 37% of total treatment time. The most used chemotherapy was capecitabine, used in 11% of the treatment time.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-01

Long term survival benefits of adjuvant zoledronic acid associated with maf status of primary tumor

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**Background:** Meta-analysis of clinical trials has shown that adjuvant bisphosphonates reduce bone metastases and improve survival in postmenopausal (PM) breast cancer and are now recommended for routine clinical use by international guidelines<sup>1</sup>. However, evaluation of menopause is imprecise and the biological rationale for lack of benefit in premenopausal women unclear. To address this, the biomarker, transcription factor MAF on 16q23 was tested retrospectively in the prospective randomized AZURE trial of standard adjuvant therapy +/- zoledronic acid (ZOL). Initial evaluation indicated that women with MAF negative tumors treated with ZOL had a lower rate of disease relapse irrespective of menopausal status.<sup>2</sup> Here we present the long-term findings of this predictive biomarker on 10 year overall survival.

**Materials and methods:** The biomarker analysis was completed on TMAs from primary tumors. Quadruplicate cores of breast tumor tissue were arrayed across replicate TMAs. MAF+ was detected using a validated (*MAF/D16Z3*) FISH test (Inbiomotion SL, Spain). A central laboratory (Targos, Germany) validated the assay for analytic and diagnostic performance, established acceptance criteria, included appropriate quality controls for each assay, and performed the analyses in a blinded fashion. A copy number cut-off  $\geq 2.5$  was preset for MAF+ for both prognostic and predictive testing. Interactions between MAF+ and effects of ZOL on Invasive disease free (IDFS), overall (OS) survival and time to bone metastases by menopausal status were evaluated using a Cox proportional hazards model.

**Results:** 1769 of the 3360 AZURE pts donated primary tumor samples. Median follow-up was 117 (interquartile range 70.4-120) months. 865 pts (49%) had 2 FISH evaluable cores and were included in the analysis. These pts had similar disease and treatment characteristics to the overall study population as well as similar IDFS and OS at 10 years. 184 (21%) had MAF+ tumors and these tumors were more likely to be of higher grade, ER-ve and HER2+. In 680 pts with MAF- tumors, ZOL was associated with improved IDFS (HR=0.75; 95%CI:0.58-0.97, [P=0.02]), reduced relapse in bone (HR=0.65; 95%CI:0.45-0.94, [P=0.022] and, most importantly, better OS (HR=0.69; 95%CI:0.50-0.94, [P=0.019]). In the 185 patients with MAF+ tumors, there was a suggestion of worse outcome (IDFS HR=1.54; 95%CI:0.96-2.47 and OS HR 1.40; 95%CI:0.83-2.33), with a strong interaction between treatment effects and menopausal status. Outcomes in ZOL treated MAF+ pts who were non-PM appeared to be much worse (IDFS HR=2.31; 95%CI:1.18-4.42] and OS HR=2.28; 95%CI:1.07-4.82) due predominantly to an excess of extra-skeletal metastases in ZOL treated patients (HR=4.47; 95%CI:1.66-12.57).

**Conclusions:** Adjuvant ZOL significantly improved disease outcomes in 79% of patients with MAF negative tumors, irrespective of menopausal status and other clinico-pathologic features. Conversely, more extra-skeletal metastases and breast cancer deaths were seen in women with MAF+ tumors who were not PM at the start of treatment. If validated in ongoing studies, the MAF FISH test could provide a clinically useful biomarker for selection of patients for adjuvant bisphosphonate treatment.

<sup>1</sup>EBCTCG, Lancet 2015; <sup>2</sup>Coleman RE et al, Lancet Oncol 2017

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-02

Outcomes and safety of paclitaxel and granulocyte-colony stimulating factor (GCSF) in breast cancer in pregnancy (BCP) - A multi-institutional retrospective analysis

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## Background

BCP is uncommon; however, the frequency is increasing due to trends in delayed childbearing. Studies have suggested that some systemic therapies, including doxorubicin and cyclophosphamide, can be delivered safely during pregnancy after the first trimester, whereas agents such as trastuzumab and endocrine therapy are contraindicated due to risk to the fetus. Data remain limited on the efficacy and safety of administering taxane chemotherapy or growth factor support during pregnancy. We retrospectively evaluated the safety of systemic therapies, including paclitaxel and GCSF, as well as clinical outcomes, in a multi-institutional cohort of patients (pts) with BCP.

## Methods

Pts treated for BCP from 1996-2018 from 3 large academic institutions were included. Demographic, oncologic treatment, and obstetric/neonatal outcomes data were obtained from medical records. Disease-free survival (DFS) and overall survival (OS) were estimated by Kaplan-Meier; Log-rank test were used to compare different groups/outcomes. Associations were calculated by Fisher's exact test.

## Results

A total of 114 pts diagnosed with BCP were included. The median age was 35 years (range 25-44) and median gestational age at diagnosis was 18 weeks (range 2-38). BCP was predominantly early stage at diagnosis (stage I 28.0%, stage II 53.5%) and ER+/HER2- negative (48.2%). Sixty-three (55.2%) women received chemotherapy, 13 (11.4%) received paclitaxel and 11 (9.6%) GCSF (daily or depot injections) while pregnant. A total of 78% of pts with HER-2-positive BCP (28/36) received trastuzumab after delivery (11% were treated before 2005 and 5.5% were T1a). With median follow-up of 67.7 months, median DFS (stage I-III) was 212.8 months (CI 95% 108.4-317.1), and median OS (stage I-IV) was not reached. Subgroup analysis suggested a higher DFS for pts diagnosed in the 1<sup>st</sup>trimester compared to the 3<sup>rd</sup>trimester among women with stage II-III (HR 0.25 CI 95% 0.09-0.70, p= 0.03). Among women who received paclitaxel, there was no significant increase in adverse obstetrical/neonatal outcomes: preterm delivery (23.1% vs 13.1%, p 0.39), low weight newborn (7.7% vs 9.1 %, p 1.0), congenital malformations (0% vs 6.1%, p 1.0) or acute neonatal adverse outcomes (7.7% vs 4.0%, p 0.51), which include NICU need and Apgar 5'<7, compared to pts who did not receive paclitaxel. Among pts who received GCSF during pregnancy, adverse outcomes were numerically but not statistically higher than women who did not receive growth factor: preterm delivery (36.3% vs 11.0%, p 0.051), low weight newborn (27.3% vs 6.9%, p 0.058), congenital malformations (9.1% vs 1.0%, p 0.18) or acute neonatal adverse outcomes (18.2% vs 3.0%, p 0.07).

## Conclusion

In this multi-institution cohort of BCP pts, despite a small number of pts, exposure to contemporary therapies including paclitaxel was not associated with unfavorable obstetrical/neonatal outcomes and these results suggest it is safe to administer during pregnancy under the care of a multidisciplinary team. Although not statistically significant, GCSF presented numerical worse outcomes and combining data from several cohorts would be helpful to provide confirmation of these findings.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-03

Statin use, site of recurrence, and survival among post-menopausal women taking bisphosphonates as adjuvant therapy for breast cancer (SWOG S0307)

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**Purpose:** Statins may mediate suppression of molecular pathways conferring benefit in cancer. Statins have shown anti-tumor effects in preclinical studies and have been associated with decreased recurrence and improved disease-specific survival. While designed to target cholesterol biosynthesis, statins can also have liver, bone and brain effects. We collected data on statin use in the S0307 adjuvant bisphosphonate trial to test the hypothesis that statin use may decrease risk of recurrence to liver, bone and brain as well as second primary (contralateral) breast cancers, and may act synergistically with bisphosphonates to decrease the risk of recurrence to bone.

**Patients and Methods:** In S0307, 6097 patients diagnosed with Stage I-III breast cancer who had undergone surgery and were receiving adjuvant systemic therapy were randomized to receive zoledronic acid, clodronate, or ibandronate for 3 years. No significant difference was found in disease-free survival (DFS) among the 3 groups, including a sub-analysis of patients  $\geq$  age 55. Statin use was infrequent in younger women in S0307, consequently we analyzed statin use in those  $\geq$  age 55. Cox proportional hazard models were used to determine which variables were independently associated with DFS and to estimate hazard ratios (HR) and 95% confidence intervals (CI).

**Results:** Among women aged  $\geq$  55 years, 684 (27%) reported taking a statin at baseline and 1,848 did not. Both groups were similar in terms of hormone receptor and HER2 status ( $p = 0.82$ ). Median age in the statin group was 64.3 versus 61.0 years in the no statin group, mean BMI 31.2 v. 29.5, mean tumor size 2.1cm v. 2.3cm, negative lymph nodes 60% v. 54%, Stage I disease 47% v. 36%, and receipt of chemotherapy 62% v. 71% (all  $p < 0.01$ ). In the statin group, 122 (17.8%) experienced a DFS event compared to 313 (16.9%) in the no statin group (HR 1.18, CI 0.95-1.46). No difference was observed by statin use in overall recurrence ( $p=0.28$ ), distant recurrence ( $p=0.64$ ), or recurrences to the bone ( $p=0.64$ ), liver ( $p=0.38$ ) or brain ( $p=0.65$ ) at initial recurrence.

Recurrence and statin use

Outcome	Group 1: On stan at baseline n=684	Group 2: No statin at baseline n=1848
DFS events	122 (17.8%)	313 (16.9%)
Died without recurrence	51 (7.5%)	97 (5.2%)
Recurrence	71 (10.4%)	216 (11.7%)
Contralateral breast cancer	9 (1.3%)	17 (0.9%)
Distant recurrence	48 (7%)	157 (8.5%)
Bone as 1st site of distant recurrence (% distant recurrence)	31 (65%)	76 (48%)
Liver as 1st site of distant recurrence (% distant recurrence)	6 (13%)	24 (16%)
Brain/CNS as 1st site of distant recurrence (% distant recurrence)	5 (10%)	17 (11%)

There was no synergy between statin use and specific bisphosphonates.

**Conclusions:** We found no evidence that statins reduce risk of second primary breast cancers or distant metastases among post-menopausal women with early-stage breast cancer. Despite promising preclinical data, they did not appear to act in synergy with a specific bisphosphonate. Though women in the statin group had less advanced disease at study entry, statin use was not associated with improved DFS. Results are limited by lack of information about type of statin used, adherence, or initiation of statin in control group.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-04

Long-term safety follow-up of patients with early stage breast cancer treated with scalp cooling on the Dignitana scalp cooling trial

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## Background

Scalp cooling has demonstrated efficacy in preventing hair loss in women with early stage breast cancer receiving neo/adjuvant chemotherapy. Data from 2 prospective trials (Rugo et al, and Nangia et al, JAMA 2017) led to FDA clearance of 2 automated scalp-cooling devices to prevent chemotherapy induced alopecia (CIA). Although scalp metastases from breast cancer are rare, historical concerns about scalp cooling included a theoretical increase in risk of recurrence in scalp due to reduced delivery of chemotherapy to the scalp.

## Methods

We conducted a multicenter prospective trial evaluating the efficacy and safety of the DigniCap in women with stage I-II breast cancer receiving neo/adjuvant chemotherapy excluding sequential or combination anthracycline/taxanes with concurrent matched controls. The primary endpoint was unblinded patient self-assessment of 5 photographs using the Dean scale to estimate hair loss 4 weeks following the last dose of chemotherapy, with success defined as a Dean score of 0-2 ( $\leq 50\%$  hair loss); additional endpoints included quality of life (QOL) and both short and long-term safety.

## Results

106 patients using the scalp cooling device and 16 concurrent controls were enrolled. As previously reported, the use of scalp cooling was associated with less alopecia and improvement in several measures of QOL (Rugo et al, JAMA 2017). 91 patients have follow-up (FU) out to 3 years; 73 with estrogen receptor (ER) positive and 18 with ER negative disease. 5 DigniCap patients have developed recurrent breast cancer in breast (n=1), liver (n=1), bone, liver and breast (n=1), bone, liver, lung, and nodes (1), and bone, breast, GI tract and bladder (n=1). Of 12 control patients with available FU, 1 developed metastases to liver. 2 patients have died of metastatic disease, one in the DigniCap arm and one in the control arm. No new safety signals have been detected.

## Conclusion

Scalp cooling using the DigniCap system in patients with early stage breast cancer receiving taxane based neo/adjuvant chemotherapy is safe and effective. No scalp metastases have been reported 3 years following completion of study treatment. 4 year FU data will be presented.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-05

The impact of clinical risk assessment versus PAM-50 ROR score on prognosis and therapeutic decision making in patients with hormone-receptor positive early stage breast cancer

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**Background:** Therapeutic recommendations for adjuvant treatment of hormone-receptor positive breast cancer patients depend on the individual recurrence risk. A number of genomic assays introduced to achieve this goal, but it's still questioned if they actually offer superior risk assessment compared to traditional risk evaluation by experienced clinicians. This study was designed to compare the prognostic accuracy of PAM-50 to clinical judgment.

**Methods:** Based on the real data of a large adjuvant trial cohort (ABCSG-8, postmenopausal HR positive breast cancer patients), we created online-questionnaires including demographic, histological, and local-therapy details, with and without results of PAM50 ROR score. Out of 14 international breast cancer experts asked for individual patient's risk evaluation (low, intermediate, high) and therapy recommendations, 9 completed the questionnaire.

Patient data were described by Kaplan-Meier estimates of distant disease free survival (DDFS) stratified by risk group. Cox regression models were compared using the Akaike Information Criterion (AIC).

**Results:** 10 years DDFS and hazard ratios for distant recurrences stratified by risk-group as estimated giving in Table 1:

10 years DDFS and hazard ratios for distant recurrences stratified by risk-group as estimated

	Low risk		Intermediate risk		High risk	
	n (%)	10y DDFS,%(95%CI)	n (%)	10y DDFS,%(95%CI)	n (%)	10y DDFS, %(95%CI)
		HR(95% CI)		HR(95% CI)		HR(95% CI)
Clinical only: AIC 817.6	269 (43)	93.0(89.8-96.2)	289 (46)	89.7(85.9-93.5)	73 (11)	76.6(66.1-87.1)
		0.68(0.39,1.20)		1		2.57(1.41,4.65)
PAM50 ROR: AIC 804.8	241 (34)	96.5(93.1-99.1)	210 (33)	89.2(84.7-93.7)	207 (33)	82.5(76.9-88.2)
		0.27(0.11,0.62)		1		1.66(0.99,2.78)
Combined: AIC 813.4	232 (37)	95.7(93.0-98.5)	282 (45)	87.8(83.7-92.0)	117 (18)	81.7(74.2-89.2)
		0.42(0.22,0.84)		1		1.90(1.11,3.24)

Adding genomic information to clinical risk factors leads to escalation of therapeutic recommendations (i.e. additional chemotherapy, extended adjuvant endocrine) in 20% of patients, and de-escalation in 13% of patients.

**Conclusions:** Clinical judgment accurately identified the patients at high risk of relapse, but was clearly inferior to multi-genomic testing using the PAM-50 ROR score in differentiating low from intermediate risk. Particularly when avoiding unnecessary escalated therapy is the strategic goal, the addition of PAM-50 testing to clinical judgment offers improved accuracy in predicting low vs. intermediate risk of breast cancer recurrence.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-06

Breast cancer in elderly women: Ageism or primum non nocere?

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**Background:** The risk of breast cancer increases with advancing age. Routine use of screening mammogram in women after 75yrs and its impact on overall survival is controversial. Studies have also found that elderly breast cancer patients are underrepresented among clinical trials and a tendency for undertreatment may result in inferior outcome.

**Method and Material:**Female patients with breast cancer who were 75 years or older and diagnosed from 2000-2015 were identified from Surveillance, Epidemiology, and End Results (SEER) 18 database. We excluded patients with unknown stage and race. We performed multivariate and survival analysis using JMP pro 13.

**Results:** 186,682 women with breast cancer of  $\geq 75$  yrs. were identified from the SEER. 167,802 patients met the inclusion criteria. Mean age was 81.27 years (CI 95% 81.25-81.30). Most of the patients were white (88%), and had Stage I/II (83%) breast cancer. 78% of patients had estrogen receptor positive cancer, while 66% had grade 1/2 disease. The 5-year overall survival was 74% for Stage I, 60% for Stage II, 38% for stage III and 11% for Stage IV cancer ( $p < 0.0001$ ). The disease specific survival (DSS) for stage I (96%), Stage II (88%), Stage III (64%), and Stage IV (23%). Out of the patients who were deceased at the time of analysis only 24% of deaths were attributed to this cancer. Cox proportional hazards regression model of overall survival [Table:1]

Cox proportional hazards regression model of overall survival

Variable	Hazard ratio( 95% CI)	p value
75—79 80-84 85+	1 1.22 1.7	<0.0001
Race White Black Others	1 1.14 0.95	<0.001
ER Positive Negative unknown	1 1.19 1.2	<0.001
Stage I Stage II Stage III Stage IV	1 1.17 1.66 3.7	<0.001

**Conclusion:** Early breast cancer continues to be the most common presentation for patients  $\geq 75$  yrs. of age. Historical prognostic factors of breast cancer like race, hormone receptor status, stage and grade continue to impact cancer outcome among elderly patients. Only 24% of deaths among the deceased were attributed to this breast cancer highlighting the concern for over diagnosis. Nonetheless once diagnoses is established a multidisciplinary comprehensive geriatric assessment should be the cornerstone of the management.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-07

Cancer management and outcome of very young non-pregnant patients with breast cancer diagnosed at 40 years or younger—GBG 29

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## Introduction

Breast cancer diagnosed in young women who are 40 years (yrs) or younger is a relatively rare disease. However, it represents the most common cause of cancer-related deaths in this age-group. Furthermore, young age at diagnosis is associated with an increased risk of recurrence and worse survival. To date, general concepts concerning oncological cancer management should be driven by clinicopathological tumor characteristics and should adhere to standardized protocols for patients in general, but little is known about the oncological cancer treatment and outcome of this very young women in today's clinical practice.

## Patients and Methods

The breast cancer in pregnancy registry study (BCP/GBG29/BIG 03-02) is a multicenter, international, observational study. BCP was established to investigate the oncological management and outcome of breast cancer in pregnancy. Since 2014 non-pregnant patients who are 40 yrs or younger are eligible if diagnosed with histological confirmed invasive breast cancer, independent of the type of treatment as control cohort. All patients received oncological treatment according to local standards. In this study the following endpoints will be analyzed descriptively for the young non-pregnant women cohort: breast cancer staging at diagnosis, biological characteristics of breast cancer at diagnosis, diagnostic procedures, treatment modalities, toxicity, pathological complete response after neoadjuvant chemotherapy, disease-free survival and overall survival.

## Results

From February 2014 until June 2018, 969 non-pregnant patients  $\leq 40$  yrs have been registered. The median age at diagnosis was 35 yrs (range 19-40). Overall, 90.1% of patients had a stage T1-2 at diagnosis and 67.1% of patients had negative lymph nodes. 86.7% of tumors were invasive ductal carcinomas and 4.1% lobular carcinomas. Grading (G) 3 was reported in 55.5%. 26.6% of tumors were luminal A-like (ER- and/or PgR-positive, HER2-negative, G1-2), 40.0% luminal B-like (ER- and/or PgR-positive, HER2-negative, G3 or ER- and/or PgR-positive, HER2-positive, any G), 7.7% HER2 positive non-luminal-like, and 25.7% triple negative breast cancers. 3.8% of young non-pregnant patients had metastatic disease at primary diagnosis.

## Conclusion

This registry comprises a large cohort of young non-pregnant patients with breast cancer diagnosed at the age of 40 yrs or younger and provides important data about a modern breast cancer treatment as well as oncological outcome in this setting of young women. Further results including oncological management, toxicity, and survival will be presented at the meeting.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-08

High-dose chemotherapy and immunotherapy in premenopausal breast cancer patients with more than 10 axillary nodes (PBC+10N). Long-term follow-up of a phase II study

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**Background:** PBC+10N have a high risk of relapse. Experimental studies suggest that high expression of vascular endothelial growth factor (VEGF) promotes tumor progression through neoangiogenesis and that natural killer (NK) cells mediate lytic activity against cancer cell lines. In a phase I-II study, we have shown that low-dose interleukin-2 (IL-2) and 13-cis retinoic acid (RA) increased NK cells and decreased VEGF, in patients with advanced cancer and a clinical benefit from chemotherapy (Clin Cancer Res 7: 1251, 2001). We assumed that IL-2 and RA, increasing NK and decreasing VEGF, could improve disease-free survival (DFS) and overall survival (OS) in PBC+10N. Primary endpoint was the evaluation of NK cells and VEGF; secondary endpoints were DFS, OS and toxicity. **Methods:** 34 patients with PBC+10N, after high-dose chemotherapy and peripheral blood progenitor cell transplantation were entered into the study. They were given hormonal therapy if needed and subcutaneous IL-2,  $1.8 \times 10^6$  IU and oral RA, 0.5 mg/Kg for 5 days/week, 3 weeks/month, until progression. NK cells, VEGF, response and toxicity were assessed every 4 months. **Results:** After a median follow-up of 120 months (range 69-209), a total of 41 courses of high-dose chemotherapy and 60 courses of immunotherapy were delivered. A statistically significant improvement of NK cells [from a mean of  $302 \pm 75/\text{mm}^3$  to a mean of  $582 \pm 72/\text{mm}^3$  ( $p < 0.001$ )] and a decrease of VEGF [from a mean of  $525 \pm 68 \text{ pg}/\text{mm}^3$  to a mean of  $155 \pm 13 \text{ pg}/\text{mm}^3$ , ( $p < 0.001$ )], were observed. 18-years DFS and OS were 29% and 32%, respectively. A significant improvement, with respect to NCI SEER data (\*), was observed in the 5-year OS rate: 55% vs. 6%. No WHO grade 3 or 4 toxicity was observed, while grade 2 cutaneous toxicity and fever occurred in 20% and 13% of patients, respectively. **Conclusions:** Our data show that immunotherapy with IL-2/RA, may determine, with an acceptable toxicity profile, a statistically significant improvement of NK cells, a decrease of VEGF, and better 5-year survival rates with respect to NCI SEER data.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-09

Use of Oncotype DX ® testing in nodal positive breast cancer patients: Real-life data from a single center in Munich, Germany

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**Introduction:** Despite a large body of prospective studies showing good prognostic and predictive value of the Oncotype DX ® testing in ER+/HER2- early breast cancer patients after breast surgery, the test is currently not reimbursed in Germany by most national health insurance companies. Nevertheless, this multigene assay has been offered to node negative (N-) and increasingly to node positive (N1) patients with 1-3 positive lymph nodes by medical- and gynecologic oncologists for several years. The aim of the present study was to analyze real life data of the test in N1 patients from a German single center with special respect to the intermediate-risk recurrence score (RS) group and event-free survival (EFS).

**Patients and Methods:** Patients with ER+/HER2- node positive breast cancer after breast surgery, to whom Oncotype DX ® testing was consecutively recommended by the interdisciplinary local tumor board from 1/2012 through 12/2016 at the Breast Center of the Red Cross Hospital Munich, Germany, were included in the study. Data was retrospectively retrieved from medical records (e.g. stage and histology), patient interviews as well as from the Munich Cancer Registry (outcome measurements). Patients were stratified according to Oncotype DX ® RS distribution (<11 low-risk, 11-25 intermediate risk and > 25 high-risk). Proportion of patients choosing adjuvant chemotherapy (CT) within the different RS groups and outcome were analyzed.

**Results:** Oncotype DX ® testing was recommended and performed in 500 (17%) out of 2942 patients with ER+/HER2- tumors. Nodal status was positive in 159 (31.8%) of these patients. Patients with more than 3 positive lymph nodes and with missing data on follow-up were excluded, leaving 121 patients for the main analysis. According to RS distribution, n=19 (15.7%) were low-risk, n=83 (68.6%) intermediate-risk and n=19 (15.7%) high-risk, respectively. Although CT was generally discussed with all N1 patients with RS>10, only 39 patients (38.2%) of the intermediate and high-risk RS-group opted for systemic chemotherapy (24.1% of RS 11-25 and 100% of RS>25). In 13 of the patients (11%) within a median follow-up of 40 months an event occurred (4 loco-regional and 2 contralateral relapses, 5 distant metastases (3 bone, 2 visceral) and 2 deaths of other causes). Of those patients with events, three presented with RS <11, six with RS 11-25 and three with RS >25. In the intermediate group (n=83) EFS was 90% for patients receiving CT followed by endocrine therapy (ET) and 91.9% for patients with ET alone (p= 0,583).

**Conclusions:** Using real life data from a large single breast center, only around 25% of patients with 1-3 positive lymph nodes from the intermediate RS group decided to undergo CT after Oncotype DX ® testing. The outcome of patients receiving ET only in this group was not inferior compared to CT followed by ET.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-17-10

The impact of age and adjuvant chemotherapy modifications on disease-free and overall survival among African American women with breast cancer

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**Background:** During chemotherapy for breast cancer, African American women receive less relative dose intensity with more dose reductions and early chemotherapy cessation compared to Caucasian women. Other research has found that older breast cancer patients are most at risk for treatment modifications; however, it is unclear if this remains true for African American patients. Furthermore, the clinical implications of treatment modifications and delays on survival is uncertain, particularly in African American patients.

**Purpose:** The purpose of this study was to investigate whether age (diagnosis <55 vs. diagnosis ≥55) was a moderator for the association between treatment modifications (dose held, dose delayed, and early cessation) and overall survival (OS) and disease-free survival (DFS) in African American women with breast cancer.

**Methods:** A retrospective cohort study of early stage African American breast cancer patients treated with adjuvant chemotherapy was employed. Dose held, dose delayed and early cessation were examined as dichotomous variables: any adjustment to the initially prescribed treatment plan was considered a modification. Medical record data extraction was utilized to gather this information. The sample was divided into two groups: those diagnosed <55 years of age and those diagnosed ≥55 years of age. A Cox's proportional hazards regression model was used to examine the interaction between age group and treatment modifications for OS and DFS, while controlling for stage and ER and HER2 status.

**Results:** In the study of 115 participants, 58 (50.4%) were diagnosed before the age of 55, and 57 (49.6%) were diagnosed age 55 or older. Across the entire sample, 43 (37.4%) patients experienced a treatment modification. There were no significant differences in the proportions of treatment modifications between the two age groups. We found no interaction between age group and treatment modifications for OS. However, there was a significant interaction between age group and held dose for DFS ( $p=0.045$ ). Specifically, those diagnosed at 55 years of age and older, who had doses of chemotherapy held, experienced worse DFS compared to those who did not (hazard ratio (HR)=3.390, 95% CI (1.013,11.34)). In contrast, there was no difference in DFS between those who did and did not have doses held in patients diagnosed below 55 years of age (HR=0.563, 95%CI (0.159, 1.986)).

**Conclusions:** African American women receiving adjuvant chemotherapy for treatment of early stage breast cancer have high levels of treatment modifications across all age groups. However, held doses of chemotherapy in older African American patients were associated with worse DFS. Further research is needed to elucidate the clinical implications of adjuvant chemotherapy treatment modifications, particularly in African American patients, and the subgroups of patients who are at greatest risk.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-18-01

Incidence of hypocalcemia in patients with metastatic breast cancer under treatment with denosumab: A non-inferiority phase III trial assessing prevention of symptomatic skeletal events (SSE) with denosumab administered every 4 weeks versus every 12 weeks: SAKK 96/12 (REDUSE)

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## Background

Monthly Denosumab (DN) has shown superiority over zoledronic acid (ZA) in delaying skeletal related events. Randomized trials have shown that ZA given every 12 weeks (q12w) is non-inferior to ZA given every 4 weeks (q4w). The primary endpoint of the REDUSE-trial is non-inferiority for SSE for DN q12w versus q4w. Here we present early data for hypocalcemia (HC), a secondary endpoint.

## Methods

Patients with bone metastasis from breast cancer (BC) not pretreated with DN or Bisphosphonates were randomized 1:1 to receive DN q4w (Arm A) versus q12w (Arm B) after a 3-month induction phase with q4w therapy for both arms. All patients received vitamin D 400 U (VitD) and calcium (Ca) 500 mg daily. Measurement of albumin-corrected serum-Ca was mandatory before each DN injection (HC defined as <2.0 mmol/l like in CTCAE V4.0). This safety interim analysis was performed after 3.5 years of accrual. Patients who received at least 1 dose of DN were considered evaluable.

## Results

351 BC-patients are currently included (177 in Arm A, 174 in Arm B). HC was the most common side effect with a rate of 20% in the first 16 weeks (during the induction phase with DN q4w for both Arms) and 19% afterwards (combined for Arms A and B). After week 16 HC-prevalence differed between the two arms: while HC was present in 25% in Arm A (q4w), the rate was only 12% in Arm B (q12w). Grade 3 HC (i.e. corrected Ca 1.5 - 1.74 mmol/l or hospitalisation indicated) was rare (0.3%), no grade 4 HC occurred. After 1 year of treatment, the rate of HC compared to the induction phase had decreased in Arm B but not in Arm A (A: 25%, B: 12%). Since HC improved in more patients in Arm B than in Arm A whereas it worsened in more patients in Arm A than in Arm B, a remarkable difference for HC resulted between the two arms.

Rates of hypocalcemia and change of severity after week 16\*

	Arm A (N = 177)	Arm B (N = 174)
<b>Rates of hypocalcemia</b>	n (%)	n (%)
Patients with hypocalcemia at any time	49 (28%)	46 (26%)
Patients with hypocalcemia after week 16*	44 (25%)	21 (12%)
<b>Change in hypocalcemia grade after week 16*</b>	for the 49 patients with hypocalcemia	for the 46 patients with hypocalcemia

Worsening	25 (51%)	8 (17%)
Stable	10 (20%)	9 (20%)
Improving	14 (29%)	29 (63%)
*week 16: i.e. the time where the schedules of DN begin to differ between Arm A and Arm B		

Arm A: DN q4w for weeks 1 - 12 and likewise thereafter / Arm B: DN q4w for weeks 1 - 12 and q12w thereafter

## Conclusions

In our trial up to 20% of all BC patients treated with DN experienced HC in the q4w induction phase despite mandatory supplementation of VitD and Ca. This rate is considerably higher than the numbers reported in the registration trials of DN (where it was 5.5% for BC). After the induction phase, HC is markedly reduced in the q12w arm compared to q4w. This suggests that DN given q12w has a more favorable long-term safety profile in terms of HC compared to DN q4w.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-18-02

Impact of the metastatic compartment on bone biomarkers and bone outcomes in patients (pts) with breast cancer (BC) and bone metastases (BM) in trial NCT00321464 of denosumab vs. zoledronic acid

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**Background:** Bone is the most common site of metastatic disease in pts with BC, and BM are frequently associated with skeletal related events (SRE), as, e.g., bone pain or fracture. Bone targeted agents (BTAs; denosumab or zoledronic acid) decrease the rate of SREs. Previous exploratory studies in pts with BM showed that the site of metastatic disease (bone-only disease [BO] vs. bone + extraskeletal disease [BES]) might impact both bone remodeling (reflected in the concentration of bone biomarkers) and the risk for SREs. In this large study of pts with BC and BM, we assessed bone biomarkers after the introduction of BTAs, time to first and subsequent on-study SRE/symptomatic SRE (SSE), and bone pain score variation according to metastatic compartment (BO vs. BES).

**Methods:** This is a retrospective analysis of the prospective, multicenter, randomized, registration clinical trial of denosumab vs. zoledronic acid in pts with BC and BM (NCT00321464). Study outcomes were variation of corrected urinary N-terminal telopeptide (uNTX) and bone alkaline phosphatase (bALP) at 3 months, time to first and subsequent SRE and SSE, and brief pain inventory (BPI) scores over time. Chi-squared test and t-test were used to compare biomarkers levels. We used the Kaplan-Meier method to describe time to event outcomes and differences were tested using the Cox proportional hazard model and Andersen–Gill model for multiple failure-time data. BPI scores were compared using mixed linear models.

**Results:** A cohort of 2046 pts was identified, 969 (47.4%) with BO and 1077 (52.6%) with BES, all treated with either denosumab or zoledronic acid. Median follow-up was 20.1 months (interquartile range 15.9-23.8; balanced between arms). Compared to pts with BO, those with BES were more frequently hormone receptor negative (20.9 vs. 15.1%) and HER2-positive (31.0 vs. 23.4%). The number of BM was similar in both groups, but those with BES had less previous SRE (31.7 vs. 42.2%). Pts with BES were more commonly treated with chemotherapy (84.0 vs. 77.5%), but less frequently with radiotherapy (59.7 vs. 65.9%) or surgery (85.0 vs. 88.1%). Absolute levels of uNTX and bALP at baseline and at 3 months, as well as normalization rates, did not differ between groups. However, when compared to those with BO and after controlling for unbalanced clinicopathological and treatment features, pts with BES presented a lower risk of first and subsequent SREs (adjusted-hazard ratio [HR] 0.73, 95% confidence interval [CI] 0.58 – 0.94; p=0.013) and first SSE (adjusted-HR 0.75; 95% CI 0.60 – 0.94; p=0.014). Hazard rates for SRE were higher in the first 6 months of treatment. Despite the small magnitude, pts with BO consistently showed slightly higher BPI scores (+0.2 points; p=0.014). Pts with BES had a shorter OS (HR 1.97, 95% CI 1.66 – 2.33).

**Conclusion:** Despite the consistent reduction in uNTX and bALP in pts with BC and BO or BES disease, pts with BO disease had a higher risk for SREs and higher pain score. Hazard rates for SREs were greater in the first 6 months of treatment. Strategies of treatment de-escalation of BTAs should consider the metastatic compartment and time variation of the hazard for SRE.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-18-03

Phase I trial to assess the safety, pharmacokinetics and pharmacodynamics of receptor activator of nuclear factor- $\kappa$ B ligand inhibitor (TK006) in patients with bone metastases from breast cancer

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## Background

Within the bone microenvironment, tumor cells secrete factors that stimulate osteoblasts to express and secrete receptor activator of nuclear factor- $\kappa$ B ligand (RANKL), which binds to its receptor RANK on the surface of osteoclasts, thus enhancing the osteoclast-mediated bone resorption and promoting skeletal complications. TK006 is a fully human monoclonal antibody that binds and inhibits RANKL, thus inhibiting osteoclast-mediated bone destruction.

## Objective

To investigate the safety, pharmacokinetics and pharmacodynamics of TK006 in patients with bone metastases from breast cancer.

## Patients and methods

In this dose-escalating study, patients were sequentially enrolled into 60 mg, 120 mg, 180 mg single-dosing and 120 multiple-dosing cohorts. Before making dose escalation decision, the safety of TK006 during the 14-day period after dosing in the prior cohort must be confirmed. In the three single-dosing cohorts, patients were followed up for 16 weeks after dosing. In the 120 mg multiple-dosing cohort, patients were treated with 120 mg TK006 every 4 weeks for 3 times totally, and followed up for 20 weeks after the first dosing. The primary outcome was safety profile, and the secondary outcomes were pharmacokinetics, pharmacodynamics and immunogenicity. Pharmacodynamics was measured by level of serum bone alkaline phosphatase (BALP) and urine creatinine corrected cross-linked N-telopeptides of type I collagen (uNTX/Cr).

Patients aged 18 to 65 years with breast cancer related bone metastasis were eligible. It was planned to enroll 10 subjects in each cohort for a total sample size of 40 subjects.

## Result

As of May 24 2018, the common adverse events (AEs) related to treatment ( $\geq 10\%$ ) included: hypocalcemia (25.0%), limbs pain (20.0%), gamma-glutamyl transferase increased (17.5%), lactate dehydrogenase increased (12.5%), alpha-hydroxybutyric dehydrogenase increased (12.5%), aspartate aminotransferase increased (12.5%), alanine aminotransferase (10.0%), osphalgia (10.0%) toothache (10.0%) and hypertriglyceridemia (10%). Most adverse reactions were mild or moderate except one case of grade 3 hypertriglyceridemia and two cases of grade 3 gamma-glutamyl transferase increasement. No osteonecrosis of the jaw or treatment-related SAE was reported.

In the 60 mg single-dosing cohort, a significant reduction in median uNTX/Cr was observed as early as day 1, the nadir of median uNTX/Cr was reach at day 28 and started to return towards the baseline level at day 112 (Table 1).

Only modest decreasing from baseline in median bone-specific alkaline phosphatase was observed

Table 1. Effects of 60 mg TK006 therapy on bone turnover markers

	uNTX/Cr, % change from baseline, median	BALP, % change from baseline, median
D1	-38.6	-4.7
D7	-63.0	1.7
D14	-55.3	0.2
D28	-69.2	-0.2
D56	-57.9	-12.1
D84	-33.4	-0.4
D112	1.3	-18.7

Ostealgia was measured by visual analogue scale (VAS). In the 60 mg single-dosing cohort, scores were reduced to 2 from 5 and 3 in two patients individually. No increasing in pain was observed in the remaining 8 patients.

### **Conclusion**

These results suggested a potential therapeutic role for TK006 in patients with bone metastases from breast cancer.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-18-04

CTCs and SUV to predict the efficacy of the bone-specific radiopharmaceutical agent radium-223 dichloride combined with hormonal therapy for hormone receptor-positive bone-dominant breast cancer metastasis

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**Background:** Radium-223 dichloride (Ra-223) is a targeted alpha particle-based radiotherapeutic that has a localized cytotoxic effect on bone metastases. We sought to determine whether the circulating tumor cell (CTC) count and the presence of CTCs in epithelial-mesenchymal transition (EMT-CTCs) along with the standardized uptake value (SUV) on positron emission tomography-computed tomography (PET/CT) scans predict the efficacy of combined Ra-223 and hormonal therapy in patients with hormone receptor (HR)-positive bone-dominant metastatic breast cancer.

**Patients and Methods:** In this single-center phase 2 study (NCT02366130), 36 patients received Ra-223 (55 kBq/kg intravenously) on day 1 and then every 4 weeks for six cycles. Patients also received a standard care endocrine monotherapy. One non-bone metastatic site was allowed. The number of prior endocrine therapies was not limited and one prior chemotherapy was allowed for metastasis. Response was evaluated using the PET Response Criteria in Solid Tumors (PERCIST) with PET/CT at baseline, 6 and 9 months (mo) later. The CTC count (CellSearch) and the presence of EMT-CTCs (AdnaTest) was determined at baseline, 6 and 9 mo later. Progression-free survival (PFS) time was calculated to evaluate efficacy.

**Results:** Seven patients (20%) had a non-bone metastatic site. The median number of prior therapies for metastasis was 1 (range, 0-4). Six patients (17%) received chemotherapy. The median CTC count at baseline was 4 (range, 0-306). Only four patients (11%) were positive for EMT-CTCs at baseline. The median follow-up time was 14.7 mo (95% confidence interval [CI], 13.2 mo-not reached [NR]). The disease control rate at 9 mo was 46% in 33 patients who reached 9 mo or progressed up to 9 mo. The tumor response rate at 6 mo was 52% (complete/partial response rate; 22/30 %) in 27 patients whose disease was evaluable using PERCIST. The SUV on PET/CT decreased significantly at 6 and 9 mo after baseline (average decreases of 1.5 (p=0.0004) and 2.5 (p=0.0054), respectively). The median PFS duration was 7.4 mo (95% CI, 4.8 mo-NR). The median bone PFS was 16 mo (95% CI, 7.3 mo-NR). Patients with bone-only metastasis (N=28, 80%) had a significantly longer median PFS duration than did patients with non-bone metastases at baseline (N=7, 20%) (13.8 mo versus 4.5 mo; p=0.017). Patients without prior treatment (N=12, 34%) tended to have longer median PFS durations than did those who underwent prior treatment (N=23, 66%) (16.8 mo versus 4.8 mo; p=0.1865). Also, patients with <5 CTCs at baseline (N=19, 54%) tended to have longer median PFS durations than did those with ≥5 CTCs (N=16, 46%) (13.8 mo versus 4.8 mo; p=0.1277). EMT-CTCs status did not predict efficacy.

**Conclusions:** Bone-only metastatic breast cancer and SUV suppression by Ra-223 are predictive of efficacy. Patients with baseline <5 CTC count tended to have better outcomes than did those with ≥5 CTCs. Combined treatment with Ra-223 and a hormonal agent is especially effective at controlling bone metastasis in patients with HR-positive breast cancer. Bone-only metastatic disease and CTC count should be factored in future clinical trial designs.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-18-05

Management of bone marrow involvement in advanced breast cancer: Retrospective multicenter cohort study

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**Background:** Optimal management of bone marrow metastasis (BMM) in advanced breast cancer (ABC) remains unknown. Associated severe cytopenias often urge treatment, but they are also a challenging factor for delivering chemotherapy (CT). Since BMM in ABC is infrequent, available data are scarce.

**Aim:** Clinical and prognostic characterization of ABC patients with BMM and its management; evaluation of the effectiveness of treating BMM with more myelosuppressive CT regimen (that we hypothesized that could be more active in the BMM) Vs. less myelosuppressive regimen.

**Methods:** Retrospective cohort study of patients with pathological confirmation of BMM (positive myelogram or osteomedullary biopsy) between Jan'2010 and Dec'2016 in the two major Portuguese cancer centers. Patients with diagnosis of a second carcinoma or active hemato-oncological condition within 5 years before BMM were excluded. We considered the more myelosuppressive regimens those with > 5% risk of febrile neutropenia according to *Truong* et al 2015<sup>(1)</sup>. Kaplan-Meier and log-rank methodology were used to estimate survival and Cox regression to identify the covariates with independent prognostic value. Statistical level of significance was 5%.

**Results:** We included 74 patients: 74% with disease stage I-III at presentation, 74% ductal and 12% lobular invasive carcinomas, 58% grade 2 and 27% grade 3, 80% hormone receptor + / HER2-, 4% HER2+ and 12% triple negative (TN). Median time from ABC diagnosis to BMM was 10 months (IQR 2-36), synchronous in 34%. At diagnosis of BMM, median age was 57 years-old (IQR 47-65), 57% were post-menopausal, 97% had bone metastasis, 50% had visceral metastasis, 53% performed ≥ 2 previous palliative systemic treatments and 53% were exposed to bisphosphonates. The most frequent immunohistochemistry change in BMM biopsy was the loss of progesterone receptor expression (37%). Bicytopenia (anemia/thrombocytopenia) was the trigger for BMM investigation in 69% of cases, with grade 3-4 anemia in 16% and grade 3-4 thrombocytopenia in 26%. Median survival after BMM diagnosis was 5 months (95% CI, 3-11); overall survival at 12 and 24 months were 35% (CI 26-48%) and 24% (CI 15-36%), respectively. First treatment after BMM was CT in 58% (median survival, 11 months) and endocrine therapy in 14% (median survival, 3 months). An anti-HER2 regimen was used in 4% and 22% did not receive any treatment after BMM. On multivariate analysis, TN subtype (HR 4.02, CI 1.46-11.01), thrombocytopenia (G0 reference; G1-2: HR 2.47, CI 1.11-5.56; G3-4: HR 4.89, CI 1.85-12.91) and ≥ 2 palliative systemic treatments (HR 2.77, CI 1.46-5.27) were associated with worse prognosis. Within those treated with CT, there was a trend for a deleterious survival effect of more myelosuppressive regimens (HR 2.19, CI 0.94-5.09; 5 months Vs. 14 months, n=31), after controlling for subtype, number of previous regimens and thrombocytopenia.

**Conclusion:** BMM was not a late event in ABC disease course and had worse prognosis in multi-treated patients, in TN subtype and in the presence of thrombocytopenia. No benefit was shown with the use of more myelosuppressive CT regimens.

<sup>(1)</sup>Truong J et al, Ann Oncol. 2016 Apr;27(4):608-18.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-18-06

First-line treatment for endocrine sensitive bone-only metastatic breast cancer: Is more always better?

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The standard first-line for endocrine sensitive metastatic breast cancer (BC) is represented by endocrine therapy. Several phase III clinical trials searched for more effective endocrine strategies. Nevertheless, the use of combinations for the first-line treatment of bone-only disease (BoD) is widely discussed, due to its indolent course. Our meta-analysis aims to explore the role of new endocrine strategies in BoD.

A systematic review of electronic databases was conducted to identify the phase III clinical trials comparing the standard AI to novel experimental strategies. The hazard ratios (HR) for PFS were pooled in a meta-analysis. The heterogeneity of the data was evaluated by Chi-square Q test and I<sup>2</sup> statistic.

8 studies were included in the analyses. 4 trials explored the role of CDK4/6 inhibitors (Monaleesa2 and 7, Monarch3 and Paloma2), 2 trials analyzed Fulvestrant + AI (SWOG and FACT), one trial studied Fulvestrant monotherapy (FALCON), while one trial evaluated the association between Bevacizumab and Letrozole (ALLIANCE). 6 trials reported data regarding the BoD, while 2 trials included the BoD in the non-visceral disease. Overall, the meta-analyses showed a PFS advantage for the experimental arms [HR 0.70 p 0.012], with a significant moderate/high heterogeneity [I<sup>2</sup> 66.48% p 0.004]. Only the FALCON and Paloma2 showed a significant improvement in PFS, respectively for Fulvestrant and Palbociclib + Letrozole. Considering only trials reporting data for BoD, the experimental arms significantly improved the PFS [HR 0.66 p 0.005], with a low/moderate non-significant heterogeneity [I<sup>2</sup> 44.95% p 0.106].

The novel strategies showed to be able to improve the PFS of BoD. Nonetheless, only Palbociclib + Letrozole provided statistically significant data of advantage in this setting. In clinical trials, BoD is often included in the non-visceral disease subgroup. Future clinical trials should take into account the differences in natural history and better prognosis of BoD, in order to define the best approach to these patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-19-01

A phase 2 study of abemaciclib in patients with leptomeningeal metastases secondary to HR+, HER2- breast cancer

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## Background

Abemaciclib is a selective CDK4 & 6 inhibitor approved to treat patients (pts) with HR+, HER2- advanced breast cancer (ABC) on a continuous dosing schedule as monotherapy or in combination with endocrine therapy.<sup>1,2,3</sup> Clinical data demonstrate that abemaciclib can penetrate the blood brain barrier resulting in comparable abemaciclib concentrations in brain metastases tissues, cerebrospinal fluid, and plasma.<sup>4</sup> We report safety and efficacy results of abemaciclib in pts with leptomeningeal metastases (LM) arising from HR+, HER2- ABC.

## Methods

Study I3Y-MC-JPBO (NCT02308020) is a multicenter, open-label, Phase 2 trial evaluating the safety and efficacy of abemaciclib in 6 cohorts of pts with brain metastases secondary to HR+ ABC, non-small cell lung cancer, or melanoma. Here we discuss a subgroup of cohort F: pts with HR+, HER2- LM from ABC, documented by positive CSF cytology or by clinical signs and symptoms associated with abnormal MRI features. Pts with concomitant parenchymal brain metastases were allowed, but must have been stable for  $\geq 4$  weeks following wholebrain radiotherapy or stereotactic radiosurgery. Abemaciclib was orally administered 200mg twice daily on a 21-day cycle. The key exploratory objectives were to assess the effect of abemaciclib on pts with LM secondary to HR+, HER2- ABC based on safety and tolerability, and radiological assessment from the Response Assessment in Neuro-Oncology leptomeningeal metastases (RANO-LM) criteria.

## Results

Between December 2015 and July 2016, 17 pts were enrolled in cohort F. This study reports on the 7 pts with HR+, HER2- ABC LM of which 4 pts were diagnosed with concurrent parenchymal brain metastases. Median duration of treatment was 3.9 months (range, 0.9-10.6), with 3 pts remaining on treatment for more than 6 months. Pts discontinued treatment due to progressive disease (PD, n=5) and adverse events (n=2). Median overall survival (OS) was 8.4 months (range, 3.3-14.2). Best investigator-assessed overall response was stable disease (SD) in 5 pts and progressive disease (PD) in 2 pts. Efficacy, as per CNS imaging, revealed SD in 4 pts (no imaging, n=1), 2 of which had stable or improved symptoms per neurological assessment response. Best overall intracranial response of parenchymal metastases was 1 complete response, 1 SD, and 2 PD. All 4 pts with extracranial disease had best overall response of SD. All pts experienced  $\geq 1$  TEAE, with the most common grade 3 TEAEs including nausea (28.6%), pain (28.6%), and vomiting (28.6%); 1 pt (14.3%) experienced grade 4 anemia and grade 4 upper gastrointestinal hemorrhage.

## Conclusion

OS for pts with LM arising from HR+, HER2- ABC is typically 4 months or less despite available therapies<sup>5</sup>. Here we report the median OS within this subgroup as 8.4 months. Concurrent intracranial and extracranial disease control was observed. Safety and tolerability results are similar to those previously reported with abemaciclib. Further study of abemaciclib in a larger pt cohort is warranted.

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# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P1-19-02

Innovation in diagnosis and treatment of brain metastases using multifunctional nanomedicines

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Systemic therapies have limited efficacy against brain metastases, largely because passive delivery of naked compounds via the bloodstream does not achieve sufficiently high or evenly dispersed intratumoural concentrations. Heterogeneous tissue architecture, abnormal perfusion, hypoxic zones and high interstitial fluid pressure are key factors limiting drug delivery, compounded by patchy blood-tumour-barrier permeability. Also, brain metastases are usually detected late, once patients become symptomatic. We are investigating whether engineered biopharmaceuticals might improve diagnostic sensitivity for earlier detection, as well as therapeutic efficacy and side-effect profiles of existing agents through active tumour targeting, delayed clearance and microenvironment-mediated activation. This study is proceeding with parallel preclinical and clinical tracks.

**Preclinical aims:** (1) Develop and characterise monoclonal antibody (mAb) fragments (scFvs) that target the brain metastasis markers HER2 and HER3; (2) Functionalise polyethylene glycol (PEG)-based nanocarriers with the scFvs, along with imaging agents to facilitate *in vivo* and *ex vivo* analysis of tissue distribution; (3) Functionalise HER2/3-targeted carriers with doxorubicin via an acid-labile hydrazone bond for release in hypoxic environments, or the endosome compartment after internalization. **Results to date.** His-tagged HER2- and HER3-targeted scFvs based on ligand-binding sequences of clinically-approved mAbs were expressed and purified from Expi293 suspension cultures. Binding affinities are an order of magnitude stronger than parent mAbs ( $K_D$  2-8x10<sup>-11</sup>M), determined using surface plasmon resonance analysis. The scFvs are cytostatic and moderately cytotoxic *in vitro*, with IC50s in order of 0.4-1.0µM. HER2 and HER3 scFvs exhibited dose-dependent, additive growth inhibition when used in combination, and induced internalisation of their receptor ligands within 4 hours in SKBr3 cells. **Conclusions.** The scFvs are strong carrier-tethering candidates in terms of both extracellular and intracellular payload release. Carrier synthesis is currently underway and preliminary *in vivo* data will be presented.

**Clinical aims:** (1) Develop and characterise <sup>89</sup>Zirconium-labelled HER2-targeted PET tracers based on parent mAb and scFv; (2) Compare uptake and retention of the tracers in breast cancer patients with brain metastases; (3) Computationally relate tumour uptake to the administered dose, perfusion, tumour size and HER2 expression; (4) Determine the uptake range within and between patients, and the minimum size for reliable detection. **Results to date.** The mAb tracer has been synthesised, characterised and labelling processes scaled for clinical production. It is stable in physiologic conditions, retains HER2-binding activity and has a favourable biodistribution profile in NOD-SCID mice bearing BT474 xenografts. **Conclusions.** Australian regulatory approvals are in place and recruitment for the mAb imaging trial ("BoNSAI") has begun. Preliminary data will be presented.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-19-03

A phase I trial of sorafenib with whole brain radiotherapy (WBRT) in breast cancer patients with brain metastases and a correlative study of FLT-PET brain imaging in patients receiving WBRT with or without sorafenib

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**Background:** WBRT is a standard therapy for metastatic breast cancer (MBC) patients (pts) with brain metastases (BM), but disease progression in the brain is common. Sorafenib, a tyrosine kinase inhibitor with anti-VEGF activity, has demonstrated anti-tumor efficacy in MBC and radiosensitizing activity preclinically. [18F] 3'-deoxy-3'-fluorothymidine (FLT) is a new PET tracer which correlates with cellular proliferation and may improve response assessment in the brain. **Methods:** A phase I trial of sorafenib with WBRT in MBC pts with BM was conducted using a 3+3 design. Sorafenib was given orally daily at the start of WBRT for a total of 21 days with 3 doses levels: 200mg, 400mg, and 600mg. The primary endpoints were to determine a maximum tolerated dose (MTD) and to evaluate safety and toxicity. The secondary endpoint was central nervous system progression-free survival (CNS-PFS). Macdonald Criteria were used for response assessment with serial MRI brain imaging. Key eligibility criteria include MBC with new or progressive  $\geq 1$ cm BM, ECOG PS 0-2, non-escalating corticosteroid dose, and no other concurrent anti-tumor therapy except trastuzumab. In parallel, we conducted a correlative FLT-PET imaging study (baseline, 7-10 days (FU1), and 10-12 weeks (FU2) after the WBRT) to assess radiographic changes among pts receiving WBRT + sorafenib and in a separate WBRT only cohort. FLT standard uptake value (SUV) and kinetic parameter data were obtained. **Results:** 13 pts were treated in the dose escalation phase and evaluable for dose-limiting toxicity (DLT). The median age was 56 years (range: 43-77). There were 4 HER2 positive (31%) and 3 triple negative (23%) pts. 2 pts had prior stereotactic radiosurgery. DLTs were: Grade (G) 4 increased lipase at 200mg (1 pt) and G3 rash at 400mg (3 pts) level. MTD was determined to be 200mg. 10 pts were evaluable for response (at least 1 follow up brain imaging). The overall response rate was 70%: 4 complete responses (CR) + 3 partial responses. All 13 pts were evaluated for CNS PFS with a median follow up of 29.7 months (min 19.6, max 57.4mo). Median CNS-PFS was 8.2 months (95%CI: 3.4-31.8). Median OS was 15.4 months (95% CI: 3.4-NR). A total of 10 pts with WBRT and sorafenib and 5 pts with WBRT only were enrolled in the FLT-PET study: all 15 pts had baseline FLT PET, 14 with FU1, and 9 with FU2. 55 baseline lesions, 38 at FU1 and 15 at FU2 were observed and analyzed. All lesions with FLT uptake had MRI correlates. Decline in average SUVmax of  $\geq 25\%$  was seen in 9/10 (90%) of WBRT+sorafenib and 2/4(50%) of WBRT only pts at FU1. A complete disappearance of FLT uptake was noted in 1 pt at FU1 and 2 more pts at FU2. **Conclusions:** Concurrent WBRT with sorafenib appears safe at 200mg daily dose with a higher rate of CR compared to historical WBRT data. We are currently enrolling patients in the safety-expansion cohort. This combination should be considered for further efficacy evaluation. Additional analysis of FLT-PET as a complementary imaging modality to MRI is currently ongoing. Clinical trial registry: NCT01724606 and NCT01621906. Support: Bayer, Susan G Komen, ASCO Gianni Bonadonna Breast Cancer Award

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-19-05

Establishment of a human neural progenitor cell microenvironment model to investigate signalling events in triple negative breast cancer brain metastases in a high-throughput setting

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**Background.** Breast cancer brain metastases (BCBM) represents a terminal diagnosis accompanied by neurological decline and diminished quality of life. Average survival is less than a year following detection of neural lesions, due in part to an absence of targeted therapeutics. BCBM affects 12-17% of all breast cancer patients and over 25% of triple negative breast cancer (TNBC) patients. In the search for improved therapies, there is a focus increasingly on the role of the unique microenvironment of the brain in tumour progression. Few studies have investigated the establishment of an in vitro breast cancer brain microenvironment model that could enable the high-throughput identification and modulation of potential targets. In order to address this issue, a novel co-culture model was developed utilising the human neural progenitor cell (NPC) line ReNcell VM and MDA-MB-468 TNBC cells.

**Methods.** ReNcell VM were differentiated as monolayers or neurospheres for four or ten days and stained for the glial marker glial fibrillary acidic protein (GFAP) and the neuronal marker  $\beta$ 3-tubulin. Cultures were imaged via automated epi-fluorescence microscopy (ImageXpress Micro, Molecular Devices) and proportions of cell types were calculated with MetaXpress image analysis software. Differentiation characteristics of neurospheres and monolayers were compared. Functionality of neural cultures was determined using calcium assays with the muscarinic agonist carbachol as a validation tool. MDA-MB-468 cells loaded with a Cell Tracker Green dye were assessed for viability on the neural matrix via bright field and epi-fluorescence microscopy.

**Results.** ReNcell VM cultured and differentiated for four days as neurospheres displayed a higher expression of  $\beta$ 3-tubulin and GFAP than those cultured as monolayers ( $\beta$ 3-tubulin 10.4% vs 7.7%, GFAP 53.6% vs 48.8%, respectively). Expression of  $\beta$ 3 tubulin was higher after four days of differentiation than after ten days for neurosphere cultures (10.4% vs 4.4%, respectively). Carbachol elicited oscillatory calcium responses from ReNcell VM differentiated as neurospheres or monolayers, indicating functionality of both populations. MDA-MB-468 and ReNcell VM stained after four days of co-culture displayed successful integration of neoplastic cells on the neural matrix.

**Conclusions.** Human NPCs provide a reproducible model of prominent cell types within the brain milieu and are suited to high-throughput automated epi-fluorescence microscopy applications. Co-culture models of TNBC and differentiated ReNcell VM represent a powerful platform for mechanistic studies and drug screening.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-19-06

The prognostic value of HER2, YKL-40 and IL-6 in cerebrospinal fluid in trastuzumab naïve breast cancer patients diagnosed with meningeal carcinomatosis

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## Background

With the improvements in treatment of patients with metastatic HER2-positive breast cancer (BC) the development of CNS metastases has become a major limitation of life expectancy and quality of life for many patients. Meningeal carcinomatosis represents approximately 10-20% of CNS metastases. The prognosis of meningeal carcinomatosis remains extremely poor. In the present study, we analysed the prognostic value of HER2, YKL-40 and IL-6 in cerebrospinal fluid (CSF) of meningeal carcinomatosis.

## Methods

Between June 1985 and August 1992 sixty-nine CSF samples were collected at Herlev Hospital from trastuzumab naïve BC patients suspected of meningeal carcinomatosis. The cohort was composed of BC patients from two previous studies. Meningeal carcinomatosis was confirmed by tumour cells in CSF (or autopsy/scans). The samples were centrifuged, frozen and kept at -80°C. The concentrations of YKL-40, IL-6 and HER2 in CSF were measured by commercial ELISA (Quidel and R&D) or Luminez (HER2 in second study). Overall survival in meningeal carcinomatosis was analysed with Kaplan-Meier curves and log rank tests using the median CSF value of HER2, YKL40 and IL-6 as cut off.

## Results

20 patients (29%) had meningeal carcinomatosis (18 patients had tumour cells in CFS, one was diagnosed by autopsy and one by CT scan; 13 of these patients participated in the first study and 7 in the second). 10 patients had concomitant brain metastasis. The median survival was 35 days (4-305 days).

The median [IQR] CSF concentration of YKL-40 was 284 ng/ml (187-597), IL-6 was 15.9 pg/ml (6.5-51.0) and HER2 was 1.5 (0.36-8.70) in the patients with meningeal carcinomatosis. The survival was significantly shorter in patients with high levels of YKL-40 ( $p=0.03$ ) and IL-6 ( $p<0.01$ ). The CSF HER2 level ( $p=0.51$ ) or presence of brain metastasis ( $p=0.77$ ) were not associated with survival.

## Conclusion

In this cohort high levels of YKL-40 and IL-6 in CSF were associated with short survival in patients with BC and meningeal carcinomatosis. This was not found for HER2 in CSF or concomitant brain metastasis.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-19-07

Real-world characteristics, treatment patterns, and overall survival in patients with metastatic breast cancer (mBC) and CNS metastases

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## Background:

CNS metastases are reported in about 10 to 15%. Knowledge about the management of these patients are limited because they are excluded from clinical trials due to its poor prognosis and morbidity. In these study, we aim to describe characteristics, treatment patterns, and overall survival (OS) of patients of mBC with CNS metastases at Instituto Nacional de Cancerología de Mexico (INCan) from january 2007 to december 2015.

## Methods:

We include patients with histological diagnosis of mBC and tumoral activity in the CNS (at diagnosis or during de follow-up). mBC subtype was defined using HER2 and hormone receptor (HR) status by immunohistochemistry; systemic treatment, and mortality data were used to characterize mBC with CNS involment.

## Results:

During the study period, we found 1272 patients diagnosed with metastatic disease, of whom 408 had CNS disease (novo/recurrence) the median follow up was 52 months. Table 1 describes the percentage of CNS metastases by subtype, clinical characteristics at diagnosis and median OS. Almost all patients (85.6%) were candidate to holocranial radiotherapy; after that, systemic treatment varied according to the subtype of mBC; 69.1% of TN received CT (26% based on platinum); 75.3% of HER2+ received systemic treatment, all included antiher2 therapy; luminal subtype, no one were treated with endocrine therapy.

## Conclusions:

HER2 positive breast cancer patients have the highest prevalence of CNS metastases, whereas luminal has the lowest. Patients with HER2+ and CNS metastases commonly receive treatment based on anti-HER2 therapy, maybe this target treatment contribute to the better survival achieved than patients with luminal or TN subtype. mBC with CNS metastases continues in the real world to be an unmet medical need.

table 1.- characteristics and Overall survival by subtype of mBC

	all mBC 1272 n(%)	her2 positive 339 (26.6) n (%)	triple negative 298 (23.4) n(%)	luminal 636 (50) n(%)
CNS metastasis at dianosis	44 (3.5)	12 (3.5)	13 (4.4)	19 (2.9)
CNS mets after prog to systemic tx	205(16.1)	63(18.6)	50(16.7)	92(14.5)
CNS as first place of recurrence	159(12.5)	75(22.2)	34(11.4)	50(7.8)
prevalence on CNS mets	408(10.7)	150(14.7)	97(10.8)	161(8.4)
median age at diagnosis of CNS mets	50(28-84)	50(29-84)	48(28-80)	51(28-80)
median OS after CNS mets (months)	14.9	27.2	9.33	16.3

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-19-08

Prognostic factors of human epidermal growth factor 2 (HER2) positive breast cancer patients with brain metastasis in the context of all available new therapies

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## Background:

Patients with advanced human epidermal growth factor 2 (HER2)-positive breast cancer are at the highest risk developing breast cancer brain metastases (BCBM), which are associated with significant morbidity and mortality. The advent of HER2-directed therapy resulted in greatly improved survival outcomes and have been widely used. In this context of a better controlled systemic disease, brain metastases are emerging as a new challenge for the oncologist. The aim of this study was to determine the clinicopathologic factors associated with the prognosis of patients with HER2 positive BCBM, in the scenario of new therapies.

## Methods:

A retrospective review of clinical data from patients who have developed HER2 positive BCBM from March 2005 to January 2018 at AC Camargo Cancer Center. Patients characteristics were collected, and overall survival (OS), time to first HER2 positive BCBM and OS after HER2 positive BCBM were estimated by the Kaplan-Meier method. Associations between OS after BCBM and clinical variables were assessed by Cox proportional hazards regression models.

## Results

Among 80 patients with HER2-positive BCBM median age was 51 years (range, 24–81 years). Of the patients, 55 patients (69%) had ER-positive/HER2-positive breast cancer, and 25 (31%) had ER-negative/HER2-positive breast cancer. The median brain metastasis-free survival period from primary breast cancer was 33.5 months. The median survival after developing brain metastasis was 28.5 months. Patients with more than 3 brain metastases had significantly shorter overall survival ( $p=0.01$ ). Nineteen patients (24%) underwent surgical treatment of metastasis in the brain and there was no association with survival duration ( $p=0.33$ ). Patients treated with radiotherapy 71 (88%) had significantly better survival ( $p=0.013$ ), if the patient received stereotactic radiosurgery (53.5%) compared to total brain radiotherapy received (46.5%) was not associated with duration of survival ( $p=0.24$ ). Treatment with other HER2-targeting agents after BCBM had significantly increased survival ( $p=0.022$ ), including trastuzumab 53 (67%), pertuzumab 21 (26%), trastuzumab emtansine (TDM1) 35 (43%) and lapatinib 37 (46%). In multivariate analysis, the presence of more than 3 brain metastases ( $p=0.005$ ) was the sole independent prognostic factor.

## Conclusions:

Our study indicates that HER2-positive patients with more than three brain metastases had a poor prognosis and that regardless of the use of new therapies. The role of new therapies in the management of patients with brain metastases is often not clearly defined. The best treatment strategy is not yet known and the study of prognostic factors may help to choose a better treatment sequence.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-20-01

Intraoperative ketorolac in high-risk breast cancer patients with and without inflammation. A prospective, randomized, placebo-controlled clinical trial

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**Introduction:** Perioperative events may affect the risk of breast cancer recurrence after surgery. Ketorolac, a non-steroidal anti-inflammatory drug routinely used to improve pain control postoperatively has been associated with better breast cancer outcome in retrospective studies. However, it is still unknown whether a single dose of pre-incisional ketorolac may be sufficient to prolong recurrence-free survival.

**Patients and methods:** The KBCt trial (NCT01806259) is a national, multicenter, prospective, double-blind, placebo-controlled, randomized phase III trial in high risk breast cancer patients. Each patient was assigned to the ketorolac or the placebo group. Patients were given one dose of ketorolac tromethamine (Taradyl®, N.V. Roche S.A., Belgium) or a matching placebo 30 minutes before surgery. Eligible patients were  $\geq 18$  and  $\leq 75$  years old with histologically or cytologically confirmed, invasive ductal or lobular breast carcinoma planned for curative breast cancer surgery, and with at least one of these 3 criteria: a Neutrophil-to-Lymphocyte Ratio  $\geq 4$ , node-positive disease (cN1-N3) or a triple-negative histology. The primary endpoint of the study was Disease-Free Survival (DFS). Secondary endpoints included safety, pain assessment and overall survival.

**Results:** Two hundred and three patients were assigned to ketorolac (n=96) or placebo (n=107). Baseline characteristics were similar between arms. Patients had a mean age of 55.7 (SD 14) years. Both intra- and post-operative blood losses were not different between groups and there was no difference in pain at day 1 after surgery. There was no difference between the 2 arms for DFS (p=0.52) nor for OS (p=0.88).

**Discussion and conclusion:** This study shows that a single administration of 30 mg of ketorolac tromethamine before surgery does not increase disease-free survival in high risk breast cancer patients. Overall survival is also comparable. No safety concerns were observed in this study.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-20-02

Incidence of hyperglycemia in non-diabetic patients with early-stage breast cancer treated with chemotherapy

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**Background:** There are shared risk factors between breast cancer (BC) and diabetes mellitus (DM). BC treatments including chemotherapy given in combination with glucocorticoids can induce hyperglycemia and steroid related DM. Patients with DM are at increased risk of developing chemotherapy related toxicities such as chemotherapy induced peripheral neuropathy (CIPN) compared to those without DM. The incidence of hyperglycemia during chemotherapy in non-diabetic patients with early-stage breast cancer is unknown.

**Methods:** We performed a retrospective analysis of non-diabetic women with stage I-III breast cancer treated with chemotherapy at Columbia University Medical Center from 9/1/2016-8/31/2017 to evaluate hyperglycemia incidence during chemotherapy and up to six months after chemotherapy completion. Eligible patients were identified in the electronic health record (EHR) by ICD9 and 10 codes (ICD9 174.x and ICD10 C50.x) and a record of chemotherapy administration. Non-diabetic patients were defined by chart review as no recorded history of diabetes and no receipt of a diabetes medication in the EHR. Breast cancer stage was determined by chart review. Glucose values were recorded prior to chemotherapy, during chemotherapy, and for six-months after chemotherapy completion. We defined hyperglycemia as a glucose value of  $\geq 200$  mg/dl. Median time to hyperglycemia was also calculated.

**Results:** We identified 82 eligible patients. The majority of patients received dexamethasone during their chemotherapy course (79 patients, 96.3%). The most frequent chemotherapy regimen was doxorubicin/cyclophosphamide and paclitaxel (32 patients, 39.0%). At baseline, 20 patients (24.4%) had a normal body mass index (BMI), 27 patients (32.9%) were overweight, and 31 patients (37.8%) were obese. Hyperglycemia occurred in 8 patients (9.8%) after initiation of chemotherapy. Among patients with hyperglycemia, the maximum blood glucose was between 200-299 mg/dl in seven patients (87.5%), and between 500-599 in one patient (12.5%). The median time to hyperglycemia was 84 days. Among patients who did not experience hyperglycemia, the maximum blood glucose was between 140-159 mg/dl in six patients (8.1%), between 160-179 mg/dl in eight patients (10.8%), and between 180-199 mg/dl in three patients (4.1%). Three patients were diagnosed with DM following chemotherapy completion.

**Conclusion:** Hyperglycemia occurred in almost 10% of non-diabetic patients who received chemotherapy for early-stage breast cancer. Additionally, over 30% of patients had a blood glucose of 140 mg/dl or higher after chemotherapy initiation. The impact of hyperglycemia on the development of chemotherapy related toxicities in this group is unknown. Future research is needed to identify effective interventions for glucose control during chemotherapy, and to determine if glucose control during treatment can reduce the risk of chemotherapy related toxicities, specifically CIPN.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-20-04

Nanoformulation of doxorubicin inside H- ferritin nanocages allows a cardio-safe combined therapy with trastuzumab:  
De-escalating cardiotoxicity in HER2-positive breast cancer

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**Background:** HER2<sup>+</sup> breast cancer (BC) accounts for 20-25 % of BCs and it is characterized by high aggressiveness.<sup>1</sup> Despite the anti-HER2 monoclonal antibody Trastuzumab (TZ) has improved BC clinical outcome, it could induce severe cardiac reversible dysfunction:<sup>2</sup> HER2 signaling is also essential for growth and survival of myocytes.<sup>3</sup> Therefore, the concurrent use of TZ with other cardiotoxic drugs as doxorubicin (DOX) is discouraged.<sup>3</sup> Both neoadjuvant and adjuvant clinical trials have challenged the notion that TZ should be administered with anthracyclines only sequentially,<sup>4</sup> despite pre-clinical studies demonstrated the significant enhancement of efficacy by their coadministration.<sup>5-7</sup> Nanomedicine answers to this clinical issue with HFn-DOX: a natural protein-based DOX nanoformulation with native tumor targeting capability that displays a self-triggered nuclear delivery of DOX improving antitumor efficacy and reducing both chemoresistance and cardiotoxicity.<sup>8</sup>

**Methods:** HER2<sup>+</sup> BC bearing mice have been treated 5 times twice a week with placebo, HFn-DOX (1 mg/Kg, i.v.), TZ (5 mg/Kg, i.p.) and with the combination of them. Main end-point were cardiotoxicity and anticancer efficacy. Tumor size was measured by caliper, while antitumor activity and cardiotoxicity were characterized by ICH, immunofluorescence, cytofluorimetry, TEM, mass spectrometry and western blot on resections. Statistical analyses were conducted using two-tailed Student's t-test (P< 0.05)

**Results:** Although single treatments with HFn-DOX or TZ display a good capability to reduce tumor progression, their combination improves antitumor potential, affecting tumor size and angiogenesis. Since the main TZ activity is the induction of the Antibody-Dependent Cell mediated Cytotoxicity, we have assessed the effect of HFn-DOX on Tumor Infiltrating Lymphocytes (TIL), revealing that both TILs enumeration and TIL activity is unaffected by HFn-DOX. On the other hand, HFn-DOX increases the induction of apoptosis, suggesting that the reduction of the tumor size observed in mice treated with the combination of TZ and HFn-DOX is attributable to the coupling of these activity. Mitochondrial morphology has been checked for cardiotoxicity. A pathological increase in mitochondria area coupled with cristae depletion has been evidenced only in mice treated with TZ alone, confirming the overall safety of the HFn-DOX formulation. Interestingly, mice treated with the TZ and HFn-DOX did not display evidences of cardiac suffering. TZ quantification in tumor and heart revealed that the combination with HFn-DOX couples the increased TZ accumulation and penetration in tumor with TZ reduction in heart, resulting in the lack of cardiotoxicity.

**Conclusions:** Our results suggest that a combined therapy with HFn-DOX and TZ allows an enhanced anticancer activity and reduced cardiotoxicity, with potential translational implications on the treatment of HER2<sup>+</sup> BC patients.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-20-05

Raman imaging as a tool for the chemical and spatial characterization of breast microcalcifications to improve lesion assessment

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**Background:** microcalcifications (MC) are common findings on screening mammography and are among the earliest signs of breast cancer. At the same time, from the use of well-known radiographic risk score systems, which include MC assessment, such as Breast Imaging-Reporting and Data System (BI-RADS), only 20% of screened patients are further associated with malignancy. This leads to repeated biopsies and to unnecessary surgeries with discomfort for patients and increased costs for the healthcare system. Furthermore, the definitive diagnosis by histological and immunohistological evaluations is still laborious and time-consuming.

Raman Spectroscopy (RS) is a photonic approach capable to provide detailed chemical information of analysed samples without complex tissue preparation or staining. RS has a proven ability to distinguish different crystal structures, including those commonly present in MC. In this context some studies based on RS suggested a correlation between MC chemical features and pathology. On the other hand, previous Raman-based studies mainly investigated the overall MC chemical composition (by single-point scans) while an extensive MC characterization by Raman imaging approaches (mapping) for diagnostic purposes is still lacking.

The aim of this study is to assess the usefulness of Raman imaging as a quick and accurate tool for a complete spatial characterization of MC detected on screening mammography and sampled by breast biopsy in order to better distinguish malignant vs. benign lesions.

**Method:** 30 patients with breast calcifications detected on mammography with radiological classification BI-RAD 3-5 where selected and evaluated by core biopsy. 10 µm formalin-fixed paraffin-embedded (FFPE) histological sections obtained from biopsies were dewaxed with a specifically developed protocol that allows the removal of paraffin in less than 15 minutes. All MC present in each tissue section (usually from 2 to >10 per section) were then characterized by a Raman microscope thus obtaining Raman maps with lateral resolution between 5 and 10 µm. After pre-processing steps the Raman maps were analysed by both clustering and multivariate analysis approaches used to produce false-colour images and to perform automated features identification.

**Results:** Our results confirm that hydroxyapatite is the prevalent form of calcium phosphate in MC and that MC composition correlates with lesion malignancy. In addition, thanks to the Raman imaging approach used here, we report for the first time that hydroxyapatite is more homogeneously distributed in malignant lesions and that, on the contrary, benign lesions show a heterogeneous distribution of hydroxyapatite, whitlockite and calcium-carbonate, inside the lesion and in the surrounding tissue.

**Conclusion:** These evidences suggest that the characterization of MC by Raman imaging is a potential tool for the definition of new diagnostic signatures of breast cancer, especially if we consider that these evaluations can be performed by the simple and relative fast scanning of dewaxed slices, without altering the clinical workflow and without the need of staining or antibodies. Further studies with a larger cohort will be done to validate these results.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P1-20-06

Association of circulating tumor DNA with clinical outcomes in metastatic breast cancer patients

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**Background:** Circulating tumor DNA (ctDNA) can be used as a non-invasive method to detect and quantify genomic alterations (GA) in blood. We evaluated the relationship between ctDNA features, radiographic disease progression (RDP) and progression-free survival (PFS) in patients (pts) with metastatic breast cancer (MBC).

**Methods:** A retrospective analysis of 32 female MBC pts with plasma ctDNA tested prior to a radiology scan was performed. Plasma ctDNA tests were run by Inivata using a 36-gene panel for detecting point mutations, short insertions/deletions, and copy number variations. ctDNA features were tabulated for each pt and included number of genomic alterations (numGA), maximum mutant allele frequency (maxMAF), and sum of mutant allele frequency (sumMAF). Univariate and multivariable logistic regression (LR) models were used to explore ctDNA features associated with RDP. Univariate and multivariable Cox proportional hazards models were used to identify ctDNA features that were associated with PFS. All models included ctDNA features as continuous variables.

**Results:** Frequency of subtypes were 38% HR+HER2-, 28% HR+HER2+, 28% triple-negative, and 6% HR-HER2+. 97% had prior chemotherapy; 92% of HR+HER2-negative pts had prior endocrine therapy. 20 of 32 samples (69%) had GAs. The most common GAs were *TP53* (50%), *ESR1* (25%), *PIK3CA* (19%), and *GATA3* (9.4%). Median numGA was 1 (0-12); median maxMAF was 1.9 (0-65.5); median sumMAF was 2.2 (0-157.2). Univariate LR analysis identified numGA ( $p=0.025$ ), maxMAF ( $p=0.034$ ), and sumMAF ( $p=0.049$ ) to be significantly associated with RDP; numGA ( $p=0.056$ ) and maxMAF ( $p=0.109$ ) were retained in the final multivariable LR model. Univariate Cox regression analysis showed that numGA (HR=1.32,  $p<0.001$ ), maxMAF (HR=1.03,  $p=0.011$ ), and sumMAF (HR=1.02,  $p=0.002$ ) were significantly associated with PFS; numGA (HR= 1.30,  $p=0.001$ ) and maxMAF (HR= 1.02,  $p=0.045$ ) were retained in the final multivariable Cox model.

**Conclusions:** Blood ctDNA profiling contributes to the prediction of RDP and PFS in MBC pts. High number of alterations and high allele fraction of these alterations were associated with worse clinical outcomes. These data provide an overview of the ctDNA dynamics in treated MBC pts.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-01-01

Early relapses in breast cancer can be prevented by a perioperative NSAID, which would be a solution to a 2000 year old problem

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A bimodal pattern of hazard of relapse among early stage breast cancer patients has been identified in multiple databases from US, Europe and Asia. We have been studying these data to determine if this can lead to new ideas on how to prevent relapse in breast cancer. Using computer simulation and access to a very high quality database from Milan for patients treated with mastectomy only, we proposed that relapses within 3 years of surgery are stimulated somehow by the surgical procedure. During the week post surgery, metastatic development is enhanced 100 fold according to the simulation. Most relapses in breast cancer are in this early category. Retrospective data from a Brussels anesthesiology group suggested a plausible mechanism. Use of ketorolac, a common NSAID analgesic used in surgery was associated with far superior disease-free survival in the first 5 years after surgery. The expected prominent early relapse events in months 9-18 are reduced 5-fold. Transient systemic inflammation accompanying surgery (identified by IL-6 in serum) could facilitate angiogenesis of dormant micrometastases, proliferation of dormant single cells, and seeding of circulating cancer stem cells resulting in early relapse and could have been effectively blocked by the perioperative anti-inflammatory agent. If this observation holds up to further scrutiny, it could mean that the simple use of this safe, inexpensive and effective anti-inflammatory agent at surgery might eliminate early relapses. Similar bimodal patterns have been identified in other cancers suggesting a general effect. Based on the writings of Galen and Celsus, metastatic development after breast tumors were removed was known to them 2000 years ago.

This effect has been demonstrated recently in a mouse model by Krall et al Science Translational Medicine and reviewed in NEJM by Komaroff. In a series of experiments in 273 mice, aggressive mouse breast cancer cells were implanted in various locations. Initially, the tumor cells grew but then became dormant. This dormancy occurred only in mice with intact immunity, which suggests that the immune system can contain certain dormant metastases. Surgery of any type (including resection of a primary tumor) led to aggressive growth of metastases in 60% of animals, compared with 10% of control animals that did not undergo surgery. Surgical procedures caused systemic inflammatory responses. Activated monocytes from the marrow traveled to the sites of the dormant metastases and became tumor associated macrophages. These macrophages suppressed the immune system near the tumor, awakening the metastases from their dormancy. Treating the animals with NSAIDs before and immediately following surgery greatly attenuated growth of these metastases.

Since this effect has by now been shown in two Belgian retrospective studies as well as a mouse model we suggest this be tested in one or more clinical trials. We also note that the bleeding potential from using NSAIDs before surgery can apparently be reduced with the use of Tranexamic Acid – currently being tested in a clinical trial for mastectomy.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-01-02

Whole exome sequencing analysis of the progression from ductal carcinoma *in situ* to invasive ductal carcinoma

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**Introduction:** Ductal carcinoma *in situ* (DCIS) is a *bona fide* non-obligate precursor of invasive carcinoma. Single cell sequencing studies have revealed intra-lesion genetic heterogeneity in DCIS and shown that progression to invasive ductal carcinoma (IDC) may occur through different mechanisms, including the selection of a subpopulation of tumor cells, acquisition of new genetic alterations or multi-clonal invasion. Here, we sought to investigate the genetic heterogeneity of DCIS, and to document further the clonal selection process accompanying progression to IDC.

**Materials and methods:** Synchronous DCIS (n=16) and IDC (n=15) samples from 14 patients were microdissected separately, and DNA samples of tumor and matched normal tissues were subjected to whole-exome sequencing (WES; n=27) or massively parallel targeted sequencing of all coding regions of ≥410 cancer-related genes (n=4). Somatic genetic alterations and mutational signatures were identified using state-of-the-art bioinformatics algorithms. PyClone was employed to define the clonal architecture of each DCIS and IDC and infer the clonal shifts accompanying progression from DCIS to IDC.

**Results:** DCIS were found to harbor recurrent somatic mutations affecting *PIK3CA* (50%), *GATA3* (44%), *TP53* (38%), *CBFB* (19%), *PTEN* (13%), and *AKT1* (13%), which are genes known to be significantly mutated in invasive breast cancers. Despite the genomic similarities between matched DCIS and IDCs, *NOTCH2* and *MYC* were found to be amplified solely in the IDC component of two cases, and *PPM1D* amplification was restricted to the DCIS component of another case. The mutational signature ascribed to aging (i.e. signature 1) was the predominant mutational signature in the DCIS and IDCs analyzed. PyClone analysis revealed that all synchronous DCIS and IDC studied here were clonally related and confirmed the previous observation that DCIS displays intra-lesion genetic heterogeneity. Evidence of clonal selection in the progression from DCIS to IDC was observed in three cases, whereby a minor DCIS subclone likely constituted the substrate for the development of IDC. In one of these cases, from a patient with a *BRCA1* germline pathogenic mutation, we observed a shift from the mutational signature associated with defective homologous recombination DNA repair (i.e. signature 3) to the APOBEC-related mutational signatures (i.e. signatures 2 and 13) in the progression from DCIS to IDC.

**Conclusion:** Intra-lesion genetic heterogeneity is a common feature in DCIS synchronously diagnosed with IDC. Our findings corroborate the notion that DCIS is a direct non-obligate precursor of IDC, and that clonal selection in the progression of DCIS to IDC may be present in a subset of cases, but is unlikely to constitute the most frequent mechanism of progression.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-01-03

Lipocalin 2 promotes inflammatory breast cancer tumorigenesis and skin invasion

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**Background:** Inflammatory breast cancer (IBC) is the most lethal form of primary breast cancer and accounts for a significant 10 % of breast cancer deaths in the USA owing to its aggressive proliferation and metastasis, and a lack of effective therapeutic options. Unraveling the underlying mechanisms of growth and metastasis of this aggressive disease could lead to effective therapeutic strategies for an improved outcome in IBC patients. We recently generated *in vitro* and *in vivo* IBC models for brain metastasis studies [Debeb *et al.* JNCI, 2016] and observed an upregulation of Lipocalin 2 (LCN2), a small, secreted iron-trafficking protein which plays a significant role in immune and inflammatory responses and the promotion of malignant progression. The purpose of this study was to investigate the function of LCN2 in IBC tumorigenesis and metastasis.

**Methods:** Stable knockdown (KD) of LCN2 in IBC cell lines was achieved with lentiviral vectors. Proteomic and gene expression profiling were performed using RPPA and Affymetrix Clariom D microarray. For *in vivo* studies, control and LCN2 KD IBC cells were transplanted into the cleared mammary fat pad of SCID/Beige mice. Tumor-skin involvement was assessed visually during primary tumor growth and tumor excision. *LCN2* gene expression levels in clinical samples were analyzed from the IBC Consortium as well as public data sets. LCN2 serum levels in IBC patients were measured using ELISA and were correlated with clinicopathological variables and outcome data.

**Results:** *LCN2* gene expression is higher in IBC versus non-IBC patients ( $p=0.00036$ ), independently of the molecular subtypes, and higher in more aggressive (TNBC and HER2+) than hormone receptor-positive subtypes ( $p<0.00001$ ). LCN2 expression in patient tissues is correlated with reduced overall survival ( $p<0.00001$ ) and metastasis-free survival ( $p=0.04$ ) in non-IBC; however, LCN2 was not associated with overall survival in IBC patient serum samples. LCN2 expression was also significantly higher in IBC cell lines, in their culture media, and in brain metastasis sublines compared to non-IBC cell lines ( $p=0.004$ ). In IBC cell lines, LCN2 KD reduced proliferation, colony formation, migration, and cancer stem cell properties. *In vivo* silencing of LCN2 in SUM149 cells inhibited primary tumor growth ( $p=0.001$ ) and resulted in a well-differentiated tumor histology. Additionally, SUM149 LCN2 KD significantly reduced skin invasion/recurrence (LCN2 control vs LCN2 KD: 88 % vs 25 %,  $p=0.01$ ) suggesting LCN2 is a mediator of tumorigenesis. Analysis of proteomics data showed changes in major signaling pathways including PI3K-Akt signaling and EGF/EGFR signaling pathways. Mechanistically, LCN2 depletion in SUM149 abrogated EGF-induced EGFR phosphorylation and ERK activation.

**Conclusions:** Our findings suggest that LCN2 may drive IBC tumor progression and skin invasion/recurrence potentially via the EGFR signaling pathway. Future studies will determine the role of LCN2 in metastasis and pinpoint the detailed mechanisms of LCN2-mediated IBC tumorigenesis and recurrence.

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Epithelial-mesenchymal transition promotes triple negative breast cancer immune suppression

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**Background:** Epithelial-Mesenchymal Transition (EMT) is a developmental process co-opted by carcinomas to facilitate metastasis. Triple negative breast cancer (TNBC), which has often undergone at least partial EMT, has a poor prognosis due to frequent and rapid recurrence as metastatic disease when compared to other breast cancer subtypes. To identify drivers of TNBC progression microRNA miR-200c was restored to the human TNBC mesenchymal-like BT549 cell line, which decreased 80% of the pan-EMT mesenchymal signature and restored 60% of the epithelial signature. In addition to decreasing proteins involved in immune-suppression via contact-dependent mechanisms, such as *CD274* (PD-L1) and (*CD273*) PD-L2, Ingenuity Pathway and GSEA analysis revealed reduced expression of genes encoding enzymes, including Tryptophan 2,3-Dioxygenase (TDO2) and Heme Oxygenase-1 (HO-1), that generate immune-suppressive metabolites that function in a non-contact dependent manner. TNBC utilizes these and other immune-suppressive factors made by fetal trophoblasts to suppress the maternal immune system to achieve fetal tolerance during pregnancy, suggesting that TNBC may mimic a physiologically relevant mechanism to promote immune evasion.

**Hypothesis:** TNBC metabolism is altered as part of an EMT program resulting in generation of immune-suppressive metabolites that promote metastasis by facilitating contact-independent immune evasion.

**Methods:** Expression and activity of TDO2 and HO-1 were determined by UHPLC-MS for kynurenine (generated by TDO2) and bilirubin (generated by HO-1) in multiple TNBC cell lines. Single cell RNAseq was performed to identify genes co-expressed with these enzymes. To develop an immune competent preclinical model, doxycycline inducible miR-200c (TripZ-200c) was stably introduced into the Met-1 mammary carcinoma cell line derived from the MMTV-PyMT mouse model developed in FVB/NJ mice. Results were confirmed via transient miR-200c expression in 66cl-4 mammary carcinoma cells (from a spontaneous BALB/c tumor).

**Results:** Restoration of miR-200c decreased *TDO2* and *HMOX1* (HO-1) by 80% and 40% respectively in Met-1 and 66cl-4 mammary carcinoma cells and reduced secretion of kynurenine and bilirubin. HO-1 was increased in lung metastasis from Met-1 and 66cl-4 cells when compared to cells prior to tail vein injection. ScRNAseq revealed specific gene expression signatures co-expressed with *TDO2* and are being analyzed to better detect TDO2 in tumor tissues. *In vivo* studies are underway to investigate the effects of inhibiting tumor TDO2 and HO-1 on infiltrating lymphocyte composition, the anti-tumor immune response, and tumor progression.

**Conclusions:** Restoration of miR-200c revealed EMT-associated alterations in tumor metabolism and consequent secretion of immune-suppressive metabolites produced by TDO2 and HO-1. We propose that targeting these contact-independent immune-suppressive factors may complement current contact-dependent therapeutic strategies (checkpoint inhibitors) to boost anti-tumor immune cell function and thereby prevent or decrease TNBC metastasis.

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Targeting of casein kinase 1 $\delta$  inhibits triple negative breast cancer metastasis

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Metastasis is responsible for most breast cancer mortalities and 30% of the patients with breast cancer will develop metastases. Despite the establishment of efficient targeted therapies for breast cancer, there is no cure for metastatic disease. Our research team have developed a potent, highly selective, small molecule inhibitor of casein kinase-1 delta (CK1 $\delta$ ), a serine/threonine kinase. We have demonstrated that our lead molecule (SR-3029) provokes tumor specific cell death and tumor regression in pre-clinical orthotopic xenograft models of triple negative breast cancer (TNBC) including, basal-like patient derived (PDX) breast cancer models. Herein, we demonstrate that targeting CK1 $\delta$  inhibits tumor cell invasion in cell based studies and markedly reduces both the number and size of metastasis lesions spreading from the breast to the lung *in vivo*. We show that invasion impairment is due to the inhibition of key effectors of the epithelial-mesenchymal transition. We have developed Lumifluor patient-derived xenograft models and assessed the effect of our inhibitors at various stages during the process of metastases. We have identified CK1 $\delta$  as an efficacious therapeutic target with dual potential for triggering apoptosis of breast tumor cells and impairing metastatic spread from the primary tumor.

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Intravital imaging of the lung reveals the efficiency of the metastatic cascade

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**Background:** Breast cancer is the leading cause of cancer related-death in women[1] with over 90% of these deaths due to metastasis. In these cases, the lung is the most common anatomical site of metastasis found at autopsy[2]. For the last 100 years, the process of metastasis has been studied through the use of an experimental metastasis (EM) assay [3] consisting of tail vein injection of tumor cells into tumor-free mice, followed by histopathological analysis of the lung weeks later to gain insight into tumor cell arrival, survival, and the growth of metastases. These studies have concluded that metastasis is an inefficient process[4]. Using a new technology developed at Albert Einstein College of Medicine, called the Window for High Resolution Imaging of the Lung (WHRIL)[5], we have directly compared EM to the more clinically relevant process of tumor cells spontaneously metastasizing (SM) from a primary tumor *in situ* to the lung and have found significant differences in metastatic efficiency between EM and SM.

**Methods:** Real-time images of tumor cell dissemination were captured using the WHRIL (Figure 1) in both EM and SM models. Metastatic potential was analyzed, and compared between the models, the percentage of tumor cells surviving in the lung over time, their endothelial crossing-time, their frequency of interaction with macrophages, the fraction of cells which are dormant, and the percent of cells that developed into metastases.

**Results:** Tumor cells which spontaneously metastasize from primary tumors show a ten-fold higher rate of survival in the lung and three times greater efficiency in forming metastases compared to those directly injected into the lung vasculature. Most of SM tumor cells are dormant indicating that the residual disease phenotype is programmed by the primary tumor either directly in the primary site or indirectly at the secondary site.

**Conclusion:** These results indicate that experimental metastasis does not accurately reflect the true clinical process and that spontaneous dissemination from a primary tumor has significant influence on the survival and growth of disseminated cells. This suggests that the tumor microenvironment of the primary tumor educates disseminating tumor cells for survival, dormancy and growth at the primary site, and/or prepares the pre-metastatic niche, in the secondary site. Understanding where and how disseminated tumor cells are educated is critical to preventing their survival and growth at secondary sites. This discovery will open the door to new strategies for the treatment of metastatic tumors to prevent metastatic progression and death.

## 2018 San Antonio Breast Cancer Symposium ®

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M2-like tumor-associated macrophages require Tartrate-resistant acid phosphatase as overall function to promote breast cancer metastasis

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Tartrate-resistant acid phosphatase (TRAP) is a member of the purple acid phosphatase family and is also known as type 5 acid phosphatase. It is mainly expressed in cells of the monocyte lineage, including osteoclasts, macrophages, and dendritic cells (DCs). There are two isoforms of TRAP enzyme: TRAP-5a and TRAP-5b, and TRAP-5b is produced by post-translational modification of TRAP-5a. TRAP-5b is the major isoform of TRAP secreted by osteoclasts and it has been shown that TRAP-5b activity correlates with the number of osteoclasts and serum activity in rat and human studies. In contrast, macrophages and DCs are thought to secrete TRAP-5a as the major subtype, whereas TRAP-5a is a non-specific marker of macrophage activation. The recent studies show that higher-grade proliferative tumors with much necrosis were associated with abundant macrophage expressing TRAP. Here we generate TRAP<sup>-/-</sup> knockout mice to study the function of TRAP in tumorigenesis. In vitro experiments have found that the expression of TRAP in macrophages is essential for the full promoting tumor function of M2-like tumor-associated macrophages (TAM) including tumor growth and metastasis. Marrow-derived macrophages (BMDM) extracted from the TRAP<sup>-/-</sup> mice lost promote tumor migration activity. The 4T1 tumor (murine breast cancer cell line) conditioned medium was used to induce TAM formation found M2-like TAM formation in wild type BMDM was promoted higher than that of TRAP<sup>-/-</sup> BMDM. In contrast, the TRAP<sup>-/-</sup> is much higher M1-like TAM formation than the wild type. Xenograft tumor formation assays were used to monitor tumor formation by combining 4T1 and different M2 polarized macrophages into BALB/c mice. M2-polarized macrophages with trap-defective BMDM showed reduced promoted neoplastic activity compared to wild type. In MMTV-PyMT transgenic mice, spontaneous tumor formation in knockout trap<sup>-/-</sup> significantly repressed tumor formation compared to TRAP<sup>+/+</sup> wild type mice. Finally, we hypothesized that TRAP is essential for the full function of M2-like tumor-associated macrophages and promotes tumor metastasis.

# 2018 San Antonio Breast Cancer Symposium ®

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The potential role of CTNND1 (catenin (cadherin-associated protein), Delta 1) in breast cancer bone metastasis

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## Background:

The bone is a frequently visited site by breast cancer cells. Most women who die of metastatic breast cancer would already have bone metastases, whether they are micro- or macro-metastases. Metastatic bone metastasis from breast cancer is mostly osteolytic, with reasons unclear and little *in vitro* and *in vivo* studies exploring the osteolytic nature of bone metastasis. In the present study, we investigated the potential role of CTNND1, Catenin (Cadherin-Associated Protein) Delta 1, in the context of bone metastasis of breast cancer.

## Materials and Methods:

In order to identify potential genes involved in bone metastasis, we established a novel *in vitro* model named Bone Matrix Extract (BME) which was extracted from human femur and used to mimic the bone environment. Full profile of gene expression in response to BME was conducted using Ampliseq technology. Potential genes associated with bone metastasis was examined in a clinical breast cohort containing both cancer and normal tissues (n = 103), collected immediately following surgery. Gene transcript levels were quantified using QPCR and analysed against patient's pathological information and clinical outcome. We generated a series of cell models by knocking down and over-expressing one of the most relevant genes, *CTNND1*, using siRNA, sh-RNA, ribozyme transgenes and insertion of full coding sequence containing plasmids. Function assays including Matrigel based-adhesion, cancer cell-osteoblastic cell contact, proliferation, transwell invasion and migration were used to investigate the changes of biological features after interfering with CTNND1 expression in relation to BME / co-culture models.

## Results:

CTNND1 was down regulated in all breast epithelial cells following BME treatment at both mRNA and protein level. From the clinical cohort, we found that compared with benign tissue, breast cancer tissues had significantly decreased CTNND1 transcript expression. Reduced CTNND1 was associated with advanced TNM stage and poor distant metastasis, local recurrence and bone metastasis. We went on to knockdown CTNND1 by siRNA, ribozyme as well as lenti-shCTNND1 transfection in MCF-10A and MDA-231 cells and overexpressed CTNND1 in MCF-7 cells. *In vitro* study demonstrated that knockdown of CTNND1 expression led to decreased capacity of Matrigel-adhesion, migration and invasion but increased cancer cell-osteoblastic cell adhesion. No effects were observed on cell proliferation after altering CTNND1 expression, in the presence or absence of BME.

## Conclusions:

In this initial study on CTNND1 in breast cancer, our current data suggests that lower transcript expression of CTNND1 associates with a poorer patient prognosis. CTNND1 reduction may play a role in the progression of breast cancer bone metastasis.

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*ESR1* mutations drive breast cancer metastasis by context-dependent alterations in adhesive and migratory properties

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**Background:** Estrogen receptor alpha (ER $\alpha$ /*ESR1*) is mutated in 30-40% of endocrine resistant ER+ breast cancer. These mutations, primarily located in the ligand binding domain, are associated with worse outcome in patients, and preclinical studies have shown that they cause ligand independent growth. An open question is whether these mutations contribute to actual metastatic process, or merely endocrine resistance.

**Methods:** Using Y537S and D538G genome-edited MCF7 and T47D cells, 3D growth was assessed in ultralow attachment plates. Cell-cell adhesion was determined using calcein-labelled adhesion assay and quantitative microfluidic fluorescence microscope (qMFM). Collagen-based adhesion and spheroid invasion assays were used to test adhesive and invasive properties. Wound scratching, spheroid collective migration and Boyden chamber transwell assays were applied to monitor cell migratory phenotypes. Mutated ER cistromes were profiled using ChIP-sequencing. *ESR1* mutations in clinical samples were characterized using ddPCR.

**Results:** Visual inspection of cells grown in suspension culture revealed more compressed multicellular spheroids in *ESR1* mutant cells, indicative of increased cell-cell interactions. This observation was confirmed in both static and microfluidic conditions. This effect was more pronounced in MCF7 than T47D cells, correlating with increased expression of desmosome and gap junction genes. Pharmacological blockade of gap junctions decreased cell-cell adhesion. Decreased attachment and increased invasion to collagen were discerned in all mutant cell types. Further functional analysis identified alterations in the TIMP3-MMP axis causing these phenotypes. The cell-cell adhesion phenotypes were restricted to MCF7-Y537S/D538G and T47D-Y537S, whereas T47D-D538G cells showed significantly increased migration. A GSEA screen identified Wnt signaling as uniquely induced in this context, and combination treatment using the Wnt inhibitor LGK974 and Fulvestrant led to synergistic inhibition of migration. ChIP-seq identified mutation-specific cistromes with an overall increased ligand-independent ER binding. However, it did not reveal binding sites in any candidate metastases genes, suggesting secondary epigenetic mechanisms. The motif analysis revealed the enrichment of FOXA1 motifs in mutated ER cistromes except T47D-D538G cells. However, knockdown of FOXA1 induced significantly higher inhibition of T47D-D538G migration than Fulvestrant treatment alone, indicating a FOXA1-dominated mechanism. Collectively, these data show that *ESR1* mutant cells gain metastatic properties, in addition to endocrine resistance. To prove this using clinical samples, we measured *ESR1* mutations in a well-defined cohort of endocrine resistant local or distant recurrence. Significant enrichment of *ESR1* mutations in distant (9/55) vs local (0/27) recurrences confirms critical role of mutant ER $\alpha$  in metastases.

**Conclusion:** Further analysis of context dependent changes in cell-cell adhesion and migration of *ESR1* mutant cells might guide the design and development of drugs targeting ER $\alpha$ -mutant tumors, such as inhibitors of gap junction, FOXA1, MMP, and Wnt signaling pathways.

**Disclosure:** The authors declare no conflict of interest.



# 2018 San Antonio Breast Cancer Symposium ®

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A peptide with the conserved amino acid residue of integrin  $\alpha 6$  inhibits metastasis through disruption of complex formation in breast cancer cells

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Metastasis inhibition may improve survival of breast cancer patients. Our previous study revealed that a transcription factor SALL4 promotes cell migration for metastasis through up-regulation of integrin  $\alpha 6\beta 1$  in metastatic basal-like breast cancer cells. In these cells, integrin  $\alpha 6\beta 1$  modulates Rho GTPase activity, which enhances focal adhesion dynamics for cell migration. We therefore focused on integrin  $\alpha 6$  as a therapeutic target for metastasis.

We investigated the expression level of integrin  $\alpha 6$  in breast cancer cell lines and a tissue microarray of breast cancer patients. In both experiments, we observed higher integrin  $\alpha 6$  expression in basal-like breast cancer cells than in luminal ones. We performed shRNA-mediated integrin  $\alpha 6$  knockdown in basal-like breast cancer cells and conducted Boyden chamber assays. In the results, reduced cell migration was observed in integrin  $\alpha 6$  knocked-down cells. Reciprocally, we introduced integrin  $\alpha 6$  overexpression in low-migratory luminal breast cancer cell lines and observed increased cell migration. These results indicate that integrin  $\alpha 6$  promotes cell migration in breast cancer cells.

To identify the functional residue of integrin  $\alpha 6$ , we analyzed its amino acid sequence by *in silico* analyses. First, we extracted integrin  $\alpha 6$  specific residues in integrin  $\alpha$  family in the human genome. Then, we compared integrin  $\alpha 6$  sequences among vertebrates. In the result, we obtained an integrin  $\alpha 6$ -specific and vertebrate-conserved sequence, Asp-358, as a candidate for the functional residue.

To inhibit integrin  $\alpha 6$  function, we designed an 8-amino acid peptide with the sequence around Asp-358. Basal-like breast cancer cells treated with the peptide showed reduced cell migration in a dose-dependent manner. The peptide did not reduce cell migration in integrin  $\alpha 6$  knocked-down cells, suggesting that the peptide inhibits integrin  $\alpha 6$  function. To determine whether the peptide inhibits metastasis, we performed zebrafish metastasis assays, and observed reduced metastasis rate in the peptide-treated group. These results indicate that the peptide inhibits metastasis through reduction in cell migration.

To understand the effect of the peptide, we performed chemical cross-linking assay in a basal-like breast cancer cell line. Protein samples from the cells treated with a cross-linker showed bands of integrin  $\alpha 6$  complexes, and the intensities of these bands were reduced in the peptide-treated group. Moreover, we immunostained cells with an antibody for focal adhesion marker, phospho-paxillin, because integrins regulate focal adhesion formation for cell migration. In the results, the peptide reduced the number of focal adhesions. These observations indicate that the peptide inhibits integrin  $\alpha 6$  function. In the future, our findings may contribute to development of a metastasis inhibition therapy.

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Detection of circulating cancer stem cells: A novel approach as a predictive marker for breast cancer metastases. This may be especially useful in patients with undetectable circulating epithelial tumor cells

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**Background:** Breast cancer is one of the most common types of cancer in women worldwide. It has been demonstrated that even localized tumors without clinically apparent metastases give rise to circulating epithelial tumor cells (CETCs). Recent studies have provided strong support for the circulating cancer stem cell (cCSC) hypothesis, which suggests that a more aggressive subpopulation of circulating tumor cells are the source of metastatic spread from the primary tumor. Measuring CETCs in blood of patients has emerged as a non-invasive diagnostic procedure for screening patients who may be at high risk for developing metastatic cancers. However, accurate detection of CETCs may provide erroneous results, since CETCs undergoing EMT during metastasis are down-regulated for the expression of epithelial cell markers.

**Methods:** 26 breast cancer patients were included into the study. The determination of CETCs and cCSC was performed using maintrac® method and tumorsphere forming assay, respectively. Cell viability, surface marker expression and ALDH 1 activity of the cells in the spheres were evaluated by fluorescence scanning microscope.

**Results:** Sphere formation was observed in 80 % of patients. We found that the number of tumorspheres depended on stage of disease. The number of tumorspheres increased significantly with tumor progression, especially with the presence of metastases. Tumorsphere formation was observed in all metastatic patients (median of 30 tumorspheres/100µl of blood), although only 27% of them had detectable CETCs. Analysis of surface marker expression profile showed that the cells in the spheres had typical phenotype of cancer stem cells. Sphere formation was not observed in healthy subjects (n=20).

**Conclusion:** There is a high correlation between the numbers of tumorspheres cultured from peripheral blood with clinical stage of disease. This may be an indicator of tumor aggressiveness, especially presence of metastases and may provide clinically useful prognostic information.

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The splicing factor PHD finger protein 5A inhibits apoptosis to promote breast cancer progression

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**Background:** All the widely accepted hallmarks of cancer are known to be affected by aberrant splicing (AS), and splicing dysregulation itself is considered a valuable therapeutic target. Understanding the AS that promote cancer progression is crucial for the development of effective strategies for treating breast cancer.

**Methods:** An *in vivo* CRISPR screen targeting RNA-binding proteins (RBPs) was performed to reveal the key splicing modulator (PHD finger protein 5A, PHF5A) of breast tumor progression. Immunohistochemistry method and survival analysis were performed using a tissue microarray (TMA) containing 450 breast carcinoma. Proliferation, transwell migration and *in vivo* tumor formation assays were utilized to assess the biological role of PHF5A. RNA sequencing and RT-PCR assay were used to identify PHF5A-regulated AS events in breast cancer cells. Biological functions and molecular pathways of the affected genes were investigated through a gene ontology (GO) analysis. Flow cytometry and Western blot analysis were used for apoptotic assessments. The correlation between PHF5A expression and AS events was further analysed using mRNA-Seq data of 40 paired breast cancer and adjacent normal breast tissues. And the correlation between the levels of PHF5A and cleaved caspase-3 were evaluated in the TMA.

**Results:** According to RNA sequencing analysis of MCF10 cell series (MCF10A, MCF10AT, MCF10DCIS and MCF10CA1a), 159 RBPs were found to be up-regulated in cancer cells compared with non-cancer cells. And the CRISPR screen targeting these 159 RBPs systematically identified highest-ranking genes including PHF5A. In TMA cohort, high PHF5A expression was correlated with poor disease-free survival. PHF5A is frequently up-regulated in breast cancer and is essential for cancer cell proliferation, migration and tumor formation. Knockdown of PHF5A induces genome-wide AS events. The RT-PCR assay of MCF10CA1a cells showed that splicing changes of nine arbitrarily selected target genes were all modulated by PHF5A. GO analysis showed that PHF5A-regulated AS events were involved in apoptotic and anti-apoptotic pathways, among which FAS-activated serine/threonine kinase (FASTK) AS showed significant PSI (percent spliced in) difference. PHF5A knockdown appeared to switch full-length FASTK (FASTK-L) to an intron 5-retained variant (herein termed FASTK short, FASTK-S) in MCF10CA1a cells. The knockdown of PHF5A resulted in cleavage of caspase-3 and poly-ADP-ribose polymerase and conversion of the FASTK-L (61 kDa) and FASTK-S (42 kDa) proteins. Intriguingly, cells transduced with exogenous FASTK-S showed the most significant apoptotic effect, whereas the FASTK-L group presented a decreased apoptotic effect. The PHF5A ratios of paired non-tumor to tumor tissue were negatively correlated with the FASTK PSI differences between non-tumor and tumor tissues. A strong negative correlation was found between the PHF5A and cleaved caspase-3 levels in TMA.

**Conclusions:** PHF5A depletion sensitizes cancer cells to apoptotic signaling partially through AS-mediated FASTK isoform conversion. This apoptotic suppressor plays a key role in breast cancer progression and acts as a prognostic indicator, and should be critically considered for optimization of the current therapeutic strategy.

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Adipocytes and cancer cell interactions promote leptin receptor expression and drive  $\beta$ -catenin-mediated progression in triple negative breast cancer

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**Background** Triple negative breast cancer (TNBC) patients suffer from poor prognosis due to short-term recurrence and/or metastasis. Obesity is reported to predict poor prognosis of TNBC. However, the mechanisms connecting obesity to progression of TNBC remain unclarified.

**Methods** We co-cultured TNBC model cells with adipocytes differentiated from human adipose-derived mesenchymal stem cells (hMSC-ad) and TNBC patients derived primary adipocytes to examine migration, invasion and metastasis abilities using Transwell assays and tail vein injection xenograft mouse model, respectively. Expression levels of leptin receptor (LEPR), epithelial-mesenchymal transition (EMT) markers and  $\beta$ -catenin signaling pathway related molecules in the control and co-cultured TNBC model were analyzed by real time quantitative polymerase chain reaction (RT-qPCR) and western blot assays. Downregulation of LEPR by LEPR specific short hairpin RNA (shRNA) and upregulation of the downstream pathway with continuous activated  $\beta$ -catenin were applied to access their roles in adipocytes and cancer cell interactions. Degree of adipocytes infiltration in tumor tissue was evaluated by histopathology. LEPR mRNA expression level in breast cancer and corresponding patients' information was retrieved from The Cancer Genome Atlas (TCGA) database.

**Results** Here we first reported that both hMSC-ad- and patients-derived adipocytes promoted migration and invasion of TNBC model cells *in vitro* and lung metastasis *in vivo*. Adipocytes were then confirmed to induce upregulation of LEPR in each TNBC model cell compared to the control groups ( $p < 0.0001$ ), resulting in higher expression of EMT markers and molecules related to  $\beta$ -catenin signaling pathway. Afterwards, LEPR knockdown led to decreased migration, invasion and metastasis capacities, as well as reduced expression of EMT markers and molecules related to  $\beta$ -catenin signaling pathway, while activation of  $\beta$ -catenin could restore robust EMT and metastatic abilities of TNBC model cells. Last, clinical specimen analyses showed that infiltration of adipocytes to tumor tissue was significantly associated with shorter DFS of TNBC patients ( $p < 0.05$ ) and upregulated LEPR in breast cancer tissue indicated poor prognosis (median OS of 7.43 vs 10.81 years of the LEPR-low group,  $p < 0.05$ ).

**Conclusions** To our knowledge, adipocytes are first shown to promote progression of TNBC via previously uncharacterized LEPR- $\beta$ -catenin signaling pathway.

# 2018 San Antonio Breast Cancer Symposium ®

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Inflammatory breast cancer cells are characterized by attenuated SMAD dependent TGF $\beta$  signaling leading to impaired cell motility responses

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**Introduction.** Inflammatory breast cancer (IBC) is an aggressive form of locally advanced breast cancer with elevated metastatic potential, characterized by the frequent presence of tumor emboli in dermal and parenchymal lymph vessels. In the past, evidence was provided that TGF $\beta$  signaling is part of the molecular biology of this disease. In this study, this relation was further examined.

**Materials and methods.** TGF $\beta$ 1 induced cell motility (i.e. XCELLigence, wound healing assays), gene expression (RNA-sequencing) and peptide phosphorylation (i.e. PAMGene technology) patterns were investigated in a panel of 3 IBC and 3 subtype-matched nIBC cell lines. In addition, a series of tissue samples from 75 and 135 patients with and without IBC was investigated for nuclear expression of total SMAD2, SMAD3 and SMAD4 using immunohistochemistry. Finally, SMAD protein expression data were related to gene expression data from patients with available Affymetrix HGU133plus2 profiles.

**Results.** The cell motility inducing capacity of TGF $\beta$ 1 was strongly abrogated in all IBC cells independent of their molecular subtype (P=0.003). Genes differentially expressed between IBC and nIBC cells post 4 hours of TGF $\beta$ 1 revealed attenuated expression of SMAD3 transcriptional regulators with concomitant overexpression of MYC target genes in IBC. Assessment of SMAD expression in patient samples demonstrated a near absence of nuclear SMAD3 expression in the primary tumors from patients with IBC (P<0.001) and a further reduction of SMAD3 staining intensity was observed in tumor emboli (P=0.019). Integrated analysis of gene and protein expression data revealed that a substantial fraction of the IBC signature genes correlated with SMAD3 and these genes (i.e. 21/24; P<0.001) carry evidence in favor of attenuated SMAD3 signaling in IBC.

**Discussion.** It is demonstrated that IBC cells are characterized by attenuated SMAD3 protein expression and transcriptional activity that obliterates the cell motility inducing capacity of TGF $\beta$ 1. Recent studies revealed that SMAD3 is essential for TGF $\beta$ 1 induced cell motility through induction of epithelial to mesenchymal transition (EMT). In the absence of SMAD3 expression, a partial EMT is induced leading to collectively invading cancer cells that are gifted with a high metastatic potential and favor lymphatic dissemination, thereby providing an intriguing explanatory model of the biology of tumor emboli in IBC.

# 2018 San Antonio Breast Cancer Symposium ®

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CRISPR-Cas9 screen identifies TMEM106A as a suppressor of breast cancer metastasis

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**Purpose:** Breast cancer is one of the most common malignancies among women. The majority of cancer-associated deaths are caused by metastasis. Many biological alterations occur during this complex process, including the epithelial-mesenchymal transition (EMT), tumor immune escape, transendothelial migration (TEM). However, the biological mechanism of metastatic disease remains poorly understood and more need to be done to unify our understanding of underpinnings for metastasis in breast cancer.

**Methods:** We performed functional screening with CRISPR-Cas9 Genome-Scale CRISPR Knock-Out (GeCKO) library to identify metastasis-associated genes in breast cancer. We confirmed the function of a set of genes by transwell assays to testify the efficacy of in vivo screening. Further, we conducted in vitro and in vivo assays to explore the mechanism of TMEM106A in suppressing metastasis of breast cancer.

**Results:** The CRISPR-Cas9 library screening identified genes with potential of suppressing metastasis in breast cancer. Further validation of COPS2, SSBP2, TDG, DYRK4, GSG1, TMEM106A and PRR5L revealed that knock down of these genes promoted cell migration and invasion ability. In MDA-MB-231 and MDA-MB-468 cells, knock down of TMEM106A promoted in vitro migration, invasion, wound healing and in vivo pro-metastasis ability. In addition, E-cadherin was down-regulated and N-cadherin was up-regulated with suppression of TMEM106A. Accordingly, the potentiality of pro-metastasis was reversed when exogenous TMEM106A was expressed in MDA-MB-231 and MDA-MB-468 cells. We found the interaction between TMEM106A and WDR77 via Co-IP and MS analysis. Down-regulation of TMEM106A promoted expression of WDR77, which primarily occurred in the cytoplasm. To verify the association between TMEM106A and WDR77, we interfere WDR77 expression in MDA-MB-231 sg-TMEM106A, and the pro-metastasis effect of TMEM106A down-regulation was reversed.

**Discussion:** CRISPR-Cas9 library screening is an effective strategy to identify genes associated with breast cancer metastasis. The positive selection provides us a set of genes with potentiality to repress migration and invasion. TMEM106A may suppress metastasis via influencing translocation of WDR77 in breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-01-17

Epigenetic and transcriptomic profiling of mammary gland development and tumor models disclose regulators of cell state plasticity

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Cell state reprogramming during tumor progression contributes to the intra-tumoral cellular heterogeneity that complicates accurate diagnosis, compromises therapeutic effectiveness, and fuels metastatic dissemination. As cancer is a caricature of normal development, we used chromatin accessibility assays and transcriptional profiling to analyze epigenetic changes that occur during mammary development as an agnostic approach to identify candidate cell state regulators that could also be involved in cancer cell state interconversions. We show that fetal and adult basal cells share epigenetic features consistent with multi-lineage differentiation potential. We find that DNA-binding motifs for SOX family transcription factors are uniquely enriched in chromatin regions that are accessible in stem/progenitor cells and inaccessible in differentiated cells. In both mouse tumor models and human tumor samples, levels of Sox10 expression are heterogeneous and correlate with stem/progenitor identity, de-differentiation, and invasive characteristics. Strikingly, we demonstrate that SOX10 binds to genes that regulate neural crest cell identity, and that SOX10-positive tumor cells uniquely exhibit neural crest cell features.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-01-19

Sphingosine-1-phosphate affects tumor-associated macrophages in breast cancer patients

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**Background:** Tumor-associated macrophages (TAMs) are considered to be one of the key players in the tumor microenvironment, which regulates cancer invasion and metastases. TAMs can be divided into two phenotypes with opposite functions. While M1 macrophages are known to exert anti-tumor activity by promoting pro-inflammatory effects and immune responses such as interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), M2 macrophages influence an anti-inflammatory response, wound healing, and pro-tumorigenic properties. A bioactive lipid mediator, sphingosine-1-phosphate (S1P) has emerged as a key regulatory molecule in cancer progression. We previously demonstrated that S1P generated by sphingosine kinase 1 (SPHK1), is a crucial mediator of breast cancer-induced angiogenesis and lymphangiogenesis, and promotes its metastasis. In particular, we found that SPHK1 is highly expressed in HER2 negative breast cancer, and the patients who developed lymph node metastasis demonstrated significantly higher levels of S1P (J Surg Res 2016). Although we have previously reported the role of S1P in recruitment of TAMs *in vivo* (Cancer Res 2018), its relevance in patients is yet to be uncovered. Here, we test our hypothesis that S1P signaling affects TAMs in human patients with breast cancer.

**Materials and Methods:** The expression level of each enzyme-encoding gene involved in S1P production was evaluated by retrieving RNA sequencing and gene expression quantification data using the Genomics Data Commons (GDC) data portal of the The Cancer Genome Atlas cohort. Gene expression levels were derived using normalization methods provided in the DESeq2 package. We compared the difference in expression levels of tumor associated macrophage related genes, including CD68, CD163, IL-6, and TNF- $\alpha$  between SPHK1-high breast tissue, and SPHK1-low breast tissue in the group of HER2 negative or positive patients. Unpaired t-tests were performed to compare expression differences between SPHK1-high and SPHK1-low breast tissue. All tests were two-sided and P values < 0.05 were considered statistically significant.

**Results:** CD68, pan-macrophage marker, is significantly increased in SPHK1-high breast cancer tissues both in HER2 negative and positive breast cancer patients ( $p$ =<0.001, <0.01). CD163 which is a scavenger receptor that is regarded as highly specific for M2 macrophages is significantly increased in SPHK1-high breast cancer tissues in HER2 negative breast cancer patients, but not in HER2 positive breast cancer patients ( $p$ =<0.001, 0.2). IL-6, which characterizes M1 phenotype is significantly increased in SPHK1-high breast cancer tissues both in HER2 negative and positive breast cancer patients ( $p$ =<0.001, <0.001). TNF- $\alpha$ , which also characterizes M1 phenotype, is significantly increased in SPHK1-high breast cancer tissues in HER2 negative breast cancer patients, but not in HER2 positive breast cancer patients ( $p$ =<0.001, 0.05).

**Conclusion:** Our results suggest that S1P affects TAMs in breast cancer patients, which implicate the important roles of S1P in the complicated immune system related to tumor progression. Our results also indicate that S1P have a large role in HER2 negative breast cancer patients. Further investigations are needed to understand the underlying mechanisms.



# 2018 San Antonio Breast Cancer Symposium ®

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LAP2alpha, a novel tumor marker candidate is related to metastasis of breast cancer

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**Background:** To date, there is no suitable serum marker for diagnosis and prognosis of breast cancer. In our early research, a new tumor marker candidate of breast cancer whose molecular weight was 5.6 kDa was screened from serum using mass spectrometry technology. It was decoded to be a fragment of isoform alpha of lamina-associated polypeptide 2 (LAP2 $\alpha$ ). The survival analysis revealed that LAP2 $\alpha$  was over-expressed in breast cancer patients and indicated poor prognosis.

**Materials and Methods:** Immunohistochemistry (IHC) was utilized to evaluate LAP2 $\alpha$  expression in paired primary breast tumor, metastasis of axillary lymph node and ipsilateral normal breast tissue of 29 breast cancer patients. An inhibition plasmid vector of LAP2 $\alpha$ -small hairpin RNA (LAP2 $\alpha$ -shRNA) was constructed and transfected into MCF-7 cells. The abilities of cell proliferation and metastasis were assessed both *in vitro* and *in vivo*. The association of LAP2 $\alpha$  with epithelial-to-mesenchymal transition (EMT) was determined by western blot.

**Results:** LAP2 $\alpha$  expression of paired tissue descended in order of metastasis of axillary lymph node (21/29, 72.4%), primary breast tumor (11/29, 37.9%) and ipsilateral normal breast tissue (4/29, 13.8%) ( $P < 0.05$  in comparisons between each two groups). CCK-8 experiments on transfected and control cells showed that inhibition of LAP2 $\alpha$  could not influence cell proliferation. Transwell and matrigel-transwell assays indicated that inhibition of LAP2 $\alpha$  could significantly reduce cell migration and invasion abilities. *In vivo* experiments utilizing subcutaneous xenograft model and tail vein-injection mouse model revealed that the down-regulation of LAP2 $\alpha$  might suppress tumorigenesis and metastasis of breast cancer. Western blot suggested that down regulation of LAP2 $\alpha$  increased the E-cadherin expression level but repressed N-cadherin and vimentin expression levels.

**Conclusions:** A novel tumor marker candidate, LAP2 $\alpha$  is related to metastasis of breast cancer both in clinical samples and tissue culture experiments. Inhibition of LAP2 $\alpha$  could suppress aggressiveness and metastasis of breast cancer probably via EMT suppression.

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## 2018 San Antonio Breast Cancer Symposium ®

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Re-activation of mutant p53 with APR-246 suppresses stem-cell like properties and lung metastasis of triple-negative breast cancer cells

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Triple-negative breast cancers (TNBC) lack ER, PR and Her2neu, proteins that are commonly targeted in breast cancer therapies. As a consequence, women who suffer from TNBC have poor prognosis and few treatment options and are usually administered toxic, non-specific chemotherapeutic agents. New therapeutic strategies are therefore urgently needed. TNBCs generally express mutant forms of tumor suppressor p53 protein (mtp53). Wild-type p53 (wtp53) induces apoptosis, restricts cell-cycle progression, and prevents metastasis, which is the major cause of death in patients afflicted with TNBC. Conversely, mtp53 lacks these properties and therefore cannot prevent the unfettered spread of metastatic TNBC. APR-246 is a recently developed small-molecule drug that reactivates mtp53 by covalently modifying the DNA-binding core domain thereby restoring wild-type conformation and function. Restoration of wtp53 renews its ability to promote apoptosis of tumor cells; however, the capacity of APR-246 to control metastasis is not known. With this in mind we conducted studies to determine whether APR-246 suppresses metastasis of TNBC to lungs. Using a well-developed lung metastasis model (MDA-MB-435 TNBC cells), we showed that low concentrations (1-5 mM) APR-246 suppress both ALDH activity and mammosphere formation *in vitro*. Thus APR-246 suppressed characteristics associated with stem-like cells and metastasis. For *in vivo* studies, female nude mice (n = 10-11/group) were injected with MDA-MB-435 cells. After 5 days APR-246 administration commenced with 100 mg/kg APR-246 every other day (11 treatments), followed by twice a week for a further 8 treatments. Compared with mice receiving no treatment, those given APR-246 exhibited a reduction in the number of metastatic colonies/lung from  $24 \pm 4$  to  $4 \pm 1$ ;  $P < 0.05$ . Our data suggests that APR-246 suppresses the formation of metastatic colonies in lungs by possibly inducing apoptosis and reducing cell cycle progression of cells lodged in the lungs, thereby preventing their expansion. No animals exhibited weight loss, indicating that APR-246 did not cause drug-induced toxicity during treatment. Using a different TNBC cell line, MDA-MB-231 we showed that APR-246 suppressed cellular migration. APR-246 also suppressed lung metastasis of MDA-MB-231 (4175) LM2, an aggressive subpopulation of clinically relevant cells (kindly provided by Dr. J. Massagué; Minn et al. Nature. 2005, 436:518-24) derived from MDA-MB-231 cells, that metastasizes to lungs efficiently. Overall these data suggest that APR-246 inhibits the growth of metastatic TNBC colonies in lungs. Its ability to also suppress the initial process of metastasis, including invasion and survival in the circulation, remains to be tested. These studies demonstrate that the use of APR-246 deserves further investigation as a treatment option for women suffering from metastatic TNBC.

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# 2018 San Antonio Breast Cancer Symposium ®

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KLF4 improve prognosis of triple negative breast cancer by suppression of epithelial mesenchymal transition

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## **Background**

Triple negative breast cancer (TNBC) is highly malignant and prone to metastasis and relapse, and therefore has poorer prognosis than other sub-types. The mechanism of higher malignancy of TNBC has not been sufficiently elucidated. KLF4 is reported to be a transcription factor that is associated with both tumor suppression and oncogenesis. We have reported that breast cancer patients with strong expression of KLF4 had better prognosis, especially in TNBC patients. And here we report that KLF4 negatively regulates the metastasis and growth of TNBC.

## **Methods**

We assessed the expression levels of KLF4 in 84 patients with TNBC by immunohistochemical staining and studied the patterns of metastasis/recurrence clinicopathologically. The overall survival (OS) rate and the disease free survival (DFS) rate after surgery was calculated by Kaplan-Maier method. In addition, circulating tumor cells (CTCs) in the peripheral blood of TNBC patients were identified and compared with primary lesions in terms of KLF4 expression. Moreover, the expression of KLF4 was inhibited by transfecting cultured TNBC cells (MDA-MB231) with the small interfering RNA (siRNA) of KLF4 to analyze the effects of KLF4 on cell proliferation and epithelial-mesenchymal transition (EMT)-like changes. For the proliferation assay, measurements were made by MTT assays. Cell migration and invasion assays of KLF4 suppressed TNBC cells were also examined. Total RNA was extracted from these cells, cDNA was synthesized, and used for the quantitative polymerase chain reaction (qPCR) analysis.

## **Results**

In the 84 TNBC patients, higher KLF4 expression was associated with significantly better OS and DFS. An analysis of KLF4 expression in CTCs of the TNBC patients showed that KLF4 expression was lower in CTCs than in primary cancer lesions. TNBC cells (MDA-MB231) that were transfected the KLF4 siRNA exhibited a greater ability to growth than controls. These cells also underwent EMT-like changes with reduced expression of epithelial factors such as E-cadherin. Treating these TNBC cells with eribulin resulted a reduction of the expression of stem cell/EMT markers.

## **Conclusion**

TNBC patients with reduced KLF4 expression had poor outcomes. The results of our experiments suggest the expression of KLF4 is one of the important factors that inhibit the EMT and growth of TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

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The role of DCC in inhibiting tumor progression in breast cancer

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## Background

Although benefiting from early diagnosis technology the prognosis of breast cancer has been improved significantly, metastasis and recurrence are now still the leading causes for cancer-related death in breast cancer worldwide. Genes, which have been proved to promote breast cancer metastasis proliferation, include *ERBB2* (alias *HER2*, *NEU*), *CTNNB1*, *KRAS*, *PI3KCA* (alias *PI3K*), *EGFR*, *MYC*, *TWIST1*, *SNAI1* (alias *SNAIL*), *SNAI2*, *MET*, and *ID1*. Thus, by studying and interfering the pathway transduced by these factors, we might find a method to intervene the tumor from progressing. DCC (deleted colorectal carcinoma) has been implied in many studies that it could play an important role in tumorigenesis. The aim of this study was to evaluate the expression level of DCC in both breast cancer tissues and cell lines. Then we discussed the clinical significance of DCC in predicting prognosis of breast cancer and the mechanism of DCC in modulating migration, invasiveness and metastasis.

## Materials and Methods

By real-time PCR and Western Blot test, we compared the expression level of DCC in MCF-10A, MCF-7, BT-549 and MDA-MB-231 cells, and by using immunohistochemistry staining and real-time PCR we compared the expression of DCC in breast cancer tissues with its matched non-cancerous tissues. Then we associated the expression of DCC mRNA with clinicopathological parameters, such as tumor size, number lymph node, vascular invasion status, breast cancer subtype, TNM stage, and biomarker (ER, PR, HER-2, and Ki67). After that, we up-regulated DCC expression with pcDNA3.1-eGFP-DCC in MDA-MB-231 cells and used Boyden Chamber assay to study migration and invasive changes. Finally, we detected the mRNA level of a series of Matrix metalloproteinase (MMP) family members related to cell migration and invasiveness by real-time PCR.

## Results

A total of 58 cases of primary breast cancers were collected by the Department of Breast Surgery of China-Japan Union Hospital of Jilin University in Jilin province of China from July 2013 to September 2014. None of these patients received preoperative chemo-, radiation-, or endocrine therapy. The mean age of these cases was 49.3 years. We observed that the expression level of DCC mRNA was lower in breast cancers while compared with that in matched non-cancerous tissue ( $P < 0.05$ ). And the level of DCC was even lower in breast cancers tissues and cells with more potential to metastasis ( $P < 0.05$ ). By Boyden chamber assay we observed that migration and invasion were decreased in MDA-MB-231 cells after DCC expression being up-regulated ( $P < 0.05$ ). The expression level of migration and invasion related genes MMP-1 and MMP-9 decreased obviously ( $P < 0.05$ ), while MMP-2, MMP-3, MMP-7 was not affected obviously. TIMP1 was up regulated obviously, while TIMP2 was not affected obviously ( $P < 0.05$ ).

## Conclusion

The level of DCC expression went down in high metastasis potential breast cancers in both cell lines and pathological tissues. Up-regulating expression of DCC would inhibit migration and invasion of breast cancer cells by modulating expression of MMP-1 and MMP-9 and TIMP1.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-01-24

Sulindac and triple negative breast cancer progression

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In 2018, a total of 266,120 new cases and 40,920 deaths from breast cancer in the United States were estimated by the American Cancer Society. Breast cancer is the most common malignancy and the second leading cause of death among American women. In this study, we will focus on triple negative breast cancer (TNBC), which is viewed by oncologists as a problematic and unpredictable sub-category of breast cancer because of higher rates of recurrence and poorer prognosis. TNBC accounts for up to 20% of all breast cancers and is highly prevalent in minority and young women. On average, 70% of women with metastatic TNBC die within 5 years, regardless of chemotherapy or other treatments. As such, there is an urgent medical need to develop more effective drugs to manage this deadly disease that already raised a health disparity concern, especially in the State of Louisiana. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used drugs for the treatment of pain, fever, and inflammation. Epidemiological studies have reported that the long term use of NSAIDs can prevent the occurrence multiple types of cancers, including breast cancer. However, their long term use for chemoprevention is not recommended because of toxicities associated with cyclooxygenase (COX) inhibition and the suppression of physiologically important prostaglandins. Our results show that the NSAID, sulindac sulfide (SS) and its non-COX inhibitory derivatives, can significantly inhibit the growth of the major subtypes of TNBC cells (basal-like, mesenchymal, and luminal). In addition, the compounds significantly inhibit tumor cell invasion. The animal experiments using Patient Derived Xenograft models supported the *in vivo* efficacy of these drugs. While studying the mechanism, we found that four oncogenic miRNAs, miR-10b, miR-17, miR-21, and miR-9 can be downregulated by SS and derivatives, and they were reported to promote tumor metastasis exclusively. Therefore, we conclude that those oncogenic miRNAs are involved in anti-metastatic activities of SS and its new non-COX inhibitory derivatives in TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

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The role of neogenin in inhibiting migration, invasion and metastasis of breast cancer

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## Background

Metastasis and recurrence are the main leading causes of cancer-related death in breast cancer worldwide, and migration of malignant cell is the basis of invasion and metastasis. Therefore, modulating the factors which have function in the pathway of migration, invasion and metastasis will help to control progression procedure. Neogenin is a multifunctional transmembrane glycoprotein, which could modulate cell migration while binding to its ligands and deliver signals into cells. Our previous study had proved that the expression level of Neogenin was lower in breast cancer tissues while compared with their matched adjacent non-cancerous tissues, and the expression of Neogenin was correlated to the pathological grade of breast cancer. So in this study, we evaluated the role of Neogenin in migration, invasion and metastasis in breast cancer.

## Materials and Methods

MCF-10A, MCF-7, BT-549 and MDA-MB-231 cell lines were obtained from China Cell Line Bank. A total of 78 specimens were collected from females, aged 26–70 years, who underwent modified radical mastectomy at the China-Japan Union Hospital of Jilin University between June 2015 and February 2016. None of these patients received preoperative therapy. This study was approved by The Ethics Committee of Jilin University and all patients provided informed consent. Tissue specimens were collected during the surgery, snap-frozen in liquid nitrogen, and stored at –80°C. We compared the expression of Neogenin in 78 cases of breast cancer tissues with different lymph node metastasis status and with different expression of E-cadherin by using immunohistochemistry staining and western blot. We compared the expression level of Neogenin in MCF-10A, MCF-7, BT-549 and MDA-MB-231 cells by real-time PCR and western blot. Neogenin mRNA was transfected into MDA-MB-231 cells by using pcDNA3.1-eGFP-Neogenin, and after up-regulating Neogenin expression we detected migration and invasiveness changes of MDA-MB-231 cells by scratch wound model and Boyden chamber assay.

## Results

The level of Neogenin was lower in breast cancer tissue with lymph node metastasis(  $P < 0.05$ ) or with E-cadherin expression positive(  $P < 0.05$ ). There was still a negatively correlation trend among three groups with different numbers of metastasis lymph node( $P = 0.07$ ). Comparing with MCF-7, BT-549 and MDA-MB-231 cells the expression level of Neogenin was significantly higher in MCF-10A cells (  $P < 0.05$ ). The expression of Neogenin in MCF-7, BT-549 and MDA-MB-231 cells showed negatively correlated to the invasive potentiality of cells. By scratch wound model and Boyden chamber assay we observed that migration and invasion were inhibited in MDA-MB-231 cells after Neogenin expression up-regulated.

## Conclusion

In this study we observed that the level of Neogenin expression went down in high metastasis potential breast cancers in both cell lines and pathological tissues. Neogenin plays an important role in migration, invasiveness and metastasis breast cancer. Up-regulating expression of Neogenin would inhibit migration and invasion potentiality of breast cancer cells.

## 2018 San Antonio Breast Cancer Symposium ®

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Inhibition of creatine kinase metabolism represses invasion and sensitizes estrogen-receptor negative breast cancer cells to standard chemotherapies

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Dysregulated tumor cell metabolism is a hallmark of cancer progression and therapeutic resistance. In a screen for Hypoxia-Inducible Factor (HIF)-dependent genes regulating metabolism, we identified creatine kinase, brain isoform (CKB) as down-regulated in HIF-1 knockout mammary tumor cells. CKB is a member of a family of cytosolic and mitochondrial creatine kinases (CKs) that reversibly catalyze the transfer of a high-energy phosphoryl group from ATP to creatine, generating local stores of phosphocreatine in the forward reaction, and re-generating ATP in the reverse reaction. Creatine kinases are up-regulated in a variety of solid tumors, including breast cancers. CK activity is inhibited by cyclocreatine (cCr), a creatine kinase substrate that represses the CK-dependent generation of ATP from phosphocreatine >170-fold. Knockdown of CKB in tumor cells derived from the polyoma middle T (PyMT) transgenic model of metastatic breast cancer did not impair cell proliferation or wound healing, but potently suppressed invasion in vitro, suggesting utility for treating stage IV disease. When female FVB/Nj mice were injected with wild type PyMT cells in a tail vein assay and then treated with cCr, lung metastasis was repressed to the same extent as CKB gene knockdown. These data were validated using a variety of human estrogen receptor (ER)-negative breast cancer cell lines. CKB loss- and gain-of-function models were created and effects on cell proliferation, survival, wound healing and ATP production were compared to cCr treatment. Overall, whereas deletion of CKB had no effect on cell proliferation or survival in either adherent or suspension conditions, either deletion of CKB or cCr therapy potently reduced intracellular ATP levels, which was associated with reduced invasive potential. In contrast, over-expression of CKB enhanced chemotaxis to EGF and invasion towards serum. Whereas cCr monotherapy is generally cytostatic, combination with either doxorubicin or paclitaxel is synergistic to kill tumor cells. Together, these data suggest that inhibition of CK activity may be effective in treating stage IV breast cancer patients with established metastatic disease. We are currently exploring the cell signaling and cell survival pathways that are altered by CKB gene deletion or cCr treatment to understand how the creatine kinase arm of metabolism promotes tumor progression, metastasis and response to conventional chemotherapies.

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The receptor tyrosine kinase EphA2 promotes glutamine metabolism in tumors by activating the transcriptional coactivators YAP and TAZ

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Malignant tumors reprogram cellular metabolism to support cancer cell proliferation and survival. Although most cancers depend on a high rate of aerobic glycolysis, many cancer cells also display addiction to glutamine. Glutamine transporters and glutaminase activity are critical for glutamine metabolism in tumor cells, and upstream regulatory signaling pathways may represent a novel mechanism for targeted therapies in glutamine-addicted breast tumors such as Her2-overexpressing and triple-negative breast cancers (TNBC). We have found that the receptor tyrosine kinase EphA2 activated the transcriptional coactivators YAP and TAZ (YAP/TAZ), likely in a ligand-independent manner, to promote glutamine metabolism in cells and mouse models of breast cancer. Overexpression of EphA2 induced the nuclear accumulation of YAP and TAZ and increased the expression of YAP/TAZ target genes. Inhibition of the GTPase Rho or the kinase ROCK abolished EphA2-dependent YAP/TAZ nuclear localization. Silencing YAP or TAZ reduced the amount of intracellular glutamate through decreased expression of SLC1A5 and GLS, respectively, genes that encode proteins that promote glutamine uptake and metabolism. The regulatory DNA elements of both SLC1A5 and GLS contain the consensus sequence of the TEAD family of transcription factors that are closely associated with YAP/TAZ and were bound by TEAD4 in an EphA2-dependent manner. In patient breast cancer tissues, EphA2 expression positively correlated with that of YAP and TAZ, as well as that of GLS and SLC1A5. Although high expression of EphA2 predicted enhanced metastatic potential and poor patient survival, it also rendered breast cancer cells more sensitive to glutaminase inhibition. The findings define a previously unknown mechanism of EphA2-mediated glutaminolysis through YAP/TAZ activation in breast cancer and identify potential therapeutic targets in patients.



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FASN inhibition as a potential treatment for therapy of endocrine resistant breast cancer

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**INTRODUCTION:** Fatty acid synthase (FASN) is a key enzyme in tumor cell biology controlling endogenous lipid biosynthesis. It is overexpressed in a biologically aggressive subset of tumors, including breast carcinoma. We previously reported prolonged stabilization of disease with TVB-2640 in patients with advanced metastatic breast cancer, including some endocrine resistant ER+ tumors. Using in vitro and in vivo models, we assessed the role of FASN inhibition by TVB-3166 (preclinical version of TVB-2640) for treatment of endocrine resistant breast cancer. **METHODS:** Breast tumor cells were incubated with TVB-3166 (200nM), imaged and analyzed by automated Live-Cell analysis system (Incucyte). For tumor growth inhibition, cells ( $2 \times 10^6$ ) were subcutaneously injected into SCID mice implanted with estrogen pellets. Once tumors were measurable, mice were divided into treatment groups: tamoxifen (4mg/kg), TVB-3166 (60mg/kg) and the combination. Patient tumor explants were incubated for 72h on gelatin sponges in culture medium in the absence or presence of 200nM TVB-3166. Tissue were fixed in 10% formalin and processed into paraffin blocks. Sections were stained with H&E, ER $\alpha$  and Ki67. **RESULTS:** The effectiveness of FASN inhibition on the growth of tumor cells has been confirmed in a number of breast cancer cell lines such as MCF7, ZR75, MDA-MB-231 and others. TVB-3166 leads to a marked inhibition of growth in tamoxifen resistant (TamR) cells, which 15% greater than in the parent line. IHC and Western blot showed FASN inhibition leads to significantly reduction of ER $\alpha$  levels. Immunofluorescent confocal microscopy showed inhibition of FASN by TVB-3166 alters subcellular localization of ER $\alpha$ . TVB-3166 was able to significantly inhibit tamoxifen resistant breast tumor growth in mice ( $p < 0.05$ ). Additionally, TVB-3166 treatment of primary tumor explants decreased their proliferation (Ki67) compared to untreated controls (21% vs 38%,  $p < 0.01$ ). **CONCLUSION:** Our preclinical data provide evidence that FASN inhibition by TVB-3166 presents a promising therapeutic strategy for treating of endocrine resistant breast cancer. RNA sequencing of tumor explants is being performed to evaluate FASN inhibition impact on canonical and non-canonical ER $\alpha$  signaling pathways.

# 2018 San Antonio Breast Cancer Symposium ®

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CD73 expression regulated by estrogen signaling associates with poor prognosis in estrogen receptor (ER)-positive breast cancer

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**Introduction:** CD73, a cell surface enzyme, catalyzes the generation of adenosine from ATP and ADP in the tumor microenvironment along with CD39. Accumulated extracellular adenosine functions as immune-suppressor, and also binds to adenosine receptors which promotes angiogenesis and cell proliferation that results in accelerate cancer progression. However, the clinical significance and molecular function of CD73 expression in breast cancer remains unclear.

**Methods:** Utilizing publicly available breast cancer cohorts of The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO), clinical significance as well as underlying mechanisms were investigated. Molecular experiments were carried out in MCF7 cells, ER-positive breast cancer cell line, to investigate the role of estrogen signaling on CD73/CD39 expression.

**Results:** In treatment naïve TCGA cohort, CD73 expression level was significantly lower in ER-positive breast cancers compared to ER-negative tumors. Higher CD73 expression was associated with worse overall survival in whole cohort ( $p=0.021$ ) and ER-positive tumors ( $p=0.003$ ), but not in ER-negative tumors. Gene Set Enrichment Analysis revealed that estrogen response gene sets (Early;  $NES=-1.57$ ,  $p=0.043$ , Late;  $NES=-1.61$ ,  $p=0.021$ ) were significantly enriched in CD73 low expressing ER-positive tumors, suggesting estrogen signaling may repress CD73 expression. To test this hypothesis, we analyzed the expression of CD73 and CD39 in MCF7 cells treated with estrogen, tamoxifen or both. Our data revealed that estrogen treatment suppressed CD73 and CD39 expression, whereas tamoxifen treatment enhanced expression of the genes. These findings suggest that CD73 and CD39 gene expression is suppressed by estrogen signaling, whereas binding of ER antagonists such as tamoxifen can remove the repressive effect on gene expression. On the other hand, epithelial-mesenchymal transition (EMT) (Normalized Enrichment Score;  $NES=2.41$ ,  $p<0.001$ ) and angiogenesis ( $NES=2.33$ ,  $p<0.001$ ) gene sets were significantly enriched in CD73 high expressing ER-positive tumors. CIBERSORT, which is an algorithm to estimate infiltrating immune cells by gene expression, demonstrated that CD73 high expressing ER-positive tumors have less infiltrating CD8-positive T cells, memory B cells and plasma cells, implying that CD73 high expressing tumors have immune suppressive environment, which is in agreement with the notion that CD73 high tumors are immunosuppressive. Finally, we found that CD73 expression was significantly elevated post-chemotherapy compared to tumors prior to the treatment ( $p=0.007$ ), and CD73 high expression patients showed worse relapse-free survival in neoadjuvant chemotherapy patients cohort ( $p=0.003$ ).

**Conclusion:** Molecular studies revealed that CD73 expression is regulated by estrogen signaling. Increased expression of CD73 significantly correlates with worse outcomes in ER-positive breast cancer patients. This may be due to upregulated pro-metastatic gene signatures such as EMT and angiogenesis as well as less infiltration of anti-cancer immune cells by adenosine generated by CD73 in the tumor microenvironment. Our data reveals an intriguing mechanism which may be responsible for recurrence and metastasis of ER-positive breast cancer.

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Targeting PFKFB3 enzyme induces cell death via reactive oxygen species-mediated toxicity in endocrine therapy-resistant breast cancers

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Reprogrammed glucose metabolism is one of the key hallmarks of cancer. High-energy demand in cancer cells leads to increased glycolysis to maintain anabolic processes that are often driven by altered enzyme levels.

6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) is an enzyme that converts fructose-6-phosphate (F6P) to fructose-2,6-bisphosphate (F2,6BP). F2,6BP is an allosteric activator of phosphofructokinase-1 (PFK1), a critical rate-limiting control within glycolysis. PFKFB3 expression and activity are upregulated in many cancers including breast cancer, facilitating the increased glycolytic activity associated with neoplasia. In ER+ breast cancer cells, PFKFB3 is further upregulated by estrogen, but whether this affects responsiveness to endocrine therapies is largely unknown.

Expression of PFKFB3 mRNA and protein was measured in estrogen-responsive MCF7 cells and in endocrine therapy-resistant LCC9 and MCF7:5C cells (estrogen-independent; 4-hydroxytamoxifen (4OHT) and fulvestrant (Fulv) crossresistant). Both MCF7:5C and LCC9 cells expressed higher levels of PFKFB3 compared to MCF7 cells. Cells with higher PFKFB3 levels also showed increased basal glucose uptake and lactate secretion, consistent with the enhanced aerobic glycolysis often referred to as the "Warburg Effect."

To evaluate the potential translational relevance of these observations, the association of PFKFB3 mRNA expression with clinical outcomes in ER+, node-negative breast cancer patients treated with an endocrine therapy was studied in two publicly available data bases. High expression of PFKFB3 was strongly associated with adverse recurrence-free survival (hazard ratio = 4.12 and  $p = 5.5 \times 10^{-5}$ ).

Next, the effect of pharmacological inhibition of PFKFB3 was investigated on the growth of endocrine-resistant breast cancer cells using PFK158, an inhibitor of PFKFB3 that is currently being evaluated in clinical trials. PFK158 treatment suppressed the basal glucose uptake in LCC9 and MCF7:5C cells. PFK158 treatment alone was effective in inducing apoptotic cell death in both cell models. However, combining a lower, sub-optimal concentration of PFK158 with either 4OHT or Fulv significantly enhanced cell death in endocrine-resistant LCC9 and MCF7:5C cells. Notably, the cytotoxic effects of PFK158 alone or in combination with 4OHT or Fulv were markedly diminished by blocking reactive oxygen species (ROS) using either of the ROS scavengers N-acetyl cysteine (NAC) or Tempol. Overall, this study reveals that (1) PFKFB3 expression drives high glycolytic activity in endocrine-therapy resistant breast cancer cells; (2) PFKFB3 mRNA expression in ER+ LN- breast cancer is a prognostic factor; and (3) targeting PFKFB3 in combination with endocrine-based therapy induces enhanced cell death mediated by high ROS levels. Based on this intriguing data, combination of the PFKFB3 inhibitor PFK158 with an antiestrogen should therefore be regarded as a potential therapeutic intervention for patients with endocrine therapy-resistant breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Deregulated lipid metabolism fuels the genesis of estrogen receptor negative breast cancer

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## **Background:**

There are no targeted pharmacologic interventions currently available for the prevention of hormone receptor negative breast cancer. Primary prevention with endocrine agents decreases the risk of ER positive disease with no effect against ER negative (ER-) disease. Thus, there is a compelling need to identify women at high risk for ER-negative breast cancer and to uncover the molecular mechanisms involved in its genesis. Our recent observation that a set of lipid metabolism (LiMe) genes are over-expressed in the contralateral unaffected breasts of women with unilateral ER- breast cancer suggests the novel hypothesis that specific lipid metabolism pathways in the breast produce a physiological milieu favoring the development of ER- breast cancer. We are now testing the specific hypothesis that lipids are the source of the acetyl-coA that is utilized to acetylate histones, an epigenetic modification that reprograms transcription.

## **Methods:**

We developed an *in-vitro* model that relies on octanoic acid, a medium chain fatty acid that freely diffuses through the plasma and mitochondrial membranes. MCF-10A cells were plated and allowed to adhere overnight and then exposed to an increasing dose of sodium octanoate for 24 hours in complete media. Acetylation of Lysine 9 of Histone 3 (H3K9) was analyzed by Western blot and RNA was extracted for qPCR and RNA-seq. Chromatin packing density at the nanoscale was quantified by partial wave spectroscopic (PWS) microscopy. Mammary organoids were prepared from breast tissue by collagenase digestion and similarly treated.

## **Results:**

We found a striking, dose-dependent increase of H3K9 acetylation in octanoate treated MCF-10A cells. The acetylation is specific to the lipids as no acetylation was observed in cells treated with the same concentration of the alcohol 1,4-Cyclohexanedimethanol. RNA-Seq revealed the differential expression of LiMe genes together with a significant upregulation of Hedgehog and Notch signaling pathways. Individual genes from various pathways were further verified by qPCR which revealed, for example, a four-fold increase in SHH expression and 25-fold increase in DLL4. The expression of two of the previously identified LiMe genes, HMGCS2 and ACSL3, was increased four-fold in the octanoate treated MCF10A cells. We repeated the octanoate treatment in organoids and found similar effects. PWS in live cells showed a dose-dependent increase in chromatin packing scaling (D) in cells exposed to octanoate, suggesting that accessibility of chromatin to transcription factors is increased upon fatty acid treatment.

## **Conclusion:**

A lipid rich microenvironment affects metabolism in ER- MCF10A cells and stimulates pro-neoplastic signaling via histone modifications. This supports our hypothesis that perturbed lipid metabolism plays an important role in the development of ER- breast cancer. Further mechanistic studies will determine if the genes differentially expressed in cell culture are also differentially expressed in antecedent benign breast biopsies from women eventually diagnosed with ER+ and ER- cancer.

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Targeting iron metabolism in breast cancer: Ferroptosis and genome stability

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Iron-sulfur clusters are cell-essential protein cofactors in at least 48 human enzymes supporting diverse cellular functions, including genomic integrity and oxidative phosphorylation, and acting as molecular sensors for oxygen and iron levels. We recently described that breast cancer cells are particularly dependent upon robust iron-sulfur cluster biosynthesis for the survival of elevated oxygen conditions, including seeding lung metastases (1). Moreover, we found that even partially blocking cluster synthesis activated an iron-starvation response, rendering breast cancer cells susceptible to oxidative stress and death by ferroptosis (1). However, the precise mechanisms underlying these phenotypes remain to be fully elucidated. Here, we find that basal-like breast cancer cells are particularly susceptible to suppression of iron-sulfur cluster biosynthesis. By systematically evaluating proteins containing iron-sulfur clusters, we discovered that this susceptibility lies in the intrinsic vulnerability of basal-like breast cancer cells to genomic instability. These results have enabled us to define specific molecular targets in basal-like breast cancer which induce DNA damage, replication fork instability, and apoptosis, with striking subtype specificity. Our findings have broad implications for the underlying differences in genomic integrity in basal-like and luminal breast cancer subsets, and will inform future novel strategies to target genomic integrity in basal-like breast cancer.

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Slit2 induced anti-tumor activity may be mediated through metabolism driven immunomodulation

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Metabolism of immune cells plays an important role in regulating tumor growth by modulating the anti-tumor M1 phenotype or pro-tumor M2 phenotype in macrophages. However, the role of bone marrow derived macrophages (BMDM) and their metabolic profile in promoting tumor growth is unknown. Slit2 is an anti-tumor molecule that is suppressed in breast cancer; however, the mechanism by which Slit2 mediates its function is not fully elucidated. We hypothesize that Slit2 mediated metabolic reprogramming of BMDMs favors anti-tumor M1 phenotype in these macrophages, which in turn reduces tumor growth. Here we assessed cellular metabolism in BMDMs from the MMTV-PyMT mouse, a model showing spontaneous tumor development. Shortly, age matched female mice with palpable tumors and control mice without tumors were treated with recombinant Slit2 (Slit2) or PBS intraperitoneally every third for 2 weeks (n=4 mice per group). Tumor volume was measured in each mouse before and at the end of treatment. Next, mice were euthanized and bone marrow was flushed from both the tibia and femur for culturing *in vitro* in the presence of macrophage chemotactic factor rich conditioned media. Rate of glycolysis was described based on extracellular acidification rate (ECAR) as measured by the Seahorse Bioanalyzer® under glycostress conditions. Lactate dehydrogenase (LDH) activity, which is linked with breast cancer progression and pro-tumor macrophage phenotype in experimental models, was also assayed using a commercially available kit. Finally, to elucidate potential pathways involved in Slit2 induced metabolic change, we assessed the expression of several proteins and factors involved in cellular metabolism.

Firstly, we observed that PyMT mice treated with Slit2 showed lower tumor volume compared to mice treated with PBS confirming Slit2 anti-tumor activity. Hematoxylin & Eosin staining of tumor sections from these mice also showed better tissue structure, with a higher cytoplasm to nuclear ratio in Slit2 treated PyMT mice compared to PBS treated mice. Furthermore, BMDMs from PyMT mice show lower aerobic glycolysis with higher lactate dehydrogenase activity (LDH) compared to control mice. Moreover, treatment with Slit2 appeared to lower LDH activity and trended to increase glycolysis in the BMDMs isolated from Slit2 treated PyMT compared to PBS treated PyMT.

Expression analyses using quantitative PCR showed a 2-4 fold decrease in PGC-1 $\alpha$  and CPT-2 in Slit2 treated BMDMs, indicating a reduction in fatty acid oxidation in these cells. This coupled with a 3 fold increase in IL-6 expression, and 2-3 fold decrease in arginase and IL-10 expression in tumor tissue suggest a potential shift from pro-tumor M2 to anti-tumor M1 phenotype. In spite of these preliminary trends, changes in metabolism and associated signals may be clearer in isolated, enriched populations of macrophages alone. Nevertheless, our findings suggest that Slit2 reduces tumor growth by affecting immune cell metabolism. Furthermore, these studies provide novel evidence of the potential immunomodulatory effects of Slit2 on macrophages. This may lead to development of Slit2 as a novel non-invasive therapeutic strategy against highly aggressive and metastatic cancers associated with high mortality and low quality of life.

# 2018 San Antonio Breast Cancer Symposium ®

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Association between obesity and firmicutes/bacteroidetes ratio in breast cancer patients

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Obesity is associated with breast cancer, especially postmenopausal obesity is a risk factor for breast cancer. Various studies have shown that obesity is correlated with microbiome. In particular, *Firmicutes* and *Bacteroidetes* occupy a significant portion of the intestinal microbiome at phylum-level. As the relation between obesity and intestinal microbiome, breast cancer may be associated with the microbiome and obesity as well. This study analyzed the microbiome of breast cancer patients and normal controls. We also investigated the relationship among breast cancer, body mass index, and eating habits. The intestinal symbiotic bacteria secretes bacterial extracellular vesicles to the blood and lymphatic fluid, and communicate with distant organs through these vesicles which are including metabolites and bacterial materials. Therefore, we analyzed the microbiome of breast cancer patients and normal controls through blood samples. The 287 blood samples in female (95 breast cancer patients and 192 normal controls) from September 2014 to August 2015 were collected and analyzed by NGS using a universal bacterial primer of 16S rDNA. A t-test was performed to find out the discrepancy between cancer and control groups. We examined the *Firmicutes/Bacteroidetes* (F/B) ratio of normal controls and breast cancer patients, and the result showed that the F/B ratio in normal group was 2.6 times higher than breast cancer group. In the normal group, *Firmicutes* were five-fold higher than *Bacteroidetes* at the phylum level. Among them, *Staphylococcus* and *Bacillus* at the genus level were especially higher. *Bacteroidetes* in breast cancer patients were elevated at the phylum level, particularly *Bacteroides* and *Parabacteroides* were elevated at the genus level. In breast cancer patients, the F/B ratio was 1.3 fold higher in the group with high BMI (BMI>30) compared to normal BMI (BMI 20-24). The F/B ratio of breast cancer patients who are meat lovers was increased (F/B ratio: 2.3), followed by vegetative diet (F/B ratio: 2.0) and omnivorous patients (F/B ratio: 1.8). Menopause did not affect the F/B ratio. However, *Verrucomicrobia* phylum was lower in breast cancer patients with premenopausal status. The most distinct genus in *Verrucomicrobia* phylum was *Akkermansia*, which was the highest in the normal group. Breast cancer patients have lower *Firmicutes* and higher *Bacteroidetes* than normal controls. Notably, the proportion of *Bacteroides* and *Parabacteroides* belonging to *Bacteroidetes* was high and may be related to the incidence of breast cancer. Conversely, *Staphylococcus* and *Bacillus* belonging to *Firmicutes* phylum may have protective effects in breast cancer. The higher BMI was related to the higher F/B ratio. This result is consistent with the higher F/B ratio in the meat lover groups with breast cancer. Menstruation did not affect the F/B ratio but influences the *Akkermansia* genus. That is, changes in intestinal bacteria which are affected by the postmenopausal status and hormonal level may have affected the prognosis of breast cancer. In the future, the research on microbiome of breast cancer patients is expected to provide microbiome-based supplements that help to treat and prevent breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

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Progesterone receptor membrane component-1 interacts with proteins of the cholesterol synthesis pathway resulting in altered cholesterol metabolism in breast cancer cells: Potential mechanism contributing to breast cancer progression

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**Background:** Progesterone Receptor Membrane Component-1 (PGRMC1) is upregulated in breast tumors and elevated expression of PGRMC1 is associated with increased tumor growth, indicating that PGRMC1 plays a role in carcinogenesis of breast cancer. However, the mechanism by which PGRMC1 contributes to breast cancer progression is not understood yet. Here we describe a potential mechanism by which PGRMC1 contributes to progression of breast cancer via deregulation of cholesterol synthesis and metabolism.

**Methods:** PGRMC1 interaction partners were identified by co-immunoprecipitation followed by mass spectrometry and validated using proximity ligation assay (PLA) in various breast cancer cell lines. To study how elevated PGRMC1 expression contributes to cholesterol balance, we generated PGRMC1 overexpressing breast cancer cells and determined levels of cholesterol-, lathosterol- and the cholesterol metabolite 27-hydroxycholesterol. Since 27-hydroxycholesterol is presumed to have estrogenic action, stimulating the growth of ER-positive breast cancer cells and limiting the effectiveness of aromatase inhibitors, we further investigated the activity of ER $\alpha$  in PGRMC1 overexpressing cell lines.

**Results:** Interaction of PGRMC1 with proteins involved in cholesterol synthesis, including Acyl-CoA desaturase (SCD), Squalene synthase (FDFT1) and Lanosterol 14- $\alpha$  demethylase (CYP51A1), was detected by mass spectrometry and verified by PLA. Elevated levels of cholesterol and its metabolites were detected in PGRMC1 overexpressing cells, suggesting a contribution of PGRMC1 to cholesterol synthesis and metabolism. Further, increased activity of ER $\alpha$  was found in PGRMC1 overexpressing cells, indicating activation of the receptor by cholesterol metabolites.

**Conclusion:** Cholesterol metabolism is involved in cancer cell proliferation as well as resistance to anti-cancer therapy. Our results indicate that elevated cholesterol metabolism induced by interaction of PGRMC1 with enzymes of the cholesterol biosynthesis pathway might contribute to malignant transformation and promote breast cancer progression. PGRMC1 might therefore present an interesting target for anti-cancer therapy.



## 2018 San Antonio Breast Cancer Symposium ®

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Combinational treatment of biguanides and fatty acid  $\beta$ -oxidation inhibitor in triple-negative breast cancers

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Among breast cancers (BCs), the driver pathways and therapeutic targets are still poorly understood for triple negative (TN) BCs. Advances in cancer metabolism research over the last decade have enhanced our understanding on metabolic reprogramming in cancer therapy. We have previously shown that metabolic reprogramming to fatty acid  $\beta$ -oxidation (FAO) is a major energy pathway in metastatic TNBC. Moreover, we reported that FAO regulates c-Src, one of the frequently upregulated oncopathways in TNBC via autophosphorylation of Src at Y419. Since FAO inhibitors alone cannot effectively control the tumor progression in TNBC, suitable combination therapies with other metabolic targets are necessary. Recently increasing evidences show that anti-diabetic biguanides have attractive anticancer effect in various cancer types including BC. However, its significance as an anticancer drug is not well established due to parallel metabolic pathways that support tumor growth.

Phenformin, a biguanide derivative similar to metformin, has a greater potency than metformin. Like metformin, phenformin also inhibits mitochondrial electron transport chain (ETC) through complex I inhibition. In addition, biguanides lead to the activation of AMPK, which plays a key role in insulin signaling and energy sensing. Importantly, AMPK is an upstream regulator of FAO pathway because it can phosphorylate ACC to activate FAO. Considering the dependency of TNBC to FAO, we evaluated the therapeutic significance of the combination of biguanides(ETC inhibitors) and FAO inhibitors in TNBC progression and metastasis. We hypothesize that blocking both 'arms' of the pathway can provide more pronounced and durable responses in TNBCs. Our different *in vitro* and *in vivo* studies using TNBC cell line and PDX models suggest that the combination of both inhibitors can provide better therapeutic significance in metastatic TNBCs. This is a rationale and cost-effective metabolic approach to manage the currently non-targetable metastatic TNBCs. Further investigation into the clinical effectiveness of this combination may provide better treatment opportunities for TNBC patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-02-12

Gain-of-function of mutant P53 elevates glucose uptake via membrane translocation of GLUT1 in breast cancer

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## Background

High rate of glycolysis exhibited by cancer cells plays a crucial role in carcinogenesis and tumor progression. Previous study showed that gain-of-function (GOF) by mutant P53 drives the Warburg effect via GLUT1 membrane translocation in malignancies. Also, in the patients with a preoperative 18F-fluorodeoxy glucose positron emission tomography (FDG-PET), which enables to estimate glucose uptake by tumor as standardize uptake value (SUV), we identified high SUV as a poor prognostic marker. In this study, we wondered whether P53 mutation promotes GLUT1 membrane translocation and increases glucose uptake in MCF10A cells and patients with breast cancer.

## Methods

Transcriptomic profiling was carried out using gene expression data from 66 breast cancer patients who underwent preoperative FDG-PET to identify a molecular signature associated with SUV. Gene network analyses revealed dysregulation of P53 pathway. We generated both MCF10A cell lines with P53-knock down and P53-mutant which include sequence variants (R175H, R273H, and R248W). Glucose uptake assay and confocal imaging studies with 2NDBG were performed. Confocal imaging system was used to detect GLUT1 membrane expression. In case-matched patients according to P53 mutation (n=114), we compared SUV. Mutational analysis of exons 5-9 of the P53 gene was carried out using Sanger sequencing.

## Results

Glucose uptake assay showed that the level of glucose uptake was increased in P53 mutant MCF10A cell lines with a 1.5-1.8 fold change compare with wild-type MCF10A. The increase of glucose uptake by mutant-P53 was reproducible with the experiments with 2NDBGD. By contrast, stable knock down of P53 did not induce an elevation of glucose uptake in MCF10A cells. The confocal imaging studies captured translocation to membrane of GLUT1 in mutant P53-MCF10A. However, in wild-type MCF10A, GLUT1 was diffusely expressed in cytoplasm. . In tumors from patients, membraneous expression of GLUT1 was significantly higher in tumors with p53 mutation than in tumors with intact p53 (P=0.022). Further, the mean SUV of p53-mutational group was significantly higher than that of wild-type p53-group (7.49 vs. 5.44, P=0.013). In multivariate analysis for recurrence-free survival, p53 mutational status carried prognostic significance (hazard ratio 3.73, 95% CI 1.15-12.07) independent of tumor size, nodal status, and estrogen receptor status.

## Conclusion

We showed that GOF by P53 mutation promotes GLUT1 translocation to membrane, which consequently induces glucose influx in MCF10A cells, elucidating that P53 mutation contributes to the increase of SUV in patients with breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-02-13

Computational metabolism modeling predicts risk of relapse in breast cancer patients

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## Introduction

Breast cancer is one of the most prevalent cancers in the world. In previous works we observed differences in glucose metabolism between breast cancer subtypes, suggesting that metabolism plays an important role in this disease. Flux Balance Analysis (FBA) is widely used to study metabolic networks, allowing predicting growth rates or the rate of production of a given metabolite.

## Material and methods

Proteomics data from 96 breast cancer tumors were obtained applying a high-throughput proteomics approach to routinely archive formalin-fixed, paraffin-embedded tumor tissue. Proteomics tumor data were analyzed using the human metabolic reconstruction Recon2 and FBA. The tumor growth rate for each tumor was calculated. In order to analyze fluxes from the different metabolic pathways, flux activities were calculated as the sum of the fluxes of each reaction in each pathway defined in the Recon2. Then, flux activities were used to build prognostic models.

## Results and discussion

Using the results obtained from FBA in the proteomics dataset, flux activities were calculated for each pathway. Employing these flux activities, a prognostic signature was built. Flux activities of vitamin A, tetrahydrobiopterin metabolism and beta-alanine metabolism pathways split our population into a low and a high risk group ( $p=0.044$ ).

## Conclusion

Vitamine A, beta-alanine and tetrahydrobiopterin metabolism flux activities could be used to predict relapse risk. Flux activities is a method proposed in a previous work to study response against drugs that now also demonstrated its utility in summarizing FBA data and is associated with prognosis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-02-14

Metabolic regulation and drug resistance in c-Src activated triple negative breast cancer

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c-Src (Src) is a proto-oncogene involved in signaling that culminates in the control of multiple biological functions. Src is also one of the most frequently upregulated pathways in triple negative breast cancer (TNBC). Dysregulation of Src has been detected in TNBC and is strongly associated with tumor metastasis and poor prognosis. However, even after promising preclinical studies, Src inhibitors did not show major clinical advantage in unselected TNBC populations. We have previously published that metastatic TNBC has high energy-dependency to mitochondrial fatty acid beta-oxidation (FAO) and FAO activates Src by inducing autophosphorylation at Y419. However, our recent analysis suggests that as observed with the Src inhibitors, TNBC tumors treated with FAO inhibitors also develop drug-resistance and continue tumor growth. Evaluation of their drug resistance mechanism revealed that while short-term inhibition of FAO or Src induces autophagic and apoptotic cell deaths, long-term inhibition results in autophagy-mediated drug resistance and survival. Further analyses suggest that FAO and Src inhibitors activate mitogen-activated protein (MAP) kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway via the induction of cellular reactive oxygen species (ROS) in TNBC. Activated MEK/ERK then induces survival pathways for drug resistance and tumor survival. Validation of *in vitro* findings using *in vivo* TNBC models confirmed that combination of FAO/Src inhibitors with MEK/ERK inhibitors can provide significant benefit to overcome the therapeutic resistance of TNBC. These findings open-up new therapeutic opportunities to manage TNBC patients with currently non-targetable metastatic tumors.

# 2018 San Antonio Breast Cancer Symposium ®

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All-trans retinoic acid perturbs the lipidomic profiles of luminal breast cancer cells characterized by sensitivity to the anti-proliferative activity of the retinoid

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**Background:** All-trans retinoic acid (ATRA) is the active metabolite of vitamin A and a promising agent in the prevention and treatment of breast cancer. We recently demonstrated that approximately 70% of estrogen-receptor-positive (ER<sup>+</sup>) breast cancer cell lines and primary tumors are sensitive to the anti-proliferative effects of ATRA (1,2). In contrast only 10-20% of the HER2-positive and triple-negative counterparts respond to the retinoid. The significance of lipids in the growth, progression and drug sensitivity of specific types of solid tumors, including breast cancer, is largely overlooked. In particular the role, if any, of specific lipids in the anti-tumor action of ATRA in breast cancer has never been studied.

**Aims and Approach:** The principal aim of the present work was to evaluate the specific perturbations induced by ATRA on the homeostasis of lipids in breast cancer cells characterized by sensitivity to the anti-proliferative action of the retinoid. To this purpose, we took a high-throughput approach and defined the lipidomic profiles of 16 breast cancer cell lines in basal conditions and following challenge with ATRA (1  $\mu$ M) for 48 hours. The panel consisted of eight cell lines characterized by a luminal phenotype and eight cell lines with a basal phenotype. Four ATRA-sensitive cell lines (*SKBR3*, *HCC1500*, *CAMA1* and *MDAMB361*) and 4 ATRA-resistant counterparts (*HCC202*, *MDAMB175VII*, *ZR75.1* and *HCC1419*) were included in the luminal group. Similarly, the basal group contained 4 ATRA-sensitive (*HCC1599*, *MB157*, *MDAMB157* and *Hs578T*) and 4 ATRA-resistant (*MDAMB231*, *CAL851*, *HCC1187* and *MDAMB436*) cell types.

**Results:** Using Lipostar, a unique and recently developed software for high-throughput LC-MS lipidomics analysis (3), we identified lipid species whose levels were modified by ATRA in each cell line. This resulted in the generation of a lipid fingerprint consisting of 530 elements. We observed that ATRA reduced the amounts of cardiolipins in luminal and ATRA-sensitive breast cancer cell lines specifically. Similar effects were not observed in luminal and ATRA-resistant cells. ATRA-dependent reduction in the amounts of cardiolipins were never observed in basal cells, regardless of their sensitivity to the retinoid. Given the role played by cardiolipins in the homeostasis of the mitochondria, we evaluated the action of ATRA on the functional activity of these organelles in the luminal and ATRA-sensitive or ATRA-resistant cell lines. In *SKBR3* and the other sensitive cell lines, we observed that ATRA modulated mitochondria-dependent oxygen consumption and ATP production. These effects were accompanied by an increase in mitochondrial membrane fluidity, which is consistent with the effects exerted by ATRA on the cellular content of cardiolipins. ATRA-dependent action on cardiolipins and mitochondrial homeostasis precedes maximal growth inhibition. The results provide new insights on the mechanisms underlying the anti-tumor action of ATRA.

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## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-02-16

Cancer/testis antigen-Plac1 promotes invasion and metastasis of breast cancer through Furin/NICD/PTEN signaling pathway

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Plac1 is a cancer-testis antigen that plays a critical role in promoting cancer initiation and progression. However, the clinical significance and mechanism of Plac1 in cancer progression remains elusive. Here we report that Plac1 is an important oncogenic and prognostic factor which physically interacts with Furin to drive breast cancer invasion and metastasis. We have shown that Plac1 expression positively correlates with clinical stage, lymph node metastasis, HR status and overall patient survival. Overexpression of Plac1 promoted invasion and metastasis of breast cancer cells *in vitro* and *in vivo*. Co-immunoprecipitation and immunofluorescence cell staining assays revealed that interaction of Plac1 and Furin degraded Notch1 and generated Notch1 intracellular domain (NICD) that could inhibit PTEN activity. These findings are consistent with the results of microarray study in MDA-MB-231 cells overexpressing Plac1. A rescue study showed that inhibition of Furin and overexpression of PTEN in Plac1 overexpression cells blocked Plac1-induced tumor cell progression. Taken together, our findings suggest that functional interaction between Plac1 and Furin enhances breast cancer invasion and metastasis and the Furin/NICD/PTEN axis may act as an important therapeutic target for breast cancer treatment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-02-17

Metabolism-driven cancer identification with GLUT5-specific molecular probes

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**Background:** Current cancer imaging agents are limited in their ability to distinguish cancers from normal cells (low cancer-selectivity) and identify cancers at different stages of development (low cancer-specificity). This limitation makes biopsy mandatory for diagnosis and continuous treatment monitoring. Analysis of biopsy samples may also have some ambiguity in clearly identifying malignant and metastatic cells, resulting in cases of mischaracterization and overdiagnosis. Hence, cancer-selective and cancer-specific imaging agents are needed. Distinguishing cancer types and stages can be achieved by addressing differences in their nutrient uptake, manifested as changes in the expression of facilitative sugar transporters (GLUTs). Here, we present a novel approach to target the enhanced metabolism in breast cancers with sugar-like fluorescently labeled probes – ManCous - engineered for specific uptake by fructose transporter GLUT5. The differential accumulation of these probes in cancer cells parallels the differential activity of GLUT5 and results in active fluorescence accumulation within cancers with the highest levels observed in premalignant phenotypes.

**Results:** Locking fructose conformation in the furanose form was found to provide a sugar-like mimics recognized explicitly by GLUT5. The corresponding coumarin conjugates – ManCous (Figure 1A) - were found to exhibit GLUT5-specific uptake and work as reporters of GLUT5 activity in cells. Differential activity of GLUT5 in cells was found to parallel the differences in the uptake of ManCous. Significant differences in accumulation of ManCou-induced fluorescence were observed between normal and cancer cells and between cancer phenotypes (Figure 1A). Namely, an 8-fold difference in ManCou accumulation was observed between normal and adenocarcinoma MCF7 cells, and 70-fold difference was observed between normal and premalignant MCF10aNeoT cells. The uptaken ManCou were found to be metabolized by hexokinase to form phosphorylated analogs and effectively compete with glucose for hexokinase II (Figure 1B). The probes were found to be non-cytotoxic at concentrations below 100  $\mu$ M, with higher cytotoxicity towards cancer cells at probe concentrations above 100  $\mu$ M

**Conclusions:** The dependence of breast cancer cells on fructose provides a firm basis for developing imaging approaches to discriminate between normal and cancer cells as well as potentially between cancer phenotypes. While current probes are restricted to in-vitro imaging, further probe evolution is expected to lead to new in vivo agents, owing to a proper modification of the scaffold with the relevant radioactive (PET) or other imaging entity and retention of transporter specificity. The development of transporter-specific GLUT5 affinity probes could further contribute to enhancing the impact of fructose uptake inhibition for approaching cancer-specific therapies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-03-01

Akt inhibition associated with change in immunophenotype of tumor microenvironment (TME) in breast cancer (BC)

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**Background:** The PI3K/Akt/mTOR pathway is a known oncogenic pathway in BC. In addition, this pathway has demonstrated capacity to modulate host immune activity and may indirectly affect tumorigenesis. Clinicopathologic studies have demonstrated that lymphocyte density within the TME is predictive of chemosensitivity and improved prognosis in BC, while myeloid infiltration may play a deleterious role. To define the impact of Akt inhibition on the TME, we analyzed tumor tissue from patients (pts) with early-stage BC treated with single agent MK-2206, an Akt inhibitor, enrolled on a presurgical trial (NCT01319539).

**Methods:** Quantitative immunofluorescence (qmIF) was performed for CD3, CD8, CD4, FOXP3, CD68, Pancytokeratin on 4uM sections from biopsy and surgical specimens of MK-2206 (n=5) and control (n=5) pts. Images were analyzed using Vectra/inForm software (PerkinElmer), allowing for multiparameter phenotyping. Transcriptomic analysis was performed on surgical specimens to assess if differences exist in mRNA expression of tumor-associated and immune genes between pts treated with MK-2206 (n=5) and untreated matched controls (n=5) (nanoString). Statistical analysis was performed using t-Test, NetBID, and multiple comparison analysis by Benjamini-Hochberg. Gene set enrichment analysis (GSEA) was performed within R with gene sets from Molecular Signatures Database (Hallmark, Reactome, GO).

**Results:** On qmIF analysis, MK-2206 treated pts exhibited a significant increase in median cytotoxic T-cell (CD3+CD8+, CTL) density between pretreatment biopsy and surgical excision specimens, as compared to the control pts (87% vs.0.2%,  $p < 0.05$ ). Mean macrophage density (CD68+) was numerically lower in surgical specimens of pts who received MK-2206 vs. control pts, although CD68+ infiltration was overall low ( $p=ns$ ). mRNA expression supports *in vivo* activity of MK-2206 with lower expression levels of cell cycle, proliferation and anti-apoptotic genes (e.g. CTNNB1, CCND2, BAX) and greater expression of pro-apoptotic genes (e.g. BAD) associated with MK-2206 treatment (raw p-value  $<0.05$ ). Additionally, greater mRNA copy number of IGF1R, a receptor tyrosine kinase (RTK) previously identified as upregulated in BC in the context of Akt inhibition, was found in post-MK-2206 surgical specimens as compared to control, non-MK-2206 specimens (raw p-value  $<0.05$ ). MK-2206 was also associated with reduced expression of myeloid markers (e.g. CSF1R, CD163) (raw p-value  $<0.05$ ). By GSEA, canonical gene sets related to interferon signaling were increased in post-MK-2206 specimens as compared to non-MK-2206 specimens, whereas monocyte chemotaxis genes were decreased in treated pts (adj p-value  $<0.05$ ). RT-PCR is currently underway to compare biopsy and surgical specimens for a subset of RTK, immune and apoptosis related genes identified above.

**Conclusion:** mRNA and qmIF analysis suggest that Akt inhibition, may increase interferon signaling, CTL density, and decrease myeloid infiltration. Thus, Akt inhibition may promote a favorable TME. At present, there are both FDA approved and investigational agents that target the PI3K/mTOR pathway. Further investigation is warranted to understand the impact of Akt inhibition on the TME and potential therapeutic implications.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-03-02

Markers of PI3K/Akt, ER & AR pathways and Ki67 expression in relation to tamoxifen outcome in ER+ breast cancer

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**Aim:** To select tumor biomarkers, included in the AKT pathway, able to predict response to tamoxifen (TAM) in patients with estrogen receptor (ER) positive breast cancer.

**Patients & Methods:** 157 endocrine treatment naïve patients with known ER and HER2 status were treated with TAM: tumour tissue was available either as tissue microarrays (TMA) (n=107) or core biopsies (n=50). After removal of duplicates, ER(-) BCs, & patients without full outcome data 103 patients were available. Ten biomarkers were evaluated by immunohistochemical staining (% positivity and/or H-Score): ER, HER2, Ki67, & phosphorylated forms of MAPK, AKT, ER (Ser118), PRAS40, and IGF1R, as well as pTEN & also androgen receptor (AR) expression. TMA cores were collected from three tumor sites, two central and one peripheral. Tumors were also analysed for AKT1 E17K somatic mutation using BEAMing technology.

Patient outcome was assessed by clinical benefit (CB) rate & survival analyses (Time To Progression (TTP) and Time To Death (TTD)). Biomarkers were selected by regression trees to be included in multivariate models for clinical outcomes. False Discovery Rate(FDR) was applied to univariate results. Combinations of pAKT & pPRAS40, Ki67 & ER and ER/AR ratio-were also analysed.

## **Results:**

**Type of Biopsy (Core versus TMA):** Generally % positivity was lower in TMAs than cores, for HER2, pER118, pIGF1R and cytoplasmic pAKT and pPRAS40. pAKT, pMAPK and Ki67 were largely unaffected by biopsy type. After FDR testing, significant differences remained for pER118 (p=0.002), pIGF1R (p<0.001), pPRAS40 (p<0.001) and HER2 (P=0.005) positivity levels.

**Biopsy Location (TMAs):** There was no significant difference in % positivity between central & peripheral tumor sites for all biomarkers examined. After FDR correction modest differences were seen for HER2 & pER118 (both p=0.044) only between the two central samples.

**IHC endpoints versus clinical outcome:** Univariate analyses identified only lower levels of pTEN and higher levels of Ki67 (% positivity) as predictive of poor outcome (TTP & TTD) following TAM treatment. In the luminal A (ER+/HER2-) subgroup only Ki67 was predictive of poor outcome (p=0.012).

The predictive power of Ki67 for TTP was not improved by combination with pTEN or ER. An inverse ER H-Score association with TTP was confined to the 'luminal A' cohort.

AR was not predictive. 71 patients had AR/ER ratio: 61 had ratio <2 (42 had CB & 19 no CB), and 10 had ratio ≥2 (3 CB & 7 no CB) (p<0.02). There was no significant difference in TTP or TTD by AR/ER ratio.

Only 2 /146 (<2%) tumors had AKT1 E17K mutations.

## **Conclusions:**

Biomarker staining cannot be assumed to be the same in TMAs & core biopsies: such data should be combined with caution. Importantly however, there was no evidence of difference in Ki67 between TMA & core samples.

There is no evidence to suggest that biomarker staining (including Ki67) at different tumor sites shouldn't be combined, or that ranking of scores differs across such sites.

The findings confirmed previous reports that Ki67 & reduced level of PI3K /AKT pathway regulator pTEN can predict resistance to TAM in ER+ BCs. The AKT mutation rate was low.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-03-03

*BRG1-SOX4* mediates a novel and essential signaling network that activates PI3K/Akt signaling in TNBC

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## **Background:**

Triple negative (TNBC) breast cancer, which is largely synonymous with the basal-like molecular subtype, is an aggressive malignancy that accounts for nearly 1 in 4 breast cancer related deaths and disproportionately affects younger women and women of African American descent. Given the lack of drug-able targets expressed by TNBC tumors, few therapeutic options exist beyond currently utilized cytotoxic therapies, and the overall prognosis for these patients remains poor. While TNBC tumors are characterized by high PI3K signaling, clinical trials targeting this pathway have had limited success. Therefore, identifying mechanisms driving key oncogenic pathways, including PI3K, is paramount to understanding the transformation process and enabling the development of rational, personalized therapeutic regimens.

## **Methods:**

We utilized a PI3K gene expression signature as a conceptual framework to analyze genome-wide mRNA expression and DNA copy number data from human breast tumors to identify genetic drivers of PI3K/Akt signaling. Kinome profiling was used to identify changes in the drug-able kinome regulated by these genes and *in vitro* studies were used to delineate mechanisms by which identified genes mediate oncogenic PI3K signaling in TNBC.

## **Results:**

Integrative proteogenomic analyses of orthogonal genome-wide data from ~3,000 human tumors from the TCGA and METABRIC studies identified amplification and overexpression of the oncogenic transcription factor *SOX4* as well as the SWI/SNF ATPase *BRG1* in tumors with high PI3K activity. These alterations were predominantly expressed in TNBC or basal-like breast tumors. Chromatin immunoprecipitation followed by DNA sequencing (ChIPseq) as well as shRNA-based studies confirmed that *BRG1* regulates *SOX4* expression in TNBC cell lines. Analyses of data from a genome-wide RNA interference (RNAi) screen in 27 breast cancer cell lines further indicated that *SOX4* is essential in cell lines with high PI3K activity and this was confirmed by colony formation and cell proliferation assays. Importantly, *in vitro* analyses confirmed that both *BRG1* and *SOX4* regulate Akt phosphorylation and down-stream signaling. Profiling of the drug-able kinome in *SOX4* depleted cell lines compared to control cells using Multiplexed kinase Inhibitor Beads coupled with quantitative Mass Spectrometry (MIB/MS) identified 21 drug-able kinases regulated by *SOX4* activity including *TGFBR2* which has been previously shown to regulate PI3K activity. RNA sequencing (RNAseq) and RT-PCR analyses confirmed that *SOX4* mediates *TGFBR2* mRNA levels and co-immunoprecipitation experiments in conjunction with ChIP assays demonstrated that *BRG1* and *SOX4* form a complex at the *TGFBR2* promoter and enhancer region to regulate *TGFBR2* expression.

## **Conclusions:**

In this study, we demonstrated that *BRG1-SOX4* constitutes a novel and essential signaling pathway which promotes PI3K/Akt activity through TGF $\beta$  signaling in TNBC/basal-like breast cancer and leads to activation of additional, drug-able kinases. Given the essentiality of *BRG1* and *SOX4*, our data suggest that targeting this interaction and/or the down-stream components of this pathway may represent a novel therapeutic strategy in TNBC.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-03-04

A novel druggable target upstream of Notch: MEK5/ERK5 signaling regulates Jagged-1 and Notch1 expression in triple negative breast cancer stem cells

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Triple negative breast cancer (TNBC) is a molecularly heterogeneous, clinically aggressive disease group that is highly prevalent among African-Americans and younger patients. Standard chemo/radio therapy often produces clinical responses, but recurrence and metastasis are unfortunately common. Metastatic disease is generally incurable. Chemo/radiotherapy has been shown to induce EMT and enrich a chemo-resistant cancer stem-like cell (CSC) population in TNBC. CSCs are thought to drive disease recurrence. Notch signaling is critical for maintenance of TNBC CSC. Expression of Notch1 and its ligand Jagged1 are correlated with poor prognosis. Efforts to pharmacologically target Notch with Gamma Secretase Inhibitors (GSIs) have been impaired by the systemic toxicity of the GSIs, and by the fact that Notch1 also plays a key role in anti-tumor adaptive immunity. Therapeutic agents that indirectly and selectively target Notch signaling in breast cancer cells would be a potentially attractive strategy. However, no such agents have been identified to date. We have found that the MAPK5-ERK5 kinase pathway, which contains at least two druggable targets, functions as a master regulator of Notch signaling in TNBC cells. ERK5 knockout TNBC cells have dramatically decreased expression of Notch receptors, ligands and transcriptional targets. In vivo, these cells form barely detectable tumors that do not metastasize and express lower levels of Notch1 and its ligand Jagged1. Using in silico screening, we identified a class of compounds that selectively target MAP2K5 (MEK5) and decrease the phosphorylation of MAPK7 (ERK5). We selected compound SC-181 for further study. Consistent with ERK5KO cells, pharmacological suppression of ERK5 phosphorylation with SC-181 decreased Notch1 and Jagged1 mRNAs and proteins. SC-181 reversed EMT and reduced the CD44<sup>hi</sup>/CD24<sup>lo</sup> CSC population in TNBC cells, but had no effect on T-cell proliferation. SC-181 decreased the number and size of mammospheres in a concentration-dependent manner. Overexpression of the Notch1 intracellular domain (N1IC) in ERK5KO cells rescues their phenotype, dramatically increasing the CSC fraction and promoting EMT. Our results suggest that targeting the MEK5-ERK5 pathway is a promising new strategy to selectively modulate Notch signaling in TNBC CSC without compromising tumor immunity.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-03-05

S100A7 enhances triple negative breast cancer growth and metastasis by cross-talk with resistin

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Triple negative breast cancer (TNBC) represents ~20% of all breast cancer cases and is highly refractory to existing therapies. Although, a number of putative functions have been proposed for S100A7, but its crosstalk with resistin in TNBC pathogenesis remains to be defined. S100A7/RAGE signaling has been shown to play an important role in inflammation-mediated breast cancer pathogenesis. S100A7/psoriasin, a member of the epidermal differentiation complex, is widely overexpressed in invasive estrogen receptor (ER) $\alpha$ -negative breast cancers. Thus, the objective of this study is to explore the cross-talk between S100A7 and resistin in TNBC cells. In this study, we observed that the treatment or overexpression of S100A7 increased the expression of resistin, cPLA2 and FASN expressions in TNBC cells. The overexpression/downregulation of S100A7 with exogenous resistin in TNBC cells also showed the marked changes in their migrating, sphere forming and colony forming abilities. The treatment of resistin also increases the effect of S100A7-mediated expansion of breast cancer stem cells (BCSCs) by increasing the ALDH activity and CD44<sup>+</sup>CD24<sup>-</sup> subpopulations. The *in-vivo* effects of resistin on breast tumor growth and metastasis were also analyzed by using MDA-MB-231 vector and S100A7 overexpressed MDA-MB-468 vector and S100A7 knock-down cells by using Xenograft NOD/SCID Il2rg null (NSG) mice model. Here, we have found that the treatment of resistin increased the tumor growth and metastases mediated by S100A7 by modulating the different myeloid cells populations. We have also shown that the S100A7 overexpressed MDA-MB-231 cells were much more sensitive to FASN and cPLA2 inhibitors as compared to vector control cells. The *in-silico* analyses also showed the clinical significance of S100A7 and resistin in TNBC pathogenesis. Collectively, these findings suggest the novel cross-talk in between S100A7 and resistin in TNBC pathogenesis and how resistin, cPLA2 and FASN could be used as potential therapeutic targets to inhibit the S100A7-mediated TNBC progression and metastasis.

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PTEN expression at the nexus of oncogenic signals in TNBC: Testing combination of p110beta-isoform-specific inhibitor with five PARP inhibitors

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**Background:** TNBC is the most aggressive form of BRCA-associated BC subtype. The loss of PTEN is a common “first event” associated with basal-like subtype (*Martins et al., 2012*) and this mode of PI3K-pathway activation (deletion/mutation/loss of PTEN) occurs more frequently (35%) than PIK3CA (5-10%) mutations in TNBC (*Ellis & Perou, 2013*). Several reports suggest upregulation of PI3K/AKT mediated by PTEN loss depends on the PI3Kbeta-isoform. PARP1 is identified as a target of BRCA-defined cancers. Inhibitors of PARP1 have been recently approved by the FDA as targeted agents in BRCA-defined breast cancers. Failure to repair damaged DNA upon PARP inhibition causes accumulation of DNA double-strand breaks and leads to apoptosis of cancer cells. Also, nuclear PTEN controls DNA repair (*Bassi et al., 2013*). Here we hypothesized that a combination of PARP inhibitor with p110beta-isoform-specific inhibitor would sensitize the effect of PARP inhibitor(s) in PTEN-deprived TNBC model. **Methods:** We tested five PARP inhibitors, Talazoparib (BMN673, B), Niraparib (N), Olaparib (O), Rucaparib (R) and Veliparib (V) in combination with p110beta specific inhibitor, AZD6482. Four PTEN-null TNBC cell lines (BRCA WT/null), MDA-MB468, HCC70 (p.F90fs\*9), BT549 (p.V275fs\*1) and SUM149 cell lines were used for the study. Proliferative, apoptotic and PARylation signals following drug combinations were demonstrated by WB in a dose and time-dependent manner. Pro-apoptotic and anti-proliferative effects were verified using complementary 3D ON-TOP assay, real-time proliferation (Incucyte), AnnexinV and cl-caspase3 analyses. As an internal control, we also compared anti-proliferative signals of GDC-0032 (p110 beta sparing inhibitor) and GDC-0941 (pan PI3K inhibitor) with that of AZD6482 at 3/6 hours in MDA-MB468 and SUM149 cells. Mode of apoptosis was tested using triple fluorescence staining in live cells. **Results:** A dose of 500 nM BMN673 alone was effective in slowing cell proliferation and induced apoptosis. AZD6482 (5-10 uM) did not have anti-proliferative or pro-apoptotic effects at 48 and 72 hours. When compared, combinations of different PARPi (100nM of B, 1uM of N, 10uM of O, 10uM of R and 10uM of V) with AZD6482 abrogated 3D growth in BT549 and MDA-MB468 TNBC cells in a time-dependent manner. The effect of PARP inhibitor was tested by PAR signals which were abrogated either alone or in combination with carboplatin in both BRCA1/2 WT and mutated cells. The most pronounced anti-tumor effect was observed with the combination of B and AZD6482 which was mechanistically explained by the robust increase of AnnexinV positive cells following a single 500nM dose of B at both 48 and 72 hours. Pan PI3K inhibitor, GDC-0941, and AZD6482 blocked the activation of PI3K and its downstream effectors in contrast to p110 alpha-specific inhibitor, GDC-0032 in MDA-MB468 and SUM149 cells. **Summary:** We demonstrated a remarkable sensitivity of tumor cells to PARP inhibition in PTEN-defined TNBC models and identified PTEN-nullness as a potential predictive biomarker for a possible co-targeting of the PI3K pathway to further sensitize TNBC to PARP inhibitors.

# 2018 San Antonio Breast Cancer Symposium ®

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Backwards translation: Exploring beta-adrenoreceptors (BAR) in triple negative breast cancer (TNBC), a novel and druggable pathway

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**Introduction:** several retrospective studies suggest that  $\beta$ AR blocking drugs (BB) are associated with improved survival in patients with a wide range of cancers. Recently, we retrospectively showed an association between BB intake and improved progression free survival in patients with HER2 negative advanced breast cancer (BC), particularly striking in triple-negative disease (TNBC) (Reference). Based on this finding we decided to conduct an *in silico* study in which we have interrogated  $\beta$ ARs in a publicly available BC sample database and the Translational Oncology Research Lab (TORL) translational platform with a genomic, transcriptomic and proteomic approach. **Methodology:** genomic and transcriptomic data sets for  $\beta$ AR 1, 2 and 3 were retrieved from cBioPortal considering all BC samples available with this information in The Cancer Genome Atlas (TCGA). Transcriptomic and proteomic data sets from 48 BC cell lines obtained from TORL were queried for  $\beta$ ARs as well and used with validation and exploratory intent. Mutations, amplifications and deletions were queried in DNA; gene expression profiles were interrogated using RNAseq data together with protein expression by RPPA. Average expression, log ratio and fold change in mRNA and Reverse Phase Protein Array (RPPA) quantitative assessments for corresponding proteins were noted. BC cell lines with top 10 mRNA and protein levels of  $\beta$ AR 1, 2 and 3 were identified. **Results:** CBioPortal DNA data shows  $\beta$ AR1 amplified in 1-10% and deleted in 0,4%;  $\beta$ -AR2 amplified in 1-3% and deleted in 0,1%;  $\beta$ -AR3 amplified in 15-20% and deleted in 2% of the BC samples. CBioPortal mRNA data shows  $\beta$ -AR1 is upregulated in 2.7% (mostly Progesterone Receptor negative BC);  $\beta$ AR2 is upregulated in 4% (mostly TNBC);  $\beta$ AR3 is upregulated in 4% (mostly HER2 negative BC) in BC samples. TORL cell line panel shows that  $\beta$ ARs are heterogeneously expressed between the BC cell lines (fold change range:  $\beta$ AR 1 2.015-3.636;  $\beta$ AR2 2.545-8.248;  $\beta$ AR3 1.809-2.444). Within the 10 BC cell lines with highest  $\beta$ AR1 and  $\beta$ AR2 expression, 7(COLO-824, HCC1937, BT-549, BT-20, HCC1599, HCC1143, HCC1806) and 6 (184A1, MDA-MB-231, 184B5, HCC1806, MCF-10A and MDA-MB-468) of them respectively correspond to the basal BC subtype. RPPA identifies caveolin 1, PAI 1, EGFR and Bax as the proteins with the higher co-expression with  $\beta$ AR1 and  $\beta$ AR2. **Conclusions:** DNA alterations are infrequent in  $\beta$ ARs in BC samples. Transcriptional mRNA data from BC samples shows  $\beta$ ARs mostly expressed in non-luminal BC subtypes, being  $\beta$ AR 2 the one with highest expression. *In silico* data results from BC cell line panel show  $\beta$ AR 1 and  $\beta$ AR 2 are highly expressed in basal BC subtype. The above data suggest that  $\beta$ AR, and  $\beta$ AR 2 in particular could be a relevant target to explore in *in vivo* BC models.

Table1: mRNA and RPPA in BC cell lines

Cell line	Average mRNA	Log Ratio	Fold Change	Caveolin 1	PAI1	EGFR
184A1	31317	3.04	8.24	4.18	3.41	1.72
SUM-190	22329	2.81	1.03	-1.37	-0.57	0.09
MDA-MB-231	15100	2.99	7.99	3.85	3.25	1.57
184B5	13012	1.97	3.93	3.90	2.36	1.82
HCC1806	12411	2.37	5.19	3.75	0.49	1.67
MCF-10A	11912	2.19	4.58	3.89	2.67	0.08
ZR-75-30	9300	1.89	3.72	-1.51	-0.61	-0.25
JIMT-1	6354	1.59	3.01	3.08	2.01	0.81
MDA-MB-415	6239	1.24	2.37	0.96	-0.39	-0.13
MDA-MB-468	6280	1.34	2.54	1.06	-0.25	1.72



## 2018 San Antonio Breast Cancer Symposium ®

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Preclinical evaluation of the PI3K $\alpha/\delta$  inhibitor, copanlisib in HER2+ breast cancer: A proof of concept study

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**Purpose:** The PI3K-AKT-mTORC1/C2 pathway is frequently activated in HER2+ breast cancer and upregulation of this pathway is a key mechanism of trastuzumab resistance. However, attempts to indirectly target this pathway by using the allosteric mTOR inhibitor everolimus have had limited clinical success. Here, we present the results of a preclinical study of the PI3K  $\alpha/\delta$  (dominant) inhibitor copanlisib alone and in combination with T-DM1, in *HER2* amplified cell lines including a model with acquired resistance to trastuzumab.

**Method:** Anti-proliferative, apoptotic, cell cycle, and intracellular signaling effects of copanlisib alone and in combination with T-DM1 were evaluated in a panel of *HER2* amplified (ER+ or ER-), and *HER2* amplified/*PIK3CA* mutated cell lines, as well as trastuzumab-resistant breast cancer cell lines.

**Results:** 1) Copanlisib inhibited PI3K, mTOR and their downstream signaling molecules in *HER2* amplified/*PIK3CA* WT or *PIK3CA* mutated as well as in trastuzumab-resistant cell lines, 2) interestingly, copanlisib also inhibited RAS-MAPK signaling in the earlier time points, 3) copanlisib caused a strong differential growth inhibition in *HER2* amplified BC cell lines by 3D-ON-TOP clonogenic assay and real-time monitoring in an IncuCyte Zoom. Inhibition was greater when copanlisib was combined with T-DM1, 4) administration of copanlisib induced cell cycle G0/G1 arrest and resulted in increased apoptosis in a dose-dependent manner, 5) unlike everolimus copanlisib blocked HIF1 $\alpha$  accumulation in hypoxic condition and this blocking effect was reversed by prior treatment with the proteasome inhibitor, carfilzomib and 6) copanlisib also attenuated *HER2* amplified cell migration, an important phenotypic feature for metastasis.

**Conclusions:** Copanlisib is highly effective (blocks proliferation, induces apoptosis, and inhibits PI3K and its downstream signaling targets) in *HER2* amplified breast cancer cell lines including trastuzumab-resistant and *PIK3CA* mutated cell lines. The addition of copanlisib to T-DM1 might represent an improved treatment strategy for patients with refractory metastatic HER2+ breast cancer.



# 2018 San Antonio Breast Cancer Symposium ®

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Autocrine motility factor signaling pathway promotes aggressive behavior and migration in breast cancer

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**Background:** Autocrine motility factor (AMF) is secreted by cancer cells and acts in an autocrine or paracrine fashion to bind to its receptor, AMFR, at the cell surface of tumor cells. The AMF-AMFR pathway has been shown to promote proliferation, anti-apoptosis, motility and migration, invasion, and metastasis pathways in various cancers. However, our understanding of the AMF-AMFR pathway in breast cancer is limited. We propose that the AMF signaling pathway is an unexplored mechanism for breast cancer tumor aggression and its receptor, AMFR, may be a potential therapeutic target.

**Methods:** Tumor tissue obtained from consented female (n = 31) and male (n = 1) patients were analyzed by the Affymetrix Oncoscan™ genome-wide microarray platform and examined for somatic copy-number alterations (SCNAs) of AMFR. cBioPortal was used to investigate SCNA of AMFR and gene expression of AMF on primary breast cancer tumors from METABRIC (n = 1,784) and TCGA Pan-Cancer Atlas (n = 981) datasets. *In vitro*, AMF and AMFR gene expression in luminal A (MCF-7) and triple-negative breast cancer (MDA-MB-231) cell lines were assessed by qPCR. Cell migration assays were performed on MDA-MB-231 cells to investigate their migration towards AMF with AMFR present or knocked down by siRNA.

**Results:** Microarray analysis of 32 tumors revealed that a single-copy loss of AMFR occurred 79% of the time in luminal A tumors (n = 19); 67% of the time in luminal B tumors (n = 6); 33% of the time in ER+, PR+, HER2+ tumors (n = 3); and 0% of the time in triple-negative breast cancer (TNBC) tumors (n = 4), suggesting that the loss of AMFR results in less aggressive tumors that have good overall prognosis. To extend our findings to a larger patient cohort, SCNA analysis of the METABRIC and TCGA Pan-Cancer Atlas datasets revealed that single-copy loss of AMFR occurred in 64.53% and 68.14% of luminal A tumors, 58.51% and 59.90% of luminal B tumors, 23.62% and 48.72% of HER2 overexpression tumors, and 29.80% and 39.77% of TNBC tumors, respectively. Therefore, AMFR appears most frequently deleted in the tumor genomes of good prognosis breast cancer molecular subtypes (luminal tumors). Gene expression analysis of AMF in the METABRIC (using z-scores) and TCGA Pan-Cancer Atlas (using batch-normalized numbers) datasets revealed median mRNA expressions of -0.3351 and 4862 in luminal A tumors, -0.05415 and 5841 in luminal B tumors, 0.5758 and 9390 in HER2 overexpression tumors, and 0.754 and 8798 in TNBC tumors, respectively, suggesting that the AMF-AMFR pathway is more active in aggressive breast cancers. Similarly, we observed that AMF and AMFR are transcriptionally overexpressed by 7-fold and 16-fold, respectively, in the TNBC cell line MDA-MB-231 compared to the luminal A breast cancer cell line MCF-7. When AMFR is knocked down in MDA-MB-231 cells, focused migration towards AMF is abolished.

**Conclusion:** AMF-AMFR pathway activity correlates with aggressive cancer cell behavior and enhanced migration in breast cancer. Single-copy loss of AMFR in tumor genomes is associated with less aggressive tumors with better overall prognosis. AMFR may be an attractive therapeutic target.

# 2018 San Antonio Breast Cancer Symposium ®

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A cholesterol-derived oncometabolite and glucocorticoid receptor signaling in triple negative breast cancer cell proliferation

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**Rational:** A role for cholesterol in the etiology of cancers has been suspected. Our recent data have shown that it is the metabolism of 5,6-epoxide cholesterol (5,6-EC) that is deregulated in breast cancers (BC), and this metabolism controls BC development (Voisin et al, PNAS 2017). Indeed, in normal breast tissues, the 5,6-EC $\alpha$  and  $\beta$  are transformed by the cholesterol epoxide hydrolase (ChEH) into cholestane triol (CT). In BC including triple-negative subtype (TNBC), CT is further transformed into the oncometabolite 6-oxo-cholestan-3 $\beta$ ,5 $\alpha$ -diol (OCDO) by the 11 $\beta$ -hydroxysteroid dehydrogenase-type 2 enzyme (11 $\beta$ HSD2). 11 $\beta$ HSD2 is known to regulate glucocorticoid metabolism by converting the active glucocorticoid receptor agonist cortisol into inactive cortisone. We established that OCDO promotes BC progression by binding to the glucocorticoid receptor (GR). Patient BC samples showed significant increased OCDO levels and greater ChEH and 11 $\beta$ HSD2 protein expression compared with normal breast tissues and 11 $\beta$ HSD2 and ChEH overexpression correlated with a higher risk of patient death, highlighting that the biosynthetic pathway producing OCDO is of major importance to BC pathology. Interestingly, data from the literature indicate that GR overexpression is correlated with poor progression-free and overall survival in patients with TNBC.

**Objective:** The aim of this study was to decipher the signaling pathways inducing the pro-proliferative activity of OCDO in TNBC cells.

**Material and methods:** OCDO was from Steraloids. Cortisol, Dexamethasone and SP600125 (SP) were from Sigma-Aldrich.

**Culture:** MDA-MB231, ShC MDA-MB231 and ShGR MDA-MB231 cells were grown in DMEM supplemented with 10% FBS and 2 mM L-glutamine. **PCR quantitative.** qRT-PCR was performed with an iCycler iQreal-time PCR detection system using SYBR Green. RNA expressions of genes of interest were determined using the  $\Delta\Delta$ CT method. **Cell Cycle Analysis.** Cell cycle analysis was performed by flow cytometry analyzer. **SPR Assays.** Binding studies were performed on a BIAcore T200 optical biosensor instrument. GST-tagged LBD of human GR (Thermo Fisher Scientific) was covalently coupled to the chip surface using amine coupling (CM5) sensor chips. A low mass weight-multiple-cycle kinetics analysis, to determine affinity constants, was carried out by injecting different concentrations of the indicated ligand (6.25  $\mu$ M–100  $\mu$ M).

**Results:** We established that OCDO binds to the GR, activates its translocation into the nucleus and the transcription of genes under the control of the AP-1 complex, while cortisol or dexamethasone represses their transcription. We showed that OCDO stimulates cell cycle progression by increasing the percent of the G2/M phase. OCDO increases the expression of cyclin D1 and c-myc proteins and the phosphorylation of c-jun forming the AP1 complex. All these effects were abolished when GR expression is knocked-down or when tumor cells are treated with OCDO and SP600125, an inhibitor of the c-Jun N-terminal Kinase pathway (JNK). These data indicate that OCDO mediates cell cycle progression by acting through the GR and by activating the JNK pathway and the oncoprotein c-jun.

**Conclusion:** Targeting this oncometabolism and GR signaling may be novel strategies to treat TNBC.

## 2018 San Antonio Breast Cancer Symposium ®

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Genetic background determines the algorithm of effectiveness of targeted drugs of RAS and PI3K pathways in TNBC: Testing a combination of MEK 1/2 inhibitor with mTOR kinase inhibitor or AKT inhibitor

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**Introduction:** Most TNBC patients with single-agent targeted therapy in multiple clinical trials develop resistance, leading to disease progression, posing a major challenge in clinical management of mBRCA. In TNBC patients there remains a lack of obvious predictive biomarkers to guide targeted therapy. Although signaling mechanisms of resistance may be either intrinsic or acquired, the role of driver pathways are known be associated with oncogenic evolution of tumor cells to a resistant state. As the PI3K-AKT-mTOR1/2 (PAM) pathway is activated in TNBC (due to alterations in EGFR, 50%; PTEN, 40%; and PIK3CA, 5-10%), the PAM pathway is a candidate for potential molecular targeting of anti-cancer therapeutics. Despite the involvement of PI3K pathway activation in TNBC tumors, single agent PI3K inhibitors show modest clinical activity. Literature references suggest extensive cross-talk exists between the (PAM) and the RAS-RAF-MEK-ERK (MAPK) pathways. Here we tested the efficacy of a combination of MEK 1/2i (AZD6244, GDC0973) with mTOR kinasei (AZD2014, TAK228) or AKTi (AKT5363) in TNBC models.

**Methods:** TNBC cell lines with alterations in KRAS/RAF (MDA-MB231), BRCA-deficient PTEN-loss (SUM149), PTEN-loss (MDA-MB468) and PIK3CA mutation (BT20) were used. Proliferation, apoptosis, cell cycle and 3D clonogenic growth were tested following drug treatment alone or in combination. Signaling events in the respective pathways following drug treatment were interrogated by WB. **Results:** MEKi led to a brief proliferation inhibition in MDA-MB231 cells. A combination of MEKi and mTORi was additive in decreasing short term proliferation and 10 day 3D growth. AKTi alone had limited effect on 3D growth but in combination with MEKi was profoundly inhibitory. A limited effect of MEKi was observed in BT20 cells; blocking 2D and 3D growth, while a profound effect of MEKi was observed in combination with mTORi (to a lesser extent with AKTi). In MDA-MB468 cells, MEKi did not have an initial appreciable effect (proliferation and G1 arrest), though 3D growth was significantly reduced at 10 days following drug alone or in combination with AKTi. SUM149 cells had G1 arrest with no appreciable change in 3D growth following MEKi. In contrast, single agent mTORi and AKTi reduced 3D growth. MEKi and mTORi doublet slowed proliferation, increased apoptosis and disrupted 3D growth. No single agent treatment with AKTi or mTORi disrupted colony formation however either agent in combination with MEKi blocked 3D growth. Single agents treatments were mostly cytostatic with no increase in apoptosis while a doublet of MEKi plus mTORi induced significant apoptosis. A combination of MEKi with PI3K pathway specific inhibitor significantly blocked phosphorylation of downstream effector molecules. Upregulation of ERK signals following PAM pathway inhibitors were abrogated by prior addition of MEKi. **Summary:** Combined blockade of the PAM and MAPK pathways (AKTi or mTORi) plus MEK1/2 inhibitor were more effective in attenuating molecular signals. This combination showed enhanced efficacy in TNBC cell models with specific PAM and or MAPK pathway alterations.

## 2018 San Antonio Breast Cancer Symposium ®

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Role of nucleus-specific intergenic long noncoding RNA-1476 in estrogen-dependent transcription in cancer

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Long noncoding RNAs (lncRNAs) are emerging as key regulators of diverse cellular processes, but their roles in cancer biology are just beginning to be elucidated. Previous work using Global Run-On sequencing (GRO-seq) to study the transcriptome of MCF-7 breast cancer cells identified a large number of unannotated noncoding RNAs. Integration of RNA-seq data from subcellular fractionated RNA (i.e., cytoplasm, nucleoplasm, and chromatin-associated) with GRO-seq data using a novel bioinformatics pipeline has yielded a comprehensive catalog of >1900 polyadenylated lncRNAs in MCF-7 cells, about half of which have not been annotated previously and about a quarter of which are estrogen-regulated. Analysis of RNA-seq data from hundreds of samples representing 13 different cancer and normal tissue types, revealed that many lncRNAs are differentially expressed in various cancers. Furthermore, a large number of lncRNAs show distinct expression patterns across molecular subtypes of cancer. In functional assays, Kaplan Meier analysis of selected lncRNA, such as lncRNA 1476, predicts clinical outcome in ER-positive cancer and is transcriptionally upregulated in MCF-7 cells upon estrogen treatment. lncRNA1476 has now been fully annotated (transcription start and stop site, 5' cap, polyA tail, and exon/intron structure), and cloned. Molecular analyses indicate that lncRNA1476 plays a critical role in ER-dependent pathway. Collectively, our results suggest that lncRNAs are essential in controlling biological processes.

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# 2018 San Antonio Breast Cancer Symposium ®

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Human papillomavirus (HPV) DNA detection in breast cancer by liquid biopsy: Something new on the horizon?

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**Background** The presence of human papillomavirus (HPV) DNA in breast cancer (BC) tissues has been widely investigated in recent years. HPV DNA has been detected in ductal lavage fluids and in serum-derived extracellular vesicles of patients with benign or *in situ* breast lesions. However, there are no data attesting to its presence in liquid biopsies of different BC subtypes or to its impact on prognosis.

**Methods** We analyzed a total of 72 serum samples for the presence of circulating HPV DNA, of which 20 were from luminal A BC (5 relapsed, 15 non relapsed), 17 from luminal B BC (5 relapsed, 12 non relapsed), 15 from triple-negative BC (6 relapsed and 9 non relapsed), 12 from HER2-positive BC (3 relapsed, 9 non relapsed) and 8 from healthy subjects. Circulating DNA was extracted and purified from 500 µl of serum by Qiamp DNA minikit (Qiagen, Milan, Italy) according to the manufacturer's instructions. HPV DNA was assessed by a high-throughput MALDI-TOF mass spectrometry-based method (Mass Array Platform, Agena Bioscience, Hamburg, Germany). The DNA target sequence was amplified by a multiplex PCR with HPV E6 or E7 gene-specific primers. A primer for primer extension annealing to the amplified product was extended at its 3' terminal base for each HPV type.

**Results** HPV DNA was detected in 5 BC patients but in none of the healthy controls. None of the serum samples analyzed showed HPV DNA types 16 or 18. Four of the 5 BC cases had high-risk HPV DNA (type 39,45,52,59) and one had low-risk HPV DNA (type 73).

Table 1. Features of BC patients positive for circulating HPV DNA.

Sample ID	Age (years)	Grading	Subtype	Death	Relapse	HPV type, risk	Cervical lesion
17	47	1	Luminal A	-	No	59, high	CIN 1
39	78	3	Luminal B	20/03/2015	No	45, high	CIN 1
45	46	2	Luminal B	-	No	39, high	CIN 1
60	58	3	TN	03/01/2011	Yes	73, low	Hyperplasia
63	52	3	HER2-positive	-	Yes	52, high	CIN 1

TN, triple negative; CIN 1:low-grade cervical intraepithelial neoplasia

The 4 patients with high risk HPV DNA had low-grade cervical intraepithelial neoplasia (CIN 1) detected by Pap smear prior to the diagnosis of BC. No relation was found between HPV infection and tumor subtype or prognosis. Our *in vitro* studies also revealed the active release of HPV DNA into the extracellular vesicle compartment of cervical cancer cells.

**Conclusions** Our findings support the feasibility of HPV DNA evaluation by liquid biopsy in BC. They also suggest that circulating HPV DNA in BC patients might be of cervical tissue origin and that the presence of HPV DNA in BC may be a consequence of its spreading from virus-infected tissue such as that of the uterine cervix.

# 2018 San Antonio Breast Cancer Symposium ®

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Chemotherapy-induced metastasis: Mechanisms and translational opportunities

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**Background:** Chemotherapy has become essential in the care of patients with advanced breast cancer. However, as we have previously discovered, chemotherapy may induce pro-metastatic changes in the breast cancer microenvironment by promoting the assembly and function of cancer cell intravasation sites called tumor microenvironment of metastasis (TMEM), and by increasing the proportion of the highly-invasive Mena<sup>INV-HI</sup> tumor cells that utilize TMEM sites for hematogenous dissemination. Consequently, chemotherapy-treated animals demonstrate increased numbers of circulating-tumor cells and micrometastases. Since the formation of metastases depends on both the presence of functional doorways for dissemination (TMEM) and cancer cells capable of using these doorways (Mena<sup>INV-HI</sup> tumor cells), we investigated the cellular and molecular contexts required for chemotherapy-mediated induction of TMEM function and Mena<sup>INV</sup> expression. Since TMEM function depends on Tie2 expressing macrophages and Mena<sup>INV</sup> expression is inducible by cancer cell -macrophage contact, we focused on inhibiting Tie2 receptors and chemotherapy-induced macrophage influx to inhibit chemotherapy-induced metastasis.

**Materials and Methods:** We used spontaneous models of mouse breast carcinoma (MMTV-PyMT) and patient-derived xenografts treated with chemotherapy with or without co-treatment with either Tie2 inhibitor (rebastinib), inhibitors of Cxcr4<sup>+</sup> macrophage chemotaxis (Cxcl12 inhibitors) or macrophage depletion agents (clodronate). Pro-metastatic endpoints were measured by intravital imaging, multichannel immunofluorescence and standard metastasis dissemination assays.

**Results:** We demonstrated, using multiple methods of macrophage suppression, that chemotherapy-mediated induction of Mena<sup>INV-HI</sup> tumor cells depends on the presence of macrophages. In particular, depletion of either the entire macrophage lineage using clodronate liposomes or the use of specific inhibitors of the Cxcl12/Cxcr4 chemotactic pathway, resulted in a significant suppression of the Mena<sup>INV-HI</sup> cancer cell subpopulation in all mammary tumors examined. Moreover, inhibition of Tie2 by rebastinib blocked TMEM function and decreased the number of circulating tumor cells and metastatic foci, despite the chemotherapy-mediated induction of Mena<sup>INV-HI</sup> tumor cells.

**Conclusions:** Our data indicate that both the Mena<sup>INV-HI</sup> disseminating cancer cell population and the TMEM doorways are necessary but not individually sufficient for metastasis. As such, suppression of either the Mena<sup>INV-HI</sup> population or TMEM function can suppress chemotherapy-induced metastasis, thus providing a target to improve clinical care and eliminate non-beneficial effects of chemotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Age-related genetic and epigenetic changes in estrogen-receptor positive breast cancer

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## Background:

Age is the main risk factor for developing breast cancer (BC) in women. The increase in incidence with age is dominated by estrogen receptor positive (ER+) BC despite the >90% reduction of estrogen levels during menopause and later life. Recent models suggest the lifetime risk of BC is correlated with the number of stem cell divisions with random mutations arising during each division and driving cancer development. However, only two genes are mutated in more than 20% of BC while several recurrent copy number alterations (CNAs) occur in more than 50%. New models of age-related risk factors need to be developed that incorporate mutations, CNAs, epigenetics, and other biological factors. As data of this type is limited in normal breast tissue, factors correlated with age of diagnosis in ER+ BC were identified to understand which variables changed with age and might impact risk of developing BC.

## Methods:

*In silico* analysis of public databases was used to identify factors in ER+ primary BC and normal adjacent tissue (NT) that are correlated with age of diagnosis including DNA mutations, CNAs, DNA methylation and gene/protein expression (TCGA n=599ER+,113NT; METABRIC n=1435ER+,144NT).

## Results:

DNA mutations accumulated with age in ER+ BC with the median mutation count below 28 in primary tumors diagnosis in patients <50yr and rising to more than 43 in patients >80yr. However, the two most frequently mutated genes (*PIK3CA* and *TP53*) showed no significant correlation with age. As previously reported, *GATA3* mutation rate was nearly twice as high in younger patients (<50yr=0.25,>80yr=0.12). There was little evidence of a general accumulation of CNAs with age, but there were higher rates of gain in 16p (<50yr=0.42,>80yr=0.51) and loss of 1p (<50yr=0.18,>80yr=0.37) in older patients and lower rates of loss in 6q (<50yr=0.34,>80yr=0.24). The most significant correlations with age related to *ESR1* including expression of *ESR1* mRNA ( $\rho=0.39, P=1.2e-23$  Spearman), ER $_{\alpha}$  protein ( $\rho=0.35, P=1.7e-15$  Spearman), and demethylation of *ESR1* promoter ( $\rho=-0.36, P=1.6e-13$  Spearman). The levels of the activated form of ER $_{\alpha}$  (pS118) also correlated with age but to a much lesser extent than total ER $_{\alpha}$  ( $\rho=0.12, P=0.01$  Spearman). Demethylation and expression of *ESR1* were highly correlated ( $\rho=-0.50, P<1e-16$  Spearman). Analysis of data from NT revealed correlation of age of diagnosis, expression of *ESR1* ( $\rho=0.34, P=0.001$  Spearman) and demethylation of the *ESR1* promoter ( $\rho=-0.22, P=0.03$  Spearman) but not with ER $_{\alpha}$  expression ( $\rho=0.32, P=0.19$  Spearman) although only 19 NT had protein data.

## Conclusions:

Analysis of DNA mutations and CNAs fit previous theoretical models with both the result of stochastic processes. In these models, the low impact on fitness from individual mutations allows the accumulation over time while the high fitness costs of CNAs prevent accumulation and are likely acquired in a single catastrophic event. The ability of ER+ BC to progress in the presence of very low postmenopausal estrogen levels may be partly explained by demethylation leading to higher *ESR1* expression and maintenance of ER $_{\alpha}$  activation. In theory, changes in expression and demethylation can be targeted to reduce the age-related increase in risk for developing BC.

# 2018 San Antonio Breast Cancer Symposium ®

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Clinical implication of APOBEC3A and 3B in Korean patients with breast cancer

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**Background:** Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) family is known to function in innate immune system that protects against retrovirus by deaminating cytosine to uracil in single-stranded DNA. APOBEC family has emerged as an endogenous mutator to contribute to the mutation burden in many cancers. We aimed to evaluate the expression of APOBEC3A (A3A), 3B (A3B) mRNA and APOBEC3A-3B deletion polymorphism in Korean breast cancer patients and investigate the correlation between their expression and clinicopathological characteristics.

**Methods:** One hundred thirty-eight patients who underwent surgery for breast cancer in Uijeongbu St. Mary's Hospital between January 2013 and December 2016 were evaluated. RNA and DNA were extracted from 138 breast cancer tissues and 10 adjacent normal breast tissues. The levels of A3A and A3B mRNA transcripts were determined using real-time quantitative PCR. Insertion and deletion PCR assays were performed to detect the APOBEC3A-3B deletion allele. Mutation hotspots in exon 2-11 of TP53 and exon 9/20 of PIK3CA were evaluated using direct sequencing method.

**Results:** The expression of A3B was increased in breast cancer tissues than in normal breast tissues. The median A3B mRNA expression levels in both triple-negative breast cancer and human epidermal growth factor 2-positive breast cancer were significantly higher than in hormone receptor-positive breast cancer. Old age and high ki-67 expression were associated with increased expression of A3A and A3B. Advanced stage, presence of lymph node involvement, and high histological grade were associated with increased expression of A3A. The APOBEC3B deletion allele was found in 78 (56%) tumor samples. There was no significant association between A3A, A3B mRNA levels and the presence of APOBEC3B deletion allele. There was no difference in clinicopathological characteristics according to the presence of APOBEC3B deletion allele except histological grade. The frequency of high histological grade was significantly higher in tumors with APOBEC3B deletion allele than tumors without APOBEC3B deletion allele. TP53 mutations were identified in 12 (8.7%) cases and PIK3CA mutations were identified in 31 (22.5%) cases. There were no significant differences in the levels of A3A and A3B mRNA expression by TP53 mutation status. The presence of a PIK3CA mutation was significantly associated with lower A3A expression.

**Conclusions:** The levels of A3B mRNA expression showed a difference according to breast cancer subtype, and triple-negative breast cancer showed the highest levels of A3B mRNA expression. The high levels of A3A and A3B mRNA expression were associated with an aggressive phenotype including high proliferation index. The APOBEC3A-3B deletion polymorphism was found in about half of the patients, but there was no difference in clinicopathological factors according to the presence of APOBEC3B deletion allele except histological grade.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-05-04

The WNT pathway gene, LBH, is critically required for breast carcinogenesis by promoting tumor initiation and survival

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**Background:** Cancer stem cells (CSCs) are a small subset of tumor cells with stem cell properties that drive tumor initiation, treatment resistance, and metastasis, thereby leading to rapid disease relapse. However, no cancer stem cell specific treatments are currently available in the clinic. Thus, there is a need to identify novel cancer stem cell-specific molecular targets.

LBH (Limb-Bud and Heart), is a poorly characterized transcription co-factor in the WNT pathway, a signaling network that is key to normal stem cell control and cancer stem cell transformation. We previously identified LBH as a basal mammary stem cell specific marker critically required for stem cell self-renewal and maintenance during normal mammary gland development. Significantly, LBH is also abnormally over-expressed specifically in worst prognosis basal-like breast cancers, a treatment-resistant breast cancer subtype that is enriched in cancer stem cells. Moreover, high inter-tumor LBH expression levels correlate with reduced metastasis-free patient survival and increased chemo-resistance. However, the role of LBH in breast carcinogenesis is not known.

**Results:** To identify the potential role of LBH in breast carcinogenesis, we abrogated LBH expression in breast cancer cell lines with high CSC populations (HCC1395, MDA-MB-231) using shRNA and CRISPR/Cas technologies. Conversely, we stably introduced LBH into more differentiated breast cancer cell lines with low CSC populations (MCF7, BT549). We found that LBH depletion significantly reduced, while LBH overexpression increased *in vitro* tumor sphere formation and clonogenic ability, suggesting LBH promotes a CSC phenotype. Modulation of LBH expression did not change proliferation rates, indicating the CSC promoting effects of LBH were not due to increased cell proliferation. However, LBH reduced apoptosis rates and increased tumor cell survival. Importantly, loss of LBH markedly decreased *in vivo* tumor initiation and metastatic potential of triple negative breast cancer cells, which are important functions of CSC. The underlying mechanisms will be discussed.

**Conclusions:** Collectively our results suggest that LBH is required for breast CSC self-renewal and maintenance. Thus, LBH inhibition may have value for future CSC-targeted anti-cancer therapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-05-05

Evaluation of pan-HER and c-MET inhibitors tested *ex vivo* in primary HER2- breast cancer cells with hyperactive c-MET and ErbB family signaling

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**Background:** To elucidate the role of c-Met signaling and its involvement with ErbB signaling as a cancer driver, a new assay using an impedance biosensor, the CELx multi-pathway signaling function (CELx MP) test, was developed. The CELx MP Test measures *ex vivo* real-time live cell response to specific ErbB and c-Met agonists to diagnose breast tumors with hyperactive HER1, HER2, HER3, HER4, and c-MET signaling activity. A recent study quantified c-MET and ErbB-driven signaling activity in epithelial cell samples derived from fresh breast tumor specimens obtained from 74 HER2- breast cancer patients. Of the cell samples tested, 20 of 74, (27.0%; 95% CI=18%-38%) had both hyperactive c-MET signaling and at least one hyperactive ErbB-family receptor signaling. Using primary breast cancer cells with hyperactive c-MET and ErbB signaling and the CELx MP test, the current study set out to: 1) determine the IC<sub>50</sub> values of six pan-HER and five c-MET inhibitors; and 2) characterize the efficacy of combinations of each pan-HER inhibitor with each c-MET inhibitor.

**Methods:** Epithelial cells from six HER2- tumor specimens with hyperactive c-MET and ErbB-driven signaling were obtained. Real-time live cell response to specific ErbB and c-Met agonists (NRG1b, EGF, or HGF) alone and in combination, with or without one of six pan-HER antagonists (neratinib, lapatinib, ibrutinib, dacomitinib, sapitinib, poziotinib) or one of five c-MET antagonists (tepotinib, cabozantinib, crizotinib, capmatinib, or savolitinib) was quantified using an xCELLigence RTCA impedance biosensor. Each individual drug IC<sub>50</sub> was determined using a 1000-fold, 5-point, dose response curve with a single fixed concentration of a corresponding agonist. For the drug combination efficacy studies, fixed concentrations of the agonist mixture and clinically relevant concentrations of combinations of the antagonists were used to determine the percentage inhibition of the ErbB and c-MET signaling.

**Results:** The IC<sub>50</sub> values for the individual c-MET and pan-HER inhibitors ranged from 3.10nM - 28nM and 2.67nM – 137.27nM, respectively. In the drug efficacy studies, an average of at least 80% of the ErbB and c-MET signaling activated by NRG1, EGF, and HGF co-stimulation was inhibited by each combination of c-MET and pan-HER inhibitors.

## c-Met Inhibitor Results

c-MET inhibitor	IC50 (nM)	Avg Inhibition (%) w/diff ErbBi's
Capmatinib	3.10	94
Savolitinib	3.32	98
Tepotinib	14.70	96
Cabozantinib	27.36	99
Crizotinib	28.21	100

## Pan-HER Inhibitor Results

Pan-HER inhibitor	IC50 (nM)	Avg Inhibition (%) w/diff c-METi's
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Pozotinib	2.67	100
Neratinib	4.81	100
Ibrutinib	13.10	99
Dacomitinib	22.06	100
Sapitinib	41.28	98
Lapatinib	137.27	80

**Conclusions:** The CELx MP test using live cells measures IC<sub>50</sub> values comparable to those derived using cell-free methods. Every combination of pan-HER and c-MET inhibitors provided comparably high (at least 80%) levels of inhibitory effect *ex vivo*. This suggests the sub-group of HER2- breast cancer patients diagnosed with coincident hyperactive c-MET and ErbB signaling by the CELx Test may respond to virtually any pan-HER and c-Met inhibitor combination. Studying combinations designed to minimize drug toxicities without sacrificing efficacy should thus be possible.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-05-06

Effects of a soy isoflavone in breast cancer treatment

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**Background :** Isoflavones are phytoestrogens that may be effective in preventing osteoporosis, reducing cardiovascular events, and improving menopausal symptoms such as hot flash due to their estrogen-like actions. Isoflavones are currently used as a supplement for improving symptoms of menopause. Epidemiological studies have shown that ingestion of soy products may also reduce the risk of breast cancer, and antitumor effects on hormone receptor (HR)-positive breast cancer cells have been shown *in vitro* for equol, an isoflavone metabolite. Equol binds to estrogen receptors (ER $\alpha$ ,  $\beta$ ) and is thought to antagonize ER $\alpha$ -estradiol binding in the presence of estradiol. Since equol shows an antiestrogenic effect, similar to that of the hormonal agent tamoxifen, it is thought that equol acts as a selective estrogen receptor modulator (SERM), but the mechanism is still unclear. In this study, we evaluated the antitumor effects of equol alone and in combination with existing therapeutic agents in HR-positive breast cancer cells, and examined the mechanism of these effects.

**Methods :** The antitumor effects of equol alone and in combination with hormone drugs (4-hydroxytamoxifen (Tam), fulvestrant (Ful)) and chemotherapeutic agents (paclitaxel (Ptx), doxorubicin (Dox)) were examined using a MTS assay. Combination indexes (CIs) were determined in HR-positive MCF-7, T-47D, and ZR-75-1 cell lines. The mechanisms of the drug effects were evaluated by Western blot for assessment of changes in chemoresistance factors at the protein level.

**Results :** In MCF-7, T-47D and ZR-75-1 cells, there was a concentration-dependent antitumor effect of equol and of the other hormonal agents. CIs showed an antagonistic effect of equol with Tam and a synergistic effect with Ful in all cell lines. Equol also had an antagonistic effect with the two chemotherapeutic agents, with the strongest antagonism occurring at a low dose of equol. Western blot showed that ER, PgR, Cyclin D1 and Bcl-2 were upregulated via ER at a low concentration of equol, similarly to the effect of 17- $\beta$ -estradiol (E2), and ER, PgR, Cyclin D1 and Bcl-2 were downregulated at a high concentration, similarly to the effect of Tam. In addition, ER, PgR and cyclin D1 were downregulated with the combination of equol and Tam, while the expression of Bcl-2, a chemoresistance factor, increased.

**Conclusion :** These results suggest that equol has a concentration-dependent antitumor effect in HR-positive breast cancer cell lines and may antagonize the effect of existing therapeutic agents (hormone drugs and chemotherapeutic agents). In particular, it was considered that in combination of equol and Tam, the apoptosis inducing action of each drug was attenuated and antagonistic effect was shown.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-05-07

Differential expression and localization of beta-catenin and HSP27 after cisplatin/doxorubicin treatment in triple negative breast cancer cells

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The treatment of triple-negative breast cancers (TNBC) involves the administration of the conventional chemotherapeutic drug doxorubicin, given the lack of specific targeted agents. Novel therapeutic strategies, such as cisplatin, are currently being tested for these patients. Many studies have demonstrated that aberrant Wnt/ $\beta$ -catenin signaling serves a role in the development of breast cancer, while others have concluded that abnormal regulation of Wnt pathway induces tumor cell chemoresistance. The small heat shock protein 27 (HSP27) is overexpressed in human breast cancer cells. As a result, cancer cells may suppress apoptosis and develop resistance to antineoplastic agents, such as doxorubicin. The present study sought to examine the role of the Wnt/ $\beta$ -catenin and HSP27 signaling pathway in response to cisplatin (CisPt)/ doxorubicin (Doxo) treatment in human triple-negative (TN) breast cancer cell lines.

**Material and Methods:** MDA-MB231 (TN) and MCF10A cell lines were used. Cell viability was measured using MTT assay and IC<sub>50</sub> values were obtained after 48 h of CisPt or Doxo exposition. Cellular senescence was assayed by measuring SA $\beta$ -galactosidase (SA- $\beta$ -Gal) activity. Total and active  $\beta$ -catenin, HSP27, phospho HSP27, GSK3 $\beta$ , phospho GSK3 $\beta$ , p38 and phospho p38 expressions were measured by western blot and immunofluorescence. Apoptosis was measured by cleaved Caspase-3 immunofluorescence.

**Results:** MDA-MB231 cells showed higher IC<sub>50</sub> values for CisPt and Doxo than the MCF10A cell line. Increased numbers of senescent cells (larger and flatter) were observed in both MDA-MB231 and MCF10A cells exposed to the IC<sub>50</sub> dose of Doxo. In MDA-MB231 cells, CisPt treatment induced caspase-3 cleavage.

In MDA-MB231 cells, the expression of  $\beta$ -catenin, active  $\beta$ -catenin, total and phospho-GSK3 $\beta$  and total HSP27 significantly decreased in the CisPt group ( $p < 0.05$ ). No changes were observed in Doxo-treated group. In MCF10A cells, the expression levels of total and active  $\beta$ -catenin did not modify with CisPt treatment, but in the Doxo group the protein have a tendency to increase. Also in MCF10A Doxo treatment significantly decreased the expression of GSK3 $\beta$  in comparison with control ( $p < 0.05$ ). In contrast, CisPt administration significantly increased phospho-GSK3 $\beta$  expression respect to the control group ( $p < 0.05$ ). Interestingly, in MDA-MB231 cells the nucleolus appeared disaggregated and active  $\beta$ -catenin increased at this subcellular localization after CisPt and Doxo treatment, in contrast, total  $\beta$ -catenin was preferentially localized in the Golgi.

**Conclusions:** CisPt treatment was associated with absence of senescence, and decreased expression of  $\beta$ -catenin and HSP27. While in Doxo-treated cells, senescence phenomenon was related to stable levels of  $\beta$ -catenin and increased expression of HSP27. The differential expression and localizations of  $\beta$ -catenin and HSP27 could be related to a differential cellular response depending on the cytotoxicity mechanism of chemotherapeutic agent used, that in turns affect the cell fate decision. Our preliminary data indicate that  $\beta$ -catenin and HSP27 may be potential therapeutic targets in TNBC. G.E.P. and M.E.C. contributed equally to this work.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-05-08

An assessment of the potential role of intracellular  $\text{Ca}^{2+}$  store regulators in breast cancer cells

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**Background:** Calcium ( $\text{Ca}^{2+}$ ) regulates many crucial cellular processes including cell survival, proliferation and death. Endoplasmic reticulum (ER)  $\text{Ca}^{2+}$  store levels are critical in the death-inducing effects of some anti-cancer agents. Modulators of ER  $\text{Ca}^{2+}$  signalling, such as neuronal calcium sensor-1 (NCS-1) may therefore represent new therapeutic opportunities to enhance the effect of some anti-cancer agents. NCS-1 is associated with poorer survival in breast cancer patients. However, the expression of NCS-1 in specific breast cancer molecular subtypes and its potential role in intracellular  $\text{Ca}^{2+}$  signalling in breast cancer cells has not been fully explored.

**Aim:** To assess expression of NCS-1 in breast cancer molecular subtypes and assess the effect of silencing NCS-1 on intracellular  $\text{Ca}^{2+}$  homeostasis and on sensitivity to doxorubicin treatment in MDA-MB-231 breast cancer cells.

**Methods:** NCS-1 levels were assessed in breast cancer molecular subtypes based on PAM50 subtyping using the TCGA public breast cancer database. Intracellular  $\text{Ca}^{2+}$  changes as a consequence of siRNA-mediated silencing of NCS-1 were evaluated using a Fluorescence Imaging Plate Reader (FLIPR) in MDA-MB-231 cells expressing the genetically-encoded  $\text{Ca}^{2+}$  indicator GCaMP6m. The effect of NCS-1 silencing on MDA-MB-231 cells treated with doxorubicin (0.03 and 1  $\mu\text{M}$ , 24 h) was evaluated by cell nuclear enumeration (Hoechst 33342 staining) and the percentage of dead cells (propidium iodide staining). Images were acquired using an automated epifluorescence microscope (ImageXpress Micro).

**Results:** Levels of NCS-1 were higher in the basal molecular subtype compared to other molecular subtypes. NCS-1 silencing promoted cell death induced by 1  $\mu\text{M}$  doxorubicin. NCS-1 silencing had no major effect on cytosolic free  $\text{Ca}^{2+}$  levels as a result of either IP3-mediated  $\text{Ca}^{2+}$  store release with the purinergic receptor activator ATP or the protease activated receptor activator trypsin. However, NCS-1 silencing suppressed constitutive  $\text{Ca}^{2+}$  influx in MDA-MB-231 breast cancer cells. The expression of NCS-1 was positively correlated with the  $\text{Ca}^{2+}$  entry channel Orai1 in breast cancers on the TCGA database. Orai1 is associated with increased migration and invasiveness in some breast cancers.

**Discussion:** These studies suggest that elevation in NCS-1 is a feature of breast cancers of the basal molecular subtype and that NCS-1 is a regulator of the cytotoxic effects of doxorubicin. Further studies are required to determine if this effect is related to changes in constitutive  $\text{Ca}^{2+}$  influx that may be mediated via Orai1.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-06-01

The effect of a subcutaneous combination of testosterone (T) and anastrozole (Ai) (HAVAHT+Ai™) on volumetric mammographic breast density (MBD); an open labelled cohort study

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MBD is the single greatest modifiable risk factor for the development of breast cancer; this risk can be lessened by reducing MBD. Previous studies suggest breast cancer risk is reduced in women treated with subcutaneous HAVAHT+Ai™. As well, HAVAHT+Ai™ has been suggested to be effective in managing hormone deprivation symptoms in women who have had breast cancer and in perimenopausal women with hormonal dysfunction.

HAVAHT+Ai™ alters the androgen/estrogen (A/E) ratio in breast tissue by delivering more bioavailable T in the presence of Ai to tissue known to contain high levels of 5alpha-reductase and aromatase, respectively facilitating production of the potent androgen dihydrotestosterone and diminishing conversion of T to estradiol. Alteration of the A/E ratio in favor of an androgenic environment reduces MBD. This study evaluates the effect of HAVAHT+Ai™ on volumetric MBD.

**Methods:** 652 women attending Wellend Health Clinic (a clinic that manages perimenopausal symptoms and/or high risk of breast cancer) received HAVAHT+Ai™ and evaluated for MBD change as determined by a fully-automated volumetric density analysis (VolparaDensity™). Women received a HAVAHT+Ai™ subcutaneous implant every 3-4 month containing 1mg/kg T and a fixed dose of Ai 1-4mg. 142 women had 2 or more mammograms within the analysis period and were included in the final analysis. A restricted analysis set (RAS) of 89 of these women were compared with a matched 65 women undergoing mammographic screening for high risk, but not receiving hormonal therapy. Safety and tolerability data were collected and analyzed.

## Results:

**Least squares mean estimated (LSME) change in % volumetric breast density (%VBD), by cumulative testosterone dose and time from first implant.**

table1

Cumulative testosterone dose	Time from first implant (yrs)	LSME (%)	Lower 95% CI	Upper 95% CI	p-value
500 - <700mg	1	-1.13	-2.42	0.17	0.087
500 - <700mg	2	-1.69	-3.01	-0.38	0.012
500 - <700mg	3	-2.26	-4.23	-0.29	0.025
700+mg	1	-1.87	-3.62	-0.12	0.036
700+mg	2	-2.34	-3.88	-0.79	0.003
700+mg	3	-2.80	-4.66	-0.95	0.003

**LSME change in absolute mammographic breast density (AVBD), by cumulative testosterone dose and time from first implant.**

table2

Cumulative testosterone dose	Time from first implant (yrs)	LSME (cm3)	Lower 95% CI	Upper 95% CI	p-value
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500 - <700mg	1	0.3	-12.3	12.8	0.965
500 - <700mg	2	-4.2	-19.8	11.4	0.595
500 - <700mg	3	-8.7	-34.1	16.7	0.499
700+mg	1	-7.8	-26.3	10.7	0.405
700+mg	2	-22.0	-39.5	-4.5	0.014
700+mg	3	-36.2	-59.7	-12.7	0.003

**Comparison with control group:** Analysis of RAS (n=89) versus untreated controls (N=65) revealed statistically significantly different from zero for both %VBD and AVBD between the 2 groups, respectively 2.36%, (95% CI on the difference, from 1.15 to 3.56) and 18.0cm<sup>3</sup>, (95% CI on the difference, from 4.8 cm<sup>3</sup> to 31.1cm<sup>3</sup>).

**Summary of safety:** The treatment was well tolerated with no serious adverse events.

**Conclusions:** HAVAH *T+Ai*<sup>TM</sup> therapy significantly reduces volumetric MBD with good safety and tolerability. An accumulative dose of greater than 700mg of T and 30mg of Ai over 2-3 years is required to achieve a similar reduction in MBD as that demonstrated with tamoxifen as a preventative agent for breast cancer.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-02

Development of a first-in-class small molecule inhibitor (EC359) targeting oncogenic LIF/LIFR signaling for the treatment of triple negative breast cancer

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**Background:** Leukemia inhibitory factor (LIF) and its receptor LIFR are over-expressed in multiple solid tumors and play a key role in tumor growth, progression, and resistance to standard anti-cancer treatments. Triple-negative breast cancer (TNBC) lacks targeted therapies and represents a disproportional share of breast cancer (BCa) mortality. TNBC exhibits autocrine stimulation of the LIF/LIFR axis and overexpression of LIF is associated with poorer relapse-free survival in BCa patients. LIF signaling also promotes maintenance of stem cells. Therefore, targeting the LIF/LIFR axis may have therapeutic utility in TNBC.

**Methods:** We rationally designed a small organic molecule (EC359) that emulates the LIF/LIFR binding site and functions as a LIFR inhibitor from a library of compounds. *In silico* docking studies were used to identify the putative interaction of the EC359 and LIF/LIFR complex. Direct binding of EC359 to LIFR was confirmed using surface plasmon resonance (SPR) and microscale thermophoresis technique (MST) assays. *In vitro* activity was tested using Cell-Titer Glo, MTT, invasion, and apoptosis assays. Mechanistic studies were conducted using Western blot, reporter gene assays, and RNA-seq analysis. Xenograft, patient-derived xenograft (PDX), and patient-derived explant (PDEX) models were used for preclinical evaluation and toxicity.

**Results:** Molecular docking studies showed that EC359 interacts at the LIF/LIFR binding interface. SPR and MST studies confirmed direct interaction of EC359 to LIFR. EC359 reduced the growth of TNBC cells with high potency (IC<sub>50</sub> 50-100nM) and promoted apoptosis. Further, EC359 treatment reduced invasion and stemness of TNBC cells. EC359 activity is dependent on the expression levels of LIFR and showed little or no activity on TNBC cells that have low levels of LIFR or ER+ve BCa cells. Further, EC359 significantly reduced the viability of cisplatin and taxane-resistant TNBC cells and enhanced the efficacy of HDAC inhibitors. Mechanistic and biochemical studies showed that EC359 interacts with LIFR and effectively blocking LIF/LIFR interactions. EC359 also blocked LIFR interactions with other LIFR ligands such as oncostatin M, ciliary neurotrophic factor, and cardiotrophin-1. EC359 treatment attenuated the activation of LIF/LIFR driven pathways including STAT3, mTOR, AKT, and MAPK. RNA-seq analysis identified regulation of apoptosis as one of the important pathway modulated by EC359. In TNBC xenograft and PDX assays, EC359 significantly reduced tumor progression. Further, using human primary BCa PDEX cultures, we demonstrated that EC359 has the potential to substantially reduce the proliferation of human BCa. Pharmacologically, EC359 exhibited high oral bioavailability and long half-life with a wide therapeutic window.

**Conclusions:** EC359 is a novel targeted therapeutic agent that inhibits LIF/LIFR oncogenic signaling in TNBC via a unique mechanism of action. EC359 has the distinct pharmacologic advantages of oral bioavailability, *in vivo* stability, and is associated with minimal systemic side effects. (DOD BCRP grant #BC170312)

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-03

Insulin receptor isoform signaling in breast cancer

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Recent data shows that insulin receptor (IR) and the type I insulin-like growth factor receptor (IGF1R) play important roles in breast cancer cell biology. Targeting only IGF1R has not been successful perhaps due to compensation by IR. IR exists in two isoforms, fetal isoform IR-A is a splice variant of IR which excludes exon 11. The adult/metabolic isoform IR-B is the predominant species expressed in normal tissues, while the fetal form IR-A is more highly expressed in breast cancer. IR-A mRNA in endocrine resistant cells is expressed at levels 24-fold higher than IGF1R expression (Gradishar, et al. Clin Cancer Res 22:301 2016 PMID: 26324738). In addition to its homodimer, IR-A can also dimerize with IGF1R to form a hybrid. Homodimer IR-A responds to IGF-II and insulin, while IGF1R/IR-A hybrid can also respond to IGF-I. Previous studies in our lab showed down regulation of IGFIR increases the sensitivity of breast cancer cells to insulin. To further investigate the roles of IR-A and IR-B in breast cancer biology, we cloned IR-A and IR-B isoforms into pLV-mCherry and pLJM1EGFP lentiviral expression vectors. Then MCF-7L breast cancer cells were infected with IR-A, IR-A vector control, IR-B and IR-B vector control. Both pooled and single clones were studied. Our results showed that both IR-A and IR-B were highly expressed in MCF-7L cells with the introduced IR tagged species present at a higher molecular weight confirmed by immunoprecipitation and immunoblot. The lower migrating species was endogenous IR. IR-A-pool/Clones had strong basal IR tyrosine phosphorylation, while IR-B-pool/clones did not. This basal phosphorylation did not activate downstream signaling as measured by pAKT and pErk1,2. IR-A basal phosphorylation was completely inhibited by BMS754807 (0.3µM), a dual IGFIR/IR tyrosine kinase inhibitor. Total levels of IRS1, IRS2, IGF1R, ERα, AKT and Erk1,2 levels were not changed in the over-expressing cells. Ligands-IGF-I (5nM), IGF-II (10nM) and Insulin (10nM) stimulated IGF1R/IR, IRS, AKT and Erk1,2 phosphorylation in IR-A-pool and high expression clone IR-A-G5. Cells expressing IR-A activated downstream signaling (IRS, AKT and Erk1,2) at 0.1nM insulin, while IR-A vector control cells required 1nM insulin. IR-B-pool overexpressing cells were not more sensitive to insulin compared to parental cells. Insulin significantly increased Erk1,2 phosphorylation in IR-A-G5 cell while IGF-I stimulation was minimal. In contrast, Erk1,2 phosphorylation in parental cells and vector control cells was primarily mediated by IGF-I, not insulin. IR-A overexpressing cells were stimulated in monolayer growth by insulin. These data show that IR-A expression, as seen in endocrine resistant breast cancer cells, sensitizes breast cancers to low concentrations of insulin. Thus, IR-A expression could serve as a target in breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-04

Enhancing abiraterone acetate efficacy in androgen receptor-positive triple negative breast cancer: Chk1 as a potential target

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**Purpose:** Our aim was to identify predictive factors of abiraterone acetate (AA) efficacy and putative new druggable targets in androgen receptor (AR)-positive triple-negative breast cancer (TNBC) treated in the UCBG 2012-1 trial.

**Material and methods:** We defined AA response as either complete or partial response, or stable disease at 6 months. Using Ampliseq, we sequenced 91 general and breast cancer-associated genes from the tumor DNA samples. We analyzed transcriptomes from the extracted RNA samples on a Nanostring platform and performed immunohistochemistry (IHC) on tumor samples using tissue microarrays. We assessed AA and CHK1 inhibitors (GDC-0575 and AZD7762) efficacies, either alone or in combination, on cell lines grown in vitro and in vivo.

**Results:** Classical IHC apocrine markers, including AR, FOXA1, GGT1 and GCDFP15, allowed identifying AA responders and non-responders. All responders have clear apocrine features. Transcriptome analysis revealed that 31 genes were differentially expressed in the two subgroups, 9 of them being linked to proliferation and DNA damage repair. One of the most significant differences was the overexpression in non-responders of CHEK1, a gene encoding Chk1, a protein kinase that can be blocked by specific inhibitors. In vitro, AA and Chk1 inhibitor combination showed additive or slightly synergistic effect on cell viability, cell cycle, apoptosis and accumulation of DNA damages. In vivo, orthotopic xenograft experiments confirmed the efficacy of this combination therapy.

**Conclusions:** This study suggests that apocrine features can be helpful in the identification of AA-responders. We identified Chk1 as a putative drug target in AR-positive TNBCs.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-05

TP-0903, an AXL kinase inhibitor, reduces inflammatory breast cancer aggressiveness and macrophage polarization through additional mechanisms that may include JAK2 and Aurora B

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**Background:** Inflammatory breast cancer (IBC) is the most lethal and aggressive type of breast cancer; it accounts for 2-4% of breast cancer cases but causes 8-10% of breast cancer deaths. Novel targeted therapy to improve the outcomes of patients with IBC is urgently needed. The receptor tyrosine kinase AXL is a driver for metastasis and drug resistance in various cancers, including breast cancer. Our previous work showed that AXL signaling contributes to the aggressiveness of IBC. In addition, emerging evidence indicates that the tumor microenvironment components, particularly tumor-associated macrophages, are critical drivers of the IBC clinical phenotype and promote IBC metastasis. AXL signaling has been shown to modulate the tumor microenvironment. In the present study, we investigated the impact of TP-0903, a small-molecule AXL kinase inhibitor, with additional activity against Aurora B and Janus kinase 2 (JAK2), on IBC cells and macrophage polarization.

**Methods:** The effects of TP-0903 on IBC cell proliferation, migration/invasion, and mammosphere formation were analyzed. The effects of TP-0903 on the polarization of human monocytic cells THP-1 were tested *in vitro*. In addition, the signaling pathways involved in TP-0903-regulated M2 macrophage polarization were investigated using Western blotting.

**Results:** The half-maximal inhibitory concentration (IC<sub>50</sub>) of TP-0903 in an array of IBC cells (including SUM149, SUM190, BCX010, FC-IBC-02, MDA-IBC-3, and KPL4) ranged from 66 nM to 346 nM, suggesting a strong cell growth inhibitory effect. TP-0903 treatment decreased the migration, invasion, and mammosphere formation of IBC cells. In addition, TP-0903 inhibited both AXL signaling and Aurora B activation, which induced a G2/M cell cycle arrest in IBC cells. Based on the importance of AXL and JAK2 in the regulation of the tumor microenvironment, we showed that TP-0903 decreased expression of CD163/CD206 and the CCL17/CCL18 cytokine, and markers of M2 macrophages, suggesting that TP-0903 treatment inhibits the polarization of THP-1 cells to M2 macrophages *in vitro*. We also found that TP-0903 treatment decreased the phosphorylation of STAT6, a critical molecule in M2 polarization, and that knockdown of STAT6 expression decreased M2 macrophage polarization, indicating that TP-0903 may regulate macrophage polarization via STAT6 signaling.

**Conclusion:** Our results demonstrated the dual functions of TP-0903 targeting of both IBC cells and macrophages, possibly via the targeting of multiple kinases, including AXL and Aurora B. Examinations of the impact of TP-0903 on the cross-talk between IBC cells and macrophages *in vitro* and *in vivo* and the related mechanisms are ongoing and will be presented at the meeting.

# 2018 San Antonio Breast Cancer Symposium ®

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Targeting loss of isoenzyme diversity as a novel therapeutic strategy in breast cancer

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**Background:** Several metabolic steps are mediated by distinct proteins or isoenzymes that catalyze the same reaction, providing redundancy of metabolic functions. Metabolic states are frequently altered in cancer to support survival and proliferation in hypoxic and otherwise hostile microenvironments, and metabolic re-wiring often involve loss of isoenzyme diversity. We hypothesize that targeting enzymes that have lost isoenzyme diversity in cancer, but not in normal cells, provides an opportunity to selectively target cancers. In this study, we assessed mRNA expression of all known human isoenzyme families in breast cancer and normal breast tissue and identified isoenzymes with loss of diversity within each breast cancer subtype.

**Methods:** We obtained RNAseq data from cancer and patient-matched normal breast tissues from the TCGA (N=66 HR+, N=24 HER2+, and N=15 TNBC tumors). We retrieved annotated human isoenzyme families from the ENZYME nomenclature database. We compared expression in cancer and matched normal samples from the same patient to identify isoenzymes that had i) same or increased expression of the target isoenzyme in cancer vs normal and ii) reduced expression of the complementary isoenzymes in cancer. We developed five scores that capture various elements of these characteristics and prioritized candidates as targets based on clustering and their combined ranking based on the five scores. We validated overexpression of the candidate isoenzymes relative to other isoforms in breast cancer microarray data from ArrayExpress (E-GEOD-76250: 33 TNBC, and E-GEOD-70951: 30 TNBC, 108 HR+, 10 HER2+).

**Results:** We identified 321 enzymes in the TCGA discovery cohort that correspond to 829 unique isoenzymes. Overall, 636, 483 and 429 isoenzymes were differentially expressed in HR+, HER2+ and TNBC cancers, respectively, compared to corresponding normal samples. Of these, 308 isoenzymes were differentially expressed relative to normal in all 3 subtypes. In all, 112 and 92, and 84 were selected as candidate isoenzyme therapeutic targets in HR+, HER2+ and TNBC, respectively. 23 isoenzymes prioritized in clustering step were further validated. Finally, 6 isoenzymes were validated in HR+ (ALDOA, GUSB, GYG1, MIF, P3H1, PCK2), 10 in HER2+ (ALDH1L2, ALDOA, GLYATL2, GUSB, GYG1, GYS1, MIF, P3H1, PCK2, PTGS1) and 12 in TNBC (ADSS, ALAS1, ALDH1L2, ALDOA, ART3, GLYATL2, GUSB, GYS1, HS3ST1, MIF, PCK2, SOAT1), as potential targets for breast cancer treatment. Of these, 5 potential isoenzyme targets (ALDH1L2, GUSB, GLYATL2, MIF, PCK2), which were mostly hydrolases and transferases, were further selected for ongoing experimental validation in the laboratory. Decreased expression of the complementary isoforms of these 5 targets were primarily due to DNA methylation of the genes in cancer.

**Conclusions:** We found that loss of isoenzyme diversity is a broad phenomenon in breast cancers that may be explored therapeutically. We identified several instances of “isoenzyme addiction” in which cancers depend exclusively on a single isoenzyme while downregulating via methylation the complementary isoenzymes, providing cancer-specific targeting opportunities. We are currently validating several of these targets in cell line models.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-07

Investigating the role of focal adhesion kinase in regulating CSC activity in invasive ductal carcinoma

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## Background

Breast Cancer Stem-like Cells (BCSCs) have been associated with tumour development, metastasis and recurrence<sup>1</sup>. Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase classically known for its role in metastasis, proliferation and survival. We have previously shown FAK plays a role in regulating CSC activity in DCIS<sup>2</sup>. We aimed to investigate FAK and CSC marker expression in a retrospective patient cohort. We aimed to evaluate the effects of FAK inhibition on CSC activity in Invasive Ductal Carcinoma.

## Methods

Using a retrospective case-control cohort of 244 patients across a range of molecular phenotypes we evaluated FAK Immunohistochemical expression alongside CSC markers; Aldehyde Dehydrogenase 1 (ALDH1) and Integrin Alpha 6 (ITGa6). FAK expression was measured in IDC cell lines and ALDEFLUOR high expressing cells. FAK was inhibited using 0.5µM VS4718 or SiRNA and CSC activity evaluated in 5 cell lines and 25 patient samples. We determined the effects of 50mg/kg VS4718 for 4 weeks as single agent or in combination with Paclitaxel 7.5mg/kg in a ER-/PR-/HER- Patient Derived Xenograft model (PDX).

## Results

Total FAK expression was associated with reduced breast cancer survival. Co-expression of FAK and either BCSC marker was associated with the poorest survival.

FAK and CSC marker expression

	pFAK	tFAK	ALDH1	ITGα6	tFAK and ALDH1	tFAK and ITGα6
Recurrence Risk	0.58 (0.31-1.08) p = 0.084	2.05 (1.23-3.43) p = 0.006	2.21 (1.20-4.05) p=0.011	1.54 (0.92-2.23) p=0.107		
Breast Cancer Death	0.41 (0.12-1.51) p=0.182	4.84 (2.33 -10.04) p = <0.001	6.58 (1.87-23.10) p=0.003	2.23 (1.08-4.58) p=0.030	16.7 (3.7-73.9) p=<0.001	12.8 (1.37-13.2,) p=0.012

Hazard ratios calculated using cox-proportional hazard regression analysis.

pFAK was higher in ALDEFLUOR expressing cells and triple negative cell lines. SiRNA knockout of FAK reduced mammosphere formation, self-renewal and ALDEFLUOR expression from 1.2% to 0.2% (p=<0.01, unpaired t-test) in MDA-MB-231 cells. VS4718 reduced primary mammosphere forming efficiency in all cell lines and reduced self-renewal in ER negative cell lines. FAK inhibition led to a reduction in mammosphere forming efficiency and self-renewal in 25 primary breast cancer specimens as outlined below:

FAK inhibition reduces MFE

	Primary Breast Cancer samples			ER negative cell lines		
	ER+/PR+/ Her2-	ER-/PR-/Her2+	ER-/PR-/Her2-	SKBr3	MDA-MB-231	SUM159

Primary mammosphere formation	51.1% (n=17)	53.2% (n=4)	49.6% (n=5)	74.1%	84.8%	67.6%
Secondary mammosphere formation	45.8% (n=8)		43.9% (n=3)	42.1%	57.9%	47.5%

Percentage of mammospheres formed given relative to control. Cell line work, minimum n=6. All above significant  $p < 0.05$ , One way Anova used with post hoc Tukeys test.

VS4718 reduced tumour growth, Ki67 staining and CSC activity in our triple negative PDX model. VS4718 administration reduced ex-vivo mammosphere formation, tumour initiating capacity and prevented ALDEFLUOR enrichment when used in combination with Paclitaxel.

## Conclusions

FAK, ALDH1 and ITGa6 are associated with increased breast cancer mortality in early breast cancer. Inhibition of FAK reduces CSC activity *in vitro* and *in vivo* in cell lines and patient samples. This data suggest that FAK inhibition may be used to reduce CSC activity in triple negative carcinoma.

1. Williams et al, Stem cells 2015.

# 2018 San Antonio Breast Cancer Symposium ®

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Identification of oncogenic gene fusions in triple-negative breast cancer metastatic to the brain

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**BACKGROUND:** As many as 24% of breast cancer (BC) patients develop metastasis to the brain and 27% of these occur in patients with triple negative (TN) BC. The brain (#2) and lung (#1) are the most common sites of TNBC metastasis. Half of women with metastatic (m) TNBC will die within one year. Novel therapies for women with mTNBC, especially to the brain, are desperately needed. TNBCs often exhibit genomic instability, resulting in gene fusions and other chromosomal rearrangements. Such alterations are long recognized as oncogenic drivers and effective drug targets in hematologic and other solid malignancies. We aimed to identify these alterations in mTNBC with Segmental Transcript Analysis (STA), a novel population-based algorithm ranking genes based on likelihood of harboring a gene rearrangement by identifying abrupt changes in transcript expression.

**RESULTS:** We performed RNAseq on 25 mTNBC, 4 matched primary BC, 2 normal cerebellum and 2 normal cerebral cortex samples. TNBC subtype analysis showed an enrichment of basal-like (BL) 2 and mesenchymal (M) subtypes among mTNBC to the brain compared to primary (p) BC in the TCGA. A principal component analysis demonstrated mTNBC and their matched primaries clustered together, indicating the pairs were more closely related to each other genetically than to other mTNBCs. When the gene expression patterns of pBC were compared with their brain metastases following adjustment for contamination with normal brain tissue, an upregulation of mesenchymal genes (p63/p73 axis) and primitive/embryonic genes (NOTCH and PAX family) were noted in the brain mTNBC. We confirmed a modest to marked upregulation of p63 expression by immunohistochemistry across all TNBC subtypes, most notably in BL1 and BL2. STA found three gene rearrangements: EHF-WT1, NF1-VWDE and JAG1-Chr20. Two of these are potential driver gene rearrangements. The first, in a tumor of M subtype, results from fusion of the 5'UTR of EHF to WT1. The resulting fusion transcript is predicted to encode a truncated WT1 protein; loss of the N-terminal regulatory region of WT1 would likely cause constitutive activation. We also identified a truncating rearrangement in the Notch ligand JAG1 in a tumor of LAR subtype. JAG1 is a membrane bound ligand for the Notch receptor and the breakpoint occurs just distal to the DSL domain essential for Notch interaction. This rearrangement occurs within an intergenic chromosomal region, introduces an abrupt stop codon and generates a truncated protein lacking a transmembrane domain. An intact signal peptide should localize the protein to the endoplasmic reticulum; however, loss of the transmembrane domain would likely lead to secretion rather than membrane insertion. This alteration is predicted to constitutively stimulate Notch activity in a paracrine fashion. If the rearrangement functions in this manner, neutralizing antibodies to the N-terminal portion of JAG1 should have therapeutic efficacy.

**DISCUSSION:** EHF-WT1 and JAG1-Chr20 are potential driver rearrangements novel to brain mTNBC. The JAG1 rearrangement predicts constitutive production of Notch transcription factors and is potentially targetable. Continued interrogation of mTNBC for discovery of additional oncogenic fusions driving BC biology is warranted.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-11

Sortilin targeted therapy in breast cancer with elevated progranulin expression

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**Background:** A major challenge concerning breast cancer therapy is the occasional lack of effects using drugs that target cancer cells unspecifically. One possible explanation for this treatment failure is the existence of the small subpopulation of breast cancer stem cells that are believed to be more resistant towards conventional therapy and possesses the ability to drive tumor formation and disease progression. Cytokines secreted by nearby cells and other factors in the surrounding tumor microenvironment further stimulate the cancer cells, contributing to a heterogeneous and potentially more treatment resistant tumor. Thus, a more specific treatment approach targeting the breast cancer stem cell niche is crucial in preventing disease recurrences. In a cytokine screen, we identified progranulin as one of the main compounds secreted from cells exposed to hypoxia, leading to cancer stem cell propagation. Progranulin is involved in biological processes such as wound healing, inflammation and cancer progression. Progranulin and its receptor sortilin are known to be highly expressed in subgroups of breast cancer and are further associated with a clinically aggressive phenotype.

**Methods/Results:** By carrying out a number of *in vitro* and *in vivo* like screening assays, we demonstrate that progranulin influences the stem cell population in breast cancer and is responsible for spreading a cancer stem cell promoting signal to normoxic tumor areas. In breast cancer, progranulin induces a dedifferentiation process in the receiving cancer cells and expression of cancer stem cell markers together with an EMT-associated gene expression profile, leading to cancer stem cell expansion. By using siRNA and pharmacological inhibition of sortilin, we show that sortilin is a functional receptor of progranulin and is responsible for driving progranulin induced breast cancer stem cell propagation. Supporting the role of progranulin in cancer progression, administration of progranulin in immunocompromised mice induce lung metastasis in our breast cancer xenograft models. The use of different approaches for blocking sortilin, such as sortilin inhibitors, down-modulators or sortilin-targeted antibodies can prevent this dedifferentiation process, both *in vitro* and *in vivo*, making the tumor cells less aggressive and metastatic.

**Conclusion:** Targeting progranulin through its associated receptors is a potential therapeutic strategy for the treatment of patients with breast tumors having elevated progranulin or sortilin expression. By inhibiting the secretion based breast cancer progression, we could possibly block the formation of metastasis and cancer cell infiltration.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-12

Oncogenic potential of Trefoil factor 3 in initiation of mammary carcinoma through suppression of p53 pathway

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## Background

Oncogenic transformation is a complex multistep process where normal cells acquire the hallmarks of cancer, leading to unrestrained outgrowth of malignant clones. Trefoil Factor 3 (TFF3) is a clinically validated and functionally potent oncogene in mammary carcinoma. Elevated TFF3 expression has been consistently observed in mammary carcinoma, being involved in cancer progression. The present study investigates the potential functional role and the underlying mechanisms of TFF3 in promoting oncogenic transformation early in the onset of mammary carcinoma.

## Material and method

Immortalized human mammary epithelial cells (HMECs): HMEC-hTERT, MCF10A and MCF12A, with forced expression of TFF3, were used as *in vitro* models and in an orthotopic xenograft model to study the oncogenic roles of TFF3. Furthermore, microarray analysis, immunofluorescence, and ubiquitination and CHX chase assays were used to examine the involvement of p53 pathway in TFF3 mediated-oncogenic transformation.

## Results

Immortalized HMECs with forced expression of TFF3 exhibited the capacity of anchorage independent growth in the soft agar colony formation assay, which is a hallmark of oncogenic transformation. The forced expression of TFF3 also enhanced 3D growth of the immortalized HMECs in matrigel. Furthermore, immortalized HMECs with forced expression of TFF3 gave rise to orthotopic xenograft tumors in nude mice, which are not observed in mice injected with immortalized HMECs. These observations suggest that TFF3 stimulates the oncogenic transformation of non-malignant immortalized HMECs. In addition, the forced expression of TFF3 promoted aberrant cell proliferation, resistance to apoptosis, and increased cell migration and invasion of the HMECs, all these being important hallmarks of cancer. Here, we showed that TFF3-mediated oncogenic transformation of the immortalized HMEC-hTERT cells is dependent on p53 signaling pathway suppression. Mechanistically, TFF3 downregulated NF-κB (p65)-mediated transcription of p53 through decreasing NF-κB (p65) expression and nuclear accumulation. TFF3 also decreased p53 protein levels through post-transcriptional regulation. The forced expression of TFF3 increased MDM2 expression, resulting in an increased ubiquitin-mediated proteasomal degradation of p53. Moreover, forced expression of TFF3 decreased the cleaved form of MDM2, which is responsible for stabilizing p53 protein. Concordantly, HMECs with forced expression of TFF3 exhibited shorter p53 protein half-life as compared to vector control HMECs.

## Conclusion

In summary, our study highlights the oncogenic potential of TFF3 in the initiation of mammary carcinoma through the suppression of the p53 pathway.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-13

A novel small molecule JMX0293 inhibits the growth of triple-negative breast cancer via suppressing STAT3 and inducing apoptosis

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Approximately 30-40% of breast cancer cases are estrogen receptor negative (ENBC), including the most aggressive triple-negative subtype (TNBC) which spread early and recur frequently with a poor prognosis. ENBCs do not respond to selective ER modulators such as tamoxifen or aromatase inhibitors, and therefore, there is an urgent need of non-ER-based therapies for ENBC and TNBC. We recently developed a novel small molecule JMX0293 based on our lead drug candidate HJC0152. Both HJC0152 and JMX0293 were able to suppress cell proliferation of both ER<sup>+</sup> (MCF-7, T-47D) and ER<sup>-</sup> (MDA-MB-231 and MDA-MB-468) breast cancer cell lines in a dose-dependent manner, with significantly lower toxicity against normal mammary epithelial cells (MCF-10A) *in vitro*. JMX0293 exhibited moderately improved anti-proliferative effects against highly invasive TNBC cell line MDA-MB-231 and ER<sup>+</sup> breast cancer cell line MCF-7 with IC<sub>50</sub> values of 0.92 and 2.24  $\mu$ M, respectively. JMX0293 was also demonstrated to induce apoptosis of MDA-MB-231 cells dose-dependently. Mechanistically, JMX0293 inhibited STAT3 phosphorylation at Tyr705 residue, leading to the down-regulation of Bcl-2 and upregulation of apoptotic proteins including Bax, cleaved-caspase 3 and cleaved-PARP. In addition, JMX0293 reduced the ratio of Bcl-2 to Bax. *In vivo*, JMX0293 treatment for 2 weeks at a dose of 10 mg/kg significantly suppressed the growth of TNBC xenograft tumors in mice with minimal impact on general health and body weight. Our results suggest that JMX0293 induces TNBC cell apoptosis and contributes to its anti-proliferative effect through inhibiting transcription factor STAT3, and JMX0293 could be further characterized and optimized for developing a promising therapeutic for treating ENBC and TNBC. This work was supported by grant (R01 CA231150) from the NIH/NCI.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-14

The role of SLC7A5 (LAT1) in endocrine therapy-resistant breast cancer

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Endocrine therapies are commonly used to treat estrogen receptor-positive (ER+) breast cancers, which comprise 70% of all new breast cancer cases. Unfortunately, emergence of resistance to these therapies presents a major clinical challenge. Cancer cells can adapt to the dysregulation of cellular metabolism induced by endocrine therapy in order to evade cell death. Central to this adaptation is the scavenging of free-formed amino acids from the tumor microenvironment. For example, we found 109 solute carrier (SLC) mRNAs to be differentially expressed between endocrine-sensitive and resistant cells. We began our mechanistic studies of these genes with SLC family 7 member 5 (SLC7A5 or LAT1). SLC7A5 is a key component of a transmembrane transporter, which can complex with CD98 and increase the uptake of large, neutral amino acids (such as leucine or tyrosine). We used a panel of endocrine therapy-resistant (LCC9) and sensitive (MCF7; LCC1) breast cancer cells. SLC7A5 expression was upregulated by estrogen in MCF7 and LCC1 cells; this induction was blocked by fulvestrant treatment. Basal expression of the SLC7A5 protein in the absence of estrogen was 2.75-fold higher in LCC9 cells compared with MCF7 cells; SLC7A5 mRNA expression was 71-fold higher. Fulvestrant treatment did not significantly alter SLC7A5 mRNA or protein expression in LCC9 cells. Inhibiting SLC7A5 function using either a pharmacological inhibitor (JPH203), or depleting expression using siRNA, led to significant suppression of LCC9 cell growth. Cell cycle analysis revealed that SLC7A5 depletion caused cells to accumulate in the G1-phase, with a concurrent reduction of cells in S-phase. In four publicly available datasets of ER+, tamoxifen treated breast cancer patients, high expression of SLC7A5 was significantly associated with poor relapse-free survival.

This study uncovers a novel adaptive mechanism in endocrine therapy-resistant breast cancer cells that is facilitated by increased expression of SLC7A5, which enables them to supplement their increased metabolic needs and promoting cell growth. Blocking the functions of SLC7A5, perhaps in conjunction with inhibition of autophagy, may therefore offer a new avenue of potential therapeutic intervention against endocrine therapy-resistant breast cancers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-15

Rational combination of Wee1 and BCL-2 inhibition in preclinical models of triple-negative breast cancer

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## Background:

Triple-negative breast cancer (TNBC) is an aggressive subtype distinguished by its lack of expression of receptors for estrogen, progesterone, and normal human epidermal growth factor 2 expression. TNBC is difficult to treat and is associated with a high risk of recurrence and mortality. In order to effectively treat TNBC, alternative therapeutic targets need to be identified. Wee1 is a tyrosine kinase that phosphorylates CDC2 to pause the cell cycle at the G2 checkpoint as a means to delay mitosis while DNA damage undergoes repair. Inactivation of Wee1 via adavosertib (AZD1775, MK1775), a highly selective inhibitor of Wee1, allows CDC25 to dephosphorylate the CDC2/cyclin B complex resulting in premature initiation of mitosis and, ultimately, mitotic catastrophe and apoptosis. An unbiased screen of adavosertib in combination with other targeted compounds in TNBC patient-derived xenograft (PDX) models demonstrated that the combination of adavosertib and navitoclax, an inhibitor of anti-apoptotic BCL-2 and BCL-XL proteins, had greater efficacy than the single agents alone. The purpose of this study was to investigate the combination of adavosertib and navitoclax in preclinical TNBC models, both *in vitro* and *in vivo*.

## Methods:

HCC1937, CAL51, MDA-MB-231 and MDA-MB-468 cells were plated in 96-well plates and exposed to increasing concentrations of adavosertib (125nM – 1000nM), navitoclax (2500nM – 10000nM), or the combination. Cellular proliferation was assessed in real-time using IncuCyte Live Cell Analysis, followed by endpoint sulforhodamine B (SRB) assay. Combination effects were analyzed using Calcsyn to determine combination indexes (CI). Apoptosis was assessed via the Caspase 3/7 assay. Western blotting was used to assess changes in expression of CDC2, phospho-CDC2, and BCL2. TNBC PDX models CU\_TNBC\_013 and CU\_TNBC\_014 were treated with vehicle, adavosertib (50mg/kg), navitoclax (100mg/kg), or the combination and assessed for tumor growth inhibition.

## Results:

The combination of adavosertib and navitoclax resulted in greater antiproliferative effects *in vitro* compared to either single agent ( $p < 0.05$ ). This effect was classified as synergistic with CI values  $< 1$ . We observed a significant increase in apoptosis with the combination treatment as measured by Caspase 3/7 ( $p < 0.005$ ). The combination of adavosertib and navitoclax treatment resulted in a decrease in phospho-CDC2, and BCL2 in cell lines. *In vivo*, the combination treatment resulted in greater tumor growth inhibition as compared to adavosertib or navitoclax alone in the CU\_TNBC\_013 and CU\_TNBC\_014 PDX models.

## Conclusions:

The combination of adavosertib and navitoclax is active in preclinical TNBC models and induces apoptosis and tumor growth inhibition. This data supports the continued development of this combination in TNBC with investigation of potential selective markers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-16

New targets in triple negative breast cancer: Role of Oncostatin M receptor pathway

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**BACKGROUND:** Triple negative breast cancer (TNBC) has poor prognosis, lack of targeted therapies and are often refractory to conventional chemotherapy treatments. Therefore, finding new therapeutic targets for those tumours is an unmet need with high clinical impact. In this context, Oncostatin M receptor (OSMR) is a promising therapeutic target as it is over-expressed in this tumour subtype and its activation promotes invasiveness (*Guo L, et al. 2013 Oncogene; West NR, et al. 2014 Oncogene*). We previously showed that OSMR is frequently copy-number gained and over-expressed in squamous cell carcinoma, where it induces migration, invasion and metastasis (*Caffarel MM, et al 2013 Journal of Pathology; Caffarel MM, et al 2014 Journal of Pathology; Kucia-Tran JA, et al. 2016 Brit J Cancer; Kucia-Tran JA, et al 2018 Journal of Pathology*). We now investigate the role of OSMR in breast cancer progression.

**METHODS:** To address this issue we use a wide array of tools including *in vitro* cell cultures and *in vivo* models. The expression of OSMR pathway was analysed in FFPE samples and large datasets of publicly available breast cancer samples (METABRIC, n=1462; and TCGA, n=547).

**RESULTS:** OSMR and its ligand Oncostatin M (OSM) are over-expressed in basal tumours, where they associate with shorter overall survival (p=0.015). While OSMR is expressed by breast cancer cells and cancer associated fibroblasts, the main source of OSM seems to be primarily macrophages. OSM treatment of breast cancer cells induces the expression of important mediators of angiogenesis and invasion. Importantly, OSMR activation accelerates tumour onset, tumour growth and metastasis in orthotopic xenografts in nude mice.

**CONCLUSIONS:** Our results support that OSMR pathway may have an important role in the initiation and progression of breast cancer and that it could be a promising candidate for therapeutic targeting in TNBC. OSMR could be blocked by antibody based inhibition, strategy that has had a major impact on breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-17

A novel interaction of AURKA with MAPK pathway in breast cancer cells as a potential therapeutic target

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**Background:** Aurora A (AURKA) is a mitotic kinase responsible for centrosome segregation and mitotic spindle formation. In normal cells, expression of AURKA is highly regulated and is predominantly restricted to G2/M phases of the cell cycle. Unlike healthy cells, cancer cells overexpress AURKA through all phases of the cell cycle resulting in the acquisition of alternate non-mitotic functions. Little is known about cellular functions regulated by AURKA and its interaction with other signaling molecules. Here, we report a novel interaction between AURKA and the mitogen-activated protein kinase (MAPK) pathway at the level of MEK1 in breast cancer cells. This interaction may serve as a novel target as well as demonstrate by an additive cytotoxic effect of AURKA- and MEK1/2-specific inhibitors against estrogen positive (*ER*<sup>+</sup>) and triple negative breast cancer (TNBC) cells.

**Results:** We show that treatment of *ER*<sup>+</sup> *HER2*<sup>-</sup> MCF-7, *ER*<sup>-</sup> *HER2*<sup>+</sup> SKBR3 and *ER*<sup>-</sup> *HER2*<sup>-</sup> BT549 cells with AURKA specific inhibitors alisertib, MK8745 and Aurora A Inhibitor I resulted in over 2-fold increase in relative levels of pMEK1/2 and pERK1/2 compared to untreated controls. The activation of the MAPK pathway was rapid with changes seen within 5 min after treatment with AURKA inhibitors and was sustained for at least 48 hours. Treatment with the pan RAF inhibitor TAK-632 did not diminish alisertib-induced pERK and pMEK1/2. Alternatively, treatment with the MEK1/2 specific inhibitor PD0325901 completely abrogated alisertib-induced phosphorylation of MEK1/2 and ERK1/2. In situ proximity ligation and pull down assays demonstrated AURKA and MEK1/2 direct interaction. In vitro kinase assay showed direct phosphorylation of MEK1 by AURKA. Combined treatment of alisertib and PD0325901 *in vitro* revealed significant additive cytotoxic effect in MCF-7 and BT549 cells when compared to either agent used alone ( $p < 0.008$  and  $p < 0.011$ ;  $p < 0.04$  and  $p < 0.028$ ) with early trend toward significance in survival in a BT549 xenograft breast cancer in vivo model.

**Conclusions:** Our data shows a novel AURKA-MEK1 interaction in breast cancer cells. In depth in vivo analysis is ongoing. The results reveal a promising new strategy for the treatment TNBC patients using a combination of AURKA and MEK1/2 inhibitors.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-19

The effect of UBE2C expression on intrinsic chemosensitivity in breast cancer cell lines

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**Background:** The ubiquitin-proteasome pathway plays a crucial role in cancer-related processes by inducing cell cycle arrest through the degradation of mitotic cyclins and other cell cycle regulatory proteins. We recently showed that elevated levels of ubiquitin-conjugating enzyme E2C (UBE2C) were associated with aggressive tumor features and unfavorable clinical outcome in breast carcinoma (BC). UBE2C suppression has been achieved using the FDA-approved proteasome inhibitor bortezomib (VELCADE®) in colorectal carcinoma, but little is known about the efficacy of UBE2C-targeted therapy with proteasome inhibitors in breast cancer.

**Methods:** Cell viability assays were used to determine the intrinsic chemosensitivity of five BC cell lines (MCF-7, MDA-MB-436, HCC38, HCC1395, and ZR-75-30; stratified by UBE2C expression and ER status) and the MCF-10A epithelial cell line to proteasome inhibitors (n=8), mitosis inhibitors (n=2), and platinum agents (n=3). UBE2C expression analysis was performed using quantitative real-time PCR and Western blot. IC<sub>50</sub> values and growth inhibition metrics (GR<sub>50</sub> and GR<sub>max</sub>) were calculated for each compound to determine drug potency and efficiency after 24 hour treatment. Proteasome activity was assessed using bortezomib-treated cells.

**Results:** Heterogeneous UBE2C expression levels were found in the different cell lines, with higher UBE2C levels in ER-negative BC cell lines (HCC38, HCC1395, MDA-MB-436) than ER-positive BC (MCF-7 and ZR-75-30) and MCF-10A control cells (ER-negative). Proteasome inhibition levels close to 50% and 100% were seen in all cell lines after 10 nM and 100 - 1000 nM bortezomib, respectively. As expected, bortezomib blocked cell cycle progression by inducing G<sub>2</sub>/M phase arrest in HCC38 cells. Due to differences in cell growth rates, calculation of the IC<sub>50</sub> value was an ineffective method to determine drug potency. In contrast, the normalized growth rate inhibition method with GR<sub>50</sub> and GR<sub>max</sub> values demonstrated a correlation between sensitivity to proteasome inhibitors in ER-negative BC cell lines and high UBE2C expression levels. However, MDA-MB-436 cells (GR<sub>50</sub>, range 1.8-286.1 nM; GR<sub>max</sub>, range -0.42- -0.93) were generally less sensitive to proteasome inhibitors than HCC38 cells (GR<sub>50</sub>, range 8.2-936.8 nM; GR<sub>max</sub>, range -0.97- -0.99) though both cell lines were ER-negative, which was possibly due to the lower expression of UBE2C in MDA-MB-436 cells. Compared with the other tested drugs, no cell line was sensitive to the mitosis inhibitors and platinum agents were most effective in HCC38 cells.

**Conclusions:** Taken together, these findings suggest an association between UBE2C expression and response to proteasome inhibition, regardless of ER status.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-20

CDC20 is a novel therapeutic target in triple negative breast cancer

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**Background:** Approximately 15% of breast cancers lack expression of Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor 2 (HER-2), and are referred to as Triple Negative Breast Cancer (TNBC). Clinical outcomes in patients with TNBC continue to be poor due to inherently aggressive biology of the disease and paucity of targeted therapies. In this study, we identified Cell Division Cycle protein 20 (CDC20), a key regulatory protein that mediates sister chromatid separation in mitosis, as a novel therapeutic target in TNBC.

**Methods:** To uncover new drivers of TNBC, we nominated genes that are significantly overexpressed in patients with TNBC, compared to non-TNBCs. After identifying CDC20 as a top hit in this analysis, we used expression microarrays, RNA-seq analyses and Western blotting to assess CDC20 expression levels in breast cancer patients and cell lines. Kaplan-Meier analyses were performed to study the correlation between CDC20 expression levels and clinical outcomes, including recurrence-free survival, metastasis-free survival and overall survival. shRNA-mediated knockdown of CDC20 in TNBC cell lines was employed to study the role of CDC20 in TNBC proliferation, invasion, migration and xenograft growth in mice.

**Results:** CDC20 was significantly overexpressed in TNBCs (compared to non-TNBCs) in The Cancer Genome Atlas (TCGA) breast dataset, and in several other independent breast cancer datasets, including Curtis, Stickeler and Kao. CDC20 expression was also significantly higher in TNBC cell lines compared to non-TNBC cell lines. Furthermore, expression levels of CDC20 were highly significantly correlated with clinical outcomes, including recurrence-free survival, metastasis-free survival and overall survival. shRNA-mediated knockdown of CDC20 expression in TNBC cell lines, MDA-MB-231 and MDA-MB-468, resulted in decreased proliferation, invasion and migration. Our preliminary findings suggest that CDC20 knockdown also results in inhibition of cell line-derived xenograft growth in mice.

**Conclusion:** We identified CDC20 is a novel therapeutic target in TNBC. Our findings support development of pharmacologic strategies to inhibit CDC20 function as a potential targeted therapy against TNBC.

## 2018 San Antonio Breast Cancer Symposium ®

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Endogenous expression of ER $\beta$  variants contributes towards chemotherapy-resistance in the triple negative breast cancer cell line HCC-1806

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Triple negative breast cancer (TNBC) still remains a challenge to treat in the clinic due to a lack of good targets for treatment. Although TNBC lacks expression of ER $\alpha$ , the expression of ER $\beta$  and its variants are detected quite frequently in this cancer type and can represent an avenue for treatment. We show that the variants of ER $\beta$ , namely ER $\beta$ 1, ER $\beta$ 2, ER $\beta$ 4, and ER $\beta$ 5, contributes to aggressiveness of the TNBC cell line HCC1806 by affecting E-Cadherin expression and chemotherapy sensitivity. We have previously shown that the HCC1806 cell line expresses all the ER $\beta$  variants. To investigate their function we used the recently developed dCas9-KRAB system with Guide RNA for both ER $\beta$  promoters 0N and 0K, using 3 guide RNA's for each promoter and we found that expression of panER $\beta$  decreased from a ct. of 28 to 33 indicating loss of total ER $\beta$ . Treatment of HCC1806 cells with chemotherapy in combination with an ER $\beta$  agonist, LY500307, is more effective in reducing cancer cell viability. Knockdown of total ER $\beta$  in HCC1806 cells using CRISPR-Cas9 technology, decreased the proliferation and increased chemo-sensitivity to docetaxel agent in cells with the 0K promoter knock down, while cells with the 0N promoter knock down were less affected. Furthermore, after ER $\beta$  knockdown, the cell morphology of HCC-1806 changed to a more epithelial phenotype and there was increase in the expression of the classical epithelial marker, E-cadherin. N-Cadherin was unchanged in the cells with the 0K promoter knock down, while E-Cadherin was slightly decreased in cells with the 0N promoter knock down. We also found that cells with the 0K promoter knock down migrated less than control cells in a migration assay, while cells with the 0N promoter knock down were less affected. These findings are in agreement with reports showing that the 0K promoter is responsible for expression of the variants ER $\beta$ 2 and ER $\beta$ 5, while the 0N promoter expresses more ER $\beta$ 1. Furthermore, we have earlier shown that ER $\beta$ 2 and ER $\beta$ 5 increase aggressiveness of TNBC via several oncogenic signaling pathways. In conclusion, knockdown of ER $\beta$  variants in HCC-1806 indicated that the variants may be responsible for conferring oncogenic properties to TNBC such as EMT, cancer cell proliferation and chemo-resistance. Therefore, treating TNBC patients with combination therapy including ER $\beta$  agonists, may prove to be extremely beneficial for securing better treatment responses and future pre-clinical studies should design appropriate experiments to confirm these findings *in vivo*.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-22

PEA15-AA, an unphosphorylatable mutant of PEA15, as a novel therapeutic gene for triple-negative breast cancer

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**Background:** Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by a high rate of metastatic recurrence and poor prognosis. Molecular mechanism underlying the metastatic behavior of TNBC has not been well elucidated, and newer approaches addressing drivers of metastasis are crucial to improving patient outcomes. PEA15 (Phosphoprotein enriched in astrocytes-15) regulates cell proliferation, apoptosis, and autophagy. In breast cancer, PEA15 expression inhibits invasion by binding to ERK and preventing its nuclear translocation. The biological function of PEA15 is tightly regulated by its phosphorylation at Ser104 and Ser116. However, the effect of PEA15 phosphorylation status on TNBC remains unknown. In this study, we tested the hypothesis that unphosphorylated PEA15 will prevent metastasis in TNBC through inhibition of the epithelial-to-mesenchymal transition (EMT).

**Method:** We established stable cells overexpressing unphosphorylatable (PEA15-AA) and phospho-mimetic (PEA15-DD) PEA15 mutants in MDA-MB-468 cells. To dissect specific cellular mechanisms regulated by PEA15 phosphorylation, we performed RT-PCR immune and metastasis arrays. *In vivo* mouse models were used to see effects of PEA15 phosphorylation on tumor growth.

**Results:** The clonogenic growth of PEA15-AA-expressing cells was significantly reduced by 80% compared with empty vector-transfected cells (PEA15-V). Anchorage-independent growth, an indicator of *in vivo* tumorigenicity, was inhibited in cells expressing PEA15-AA by 60% compared with PEA15-V. PEA15-AA upregulated the expression of E-cadherin and decreased the expression of mesenchymal markers, suggesting that PEA15-AA reverses EMT. Compared with PEA15-V, migration and invasion of cells expressing PEA15-AA were reduced by 65% and 72%, respectively. In contrast, PEA15-DD promoted migration, invasion, and expression of mesenchymal markers. To determine the *in vivo* effect of PEA15-AA, we injected stable PEA15 transfectants of MDA-MB-468 cells into the mammary fat pad of NOD/SCID mice. The PEA15-DD-injected group showed greater tumor volumes than PEA15-V and PEA15-AA groups, suggesting that PEA15-AA has antitumor effects both *in vitro* and *in vivo*. From the immune and metastasis arrays, we found that expression level of IL-8, which is known to induce EMT, was greatly decreased by PEA15-AA, while IL-8 was highly expressed in PEA15-DD cells. Addition of recombinant IL-8 to the cells expressing PEA15-AA partially rescued mesenchymal characteristics, increasing migration and expression of mesenchymal markers. By contrast, IL-8 knockdown in PEA15-DD-expressing cells decreased the mesenchymal phenotype. These findings indicate that IL-8 may play an important role as a mediator of phosphorylation of PEA-15 in breast cancer cell migration and invasion and suggest that PEA15-AA inhibits the expression of IL-8, thereby reversing EMT.

**Conclusion:** Taken together, our results show that PEA15 phosphorylation serves as an important regulator, having a dual role as an oncogene or tumor suppressor. Further studies are warranted to evaluate the impact of PEA15 phosphorylation status on metastasis *in vivo*. These findings support the development of PEA15-AA as a potential therapeutic strategy for TNBC.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-23

New biomarkers for adjuvant bisphosphonate use in triple-negative breast cancer

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We first showed that triple-negative breast cancer (TNBC) patients with low Toll-like receptor 9 (TLR9) expression have significantly worse prognosis than patients with high TLR9 expression. TLR9 is an innate immunity DNA receptor which is also expressed in several cancers, including TNBC. Later, we showed that low-TLR9 cells and tumors are more sensitive to amino-bisphosphonates, especially to zoledronate. Bisphosphonates (BP) inhibit osteoclasts and thereby effectively prevent bone fractures in osteoporosis and in cancer-induced bone disease. BPs also have direct anticancer effects. Interestingly, adjuvant BP use is associated with significantly improved progression-free and overall survival, but only among post-menopausal breast cancer patients. The molecular mechanisms behind this observation is unclear.

CD73 is a 70 kDa glycoprophosphatidylinositol-anchored cell protein, which converts adenosine monophosphate to adenosine and organic phosphate. High CD73 expression has been shown to have cancer cell invasion-promoting properties. On the other hand, CD73 is associated with poor prognosis in TNBC. Interestingly, TLR9 and CD73 have been shown to have inverse relationship in T cells and during colonic inflammation. Currently, we study the relationship of CD73 and TLR9 *in vitro* and *in vivo* upon BP treatment.

Our preliminary results show that TLR9 and CD73 have an inverse relationship also in TNBC cells. We use both nitrogen-containing (zoledronate and alendronate) and pyrophosphate-like (clodronate) BPs together with doxorubicin to study TNBC cell responses. We have seen that zoledronate and alendronate, but not clodronate, inhibited 4T1 murine triple-negative mammary tumor cell proliferation in a dose-dependent manner. Also, high concentrations of zoledronate and alendronate significantly inhibited 4T1 cell migration. Additionally, CD73 shRNA cells are more sensitive to zoledronate and alendronate, showing morphological changes and disruption in actin organization.

Our results suggest that CD73 expression may affect treatment responses to BPs in TNBC. Further, low-CD73 tumors could benefit more of BP therapy, compared with high-CD73 tumors.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-06-24

Cannabinoid receptors agonist induces lysosome-mediated cell death in TNBC

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Breast cancer is one of the leading causes of death among women in the United States. Among all the breast cancer subtypes, triple-negative breast cancer (TNBC) is denoted as having the highest rate of mortality due to inefficacious therapy, drug resistance, and high recurrences rate. Despite many therapeutic advances over the past decades, novel therapeutic approaches are required to treat TNBC. Although lysosomes have recently been identified as novel targets in a variety of cancers, not much known about their role in breast cancer especially in TNBC. Due to the altered features of lysosomes in cancerous cells, its functional ability affects cell proliferation, metabolism, adaptation, and autophagy in tumor cells. Thus, the induction of lysosomal membrane permeabilization (LMP) may be used as a novel approach in controlling the aberrant cellular functions in TNBC. Cannabinoid receptor 2 (CB2) is expressed in breast cancer and its agonist JWH-015 has been shown to inhibit TNBC growth and metastasis. However, not much is known about the mechanisms by which JWH015 inhibit growth and metastasis. To investigate JWH015-mediated therapeutic mechanisms, we examined the lysosome-mediated cell death in TNBC cells. We found that JWH015 induces cell death in TNBC cells through lysosomal disruption mechanism. We also showed that JWH-015 treatment increases releases of lysosomal proteins, cathepsin D/B, and autophagy marker LC3B and that leads to activation of apoptotic cell death signaling in TNBC. These alterations result in the formation of autolysosomes to promote LMP. Our findings elucidate the mechanism by which JWH-015 promotes LMP induced autophagy and regulates TNBC cell proliferation. These studies elucidate novel lysosomal-mediated cell death mechanisms through activation of CB2 receptors in TNBC.

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A phenotypic screening and machine learning platform efficiently identifies triple negative breast cancer-selective and readily druggable targets

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Identifying effective oncogenic targets is challenged by the complexity of genetic alterations in cancer and their poorly understood relation to cell function and survival. This, combined with the fact that the majority of the proteome is predicted to be undruggable, may explain why genomic approaches have had only moderate success in identifying and translating oncogenic drug targets. There is a need for methods that rapidly and accurately identify “pharmacologically effective” targets without the requirement for priori knowledge of complex signaling networks. We developed an approach that uses machine learning to relate results from unbiased phenotypic screening of kinase inhibitors to their biochemical activity data. This process, which we call idTRAX (Identification of **D**rug **T**ar**G**ets and **A**nti-targets by Cellular and Molecular **C**ross-referencing), identifies targets that are pharmacologically responsive and readily druggable. Additionally, the identified targets are not typically overcome by the robustness of signaling networks, because only targets that effectively induce a phenotype upon pharmacological engagement are selected. We applied this methodology to triple negative breast cancer, which still lacks targeted therapy. We screened 19 breast cancer cell lines with ~500 small-molecule kinase inhibitors with annotated kinase activity data and identified cell-line specific kinase targets, i.e. kinases whose inhibition causes cell line-specific cytotoxicity or cytostaticity. We were able to identify various unique cell line selective kinase addictions along with well-known driver kinase addictions, for example, dependence on FGFR2 in the MFM-223 cell line and AKT kinases in MFM-223, CAL-148, and others. We also found that triple negative breast cancer cell lines exhibit heterogeneous target patterns, indicating the need of personalized medicine approach to tackle them. We further compared candidate targets identified by idTRAX with those reported from RNAi- and CRISPR-based screens. Strikingly, the correlation between targets identified by the pharmacological approach and those identified by knockdown/knockout-based screens was low [average rank correlation (&#120762;) = 0.0273- 0.1074], suggesting that gene-silencing approaches may not be the most efficient at identifying targets for small-molecule drug discovery or repurposing. Our approach provides a platform for rapidly identifying sample-specific drug targets and potentially guiding personalised therapy regimens.

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Prediction model for lymphoedema, and effect of Lymphoedema diagnosis on quality of life (QoL) and distant recurrence from breast cancer

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## Introduction

Lymphoedema develops after surgery in 30% patients. In a prospective, multi-centre UK study, we used a validated perometer arm measurement to determine 1) the factors predicting lymphoedema development and 2) the effect of a lymphoedema diagnosis on QoL and survival.

## Methods

Participants (n = 1100) undergoing axillary clearance at 9 centres underwent arm volume measurements pre and post-surgery by perometry, and completed FACT-B+4 and Lymphoedema checklist questionnaires. Relative arm volume increase (RAVI) of  $\geq 10\%$  diagnosed lymphoedema. Predictors of lymphoedema development were determined using logistic regression, and changes in QoL were assessed using Generalised Estimating Equation (GEE) analyses.

## Results

Median patient age was 56 (range 22 to 90) years; 78% received radiotherapy and 65% chemotherapy. Lymphoedema was detected in 21.4% of women by perometry and 24.4% underwent sleeve application by 24 months.

Initial decreases in QoL scores post-surgery were greater in patients with lymphoedema and took longer to return to baseline values (FACT-B p=0.014, TOI p=0.008, ARM subscale p<0.001).

RAVI at 1 month (p<0.001), BMI in three categories ( $\leq 25$ ,  $>25$ - $\leq 30$  and  $>30$ , p=0.05), ER status (p=0.05) and number of positive nodes (p<0.001) were used to develop a novel scoring model (AUROC 0.80) to predict lymphoedema. Out of 826 patients used for the model, 75% of patients had low scores ( $\leq 1$ ) at 1 month and 11.6% developed lymphoedema by 24 months, whereas 20% who scored moderate risk (1.5-2) had a 31.3% risk and 5% who scored high risk (2.5-4) and had a 66.7% risk. Using the model scores, 75% of patients could be reassured regarding their lymphoedema risk.

Local recurrence was 1.7%. One hundred and twenty-nine patients had died of breast cancer (n=88) or had distant recurrence (n=41) across the study.

Lymphoedema (RAVI $\geq 10\%$ ) by 9 months was an independent predictor of post 9 months distant disease-free cancer survival (Table).

Distant Disease Free Survival (DDFS) after Lymphoedema diagnosis

	Single variable			Multivariable	
Variable (between 3 and 9 months)	n	Hazard ratio (HR) (95% CI)	p-value	HR (95% CI)	p-value
RAVI $\geq 10\%$ Yes: 105 vs No	955	2.30 (1.39-3.81)	0.001	1.73 (1.01-2.96)	0.047
ER Status Negative: 171	953	2.94 (1.92-4.49)	<0.001	1.734 (1.06-2.83)	0.028
No. positive nodes*	969	1.07 (1.05-1.09)	<0.001	1.05 (1.03-1.08)	<0.001
Adjuvant CT Yes: 654 vs No	963	0.83 (0.54-1.27)	0.39	0.60 (0.37-0.964)	0.035
Hormone treatment Yes: 808 vs No	964	0.45 (0.28-0.72)	0.001	-	-

Tumour size*	959	1.02 (1.01-1.024)	<0.001	1.02 (1.01-1.03)	<0.001
Grade					
0/1: 67	961	1 (-)	<0.001	1 (-)	<0.001
2: 428		3.96 (0.54-29.324)		2.69 (0.36-20.33)	
3: 436		11.59 (1.61-83.54)		6.91 (0.94-50.90)	
Undiff: 30		17.58 (2.12-146.184)		12.54 (1.43-109.96)	

\*Per unit increase

## Conclusions

Lymphoedema is associated with lasting quality of life deficits and early distant relapse.

Women at low risk of lymphoedema (75%) can be reassured using the scoring model. Early Arm measurements at 1 month post-surgery a useful measurement time to determine lymphoedema risk and enable patients to be reassured (75%) or plan for lymphoedema monitoring.



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A newly derived combined clinical treatment score and immunohistochemical-4 prognostic tool

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## AIM

To determine whether a modified Clinical Treatment Score (CTS) based on continuous tumor size and 5 lymph node categories provided more prognostic information in an independent test set than the original CTS with and without the Immunohistochemical-4 (IHC4) algorithm for prediction of residual distant recurrence risk over 10-years.

## BACKGROUND

Risk of recurrence information in patients with estrogen receptor-positive (ER+), early breast cancer informs decision-making on chemotherapy use. The CTS and IHC4 algorithms provide such information, particularly when used in combination (IHC4+C). Their derivation in the translational cohort of the Arimidex Tamoxifen Alone or in Combination trial (TransATAC) was described by Cuzick et al in 2011. In the original model tumor size and nodal status were each classified into three categories, causing prognostic information to be lost.

## METHODS

We modeled a novel CTSn algorithm on outcome data from patients in the anastrozole and tamoxifen arms in ATAC incorporating tumor size as a continuous variable and sub-dividing nodal status into five categories. IHC4n was re-derived in the TransATAC cohort independent of CTSn. Patients were chemotherapy-naïve. We compared ability to predict risk of residual distant recurrence of the new IHC4n+Cn model with that of the original one when tested in a training cohort and in a validation set of chemotherapy-naïve patients from the Tamoxifen vs. Exemestane Adjuvant Multicentre (TEAM) trial using Cox regression models and the C-index.

## RESULTS

The ATAC training set for CTSn comprised 4056 patients, the TransATAC training set for IHC4n comprised 1125 patients; 2591 patients were in the TEAM validation set. Patients in the TEAM set were older (median age in TransATAC: 63.5, TEAM: 68.3 years), had a higher nodal-burden (node-positive in TransATAC: 29.4%, TEAM: 51.8%) and had more Grade 3 tumors (TransATAC: 18.3%, TEAM: 32.2%).

The new IHC4n+Cn was significantly prognostic, and non-significantly more prognostic than the original IHC4+C in both the training and validation cohorts. When assessed using the C-index statistic, IHC4n+Cn had a higher discriminatory ability than the original algorithm (Table 1).

Table 1

	TransATAC (N=1125)		TEAM (N=2591)	
	HR* (95% CI)	C-index	HR* (95% CI)	C-index
Old Models				
CTS	2.26 (2.01-2.53)	0.681	1.88 (1.73-2.03)	0.650
IHC4	1.67 (1.46-1.91)	0.630	1.49 (1.35-1.63)	0.604
IHC4+C	2.76 (2.40-3.18)	0.724	2.03 (1.87-2.21)	0.671
New Models				
CTSn	2.64 (2.26-3.09)	0.721	2.16 (1.96-2.39)	0.687
IHC4n	1.74 (1.52-2.01)	0.642	1.51 (1.36-1.68)	0.603
IHC4n+Cn	2.91 (2.47-3.42)	0.738	2.28 (2.06-2.51)	0.695

(\*Hazard Ratio for change in one Standard Deviation).

## CONCLUSION

By separately remodelling the part of the IHC4+C score based on clinicopathological characteristics using the whole ATAC chemo-naïve cohort, and the part that uses IHC-derived information in chemo-naïve TransATAC patients, we increased the precision of the individual risk estimates produced by both CTSn and IHC4n compared to those given by the original algorithms. The new IHC4n+Cn shows a trend for improved prognostic ability compared to the original IHC4+C. Like its predecessor, it relies on information that is readily available to clinicians and integrates it in an evidence-based way to improve prognostication in ER+ early breast cancer.

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Refining neoadjuvant predictors of three year distant metastasis free survival: Integrating volume change as measured by MRI with residual cancer burden

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**Background:** Patients achieving a pathologic complete response (pCR) following neoadjuvant therapy have significantly improved event-free survival relative to those who do not; and pCR is an FDA-accepted endpoint to support accelerated approval of novel agents/combinations in the neoadjuvant treatment of high risk early stage breast cancer. Previous studies have shown that recurrence risk increased with increasing burden of residual disease (as assessed by the RCB index). As well, these studies suggest that patients with minimum residual disease (RCB-I class) also have favorable outcomes (comparable to those achieving a pCR) within high risk tumor subtypes. In this study, we assess whether integrating RCB with MRI functional tumor volume (FTV), which in itself is prognostic, can improve prediction of distant recurrence free survival (DRFS); and identify a subset of patients with minimal residual disease with comparable DRFS as those who achieved a pCR. Imaging tools can then be used to identify the subset that will do well early and guide the timing of surgical therapy.

**Method:** We performed a pooled analysis of 596 patients from the I-SPY2 TRIAL with RCB, pre-surgical MRI FTV data and known follow-up (median 2.5 years). We first assessed whether FTV predicts residual disease (pCR or pCR/RCB-I) using ROC analysis. We applied a power transformation to normalize the pre-surgical FTV distribution; and assessed its association with DRFS using a bi-variate Cox proportional hazard model adjusting for HR/HER2 subtype. We also fitted a bivariate Cox model of RCB index adjusting for subtype; and assessed whether adding pre-surgical FTV to this model further improves association with DRFS using a likelihood ratio (LR) test. For the Cox modeling, penalized splines approximation of the transformed FTV and RCB index with 2 degrees of freedom was used to allow for non-linear effects of FTV and RCB on DRFS.

**Result:** Pre-surgical MRI FTV is significantly associated with DRFS (Wald  $p < 0.00001$ ), and more effective at predicting

pCR/RCB-I than predicting pCR alone (AUC: 0.72 vs. 0.65). Larger pre-surgical FTV remains associated with worse DRFS adjusting for subtype (Wald  $p < 0.00001$ ). The RCB index is also significantly associated with DRFS adjusting for subtype (Wald  $p < 0.00001$ ). Adding FTV to a model containing RCB and subtype further improves association with DRFS (LR  $p = 0.0007$ ). RCB-I patients have excellent DRFS (94% at 3 years compared to 95% in the pCR group). Efforts are underway to identify an optimal threshold for dichotomizing pre-surgical FTV and FTV change measures for use in combination with pCR/RCB-I class to generate integrated RCB (iRCB) groups as a composite predictor of DRFS.

**Conclusion:** Pre-surgical MRI FTV is effective at predicting minimal residual disease (RCB0/I) in the I-SPY 2 TRIAL. Despite the association between FTV and RCB, FTV appears to provide independent added prognostic value (to RCB and subtype), suggesting that integrating MRI volume measures and RCB into a composite predictor may improve DRFS prediction.

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Prognostic value of residual cancer burden (RCB), neo-bioscore and neoadjuvant response index (NRI) to evaluate response to neoadjuvant trastuzumab-based therapy in HER2-positive breast cancer (BC)

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**Intro** Pathological complete response (pCR) to neoadjuvant systemic therapy is associated with favorable long-term outcome. As pCR is not an optimal surrogate marker for outcome, other tools were developed to predict long-term outcome more accurately, including the RCB<sup>4</sup>, NRI<sup>3</sup>, and Neo-Bioscore<sup>5</sup>. We evaluated the prognostic value of these tools in a cohort of patients with HER2+ BC with the aim of selecting a group of patients with residual disease but a similar long-term outcome as patients achieving pCR.

**Methods** We included all patients with stage II-III HER2+ BC who were treated with trastuzumab-based neoadjuvant therapy and surgery in the Netherlands Cancer Institute between November 2004 and December 2016. Patients were identified from the institutes' tumor registry and data was collected from the patients' records. To assess RCB scores surgical specimens (breast and axilla tissue) of patients without pCR were retrospectively reviewed. NRI and Neo-Bioscore were calculated based on original pathology reports.

Primary endpoint was recurrence-free interval (RFI), defined as time since diagnosis of BC till locoregional or distant recurrence or death from BC, whatever came first. Cox proportional models were used with transformations of RCB, NRI, and Neo-Bioscore. In addition, we evaluated at which cut-off point the NRI could select patients with a similar good prognosis as patients who achieved a pCR, defined by the same lower bound of the 95%CI of the 5-year RFI estimate for the pCR-group.

**Results** 283 women were included, 149 (53%) with HER2+/ER+ BC. 28% received dual HER2-blockade. Median follow-up was 66 months (range 11-148). 157 patients (55%) achieved a pCR in breast and axilla; predicted 5-year RFI for this group was 91% (95%CI 86-96), HR no-pCR vs pCR 2.19, 95%CI 1.07-4.47. Table 1 shows the predicted 5-year RFI and HR for RCB classes. The HR of an RFI event increases gradually for lower NRI values compared to NRI of 1 and gets more steep near NRI values of 0. Patients with a NRI of ≥0.80-0.99 have a 5-year RFI estimate of 90% (95%CI 86-96), HR 1.1 (95%CI 0.6-1.9) compared to patients with NRI of 1 (which is pCR). Table 2 shows the predicted 5-year RFI and HR for the Neo-Bioscore.

Table 1 RCB classes, estimated 5-year RFI and HR

RCB	n	% 5-year RFI	95% CI		HR	95% CI	
0	163	92.6	88.3	97.1	1	1	1
1	39	90.3	85.2	95.6	1.33	0.67	2.65
2	62	78.4	69.4	88.5	3.18	1.42	7.11
3	11	35.3	16.4	76.1	13.60	5.30	34.81

Table 2 Neo-Bioscore classes, predicted 5-year RFI and HR

Neo-Bioscore	n	% 5-year RFI	95% CI		HR	95% CI	
0	19	98.7	95.5	100	1	1	1
1	53	92.4	86.0	99.3	6.10	0.92	40.52

2	93	84.9	77.4	93.0	12.67	0.76	210.40
3	72	89.9	83.8	96.5	8.20	0.62	108.20
4	19	74.9	62.9	89.2	22.33	1.76	283.44
5	3	29.4	10.3	84.0	95.20	6.27	1446.64
6	1	0.6	0	1.00	406.26	19.55	8442.21

**Conclusions** We show that in a HER2+ BC cohort the RCB and NRI are able to identify a subgroup of patients with limited residual disease after neoadjuvant therapy with similar good prognosis as patients with pCR and therefore may not benefit from additional adjuvant therapy.

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# 2018 San Antonio Breast Cancer Symposium ®

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A clinical calculator to predict disease outcomes in women with hormone receptor-positive advanced stage breast cancer treated with first-line endocrine therapy

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**Purpose:** Endocrine based therapy is an effective strategy to manage hormone receptor-positive, human epidermal growth factor receptor 2-negative (HR+/HER2-) advanced breast cancer (ABC). However, nearly all patients exhibit/develop either *de novo* or acquired resistance. While prognostic biomarkers of endocrine responsiveness are well established for the adjuvant treatment in ER+ breast cancer, less is known regarding prognostic and predictive biomarkers of response in the first line ABC setting. We sought to develop a clinical calculator based on clinical criteria for predicting progression-free survival (PFS) and overall survival (OS) of women with HR+/HER2- ABC who will be receiving endocrine monotherapy as first-line treatment for ABC.

**Methods:** The development of the clinical calculator will be based on data from modern clinical trials in women with HR+/HER2- ABC. The studies to be included in the final analyses are given in Table 1. The control arm data from trials 1-6 will form the training dataset (N = 1,223) and be used to construct the clinical prediction models. Variables considered include age, race, ECOG status, disease measurability, body mass index, disease-free interval, number of metastatic sites, locations of metastatic sites, prior endocrine therapy, and prior chemotherapy. Missing values will be imputed using single imputation with all variables included in the imputation model. For continuous variables, restricted cubic splines will be used to determine if non-linear effects may be more appropriate. The Lasso regression will be used as a variable selection technique to reduce the dimensionality of covariates; initially all pairwise interactions will be included in the model. Following Lasso regression, the multivariable Cox proportional hazards models will be constructed for PFS and OS including only variables retained in Lasso. The final model will be internally validated for discrimination and calibration using 10-fold cross-validation. External validation will be performed using control arm data from EGF 30008 (N = 536).

**Results:** To date, control arm data from four trials (trials 1-4) have been received. The preliminary results presented here are based on pooled data from C40503 and LEA, for which data elements have been harmonized. Models for predicting PFS and OS have good calibration and are associated with bias-corrected C-indices of 0.61 and 0.65, respectively. These models will be updated using pooled data from trials 1-6.

**Conclusions:** Our preliminary data demonstrate that clinical calculators based on baseline clinical factors can provide accurate prediction of PFS and OS in patients with HR+/HER2- ABC treated with first-line ET. If validated, these tools may be used for risk stratification in future clinical trials and to identify patients who may require more or less aggressive therapy.

Table 1: Studies to be included

Trial Number	Trial Name	Trial PI	Sample Size in Control Arm
1	C40503	Maura Dickler	152 (letrozole)
2	LEA	Miguel Martin	179 (letrozole)
3	FACT	Jonas Bergh	188 (anastrozole)
4	FALCON	John Robertson	194 (anastrozole)
5	S0226	Rita Mehta	345 (anastrozole)
6	MONARCH 3	Matthew Goetz	165 (nonsteroidal AI)
7	EGF 30008	Stephen Johnston	536 (letrozole)





# 2018 San Antonio Breast Cancer Symposium ®

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Cumulative copy number imbalances after neoadjuvant chemotherapy residual breast tumor is an independent predictor of relapse

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**Background:** Identifying breast cancer patients after neoadjuvant chemotherapy (NAC) at greatest risk of recurrence would enhance selection of patients who may benefit from novel adjuvant treatments.

**Patients.** 243 stage I-III breast cancer patients who underwent NAC with  $\geq 10\%$  residual tumor cellularity were identified from the MD Anderson Cancer Center and Ben Taub General Hospital, Harris County hospital. Tumor DNA was isolated for DNA copy number using OncoScan CNV FFPE, Affymetrix. Median follow-up was 67.8 months. Continuous residual cancer burden (RCB) scores with CNI data were available for 152 cases. To test if CNIs covering large regions were associated with recurrence after adjusting for prognostic variables and study site, data were summed to a chromosome-arm level. Eleven chromosome arms with false discovery rate  $< 0.05$  for breast cancer recurrence were identified. A stepwise multivariable model including age at diagnosis, tumor subtype, histologic grade, pre- and post-treatment stage, study site, and the 11 chromosomal arms were used to fit a parsimonious multivariate model for recurrence. Minimizing the Akaike Information Criterion yielded a final model with post-stage and a 5-arm CNI (5A-CNI) indicator including 2q, 3q, 4q, 10p, and 18p. Tumors were classified on 5A-CNI as 0 [no CNI], 1 [1- 2] and 2 [ $> 2$ ].

**Results.** The study population included 76 non-Hispanic White, 89 Hispanic, and 68 African American patients with a mean age of 49.1 years. 105 patients were classified as 5A-CNI-0, 97 as 5A-CNI-1 and 41 as 5A-CNI-2. A higher 5A-CNI score was associated with tumor grade, ER-negative tumors ( $p < 0.002$ ) and tumor subtype ( $p = 0.014$ ). For 5A-CNI scores of 0, 1 and 2, recurrence rates of 14%, 34% and 58.5% were observed, respectively. In the final multivariable model adjusted for post-stage, RCB and study site, when compared to 5A-CNI-0, the hazard of recurrence was elevated for 5A-CNI-1 (HR= 2.27 [95% CI, 1.01-5.1]) and 5A-CNI-2 tumors (HR=7.43 [95% CI, 2.85-19.39]). Further, while the sample size is limiting, of 10 patients who were RCB3 and 5A-CNI-2, 9 relapsed (90%) during follow-up compared to only 6 of 43 (14%) of RCB3 patients with 5A-CNI-0 ( $p < 10^{-6}$ ). For patients with RCB1 or 2, relapse did not differ by 5A-CNI score. Neither race nor ethnicity were found to be independently associated with recurrence or tumor subtype. However, African American, followed by Hispanic patients, were more likely than non-Hispanic White patients to be classified as 5A-CNI-2 ( $p = 0.013$ ).

Table 1. Significant difference in distribution of 5 arm CNI classifier by Race/Ethnicity in Study Sample ( $p = 0.013$ ).

5A-CNI	0	1	2
Non-Hispanic White	n=44; 57.9%	n=25; 32.9%	n=7; 9.2%
Hispanic	n=32; 36%	n=42; 47.2%	n=15; 16.9%
African American	n=28; 41.2%	n=23; 33.8%	n=17; 25%

**Conclusion.** The 5A-CNI score in post NAC tumor identifies a patient population with very poor prognosis independent of current clinical prognostic factors including RCB. Validation of these findings may lead to a post NAC genomic test that identifies patients who would benefit from additional treatment Further investigation of the nature of the association between the 5A-CNI score and race/ethnicity, which appears independent of tumor subtype, is warranted.

# 2018 San Antonio Breast Cancer Symposium ®

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Development of a machine learning-based classifier for Oncotype DX® category prediction in a population of lymph node positive breast carcinoma patients

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**INTRODUCTION:** Oncotype DX® (ODX) Assay is a valuable prognostic and predictive tool in ER+, Her2- invasive breast cancer (IBC). Initially tested and validated in lymph node (LN) negative patients, the indications of this test have been expanded to include patients with limited LN-positive disease. Several prediction systems have been developed to predict the ODX Recurrence Score (RS) with substantial performance in predicting a high vs low RS score but with low performance when predicting the 3 classes that compose the standard ODX. Additionally, many of these prediction systems have not been developed and/or tested for a population of LN+ patients.

**OBJECTIVES:** The primary objective of this study was to evaluate the performance of several previously published ODX RS predictive systems in a population of LN+ patients. Furthermore, we developed a machine-learning based classification system to accurately predict the ODX 3 category RS for this specific population.

**METHODS:** We conducted a retrospective search of Stanford's pathology database for all patients with LN+ IBC diagnosed between January 2013 and December 2017 with an ODX RS available. A total of 119 patients were identified for inclusion in this cohort. Our multivariate pathologic feature-based discriminatory model aimed to classify each case as belonging to the low, intermediate or high ODX RS category. We performed model validation by the 10-fold cross validation (10F-CV) method. The model's performance was assessed by comparing simple accuracy, balanced accuracy, F1 score (harmonic average of the precision and recall) and several concordance classification metrics.

**RESULTS:** Of the evaluated methods, Magee equations performed well in this population of LN+ patients with the modified Magee equation 2 displaying the best accuracy (70.9%) which was surprisingly better than originally reported (55.8%, in Klein et al. Mod Path. 2013). After an initial screen of methods and tuning of the best performing model, our model achieved an overall accuracy of 78.1% on 10F-CV with a 79.1 % balanced accuracy and no two-step discordances. This corresponded to an increase of weighted Cohen's kappa of 30% versus the best performing Magee equation in this cohort and an increase of 103% versus the modified Magee 1 equation (which uses the same features as our model except for tumor grade).

**DISCUSSION:** Classifiers aimed at providing an alternative to Oncotype DX testing are available and perform consistently across datasets. We are currently validating our approach in a population of 1000 LN-negative patients from the MSKCC and the SEER database. Due to the substantial performance of our machine learning-based classifier based on standard reported pathologic features, our model may be considered an alternative to the ODX standard testing or a screening method for ODX testing, especially for cases with where the cost and availability of the ODX test are a concern.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-07-08

Standardized prediction of Oncotype DX® risk classes by local RT-qPCR

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**Background:** Recent results from the prospective validation of the Oncotype DX® recurrence score (RS) have underlined the clinical validity of the assay for the prediction of chemotherapy benefit in ER+/HER2- early stage breast cancer patients. Due to health economic restrictions, some patients have no easy access to the test. A pre-selection of tumor samples may help identify patients with a high likelihood to be spared chemotherapy. Histology and semi-quantitative IHC are hence used to select samples for Oncotype testing, but these suffer from intra- and inter-observer variability, especially for Ki-67 which is a main factor in most RS prediction algorithms. We have established and validated a tool for the prediction of RS risk classes (TAILORx cutoff RS  $\leq 25$ ) based on highly standardized, reproducible and locally performed RT-qPCR measurements of *ERBB2*, *ESR1*, *PGR* and *MKI67* mRNA using the CE-marked IVD MammaTyper®.

**Methods:** Total RNA was extracted from whole surface 10µm sections from FFPE breast cancer samples with a known RS result and a tumor cell content  $\geq 20\%$ . *ERBB2*, *ESR1*, *PGR* and *MKI67* mRNA expression was measured by RT-qPCR on a CFX96 qPCR cycler using the MammaTyper® kit. A prediction model for an RS  $\leq 25$  result was established using multivariable logistic regression. Based on this model and the training data two cutoffs for confident prediction of low chemotherapy benefit patients in a clinical setting were established at 95% and 97.5% specificity. The model and the cutoffs were then fixed and validated in a second, separate set of breast cancer samples. ROC analysis was used to characterize predictive power of the continuous values resulting from the prediction model. Positive and negative predictive values for detection of an RS  $\leq 25$  result were also determined on the validation samples using the two pre-defined cutoffs.

**Results:** The sample set for training of the prediction model encompassed 202 samples including 29 samples (14.4%) with an RS  $> 25$ . In an initial multivariable model with all four markers, *PGR* and *MKI67* were the strongest predictors while the influence of *ESR* in the model was lower, but still significant. *ERBB2* was no significant predictor in this set of *ERBB2* negative samples and was therefore excluded from the final model which was based on three markers only. This three marker model achieved an AUC of 0.920 (95% CI: 0.871-0.968) in the training samples. When applying the fixed model from the training dataset to a second separately collected set of 104 samples containing 20 samples (19.2%) with an RS  $> 25$ , an AUC of 0.883 (95% CI: 0.810-0.955) was documented. When further applying the two predefined cutoffs established in the training set, 45 and 36 of the 104 validation samples (43.3% and 34.6%) had a predicted low chemotherapy benefit result (RS  $\leq 25$ ). Even with the less stringent cutoff, not a single one of the RS  $> 25$  cases from the validation cohort was falsely predicted as RS  $\leq 25$  sample.

**Conclusion:** We have established a highly reliable method for prediction of Oncotype DX® low chemotherapy benefit results based on local and cost effective mRNA measurements. This method enables local pathologies to pre-assess routine samples using a highly precise molecular tool and thereby reserve the Oncotype DX® test for cases with ambiguous cancer biology.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-07-09

Immune recurrence score (IRS) using 7 immunoregulatory protein expression can predict recurrence in stage I-III breast cancer patients

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**Background:** Immune cells in the tumor microenvironment play an essential role in tumor progression and regression. However, immunologic characteristics and their prognostic role have not been clearly identified in breast cancer patients. This study aimed to evaluate the immunologic characteristics and their prognostic role in breast cancer patients.

**Methods:** This study enrolled pathologically proven stage I-III breast cancer patients. We performed immunohistochemical staining in stromal tumor-infiltrating lymphocytes (TILs) using 10 immune markers (PD-1, PD-L1, PD-L2, IDO, TIM3, OX40, OX40L, B7-H2, B7-H3, B7-H4) with known/possible clinical relevance. Expression of PD-1, PD-L1, and PD-L2 was also measured in adjacent tumor tissue. Intensity and proportion of the staining was measured for each immune marker. The intensity of immunohistochemical staining (IS, intensity score) was graded as follows: 0 (negative), 1 (weak), 2 (moderate), 3 (strong). The proportion of staining (PS, proportion score) was graded as follows: 0 (stain under <1%), 1 (1% to 5%), 2 (5% to 10%), 3 (10 to 25%), 4 (25% to 50%), Grade 5 (> 50%). Immune markers were defined as positive with one of the following; IS 1 with PS over 3, IS 2 with PS over 2, IS 3 with PS over 1.

**Results:** A total of 392 patients, 271 (69.1%) luminal A, 36 (9.2%) luminal B, 32 (8.2%) HER2-positive, and 53 (13.5%) triple negative breast cancers were included. In total, PD-1 was expressed by stromal TILs in 130 (33.2%) patients, PD-L1 in 47 (12.0%), PD-L2 in 109 (27.8%), B7-H2 in 225 (57.4%), B7-H3 in 227 (57.9%), B7-H4 in 106 (27.0%), TIM3 in 111 (28.3%), IDO in 96 (24.5%), OX40 in 137 (34.9%), and OX40L in 165 (42.1%). In addition, PD-L1 was expressed in 15 (3.8%) tumor tissue, PD-L2 in 237 (60.5%), and PD-1 was not expressed in tumor tissue. Each breast cancer subtype showed different immunologic characteristics. Expression of PD-1 (stromal TILs) and PD-L1 (Tumor and stromal TILs) was higher in HER2-positive and triple negative breast cancer. By contrast, expression of TIM-3, OX40, and OX40L by stromal TILs were higher in luminal A and luminal B breast cancer. In the univariate analysis, expression of B7-H3 was associated with worse DFS and expression of OX40 and B7-H4 was associated with favorable DFS. Expression of PD-L1, PD-L2, OX40L, and B7-H2 had a tendency of favorable DFS. We devised an immune recurrence score (IRS) using 7 markers with prognostic value (B7-H2, B7-H3, B7-H4, OX40, OX40L, PD-L1, and PD-L2). Patients were classified as high-risk (31 patients, 7.9%), intermediate-risk (265, 67.6%), or low-risk (96, 24.5%) according to 7 immune marker expression. In the multivariate analysis, IRS low-risk (adjusted HR, 0.14; 95% CI, 0.04 – 0.45;  $p = 0.001$ ) and intermediate-risk (adjusted HR, 0.32; 95% CI, 0.16 - 0.65642;  $p = 0.002$ ) had significantly lower risk of recurrence compared with high-risk. In the subgroup analysis, the prognostic role of IRS were maintained in both luminal A patients and non-luminal A patients.

**Conclusions:** This study identified immunologic characteristics of breast cancer patients using 10 immune markers. In addition, we devised an IRS with 7 immune markers which may predict recurrence in stage I-III breast cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-07-10

Development of an NGS-based multigene assay to predict recurrence risk in hormone receptor-positive, HER2-negative, node-negative breast cancer

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**Introduction:** Multigene assays provide prognostic information in hormone receptor (HR)-positive breast cancer. Compared to reverse transcription polymerase chain reaction (RT-PCR) or microarray used in currently available assays, a next-generation sequencing (NGS)-based RNA-sequencing allows for a more precise expression analysis with a broader coverage in a larger number of genes at lower costs. The purpose of this study was to develop and validate an NGS-based multigene prognostic assay to predict distant recurrence risk in HR-positive, HER2-negative, node-negative breast cancer.

**Methods:** We selected genes that are well correlated with the 21-gene assay recurrence score (RS) calculated from seven public RNA datasets. Using formalin-fixed, paraffin-embedded (FFPE) tissue from 343 consecutive patients with known RS (range 2-71), RNA-sequencing was performed for the selected genes and expression data was developed. A training dataset of 250 samples was used to develop an algorithm that predicts RS, and an independent dataset of 93 samples was used to verify its predictive ability. We validated the prognostic ability of the developed algorithm using 482 FFPE samples with long-term follow up, 78 of which had developed distant metastasis. All cases with no distant metastasis during follow up had not received adjuvant chemotherapy. Kaplan-Meier survival analysis and Cox proportional hazards model was used for validation.

**Results:** We developed an algorithm that predicts RS using expression data of 149 genes. A verification set of 93 samples demonstrated an accurate classification of predicted RS $\geq$ 25 vs <25 in 92.5% and an R<sup>2</sup> of 0.875 on regression analysis. Median follow up of 482 samples used for validation of prognostic ability of the algorithm was 110 months. Five-year metastasis-free survival (MFS) according to predicted value cut-offs of 25, 20, and 18, as well as their hazard ratios from Cox proportional hazards model are shown in Table 1.

Table 1. Metastasis-free survival according to prognostic scores derived from the NGS-based multigene assay.

cut-off value	prognostic score	5yr MFS	HR	95% CI
25	$\geq 25$	98.0 $\pm$ 0.8%	2.917	1.761-4.832
	<25	78.8 $\pm$ 3.3%	1	
18	$\geq 18$	98.4 $\pm$ 0.9%	4.607	2.567-8.269
	<18	79.2 $\pm$ 4.2%	1	
20	$\geq 20$	98.0 $\pm$ 0.8%	5.776	2.869-11.575
	<20	78.8 $\pm$ 3.3%	1	

MFS, metastasis-free survival; HR, hazard ratio; CI, confidence interval

The prognostic value is greatest when using the cut-off value of 20, and is consistent for 283 patients with age <50 (5yr MFS 98.4 $\pm$ 0.9% vs 79.2 $\pm$ 4.2%; HR 5.763; 95% CI 2.869-11.575).

**Conclusions:** We developed an NGS-based multigene assay that accurately predicts distant recurrence risk in HR-positive, HER2-negative, node-negative breast cancer. The prognostic ability is consistent for younger patients under age 50. This assay may be an alternative to commercially available assays.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-07-11

Prediction of the Oncotype Dx recurrence score (RS) from clinicopathologic factors

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**Background:** The Oncotype Dx assay is currently used as an aid to therapeutic decisions for the adjuvant treatment of women with ER-positive, Her2-negative, lymph node-negative or -micrometastatic breast cancer. The recently reported TAILORx study showed that no or minimal benefit is derived from adjuvant chemotherapy in patients with an Oncotype Dx Recurrence Score (RS) of 25 or less.

**Methods:** Charts of breast cancer patients that had the Oncotype Dx test in our cancer center in a nine-year period were reviewed. Data on demographic, and cancer-specific characteristics of the included patients were extracted. Predicted disease recurrence from the Oncotype Dx test was recorded and correlated with select clinicopathologic characteristics.

**Results:** Two hundred and thirty patients with ER-positive, Her2-negative, lymph node-negative or micrometastatic breast cancer were included. Mean age was 65 years-old (SD 9.9). Two hundred and three patients (88.3%) were post-menopausal and one hundred and thirty-three patients (57.8%) were 65 years-old or older. Two hundred and nine patients (90.9%) had lymph node-negative disease. Oncotype Dx recurrence score was low (<11) in sixty-four patients (27.8%), intermediate (11-25) in one hundred and forty patients (60.9%) and high (>25) in twenty-six patients (11.3%). High tumor grade and low progesterone receptor (PR) staining by IHC were the two clinicopathologic factors most associated with a high Oncotype Dx RS ( $\chi^2$  test  $p < 0.00001$  and Fisher's exact test  $p < 0.0001$ ). A predictive index (PI) was constructed, assigning one point each for grade 3 and PR staining in 20% or less of tumor cells. A PI of 0 was observed in one hundred and thirty-eight patients (60%), a PI of 1 was observed in seventy-one patients (30.9%), and a PI of 2 was observed in twenty-one patients (9.1%). One hundred and thirty-four patients (97.1%) with a PI of 0 had a RS of 25 or less. Patients with a PI of 1 and 2 had a RS of >25 in 12.7% and 61.9% of cases, respectively.

**Conclusion:** The PI based on tumor grade and PR we propose is a simple predictor of Oncotype Dx RS. 97.1% of patients with a grade 1 or 2 tumor and PR positivity in >20% of tumor cells had a RS of 25 or less. The Oncotype Dx test and its associated cost can therefore be avoided in these patients, especially in low-resource settings.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-07-12

A prognostic prediction nomogram (PDIDC) for breast Paget's disease with infiltrating ductal carcinoma patients: A SEER cohort analysis

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## Purpose

The aim of the study was to develop a specific nomogram for prediction of prognosis for breast Paget's disease with infiltrating ductal carcinoma (PD-IDC) patients.

## Patients and Methods

Patients data were obtained by the Surveillance, Epidemiology, and End Results (SEER) program (N=2502). Study outcome was Breast Cancer Specific Survival (BCSS). Cox proportional hazards model was applied to identify risk factors and develop predictive model. For internal validation, discrimination was calculated with the concordance index (C-index) using the bootstrap method and calibration assessed.

## Results

NPI classification, skin symptom, tumor site and age showed significant association with BCSS(table.1)and were used to build the PDIDC nomogram and to calculate risk score.

Variable finally selected for risk predicted model.

Predictor	Hazard Ratio	P Value	95% CI
NPI classification			
Good	1		
Moderate	2.17	0.000	1.51-3.14
Poor	7.26	0.000	4.96-10.63
Skin symptom			
Without	1		
With	1.76	0.000	1.34-2.32
Tumor site			
Centrally located	1		
Non-centrally located	1.25	0.042	1.07-1.56
Age*	1.01	0.000	1.01-1.03

\* Continuous variable.

PDIDC nomogram's C-index (0.791, 95%CI 0.783-0.818) showed better discrimination power than NPI classification (0.691, 95%CI, 0.650-0.735,  $P=0.000$ ) and AJCC staging (0.718, 95%CI, 0.695-0.741,  $P=0.000$ ). Patients were divided into high-risk (1882/2502, 75.21%) and low-risk (620/2502, 24.78%) subgroups with the optimal cut-off of risk scores (4.28). The total BCSS of low-risk subgroup was 77.8% (95%CI 74.4%-81.4%) vs. 31.1% (95%CI 19.4-49.8) of high-risk group ( $P=0.000$ ). Bootstrap internal validation demonstrated an average C-index of 0.739 (95% CI, 0.692-0.746). The nomogram calibration was validated to be accurate in predicting 5-year and 10-year survival.

## Conclusion

Utilizing NPI classification, skin symptom, tumor site and age, we developed the PDIDC nomogram to predict the 5-year and

10-year BCSS of breast PD-IDC patients.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-07-13

OncoproMex®: An intelligent decision support system for Mexican breast cancer patients

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**Background:** Breast cancer in Mexico is the first cause of mortality due to malignant tumors among women. The five-year overall survival among Mexican breast cancer patients (**MexBCP**) treated at governmental facilities is about 75-80% as a result of an increased access to oncology treatments (WHO 20<sup>th</sup> model list essential medicines) in the Public Health Insurance called "Seguro Popular". Expert systems are computer programs that are derived from a branch of computer Science research called Artificial Intelligence (AI). We do not have a system based on artificial intelligence for MexBCP prognostic and predictive evaluation. The aim was develop an expert system that generates a model based on data mining techniques, which allowed predict the MexBCP survival

**Patients and Methods:**

This study was carried out by the methodology currently used in the processes of Knowledge Discovery from Databases (KDD), supported by the WEKA free distribution tool for the modeling of data mining techniques. The breast cancer data of 4,773 were provided by INCAN cohort of 4300 patients diagnosed from 2006 to 2013 with a median follow-up of 40.5 months and by INCMNSZ cohort of 473 patients from 2011 to may 2018 with a median follow-up of 39 months. The clinical and pathologic variables were: age, TNMc stage, hormonal status (pre or perimenopause or postmenopause), ER, PR, HER2, Ki67, nuclear grade. Date of histological diagnosis, date of recurrence or last medical consultation, date of death, date of death, specific cancer were used for Survival analysis.

**Results:** The knowledge base for the expert system was based on the rules generated by the different data mining techniques. The rules used were generated by the Prism classification algorithm, which classify with a 97% percentage of instances correctly and a Kappa statistic of 0.9208. These rules obtained characteristics in each of the attributes, as well as the percentage of certainty of each of those rules. In addition to determining the average life of the group of patients that was classified in each of the generated rules. Finally, the basic elements that formed part of the architecture of the expert system carried out were the knowledge base, the inference engine, the database and the interface with the user. An on-line expert system was created, which allows users to interact and thus allow decision-making based on the results presented.

**Conclusion:**

As far as we know this is the first expert system that allows calculate prognosis according to clinical-pathological variables. It is of great relevance know the survival of a Mexican patient with breast cancer in the public health system with access to essential treatment. The applications of the system can be multiple in the usual clinical practice, education and in the taking of public policies for breast cancer in Mexico. We are currently working on a predictive model of oncological treatment benefit based also on an expert system.

# 2018 San Antonio Breast Cancer Symposium ®

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Factors predicting relapse in early breast cancer patients with a pathological complete response after neoadjuvant therapy – Results of a pooled analysis based on the GBG meta-database

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## Background

Even though patients with a pCR following neoadjuvant chemotherapy have an excellent prognosis still some of these patients will eventually relapse. A better identification of pts with an increased risk of relapse despite a pCR would be helpful to select these patients for additional post-neoadjuvant treatment strategies. Thus, the rationale of this retrospective analysis was to identify factors predicting relapse despite a pCR.

## Methods

This pooled retrospective analysis based on the GBG meta-database includes the neoadjuvant trials GeparTrio, GeparQuattro, GeparQuinto, GeparSixto and GeparSepto. In these trials 2188 (27%) of 7933 pts had a pCR according to ypT0/ypTis ypN0 Definition and were included. The primary endpoint was disease-free survival (DFS), secondary endpoints were distant DFS (DDFS) and overall survival (OS). A multivariate Cox proportional hazards model was used to report hazard ratios with 95% confidence interval (CI). The two-sided significance level was set to  $\alpha=0.05$ . Endpoints were analysed for all pts and in subgroups defined by intrinsic subtypes. The potential risk factors intrinsic subtype (HER2 negative/hormone receptor (HR) positive, triple negative, HER2 positive/HR positive, HER2 positive/HR negative), histological tumor type (lobular vs other), grade (G1/G2 vs G3), Ki67 ( $\leq 20\%$  vs higher), initial cT and cN stadium (cT1 vs cT2 vs cT3/4; cN0 vs cN+), age ( $\leq 40$  vs 41-59 vs  $\geq 60$ ), BMI ( $< 25$  vs 25-29 vs  $\geq 30$ ), planned number of cycles of chemotherapy ( $\leq 6$  vs  $> 6$ ), menopausal status (pre- vs postmenopausal) and clinical response after 2-4 cycles (SD vs PR vs CR vs PD) were included as covariates in multivariate Cox regression models as well as study identification.

## Results

From 2188 evaluable patients DFS, DDFS and OS events were observed in 290/197/130 pts respectively; the median follow-up over all studies was 59 months. In multivariate analysis including study and all potential risk factors DFS was significantly different with regard to the initial cN status (cN+ vs cN0, hazard ratio (HR) 1.70; 95% CI [1.2, 2.4],  $p=0.002$ ). Of borderline significance was histological type (non-lobular vs lobular, HR 0.52 95% CI [0.3, 1.1];  $p=0.076$ ) and initial tumor stage (cT3/4 vs cT1, HR 1.61 95% CI [1.0, 2.7];  $p=0.064$ ). In terms of DDFS significant differences were seen for the initial cN status (cN+ vs cN0, HR 2.34; 95% CI [1.5, 3.6],  $p<0.001$ ) and initial tumor stage (cT3/4 vs cT1, HR 1.83 95% CI [1.0, 3.3];  $p=0.044$ ); histological type was again close to significance (non-lobular vs lobular, HR 0.46 95% CI [0.2, 1.1];  $p=0.067$ ). Multivariate analysis showed significantly worse OS with initial cT3/4 tumors (cT3/4 vs cT1, HR 2.48 95%CI [1.1, 5.7];  $p=0.030$ ), and the lobular type (non-lobular vs lobular, HR 0.35 95% CI [0.1, 0.9];  $p=0.026$ ) and a trend for worse OS in pts with cN+ (cN+ vs cN0, HR 1.67 95% CI [1.0, 2.9];  $p=0.067$ ).

## Conclusions

Initial tumor load before start of neoadjuvant chemotherapy (tumor stage and nodal status) and lobular subtype were predictors of long term outcome after a pCR following neoadjuvant chemotherapy. Intrinsic subtype, Ki67, grade and planned number of cycles were not predictive for a relapse.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-02

Interaction of PIK3CA mutation subclasses with response to preoperative treatment with the PI3K inhibitor pictilisib in patients with estrogen receptor-positive breast cancer

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**Background:** Although preclinical data suggest that combining PI3K inhibitors with endocrine therapy may overcome resistance, results from randomized clinical trials have failed to identify a subgroup of patients that derive a substantial benefit. This preoperative window study assessed whether adding the PI3K inhibitor pictilisib can increase the anti-tumor effects of anastrozole in primary breast cancer and aimed to identify the most appropriate patient population for combination therapy.

**Methods:** In this randomized, open-label, phase 2 study, 167 postmenopausal women with newly diagnosed, operable, ER-positive, HER2-negative breast cancers were recruited. Participants were randomly allocated (2:1, favoring the combination) to two-weeks of preoperative treatment with anastrozole 1 mg once daily or the combination of anastrozole 1mg with pictilisib 260 mg once daily. The primary endpoint was inhibition of tumor cell proliferation, as measured by change in Ki-67 protein expression between tumor samples taken before and at the end of treatment. Secondary endpoints include induction of apoptosis (Caspase3) and safety. Comprehensive biomarkers analyses included targeted NGS of a comprehensive cancer panel of >400 genes (Ampliseq Comprehensive Cancer panel), copy number variation analyses, and pre- and post-treatment reverse-phase protein arrays (RPPA) and RNA profiling (NanoString nCounter platform).

**Results:** There was significantly greater geometric mean Ki67 suppression of 82.5% (90% CI, 78.3%-85.8%) for the combination vs 70.7% (61.0%-78.0%) for anastrozole [geometric mean ratio (combination/ anastrozole) 0.60 (0.58-0.85); p=0.01]. Higher baseline Ki67, Luminal B status and/or negative PR status were associated with increased benefit from adding pictilisib. A significant interaction was observed between PIK3CA mutation subtypes [helical domain mutations (HD), kinase domain mutations (KD), wildtype (WT)] and mean Ki67 suppression; the combination/anastrozole geometric mean ratio of Ki67 suppression was 0.48 (0.27-0.84; p=0.02) for patients with HD mutations and 0.63 (0.39-1.0; p=0.05) for patients with PIK3Ca WT, compared to 1.17 (0.57-2.41; p=0.64) for patients with KD mutations. This was largely due to patients with HD mutations showing a particularly poor response to anastrozole alone [mean Ki67 suppression 53.9% (9.5%-76.5%)], that was reversed by the addition of pictilisib [mean Ki-67 suppression 78.1% (71.0%-83.4%)]. On the other hand, patients with KD mutations responded well to anastrozole alone [mean Ki-67 suppression 77.7% (57.0%-88.4%)] and showed no benefit from the addition of pictilisib [mean Ki-67 suppression 73.9% (59.8%-83.0%)]. There was no significant difference in induction of apoptosis between treatment groups. Comprehensive pre- and post-treatment biomarkers analyses will be presented.

**Conclusions:** Adding pictilisib to anastrozole significantly increases the anti-proliferative response to preoperative treatment with anastrozole. A significant interaction was observed between PIK3CA mutation subtypes, with patients with helical domain mutations showing a particularly poor response to anastrozole alone that was reversed by the addition of pictilisib.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-03

Multiple foci of microinvasion is associated with an increased risk of invasive local recurrence and an increased risk of breast cancer mortality in women with ductal carcinoma in situ treated with breast-conserving therapy

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**Purpose:** Ductal Carcinoma in Situ (DCIS) with microinvasion (MI) ( $\leq 1$ mm) includes a spectrum of cases with a single focus of MI and those with multiple foci (2 or more) of MI. The impact of *multiple* foci of MI on the risks of local recurrence (LR), invasive LR and breast cancer-specific survival (BCSS) is unknown, leading to uncertainty if DCIS with multiple foci of MI requires more aggressive treatment than those with a single focus of MI or pure DCIS. We examined the impact of multiple foci of MI, confirmed by expert pathology review, on the 15-yr risks of LR, invasive LR and breast-cancer specific survival (BCSS) in a population cohort of DCIS (+/-MI) treated with breast-conserving surgery (BCS) +/- radiotherapy (RT).

**Methods:** The cohort includes all women diagnosed with DCIS +/- MI in Ontario from 1994-2003 treated with BCS+/-RT. Cases with prior malignancy were excluded. Treatment and outcomes were ascertained by deterministic linkage with chart validation. Cause of death was determined from the provincial cancer registry or terminal hospital admission records. Cox proportional hazards model was used to evaluate the impact of multiple foci of MI on the risks of ipsilateral invasive LR and ipsilateral DCIS LR, adjusting for significant co-variables. The 15-yr invasive local recurrence-free survival (LRFS), DCIS LRFS and 15-yr BCSS risks were calculated using the Kaplan-Meier method with differences compared using the log-rank test.

**Results:** The cohort includes 3529 women; 2988 (85%) with pathology review are included in this analysis. 2,721 had pure DCIS (51% received RT), 267 had DCIS with MI (1 focus, N=156; multiple foci, N=111 (58% had RT)). Median follow-up was 13 years. Median age at diagnosis was 58 years. LR developed in 571 cases (21%) of pure DCIS, 33 cases (21%) with 1 focus of MI and 23 cases (27%) with multiple foci of MI. On multivariable analyses, the presence of multiple foci of MI was associated with an increased risk of invasive LR (HR=1.59, 95%CI: 1.01-2.49, p=0.04) but not DCIS LR (HR=0.89, 95%CI: 0.46, 1.76, p=0.7). Women with multiple foci of MI had higher risks of invasive LR and lower BCSS at 15 years compared to those with pure DCIS. The 15-year *invasive* LRFS risks for cases with pure DCIS, with 1 focus or multiple foci of MI were 85.7%, 85.6%, 74.7% for cases treated by BCS alone and 87.2%, 89.9% and 77% following BCS+RT without boost; however, women treated with boost RT had substantially higher 15-yr invasive LRFS risks than those who did not receive boost RT. The 15-yr invasive LRFS risks for those with pure DCIS, 1 or multiple foci of MI, were 89.2%, 91.3% and 95% (all p values>.05, limited by low event rate). The 15-yr BCSS risks for cases with 1 focus, multiple foci of MI or pure DCIS were 92.1%, 93.1%, 96.4% (p=.006).

**Conclusions:** The presence of multiple foci of MI in DCIS is associated with higher 15-year risks of invasive LR and lower breast-cancer specific survival after breast-conserving therapy compared to women with pure DCIS but treatment with whole breast and boost RT can mitigate this risk.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-08-04

Prediction of distant recurrence by EndoPredict in patients with estrogen receptor-positive, HER2-negative breast cancer who received adjuvant endocrine therapy plus chemotherapy (ET+C) or endocrine therapy alone (ET)

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**Background:** EndoPredict (EPclin) is a validated prognostic test combining expression of 12 cancer-related genes for breast cancer patients with estrogen receptor (ER) positive, HER2-negative disease who received 5 years of endocrine therapy (Buus *et al.*, 2017; Dubsky *et al.* 2012) and for women who received chemotherapy (Martin *et al.*, 2014). Here, we determine the EPclin and 10-year distant recurrence free interval (DRFI) rates for patients who received adjuvant endocrine therapy plus chemotherapy (ET+C) or endocrine therapy alone (ET) using data from five large clinical trials.

**Methods:** A total of 3746 women with ER-positive, HER2-negative disease were included in this analysis. 2630 patients received 5 years of ET alone (ABCSG-6/8, TransATAC) and 1116 patients received ET+C (GEICAM 2003-02/9906). EPclin incorporates tumor size and nodal status and accounts for different EPclin scores between ET+C and ET alone cohorts. The primary objective was to evaluate the 10-year DRFI rates as a continuous function of EPclin separately for patients in ET+C and ET. Secondary objectives included assessing the difference in the prognostic ability of EPclin between ET+C and ET overall (years 0-10) and for specific follow-up periods (years 0-5 and years 5-10). The primary endpoint was DRFI and the secondary endpoint was breast cancer free interval (BCFI). Cox proportional hazard models were used to estimate 10-year DRFI rates and to assess the prognostic information provided by EPclin.

**Results:** All of the women on ET alone and 49% of those on ET+C were postmenopausal. Women who received ET+C had more node positive disease, more poorly differentiated tumours, and higher EPclin scores than those who received ET alone. Women who received ET+C had significantly smaller increases in 10-year DRFI rates with increasing EPclin score than those receiving ET alone (Table). EPclin was highly prognostic for DRFI in all women who received ET alone (HR=2.79 (2.49-3.13), P<0.0001) as well as in those who received ET+C (HR=2.27 (1.99-2.59), P<0.0001), both in the overall cohort and in postmenopausal women only (ET+C: HR=2.64 (2.07-3.37), P<0.0001). We observed a significant interaction between EPclin and treatment for DRFI at 10 years (P<sub>interaction</sub>=0.022). EPclin was highly prognostic in ET alone and ET+C in years 0-5 and in particular in years 5-10. Similar results were observed when BCFI was the endpoint.

**Conclusion:** In our results from a non-randomized analysis, we observed significantly smaller increases in 10-year DRFI rates with increasing EPclin scores for women who received ET+C compared to those who received ET alone. Our indirect comparisons suggest that a high EPclin score can predict chemotherapy benefit in women with ER-positive, HER2-negative disease.

10-year DRFI risks (%) (95% CI) according to EPclin score for patients who received ET+C versus ET alone.

	ET+C	ET
EPclin	10-year DR risk (%)	10-year DR risk (%)
1	1.1% (0.5-1.7)	1.0% (0.6-1.4)
2	2.5% (1.5-3.5)	2.8% (2.1-3.5)
3	5.7% (4.1-7.2)	7.6% (6.4-8.8)
4	12.4% (10.1-14.6)	19.8% (17.6-22.0)

5	25.8% (22.0-29.5)	46.1% (40.2-51.4)
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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-06

Standardized nCounter-based determination of estrogen receptor (ER), progesterone receptor (PR) and HER2 receptor status in breast cancer

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**Background:** The expression of ER, PR and HER2 in breast cancer is determined by routine pathology assessment to indicate systemic treatment. Here, we investigated the ability of a standardized mRNA-based assay to predict ER, PR and HER2 status and treatment response.

**Methods:** ESR1, PGR and ERBB2 expression was analyzed from the standardized nCounter-based PAM50 assay in 1,544 FFPE breast tumors obtained from 13 independent studies (NeoEribulin, GEICAM 2012-09, TBCRC023/006, LPT109096, EGF117165, PAMELA, PerELISA, GEICAM 2003-11, VENTANA, IBIMA and HCB). All immunohistochemical and in situ hybridization analyses followed ASCO/CAP criteria and were performed in central labs except for Neoeirbulin and VENTANA studies. To explore the best cutoff for each gene, we used Monte Carlo cross validation (repeated 1000 times, 2/3 training, 1/3 testing) to achieve the highest kappa values when gene expression was compared to pathology assessment. Receiver operating characteristic analysis and area under the ROC curve (AUC) was used to evaluate the performance of each gene to predict ER, PR and HER2 status. Finally, the association of each gene (using the pre-established cutoffs) with pathological complete response (pCR) was evaluated using univariate logistic regression analyses in 2 neoadjuvant cohorts: 1) A combined HER2+ cohort using 191 tumor samples from PAMELA/PerELISA phase II trials, where patients received neoadjuvant dual HER2 blockade without chemotherapy for 15-18 weeks and 2) a combined cohort of hormone receptor-positive/HER2-negative using 205 tumor samples from IBIMA/HCB consecutive series, where patients received anthracycline/taxane-based chemotherapy.

**Results:** Concordance between ESR1 and ER was 95.3% (95% confidence interval [95CI] 94.0-96.4%; AUC=0.98). ER+ and ER- cases were classified as ESR1- and ESR1+ in 5.5% and 4.8% of the cases, respectively. Concordance between PR and PGR was 85.2% (95CI 83.1-87.1%; AUC=0.95), and between ERBB2 and HER2 was 92.8% (95CI 91.4-94.1%; AUC=0.95). HER2+ and HER2- cases were classified ERBB2- and ERBB2+ in 16.7% and 2.9% of the cases, respectively. In the neoadjuvant HER2+ cohort, the pCR rates were 44.6% in ESR1- and 18.8% in ESR1+ (odds ratio [OR] 3.9; 95CI 1.9-8.4; p<0.001), 38.5 in PGR- and 15.8% in PGR+ (OR 3.2; 95CI 1.6-6.7; p=0.001), 2.6% in ERBB2- and 35.3% in ERBB2+ (OR 19.7; 95CI 2.6-149.1; p<0.001). In the neoadjuvant HR+/HER2- cohort, the pCR rates were 40% in ESR1- and 5.12% in ESR1+ (OR 11.5; 95CI 2.7-48.5; p<0.001), 15.5% in PGR- and 4.3% in PGR+ (OR 3.8; 95CI 1.3-11.7; p=0.019), 7.36% in ERBB2- and 0% in ERBB2+ (OR NA). Finally, ESR1, PGR and ERBB2 expression as continuous variables were found significantly associated with pCR in both cohorts.

**Conclusion:** ESR1, PGR and ERBB2 standardized mRNA levels show high concordance with ER, PR and HER2, and might provide a more objective and quantitative prediction of response to chemotherapy and anti-HER2 therapy. The level of concordance between GE and pathology-based assessments is similar to 2 FDA 510(k) cleared pathology-based ER assays (e.g. SP1 versus 6F11 antibody clones), local versus central HER2 testing, and ER or PR status when determined by the

ligand-binding assay versus immunohistochemistry.



# 2018 San Antonio Breast Cancer Symposium ®

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Prognostic impact of the 21-gene recurrence score assay among young women with node-negative and node-positive ER+/HER2- breast cancer

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**Background:** The 21-gene Recurrence Score (RS) assay is prognostic among women with early-stage estrogen receptor (ER) positive and human epidermal growth factor receptor 2 (HER2) negative breast cancer (BC) and is used to select patients for chemotherapy (CT). Young women (age  $\leq 40$ ) have represented a minority in studies evaluating gene expression assays, including TAILORx, and additional data in young women are needed.

**Methods:** In the Young Women's Breast Cancer Study, a prospective cohort study of women diagnosed with BC at age  $\leq 40$  enrolling between 2006-2016 (N=1302), we identified those with stage I-III ER+/HER2- BC. Disease and treatment information were obtained through serial surveys and medical record review. The RS was performed on banked specimens for those not tested clinically. Distant recurrence free interval (DRFI), defined as distant recurrence or BC specific death, by risk group was assessed using Cox regression and Kaplan-Meier survival estimates. Outcomes by receipt of CT were explored in the RS 11-25 group, and due to small number of events, reported descriptively.

**Results:** Among eligible women (N=577), 189 (33%) had undergone RS testing and 320 (56%) had banked specimens sufficient for testing. Median follow-up was 6 years. Median age at diagnosis was 37, most had N0 BC (300/509, 59%), and the majority had RS 11-25 (306/509, 60%).

Table 1

	N0		N1		Total Cohort	
	N	%	N	%	N	%
	300	59	163	32	509	100
Median Age	37.1		37.5		37.2	
Tumor Stage						
T1	208	69	69	42	293	58
T2	82	27	78	48	176	35
T3	10	3	15	9	35	7
T4	0	0	1	1	5	1
Grade						
I	47	16	8	5	57	11
II	165	55	79	49	266	52
III	88	29	75	46	185	36
Not assessed			1		1	
PR status by IHC						
Negative (<1%)	20	7	17	10	39	8
Positive ( $\geq 1\%$ )	280	93	146	90	470	92
Chemotherapy						
No	141	47	12	7	154	30

Yes	159	53	151	93	355	70
Ovarian Suppression						
No	263	88	149	91	452	89
Yes	37	12	14	9	57	11
TAILORx RS Groups						
RS <11	33	11	14	9	54	11
RS 11-25	195	65	88	54	306	60
RS ≥26	72	24	61	37	149	29
Conventional RS Groups						
RS <18	127	42	54	33	199	39
RS 18-30	125	42	69	42	211	42
RS ≥31	48	16	40	25	99	19

RS result was significantly associated with DRFI in N0 BC, with hazard ratio (HR) (95% CI) of 0.29 (0.07,1.30) and 0.21 (0.09,0.50) for RS<11 and RS 11-25, respectively, relative to RS≥26 (and trended towards significance in N1 BC).

Table 2

	6-year freedom from distant recurrence or breast cancer death		DRFI HR (95% CI)	
	N0	N1	N0	N1
TAILORx RS Groups				
RS <11	94.4%	92.3%	0.29 (0.07,1.30)	0.21 (0.03,1.61)
RS 11-25	96.9%	85.2%	0.21 (0.09, 0.50)	0.55 (0.27,1.12)
RS ≥26	85.1%	71.3%	Ref	Ref
Conventional RS Groups				
RS <18	97.5%	85.9%	0.19 (0.06,0.59)	0.31 (0.13,0.74)
RS 18-30	93.1%	87.3%	0.39 (0.16,1.00)	0.32 (0.14,0.73)
RS ≥31	86.4%	62.8%	Ref	Ref

Results were similar using conventional RS groups. Among women with N0 BC and RS 11-25, 44% received CT, with two events in the 86 receiving CT (2.3%) and 6 events in the 109 without CT (5.5%); 5/8 (63%) occurred in those with RS 20-25.

Conclusions: The RS is prognostic among young women with node-negative and node-positive BC, and is a valuable tool for risk stratification. Disease outcomes among young women with N0 disease and RS 11-25, a minority of whom received CT, are very good. Evaluation of the effect of ovarian suppression/CT-induced amenorrhea by RS/treatment strata is ongoing.

# 2018 San Antonio Breast Cancer Symposium ®

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Isolated ipsilateral local recurrence of breast cancer: Predictive factors and prognostic impact

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## Background

Tumour features associated with isolated invasive breast cancer ipsilateral local recurrence (ILR) after breast conservative treatment (BCT) and consequences on overall survival (OS) are still debated. The aim of our study was to examine predictive factors of isolated ILR after BCT with *in sano* resection and whole breast irradiation as well as the impact of such an ILR on overall survival in a large multi-institutional cohort.

## Methods

Patients were retrospectively identified from a large cohort of 23,375 consecutive patients who underwent BCT for invasive breast cancer in 16 cancer centres. End-points were ILR rate and OS. The impact of ILR on OS was assessed through multivariate analysis by logistic regression and Cox model, adjusted on ERs/Grade status (ERs+/Grade 1, ERs+/Grade 2, ERs+/Grade 3 and ERs-) and then on tumour subtypes.

## Results

Of 15,570 patients, ILR rate was 3.1%. Cumulative ILR rates differed according to ERs/grade (ERs+/Grade2: HR=1.42,  $p=0.010$ ; ERs+/Grade3: HR=1.41,  $p=0.067$ ; ERs-: HR=2.14,  $p<0.0001$ ), endocrine therapy (HR=2.05,  $p<0.0001$ ) and age<40-years old (HR=2.28,  $p=0.005$ ) in multivariate analysis. When multivariate analysis was adjusted on tumour subtype, the latter was the only independent factor. OS-after-ILR was significantly different according to ILR-free intervals (HR=4.96 for ILR-free interval between 2-5-years and HR=9.00 when <2-years, in comparison with  $\geq 5$ -years)

Impact of free interval time on OS among patients with ILR and among all patients

		p-value	HR	Inf	Sup
ILR	no ILR		1		
	<2 years	0.017	2.255	1.159	4.388
	2-5 years	0.001	2.45	1.42	3.89
	$\geq 5$ years	0.103	0.555	0.274	1.126
Tumor subtypes	Luminal A G1		1		
	Luminal A G2	0.003	1.431	1.132	1.810
	Triple negative	<0.0001	2.699	2.055	3.544
	Luminal B ER-	<0.0001	3.195	2.414	4.229
	Luminal B ER+	0.02	1.608	1.076	2.401
	HER2+	<0.0001	2.279	1.452	3.579

### ***Conclusion***

ERs/Grade status, lack of endocrine therapy and tumour subtypes predict isolated ILR risk in patients treated with BCT. Short ILR-free-intervals represent a strong pejorative factor for OS. These results may help selecting initial treatment as well as tailoring ILR systemic chemotherapy.

## 2018 San Antonio Breast Cancer Symposium ®

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Nomogram update based on TAILORx clinical trial results - Oncotype DX breast cancer recurrence score can be predicted using clinicopathologic data

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Oncotype DX (ODX) recurrence score (RS) breast cancer (BC) assay provides prognostic and predictive BC recurrence information for hormone(+)/node(-) patients (pts). Pts with low ODXRS (0-10) can safely forego adjuvant chemotherapy (ACH), while ACH is recommended for high ODXRS ( $\geq 26$ ). No ACH recommendations were previously available for intermediate (11-25) ODXRS until 6/3/2018, when the TAILORx clinical trial results were presented and e-published. According to the new data, pts with ODXRS 11-25 can now safely forego ACH, although some benefit of ACH was found in pts  $\leq 50$  with ODXRS 16-25. These new data now allow us to categorize ODXRS as a binary variable. Since ODX is a costly assay, we previously developed and published a user-friendly nomogram based on clinicopathologic characteristics (CPC) of ODX tested patients captured by the National Cancer Data Base (NCDB) as a surrogate prediction model for the ODX assay. As intermediate score patients were excluded from our previously created nomogram, the objective of this update is to test the predictive value of CPC variables for forecasting the new TAILORx binary ODXRS stratification using the entire NCDB population of ODX tested pts.

Five CPC variables (age, tumor size, grade, progesterone receptor status (PR) and the 4 most frequent BC histologic types) were assessed with logistic regression to predict for a low- or high-risk ODXRS test results using 0-15 or 0-25 and 16-100 or 26-100 for a low- and high-ODXRS, respectively. These ranges were used in the TAILORx trial. A training cohort consisted of 65,754 ODX tested ER+/HER2-/lymph-node-negative pts with 6-50mm tumor size, captured by the NCDB from 2010-2014; 18,585 ODX tested pts in 2015 served as an external validation cohort. The predictive accuracy of the regression model was yielded using a Receiver Operator Characteristic (ROC) analysis. Model fit was analyzed by plotting the predicted probabilities against the actual probabilities.

Grade and PR are the most significant predictors for a low- or high-risk ODXRS, followed by tumor size, histologic tumor type and age for any of the tested cut-off values. The ROC curves showed the best agreement between the nomogram prediction and actual observation for 0-25 (low) and 26-100 (high) ODXRS cut-off value (C-index=0.81). Overall, our model correctly predicted for 99.2% of low-risk and 18.6% of high-risk ODX cases. These cut-off values were used for building the updated nomogram model.

### Predicting for Low-risk (LR) ODXRS

Training cohort N=65,754	Points assigned
Age (19-90)	0-9
Tumor Size (6-50mm)	31-0
G1	100
G2	62
G3	0
PR+ $\geq 1\%$	70
PR- $< 1\%$	0
IDC	0
ILC	31
IDC+ILC	21
IDC+other	6

Total Points 0-241	Probability LR ODXRS	% accurately predicted LR ODX
160	.91	95
132	.8	93
112	.7	92
98	.6	90
24	.15	95% accurate for High-risk ODXRS

**IDC**=Invasive ductal BC **ILC**=invasive lobular BC **G**=grade

An updated nomogram is now available, created and validated based on the entire population of ODX tested pts (84,339) captured by the NCDB from 2010-2015. The updated nomogram correctly predicts for 99.2% of a low ODXRS with a 0.81 C-index. This revised calculator will continue serving as a surrogate for BC pts older than 50 for which ODX testing is not necessary, affordable or available.

# 2018 San Antonio Breast Cancer Symposium ®

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Similarities between low hormone receptor positive and hormone receptor negative breast cancer: An analysis of 4366 patients from multicenter clinical trials

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## **Background:**

Currently, patients with breast cancer (BC) with hormone receptor (HR) immunohistochemical expression between 1-9% are eligible to receive endocrine therapy. However, recent data suggest that these tumors express a basal-like molecular phenotype associated with triple negative BC (TNBC) rather than luminal phenotype associated with HR positive BC. Here, we aimed to determine the differences between strong HR positive, low HR positive and negative HR BC, in regard to responsiveness to neoadjuvant chemotherapy (NACT) and disease free survival (DFS) in large cohorts from GBG clinical trials.

## **Methods:**

In this retrospective analysis of data from women with BC treated in the neoadjuvant GeparQuinto (n=2572), GeparSixto (n=588) and GeparSepto (n=1206) clinical trials, we compared patients with three HR phenotypes: low positive (ER and/or PR= 1-9%), strong positive (ER or PR= 10-100%), and negative (ER and PR= <1%), regarding pathological complete response (pCR, ypT0 ypN0) and DFS. A logistic regression model for endpoint pCR was performed on pooled data from all trials. Cox regression was used to model DFS for patients participating in GeparQuinto and GeparSixto trial, including 71 with low HR positive phenotype. The models were adjusted by age, tumor and nodal status, grading, Her2 status, histological type, stromal and tumor infiltrating lymphocytes and clinical trial. The survival model was additionally adjusted by pCR after NACT.

## **Results:**

Patients median age was 49 years, the majority had clinical tumor stage 2 (54.1%), negative nodal status (54.7%), and Her2 negative tumors (72.4%). 85.1% of women had BC classified as no special histological type. The pCR rate across the studies was 26.2%. 145 (3.4%) patients had low HR positive, 2417 (57.3%) strong HR positive and 1658 (39.3%) HR negative tumors. After NACT, 16.3% of patients with strong HR positive BC achieved a pCR, while among those with HR negative and low HR positive tumors, pCR rates were 40.2% and 37.9%, respectively ( $p<0.001$ ). In the adjusted logistic regression model, there was no statistically significant difference between low HR positive and HR negative tumors (OR: 1.34, 95%-CI: (0.84-2.13),  $p=0.222$ ). But strong HR positive tumors had a significantly lower chance of achieving a pCR compared to low HR positives (OR 0.48, 95%-CI: 0.30-0.76,  $p=0.002$ ). Patients with strong HR positive BC had a better DFS than patients with low HR positive tumors (hazard ratio 0.35, 95%-CI: 0.18-0.70,  $p=0.003$ ). DFS was not significantly different between patients with HR negative and low HR positive tumors (hazard ratio 0.74, 95%-CI: 0.38-1.43,  $p=0.370$ ).

## **Conclusions:**

Similarly to patients with negative HR tumors, patients with low HR positive tumors have a better responsiveness to NACT and worse survival rates, compared to patients with strongly HR positive BC. We suggest that studies on treatment options for basal-like/TNBC, should also consider including patients with low HR positive tumors.

# 2018 San Antonio Breast Cancer Symposium ®

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Tumor infiltrating lymphocytes (TILs) in ER+/HER2- breast cancer

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**Background:** The prognostic role of tumor-infiltrating lymphocytes (TILs) in ER+/HER2- breast cancer (BC) is debated. We evaluated the association of TILs and clinico-pathological features with distant disease-free survival (DDFS) in a large series of patients (pts) with ER+/HER2- BC treated at a single Institution.

**Methods:** This case-cohort study was constructed using data from a previous mono-institutional study (Maisonneuve, BCR 2014). The initial cohort of 9415 pts included all women who underwent breast surgery for early ER+/HER2- BC at IEO. Then, the cohort was restricted to 3986 pts who underwent surgery in the period 1998-2002, and for whom long-term follow-up data was available. A case-cohort was built by randomly selecting approximately 17% of the above cohort (680 pts). 307 additional pts with an event (distant metastasis or death due to BC) were added to this cohort. TILs were assessed for these 987 cases on centralized H&E-stained slides according to recommendations (Salgado, Ann Onc 2015). TILs were considered both as continuous variable, and dichotomized in low (<5%) vs high (≥5%). The main outcome was DDFS and was calculated from the date of surgery to the date of any first event or the date of last contact with the patient. Median follow-up was 7.5 years (0.1-10). Differences between BC subtypes were assessed using the log-rank test. Univariable and multivariable Cox proportional hazards regression with inverse sub-cohort sampling probability weighting were used to evaluate the risk across groups. Analyses were carried out with the SAS software version 9.4 (Cary NC).

**Results:** Median TILs was 2% (Q1-Q3 1-4%). Higher TILs were positively associated with pN (p=0.003), tumor grade (p<0.0001), peritumoral vascular invasion (PVI) (p=0.003), Ki-67 (p=0.0001), luminal B subtype (p<0.0001), and chemotherapy (p<0.0001), while they were inversely associated with ER expression (p<0.0001) and age (p=0.02). There was no association with type of endocrine therapy. In multivariable regression analysis, only Ki-67 expression retained significant association with TILs. Age and ER showed a trend towards negative association with TILs. In univariate Cox regression, TILs expression (≥5% vs. <5%) was not associated with DDFS (HR 1.08, 95% CI 0.80-1.46, p=0.62). At stratified cox exploratory analyses, we found an association between high TILs and low risk in very young women (p=0.03) and grade 3 tumors (p=0.047); conversely high TILs were associated with worse outcome in grade 1 tumors (p=0.05). We evaluated TILs by treatment group (chemo vs no chemo). TILs were not associated with DDFS in the group that did not receive chemotherapy. Instead, in the group treated with adjuvant chemotherapy, high TILs were associated with better DDFS (HR 0.52, 95%CI 0.33-0.83, p=0.006), particularly in the group with ki67≥20% (HR 0.50, 95%CI 0.29-0.86, p=0.01).

**Conclusion:** High TILs in ER+/HER2- BC are significantly associated with several clinico-pathological features of dismal outcome. In this group, treatment escalation might be worthy. Our findings suggest that this subgroup might be more immunogenic, thus deserving the exploration of immunotherapy approaches. The prognostic value of TILs seems to be different in patients treated with or without chemotherapy.



# 2018 San Antonio Breast Cancer Symposium ®

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Development and validation of prognostic gene signatures for basal-like breast cancer and high grade serous ovarian cancer

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**Background:** Basal-like breast cancer (BLBC) have poor prognosis. Molecular similarities have been reported between BLBC and high grade serous ovarian cancer (HGSOC). To date, there have been no prognostic biomarkers specifically developed for BLBC or HGSOC that can provide risk stratification and inform treatment selections. In this study, we utilized RNA-seq data available from The Cancer Genome Atlas (TCGA) project to develop molecular signatures for risk stratification in BLBC, and further validated these signatures in HGSOC RNA-seq data from TCGA.

**Methods:** Raw count of RNA-seq data were downloaded from TCGA for 190 BLBC and 374 HGSOC patients. The datasets were annotated with 56963 Ensembl gene IDs. Excluding 31375 gene IDs with no greater than 10 counts in at least 90% of the samples, totally 25228 unique Ensembl gene IDs were used. Progression-free interval (PFI) is the primary study endpoint. Analyses of differentially expressed genes were performed using 3 bioconductor packages: DESeq2, edgeR and voom/limma. Signatures based on commonly identified genes among the 3 analytic methods were established using weighted linear combination of gene expression levels. Their performance was evaluated in the BLBC and HGSOC datasets using Kaplan-Meier survival analysis with log-rank tests and Cox proportional hazard regressions.

**Results:** Among 190 TNBC patients, 18 had recurrences within 2 years and 40 showed no recurrences for at least 5 years. These patients were used as recurrent vs. non-recurrent cases for differential expression analysis. 307 and 343 genes were differentially expressed based on adjusted p value threshold 0.05 and 0.01 in DESeq2 and edgeR analysis, respectively. voom/limma identified no genes differentially expressed based on adjusted p values, but 228 genes had unadjusted p values < 0.01 and were used in the following analysis. Taken together, 63, 58 and 21 genes were commonly identified by DESeq2/edgeR, DESeq2/limma and edgeR/limma analysis, respectively. All 3 signatures were able to significantly stratify the TNBC full dataset (n=190) using either 20-, 50- or 80-percentile as the cut-points. When evaluated in HGSOC patients using 80-percentile cut-point, both 63- and 58-gene signatures were able to significantly stratify patients into different risk groups (HR 2.16, 95% CI: 1.4-3.34, p < 0.001; HR 2.06, 95% CI: 1.36-3.11, p < 0.001). Multivariate Cox regression adjusting for age, grade and stage showed 63- and 58-gene signatures remained to be statistically significant in stratifying HGSOC patients (p = 0.0005 and 0.001, respectively).

**Conclusion:** Gene signatures were specifically identified to prognosticate BLBC patients based on RNA-seq data from TCGA project. Which were able to classify HGSOC patients into differential risk groups. With further validations, these signatures may provide additional prognostic tools for clinicians to better manage triple-negative breast cancer that mostly overlap with BLBC, and HGSOC patients who are difficult-to-treat currently.

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# 2018 San Antonio Breast Cancer Symposium ®

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Survival in women with de novo metastatic breast cancer: Comparison of real-world evidence from publicly-funded Canadian province and the United States by insurance status

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**Background:** Access to cancer screening, diagnosis and treatment in the United States (US) is affected by insurance status; whereas, access within a publicly-funded health care system is similar across the whole population. The aim of this study was to compare overall survival (OS) of de novo stage IV (metastatic) breast cancer (BC) pts in a Canadian province and in the US according to insurance status.

**Methods:** All female pts 18-64 yrs of age diagnosed with de novo stage IV BC from Jan 1, 2010 through Dec 31, 2014 with available biomarker information were included. Pts diagnosed by death certificate or autopsy and pts  $\geq 65$  yrs were excluded due to unreliable insurance status classification in the US SEER database. The Alberta cohort (AB) was obtained from the Alberta Health Services CancerControl Breast Data Mart (BDM), a repository of information on all pts diagnosed with their first BC diagnosis from Jan 1, 2004 onwards in the province of Alberta, Canada. The U.S. cohort was obtained from the US Surveillance, Epidemiology, and End Results (SEER) program cancer database. A total of 9,604 pts from the SEER database and 294 pts from the BDM were analyzed. OS was evaluated over a 2 yr period and median and 2 yr OS were estimated. Unadjusted associations were compared using the log-rank test, and hazard ratios (HR) were estimated using the Cox proportional hazards model with US insured set as reference group.

**Results:** Comparison of AB and US cohorts showed no differences based on age group (18-49 vs 50-64), yr of diagnosis or receipt of primary surgery. The AB cohort had a higher incidence of hormone receptor positive (HR+), similar frequency of HER2+, and a lower incidence of triple negative (TN) BC relative to the US cohort: HR+ 60.5% vs 56.4%; HER2+ 30.6% vs 28.8%; and TN 8.8% vs 14.8%, respectively [p=0.017]. The distribution of HR+, HER2+ and TN BC was consistent between the SEER insured, Medicaid and uninsured groups. AB cohort estimated 2 yr OS was 70.1%, similar to the insured group of 66.0% and significantly better than the Medicaid or uninsured pts [53.2% and 50.9%; p<0.0001]. Subgroup analysis based on biomarker status, surgery and age group showed similar results

	HR+	HER2+	TN	No Surgery	Surgery	18-49	50-64
<b>Insured</b>	Reference Group: HR=1.00						
<b>AB</b>	0.92 (0.68-1.24) p=0.584	1.15 (0.76-1.72) p=0.509	0.84 (0.49-1.42) p=0.507	0.91 (0.71-1.17) p=0.466	0.71 (0.45-1.11) p=0.133	0.84 (0.56-1.27) p=0.408	0.87 (0.67-1.12) p=0.284
<b>Medicaid</b>	1.43 (1.27-1.61) p<0.001	1.57 (1.32-1.87) p<0.001	1.37 (1.18-1.60) p<0.001	1.46 (1.33-1.59) p<0.001	1.55 (1.33-1.81) p<0.001	1.75 (1.52-2.00) p<0.001	1.47 (1.34-1.61) p<0.001
<b>Uninsured</b>	1.76 (1.44-2.15) p<0.001	1.64 (1.20-2.22) p<0.001	1.56 (1.20-2.05) p=0.001	1.56 (1.34-1.80) p<0.001	1.59 (1.16-2.18) p=0.004	1.76 (1.35-2.29) p<0.001	1.67 (1.43-1.94) p<0.001

. Adjusting for these variables, AB OS remained similar to the insured group [HR=0.92 (0.74-1.15) p=0.474] with worse OS noted in the Medicaid and uninsured populations [HR=1.44 (1.32-1.56) and HR=1.53 (1.33-1.77) p<0.001, respectively].

**Conclusion:** OS in women with de novo stage IV BC in AB was similar to US insured. AB and US insured experienced superior OS compared with US Medicaid and uninsured.

# 2018 San Antonio Breast Cancer Symposium ®

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Dissecting the effect of hormone receptor (HR) status expression in patients (pts) with HER2-positive (HER2+) early breast cancer (EBC): Exploratory analysis from the ALTTO (BIG 2-06) trial

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## Background

We investigated the clinical outcomes and behavior of HER2+ EBC according to centrally tested HR status in pts treated with modern chemotherapy (CT) and trastuzumab (T)-based regimens.

## Patients and methods

ALTTO is an international phase 3 trial in HER2+ EBC pts randomized to 4 adjuvant anti-HER2 treatment arms: T alone, lapatinib (L) alone, their sequence (T->L) or their combination (T+L). Pts in the L alone arm were excluded from the present analysis. HER2 and HR status were centrally tested for all patients. HER2 status was defined based on the 2007 ASCO/CAP guidelines. HR-positive (HR+) was defined as  $\geq 1\%$  tumor cells with expression of estrogen (ER) and/or progesterone receptors. We investigated the prognosis of pts with HR+ or negative (HR-) HER2+ EBC, the risk of developing early (years 0-5) and late (years 6-8) recurrences, the factors impacting this risk, and the patterns of relapse according to HR status.

Univariate and multivariate Cox proportional hazard models were used to compare hazard rates within the HR subgroups; a conditional landmark analysis was conducted to investigate late recurrences. For the hazard ratios for HR status, an interaction between log(time) and HR status was included as proportional hazard assumption was not satisfied.

## Results

This analysis included 6,271 HER2+ pts of whom 3,601 (57%) had HR+ and 2,670 (43%) HR- EBC. Median follow-up was 6.93 years (range 6.81-6.96).

5-year disease-free survival (DFS) was 86% (85-87) in HR+ and 83% (82-85) in HR- pts; 8-year DFS was 80% (78-81) in HR+ and 79% (77-81) in HR- pts. The mean annual hazards of recurrence in years 0-5 were 3% in HR+ and 4% in HR- pts, while in years 6-8 they were 3% in HR+ and 2% in HR- pts. The hazard ratios for HR status (ref=HR-) were 0.60 (0.51-0.72) overall, 0.74 (0.65-0.85) for years 0-5 and 1.61 (1.17-2.24) for years 6-8.

In HR+ pts, risk factors for recurrence overall were age  $\geq 65$  ( $p=0.01$ ), tumor size pT3-4 ( $p<0.001$ ), nodal status pN1-3 ( $p<0.001$ ), tumor grade 2 or 3 ( $p<0.001$ ), anthracycline-only CT ( $p<0.001$ ), no use of aromatase inhibitors ( $p<0.001$ ), and ER<10% ( $p=0.01$ ). Main risk factors for recurrence in both years 0-5 and years 6-8 were pN2-3 ( $p=0.04$  for both) and high ( $\geq 5$ ) HER2 FISH ratio ( $p=0.02$  for both).

In HR- pts, risk factors for recurrence overall were BMI (obesity or underweight;  $p=0.01$ ), pT3-4 ( $p<0.001$ ), pN1-3 ( $p<0.001$ ) and anthracycline-only CT ( $p<0.001$ ). No significant risk factors for recurrence in years 0-5 were identified. Main risk factor for recurrence in years 6-8 was BMI (obesity;  $p=0.002$ ).

HR+ pts had lower incidence of visceral disease (58.8% vs. 69.6%;  $p=0.005$ ) and lung metastasis (18.5% vs. 27.8%;  $p=0.006$ ); bone was the most common site of first recurrence (30%).

## Conclusions

This large analysis of centrally tested HR+ and HR- HER2+ EBC provides strong evidence on the existence of two different diseases within the HER2+ population based on HR expression. These up-to-date estimates of pts outcomes after modern CT and T-based regimens may be used as "historical control" for the design of future de-escalation or escalation trials separately in HR+ and HR- HER2+ EBC pts.

# 2018 San Antonio Breast Cancer Symposium ®

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Metabolic syndrome and early-stage breast cancer outcome: Results from a prospective observational study

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**Background:** Previous studies suggested a link between obesity, insulin-resistance and breast cancer outcome. The aim of the present prospective observational study was to investigate the role of metabolic syndrome (MetS) and its components on early breast cancer (EBC) patients' outcome.

**Methods:** MetS was defined by the presence of 3 to 5 of the following components: waist circumference (WC) > 88 cm, blood pressure  $\geq 130/\geq 85$  mmHg, serum levels of triglycerides (TG)  $\geq 150$  mg/dL, high-density lipoprotein (HDL) < 50 mg/dL and fasting glucose (FG)  $\geq 110$  mg/dL (National Cholesterol Education Program Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults - NCEP-ATPIII criteria). Overall, 955 EBC patients were prospectively enrolled between January 2009 and December 2013 at University Hospital Federico II and National Cancer Institute G.Pascale, Naples, Italy. Clinical and tumor characteristics were collected for all the patients. A total of 494 patients (51.7%) had complete data on all the components of MetS at first diagnosis and thus were included in the current analysis. Study population was divided into 2 main groups: (1) patients with less than 3 components (No MetS); (2) patients with 3-5 components (MetS). Categorical variables were analyzed by the chi-square test and survival data by the log-rank test and Cox proportional hazards regression model.

**Results:** Overall 366 (74.1%) and 128 (25.9%) women were categorized as No MetS and MetS, respectively. MetS patients were more likely to be older and postmenopausal compared to No MetS patients. In detail, 46% vs 38% were older than 55 yrs ( $p < 0.0001$ ) and 87% vs 54% were postmenopausal ( $p < 0.0001$ ) in MetS vs No MetS groups, respectively. No statistically significant differences in tumor stage, type of adjuvant therapy or tumor subtypes defined by immunohistochemistry (IHC) were identified among the two groups. At univariate analysis, stage, tumor subtypes, TG and FG values, number of components of MetS, and presence of MetS were significantly associated to both disease free survival (DFS) and overall survival (OS). Age, BMI, WC, and HDL levels were correlated to OS only. At the multivariate Cox proportional hazards model (adjusted for age, menopausal status, stage, IHC subtypes and adjuvant therapy) MetS patients had numerically higher risk of relapse and significantly higher risk of death compared to No MetS patients [DFS hazard ratio (HR): 1.64 95% confidence interval (CI): 0.94-2.86,  $p = 0.07$  and OS HR: 3.83, 95% CI 1.7-6.77  $p = 0.001$ ]. Additionally, of the 366 No MetS patients included in the analysis, 122 (33.3%) had 0 and 244 (66.7%) had "1 to 2" components of MetS. Interestingly, patients with "1 to 2" components of MetS had increased risk of dying compared to patients with 0 components (OS HR: 4.39, 95% CI: 1.26-15.36,  $p = 0.02$ ). No significant difference among these two groups was observed in terms of DFS.

**Conclusions:** MetS is correlated with poor outcome in EBC patients. Among patients without full criteria for MetS diagnosis, the presence of 1 or 2 components of the syndrome may predict for worse survival. Testing for components of MetS in BC patients is recommended to predict outcome and to eventually suggest lifestyle changes, exercise and diet.

# 2018 San Antonio Breast Cancer Symposium ®

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Impact of germline *BRCA* mutation status on survival in women with metastatic triple negative breast cancer

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**Background:** 15-20% of patients with triple negative breast cancer (TNBC) harbor deleterious germline (g) BRCA1/2 mutations. Recent data suggests that in metastatic TNBC (mTNBC) gBRCA1/2 mutations are associated with response to PARP inhibitors (PARPi) and platinum chemotherapy. However, diagnosis of mTNBC is associated with short overall survival (OS) with no available biomarkers that can identify mTNBC patients with better prognosis.

**Aim:** Utilizing data from a prospective registry, the objective of this study was to investigate whether presence of gBRCA1/2 mutation impacts overall survival for patients with mTNBC treated prior to clinical availability of PARPi.

**Methods:** 643 patients with stage I-IV TNBC were enrolled in an IRB approved multisite prospective registry between 2011 to 2018. Clinical, demographic, and treatment information was collected and patients were followed for recurrence and survival. 100/643 patients had metastatic breast cancer (de novo stage IV disease or metastatic recurrence). OS (from the time of diagnosis of metastatic disease to death from any cause) was estimated according to the Kaplan-Meier method and compared among groups by log-rank test.

**Results:** For the 100 mTNBC patients, the median age at diagnosis of metastatic disease was 55 years, 17% were African American, 20% had novo stage IV and 80% had relapsed disease. 84% had visceral disease, 12% had bone-only disease, and 4% had lymph node only disease. Metastatic treatment: 87% received chemotherapy, 7% received radiation only without chemotherapy and 6% did not receive any treatment. No patients received treatment with PARP inhibitor. Among de-novo stage IV patients, 35% (7/20) had breast surgery for removal of primary tumor during their course of metastatic treatment. For all 100 patients, 12% (n=12) had gBRCA mutation; 72% (n=72) had no gBRCA mutation; and 16% (n=16) had unknown BRCA mutation status. When compared with non-carriers, gBRCA carriers were younger at time of metastatic diagnosis (median age 49 vs. 57 years,  $p=0.02$ ). There was no difference in prevalence of visceral disease, de-novo stage IV disease or median lines of metastatic chemotherapy among gBRCA carriers and non-carriers. At a median follow up of 31 months, median OS for all patients is 21 months (95% CI 13-23 months). Median OS is 18 months (95% CI 15-27 months) for non-carriers and has not yet been reached for gBRCA mutation carriers ( $p=0.023$ ). 3-year estimated OS is 63% in gBRCA carriers compared to 28% in non-carriers ( $p=0.02$ ). On multivariate Cox regression analysis, gBRCA carrier status was associated with reduced risk of death (HR=0.33; 95%CI [0.23-0.91],  $p=0.033$ ).

**Conclusions:** gBRCA mutation associated mTNBC patients have a clinically significant improved OS at 3 years compared to mTNBC patients without BRCA mutations (3-year OS of 63% vs 28%). Further research is needed to understand tumor and host biological reasons for this observation. Outcomes of gBRCA mutation associated mTNBC are likely to be further improved with availability of PARPi. Given that patients with gBRCA mutation are at risk for second breast/ovarian cancers, these findings also underscore need for further research regarding the role of prophylactic surgeries mTNBC with gBRCA mutation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-17

Tumor inflammation signature (TIS), intrinsic subtypes and chemo-endocrine score (CES) in metastatic triple-negative breast cancer (mTNBC): A SOLTI biomarker program study

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**Background:** The TIS is a clinical research gene expression-based assay that enriches for response to anti-PD1 monotherapy in multiple cancer-types (Ayers et al. JCI 2017). However, the expression of the TIS in mTNBC and its relationship with other biological classifications and overall survival (OS) is currently unknown.

**Methods:** A comprehensive RNA-based characterization of 45 patients with mTNBC treated at 2 SOLTI sites was performed. RNA from metastatic biopsies was analyzed on the nCounter system using the Breast Cancer 360<sup>TM</sup> panel, which includes 752 breast cancer-related genes, including the TIS and the PAM50 and TNBCtype subtype classifications. The 1<sup>ary</sup> objective was to estimate the proportion of TIS-high tumors (defined as a score above its median expression in the PanCancer TCGA dataset) within PAM50 Basal-like disease. 2<sup>ary</sup> objectives were 1) to explore TIS distribution across the other molecular subtypes, 2) to evaluate the distribution of the PAM50 and TNBCtype subtype classifications in mTNBC, 3) to explore the association between OS and TIS, subtypes and 38 additional signatures tracking multiple tumor biological processes. OS was defined as the time from the date of metastatic diagnosis to death or last follow-up. Descriptive statistics, log-rank tests and univariate cox models were performed using R code.

**Results:** Most tumor samples (77.3%) were obtained at first recurrence or diagnosis of metastatic disease. Metastatic biopsies were obtained from 10 different sites, being skin (31.3%), breast (22.2%) and lung (8.9%) the most frequent. PAM50 subtype distribution was as follows: Basal-like (73.3%), Luminal A (13.3%), HER2-enriched (11.1%) and Luminal B (2.2%). Similarly, all the TNBCtype subtypes were identified: Mesenchymal (MSL, 48.8%), Basal-like Immune-activated (31.2%), Luminal Androgen Receptor (LAR, 15.6%) and Basal-like Immune-Suppressed (4.4%). The vast majority of non-Basal-like tumors were identified as LAR or MSL (81.8%). The proportion of TIS-high within Basal-like disease was 73% (95% confidence interval [CI] 59-84%) and similar to the proportion of TIS-high within TNBC from TCGA (70%, CI 61-78%). The proportion of TIS-high was similar across the PAM50 and TNBCtype molecular subtypes. The median OS was 25.3 months (CI 19.2-36.8). TIS, PAM50 and TNBCtype subtypes were not found associated with OS. Among the 41 biological classifications, expression of 6 signatures were found significantly associated with OS: CES (hazard ratio [HR] 0.44; p=0.015), Basal-like score (HR=5.52; p=0.011), FOXA1 (HR=0.83; p=0.014), mast cells (HR=0.77; p=0.017), differentiation score (HR=0.63; p=0.039) and Luminal A score (HR=0.27; p=0.039). Compared to tumors with a CES-low score, tumors with a CES-high score were found enriched for luminal-related genes, including AR.

**Conclusions:** ~50% of mTNBC tumors are PAM50 Basal-like and are enriched for the TIS. Future studies should determine the ability of this biomarker to predict response to anti-PD1 monotherapy or in combination with chemotherapy. In addition, mTNBC with a high luminal-profile or CES might help identify patients who might benefit from anti-androgen therapies alone or in combination with immunotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Tumor microenvironment of metastasis (TMEM) score in residual breast carcinoma post-neoadjuvant chemotherapy as an independent prognosticator of distant recurrence

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**Background:** Tumor microenvironment of metastasis (TMEM) is a microanatomical structure composed of 3 cells in direct contact, including a tumor cell expressing the actin-regulatory protein Mammalian-enabled (Mena), a perivascular Tie2<sup>hi</sup>/Vegf<sup>hi</sup>-expressing macrophage, and an endothelial cell. TMEM are intravasation sites that function as doorways for hematogenous tumor cell dissemination and metastases (Harney et al. Cancer Discovery 2015). TMEM may be identified and enumerated by triple immunohistochemistry in mouse and human mammary carcinomas. High TMEM score is associated with increased risk of distant metastasis in early stage breast cancer, and provides complementary prognostic information to IHC4 (Rohan et al. JNCI 2014) and Oncotype DX Recurrence Score in ER+, HER2-negative breast cancer (Sparano et al. NPJ Breast Cancer, 2017). Neoadjuvant chemotherapy (NAC) increases TMEM score in breast carcinoma in animal models and humans, indicating a previously unrecognized mechanism of resistance to cytotoxic therapy (Karagiannis et al. Science Trans Med 2017). Intravasation at TMEM sites may be inhibited using agents that block release of VEGF from TMEM-associated Tie2<sup>hi</sup>, VEGF<sup>hi</sup> macrophages (Harney et al. Mol Cancer Ther, 2017). Here we investigated whether TMEM score in post-NAC treated breast carcinoma is prognostic of distant recurrence in localized breast cancer after NAC, and thus provides a foundation for testing agents that block TMEM function in combination with NAC.

**Methods:** We determined TMEM score in 80 evaluable patients' post-NAC specimens with residual invasive ductal carcinomas of at least 0.5 cm. Approximately 60% of patients had ER+/HER2-negative, 28% had triple negative and 12% had HER2+ disease. Most of the patients received doxorubicin/cyclophosphamide + taxane and an anti-HER2 therapy if applicable. Tissue sections from residual tumors were stained for TMEM using triple immunohistochemistry for Mena-expressing cancer cells, CD31-expressing endothelial cells and CD68-expressing macrophages. The stained slides were scanned, and the images were analyzed by three pathologists, blinded to outcome, who independently determined the tissue areas appropriate for TMEM scoring. TMEM was scored within these areas using an automated algorithm.

**Results:** TMEM score was significantly higher in patients with distant recurrence (average TMEM=106), compared to patients without distant recurrence (average TMEM=71) (p<0.01, two-sided t-test). Moreover, in a Cox proportional hazards model that included TMEM score (upper tertile vs. lower 2 tertiles), age (>50 yrs. vs. <50), race (black vs non-black), tumor stage (T 1-3), estrogen receptor (ER) status (+ vs -), high TMEM score was associated with an increased risk of distant recurrence (HR=2.2, 95% CI=1.0 to 4.9, p=0.05)

**Conclusion:** TMEM score may provide independent prognostic information for distant recurrence in patients with residual invasive carcinoma after NAC. These results support the use of agents that block TMEM function in combination with NAC, as planned in the I-SPY2 trial.

# 2018 San Antonio Breast Cancer Symposium ®

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Exploratory biomarker analyses of FAIRLANE, a double-blind placebo (PBO)-controlled randomized phase II trial of neoadjuvant ipatasertib (IPAT) + paclitaxel (PAC) for early triple-negative breast cancer (TNBC)

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**Background:** The oral AKT inhibitor IPAT is being evaluated in cancers with a high prevalence of PI3K/AKT pathway activation. In the PBO-controlled randomized phase II FAIRLANE trial (NCT02301988), adding IPAT to PAC as neoadjuvant therapy for TNBC led to a numerical increase in pathologic complete response (pCR) in unselected patients (17.1% vs 13.3%), with a greater treatment effect in patients with *PIK3CA/AKT1/PTEN*-altered tumors (17.9% vs 11.8%). The addition of IPAT also led to an increase in complete response (CR) by MRI (27.6% vs 13.3%) that was enhanced in patients with *PIK3CA/AKT1/PTEN*-altered tumors (39.3% vs 8.8%) [Oliveira, AACR 2018]. We report an exploratory analysis performed to provide better understanding of potential biomarkers for response.

**Methods:** Pretreatment tumor samples were evaluated for genomic alterations using the FoundationOne® (Foundation Medicine) assay (n=144) and gene expression by RNA-Seq (n=92). Samples were classified into TNBC subtypes based on the method developed by Lehmann and Pietersen [Lehmann, J Clin Invest 2011]. Tumor-infiltrating lymphocytes (TILs) were quantified using the Salgado method [Salgado, Ann Oncol 2015] (n=135).

**Results:** Of 62 patients (43%) with *PIK3CA/AKT1/PTEN*-altered tumors, 21 had an activating mutation in *PIK3CA* or *AKT1* and 47 had an alteration in *PTEN* (6 [3 in each arm] had both *PIK3CA* mutation and *PTEN* alteration). Although only 3 patients with *PIK3CA/AKT1*-mutant tumors achieved a pCR, there was an increased rate of MRI CR with the addition of IPAT to PAC [Table]. In patients with *PTEN* alterations, both pCR rate and MRI CR rate were increased with IPAT. In patients treated with PBO + PAC, all 4 pCR patients evaluable by RNA-Seq were of the immunomodulatory (IM) subtype. However, in the IPAT + PAC arm, pCRs were also seen in patients with basal-like 1 (BL-1), mesenchymal (M), and mesenchymal stem-like (MSL) subtypes. Consistent with this observation, in the PBO + PAC arm, samples from patients achieving a pCR had significantly higher levels of stromal TILs than those from patients who did not have a pCR, while no difference was observed in the IPAT + PAC arm.

Response, n (%)	<i>PIK3CA/AKT</i> mutation (n=21)		<i>PTEN</i> alteration (n=47)	
	IPAT + PAC (n=11)	PBO + PAC (n=10)	IPAT + PAC (n=21)	PBO + PAC (n=26)
pCR	1 (9%)	2 (20%)	4 (19%)	3 (12%)
CR by MRI	5 (45%)	1 (10%)	8 (38%)	2 (8%)

**Conclusions:** This retrospective exploratory biomarker analysis of the phase II FAIRLANE trial of neoadjuvant IPAT for TNBC provides insight into the potential heterogeneity of response and resistance to taxane therapy. The results also hint that response to PAC alone is dependent on baseline immune infiltration and that this dependency might be relieved with the addition of AKT inhibition.



# 2018 San Antonio Breast Cancer Symposium ®

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Prognostic impact of SRC, CDKN1B and JAK2 expression in metastatic breast cancer patients treated with trastuzumab

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**Background-aim:** SRC, CDKN1B and JAK2 play a crucial role in the coordination and facilitation of cell-signaling pathways controlling a wide range of cellular functions. In the present study, we investigated the prognostic significance and clinical utility of these biomarkers in metastatic breast cancer (MBC) patients treated with trastuzumab (T). **Methods:** We assessed SRC, CDKN1B and JAK2 mRNA expression with qRT-PCR (Taqman-MGB assays) on 197 paraffin tumors. PIK3CA mutation status was previously assessed. Relapsed (RMBC) and de novo MBC (dnMBC) patients had received T for metastatic disease only. Tumors were centrally re-assessed for HER2 status. **Results:** Only 133/197 patients (67.5%) were found to be truly HER2(+). CDKN1B mRNA expression strongly correlated with SRC ( $\rho = 0.71$ ) and JAK2 ( $\rho = 0.54$ ); high CDKN1B was more frequent in RMBC compared to dnMBC ( $p = 0.001$ ) and in PIK3CA wild-type tumors ( $p = 0.005$ ). In HER2(+) patients, low CDKN1B conferred higher risk for progression (HR 1.58, 95% CI 1.08-2.32,  $p = 0.018$ ). In HER2(-) patients, low SRC was associated with longer survival (HR 0.56, 95% CI 0.32-0.99,  $p = 0.045$ ) and, as a trend, with increased progression-free survival (PFS) ( $p = 0.067$ ). For PFS, in RMBC, we observed trends for unfavorable low CDKN1B ( $p = 0.068$ ) and JAK2 ( $p = 0.086$ ); similarly, in dnMBC for unfavorable low CDKN1B ( $p = 0.072$ ). Low SRC showed a trend for better survival in RMBC ( $p = 0.087$ ). Upon multivariable analyses, only PIK3CA mutations strongly predicted for unfavorable PFS in HER2(+) patients (HR 3.37, 95% CI 1.98-5.73,  $p < 0.001$ ). Low CDKN1B and JAK2 mRNA expression remained unfavorable factors for PFS in dnMBC and RMBC patients (HR 2.36, 95% CI 1.01-5.48,  $p = 0.046$  and HR 1.76, 95% CI 1.01-3.06,  $p = 0.047$ , respectively). **Conclusions:** Low CDKN1B and JAK2 mRNA expression were unfavorable prognosticators in a cohort of T-treated MBC patients previously unexposed to this agent, with distinct impact in de novo and RMBC. Our results highlight biological and clinical differences between de novo and RMBC and suggest that CDKN1B and JAK2, if validated, may serve as prognostic factors potentially implicated in T-resistance, which seems to be associated with distinct pathways in the two MBC settings.

# 2018 San Antonio Breast Cancer Symposium ®

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Mismatch repair protein loss is a prognostic and predictive biomarker in breast cancers regardless of microsatellite instability

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Despite the approval of pembrolizumab in all tumors showing mismatch-repair (MMR) deficiency and/or microsatellite instability (MSI), there are currently no companion diagnostics for MMR status assessment in breast cancer. Here, we sought to define the diagnostic and prognostic role of MMR and MSI testing in breast cancer patients.

We subjected 444 breast cancers to MMR immunohistochemistry (IHC) and MSI analysis. Cases were classified as MMR-proficient (pMMR), MMR-deficient (dMMR), and MMR-heterogeneous (hMMR) based on the loss of immunoreactivity; MSI was defined by the instability in the five indicators recommended by the National Cancer Institute for endometrial and colorectal cancers. Correlation of MMR status with patients' survival was assessed using the Kaplan-Meier estimator.

In 75 patients (17%) the loss of MMR proteins was homogeneous, classified as dMMR, while 55 cases (12%) were hMMR. The prevalence of cancers with loss of the MMR proteins was homogeneous across ER+ breast cancers (15-19% for dMMR and 10-18% for hMMR tumors). The level of overlap between IHC and MSI analysis was 9% ( $p < 0.0001$ ). Among ER+/HER2- carcinomas, pMMR and hMMR patients displayed better survival rates ( $p = 0.008$ ). In chemo-treated ER-/HER2- breast cancers, the dMMR status was a marker of good prognosis ( $p < 0.001$ ).

Our study documents the clinical impact of MMR testing in a large series of breast cancers, using the most commonly adopted diagnostic tools and criteria. We show that MMR protein loss is a rather common event in breast cancer and has a remarkable degree of intra-tumor heterogeneity, therefore making the analysis of a small area of the tumor, or a small biopsy, of little clinical value. Our investigation supports the concept that MSI occurs rarely in breast cancer and demonstrate that this condition is restricted to a minority of tumors with MMR protein loss. These data suggest that MMR IHC and MSI analysis should not be considered as interchangeable tests in the diagnostic workup of breast carcinomas. Finally, our observations indicate that the complete loss of at least one of the MMR proteins assessed by IHC is able to identify high-risk ER+/HER2- breast cancers that can potentially benefit from pembrolizumab therapy, whereas first-line chemotherapy shows comparatively good results in dMMR ER-/HER2- breast cancers.

# 2018 San Antonio Breast Cancer Symposium ®

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Clinical implication of HER2/neu status in hormone receptor positive pure mucinous breast cancer

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**Background:** Mucinous carcinoma of the breast is a rare type of breast cancer with favorable outcome compared with other types of breast cancer. The current guideline does not recommend chemotherapy/anti-HER2 therapy for mucinous breast cancer with hormone receptor-positive subtype regardless of HER2/neu status. In this study, we evaluated the survival of pure mucinous breast cancer according to tumor stage and subtype.

**Methods:** Between 1989 and 2014, in Asan Medical Center, Korea, total 473 pure mucinous carcinomas (stage I-III) undergone curative surgery were reviewed retrospectively. 5yr disease-free and overall survival were analyzed according to size, lymph node metastasis, hormone receptor/HER2 status and given therapy.

**Result:** Total of 473 patients with pure mucinous breast cancer were analyzed and median follow-up duration was 78.00 months. 439 patients were hormone receptor-positive, 374 were node negative, 55 were HER2/neu positive. Among 374 patients with hormone receptor-positive and node-negative, tumor size was <1cm in 46 patients, 1-2.9cm in 259 patients, ≥3cm in 69 patients. In HR-positive/Node-positive BCs, 90.8%(59/65)were given chemotherapy and 35.3%(6/17) were also given trastuzumab. Sixteen patients given trastuzumab were only included in the analysis to assess the benefit of trastuzumab among HER2 positive BCs.

Overall, 5-year disease-free survival (DFS) rate was 94.1% and the 5-year overall survival (OS) rate was 95.9%. Using Cox regression analysis, lymph node metastasis was the only significant prognostic factor for both DFS (HR4.0, 95%CI:1.8-9.0,  $p=0.001$ ) and OS (HR3.5, 95%CI:1.3-8.9,  $p=0.008$ ). Among HR-positive/node-negative with tumor size ≥3cm, HER2/neu positivity was only significantly associated with 5yr-DFS (71.4% in HER2/neu+ vs. 96.4% in HER2/neu-, HR9.5, 95%CI:1.3-67.5,  $p=0.024$ ). This observation was consistently combining both 'HR-positive/node-negative/>3cm' and 'HR-positive/node positive' BCs (N=127) that HER2 positive tumors showed worse survival (HR 3.7, 95%CI:1.2-10.8,  $p=0.015$ ). Intriguingly, within this subgroup of HR-positive/node-negative/>3cm' and 'HR-positive/node positive' BCs, among HER2 positive tumors, while 5yr-DFS was 63.7% in patients who didn't receive trastuzumab, 100% were disease free in patients who were given trastuzumab.

**Conclusions:** Overall, nodal status was the most significant prognostic factor for pure mucinous breast cancer. In hormone receptor-positive, lymph node negative mucinous breast cancer with tumor of ≥3cm, HER2 positive BCs showed worse survival, suggesting a potential role of anti-HER2 strategy in this subgroup.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-23

A combined score of tumour budding and tumour necrosis has prognostic value for cancer specific survival in both ER positive and ER negative primary operable breast cancer

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**Background:** As new systemic therapies emerge for the treatment of breast cancer, new prognostic markers are required to help stratify patients into higher and lower risk groups to aid treatment decision making. Features of the tumour microenvironment, such as tumour necrosis, tumour-stroma percentage (TSP), and tumour budding have been shown to have prognostic value in some cancers. However, their role in breast cancer is unclear.

**Methods:** Patients who underwent surgery for primary operable breast cancer in 2 centres between 1995-2007 and who had paraffin-embedded tissue blocks available were identified. Clinicopathological details and survival data were obtained from patient records. Haematoxylin & Eosin-stained slides were visually assessed within a set visual field for TSP ( $\leq 50\%$  or  $>50\%$  tumour stroma), tumour necrosis ( $<25\%$  or  $>25\%$  necrosis) and tumour budding ( $\leq 20$  buds or  $>20$  buds). A combined score of tumour necrosis and tumour budding was then created. A score of 0 was assigned to tumours where both components were low, 1 to those where only one component was high, and 2 to those where both were high. Multivariate cox regression analysis was carried out for cancer specific survival (CSS).

**Results:** A breast cancer cohort of 1301 patients was utilised, from which 1186 H&E slides were scored for necrosis, TSP and tumour budding. Median follow up was 158 months (26-183) and there were 234 breast cancer deaths. In the full cohort, necrosis ( $p<0.0001$ ), high TSP ( $p=0.010$ ) and high budding ( $p<0.0001$ ) were associated with CSS and all 3 were independently prognostic on multivariate analysis (necrosis HR 1.54, 95%CI 1.15-2.07,  $p=0.004$ ; high TSP HR 1.49, 95%CI 1.12-1.98;  $p=0.006$ ; high budding HR 1.38, 95%CI 1.02-1.87,  $p=0.035$ ). In ER positive disease ( $n=826$ ), necrosis was associated with worse CSS ( $p<0.0001$ ) and was independently prognostic (HR 1.46, 95%CI 1.03-2.08,  $p=0.033$ ). In ER negative disease ( $n=359$ ), necrosis, high TSP and high budding were associated with worse CSS ( $p=0.001$ ,  $p=0.002$ ,  $p<0.0001$  respectively) and were independently prognostic (necrosis HR 2.44, 95%CI 1.34-4.43,  $p=0.003$ ; high TSP HR 1.64, 95%CI 1.06-2.53,  $p=0.026$ ; high budding HR 2.47, 95%CI 1.56-3.89,  $p<0.0001$ ). To assess if combining these markers added additional prognostic power a combined budding/necrosis score was established. This was associated with worse CSS in ER positive disease ( $p<0.0001$ ) and a score of 2 was independently associated with worse CSS compared to a score of 0 (HR 1.96, 95%CI 1.19-3.23,  $p=0.008$ ). This was potentiated in node-negative patients (HR 5.14, 95%CI 2.18-12.08,  $p<0.0001$ ). In ER negative disease, an increasing score was associated with worse CSS ( $p<0.0001$ ) and was independently prognostic (combined score 1 vs. 0: HR 2.37, 95%CI 1.13-5.00,  $p=0.023$ ; score 2 vs. 0: HR 5.93, 95%CI 2.62-13.40,  $p<0.0001$ ).

**Conclusions:** A combined score of tumour necrosis and budding shows promise as a readily-available prognostic tool to aid treatment decision making in primary operable breast cancer, both by stratifying risk in ER negative disease, and by identifying a high-risk group in ER positive, node negative disease.

# 2018 San Antonio Breast Cancer Symposium ®

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The average modified Magee score can be helpful in predicting an Oncotype DX recurrence score  $\leq 25$

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**Introduction:** The recent TAILORx results suggests that adjuvant endocrine therapy and chemoendocrine therapy had similar efficacy in women with hormone-positive, HER2-negative, axillary node-negative breast cancer who had an Oncotype Dx recurrence score (ODXRS) between 11 and 25. These findings, along with updated results that patients with an ODXRS  $< 11$  have a 9-year recurrence rate of only 3%, suggest that certain populations of patients with an ODXRS  $\leq 25$  may not benefit from additional systemic chemotherapy. Oncotype Dx is an expensive test (current list price of \$4,650.00), and cost has been an impediment to its adoption in many centers throughout the United States, and internationally. The test can be performed only at a commercial specialized laboratory. Based on a modification of the new Magee equations (Klein ME, et al. *Mod Pathol.* 2013;26(5):658–664) we published an algorithm based on 283 patients with ODXRS's (Turner BM, et al. *Mod Pathol.* 28(7):921-31) that suggested our algorithm offered a less expensive alternative to Oncotype DX testing. We have validated this algorithm in a multi-institutional study on an additional 619 patients with ODXRS's (in preparation for publication). Our validation data, and our data from the original study, also suggests that the average modified Magee equation can reliably predict patients who will have an ODXRS  $\leq 25$ . **Methods:** 903 cases with an available ODXRS (2006-2018) were identified from the pathology files at the University of Rochester Medical Center (n = 752) and the University of Louisville (n = 151). Information required to calculate the average modified Magee score, which includes estrogen receptor (ER) and progesterone receptor (PR) status, Her-2 status, Nottingham score, tumor size, and Ki-67 were also extracted from the medical record. 78 patients did not have an available Ki-67, leaving 825 patients for inclusion in this study. **Results: 478/488 (98%) patients with an average Modified Magee score  $\leq 18$  had an ODXRS  $\leq 25$**  (only 2% had an ODXRS  $> 25$  - Table 1). 125/337 (37%) patients with an average Modified Magee score  $> 18$  had an ODXRS  $> 25$  (Table 1). **Conclusions:** The average Modified Magee Score can be helpful in predicting an ODXRS  $\leq 25$ . Patients with an average Modified Magee score  $\leq 18$  may not need to be sent out for Oncotype Dx testing. The potential cost savings to the health care system would be enormous.

Table 1: Average Modified Magee score and risk of an Oncotype Dx recurrence score (ODXRS)  $> 25$

Magee score	n	Average ODXRS	Minimum ODXRS value	Maximum ODXRS value	Cases with ODXRS $> 25$ [n (%)]
$\leq 9$	18	11.0	2	19	0 (0)
10	21	10.9	0	26	1 (4.8)
11	37	12.1	0	25	0 (0)
12	60	12.0	0	28	1 (1.7)
13	64	12.0	0	23	0 (0)
14	70	12.9	0	27	1 (1.4)
15	65	13.6	1	31	3 (4.6)
16	56	14.1	2	29	2 (3.6)
17	52	14.5	0	27	2 (3.8)
18	45	14.3	0	25	0 (0)
19	54	18.9	6	37	9 (16.7)
20	43	19.3	0	34	8 (18.6)
21	59	21.1	9	37	15 (25.4)
22	38	19.3	2	49	6 (15.8)

23	25	23.3	8	38	11 (44)
24	29	20.8	3	32	9 (31)
25	23	25.0	14	40	11 (47.8)
> 25-27	27	29.2	17	46	20 (74.1)
> 27-30	16	34.9	22	51	13 (81.3)
> 30	23	45.7	31	78	23 (100)
≤ 18	488	12.9	0	31	10 (2)
> 18	337	23.7	0	78	125 (37)

# 2018 San Antonio Breast Cancer Symposium ®

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PD-L1 expression at the protein and RNA levels as prognostic factor in early breast cancer

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**Introduction:** PD-L1 expression as assessed by immunohistochemistry (IHC) is a clinically relevant biomarker in certain malignancies such as lung cancer, since it selects appropriate candidates for PD-1 blockade. Since these agents are under evaluation for breast cancer, discovering and validating predictive biomarkers is of outmost importance. However, the clinical utility of PD-L1 expression in breast cancer is questionable, in light of prior inconclusive reports which have used various IHC antibodies, scoring methods and cut-offs. Moreover, there are only few previous studies on comparing IHC and RNA data at the same cohort, not limited to a single subtype. **Methods:** Our cohort is derived from a nested case-control study consisting of 619 patients diagnosed with primary breast cancer between 1997-2005 in Stockholm health care region. Tissue microarrays from epithelial tumor areas have been constructed using duplicate cores from primary tumors and tissue sections were used for IHC with PD-L1 (Ventana; clone SP263) antibody. Positivity was defined as the presence of any single cell with membranous expression of PD-L1. Gene expression profiling was performed using DNA microarrays (GSE48091). Data on clinical and pathological tumor characteristics, survival, loco-regional and systemic treatments, and follow-up have been collected. Correlations between transcript and protein expression levels were estimated using Mann-Whitney test, while survival analyses were conducted using the Kaplan-Meier method. Furthermore, we associated an immune gene module score (IMS) –whose predictive power in neoadjuvant and metastatic settings has been previously demonstrated- with PD-L1 transcript levels by using Spearman's rank correlation coefficient. **Results:** IHC data were available for 87.4% (541/619) of the patients. PD-L1 was expressed on tumor cells in 9.6% (52/541) of the patients while it was also expressed by immune cells in 23.1% (125/541) of the patients. Any PD-L1 expression (tumor and/or immune cells) was noted in 24.2% (131/541) of the patients. PD-L1 transcript levels and protein expression on tumor, immune and/or both cell types were statistically significantly associated ( $p < 2.2 \times 10^{-16}$ ). In the whole cohort, patients with higher PD-L1 transcript levels were associated with better breast cancer-specific survival (BCSS) ( $p = 0.0061$ ). In addition, within intrinsic subtypes, high PD-L1 transcript expression was significantly associated with better BCSS only in basal-like ( $p = 0.019$ ) disease. There was no significant correlation between improved BCSS and PD-L1 protein expression by tumor ( $p = 0.13$ ), immune ( $p = 0.12$ ) or both types of cells ( $p = 0.2$ ). PD-L1 transcript levels were also positively associated with the IMS (Spearman's  $\rho = 0.85$ ). **Conclusions:** The prognostic value of PD-L IHC expression in breast cancer remains inconclusive. However, RNA expression of PD-L1 may be more informative as a prognostic factor, especially in basal-like disease and merits further validation.

# 2018 San Antonio Breast Cancer Symposium ®

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High expression of CYP27A1 in breast cancer is associated with poor tumor pathological features and may differentially predict prognosis depending on menopausal status

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**Background:** Pre-clinical and epidemiological data strongly link high cholesterol with breast cancer progression and poor prognosis. It was recently uncovered that the pathogenicity of cholesterol in breast cancer is directly propagated by 27-hydroxycholesterol (27HC), an oxysterol produced when cholesterol is hydroxylated by cytochrome P450, family 27, subfamily A, polypeptide 1 (CYP27A1) during bile acid synthesis. 27HC promotes breast tumor growth and metastasis via interactions with the estrogen receptor (ER) and liver x receptors respectively. Consequently, pharmaceutical approaches that directly interfere with CYP27A1 activity have been proposed to mitigate the adverse impact of 27HC in breast cancer. However, CYP27A1 expression or deregulation in clinical breast cancer is not well characterised. The aim of this study was to comprehensively describe the impact of tumor-specific expression of CYP27A1 protein on clinical breast cancer pathobiology and prognosis.

**Methods:** CYP27A1 expression in tumor cells was evaluated by immunohistochemistry in two independent population based cohorts including female patients with primary invasive breast cancer diagnosed between 1991 and 2010 (cohort 1) and between 2002 and 2012 (cohort 2). Staining was evaluable in 645 and 813 cases in cohort 1 and cohort 2, respectively. Associations between CYP27A1 expression with tumor pathological factors and survival were assessed by using logistic and Cox regression models respectively. Multivariable models adjusted for age at diagnosis, nodal status, histological grade, tumor size, ER and BMI.

**Results:** CYP27A1 was overexpressed in 21% and 28% in cohort 1 and cohort 2 respectively. High CYP27A1 expression was significantly associated with adverse tumor pathological features including negative hormone receptor (ER and PgR) status and histological grade 3 in both cohorts and with larger tumors (>20 mm) in cohort two only ( $p < 0.05$ , for all comparisons). In multivariable Cox regression analyses, overexpression of CYP27A1 was neither independently prognostic for recurrence-free survival (cohort 2: HR=1.3, 95% CI= 0.88 – 1.9) nor overall survival (cohort 1: HR=1.3, 95% CI= 0.88 – 1.9 and Cohort 2: HR=1.3, 95% CI= 0.81 – 2.0, respectively). Upon stratification for menopausal status using age at diagnosis (< 50 years vs  $\geq$  50 years) as surrogate, the relationship between CYP27A1 expression and prognosis remained non-significant for older (postmenopausal) patients. Interestingly, among younger (premenopausal) women, elevated CYP27A1 expression was independently prognostic for shorter time to recurrence or death (HR=3.3, 95% CI= 1.5 – 7.4; cohort 2).

**Conclusions:** Collectively, these results indicate that intratumoral CYP27A1 expression supports the notion that 27HC plays an important pathological role in breast cancer progression but tumor cell-specific CYP27A1 expression is not sufficient to independently predict overall survival in postmenopausal patients. Further sufficiently sized studies are needed to clarify the prognostic significance of CYP27A1 in younger and presumably premenopausal patients and evaluate its role as a treatment predictive factor.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-27

Does a large breast protect against axillary nodal spread in breast carcinoma?

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## Introduction

Prognostic scoring systems in breast cancer such as TNM and NPI (Nottingham prognostic index) use maximal diameter for size. Breast cancer is three-dimensional therefore volume and weight are more accurate biological predictors

Tumour size is an important predictor of axillary node positivity and is used in the Memorial Sloan Kettering Cancer Centre (MSKCC) nomogram.

It has been suggested that positivity rate is related more to increasing tumour size rather than grade.

Nodal spread may be different for tumours occupying a greater or lesser proportion of the breast. An association has previously been identified between a high tumor-to-breast volume ratio and poor prognosis.

The aim of this study was to measure breast cancer volume as a proportion of the mastectomy specimen volume and compare node positivity rates in high and low proportion specimens.

## METHODS

Data was retrieved from the CMDHB (Counties Manukau District Health Board) breast clinic database. Size, grade, node positivity (macro, micro, ITC (isolated tumour cells) ), LVI (lymphovascular invasion), receptors, quadrant and tumour type were recorded from concertina pathological reports.

The weights of carcinomas from 382 mastectomy specimens were estimated as a proportion of the entire mastectomy weight.

Carcinoma volume and weight was estimated using the ellipsoid model ( $V = \frac{4}{3} \pi \cdot \frac{a}{2} \cdot \frac{b}{2} \cdot \frac{c}{2}$ )

The cohort was then divided at the medians into “low” and “high” tumour proportionality and “large” and “small” tumour.

Logistic regression was applied for node positivity and multivariate analysis was used to standardise by tumour size and grade.

## RESULTS

The overall node positivity rate was 194/382 (51%).

In tumours with a low proportionality, 37.6% (72/191) were node positive and in the high proportionality group, 63.8% (122/191) were node positive ( $p = <0.00001$ ).

Grade 1 tumours were node positive in 16/44 and grade 3 tumours were positive in 84/155 ( $p = 0.082$ ).

Small volume tumours were positive in 70/191 and large volume tumours were positive in 124/191.

The smallest tumour to breast weight ratio was 0.00001 and the largest was 0.709.

## CONCLUSION

Prediction of node positivity preoperatively is essential in all malignant primary breast surgery.

Larger tumour volumes and greater proportion of cancer leads to increased node positivity.

Surgeons should calculate the three-dimensional proportion of the breast that is occupied by cancer, particularly when the patient is on the cusp of sentinel node vs axillary dissections.

Since breast volumes and breast cancer size vary by race, ethnic disparities in risk of lymph node spread may exist.

## 2018 San Antonio Breast Cancer Symposium ®

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Relationship between FDG uptake and neutrophil/lymphocyte ratio in patients with breast invasive ductal cancer

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**Background:** <sup>18</sup>F-Fluorodeoxyglucose-positron emission tomography (FDG-PET) is used to evaluate the glucose metabolic rates of cancers. Several studies have reported that high FDG uptake is predictive of poor prognosis and aggressive features in patients with breast cancer. FDG uptake is influenced by many factors, including inflammation. In this study, we investigated the relationship between FDG uptake and neutrophil/lymphocyte ratio (NLR), which is an indicator of systemic inflammation. **Patients and Methods:** We retrospectively investigated the cases of 143 consecutive invasive ductal carcinoma patients who had undergone surgery and FDG-PET preoperatively. PET was evaluated using standardized uptake value max (SUVmax). The median SUVmax was 2.5 (range 0-10.5). Thus, we divided the cases into two groups based on the value of SUVmax; low (<2.5) and high (≥2.5). The relationships between SUVmax and clinicopathological features, including NLR, were investigated. **Results:** Among the 143 patients, 73 (51.0%) had high SUVmax in the primary tumor. The analysis revealed that large tumor size ( $p<0.001$ ), high nuclear grade ( $p<0.001$ ), the presence of lymphovascular invasion ( $p<0.001$ ), CRP ( $p=0.046$ ) and high NLR ( $p<0.001$ ) were significantly associated with high SUVmax in the primary tumor. There were associations between SUVmax and NLR ( $r=0.323$ ,  $p<0.001$ ). Among the 70 cases with low SUVmax, there was no recurrent disease, while 6 cases among the 73 cases with high SUVmax had disease recurrence. It is interesting to note that the group with high SUVmax and low NLR had no recurrent disease. **Conclusions:** To the best of our knowledge, this is the first report to describe the relationship between FDG uptake and NLR in breast cancer. The present study demonstrated that the finding of preoperative high FDG uptake in breast cancer may be reflective of poor prognosis and that high NLR may be predictive of aggressive features among patients with breast cancer. On the other hand, among breast cancer patients with high SUVmax in the primary tumor, it will be useful to identify the subset of patients with low NLR in order to improve prognostic accuracy.

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The impact of time interval between diagnosis and surgery in each type and stage of breast cancer

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**Background:** There are many factors that might contribute to the delay of surgery in patients with breast cancer. Previous studies investigate the influence of delay of surgery, but they reported inconsistent results. The purpose of this study was to evaluate the impact of time of surgery on prognosis of breast cancer.

**Methods:** We performed a retrospective review of the patients with breast cancer, who received surgery between 1992 and 2009, by using data from Korea Breast Cancer Society Registry. Kaplan-Meier survival analysis and Cox regression model were used to evaluate the impact of time to surgery in breast cancer and subgroup analyses were performed for each disease stage and molecular subtype.

**Result:** A total 14727 patients were included for analysis. Delay of surgery more than 31 days was associated with worse survival for breast cancer [hazard ratio (HR) = 2.16; 95% confidence interval (CI), 1.936-2.408,  $p < 0.001$ ]. Subgroup analyses revealed that over 31 days of surgical delay were significantly associated with worse survival in hormone receptor positive and HER-2 negative ( $p < 0.001$ ), hormone receptor positive and HER-2 positive ( $p < 0.001$ ), hormone receptor negative and HER-2 positive ( $p < 0.001$ ), triple negative ( $p < 0.001$ ) and stage II, III breast cancer patients ( $p < 0.001$ ).

**Conclusion:** Surgical delay of more than 31 days were independent risk factors for worse outcome of breast cancer in each molecular subtype and breast cancer group except stage 0 and I. Although preoperative evaluation is required, surgical delay should be shortened to enhance survival of breast cancer, especially in patients with tumor size more than 2cm or presence of lymph node metastasis.

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Vitamin D as a prognostic factor in triple negative early breast cancer

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**Background:** Triple negative breast cancer is a special phenotype where the pathological complete response (pCR) remains the most important factor but only around a 40% of patients achieve this response. We analyze if the vitamin D receptors (VDR) could be a prognostic factor not related with the histological response.

**Methods:** A series of 160 patients with early or locally advanced triple negative breast cancer that received neoadjuvant chemotherapy were retrospectively reviewed from 2007 to 2017. Clinicopathological and vitamin D receptors analysis were correlated to pCR.

**Results:** Median age was 53, median tumor size 30mm, 48% had nodal involvement, and median ki67 expression was of 70%. Androgen receptor was expressed in 28% of tumors analyzed, EGFR in 89%, CK5/6 in 63%. VDR (cytoplasmatic and nuclear) expression was determined in 56 patients, finding in 45 (80%) an expression that was considered as high. We achieved a total of 73/160 pCR (45%) with a significant median disease free survival of 178 months (log rank p: 0,0001). In the univariate analysis, the initial tumoral size, initial nodal involvement and expression of VDR was significant; and in the mutivariate model the expression of VDR maintains the level of significance ( HR: 0,138 IC 95% 0,039-0,493 p:0,002).

**Conclusions:** The expression of VDR in the initial tumor before starting neoadjuvant chemotherapy is a strong predictor of recurrence independently of histological response with a median disease free survival of 149 months in patients with high expression versus 62 month. Furthermore, in patients without histological response, progression-free median survival is 86 months for patients with high expression versus 35 months.

# 2018 San Antonio Breast Cancer Symposium ®

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Predictive and prognostic value of stromal tumor-infiltrating lymphocytes before and after neoadjuvant therapy in triple negative and HER2-positive breast cancer

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**Background:** Lymphocyte predominant breast cancer subgroup, defined as  $\geq 50\%$  stromal tumor-infiltrating lymphocytes (sTILs), is associated with high pathological complete response (pCR) rate after neoadjuvant therapy (NAT) and favorable outcome. In a cohort of triple negative (TNBC) and HER2+ breast cancer (BC) patients treated with NAT, we aimed to assess the predictive and prognostic value of pre- and post-NAT sTILs and the information provided by the change in sTILs during NAT.

**Materials and methods:** Two-hundred and nine consecutive patients (n=80 TNBC; and n=129 HER2+) who received NAT between 2001 and 2009 in our institution were evaluated. Pre-NAT sTILs were assessed on biopsy sample (baseline) and post-NAT sTILs on surgical specimens just for non-pCR patients. sTILs level was categorized as low 0-9%, intermediate 10-49%, and high  $\geq 50\%$ . The change in sTILs during NAT was calculated as the absolute difference between pre- and post-NAT sTILs. We evaluated the association of pre-NAT sTILs and pCR, and the association between pre- and post-NAT sTILs, and their change with relapse-free survival (RFS).

**Results:** Overall pCR rate was 37.8% (31.3% for TNBC, 41.2% for ER+/HER2+BC, 42.3% for ER-/HER2+BC). In each subtype, pre-NAT low sTILs group was significantly associated with lower pCR rate. During the median follow-up period of 98 months, 44 recurrences (21.1%) were observed. For TNBC, low pre-NAT sTILs group was associated with higher recurrence risk compared with int/high sTILs (HR=4.675 [2.013-10.859],  $p<0.001$ ). For only non-pCR patients, both pre- and post-NAT sTILs were significantly associated with RFS. The risk of recurrence was higher in the group with low pre-NAT sTILs (HR=5.333 [1.731-16.427],  $p=0.004$ ), and the group of low post-NAT sTILs (HR=4.271 [1.498-12.173],  $p=0.007$ ). Patients with the change of sTILs increase during NAT were not associated with RFS, compared with decrease or equal group (log-rank  $p=0.163$ ). In multivariate analysis including both pre- and post-NAT sTILs, only pre-NAT sTILs retained significance (HR=3.844 [1.190-12.421],  $p=0.024$ ). Low post-NAT sTILs group showed only a borderline significant association with shorter RFS (HR=2.836 [0.951-8.457],  $p=0.061$ ), but it suggests that both pre- and post-NAT sTILs might provide independent prognostic information. In ER+/HER2+BC, low pre-NAT sTILs were associated with short RFS ( $p=0.036$ ), but this association was not significant when only non-pCR patients were considered. In ER-/HER2+BC, sTILs were not significantly associated with RFS.

**Conclusion:** In TN and HER2+ BCs, tumors with low pre-NAT sTILs have a low likelihood to achieve a pCR (predictive marker). In TNBC, low pre-NAT sTILs were associated with higher recurrence risk. In non-pCR TNBC patients, both low pre- and post-NAT sTILs were associated with shorter RFS. These results suggest that sTILs information should be taken into account when additional post-surgery treatments are considered in non-pCR patients.

# 2018 San Antonio Breast Cancer Symposium ®

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Trends in mortality and prognostic significance of hypercalcemia in breast cancer: A nationwide study

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## Background

Hypercalcemia(HC) in widespread breast cancers results from various mechanisms including increased bone turnover and secretion of Parathyroid hormone-related peptide (PTH-rP). Among patients affected with breast cancer, the presence of HC has been associated with poor prognosis. Recent advances in treatment of HC with bisphosphonates and RANK-L inhibitors, and widely metastatic breast cancers with systemic chemotherapy and immunotherapy, have caused a paradigm shift in the symptoms and progression free survival among these patients. However, with these recent advances, the trends and changes in prognostic significance of HC have not been investigated. Our study aims to examine the trends in the mortality of HC in Breast Cancer(BC) patients.

## Methods

Adult admissions (adm) (>18 years of age) between 1999 and 2014 with a primary diagnosis of BC were extracted from the Nationwide Inpatient Sample using the ICD-9 code 174.9. These were stratified into three categories based on the years of admission to minimize the effect of changes over the time interval studied. Adm were filtered for the presence of HC using the ICD-9 code 275.42. We performed bivariate analysis to determine the in-hospital mortality percentage across various categorical variables. Multivariate analysis using the cox proportional hazard regression was employed to estimate the hazard ratio(HR) of mortality within 30 days of admission during the hospitalization in patients with and without HC after controlling for confounders like age, race, income, insurance status, comorbidities, geographic location etc.

## Results

A total of 484,859 (N=98,631, weighted N=484,859) adm with a primary diagnosis of BC were extracted and stratified according to the year of admission into three year groups: Group1: 1999-2004, Group 2: 2005-2009 and Group 3: 2010-2014. The percentage of adm with HC increased from 0.52% in 1999 to 1.32% in 2014. The in-hospital mortality percentage and adjusted Hazard Ratios (HR) of mortality among different year groups based on the presence of HC are shown in the table.

Table 1: Inhospital Mortality and Hazard Ratios(HR)

Year Group	In hospital mortality percentage		P value	HR of Mortality With hypercalcemia vs without hypercalcemia(95% CI)	P Value
	With hypercalcemia	Without hypercalcemia			
Group 1: 1999 to 2004	28.65	4.05	<0.0001	1.96 (1.75-2.18)	<0.0001
Group 2: 2005 to 2009	20.13	4.25	<0.0001	1.39 (1.21-1.60)	<0.0001
Group 3: 2010 to 2014	13.90	2.83	<0.0001	1.22 (1.08-1.39)	0.001

## Discussion:

The mortality percentage and hazard ratio of death during hospitalization is significantly higher among the patients with HC when compared to their counterparts. The in-hospital mortality percentage declined over the course of 15 years. While examining the HR, there was a 96% increase in risk of mortality in group 1, 39 % increase in group 2, 22% increase in group 3.

## **Conclusion**

Despite the consistent decrease in mortality over the interval studied, the presence of HC continues to be an independent risk factor of mortality among patients with breast cancer after controlling for potential confounders. Considering the limitations of our study, there is further need to evaluate the prognostic significance of HC in breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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A novel seven-gene signature predicts prognosis in early-stage triple-negative breast cancer

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## **Purpose:**

Chemotherapy remains the only systemic treatment option for patients with triple-negative breast cancer (TNBC). However, due to the heterogeneity of TNBC, not all patients benefit from chemotherapy, especially those with early-stage disease. In order to improve prognostic assessment and reduce unnecessary adjuvant systemic therapy in these patients, we have developed a novel seven-gene signature.

## **Experimental Design:**

With the ComBat method, we integrated the results from 150 transcriptome microarrays samples and 246 RNA-seq samples of early-stage TNBC patients, and identified mRNAs associated with recurrence-free survival (RFS) using Lasso-Cox model. We further analyzed these TNBC samples and compared them with 60 paired normal breast tissues (40 samples from RNA-seq and 20 samples from microarrays) to identify tumor-specific mRNAs. Twenty-one overlapped mRNAs of the RFS-associated mRNAs and the tumor-specific mRNAs are selected as candidate mRNAs. An additional 371 samples of frozen primary tumors were then collected from early-stage TNBC patients (mean follow-up of 45 months) and randomly divided into two sets: a training set (n = 186) and a validation (n = 185) set. Expression level of candidate mRNAs in these samples were measured using RT-qPCR assays, and a seven-gene signature was built through all subset regression in the training set. The prognostic and predictive accuracy of our signature was tested in the validation set and other public databases (GSE5327, GSE2034 and METABRIC).

## **Results:**

Twenty-one candidate mRNAs were identified in early-stage TNBC patients, from which we developed a novel seven-gene signature (recurrence risk score [mRNA signature] =  $1.108 \times \text{TMEM101} - 0.213 \times \text{KRT5} - 0.315 \times \text{ACAN} - 0.464 \times \text{LCA5} + 0.446 \times \text{RPP40} - 0.373 \times \text{LAGE3} - 0.257 \times \text{CDKL2}$ ). Patients in the training set were classified into high- or low-risk group based on our seven-gene signature and an optimum cut-off score derived from x-tile. The patients in high-risk group were more likely to suffer from recurrence (HR, 2.718; 95% confidence interval [CI], 1.928–3.726, P= 0.001), and a time-dependent receiver operating curve showed that the seven-gene mRNA signature had a better prognostic value than the clinicopathologic risk factors in both training set and validation set. The prognostic and predictive accuracy of the signature was also validated in the METABRIC and two other public GEO databases (GSE5327 and GSE2034). The time-dependent receiver operating curve showed that this signature had an area under the curve (AUC) of 0.742 (95% CI, 0.705-0.773) in METABRIC, 0.716 (95% CI, 0.682-0.739) and 0.723 (95% CI, 0.683-0.756) in GSE5327 and GSE2034 respectively.

## **Conclusion:**

In this study, we developed a novel seven-gene signature which can provide additional prognostic information and may guidance in identifying early-stage TNBC patients eligible for adjuvant therapy or reduction of chemotherapy. To our knowledge, this is the first study investigating the prognostic potential of mRNA signature in early-stage triple-negative breast cancer. Our novel signature may provide an opportunity for de-escalating treatment in early-stage TNBC patients in the future.



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The network metanalysis of data from PALOMA 2, MONALEESA 2, MONARCH 3, FALCON, SWOG and FACT trials: Progression free survival (PFS) benefit from first-line endocrine-based therapies in postmenopausal women with HR+ HER2- metastatic breast cancer (MBC) according to different prognostic subgroups

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## Background

The three classes of Cyclin Dependent Kinase (CDK) 4/6 inhibitors, Palbociclib (P), Ribociclib (R) and Abemaciclib (A), in combination with non-Steroidal Aromatase Inhibitors (nSAIs) showed improvement on Progression Free Survival (PFS) in patients with HR+/HER2- MBC compared to AIs monotherapy.

Fulvestrant (F) also showed a PFS benefit over AIs in first-line setting of endocrine naive patients (pts) which was even greater in pts without visceral disease.

The absence of direct comparison between F and CDK 4/6 combination therapies and their less favorable toxicity profile generated great interest in the identification of a specific subgroup of pts based on clinical and pathological factors for decision-making in the use of endocrine monotherapy.

This analysis combines data from six randomized phase III trials investigating the role of endocrine-based therapies in the first-line setting of HR+/HER2- MBC to identify clinical factors in the choice among available drugs.

## Methods

A Bayesian network meta-analysis was carried out for PFS; Hazard Ratio (HR) and 95% CI were reported. Potential treatment effect modifying covariates were investigated using sub-group analysis, stratifying by age, ECOG, ethnicity, prior chemotherapy or endocrine therapy exposure, measurable disease at the time of metastasis occurrence, visceral or bone only disease, time from the initial diagnosis of breast cancer to the metastasis onset. Data analysis was performed using R Statistical Software version 3.5.0

## Results

In the absence of direct comparison between CDK 4/6 inhibitors + nAIs and F endocrine-based therapies, all these therapeutic options resulted in significant PFS benefit compared to nAIs monotherapy (HR: 0.74; 95% CI 0.67-0.80). However, among the three classes of CDK 4/6 inhibitors and F a significant longer PFS was observed according to some clinical-pathological factors as followed reported: from P + nAIs in "bone only" disease (HR 0.47; CI 0.25-0.86); from A + nAIs in "de novo" subgroup (HR 0.60; CI 0.37-0.97), in "Asian" population (HR 0.37; CI 0.16-0.85) and "non visceral" disease (HR 0.48 CI 0.25-0.89); from R + nAIs in "de novo" subgroup (HR 0.55; CI 0.32-0.95) and in "visceral" disease (HR 0.66 CI 0.45-0.96); from all the three combination strategies (A, P and R) in "prior endocrine" exposure subgroup (HR 0.47 CI 0.25-0.87; HR 0.60 CI 0.45-0.80; HR 0.64 CI 0.41-1.0, respectively). Even though no significant PFS benefit was observed in the remaining subgroups, combined CDK 4/6 strategies appeared more effective than F according to relative HR.

## Conclusions

CDK 4/6 inhibitors endocrine-based therapies as first-line treatment for postmenopausal women with HR+/HER2- MBC showed PFS improvement, regardless of prognostic subgroup and additionally advantage was emerged by indirect comparison with F. Further direct comparative studies are needed to explore greater benefits from different therapeutic options.

## 2018 San Antonio Breast Cancer Symposium ®

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A significance of SUVmax levels on FDG-PET as a prognostic factor may be mediated by local immune environment of breast cancer

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**(Background)** SUVmax levels (maximum radioactivity concentration per a pixel) on FDG-PET reflect glucose uptake and it is clinically useful as a prognostic factor. It is reported that breast cancer with high levels of SUVmax causes insufficient glucose concentration in stromal tissue, which results in suppressed cytotoxic T-lymphocytes function. These data may indicate that the prognostic significance of SUVmax levels is influenced by local immune environment of breast cancer. The aim of this study is to investigate whether local immune responses of breast cancer affect correlation of SUVmax levels and prognosis.

**(Method)** The 278 invasive breast cancer patients were recruited who underwent surgery at Hyogo College of Medicine Hospital and whose SUVmax levels in the breast were examined before surgery or neo-adjuvant therapy. The cutoff value of SUVmax levels was set at 3.585. Tumor infiltrate lymphocytes (TILs) were evaluated as a local immune response and the distributions of TILs were divided into three groups, inflamed (intra-tumoral lymphocytes, Inf), immune excluded (peri-tumoral lymphocytes, IE) and immune desert (very few lymphocytes, ID). During follow-up period (median 39 months), 21 patients relapsed.

**(Results)** Relapse free survival (RFS) in the SUVmax-high group was significantly worse than in the SUVmax-low group ( $p=0.0026$ ). There was no correlation between TILs distribution patterns and RFS. In the IE+ID group (175 patients) SUVmax levels were not correlated with prognosis, but in the Inf group (103 patients) RFS of SUVmax-high was significantly worse than of SUVmax-low ( $p=0.0051$ ). In the multivariate analysis including nodal status and nuclear grade, SUVmax levels of the Inf group was an independent prognostic factor.

**(Discussion and conclusion)** SUVmax levels in primary lesions were correlated with prognosis only in the Inf group and were not in the IE+ID group. A significance of SUVmax levels as a prognostic factor may be diverse depending on the local immune environment of breast cancer. A novel therapeutic strategy such as inducing suppression of glucose uptake in cancer cells is suggested for breast cancer with immune inflamed.

# 2018 San Antonio Breast Cancer Symposium ®

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Comparing prognostic performance of different lymph node staging systems among patients with breast cancer

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**Background:** Metastatic regional lymph nodes (LN) is a strong predictor of worse long-term outcome after resection of breast cancer. This study aimed to compare the prognostic performance of American Joint Committee on Cancer (AJCC) N stage relative to lymph node ratio (LNR), log odds of metastatic lymph nodes (LODDS), number of removed lymph nodes (NRLNs), and number of negative lymph nodes (NNLNs) in breast cancer patients.

**Methods:** All of the breast cancer patients who underwent surgery between 2004 and 2012 were identified from the Surveillance, Epidemiology, and End Results database. Restricted cubic spline functions were used to characterize the association between continuous variables and the risk of death and determine the optimal cut-off points. The Cox proportional hazards models were constructed, and the relative discriminative abilities of the different LN staging systems were assessed using the Akaike's Information Criterion (AIC) and the Harrell's concordance index (C-index).

**Results:** A total of 264,096 breast cancer patients were enrolled, and 177,598 (67.2%) had no lymph node metastasis, whereas 86,498 (32.8%) had lymph node metastasis. 187,785 (71.1%) patients had a limited number of LNs harvested (NRLN <10). The median follow-up time was 73 months, and the 8-year overall survival (OS) and breast cancer-specific survival (BCSS) were 82.6% and 90.4%, respectively. LNR, LODDS, NRLNs, and NNLNs were all nonlinearly associated with OS and BCSS. Patients with metastatic LN had an increased risk of OS (hazards ratio: 2.32, 95% confidence interval: 2.27–2.37;  $P < 0.001$ ) and BCSS (hazards ratio: 4.53, 95% confidence interval: 4.40–4.66;  $P < 0.001$ ). When LNR was equal to 0 or 1, there was a heterogeneity of outcomes, and LODDS still yielded informative values compared to LNR. Among the entire cohort, LNR modeled as a continuous variable had a somewhat better prognostic performance (AIC: 923231.4 and C-index: 0.722 for OS; AIC: 482962.3 and C-index: 0.817 for BCSS) than any of other LN staging systems. However, a model with AJCC N stage showed the best fit in patients with a limited number of LNs harvested (AIC: 501321.8 and C-index: 0.699 for OS; AIC: 212605.6 and C-index: 0.809 for BCSS). When assessed among patients with metastatic LN, LODDS outperformed other staging systems including AJCC N stage, LNR, NRLNs and NNLNs, whenever assessed using continuous (AIC: 428626.2 and C index: 0.728 for OS; AIC: 296886.8 and C index: 0.770 for BCSS) or categorical (AIC: 429527.5 and C index: 0.722 for OS; AIC: 297796.6 and C index: 0.762 for BCSS) cutoff values.

**Conclusions:** Although LNR assessed as a continuous variable was the most potent method to stratify patients regardless of LN status, the prognostic superiority of LNR is confounded by a limited LN harvest. LODDS was a better and more powerful predictor of survival when patients were LN positive, especially among those patients with either very low or high LNR.

# 2018 San Antonio Breast Cancer Symposium ®

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The prognostic impact of synaptojanin 2 expression in estrogen receptor  $\alpha$ -positive breast cancer patients

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**Background:** Synaptojanin 2 (SYNJ2) was reported to be a druggable mediator of metastasis. It is overexpressed and amplified in breast cancer, particularly estrogen receptor  $\alpha$  (ER $\alpha$ )-positive breast cancer. *SYNJ2* was also shown to promote cell migration and invasion in breast cancer xenograft cultures and lung metastasis in mice. Here, we investigated *SYNJ2* mRNA expression in breast cancer patients during long-term follow-up.

**Materials and methods:** A total of 434 invasive breast cancer tissues were analyzed for *SYNJ2* mRNA expression using TaqMan PCR, and the correlation of this expression with patient clinicopathological factors was determined. We also examined the expression of markers associated with tumor-initiating capacity, such as *SNAI1*, *SNAI2*, and *VIM*. Survival curves were analyzed using the Kaplan–Meier method. Cox proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values.

**Results:** The median follow-up period was 10.7 years. We found positive correlations between high expression of *SYNJ2* mRNA and shorter disease-free survival in breast cancer patients (P=0.049), which was limited to ER $\alpha$ -positive patients (P=0.020) and not seen in ER $\alpha$ -negative patients (P=0.863). High *SYNJ2* mRNA levels were positively correlated with high tumor grade, ER $\alpha$  negativity, and node positivity. Multivariate analysis indicated that high *SYNJ2* mRNA expression was an independent factor for poor disease-free survival in breast cancer patients.

Multivariate analysis of poor disease-free survival

			DFS	
			Multivariate	
		All patients	p value	HR (95%CI)
Tumor size	2cm and fewer	154	0.4315	1 (Reference)
	more than 2cm	280		1.19 (0.78-1.85)
Lymph node metastasis	Negative	219	0.0001 and fewer	1 (Reference)
	Positive	184		5.61 (3.59-9.05)
	Unknown	31		2.40 (0.89-5.48)
Grade	1,2	233	0.3847	1 (Reference)
	3	195		1.20 (0.79-1.81)
	Unknown	6		0.17 (1.78-3.49)
ER status	Positive	337	0.0262	1 (Reference)
	Negative	97		2.06 (1.87-4.07)
PgR status	Positive	293	0.3951	1 (Reference)
	Negative	141		1.30 (0.70-2.28)
SYNJ2 mRNA expression	low	217	0.0225	1 (Reference)
	high	217		1.57 (1.07-2.34)

**Conclusion:**High *SYNJ2* expression was shown to be an independent predictive factor of poor prognosis in ER $\alpha$ -positive breast cancer patients. *SYNJ2* could therefore be used as a candidate biomarker and therapeutic target in breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Influence of prognostic factors on outcomes among metastatic breast cancer patients treated with CDK4&6 inhibitors in routine clinical practice

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**Background:** Evidence suggests that there are clinical features associated with a less favorable prognosis among patients with HR+/HER2- metastatic breast cancer (MBC) such as metastases to non-bone sites, including liver and lung, and negative progesterone receptor (PgR-) status. The objective of this study was to compare baseline characteristics and outcomes between those with and without these clinical factors among a cohort of HR+/HER2- MBC patients treated with a CDK4&6 inhibitor (CDK4&6i).

**Methods:** This was a retrospective analysis of the Flatiron Health electronic health records-derived database for US patients diagnosed with MBC between 1/1/2011 and 9/30/2017. The study included a random sample of patients with HR+/HER2- MBC who were treated with a CDK4&6i on or after 6/30/2016. Baseline variables, including demographics, comorbidities, and sites of metastasis, were recorded at start of the first CDK4&6i containing line of therapy in the metastatic setting on or after this date. Dates of real-world progression were abstracted from patient charts. Descriptive statistics and appropriate statistical tests were used to compare baseline characteristics between patients with or without select clinical factors associated with unfavorable outcomes. In patients who received a CDK4&6i-based therapy, Kaplan–Meier methods and univariable Cox proportional hazards models were used to assess real-world progression free survival (rwPFS) by line from start of line to the date of first progression or death within line (unadjusted for treatment and other potential confounders).

**Results:** 518 patients were included in this study. Median age at metastatic diagnosis was 66y (IQR; 59-73y); 99% female and 11.4% had PgR- status. At baseline, 20.5%, 46.3% and 65.8% of patients had liver, visceral (defined as liver and/or lung), and non-bone only metastases, respectively. Among a total of 207 patients who received a CDK4&6i as initial therapy in the metastatic setting, 69.1% received it in combination with an aromatase inhibitor, 29.5% received it in combination with fulvestrant, and 1.4% as monotherapy. Within the same group, 58 had disease progression or died during first line (1L); median rwPFS measured from start of 1L was not reached (95% CI: 10.7 months, NA). Univariable analyses revealed the presence of liver metastases was associated with a higher risk of progression or death compared to no liver metastases (HR: 2.04, 95% CI: 1.13 - 3.68). Having non bone-only metastases was associated with a higher risk of progression or death compared to having bone-only metastases (HR: 2.23, 95% CI: 1.20 – 4.15). Univariable analyses did not reveal any statistically significant differences in first-line rwPFS by PgR status or presence of visceral metastases. Results from other lines of therapy are forthcoming.

**Conclusion:** In a real world data set, and consistent with prior prospective data, presence of liver and non-bone only metastases were associated with a higher risk of progression among patients with HR+/HER2- MBC receiving initial therapy with a CDK4&6i. The heterogeneity of prognoses among this population reinforces the need to consider these clinical features in treatment decisions for optimal patient outcomes.

# 2018 San Antonio Breast Cancer Symposium ®

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Predictors of distant metastasis in patients with triple negative breast cancer who failed to achieve a pathological complete response after neoadjuvant chemotherapy

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## Background

Patients with triple-negative breast cancers (TNBC) are at an increased risk of distant metastasis compared to patients with other subtypes of breast cancer. While TNBCs are aggressive as a group, many are potentially curable, reflecting an underlying heterogeneity. As such, there is an interest in identifying factors that may allow further stratification of patients in relation to the risk of distant metastasis and, ultimately, better tailor treatment plans to individual patients. With the increasing use of neoadjuvant chemotherapy (NAC), past studies have shown that patients who achieve a pathological complete response (pCR) following NAC have a decreased risk of distant metastasis. However, beyond the presence or absence of pCR, other risk factors for distant metastasis have not been well characterized.

## Methods

This is a single institution, retrospective study of women with TNBC treated with NAC, surgery, and radiation therapy (RT) between 2000 and 2013. The rate of distant metastasis was estimated and compared between patients who achieved pCR versus those who did not achieve pCR using Kaplan-Meier method. In patients who failed to achieve pCR, patient-specific and treatment-specific factors including age, race, menopause status, family history, smoking history, clinical stage, histology, NAC regimen, whether breast conserving surgery was performed, response to NAC, treatment with adjuvant chemotherapy, and use of RT boost were analyzed using multivariable cox proportional hazards method to evaluate factors associated with distant metastasis.

## Results

A total of 153 patients with a median follow up of 48.6 months were included. Of the 153 patients, 108 (70.9%) were identified as not having pCR following NAC. Among those 45 patients that did achieve a pCR, only 1 patient (2.2%) went on to have distant metastasis. In contrast, of the 108 patients that failed to achieve a pCR, 47 (43.5%) went on to have distant metastasis. On univariable analysis, factors associated with distant metastasis in patients that did not achieve a pCR included increasing clinical and pathological T and N stage, positive pathologic lymph node status, multifocality, lymphovascular space invasion (LVSI), extranodal extension, and failure of downstaging after NAC. After controlling for potential confounders in multivariable analysis, higher pathological N stage (HR 2.18, 95% CI 1.12 - 4.22), positive pathologic lymph nodes (HR 2.21, 95% CI 1.02 - 4.80), LVSI (HR 1.87, 95% CI 1.04 - 3.37), and multifocality (HR 2.05, 95% CI 1.05 - 4.03) were found to be independent predictors of distant metastasis.

## Conclusions

Approximately 43.5% of patients with TNBC that did not achieve a pCR went on to develop distant metastasis, perhaps reflecting an underlying chemo-resistance of these non-pCR tumors. Here we identify multiple risk factors associated with distant metastasis among patients not achieving a pCR, including positive lymph nodes, LVSI, high pathologic N stage, and multifocality. This data can be used to inform prognoses and treatment decisions in this high-risk cohort of patients and future clinical trials are warranted to lower the risk of distant metastasis in this population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-40

Reconsidering “at risk” criteria for breast cancer recurrence in hormone positive patients: Risk stratification is still important in patients with an Oncotype Dx recurrence score  $\leq 25$ !

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**Introduction:** The recent TAILORx results suggest that additional systemic chemotherapy may not be necessary in certain hormone +, HER2 -, node negative breast cancer patients with an Oncotype Dx recurrence score (ODXRS)  $\leq 25$ . ODX is an expensive test (current list price of \$4,650.00), and cost has been an impediment to its adoption in many centers throughout the world. Based on a modification of the new Magee equations (Klein ME, et al. *Mod Pathol.* 26[5]) we published data based on 283 patients with ODXRS's (Turner BM, et al. *Mod Pathol.* 28[7]), suggesting that the modified Magee equation (MME) offered a less expensive alternative to ODX testing in certain breast cancer patients. We now have outcome data suggesting that the MME along with progesterone receptor (PR), Ki-67, lymph node (LN) status, and lymphovascular invasion (LVI) status can be helpful in predicting which patients with an ODXRS  $\leq 25$  are more likely to recur. **Methods:** 248 patients with information on estrogen receptor (ER), PR, Ki-67, Her-2 status, Nottingham score, tumor size, LN status, LVI status, and an available ODXRS (2008-2018) were identified from the pathology files at the University of Rochester Medical Center. **Results:** All of the patients that recurred had an average modified Magee score (MMS)  $\geq 14$  (Table 1). Patients with LN involvement (5/43, 12%) or with LVI (5/27, 19%) had a higher percentage of recurrence than patients without LN involvement (8/197, 4%) or without LVI (8/216, 4%). Patients that recurred had a significantly ( $p < 0.05$ ) lower PR and higher Ki-67 than patients in the same risk class that did not recur (Table 2). Neither grade nor ER status was significantly different between patients that recurred and did not recur (Table 2). All of the patients that recurred had at least a lowered PR, higher Ki-67, LN involvement, or LVI, and most had some combination of these variables (Table 1). 8 of the 13 patients that recurred in our population (61.5%) had an ODXRS of  $\leq 25$ . **Conclusions:** Risk stratification is still important in patients with an ODX score  $\leq 25$ . The MMS along with PR, Ki-67, LN, and LVI status can be helpful in predicting patients with a higher risk of recurrence.

Table 1: Recurrence data

ODXRS	MMS	Type of therapy*	Nodal and LVI status**	Nottingham score	ER-H score***	PR-H score***	Ki-67
11	19.1	NONE	NONE	6	240	180	27
13	19.7	H	NONE	5	300	15	20
15	21.6	H	N	5	120	90	5
15	14	H	NONE	5	270	210	UNKNOWN
16	15	H	LVI	5	270	180	5
17	27.2	H	B	6	270	1	60
19	16.3	H	NONE	4	285	45	UNKNOWN
24	24.5	C	LVI	8	240	105	35
27	23.9	B	NONE	7	210	100	35
28	21	H	N	5	270	21	25
28	23.4	H	B	5	285	30	35
31	28.7	H	B	8	270	1	45
44	32.3	H	NONE	9	210	60	70
<b>21.4****</b>	<b>21.4****</b>	-	-	<b>5.9****</b>	<b>250.7****</b>	<b>93.4****</b>	<b>31.4****</b>

\*H = Hormone only;C = Chemo only;B = Both; \*\* N = Nodal involvement;L = LVI;B = Both; \*\*\*modified (Turner BM, et al. Mod Pathol. 2015;28(7):921-31); \*\*\*\* average

Table 2: Recurrence data in specific populations

Population type	N	Grade	ER*	PR*	Ki-67
≤ 25 no recurrence	216	5.4	249.7	185.7	12.8**
≤ 25 recurrence	8	5.5	249.4	103.3	25.3***
> 25 no recurrence	26	7.3	211.7	76.8	34.1****
> 25 recurrence	5	6.8	249.0	42.5	42.0

\* modified (Turner BM, et al. Mod Pathol. 2015;28(7):921-31); \*\* n = 181; \*\*\* n = 6; \*\*\*\* n = 23



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-41

Biology, metastases pattern and survival of triple negative breast cancer (TNBC) – A comparison between younger (<40 years) and elderly (>74 years) patients

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**Background:** TNBC remains a poorly defined and heterogeneous subset of breast cancer with an adverse prognosis that more often affects younger patients. The biology and outcome of TNBC in elderly patients are less studied.

**Aims:** To determine the biology, recurrence rate, metastases pattern and survival from diagnosis of primary BC, as well as from diagnose of recurrences in a population based cohort of primary TNBC with focus on the comparison between younger and elderly patients.

**Material and Methods:** Patients with primary TNBC stage I-IV diagnosed from 2007 through 2015 were identified through the regional cancer registry and detailed information on tumor biology: histological grade, lymphovascular invasion (LVI) and Ki67; stage, type of adjuvant/neoadjuvant treatment, disease-free interval, type of recurrences (involved organs), and survival times were extracted from patients' charts. The definition of younger (<40 years) and elderly (>74 years) patients was chosen as these patients not participate in mammogram screening programs. For comparisons all factors but Ki67 were run as dichotomous variables.

**Results:** A total of 525 patients median age 60 years (range: 24-94) were identified. Stage at diagnose were: stage I (24.0%); II (44.6%); III (23.8%) and IV (3.4%). Biology parameters are as follows: Histopathological grade I (1.7%); grade II, (18.5%) and grade III (77.8%) respectively. LVI was present in 24.4%; median Ki67 was 70% (range 1-100%) and median follow-up time is 55.9 months (range: 24-94). In the whole cohort, 396 (75.5%) patients received adjuvant/neoadjuvant chemotherapy and 373 (71%) patients are free from recurrences. The median DFI was 14.9 months (range: 2.0-64.7).

The clinical stage at diagnosis did not differ between younger (n=58) and elderly (n=96) patients; stage I (12% vs 11.5%; stage II (63.8% vs 48.7%; stage III (22.4% vs 30.8%); stage IV (1,7% vs 9,0%) (p=0.17). A statistically significant difference was found concerning histopathologic grade (grade III 77.0% vs 53.0%; p=0.006) and Ki67 (median 74.5% versus 62.7%; p=0.003) but not for LVI (p=0.915) with a higher proportion of poorly differentiated high proliferative tumors among younger patients. A larger proportion of younger patients received (neo-)adjuvant chemotherapy compared with elderly (94.8% versus 11.5%; p>0.001). Pattern of recurrence was similar concerning lung (p=0.38); liver (p=0.1); distant lymph nodes (p=0.35) but breast cancer brain metastases were statistically significantly more frequently registered among younger patients (p=0.009). Shorter survival times were found among elderly patients; RFS (p=0.011); BCSS (p=0.002); OS (p<0.001), as well as survival following diagnose of recurrence (p=0.034).

**Conclusions:** We show that primary TNBC is more aggressive in terms of poor differentiation grade and high proliferation rate with more frequently development of brain metastases in younger compared with elderly patients. The majority of elderly patients still have grade III tumors with a Ki67 > 60% and survival following diagnosis of both primary BC and metastatic BC is short. Our results underline the need for novel treatment options suitable also for an elderly patient population.

# 2018 San Antonio Breast Cancer Symposium ®

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Use of breast cancer index to analyze tumor proliferation and endocrine responsiveness in genomic intermediate risk patients

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**Background:** Breast Cancer Index (BCI) is a genomic assay that stratifies patients (pts) for cumulative 10-year and late (post-5-year) risk of distant recurrence and predicts the likelihood of extended endocrine therapy (EET) benefit based on the algorithmic analysis of gene expression from two functional gene cassettes: 1) The Molecular Grade Index (MGI), which contains 5 proliferation genes and 2) The HoxB13/IL17BR (H/I) ratio, an endocrine response biomarker. BCI more precisely resolves genomic intermediate risk patients into low- and high-risk groups (Sestak, *Clin Cancer Res* 2016) and adds significant prognostic data beyond clinical features (ie, clinical treatment score; Sestak, *JAMA Oncol* 2017) by interrogating different aspects of tumor biology such as proliferation and endocrine response. The objective of this study was to measure tumor proliferation and endocrine responsiveness using MGI and H/I respectively and evaluate their correlation with age in genomic intermediate risk pts.

**Methods:** This study utilized a subset of cases from the BCI Clinical Database for Correlative Studies, an IRB-approved de-identified database that contains clinicopathologic and molecular variables from clinical cases submitted for BCI testing. Genomic intermediate risk cases were defined as LN-, HER2- (or HER2 status unknown) with 21-gene recurrence scores (RS) of 11 to 25 using cut-points from the TAILORx study. Quantitative scores for MGI and H/I were derived by algorithmic analysis of BCI gene expression. Age groups ( $\leq 50y$  and  $\geq 51y$ ) were determined using the date of diagnosis. MGI and H/I were evaluated in two different genomic intermediate risk groups: RS 11-15 and RS 16-25. Pearson correlation coefficients were used to determine the correlation between MGI, BCI, H/I, and genomic intermediate risk scores for patients  $\leq 50y$  and  $\geq 51y$ .

**Results:** Of the 441 pts with RS and BCI results, 303 (69%) with genomic intermediate risk were analyzed. The median MGI score in pts  $\geq 51y$  was higher in the RS 16-25 group compared to RS 11-15, but there was no difference in MGI score between the two genomic intermediate RS risk groups for pts  $\leq 50y$ . In contrast to MGI, median H/I was higher in the RS 16-25 group irrespective of age, with 37% of pts  $\leq 50y$  and 41% of pts  $\geq 51y$  having tumors predicted as more likely to benefit from EET using validated cut-points for H/I. There was no significant correlation between tumor proliferation (MGI:  $r=0.166$ ) or endocrine responsiveness (H/I:  $r=0.244$ ) with genomic intermediate-risk RS 11-25 group.

**Conclusion:** These data, which show variations in tumor proliferation and endocrine signaling based on age and genomic intermediate risk group, highlight the importance of measuring different features of tumor biology for risk classification and prediction of therapy response. The absence of correlations between tumor proliferation and genomic intermediate risk and age, and between estrogen signaling and genomic intermediate risk and age, suggests that assays such as BCI that combine distinct aspects of tumor biology for prognosticating risk of recurrence and prediction of benefit from endocrine therapy provide additional value for individualizing the management of patients with early-stage ER+ breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-43

Can optoacoustic imaging combined with ultrasound non-invasively offer prognosis for breast cancer molecular subtypes?

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**Aim:** To investigate the role of optoacoustic imaging combined with gray-scale ultrasound (OA/US) to better differentiate between breast cancer molecular subtypes.

**Materials and Methods:**

This prospective 5-center study was performed in the Netherlands between March 2015 and February 2016. Only masses considered suspicious at conventional diagnostic breast ultrasound (US) were included. The study was approved by the institutional ethical boards of the participating hospitals and written informed consent was obtained from all patients. Dedicated breast radiologists evaluated the included masses using OA/US and scored the internal and external OA/US features accordingly. Spearman Correlation was used to analyze the relationship between OA/US features and mitotic figures. The same statistical method was also used to evaluate the correlation between OA/US features and percentages of ER, PR and Ki67. Wilcoxon-Mann-Whitney tests were used to analyze the relationship between OA/US features and molecular subtypes of breast cancer (Luminal A, Luminal B, Triple Negative and HER2-enriched breast cancers).

**Results:** Overall, 209 patients with 215 breast lesions were included in this study. Sixty-seven masses were considered malignant and the 59 masses classified as invasive breast cancers were included in the final mitotic figures, ER, PR, Ki-67 and molecular subtype analyses. Significant correlations were found between OA/US Total Internal Features and ER ( $p = 0.0333$ ) and Ki-67 ( $p = 0.0092$ ) percentages. Regarding molecular subtypes, Internal Vessels ( $p = 0.0257$ ), Total Internal Features ( $p = 0.0196$ ) and combined Total Internal and External Features ( $p = 0.0289$ ) helped to differentiate between Luminal A and Luminal B cancers. Internal Vessels ( $p = 0.0030$ ), Internal Blush ( $p = 0.0044$ ), Total Internal Hemoglobin ( $p = 0.0053$ ), Total Internal Features ( $p = 0.0010$ ), Total Internal divided by Total External Features ( $p = 0.0255$ ) and combined Total Internal and External Features ( $p = 0.0108$ ) helped to differentiate between Luminal A and Triple Negative breast cancers. Total Internal Features showed a borderline result ( $p = 0.0551$ ) regarding the differentiation between Triple Negative and HER2-enriched subtypes.

**Conclusions:** The use of OA/US features to non-invasively differentiate between breast cancer molecular subtypes may help to establish an earlier prognosis and treatment planning, potentially decreasing costs and facilitating larger scale diagnosis. Future research with larger sample sizes may confirm these preliminary results.

# 2018 San Antonio Breast Cancer Symposium ®

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Tumor infiltrating lymphocytes in triple negative breast cancer: Our experience at an Argentine breast unit

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## Introduction

The assessment of Tumor Infiltrating lymphocytes (TILs) in primary breast cancer (BC) has been well established as a clinically relevant immunologic biomarker. The available evidence suggests that the extent of the lymphocytic infiltrate has prognostic and predictive significance, especially in triple negative and Her2 positive breast cancers. The presence of TILs has been associated to better response to adjuvant and neoadjuvant treatment as well as better overall long-term prognosis. The goal of this study was to establish the prognostic implications of the presence of TILs in triple negative breast cancer (TNBC) in our patient population.

## Materials and Methods

We retrospectively analyzed data collected from all patients who underwent treatment for triple negative breast cancer (TNBC) at the Hospital Italiano at Buenos Aires between January 2007 and December 2016. The presence of TILs was defined as the proportion of mononuclear cells (lymphocytes and plasma cells) in the stromal compartment and within the tumor surface area. The TILs were evaluated on hematoxylin and eosin (H&E) stained tumor sections, measuring 4-5 µm in thickness. Hotspots were not considered. Absence of TILs was defined as less than 10% of mononuclear cells in the stromal compartment. We stratified patients into two groups according to whether the presence of TILs was greater or less than 50%.

## Results

A total of 2350 patients underwent treatment for breast cancer (BC) at the Hospital Italiano de Buenos Aires over this period. Among these, 169 (7,19%) were TNBC. The mean patient age was 59.4 years. Seventy percent of patients were postmenopausal. Breast conserving therapy (BCT) was feasible in 61% of patients. The rate of axillary metastasis was 43.7%. Thirty-three patients (19.5%) presented TILs greater than 50%. Mean follow up was 55 months (1-133 months). Patients were stratified according to percentage of TILs. Among the group with TILs greater than 50%, 73 patients (53%) remain disease-free. On the contrary, patients with TILs greater than 50%, 25 (75%) remain without disease, which resulted in a statistically significant difference in survival.

## Events in TNBC

TILs	Disease Free	(%)	Distant Disease	(%)	Death (Breast Cancer)	(%)
TILs <50	73	53	4	2	50	36
TILs >50	25	75	2	6	4	12

Upon statistical analysis (cox proportional hazard risk model), patients with TILs above 50% had a hazards ratio (HR) of 0.47 for events ( $p = 0.046$ ; CI<sub>95%</sub> 0.23 – 0.98). The Odds Ratio (OR) for death associated with high TILs was 0.24 (CI<sub>95%</sub> 0.08 – 0.72). After adjusting for tumor size and axillary status, the difference continues to be statistically significant: OR 0.26 ( $p = 0.01$ ; IC 95% 0.08 – 0.81).

## Conclusions

In our experience, patients presenting TILs showed significantly less events during follow up. The presence of TILs in this subgroup appears to behave as an independent prognostic factor in our patient population, which reproduces the data currently published.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-45

Malic enzyme 1 is a potential metastasis-related biomarker of breast cancer

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**Background:** Malic enzyme 1 (ME1) catalyzes malate to pyruvate and thus promotes glycolysis, playing a part in the Warburg effect. Recently, several researches have revealed its crucial role in cancer metastasis. KM-plotter, an online analysis tool, showed high ME1 mRNA level was related to poorer RFS and OS. Herein, we explore the prognostic effect of ME1 on invasive breast cancer in Chinese patients and its effect on metastasis *in vitro*.

**Methods:** 220 patients with early breast cancer were included in this study. ME1 expression was evaluated semi-quantitatively with tissue microarray-based immunohistochemistry. The relationships between ME1 expression level and clinicopathological features were explored. Survival analyses were carried out by Kaplan-Meier test and COX proportional hazard model. MCF-7 and MDA-MB-468 cell lines were then used for *in vitro* cell migration and invasion assays.

**Results:** High expression of ME1 was observed in 51.5% patients. The median follow-up period was 28.9 months (range 0.5-34.0). In correlation analyses, compared with ME1-low cases, ME1-high cases were significantly associated with larger tumor size ( $P=0.036$ ), positive lymph nodes ( $P<0.001$ ) and positive lymph-vascular invasion ( $P=0.003$ ), and tended to be HER2 positive ( $P=0.094$ ). Survival analysis by Kaplan-Meier test showed high ME1 expression was significantly correlated with poorer recurrence free survival (RFS) ( $P=0.015$ ). Multivariate analysis identified high ME1 expression as an independent prognostic factor for RFS ( $P=0.035$ , HR=5.072 [1.121, 22.942]). Stratified analysis revealed high ME1 expression was related to poorer RFS among cases more than 45 years old ( $P=0.029$ , HR=9.833 [1.269, 76.164]) and among those with Ki67 index  $\geq 20\%$  ( $P=0.038$ , HR=3.805 [1.074, 13.486]). *In vitro*, HER2 positive cell line (SKBR3) and TNBC cell lines (MDA-MB-231, MDA-MB-468) showed high expression of ME1, while luminal cell line (MCF-7) showed low expression of ME1. Upregulation of ME1 in MCF-7 cell line remarkably enhanced its ability in migration and invasion, while knockdown of ME1 in MDA-MB-468 cell line had a profound inhibitory effect on migration and invasion.

**Conclusions:** ME1 enhances migration and invasion of breast cancer cell lines and may be involved in early development of breast cancer metastasis. Thus, ME1 is a promising prognostic indicator and a potential therapeutic target.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-46

Prognostic value of tumor-infiltrating lymphocytes (TILs) and clinical values for prediction of breast cancer recurrence in HER2 positive early breast cancer after surgery in King Chulalongkorn Memorial Hospital

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**Background:** Tumor-infiltrating lymphocytes (TILs), one of immunological biomarkers have been investigated in breast cancer and other cancers. High levels of TILs or lymphocyte-predominant breast cancer subgroup (LPBC) were associated with better prognosis in HER2 positive and triple negative breast cancers from various studies. Recently, TILs is also the predictive biomarker for response to neoadjuvant chemotherapy. Clinical utility of TILs has been recommended in routine clinical practice for breast cancer patients. However, limitation of TILs in HER2 positive women in Thai ethnicity was reported. So, the aim of this study was to evaluate TILs in combination with clinical values as the prognostic value for predicting the recurrent breast cancer in Thai population.

**Methods:** Four hundred and eighty-six patients with early stage HER-2 positive breast cancer who were diagnosed and treated at King Chulalongkorn Memorial Hospital from January 2005 to December 2016 were reviewed retrospectively. Clinico-pathological features, stromal TILs classified as low, intermediate and high, and survival outcomes were analyzed.

**Results:** The median age was 52 years (26-85). Of the 486 HER2 positive women, 56% had postmenopause, 54.5% had T2 tumor, 47.7% had node negative, 66.8% had stage I-II, 44.6% had lymphovascular invasion, and 47.5% had positive hormonal receptor. For the primary treatment, 67.3% underwent modified radical mastectomy, 96.5% received neoadjuvant/adjuvant chemotherapy, 69.7% received adjuvant trastuzumab, 46.7% received adjuvant hormonal therapy. In 92 recurrent patients (18.9%), distant metastasis was identified in 67.4%. In 100 available tissues for evaluating stromal TILs, only 14 (14%) had high stromal TILs (at least 50%) and 46 patients (46%) had recurrent disease. Twenty three of 39 (59%) in low stromal TILs group had disease recurrence while only 4 out of 14 (28.6%) in high stromal TILs had recurrent disease. Median percentage of stromal TILs in recurrent and non-recurrent group was 17.5% and 27.5%, respectively. From multivariate analysis, high stromal TILs (HR 0.52 [95%CI 0.32-0.85]; p = 0.01) and trastuzumab used (HR 0.39 [95%CI 0.25-0.61]; p <0.001) were associated with decreased risk of recurrences. After median follow up of 4.1 years, 5-years disease free survival (DFS) and 5-years overall survival (OS) were 80.9%, 86.8%, 69% and 92.6%, 91.5%, 94.5% in overall populations, trastuzumab and non-trastuzumab used group, respectively. Additionally, 5-year DFS and 5-year OS were 34.3%, 60.8%, 75.7% and 86.2%, 82.5%, 83.1% in low, intermediate and high stromal TILs subgroup, respectively.

**Conclusion:** High stromal TILs and trastuzumab used were statistically significant prognostic values for predicting disease recurrence in HER-2 positive early breast cancer. Stromal TILs together with clinical values established clinical utility in Thai HER2 positive breast cancer women.

## Level of Stromal TILs in Overall Patients

Stromal TILs	Total (n = 100)	No recurrence (n = 54)	Recurrence (n = 46)	P value
Low (0-15%)	39(39%)	16(41%)	23(59%)	0.03
Intermediate (20-40%)	47(47%)	28(59.6%)	19(40.4%)	
LPBC (50-90%)	14(14%)	10(71.4%)	4(28.6%)	

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-47

Evaluation of stromal tumor-infiltrating lymphocytes (sTIL) and tertiary lymphoid structures (TLS) in early breast cancer patients with triple negative breast cancer (TNBC) included in a prospective study of neoadjuvant chemotherapy (NAC) with Epirubicin and cyclophosphamide (EC) and carboplatin-paclitaxel (PC) (BSMO 2014-01)

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**Background:** BSMO 2014-01 is a completed prospective phase 2 study evaluating the efficacy of neoadjuvant EC and PC. One of the secondary endpoints was the correlation of sTIL with response, pCR and survival. We also assessed the relationship between sTIL and TLS in the diagnostic biopsies.

**Methods:** Stromal TIL (sTIL) were evaluated on H&E stained tumor biopsies before the start of the NAC according to the criteria described by Salgado et al(1). Scores were defined as "low" or "high" if lymphocytic infiltration in the stroma around the tumor was  $\leq 10\%$  or  $> 10\%$ . TLS are ectopic lymph node-like structures recently identified in breast cancer. TLS were counted using a dual IHC stain for CD3 (T cells) and CD20 (B cells) and categorized as "little" if the TLS occupied  $< 10\%$  or "moderate to abundant" if they occupied  $\geq 10\%$  of the adjacent tissue. The correlation between sTIL and pathologic parameters was analyzed using the chi-square test; DFS and OS between the groups was estimated by using the log-rank test.

**Results:** So far we could quantify the number of sTIL in 38 out of 63 TNBC pts treated with neoadjuvant EC-PC. Twenty eight pts had a high sTIL score and 10 pts had a low sTIL score. The high-sTIL group (19/28) achieved a numerical higher pathologic complete remission (pCR) rate than the low-sTIL group (5/10) ( $p=0.3$ ); both groups had a comparable disease free survival of 28.6 mths and 26.7 mths respectively ( $p=0.7$ ). The overall survival was similar: 29 mths and 27.8 mths respectively ( $p=0.8$ ). Stromal TLS were identified in 10 out 23 samples available for this analysis and we could demonstrate a positive correlation between high levels of sTIL and high levels of moderate to abundant TLS (CD3) in the adjacent tissue in six out of the ten samples in which TLS were present ( $p=0.1$ ).

**Conclusion:** These preliminary results could not confirm the results published by Denkert *et al* earlier this year(2). A trend for correlation of the presence of high sTIL with moderate to abundant levels of TLS was found. Analysis on the remaining samples of all patients included in the study and correlation with outcome is ongoing and these completed results will be presented.

(1) Assessing Tumor-infiltrating lymphocytes in solid tumors. Hendry S, Salgado R et al. *Adv Anat Pathol* 2017; 235-251.

(2) Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer. Denkert C et al. *Lancet Oncol* 2018; 40-50.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-48

Features associated with long-term survival in metastatic breast cancer

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**Background:** 5-10% of women with metastatic breast cancer (MBC) survive  $\geq 5$  years. Predictors of long-term survival are not clearly elucidated. We used data from 122 long-term MBC survivors ( $\geq 5$ -year survival from date of MBC diagnosis) and 191 short-term MBC survivors ( $\leq 2$ -year survival from date from MBC diagnosis) to identify clinico-pathologic and socioeconomic features associated with MBC survival.

**Methods:** Women initially diagnosed with breast cancer (BC) in or after 1999, and diagnosed with MBC at Magee Women's Cancer Program of UPMC were included (N=313). Data abstracted from medical records included: stage at initial BC diagnosis, body mass index (BMI), Charlson Comorbidity Index (CCI), age, menopausal status at initial BC diagnosis, tumor receptor status at initial BC diagnosis, site of initial metastases, time to recurrence between initial diagnosis and MBC, household income, race, employment status, and partner status. Differences between groups were assessed using t-tests and Chi-square or Fisher's exact tests. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using multivariate logistic regression models.

**Results:** Long-term survivors were significantly ( $P < 0.05$ ) younger, had more ER positive, PR positive, and Her2 positive disease, lower CCI, more often premenopausal at initial diagnosis, lower rates of visceral metastases, higher household income, and more often partnered than short-term survivors. The association of premenopausal status at initial diagnosis with long-term survival remained significant after adjustment for stage at initial diagnosis, tumor receptor status, and CCI (OR: 1.96, 95% CI 1.02- 3.79). Long-term survivors were also significantly more often diagnosed with de novo MBC compared to short-term survivors. The association of de novo MBC with long-term survival remained significant after adjustment for age, tumor receptor status, and CCI (OR: 3.0, 95% CI 1.6-5.4). Time to recurrence between initial diagnosis and MBC, BMI, race, and employment status were not associated with survival.

**Conclusions:** Diagnosis of de novo MBC, ER-, PR- and/or Her2-positive primary tumor, lower rates of visceral metastases, higher household income, younger age, lower CCI, premenopausal status, and having a partner are associated with long-term survival after diagnosis of MBC. This is one of the first studies to show a survival benefit in MBC for patients with de novo MBC, premenopausal status at initial diagnosis, positive partner status, and higher household income.



# 2018 San Antonio Breast Cancer Symposium ®

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The role of neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR) and monocyte – lymphocyte ratio (MLR) in prognosis of breast cancer patients

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**Introduction:** Breast cancer (BC) is common malignancies in women. Biomarkers such as neutrophils, lymphocyte, neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), monocyte – lymphocyte ratio (MLR) and PLT have been demonstrated to be closely related to poor prognosis in several solid tumors. The objective of this study was to evaluate the blood PLR, NLR and MLR for its prognostic value in patients with breast cancer. **Material and Methods:** We retrospectively reviewed 436 breast cancer patients (all woman) diagnosed and treated in MSC Memorial Cancer and Institute of Oncology, Gliwice Branch in years 2005 - 2018. The median age of patients was 52.5 years (range from 25 to 78). We assessed the prognostic value (overall survival) of pretreatment PLR, NLR and MLR based on univariate and multivariate analysis. The cut-off value of NLR was 'elevated' as  $>2.65$ , MLR value was 'elevated' as  $>0.28$  and PLR cut-off value was 'elevated' as  $>190.9$ . **Results:** Median follow-up was 71 months (range, from 3 to 165 months). The 5-year and 10-year OS rates were 88.1% and 80.2%, respectively. The 5-year OS was lower in NLR  $> 2.65$  in comparison to NLR  $\leq 2.65$  (82.5% vs. 89.6%,  $p=0.053$ ), especially in subgroup of triple negative breast cancer (TNBC) (70.3% vs. 89.3%,  $p=0.034$ ) and in patients with estrogen receptor negative status tumors (66.6% vs. 83.6%,  $p=0.018$ ). Similarly, the 5-year OS was lower in patients with PLR  $> 190.9$  in comparison to PLR  $\leq 190.9$  (78.7% vs. 89.4%;  $p=0.020$ ). The worse OS rate was also observed in subgroup with TNBC and PLR  $> 190.9$  (68.2% vs. 88.5%,  $p=0.032$ ) or in subgroup with ER negative steroid receptor status tumors with 'elevated' PLR (57.7% vs. 83.6%,  $p=0.002$ ). The 'elevated' value of MLR ( $>0.28$ ) was not associated with overall survival time in our group of patients ( $p=0.830$ ), also in TNBC ( $p=0.219$ ) and ER (-) ( $p=0.453$ ) subgroups of patients. Multivariate analysis has showed that NLR and PLR were insignificantly negative prognostic factors in all analyzed group. However the analysis in subgroup of patients with ER (-) negative tumors has showed that higher NLR ( $p=0.013$ ; HR=2.40; 95%CI 1.20-4.80) and higher PLR ( $p=0.012$ ; HR=2.51; 95%CI 1.23-5.14) were an independent factors for lower OS together with metastatic lymph nodes ( $p=0.0001$ ). **Conclusion:** Elevated pre-treatment NLR ( $>2.65$ ) and PLR ( $>190.9$ ) are associated with lower OS in breast cancer patients. In ER (-) subgroups of patients elevated NLR and PLR were significant independent prognostic factors. MLR did not affect overall survival.

# 2018 San Antonio Breast Cancer Symposium ®

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Elevated levels of serum tumor marker p53 is a prognostic parameter and a monitoring biomarker for patients who had undergone surgical resection in breast cancer

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## **Background**

Elevated levels of serum tumor marker p53 antibody is expected as an indicator of early diagnosis and a parameter of recurrence in breast cancer. P53 mutation accounts for 20% to 35% in all breast cancer patients. Preoperative high level of anti-p53 antibody in breast cancer patients tend to associate with worse prognosis. This study investigated the prognostic value of preoperative serum p53 levels, and the significance as a biomarker to evaluate a recurrence after surgical resections in breast cancer.

## **Methods**

Preoperative serum p53 concentration levels were measured in total of 259 breast cancer patients, who had undergone either a total mastectomy or a partial mastectomy, through 2010 to 2015 in our facility. Patients with elevated levels of p53 (29 patients) and normal levels of p53 (230 patients) were compared to analyze the association of a marker level with the prognosis and the indication to diagnose recurrence in breast cancer.

## **Results**

Elevated serum level of p53 mutation was identified in 29 (11%) patients. The size of tumor, staging, and pathology did not associate with the level of p53. Patients with elevated serum level of p53 correlated to the high score of nuclear grade (NG2 and NG3) and the high percentage of Ki-67 (>14%), which leading to the worse prognosis. Triple negative breast cancer was the major molecular subtype in the group of high level of p53 comparing with the group of low level of p53. Survival analysis using the Kaplan-Meier method were performed to examine DFS and OS of high serum level of p53 patients. Patients with high level of p53 were significantly showed worse DFS than a normal p53 group. Serum level of p53 was also reflected to the recurrence and metastasis of postoperative breast cancer. There were three patients, who had local recurrences and metastasis, in the group of high levels of p53. Their serum levels of p53 were re-elevated as emerging the local recurrence and metastasis once they had gotten the normal serum levels of p53 after surgical resections. It showed that the increasing of the level of p53 was reflected to the recurrence and metastasis of tumors after surgical resections in breast cancer.

## **Conclusions**

This study suggests that preoperative serum level of p53 can be an independent prognostic parameter and a monitoring biomarker for breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-51

Residual Ki67 index and PRPTD mutational status after neoadjuvant chemotherapy containing platinum salts predicts the survival in triple negative breast cancer

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**Background:** Platinum salts have demonstrated sufficient efficacy and safety to consider their use in a neoadjuvant setting for triple negative breast cancer (TNBC). In this study, we focused on the prognostic value of Ki-67 and PRPTD in TNBC patients who received neoadjuvant chemotherapy containing platinum salts.

**Patients and Methods:** We retrospectively analyzed 145 TNBC cases to compare the activity and tolerability of cisplatin and carboplatin. Two groups received weekly paclitaxel and platinum salts for 4 cycles. Immunohistochemistry assessment of EGFR, Ki67, CK5/6, CK14 was conducted in paraffin-embedded tumor samples after neoadjuvant treatment. Nonsense/missense mutations of PTPRD were also evaluated in these tumor samples determined by using next generation sequencing.

**Results:** In total, 87% of patients in the cisplatin group and 82% of patients in the carboplatin group experienced a clinical objective response after 4 cycles (P=0.570). Pathological complete response (pCR) occurred similarly in both groups (44% versus 42%, P=0.789).

Clinical and pathological evaluation

Category	Cisplatin group	Carboplatin group	OR [95% CI]	P value
ypT0/is,ypN0	-	-	0.911 (0.459-1.806)	0.789
No	29 (56%)	54 (58%)	-	-
Yes	23 (44%)	39 (42%)	-	-
ypT0/is,ypN0/+	-	-	1.031 (0.523-2.034)	0.930
No	25 (48%)	44 (47%)	-	-
Yes	27 (52%)	49 (53%)	-	-
MP grade for breast	-	-	NA	0.448
MP 5	27 (52%)	49 (53%)	-	-
MP 4	8 (15%)	8 (9%)	-	-
MP 3	14 (27%)	24 (26%)	-	-
MP 2	3 (6%)	9 (10%)	-	-
MP 1	0 (0%)	3 (3%)	-	-
Clinical response after 2 cycles	-	-	NA	0.352
CR	3 (6%)	2 (2%)	-	-
PR	43 (82%)	84 (90%)	-	-
Overall (CR or PR)	46 (88%)	86 (92%)	-	-
SD or PD	6 (12%)	7 (8%)	-	-
Clinical response after 4 cycles	-	-	NA	0.570
CR	8 (15%)	11 (12%)	-	-
PR	37 (72%)	65 (70%)	-	-
Overall (CR or PR)	45 (87%)	76 (82%)	-	-
SD or PD	2 (4%)	7 (8%)	-	-

In survival analysis, there was no difference between two regimens. The pathological variables of univariate Cox analysis were

obtained from EGFR, Ki67 index, CK5/6, CK14, PTPRD mutational status in residual tumor after NAC. Multivariate Cox analysis identified two significant prognostic factors, including Ki67 index and PRPTD mutational status.

Prognostic value of pathological variables in predicting disease free survival using univariate and multivariate Cox model

Category	Univariate Cox Analysis Odds ratio (95% CI)	P-value	Multivariate Cox Analysis Odds ratio (95% CI)	P-value
EGFR negative vs. positive	1.353 (0.548-3.342)	0.512	-	-
Ki67 ≤20% vs. >20%	3.951 (1.291-12.094)	0.016	6.239 (1.614-24.114)	0.008
CK5/6 negative vs. positive	0.822 (0.324-2.085)	0.680	-	-
CK 14 negative vs. positive	1.396 (0.541-3.604)	0.490	-	-
PTPRD wide type vs. mutation type	3.219 (1.117-9.277)	0.030	7.362 (2.305-23.513)	0.001

Abbreviation: EGFR. epidermal growth factor receptor; PTPRD. protein tyrosine phosphatase receptor type delta

The most common grade 3/4 adverse events were neutropenia and leukopenia.

Conclusion: There was no significant difference between the groups in adverse events. Both types of platinum salts and weekly paclitaxel are feasible therapies that achieved high pCR rates and tolerability in TNBC patients. Ki67 index and PRPTD mutational status in residual tumor should be suggested as promising biomarkers to predict the prognosis.

# 2018 San Antonio Breast Cancer Symposium ®

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A predictive model for distant metastasis in breast cancer patients using machine learning

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## Introduction

Tumor metastasis is a major clinical challenge accounting for the vast majority of cancer related deaths. In previous studies, prediction of distant metastasis was based on subtypes, clinical status and sometimes gene expression were used however clinical application was difficult.

In this study, we develop the easy to use prediction tool for distant metastasis using clinical characteristics and gene profiles which came from CancerSCAN<sup>TM</sup>, Next Generation Sequencing based targeted-sequencing platform designed at Samsung Medical Center (SMC).

## Methods

We performed a retrospective chart review of 326 breast cancer patients who underwent surgery and CancerSCAN<sup>TM</sup> between Jan 2001 and Dec 2014 at SMC. Median follow up period was 83 months (Range 1~190). Cancer scan<sup>TM</sup> cover 381 genes but 27 genes and 34 occasions (loss of function, mutation or copy number variation) were selected for analysis through gradient boosting and Wilcoxon Signed rank test. Azure Machine Learning is a cloud service that enables the execution of machine learning processes. This was accomplished using the steps of (1) edit the data, (2) split the data, (3) train the model, (4) score the model, and (5) evaluate the model. We split the modeling data into training and testing sets using a randomized 50–50 split. Two-class Decision Forest method was used. After deploying the Azure ML predictive model as a web service, we used a Representational State Transfer application programming interface to send data and obtained predictions in real-time.

## Results

No distant metastasis group and distant metastasis group consisted of 267 and 59 patients, respectively. HR-/HER2+ and 50 years old and over patients were higher in metastasis group ( $p$ -value = 0.003 and  $p$ -value = 0.000). Nuclear grade 3 and N2,3 were higher in metastasis group ( $p$ -value = 0.010 and  $p$ -value = 0.000,  $p$ -value = 0.001 respectively). Stage III was also higher in metastasis group ( $p$ -value = 0.000). Among 59 patients with distant metastasis, multiple sites metastasis was 21 cases (35.6%) and then lung metastasis was 19 cases (32.2%). In the 21 cases of multiple sites metastasis, triple sites was 6 cases (28.6%) and double sites was 15 cases (71.4%). PIK3CA mutation was the most frequent gene variation in all patients (34.5% of no metastasis group and 27.1% of metastasis group) but there was no difference between two groups ( $p$ -value = 0.278). BRCA 1 loss of function and BRCA2 loss of function were more frequent in metastasis group than no metastasis group ( $p$ -value = 0.033 and  $p$ -value = 0.024, respectively) but total counts was too small. We assessed the area under the curve (AUC) of the receiver operating characteristic (ROC) curve for predictive value. The AUC of ROC curve was 1.000 and also accuracy, precision, recall were 1.000. In addition, we conducted internal validation using 83 patients during 2015. When we applied a 0.5 threshold value with our predictive model, true negative was 81 and true positive was 2 among 83 patients. Finally, the accuracy of validation was 1.000.

## Conclusion

Our predicted model could represent a useful and easy-to-access tool for the selection of patients with distant metastasis. After additional evaluation with large data and external validation, worldwide use of our model could be expected.

# 2018 San Antonio Breast Cancer Symposium ®

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Tumor elasticity and clinicopathologic factors affecting neoadjuvant chemotherapy response in breast cancer patients

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**Background:** Neoadjuvant chemotherapy for breast cancer has been increased. Many studies have reported on clinicopathologic factors to predict neoadjuvant chemotherapy response. Elastography, which is usually used to differentiate benign and malignant tumors, can be performed to evaluate tissue elasticity during conventional ultrasonography. The purpose of this study was to determine the clinicopathologic factors, including tumor elasticity, that affect neoadjuvant chemotherapy response in stage II or III breast cancer patients.

**Methods:** From April 2014 to March 2017, 95 patients received neoadjuvant chemotherapy for clinical stage IIa-IIIc primary breast cancer. To evaluate tumor elasticity, strain elastography was performed in 74 patients before neoadjuvant chemotherapy. Patients were divided into two groups by the Tsukuba elasticity scoring system (soft group  $\leq 3$  vs. hard group  $\geq 4$ ). Histologic type, nuclear grade, tumor infiltrating lymphocytes (TILs), tumor cellularity, characteristics of stroma, and hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status were evaluated using core needle biopsy specimens obtained before neoadjuvant chemotherapy. Pathologic complete response (pCR) was defined as the absence of invasive carcinoma in breast (ypT0 and ypTis) and axillary lymph node (ypN0). Residual cancer burden (RCB) was also calculated in 79 cases and the cases were categorized into 2 groups; favorable RCB group (RCB-0 and I) and unfavorable RCB group (RCB-II and III).

**Results:** The mean age of patients was  $46.43 \pm 8.62$  years (range, 27-71 years) and the mean initial tumor size was  $3.63 \pm 1.95$  cm (range, 2.1-12.8 cm). Twenty-four patients (32.4%) were categorized into the soft group and 50 patients (67.6%) into the hard group. The mean tumor cellularity on core needle biopsy specimens and characteristics of stroma were not significantly different between the two groups ( $p=0.35$  and  $p=0.79$ , respectively). Twenty-two patients achieved pCR (23.2%). The patients with pCR were more likely to have estrogen receptor (ER) or progesterone receptor (PR) negative breast cancer ( $p=0.04$  and  $p=0.03$ ). The rate of nuclear grade 3 was higher in patients with pCR than those without ( $p=0.03$ ). Tumor elasticity was not correlated with pCR ( $p=0.28$ ). Thirty patients (38.0%) achieved favorable RCB and forty-nine patients (62.0%) had unfavorable RCB. Not only the rates of ER negativity ( $p=0.05$ ), PR negativity ( $p=0.03$ ), nuclear grade 3 ( $p=0.01$ ), and high TILs level ( $\geq 10\%$ ) ( $p=0.04$ ) but also the mean TILs level ( $p=0.05$ ) were significantly higher in the favorable RCB group compared with the unfavorable RCB group. No significant difference in tumor elasticity was observed between the two groups ( $p=0.30$ ). In univariate analyses, nuclear grade 3 ( $p=0.03$ ), and high TILs level ( $\geq 10\%$ ) ( $p=0.04$ ) were significantly correlated with favorable RCB. HR negativity was an independent predictor of favorable RCB in multivariate analysis (odds ratio, 2.93; 95% confidence interval, 1.04-8.28;  $p=0.04$ ).

**Conclusion:** Tumor elasticity was not associated with pCR or RCB. HR negativity was an independent predictor for favorable RCB. Nuclear grade and TILs were also potential predictive factors for neoadjuvant chemotherapy response.

# 2018 San Antonio Breast Cancer Symposium ®

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Change in therapeutic management after EndoPredict assay in a prospective decision impact study of Mexican premenopausal patients

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**Background:** Often, therapeutic decision-making in breast cancer (BC) patients is deeply influenced by age, with young women receiving highly aggressive systemic therapy, even when low clinical risk features are present. Currently, genomic signature studies have included a limited number of young patients, thus hindering their use in this population. Furthermore, significant data about their impact on clinical decision-making is lacking. Consequently, the medical community should strive to identify young patients who would not benefit from chemotherapy (CT). The aim of this prospective study was to determine the impact of the EndoPredict assay on adjuvant decision-making by a multidisciplinary team in premenopausal women.

**Patients and method:** A total of 92 premenopausal women with hormone receptor-positive, HER2 negative, T1-T2, and N0-N1 BC have been included. Clinicopathological characteristics were recorded and each case presented in a multidisciplinary tumor board. Consensual therapeutic decisions before and after EndoPredict results were registered. Pearson chi-square was used to analyze differences between groups.

**Results:** Median age at diagnosis was 43.5 years (y), with 68% being  $\leq 45$ y. 28% had node-positive disease. Stage at diagnosis was: I (40%), IIA (36%), and IIB (23%). 11%, 72%, and 10% of tumors were low-, intermediate-, or high-grade, respectively. A total of 39 patients (46%) had a low-risk EPclin result. Notably, 61% of patients in the N0 group had a low-risk result compared to 9% with N1 status ( $p < .001$ ). Additionally, 30% of patients  $\leq 40$ y-old had a low-risk EPclin, compared with 53% in older patients ( $p = .0421$ ). Tumor grade was significantly associated with EPclin, with 90% of low-grade tumors being low-risk, while 89% of grade 3 BC had a high-risk result ( $p = .0023$ ). Grade 2 BC was not predictive of EPclin results, with 45% of patients being classified as low risk. No significant association was found between Ki67 or LVI, and EPclin.

Change in CT decision was recorded in 14/85 patients (17%), with the greatest impact in CT reduction (table 1). Furthermore, 22/85 (26%) and 19/85 (22%) had a change in CT and anti-hormonal therapy regimen, respectively. Overall therapeutic compliance with EndoPredict results was 95%: 100% of patients with a high-risk EPclin received CT, while 87% with a low-risk result did not.

Table 1. Pre- and Post-EndoPredict CT consensus

		Post-Test	Post-Test	
		No CT	CT	Total
Pre-Test	No CT	24 (28%)	4 (5%)	28 (33%)
Pre-Test	CT	10 (12%)	47 (55%)	57 (67%)
	Total	34 (40%)	51 (60%)	85 (100%)

**Conclusions:** This is the first study that evaluates the EndoPredict assay effect in decision-making in a premenopausal BC patient cohort. The less overall therapy change observed, compared to previous studies, could be the result of a reliable clinical judgment for identifying high-risk patients, which represent a large proportion in this young group. Overall, the test's greatest impact was aiding the discrimination of patients that would not benefit from CT, thus avoiding unnecessary adverse effects. In conclusion, the EndoPredict test successfully assisted the clinical decision-making process in premenopausal patients, with a high rate of therapeutic compliance and a significant change in overall decision.

# 2018 San Antonio Breast Cancer Symposium ®

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Expression of MTH1 correlates with prognostic factor in breast cancer

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**Purpose:** Genetic mutations are widely found in breast cancer tissues. 8-oxo-7,8-dihydroguanine(8-oxoGsn) in RNA and 8-oxo-7,8-dihydro-2' deoxyguanosine (8-oxodGsn) in DNA are productions of oxidative Stress ,which can induce transversion mutations. MTH1, as the most important repairase of nucleic acid oxidation, degrades 8-oxodG and 8-oxoG to monophosphates in nucleotide pools, thus prevents the occurrence of transversion mutations. This study mainly investigate the relationship between MTH1 expression and prognosis in breast cancer.

**Materials and methods:** MTH1 expression of 140 breast cancer tissues were evaluated by immunohistochemical staining of tissue microarrays. The clinical and pathological data of patients were analyzed by SPSS.

**Results:**Breast cancer patients with high expression of MTH1 had an extremely poor overall survival after surgical resection [figure1]. This phenomenon was significantly associated with American Journal of Critical Care (AJCC) stage (p=0.016), lymph node metastasis (p=0.023) and Lymphatic vessel invasion (p=0.009) of breast cancer specimens.

Clinicopathologic findings and correlation with the MTH1 expression (n = 140)

	Total(%)	MTH1 Low (%)	MTH1 High (%)	P
Age, y				0.96
≤55	78	27	50	
>55	62	22	40	
Gender				
Male	0	0	0	
Female	140	50	90	
Tumor location				0.096
LEFT	72	21	51	
RIGHT	68	29	39	
AJCC stage				0.016*
I—II	94	40	54	
III—IV	46	10	36	
TNM stage				
T stage				0.671
T1—T2	138	49	89	
T3—T4	2	1	1	
N stage				0.023*
N0	75	33	43	
N1	19	7	12	
N2	38	6	32	
N3	8	4	4	
Lymphatic vessel invasion				0.009*
NO	124	49	75	
YES	16	1	15	



Recrudescence				0.308
NO	102	39	63	
YES	38	11	27	
Preoperative Her-2#				0.123
(-)	61	18	43	
(+)	58	25	33	
Preoperative ER#				0.579
(-)	42	14	28	
(+)	94	36	58	
PR#				0.422
(-)	65	21	44	
(+)	72	28	44	
P53#				0.328
(-)	66	28	38	
(+)	17	5	12	
Bcl-2#				0.989
(-)	26	11	15	
(+)	19	8	11	

\*: Statistically significant. #: Several data points from the 140 cases were not collected.

Cox univariate analysis shows that the MTH1 was one of prognostic factors in patients (p=0.0173).

COX Univariate analyses of the overall survival

Factor	Examination object	Comparable object	Univariate analysis		
			HR	95% CI	P value
Age, y	>55	>55	0.48	0.225-1.026	0.0583
Gender	Female	Male			
Tumor location	RIGHT	LEFT	1.132	0.539-2.380	0.7431
AJCC stage	III—IV	I—II	0.262	0.122-0.560	0.0005*
Tumor depth	T3—T4	T1—T2	20.581	0.000-12417080.093	0.6561
Lymph node metastasis	N (+)	N (—)	0.302	0.133-0.687	0.0043*
Lymphatic vessel invasion	YES	NO	0.528	0.201-1.389	0.1955
Recrudescence	YES	NO	0.057	0.023-0.142	<0.0001*
Preoperative Her-2	(+)	(-)	2.388	1.631-7.296	0.0526
Preoperative ER	(+)	(-)	3.450	1.631-7.296	0.0012*
PR	(+)	(-)	1.447	0.685-3.060	0.333
P53	(+)	(-)	2.957	1.159-7.540	0.0232*
Bcl-2	(+)	(-)	0.65	0.163-2.602	0.5431
MTH1	High	Low	0.28	0.096-0.797	0.0173*

\*: Statistically significant. #: Several data points from the 140 cases were not collected.

**Conclusions:** MTH1 plays an important role in breast cancer prognosis. MTH1 will be a powerful prognostic factor and a potential therapeutic target in breast cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

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Effects of young age on prognosis in patients with node-negative tumors 2 cm or smaller breast cancer

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**Background** It is still controversial to consider age as a prognostic factor into the treatment strategy of patients with T1N0M0 breast cancer.

**Aim** The main purpose of this study was to evaluate the effect of age on recurrence risk in patients diagnosed with T1N0M0 breast cancer as well as compare the prognosis of young aged patients(YA, ≤40 years old) to non-young aged patients(non-YA, >40 years old) by using a propensity score matching(PSM) analysis.

**Methods** 365 patients with T1N0M0 breast cancer diagnosed between 2003 and 2016 who received surgery in Sun Yat-sen Memorial Hospital Breast Cancer Center were included. The recurrence free survival (RFS) and risk factors for recurrence were identified by using Kaplan-Meier method and Cox proportional hazards models. PSM was then used to reduce the confounding effect of known risk factors on prognosis and then to compare 5-year RFS rates in patients between two age groups.

**Results** After a median follow up of 79 months, 54 patients developed recurrences and 5-year RFS was 87.6%. YA patients had lower RFS estimates (80.6%), compared to patients diagnosed in a later age (89.1% if older than 40-years old;  $P = 0.049$ ). YA patients tended to have Her-2 positive, TNBC tumors, higher rate of Ki-67 expression and nuclear grade tumor. At multivariate analysis, Her-2 positive (HR 2.115; 95% CI 1.103-4.055,  $p=0.024$ ) and TNBC (HR 2.963; 95% CI 1.485-5.914,  $p=0.002$ ) resulted independent prognostic factors of patient with T1N0M0 breast cancer. In the subgroup analysis, we found significant poor RFS for YA patients with Her-2 positive breast cancer compared to the older counterparts( $p=0.006$ ) and YA patients were associated with significantly higher rates of the locoregional recurrence rather than metastasis( $p=0.004$ ), especially in first 5 years after diagnosis. After PSM, the baseline level and treatment status including tumor size, grade, HR status, Her-2 status, Ki67 expression breast surgery type and systemic adjuvant treatment(AST) of patients in the two age groups tended to be equal. As result, we found significant difference in the 5-year RFS between two age groups( $p=0.008$ ).

**Conclusion** Based on equal treatment condition, young age at presentation conferred a worse prognosis in patients with T1N0M0 breast cancer is independent on other pathological features.

# 2018 San Antonio Breast Cancer Symposium ®

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A biologic signature to predict ipsilateral breast event risk at 10 years for early breast cancer

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## Background

Outcomes for women with early breast cancer have continually improved. A biologic signature to identify those patients that have elevated ipsilateral breast event (IBE) risk after breast conserving surgery (BCS) treated with or without radiation therapy (RT) is needed. More aggressive systemic or surgical options may be warranted for patients with elevated risk while BCS alone may be an option for very low risk patients. We report early results for a biologic signature interrogating critical pathways.

## Material and Methods

This study includes patients from Uppsala University Hospital and Åström's Hospital diagnosed with early breast cancer, 20mm or less, treated surgically between 1987 and 2004. Women with lymph node metastases or treated with mastectomy or chemotherapy were excluded. A panel of biomarkers (HER2, PR, Ki67, COX2, p16/INK4A, FOXA1 and SIAH2) were assayed and scored in PreludeDx's CLIA lab by board-certified pathologists. There were 171 eligible patients with biomarker data; 131 received RT and 9 received hormone therapy.

Risk groups were calculated using biomarkers and clinical factors age and size. Absolute 10-year IBE risk was assessed using Kaplan-Meier survival analysis. Hazard ratios (HR) were determined using Cox proportional hazards analysis.

## Results

There were 49 IBEs recorded. The biologic signature classified 41% of women into a low risk group. Patients in the elevated risk group had a significantly increased risk of 10-year IBE compared to those in the low risk group (Table 1). The HR for elevated vs. low risk group was 5.0 [2.2-11],  $p < 0.001$ , in a multivariate analysis of risk group and RT. Patients in the elevated risk group treated with BCS and RT had an 18% apparent risk difference in 10-year IBE. Patients in the low risk group had similar low 10-year risks of IBE, when treated with BCS, with or without RT. The low risk women had somewhat increased prevalence of low grade tumors (58% vs. 41%). Women with low grade and small tumors (up to 10mm) were classified into both risk groups (54% low vs. 38% elevated risk).

Table 1: 10-year Risks of Local Recurrence by Risk Group

	BCS without RT		BCS plus RT	
	N	10-Yr local IBE Risk, 95%CI	n	10-Yr local IBE Risk, 95%CI
Baseline	40	28%, [11% - 31%]	131	22% [12% - 24%]
Low Risk Group	19	6% [0%-14%]	51	6% [0%-12%]
Elevated Risk Group	21	49% [20%-68%]	80	31% [21% - 41%]

## Discussion

A biologic risk signature identified early breast cancer patients with low and elevated 10-year IBE risks for women treated with BCS with or without RT and no chemotherapy. Approximately 40% of women were classified into a low risk group with a 0.5% IBE risk per year. Women in the elevated risk group had 3% to 5% IBE risks per year depending on treatment. Treatment for women in this observational study was neither randomized nor strictly rules based. With further prospective validation, the biologic signature identified herein may provide a tool enabling improved management for women diagnosed with early breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Prognostic factors of survival in node positive breast cancer patients after neoadjuvant chemotherapy in a large series after 5y follow-up: Can response overcome the poor prognosis of nodal stage?

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**Background:** Status of the axilla is one of the most significant prognostic factors in breast cancer (BC) patients. On the other hand, response to neoadjuvant chemotherapy (NACT) is related to survival. The aim of the present study is to analyze which prognostic factors impact most on Node positive (N+) BC patient survival after NATC. **Material and methods:** Retrospective analyses on a series of N+ BC patients treated with NATC based on anthracyclines and taxanes +/- trastuzumab if HER2 positive tumors, between June 2008 and December 2016. Clinical, radiological and pathological outcomes have been evaluated. Residual cancer burden (RCB) <sup>1</sup> and the neoadjuvant response index (INR) <sup>2</sup> have been recorded. Survival was calculated with Kaplan-Meier survival curve since the start of NATC to the first documented disease recurrence (DFS) or death (OS). Hazard ratios (HRs) with 95% CIs were estimated with cox proportional hazards regression analysis and subgroups were compared with a two-sided log-rank test. **Results:** A total of 345 N+ BC patients were included. Pathological complete response was achieved in 72 (20.8%) patients. After NACT, 137 (39.6%) become ypN0, 9 (2.6%) ypN1 mic, 113 (32.7%) ypN1, 60 (17.3%) ypN2 and 26 (7.6%) N3. Those independent predictive factor of ypN0 were molecular subtype (TN and Her2+) with OR: 7.7, p<0.001 and clinical response with OR 6.88, p: 0.04. At a mean follow-up of 58 months there have been 73 (21.1%) recurrences: 9 (2.3%) local, 45 (13%) systemic, 15 (4.3%) systemic+ local, 3 (0.9%) axilla, 1 (0.3%) supraclavicular. The estimated 5y OS was 87.8%. The univariate analysis according to DSF is detailed in Table1.

Adjusted univariate anaalysis cox regression of clinical and pathological factors of disease free survival

BMI	1	0.989-1.01	0.963
AGE	0.996	0.953-1.042	0.876
Dose NATC	0.994	0.979-1.008	0.402
Clinical Stage	1.402	1.077-1.826	0.012
Rx Image	1.26	0.803-1.994	0.311
Rx size	1.009	0.995-1.024	0.217
Number suspicious ALN	1.095	0.801-1.497	0.57
Molecular subtype TN,HER2	0.880	0.534-1.45	0.616
Nottingham grade	1.046	0.753-1.453	0.789
Histological subtype	1.465	1.044-2.057	0.27
MOlecular subtype	1.151	0.956-1.385	0.137
Vascular invasion	1.676	1.137-2471	0.009
Clinical response	2.369	1.709-3.284	<0.001
Fibrosis tumor bed	0.98	0.972-0.989	<0.001
Nodal fibrosis>50%	1.795	0.874-3.686	0.111
Pathological tumoral response	1.686	1.175-2.418	0.005
ypN0	3.56	1.853-6.838	<0.001
NRI	0.33	0.192-0.565	<0.001
RCB	1.274	1.106-1.468	0.001

In the multivariate model those parameters that were independently prognostic were clinical response HR: 5.44 (IC95% 2.275-13.042,  $p < 0.001$ ) and clinical stage HR: 2.364 (IC95% 1.018-5.490,  $p: 0.045$ ). **Conclusions:** The most significant prognostic factor in our N+ series was response to NATC, followed by clinical stage. Those independently predictive factors of axillar response (ypN0) were molecular subtype (TN and Her2+) and clinical response. In conclusion, in those patients with chemo sensitive tumors, lymphadenectomy could be safely spared with a more selective axillary approach.

# 2018 San Antonio Breast Cancer Symposium ®

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Different predictive and prognostic impact of intra-tumor heterogeneity, tumor biology, and microenvironment in triple negative breast cancer

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## Background

Intra-tumor heterogeneity, tumor biology, and microenvironment in triple negative breast cancer (TNBC) has been reported to be predictive and prognostic factors. However, it is not well known how these factors are correlated each other according to response to chemotherapy and their prognosis. The aim of this study was to assess the predictive and prognostic impact of these factors in TNBC.

## Method

Biopsy samples before neoadjuvant chemotherapy (NAC) from 59 TNBC patients who underwent surgery after NAC from 2001 to 2007 were retrospectively assessed. For tumor biology, tumors were classified as Hormonal related luminal androgen receptor (LAR) if >10% staining of AR, Basal-like if positive for cytokeratin 5/6 and EGFR, and Others. Claudin 1 and p16 expression levels were assessed for intra-tumor heterogeneity. and stromal tumor-infiltrating lymphocytes (Str-Tils) levels for tumor-microenvironment were also assessed as low for ≤10%, Intermediate for 10-49%, and high for >50%. The predictive and prognostic impact of clinicopathological factors including age, nuclear grade (NG), lymph node status, were also assessed. Overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method and were compared between groups using the log-rank test.

## Results

A median overall survival period of the 59 patients was 98 month (6 -172 month). Eighteen (30.5%) were classified in LAR, 16 (27.1%) in Basal-like, and 25 (42.4%) in Others. According to response to NAC, 10 patients (16.9%) achieved pathologic complete response (pCR). These biological classifications were not associated with pCR rate ( $p=0.135$ ). high-p16 had significantly high pCR rate ( $p=0.046$ ). However, Str-Tils level was not associated with pCR rate. Patients with lymph node metastasis had significantly low pCR rate ( $p=0.017$ ). In terms of their prognosis, age<50 had significantly shorter OS and DFS than that of age>50 (OS:  $p=0.023$ , DFS:  $p=0.027$ ). NG3 had a trend of short OS compared to NG 1 or 2 (NG 1 vs 3, OS:  $p=0.053$ , and NG 2 vs 3, OS:  $p=0.073$ ). There were no difference of their prognosis among three tumor biology classifications except Basal-like had significantly shorter OS than that of LAR (LAR vs Basal OS:  $p=0.041$ , DFS:  $p=0.574$ , LAR vs Others OS:  $p=0.407$ , DFS:  $p=0.866$ , Basal vs Others OS:  $p=0.162$ , DFS:  $p=0.713$ ). Claudin 1 and p16 expression levels were not associated with OS and DFS. Low-Str-Tils had a trend of shorter OS and DFS than that of intermediate- or high-Str-Tils (low vs int; OS:  $p=0.085$ , DFS:  $p=0.026$ , low vs high; OS:  $p=0.062$ , DFS:  $p=0.055$ ). In multivariate analysis, age<50 was only independent prognostic factor ( $p<0.05$ ).

## Conclusion

We showed that intra-tumor heterogeneity, tumor biology, and microenvironment had different predictive and prognostic impact in TNBC. These results might suggest the strategy of additional targeting treatment to non-pCR patients.

# 2018 San Antonio Breast Cancer Symposium ®

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FOXA1, Nestin, GATA3 and mammaglobin expression in 164 breast cancer metastases – A retrospective immuno-histochemical study of a 10-year period (2004-2014)

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**Background.** Including additional genes in standard immunohistochemical panels might be helpful in determining the prognosis of breast cancer patients. Previous studies have shown that **FOXA1** is a significant predictor of good outcome in breast cancer, while **Nestin** expression was preferentially found in triple-negative breast cancers, revealing a strong association with germline BRCA1-related breast cancer, a basal-like phenotype, stemness characteristics, high proliferation rates, p53 nuclear expression, increased rate of nodal metastases, and reduced survival (1,2,3,4). **ATA3** was not only an important luminal marker, but a reliable immunohistochemical marker, which plays a crucial diagnostic role in verification of breast cancer metastases (**Figure 1.** and **Figure 2**). In a cohort containing 164 breast cancer metastases, we found GATA3 to be a reliable and sensitive diagnostic marker. We verified that the metastases originate from breast carcinoma with 95% GATA3-positivity, while mammaglobin showed only 51.2% positivity (5). According to the **mammaglobin** immunohistochemical profile, we evaluated the expression of FOXA1, Nestin and GATA3 in mammaglobin-positive (n=84) and mammaglobin-negative (n=80) samples. **Materials and method** Full-face formalin-fixed paraffin-embedded (FFPE) specimens for 164 breast cancer metastases (from various anatomical sites) diagnosed between 2004 and 2014 were obtained from the Department of Clinical Pathology and Genetics at Sahlgrenska University Hospital (Gothenburg, Sweden). Each FFPE specimen was examined for mammaglobin, ER/PR, CK7, CK20, and HercepTest at the time of diagnosis and retrospectively analyzed for FOXA1, Nestin and GATA3 expression by immunohistochemistry (IHC). The statistical analyses were performed using a .05 P-value cutoff in R/Bioconductor (version 2.15.0) and all P-values are two-sided. The relationship between clinicopathological features and mammaglobin/GATA3 protein expression patterns was evaluated using two-tailed Fisher's exact test. **Results** In mammaglobin-positive metastases, FOXA1-positivity was associated with ER+ (83%) and negatively associated with triple-negativity (81%; **Figure 3.**). Moreover, there was no association between Nestin-positivity and the established clinicopathological features in mammaglobin-positive samples (**Table 1 and Figure 4**). In mammaglobin-negative samples, FOXA1-positivity was associated with GATA3+ (100%), ER+ (77%), HER2+ (36%), and triple-negative status (82% were non-triple negative). In addition, Nestin-positivity was associated with specific metastatic sites (mostly brain, but even gynecological sites and skin), GATA3- (29%), ER- (50%), PR- (79%), and triple-negative status (50% were triple negative) in mammaglobin-negative samples (**Table 2**). **Conclusion** In the present study, we found that **FOXA1** was expressed mostly in ER-positive breast cancer metastases and **Nestin** expression was associated with breast cancer metastases with a triple-negative immune profile, where the brain was the most frequent metastatic site.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-62

Predicting survival of triple negative breast cancer using artificial neural networks

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## Background:

Breast cancer is a major health problem with 1.7 million estimated new cases and nearly 459,000 deaths every year worldwide. Triple Negative Breast Cancer (TNBC) is a subtype of breast cancer based on immunohistochemistry in which estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) are negative. Among all the breast cancer subtypes, TNBC is associated with a worse prognosis. TNBC is highly aggressive cancer with unique molecular profile, aggressive nature, distinct metastatic patterns and lack of targeted therapies. Due to these heterogeneous characteristics, predicting TNBC survival is challenging. Reliable predictions can help in achieving more personalized care and better management. Here, we test the ability of artificial neural networks to predict TNBC survival.

## Methods:

Female patients with TNBC were identified through the Surveillance, Epidemiology and End Results database (SEER). Clinical data of the patients were extracted including: age, race, ethnicity, tumor site, tumor histology, grade, tumor size, tumor extension, lymph nodes involvement, metastasis at diagnosis, site of metastasis, laterality, cancer sequence number, TNM stage, surgery, radiotherapy, chemotherapy, radiation sequence with surgery, state, county, survival months. Patients' records were randomly divided into a training set (80%) and a validation set (20%). Artificial neural networks were used to predict patients' survival.

## Results:

A total number of 9,880 patients were identified through SEER from 2010 – 2011 with median age of 57 years and a median survival of 54 months. Patients with negative ER, PR and HER2 were selected. The parameters of artificial neural networks were tuned to achieve better results. For evaluating model performance, the Area under the Receiver Operating Characteristic Curve (AUC), precision, accuracy and sensitivity were calculated. Artificial neural networks yielded AUCs of 87.6% at 6 months, 80.8% at 12 months, 79.8% at 24 months and 77.7% at 24 months. The trained model achieved an average accuracy on the validation dataset of 94.8%, 91.8%, 85.3% and 80.1% at 6, 12, 24 and 36 months, respectively. Sensitivity of prediction of patients with survival months less than 6, 12, 24 and 36 ranged from 41% to 56%. However, sensitivity for patients with survival months less than 6, 12, 24 and 36 ranged from 88% to 96%.

## Conclusion:

Artificial neural networks achieved a good performance in predicting survival of patients with TNBC based on clinical data. High performance of prediction is essential especially for cancers with bad prognosis like TNBC because it can help in making better treatment decisions and planning social and care needs.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-08-63

The relationship between serum level of copper and ceruloplasmin and pathologic and clinical characteristics in early breast cancer patients

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**Purpose:** The increase of serum copper and ceruloplasmin has been reported to positively related with the progress of advanced breast cancers. However, the role of them in early breast cancer(EBC) is unknown.

**Methods:** 209 female patients that been diagnosed as EBC within Xijing Hospital from Oct 2016 to Apr 2017 were included. The level of serum copper and ceruloplasmin were assayed using atomic absorption spectroscopy and immunoturbidimetry assay, respectively. The pathologic and clinical characteristics were analyzed by X test, t test and Pearson correlation analysis.  $p<0.05$  is significant.

**Results:** Table 1 show the primary characteristic of patients. After transforming copper and ceruloplasmin into binary variable (elevated and normal), age was both significant ( $p<0.01$ ). The different expression level of ER, PR, Ki67 and molecular type, number and percent of metastatic lymph nodes were significant ( $p<0.01$ ). After correlation analysis, negative relationship existed between age and copper or ceruloplasmin (coefficient = 0.265 or 0.233,  $p<0.01$ ). And copper was positively related to the level of Ki67(coefficient = 0.169,  $p=0.018$ ). While ceruloplasmin was negatively related to PR (coefficient = -0.273,  $p<0.01$ ) and molecular type (coefficient = -0.217,  $p=0.05$ ).

**Conclusions:** This is the first study that revealed the abnormal escalation of serum copper and ceruloplasmin in Chinese EBC patients. The levels of both them were significantly higher in patients of young age, high Ki67 index and Luminal type. It is inferred that they might play an important role in early procedure of breast cancer, and it need more data in the future to validate.

Table 1 The primary characteristic of patients

Characteristic	Results(N=209)
Median age, year(range)	51.3(29-83)
AJCC stage, N	190*
Stage 1	79
Stage 2	81
Stage 3	30
Histological grade, N	160*
G1	19
G2	101
G3	40
Histopathologic characteristic, N	206*
DCIS	13
IDC-NOS	151
ILC	7
IDC-NOS + DCIS	14
Specific IDC	21
Primary tumor characteristic, N	188*
Median tumor size, cm(range)	1.7(0.5-6.7)
Median positive number of lymph nodes, N(range)	2(1-23)

Median percent of positive lymph nodes, %(range)	13.3 (3.4-100)
Molecular type, N(%)	176*
Luminal A (ER+/PR+/HER2-/Ki67<15%)	29(16.5)
Luminal B1 (ER+/PR+/HER2-/Ki67≥15%)	85(48.3)
Luminal B2 (ER+ and/or PR+/HER2+)	19(10.8)
HER2 (HER2+)	12(6.8)
Triple Negative (ER-/PR-/HER2-)	31(17.6)
Copper (Reference Value)	11.8-21.28umol/L
Median of Copper (Range)	17.7(12-32.9)
Elevated cases, N (%)	30(14.4)
Normal cases, N (%)	179(85.7)
Ceruloplasmin (Reference Value)	23-43mg/dL
Median of Ceruloplasmin (range)	43.2(23.4-82.5)
Elevated cases, N (%)	88(42.3%)
Normal cases, N (%)	120(57.7)

DCIS ductal carcinoma in situ;IDC-NOS invasive ductal carcinoma non special; ILC invasive lobular carcinoma; ER estrogen receptor; PR progesterone receptor; HER2 human epidermal growth factor receptor-2 \* Excluding missing data

# 2018 San Antonio Breast Cancer Symposium ®

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Patterns and predictors of early failure in women with triple negative breast cancer

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**Introduction:** Triple negative breast cancer accounts for approximately 15% of breast cancer in the United States, but behaves much more aggressively. It occurs more commonly in younger, black female patients and death within two years of diagnosis is much more common in this subset of breast cancer compared to hormone receptor positive patients. We see a high proportion of triple negative breast cancer patients in our urban academic comprehensive cancer center and present an evaluation of our patients who develop failures within 12 months of completion of definitive therapy in an effort to optimize screening strategies and therapy for women with triple negative breast cancer treated at our institution.

**Materials/Methods:** A retrospective database of 198 women with triple negative breast cancer treated at our institution from 2005-2017 was constructed. Patient characteristics are as follows: 100% female, median age of 54 years, 64% black, 40% married, 93% infiltrating ductal carcinoma, 68% grade 3, 18% with lymphovascular space invasion, 7% BRCA mutated, and 3% HIV positive. Stage breakdown is as follows: Stage I (33%), Stage II (47%), Stage III (16%) and Stage IV (4%). Thirty percent of patients had neoadjuvant chemotherapy. Adjuvant chemotherapy was given in 67% of patients. Ninety-eight percent of patients underwent surgical resection, 55% of whom underwent lumpectomy with 61% having sentinel lymph node biopsy. Adjuvant radiation was received in 56% of patients with a median dose of 60 Gy. Chi-square testing was used to compare variables, while logistic regression with Kaplan-Meier estimate was used to calculate overall survival (OS) and freedom from recurrence (FFR).

**Results:** With a median follow up of 45 months, 33 (17%) documented failures occurred. 63% of patients in this cohort were AJCC 7th edition stages II or III. In the women who suffered failures, these occurred at a median of 16 months after initiation of therapy, with a median OS of 29 months. Complete records including the date of last radiation therapy (signaling completion of definitive treatment) were available for 25 (76%) of patients. 10/25 failures (40%) occurred within 6 months of completion of radiation therapy while 14/25 (56%) occurred within 12 months of completion of radiation therapy. In women who failed within 6 months of completion of definitive therapy, site of first failure was local in 4 and distant in 6 patients. In the four additional patients who failed between 6 and 12 months following completion of definitive RT, all were considered local or locoregional. Univariate and multivariate analyses were performed which in this small group of women did not show any statistically significant predictors of early failure.

**Conclusion:** This institutional analysis shows that a large proportion of women with TNBC fail within 12 months of completion of definitive therapy with 57% of failures occurring locoregionally and the remainder occurring distantly. This data suggests that consideration of systemic imaging should occur to better detect these failures and additional study is warranted to determine if predictive factors can be identified for therapy escalation.

# 2018 San Antonio Breast Cancer Symposium ®

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A single-center real-world observational study to explore clinical treatments and prognoses of the Chinese patients with breast cancer complicated with brain metastases

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**Introduction:** The incidence of brain metastasis complicated with breast cancer (BCBM) is approximately 10-15%. Given that the survival status of the BC patients is not optimistic, the choice of clinical treatments should be based on individualized clinical characteristics of the BC patients. Thus understanding of the patient clinical characteristics is critical for patient management and forecasting the prognoses of the patients.

**Methods:** The trial was designed as a real-world observational study. The BCBM patients who were enrolled in the clinics from 2012 to 2017 were recruited and the study data on the patient demographic, tumor biological characteristics, and clinical treatments were retrospectively collected for evaluating overall survival (OS), and the OS impact factors. The Kaplan-Meier and Log-rank tests were performed to assess patient survivals. The Cox regression analyses were applied to explore the OS impact factors. All the statistical significance was set as  $\alpha=0.05$  except as other specified.

**Results:** A total of 117 female BCBM patients (mean age:  $48.28 \pm 9.49$  years) were enrolled in this study including 32 patients (27.4%) with extracranial metastases and 85 patients (72.6%) without extracranial metastases. Of all the subjects, by classification of the molecular types, 13.3% of the patients were diagnosed with luminal A tumor and 45.8% were observed with luminal B tumor. The patients with HER2 positive accounted for 19.3% of all the subjects and 21.7% of the subjects were triple-negative BC patients. In the clinical treatments, 65.8% of the patients were administered with chemotherapies, 14.5% with target therapies, and 11.1% with radiotherapies.

The median of the OS duration was approximately 38.57 months. The OS rates were decreased along with the observation period: The 18-month OS rate was 73.6% (95% confidential interval, CI 0.558, 0.852) and the 48-month OS rate was 24.9% (95% CI 0.045, 0.537), respectively. In the patients with  $>3$  metastatic brain tumors, the median of the OS duration was 20.27 months and the median of the OS duration was 38.57 months in the patients with  $\leq 3$  metastatic brain tumors. The OS durations in the patients with  $\leq 3$  metastatic brain tumors were longer than the durations in the patients with  $>3$  metastatic brain tumors from 12 month- to 36 month- observation period. In the patients with  $Ki-67 \leq 14\%$ , the OS durations were longer than the durations of the patients with  $Ki-67 > 14\%$ . In the 18 month-observation period, the OS duration was longer in the patients with HER2 positive as compared with that of the patients with HER2 negative. In the univariate and multivariate analyses, those patients with chemotherapies were significantly associated with longer OS durations ( $p < 0.05$ ). The multivariate analyses showed that the menopausal patients were related to shorter OS durations ( $p < 0.05$ ).

**Conclusions:** The real-world study offered the clinical evidences on the clinical characteristics of the BCBM patients, patient management, and patient survivals. The patient survivals improved significantly after clinical treatments with BCBM. The tumor biological characteristics and chemotherapies were predictive for the status of patient survivals.

# 2018 San Antonio Breast Cancer Symposium ®

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Outcomes among metastatic breast cancer patients with characteristics that confer a less favorable prognosis

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**Background:** Recent advances in the treatment of hormone receptor positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) metastatic breast cancer (MBC) have contributed to increased overall survival (OS). Despite advances, MBC remains incurable and there is a subset of patients with clinical features that are associated with poorer prognosis. This study described the patient characteristics, treatment patterns, and outcomes of a cohort of US patients with HR+, HER2- MBC as a function of various factors associated with poor prognosis, including presence (vs. absence) of liver metastases (LM).

**Methods:** This retrospective study used US community oncology electronic health record data from the Vector Oncology Data Warehouse. Eligible women who received systemic treatment for MBC, had a diagnosis of MBC in 2008 or later, and had completed at least three Patient Care Monitor (PCM) surveys, (a patient-reported outcomes survey collected as a part of clinical care), were included. OS was measured from the start of the first three regimen-based lines (1L, 2L and 3L) of treatment; patients without evidence of death were censored at the last observed visit. The statistical significance of differences in categorical and continuous variables between LM positive (LM+) and LM negative (LM-) were evaluated with chi-square ( $\chi^2$ ) tests, and t-tests, respectively. Kaplan-Meier and Cox analyses were applied to evaluate differences in OS by LM status and by line of therapy at the start of MBC treatment (unadjusted for treatment).

**Results:** A total of 378 women, 98.4% residing in the South and 40.5% African-American, were included; 295 (78.0%) were LM- at the time of diagnosis. Following 1L, approximately 82.8% and 60.8% of patients received 2L and 3L, respectively. Patients with a LM+ status had a lower mean age (mean: 57.2, SD: 13.8 vs. 61.2, 13.1;  $p=0.016$ ) and a higher percentage had a grade 3 tumor (36.1 vs. 24.7%;  $p=0.039$ ) compared to patients with LM-status. Table 1 shows the OS results for 1L-3L. For all 3 lines, median OS for LM+ was shorter than the LM- median OS. LM+ patients had a poorer prognosis as they were more likely to have an OS event across 1L-3L compared to LM- patients.

**Conclusions:** Among this community oncology cohort, median OS in 1L was 14 months shorter in LM+ patients compared to LM- patients. It is important to note that the sample size and selection criteria may limit generalizability of these results. Despite progress in treating women with MBC, treatment options are lacking for patients with less favorable prognosis, including those with LM. Other potential indicators of poor prognosis, such as high tumor grade, are being explored.

Table 1. OS (months) by regimen-based line of therapy

Measure	Liver Mets (LM+)	No Liver Mets (LM-)	HR	p-value
<b>1L, # of Events/ # of Patients</b>	55/83	168/295		
Median (95% CI)	23.9 (15.5-28.6)	35.2 (30.1-42.3)		<0.0001*
Cox Hazard Ratio			1.93	<0.0001
<b>2L, # of Events/ # of Patients</b>	48/72	149/241		
Median (95% CI)	16.6 (12.0-22.6)	24.2 (21.3-29.0)		0.002*
Cox Hazard Ratio			1.49	0.040
<b>3L, # of Events/ # of Patients</b>	35/52	118/178		
Median (95% CI)	11.5 (7.0-21.0)	17.4 (14.7-20.0)		0.038*
Cox Hazard Ratio			1.54	0.060

CI=Confidence Interval; \*p-value was derived using log rank test.

# 2018 San Antonio Breast Cancer Symposium ®

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Subgroups analysis of a multicenter, prospective, randomized, blinded phase 2b trial of trastuzumab + nelipeptimut-S (NeuVax) vs trastuzumab for prevention of recurrence in breast cancer patients

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**Background:** HER2 low-expressing (LE) (IHC 1-2+, FISH non-amplified) breast cancer (BC) patients (pts) have not benefited from HER2-directed therapy despite HER2 antigen availability. Triple negative BC (TNBC), in particular, is immunogenic and in need of additional therapeutic options. We have previously shown the HER2-derived nelipeptimut-S (E75) + GM-CSF (NeuVax) to be synergistic with trastuzumab (Tz) in pre-clinical and pilot clinical studies. In a planned interim analysis of a multi-center, prospective, randomized, single-blinded, placebo-controlled phase 2b trial of Tz + NeuVax vs Tz to reduce recurrence in HER2 LE, node-positive (NP) and/or triple negative BC (TNBC) pts, we previously reported that the NeuVax + Tz was safe without added cardiac toxicity and demonstrated a significant reduction of recurrences in TNBC pts. This analysis examines additional subsets in this trial.

**Methods:** HER2 LE, NP and/or TNBC pts who were clinically disease-free after standard therapy were randomized to receive Tz+NeuVax (vaccine group; VG) or Tz+GM-CSF (control group; CG). All pts received 1 yr of Tz per label. NeuVax or GM-CSF was given every 3 weeks x 6 starting with the 3<sup>rd</sup> Tz dose, and then boosted every 6 months x 4. This pre-specified interim analysis was triggered 6 months after last enrollment. The primary endpoint is intention-to-treat 24 month disease-free survival (DFS) evaluated by log rank.

**Results:** Of 275 pts randomized in the study (VG n=136, CG n=139), 98 had TNBC (VG=53, CG=45). In the interim analysis, estimated disease-free survival (DFS) was assessed with a median follow up of 18.8 months. No significant clinicopathologic differences were seen between treatment groups. In the TNBC group, estimated DFS was higher overall in VG vs CG (91.9% v 69.9%, p=0.023; hazard ratio [HR] 0.29, 95% confidence interval [CI] 0.09-0.90). On TNBC subgroup analysis, estimated DFS was higher in VG vs CG among pts who received neoadjuvant chemotherapy (VG n=35, CG n=31; HR 0.26, CI 0.07-0.93; p=0.03), HER2 IHC 1+ BC (VG n=34, CG n=28; HR 0.20, CI 0.04-0.96; p=0.03), pts who were AJCC 7<sup>th</sup> edition stage I/II (VG n=37, CG n=27; HR incalculable, no recurrences in the VG, p=0.008), and pts <sup>≥</sup>51yr of age (VG n=32 & CG n = 26; HR 0.26 CI 0.07,0.94; p=0.009). HRs did not appreciably vary based on the histologic grade or presence of lymphovascular invasion.

**Conclusion:** Examining the subgroups from the pre-specified interim analysis demonstrates a highly significant clinical benefit in TNBC pts overall. Within the TNBC cohort, specific benefit was seen in pts who received chemotherapy neoadjuvantly, expressed lower HER2, were earlier stage, and were older in age. These factors may help enrich the TNBC population targeted in a definitive Phase 3 study in TNBC patients with residual disease after neoadjuvant chemotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Modulation of the immune microenvironment in high risk DCIS by intralesional injection of anti-PD-1 (pembrolizumab)

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**Background.** Ductal carcinoma in situ (DCIS) is a risk factor for the subsequent development of invasive breast cancer. Features of DCIS that are associated with a high risk of a subsequent event include large size (> 5 cm), high grade, comedo necrosis, palpable mass, hormone receptor negativity, and HER2 positivity. We have previously shown that immune infiltrates are positively associated with these high-risk features, suggesting that manipulating the immune microenvironment in high-risk DCIS, for example via checkpoint blockade, could potentially alter disease progression.

**Methods.** In this phase 1 pilot study we investigated changes in the immune microenvironment of high risk DCIS after intralesional injection of anti-PD-1 (pembrolizumab). Study participants received 2 intralesional injections of pembrolizumab, 3 weeks apart, with surgery approximately 3 weeks after the 2nd dose. The study started with a dose of 2 mg/injection (1/100 of the standard 200 mg iv dose), then escalated to 4 mg and 8 mg, with 3 patients at each dose. Tissue samples from pre-treatment biopsies and post-treatment surgical resections were stained with two 6-plex immune panels using Opal immunofluorescence reagents (Perkin Elmer) on a fully automated Ventana Discovery platform, imaged with a Vectra 3 system and analyzed with inForm software (Perkin Elmer). An algorithm for tumor/stroma segmentation developed in inForm was used to randomly select 10 high power fields (hpfs) for imaging. Cell phenotype maps were generated for each of these hpfs for each sample. Cell densities were determined per area of stroma, DCIS, or total tissue and averaged across all hpfs for a given case. Spatial analyses were performed to quantitate co-localization of immune cells with DCIS cells.

**Results.** The intralesional injections were easily administered and well tolerated. No systemic toxicities were observed at any dose. MRI imaging demonstrated no change in the size of lesions after treatment. Multiplex immunofluorescence analyses demonstrated heterogeneous responses ranging from dramatic increases in T cells, in particular CD8<sup>+</sup> T cells, in cases which had a T cell infiltrate prior to therapy, to no post-therapy T cell infiltrate in cases with a pre-therapy immune desert. We also observed increases in B cells and macrophages and a decrease in the ratio of FoxP3<sup>+</sup> T cells to CD8<sup>+</sup> T cells, the latter mainly due to a significant increase in CD8<sup>+</sup> cells, as opposed to a decrease in FoxP3<sup>+</sup> cells. Spatial analyses indicated that in some cases, despite a marked increase in T cells post therapy, these cells did not co-localize with DCIS cells, indicating a state of immune exclusion.

**Conclusions.** We have demonstrated the safety and feasibility of intralesional injection of an immune checkpoint inhibitor (pembrolizumab) in high risk DCIS. In some patients we observed a dramatic change in the immune microenvironment, with an increase in T cells, B cells, and macrophages, and a decrease in the FoxP3:CD8 ratio, even at a dose that is 1/100 of the standard intravenous clinical dose. An expansion study is underway in which patients will receive 4 injections of pembrolizumab at 3 week intervals prior to going to surgery to determine if more injections/time will increase response rate.

# 2018 San Antonio Breast Cancer Symposium ®

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Updated efficacy of first or second-line pembrolizumab (pembro) plus capecitabine (cape) in metastatic triple negative breast cancer (mTNBC) and correlations with baseline lymphocyte and naïve CD4+ T-cell count

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**Background:** In mTNBC, anti-PD-1/L1 monotherapy is most effective when administered early in the course of disease, with recent trials demonstrating overall response rates (ORR) of 23-26% in the first-line setting and 5-6% in later lines. This may reflect iatrogenic lymphopenia from preceding cytotoxic chemotherapy. Furthermore, curative-intent chemotherapy is associated with prolonged suppression of naïve CD4+ cells, a T-cell subset that may play a critical role in the generation of *de novo* anti-tumor immune responses. We present the final clinical results of a pilot study evaluating the safety and efficacy of combining pembrolizumab plus standard-of-care capecitabine in the first/second-line mTNBC setting. We also explore potential associations between clinical benefit and lymphopenia, preceding chemotherapy, and absolute naïve CD4+ counts.

**Methods:** In a pilot study, we evaluated the tolerability and preliminary efficacy of concurrent pembro (200mg IV q21 day) plus investigator-selected 1<sup>st</sup>/2<sup>nd</sup> line paclitaxel (80mg/m<sup>2</sup> IV weekly) or oral cape (2,000mg BID, weekly 1 on/1 off). The primary endpoint was tolerability, defined as the proportion of subjects receiving >6 weeks concurrent therapy without dose discontinuation with toxicities reported per CTCAE v4.0. The secondary endpoint was 12-week objective response rate (ORR) by RECIST1.1. Exploratory endpoints included peripheral blood cell enumeration by real-time flow cytometry and routine clinical laboratory. Naïve CD4+ cells were defined as CD45+ CD3+ TCRab+ CD4+ CD45RA+ CCR7+. Here, we report the results of the pilot phase of the cape cohort (NCT02734290).

**Results:** Twelve of 14 subjects were treated in the first-line setting. All subjects (14/14, 100%) tolerated cape+pembro for >6 weeks, with toxicities consistent with monotherapy cape experience (diarrhea: grade I-II 50%, grade III 7%; hand-foot: grade I-II 71%) that improved with dose-reduction as needed. At 12 weeks, the ORR was 6/14 (42.9%), and the clinical benefit rate (ORR + stable disease) was 8/14 (57.1%). Depressed absolute lymphocyte count at baseline (ALC<1.0/uL: 33% CBR; ALC≥1.0/uL: 75% CBR) and recent exposure to cytotoxic chemotherapy (<6 months: 33% CBR; >6 months: 75% CBR) were associated with reduced clinical benefit. By flow cytometry, subjects experiencing clinical benefit had higher baseline absolute naïve CD4+ counts (average 283 cells/uL v. 93 cells/uL, p=.069).

**Conclusions:** This study met the primary endpoint of safety for cape plus pembro in mTNBC, with encouraging clinical activity. These data are supportive of further studies evaluating combination chemotherapy plus anti-PD-1/L1 mTNBC. We observed greater clinical benefit in subjects with non-suppressed ALC, less exposure to recent chemo, and higher baseline naïve CD4+ counts, suggesting that iatrogenic immunosuppression can impair response to immune checkpoint therapy in mTNBC. These findings should be confirmed in ongoing randomized trials of immune checkpoint +/- chemotherapy in mTNBC, and should be considered in the design of future clinical trials.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-04

Identification of a neoantigen targeted by tumor-infiltrating lymphocytes in a patient with Her2+ breast cancer

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**Background:** Recent studies have demonstrated that the number of tumor infiltrating lymphocytes (TILs) positively correlates with outcome and response to chemotherapy in patients with HER2+ and Triple-Negative Breast Cancer (TNBC). Furthermore, first studies of immune-checkpoint inhibitors showed promising results in those patients. However, the targets of those TILs remain unknown. Neoantigens, which arise in the process of tumorigenesis, appear as potential targets. They can elicit high avidity, tumor-specific T-cell responses. Thus, it is the aim of our study to ascertain if these TILs are directed against tumor-specific mutations.

**Methods:** TILs from breast cancer biopsies taken at the time point of diagnosis were expanded by unspecific stimulation. Additionally, we used the Gentle Macs Dissociator in combination with flow cytometry to investigate the number of TILs in the tumor tissue. Furthermore, we performed whole-genome sequencing of tumor tissue and as reference autologous blood cells to determine tumor-specific mutations. Mutations leading to a non-synonymous amino acid change were analyzed for RNA expression of the encoding gene as well as to determine potential neoantigens. Neoantigens were evaluated for their potential binding to the patient's specific HLA molecules. Peptides for potential neoantigens were synthesized, loaded onto autologous antigen presenting cells (APCs) and cocultured with TILs. All IFN $\gamma$  producing T-cells were clonally expanded and retested for peptide specificity to identify neoantigen specific T-cell clones.

**Results:** Our flow cytometric analysis of the tumor biopsy for more than 300 patients showed higher frequencies of TILs in TNBC as compared to other types of breast cancer or patients without malignancy. Screening for neoantigen specific T-cells in one patient led to identification of three peptide-specific CD4+ T-cell clones isolated from HER2+ breast cancer tissue taken at the time point of diagnosis. All T-cell clones specifically recognized the same tumor-specific mutation and not the wildtype counterpart. Furthermore, we demonstrated that these T-cell clones also recognized the endogenously expressed mutated antigen. This verified the ability of processing and presentation of the respective protein. Interestingly, we could also isolate a T-cell clone recognizing the same neoantigen in the resected tumor tissue after neoadjuvant therapy. Based on CDR3 sequencing we could prove that the four T-cell clones represented individual clones. This confirms the polyclonal nature of the immune response. Moreover, we showed that the same neoepitope was presented in two different HLA restriction molecules of the patient with three of the clones recognizing it in HLA-DPB1\*0401 and one in HLA-DPB1\*0201. These results further underline the immunogenicity of this neoantigen.

**Conclusion:** In conclusion, our data demonstrate tumor-specificity of TILs in a patient with HER2+ breast cancer. Furthermore, we show the feasibility to identify individual cancer specific T-cell targets in breast cancer patients. These results may contribute to the development of targeted patient-specific immunotherapies in the future.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-05

**LCCC 1525:** A phase II study of a priming dose of cyclophosphamide prior to pembrolizumab to treat metastatic triple negative breast cancer (mTNBC)

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**Background:** While immunotherapy holds promise in the treatment of mTNBC, response rates (RR) in unselected patients (pts) are approximately 20%. Strategies to augment response to immunotherapy include depletion of regulatory T cells (Tregs). A single dose of cyclophosphamide (Cy) given prior to checkpoint inhibition achieved this goal in preclinical models of TNBC (Taylor et al., JCI, 2017). Thus, we designed a phase II study to evaluate this strategy in the clinical setting of mTNBC.

**Patients/Methods:** In cycle 1 (C1), eligible pts with mTNBC received a single dose of Cy 300mg/m<sup>2</sup> IV on day 1 (C1D1) followed by pembrolizumab 200mg IV on day 2 (C1D2), then every 3 weeks thereafter. The co-primary objectives were (1) progression free survival (PFS, null 1.9 mos vs. 2.9 mos, 80% power, alpha 0.05) and (2) reduction in Tregs in peripheral blood measured by flow cytometry. Secondary endpoints were response rate (RR), survival (OS), and RNA-based correlative endpoints.

**Results:** 40 patients were evaluable for efficacy: mean age 54.5 yrs (33 – 82 yrs), 75% white, 22% black, 3% American Indian. All patients had received 1 prior line of chemotherapy in the metastatic setting; 29% received 5 or more prior lines. The most common grade 3 adverse events (AE's), all 5%, were neutropenia, anemia, elevated AST, and fatigue. Immune-related grade 3 AE's, all 3%, included colitis, dry mouth, pneumonitis. Overall RR was 21% (0 CR, 8 PR), 3 pts had stable disease. Median PFS was 1.8 months (mos) (95% CI 1.4–2.5) and OS was 6.3 mos (95% CI 2.8–8.4). There was a non-significant decrease in Tregs from C1D1 to C1D2 (-3.3%, p=0.19); but from C1D2 to C2D1, Tregs increased 21.7% (p=0.005). There was no association between changes in Tregs or number of prior lines of therapy with RR (p>0.09), while immune-related AE's were associated with response (p=0.02). Correlative studies illustrate B cell immune gene signature expression and B cell receptor repertoire diversity were enriched in responders, while genes/pathways associated with neutrophils, anti-apoptosis, PI3K/AKT and down-regulation of MHC class 1 were associated with non-response.

**Conclusions:** While pembrolizumab plus Cy was well-tolerated among pts with mTNBC, efficacy was similar to historical control, likely due to minimal effect of Cy on Tregs. Correlative analyses illustrate that study of adaptive immune features, including B cell biology, is a promising strategy for understanding response to PD-1 inhibition in breast cancer. Further strategies to deplete Tregs in a more sustained manner are worthy of future exploration (NCT02768701).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-06

Safety and efficacy of stereotactic body radiotherapy and Pembrolizumab in advanced breast cancer patients with 1 to 5 metastases

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## Background

Pre-clinical studies have demonstrated that stereotactic body radiotherapy (SBRT) induces immunogenic cell death and tumor antigen release promoting anti-tumor immunity. We hypothesized that the efficacy of SBRT could be improved with the addition of PD-1 blockade in advanced breast cancer (BC) patients with oligometastatic disease.

## Methods

Advanced BC patients with 1 – 5 metastatic sites of disease received SBRT at a dose of 20Gy in 1 fraction to at least 1 metastasis followed by pembrolizumab 200mg IV (within 5 days of SBRT), once every 3 weeks for a total of 8 cycles. The primary endpoint was safety of the combination. The secondary endpoints were response using RECIST 1.1 and PERCIST 1.0 using 18F-Fluorodeoxyglucose (FDG)- positron emission tomography (PET) scans as well as local progression-free survival and distant progression-free survival (d-PFS). Correlatives included deep sequencing of the T cell receptor (TCR) CDR3 regions of archival metastatic tumor material and in peripheral blood as well as evaluation of systemic markers of immune activation.

## Results

15 patients were enrolled between March 2016 and Nov 2017. The number of patients with 1, 2, 4 and 5 metastases treated with SBRT was 9 (60%), 3 (20%), 2 (13%) and 1 (7%), respectively. There were 3 (20%) TNBC, 2 (13%) HER2+ and 10 (67%) ER+/HER2- (Luminal) BC patients. Five (33%) patients experienced Grade 3 or higher Aes. Immune related Aes were reported in 10 (67%) patients, the most common being rash (n=4), thyroid (n=3) and pneumonitis (n=3) with pembrolizumab delayed in 3 (20%) patients and discontinued in 2 (13%) patients. Nine (60%) patients had a complete response or partial response according to RECIST 1.1 criteria and 13 (86%) patients had a complete metabolic response (CMR) or partial metabolic response (PMR) according to PERCIST 1.0 at the 3-month FDG-PET scan. At time of reporting (median follow-up 15 months; range 9-26 months) no deaths or local progression have been observed. Five patients (33%) progressed distantly at 3, 8, 8, 12 and 18 months: 2 of 3 (66%) TNBC, 2 of 10 (20%) Luminal and 1 of 2 (50%) HER2+. Estimated dPFS at 9 months was 80% (95% CI [62% – 100%]). Three patients have reached the 2-year mark without progression (all Luminal). In a patient who remained progression free at 24 months, TCR profiling showed that TCR sequences in a pre-treatment metastatic tumor biopsy were found in peripheral blood and became elevated after combination treatment, along with the emergence of new TCR sequences not seen in the tumor sample. Markers of increased CD8+ and CD4+ T cell activation using flow cytometry of peripheral blood lymphocytes were observed. This data suggests that the treatment induced peripheral expansion of tumor-specific T cells in this responding patient. Further correlatives are pending.

## Conclusion

The combination of SBRT and 6 months of pembrolizumab in advanced breast cancer patients with 1-5 metastases showed an acceptable toxicity profile and promising clinical benefit especially in Luminal BC patients. Evidence of peripheral anti-tumor T cell responses was observed in a patient who remains progression free at 24 months. The potential synergy between these treatment modalities requires further study.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-07

Preoperative pembrolizumab (Pembro) with radiation therapy (RT) in patients with operable triple-negative breast cancer (TNBC)

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**Background:** Radiation therapy (RT) induces immune-mediated cell death and could generate a rich supply of tumor antigens if administered in the pre-operative, curative-intent setting. The addition of PD-1 mediated checkpoint blockade to pre-operative RT could thus, generate robust anti-tumor immune responses, induce long-term tumor-specific memory, and ultimately, improve cure rates. This study aims to establish the safety of pre-operative pembrolizumab (pembro)-mediated immune modulation with a RT “boost” equivalent in patients with operable triple negative breast cancer (TNBC) for whom lumpectomy and adjuvant RT are planned (NCT03366844). Serial research biopsies permit interrogation of conventional biomarkers including tumor infiltrating lymphocytes (TILs) and novel immune correlates as potential predictors of response to pembro alone versus pembro with RT.

**Methods:** Ten women with operable, primary TNBC >2cm for whom breast-conserving therapy is planned are being enrolled in this single-institution pilot study. Study treatment consists of 1 cycle of pre-operative pembro (200 mg IV) alone, followed 3 weeks later by a RT boost (24 Gy/3 fractions) to the primary breast tumor concurrently with pembro (+/- 5 days). Curative-intent, standard-of-care, neoadjuvant chemotherapy (NAC) or breast-conserving surgery is then undertaken within 8 weeks of study enrollment (i.e. within 5 weeks of pembro #2). Adjuvant RT is administered per standard-of-care after surgery, but without a boost dose. Research blood and fresh tumor biopsies are obtained at baseline and after cycles 1 and 2 of pembro. Co-primary endpoints are: 1) safety/tolerability, as defined by the number of patients who do not necessitate a delay in standard-of-care chemotherapy or surgery and 2) change in TIL score. Secondary endpoints include safety/toxicity up to 19 weeks after study enrollment, pCR rates and disease-free survival. Correlative analysis will include single-cell RNA sequencing of the tumor immune infiltrate and multispectral immunohistochemistry

**Results:** Seven patients enrolled between 12/19/17 and 7/1/18. As of 7/1/18, 5 patients have completed the experimental pembro/RT phase of the trial and are currently completing standard-of-care NAC; 1 patient is currently being treated in the experimental pembro/RT phase; and 1 patient with a cT2N0 tumor at baseline achieved a pathologic complete response (pCR, ypT0/Tis ypN0) after completing the experimental pembro/RT phase followed by anthracycline- and taxane-based NAC. No grade 3 or 4 toxicities have been observed during pembro/RT in the 6 patients completing the experimental phase to date. Three additional patients will be enrolled

**Conclusions:** This is the first trial of curative-intent, pre-operative checkpoint blockade with RT in breast cancer and the strategy appears to be well tolerated to date. At the time of presentation, safety, change in TIL score, and pCR rates for all patients completing the experimental and NAC phases of the study will be reported.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-08

Imprime PGG, a novel innate immune modulator, combined with pembrolizumab in a phase 2 multicenter, open label study in chemotherapy-resistant metastatic triple negative breast cancer (TNBC)

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**Background:** CPI monotherapy provides substantial clinical benefit to patients (pts) in multiple cancers, yet response rates are limited (~15-30%) and fails to benefit the majority. In these pts there is limited or no ongoing T cell-based immune response. Imprime PGG (Imprime), a novel beta glucan derived from *Saccharomyces*, may expand the clinical benefit of CPI therapy by stimulating an anti-cancer immune response. Acting as a pathogen-associated molecular pattern (PAMP), Imprime enlists innate immune functions including cytotoxic effector mechanisms, reversal of immunosuppression and cross-talk with the adaptive immune system. Imprime-mediated innate immune activation requires formation of an immune complex with naturally-occurring anti-beta glucan antibodies (ABA); sufficient ABA levels is required for complex formation. Imprime is now being studied in combination with pembrolizumab (KEYTRUDA®, Pembro), a humanized mAb against PD-1 which has been previously studied in TNBC pts.

**Methods:** In this study of patients who previously failed chemotherapy for metastatic TNBC, Imprime is being used in combination with Pembro in a Simon 2 stage design. A sample size of 12 evaluable pts in Stage 1 was planned. Evaluable pts received at least one dose of study treatment (tx), had measurable disease at baseline per RECIST v1.1, had at least one post-baseline scan or discontinued tx as a result of progressive disease, death, or a tx-related adverse event before the first post-baseline scan. Pts received Imprime (4 mg/kg IV days 1, 8, 15 of each 3-week cycle) + Pembro 200 mg on D1 of each cycle. Criteria to advance to Stage 2 were  $\leq 4$  grade 3/4 AEs during the first tx cycle (other than infusion reactions) and  $\geq 1$  objective response. Study primary endpoints are ORR and safety; secondary endpoints are TTR, CRR, DoR, PFS, and OS. Exploratory endpoints include ORR and PFS per irRECIST. Biopsies and blood samples are being collected to assess tx impact on immune activating events at the tumor site and in the periphery.

**Results:** A review of efficacy and safety data was conducted at the end of Stage 1. Thirteen pts (12 evaluable) were enrolled into Stage 1. Safety review noted 2 grade 3 adverse events that met protocol definition of Stage 1 events (1 pt: cellulitis and 1 pt: pleural infusion; both unrelated to treatment). Two events lead to 2 pts discontinuing treatment (infusion reaction and pancreatitis) and only 1 autoimmune event was observed (pancreatitis). Observed efficacy responses in the evaluable pts included 1 complete response (CR; ongoing) and 2 partial responses (PR; ongoing). Secondary efficacy endpoints have not been assessed. Early translational results support proposed MOA and analysis of Stage 1 translational data is ongoing.

**Conclusion:** The use of Imprime with Pembro was well tolerated and met both safety and efficacy requirements to move forward with Stage 2 of the study. No significant safety concerns were identified in Stage 1. Further investigation is thus warranted and enrollment into Stage 2 is ongoing. Updated data will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-09

Initial safety and efficacy of a phase I/IIa trial of a modified whole tumor cell targeted immunotherapy in patients with advanced breast cancer

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**Background:** SV-BR-1-GM is a GM-CSF transfected breast cancer cell line which expresses HLA class I & II antigens. In a previous clinical trial, a partial response of widely metastatic breast cancer was seen in a patient who matched SV-BR-1-GM at *HLA-DRB3\*02:02*. Here we report the safety and efficacy analysis with immunologic correlates of response in the initial patients in a phase I/IIa trial of SV-BR-1-GM in patients with advanced breast cancer

**Methods:** This phase I/IIa trial enrolled patients with recurrent and/or metastatic breast cancer refractory to standard chemotherapy/targeted-therapy. Patients received low-dose cyclophosphamide 2-3d prior to intradermal injection of SV-BR-1-GM (20x10<sup>6</sup> cells divided into 4 sites) and interferon- $\alpha$  into the inoculation sites (10,000 IU/site) ~2 & 4 days subsequently. Cycles were 2 weeks x3 then q mo x 3. Adverse events (AE) were evaluated after each inoculation and graded via CTCAE v4.03. Immunologic response was measured by delayed type hypersensitivity (DTH) after each inoculation. Disease response was evaluated radiographically q3 mo and as clinically indicated (clinical trial NCT03066947).

**Results:** To date, twenty-two patients have been enrolled and 17 have been inoculated for a total of 39 SV-BR-1-GM inoculations given. Per inoculation, the maximum related AE was grade 1 in 64%, grade 2 in 7.7%, and grade 3 in 7.7%. There were no related grade >3 or unexpected AE. Efficacy data is available on the first six (Table). Tumor regression was seen in 2 patients. 01-002 presented with liver, bone and 20 classic miliary lung metastases (up to 9mm). This subject previously received 7 chemotherapy regimens. She matched SV-BR-1-GM at Class I & II HLA loci. Imaging at 3 mo showed virtually complete regression of all 20 identifiable lesions in the lungs. This response was maintained at 6 mo but the subject was taken off protocol because of disease progression (liver and bone). 01-005, matching *HLA-A\*24:02*, had notable regression of cutaneous lesions, but progressed in pleural and pericardial effusions, had irreversible cardiac arrest (unlikely related). DTH increased in 01-002 from 4mm (first dose) to 47mm (8<sup>th</sup> dose). Three of 3 patients evaluated developed antibodies responses (as measured by flow cytometry with SV-BR-1) including 01-002. Interleukin 8 also increased in 01-002.

**Conclusions:** SV-BR-1-GM in this regimen appears to be safe and well-tolerated. In this initial exploratory analysis, SV-BR-1-GM can produce regression of pre-treated metastatic breast cancer correlating with an immunologic response. HLA matching is being evaluated as a predictor of response.

Patient	Age	Metastatic Sites	# Prior Regimens	HLA Matches	# of Cycles	Tumor Regression?
01-001	46	Pleura, Lymph Nodes	7 chemo/bio, 5 hormonal	DRB3*02:02	1	No
01-002	73	Lung, Liver, Bone	6 chemo, 1 hormonal	A*24:02, DRB3*02:02	8	Lungs
01-005	54	Lymph nodes, Pleura, Skin	3 chemo/bio	A*24:02	2	Skin
02-001	70	Lymph nodes	1 chemo/bio	None	1	No
02-003	61	Bone, Brain	3 chemo	None	6	No
02-004	74	Lymph nodes, Cutaneous	3 chemo/bio, 1 hormonal	DRB3*02:02	2	Lost to Follow-up

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-10

Epigenetic modulation—unlocking the potential of checkpoint inhibition in breast cancer

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Immune checkpoint inhibition (ICI) has revolutionized treatment in cancers that are naturally immunogenic by enabling infiltration of T cells into the tumor microenvironment (TME) and promoting cytotoxic signaling pathways. Tumors possessing complex immunosuppressive TME's such as breast cancer present unique therapeutic obstacles as response rates to ICI remain low. Such tumors often recruit myeloid-derived suppressor cells (MDSCs) whose functioning prohibits both T cell activation and infiltration. To date, most studies focus on use of ICI in triple negative disease. Our work aims to uncover the efficacy of ICI in both early and advanced HER2 positive (HER2<sup>+</sup>) disease and to advance our understanding of how to improve response rates to these new promising therapies.

We are using a HER-2/neu transgenic mouse model with tumor challenge of syngeneic cell lines to test the efficacy of different combinations of an epigenetic agent, the histone deacetylase inhibitor entinostat (ENT), checkpoint inhibitors anti-PD-1 and anti-CTLA-4, on primary and metastatic disease. We are examining treatment effects on primary tumor growth, metastatic burden, and survival. Characterization of tumor infiltrating lymphocytes and their functional capabilities are being investigated using fluorescence-activated cell sorting, gene expression profiling, and ex vivo suppression assays. Western blots, qPCR and other in vitro assays will be performed on MDSCs to investigate mechanisms behind response.

In the HER2<sup>+</sup> mouse model of early stage disease, we show that combining ENT, with ICIs significantly improves survival and delays tumor growth. Preliminary data in models of advanced disease, show only ENT + a-PD-1 improves survival and metastatic burden. Conversely, in the metastatic model, ENT + a-CTLA-4 negatively effects survival and metastatic burden. In primary tumors, ENT + ICIs leads to significantly decreased suppression by granulocytic-MDSCs. However, MDSC infiltration and function is not affected in lungs containing macrometastatic disease. Interestingly, we found an increase in activated granzyme-B-producing CD8<sup>+</sup> T effector cells in mice treated with combination therapy in both primary and metastatic tumors. Finally, gene expression profiling of MDSCs from primary tumors identified significant changes in immune-related pathways, and identified a common downstream regulator –STAT3. Studies are ongoing to evaluate the mechanistic role of STAT3 in the response observed in primary tumors and to determine if STAT3 is involved in response in the metastatic setting.

In summary, addition of ENT to ICIs significantly affects overall survival in early stage models of HER2<sup>+</sup> breast cancer however, only addition of a-PD-1 to ENT is beneficial in models of advanced disease. Additionally, the mechanism of action in early stage disease involves altered infiltration and function of MDSCs, allowing for a more robust adaptive immune response. However, a different mechanism of action is likely responsible for the responses seen in advanced stage disease. These novel findings provide a rationale for combination therapy in patients with HER2<sup>+</sup> breast cancer and suggest responses to this combination therapy are linked to stage of disease likely due to different mechanisms of action.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-11

Intratumoral tavokinogene telseplasmid and electroporation in pre-treated inoperable locally advanced or recurrent triple-negative breast cancer

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**Introduction:** Triple negative breast cancer (TNBC) accounts for approximately 15% of breast cancer diagnoses and is associated with a higher risk of recurrence and more aggressive course in the metastatic setting. Emerging data suggest that some patients with TNBC benefit from immune-based therapies targeting the anti-programmed cell death protein 1 (PD-1)/PD-1 ligand 1 (PD-L1) axis, but success has been limited in poorly immunogenic tumors. Thus, combination therapies that drive an influx of CD8<sup>+</sup> tumor infiltrating lymphocytes (TILs) and/or upregulate PD-L1 expression are required to increase response rates to these therapeutics. Intratumoral injection of plasmid IL-12 (tavokinogene telseplasmid; tavo) followed by electroporation (IT-tavo-EP) is a gene therapy approach that drives local expression of the proinflammatory cytokine, interleukin-12 (IL-12). Local expression of IL-12 is hypothesized to result in increased TILs and enhanced expression of proinflammatory cytokines, resulting in conversion of poorly-immunogenic/low TIL TNBC tumors into highly inflamed immunologically active lesions while demonstrating a high safety profile.

**Methods:** OMS-I140 is a phase I, non-randomized, open-label study of IT-tavo-EP in patients with inoperable locally advanced, metastatic and/or treatment-refractory TNBC (NCT02531425). Eligible patients have pre-treated TNBC and at least 2 anatomically distinct cutaneous or subcutaneous lesions accessible for injection and electroporation, with or without other regional or distant metastases. 10 patients are planned for enrollment. IT-tavo-EP is administered on Days 1, 5 and 8 of a single 28-day cycle. Tavo is injected intratumorally (based on tumor volume) at a concentration of 0.5 mg/mL and immediately followed by co-localized electroporation (6 pulses at 1500 V/cm with 1-second intervals). Tumor biopsies are obtained at baseline and post-treatment on day 28 of both treated and untreated lesions to determine if this therapy can promote a pro-inflammatory molecular and histologic signature. Pain scores and adverse events are recorded.

**Results:** To date, nine patients have completed study therapy. Reported treatment-related adverse events include pain associated with electroporation (grade 1) in 8 patients and fatigue (grade 1) in 1 patient. Median pain score (range 0-10) immediately after treatment was 2 (range 0-10) and 5 minutes post-treatment was 0 (range 0-6). In some patients, treatment-related increases in CD8<sup>+</sup> TIL density have been observed by intratumoral chromogenic staining. Further immune profiling is being conducted to characterize the tumor microenvironment pre- and post-therapy. A subset of patients with treatment refractory TNBC received anti-PD-1 monotherapy as their immediate next therapy with clinical response observed. Updated data will be presented.

**Conclusions:** Our data suggest that IT-tavo-EP is a safe and tolerable TIL stimulating therapy in TNBC. Further study of IT-tavo-EP in combination with pembrolizumab in pretreated metastatic TNBC is planned.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-12

Perilymphatic IRX-2 cytokine therapy to enhance tumor infiltrating lymphocytes (TILs) and PD-L1 expression preceding curative-intent therapy in early stage breast cancer (ESBC)

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**Background:** Cytokines are being explored as a therapeutic strategy to modulate the tumor microenvironment and facilitate immunotherapy benefit in breast cancer. Here, we investigate a locoregional therapeutic approach whereby cytokines (IRX-2) are administered into the subcutaneous peri-areolar tissue (in an anatomic distribution similar to sentinel lymph node mapping) to facilitate immune cell recruitment/activation within the draining lymph nodes and tumor in ESBC. IRX-2 is derived from *ex vivo* phytohemagglutinin-stimulated lymphocytes and contains multiple cytokines including IL-1 $\beta$ , IL-2, TNF- $\alpha$ , IFN- $\gamma$ , IL-6, IL-8, and GM-CSF, with stable concentrations from lot to lot. Preclinically, IRX-2 activates T-cells and natural killer (NK) cells, facilitates antigen presentation, and enhances activity of anti-PD-1/L1 in a SCC7 model. In a preceding head/neck squamous cell carcinoma phase I trial, perilymphatic IRX-2 was safe and increased TILs. Here, we report the final clinical results of a phase Ib trial evaluating the feasibility and immunologic activity of IRX-2 in ESBC.

**Methods:** Beginning 21 days prior to surgical resection, enrolled operable patients with stage I-III ESBC (all subtypes) received the pre-operative IRX-2 regimen consisting of a single low-dose cyclophosphamide (300 mg/m<sup>2</sup> to facilitate T-regulatory cell depletion), followed by 10 days of subcutaneous peri-areolar IRX-2 injections into the affected breast (1 mL  $\times$  2 at tumor axis and at 90°). Endpoints were feasibility (primary endpoint), stromal TIL (sTIL) count (pre-treatment versus post-treatment, blinded average of two pathologist reads using San Antonio H&E sTIL guidelines), PD-L1 expression (Nanostring) and enumeration of peripheral immune cells by flow cytometry.

**Results:** All patients (n=16/16) completed and tolerated the regimen with no surgical delays or treatment-attributed grade III/IV toxicities. Common adverse events (occurring in >15% subjects) attributed to IRX-2 injections were: injection site reaction (grade 1, n=8/16), bruising (grade 1, n=7/16), and pain (grade 1, n=3/16). Common adverse events attributed to low-dose cyclophosphamide were: fatigue (grade 1, n=5/16) and nausea (grade 1/2, n=3/16). Treatment was associated with an increase in sTIL score (Wilcoxon signed-rank p=.04), with 4/10 sTIL-low tumors (0-10% score) re-categorized to sTIL-moderate (11-50% score). Increases in PD-L1 RNA expression were observed (Wilcoxon signed-rank p=.04) in 12/16 tumors (median 57% increase, range: -53% to 185% increase), as well as increases in Nanostring NK and Th1 cell signatures. In blood, increases in CD4 and CD8 effector T-cell activation (ICOS, HLA-DR, and CD38) and T-reg depletion were observed.

**Conclusions:** IRX-2 was well tolerated with preliminary evidence of sTIL increase, PD-L1 upregulation, and peripheral lymphocyte activation. Based upon these data and preclinical evaluations demonstrating synergy with checkpoint inhibition, the IRX-2 regimen is being evaluated for clinical efficacy in conjunction with pembrolizumab and neoadjuvant chemotherapy (doxorubicin, cyclophosphamide, paclitaxel) in patients with stage II-III triple negative breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-13

A phase I study of adoptive immunotherapy for ROR1+ advanced triple negative breast cancer (TNBC) with defined subsets of autologous T cells expressing a ROR1-specific chimeric antigen receptor (ROR1-CAR)

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**BACKGROUND:** ROR1 is a type 1 transmembrane tyrosine kinase receptor that plays a critical role in embryonic and fetal development. ROR1 has been described as a possible oncogene and is expressed in numerous malignancies including TNBC and non-small cell lung cancer (NSCLC). We are conducting a first-in-human trial targeting ROR1 with CAR-T cells in patients with advanced TNBC and NSCLC. The cellular construct employed targets the Ig/Fc portion of the extracellular domain of ROR1 and contains 4-1BB/CD3ζ intracellular signaling domain. The manufacturing process utilizes autologous peripheral blood lymphocytes, separated into CD4 and CD8 subsets, which are independently cultured with anti-CD3/anti-CD28 beads and IL-2, then transduced with a lentiviral vector encoding the ROR1 CAR. The CAR-T cell product is formulated in a 1:1 ratio of CD4+ and CD8+ CAR-T cells.

**METHODS:** This ongoing phase I trial (NCT02706392) is evaluating the safety of administering ROR1 CAR-T cells in escalating doses ( $3.3 \times 10^5$ ,  $1 \times 10^6$ ,  $3.3 \times 10^6$  and  $1 \times 10^7$  cells/kg) following lymphodepletion with cyclophosphamide-containing regimens using a continual reassessment method (CRM) for dose escalation. TNBC patients with adequate organ function and performance status, measurable disease, and tumors expressing ROR1 (>20% by IHC) are eligible for enrollment. Persistence of CAR-T cells in blood, cytokine levels, measures of immunogenicity and multi-parametric flow cytometry are being evaluated at multiple time points. Imaging assessments by RECIST 1.1 are performed day 28 - 90, then at 6 and 12 months, and every 6 months as clinically indicated to estimate efficacy.

**RESULTS:** To date, 4 TNBC patients (age range 38-67) have been enrolled, treated and are evaluable for response. Patients had received prior therapies for metastatic disease (range 3-11). 3 of 4 had visceral metastases. No dose-limiting toxicities, severe neurotoxicity or severe cytokine release syndrome (sCRS) were observed at dose levels 1 and 2. Two patients experienced grade 1 CRS. 2 of 4 patients had evidence of CAR-T cell expansion between days 14 and 20, with peak CD8+ CAR-T up to 232.1 cells/uL. Analysis of surface phenotype revealed upregulation of inhibitory receptors on CAR-T cells at the peak of expansion, confirmed by RNA seq. Post-treatment tumor biopsy in patient with partial response revealed an influx of CD3+ T cells and macrophages suggesting ROR1 CAR-T cell trafficking. Two patients received 2 CAR-T cell infusions. Two patients had confirmed stable disease at 15 weeks and 19 weeks, respectively. One patient has stable disease after 1<sup>st</sup> CAR-T cell infusion, then confirmed partial response after 2<sup>nd</sup> CAR-T cell infusion which has persisted for 14 weeks. Results will be updated.

**CONCLUSIONS:** ROR1+ CAR-T cells can be safely transferred, expand *in vivo* in patients with TNBC. Current efforts are directed at understanding and overcoming the mechanisms that limit homing, persistence, and/or function at tumor sites. The trial is continuing with dose-escalation. Funding provided by 8RO1 CA114536-11 and Juno Therapeutics.

# 2018 San Antonio Breast Cancer Symposium ®

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Immunogenomic pathway and survival analysis in breast cancers based on tumor location and molecular subtypes

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**Introduction:** Most breast tumors respond poorly to immunotherapy. Triple-negative breast cancer (TNBC) breast tumors are thought to be more immunogenic than other breast cancer subtypes (luminal A/B or HER2+). Increased immune response in TNBC are characterized by high levels of tumor infiltrating T lymphocyte (TIL) composition that would predict excellent response to immune checkpoint blockade. For all breast cancers, tumors appear more commonly in the upper outer quadrant. However, it is not clear whether expression of immune response genes vary with tumor location among the subtypes. Here, we hypothesized that by analyzing differential gene expression associated with immune response pathways among molecular subtypes of breast cancer such as luminal A/B, HER2+ or TNBC, we can identify targetable pathways to improve therapy with breast cancer.

**Methods:** Using the Cancer Genome Atlas (TCGA) dataset, we have identified 918 breast cancer tumor samples and compared RNAseq gene expression based on molecular subtypes and anatomic locations of biopsies (i.e., right, left, lower inner quadrant, lower outer quadrant, upper inner quadrant or upper outer quadrant). Genes with significantly different expression ( $p < 0.01$ ) were selected for survival analysis. R, Reactome Pathway Browser were used to retrieve and analyze data.

**Results:** In TNBC, tumors from lower outer quadrant, lower inner quadrant demonstrated significantly higher CD8B mRNA expression compared with luminalA/B and HER2 ( $p = 2.93E-04$ ,  $2.73E-04$ ) from same locations. CD8B mRNA was not significantly higher in TNBC tumors of other sites compared with luminalA/B and HER2. However, pathway/genes associated with CTL function remained significantly different between the different sites for TNBC compared with other subtypes. The metastasis suppressor gene, CD82, was significantly higher in TNBC samples from the right side ( $p = 4.83E-05$ ), lower outer quadrant ( $p = 4.33E-05$ ), lower inner quadrant ( $p = 3.32E-03$ ) and upper inner quadrant ( $p = 4.51E-07$ ), but this gene was not significantly expressed in the upper outer region, where tumors are prevalent.

From immune pathway analysis, genes involved in the antigen activates B cell receptor pathway ( $p < 0.05$ ) were associated with overall survival (OS) in right and left sided Luminal A/B and HER2 tumors and right sided TNBC tumors. Finally, genes from pathway involved in immune-regulatory interactions between a lymphoid and a non-lymphoid cells were associated with OS in lower outer quadrant, upper outer quadrant tumors in luminal A/B and HER2 cases and right sided tumors in TNBC ( $p < 0.05$ ).

**Conclusion:** While previous studies have reported that tumor infiltrating lymphocytes and lymphoid aggregates in tumors are associated with survival, following more complex analysis, we reveal novel genes and immune pathways that demonstrate improved survival prediction in the TCGA dataset for breast cancers. Furthermore, as expected, we confirm that different immune pathways are associated with survival in luminalA/B, HER2 and TNBC tumors. Our findings demonstrate the importance of a patient-centered approach to the treatment of patients with breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

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A phase I study of interferon-gamma ( $\gamma$ ) plus weekly paclitaxel, trastuzumab and pertuzumab in patients with HER-2 positive breast cancer

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**Background:** IFN- $\gamma$ , a cytokine that plays diverse roles in innate and adaptive immunity, has been shown to be essential in anti-tumor immune response. *In vitro* and *in vivo* studies have shown the synergistic effect of IFN- $\gamma$  in combination with HER2-targeting monoclonal antibodies with or without taxane chemotherapy. We have conducted a phase 1 clinical trial of systemic IFN- $\gamma$  in combination with trastuzumab, pertuzumab, and paclitaxel in HER2-positive metastatic breast cancer.

**Methods:** Two dose levels (DL) of IFN- $\gamma$ , 50 (DL1) and 75 mcg/m<sup>2</sup> (DL2), were evaluated. IFN- $\gamma$  was given as subcutaneous injection three times weekly starting on day 1 of therapy for 12 weeks. Paclitaxel was administered intravenously (IV) weekly at 80mg/m<sup>2</sup> in combination with trastuzumab IV (8 mg/kg loading dose, then 6 mg/m<sup>2</sup> q 21 days) and pertuzumab IV (840 mg loading dose, then 420 mg q 21 days). Eligible patients had measurable metastatic HER2-positive breast cancer, were candidates to receive paclitaxel chemotherapy, and had an ECOG PS 0-1. The primary objective of this study was to evaluate the safety and tolerability of the combination therapy during the 12 weeks of treatment and to determine the recommended phase II dose (RP2D). Dose-limiting toxicity (DLT) during cycle one was defined as follows: Non-hematologic or hematologic toxicities that are  $\geq$  grade 3 and probably or definitely related to study therapy which lead to chemotherapy treatment delays > 14 days.

**Results:** A total of nine patients (3 on DL1 and 6 on DL2) were enrolled between 2/2017 and 11/2017. No DLT was observed. For DL1, no serious adverse events (SAE) or significant adverse events (AE) were observed among 3 patients who completed 12 weeks of treatment. For DL2, two out of 6 patients had SAEs including grade 3 pneumonitis (at week 8; treatment was subsequently discontinued) and grade 3 non-neutropenic fever (at week 6), which were possibly related to study treatment. These toxicities, however, did not meet the protocol definition of DLT. Based on these findings suggesting an improved tolerability of DL1 (50 mcg/m<sup>2</sup>), DL1 was selected as the RP2D. The most frequently observed grade 1 and 2 AEs that were at least possibly related to IFN- $\gamma$  were fatigue (45%) nausea (36%), myalgia (36%), and fever (27%) diarrhea (18%). No grade 4 AE was noted. Grade 3 AEs included diarrhea, nausea, pneumonitis, non-neutropenic fever. Three out of 9 patients achieved partial response and 6 patients had stable disease per RECIST criteria.

**Conclusion:** IFN- $\gamma$  in combination with trastuzumab, pertuzumab, and paclitaxel was well tolerated in patients with HER2-positive metastatic breast cancer. Updated results will be presented and the phase 2 neoadjuvant trial is ongoing to further assess the efficacy of this approach.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-09-16

CD8 T cells induced by novel alphaviral vector predict improved progression free survival in advanced HER2+ breast cancer patients

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**Background:** Immune-based therapy for metastatic breast cancer has had limited success. Strategies to augment adaptive immunity include vaccines targeting genomic amplifications like Human Epidermal Growth Factor Type 2 (HER2), an established driver of malignancy. Using a novel alphaviral vector, we constructed a vaccine encoding a portion of HER2 (VRP-HER2).

**Methods:** In preclinical studies, mice were immunized before or after implantation of hHER2+ tumor cells and HER2-specific immune responses and anti-tumor function were assessed. We then translated this vaccine into a phase I clinical trial in which subjects with advanced HER2-overexpressing breast cancers received VRP-HER2 every 2 weeks for a total of three doses (cohort 1). In cohort 2, subjects received the same dose of VRP-HER2 along with a standard HER2 targeted therapy.

**Results:** VRP-HER2 induced HER2-specific T cell and antibody responses while controlling tumor growth in murine models. Vaccination with VRP-HER2 was well tolerated in both patient cohorts. PFS was modest, while median OS was 50.2 months in cohort 1 and 32.7 months in cohort 2. In cohort 2, there is one partial response and two patients with continued stable disease. Vaccine induced anti-HER2 antibodies and T cells were identified. Increased perforin expression by memory CD8 T cells post vaccination significantly correlated with improved PFS.

**Conclusions:** VRP-HER2 led to an increase in perforin expressing HER2-specific memory CD8 T cells in preclinical and clinical studies, and had profound antitumor effects in murine models. The generation of HER2-specific memory CD8 T cells was significantly correlated with increased PFS in patients. Subsequent studies will seek to enhance T cell activity by combination with anti-PD-1/PD-L1 antibodies.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-10-01

Phase II study of antiangiogenic tyrosine kinase inhibitor apatinib in combination with oral vinorelbine in heavily pretreated HER2-negative advanced breast cancer

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**Background:** Metastatic breast cancer remains an incurable disease, and clinical benefit and progression-free survival are the main end points in advanced setting. Targeted therapies have shown promising potentials in HER2-positive breast cancer, but with uncertain effects in HER2-negative breast cancer, especially when the disease is progressing rapidly. The regimen of antiangiogenic therapy in combination with chemotherapy had been studied for years and gained improved efficacy. Apatinib is an oral, highly potent tyrosine kinase inhibitor targeting vascular endothelial growth factor receptor 2. Phase II clinical trials of Apatinib single agent had presented objective response and manageable toxicity in heavily pretreated, metastatic breast cancer. Oral vinorelbine represents a good choice for its toxicity and activity in anthracycline and taxane-pretreated breast cancer patients. This all-oral study aims to investigate the efficacy and safety of the oral vinorelbine-apatinib combination in pre-treated metastatic breast cancer. **Methods:** This study enrolled patients with HER2-negative advanced breast cancer, pretreated with anthracycline/taxanes, and who failed in the metastatic setting at least one prior chemotherapy or endocrine therapy when hormone receptor is positive. Patients were treated with apatinib 500mg/425mg daily plus oral vinorelbine 60mg/m<sup>2</sup> day 1, 8, 15 every 3 weeks/cycle. Patients eligible were evaluated by CT or MRI scan at baseline and every 2 cycles (6 weeks) thereafter until disease progressed. The primary endpoint was PFS. The secondary endpoints were objective response rate, clinical benefit rate, OS, and safety. **Results:** 40 patients were enrolled with a median age of 55 (30-70) years. First 17 patients started apatinib at the dose of 500mg/day. Considering safety issues, a lower dose of apatinib 425mg/day was subsequently started as the initial dose after these 17 patients recruited. 26(65.0%) patients experienced treatment delay and 20(50.0%) patients experienced dose modification during treatment. Median follow-up time was 10.3 months. Of all 40 patients, median PFS was 5.4 months (95% CI, 3.4m–7.3m). Median OS was not reached. 32 patients were eligible for efficacy analysis. ORR was 15.6% (5/32). CBR was 46.9% (15/32). Patients with triple-negative breast cancer or who received combined therapy as second line treatment gained better ORR and longer median PFS. The most common adverse events of all grades included gastrointestinal reaction (70.0%), myelosuppression (67.5%), hypertension (62.5%), pain (60.0%), malaise (52.5%), anorexia (50.0%), elevated transaminase (47.5%), hand-foot reaction (47.5%), proteinuria (37.5%), and elevated bilirubin (32.5%). Proteinuria, treatment delay, and ECOG performance status were independent predictive factors for PFS. **Conclusions:** The all-oral therapy of antiangiogenic tyrosine kinase inhibitor apatinib plus vinorelbine presented objective efficacy in advanced HER2-negative breast cancer who failed from first-line therapy, with acceptable and manageable toxicity.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-10-02

**AVASTEM – Stem cells inhibition by bevacizumab in combination with neoadjuvant chemotherapy for locally advanced breast cancers: A prospective proof of concept randomized phase II trial**

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**Background.** Preclinical works have suggested that conventional cytotoxic chemotherapies may increase the number of cancer stem cells. Angiogenesis inhibition has been described *in vitro* to have an impact on stem cells proliferation. We developed a proof of concept clinical trial to explore Bevacizumab-chemotherapy activity on breast cancer stem cells for patients treated in the neoadjuvant setting.

**Patients and Methods.** Breast cancer patients requiring preoperative chemotherapy were included in this open-label, randomized, prospective, multicentre phase II trial. All received FEC-docetaxel combination for a maximum of 8 cycles, and patients randomized in the experimental arm received concomitant Bevacizumab (15 mg/kg Q3W). The primary endpoint was to describe aldehyde dehydrogenase (ALDH1, identified by immunohistochemistry) positive tumour cells rate before treatment and after the 4<sup>th</sup> cycle. Secondary objectives included safety, pathological complete response (pCR) rate, disease-free survival (DFS), relapse-free survival (RFS), and overall survival (OS).

**Results.** Seventy-five patients were included from March 2010 to July 2012, including 50 in the experimental arm. More than 80% of patients received all planned chemotherapy cycles. ALDH1 expression could be assessed both before treatment and after the fourth cycle of chemotherapy for 32 patients. The absence of a significant increase (> 5%) in ALDH1+ cells rate after chemotherapy was demonstrated in the Bevacizumab arm (n=19, Median=-0.125, one-sided 95%CI=[-∞-0], p=0.001). Yet, the same was observed in the control arm (n=13, Median=-0.25, one-sided 95%CI=[-∞-0], p=0.006). Grade 3 or 4 adverse events, including haematological, digestive, and cutaneous disorders, were observed for 94% of the patients in the experimental arm and 88% in the control arm. A non-significant increase in pCR was observed in the Bevacizumab arm (OR=2.24, 95CI [0.77-6.54], p=0.14), but survival was not improved (OS: p=0.89 for the whole cohort; DFS: p=0.45; and RFS: p=0.68 for non-metastatic cases). ALDH1 status at inclusion was not correlated to efficacy.

**Conclusions.** We observed that the rate of ALDH1+ tumour cells did not increase after Bevacizumab-based chemotherapy. However, as similar results were observed with chemotherapy only, Bevacizumab impact on breast cancer stem cells cannot be confirmed.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-11-01

Safety and efficacy of sacituzumab govitecan (anti-Trop-2-SN-38 antibody-drug conjugate) as  $\geq 3^{\text{rd}}$ -line therapeutic option for treatment-refractory HER2-negative metastatic breast cancer (HER2Neg mBC)

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**Background:** Sacituzumab govitecan is an antibody-drug conjugate consisting of SN-38, the active metabolite of irinotecan, conjugated to a humanized mAb targeting Trop-2 (trophoblastic antigen-2), which is highly expressed in many epithelial cancers. A phase I/II basket trial (NCT01631552) investigated its activity in patients (pts) with advanced epithelial cancers. Herein, we summarize pooled safety and efficacy findings in 162 pts with HER2-negative metastatic breast cancer (mBC) accrued between 7/2013 and 6/2017 who received at least 2 prior therapies for metastatic disease and were treated with sacituzumab govitecan at the 10 mg/kg dose level.

**Methods:** Patients with triple-negative (N=108) and patients with hormone-receptor positive (N=54) mBC received 10 mg/kg sacituzumab govitecan on days 1 & 8 of a 21-day cycle continued until progression or unacceptable toxicity. All pts had measurable disease by CT or MRI. Efficacy was assessed locally by RECIST 1.1 including overall response rate (ORR) and Kaplan-Meier estimates of duration of response (DOR), progression-free survival (PFS) and overall survival (OS). Adverse events (AE) were evaluated according to CTCAE v4.0

**Results:** The patient cohort (161 female /1 male; median age 55 yrs, range 31-80) received a median of 4 prior therapies for metastatic disease (range 2-17), with prior chemotherapy agents in the metastatic setting including taxane (68%), capecitabine (60%), platinum (59%), gemcitabine (44%), eribulin (41%), and anthracycline (38%). 77 pts have died, with 57 in long-term follow-up and 28 still on treatment at data cutoff. The median number of administered sacituzumab govitecan doses was 14 (range 1-88). Treatment was generally well tolerated. 29% of pts had dose reductions, 3% discontinued treatment due to drug-related AEs, and there were no treatment-related deaths. Based on currently available AE data, grade  $\geq 3$  toxicity included neutropenia (43%), anemia (9.5%), diarrhea (7.0%) and febrile neutropenia (6.3%). For the TNBC subgroup, with a median follow-up of 9.3 months, the ORR was 33% (3 CRs + 33 PRs /108) with a median DOR of 8.3 months (95% CI: 4.8 – 11.6). For the ER+ subgroup, with a median follow-up of 10.0 months, the ORR was 31% (17 PRs/54) with a median DOR of 7.4 months (95% CI: 4.4 – 18.3). The combined HER2Neg ORR was 33% (3 CRs+50 PRs/162), with a median DOR of 8.3 months (95% CI: 4.9 - 10.8), PFS of 5.6 months (95% CI: 5.1 – 6.9) and OS of 13.0 months (95% CI: 11.5 - 15.0). The ORR was comparable for pts  $\leq 50$  yrs. old [32.2% (19/59)] vs.  $> 50$  yrs old [33.0% (34/103)] and little different for pts with 2 prior therapies [35.4% (17/48)] vs.  $>2$  prior therapies [31.6% (36/114)].

**Conclusions:** Monotherapy with sacituzumab govitecan was well tolerated with a manageable safety profile, and achieved a 30+% objective response rate among heavily pre-treated patients with HER2-negative metastatic breast cancer regardless of ER status.



# 2018 San Antonio Breast Cancer Symposium ®

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Dose- and exposure-response relationship and biomarker correlation analysis in breast tumors from patients treated with capivasertib, an AKT inhibitor, in the STAKT randomized, placebo controlled pre-surgical study

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**Background:** Capivasertib (AZD5363), an AKT1,2,3 inhibitor, significantly improved progression-free and overall survival when added to paclitaxel in triple negative breast cancer (BC) patients (Schmid et al. ASCO 2018). We have previously reported in STAKT, robust target inhibition at 480mg BD versus placebo, including significant decreases in the primary biomarkers (PBs) - Ki67, pPRAS40 & pGSK3 $\beta$  - in primary BCs (Robertson et al. SABCS 2017). We now report the dose- and exposure-response relationship of capivasertib and the correlation between primary and secondary (pAKT, pS6, nuclear FOXO3a) tumor biomarkers.

**Design:** STAKT was a two-stage, double blind, randomized, placebo controlled 'window-of-opportunity' trial in newly diagnosed ER+ BC patients. Stage 1 assessed capivasertib at a dose of 480mg BD p.o. versus placebo. Stage 2 assessed capivasertib at two lower doses 360mg and 240mg BD. Tumor biopsies were taken prior to 1<sup>st</sup> dose and after 4.5 days of dosing. Evaluable patients (who required pre-defined minimum baseline PD values for PBs) included placebo (n=11), capivasertib at 480mg (n=17), 360mg (n=5) and 240mg (n=6). Blood samples for pharmacokinetic (PK) studies were scheduled at pre-dose; 2, 4, optional 6 & 8 hrs post first dose on Day 1; ~2-4 h post last dose on Day 5 (before biopsy). The % change from baseline for PBs were evaluated against the following exposure variables (placebo=0): i) Dose, ii) Observed C<sub>max</sub> Day 1 (~2h post-dose), iii) Observed plasma concentration on Day 5, iv) Model-predicted plasma concentration Day 5 at time of biopsy, and v) Model-predicted AUC on Day 5. Spearman correlation coefficient measured the strength and direction of association between biomarkers.

## Results:

- Significant mean reductions in % change from baseline were observed for the PBs pGSK3 $\beta$  (-39%; p<0.006), pPRAS40 (-50%; p<0.0001) and Ki67 (-23%; p=0.052) at 480mg versus placebo. At 360mg and 240mg, mean % changes from baseline in pGSK3 $\beta$  were -27% and -9%, respectively; in pPRAS40 -45% and -28%, respectively; and in Ki67 0% and +22%, respectively.
- Dose-response relationships for individual % change from baseline could be described by an E<sub>max</sub> model for all PBs. Overall, the correlation to PK exposure (observed or predicted) was similar to the correlation to dose.
- Correlation coefficient analyses between biomarkers at capivasertib 480mg BD identified- i) Positive correlations for pGSK3 $\beta$  with Ki67 ( $\rho$  = 0.52, p-value < 0.05) & with pS6 ( $\rho$  = 0.54, p-value<0.05); ii) Negative correlations between FOXO3a and Ki67 ( $\rho$  = -0.75, p-value<0.001) pGSK3 $\beta$  ( $\rho$  = -0.71, p-value<0.001) & also pS6 ( $\rho$  = -0.61, p-value<0.001). Correlation coefficients for lower doses are not robust due to small sample size in these groups.

## Conclusions

- Capivasertib caused dose- and concentration- dependent effects on biomarkers after only 4.5 days.
- Significant changes in the PBs were demonstrated at 480 mg BD. Biomarker changes was observed at 360mg and 240mg BD, but statistical analysis was limited by the small sample size at lower doses.
- Correlation between a number of tumor biomarkers (relative changes) were identified for capivasertib 480mg BD.

## 2018 San Antonio Breast Cancer Symposium ®

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discovery of the  $\beta$ -catenin/Tcf inhibitors for treatment of triple negative breast cancer

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In triple negative breast cancers (TNBCs), the autocrine activation of Wnt ligands, the epigenetic silencing of Wnt suppressor genes, and the cross talks between signaling pathways stabilize  $\beta$ -catenin in the dephosphorylated state, increase the level of nuclear  $\beta$ -catenin, and aberrantly activate the Wnt/ $\beta$ -catenin signaling pathway. The design of inhibitors for the upstream effectors of the Wnt/ $\beta$ -catenin signaling pathway cannot confer the inhibitory activities to cancer cells that harbor downstream APC, Axin, or  $\beta$ -catenin activation mutations and can perturb the function of  $\beta$ -catenin in cell–cell adhesion. The formation of the  $\beta$ -catenin/T-cell factor (Tcf) complex in the cell nucleus is the penultimate step of the Wnt/ $\beta$ -catenin signaling pathway. The aberrant formation of this protein–protein interaction (PPI) complex has been recognized as a key driving force for many cancers including TNBCs. Since  $\beta$ -catenin was reported in 1992, significant interest has been paid to screen compound libraries to discover small-molecule inhibitors that can bind  $\beta$ -catenin and disrupt the  $\beta$ -catenin/Tcf interaction. Many high-throughput screening campaigns were conducted, but little to no success was obtained. None of the reported compounds were able to deliver any drug candidates to preclinical and clinical trials. We decided to take a rational drug design approach to design new inhibitors based on the electronic properties of the key structural features for  $\beta$ -catenin/Tcf recognition. Our crystallographic and biochemical analyses revealed that the Tcf4 G<sup>13</sup>ANDE<sup>17</sup> binding site of  $\beta$ -catenin could be targeted to design potent small-molecule inhibitors selective for the  $\beta$ -catenin/Tcf interaction. In combination of peptidomimetic strategy, structure-based drug design, and chemical synthesis, and biochemical and cell-based characterizations, we successfully designed and synthesized potent and selective small-molecule inhibitors for the  $\beta$ -catenin/Tcf interaction. The most potent inhibitor exhibited submicromolar inhibitory potency for disruption of the  $\beta$ -catenin/Tcf interaction. This potent inhibitor also exhibited dozens to hundreds folds of selectivities for the  $\beta$ -catenin/Tcf over the  $\beta$ -catenin/E-cadherin and  $\beta$ -catenin/APC interactions. The binding mode of new inhibitors was characterized by the site-directed mutagenesis and structure-activity relationship studies. The cell-based studies demonstrated that new inhibitors passed the cell membrane, significantly attenuated Wnt/ $\beta$ -catenin signaling in TNBC cells, and suppressed growth of Wnt/ $\beta$ -catenin-dependent TNBC cells. These inhibitors also exhibited cell-based selectivities for the  $\beta$ -catenin/Tcf over  $\beta$ -catenin/cadherin and  $\beta$ -catenin/adenomatous polyposis coli (APC) interactions.

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Efficacy of neratinib in hormone receptor-positive patients who initiated treatment within 1 year of completing trastuzumab-based adjuvant therapy in HER2+ early-stage breast cancer: Subgroup analyses from the phase III ExteNET trial

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**Background:** The international, randomized, placebo-controlled phase III ExteNET trial showed that 1 year (yr) of neratinib 240 mg/day after trastuzumab-based adjuvant therapy significantly improved invasive disease-free survival (iDFS) in 2840 patients with early-stage HER2+ breast cancer at 2 yr (hazard ratio 0.67; 95% CI 0.50–0.91; p=0.009) [Chan 2016] and 5 yr (hazard ratio 0.73; 95% CI 0.57–0.92; p=0.008) [Martin 2017]. A prespecified subgroup analysis by hormone receptor (HR) status suggested enhanced efficacy with neratinib in patients with HR+ (2-yr hazard ratio 0.51; 95% CI 0.33–0.77) vs. HR– tumors (2-yr hazard ratio 0.93; 95% CI 0.60–1.43). The efficacy of neratinib was also greater in patients who initiated treatment within 1 yr of prior trastuzumab compared with those who started neratinib later. The European Medicines Agency's Committee for Medicinal Products for Human Use recently recommended neratinib for use in patients with HR+ tumors who initiate treatment within 1 yr of completing trastuzumab-based adjuvant therapy. Subgroup analyses from ExteNET examining iDFS benefits in this patient population are presented here.

**Methods:** Patients with early-stage HER2+ breast cancer who completed trastuzumab-based (neo)adjuvant therapy were assigned to oral neratinib 240 mg/day or placebo for 1 yr. Randomization was stratified by HR status (determined locally before trial entry), nodal status, and trastuzumab regimen. Endocrine therapy was allowed in patients with HR+ disease. The primary endpoint, iDFS, was tested by 2-sided log-rank test and hazard ratios (95% CI) were estimated using Cox proportional hazards models. Kaplan-Meier methods were used to estimate iDFS rates. Secondary endpoints were DFS-DCIS, time to distant recurrence, distant DFS, and CNS recurrences. The primary analysis was conducted at 2 yr, and a sensitivity analysis conducted at 5 yr. Clinicaltrials.gov:NCT00878709.

**Results:** Of the 2840 patients (neratinib, n=1420; placebo, n=1420), 1631 (57%) had HR+ disease (neratinib, n=816; placebo, n=815). Most (93%) HR+ patients were receiving endocrine therapy at baseline. 1334 of 1631 (82%) patients with HR+ tumors were randomized to start neratinib within 1 yr of last trastuzumab dose (neratinib, n=670; placebo, n=664). iDFS benefits from neratinib in this population are shown in the table. Secondary endpoints were also improved with neratinib vs. placebo in this population. Safety data in this subset will be presented at the meeting.

**Table.** iDFS in patients with an interval between last trastuzumab dose and randomization of ≤1 yr

	HR+ population (N=1334)			ITT population (N=2297)		
		Hazard ratio <sup>b</sup>			Hazard ratio <sup>b</sup>	
	$\Delta$ , % <sup>a</sup>	(95% CI)	P-value	$\Delta$ , % <sup>a</sup>	(95% CI)	P-value
2-yr analysis <sup>c</sup>	+4.5	0.49	0.002	+2.9	0.63	0.006
		(0.30–0.78)			(0.45–0.88)	
5-yr analysis <sup>d</sup>	+5.1	0.58	0.002	+3.2	0.70	0.006
		(0.41–0.82)			(0.54–0.90)	

<sup>a</sup>Difference in iDFS rates between neratinib vs. placebo; <sup>b</sup>Neratinib vs. placebo; <sup>c</sup>Data cut-off: July 2014; <sup>d</sup>Data cut-off: March 2017

**Conclusions:** Neratinib may have enhanced and sustained efficacy in patients with HR+ disease who initiate treatment within 1 yr of trastuzumab-based adjuvant therapy.

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Patient-reported outcomes with trastuzumab monotherapy versus trastuzumab plus standard chemotherapy as a postoperative adjuvant therapy in HER2-positive elderly breast cancer patients (RESPECT): A randomized, open-label, phase 3 clinical trial

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**OBJECTIVE:** The RESPECT trial compared 1-year trastuzumab monotherapy with trastuzumab plus standard chemotherapy as a postoperative adjuvant therapy in HER2-positive elderly breast cancer patients. Primary objective of this study was to verify the noninferiority of 1-year trastuzumab monotherapy to trastuzumab plus chemotherapy in terms of disease free survival, and the planned analysis showed that the difference of restricted mean survival time between two groups at 3 years was 0.45 months (reported by Sawaki at ASCO2018). This report assesses the patients-reported outcomes and health-related quality of life (HRQoL).

**PATIENTS AND METHODS:** The study was done at 99 hospitals in Japan. Elderly women (70 to 80 years old) with HER2-positive, stageI-IIIa invasive breast cancer treated by surgery with clear resection margins were randomly assigned to receive either 1-year trastuzumab or 1-year trastuzumab plus standard chemotherapy, stratified by age, hormone-receptor status, pathological lymph node metastasis and institution. Patients completed questionnaires at baseline, 2 months, 1year, and 3 years after protocol treatment started. The primary outcome was global HRQoL assessed using Functional Assessment of Cancer Therapy-General (FACT-G) total score, and secondary outcomes were chemotherapy-induced peripheral neuropathy (CIPN), instrumental activities of daily living (IADL), anxiety, depression, and subjective happiness. We did the analyses by intention to treat, including patients who completed questionnaires at baseline before start of protocol treatment, and 5point or more change is meaningful in FACT-G total score. This study is registered with ClinicalTrials.gov, NCT01104935.

**RESULTS:** Between Oct 2009 and Oct 2014, 275 patients were enrolled in the study, of whom 9 patients were excluded: 135 assigned to trastuzumab monotherapy and 131 assigned to trastuzumab plus chemotherapy. We detected significant difference between treatment groups for: clinically meaningful HRQoL deterioration rate at 2 months (31% for trastuzumab monotherapy vs 48% for trastuzumab plus chemotherapy; p=0.016) and at 1year (19% vs 38%; p=0.009), clinically meaningful HRQoL improvement rate at 2 months (38% for trastuzumab monotherapy vs 15% for trastuzumab plus chemotherapy; p<0.01) and at 1year (43% vs 25%; p=0.021), severe sensory CIPN rate at 2months (1.9% for trastuzumab monotherapy vs 14.4% for trastuzumab plus chemotherapy; p=0.001), IADL score at 1year (11.97 for trastuzumab monotherapy vs 11.54 for trastuzumab plus chemotherapy; p<0.042), Hospital Anxiety and Depression Scale score at 2months (8.92 for trastuzumab monotherapy vs 10.79 for trastuzumab plus chemotherapy; p<0.003), and subjective happiness score at 1year (12.8 for trastuzumab monotherapy vs 11.8 for trastuzumab plus chemotherapy; p<0.024).

**CONCLUSION:** Given the small advantage of adjuvant trastuzumab plus chemotherapy compared to trastuzumab monotherapy for elderly HER-2 positive breast cancer women, decisions about treatment should be informed by the risk for adverse health effects associated with chemotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

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The impact of neratinib with or without anti-diarrheal prophylaxis on health-related quality of life in HER2+ early-stage breast cancer: Analyses from the ExteNET and CONTROL trials

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**Background:** Neratinib is an irreversible pan-HER tyrosine kinase inhibitor. ExteNET, a randomized placebo-controlled phase III study, showed that neratinib given for 12 months after trastuzumab-based adjuvant therapy significantly improved 2-year (HR 0.67; 95% CI 0.50–0.91; p=0.0091) and 5-year (HR 0.73; 95% CI 0.57-0.92; p=0.008) iDFS in pts with early-stage HER2+ breast cancer. Anti-diarrheal prophylaxis was not mandated by protocol; grade 3/4 diarrhea occurred in 40% of pts with a median cumulative duration of 5 days. The phase II CONTROL study was initiated to investigate the effectiveness of various prophylactic regimens in the prevention of neratinib-associated diarrhea. Loperamide (L) alone or in combination with add-on agents targeting underlying inflammation [i.e. budesonide (BUD)] or bile acid malabsorption [i.e. colestipol (COL)] were tested. We report longitudinal HRQoL findings from both ExteNET and CONTROL.

**Methods:** Pts with early-stage HER2+ breast cancer who had received trastuzumab-based adjuvant therapy were eligible for both studies. In ExteNET, pts received neratinib or placebo for 12 months. In CONTROL, pts received neratinib for 13 x 28-day cycles combined with L, L + BUD or L + COL for 1 or 2 cycles (see table for schedules). HRQoL was assessed using Functional Assessment of Cancer Therapy–Breast (FACT-B), v4.0, at baseline, months 1, 3, 6, 9, 12 (ExteNET) or baseline, cycles 2, 4, 7, 10, 13 (CONTROL). Changes in scores from baseline were considered to be clinically meaningful if greater than the lowest estimate for an 'important difference' (ID) reported in the literature. Evaluable pts were required to have HRQoL assessments at baseline and at least 1 post-baseline. ClinicalTrials.gov: NCT00878709 (ExteNET); NCT02400476 (CONTROL).

**Results:** HRQoL findings are summarized in the table. Hospitalization rates due to diarrhea: 1.5% (neratinib + L), 0% (other cohorts) in CONTROL; and 1.4% (neratinib), 0.1% (placebo) in ExteNET.

Study	Cohort/Group	Mean change from baseline					
		M1	M3	M6	M9	M12	
		FACT-B TOTAL					
		(ID range: 7–8 points)					
CONTROL	N + L <sup>a,b</sup> (N=40)	–3.8	–4.5	–1.5	–2.5	–3.3	
	N + L + BUD <sup>a,b,c</sup> (N=62)	–6.0	–4.9	–1.6	–3.6	–4.5	
	N + L + COL <sup>a,b,d</sup> (N=125)	–3.8	–2.0	–4.0	–4.6	–3.6	
	N + L prn + COL <sup>a,d</sup> (N=85)	–1.8	–1.5	4.0 <sup>e</sup>	–	–	
ExteNET	N + L prn <sup>a</sup> (N=1124)	–4.6	–3.4	–3.5	–3.3	–3.7	
	P (N=1188)	–1.7	–3.5	–2.9	–2.9	–2.8	
		FACT-B PWB					

		(ID range: 2–3 points)					
CONTROL	N + L <sup>a,b</sup> (N=40)	–4.0	–2.3	–1.9	–2.4	–2.3	
	N + L + BUD <sup>a,b,c</sup> (N=62)	–3.2	–2.1	–1.4	–1.7	–1.7	
	N + L + COL <sup>a,b,d</sup> (N=125)	–2.8	–2.0	–2.4	–2.5	–2.4	
	N + L prn + COL <sup>a,d</sup> (N=85)	–2.8	–1.8	0.0 <sup>e</sup>	–	–	
ExteNET	N + L prn <sup>a</sup> (N=1124)	–2.9	–1.9	–1.7	–1.6	–1.5	
	P (N=1188)	–0.6	–0.8	–0.7	–0.6	–0.4	

C, cycle; L, loperamide; M, month; N, neratinib; prn, as needed; PWB, physical well-being. CONTROL cut-off: 1 May 2018. <sup>a</sup>N 240 mg qd for 13 x 28d cycles or 12 months; <sup>b</sup>L 4 mg, then 4 mg tid d1-14, then 4 mg bid d15-28 or d15-56, then prn; <sup>c</sup>BUD 9 mg qd d1-28; <sup>d</sup>COL 2 g qd d1-28; <sup>e</sup>n=1.

**Conclusions:** Adjuvant neratinib with or without anti-diarrheal prophylaxis was associated with small decreases in HRQoL. With the exception of the FACT-B PWB subscale, HRQoL changes did not reach clinically meaningful thresholds. Follow-up in CONTROL is ongoing.

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Impact of the adjuvant treatment with trastuzumab in HER2 positive breast cancer in the real-world setting. Analysis of two cohorts (1997-2005/2006-2015) in 1970 patients

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**INTRODUCTION:** Although large randomized trials assessing the benefit of adjuvant trastuzumab in HER2-positive (HER2+) breast cancer have clearly demonstrated a significant improvement in long-term survival, it is necessary to know the impact of the use of trastuzumab adjuvant in the real life population, which includes patients frequently excluded from clinical trials, such as those with very small tumors without node involvement, or advanced age. The objective of this study is to describe the outcomes of women who received adjuvant trastuzumab for HER2+ cáncer since it was approved in 2006, compared with a previous cohort of HER2+ patients not treated with trastuzumab in 7 Spanish centers.

**METHODS:** Women with newly diagnosed stage I-III, HER2+ breast cancer, between 1997 and 2015 were included in the study. Two cohorts were considered: The No-Trastuzumab cohort (No-T), between 1997 and 2005, and the Trastuzumab cohort (T) with trastuzumab-treated women between 2006 and 2015. Kaplan-Meier estimates were used to evaluate DFS and OS. Additionally, cohorts were analyzed by pathologic tumour size, lymph node involvement and hormonal receptor status to stratify outcome measures.

**RESULTS:** A total of 2134 patients were identified. In 164 cases, data were insufficient or the follow-up incomplete. Therefore, the final analysis included 1970 patients, of whom 539 belong to the "No-T" cohort and 1431 to the "T" cohort. The median follow-up was 81 months. Median age: 53 years [22-98]. A total of 699 patients had T1 tumors [43% in the "No-T" cohort vs 33% in the "T" cohort]. 55% of the cases were N0 [58% and 54% in the "No-T" and "T" cohorts respectively]. The status of the hormonal receptors was well balanced between groups [36% ER negative in both]. Regarding the type of adjuvant treatment administered, in the "T" cohort more patients received adjuvant chemotherapy [65% vs 97%] and also in the "T" group combinations of taxanes and anthracyclines were more frequent [14% vs 72%]. The proportion of adjuvant endocrine therapy was similar in both groups [37% vs 34%].

In the "T" cohort, median Disease Free Survival (DFS) was not-reached, compared with 149 months in the "No-T" group. 5-year DFS was 83% vs 65% respectively [ $p < 0.001$ ]. 5-year DFS was also superior and statistically significant in all the subgroups analyzed, including patients with T1 tumors (87% vs 57%), N0 (87% vs 78%), patients T1N0 (88% vs 74%) and HR positive (86% vs 71%) or negative (78% vs 50%). Similarly, Overall Survival (OS) was increased in patients treated with Trastuzumab (median: 224 months vs not-reached, 5-year OS: 92% vs 75% [ $p < 0.001$ ].) 5-year OS was also statistically superior in the T1 subgroup (92% vs 72%), and N0 (95% vs 88%). [ $p < 0.001$  in all subanalysis].

**CONCLUSIONS:** Adjuvant treatment with Trastuzumab under conditions of real clinical practice in HER2+ early breast cancer, shows a highly significant benefit in terms of DFS and OS, regardless of the stage of the disease or other clinical variables. A very important benefit was reached in patients with small tumors, node-negative disease, or both conditions (T1N0). The benefit was also obtained regardless of the expression of hormonal receptors.



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Prevalence of trastuzumab-induced cardiotoxicity in a real-world setting

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**Background:** Trastuzumab treatment plus adjuvant or neoadjuvant chemotherapy is the standard of care for women with HER2 positive breast cancer. Despite relative low rates of cardiotoxicity observed in randomized clinical trials, trastuzumab interruption driven by LVEF reduction is a major concern in current clinical practice.

**Patients and methods:** We retrospectively identified women with stage I-III HER2 positive breast cancer who received 12 months of trastuzumab treatment after adjuvant or neoadjuvant chemotherapy at Instituto Nacional de Cancerología (INCan, Mexico City), between 2006 and 2018. Patients were eligible if a pre-therapy MUGA scan and  $\geq 2$  subsequent follow-up scans were available. Cardiotoxicity was defined as a  $\geq 10\%$  LVEF reduction to a value  $< 50\%$ , associated with trastuzumab interruption.

**Results:** 910 patients were included, with a median age at diagnosis of 50 (24-85) years and a median follow up of 7 (2-11) years. Among the whole cohort, 10.3% of patients had diabetes, 15.4% had hypertension, 78% were obese/overweight, and 40% had positive estrogen and/or progesterone receptor status. Anthracycline-based therapy was used in 819 (90%) patients, with a median (doxorubicin equivalent) cumulative dose of 200 mg/m<sup>2</sup> (IQR 180-240). The median baseline LVEF was 61.8% (50-88.9). In total, 94 (10.3%) patients developed cardiotoxicity, but symptomatic heart failure was observed in only 31 (3.4%) individuals. In univariable analyses, the development of cardiotoxicity was not associated significantly with cardiovascular risk factors.

**Conclusions:** In this large single-center cohort, cardiotoxicity rates remain high, thus, interventions to minimize the risk of cardiotoxicity and trastuzumab treatment interruption should be considered.

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Long term clinical follow up of real world HER2-positive patients since the introduction of trastuzumab

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**Background:** The prognosis for patients with HER2-positive early breast cancer (EBC) has improved dramatically since the introduction of adjuvant trastuzumab therapy. With the addition of pertuzumab the prognosis has improved further. However, there is a need to study how these results from clinical controlled trials are transferred to the real-world clinical setting. In this study we aim to investigate all patients with early HER2-positive breast cancer in our region since the introduction of adjuvant trastuzumab to evaluate the implementation of trastuzumab treatment regarding treatment coverage, prognosis and survival.

**Method:** All patients with HER2-positive EBC, diagnosed between 2006 and 2014 in South-east Sweden were included in the study. The patients were identified using the Swedish national breast cancer register and then cross-referenced with data from the pathology department at each hospital in order to obtain complete coverage in a retrospective clinical follow up. In addition, data were collected from medical records for each patient to verify the actual given treatments and survival data.

**Results:** Preliminary data is available. 611 patients were included with a median follow-up time of 5 years. During the follow-up period the number of patients diagnosed with HER2-positive EBC cancer doubled. 73% of all patients received trastuzumab treatment; however the coverage increased successively from 56% in 2006 to 83% in 2013. ER-positive patients did receive trastuzumab therapy to the same extent as ER-negative patients. Local recurrence-free survival (LRFS), distant recurrence-free survival (DRFS) and breast cancer specific survival (BCSS) at 5 years were 85%, 76%, and 75% for patients not receiving trastuzumab. In the trastuzumab treated group LRFS, DRFS and BCSS was 95%, 85% and 83% respectively. The group not receiving trastuzumab was significantly older, had more frequently node negative disease and was not treated with chemotherapy to the same extent.

**Conclusion:** A significant amount of early HER2-positive breast cancer patients did not receive adjuvant trastuzumab therapy between 2006 and 2014. In this group fewer patients received chemotherapy and despite less nodal involvement LRFS, DRFS and BCSS were poor for these patients.

# 2018 San Antonio Breast Cancer Symposium ®

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Efficacy of short-course adjuvant trastuzumab in early stage breast cancer

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**Background:** A total of 12 months adjuvant Trastuzumab is still the optimal choice for patients with human epidermal growth factor receptor 2 (HER2)-positive early breast cancer. Still, the adjuvant short course 9-week trastuzumab may be considered as a cost-effective and safe option in developing countries such as Syria.

**Purpose:** The primary objective was disease-free survival after short course adjuvant Trastuzumab therapy in early breast cancer at Al-Bairouni university hospital compared with 6 month of adjuvant trastuzumab , the secondary objective was assessing toxicities especially cardiac toxicity.

**Patients and methods:** Women with histologically confirmed HER2 positive BC with either positive or negative hormone receptor of stages IA,IIA,IIB,IIC,IIIA,IIIB and IIIC were eligible for the study . Patients were assigned to 2 groups . The initial systemic treatment was similar in the 2 groups . Thereafter, no further Trastuzumab or chemotherapy was administered in Arm A; 90 patients (loading dose 4 mg/kg; then 2mg/kg weekly to complete 9 weeks of T), whereas in Arm B that included 157 patients single-agent T was administered to complete 6 months (loading dose 8 mg/kg then 6 mg/kg every 3 weeks) . The LVEF was measured pretreatment, and every 3 months afterword .

**Results:** After a median follow up of 24 months DFS was 89.06% and 89.81% in groups A and B respectively (P=0.11).No obvious side effects have been noticed during the follow up period in group A. otherwise congestive heart failure was reported in 2.55% of group B patients .

**Conclusion:** Short course **9 weeks** adjuvant Trastuzumab achieved a good 2 years disease-free survival rate (DFS) compared to 6 month of treatment, with acceptable toxicity and good tolerability for treated patients with early-stage invasive breast cancer. Patients are being monitored for a longer follow up period.

# 2018 San Antonio Breast Cancer Symposium ®

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The impact of local therapy on locoregional recurrence in women with high risk breast cancer in the neoadjuvant I-SPY2 TRIAL

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**Background:** In women with breast cancer receiving neoadjuvant chemotherapy, residual cancer burden (RCB) predicts distant recurrence and survival. In those with high risk tumors, locoregional recurrence (LRR) remains a concern, and has been associated with type of local therapy received. We evaluated the impact of local therapy on LRR in the I-SPY-2 TRIAL.

**Methods:** Data were analyzed in Stata 14.2, using Chi2 test, log rank test, and a Cox proportional hazards model. RCB was considered a categorical variable (0/1 versus 2/3), as described in prior publications. Breast surgery categories were lumpectomy +/- radiotherapy, or mastectomy +/- radiotherapy. Axillary surgery was defined as sentinel lymph node (SLN) surgery ( $\leq 6$  nodes removed) or axillary dissection ( $> 6$  nodes).

**Results:** Follow up data from the I-SPY2 TRIAL were available for 630 patients (median follow up 2.76 yrs, range 0.4-7.2). Type of local therapy was significantly associated with clinical stage at presentation, with stage III patients most frequently undergoing mastectomy + radiation ( $p < 0.001$ ). Women with higher RCB were more likely to undergo mastectomy than those with lower RCB (61.3% vs 48.8% mastectomy rate,  $p = 0.002$ ), and more likely to receive adjuvant radiotherapy (62.0% vs 53.9%,  $p = 0.048$ ). There was no association between clinical stage, type of surgery, or radiotherapy and LRR (Table). Higher RCB was significantly associated with LRR, with 3 year locoregional recurrence free rate of 95.1% in RCB 0/1 versus 89.9% in RCB 2/3 ( $p = 0.003$ ). In a Cox model adjusting for clinical stage, tumor subtype, surgical therapy, RCB status, nodal radiation, and age, significant predictors for LRR were tumor subtype and RCB status. Hazard ratio (HR) for LRR in those with RCB 0/1 was 0.39 compared to those with RCB 2/3 (95% CI 0.17-0.87,  $p = 0.021$ ). There was no difference in LRR between breast conservation and mastectomy; within the breast conservation group, those who had lumpectomy alone had higher hazard of LRR compared to those having lumpectomy + radiation (HR 3.1, 95% CI 1.1-9.2,  $p = 0.043$ ).

**Conclusions:** Extent of surgical therapy was not associated with local tumor control, regardless of advanced tumor stage at presentation. Rather, tumor biology and response to therapy were the best predictors of LRR. These data highlight the opportunity to minimize the morbidity of extensive surgical therapy for patients with excellent response to systemic therapy.

LRR rates by clinical features and treatment status

	Frequency	LRR Rate	P value
Clinical Stage			0.5

	I	240 (47.5%)	5.8%	
	II	185 (36.6%)	8.7%	
	III	80 (15.8%)	6.3%	
<b>Tumor Subtype</b>				0.014
ER+PR+Her2-		161 (26.4%)	3.1%	
ER+PR-Her2-		56 (9.2%)	3.6%	
Her2+		176 (28.9%)	6.3%	
Triple negative		216 (35.5%)	11.1%	
<b>Local therapy</b>				0.169
Lumpectomy		85 (13.5%)	11.8%	
Lumpectomy with radiation		198 (31.4%)	5.6%	
Mastectomy		173 (27.5%)	5.2%	
Mastectomy with radiation		174 (27.6%)	8.6%	
<b>Axillary surgery</b>				0.23
None		5 (0.8%)	20%	
SLN		329 (52.2%)	5.8%	
ALND		296 (47%)	8.5%	
<b>Axillary radiation</b>				0.535
Yes		42 (6.7%)	9.5%	
No		588 (93.3%)	7.0%	
<b>Axillary management</b>				0.2
No surgery or radiation		5 (0.8%)	20.0%	
SLN		312 (50%)	5.3%	
SLN+Axillary radiation		17 (2.7%)	8.3%	
ALND		271 (43%)	10.3%	
ALND+Axillary radiation		25 (4%)	5.4%	
<b>RCB</b>				0.002
0/1		293 (50.1%)	3.8%	
2/3		292 (49.9%)	10.3%	

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-02

Get skin sparing mastectomy right: Reduce local recurrence rate by meticulous removal all breast tissue

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## **Background:**

Skin sparing mastectomy (SSM) was introduced to allow aesthetically better results when combined with breast reconstruction. However, preservation of a larger skin envelope than that left after standard mastectomy could lead to retention of significant amounts of residual breast tissue in the subcutaneous fat, increasing the risk of local recurrence or new breast cancers developing.

## **Study method:**

Over a 15 year period, subcutaneous fat samples were routinely sampled during SSM and reconstruction performed by one surgeon. SSM was offered to all patients bar those with inflammatory cancers unresponsive to primary chemotherapy and those medically unfit for surgery. Hospital and personal databases of these cases were interrogated for:

presence of breast epithelial tissue in subcutaneous fat samples

local recurrence

systemic recurrence

aesthetic grade by independent surgeons

## **Results:**

402 SSM were reviewed. Results of subcutaneous samples were recorded for 357.

3 patients had residual breast tissue in subcutaneous fat samples (0.75%),

4 developed a local recurrence (1%), 42 systemic recurrence (11%).

238 patients had G1-3 invasive ductal carcinoma, size range 3-100mm, 25 invasive lobular carcinoma, 12.5-88mm and 87 DCIS, 20-116mm. These included patients who responded to preoperative chemotherapy and hormone therapy. 52 patients had prophylactic procedures.

The aesthetic outcome was judged as good for 76%, moderate for 20%.

The median follow up period was 9 years, giving annual local and systemic recurrence rates of 0.11% and 1.16% respectively.

## **Conclusions:**

There was a low incidence of residual breast tissue in random subcutaneous fat samples collected during SSM (0.75%)

The local recurrence rate was one tenth of the systemic recurrence rate, 1% vs 11%, and annual rates of 0.11% and 1.16% respectively.

## **Discussion:**

Surgery for breast cancer is a local treatment, and though removal of the main tumor will contribute to systemic control, that will be achieved by a combination of local and systemic therapies based on the biology of the individual's disease. Once surgery is performed, it can have no further influence on systemic control. However, the meticulousness of the surgical procedure can influence subsequent local control. Residual breast tissue left after mastectomy may harbor areas of disease that may not respond to other adjuvant therapies leading to local recurrence, or separate new cancers. Traditionally it has been considered that some breast tissue will be retained in skin flaps post mastectomy, up to 5% with standard mastectomies. This study suggests that this can be avoided, even when a large skin envelope is retained for an aesthetic outcome, and lead to a marked reduction in local recurrence.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-03

Longitudinal changes in psychosocial health in young women following breast cancer surgery: Results from a multi-center cohort study

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**Background:** Young women with breast cancer (BC) are increasingly choosing contralateral prophylactic mastectomy (CPM), yet little is known about the impact of surgical choices on quality of life (QOL) and psychological health. Using a large, prospective cohort of young women with BC, we sought to evaluate psychosocial outcomes following surgery.

**Methods:** Among participants of the Young Women's BC Study, a multi-center cohort of women dx'd with BC at age  $\leq 40$ , we identified women with Stage 0-3 unilateral BC who had surgery and completed surveys that included measures of QOL (CARES) and psychological health (HADS). Linear mixed-effects models were fit to assess changes from 1 to 3 years (yrs) post-dx in anxiety, depression, psychosocial, body image, and sexual scores. Adjusted (stage, hormone receptor status, chemotherapy, age) means were estimated and differences compared (Bonferroni adjusted p-values) between CPM vs breast conserving surgery (BCS) and unilateral mastectomy (UM) at 1, 2, and 3 yrs.

**Results:** Of 863 women, 30% had BCS, 24% UM, 46% CPM. Median age at dx was 37 (range: 22-40). Of women who had UM/CPM, 84% had reconstruction. Among women who had CPM, mean body image ( $p=.02$ ), psychosocial ( $p<.0001$ ), sexual ( $p<.0001$ ), and depression ( $p=.0007$ ) scores decreased, indicating improvement, from yr 1 to 2 but remained stable from yr 2 to 3 (Table). Anxiety decreased from yr 1 to 2 for women who had BCS ( $p=.0007$ ) and M ( $p=.03$ ), and from yr 2 to 3 for women who had CPM ( $p=.003$ ). Body image scores did not change significantly between any time points among women who had M or BCS. Overall change trajectories for sexual ( $p=.03$ ) and anxiety scores ( $p=.008$ ) differed by surgery. Compared to BCS and UM, psychosocial scores were higher in women who had CPM at 1 yr ( $p<.05$ ) and remained higher compared to BCS at 2 yrs ( $p=.04$ ). Anxiety was higher among women who had CPM vs UM at 1 and 2 yrs ( $p<.01$ ), vs BCS at 2 yrs ( $p=.004$ ). Depression was higher among women who had CPM vs UM in yr 1 ( $p=.05$ ). By yr 3, there were no significant differences in anxiety, depression, and overall psychosocial scores between groups. Compared to BCS, women who had CPM had higher sexual and body image scores ( $p<.01$ ), indicating worse QOL, at all timepoints. Compared to UM, women who had CPM had higher sexual scores at 1 and 3 yrs ( $p<.05$ ) and body image scores at 3 yrs ( $p=.02$ ).

**Conclusions:** While psychosocial health improves over time, differences by surgery persist, with women who have CPM experiencing more sexual and body image issues compared to women who undergo BCS or M in the years following surgery. Given that surgical choices may be affected by distress experienced before or at dx, ensuring young women receive adequate support when making surgical decisions as well as after surgery is warranted.

Mean CARES and HADS scores

		Year		
		1	2	3
Psychosocial*	CPM	.89	.78	.76
	UM	.75	.69	.66
	BCS	.72	.65	.66
Sexual*	CPM	1.64	1.37	1.40
	UM	1.41	1.29	1.08

	BCS	1.18	1.07	1.04
<b>Body image*</b>	CPM	1.33	1.22	1.30
	UM	1.16	1.13	1.04
	BCS	.64	.57	.56
<b>Anxiety**</b>	CPM	7.65	7.40	6.79
	UM	6.50	5.91	6.39
	BCS	7.03	6.22	6.50
<b>Depression**</b>	CPM	3.72	3.21	3.25
	UM	3.02	2.80	2.70
	BCS	3.33	2.82	3.13

\*\*CARES range: 0-4;higher scores=worse QOL \*\*HADS range 0-21;higher scores=more anxiety/depression



# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-14-04

Genetic testing and bilateral mastectomy for women with breast cancer: Does testing matter more than the test result?

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**Background:** Genetic testing for hereditary breast cancer may allow women with breast cancer to make informed and individualized decisions regarding breast cancer treatment and strategies to reduce the risk of contralateral breast cancer. While rates of bilateral mastectomy (therapeutic mastectomy + contralateral prophylactic mastectomy) among women with unilateral breast cancers are known to be increasing, the factors influencing this decision and the role of genetic testing are poorly understood. We examined bilateral mastectomy rates and factors associated with bilateral mastectomy among women with unilateral breast cancer.

**Methods:** We identified patients diagnosed with unilateral breast cancer from 2000-2015 from The Health of Women Study, a cohort study led by the Dr. Susan Love Research Foundation. We calculated rates of bilateral mastectomy among women who underwent surgical treatment for breast cancer. We used multivariable logistic regression models to evaluate factors associated with bilateral mastectomy.

**Results:** Among 2,028 patients who underwent surgery for unilateral breast cancer, 19% underwent bilateral mastectomy. Forty-one percent (n= 84 of 205) of patients who underwent testing and tested positive for a deleterious mutation underwent bilateral mastectomy, compared to 26% (n= 439 of 1,689) of women who tested negative and 12.5% (n= 274 of 2,197) of those not tested. Among those with a negative genetic test, pre-test risk of harboring a genetic mutation (based on family history and age at diagnosis) was not associated with bilateral mastectomy (p=0.250). After adjusting for race, age, level of education, and pre-test mutation risk, the odds of bilateral mastectomy were 69% higher among patients who underwent genetic testing (OR 1.69, 95% CI 1.29–2.22, p<0.001) versus those who did not undergo genetic testing. This finding was consistent regardless of genetic test result

Multivariable analysis of factors associated with bilateral mastectomy

	Odds ratio	95% Confidence Interval	p value
Risk Category			
Low risk	Reference	-	-
Moderate risk	1.47	0.96 – 2.42	0.078
High risk	1.46	0.92 – 2.32	0.111
Genetic testing	1.69	1.29 – 2.22	<0.001
Genetic test result			
Not tested	Reference	-	-
Positive test	2.71	1.66 – 4.46	<0.001
Negative test	1.56	1.18 – 2.07	0.002

\*Adjusted for age, race, and level of education.

**Conclusions:** Genetic testing among breast cancer patients, regardless of test result or pre-test mutation risk, is significantly associated with bilateral mastectomy. Many women (~1/4) with a negative genetic test and at low risk of hereditary breast cancer, by family history and age at diagnosis, currently undergo bilateral mastectomy with unclear benefits. Further qualitative research will aim to elucidate reasons driving choice of bilateral mastectomy among women with breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-05

Can an internal surgical adhesive facilitate drain-free mastectomy and reduce overall invasiveness?-A prospective, randomized, controlled, multicenter non-inferiority trial

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**Introduction:** Mastectomy closure without drains has many potential advantages. Flap fixation techniques have shown to be an effective alternative to drains. This study tested the non-inferiority of a surgical adhesive in overall invasiveness compared to standard wound closure with drains. **Methods:** This trial (ClinicalTrials.gov Identifier: NCT02958449) recruited seventy-seven patients undergoing eighty-four mastectomies +/- SLNB (n=84) at eleven international centers. Procedures were prospectively randomized to standard wound closure with drains (SWC; n=41) or wound closure without drains using a high strength lysine-based adhesive named TissuGlu® (TG; n= 43). The primary outcome measured assessed overall invasiveness using the number of post-operative clinical interventions, including drain removals and needle aspirations. Secondary endpoints included total wound drainage, cumulative days of treatment, days to drain removal and wound healing related complications. A patient questionnaire evaluating quality of life measures was also administered. **Results:** Subjects in the TissuGlu® group required significantly fewer post-operative clinical interventions ( $1.25 \pm 1.39$  TG vs.  $2.03 \pm 1.45$  SWC,  $p = <.0001$ ) compared to the Control group and had fewer cumulative days of treatment (defined as days of drains being in place and / or days on which an aspiration occurred;  $2.14 \pm 4.15$  TG vs.  $5.76 \pm 4.02$  SWC,  $p = <0.0001$ ). Presence of a drain was associated with significantly higher pain and lower mobility scores. **Conclusion:** The study demonstrates that flap fixation with this adhesive can permit drain-free mastectomy closure, reducing overall invasiveness and patient morbidity.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-07

Use of an activatable cell penetrating peptide-fluorescent imaging agent (AVB-620) to identify positive and close margins intraoperatively

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## Background:

Intra-operative identification of positive margins and lymph nodes continues to be a challenge for breast cancer surgeons. Current techniques such as frozen section or touch prep cytology are time consuming and vary in accuracy across institutions. Our study is the first part of a phase 2 trial of a systemically administered, ratiometric, activatable, cell-penetrating fluorescent peptide dye conjugate that visualizes breast cancer tumor tissue *in vivo* and *ex vivo*. We hypothesized that this method would be able to identify breast cancer close to the margin of lumpectomy specimens.

## Methods:

AVB-620 is a cell penetrating peptide-fluorescent imaging agent which undergoes proteolytic cleavage by proteases in the matrix metalloproteinase family that have higher activity in cancerous tissue. After cleavage, the predominant fluorescent emission wavelength changes and fluorescent intensity increases, generating a ratiometric readout. AVB-620 was administered 3- 24 hours prior to operation via intravenous infusion to stage 0-III breast cancer patients. Patients were monitored for safety followed by primary breast surgery and either sentinel lymph node biopsy (SLNB) with radiotracer only or axillary lymph node dissection (ALND). Using a near-infrared camera system, fluorescent intensity of two wavelengths was measured intraoperatively both *in vivo* and *ex vivo*, including primary tumors, shave margins, and lymph nodes. The ratio of the intensity of these two wavelengths was utilized to distinguish between malignant and non-malignant tissues and visualized on a video monitor. Pathology reports were correlated to a computer-assisted human review of the fluorescent images.

## Results:

Thirty-two patients were dosed without adverse events attributable to the agent; 31 were evaluable for fluorescent imaging. The average age of patients was 60 years (range 32-79). All patients underwent lumpectomy and axillary surgery. Two patients received neoadjuvant chemotherapy; 10% of patients had pure DCIS tumors. Among invasive tumors: 94% were ER+; 13% were high grade. All primary tumor images demonstrated fluorescent intensity and ratiometric changes that differentiated malignant and non-malignant tissue. 35% of patients had at least one positive margin (invasive tumor on ink or DCIS  $\leq 2$ mm from ink). 61% of cases had at least one margin that was considered either close ( $\leq 2$ mm from ink for invasive tumor) or positive on H&E staining. 84% of patients with positive/close margins were identified utilizing fluorescent imaging. Four of the 5 patients with positive nodes had a positive fluorescent image.

## Discussion:

These results demonstrate the ability of AVB-620 to identify and visualize malignant tumor and lymph nodes intraoperatively, illustrating the potential of such a tool to decrease reoperation rates. The second part of this phase II study is underway where intraoperative fluorescent imaging will be utilized to guide removal of additional shave margins.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-08

Clinicopathological features and prognosis of nipple–areola and skin flap recurrence after nipple-sparing mastectomy for breast cancer over 20 years

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**Background:** Nipple-sparing mastectomy (NSM) is an increasingly popular surgical procedure for treatment of breast cancer. However, NSM is controversial because of its association with locoregional recurrence. We started performing NSM in 1978. **Methods:** We investigated the surgical safety including nipple necrosis, and nipple–areola recurrence (NAR) and skin flap recurrence (SFR) after NSM for 1071 patients with breast cancer, including 31 with stage 0, 414 with stage 1, 479 with stage 2, 141 with stage 3 and 6 with stage 4, from 1985 to 2017. Our NSM method involved creating a thick skin flap to avoid surgical complications. No patients received radiotherapy. In 1034 patients with stage 1–3 breast cancer treated with NSM who developed NAR or SFR, we evaluated cancer stage, nuclear grade, lymph node metastasis, tumor–nipple–areola distance, and histological classification as tubule forming, solid and scirrhous type. In 748 patients with early stage 1 and 2A breast cancer treated with NSM, NAR and SFR were evaluated for estrogen receptor and HER2 expression. We evaluated disease-free interval and frequency of late NAR and SFR. **Results:** Median follow-up after NSM was 87 (3–397) months. There was only one case of total nipple necrosis among all 1071 patients. There were 96 patients (9.0%) with local recurrence, including 44 (4.0%) with NAR and 52 (4.8%) with SFR. NAR was seen in 1 (3.1%), 14 (3.4%), 17 (5.1%), 5 (3.4%), 7 (5.0%) and 0 patients with stage 0, 1, 2A, 2B, 3 and 4 cancer, respectively. SFR was seen in 0, 15 (3.6%), 8 (2.7%), 7 (4.8%), 22 (15.6%) and 0 patients with stage 0, 1, 2A, 2B, 3 and 4 cancer, respectively. Median disease-free interval of NAR and SFR was 3.4 (0.96–22.3) and 2.5 (0.21–21.2) years, respectively. Twenty-three (53%), 12 (27.9%) and 6 (14%) patients had NAR at more than 3, 5 and 10 years after NSM, respectively. Twenty (38%), 13 (25%) and 6 (11.5%) patients had SFR at more than 3, 5 and 10 years after NSM, respectively. Therefore, late NAR and SFR were observed. Patients with stage 1–3 cancer treated with NSM who had significantly more frequent NAR, were characterized by high nuclear grade and tubule-forming type cancer. Patients with significantly more frequent SFR were characterized by stage 3 cancer, positive lymph node metastasis and age  $\leq 40$  years. Patients with early stage breast cancer treated with NSM with significantly more frequent NAR had negative estrogen receptor expression, positive HER2 expression and shorter tumor–nipple–areola distance ( $\leq 2$  cm). Overall survival was significantly better in patients with NAR (97% at 5 years and 80% at 10 years) than SFR (71% at 5 years and 50% at 10 years). Regarding SFR, overall survival was significantly worse for multiple ( $\geq 2$ ) and diffuse (clinical inflammatory syndrome) recurrence than for single-nodule recurrence. There was no significant difference in prognosis between NAR and single-nodule SFR. **Conclusions:** Our data showed that clinicopathological features and prognosis differed between patients with NAR and SFR. There was no significant difference in prognosis between NAR and single-nodule SFR. Late NAR and SFR were seen, and careful long-term follow-up observation is necessary after NSM.

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A novel nipple areolar complex involvement predictive index (NACPI) for indicating nipple sparing mastectomy in breast cancer patients

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**Background:** While Preservation of NAC is concerned to increase the risk of local recurrences in the retroareolar glandular tissue, nipple sparing mastectomy (NSM) is increasing in patients with breast cancer and has been shown to result in better cosmetic outcome and the benefit for quality-of-life. It is necessary to predict accurately NAC involvement in order to select which patients may be candidates to NSM. The distance from the nipple to the tumor (DNT) is proposed as one of the best criteria to select the patient. The purpose of this study is to identify the predictors of NAC involvement retrospectively and to develop a clinical predictive model to select the patients who can be offered preservation of NAC. **Methods:** A total of 168 patients with primary operable breast cancer who received subcutaneous mastectomy for breast reconstruction at Saitama Medical Center during July 2013 to December 2017 were selected from the hospital's surgical database. NAC involvement was defined by the presence of invasive carcinoma and/or ductal carcinoma in situ at the subareolar margin.

**Results:** Of the 148 patients who were preserved NAC, 89.9% (133/148) were NAC involvement negative and 10.1% (15/148) were positive in permanent pathological specimens. Of the 20 patients who were resected NAC, NAC involvement positivity was only 50.0% (10/20). This revealed that NAC involvement with a sensitivity (SN) of 40.0%, a specificity (SP) of 93.0%, a positive predictive value (PPV) of 50.0% and a negative predictive value (NPV) of 89.9% (AUC=0.665, 95%CI: 0.5345-0.796). In 140 patients who were performed intraoperative sub-nipple frozen section biopsy, the findings was significantly associated with NAC involvement ( $P<0.001$ ), and which predict the NAC involvement with a SN of 93.3%, a SP of 89.6%, a PPV of 51.9%, and a NPV of 99.1% (AUC=0.915, 95%CI: 0.835-0.994). In the concordance rate between frozen section findings and definitive pathologic results, DCIS was 53% (9/17), atypical cell was 50% (2/4) and invasive ductal carcinoma was 100% (3/3). Correlation between NAC involvement and clinicopathological factors, tumor size  $\geq 4$ cm ( $P<0.001$ ), DNT  $<1$ cm by MMG ( $P=0.002$ ), DNT  $<1$ cm by MRI ( $P<0.001$ ), nipple contrast findings by MRI ( $P<0.001$ ), tumor in central portion ( $P<0.001$ ), multicentric/focal lesion ( $P<0.001$ ), c(N) positive ( $P=0.014$ ) were significant relation with NAC involvement. Each predictors were scored 0 or 1, and the total score of 0-3 points was defined as low risk, 4 points as intermediate risk, and 5-7 points as high risk. Depending on this categorized classification, the NAC involvement rate was 3.5% (5/142) in low risk, 68.7% (11/16) in intermediate risk, 90.0% (9/10) in high risk and there was a significant correlation between the risk group and NAC involvement ( $P<0.001$ ). Notably, assuming that NAC is preserved for low risk patients and is resected for intermediate and high risk patients, NACPI contributes to improve the accuracy of selecting the surgical procedures (SN 80.0%, SP 95.8%, PPV 76.9%, NPV 96.5% (AUC=0.879, 95%CI: 0.784-0.974)). **Conclusion:** This study suggests that NACPI can help us indicating subcutaneous mastectomy for the breast cancer patients who request preserve NAC with more oncological safety.

# 2018 San Antonio Breast Cancer Symposium ®

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Metastatic pattern discriminates survival benefit of primary surgery for de novo stage IV breast cancer patients: A longitudinal cohort study

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**Background:** Primary tumor resection for patients(pts) with de novo stage IV breast cancer(BC) was controversial among recent clinical trials. Indeed, considerable discrepancy in metastatic pattern between those studies was considered as a potential reason for their inconsistent results. We sought to determine the survival benefit of primary surgery on the basis of metastatic pattern.

**Methods:** A retrospective cohort study based on the SEER database was conducted to identify pts with de novo stage IV BC diagnosed between 2010 and 2015. Pts were divided into a surgery and a non-surgery group, and propensity score weighting was used to balance clinicopathologic factors between groups.

**Results:** Of 8,142 de novo stage IV BC pts with a total of 12,737 sites of distant metastases identified in this study, 1,891(23%) cases were managed with surgery and 6,251(77%) cases were managed without surgery. There were 3,821 all-cause deaths and 3,291 BC specific deaths over a median follow-up of 22 months. The weighted 3-year overall survival(OS) for the surgery group was 54.5%, compared to 47.7%(HR=0.82;0.78 to 0.86,P<0.001) for the non-surgery group. The magnitude of the survival difference with surgery was significantly correlated with metastatic patterns( $P_{interaction}<0.05$ ). Significant survival improvements in surgery group compared with non-surgery group were observed in pts with bone-only metastasis(adjusted HRos=0.83;0.76 to 0.90,P<0.001) or multiple metastases with bone involved (adjusted HRos=0.76;0.70 to 0.83,P<0.001), whereas survival inferiority of surgery was found for pts with multiple visceral organs-only metastases(adjusted HRos=2.08;1.39 to 3.11,P<0.001).

Hazard ratios comparing survival between surgery group and non-surgery group according to metastatic patterns.

Metastatic pattern	No. of Patients (Non-surgery group/Surgery group)	Weighed* 3-year OS (%) (Non-surgery group/Surgery group)	Weighed Multivariable*† HR of OS	P	Weighed* 3-year BCSS (%) (Non-surgery group/Surgery group)	Weighed Multivariable*† HR of BCSS	P
Distant Lymph nodes-only	285/192	55/55	0.96	0.71	64/58	1.16	0.2
Bone-only	2409/704	54/64	0.83	<0.001	57/67	0.8	<0.001
Brain-only	34/16	15/9	0.42	0.01	19/15	0.52	0.08
Lung-only	434/270	46/52	0.9	0.19	50/60	0.75	0.001
Liver-only	378/203	53/51	1.16	0.14	58/55	1.13	0.23
Bone ± non-visceral ± visceral organs	2305/388	41/50	0.76	<0.001	45/53	0.74	<0.001
Non-visceral ± visceral organs	302/89	43/50	0.78	0.06	46/53	0.75	0.03
Visceral organs-only	104/29	31/22	2.08	<0.001	36/28	1.29	0.25

(\*)Weighted by inverse propensity score.(†)Aadjusted for age,year of diagnosis,race,marital status,grade,histologic type,tumor size,ER,PR,HER2 status,chemotherapy. BCSS, breast cancer specific survival.

**Conclusion:** The survival benefit offered by surgery for de novo stage IV BC varies by metastatic patterns. Local surgery for pts

with bone-only metastasis or high metastatic burden accompanied by bone metastasis offers a significant survival advantage over non-operative management, whereas the opposite effect is observed among simultaneous liver and lung metastasis pts. Decisions for primary surgery of de novo stage IV BC pts should be tailored according to metastatic pattern, and mechanisms of bone/visceral organs-only/first metastasis BC are needed further understanding.

# 2018 San Antonio Breast Cancer Symposium ®

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Mechanisms involved in hypnosis analgesia explaining potential benefits observed among breast cancer patients undergoing breast surgery

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**Background:** Our team has previously highlighted the benefits of hypnosis analgesia on different modalities of breast cancer treatment. In order to confirm these benefits and -in order to try to explain the mechanisms implicated in hypnosis sedation -, we have initiated this new study. **Material and methods:** This study is a prospective non -randomized multicentric study approved by our local ethics committee which has planned to include 450 consecutive non -metastatic breast cancer patients treated in our breast clinic. The study is divided in 3 arms. In the first arm, patients undergo oncologic breast surgery while on general anesthesia. In the second arm , general anesthesia is preceded by a session of hypnosis relaxation mediated by virtual reality (Aqua program, Oncomfort ). In the third arm , the patients undergo breast surgery while on hypnosis analgesia and local anesthesia. To decrease the impact of local anesthesia, on the measured outcomes , this procedure is added to general anesthesia in the first two arms. Different parameters are measured for every patient : anxiety scales (– evaluated by the NCCN DT-National Comprehensive Cancer Network Distress Thermometer- on days 0, 1 and 8), pain scores (measured by NRS (- numerical rating scale-) and VAS (-visual analogue scale-) on days 0, 1 and 8 and biological parameters : NLR( - Neutrophils to lymphocytes ratio- and CRP – C reactive protein- measured on days 0, 1 and 8.) **Results:** We here present the results of the first 150 patients included in the study between October 2016 and April 2018, i.e 50 patients in each arm. The groups are well balanced for age (mean age of 60 years in the 3 arms), surgical procedures (lumpectomies, mastectomies and sentinel biopsies), tumor and patients' characteristics. Anxiety scales are high and do not differ statistically between the 3 groups on day 0. On the contrary , on days 1 and 8, anxiety scales are lower in the groups of hypnosis analgesia and hypnorelaxation - compared with the group of general anesthesia alone - (p value= 2. 2 e-16). No - statistically significant differences- are observed between the hypnosis and hypnorelaxation groups . Pain scores and analgesics consumption are lower in the groups - using hypnosis analgesia and hypnorelaxation at each time point -. The group using hypnosis analgesia is the most efficient in pain reduction. The variations are statistically significant : p- value -0.009 -on day 0, 0.002 on day 1 and 0.0009 on day 8. The NLR and CRP values do not differ between the 3 groups on day 0. They are statistically lower on day 1 in the arm of hypnosis analgesia compared with the two groups with general anesthesia (p-values 0.00037 for NLR and 0.024 for CRP). On day 8, the variations are not statistically different between the 3 groups.**Discussion:** Inflammatory reactions associated with breast cancer surgery could exert a negative impact on quality of life (pain, anxiety) and perhaps on breast cancer survival. On the contrary, interventions associated with a decreased inflammatory reaction could be beneficial for breast cancer patients.**Conclusion:** This preliminary report suggests that hypnosis analgesia can modulate the immune system and exert benefits in this way.



# 2018 San Antonio Breast Cancer Symposium ®

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Impact of the time interval between neoadjuvant chemotherapy and surgery in Latin-Americans breast cancer patients

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**Background:** Few studies have evaluated the impact of the time interval between neoadjuvant chemotherapy (NAC) and surgery in breast cancer. In Latin America, where the vast majority of hospitals are oversaturated, it is important to define which patients to give priority and to be clear about ideal time or maximum to schedule surgery after NAC without altering the prognosis. The objective of this work is to establish the ideal time interval for post-neoadjuvant surgery and evaluate the impact on patient survival.

**Methods:** We reviewed the clinical histories of breast cancer with clinical stage II and III who received NAC between 2005 and 2014. Patients were divided into 3 groups according to the time interval to surgery: <4, 4-8 and >8 weeks. Overall survival (OS) and recurrence-free survival (RFS) were estimated using the Kaplan-Meier method, and comparisons of survival curves using the logrank or Breslow test, both globally and by molecular subtypes. The optimal time to surgery has been determined by the Cox model.

**Results:** During the study period, 583 patients who had post NA surgery before six months were registered. The median age was 49 years (range: 24-85), 82% had clinical stage III, 53% histological grade III, 32.7% were luminal A, 15.6% luminal B, 24.4% Her2 and 27.3% TN. According to the time interval to surgery, 67 (11.5%) patients had surgery before 4 weeks, 204 (35.0%) between 4 to 8 weeks, and 312 (53.5%) after 8 weeks. The groups do not present differences in relation to the clinical characteristics (p> 0.05). The median follow-up time was 4.8 years. The 5-year OS rate according to the time interval was 57.9, 61.5, and 62.7% (p = 0.581) and the RFS rate was 40.6, 52.3, and 51.1% (p = 0.411)

TABLE 1 : Time Interval :OS - RFS

		RFS			OS	
	MEDIAN	5 - years	P	MEDIAN	5 - years	P
Weeks for NAC to Surgery						
<4 weeks	3.2	40.6		6.1	57.9	
4-8 weeks	6.3	52.3		9.1	61.5	
>8 weeks	5.1	51.1	0.41	6.7	62.7	0.581
Weeks for NAC to Surgery						
<8 weeks	5	49.5		9.1	60.7	
>8 weeks	5.1	51.1	0.585	6.7	62.7	0.414

. No differences were found in the survival curves by molecular subtypes , except for luminal b like

TABLE 2 : Time Interval - Molecular Subtype

		RFS			OS	
	MEDIAN	5 - years	P	MEDIAN	5 - years	P
LUMINAL A LIKE						
< 8 weeks	----	74.1		----	84	

>8 weeks	7.1	63.8	0.71	9.9	73.9	0.236
*LUMINAL B LIKE						
< 8 weeks	2.3	36.8		5.4	60.5	
>8 weeks	5.8	57.6	0.46	----	82	0.08
HER2						
<8 weeks	2.2	28.7		3.9	38.4	
>8weeks	3.6	45.9	0.57.2	6.2	61.3	0.616
TRIPLE NEGATIVE						
<8 weeks	3.1	44.2		3.4	48.4	
>8 weeks	2.0	41.8	0.914	3.7	43.9	0.516

\* ER + PR >20% KI67>14% , HER2 NEGATIVE

. In the multivariate analysis, the effect of the time interval to surgery was not significant in OS and RFS; however, the HR curve suggests that the appropriate cut-off point for surgical time would be 8 weeks.

Conclusion: The time interval between the end of neoadjuvant period and surgery has no impact on recurrence-free survival or on overall survival, despite this we suggest that the period of time between neoadjuvant and surgery not be greater than 8 weeks.

More studies will be required to determine the ideal time interval and which cases should be prioritized according to the characteristics of our patients.

# 2018 San Antonio Breast Cancer Symposium ®

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Single institute data to assess timing of surgery post neoadjuvant in breast cancer patients

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**Background:** This study aims to analyze the impact of the time taken from the completion of neoadjuvant chemotherapy to surgery on patients' outcomes in terms of pathological response, overall survival and disease-free survival. There is no specific guideline for timing of the surgery. This study presents the experience of our institute's unique and large data of locally advanced breast cancer patients who received neoadjuvant systemic therapy.

**Methods:** This retrospective study evaluated patients diagnosed with Stage II and III breast cancer patients who received neoadjuvant chemotherapy, which was FEC and Taxotere +/-Herceptin depending on Her2 status of disease. Evaluation of the treatment outcome was based on the time interval between completion of neoadjuvant chemotherapy and surgery. Patients were selected from the time frame of January 2004 to December 2014. The effect of time interval was studied using two types of stratification. First stratification included time interval less than 4 weeks, 4-6 weeks and more than 6 weeks. Second stratification included patients with time interval <4 weeks, 4-7 weeks, and  $\geq 8$  weeks. Patients were also evaluated on the basis of receptor status ER, PR and Her2, and their outcomes.

**Results:** A total of 611 patients were identified. The patients were divided into two cohorts for better analysis. The first cohort showed 94 patients (15.4%) who had surgery within 4 weeks of their last dose of neoadjuvant chemotherapy, 378 (61.9%) within 4-6 weeks, and 139 (22.7%)  $\geq 6$  weeks. For the second cohort 94 patients (15.4%) had surgery within 4 weeks, 424 (69.4%) within 4-7 weeks, and 93 (15.2%)  $\geq 8$  weeks. Median OS and median DFS is not reached. OS at 5 years was 89.6% and DFS at 5 years was 74%. In both cohorts, OS and DFS were not significant when stratified to timing of surgery but the trend of DFS, although not statistically significant, was poor when patients had surgery more than 6 and 8 weeks. When patients were assessed on pathologic response stratified with timing of surgery, about 15% of patients had surgery  $\geq 8$  weeks, only 12.9% of those had complete pathological response compare to patients whose surgery was 6-7 weeks and complete pathologic response was 26% ( $p=0.02$ ). In terms of receptor status, (ER-/HER-2+) patients had a statistically significant decrease in complete pathologic response if surgery was  $\geq 8$  weeks. However, ER+/HER-2-, (ER+/HER-2+), ER-/HER-2- had no difference in complete pathological response.

**Conclusion:** The above data indicates that our patients showed improved complete pathologic response if the surgery was performed within 8-weeks, especially for (ER-/HER-2+) patients. All patients post neoadjuvant had better OS and DFS trends if the surgery was performed between 4-6 weeks. The data suggests that early surgery helps complete pathologic response, and the necessary measures must be taken to identify any obstacles leading to delay in surgery and eliminating these obstacles.

# 2018 San Antonio Breast Cancer Symposium ®

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Voice of cancer patients (VoCP): Analysis of experiences of cancer patients undergoing breast cancer surgery

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Surgery is an important part of breast cancer treatment. Most patients either go for mastectomy or lumpectomy + radiation (i.e., breast conserving surgery, BCS). It is well known that mastectomy and BCS have equal long-term outcomes, and more patients are choosing to get breast reconstruction.

In this study, we analyzed experiences, concerns, complications and side effects in patients who have undergone breast cancer surgery and who shared their views on various online forums. Such forums have millions of freely shared messages and are rich sources of such information. However, this data is unstructured. We used our automated system, VoCP, that used techniques from Big Data science and artificial intelligence (e.g., deep learning, topic modeling, information retrieval, natural language processing) to analyze concerns and experiences of patients undergoing breast cancer surgeries.

**Methods:** We collected 5.5 million messages from 174556 distinct users in 21 unrestricted breast cancer forums. We built specific ontology for different surgeries, reconstruction, side effects and sentiments and used our system, VoCP, to extract relevant information from these messages.

**Results:** 52172 users shared 307966 messages regarding surgery and their views are summarized below.

## **Lumpectomy (BCS):**

- 25850 users provided 98499 messages
- 15771 users had lumpectomy and shared 50390 messages
- 3760 users shared 6322 messages with complications
- 2760 users mentioned need for additional surgery
- 1447 users mentioned satisfaction with the outcome whereas 117 were dissatisfied

## **Mastectomy:**

- 37544 users shared 198494 messages
- 22716 users had mastectomy and shared 94595 messages
- 5065 users shared 8983 messages regarding complications
- 2730 patients expressed satisfaction with outcome whereas 286 patients were dissatisfied

## **Reconstruction:**

- 23164 users provided 113568 messages regarding reconstruction
- 9850 users had reconstruction and shared 32472 messages
- 1904 users shared 2734 messages with complication
- 1982 users had immediate reconstruction; 353 users mentioned delayed reconstruction
- 2686 users had autologous tissue reconstruction and 3105 repot implant reconstruction
- Type of Autologous Reconstruction reported : 759 TRAM flap, 296 Diep flap, 820 fat grafting, 108 Latissimus Dorsi flap, 89 SGAP Flap/hip Flap
- 418 users expressed satisfaction whereas 40 were dissatisfied with outcome

## Complications reported

Complications	Reconstruction #	Lumpectomy #	Mastectomy#	Overall#
Scars	107	1,505	1,416	3,028
Pain	710	1,410	405	2,525
Infection	282	310	652	1,244
Necrosis	229	51	167	447
Numbness	35	145	369	549

Lymphedema	80	136	334	550
Lumps	60	115	196	371
Seroma	56	222	462	740
Swelling	83	290	385	758
Frozen Shoulder	37	83	151	271
Bleeding		44	55	99
Hematoma		178	139	317
Itching/Rash		130	253	383
Anxiety		82	145	227
Blood Clot			64	64
Bruising			60	60
Drainage			54	54

### Conclusions:

- Despite reports of equal long term outcomes between BCS and mastectomy, more patient had mastectomy.
- Scar issues and pain is the most common complication from any surgery.
- Most patients have expressed satisfaction from their chosen surgery.
- VoCP reliably provides meaningful insights from the patient's point of view; it also gives insight into unmet needs where more resources and research should be focused.

# 2018 San Antonio Breast Cancer Symposium ®

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Breast cancer-related lymphedema: Morbidity of sentinel node biopsy

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## Purpose:

Sentinel lymph node biopsy (SLNB) lowers morbidity of lymphedema then axillary lymph node dissection (ALND). However, there has been concern about incidence of lymphedema after SLNB especially when the number of harvested nodes during sentinel node biopsy procedure is more than a few. In this study, we assessed lymphedema incidence and its risk factors including the number excised lymph nodes in patients who underwent SLNB.

## Methods:

Between January, 2011 and April, 2012, the records of 910 consecutive patients who underwent breast surgery with axillary staging (SLNB/ALND) for breast cancer at Seoul National University Hospital were reviewed. Lymphedema was assessed by circumferential upper extremity measurements. The lymphedema was defined as > 1cm for either the upper arm or the forearm. Patients with clinical records of the treatment for lymphedema in the rehabilitation clinic were regarded as having lymphedema. Univariate and multivariate analyses were performed to identify potential risk factors associated with lymphedema. Association of number of excised lymph nodes with lymphedema was analyzed by Spearman rank correlation coefficient.

## Results:

At median follow-up of 69.8 months, 231 patients (25.4%) presented with lymphedema. In univariate analysis, body mass index (BMI) ( $P<0.001$ ), T stage ( $P<0.001$ ), N stage ( $P<0.001$ ), type of surgery ( $P<0.001$ ), ALND ( $P<0.001$ ), neoadjuvant chemotherapy ( $P<0.001$ ) and adjuvant chemotherapy ( $P=0.027$ ) were significantly associated with lymphedema. In multivariate analysis BMI ( $P<0.001$ ), ALND ( $P<0.001$ ), neoadjuvant chemotherapy ( $P=0.044$ ), and radiation therapy ( $P=0.046$ ) were significantly associated with lymphedema. In patients treated with SLNB only ( $n=595$ ), the incidence of lymphedema was 16.3% ( $n=97$ ). In SLNB only subgroup, BMI was only significant risk factor of lymphedema. There was no correlation between number of excised lymph nodes during sentinel lymph node biopsy procedure with incidence of lymphedema ( $P=0.138$ ).

## Conclusion:

The risk of lymphedema is multifactorial in breast cancer surgery and adjuvant treatments. In SLNB alone patients, higher BMI was only significant factor correlated with lymphedema. Excised number of lymph nodes during sentinel biopsy procedure was not associated with lymphedema.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-16

Comparison of robotic nipple sparing mastectomy (R-NSM) to endoscopic assisted nipple sparing mastectomy (E-NSM) in the management of breast cancer

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**Background:** Endoscopic assisted nipple sparing mastectomy (E-NSM) alone or followed by immediate breast reconstruction (IBR) with implants or autologous flaps were reported to be associated with small inconspicuous incision and good cosmetic outcome. Robotic nipple sparing mastectomy (R-NSM), which introduce da Vinci surgical platform through a small axillary wound to perform NSM with (or without) IBR, was reported to have potential to overcome the technique difficulty of E-NSM and showed promising cosmetic outcome. However, few evidence was available compared the effectiveness and safety of R-NSM compared with E-NSM in the management of breast cancer.

**Methods:** Patients with breast cancer received E-NSM or R-NSM performed from July 2010 to June 2018 were searched from breast surgery database at Changhua Christian Hospital (CCH), Taiwan. Data on clinicopathologic characteristics, type of surgery, complications and recurrence were analyzed to determine the effectiveness and oncologic safety of R-NSM and E-NSM. Patient-reported cosmetic outcome result was also obtained and compared.

**Results:** A total of 127 E-NSM and 36 R-NSM procedures were found and data collected for analysis. About 77.8% of R-NSM group received breast reconstruction, and 78% of E-NSM group received breast reconstruction ( $P=0.982$ ). The surgical margin involved rate was 2.8%(1/36) in R-NSM versus 3.4%(5/127) in E-NSM ( $P=1$ ). The overall operation time was  $281.5 \pm 77.0$  mins in R-NSM group versus  $210.8 \pm 55.5$  mins in E-NSM group ( $P < 0.001$ ). Blood loss was mean  $36.5 \pm 33.7$  ml in R-NSM group versus  $88.0 \pm 61.0$  ml in E-NSM group ( $P < 0.001$ ). The hospital stay was  $6.8 \pm 1.3$  days in R-NSM group versus  $5.1 \pm 1.3$  in E-NSM group ( $< 0.001$ ).

-From learning curve analysis, about 15-17 cases needed to significantly decrease operation time in E-NSM group, and in R-NSM group around 10-12 cases needed.

-About 50 E-NSM and 25 R-NSM patients received post-operative questionnaire survey for cosmetic outcome evaluation and acceptance of operations. Patient-reported outcome survey showed that the satisfaction rate of R-NSM 96.4% group versus 94.8 in E-NSM group ( $p=0.96$ ). The will to receive the same operation again if they could chose again: 100% in E-NSM group versus 96.4% in R-NSM group.

-Cost analysis- The breast cancer operation cost was reimbursed by national insurance in Taiwan. The additional cost of E-NSM and IBR with Gel implant was 4,000-6,000 USD (according to different type of implants used). The cost of R-NSM and IBR with Gel implant was 10,000-12,000 USD (according to different type of implants used). The cost difference was about 2,500-3,300 USD higher in R-NSM group than in E-NSM group.

**Conclusion:** Both E-NSM and R-NSM were equally effectively in the management of breast cancer with no different surgical margin involved rate, however, longer follow-up remained mandatory for oncologic safety evaluation. Shorter learning curve indicated more friendly operation platform of robotic surgery in performing NSM. Relative longer operation time, and higher cost of R-NSM compared with E-NSM was observed. Longer hospitalization is biased from personal insurance consideration due to higher cost of R-NSM.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-17

Long-term oncologic safety of nipple-sparing mastectomy with immediate reconstruction

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## Background

Nipple-sparing mastectomy (NSM) is an alternative procedure to skin-sparing mastectomy (SSM) for selected patients who undergo immediate reconstruction. However, the evidence of long-term oncologic safety of NSM has not been established. In this study, we aimed to compare the prognosis of breast cancer patients who underwent NSM to those who underwent SSM with immediate reconstruction.

## Methods

The clinicopathological factors including recurrence site, pathologic stage, nipple-tumor distance, histological type, lymphovascular invasion, margin status, ER, PgR and HER2 status of stage 0–III primary breast cancer patients who underwent NSM or SSM with immediate primary reconstruction with tissue expander from our breast center database was retrospectively assessed. Patients with a nipple tumor distance of <1 cm who underwent NSM were excluded. 190 patients who underwent NSM and 729 patients who underwent SSM were included in the analysis. All patients underwent MRI or US before treatment. Nipple-tumor distance was mainly measured by MRI.

## Results

The median follow-up period was 71 months (range: 10 - 131 months) for the NSM group and 79 months (range: 9 - 140 months) for the SSM group. There were no significant difference of clinicopathological factors between the NSM group and the SSM group, except of the larger diameter of tumor in the SSM group. NSM was performed for 60 patients (32%) with stage 0, 71 patients (37%) with stage I, and 59 patients (31%) with stage II/III. SSM was performed for 185 patients (26%) with stage 0, 268 patients (37%) with stage I, and 276 patients (37%) with stage II/III. Local recurrence was found in 11 (5.8%) patients in the NSM group and in 44 (6.0%) patients in the SSM group. In the NSM group, only one (0.5%) patient had local recurrence in the nipple areola complex. In terms of DFS and overall survival (OS) rate, there was no difference between the NSM group and the SSM group (DFS; 89.5% vs 89.2%, HR, 1.044;  $p = 0.8992$ ; 95% CI, 0.5116–1.9519, and OS; 98.4% vs 96.4%, HR, 0.963;  $p = 0.9116$ ; 95% CI, 0.473–1.793). According to breast cancer subtype, in the NSM group, all of the 11 patients (100%) who developed local recurrence in the NSM group was hormone receptor (HR)-positive/HER2-negative breast cancer. 29 of the 44 patients (65.9%) who developed local recurrence in the SSM group was HR-positive/HER2-negative, 6 patients (13.6%) was HR-negative/HER2-positive, and 7 patients (15.9%) was triple-negative breast cancer. Among patients who had received neoadjuvant chemotherapy, the NSM group (3 of 14 patients, 21.4%) had a trend for higher local recurrence rate than the SSM group 7 of 116 patients (6.0%) ( $p = 0.0813$ ). However, no local recurrence in the nipple areola complex was observed for the NSM group. In addition, there was no difference of OS between the NSM group (92.9%) and the SSM group (90.5%) (HR, 0.903;  $p = 0.9943$ ; 95% CI, 0.049-4.739).

## Conclusions

Our results suggested that NSM with immediate reconstruction might be safe as well as SSM for breast cancer with the nipple–tumor distance of >1 cm with respect to their prognosis and local control regardless of breast cancer subtype or invasiveness. Further studies with a large sample size to assess the risk of local recurrence for NSM after neoadjuvant chemotherapy.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-18

Achieving rapid intraoperative diagnosis during breast cancer surgery using high-resolution full-field optical coherence imaging and dynamic cell imaging

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**Background:** Intraoperative pathological diagnosis such as frozen section and imprint cytology is not routinely recommended in clinical practice because of time and accuracy concerns. Full-field optical coherence tomography (FF-OCT) is a new optical imaging technique that could generate sectioning tomogram from fresh tissue and provide depiction of the morphological structure and pathological changes in minutes without conventional tissue preparation, slicing, and staining, and dynamic cell imaging (DCI) added the viability information of cells/tissue, which could be more important in cancer diagnosis. This study was to evaluate the feasibility and diagnostic value of FF-OCT and DCI in breast lesions and lymph node specimens during breast cancer surgery.

**Methods:** We evaluated normal breast tissue, benign breast lesions, breast cancer and axillary lymph node specimens from 107 patients using FF-OCT and DCI. After the optical assessment, the tissue was paraffin embedded and sent to conventional H&E diagnosis. The similar layer of OCT and H&E images were compared and diagnostic criteria were generated. The diagnostic sensitivity and specificity by two trained surgeons without pathology diagnosis experience were evaluated.

**Results:** A total of 194 specimens were examined, including 143 breast tissue (101 malignant and 42 benign/normal) and 51 lymph nodes (26 metastatic and 25 non-metastatic). On FF-OCT and DCI, normal morphological structures such as adipose, collagen, mammary ducts, and lobules in breast tissue and lymphoid follicle and hilum in lymph nodes were easily recognized. Breast cancer characteristics on H&E imaging correspond to collagen distortion, focal hypointensity, micro-calcification, clustered or linear lively cells et etc on FF-OCT or DCI, which could also be easily distinguished. We included the previously mentioned features to build diagnosis criteria for cancer on FF-OCT and DCI. The average acquisition time is 14±11 minutes. The sensitivity and specificity for breast cancer diagnosis were 92.1% and 94.3% respectively. The sensitivity and specificity for lymph node involvement were 92.3% and 84% respectively.

**Conclusion:** The time- and tissue-saving optical imaging technique yielded high accuracy that was comparable to that of traditional intraoperative and postoperative pathological diagnosis in breast cancer and lymph node metastasis. These results implied the promising application in the intraoperative evaluation and possible decrease of the re-excision rate for breast cancer surgery.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-19

Surgical and long-term outcomes of patients receiving neoadjuvant pertuzumab-containing regimens for HER2-positive localized breast cancer

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**Background:** The addition of pertuzumab to trastuzumab and chemotherapy significantly improves the pathologic complete response (pCR) rate in HER2+ localized breast cancer in the preoperative setting. Although many patients are converted to breast conserving therapy (BCT) candidates by neoadjuvant HER2-directed therapy, a significant proportion opt for a mastectomy for various reasons. Among mastectomy procedures, nipple sparing mastectomy (NSM) is frequently chosen instead of non-nipple sparing mastectomy (NNSM). In this study, we evaluated the surgical and long-term outcomes of HER2+ patients receiving neoadjuvant pertuzumab-containing regimens.

**Methods:** We performed a retrospective review of localized breast cancer patients treated with neoadjuvant pertuzumab-containing regimens from 2011 to 2016, who underwent BCT or mastectomy at an academic institution and two community-based practices. Disease characteristics, treatment regimens, surgical outcomes, and recurrence data were extracted from the electronic medical records.

**Results:** Among 90 patients with stage II-III HER2+ breast cancer, 45 received AC-THP (50.0%), 26 received THP (with adjuvant AC) (29.0%), and 19 received TCHP (21.0%). The majority of patients had grade 3 tumors (61.1%), clinical stage II disease (80.0%), invasive ductal carcinoma (86.7%), and ER+ disease (65.6%). Thirty-seven (41.0%) patients underwent BCT and 53 (59.0%) patients underwent mastectomy. Among the mastectomy patients, 38 (71.7%) patients underwent bilateral mastectomies, specifically 33 (62.0%) patients underwent a NSM and 20 (38.0%) patients underwent a NNSM. The type of surgery that patients underwent stratified by type of neoadjuvant regimen is outlined in the Table 1 below. Most patients who underwent BCT and mastectomy received radiation, including 36 (97.3%) BCT, 24 (72.7%) NSM, and 18 (95.0%) NNSM. Over a median follow-up period of 33 months, 6 patients (6.7%) had recurrences with 2 (2.2%) local recurrences and 4 (4.4%) distant recurrences. The 2 local recurrences occurred in one patient who underwent BCT and one patient who underwent NNSM followed by post-mastectomy radiation.

**Conclusions:** Among mastectomy patients, NSM was more commonly pursued than NNSM. Rates of local recurrence following pertuzumab-containing regimens for HER2-positive localized breast cancer were low overall, regardless of the type of surgery. Data on plastic surgery approaches and complication rates will be presented at the meeting.

Table 1. Type of surgery in patients receiving neoadjuvant HER2-directed therapy.

	AC-THP (N = 45)	TCHP (N = 19)	THP (N = 26)
BCT	46.7%	47.4%	26.9%
NNSM	26.7%	10.5%	23.1%
NSM	26.7%	42.1%	50.0%

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-14-20

Early locoregional breast surgery improves overall and progression-free survival in oligometastatic breast cancer

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## Background

Four percent of patients have Metastatic Breast Cancer (MBC) at the time of diagnosis. Surgery in this context is generally not recommended. However, tumour resection may have an effect on tumour load influencing metastatic growth. Some studies have suggested an advantage of locoregional surgery in MBC. The objective was to evaluate the effectiveness of surgery in the year following diagnosis of metastatic disease.

## Methods

Data were collected within the ESME breast cancer data platform. Stage IV patients diagnosed between 2008 and 2014 without further personal cancer were included. Patients who died or progressed in 1-year post-diagnosis were excluded. We compared patients operated within the first 12 months after diagnosis with the others.

## Results

Among 1977 patients with MBC at diagnosis, alive and progression-free at 12 months, 530 (26.8%) had surgery within this interval. Patients operated in the year of diagnosis had less oligometastatic disease (less than 3 metastases; 9.2% vs 21.8%,  $p<0.01$ ) compared to patients with no surgery. They had less bone metastasis (57.7% vs 74.4%,  $p<0.01$ ), more lymph node (33.2% vs 27.8%,  $p=0.02$ ), lung (19.8%,  $p<0.01$ ) and less liver metastasis (17.9% vs 26.8%,  $p<0.01$ ) sites. Other treatments included more chemotherapy and HER2-targeted therapy (89.1% vs 69.6%,  $p<0.01$ ), locoregional radiotherapy (81.7% vs 32.5%,  $p<0.01$ ) and the same frequency of hormone therapy (79.8% vs 78.6%,  $p=0.57$ ). Multivariate survival analysis based on Cox model showed that surgery of primary breast lesion performed within 12 months (Hazard Ratio(HR)=0.75,  $p<0.01$ ), HER2 positive status (HR=0.42,  $p<0.01$ ) and the non-visceral metastases (HR=0.80,  $p=0.02$ ) improved overall survival (OS) whereas an older age than 50 (HR=1.50,  $p<0.01$ ) and 3 or more metastases (HR=1.45,  $p<0.01$ ) were associated with poorer survival. Progression-Free Survival (PFS) was also improved for locoregional surgery (HR=0.70,  $p<0.001$ ) after adjustment on HER2 positive status (HR=0.70,  $p<0.01$ ), age greater than 50 (HR=1.18,  $p<0.01$ ) and 3 or more metastases (HR=1.27,  $p<0.01$ ). Propensity score matching analyses confirmed these results.

## Discussion

We demonstrated that surgery of primary breast lesion had a benefit on OS (HR=0.75,  $p<0.01$ ), and PFS (HR=0.70,  $p<0.01$ ) in metastatic patient. Metastasis number less than 3 was a complementary protective factor to surgery. Studies have sought to evaluate the population that could benefit from surgery of primary breast lesion in the event of metastatic disease. They showed an increasing morbimortality in plurimetastatic patients. In our study, patients with more than 3 metastases had poorer OS and PFS (HR=1.45,  $p=0.01$  and HR=1.27,  $p=0.03$  respectively).

## Conclusion

Results of our study show that surgical control of primary breast lesion could be considered as an option in the locoregional treatment of MBC, particularly in non-visceral oligometastatic breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-21

The effects of poloxamer and sodium alginate mixture (Guardix-SG®) on range of motion after axillary lymph node dissection: A single-center, prospective, randomized, double-blind pilot study

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## **Purpose:**

Restricted shoulder mobility is a major upper extremity dysfunction associated with lower quality of life and disability after breast cancer surgery. We hypothesized that a poloxamer and sodium alginate mixture (Guardix-SG®) applied after axillary lymph node dissection (ALND) would significantly improve shoulder range of motion (ROM) in patients with breast cancer.

## **Methods:**

We conducted a double-blind, randomized, prospective study to evaluate the clinical efficacy and safety of Guardix-SG® for the prevention of upper extremity dysfunction after ALND. The primary outcome measure was shoulder ROM at baseline (T0) and 3 (T1), 6 (T2), and 12 months (T3) after surgery. Secondary outcome measures were the Disabilities of the Arm, Shoulder, and Hand score (DASH), pain associated with movement, which was assessed using a numeric rating scale, and lymphedema assessed using body composition analyzer.

## **Results:**

A total of 83 women with breast cancer were randomly assigned to either the Guardix-SG® group or the control group. In the Guardix-SG® group (n=37), Guardix-SG® was applied to the axillary region after ALND. In the control group (n=46), ALND was performed without using Guardix-SG®.

Compared to the control group, the Guardix-SG® group displayed improved shoulder ROM at 12 months (shoulder abduction of Guardix-SG® 175.5°,  $p=0.037$  vs. control group; 168.1°,  $p=0.117$ ). No adverse effect was observed in either group.

## **Conclusions:**

The results indicate that Guardix-SG® tends to relieve pain and improve shoulder ROM. A further large-scale study is needed to obtain a more conclusive conclusion.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-22

Is sentinel lymph node biopsy necessary in breast cancer patients who were diagnosed as initially clinically node-negative before neoadjuvant chemotherapy?

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## Background

Neoadjuvant chemotherapy (NAC) is established for treatment of locally advanced disease and is being used increasingly for early-stage breast cancer. And sentinel lymph node biopsy (SLNB) for clinically node-negative breast cancer patients after NAC is performed as a daily procedure. The purpose of this study was to identify the group that can omit SLNB in patients with clinically node-negative breast cancer at diagnosis before NAC.

## Materials and Methods

A single institutional retrospective analysis was performed. 126 patients were diagnosed as clinically node-negative before NAC and underwent SLNB between 2005 and 2017.

Before NAC, all patients underwent clinical assessment of lymph-node status by palpation and axillary ultrasound. We judged patients to be node-negative when palpation and ultrasound showed no suspicious nodes. If axillar lymph nodes were swelling, fine-needle aspiration cytology was performed. After NAC, clinical assessment of lymph-node status was performed, again. 119 patients received anthracycline-based (anthracycline alone: 11, anthracycline followed by taxane: 108) chemotherapy and 7 patients received taxane alone. Clinical complete response (cCR) was defined if there was no evidence of tumor in the breast and axillary lymph nodes by US and MRI. SLNB procedure was done after NAC in all patients. Lymphatic mapping was performed with radioactive colloid and/or lymphatic blue dye.

## Results

Median follow-up time was 51.2months. cCR rate was 16.7% (21/126).

Success rate for the identification and removal of a SLN was 96.8% (122/126). The median number of SLNs removed was 1.9 (1-5).

15 patients (12.3%) represented SLN positive in patients who were diagnosed as clinically node-negative before NAC. They were performed axillar dissection and the SLNs were the only positive nodes in 9 patients.

No SLNs metastases were observed in 21 patients who were diagnosed as cCR by ultrasound and MRI. And 15 patients (14.9%) had positive SLN in the 101 patients who were diagnosed as clinical partial response or clinical stable disease ( $p=0.071$ ).

Tumor size, Surgical procedures, hormone receptor status and HER2 status did not influence the positive rate of SLN metastases.

No axillar lymph node recurrence was observed within follow-up period.

## Conclusion

Our results show that SLNB is necessary even in breast cancer patients who were diagnosed as initially clinically node-negative before NAC.

However, SLNB may be omitted in breast cancer patients who were diagnosed as clinically node-negative by palpation and axillary ultrasound before NAC and as cCR by ultrasound and MRI after NAC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-23

Successful intraoperative margin assessment in DCIS and invasive breast cancer with diffusion-weighted MRI using the ClearSight™ system

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## **Aim**

Aim of our study is to evaluate the performance of the ClearSight™ system (ClearCut Medical, Ltd.) in assessing surgical margins for DCIS and IBC in breast conserving surgery

## **Material and Methods**

The ClearSight™ system utilizes a diffusion-weighted-imaging (DWI) protocol to create 2D surface maps showing T2\*, a MR parameter related to the tissue's apparent diffusion coefficient (ADC), with a depth penetration of 1.5mm. ADC is a highly accurate differentiator for irregular versus normal tissue.

From November 2017 a prospective, blinded post marketing study (N=63), evaluating the performance of the ClearSight™ system has been conducted in the Breast Centre at the Agaplesion Markus Krankenhaus, Frankfurt, Germany. After standard evaluation with ultrasound and/or X-ray, the specimens were scanned with the ClearSight™ system, and results were compared with the final histopathology results on a margin per margin bases, applying a simple T2\* threshold to flag irregular tissue.

## **Results**

Breast specimens' margins from 60 patients were analyzed. Pursuant to the breast conserving surgery (BCS), 348 margins were scanned by the ClearSight™ system. A rigid T2\* threshold comparison with the pathology findings resulted in a sensitivity of 80% and specificity of 84%. Accuracy for invasive and in-situ cancers was found to be similar for tissues scanned within one hour after excision. After that sensitivity for DCIS started to drop and was found to be 56% after 2 hours. Further accuracy improvements can be achieved if the reading physician is free to apply an intuitive diffusion map interpretation rather than adhere to a fixed threshold.

## **Conclusion**

The data suggest that ClearSight™ can reduce excision rates by a robust 80% if the excised breast tissue is scanned within one hour. Tissue drying impacts sensitivity for DCIS negatively. Free map interpretation leads to better results than a fixed threshold.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-14-24

Argon scalpel in the surgical treatment of breast cancer

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**Summary:** Surgery is a key part of the treatment of breast cancer. The adoption of electric scalpel began to be used in breast surgeries in 1970 and this equipment uses high frequency electric current to create the following effects: cutting, coagulation or mixing of the two. Data show a decrease in the intraoperative bleeding, however, can also increase complications, such as seroma and thermal lesions in the surgical flaps. A new technique that could be used is the coagulation with argon plasma which is a method of non-contact thermal hemostasis.

**Objectives:** to compare the electric scalpel with the scalpel by coagulation with argon plasma about aspects surgical and pathological.

**Methods:** this is a prospective cohort study in which 60 patients with breast cancer were selected at the Discipline of Breast Diseases of the Department of Gynecology of the Federal University of São Paulo (UNIFESP) at any clinical stage where the surgical treatment was indicated, from March 2014 to August 2014. The patients were consecutively selected and randomized into two groups: electric scalpel surgery (ES) and argon plasma coagulation surgery (APC). Inclusion criteria were: 18 to 90 years old patients with breast cancer at any clinical stage where surgical (conservative or radical) treatment was indicated. Intraoperative bleeding was assessed by measuring the weights of the compresses. The patients who underwent surgery were evaluated at 7, 14 and 30 postoperative days. In these returns, the appearance of the surgical wound, the presence and amount of seroma (in mL), hematoma or infection were analyzed. Surgical site infection was considered when there was erythema, increased local or systemic temperature, pain, suture dehiscence or presence of purulent exudate. The surgical specimen was studied in the Department of Pathological Anatomy of UNIFESP. The pathological analysis as recommended by the WHO and particular evaluations were carried out in order to observe the extent and degree of the thermal effect produced in surgical specimens by the two hemostatic techniques (ES and APC).

**Results:** The mean age of the patients was 56.0 years for the ES group and 54.9 for the APC. There was no significant difference between the groups regarding intraoperative bleeding. However, a statistically significant difference was observed when the days with drain were compared in the postoperative period, with a mean of 10.1 days for the SE group and 7.1 days for the APC group. The study demonstrated that the APC group had a significant greater thermal effect on the margins of the surgical specimen

**Table 1. Thermal effect on the margins of the surgical specimens by study group (p=0.032)**

Thermic Effect	Electric N (%)	Argon N (%)	Total N (%)
Absent	7 (23.3)	0 (0)	7 (11.7)
G1	10 (33.3)	12 (40.0)	22 (36.4)
G2	11 (36.7)	13 (43.3)	24 (40.0)
G3	2 (6.7)	5 (16.7)	7 (11.7)

**Conclusions:** the use of argon scalpel, when compared to the electric scalpel, allowed hemostasis to be performed adequately without altering the rates of bleeding, surgical time and postoperative complications, and reduced the number of days with the drain. The thermal effect on the surgical specimen was significant greater with the argon scalpel.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-25

Application of robotic surgery (da Vinci) in the management of breast cancer- Preliminary results and experience sharing

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**Background:** The experience of application of robotic surgery platform in the management of breast cancer was limited. The preliminary results of robotic surgery (da Vinci) in the management of breast cancer was reported in current study.

**Methods:** Patients with breast cancer received robotic breast surgeries from March 2017 to June 2018 were searched from robotic breast surgery database at Changhua Christian Hospital, Taiwan. Data on clinicopathologic characteristics, type of surgery, complications and recurrence were analyzed to determine the effectiveness and oncologic safety of robotic breast surgery. Patient-reported cosmetic outcome result was also obtained.

**Results:** During the study period, a total of 41 robotic breast surgeries were performed in 35 female breast cancer patients, including 6 patients with bilateral disease. Among these 41 robotic breast procedures, 39 were R-NSM related. Four patients with bilateral R-NSM (two patients with bil. breast cancer, and another two patients received contralateral prophylactic mastectomy (CPM)) without breast reconstruction. The other 31 R-NSM were associated with immediate breast reconstruction (IBR). Two patients received R-NSM and IBR with robotic assisted harvest of latissimus dorsi flap (RAHLDF), and 29 patients received R-NSM and IBR with Gel implant procedures. One patient received robotic assisted quadrantectomy for upper outer located large breast cancer and immediate partial breast reconstruction with RAHLDF. The other patient received endoscopic assisted partial mastectomy for upper inner located multifocal breast cancer and immediate partial breast reconstruction with robotic assisted harvest of omentum flap.

Among those patients who received R-NSM, the mean operation time for R-NSM (after set-up of robotic breast surgery system) was  $115.6 \pm 50$  mins, and  $70.2 \pm 23.2$  mins for Gel implant reconstruction. The docking time was quickly dropped from 20 mins to 6-8 mins, and the time needed to complete R-NSM could usually be completed within 100mins after accumulated cases' experience. The mean blood loss was  $35 \pm 37.2$  ml. The positive surgical margin rate for R-NSM was 2.6%(1/39), which was superficial margin involvement, and no further surgery was performed. About 13% patients suffered from transit nipple ischemia change, and no total nipple areolar complex necrosis case was observed.

Among those 3 patients who received RAHLDF, it took about 267 mins, 97 mins, and 90 mins to complete the 1st, 2nd, and 3rd RAHLDF, separately. All of them were event free, except seroma formation over the back, which relived after repeat aspiration. No local recurrence, or mortality was found among these 35 patients during mean  $8.9 \pm 4.2$  months follow-up. The patient-reported survey shows that 97%(32/33) of the patients who received robotic breast surgery with breast reconstruction satisfied the cosmetic outcome.

**Conclusion:** From our preliminary experience, robotic breast surgery is a feasible and safe option for some selected indications of breast cancer patients. R-NSM and IBR with Gel implant or RAHLDF were the most frequent performed operations. Bilateral R-NSM could be safely performed in bilateral breast cancer patients or unilateral breast cancer patients combined with CPM.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-26

Management of infected breast prosthesis with negative pressure wound therapy: A novel technique

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**Background:** Although treatment of mild peri-prosthetic infection in implant-based breast reconstruction results in high rates of resolution, successful management of severe peri-prosthetic infection remains a significant challenge. Severe infection is defined when the patient has a significant systemic infection, purulent drainage or aggressive or atypical organisms in peri-prosthetic fluid (e.g., *Pseudomonas* or Gram-negative rods). The traditional management of patients with severe peri-prosthetic infection has been explantation and delayed breast reconstruction.

**Methods:** A protocol utilising a novel dressing - negative pressure wound therapy with instillation (NPWTi) - for the management of peri-prosthetic infection in breast reconstruction patients has been developed by surgeons in Westmead Breast Cancer Institute. This is an operative technique involving: (1) explantation of the breast prosthesis and application of the NPWTi dressing to the implant pocket, (2) change of the NPWTi dressing, (3) serial intra-operative fluid/tissue cultures, and (4) re-implantation of the breast prosthesis when cultures revealed no growth.

**Results:** This protocol was utilised in 22 of severe peri-prosthetic infection in 20 patients with immediate breast reconstruction for breast cancer or risk-reducing surgery. Peri-prosthetic infection occurred during the Direct to implant reconstruction in 9 cases, first-stage (expander/implant) in 11 cases and following the second-stage in 2 cases. Cultures of fluid/tissue grew *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia Marcescens*, *Staphylococcus haemolyticus*, *Staphylococcus aureus* and *Klebsiella oxytoca*. Only 3 cases did not yield an organism on culture. Based on the type of microorganisms, different solutions including normal saline, Prontosan® and 1% acetic acid were used for instillation of the breast prosthesis pocket. Successful implant salvage was achieved in 18 of 22 cases. The median length of stay and time to achieve negative culture were 4(7-18) days and 3(0-14) days respectively.

**Conclusions:** In patients with severely infected breast prostheses, timely operative intervention and usage of NPWTi can salvage the previously "unsalvageable" implant. This treatment option could be considered for management of suitable candidates with severe peri-prosthetic infection.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-27

Impact of surgery on survival in patients with stage IV breast cancer: A population-based study from the SEER database

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Surgical treatment for stage IV breast cancer remains controversial. Therefore, we aimed to further explore the impact of surgery on survival in patients with stage IV breast cancer using the Surveillance, Epidemiology, and End Results (SEER) database. We selected 3,822 patients diagnosed with stage IV breast cancer from 2010 to 2012 in the SEER database who were divided into surgery and non-surgery groups. We assessed the breast cancer-specific survival (BCSS) and overall survival (OS) of the two groups using Kaplan-Meier plots and Cox proportional hazard regression models. In addition, we performed stratification analyses of breast subtype, tumor size and status of distant metastasis to identify the effects of these factors on surgical outcomes. The median survival times were 30 and 24 months in the surgery and non-surgery groups, respectively. The Kaplan-Meier curves showed that the surgery group survived longer than the non-surgery group. The hazard ratios (HR) of the surgery group were 0.504 for BCSS and 0.507 for OS ( $P<0.001$ ). Furthermore, patients in the surgery group still experienced significantly better survival than those in the non-surgery group when stratified according to breast subtype, tumor size and status of distant metastasis. Surgical treatment appears to be associated with improved survival in patients with stage IV breast cancer, independently of breast subtype, tumor size and status of distant metastasis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-28

Local surgery improves survival in patients with primary metastatic breast cancer: A population-based study

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## Background

The aim of this study was to assess the prognosis of breast cancer patients with distant metastasis after surgery of the primary tumor and provide new understanding of treatment for these patients.

## Methods

We retrospectively analyzed 8922 primary metastatic breast cancer (PMBC) patients from Surveillance, Epidemiology, and End Results (SEER) Database. The patients' clinical and pathological characteristics were investigated. Overall survival (OS) and breast cancer-specific survival (BCSS) were calculated by Kaplan-Meier method. Independent risk factors associated with disease special mortality were identified by Cox regression analysis.

## Results

A total of 8922 PMBC patients from SEER database were identified, while 1724 (19.3%) of them received surgery therapy (ST) and 7198 (80.1%) patients were identified as no surgery therapy group (NST). Patients' underwent ST had dramatically increased OS ( $P < 0.001$ ) and BCSS ( $P < 0.001$ ) compared with those in NST group. And the result figured out that the patients with 1 or 2 distant metastatic sites might benefit most from local surgery, while the prognosis of patients with 3 or more showed no significant difference ( $p = 0.073$  for OS,  $p = 0.091$  for BCSS).

Furthermore, no differences were found in the survival rates between different surgical procedure groups ( $p = 0.886$  for OS,  $p = 0.943$  for BCSS). PMBC patients with ST tended to have increased OS (hazard ratio [HR] = 0.642; 95% confidence interval [CI], 0.583-0.707,  $p < 0.001$ ) and BCSS (HR = 0.649; 95% CI, 0.587-0.718,  $p < 0.001$ ), while age, grade, T stage, breast subtype, radiation, the number of metastatic site were also independent prognostic factors.

## Conclusions

The current study demonstrated the survival benefit of ST in PMBC patients, especially those has 1 or 2 distant metastatic sites. Enlarged surgical range could not prolong survival. However, a large randomized clinical trial to validate the efficacy of ST in PMBC is essential in the future.

**Key words:** primary metastatic breast cancer; SEER Database; survival; surgery

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P2-14-29

Image guided surgery for tumor detection in breast cancer using the PH activated micellar tracer ONM-100: The SHINE study

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**Background:** Currently, no reliable intra-operative tumor detection and margin assessment technologies during breast conserving surgery are available. Fluorescence-guided surgery (FGS) using tumor specific fluorescent tracers can improve intra-operative tumor detection. However, a major limitation is the lack of broad tumor applicability due to complex oncogenotypes and histologic phenotypes. A strategy to overcome this challenge is targeting metabolic vulnerabilities that are more ubiquitous and regarded as generic hallmarks of cancer. The extracellular environment of tumors is relatively acidic compared to healthy tissue due to aerobic glycolysis, the so-called Warburg effect. ONM-100, a micellar polymer tracer labeled with the fluorescent imaging dye Indocyanine Green (ICG), has an exquisitely pH-sensitive binary on/off mechanism. The micelles dissociate in acidic environments causing the unquenching and fluorescent activation of the ICG dye. As most solid cancer types are acidotic, ONM-100 acts as a generic tracer targeting a broad range of tumors. This proof of concept, first in-human study, investigates the safety and feasibility of ONM-100 as an intra-operative fluorescent tracer in breast cancer (BC) patients.

**Methods:** In this phase 1 study, the pH-activated fluorescent tracer ONM-100 was administered 24±8h prior to surgery in a dose escalation scheme ranging from 0.1 mg/kg to 0.8 mg/kg in groups of 3 patients each. Patients with biopsy proven BC were included. Patients that had undergone neoadjuvant therapy were excluded. Blood was drawn up to day 10 to assess safety and pharmacokinetic data. Intra-operative images were collected of the tumor before and after excision and of the wound bed. Immediately after excision ex vivo fluorescence images were obtained from the serially sliced specimen and the formalin fixated paraffin embedded tissue blocks. Fluorescence images were correlated with histopathological assessment on Hematoxylin and Eosin (H/E) stained sections.

**Results:** In this ongoing clinical trial, 4 patients with BC were enrolled between March and May 2018. No tracer related (serious) adverse events were observed. A strong and sharply demarcated fluorescent signal in tumor tissue was observed in all 4 patients with in- and ex vivo imaging (median Contrast to Noise Ratio 6.5; IQR 7.25), which correlated with areas of tumor involvement on histopathology. In one BC patient, an intra-operatively unnoticed tumor positive margin was detected using fluorescence imaging. Additionally, a BC satellite lesion was detected, which was otherwise missed by the pathologist.

**Conclusion:** Preliminary results of this ongoing first in-human study with the pH-activated tracer ONM-100 shows that ONM-100 is well tolerated and safe and allows fluorescent tumor visualization both in- and ex vivo. Here, we provide the first data that this pH-sensitive optical tracer can be used as a tracer for FGS and for margin detection. Further analysis on microscopic biodistribution of ONM-100 is currently being performed and possibilities for metastatic lymph node detection will be explored.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P2-14-30

Survival outcomes of breast conserving surgery versus mastectomy for ultrasound detected non-palpable breast cancer in hospital-based screening among Chinese women

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**Background:** Some population-base studies have reported similar or improved survival for breast-conserving surgery (BCS) plus radiotherapy compared with mastectomy (Mx) in early breast cancer [PMID: 22373563, 27344114]. Among the screening detected early breast cancer, ultrasound (US) could detect more invasive non-palpable breast cancer (NPBC) with positive lymph nodes in hospital-based asymptomatic Chinese women, who could achieve comparable 10-year DFS and OS as mammography (MG)-detected NPBC [2016 SABCS P5-02-05, PMID: 27689334]. However, there is little data about the surgical outcomes of BCS verse Mx in the low-risk screening detected NPBC with US as the initial imaging test.

**Methods:** From 2001 to 2017, 6,423 consecutive asymptomatic women underwent mammography or ultrasound guided biopsy in Peking Union Medical College Hospital. Among them, 1130 NPBC including 914 US-detected and 216 MG-detected NPBC were diagnosed and treated. There were 349 (30.9%) patients underwent BCS including 286 (25.3%) patients received radiation therapy and 63 (5.6%) elderly patients (>70 years) who did not. The clinicopathological features, treatment choice, 10-year disease-free survival (DFS) and overall survival (OS) were compared between breast conservingsurgery (BCS) versus mastectomy(Mx) in all NPBC and between the US-detected and MG-detected NPBC.

**Result:** Compared to those who received BCS, the 781 (69.1%) patients who underwent Mx had more cancers with relatively higher histologic grade ( $p=0.003$ ), positive lymph node (18.8% vs 12.0%,  $p=0.005$ ), ER-negative (22.5% vs 11.5%,  $p<0.001$ ), PR-negative (29.6% vs 16.3,  $p<0.001$ ), Her2-positive (16.3% vs 8.9%,  $p=0.001$ ), and received chemotherapy (37.6% vs 28.7%,  $p=0.003$ ). The breast conserving rates of US-NPBCwere higher than that of MG-NPBC (32.6% vs 23.6%,  $p=0.010$ ), but the breast conserving rates were similar between ductal carcinoma in situ (DCIS) and invasive cancers. The 10-year DFS and OS were similar among BCS with radiation therapy, BCS without radiation therapy and Mx as well as among US-NPBC with BCS, US-NPBC with Mx, MG-NPBC with BCS and MG-NPBC with Mx.

Table 1. Kaplan-Meier estimated 10-year DFS and OS of all NPBC§

Patients (No.)	NPBC Group	Number (%)	10-year DFS (%)	P value	10-year OS	P value
All NPBC (1130)	BCS without Radiotherapy	63 (5.6)	85.0	0.105	92.3	0.722
	BCS with Radiotherapy	286 (25.3)	92.7		99.5	
	Mastectomy	781 (69.1)	93.2		98.7	
All NPBC (1130)	US+BCS	298 (26.4)	90.4	0.248	96.3	0.542
	US+Mx	616 (64.5)	92.4		98.4	
	MG+BCS	51 (4.5)	90.3		100.0	
	MG+Mx	165 (14.6)	96.1		100.0	

§ Kaplan-Meier survival curves would be displayed in the poster.

However, MG-NPBC with Mx had favorable 10-year DFS than that of MG-NPBC with BCS ( $p=0.041$ ).

**Conclusion:** The 10-year DFS and OS of breast conserving surgery versus mastectomy were similar among all NPBC patients. As the current initial imaging test, US-detected NPBC patients would receive significantly more BCS compared to MG. There was no significant difference in surgical outcomes among BCS and Mx in US-detected NPBC. However, among MG-detected NPBC, patients with Mx reached a better DFS but a similar OS than those with BCS. The radiation therapy could be safely omitted in the elderly patients (>70 years) with NPBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-01

Circulating tumor cells (CTCs) after neoadjuvant chemotherapy for triple negative breast cancer (TNBC)

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**Background:** ARTEMIS (A Randomized, TNBC Enrolling trial to confirm Molecular profiling Improves Survival) is a randomized trial to determine if precision guided neoadjuvant chemotherapy (NAC) impacts rates of pathologic complete response in the breast and axillary nodes (pCR). We hypothesized that CTCs in peripheral blood after completion of NAC would provide prognostic information beyond pCR alone in TNBC patients.

**Methods:** Blood was assessed for CTCs after NAC as part of two IRB approved studies, ARTEMIS (2014 – 0185/PA15-1050), and LAB04-0698. CTCs were identified using the Cell Search® System (Menarini Silicon Biosystems). Samples with one or more cells, also having morphologic criteria for malignancy, were deemed CTC positive. Log-rank test and Cox regression analysis were applied to evaluate associations between CTC positive, pCR, and overall survival.

**Results:** pCR was achieved in 24/68 (35%) patients with TNBC. Twenty four patients (35%) were CTC positive. Three year overall survival was evaluated in 4 groups of patients: pCR and no CTCs (n=20), pCR and CTC positive (n=4), non-pCR and no CTCs (n=24) and non-pCR and CTC positive (n=20). Three year overall survival was higher in the pCR and no CTCs cohort (100%), compared to pCR and CTC positive (50%), non-pCR and no CTCs (83%), non-pCR and CTC positive (19%); log rank  $p < 0.0001$ . In the non-pCR and CTC positive patient cohorts, the presence of CTCs was associated with significant risk of death at 3 years [hazard ratio of 12.3 (95% CI 3.4-454,  $p = 0.00002$ )], whereas a favorable, but non-significant trend was noted for pCR [hazard ratio of 0.2 (95% CI 0.0, 1.4,  $p = 0.11$ )].

**Conclusion:** The identification of CTCs after NAC has prognostic significance beyond that of pCR, and should be considered in evaluation of patients for clinical trials of adjuvant therapies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-02

Detection of circulating tumor cells (CTC) in blood and disseminated tumor cells (DTC) in bone marrow at surgery identifies breast cancer patients (pts) with long-term risk of distant recurrence and breast cancer-specific death

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We examined the prognostic impact of CTCs and DTCs detected at the time of definitive surgery in pts diagnosed with early breast cancer (EBC).

**Methods:** Blood and bone marrow samples from 742 treatment-naïve EBC pts, not eligible for neoadjuvant therapy, were collected immediately prior to surgery. 87% were hormone receptor (HR)-positive, and 71% were node-negative. DTCs (n=584) were enumerated using an EPCAM-based method involving immunomagnetic enrichment and flow cytometry (IE/FC). CTCs were enumerated either by IE/FC (n=288) or CellSearch (n=380). Optimal cutoffs for CTC-/DTC-positivity were selected using Monte-Carlo cross validation. Multivariate Cox regression analysis was performed to determine correlation between levels of CTCs/DTCs vs. distant recurrence-free survival (DRFS) and breast cancer-specific survival (BCSS). The overall median follow-up was 7.1 years for DRFS and 9.1 years for BCSS, but extended up to 13.3 years in subset analyses (Table 1).

**Results:** CTC-positivity by CellSearch was associated with HER2-positivity (Fisher p=0.01). Using optimized cutoffs in multivariate analyses, we found that CTC-positive pts by CellSearch had a statistically significant increased risk of distant recurrence (HR 4.93, p=0.0067). Moreover, pts who were CTC-positive by IE/FC had a statistically significant increased risk of breast cancer-specific death (HR=3.54, p=0.0138). DTC status, by itself, was not prognostic; however, when combined with CTC status by IE/FC (n=273), positive detection for both (CTC+DTC+) was significantly associated with increased risk of distant recurrence (HR=3.09, p=0.0270) and breast cancer-specific death (HR=4.55, p=0.0205).

Table 1. Multivariate analysis to determine the prognostic significance of CTCs and DTCs detected at the time of surgery in treatment naïve early breast cancer patients. Adjusted for age at diagnosis, tumor size, pathologic stage, HR and HER2 status, node status and grade.

		DRFS			BCSS		
Variable and Method	% positive	HR [95% CI]	Wald p-value	Median f/u [range] Years*	HR [95% CI]	Wald p-value	Median f/u [range] Years*
CTC+ vs. CTC- by CellSearch	9	4.93[1.56-15.6]	<b>0.0067</b>	6.4 [0.16-13.8]	4.50[0.76-26.5]	0.0962	7.5 [0.71-15.0]
CTC+ vs. CTC- by IE/FC	40	1.92[0.93-3.95]	0.0759	9.8 [0.09-18.5]	3.54[1.29-9.72]	<b>0.0138</b>	13.3 [1.93-18.5]
DTC+ vs. DTC- by IE/FC	18	1.46[0.75-2.81]	0.2631	7.5 [0.09-18.5]	1.48[0.64-3.42]	0.3542	9.8 [1.55-18.5]
CTC+DTC+ vs. CTC-DTC- by IE/FC	8**	3.09[1.14-8.40]	<b>0.0270</b>	9.8 [0.09-18.5]	4.55[1.26-16.39]	<b>0.0205</b>	13.3 [1.93-18.5]

\*f/u - follow-up; \*\*double positive

**Conclusions:** We demonstrate the impact of quantitative evaluation of CTCs and DTCs by IE/FC. Our large single institution dataset, in which CTCs and DTCs have been contemporaneously quantitated, has the longest patient follow-up. Simultaneous detection of CTCs and DTCs at the time of definitive surgery in treatment naïve EBC pts is an independent prognostic factor associated with increased long-term risk of distant recurrence and death due to breast cancer. Given the lack of early endpoints for low-risk patients, liquid biopsy may be an important consideration for future studies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-03

Circulating tumor cells (CTCs) with epithelial to mesenchymal transition (EMT) phenotype are associated with inferior outcome in primary breast cancer

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**Background:** CTCs comprise heterogenous population of cancer cells with different clinical and biological value. Epithelial-mesenchymal transition (EMT) leads to generation of cells with cancer stem cell properties and increased resistance to chemotherapy and radiation therapy. While the prognostic value of CTCs with epithelial phenotype was repeatedly demonstrated in primary as well as metastatic breast cancer, prognostic value of CTCs with EMT phenotype (CTC\_EMT) remained unknown. The aim of this study was to evaluate the prognostic value of CTCs with EMT phenotype in primary breast cancer (PBC) patients.

**Methods:** This study included 432 primary breast cancer patients treated by surgery and adjuvant therapy from March 2012 to February 2015. CTC\_EMT were detected before surgery by quantitative RT-PCR assay. Peripheral blood mononuclear cells (PBMC) were depleted of hematopoietic cells using RossetteSep<sup>TM</sup> negative selection kit. RNA extracted from CD45-depleted PBMC was interrogated for expression of EMT transcription factors (TWIST1, SNAIL1, SLUG, ZEB1) by qRT-PCR. Patient samples with higher EMT genes transcripts than those of healthy donors (n=60) were considered as CTC positive. Herein, we report the impact of CTC\_EMT on disease-free survival (DFS).

**Results:** CTC\_EMT were detected in 76 (17.6%) patients. Patients CTC\_EMT had significantly inferior DFS compared to patients without CTC\_EMT (HR = 2.46, 95%CI 1.29 – 4.68, p = 0.0003). Estimated 2- and 5-year DFS for CTC\_EMT negative vs. CTC\_EMT positive patients was 93.4% and 85.5% vs. 86.9% and 58.1%, respectively. Prognostic value of CTC\_EMT was demonstrated in all subgroups of patients, most pronounced in hormone receptor positive, HER2 negative subgroup. In multivariate analysis, presence of CTC\_EMT, axillary nodal involvement and hormone receptor status were independently associated with DFS (**Table 1**). Presence of CTC\_EMT was not associated with any patients/tumor characteristics except p53 status (CTC\_EMT were present in 20.7% of p53 negative vs. 12.4% p53 positive tumors, p = 0.04).

**Conclusions:** In this translational study, we demonstrate for the first time the prognostic value of CTC with EMT phenotype in primary breast cancer. Presence of CTC\_EMT could lead to better identification of patients with increased risk of recurrence, especially in hormone receptor positive, HER-2 negative primary breast cancer patients.

## Multivariate analysis of factors associated with disease free survival

Variable	HR (95%CI)	P – value
CTC with EMT phenotype		
Present vs. absent	2.46 (1.48-4.10)	0.0005
N stage		
N+ vs.N0	2.92 (1.78-4.76)	0.00001
ER/PR status		
Positive for either vs. Negative for both	0.40 (0.23-0.71)	0.001



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-04

**DETECT V – Expression analysis of human epidermal growth factor receptor 2 and estrogen receptor on circulating tumor cells of metastatic breast cancer patients**

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**Introduction:** The prognostic relevance of Circulating Tumor Cells (CTCs) in patients with metastatic breast cancer (MBC) has been shown in multiple clinical trials. Beside their quantification it is of particular interest to analyze the predictive value of these cells and investigate if therapeutic decisions can be based on the phenotype of CTCs. The DETECT study concept evaluates this possible implication of CTCs. The DETECT V study compares endocrine therapy vs. chemotherapy, both combined with Trastuzumab and Pertuzumab in MBC patients with a HER2-positive (Human Epidermal Growth Factor Receptor 2), hormone receptor-positive tumor. Translational projects to identify potential predictive markers are associated to the study program. The expression of predictive markers HER2 and estrogen receptor (ER) on CTCs is analyzed to establish and validate an “Endocrine Responsiveness Score” (ERS). The ERS is aiming to estimate the potential benefit of endocrine therapy.

**Methods:** Quantification and characterization of CTCs was performed using Cell Search® CXC Kit. The staining protocol was validated using cell lines with known expression of ER and HER2. CTCs were quantified at randomization, after six weeks and at the end of treatment in two samples. Staining intensities of both markers were specified.

**Results:** Staining was successfully established. ER staining intensity was specified as negative or positive; HER2 staining intensity as negative, weak, moderate or strong. Detection and characterization of CTCs was analyzed for the first 30 patients. At time of randomization 17 of 26 samples were CTC positive with  $\geq 1$  CTC in one sample (HER2 and ER), 6/25 in both samples. After six weeks 8 of 26 patients contained  $\geq 1$  CTC in one sample and 5 of 26 in both samples. 11 of 15 CTC positive patients at time of randomization were CTC negative in the subsequent sample after six weeks. The cumulative frequency of ER- and HER2-positivity regarding CTCs detected in all samples was analyzed. 13% of all CTCs detected were ER positive (14/115). The percentage of HER2 3+ CTCs decreased from 27% at time of randomization (25/92) to 0% after six weeks (0/87). Analyzing the distribution of the expression of ER and HER2 in all CTCs of one sample, 10 of 19 samples containing  $\geq 2$  CTC showed heterogenic intensity of HER2-specific immunofluorescence. 8 of 14 samples containing  $\geq 2$  CTC showed heterogenic intensity of ER-specific immunofluorescence, respectively.

**Conclusion:** The implementation of “Endocrine Responsiveness Score” ERS aims to predict the potential benefit of endocrine therapy in patients with a HER2-positive, hormone receptor-positive tumor. Descriptive analysis of first patient samples has shown to detect heterogenic expression of HER2 and ER in CTCs of patients participating in the DETECT V study.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-05

Circulating tumor cell detection predicts early recurrence in patients with non-metastatic breast cancer

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## Background:

Our group and others have shown that CTCs are identified in 20-25% of non-metastatic breast cancer patients. Recent data suggests that circulating tumor cell (CTC) detection at 5 years follow-up predicts late recurrence for non-metastatic, estrogen receptor positive, HER2/neu negative (ER+/HER2-) breast cancer patients. The aim of this study was to determine if CTC detection at time of surgery in ER+/HER2- predicts early (less than or equal to 5 years) recurrence, and to compare the median recurrence-free survival (RFS) to triple negative (TN) patients.

## Methods:

We performed CTC enumeration on 506 patients with non-metastatic breast cancer just prior to surgical resection as part of an IRB approved study. CTCs (per 7.5 ml blood) were identified using the Cell SearchSystem (Menarini Silicon Biosystems). The presence of one or more CTC meeting morphological criteria for malignancy was considered a positive result. Patients with inflammatory breast cancer were excluded from our analysis. Log-rank test and Cox regression analysis were applied to establish the association of CTCs with RFS.

## Results:

Median follow-up was 68 months and mean age was 53 years. Eighty-four percent of patients (417/498) had T1/T2 tumors, 63% (307/487) had grade 1 or 2 tumors, and 58% (292/501) had negative lymph nodes. Eighty-three percent (419/506) had ER+/HER2- tumors, and 17% (87/506) were TN. One or more CTC was identified in 22% (91/419) ER+/HER2- patients, and in 28% (24/87) of TN patients. Nine percent (45/506) of patients recurred within 5 years of CTC assessment. Of the 45 patients who relapsed, detection of one or more CTCs predicted shortened RFS. Median RFS for CTC-positive patients was 1.2 years, vs. 2.5 years for CTC-negative patients, irrespective of ER+/HER2- or TN subtype (log-rank  $P < 0.001$ , HR = 2.71, 95% CI, 1.50 to 4.87).

**Conclusions:** One or more CTCs identified at surgical resection predicted shortened RFS, irrespective of subtype, in non-metastatic breast cancer patients. This data warrants larger studies to determine if CTC positivity can be used to stratify both ER+ and TN patients who are at increased risk for early recurrence.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-06

Correlation of disseminated or circulating tumor cells with the OncotypeDX recurrence score

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**Background:** New biomarkers continue to emerge to predict the risk of recurrence in women with early stage breast cancer. A high OncotypeDX Recurrence Score (RS)® has been found to be associated with worse disease-free and overall survival in patients with early stage breast cancer. Similarly, circulating tumor cells (CTCs, blood) and disseminated tumor cells (DTCs, bone marrow) have prognostic value in patients with breast cancer. We sought to evaluate the association between high RS and CTCs and DTCs.

**Methods:** We evaluated patients with hormone receptor positive, HER2 negative, node-negative invasive breast cancer from a prospective database from 1/2005 to 1/2017. RS was classified based on the new TAILORx study cutoff points as low (<11), intermediate (11-25), and high (>25). CTCs were assessed using CellSearch. For DTCs cytospin specimens of bone marrow aspirates, enriched for epithelial cells by density gradient separation, were immunostained using a pancytokeratin cocktail of antibodies, including AE1/AE3, CAM5.2, MNF116, cytokeratin 8 (CK8), and CK18. CTCs and DTCs were considered to be positive if one or more CTCs or DTCs were identified, respectively. Chi square analyses were utilized to evaluate for a relationship between OncotypeDX RS and CTCs or DTCs. Statistical analyses were performed in SPSS version 24 with p value <0.05 considered significant.

**Results:** Two hundred and thirty patients meeting the above criteria were identified from a prospective database, of which 106 had OncotypeDX testing results. Of the patients with available OncotypeDX data, 93 patients had CTC results and 60 patients had DTC results. CTCs were detected in the blood of 18/93 (19.4%) patients, while DTCs were detected in the bone marrow of 20/60 (33.3%) patients. Patients with high RS were not more likely to have CTCs as compared with patients who had low or intermediate RS (16.7% vs 19.8%, p=0.801). Similarly, high RS was not associated with the detection of DTCs, with DTCs present in 44.4% of patients with high RS, compared with 31.4% of patients with low or intermediate RS (p=0.443). In the subgroup of patients ≤50 years of age no associations were found between high RS and CTCs (p=0.720) or DTCs (p=0.151).

**Conclusions:** High OncotypeDX RS did not correlate with CTCs in the blood or DTCs in the bone marrow in our study.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-07

Enabling HER2 and androgen receptor (AR) protein expression and localization in circulating tumor cells (CTCs) of ER(+/-)HER2(-) metastatic breast cancer (MBC) patients (pts)

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## **Background:**

Upregulation of HER2 and AR are mechanisms of acquired resistance to endocrine therapy, and are being investigated as treatment-guiding biomarkers. However, measurement of these proteins and their localization requires metastatic biopsies, which are costly, invasive, and prone to under-sampling which limits their utility to guide treatment in late stage metastatic patients. A CTC-based test could expand the clinical utility of these biomarkers. Here we utilized the Epic Sciences CTC platform for CTC detection and characterization. MBC blood samples were characterized for CTC prevalence, HER2 and AR expression at time of disease progression.

## **Material and methods:**

HER2 and AR expression levels were determined based on model cell lines. A total of 72 blood samples were acquired from ER(+/-)/HER2(-) patients (by standard tissue pathology) at disease progression. 72 samples were analyzed for HER2 and 64 were analyzed for AR using the Epic Platform. Single-cell whole genome sequencing was performed to assess clonality and inter-patient heterogeneity of CTCs detected.

## **Results:**

55/72 (76.4%) of patients had CTCs detected across two slides. 13/72 (18.1%) had at least one HER2(+) CTC, 14/64 (21.9%) had at least one AR(+) CTC, and 7/64 (10.9%) had at both AR(+) and HER2(+) CTCs detected on replicate slides. HER2 expression on individual CTCs showed distinctive cytoplasmic membrane staining, and AR expression on individual CTCs showed frequent nuclear localization. Most patient samples showed heterogeneous expression of these markers at disease progression indicating subclonal sensitivity to targeted therapies. Subsequently, these cells will be individually sequenced to better determine the clonality of resistance.

## **Conclusions:**

CTCs are detected in most MBC pts upon disease progression, with expression of known endocrine therapy resistance markers, HER2 and AR, observed that CTCs could guide subsequent therapy selection. Prospective evaluation of HER2 and AR on MBC pts' CTCs as predictive biomarkers of benefit from inhibitors of these proteins is needed.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-08

HER2-negative metastatic breast cancer with HER2-positive circulating tumor cells (CTCs): A new CTC-defined HER2-positive subgroup

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**Introduction:** CTCs can overexpress HER2 discordant from tumor HER2 expression. We aimed to describe characteristics of a CTC-defined group of pts with metastatic breast cancer (MBC) that is tumor HER2- and CTC HER2+ (HER2 tumor- CTC+).

**Methods:** We retrospectively analyzed data from pts treated at Northwestern University who had serial evaluation of CTCs and circulating tumor DNA (ctDNA). We included pts with pathologically confirmed HER2- MBC and HER2+ CTCs. CTCs were enumerated with the CellSearch immunomagnetic kit (Menarini Silicon Biosystems), HER2 expression on CTCs was determined using the CellSearch CXC Kit in 7.5 cc whole blood, and ctDNA was analyzed using the Guardant360 NGS assay (Guardant Health).

**Results:** Among 98 pts with HER2- MBC and CTC analysis, 46 (47%) had at least 1 HER2+ CTC. In this cohort the median age was 53. At initial BC diagnosis, 80% had early stage or locally advanced BC and 20% had *de-novo* metastatic disease. Baseline histology was 65% ductal, 20% lobular, 2% mixed ductal and lobular, and 13% unknown. Pathology of metastatic tumor was hormone receptor positive (HR+)/HER2- in 78% and triple negative in 22%. Detailed HER2 immunohistochemistry (IHC) and FISH results from metastases were available from 63% of pts of whom 72% had an IHC score of 0 or 1 and 28% had an IHC score of 2 with negative FISH testing. The median time from the most recent pathologic metastatic tumor assessment to the detection of a HER2+ CTC was 6.5 mo. Twenty-two pts had simultaneous (within 8 weeks) HER2- tumor confirmation and HER2+ CTC detection. The median lines of endocrine therapy (ET) for MBC prior to detection of HER2+ CTCs was 1 (range 0-5, 41% no ET, 17% 1 line, 41%  $\geq 2$  lines). Pts received a median of 2 (range 0-10) prior systemic therapies for MBC prior to detection of HER2+ CTCs, (20% 0 lines, 41% 1-3 lines, and 39%  $\geq 4$  lines). Among these 46 pts, CTCs were analyzed longitudinally in 104 samples, with HER2+ CTCs detected in 77 samples. Number of HER2+ CTCs at initial detection ranged from  $<5$  in 24%, 5-50 in 43%, and  $>50$  in 33%, with a median of 11.5 HER2+ CTCs. CTC clusters were noted in 37% of pts. At initial detection the proportion of CTCs that were HER2+ was 0-25% in 13% of pts, 26-50% in 46% of pts, and 51-100% in 41% of pts. Seven pts had *ERBB2* aberrations in ctDNA. Of 12 pts with tumor sequencing, 2 had *ERBB2* mutations, 1 had *ERBB3* amplification, and 1 had overexpression of *ERBB3* RNA. After detection of HER2+ CTCs, 18 pts received HER2 directed therapy (with chemotherapy in 13 pts, with endocrine therapy in 4 pts, and as monotherapy in 1 pt). Imaging demonstrated a partial response or stable disease in 9 pts (clinical benefit rate 50%), including in 1 pt with trastuzumab monotherapy, progressive disease in 8 pts, and not evaluated in 1 pt.

**Conclusions:** HER2+ CTCs are frequently detected simultaneously or soon after HER2- tumor assessment in MBC. Within this newly defined subgroup, the several responses seen with HER2 targeted therapy serve as a proof of concept that HER2 tumor-CTC+ patients can benefit from HER2 targeted therapy. Future studies are needed to determine a clinically relevant threshold for HER2+ CTCs to guide further study of HER2 therapy combinations in HER2 tumor- CTC+ pts.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-09

Tracing clonal evolution of circulating tumor cells and cell-free DNA in a metastatic breast cancer patient

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**Background:** Copy number variation analysis has been shown as a valuable tool to infer clonal evolution of tumor cells. While bulk genomic analysis of FFPE tissue can provide information about the characteristics of a primary tumor or a metastatic nodule, these biopsies are rarely repeated due to their invasive nature. Liquid biopsies on the other hand, provide minimally invasive access to tumor derived cells and DNA and can therefore be used to monitor genomic changes of the tumor longitudinally and in real time. Here we evaluate the kinetics of liquid biopsy biomarkers and monitor genomic changes in single circulating tumor cells (CTCs) and cell-free DNA (cfDNA) from 17 longitudinal blood draws collected from a metastatic breast cancer patient over seven treatment regimes. FFPE tissue from the primary tumor and two metastatic sites in liver and bone are available for comparative analysis.

**Methods:** We used the high definition – single cell assay (HD-SCA) platform to detect and enumerate CTCs of the blood of one metastatic breast cancer patient followed for 4 years. CTCs were characterized morphometrically and single CTCs of each draw were sequenced for copy number variation (CNV). cfDNA was extracted from plasma and tissue samples of the primary breast, bone and liver metastasis were micro-dissected. Extracted DNA from tissue samples and plasma underwent CNV analysis and were compared with CNV data of CTCs.

**Results:** CTCs were identified in 14/17 draws and varied from 0 to >3000 cells/ml, spiking at two different times concordant with clinical progression. Changes in cell free tumor DNA fraction (ctDNA) correlated strongly with CTC count. Comparative CNV analysis of CTCs, cfDNA and FFPE tissue confirmed the tumorous origin of both cells and cfDNA. Single cell CNV analysis found four genomically related sub-clones in the patient's blood, three of which were present at time of enrollment, while the fourth clone emerged 3.5 years later concordant with the decline in the patient's health. Genomic copy number losses included tumor suppressor genes such as ARID1A, FOXP1, ATM, BRCA2, RB1, CDH11 and CDH1. Genomic gains were limited but included the proto-oncogenes BCL9, ABL2 and MDM4. It is striking that the sub-clonal structure persisted nearly unchanged for 3.5 years throughout various hormone and cytotoxic treatments, indicating high genomic stability. Despite their genomic differences, cells from the different clones are indistinguishable by immunofluorescence or morphometric analysis. CNV profiles of cfDNA reflect the most abundant CTC clone at each time-point.

**Conclusion:** Using the HD-SCA assay cellular (CTC), and soluble (cfDNA) fractions of liquid biopsies can be compared at the molecular level over a long time course, and verified by comparison with tissue biopsy. This work confirms that liquid biopsies can be used to access the molecular state of a patient's cancer in near real-time and provide insight into the response to treatment years after the original characterization of the primary tissue. Here we show in a single patient example the power of single CTC analysis and highlight how CNV analysis of liquid biopsies could serve as minimally invasive tool to monitor tumor evolution longitudinally.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-10

Associations between plasma Interleukin 2 (IL-2) and HER2 expression in circulating tumor cell (CTC) and MYC alterations in circulation tumor DNA (ctDNA) open a new insight on immune microenvironment for patients with metastatic breast cancer (MBC)

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**Introduction:** Overexpression of HER2 has been reported to be associated with metastasis and poor prognosis of patients with MBC. We reported in AACR 2018 that HER2 overexpression is associated with CTC-cluster. Preclinical data suggested that MYC and HER2 cooperate to drive stem cell phenotype and poor prognosis in MBC (Nair R). Furthermore, IL-2 upregulates the transcription of MYC (Grigorieva I) and gets involved into its alterations. We reasoned that further understanding of interactions of HER2 in CTC and MYC will be important to elucidate the mechanism of metastasis of MBC. Herein, we report a significant correlation between the plasma IL-2 level and HER2 expression in CTCs, and the IL-2 related MYC ctDNA alterations in MBC.

**Methods:** This study enrolled 43 patients with stage III/IV BCa at the Northwestern Memorial Hospital (2016-2017) that had longitudinally detection of CTCs and ctDNA. Whole blood samples (7.5ml/each) were collected for CTCs enumeration by using CELLTRACKS ANALYZERII® System (Menarini) contains antibodies of anti-EpCAM for capturing CTCs, anti-CK-PE for epithelial cells, DAPI for nucleus, anti-CD45-APC for leukocytes and anti-HER-2/neu-FLU. The CTCs were classified based on phenotype as CK<sup>+</sup>, EpCAM<sup>+</sup>, DAPI<sup>+</sup> and CD45<sup>-</sup>. Plasma ctDNA was analyzed using the Guardant360™ NGS-based assay (Guardant Health), a 73 genes panel. ELISA for IL-2 was performed by using patients' plasma. Database of IL-2, HER2, CTCs and ctDNA was linked with clinical database and analyzed by Kruskal-Wallis test.

**Results:** CTCs  $\geq 5$  were found in 20 patients (46%). There were 15 patients that had HER2 negative CTCs (Group 1), and 5 patients had HER2 positive (Group 2) CTCs. The level of IL-2 was much higher in Group 1 (88.17pg) compared to Group 2 (66.81pg), indicating that patients with HER2 positive CTCs have significant lower IL-2 than patients with negative CTCs ( $P=0.02$ ). Meanwhile, ctDNA MYC alterations were detected in 10 patients (including 1 L114R mutation, 7 CNV and 2 SNV) who have the average IL-2 level as 94.00pg. There were 11 patients without any alterations of MYC had average IL-2 level of 70.17pg, which indicated that patients with alterations in the ctDNA MYC have significant higher level of IL-2 in compared with patients without MYC alterations ( $P=0.02$ ).

**Conclusions:** Findings of the correlation between overexpression of HER2 in CTCs and low IL-2 level indicated that low immunity may contribute to more aggressive MBC. And the higher level of IL-2 appear associated predominantly with MYC genomic alterations indicated that overexpression of MYC may also stimulate the immune response by upregulating IL-2 via a reverse feedback pathway. We postulated that increasing IL-2 suppresses the HER2 expression in CTC and breaks cooperation between HER2 and MYC. Although the interactions between them still unknown, our results suggest that IL-2 related immune microenvironment acts as a key player to suppress HER2- and MYC-mediated progress in MBC, including the formation of CTC-cluster. Monitoring and administration of IL-2 may benefit pretreated MBC patients and predict disease metastasis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-11

A novel six-parameter assay for comprehensive phenotyping of circulating tumor cells

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**Background.** The presence and number of circulating tumor cells (CTCs) are prognostic for breast cancer treatment outcome. Direct imaging assays traditionally employ four markers to identify canonical epithelial CTCs: nucleus, exclusion (CD45), and inclusion (EpCAM and cytokeratin). There is intense interest in the ability to phenotype CTCs in order to provide a noninvasive means by which to predict treatment benefit from endocrine therapy and/or HER2-directed therapy in breast cancer. To address this, a 6-parameter assay for detection of ER and HER2 expression on CTCs was developed. We applied this assay to four well characterized breast cancer cell lines representative of various ER and HER2 phenotypes. **Methods.** BT474, MCF-7, SKBR3, or MDA-MB-231 cells were spiked into peripheral blood from healthy donors and processed using the AccuCyte® sample preparation system; nucleated cells, including CTCs, are captured onto glass slides (8 slides per 7.5 mL blood sample) for subsequent immunofluorescent staining. Slides were stained using the combined epithelial marker and ER/HER2 CTC assay and then analyzed with the CyteFinder® imaging system. CTCs were identified as nucleated cells with positive EpCAM and/or cytokeratin staining, and negative CD45 staining. ER and HER2 expression were assessed as present or absent. **Results.** All cell lines expressed both cytokeratin and EpCAM, except for MDA-MB-231 which was EpCAM-negative. The ER / HER2 expression patterns observed were consistent with reported phenotype: BT474 (+/+), MCF-7 (+/-), SKBR3 (-/+), and MDA-MB-231 (-/-). **Conclusions.** Identification of epithelial CTCs and phenotypic characterization of ER and HER2 status are feasible in a combined assay applied to a single blood sample. This approach has implications for efficiency and cost effectiveness, which are of particular importance given the interest in longitudinal testing. Assay evaluation is currently underway using blood samples from breast cancer patients with known receptor status, treatment history, and clinical outcomes. Results will be available for presentation at the meeting.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-12

Clinical safety of diagnostic leukapheresis as a liquid biopsy to collect circulating tumor cells in primary and metastatic breast cancer patients

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**Introduction:** The enumeration of circulating tumor cells (CTCs) has been shown to be of prognostic relevance for neoadjuvant, adjuvant and metastatic setting of breast cancer in multiple clinical trials. Moreover, the serial determination of CTCs enables therapy monitoring in the metastatic setting. One major caveat is the low number of CTCs detected by established methods which limits the possibility for further evaluation including phenotyping and genotyping. Therefore, the clinical use of CTCs as liquid biopsy for making therapy decisions is still under discussion. Diagnostic leukapheresis (DLA) has been previously established by our research group and implemented in the workflow for isolation and detection of CTCs enabling a reliable detection of CTCs at high frequency. The aim of this clinical study was to assess the safety of leukapheresis in 39 patients with primary and metastatic breast cancer.

**Methods:** DLA was performed at least 1d before surgery or chemotherapy. A median blood volume of 2.7 L (range, 1.0 L–5.3 L) was processed. Citrate dextrose solution A was used for anticoagulation with ratios ranging from 11:1 to 24:1. Complete blood count as well as measuring blood pressure and heart rate was performed before start of DLA and immediately after DLA. CTCs were enumerated using the CellSearch system. DLA products containing a median number of  $1.8 \times 10^8$  MNCs were processed.

**Results:** 41 patients were eligible for DLA. Only in two patients DLA could not be performed due to technical problems. Thirty-nine patients underwent leukapheresis. Twenty-six patients had non metastatic breast cancer. Thirteen patients were diagnosed with metastatic breast cancer. Severe adverse events including hypotension, nausea, tingling e.g. resulting in interruption of apheresis were not observed. The DLA did not interfere with the start of chemotherapy or surgery. Complete blood count before and after DLA showed statistic significant but clinically irrelevant decrease in numbers of leukocytes, thrombocytes, hemoglobin and the percentage of hematocrit. In 11/21 DLA samples (52%) of patients with primary breast cancer CTCs were detected. Number of CTCs ranged from 1 to 51. In 11/13 DLA samples (85%) of patients with MBC CTCs were detected. Number of CTCs ranged from 1 to 2913.

**Conclusion:** Establishing a routine DLA protocol we demonstrated that this procedure is clinically safe and can be implemented into the clinical workflow of breast cancer patient care.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-13

Co-expression of molecules associated with innate and adaptive immune response on single CTCs of patients with metastatic breast cancer (mBC)

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## Introduction

The expression of CD47 on tumor cells can act as a "don't eat me signal" against phagocytosis by macrophages and dendritic cells. Moreover, PD-L1-expressing tumor cells inhibit the anti-tumor activity of cytotoxic T cells. Circulating tumor cells (CTCs) expressing these molecules could overcome elimination by the immune system. In the current study, we evaluated for the first time the co-expression of CD47 and PD-L1 on single CTCs from patients with metastatic breast cancer (mBC).

## Methods

Triple immunofluorescence staining was performed on peripheral blood mononuclear cells (PBMC) cytospin preparations from 18 CTC-positive patients with mBC, using antibodies against cytokeratins (for CTC detection), CD47 and PD-L1. Blood samples were obtained before the initiation of first-line chemotherapy. A total of  $1 \times 10^6$  PBMCs were analyzed per patient using the Ariol microscopy system. The expression levels of CD47 and PD-L1 were characterized as high or low/-, after quantification by the Ariol system, using the MDA.MB.231 breast cancer cell line as positive control.

## Results

A total of 23 CTCs (median: 1, range: 1-4) were identified. CD47-expressing CTCs were detected in 94.4% of patients and represented 91.3% of total CTCs. However, high CD47 expression was confirmed in 38.9% and 43.5% of patients and CTCs, respectively. PD-L1 expression was evident in 27.8% of patients and in 21.7% of CTCs, whereas CTCs expressing high levels of PD-L1 were identified in 16.% of patients and represented 13% of total CTCs. Co-expression of CD47 and PD-L1 (CD47<sup>+</sup>/PD-L1<sup>+</sup>) was observed in 21.7% of CTCs, whereas 69.6% of CTCs expressed CD47 only (CD47<sup>+</sup>/PD-L1<sup>-</sup>). No CTCs expressing only PD-L1 (CD47<sup>-</sup>/PD-L1<sup>+</sup>) were detected and 2 of 23 cells were negative for both markers (CD47<sup>-</sup>/PD-L1<sup>-</sup>). Regarding the differential expression levels of CD47 and PD-L1, the phenotype CD47<sup>low/-</sup>/PD-L1<sup>low/-</sup> was the most abundant both at the patient (61.1%) and the CTC level (52.2%). CD47<sup>high</sup>/PD-L1<sup>low/-</sup> CTCs were observed in 33.3% and 34.8% of patients and CTCs, respectively whereas only 1 CD47<sup>low/-</sup>/PD-L1<sup>high</sup> CTC was detected in one patient. Interestingly, CD47<sup>high</sup>/PD-L1<sup>high</sup> CTCs were identified in only 8.7% of CTCs.

## Conclusions

CD47 expression is identified in the great majority of CTCs in mBC and could represent a potent signal to facilitate the escape from innate immune response. PD-L1 expression on CTCs is less commonly observed and could serve for the attenuation of an adaptive anti-tumor immune response. Interestingly, CD47 and PD-L1 are co-expressed in a subset of CTCs, whereas simultaneous high expression on single CTCs is even less common. The expression of these molecules is currently further investigated in a larger cohort of patients with mBC and in patients with early disease, in order to evaluate their differential distribution that potentially reflects the equilibrium and/or escape from the immune surveillance.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-01-14

Diverse inter- and intra-patient circulating tumor cells (CTCs) phenotypic heterogeneity identified in metastatic breast cancer (MBC) cohort

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**Background:** There is unmet need for biomarkers to guide the treatment selection in MBC. We described phenotypic CTC heterogeneity in metastatic castrate resistant prostate cancer(mCRPC), and showed that pts with CTC high heterogeneity have improved OS with chemotherapy, while with pts low CTC heterogeneity pts have longer OS with AR inhibitors (Scher et al. 2017 Cancer Research). Here, the same methodology was applied to evaluate the feasibility of CTC heterogeneity analysis in MBC pts.

**Material and methods:** 92 blood samples from MBC pts were processed for HER2 CTC analysis utilizing the Epic Sciences platform. Following enumeration, multi-dimensional phenotypic characterization analysis was performed utilizing protein expression and digital pathology features. 20 features from each CTC were clustered using unsupervised approach (K-means) and the optimal number of clusters was determined using the elbow method with greater than 85% of variance taken into account. Shannon Index was used to score intra-patient CTC heterogeneity.

**Results:** CTCs were detected in 77.2% (71/92) of the MBC pts. 1501 CTCs from 60 pts were clustered into phenotypic subtypes which are distinctively different in nuclear size, circularity, CK and HER2 intensity etc. HER2 expression was enriched in cell types that had higher CK intensity and was more circular morphologies often associated with replicative stress. A wide range of CTC phenotypic heterogeneity was observed across pts, Shannon Index scores from 0 (low heterogeneity) to 1.67 (high heterogeneity) with a median of 0.60. CTC heterogeneity was independent of CTC counts, some low count pts had heterogeneous CTC subtypes while some high count pts had homogeneous CTC subtypes.

**Conclusions:** Diverse inter- and intra-patient phenotypic CTC heterogeneity were observed in this MBC cohort, consistent with that seen in mCRPC and other MBC cohorts (Beverly H et al, ASCO 2018). Studies linking degree and patterns of CTC heterogeneity to therapeutic outcomes are ongoing.

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Circulating tumor cell subset analysis to assess lifestyle interventions for breast cancer patients after neoadjuvant chemotherapy

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**Background:** Circulating tumor cells (CTCs) are an independent predictor of survival in patients with breast cancer. In addition, mesenchymal (EMT-CTC) and stem-like (Stem-CTC) CTCs contribute to disease progression. The objective of the overall study is to determine whether a comprehensive lifestyle intervention program started prior to radiotherapy can modulate changes in CTC subsets that are correlated with disease recurrence and progression. For these analyses we examined the association between medical and treatment-related factors and CTCs.

**Patients and Methods:** Seventy-eight patients with stage II/III breast cancer were recruited and randomized to either the intervention group or a standard care group. The intervention group (n=42) had in-person lifestyle counseling across the 4-6 weeks of radiotherapy (XRT) followed by video counseling for the subsequent 12 months. The standard care group (n=36) was provided patient-education materials for cancer prevention including information on diet, exercise, and stress management, without counseling. Blood samples were collected prior to initiation of XRT, end of XRT, and at 3-month intervals thereafter for up to 5 years. CTC subsets were detected by AdnaTest EMT2 kit (Qiagen, Venlo, Netherlands). Samples were considered positive for CTCs if any one of breast (EPCAM, MUC1, and HER2), EMT (TWIST1), or stem cell-related (ALDH1, AKT2, and PI3Kalpha) genes were detected by PCR above the manufacturer's suggested threshold.

**Results:** The median age of patients was 49 years (range 26-82 years). Thirty-four patients were overweight (BMI 24.4-30) and 44 patients were obese (BMI >30). Forty-five patients were HR<sup>+</sup>Her2<sup>-</sup>, 12 patients were HR<sup>+</sup>Her2<sup>+</sup>, 5 patients were HR<sup>-</sup>Her2<sup>+</sup>, and 16 patients were TNBC. Sixteen patients were stage IIA or IIB, 34 patients were stage IIIA or IIIB, 27 patients were stage IIIC, and 1 was stage IV. Sixty-seven of 78 patients received neoadjuvant chemotherapy (NACT); 13 patients achieved a complete pathological response (pCR). The median follow-up was 21.6 months. CTC data of both intervention and standard groups were similar at baseline. Presence of CTCs at baseline or follow-up time points was not correlated to HR/Her2 status, stage, obesity, or pCR, but was significantly correlated with receiving NACT. Patients without NACT had significantly higher CTCs than patients who underwent NACT (Fisher Exact Test p=0.010). Furthermore, CTCs by the detection of any gene 3 months after completing XRT was associated with shorter PFS (log-rank p=0.016) and OS (p=0.03).

**Conclusions:** This is an interim analysis of the prognostic potential of CTCs detected by AdnaTest EMT2 kit in non-metastatic breast cancer. We observed a lower proportion of patients with CTCs following neoadjuvant chemotherapy. However, the relative small sample size and short follow-up time preclude drawing conclusions to the efficacy of using CTCs as surrogate measures for lifestyle interventions, although the presence of CTCs in peripheral blood of patients 3 months after radiation therapy can be a promising indicator of disease relapse and overall survival.

# 2018 San Antonio Breast Cancer Symposium ®

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Micro-cavity array system for size-based enrichment of circulating tumor cells and circulating cancer associated fibroblasts from blood of patients with breast cancer

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**Background:** Circulating Tumor Cells (CTCs) have prognostic implications in patients with metastatic breast cancer (MBC). During the Epithelial Mesenchymal Transition (EMT), CTCs acquire a more mesenchymal phenotype. Hence, methodologies such as the Cell search that rely on the expression of an epithelial marker EpCAM in CTCs fail to capture a subset of CTCs undergoing the process of EMT and therefore do not adequately represent the true circulatory metastatic load. Hitachi chemicals has invented a size based micro cavity array (MCA) system that allows for the isolation of tumor cells based on the differences in size and deformability between tumor and blood cells. Photolithography and the metal plating can precisely control the filter pore size of our system. Our platform is more sensitive than the Cell Search method in detecting CTCs in Lung Cancer. Cancer Associated Fibroblasts (CAFs) are a major component of the breast tumor microenvironment. Using a micro filter capture technique, our co-authors have demonstrated that CAFs can be enumerated as circulating CAFs (cCAFs). Hitachi's Micro cavity Array System has not been evaluated in the detection of CTCs and cCAFs in patients with Breast Cancer. The purpose of this study is to demonstrate that CTCs and cCAFs can be enumerated using our platform and the cCAFs can serve as biomarkers of metastasis simultaneously with CTCs.

**Method:** We undertook a Pilot study of 20 patients each with breast cancer across Stage I, Stage II, Stage III and Stage IV. A total of 10ml of peripheral blood was obtained from each patient. Enumeration of CTCs and cCAFs was carried out by the size based microcavity array system invented by Hitachi Chemicals. Identification of these cells was done by a triple Immunofluorescence staining for pan-CK (cytokeratin), FAP (Fibroblast Activated Protein) and CD45. CTCs were identified as CK+, CD45-, FAP- cells and cCAFs were identified as FAP+, CK- and CD 45 negative cells.

**Result:** Our method had a high cell recovery rate (90% or higher) and efficient white blood cells depletion rate (99.99%). We present the data from a total of 13 patients in this abstract, (two with stage III and eleven with stage IV breast cancer). Data from rest of the subjects will be presented at the actual meeting. We detected the presence of CTCs in 11/11 (100%) in patients with stage IV (mean of 44) and in 2 out of 2 (100%) patients with Stage III Breast Cancer. We detected the presence of cCAFs in 1 out of 2 patients (50%) with stage III and in 8 of 11 (81.8%) (mean of 9) patients with stage IV breast cancer (Fisher's exact test p-value = 0.42). The number of CTCs and cCAFs was significantly elevated in patients with MBC and the number was clinically associated with a high metastatic burden.

**Conclusions:** CTCs and cCAFs can be enumerated using a size based size based micro cavity array invented by Hitachi Chemicals that does not rely on the expression of epithelial markers in CTCs. CTCs and cCAFs can be detected in patients with stage III and stage IV breast cancer. CTCs and cCAFs were associated with high metastatic burden and their numbers were significantly elevated in patients with MBC. cCAFs could serve as biomarkers alongside of CTCs in MBC.

## 2018 San Antonio Breast Cancer Symposium ®

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PD-L1 expression on circulating epithelial tumor cells (CETCs) correlates with aggressiveness of tumor in breast cancer patients

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**Background:** Strategies to improve the efficacy of the immune system against malignant tumors represent a major innovation focusing on the programmed death-1 receptor (PD-1), with its two ligands PD-L1 and PD-L2. The expression of PD-L1 has been evaluated in a number of different tumor types and can be used as a predictive biomarker for PD-1/PD-L1 checkpoint inhibitor treatment response. We used a non-invasive, real-time liquid biopsy to better characterize PD-L1 expression on circulating epithelial tumor cells (CETCs) in breast cancer patients.

**Methods:** CETCs were determined from blood of 72 patients suffering from breast cancer. The number of vital CETCs and their expression of PD-L1 was investigated using the maintrac® method.

**Results:** PD-L1 expressing CETCs were detected in 94.5 % of breast cancer patients. Breast cancer patients with metastatic disease had significantly more PD-L1 positive CETCs as compared to patients without metastasis (median 75% vs. 61.1%;  $p < 0.05$ ). Furthermore, patients with positive lymph node status had significantly more PD-L1 positive CETCs as compared to patients with negative lymph nodes (median 60% vs. 93%,  $p < 0.05$ ). Moreover, we observed a significant heterogeneity in PD-L1 immunostaining intensity across CETCs from the same patients.

**Conclusion:** Breast cancer patients have detectable CETCs with a high frequency of PD-L1 which correlates with progression of cancer disease. PD-L1 seems to be a major factor in immune evasion and may be a promising target of anticancer therapies. Monitoring the frequency of PD-L1 positive CETCs could reflect individual patient's response to an anti-PD-1/PD-L1 therapy.

# 2018 San Antonio Breast Cancer Symposium ®

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*In vivo* isolation of circulating tumour cells using CellCollector and detection of gene mutations in different metastasis organ sites in breast cancer

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**Introduction:** Metastasis is thought to result from tumour cell entry into the circulation and migration to distant organs, where the mutation landscape of metastatic breast cancer (MBC) may shift and vary. The genotypic features of circulating tumour cells (CTCs) typically differ from those of primary breast cancer (BC) cells. Gene mutation analysis of CTCs in MBC may benefit patients by identifying those amenable to specific therapies. Currently, CTCs are primarily isolated *in vitro* from small volumes of blood. The aim of this study was to isolate CTCs *in vivo* using CellCollector and screen for specific gene mutations in cells from different metastasis organ sites and molecular subtypes in MBC patients.

**Methods:** In this study, we used a novel technology, CellCollector, to collect peripheral CTCs. Thirty MBC patients were enrolled, and 17 were analysed with next-generation sequencing (NGS) methods. Clinical characteristics were analysed along with CTC enumeration and detection rates. Whole-genome amplification (WGA) was used to amplify the CTC genomic DNA of 127 genes.

**Results:** We isolated CTCs *in vivo* from 20 of 30 MBC patients (66.7%), with a median and mean (range) of 2 (0-15) CTCs. In non-cancer patients, no CTCs were detected. We analysed CTC enumeration and the detection rate in different clinical characteristic subgroups. We found that in their corresponding subgroups, patients younger than 45 years old, with brain metastasis, with three or more metastasis organ sites, or with HER2-positive subtypes had the highest CTC medians and means. As far as clinical characteristics were concerned, the number of CTCs seemed correlated with more advanced clinical characteristics. In the one metastasis organ, two metastasis organs and three or more metastasis organs subgroups, the CTC detection rates were 38.5% (5/13), 77.8% (7/9), and 100.0% (8/8), respectively. The CTC detection rate correlated with the number of metastasis organs; patients with more metastasis organ sites had higher CTC detection rates. We also found that different metastasis organs and molecular subtypes contain high-frequency mutation genes, and also contain unique gene mutations.

**Conclusions:** In MBC, CellCollector can be used to collect intact CTCs, from which we can obtain gene mutation information. Different metastasis organs and molecular subtypes may have corresponding unique mutations, which may provide a basis for future gene therapy.

CTCs enumeration data and correlations to clinicopathologic characteristics

Patients' characteristics	CTCs enumeration		
	Range	median	mean
Total	0-15	2	2
Age (years)			
<45	0-4	2	2
≥45 and <60	0-15	1	2
≥60	0-2	1	2
Metastatic location			
Brain	0-15	5	6
Lung+liver	0-7	1	2
Bone+local recurrence	0-4	1	1
Number of metastatic locations			
1	0-4	0	1
2	0-15	1	3

3-4	1-7	2	3
Molecular subtypes			
Luminal A	0	0	0
Luminal B	0-5	1	1
HER2 positive	0-15	2	3
Triple negative	0-7	1	2

HER2, human epidermal growth factor receptor 2



# 2018 San Antonio Breast Cancer Symposium ®

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HER2-positive circulating tumor cells (CTCs) in advanced breast cancer (BC): A feature independent of BC subtype

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**Introduction:** HER2 overexpression is observed on CTCs in advanced BC (ABC), but their significance is not known. We aimed to describe clinical, pathologic, and molecular associations with HER2 overexpression on CTCs in ABC patients (pts).

**Methods:** We conducted a retrospective analysis of data from ABC pts treated at Thomas Jefferson University and Northwestern University who had evaluation of CTCs and circulating tumor DNA (ctDNA). CTCs were enumerated with the CellSearch immunomagnetic kit (Menarini Silicon Biosystems), HER2 expression on CTCs was evaluated using the CellSearch CXC Kit, and ctDNA was analyzed using the Guardant360 NGS assay (Guardant Health). Associations with the presence of HER2+ CTCs were explored through univariate and multivariate logistic regression. Kruskal-Wallis testing evaluating HER2+ CTCs as a continuous variable was also conducted to confirm consistency of findings. Time to development of HER2+ CTCs was evaluated using Cox proportional hazards regression analysis.

**Results:** Baseline CTCs were evaluated in 209 pts (10% stage III, 90% stage IV) of whom 41% had no detectable CTCs, 23% had 1-4 CTCs, and 36% had  $\geq 5$  CTCs (stage IV aggressive). Twelve percent had CTC clusters. At least 1 HER2+ CTC was seen in 33% of pts at baseline draw. Of 39 patients with HER2+ BC, only 18% had HER2+ CTCs. Of patients with HER2+ CTCs, 55% had hormone receptor positive BC, 28% had triple negative BC, and 18% had HER2+ BC. On univariate logistic analysis, BC subtype or HER2 status was not associated with the presence of HER2+ CTCs. IBC pts represented 52% of pts and were less likely to have HER2+ CTCs (OR 0.40 95% CI 0.19-0.84). Bone metastases were associated with an increased likelihood of HER2+ CTCs (OR 2.46, 95% CI 1.12-5.38); however, other sites of metastases and number of metastatic sites were not correlated with HER2+ CTCs. Aggressive disease features including  $\geq 5$  CTCs and presence of CTC clusters were strongly associated with HER2+ CTCs (OR 15.72, 95% CI 6.89-35.8 and 8.97, 95% CI 3.23-24.89, respectively). Of 168 pts with ctDNA analysis, *ERBB2* aberrations were seen in 22% of pts and were significantly associated with HER2+ CTCs (OR of 3.74, 95% CI 1.45-9.63). On multivariate analysis, the associations with  $\geq 5$  CTCs and *ERBB2* alterations in ctDNA remained statistically significant. The associations of HER2+ CTCs with bone disease,  $\geq 5$  CTCs, CTC clusters, and *ERBB2* alterations in ctDNA, and the inverse relationship with IBC were consistent when HER2+ CTCs were evaluated as a continuous variable with Kruskal-Wallis testing. Among pts without HER2+ CTCs at baseline, the time to detection of HER2+ CTCs correlated with the presence of bone metastases (HR 3.40, 95% CI 1.14-10.19),  $\geq 5$  CTCs (3.77, 95% CI 1.33-10.70), and visceral disease (HR 3.00, 95% CI 1.07-8.44).

**Conclusions:** HER2+ CTCs are common in ABC, independent of HER2 status of the tumor, and, in fact, common in the luminal BC. HER2+ CTCs were also strongly associated with CTC characteristics of aggressive disease with poor survival (CTCs clusters and  $\geq 5$  CTCs) and *ERBB2* aberrations in ctDNA. Further studies will be investigating the role of HER2+ CTCs in endocrine resistance and the potential of anti-HER2 therapy in this unique CTC-defined setting.

# 2018 San Antonio Breast Cancer Symposium ®

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Proof-of-concept of a 4-marker system for improved CTC analysis of metastatic triple-negative breast cancer

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**Introduction:** Circulating tumor cells (CTCs) represent a potent opportunity to glean important information about *in vivo* breast cancer biology in a non-invasive fashion. A current limitation to CTC analysis is the inability of positive-selection systems to capture EpCAM-low/negative CTCs, a phenotype that is enriched in the CTCs of metastatic triple negative breast cancers (mTNBCs). This proof-of-concept study aims to increase CTC capture for downstream molecular analysis through the inclusion of additional markers specifically relevant to TNBC.

**Methods:** For inclusion in the analysis, marker candidates were: (1) sufficiently characterized in TNBC, (2) exclusively surface markers to avoid permeabilization, (3) not reported on leukocytes if a cluster of differentiation (CD) nomenclature was associated, and (4) targetable with commercially available antibodies. Cell lines were purchased as part of the TNBC Panel 3 (ATCC) and were characterized as positive or negative across the selected markers by flow cytometry. Capture efficiency of cell lines from culture medium was conducted using antibodies conjugated to magnetic beads in concert with an immunomagnetic detection system developed by the Savran Research Group at Purdue University. Comparisons to EpCAM-only based detection in capture efficiency experiments were completed using Student's t-test. EDTA-anticoagulated blood drawn from normal subjects was assessed for CTCs using the 4-marker capture and parallel 4-marker fluorescent cross-stain. This study was approved by the Institutional Review Board at Indiana University.

**Results:** We assessed surface expression of 4 markers (TROP2, N-Cadherin, EGFR, EpCAM) across 11 TNBC cell lines using flow cytometry. 100% of cell lines were positive for at least 1 of 4 markers in the panel. 7 of 11 cell lines were characterized by EpCAM positivity. The remaining 4 EpCAM-negative cell lines were positive for N-Cadherin, EGFR, or both in the absence of EpCAM. Immunomagnetic capture experiments performed across the 4 EpCAM-negative cell lines revealed a significant increase in capture efficiency yielded by the 4-marker panel as compared to EpCAM-only capture ( $p=0.0006$ ). Capture efficiency for EpCAM-positive cell lines with the 4-marker panel was equivalent to EpCAM-only. The 4-marker panel was highly specific as CTC assessment of blood samples collected from 5 normal women without cancer yielded 0 positive cells.

**Discussion:** Compared to EpCAM-only based capture, the 4-marker experimental system presented here has potential to enhance CTC analysis by more completely representing the heterogeneity of TNBC. This results in better overall capture efficiency, while still maintaining sufficient specificity. The primary limitation of our study is that *in vivo* characterization of the system is incomplete. To address this, a clinical protocol for the performance assessment of this method compared to EpCAM-based detection in patients with mTNBC has been initiated at Indiana University and will begin enrolling patients in July 2018.

## 2018 San Antonio Breast Cancer Symposium ®

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Circulating tumor cells of breast cancer origin identified by fluorescence in situ hybridization and may be an early predictor of therapy failure in early breast cancer

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**Background:** Most modern methods of detecting circulating tumor cells (CTCs) involve identifying cells with epithelial markers. This approach presents challenges, as not all epithelial cells found in circulation originate from the tumor and not all CTCs express epithelial markers. We propose using a size-exclusion filtration system to enrich for CTCs in peripheral blood followed by fluorescence in situ hybridization (FISH) of the filtered cells to identify cells of tumor origin in the early-stage breast cancer patients. We further hypothesize that the presence of CTCs may be indicators of therapy failure in early-stage breast cancer patients.

**Methods:** Patients diagnosed with breast cancer (n = 9) were consented for CTC evaluation. Primary tumor DNA was analyzed by the Affymetrix Oncoscan™ genome-wide microarray platform and investigated for somatic copy-number alterations (SCNAs). For each patient, two FISH probes were then identified for two regions of gain or a region of gain and a region of loss from the microarray results. Blood samples from patients were obtained before surgery, radiation therapy, endocrine therapy, and at 6-month or 1-year follow-up visits. Blood samples were filtered using ScreenCell® Cyto V2 devices, and FISH was performed. Cells were categorized as normal (diploid for all FISH probes), suspicious (single SCNA detected by FISH), or CTC (two SCNAs detected by FISH). Patients were identified as having CTCs present in their circulation when  $\geq 2$  CTCs were observed or when one CTC and  $>15$  suspicious cells were observed.

**Results:** The microarray data revealed that luminal A tumors ranged from 2-43 SCNAs; luminal B tumors ranged from 15-20 SCNAs; and ER+, PR+, HER2+ tumors ranged from 46-98 SCNAs. Although a correlation appears to exist between tumor genetic complexity and molecular subtype, the degree of complexity was highly varied within each subtype. We found that neither complexity of tumor profile, molecular subtype, nor stage could predict the presence of CTCs in patients.

Molecular Subtype	SCNAs	CTCs	Suspicious Cells Only
Luminal A	2-43	3/5	2/5
Luminal B	15-20	0/2	1/2
ER+, PR+, HER2+	46-98	2/2	0/2

In pre-surgical blood samples, we detected CTCs in 63% of patients with stage 1 disease and in 60% of patients with luminal A tumors, 0% of patients with luminal B tumors, and 100% of patients with triple-positive tumors.

Stage	CTCs	Suspicious Cells Only
IA/B	5/8	2/8
IIA	0/1	1/1

Although limited in number, ongoing investigation revealed that one of our patients in early follow-up with a luminal A, stage IB tumor was identified to have persistent CTCs at 1-year after starting hormonal adjuvant therapy, suggesting residual tumor burden not detected by standard clinical modalities; this finding also suggests that this patient may be at highest risk for relapse and should be considered for additional therapies.

**Conclusion:** Size-exclusion filtration followed by FISH analysis can accurately identify CTCs in early-stage breast cancer patients. Tumor complexity, molecular subtype, and stage did not predict the presence of CTCs in circulation. Our method for CTC detection may be able to serve as a diagnostic tool for treatment failure.

# 2018 San Antonio Breast Cancer Symposium ®

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Isolation and expansion the circulating tumor cells from patients' peripheral blood *in vitro*

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**Background:** Circulating tumor cells (CTCs) are rare tumor cells disseminated in peripheral blood from either original sites or metastatic sites. Current studies on CTCs are mainly focused on its diagnosis and prognosis significances by liquid biopsy, although controversial results were reported elsewhere. Actually, as a few studies showed that CTCs would be a kind of intriguing drug targets and more beneficial for cancer patients if there genomic and proteomic information could be deciphered dynamically. However, the rarity, challenging isolation as well as the primary culture procedures limited the analysis of CTCs insightfully.

**Methods:** All patients' samples were referred to the regulation of ethnic commission of Shenzhen People's Hospital and informed parental consent was then collected also for this study. The density gradient centrifugation was used to obtain PBMCs. Cells adherent and suspension culture for collected the circulating tumor cells. Immunofluorescence, karyotyping, nude mouse tumorigenesis were verify the tumor cells. Also characteristic and functional of CTCs were analyzed.

**Results:** It was showed that the morphology of CTC-3 is clustered growth and the CTCs were DAPI-positive, Cytokeratin-positive, and CD45-negative with the immunofluorescence identification. The karyotyping results showed that the chromosome number of CTC-3 varied from 56-60 and the chromosomes were aneuploid. This cell line, which was named as CTC-3, formed clones and displayed stable epithelial phenotype and showed stem-cell like properties. CTC-3 had larger nuclear/cytoplasmic ratio and limitless replicative potential. The growth curve of CTC-3 was similar with that of MCF-7, and CTC-3 grew faster than MCF-7 since the fifth day. Furthermore, the sphere formation ability of CTC-3 was significantly higher than MCF-7 *in vitro*. Besides, the tumorigenesis of CTC-3 cells was almost the same as MCF-7 *in vivo* by the analysis of xenografting in immunodeficient mice. STR analysis showed that the similarity of CTC-3 and MCF-7 on the standard site alignment is 20%, which indicated that CTC-3 was a totally new breast cancer cell line. For drug resistance, CTC-3 cells were more resistant to anti-tumor drugs after 3D culture compared with 2D, survive rates of 3D-CTC-3 cells after treatment were more than 80%. Finally, the RT-PCR results shows that the expression of Nanog, E-cadherin and ABCG2 were significantly higher in CTC3, also Oct3/4, CD44, CD133, Vimentin, MMP2, MMP9 no significant differences were found between other genes.

**Conclusion:** We have successfully isolated and expanded a new breast cancer cell line from the breast cancer patient, which could provide more drug sensitivity information of the patient and open up novel avenues towards the understanding of breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Targeting of endocrine therapy-induced estrogen-receptor/HER2-cross-talk in circulating tumor cells from metastatic ER+/HER2- breast cancer: Implications for treatment of ER+/HER2- breast cancer

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**Background:** Transient induction of HER2 expression upon inhibition of the estrogen-receptor (ER $\alpha$ ) might be an underestimated rescue mechanism that enables ER+/HER2- breast cancer cells to survive and to grow in the presence of endocrine therapies. Besides protecting ER+/HER2- BC cells from endocrine treatment, transient HER2 expression is also considered to save circulating, disseminated and dormant ER+/HER2- BC cells from apoptotic cell death in hostile environments and might therefore be of importance for ER+ BC progression, metastasis formation and recurrence. Thus therapeutic strategies that efficiently target both ER $\alpha$  and HER2 simultaneously might improve treatment of patient with ER+/HER2- BCs.

**Methods:** Circulating tumor cells (CTCs) were originally isolated from a patient with bilateral ER+/HER2- metastatic BC. These cells have been treated with either the proteasome inhibitor (PI) carfilzomib or fulvestrant alone or with both drugs in combination. The consequences of these drugs upon ER $\alpha$  and HER2 expression were monitored by western blotting or qPCR. Induction of cell death upon treatment was measured by PARP and caspase cleavage or by quantification of SubG1 cells using propidium iodide staining or by the use of colony forming assays.

**Results:** Fulvestrant treatment of CTCs decreases the amount of ER $\alpha$  but immediately increases HER2 expression whereas carfilzomib markedly inhibits the expression of both ER $\alpha$  and HER2 simultaneously. Combined treatment of CTCs with carfilzomib + fulvestrant cause reduced expression of HER2 and lead to a much stronger decrease of ER $\alpha$  than carfilzomib or fulvestrant alone. Fulvestrant causes no significant reduction of proliferation and no induction of cell death. Conversely the combination of carfilzomib and fulvestrant causes a significant induction of apoptotic cell death and a massive reduction of colonies in colony forming assays.

**Conclusion:** These findings suggest that rapid and transient up-regulation of HER2 expression following endocrine treatment might be an important so far underestimated adaptive mechanism which enables ER+/HER2- BC cells to sustain proliferation in the presence of ER $\alpha$ -inhibitory drugs and to stay alive during the metastatic process. Furthermore these data also lead to the assumption that PIs such as carfilzomib in combination with ER $\alpha$  degraders could be a potential therapeutic strategy for efficient targeting of metastatic ER+/HER2- BC cells.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-02-02

Sensitivity and dynamic range of CellSearch based DTC enumeration in the CSF in patients with leptomeningeal metastases (LMM)

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Improvements in the treatment of patients with MBC and the inefficiency of many drugs in crossing the BBB have led to an increased incidence of symptomatic CNS metastasis. CNS metastases are clinically characterized by two distinct phenotypes, although their combined occurrence is possible. Most patients suffer from solid CNS metastases, but metastatic deposits limited to the leptomeningeal surfaces occur (LMM). In addition to its poor prognosis, neurological impairment in LMM is devastating.

**Methods:** 27 patients with breast cancer (MBC) were diagnosed with LMM and treated with intrathecal (IT) therapy from 2008 to 2018 in a single centre (GZA Hospitals Antwerp). Clinicopathologic and treatment information were collected. Conventional diagnosis was based on positive cytology on CSF and/or on the combination of clinical signs and neuro-imaging findings. For a subset of patients (n=12), we have enumerated circulating tumor cells (CTCs) and CSF DTCs at time of LMM diagnosis. Whole blood and CSF was processed on the CellSearch platform according to the standard method for CTC enumeration in blood.

**Results:** The 27 MBC pts diagnosed with LMM had a median time to LMM of 70.5 months (6.5-241) from the diagnosis of BC. Median age at LMM diagnosis was 56 y (36-75). At time of last follow-up, 23 patients (85%) had died. The median OS from LMM diagnosis was 27 months (4.5-128). From the 27 patients, 52% (14/27) were hormone receptor-positive (HR+), 33% (9/27) were HER2 overexpressing and 15% (4/27) were triple-negative. Coexisting brain metastases are present in 37% of patients. In 11 cases (41%), LMM was not accompanied by extra cranial disease at time of diagnosis. The biochemical data matched the classic CSF findings of LMM including a high protein concentration (75%, 9/12) and a low glucose concentration (42%, 5/12) (Table 1.). CSF lactate was elevated (>2.2 mmol/l) in 100% (10/10). In 70% (7/10) CSF lactate was >3.5 mmol/l. Cytologic evaluation of the CSF demonstrated unequivocal malignant cells in 10/12 (83%). DTC enumeration in the CSF of these patients, however, revealed the presence of tumor cells 12/12 (100%).

Results off the CSF analysis in 12 patients with LMM

Patient	WBC	Glucose	Protein	CTC	Cytology	DTC	Volume mL
1	25	<20	185	66	pos	21	2
2	185	34	221	49	pos	2304	7
3	170	68	55	0	pos	> 1000	5
4	133	39	60	8	neg	> 1000	7.5
5	40	42	28	0	pos	11442	7.5
6	77	206	441	0	pos	6366	7.5
7	56	26	121	1151	pos	26423	7.5
8	16	75	161	NA	pos	>1000	3
9	8	94	84	0	pos	393	1
10	62	31	320	0	pos	19165	7.5
11	296	80	2.4	0	pos	5914	4
12	25	60	5,11	3	neg	799	4

Furthermore, the cell count ranged between 10 to 3523 Epcam-positive cells/mL CSF. Additional evaluation proved these to be cancer cells.

**Conclusion:** Symptomatic LMM has a variable prognosis, with some patients experiencing prolonged survival. LMM occurs in all

breast cancer subtypes and can be the sole location (40%). CSF lactate is a sensitive but aspecific marker. DTC enumeration in the CSF is a robust tool to diagnose LMM with apparently a superior sensitivity over conventional cytology. It furthermore has a broad dynamic range rendering it more suitable for treatment effect evaluation. This methodology should be considered in routine workup in MBC patients with suspected LMM.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-01

Comparison of sentinel lymph node detection performances using methylene blue in conjunction with indocyanine green or radioisotope in breast cancer patients: A prospective single-center cohort study

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**Aim:** This cohort study aimed to compare the clinical efficacies between the novel dual tracer composed of indocyanine green (ICG) and blue dye (BD) and the conventional dual tracer composed of radioisotope and BD for sentinel lymph node (SLN) mapping in breast cancer patients.

**Methods:** This study enrolled 471 clinically lymph node-negative patients with primary breast cancer. All the patients received mastectomy, while, for the sentinel lymph node biopsy (SLNB), they were randomized to receive BD plus radioisotope or BD plus ICG. Two hundred and twenty-seven patients received radioisotope plus BD (Control, RB group) for SLNB, while, 200 patients underwent the ICG plus BD (IB group) for SLNB. The detection performances on SLN identification rate, positive SLN counts, detection sensitivity, and false-negative rate were compared between the two groups. Following SLNB, axillary lymph node dissection (ALND) was performed only on patients with metastatic SLNs. Injection safety and potential side-effects of the two dual tracers were evaluated by a 24-month follow-up after the SLNB procedure.

**Results:** In the IB group, 97% (194/200) of the patients who underwent the ICG and BD dual tracer injection showed fluorescent-positive lymphatic vessels within 2-5 minutes. The identification rate of SLNs was comparable between the IB group (99.0%, 198/200) and the RB group (99.6%, 270/271) ( $P=0.79$ ). No significant differences were observed in identification rate of metastatic SLNs (22.5% versus 22.9%,  $P>0.05$ , RB group versus IB group, the same below), positive SLN counts ( $3.72\pm 2.28$  versus  $3.91\pm 2.13$ ,  $P>0.05$ ), positive metastatic SLN counts ( $0.38\pm 0.84$  versus  $0.34\pm 0.78$ ,  $P>0.05$ ), SLNB detection sensitivity (94.4% vs. 92.5%,  $P>0.05$ ), or false-negative rate (5.6% versus 7.5%,  $P>0.05$ ) between the two groups. No obvious allergic reaction or anaphylaxis, infection at the injection sites, skin necrosis or distal metastasis was reported in either group during the 24-month follow-up.

**Conclusions:** ICG can be used as a promising alternative tracer for radioisotope in SLN mapping, and when it is combined with BD in lymphangiography, it offers comparable detection sensitivity compared to the conventional lymphatic mapping strategies that are widely used in clinical practice.

Efficacy of indocyanine green vs. radioisotope + blue dye for sentinel lymph node mapping in breast cancer patients.

Parameters	99Tcm-Dx+BD(N=271)	ICG+BD (N=200)	P-value
SLN identification rate (%)	99.6(270/271)	99.0(198/200)	0.79
SLN counts	3.91±2.13	3.72±2.28	0.09
Metastatic SLN rate (%)	22.9(62/271)	25.5(51/200)	0.51
Metastatic SLN counts	0.34±0.78	0.38±0.84	0.46
Sensitivity	92.5(62/67)	94.4(51/54)	0.96
False-negative rate (%)	7.5(5/67)	5.6(3/54)	

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-02

Redo sentinel lymph node biopsy for ipsilateral breast tumor recurrence after breast conserving surgery with negative sentinel nodes: A pooled analysis from a systematic review and two institutes

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**Background:**Cases of redo sentinel lymph node biopsy (SLNB) are growing according to the increase of ipsilateral breast tumor recurrence (IBTR) after breast-conservative surgery (BCS). To evaluate a feasibility of redo SLNB in patients with IBTR after negative SLN, we conveyed a pooled analysis using data from a systematic review and two institutes.

**Materials and methods:**A systematic search of PubMed was conducted to identify data of patient level from publications evaluating redo SLNB for cases with IBTR. Eligible patients who underwent BCS and were confirmed as negative axilla after SLNB were identified. An identification rate (IR) and a false-negative rate (FNR) were calculated. To identify FNR, we only included cases with back-up axillary node dissection (ALND) from retrieved data.

**Results:**In a systematic review, a total of 197 peer-reviewed publications were retrieved, of which 19 papers included patients who met eligibility criteria. Data from 464 patients were collected. In two-institutes, 38 cases with same criteria were identified. A total of 502 patient's data were pooled. The IR of redo-SLNB was 71.7% (360/502) in pooled data. For the FNR, data from 147 patients with back-up ALND after SLNB was analyzed. The FNR and accuracy of redo-SLNB were 9.8% (5/51) and 97% (142/147).

**Conclusions:**We found that the IR and the FNR of redo SLNB were 71.7% and 9.8%, respectively. Redo SLNB is reliable procedure for axillary staging in patients with IBTR after negative SLNB.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-03

Sentinel node biopsy (SNB) vs Low axillary sampling (LAS) in predicting nodal status of post-chemotherapy axilla in women with breast cancer

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## Introduction

There is no safe method of avoiding complete axillary lymph node dissection in women with breast cancer after neo-adjuvant chemotherapy. sentinel node biopsy (SNB) has had prohibitively high false negative rate. We tested low axillary sampling (LAS) and SNB performed in same patient to predict axillary lymph node status in clinically node negative women undergoing breast conservation or modified radical mastectomy after neo-adjuvant chemotherapy.

## Methodology

Post neo-adjuvant chemotherapy 751 women who had no palpable axillary lymph node underwent LAS (all lymph nodes below intercosto-brachial nerve). Of these 751 women, 730 also underwent SNB by dual technique after injection of blue dye as well as radio-isotope. SN was identified within and outside axillary sampling specimen. SN as well as LAS specimens were distinctly examined for nodal metastasis. The rest of the axillary dissection was completed in all patients. Post NACT 292/751 (38.9%) had residual positive lymph nodes on pathology. The identification rate, false negative rate (FNR), and negative predictive value (NPV) of SNB and LAS were compared for predicting negative axillary lymph node status.

## Results

The median clinical tumor size was 5cm (1-15cm) and 533(71%) patients were N1 or N2 at presentation. The SNB identification rate was 87.1% (636 of 730), with a median of 5 nodes and node positive in 238 of 636 (37.4%). LAS identification rate was 98% (736 of 751), with a median of 7 nodes and node positive in 292 of 736 (39.6%). In all but one case, the SN was found within the LAS specimen. The FNR of SNB (blue, hot and adjacent palpable nodes) was 19.7% (47 of 238, one sided 95% upper CI 24.0) compared to LAS with FNR of 9.9% (29 of 292, one-sided 95% upper CI 12.8) ( $p < 0.001$ ). Comparative NPV for SNB and LAS were 89.4% and 93.9% respectively. If SNB was confined to blue/hot node excluding adjacent palpable nodes, FNR was 31.6% (74 of 234, 95% upper CI 36.6).

## Conclusions

LAS is superior to SNB in identification rate, FNR and NPV in predicting node negative axilla post-neoadjuvant chemotherapy. LAS can be safely used to predict negative axilla with less than 10% chance of leaving residual disease.

# 2018 San Antonio Breast Cancer Symposium ®

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Intraoperative touch imprint cytology in targeted axillary dissection after neoadjuvant chemotherapy among breast cancer patients with initial axillary metastasis

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**Background:** A false-negative rate of <10% can be achieved if targeted axillary dissection (TAD) is performed, which includes the excision of both biopsy-proven positive lymph nodes (BxLNs) and sentinel lymph nodes (SLNs). However, little evidence exists on the accuracy of intraoperative touch imprint cytology (ITPC) applied in TAD after neoadjuvant chemotherapy (NAC) among breast cancer patients with initial axillary metastasis. We aimed to investigate the accuracy of ITPC in TAD after NAC.

**Methods:** Breast cancer patients with biopsy-confirmed nodal metastasis were prospectively enrolled. After the completion of NAC, all patients underwent TAD followed by axillary lymph node dissection. ITPC was carried out to evaluate BxLNs and SLNs. The accuracy of TAD and ITPC was calculated in comparison with haematoxylin and eosin staining of ALNs. The results of ITPC over 6 months in our centre in the adjuvant setting were used for comparison.

**Results:** Overall, the false-negative rate of TAD was 10.8%.ITPC was tested in 92 patients. Accuracy, sensitivity, and specificity of ITPC were 92.4%, 87.9% and 94.9%, respectively

Table 1 ITPC results among patients with and without NAC

	ITPC with NAC (n=92)	ITPC without NAC (n=859)	P value
Including Micrometastases or ITCs			
Accuracy	92.4% (85/92)	91.2% (783/859)	0.69
Sensitivity	87.9% (29/33)	69.9% (144/206)	0.03
Specificity	94.9% (56/59)	97.9% (639/653)	0.33
NPV	93.3% (56/60)	91.2% (639/701)	0.57
PPV	90.6% (29/32)	91.1% (144/158)	1.00
Excluding Micrometastases or ITCs			
Accuracy	96.7% (89/92)	96.3% (827/859)	1.00
Sensitivity	100.0% (33/33)	91.3% (188/206)	0.16
Specificity	94.9% (56/59)	97.9% (639/653)	0.33
NPV	100.0% (56/56)	97.3% (639/657)	0.42
PPV	91.7% (33/36)	93.1% (188/202)	1.00

Abbreviations: NPV, negative predictive value; NAC, neoadjuvant chemotherapy; ITPC, intraoperative touch imprint cytology; PPV, positive predictive value

. In the non-NAC group, ITPC showed a similar accuracy (91.2%) and specificity (97.9%) but a significantly lower sensitivity (68.9%, P=0.03). Multivariate analysis indicated that NAC, age and size of metastases was independent risk factors associated with false-negative ITPC (P<0.05)

Table 2 Univariate and multivariate analysis for the factors of false negative cases in ITPC

Factors	Univariate		Mltivariate	
	OR(95%CI)	P value	OR(95%CI)	P value
Age				
≤40	1.00		1.00	
>40	1.19 (0.59-2.40)	0.63	2.78 (1.13-6.87)	0.03

Clinical T status				
1-2	1.00		1.00	
3-4	0.21 (0.03-1.62)	0.13	0.50 (0.04-6.58)	0.60
Clinical N status				
0	1.00		1.00	
1-2	0.42 (0.18-1.00)	0.05	1.28 (0.24-6.70)	0.77
Number of biopsied LNs				
≤2	1.00		1.00	
>2	1.15 (0.57-2.32)	0.70	1.01 (0.42-2.41)	0.99
Histologic type				
Ductal	1.00		1.00	
Lobular	1.33 (0.32-5.46)	0.70	2.65 (0.53-13.34)	0.24
Size of metastases				
Macrometastases	1.00		1.00	
Micrometastases or ITCs	14.42 (7.31-28.46)	<0.01	19.68 (9.19-42.12)	<0.01
NAC				
No	1.00		1.00	
Yes	3.12 (1.05-9.26)	0.04	5.24 (1.48-18.57)	0.01

Abbreviations: NAC, neoadjuvant chemotherapy; ITC, isolated tumor cell; LN, lymph node

**Conclusions:** ITPC was feasible in TAD among breast cancer patients with biopsy-confirmed axillary metastasis who were treated with NAC. All the misses in the ITPC were in patients with micrometastases or isolated tumour cells. ITPC can help decrease the number of second operations in patients with residual disease in ALNs after NAC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-05

Comparison of sentinel lymph node biopsy by dual method of indocyanine green fluorescence and radioisotope versus radioisotope only in breast cancer patients after neoadjuvant chemotherapy: A randomized controlled trial

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**Objectives:** This study aimed to evaluate the identification rate of sentinel lymph node (SLN) in breast cancer patients after neoadjuvant chemotherapy (NAC) by dual method (DM) using a mixture of indocyanine green (ICG) and radioisotope (RI) compared with the RI alone.

**Methods:** In this randomized study, 130 patients after NAC for breast cancer were enrolled and 122 patients received SLNB with either DM (N=58) or RI only (N=64). We compared the identification rate, the number of SLNs, and detection time of SLN biopsy (SLNB) and evaluated the safety.

**Results:** The mean age of the DM group and RI group was 49.9 and 47.8 years ( $p=0.22$ ), respectively. Among 122 patients, 113 (92.6%) were clinically node positive before NAC. There were no differences in clinical T stage, hormone receptor positivity, and type of operation between groups. The identification rates of SLNs were 98.3% in DM group and 93.8 % in RI group ( $p=0.14$ ), respectively. The average number of SLNs in DM group was similar with that in the RI group ( $2.19 \pm 1.13$  vs.  $1.94 \pm 1.33$ , respectively;  $p=0.26$ ). The time to detect the first sentinel lymph node was similar in each group ( $8.7 \pm 4.98$  vs.  $8.3 \pm 4.31$  min;  $p=0.30$ ). In the DM group, transcutaneous lymphatic drainage was visualized by fluorescent imaging in 65.5% (38 of 58 patients) and 94.7% of first SLN were detected by ICG-F and 93.0% by RI ( $p=0.79$ ). During and after the operation, there were no complications, including allergic reactions, skin staining, or necrosis.

**Conclusions:** This study is the first randomized trial that compared DM using ICG-F and RI and the conventional RI method for SLNB in breast cancer patients after NAC. DM could be a feasible and safe method for SLNB in initially node positive breast cancer patients with NAC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-06

Internal mammary chain sentinel nodes in early stage breast cancer patients: Towards selective removal

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## Background

Internal mammary chain (IMC) sentinel nodes (SN) are visible in 1 out of 5 breast cancer patients on lymph scintigraphy after intra- or peritumoral injection of a radiopharmaceutical. The IMC SN status affects prognosis and treatment of breast cancer and IMC radiotherapy improves survival in selected patients. In contrast to the axillary SN, removal of the IMC SN is not routinely performed and often technically challenging. This study aims at determining the effect of IMC SN biopsy on recurrence-free survival (RFS) and overall survival (OS) and the identification of predictive factors for the development of IMC- and distant metastases.

## Methods

All patients with IMC SNs were selected from a prospective database from 1999 to 2007. Following intratumoral injection of technetium-99m, conventional lymphoscintigraphy was performed. Sentinel nodes were removed in all regions with lymphatic drainage on scintigraphy. The RFS and OS were calculated for the total group and subgroups with tumor-positive, tumor-negative or non-removed IMC SN. Predictive factors were identified for tumor-positive IMC SN and for distant metastasis by regression analysis.

## Results

Internal mammary chain SN biopsy was performed in 287 out of 336 patients (85%). The IMC SN was tumor-positive in 38 patients (13%). Patients with IMC metastasis had poorer OS compared to patients without IMC metastasis or a non-removed IMC SN (57%, 82% and 59% 10- year OS, respectively,  $p = 0.002$ ). These patients also had worse RFS, mainly due to by the development of distant metastases (68%, 84% and 61% RFS, respectively,  $p = 0.002$ ). Multivariable predictive for tumor-positive IMC SN were axillary metastases (PPV = 38.5%). Predictive factors for distant metastasis were tumor-positive IMC SN (HR 2.5, 95% CI; 1.0 - 5.8,  $p = 0.04$ ), not removed IMC SN (HR 2.3, 95% CI; 1.0 - 5.1,  $P = 0.05$ ), tumor diameter >1.5cm (HR 3.5, 95% CI; 1.6 - 8.4,  $p < 0.00$ ) and age >65 years (HR 3.1, 95% CI; 1.2 - 7.7,  $p = 0.02$ , reference <50 years).

## Conclusion

Breast cancer patients with tumor-positive IMC SN have worse 10- year survival than patients with tumor-negative IMC SN, mainly due to the development of distant metastasis. The clinically relevant predictive factor for distant metastasis is tumor size >1.5cm. Radiotherapy of the IMC can improve survival. However, the cardiotoxicity of parasternal radiotherapy must be weighed against the expected survival benefit. Therefore, our current protocol is to perform IMC SN biopsy in patients younger than 70 years with a tumor diameter >1.5cm.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-07

Indocyanine green (ICG) fluorescence mapping for sentinel lymph node (SLN) localisation in early breast cancer

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**Background:** Dual localization methods with blue dye and radioisotope are commonly employed for SLN identification but allergic reactions together with staining of skin and surgical tissues disadvantage blue dye. Fluorescence mapping is consistently associated with high rates of identification (exceeding 95%) and sensitivity compared with standard agents. A feasibility study using blue dye, radioisotope and ICG confirmed high sensitivity of ICG fluorescence for SLN detection with three-quarters of nodes both radioactive and fluorescent (Wishart GC, et al. . EJSO 2012; **38**: 651 - 656). This follow-on study has specifically evaluated a combination of ICG with radioisotope for SLN identification.

**Methods:** In a prospective observational study, 50 patients with unilateral clinically node negative patients scheduled to undergo routine SLN biopsy for core-biopsy proven invasive (n= 49) or non-invasive (n = 1) breast cancer were identified at the multidisciplinary team meeting [26 screen-detected; 24 symptomatic]. All patients received dual localization with radiocolloid (Technetium nanocolloid, 4MBq) and ICG (0.5%). All patients had pre-operative axillary ultrasound and the number of nodes recorded numerically and whether radioactive, fluorescent or both. Subcutaneous lymphatics and nodal tissue were visualized with a Photodynamic Eye camera and sensitivity of individual tracers alone and in combination calculated. Approval was granted by the Joint Committee on Drugs and Therapeutics for use of ICG as a replacement tracer for blue dye.

**Results:** A total of 102 nodes were retrieved from 50 patients with an average nodal count of 2.04 (range 1 – 4) and an overall identification rate of 98% (49/50). Amongst these excised nodes, 94 displayed uptake of tracer and were either fluorescent, radioactive or both (8 nodes were removed incidentally or were palpably suspicious and tracer negative). More than 90% (94/102) of nodes were fluorescent and 61.8% (63/102) were radioactive with at least 10% activity of the hottest node. These results yielded an overall concordance rate of 69.6%. Nodal detection rates for ICG alone or combined with radioisotope were 92.2% (94/102) and 61.8% (63/102) respectively whilst procedural detection rates were 98% (49/50) for radioisotope and 100% (50/50) for ICG. Metastases were present in 9 nodes (all fluorescent and hot) with 9 patients having a single positive node containing macrometastases (n= 5), micrometastases (n = 2) or isolated tumor cells (n = 2). The node positivity rate was 14% (macro- or micrometastases) and the 4% of patients with isolated tumor cells testifies to removal of biologically important nodes. No serious adverse reactions were recorded.

**Conclusion:** ICG fluorescence imaging permits real-time visualization of lymphatics and provides an additional dimension to SLN biopsy that appears to be safe and effective. These results confirm high sensitivity for fluorescence in SLN identification with comparable performance parameters to the gold standard of radioisotope localization. With further refinements in technique may it may be possible to eventually rely on ICG as a sole tracer agent that avoids potential drawbacks of standard tracer agents including availability and costs of radioisotope.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-08

Preoperative positive axillary lymph node biopsy, node metastasis burden, and possible sentinel lymph node biopsy for breast cancer patients in the post-ACOSOG Z0011 trial era

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**Background.** Breast cancer patients with suspicious axillary lymph node (ALN) at ultrasound and positive fine needle aspiration (FNA) results were required to receive ALN dissection (ALND), which was not certain in the post-ACOSOG Z0011 era. We aim to evaluate the ALN metastasis burden in these patients, thus to illustrate whether they can follow the ACOSOG Z0011 trial procedure.

**Methods.** Clinically T1-2N0 breast cancer patients with positive preoperative ALN biopsy (FNA group) or 1-2 positive sentinel nodes (SLNB group) were retrospectively analyzed. ALN metastasis burden were compared between two groups, which were further analyzed in certain subtypes. Association between clinicopathological factors and  $\geq 3$  ALNs metastasis were also analyzed.

**Results.** A total of 388 patients were included: 202 in the FNA group and 186 in the SLNB group. The FNA group had a significantly higher number of positive ALN (5.18 vs. 1.77,  $P < 0.001$ ) and a larger proportion of patients with  $\geq 3$  ALNs metastasis (58.42% vs. 11.83%,  $P < 0.001$ ) than the SLNB group

ALN metastasis burden between the FNA and SLNB groups

	FNA group (N = 202)	SLNB group (N = 186)	P value
Mean No. of ALN removed	19.96 (19.20-20.71)	19.03 (18.28-19.78)	0.086
Mean No. of positive ALN	5.18 (4.44-5.92)	1.77 (1.50-2.04)	<0.001
No. of positive ALN			<0.001
1+	33 (16.34)	119 (63.98)	
2+	51 (25.25)	45 (24.19)	
$\geq 3+$	118 (58.42)	22 (11.83)	

ALN axillary lymph node, FNA fine-needle aspiration, SLNB sentinel lymph node biopsy

, which was not influenced by different tumor size stage and molecular subtypes. ALN metastasis identified by FNA was independently associated with high rate of ALN  $\geq 3$  metastasis (OR = 6.98, 95% CI 1.95-25.02,  $P = 0.003$ )

table 2

Characteristics	ALN 1-2+ (N = 248)	ALN $\geq 3+$ (N = 140)	P value	OR	95% CI	P value
ALN metastasis identified by			<0.001			0.003
FNA	84	118		6.98	1.95-25.02	
SLNB	164	22		1.0		
Tumor stage			0.005			0.738
T1	101	37		1.0		
T2	147	103		1.12	0.57-2.21	
Number of suspicious ALNs at US			<0.001			<0.001
$\leq 1$	175	32		1.0		
> 1	73	108		5.38	2.31-12.56	

Thickness of the cortex at US (mm)			0.037			0.068
≤ 3.5	43	34		1.0		
> 3.5	61	87		1.81	0.96-3.41	
Histological grade			0.002			0.233
I	12	3		1.0		
II	130	52		0.31	0.03-3.36	0.335
III	106	85		0.50	0.05-5.40	0.570
LVI			<0.001			<0.001
Negative	225	100		1.0		
Positive	23	40		4.78	2.04-11.24	
Multifocality			0.003			0.013
Unifocal	230	116		1.0		
Multifocal	18	24		3.93	1.33-11.63	
HER2 status			0.022			0.932
Negative	196	96		1.0		
Positive	52	44		1.03	0.51-2.11	
Ki67 (% , mean)			0.019			0.567
< 14%	83	31		1.0		
≥ 14%	165	109		0.79	0.35-1.79	

Abbreviation: ALN axillary lymph node, OR odds ratio, CI confidence interval, FNA fine-needle aspiration, SLNB sentinel lymph node biopsy, US ultrasound, LVI lymph vascular invasion, ER estrogen receptor, PR progesterone receptor, HER2 human epidermal growth factor receptor type 2. \*225 patients with suspicious ALN at US were evaluated for this characteristic

**Conclusions.** Patients with positive preoperative ALN biopsy had a higher ALN metastasis burden than patients with 1-2 positive SLNs, which was also the strongest factor associated with ≥ 3 ALNs metastasis, indicating these patients are not appropriate to receive SLNB in the post-ACOSOG Z0011 trial era.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-09

A nomogram predicting lymph node metastasis in T1 breast cancer based on the surveillance, epidemiology, and end results program

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**Background:** In developed countries, T1 stage breast cancers have become the most frequently diagnosed invasive breast diseases. Patients with early stage breast cancer with lymph nodes metastasis have been proven to have more aggressive biologically phenotypes. The risk of missing metastases using sentinel lymph node biopsy can range from 1% to 4%, with a false negative rate of 10%. This evidence implies that SLNB might not be sufficient for the diagnosis of lymph node metastasis in T1 breast cancer patients. The aim of this study was to build a nomogram to predict lymph node metastasis in patients with T1 breast cancer.

**Methods:** We identified female patients with T1 breast cancer diagnosed between 2010 and 2014 in the Surveillance, Epidemiology and End Results database. The patients were randomized into training and validation sets. Univariate and multivariate logistic regressions were carried out to assess the relationships between lymph node metastasis and age, race, and tumour size, primary site, pathological grade, histologic type, and molecular subtype. A nomogram was developed in the training set and validated by a calibration curve with the bootstrapping method and receptor operating characteristic curve analysis.

**Result:** A total of 91,364 T1 breast cancer patients were included in the present study. For patients with T1 breast cancer, age, race, tumour size, tumour primary site, pathological grade, oestrogen receptor status, progesterone receptor status and human epidermal growth factor receptor 2 status were independent predictive factors of positive lymph node metastasis ( $P < 0.001$ ). Increasing age, tumour size and pathological grade were positively correlated with the risk of lymph node metastasis. Based on multivariate logistic regression analysis, we successfully developed a nomogram to predict lymph node metastasis by summing the scores of each variables. The nomogram was further validated it in a validation set, with areas under the receiver operating characteristic curves of 0.733 (95% CI: 0.722-0.744) and 0.741 (95% CI: 0.731-0.752) in the training and validation sets, respectively. We determined the cut-off value of total points to predict lymph node metastasis according to Youden's index in the training set. Both the training set and validation set were divided into two groups: the low score group (total points  $\leq 182$ ) and the high score group (total points  $> 182$ ). We found a significant difference in the probability of lymph node metastasis between the high and low score groups in univariate analysis in both the training set (OR=4.15, 95% CI: 3.77-4.57,  $P < 0.001$ ) and the validation set (OR=4.53, 95% CI 4.10-5.00,  $P < 0.001$ ).

**Conclusions:** A better understanding of the clinicopathological characteristics of T1 breast cancer patients could be vital for the assessment of their metastatic lymph node status. The nomogram developed here, if further validated in other large T1 patient cohorts, might provide additional information regarding lymph node metastasis. Together with sentinel lymph node biopsy, this nomogram can help comprehensively predict lymph node metastasis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-10

Pre-operative lymphoscintigraphy for sentinel lymph node localisation: Is it necessary?

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## Introduction

Sentinel lymph node biopsy (SLNB) has replaced lymph node clearance for staging of the axilla in patients with early invasive breast cancer and no evidence of lymph node involvement on ultrasound or needle biopsy. It is recommended that a dual technique, using isotope and blue dye, is used to locate the sentinel lymph node (SLN) intra-operatively. Often, at the time of radioisotope injection, a lymphoscintigram (nuclear medicine scan) is obtained to demonstrate the 'hot' sentinel lymph node with or without skin marking of its anatomical position.

Performing a lymphoscintigram adds time and cost to the localisation process. In our centre, this investigation costs £899-999 (~US \$1180-1300).

## Aims

The aim of this study is to find out whether obtaining a pre-operative lymphoscintigram aids the surgeon in the localisation of the SLN or affects the number of sentinel lymph nodes biopsied in the axillary staging of patients with early invasive breast cancer.

## Methods

We carried out a retrospective study of patients who underwent SLNB for breast cancer in our hospital Trust between March 2012 and November 2017. We identified those patients who had a lymphoscintigram performed pre-operatively for SLN localisation. We recorded the number of SLNs identified on imaging and compared this with the number of SLNs biopsied during the operation.

## Results

349 patients underwent 354 SLNBs during the study period. One patient was male, the remainders were female. The mean age of patients was 57.2 years (range 25 to 98 years).

In 295 (83.3%) cases, a lymphoscintigram was obtained prior to SLNB for node localisation, and 268 (90.8%) of these scans were able to identify one or more SLNs. In 173 (58.6%) scans, a single SLN was identified. In 27 (9.1%) scans it was either unclear how many SLNs were demonstrated or no SLNs were seen (16/295 no SLN identified, 11/295 unclear how many SLNs).

In 102 (34.6%) cases, the number of SLNs biopsied matched the number of SLNs identified on imaging. Of those that did not match, 76.2% had more and 15.0% fewer SLNs excised than shown on imaging. In 8.8% it was unknown if the number of SLNs matched that seen on imaging due to lack of histopathology results.

## Conclusion

Lymphoscintigraphy for SLN localisation is costly and time consuming. In a high proportion of cases, number of SLNs identified on imaging does not match the number biopsied and thus, we suggest, that it is not required prior to SLNB and should be removed from practice.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-11

Intraoperative assessment of the sentinel node in breast cancer by one step nucleic acid assay: Experience of over 1100 patients

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## Introduction

The intraoperative assessment of the sentinel node in women with breast cancer enables an immediate axillary node clearance to be done as part of the same operative procedure if the sentinel node is positive. This has significant benefits for the Patient, the Surgeon and the Health Care Provider. There are a variety of methods for the intra-operative assessment of the sentinel node which include: Touch Imprint Cytology, Frozen Section Analysis and Polymerase Chain Reaction (PCR) based molecular assays. OSNA is an automated molecular assay using a Polymerase Chain Reaction (PCR), which detects the presence of cytokeratin 19 in the sentinel node. We report our experience of OSNA for the intraoperative assessment of the sentinel node in our institution with 1148 patients.

## Methods

All patients with operable breast cancer who were found to be node negative on clinical and radiological assessment of the axilla, and who had their axilla staged by a sentinel node biopsy at the Breast Unit at Warwick Hospital, UK over a 7 year period were included in this study. Data was collected from a prospective database maintained at the Breast Unit. The axillary node positivity rate and the number of patients with macrometastatic and micrometastatic disease as detected by OSNA was collected and compared with a group of 411 patients who had the intraoperative assessment by Touch Imprint Cytology and final histology by conventional Haematoxylin & Eosin (H&E) assessment, prior to the introduction of OSNA. The Chi-square test were used for statistical significance.

## Results

1148 patients had their sentinel node assessed intraoperatively using OSNA in this 7 year study period. The sentinel node was positive in 376 patients (32.8%). Of those who had a positive node, 183 (15.9%) had macro-metastatic disease and 193 (16.8%) had micro-metastatic disease. When compared to 411 patients in the pre-OSNA period, that were assessed by Touch Imprint Cytology and H&E sections, the node positivity rate increased from 23.8% to 32.8% ( $p < 0.05$ ) with the introduction of OSNA. Whilst there was no significant increase in the rate of macrometastatic disease – 20.4% versus 15.9 % ( $p = 0.038$ ), there was a significant increase in the patients who had micrometastases detected on OSNA - 3.4% versus 16.8 % ( $p < 0.05$ ) as shown in the table.

## Conclusion

Our results demonstrate that OSNA is a more sensitive test for picking up metastatic disease, especially micrometastatic disease, in the sentinel node. Whilst this did cause some anxiety initially, the results of recent trials like ACSOG Z-11 and IBCSG 23-01 have shown that small volume disease or micrometastases in the sentinel node do not require an axillary node clearance. Intraoperative assessment of the sentinel node with OSNA significantly upstages the axillary nodal status, especially with regard to micrometastatic disease, but the ability to proceed to an axillary node clearance at the same operation as the sentinel node biopsy, still has significant advantages for the Patient, Surgeon and Health Care Providers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-12

SentiDose interim analysis. A dose optimizing study with a super paramagnetic iron oxide for sentinel node detection

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## Background

Superparamagnetic iron oxide nanoparticles (SPIO) is a novel tracer for axillary mapping in breast cancer with comparable performance to the dual standard of isotope and blue dye. The earlier SPIO (Sienna+®) required 2 ml of SPIO diluted in 3 ml NaCl and was injected retro-areolarly. This was considered to be associated with the discoloration observed in 40% of breast conservation cases. Subsequently, a new form was developed (SiennaXP™) in a volume of 2 ml without dilution. The aim of the ongoing SentiDose study is to compare smaller doses of SiennaXP™ injected in different time-frames (1.5 ml periareolarly on the operation day vs 1 ml peritumourally 1-7 days preoperatively) and compare it to the performance of the original SPIO (Sienna+®). A background mapping with isotope and blue dye was performed for assessment of concordance.

## Method

In all, 330 patients will be recruited from six sites in Sweden, divided in two isonumerical cohorts injected as described above. Results from the 1.5 ml cohort are presented and compared on a patient-level analysis to the SentiMag Nordic trial that used Sienna+®, on a 2-sided non-inferiority margin of 5%. Study endpoints are detection rate per patient, number of sentinel nodes (SN) retrieved and discoloration at 3 weeks postoperatively.

## Results

Detection rate for SiennaXP™, 1.5 ml, was comparable with Sienna+® (96.9 vs 97.6%, p=0.76), even in multivariate analysis adjusting for age and metastasis rate (Exp(B)=0.68; 95% CI; 0.18-2.60, p=0.58). with a high concordance between isotope and SiennaXP™. The number of SNs were similar (1.91 vs. 1.83, p=0.08) for Sienna+® and SiennaXP™. Discoloration rate was lower for SiennaXP™ compared to Sienna+® (14.3% vs. 38.2%, p<0.001) after breast conserving surgery. Furthermore, two patients were excluded in the SentiDose cohort due to protocol violation.

Demographics and outcomes are illustrated in

Table 1

	Nordic SentiMag Trial (n=206)	SentiDose 1.5ml Cohort (n=163)	p-value
Age (yrs)	61.8	64.3	0.03
BMI (kg/m <sup>2</sup> )	26.9	27.2	0.84
Size (mm)	19.2	20.0	0.64
Type of Surgery (BCS/Mx)	154 (74.8%) / 52 (25.2%)	130 (79.8%) / 33 (20.2%)	0.26
SPIO Detection Rate (per patient)	97.6%	96.9%	0.76
SPIO-Tc Concordance	97.5%	97.5%	0.42
Mean SPIO detected SN	1,83	1,91	0.08
Metastasis Rate	26.2%	16.0%	0.01
SPIO nodal rate in malignancy	91.2%	81.6%	0.21
Discoloration in BCS	38.2%	14.3%	<0.001

## Conclusion

The periareolar injection of 1.5 ml SiennaXP™ on the day of the operation provides comparable detection rates with much less

skin discoloration, providing effectivity and flexibility. The completion of the SentiDose study will allow for more definitive results on the dose, timeframe and injection site of SPIO.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-13

Preoperative positive axillary lymph node biopsy, predictors of node metastasis burden, and possible sentinel lymph node biopsy for breast cancer patients in the post-ACOSOG Z0011 trial era

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**Background.** Breast cancer patients with suspicious axillary lymph node (ALN) at ultrasound and positive fine needle aspiration (FNA) results were required to receive ALN dissection (ALND), which may lead to overtreatment for patients with 1-2 ALN metastasis in the post-ACOSOG Z0011 era. We aim to identify clinicopathologic and imaging characteristics associated with  $\geq 3$  ALNs metastasis in these patients, thus to illustrate whether FNA positive patients could be selected to follow the ACOSOG Z0011 trial procedure.

**Methods.** Clinically T1-2N0 breast cancer patients with positive FNA results were retrospectively analyzed. Clinicopathologic and imaging characteristics were compared between patients with 1-2 or  $\geq 3$  ALNs, association between characteristics and  $\geq 3$  ALNs metastasis were also analyzed.

**Results.** A total of 165 patients were included. Having lymphovascular invasion (LVI) positivity (OR = 4.96, 95% CI 1.79-13.73, P = 0.002),  $> 1$  suspicious ALNs at ultrasound (OR = 6.21, 95% CI 2.24-17.23, P < 0.001), MRI (OR = 2.57, 95% CI 1.28-5.15, P = 0.008), and cortical thickness of the suspicious ALN at ultrasound  $> 4.5$  mm (OR = 2.28, 95% CI 1.11-4.67, P = 0.025) were independently associated with  $\geq 3$  ALNs metastasis

table 1

Characteristics	ALN 1-2+ (N = 69)	ALN $\geq 3+$ (N = 96)	P value	OR	95% CI	P value
Histological grade			0.099			0.221
I	1	3		1.0		
II	35	33		0.19	0.02-2.16	0.182
III	33	60		0.31	0.03-3.51	0.345
LVI			0.001			0.002
Negative	61	64		1.0		
Positive	8	32		4.96	1.79-13.73	
No. of suspicious ALNs at US			0.002			<0.001
1	19	9		1.0		
$> 1$	50	87		6.21	2.24-17.23	
Fatty hilum of the suspicious ALN at US			0.881			
Absent	31	42				
Present	38	54				
Cortical thickness of the suspicious ALN at US (mm)			0.019			0.025
$\leq 4.5$	43	42		1.0		
$> 4.5$	26	54		2.28	1.11-4.67	
No. of suspicious ALNs at MRI			<0.001			0.008
$\leq 1$	43	33		1.0		
$> 1$	26	63		2.57	1.28-5.15	
No. of suspicious ALNs at CT			0.003			0.841
$\leq 1$	44	39		1.0		



> 1	25	57		1.08	0.50-2.35	
No. of suspicious ALNs at MMG			0.530			
≤ 1	45	58				
> 1	24	38				

Abbreviation: ALN axillary lymph node, OR odds ratio, CI confidence interval, LVI lymph vascular invasion, US ultrasound, MRI magnetic resonance imaging, CT computed tomographic, MMG mammogram

**Conclusions.** For patients with positive FNA results, having LVI positivity, > 1 suspicious ALNs at ultrasound, MRI, and cortical thickness of the suspicious ALN at ultrasound > 4.5 mm were associated with ≥ 3 ALNs metastasis. Indicating these factors can help select patients to receive SLNB in the post-ACOSOG Z0011 trial era.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-14

Clinical utility of one-step nucleic acid amplification (OSNA) in axillary surgery after neoadjuvant chemotherapy (NAC)

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## Introduction

NAC has been used for downsizing of the tumour in breast and axilla to allow more conservative surgery. In the NAC setting, intraoperative assessment of sentinel lymph node(s) (SLN) is still considered necessary<sup>1</sup>. Current awareness of the prognostic value for axillary nodal down-staging has renewed interest in analysis of SLN post-NAC.

In this study we want to examine the clinical utility of OSNA (based on CK19 mRNA detection) as a method of intra-operative analysis of SLN to assist real-time decision-making for axillary surgery post-NAC in early breast cancer (EBC).

## Methods

Retrospective analysis of prospective data on 399 consecutive patients with EBC who received NAC followed by breast surgery with SLN biopsy (408 axillae) and assessment by OSNA, from September 2011 to January 2018 at the Royal Marsden Hospital (UK). OSNA readouts from the Sysmex RD-100i were collected separate to and blinded from clinico-pathological data. A negative or benign pre-treatment axillary ultrasound scan or indeterminate ultrasound with negative or benign axillary cytology/histology prior to NAC was considered cN0. Univariate analysis (significance at  $p < 0.05$ ) was used to identify risk of recurrence. Patients had a median (mean) follow up of 32.5 (36) months.

## Results

The median age at diagnosis was 49 years, median BMI 26, 41 EBC (10%) were screen-detected, 292 (72%) were grade 3 and the most frequent phenotype was receptor triple negative ( $n=132$ , 32%).

Of 408 axillae, 248 (60%) were initially cN0, of which 113 (46%) had a pathological complete response (pCR) in the breast. SLN in 54 (22%) cN0 patients were positive on OSNA, of which only 6 (9%) had further involved axillary nodes all 6 of which were ER+ Her2-.

The remaining 160 (40%) axillae were cN1 of which 87 (54%) had conversion to ypN0 including 55 (34%) with both ypT0ypN0. Axillary lymphadenectomy (AL) was performed in 79 (19%) patients overall, of which  $n=22$  (28%) were cN0 and 57 (72%) were cN1. Of these, 30 (53%) of the cN1 and 6 of 22 (45%) of cN0 had at least 1 additional positive AL node.

Overall 59 (14.4%) patients relapsed. A significantly worse rate of relapse was observed in cN1 compared to cN0 patients (37/159 (23.3%) versus 22/244 (9%),  $p < 0.001$ ). Combined pCR of both breast and axilla (in cN1,  $n=54$ ) was associated with a significantly reduced risk of relapse and death ( $p < 0.001$ ) compared to those without pCR of either breast or axilla ( $n=62$ ). Of the latter 18 (29%) relapsed (including 10 deaths).

The mean of both the single highest node tumour load (and total nodal tumour load), as measured by CK19mRNA copies/ul on OSNA, were significantly higher at 90,000 (98,300) for those who relapsed versus 23,100 (25,100) for those without relapse ( $p=0.027$ ).

## Conclusions

The OSNA assay is an accurate tool for axillary SLN analysis in patients after NAC and was helpful in intra-operative axillary management. OSNA reduces the need for a second surgery for AL in 20% of breast cancer patients with a positive-SLN after NAC and might offer additional prognostic value.

## Reference

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## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-15

Patient reported outcomes in women undergoing sentinel lymph node biopsy in the SUNRISE randomized trial evaluating different doses of superparamagnetic iron oxide

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**Background.** Sentinel lymph node biopsy (SLNB) with 2 mL of superparamagnetic iron oxide (SPIO) tracer has shown to be non-inferior to the standard radioisotope technique in several studies. The SUNRISE randomized trial has shown non inferiority of 1mL vs. 1.5 mL vs. 2 mL dose of SPIO with the standard use of radioisotope <sup>99m</sup> Tc. We present patient reported outcomes from this randomized trial.

**Material and methods.** Patients with stage I breast cancer who underwent breast conservative surgery and sentinel lymph node biopsy were assigned consecutively (1:1:1) to one of the three groups defined by the different SPIO dose, group 1 (1mL), group 2 (1.5 mL) and group 3 (2 mL). Patients filled a questionnaire related to the presence of skin staining, staining intensity ( mild, moderate, intense) and whether the stain worried them. Patient also completed a quality of life (QoL) EORTC C30 questionnaire at 1 month postoperative visit, at 6 months and at 12 months. First analysis from the 1 month postoperative outcomes are reported here. Follow up on the 6 months will be available for the meeting.

**Results.** One hundred and thirty five patients were included in the trial, 45 in each group. Median age in group 1 was 58 years old, 63 y/o in group 2 and 65 y/o in group 3. (p=0.03) At 1 month follow up, patients in group 1 had less skin tattoo when compared with patients in group 2 and 3 (p= 0.02). There were no significant differences related to skin staining intensity by doses. Seventy percent of patients felt no concern about the tattoo.

On the multivariate analysis including age, SPIO dose, body mass index and breast density, only younger age (p = 0.037), and higher SPIO doses (p= 0.029) were significantly associated with increased skin staining.

There were no statistically significant differences in responses in the QoL questionnaire at 1 month postoperative visit. There were no severe reactions to the procedure or complications in any patient.

**Conclusions.** SLN with 1mL dose of SPIO has shown non-inferiority in the detection of SLN when compared to 1.5 and 2 mL. Being young and the use of 1.5 and 2 mL increased the risk of developing skin staining. Even though, most of the patients were not concern about the skin staining. Rates of discontinuation of skin discoloration will be assessed at 6 months follow up.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-16

Intraoperative evaluation of sentinel lymph nodes after neoadjuvant systemic therapy in breast cancer

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## Introduction:

Intraoperative evaluation of sentinel lymph nodes (SLN) in breast cancer patients are performed using Touch preparation (TP) and/or frozen section (FS). Touch preparation for intraoperative evaluation of SLN is quick and known to be a highly sensitive and specific method for detection of metastasis. Detecting metastases in SLN intraoperatively can be challenging in patients who receive neoadjuvant systemic therapy (NST). In our hospitals, we have been routinely evaluating SLN intraoperatively in patients who have undergone (NST), including those with known metastasis to an axillary lymph node (LN) prior to therapy.

## Objective:

To compare the sensitivity and specificity of TP and frozen section (FS) in the intraoperative evaluation of SLN in the neoadjuvant setting.

## Material and Methods:

This retrospective review study was approved by the institutional review board. Four hundred ninety-eight SLN from 142 patients were included in this study. The intraoperative results for TP and FS were compared with the final pathology results. Relevant clinical and pathological findings such as type of surgery, tumor grade, histologic subtype, and size of metastasis were reviewed.

## Results:

Of the 498 SLN evaluated intraoperatively, 341 were by TP only, 57 by FS only and 100 by both.

Of the 341 SLN examined by TP only, 313 (92%) were interpreted as negative and 28 (8%) as positive for carcinoma intraoperatively. Eighteen LN turned out to be false negative (FN) with no false positives (FP) (sensitivity=62%, specificity=100%). In the false negative cases, 12 LN had micrometastasis, 6 macrometastasis and 1 showed isolated tumor cells (ITC). The size of the macrometastatic ranged from 3 mm to 10 mm.

Of the 57 LN examined by FS only, 48 were true negative and 9 were true positive (sensitivity=100%, specificity=100%).

Of the 100 LN evaluated by both TP and FS, 59 were interpreted as negative and 41 as positive for carcinoma. There were 8 false negatives and 1 false positive (sensitivity=83%, specificity=98%). Of the 8 false negatives, 7 showed micrometastasis and 1 LN had ITC.

## Discussion:

In neoadjuvant cases, both the primary tumor as well as lymph node metastases can show therapy effect such as fibrosis, necrosis and/or histiocytic aggregates. Evaluating SLN in NST cases can be challenging secondary to these effects. The TP slides are often paucicellular in SLN with treatment effect. Residual tumor cells are often trapped in a fibrotic scar and do not transfer onto the TP slide leading to low sensitivity. Therefore, for optimal intraoperative evaluation of SLN in NST cases, frozen section with or without touch preparation, is recommended.

# 2018 San Antonio Breast Cancer Symposium ®

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No relationship of axillary total tumor load (TTL) by PCR (OSNA) in early breast cancer and local and distant clinical outcomes

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## BACKGROUND

The study of sentinel lymph nodes (SLN) assessed by One Step Nucleic Acid Amplification (OSNA, Sysmex, Kobe, Japan) creates a new variable, Total Tumor Load (TTL). This variable is defined as the total number of CK19 mRNA copies in all positive SLN (copies/microL). The latest edition of the Spanish Oncological Gynecology Society (SEGO) Guideline (2017) proposes complete axillary lymph node dissection (ALND) when TTL is 15,000 copies or more in early breast cancer. In our center we are using OSNA to ascertain if there is axillary node involvement but the decision to proceed to ALND is based on Z0011 criteria. We want to determine if there is a correlation between clinical outcomes and TTL values, between TTL and pathological variables and if TTL is a useful tool to decide when to complete an ALND.

## METHODS

Clinicopathological and follow up data were obtained from all patients with invasive breast cancer and SLN assessed by OSNA between 2011 and end of 2016 at our center.

## RESULTS

A total of 277 patients underwent SNB assessed by OSNA with an average follow-up of 56.4 months. 276 were female and 1 male. Age range 27-88 years (mean 58,7). 86,2 % were ductal, 10,8 % lobular and 2,8 % other. 51.9% were luminal A, 51.98% luminal B, 28.51%, triple negative, 5% Her2 positive and 5% luminal B-Her2 positive. TTL was equal to 0 in 155 cases and greater than zero in 122 cases. 68 cases showed a TTL higher than 15,000 copies. Only 19 cases met Z0011 criteria and had ALND. As of now, 3 patients have had locoregional relapse (TTL = 0 in 2 cases and 18,000 copies in one) and 5 metastatic disease (none with simultaneous locoregional recurrence). 7 patients have died (one from metastatic breast cancer, 1 febrile neutropenia, 2 septic shock unrelated to chemotherapy, 2 from other tumors, 1 encephalopathy).

BASELINE DATA N=277

VARIABLE		N	%
AGE, MEDIAN RANGE		58,7 (27-88)	
TUMOR TYPE	Ductal	239	86,2
	Lobular	30	10,8
	Others	8	2,8
IMMUNOPHENOTYPE	Luminal A	144	51,98
	Luminal B	79	28,51
	Luminal B-HER2	14	5
	HER2 positive	14	
	Triple Negative	26	9,3
TOTAL TUMORAL LOAD (TTL)	=0	155	56
	>0	122	44
AXILLARY LYMPH NODE DISECTIONS		19	6,8
TTL>15000		68	24,5
LOCOREGIONAL RELAPSES		3	1

## CONCLUSIONS

1. Using Z0011 criteria, we have adequate clinical outcomes with a very low rate of ALND and locoregional recurrences.
2. If we had based the axillary management on TTL values we would have multiplied the number of ALND by a factor of 2,7 (from 18 to 50).
3. We have observed a tendency to higher TTL in luminal phenotypes and to lower TTL in HER2 positive and triple negative subtypes.
4. Work is in progress to increase our sample size.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-18

Invasive lobular carcinoma does not fit to axillary lymph node management according to NCCN guideline influenced by ACOSOG Z0011 criteria

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[Introduction] Surgery for breast cancer (BC) became less invasion, from radical mastectomy to modified or breast conserving surgery (BCS). Axillary lymph node (ALN) management for cN0 also became less, from ALN dissection (ALND) to sentinel lymph node (SLN) biopsy. In some cases, management without ALND is allowed even if ALN macro-metastasis exist.

[Background] ALND for SLN metastasis positive case is useful for local control, staging and decision making for post-operative treatment. Since the ACOSOG Z0011 trial (Z11) result was reported, however, the necessity of ALND, even if SLN metastasis, became less. In the NCCN guideline (NCCN), strongly influenced by Z11, for cN0 BC with SLN metastasis, operations without ALND are allowed in cases of T1 or T2, the number of metastatic ALN 1 or 2, BCS with whole breast radiation and no-neoadjuvant therapy. This does not mean ALND was abolished but the position of ALND changed, from the perspective that over invasive procedure must be prohibited with appropriate pre-operative and intra-operative diagnosis. If there are some discordance between clinical and pathological diagnosis of tumor size or ALN metastasis, however, the criteria for axillary operation by NCCN will not be recommended. We have major two types of invasive carcinoma, ductal (IDC) and lobular (ILC). Z11 or NCCN did not describe about these two phenotypes. We compared these at the point of suitable axillary management.

[Subjects] Out of 1320 invasive BC (IDC; 1212, ILC; 108) cases in our hospital from January 2008 to January 2018, 1210 cases (IDC; 1113, ILC; 97) with T1/T2 and cN0 were reviewed in two points, the judgment of the competence for BCS was appropriate or not, and cN0 reflected the condition for the omission of ALND (ALN metastasis within 2) or not.

[Results] The difference of diameter between cT and pT; dT (=pT-cT) were measured significantly larger in ILC ( $0.68 \pm 1.97\text{cm}$ ) than IDC ( $0.01 \pm 1.08\text{cm}$ ) ( $p < 0.01$ , t-test) with the wide scattering. We can make proper evaluation for the cT of IDC, but underestimate for ILC. The conversion rate from cN0 to pN1 was significantly higher in ILC (33/97; 34.0%) than IDC (238/1113; 21.4%) ( $p < 0.01$ ,  $\chi^2$  test). In addition, the cases with 3 or more ALN metastasis, this means ALND is necessary, was observed with significantly higher frequent in ILC (13/97; 13.4%) than IDC (74/1113; 7.1%) ( $p = 0.02$ ,  $\chi^2$  test). Clinical evaluation for ALN in ILC was difficult and inaccurate.

[Discussion] Commonly, ILC makes diffuse spread into the breast tissue. This feature will make it difficult to evaluate the clinical appropriate tumor size. Because of not only underestimation but wide scattering, the diagnosis for safety BCS may not be guaranteed in ILC. For ALN, cN0 did not reflect adequately the condition of omission for ALND in ILC compared with IDC. ILC patients with SLN metastasis have to be performed ALND at higher risk. These facts will mean that ILC does not fit to ALN management according to NCCN. Few guidelines separate ILC from IDC for the axillary management. The validation of clinical trials for ALND should be done in histological subtype as well as intrinsic again. Clinically, we must observe carefully in cases of ILC without ALND according to Z11.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-19

Tumor cell detection and immune profiling of lymph nodes from breast cancer patients by mass cytometry

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## **Introduction:**

A sentinel lymph node (SN) is the primary node draining the tumor and is assumed to be affected early in the metastatic process. Detection of metastases in SN is a standard procedure in breast cancer diagnostics based on microscopic evaluation (morphology and immunohistochemistry), determining the need for removal of all axillary glands for inspection which again is crucial for tailoring adjuvant therapy. The identification by microscopy is time-consuming and has a risk for false negative results. We hypothesize that the immune profile of SN changes with the presence of tumor cells, even at very low frequencies (micrometastases). By using a multi marker approach to characterize millions of cells from sentinel lymph nodes with and without metastases we aimed at identifying both tumor cells but also characterize a tumor specific immune response. This dual approach might provide an opportunity for a more sensitive test for SN diagnostics.

## **Material and Methods:**

We established a mass cytometry assay containing 38 markers (antibodies) using CyTOF technology to combine immune profiling with identification of breast cancer cells. Cell suspensions from 14 metastatic axillary lymph nodes (ALNmet), 16 metastatic sentinel lymph nodes (Snmet) and 14 non-metastatic sentinel lymph nodes (SN) from breast cancer patients from the clinical observational trial Oslo2 (early, operable breast cancer patients representing all subtypes) were successfully analyzed by the multimarker panel (single cell resolution).

## **Results:**

By using mass cytometry, we detected tumor cells (gated as PanKeratin<sup>+</sup>/CD45<sup>-</sup> cells) in 86% (26/30) metastatic lymph nodes (ALNmet and Snmet) and in 14% (2/14) non-metastatic lymph nodes (SN). Further, the leukocyte population, identified as CD45<sup>+</sup> cells, was gated into 15 subpopulations, mainly comprising different subsets of B and T cells, monocytes and NK cells. By comparing the leukocyte composition in the ALNmet with those in SN samples we identified a significant increase in the abundance of CD8<sup>+</sup> memory phenotype, TFH and TCRγδ cells and a decrease in the CD4<sup>+</sup> subpopulation in ALNmet compared to the SN samples. The Snmet samples had smaller deposits of tumor cells than the ALNmet samples, and we found no significant differences in leukocyte composition between Snmet and SN samples. Interestingly, when looking at the activation marker CD56, we observed a significant higher expression in the CD4RO, CD8RO, TFH and Treg subpopulations of Snmet samples compared to SN samples.

## **Conclusion:**

In this study we identified a significant difference in immune cell composition in lymph nodes with and without metastases (ALNmet compared to SN samples). We also identified activation markers unique for subpopulations of lymphocytes in Snmet, but not in negative lymph nodes (SN). We were also able to detect and identify micrometastases in most lymph nodes where morphological examination had identified them, but in addition found tumor cells in two samples scored as negative. The results will be validated in a larger sample series.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-20

Which biopsy is preferred to stage the axilla under ultrasound guidance in early breast cancer patients: Fine-needle aspiration cytology or core needle biopsy?

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## Background:

Preoperative axillary ultrasound (AUS) offers the potential to identify clinically axillary negative patients with axillary lymph node (ALN) metastasis directly to axillary lymph node dissection (ALND) with avoid of unnecessary sentinel lymph node biopsy (SLNB) except Z0011 candidates. Which biopsy should be preferred is pendent with lack of enough prospective studies. The purpose of our study was to compare the accuracy of ultrasound guided fine-needle aspiration cytology (FNAC) and core needle biopsy (CNB) for the designated ALN prospectively.

## Methods:

Consecutive patients with histologically confirmed breast cancer from April, 2010 to December, 2014 were preliminary screened for our study. All the candidate patients were prospectively assessed by ipsilateral AUS before initial treatment regardless of clinical ALN status. Abnormal lymph nodes were characterized by round shape with uniform or eccentric thickening, focal bulging or irregularity of the cortex displacement, obliteration of the hilum; or total loss of recognizable lymph node architecture. If suspicious ALN was identified, both ultrasound-guided FNAC and CNB were performed for the same designated ALN. The diameter in longitudinal section, transverse section and maximum cortical thickness of the target node was recorded. When more than one abnormal lymph node was present, the most abnormal-appearing node was sampled. The target tissue for biopsy was hypoechoic thickened cortex. Patients with a positive FNAC/CNB underwent ALND, and those with a negative FNAC and CNB biopsy or without suspicious ALN by AUS underwent SLNB. The paired parameters of FNAC and CNB were compared using McNemar's exact test.

## Results:

705 consecutive patients were screened and 558 patients (561 axillae) underwent AUS evaluation with confirmed ALN pathological results. 145 patients (146 axillae) with both FNAC and CNB were finally enrolled. Final histopathological results showed that 83.6% (122/146) were node positive (5 micro-metastases were considered negative). Sensitivity of FNAC and CNB was 67.2% (82/122) and 91.8% (112/122). Overall accuracy of FNAC and CNB was 72.6% (106/146) and 93.2% (136/146). Negative predictive value (NPV) of FNAC and CNB was 37.5% (24/64) and 70.6% (24/34). There was significant difference in sensitivity, overall accuracy and NPV between FNAC and CNB ( $p < 0.001$ ). 80.6% (29/36) FNAC negative and CNB positive patients have ALN cortical thickness of 3.3-7.2 mm.

## Conclusion:

CNB is more sensitive and accurate than FNAC to stage the axilla under ultrasound guidance in operable breast cancer patients, especially in patients with ALN cortical thickness of 3.3-7.2 mm.

# 2018 San Antonio Breast Cancer Symposium ®

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Usefulness of sentinel lymph node biopsy by indocyanine green fluorescence method for cN0 breast cancer patients

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**Background.** Indocyanine green (ICG) fluorescence method (ICG-f) has been recently widely used in sentinel lymph node (SLN) detection. The advantages of ICG-f are no radiation exposure, no limitation to use in high-volume medical centers without radioactive facility, and to confirm lymph flow as a real-time image from outside the body. ICG-f identified an average of 2.3-3.4 SLNs and the detection rate was 99%, compared to 1.7-2 SLNs by RI methods. Long-term observation after SNB using ICG-f has not been reported, including arm lymphedema as the complication of this method. We evaluate the usefulness of SLN biopsy (SNB) for cN0 breast cancer patients from data of multicenter cohort study on long-term results after negative SNB by ICG-f.

**Methods.** Eleven hundred and thirty-two women were enrolled who had histologically proved clinical stage T1-4, pN0, M0 primary invasive breast cancer with SNB using ICG-f (ICG alone or combination of RI/blue dye method) sparing axillary lymph node dissection from May 2007 to December 2015. This study is retrospective, multicenter cohort study conducted at 6 centers in Japan. Primary endpoint is axillary recurrence rate. We analyzed the correlation with the axillary recurrence and adjuvant systemic therapy, adjuvant radiotherapy, and the clinicopathological characteristics. Secondary endpoint is lymphedema.

**Results and Discussion.** The median follow-up time was 41 (range 21-117) months, and axillary recurrence was found in 6 patients (0.53%). Five out of 6 patients were not received standard adjuvant systemic therapy or adjuvant radiation therapy after breast conserving surgery because of patient's preference or old age. Lymphedema was identified only 4 patients in 632 patients. It is reported that axillary recurrence after SNB was 0.3-1.65%, which was consistent with our result. Lymphedema was not frequent in patients received SNB using ICG-f, because SLNs are removed along with lymphatic ducts in the limited area of axillary adipose tissue.

**Conclusion.** Axillary recurrence after negative SNB using ICG-f was comparable to RI or blue dye method. It might be important to perform appropriate adjuvant medication or radiation therapy for preventing axillary recurrence after SNB using ICG-f. Next, ICG-f after neoadjuvant chemotherapy is to be investigated, because it is reported that removing more than 2 SLNs were associated with a lower likelihood of false negative ratio in patients with clinically node-positive disease converted to clinically node-negative after chemotherapy, and ICG-f might overcome this issue.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-22

Prognostic impact of axillary lymph node status after neoadjuvant chemotherapy for patients with breast cancer

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## BACKGROUND:

Patients were stratified by final pathological axillary status: ypN0, ypN1, pN0 or pN1.

The prognostic impact of lymph node involvement after neoadjuvant chemotherapy (NAC) for breast cancer is not straightforward.

The aim of this study was to compare overall survival (OS) between pathologically node-positive T1,T2 T3 breast cancer patients treated with NAC, with ypN0 or ypN1 and those treated without NAC with pN0 or pN1.

## METHODS:

A total of 3903 consecutive patients with operable breast cancer were prospectively identified at our institution between April 2006 and December 2017. Patients with suspicious axillary LN of breast cancer were assessed using preoperative imaging, underwent fine-needle aspiration cytology or core needle biopsy.

The patients in this study were divided into four groups as follows: the ER(+), the ER(+)/HER2(+) , the HER2(+) and the Triple negative (TN) group.

We evaluate the prognostic impact of the ypN0, ypN1 (with one to three positive lymph nodes after NAC), pN0 and pN1 with no NAC.

The main outcome measures DFS and OS were analyzed using Kaplan–Meier survival analysis.

## Result

A number of 270 and 3633 patients were included for NAC and non NAC, respectively. Pathologic nodal status was ypN0 in 58%, ypN1 in 42% for NAC and pN0 in 76%, pN1 in 24% of patients for non NAC.

Overall, 10-year DFS and OS was 81%, 93% in ypN0, 67%, 80% in ypN1, in 90%, 97%, in pN0 and 83%, 94% in pN1 ( $p < 0.001$ ).

In subgroup analysis, 10-year DFS of ypN0, ypN1, pN0 and pN1 was 86%,77%,95% and 80% in the ER group, 91%,56%,93%,and 76% in the ER/HER2 group,89%,55%,91% and 80% in the HER2 group, 85%,59%,92% and 80% in the TN group.

10 years DFS for the ER group were significantly different between ypN0 and pN0 (HR, 2.42 (1.03–4.86,  $p = 0.04$ ) but were not significantly different between ypN0 and pN0 for the ER/HER2 group (HR 3.58 (0.2–6.88,  $p = 0.66$ ), for the HER2 group (HR 2.6 (0.78–7.65,  $p = 0.10$ ) and for the TN group (HR 1.22(0.56–2.38,  $p = 0.58$ ), respectively).

In all group, DFS for ypN1 was inferior to ypN0.

## Conclusions

In the ER group treated with NAC, DFS for ypN0 be inferior to pN0 with adjuvant treatment. In the HER2, the ERHER2 and the TN group treated with NAC, ypN0 is similar to pN0 with adjuvant chemotherapy.

Axillary nodal status ypN1 in each subgroup is associated with a less favorable prognosis compared to ypN0. In conclusion, the HER2 or TN group is highest for predicting ypN0, shown to be most prognostic of long-term survival similar to the patients with pN0. They could be omitted the axillary dissection.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-23

Which factor of metastatic lymph nodes—The number, tumor volume or anatomical location—Is independently prognostic in breast cancer? - A prospective cohort study using molecular whole-node analysis of all removed axillary nodes

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**Background:** Axillary lymph node status is one of the most powerful prognostic factors in breast cancer. However, it remains unknown which factor of metastatic lymph nodes—the number, tumor volume or anatomical location—is independently prognostic. Conventional pathological examinations of lymph nodes have limited ability to accurately measure metastatic tumor volume due to the partial evaluation of nodes. On the other hand, the one-step nucleic acid amplification (OSNA) assay, a novel molecular method, can quantify the tumor volume in a whole node based on cytokeratin 19 (CK19) mRNA copy number. In this prospective cohort study using the OSNA whole-node analysis, we aimed to elucidate the independent prognostic factor of lymph node metastasis in breast cancer.

**Patients and Methods:** The subjects consisted of 307 cN0 patients with invasive breast cancer, who underwent axillary dissection after a metastatic sentinel node (SN) biopsy and whose SNs and non-SNs were all examined using the OSNA whole-node assay between 2009 and 2012. The cut-off values of the OSNA assay for negative/positive results and micro/macrometastasis were defined at 250 and 5,000 copies/μL of CK19 mRNA, respectively. The total tumor volume in the SN or non-SN was defined as the sum of CK19 mRNA copy numbers from all samples in the SN or non-SN. The cut-off value for the total tumor volume in the SN was set at 2,810 copies/μL according to our previous study (Osako *et al.* Br J Cancer 2017). The anatomical location of metastasis was classified into Level I (confined to SN), Level I (spread to non-SN), or Level II/III. Predictive factors for distant disease-free survival (DDFS) were investigated using the univariate log-rank tests and multivariate Cox proportional hazards models. The median follow-up time was 6.1 years (range, 0.2–8.6).

**Results:** Of the 307 patients, 130 (42.3%) and 177 (57.7%) had the total tumor volume <2,810 and ≥2,810 copies/μL in the SN, respectively. Five-year DDFS was 96.0% in the entire cohort. In the univariate analysis, DDFS was significantly related to the pT classification, grade, hormone receptor status, triple-negative subtype, total tumor volume in the SN and cytotoxic chemotherapy. However, DDFS was not significantly related to the number of metastatic or macrometastatic nodes in the SN, non-SN, or all nodes (i.e. SN + non-SN); the total tumor volume in the non-SN or all nodes; the AJCC pN classification; or the anatomical location of metastasis. In the multivariate analysis, the total tumor volume in the SN (<2810 vs. ≥2810 copies/μL, hazard ratio 5.2, 95% confidence interval 1.2–23.2,  $P=0.03$ ) and cytotoxic chemotherapy (- vs. +, hazard ratio 0.05, 95% confidence interval 0.02–0.17,  $P<0.001$ ) remained significant.

**Conclusions:** The total tumor volume in the SN was the independent prognostic factor of lymph node metastasis in SN-positive invasive breast cancer. Accurate evaluation of metastatic tumor burden in the SN can be important for predicting prognosis and may help to guide the precise therapeutic decision making for breast cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-24

ABCSG 33 - A multi center registry to evaluate the affect of macro metastasis in sentinel lymph node on survival

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## **Background:**

Sentinel lymph node dissection identifies nodal positivity in early breast cancer. Trials like the ACOSOG Z0011 trial tried to show that the waiver of axillary dissection in nodal positive breast cancer (BC) has no effect on the oncologic outcome, however real world data are rare and the role of adjuvant regional radiotherapy is still disputed in this respect.

## **Objective:**

We initiated a multicenter observational registry to investigate omission of axillary lymph node dissection in nodal positive early BC.

## **Design and Setting:**

The 18 sites participating in Austria and Switzerland included from 2014 to 2017 178 patients in this trail.

## **Patients:**

Women with unilateral invasive lymph node positive BC with one or two sentinel lymph node makrometastases, who did not undergo axillary lymph node dissection were included.

## **Results:**

We had a median follow up time of 3.1 years (range between 0.5 and 10.5 years), the median patient age is 63.6 years (range between 33 – 93 years). In 16.9% women had a G1 Grading, 53.1% had G2 tumor and 29.9% had a G3 tumor. Multifocality was seen in 18.1% of the patients. Luminal A tumors were seen in 16 (8.9%) and Luminal B in 82 (46.1%). Fourteen (7.8%) patients in this cohort had HER2 positive BC. In one (0.5%) local recurrence of the axilla occurred. Three (1.7%) of 178 patients died due to BC recurrence.

## **Conclusion:**

Patients with macro metastasis in the sentinel lymph node, treated with breast conserving surgery and whole breast radiation did not have an increased risk of BC recurrence.

Therefore the authors assume that axillary dissection in patients with early stage BC and macro metastasis is not necessary in this patient cohort.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-25

Quality of life and cost-effectiveness in patients undergoing level I versus level II and III axillary lymph node dissection for breast cancer: A double blind randomised controlled trial with non-inferiority hypothesis

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**Background:** Asian patients present with advanced breast cancer, necessitating full, level III axillary lymph node dissection (ALND). Full axillary dissection is associated with complications like seroma, surgical site infection, shoulder dysmobility and lymphedema. These complications lead to impaired activities of daily life and poor quality of life (QOL). Management of these morbidities escalates the cost of care. We designed this trial to ascertain and compare the quality of life and cumulative incidence and severity of complications of patients undergoing a level I vs level III axillary dissection.

**Methods:** In this two-group parallel design randomized trial with non-inferiority hypothesis, 70 patients were randomized. Patients were scheduled for level I axillary clearance. Those with no palpable nodes in the remaining axilla after level I clearance were randomized in OR to receive either complete ALND(level II,III clearance) or limit to level I only. Patients were followed for 3 months postoperatively(week 1,2,3,12). Baseline and postoperative quality of life scores calculated by EORTC-QLQ-C30 and BR23 module were compared between the two groups. Indirect cost for patients in each group was calculated based on the duration of OR time consumed and direct costs included the expenditure in the treatment of complications following surgery. Number of successfully managed patients was identified in each group(those patients not associated with incidence of seroma requiring aspiration and SSI). Cost effectiveness was calculated for each group by dividing the total cost of patient care in each group by the number of successfully managed patients.

**Findings:** There was a significant difference in the duration of procedure between the two groups[level I: 18.57 min vs level III: 32.48 min, p value = 0.000]. Significant differences with worse trend was found in level III patients for QOL[ Global Health Status/QOL- Level I: 77.25(8.92) vs Level III: 71.71(6.89), p value= 0.005; Physical functioning- Level I: 96.21(3.34) vs Level III: 92.92(2.32), p value= 0.000; Role functioning- Level I:91.44(8.44) vs Level III:78.78(11.23), p value= 0.000]. Significant differences were also found in symptom scales of fatigue, pain and arm symptoms. 72.9 % and 54.5 % of patients were successful in level I and level III groups, respectively(p value= 0.137), which yielded a cost-effectiveness ratio of Rs 2,240.46 for level I group and Rs 5.299.12 for level III group.

Comparison of different domains of QOL between 2 groups

Outcome	Level I	Level III	p value
Global Health Status	77.25 (8.92)	71.71 (6.89)	0.005
Physical Functioning	96.21 (3.34)	92.92 (2.32)	0.000
Role Functioning	91.44 (8.44)	78.78 (11.23)	0.000
Fatigue	11.11 (0-33.33)	22.22 (0-44.44)	0.000
Pain	0 (0-33.33)	33.33 (0-33.33)	0.000
Arm Symptoms	0 (0-22.22)	33.33 (11.11-44.44)	0.000

**Conclusion:** Level I dissection was associated with significantly better quality of life and was more cost-effective method of ALND as compared to level III ALND.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-26

Challenging dual modality as the gold standard for sentinel lymph node biopsy in breast cancer: A systematic review and network meta-analysis of novel and conventional techniques

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The current gold standard for sentinel lymph node biopsy in breast cancer patients is the dual modality technique (radioisotope and blue dye). Owing to the limitations inherent to the use of radioisotopes, the uptake of this method is limited to approximately 50-60% of breast cancer patients in developed countries. In most centers worldwide with no access to radioisotopes, blue dye is the most commonly employed technique as it is relatively cheap, easy to administer with no radiation exposure risk. However, the use of blue dye is associated with a high false negative rate. We performed a systematic review and network meta-analysis to compare the performance of blue dye and radioisotope against three novel techniques, namely indocyanine green fluorescence (ICG), superparamagnetic iron oxide (SPIO) nanoparticles and contrast enhanced ultrasound (CEUS) using microbubbles. This is the first network meta-analysis synthesizing direct as well as indirect comparisons of performance among different techniques in terms of sentinel lymph node detection and false negative rate, thereby allowing a more robust quantitative analysis. In comparison to a published systematic review on this topic in 2014, the current review had almost double the number of patients and trials included with a total of 35 cohort studies and 4,244 patients. Our systematic review suggested that two of the techniques, indocyanine green fluorescence (ICG) and superparamagnetic iron oxide (SPIO) nanoparticles have consistently performed better than the blue dye technique and similar to gold standard dual modality.

Table 2 Pooled estimates of RR and averages on detection rate and false negative rate from network meta-analysis (RR)

ICG	1.03 (0.96, 1.11)	1.08 (1.00, 1.15)	1.03 (0.99, 1.06)	1.05 (0.96, 1.14)	1.12 (1.07, 1.16)*
0.67 (0.21, 2.08)	SPIO	1.03 (0.95, 1.12)	0.99 (0.93, 1.06)	1.01 (0.95, 1.07)	1.09 (1.01, 1.18)*
0.16 (0.52, 0.05)	0.24 (0.05, 1.08)	CEUS	0.96 (0.89, 1.02)	0.98 (0.90, 1.06)	1.03 (0.98, 1.11)
0.64 (0.36, 1.13)	0.96 (0.34, 2.75)	4.00 (1.17, 13.7)*	Tc	1.00 (0.91, 1.10)	1.09 (1.04, 1.15)*
0.56 (0.14, 2.23)	0.84 (0.36, 1.93)	3.49 (0.67, 18.2)	0.87 (0.24, 3.23)	Tc/BD	1.09 (0.98, 1.16)
0.29 (0.16, 0.54)*	0.45 (0.14, 1.45)	1.85 (0.68, 6.06)	0.44 (0.20, 0.96)*	0.57 (0.13, 2.51)	BD

BD: Blue dye; CEUS: Contrast-enhanced ultrasound; ICG: Indocyanine green; SPIO: Superparamagnetic iron oxide; Tc: Technetium-99; Tc/BD: Combined used of Technetium-99 and blue dye Top right panel reported the pooled RRs of detection rate from network meta-analysis, and the technique name below each RR was the reference group; Bottom left panel reported the pooled RRs of false negative rate from network meta-analysis, and the technique name to the right of each RR was the reference group; \*: Statistical significant with P value < 0.05

Hence, in centres where blue dye was used as a single modality, perhaps ICG and SPIO can offer a viable alternative with improved performance. Future research should focus on the economic evaluations of various techniques as well as to explore the cost-effectiveness and cost-utility of adopting new techniques in the clinical setting.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-27

Can we select patients suitable for targeted axillary dissection after neoadjuvant chemotherapy who originally presented with involved axillary nodes?

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## **Introduction:**

The current recommended management for patients with involved lymph nodes at diagnosis of breast cancer is to perform an axillary node dissection after receiving neoadjuvant chemotherapy (NAC), regardless of response. A number of studies have proposed de-escalation of this practice by performing sentinel node biopsies and targeted axillary dissections in those with response to chemotherapy. We have audited our practice to assess the safety of introducing management change.

## **Methods:**

Cancer data was collected between 2014 and 2018 for patients who had NAC and further selection criteria for those with lymph node involvement at diagnosis and subsequently underwent NAC. Assessment of radiological response at NAC completion, tumour hormone receptors and HER2 status along with axillary nodal response on final histopathology were reviewed to assess whether patients can be stratified to less extensive axillary management.

## **Results:**

290 patients underwent NAC, 60 of whom had nodal involvement at diagnosis:

- All had USS axilla, MRI at baseline and completion of NAC
- All 60 node positive patients underwent axillary clearance, as per current local protocol
- Out of those 60 node positive patients, 39 had breast conserving surgery, whilst 21 patients had mastectomy
- 20 (33%) patients showed complete radiological response (CRR) on MRI in both breast and nodes
- 23 (38%) patients achieved pathological complete response (PCR) in their nodes
- 17 patients showed both CRR and PCR
- 4 patients showed CRR but did not achieve PCR:
  - o patient 1: 1 macromet / 7 nodes - NEG/NEG/NEG
  - o patient 2: 1 micromet / 14 nodes - POS/POS/POS
  - o patient 3: 7 macromet / 10 nodes - POS/POS/NEG
  - o patient 4: 5 macromet / 19 nodes - POS/POS/NEG
- Large volume of nodal disease remained in 2 patients with POS/POS/NEG despite CRR

## **Conclusion:**

MRI can be safely and reliably used in patients who show CRR with TNBC and HER2+ cancers to select patients for de-escalating axillary surgery. Caution in those patients with POS/POS/NEG cancers as MRI may show CRR but large volume disease in nodes may still persist. Further prospective audit of de-escalating treatment will be essential to ensure locoregional control and long-term disease-free survival outcomes.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-29

Evaluation of a direct reverse transcription loop-mediated isothermal amplification method without RNA extraction (direct RT-LAMP) for the detection of lymph node metastasis in early breast cancer

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**Background:** The detection of lymph node metastasis by reverse transcription loop-mediated isothermal amplification method (RT-LAMP) had been studied previously. Even though, RT-LAMP method provides improved performance compared to intraoperative histology sentinel lymph node (SLN) evaluation, direct RT-LAMP method without RNA extraction can be more efficient and easily accessible process. Therefore, we evaluated the performance and efficacy of a direct reverse transcription loop-mediated isothermal amplification (direct RT-LAMP) assay for visual detection of *CK19*, *CK20*, and *CEA* mRNAs to identify lymph node metastasis in patients with early breast cancer.

**Methods:** A total of 92 lymph nodes dissected from 40 patients with breast cancer were collected at the breast cancer center of Kyungpook National University Chilgok Hospital between November 2015 and February 2016. All of the samples were analyzed by direct RT-LAMP assay and routine histopathology examination. Cutoff values to distinguish metastasis and nonmetastasis were determined by measuring cytokeratin 19 (CK19) mRNA in histopathologically positive and negative lymph node using direct RT-LAMP.

**Results:** We set the cutoff value of direct RT-LAMP assay for CK 19 mRNA at 1ng to distinguish status of LN metastasis. The sensitivity and specificity of the RT-LAMP assay were 85.7% and 100%, respectively. The positive predictive value and negative predictive value were 100% and 94.4%.

**Conclusion:** Direct RT-LAMP assay can allow detection of SLN metastasis in breast cancer patients intraoperatively with a good sensitivity through cost-effective and time-saving manner.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-30

Validity and safety of omission of axillary lymph node dissection among sentinel lymph node-positive breast cancer patients treated with mastectomy

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**Background:** American College of Surgeons Oncology Group Z0011 trial showed that axillary lymph node dissection (ALND) had no impact on recurrence and survival in patients with positive sentinel lymph node (SLN) after breast-conserving surgery. However, it is still unknown if the omission of ALND can be applicable to patients treated with mastectomy. The aim of this study was to evaluate whether ALND could be safely omitted for patients with SLN-positive breast cancer after mastectomy.

**Methods:** From a prospective database of 296 patients with clinically node-negative breast cancer who underwent mastectomy and sentinel lymph node biopsy (SLNB) from March 2006 to December 2016, 81 patients who had positive SLNs were selected. Patient characteristics and prognosis were compared between SLN-positive patients with and without ALND. Patients treated with neoadjuvant chemotherapy were excluded from the analysis. Lymphatic mapping was performed using a combined method of blue dye and radioisotope.

**Results:** The median age of entire patients was 57.0 (range: 32-85) years and the median tumor size was 2.5 (range: 0.6-7.9) cm. Of 81 patients, 23 (28.4%) patients omitted ALND. Patients with SLNB alone were more likely to have smaller SLN involvements ( $p < 0.001$ ): micrometastasis was identified in 13 (56.5%) patients in SLNB-alone group and 9 (15.5%) patients in ALND group. The number of positive SLN was comparable between SLNB-alone (median: 1.0, range: 1-6) and ALND groups (median: 1.0, range: 1-5) ( $p = 0.063$ ). There was no significant difference in characteristics including age, tumor size and tumor subtypes between the two groups. Post-mastectomy radiotherapy was performed in 5 (21.7%) patients with SLNB alone and 16 (27.6%) patients with ALND ( $p = 0.588$ ). The majority of patients with macrometastatic SLN received adjuvant chemotherapy in both groups (83.3% vs. 75.5%,  $p = 0.562$ ). Twenty (87.0%) and 51 (87.9%) patients received adjuvant endocrine therapy in SLNB-alone and ALND group, respectively ( $p = 0.584$ ). After a median follow-up of 54.7 months, no axillary recurrence was observed in both groups and 5-year disease-free survival was not significantly different between the two groups (75.0% vs. 88.8%,  $p = 0.489$ ). Lymphedema was observed significantly more often after ALND than after SLNB (22.4% vs. 4.3%,  $p = 0.045$ ).

**Conclusions:** These data suggested that ALND could be safely omitted in SLN-positive breast cancer patients treated with mastectomy and appropriate systemic therapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Is sentinel lymph node biopsy necessary in all patients with early breast cancer?

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**Background and objectives :** Since the results of the American College of Surgeons Oncology Group Z0011 published, the criteria for applying axillary lymph node (ALN) dissection was relaxed among early breast cancer patients who were scheduled for breast conserving surgery, adjuvant chemotherapy therapy, and adjuvant radiation therapy. SLNB criteria may be established if pathologic nodal status can be predicted. The aim of this study was to develop a nomogram for preoperative prediction of axillary node metastasis.

**Methods:** The records of 1650 patients with T1, T2 primary invasive breast cancer who were treated between January 2013 and September 2016 were selected from the medical database of Yonsei University (Seoul, South Korea). Those whom a preoperative diagnosis of axillary node metastases were excluded. Two nomogram that predicted three or more axillary metastasis and one or more axillary metastasis were developed using a binary logistic regression model with a training cohort. Internal validation was carried out adopting bootstrap method by validation cohort 500 times resampling.

**Result:** A total of 82 (4.8%) patients had three or more ALNs metastasis. Three hundred seventy five (17.4%) patients had one or more ALNs metastasis. Axillary metastasis was associated with Preoperative ALN suspicious image findings, clinical tumor size, Number of neoplastic foci, estrogen receptor status, Ki-67 expression, tumor marker. The nomogram was developed based on the clinical and statistically significant predictors. It had good discrimination performance (AUC 0.79, 95% CI, 0.73–0.85), (AUC 0.71, 95% CI, 0.67–0.74) and calibration fit.

**Conclusion:** Our nomogram might help predict the ALN metastasis in breast cancer patients. Patients with a low probability of ALN metastasis could be spared SLNB.

# 2018 San Antonio Breast Cancer Symposium ®

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Monocentric experience with the sentinel lymph node biopsy prior to neoadjuvant chemotherapy in clinically lymph node negative early breast cancer

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## Background

In patients with clinically lymph node negative (cN0) early breast cancer (EBC) treated with neoadjuvant chemotherapy (NACT), the sentinel lymph node biopsy (SLNB) can be performed before or after NACT. We report safety of axillary staging performing the SLNB prior to NACT in cN0 EBC and estimate NACT-induced downstaging to ypN0 in previously NACT-treated cN1 EBC, to make an assumption for avoiding axillary lymph node dissection (ALND) if SLNB was done after NACT.

## Patients and Methods

Monocentric retrospective study of consecutive triple negative (TNBC) and HER-2 amplified BC patients treated with standard NACT. cN0 patients had SLNB before NACT followed by local therapy. Axillary lymph node dissection (ALND) post-NACT was performed in all cN1 and in cN0 cases with a positive or failed SLNB. Using descriptive statistics, we here report SLNB-detection and SLNB-positive rate, SLNB-operative complications, complete tumor regression in the breast (ypT0/is) and disease-free survival (DFS) for cN0 cases and NACT-induced downstaging to ypN0 in previously NACT-treated cN1 EBC.

## Results

We included 245 NACT-treated patients; 119 cN0 and 126 cN1. SLNB-detection rate in cN0 cases was 99,2%; 25 or 21% had  $\geq 1$  involved SLN, 21.8% experienced SLNB related-complications (e.g. infection, seroma, hematoma) leading to NACT-delay in 3 and interruption in 1 patient. Median start of NACT after SLNB was 7 days (range 1-20 days). In patients with a positive SLNB, there were no additional involved nodes in the ALND. In 5 of these patients, therapy response in a lymph node was described. Complete tumor regression in the breast (ypT0/is) was 52% in SLNB-positive and 59,1% in SLNB-negative cN0 cases. NACT-induced ypN0 was 61% in cN1 cases. At 30 months of median follow-up (range 1-86 months), DFS was 93,2% (4.2% metastatic; no axillary relapse) in cN0 cases. Median DFS was better for patients with complete tumor regression in the breast as compared to those with partial response; 95.6% and 90% respectively.

## Conclusion

In conclusion, performing SLNB before NACT in cN0 cases is a safe and accurate method. While some pN1(sn) could have avoid ALND by NACT-induced axillary down-staging, based on our assumption, long term follow-up is needed to conclude whether SLNB after NACT is safe.

**Keywords:** Breast cancer, neoadjuvant chemotherapy, timing sentinel lymph node biopsy

# 2018 San Antonio Breast Cancer Symposium ®

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Sentinel lymph node biopsy is unnecessary in ductal carcinoma *in situ* patients diagnosed by biopsy

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**Background:** Japanese Breast Cancer Society Clinical Practice Guidelines for Breast Cancer 2018 state that sentinel lymph node (SN) biopsy is unnecessary for patients treated with breast-conserving therapy (BCT) and with an expected final pathological diagnosis of ductal carcinoma *in situ* (DCIS). Regardless of whether they were diagnosed with DCIS by biopsy before surgery, 78% of patients currently undergo axillary procedures in Japan because invasive lesions may be detected in surgical specimens. This study examined whether SN biopsy can be omitted in DCIS patients diagnosed by biopsy and which factors are associated with invasion.

**Methods:** Patients who underwent definitive surgery for DCIS diagnosed by preoperative biopsy at our institution from May 2004 to January 2018 were investigated retrospectively. The factors associated with upstaging to invasive cancer from DCIS were examined with Fisher's exact test and the t-test. (Age, Tumor size, Operation (Mastectomy or BCT), Biopsy method (Core Needle Biopsy or Vaccume-Assisted Biopsy), Mammography (detected or not-detected), Ultrasound (detected or not-detected, mass or non-mass), Comedo, ER, PgR, HER2)

**Results:** A total of 311 patients were enrolled in this study, of whom 277 (89.1%) underwent SN; six of these (2.2%) had SN metastasis. All six cases were upstaging to invasive cancer: five (1.8%) had micrometastasis and one had macrometastasis (0.4%). From a surgical viewpoint, SN metastasis were detected in 3/161 (1.9%) cases treated with mastectomy and 3/150 (2.4%) cases treated with BCT. Although all three cases treated with BCT had micrometastasis, one case treated with mastectomy had macrometastasis (the other two cases had micrometastasis). A total of 80/311 cases (25.7%) upstaged to invasive cancer and the only predictor of invasion was tumor size on images ( $p=0.0002$ ). We could not determine the effective cut-off for tumor size because the area under the receiver operating characteristic curve was  $0.63 < 0.70$ .

Table 1. Tumor size on images was the only predictor of invasion.

	Upstaging (N=80)	DCIS (N=231)	P value
Tumor size: mm (95% Confidence Interval)	47.5 (41.9-53.2)	33.9 (30.5-37.3)	0.0002

**Conclusion:** Tumor size was found to be the only predictor of invasion. Only 2.2% of DCIS patients had SN metastasis despite the fact that 25.7% patients were upstaged to invasive cancer. We conclude that SN biopsy is not necessary for DCIS patients diagnosed by biopsy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-35

Prevalence and risk factors associated with development of lymphedema after axillary lymph node dissection among breast cancer patients: Single center retrospective study

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**Background:** Lymphedema in breast cancer is one of the most important complications, and causes symptoms of arm swelling, heaviness and limited movement. Once lymphedema has occurred, it is difficult to cure. Nowadays, treatment with axillary lymph node dissection (ALND) has been decreasing among breast cancer patients as a result of the ACOSOG Z0011, AMAROS and IBCSG 23-01 trials. However, some cases require ALND for ALN metastasis. ALND increases the risk of lymphedema and detracts from quality of life, but the surgical procedure based on anatomical landmarks has not been changed for several decades. The upper borderline for ALND might cause injury to lymph ducts from arms, and incidence and risk factors for lymphedema after ALND are still unclear. Our aim was to identify prevalence and risk factors associated with development of lymphedema after ALND among breast cancer patients.

**Methods:** This retrospective study was based on data collected from 178 breast cancer patients who underwent ALND in Nagasaki University Hospital, Japan, between 2005 and 2017. Lymphedema was defined as symptomatic arm swelling with >2 cm difference in circumference of the arm compared with that of the contralateral arm. We classified the patients with and without lymphedema, and compared them regarding surgical and pathological findings. Univariate and multivariate analyses were performed to evaluate the risk factors, using the  $\chi^2$  test, Student's *t*-test and Cox logistic regression analysis.

**Results:** Prevalence of lymphedema was 16% (28/178 patients) and mean time interval from surgery to development of lymphedema was 463 days. In univariate analysis, there was a significant difference in postmastectomy radiation therapy (PMRT) ( $p = 0.02$ ) and the number of patients with >18 dissected ALNs ( $p = 0.02$ ). Body mass index ( $p = 0.6$ ), administration of docetaxel ( $p = 0.2$ ), and smoking ( $p = 0.07$ ) did not significantly increase lymphedema. In multivariate analysis, PMRT ( $p = 0.01$ ) and dissection of >18 ALNs ( $p = 0.001$ ) significantly increased the risk of lymphedema, whereas smoking did not ( $p = 0.4$ ).

**Conclusion:** Our study suggested that PMRT and number of dissected ALNs were risk factors for lymphedema. Aggressive and empiric ALND might be associated with axillary lymph duct damage. Therefore, we plan to introduce axillary reverse mapping using indocyanine green to reduce the risk of lymphedema in breast cancer patients who undergo ALND.

## 2018 San Antonio Breast Cancer Symposium ®

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Extending ACOSOG Z0011 to encompass mastectomy patients: A retrospective review

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**INTRODUCTION:** Axillary nodal status in breast cancer patients is a paramount prognosticator, next to primary tumor size and grade. It has been well established that patients with negative sentinel lymph node biopsy can safely avoid axillary lymph node dissection. A positive sentinel lymph node has traditionally required subsequent axillary dissection. The ACOSOG Z11 trial of sentinel lymph node positive patients undergoing breast-conserving surgery found no difference in Overall Survival (OS) and Disease Free Survival (DFS) in those patients who underwent subsequent Axillary Lymph Node Dissection (ALND) vs. observation. The Z11 trial excluded patients who underwent mastectomies. The purpose of this study is to determine whether Z0011 can be applied to mastectomy patients as well in 1-3 positive sentinel lymph nodes and avoid unnecessary ALND.

**METHODS:** A retrospective review was conducted at Shaukat Khanam Memorial Cancer Hospital Pakistan from Jan 2015 to Dec 2017 including patients who were treated for invasive breast cancer and required upfront mastectomy. They were clinically node negative so sentinel lymph node biopsy was performed. Patients underwent ALND with positive sentinel lymph node. A total of 156 breast cancer patients with mastectomies were reviewed.

**RESULTS:** 95% of the patients were female while 3% were male. Average age was 44 years. There was no difference in race, comorbidities, histology, T stage, N stage, overall stage, use of adjuvant chemotherapy and radiation therapy. 64 patients underwent ALND for positive lymph node while 92 patients were spared of axillary dissection due to negative SLNBx. Out of 64 patients 38 patients (59%) had only 1 lymph node positive which was the sentinel node. 18 patients (28%) had 2 lymph nodes positive including the sentinel node while only 8 patients (13%) had 3 or more positive nodes.

**CONCLUSION:** Keeping in mind the complications related to ALND, above results clearly show that ALND could have been avoided in 87% of patients in the setting of adjuvant radiation, possibly avoiding the morbidity associated with axillary lymphadenectomy although a prospective randomized trial needs to confirm these results.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-37

A clinical evaluation of performing total axillary lymph node clearance in breast cancer patients after positive sentinel lymph node biopsy in light of the ZOO11 randomised control trial, based at one centre in the UK

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## Introduction

Axillary nodal involvement is a poor prognostic indicator in breast cancer<sup>1</sup>. Sentinel lymph node biopsy (SLNB) is known to have a >95% rate for identification of nodal metastatic spread, its use has significantly reduced common complications of total axillary lymph node clearance (TALNC)<sup>1</sup>. However, a recent large American study (ZOO11 RCT)<sup>2</sup> demonstrated that in cases of 1- 2 positive sentinel lymph nodes (SLN) in patients with T1/T2 tumours, conservative management is non- inferior to TALNC and does not impact on the 10 year survival rate<sup>2</sup>. In this retrospective study we have analysed a cohort of patients from our centre to clinically evaluate the need for TALNC in patients who have 1-2 positive SLN.

## Methods

Retrospective analysis of histopathology data within our centre identified 1100 patients who had a breast surgery procedure recorded between 2012- 2017. Patients were excluded from this original data set due to duplication of results, lack of electronic patient records as well as coding for non breast surgery related procedures. This left a total of 774 patients. A data collection tool was used to identify and record those patients who had SLNB performed. We recorded the number of nodes yielded as well as the number found to be positive for both SLNB and TALNC.

## Results

From 774 patients 47.5% (368) patients had SLNB performed. The remaining 52.6% (407) patients had a core biopsy, no biopsy or radiological identification of lymphatic spread. A total of 82% (635) patients had TALNC. There were 30.4% patients who had a TALNC based on positive SLNB. The percentage of patients who had TALNC that yielded positive lymph nodes was 13.2%. There were 9.56% (74) patients with only 1-2 positive SLN excised that went on to have TALNC. There were 6.71% patients who had only 1 positive SLN (mean no. of nodes removed = 2.3) and 2.84% patients with 2 positive SLN (mean no. of nodes removed = 3.2). Interestingly there were 2 patients who had 0 positive SLN but had TALNC.

## Conclusion

Our study demonstrates that the number of patients who had SLNB performed with only 1-2 positive nodes identified and then went on to have a TALNC, was very low (9.56%). It brings in to question whether performing a TALNC on this cohort of patients is a necessary routine procedure. Especially given that the ZOO11 RCT demonstrated no difference in 10 year survival (in a similar group of patients) between TALNC vs no TALNC, with both groups receiving radiotherapy post operatively. We could possibly suggest that a change in routine management for patients with only 1-2 positive SLN is that they do not undergo further operative TALNC but proceed straight to radiotherapy treatment.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-38

Sentinel lymph node biopsy procedure: using surgical pliers eliminate radiation exposure risk

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## Introduction

The use of radioactive compounds for sentinel lymph node biopsy (SLNB) is now generally accepted for surgical treatment of breast cancer albeit the risk of radiation exposure to the surgeon. The purpose of our study was to compare the theoretical maximal radiation dose (TMRD) at the injection site of the breast versus fingers radiation dose (FRD) of the surgeon performing SLNB.

## Patients, Material and Methods

This is a monocentric, prospective study on a single surgeon performing SLNB with different dosimetric measurements. Periareolar intradermal injections of technetium-99m colloidal rhenium sulphide (Nanocis® of Curium Laboratory) were administered in the nuclear medicine unit. For one and two day protocols, the activity was 25 MBq and 40 MBq  $\pm$  10%, respectively. During surgery, the nodes in the axilla were visualized with the injected blue dye and by a hand-held gamma probe. During surgery, the absorbed dose to the surgeon's right (dominant) and left hands were recorded using thermoluminescent dosimetry in a ring form From Landauer Laboratory. This lithium fluoride dosimeter enables monitoring equivalent skin dose "Hp007" with a minimum dose threshold of 0.1 mSv. On the hands, one ring was placed on each thumb under the surgical glove and another dosimeter on the nipple of the breast, covered in two thin surgical gloves. The radiation exposure to the hands of the surgeons (FRD) and injection site of the breast (TMRD) were measured by the rings' dosimeters from incision to the cutaneous stitch.

## Results

Between 02-01-2018 and 04-30-2018, a total of 38 SLNBs were included in this study: 9 mastectomies, 28 lumpectomies and 1 sentinel node alone were performed by the same surgeon. The mean patient age was 57 years (range, 31-81). For one day protocol, 36 patients received 22.8 MBq (range, 15-26), and for the last two patients in the 2 day protocol, the activity was 39 and 40 MBq.

The mean exposure time was 57 min (range, 19-73) and the overall time of exposure was 33 h and 31 min. In our study, the TMRD is around 8.8 mSv for 3 months and represents approximatively 35 mSv per year for a breast surgeon. The FRD is similar between right and left hand, 0.16 and 0.15, respectively for 3 months and about 0.6 mSv per year.

## Discussion

It is evident that the surgeon received the highest radiatiodose in the operating theatre, the 3 months TMRD was 8-fold higher in injection site than the FRD, 35-fold per year. Nevertheless, the maximal dose at the injection site was below the critical dose (150 mSv) recommended by the French Nuclear Safety authority (ASN). Surgical techniques using tools such as pliers significantly decrease the dose on surgeon's finger (8.8 vs 0.15mSv for 3 months). According to a French Directive, workers exposed to radiations must be monitored if the effective extremity's annual dose is likely to exceed 50 mSv. Radioactivity in operating room is systematically monitored at our institute. We encourage young surgeons to perform surgery using surgical pliers to manipulate the radioactivity injection site of in the breast, to minimize exposure to radioactivity.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-39

Sentinel lymph node biopsy after surgery with removal of the primary tumor

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Nowadays, sentinel lymph node biopsy (SLNB) is one of the basic methods for diagnosing the lesion of regional lymph nodes (LN) and it is recommended by experts as a standard method in determining the prevalence of the disease in breast cancer patients (BC).

The aim of the study was the possibility of sentinel lymph node biopsy after surgery with removal of the primary tumor.

## Materials and methods

The study was conducted in the N.N. Petrov's National Medical Center of Oncology from 2013 to 2018. SLNB was performed in patients who had a primary tumorectomy in another medical institution. The study included patients with the stage cN0. Radionuclide imaging of the sentinel lymph nodes (SLN) was performed in 37 patients with breast cancer. The injection of radiopharmaceuticals in postoperative scar was performed before the biopsy. Median of the period between the operations was 21 days (14 to 30 days).

## Results

After injection, the image of SLN was obtained in 91.9% (34 of 37) of patients. During the operation, 1-5 sentinel lymph nodes were visualized. The lesion of the sentinel lymph nodes (N+) was detected in 21.6% (8) patients, N0 in 78.4% (29) patients, respectively.

There is no data for locoregional relapse for the period of observation from 2014 to nowadays.

## Conclusions

The obtained results are comparable with the results of sentinel lymph node biopsy in primary patients with breast cancer stage cN0. This indicates the possibility of biopsy after surgery with removal of the primary tumor.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-40

A prospective two arm comparative study of indo-cyanine green (ICG) enhanced fluorescence imaging vs conventional methods (blue dye and radiocolloid/hand held gamma probe) for sentinel lymph node detection in breast cancer - Going beyond the horizon

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## Background:

The dual technique with radio colloid and blue dye is the gold standard in sentinel lymph node biopsy (SLNB) to stage axilla in breast cancer. However due to cost & infrastructural demands of nuclear medicine department most of the oncology centers are not doing slnb or are doing SLNB with blue dye which is not a standard of care. Indocyanine green (ICG) has recently been used as a method of identifying sentinel lymph nodes. Studies have shown that ICG fluorescence imaging alone or in combination with the blue dye method or the radionuclide method is a safe and easy technique. The objective of the present study was to assess the diagnostic performance of sentinel lymph node (SLN) biopsy using the indocyanine green (ICG) fluorescence method compared with that using the conventional method in detection of sentinel lymph nodes.

## Material & Methods:

60 patients diagnosed with early breast cancer underwent the SLNB procedure using technetium 99m radio colloid (R), methylene blue dye (MB), and ICG. Fluorescence imaging was done using an indigenously designed , very economical fluorescence imaging system, Irilic.nm fluorescence imaging along with Indocyanine green. All SLNs that were removed during surgery were labelled as hot, blue or/and fluorescent and sent for pathological examination. The detection rate of SLNs and positive SLNs, and the number of SLNs of ICG, MB+ R, ICG + MB, ICG + R were compared. Injection safety of ICG and MB was evaluated.

## Results:

Sentinel Lymph Node was identified in all 60 cases. Total Sentinel lymph nodes removed was 145 (Mean=2, Range 2-5), ICG was able to identify more nodes than the dual dye technique. The identification rate with the dual dye technique was 95%, with blue dye alone 93.6% and with radioisotope alone 96.8% whereas with ICG alone was 100%, with ICG + MB was 96.6% & ICG + R was 96.6 %. 28(46.6%) out of 60 patients had positive nodes which was identified by both dual dye & ICG. None of the patients had any local or systemic reaction with ICG, 3 patients with blue dye had tattooing & staining of skin.

## Conclusion:

ICG is as effective as the dual dye for SLNB. ICG is safe & reliable. In addition, as a near-infrared dye, it has the advantages of real-time visualization, lower cost, and wider availability. It can be a boon for developing countries & second tier referral centers of developed country where there is limited access to nuclear medicine department & radiocolloid and even if its accesible the cost involved is too high which comes with added radiation exposure to medical personnel handling them. A combination of blue dye and ICG is useful dual approach when radioisotope is unavailable.

## ICG versus Conventional Dye Clinical Profile

	ICG	Radio-colloid+ B;ue Dye
Detection Rate	100%	95%
Sensitivity	100% (CI 83.16% to 100.00%)	100% (CI 83.16% to 100.00%)
Positive Predictive Value	100%	100%
Accuracy	100%	100%

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-41

Preoperative wirefree localization of positive axillary lymph nodes 31-365 days prior to surgery: A proposed practical approach to supplement SLN in neoadjuvant therapy patients

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The study objective was to evaluate preoperative Wirefree Localization (WFL) placement success rate and device stability 31-365 days prior to successful surgery in patients with node-positive breast cancer prior to neoadjuvant treatment (NAT).

**Background:** Wirefree nonradioactive Localization (WFL) has become a standard of care in over 40,000 breast cancer patients in 300 US sites. The radiologist/surgeon performs preoperative WFL of the positive breast or axillary lymph node (LN) using Mammography (MG), Ultrasound (US) or CT guidance.

In August 2018, the FDA expanded clearance of long-term breast WFL to soft tissue and LN. Results of this study further support the ACOSOG-Z1071 subset findings that selected patients with node-positive disease and NAT may be eligible for sentinel lymph node (SLN) surgery and may potentially require less extensive axillary surgery. Long-term (31-365 day) preoperative localization of the biopsy proven positive LN may represent a more practical approach to supplement SLN in NAT patients.

**Methods:** This prospective pilot study enrolled 33 breast cancer patients aged 28-74 (10 Caucasian, 12 African American, 11 Hispanic), with clinical T1-4, N0-2, M0 disease who planned NAT. WFL was performed prior to NAT response and 31 - 365 days preoperatively in the breast and/or positive axillary LN (19 LN only, 4 both breast and LN, 10 breast only). Descriptive statistics were used.

**Results:** This subset analysis showed 23/33 patients were node-positive (10-51 mm size). WFL placements were successful (0-10 mm from center) in all 23/23 patients via US guidance (22 patients, LN 8-35 mm deep to skin) and CT guidance (1 patient, LN 90 mm deep to skin).

WFL stability (0 mm migration) throughout NAT was documented on all standard of care (SOC) preoperative surveillance imaging MG, US, MRI, CT and specimen X-rays (0-222 days). Both the target LN and WFL were well visualized on 9/9 MRI and 8/8 PET/CT SOC imaging. WFL successfully supplemented SLN final surgery in 11/23 subjects to date.

**Conclusions:** WFL of positive LN may be successfully performed prior to NAT response, when the lesion is clearly visualized on imaging. Since successful NAT can result in a complete or partial imaging response, a simpler pre-NAT image-guided WFL may replace the more difficult and less reliable localization post-NAT response. The latter can contribute to incomplete removal of the targeted LN, and unintended larger, more disfiguring cancer surgery.

This subset analysis provides preliminary information to suggest that up front WFL of positive LN may be performed long-term prior to NAT response with no significant adverse events or device migration. Long-term (31-365 day) preoperative WFL of the biopsy proven positive LN may represent a more practical approach to supplement SLN in NAT patients. If larger scale studies confirm these findings, this may prove a clinically relevant paradigm shift for future LN positive patients to ensure that the targeted LN is successfully removed, potentially requiring fewer and/or less extensive radiology and surgical procedures.

**ClinicalTrials.gov NCT03015649 accrual: 33/33**

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-42

Prevention of lymphoedema after axillary clearance (ANC) by external compression sleeves randomised trial: Results of PLACE trial

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Up to 30% of women with early arm swelling (RAVI 4-9%) develop lymphoedema by 24 months post ANC (based on a Relative Arm Volume Increase (RAVI) of >10% criterion). Intervention with a compression garment before arm swelling becomes chronic is claimed to prevent lymphoedema but there is no randomised evidence to support the value of compression garments in preventing lymphoedema after ANC.

## Methods

The PLACE trial was a multicentre randomised open controlled trial testing (1)standard management (written advice, arm elevation, exercises and massage) versus (2)application of whole arm graduated compression garments (pressure 20-25mmHg:Sigvaris) to affected arm, and standard management for 1 year. Women randomised to compression garments were given 4 compression garments (type 2 20 -25mmHg) for 12 months.

The aim was to compare the 1)Time to development of lymphoedema (>10% RAVI) from randomisation, 2)Quality of life in each group (TOI and FACT B+4 ARM sub-scale).

Women with node positive, early breast cancer(n=1300) undergoing ANC consented to pre and postoperative arm measurements with a perometer and those developing a 4-9% increase in arm volume up to 9 months post-surgery entered into the trial and had follow-up measurements.

## Results

Between October 2010 and August 2016, 143 patients were randomised and 38 (27%) patients developed lymphoedema after randomisation. A total of 15 patients died during the study.

Randomised trial arm		No sleeve (n=74)	Sleeve (n=69)
Age at randomisation		55.5 (33.5, 89.9)	55.8 (32.0, 86.9)
BMI (pre op)		27.8 (17.2, 45.3)	28.7 (16.9, 60.9)
BMI (at PLACE entry)		26.9 (18.0, 47.0)	28.4 (20.7, 58.4)
RAVI % change (at P entry)		5.9 (4.1, 8.9)	6.4 (4.0, 8.5)
Follow-up (months)		23 (0, 59)	21 (0, 61)
Time to Lymphoedema (months) from entry		6.7 (interquartile range (IQR):2.0-16.5)	9.9 (IQR: 4.7-14.8)
Type of surgery	ANC	13	15
	WLE+ANC	25	19
	Mastectomy+ANC	36	35
Post Surgery RT Dose (cGy)		N=63 4005 (3660, 5605)	N=59 4005 (1068, 6010)
# Fractions		15 (15, 25)	15 (4, 30)
Site of Radiotherapy	Breast	29	25
	Breast+SCF	20	22
	Breast+Axilla	3	2

	Breast+SCF+Axilla	3	2
	Other	7	8

The lymphoedema rate by 24 months (as predefined by RAVI  $\geq 10\%$  after randomisation) for patients randomised to 'no sleeve' was 30% (21/71) compared to 26% in patients randomised to 'sleeve' (17/66:  $p=0.62$ ). A total of 26 patients randomised to the 'no sleeve' group had a sleeve applied within 24 months; 13 (50%) of these patients subsequently developed RAVI $\geq 10\%$ .

Body Mass Index (BMI) pre-surgery predicted lymphoedema at any time point HR 1.08 (CI 1.02-1.14;  $p=0.011$ ) with BMI  $>30$  HR 3.09 (CI 1.11-8.54 compared to BMI  $\leq 25$ ) patients having the highest lymphoedema rate in both arms of the trial (sleeve: 10/23, 43.5%; no sleeve: 10/22, 45.5%).

There was no significant difference between patients in the two groups in their change in QoL (FACT-B, TOI and ARM subscale) from baseline to 12, 18 or 24 months.

Compression sleeves applied after development of Lymphoedema improved QoL scores (FACT-B  $p=0.007$ :TOI  $p=0.042$ ).

### Conclusions

Early intervention with Type 2 External Compression garments does not prevent lymphoedema, particularly in women with a BMI $>30$ . The use of prophylactic compression garments in "subclinical" lymphoedema (RAVI $<10\%$ ) is unwarranted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-03-43

Incorporation of MSKCC nomogram to guide the application of intra-operative sentinel lymph node frozen section evaluation in patients with early breast cancer

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## **Background and Objectives:**

Although de-escalation of axillary surgery becomes more popular, axillary lymph node dissection (ALND) is still the standard care for sentinel lymph node (SLN) positive patients not meeting the criteria of ACOSOG Z0011 in many hospitals, and frozen section (FS) of SLN is one of the valuable intra-operative assessments to avoid axilla re-operation although it was controversial due to accuracy and efficiency concerns. This study was to assess the performance of selective use of frozen section evaluation guided by MSKCC lymph node metastasis risk prediction nomogram to optimize the procedure to be more accurate and cost effective.

## **Methods:**

Surgical pathology records of consecutive 2582 biopsies in 2552 patients breast cancer patients from 2011 to 2017 were reviewed, intra-operative frozen section diagnosis were compared to post-operative paraffin reports. We calculated the sensitivity, specificity, accuracy and FNR for different MSKCC risk, the axilla re-operation rate with or without FS and the number needed to treat (NNT) to avoid second ALND was also analyzed.

## **Results:**

The sensitivity, specificity, and FNR of FS were 84.7%, 99.9%, and 15.3% respectively. The axilla re-operation rates were significantly decreased if FS was done ( $4.7\% \pm 0.4\%$  with FS versus  $35.8\% \pm 5.8\%$  without FS,  $P < 0.001$ ). The estimated axilla re-operation rate without FS was positively correlated with MSKCC risk ( $r = 0.99$ ,  $P < 0.001$ ), while NNT to avoid second ALND by FS were negatively correlated with MSKCC risk ( $r = -0.98$ ,  $P < 0.001$ ). When patients were divided into four groups according to quartile MSKCC risk, the axilla re-excisional rates were 18.4%, 25.1%, 38.7%, 58.7% without FS, while 4.8%, 3.2%, 5.6%, 3.2% with FS, and NNT correspondingly decreased from 7.3, 4.5, 3.0 to 1.8. An decision-making algorithm for application of FS was proposed.

## **Conclusion:**

Stratified decision-making algorithm based on MSKCC prediction model improved the efficiency of FS to avoid axilla re-operation in patients undergoing sentinel lymph node biopsy. We recommend FS be restricted to patients with MSKCC risk higher than 0.5 who do not meet ACOSOG Z0011 criteria.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-04-01

The value of stereotactic vacuum-assisted biopsy in the investigation of microcalcifications in 1354 patients in public Brazilian hospital

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**Background:** The gold standard for breast biopsy procedures is currently an open excision of the suspected lesion. However, an excisional biopsy inevitably makes a scar. The cost and morbidity associated with this procedure has prompted many physicians to evaluate less invasive, alternative procedures. More recently, image-guided percutaneous core-needle biopsy has become a frequently used method for diagnosing palpable and non-palpable breast lesions. Although sensitivity rates for core-needle biopsy are high, it has the disadvantage of histological underestimation, which renders the management of atypical ductal hyperplasia, papillary lesions, and fibroepithelial lesions somewhat difficult. Stereotactic vacuum assisted breast biopsy (VABB) was developed to overcome some of these negative aspects of core-needle biopsy. VABB allows for a sufficient specimen to be obtained with a single insertion and can provide a more accurate diagnosis and completely remove the lesion under real-time ultrasonic guidance. The advantage of complete lesion removal with VABB is to reduce or eliminate sampling error, to decrease the likelihood of a histological underestimation, to decrease imaging-histological discordance, to decrease the re-biopsy rate, and to diminish the likelihood of subsequent growth on follow-up, especially when stereotactic VABB is used to investigate microcalcifications. This method is expensive but cost effective when used to investigate microcalcifications classified as *BI-RADS* 4 and 5.

**Methodology:** We performed a review in 1,354 patients with suspicious mammography microcalcifications, classified as *BI-RADS* 4 or 5 that were seen in Perola Byington Hospital from July 2012 to July 2017 in São Paulo-Brazil. We have used aHologic Lorad Multicare Platinum Stereotactic Prone Breast Biopsy and a Surus Pearl (Hologic, Malbolrough, Massachusetts, USA), with *gauge* 9 probe. Four to eight fragments (median of 6) were obtained with the placement of a metal clip in the bed that the biopsy was performed, and histopathological analysis was made.

**Results:** The histopathological study of the lesions revealed benign alterations in 956 (68%) of our patients. The findings were positive for malignancy in 358 patients (29%) and the precursor lesions were diagnosed in 40 (3%). In 81 cases (5.9%) the findings were discordant. The sensitivity of the method was 84.4%, specificity was 96.1%, false negative rate was 4.5%, positive predictive value (PPV) was 89.8%, negative predictive value (NPV) was 93.8%. In literature review the sensitivity varies 91.5-100%, specificity 81.9-110%, false negative rate 0-3.3%, PPV 92.2-100% and NPV 80.5-99.5%. All patients with positive or discordant cases underwent surgical treatment to increase the margin or complete removal of the lesion. **Conclusions:** The VABB is an outpatient procedure that avoids hospital admissions for diagnostic elucidation in most of cases suspected of malignancy. It has high predictive value in both benign and malignant lesions, guiding therapeutic planning. In addition to presenting the cost lower than the surgical biopsy it indirect increases the supply of hospital beds for cancer treatment.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-04-02

High ki67 increasing after core needle biopsy was associated with worse disease outcome in estrogen receptor negative breast cancer patients

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Ki67 level would increase after core needle biopsy (CNB) in invasive breast cancer. However, we don't know whether this Ki67 increasing will influence disease outcome. In current study, we enrolled 2029 invasive breast cancer patients with paired CNB and surgical removed samples (SRS) between Jan-1<sup>st</sup>, 2009 and Jun-30<sup>th</sup>, 2016. Estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki67 status were both tested in CNB and SRS samples. Ki67 change ( $\Delta$ Ki67) was calculated as Ki67 expression level in SRS minus CNB. Disease free survival (DFS) and overall survival (OS) were compared between patients with different  $\Delta$ Ki67. The concordance rate between CNB and SRS was 96.55%, 91.42%, 98.62%, and 81.62% for ER, PR, HER2, and Ki67, respectively. Mean Ki67 expression level was much higher in SRS compared with CNB samples: 30.37% vs. 26.55% ( $\Delta$ Ki67 = 3.82%,  $P < 0.001$ ). High Ki67 increasing after CNB was defined as  $\Delta$ Ki67 > 3.82%. Both univariate and multivariate analysis found that surgery time interval after CNB, surgery type, pathological type and molecular subtype status were associated with  $\Delta$ Ki67 ( $P < 0.05$ ). In whole population, there was no DFS ( $P = 0.679$ ) or OS ( $P = 0.974$ ) difference for patients with high or low  $\Delta$ Ki67 ( $\Delta$ Ki67  $\leq$  3.82%). Moreover, ER status interacted with disease outcome and  $\Delta$ Ki67 value ( $P < 0.001$ ). For patients with ER positive breast cancer,  $\Delta$ Ki67 level was still not related with DFS ( $P = 0.206$ ) or OS ( $P = 0.112$ ). However, in ER negative breast cancer patients, the 5-year DFS was 84.7% in the low  $\Delta$ Ki67 group, which was much higher than those in the high  $\Delta$ Ki67 group (77.5%,  $P = 0.025$ ). The 5-year OS was 91.3% and 87.0% for ER negative patients with low or high  $\Delta$ Ki67, respectively ( $P = 0.079$ ). Furthermore, multivariate analysis demonstrated that high  $\Delta$ Ki67 was associated with worse DFS (hazard ratio (HR) = 1.68, 95% confidence interval (CI): 1.06-2.65,  $P = 0.027$ ) and OS (HR = 1.90, 95% CI: 0.97-3.73,  $P = 0.060$ ) in ER negative patients. In conclusion, Ki67 value would increase after CNB for invasive breast cancer. High  $\Delta$ Ki67 didn't influence disease outcome in ER positive breast cancer but was significantly associated with worse disease outcome in ER negative patients, which warrants further study.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-05-01

Age-related methylation signals of breast cancer risk in blood

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**BACKGROUND:** Age is the biggest risk factor for developing breast cancer, which suggests that the biological aging process is a direct driver of cancer etiology. In all normal tissues, DNA methylation status changes systematically with age and is believed to mediate the biological consequences of aging. DNA methylation patterns, commonly referred to as 'Epigenetic Clocks', can be used as measures of aging. However, the chronologic and epigenetic ages can have subtle differences in different individuals. We hypothesize that accelerated epigenetic aging (i.e. DNA methylation indicating an older age than the chronologic age of the individual) is a risk factor for breast cancer development.

**METHODS:** We used DNA methylation data from blood samples and clinical data from n=2,107 participants in the Women's Health Initiative (WHI) and tested the association between breast cancer risk and two of the most commonly used epigenetic clocks by Horvath (based on 353 CpGs) and Hannum et al. (based on 71 CpGs). DNA methylation in whole blood was measured using the Illumina HumanMethylation450 BeadChip. We used Cox proportional hazard models to assess the association between two epigenetic age predictors (calculated using the algorithms by Horvath and Hannum et al.) and subsequent risk of breast cancer. The model was adjusted for several breast cancer risk factors including: chronologic age at the time of blood sampling, observational vs. clinical trial, clinical trial arm, race/ethnicity, education, BMI, waist-hip ratio, smoking, alcohol, age at menopause, age at menarche, number of pregnancies, age at first birth, previous mastectomy, months breastfed, and cell count estimates. Family history data and BRCA mutation status was incomplete in the WHI and therefore could not be included in our analysis.

**RESULTS:** Increased epigenetic age determined by the Horvath clock relative to chronological age was associated with increased future incidence of invasive breast cancer, even after adjusting for known risk factors (HR=1.04, P=0.03). Utilizing the Hannum clock, we found no significant association between epigenetic age and breast cancer risk (HR=1.01, P=0.568). When we included both age predictors as independent variables in a single model, the strength of the association between the Horvath epigenetic age and breast cancer risk increased (Horvath HR=1.09, P=6.3e-5; Hannum HR=0.95, p=0.077), such that every one year increase in epigenetic age relative to chronological age was associated with a 9% increased risk of future breast cancer. These results suggest that the aging signal in the Horvath clock that is unique from that captured by Hannum is what drives the specific association with breast cancer.

**CONCLUSIONS:** Our results support the hypothesis that "accelerated" epigenetic aging measured in the blood increases breast cancer risk. We also demonstrate that the two epigenetic clocks capture different aspects of aging, only some of which have implications for breast cancer risk. Epigenetic clocks may assist in targeting breast cancer screening to higher risk populations in the future, and understanding the biological mechanisms that are altered by the epigenetic changes may lead to new risk reduction strategies.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-05-02

Epigenetic remodeling in response to BRCA2-crisis

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Individuals with a single functional copy of the *BRCA2* tumor suppressor have elevated risks for breast, ovarian and other solid tumor malignancies. The exact mechanisms of carcinogenesis due to *BRCA2* haploinsufficiency remain unclear, but one possibility is that at-risk cells are subject to acute periods of decreased BRCA2 availability and function ("BRCA2-crisis"), which may contribute to disease. Here we establish an *in vitro* model for BRCA2-crisis that demonstrates novel epigenetic remodeling and activation of an NF- $\kappa$ B survival pathway in response to transient BRCA2-depletion. Mechanistically, we identify BRCA2 chromatin binding, histone acetylation and associated transcriptional activity as critical determinants of the epigenetic response to BRCA2-crisis. These epigenetic alterations are reflected in transcriptional profiles of pre-malignant tissues from *BRCA2*-carriers and therefore may reflect natural steps in human disease. By modeling BRCA2-crisis *in vitro* we have derived insights into pre-neoplastic molecular alterations that may enhance the development of novel preventative therapies.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-05-03

Identification of epigenetically silenced breast cancer driver genes

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Breast cancer is clinically and molecularly complex disease driven by aberrant genetic and epigenetic alterations. Epigenetic alterations in particular DNA methylation changes are one of the most important events involved in breast cancer initiation and progression. Previous reports identified many aberrant DNA methylation signatures associated with molecular subtypes of breast cancer and over 100 candidate genes with promoter hypermethylation in breast cancer. However, it remains elusive which of these genes with promoter hypermethylation play “driver” role in tumorigenesis. In previous studies, the average gain of DNA methylation across all cancer samples compared to the average DNA methylation in normal samples has been the criterion to select for potential targets. However, known tumor suppressor driver genes regulated by methylation are relatively infrequently altered in target cancers. Therefore, we propose the paradoxical hypothesis that identifying hypermethylated cancer drivers require focusing on infrequent rather than frequent events. Hence, to identify these potential driver genes, we developed an algorithm with two unique properties. First, unlike previous studies we focused on targets that gained DNA methylation relatively infrequently (10-40%) and that lost expression in breast cancer. Second, using this algorithm, we distinguished cancer dependent gain of DNA methylation from age-dependent gain of methylation. To discern age dependent and independent DNA methylation changes, we generated DNA methylation sequencing data on 29 normal purified breast epithelium (age range 33-82 years old). Furthermore, to study the biological effects of the overexpression or downregulation of these genes, we generated DNA methylation sequencing data on 6 breast cancer cell lines. We also used DNA methylation and RNA expression datasets (675 cancer, 100 normal) available through the TCGA. Using our algorithm, we identified 53 genes with age independent promoter hypermethylation and loss of expression in TCGA tumor samples. To begin testing the biological effects of these driver genes, we performed canonical pathway enrichment analyses using Ingenuity Pathway Analysis software. We also investigated the mutational status of these genes and their molecular subtype enrichment. Based on these analyses, we picked 12 genes (C10orf125, RUNX3, YOD1, FXYD5, SMOC1, SLC16A5, RNLS, DKK1, PNPLA3, FZD10, RND2, and PLCB1) for further study. We stably overexpressed these potential driver genes in different breast cancer cell lines. Twelve genes out of the 12 tested, slowed cell proliferation and 9 decreased anchorage independent growth. We further validated these driver genes by knocking them out in normal human mammary epithelial cells using CRISPR/Cas9 tool. The loss of these genes, increased cell proliferation rate in normal human mammary epithelial cells compared to the control cells. In conclusion, based on our preliminary data, using bioinformatics tools as well as functional assays, we identified epigenetically altered breast cancer driver genes. Identifying and deciphering true epigenetic cancer drivers could potentially lead to the development of therapeutic drugs targeting these genes and/or targeting pathway dependence.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-06-01

Clonal evolution and heterogeneity in breast tumors treated with neoadjuvant HER2-targeted therapy

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**Background:** Understanding to what extent a breast tumor's genetic composition may change over the course of a few months of neoadjuvant therapy has implications for optimal therapeutic approach. However, genomic changes observed across treatment may result from either treatment-induced clonal evolution or geographically disparate sampling of a heterogeneous tumor. We sought to characterize the geographic heterogeneity in primary breast tumors, and to incorporate this information into analysis of clonal evolution with neoadjuvant therapy.

**Methods:** We assembled the largest cohort to date of multi-region (n=2-3) whole-exome sequenced (WES) or whole-genome sequenced untreated primary breast tumors with matched normal and adequate tumor purity for analysis: four tumors with data generated for this study and five tumors compiled from three previous studies. We also generated the first cohort of multi-region (n=2-6) WES breast tumors post-neoadjuvant HER2-targeted therapy and chemotherapy, sequencing one region from a pre-treatment diagnostic specimen, multiple regions from the post-treatment surgical specimen, and matched normal for five HER2+ breast tumors that did not achieve a pathologic complete response. We used an agent-based model of spatial tumor growth to investigate whether the mutational patterns we observed with treatment were consistent with pre-existing heterogeneity or treatment-induced selection.

**Results:** In untreated primary breast tumors, on average 30% (range 1-70%) of apparently clonal mutations from a single region were absent or rare in a second, spatially disparate region (high-frequency regional, or HFR). Intra-tumor heterogeneity was similar post-treatment (HFR 28%, range 10-54%), and was higher in breast tumors than in previously analyzed colon, brain, lung, and esophageal tumors. Simulation studies confirmed that with high heterogeneity as observed in breast tumors, analysis of one pre-treatment and one post-treatment region could not distinguish treatment-induced clonal evolution from pre-existing heterogeneity; however, obtaining at least two post-treatment regions allowed for detection of clonal shifts with treatment. Analysis of multi-region data revealed that clonal replacement occurred with neoadjuvant therapy in two of the five tumors. Candidate causes of therapeutic resistance included amplifications in *CCND1*, *ERBB4*, and *MYC* in one subclone, and functional protein-altering mutations in *ERCC2*, *SMO*, and *WT1* in another. Mathematical modeling suggested that these putative resistant subclones comprised 0.02-12.5% of the overall pre-treatment cell population, substantially larger than previous estimates of resistant tumor clone size.

**Conclusions:** WES data from multiple regions of untreated and treated primary breast tumors revealed considerable heterogeneity that remained present throughout treatment with chemotherapy and HER2-targeted therapy, even while major clonal sweeps took place in a minority of tumors. Obtaining at least two samples for analysis from breast tumors post-neoadjuvant therapy may reveal the tumor's evolutionary path and, especially as increasing numbers of molecular and immune therapeutic targets are identified, inform new clinical strategies.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-06-02

Imprint of parity and age at first pregnancy on the genomic landscape of subsequent breast cancer

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**Background:** Although parity and age at first pregnancy are among the most known extrinsic factors that modulates breast cancer risk, their impact on the biology of subsequent breast cancer has never been explored in depth. In this study, we investigated the imprint of parity and age at first pregnancy on the pattern of somatic mutations, somatic copy number alterations (SCNAs), transcriptomic profiles, and tumor immune microenvironment by assessing infiltrating lymphocytes (TILs) levels of subsequent breast cancer.

**Methods:** A total of 313 patients with primary breast cancer with available whole genome, RNA sequencing and TILs data were included in this study. We used a multivariate analysis adjusted for age at diagnosis, pathological stage, molecular subtypes and histological subtypes. We compared nulliparous vs. parous, late parous vs. early parous, and nulliparous vs. pregnancy associated breast cancer (PABC) patients. Late and early parous patients were grouped by using the median age at first pregnancy as a cut-off value. PABC was defined as patients diagnosed up to 10 years postpartum.

**Results:** Genomic alterations of breast cancer were associated with age at first pregnancy but not with parity status alone. Independently of clinicopathological features, early parous patients developed tumors characterized by a higher number of Indels ( $P_{adj} = 0.002$ ), a lower frequency of *CDH1* mutations (1.2% vs. 12.7%;  $P_{adj} = 0.013$ ), a higher frequency of *TP53* mutations (50% vs. 22.5%;  $P_{adj} = 0.010$ ), *MYC* amplification (28% vs. 7%;  $P_{adj} = 0.008$ ), and a lower prevalence of mutational signature 2, putatively associated with APOBEC activity. PABC were associated with increased TILs infiltration ( $P_{adj} = 0.0495$ ).

**Conclusions:** These findings highlight an unprecedented link between reproductive history and the genomic landscape of subsequent breast cancer. This work advocates that reproductive history should be routinely collected in future large scale genomic studies addressing the biology of female cancers.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-06-03

Copy number analysis identifies *ESR1* and *MDM4* as drivers of progression in invasive lobular breast carcinoma

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**Background:** Invasive lobular carcinoma (ILC) is the second most common histological subtype of breast cancer after invasive ductal carcinoma (IDC). While specific clinical and pathological features differ between ILC and IDC, both histologies are treated the same, due to a lack of knowledge of targetable pathways underlying the observed differences. To identify potential genetic drivers of ILC progression, we set out to identify genes with copy number (CN) alterations, comparing tumors with good outcome to those with poor outcome.

**Method:** We designed probes for a total of 67 genes known to be frequently altered in breast cancer and used sensitive nanoString technology to comprehensively investigate CN alterations of these genes in 70 well-curated primary ILCs. ILC cell lines MDA-MB-134-VI, SUM44PE, and BCK4 were used for functional studies including proliferation, apoptosis, colony formation, and analysis of gene expression.

**Results:** Our studies reveal that *ESR1* is frequently amplified in primary ILC (14% gains and 10% amplification), and that tumors with amplified *ESR1* are more likely to recur compared to those with normal CN. Our analysis also identified a subset of ILCs with *HER2* amplification (19%) despite a negative clinical IHC score, and these tumors expressed high *HER2* mRNA, protein, and demonstrated enrichment of a molecular *HER2* signature. The other most frequently amplified genes included *CCND1* (33%), *MDM4* (17%), and *MYC* (17%), and most frequently lost genes were *NCOR2* (7%), *FGFR4* (6%) and *TP53* (6%). *MDM4*, a negative regulator of p53, has previously been reported to play a role in breast cancer, though little is known about its role in ILC. We demonstrate that decreasing *MDM4* levels in p53 wild type ILC cell lines results in increased apoptosis, decreased proliferation associated with cell cycle arrest, and activation of p53 target genes. Intriguingly, a similar induction of G0/G1 cell cycle arrest and increase in apoptosis was observed in p53 mutant ILC cells after *MDM4* downregulation, suggesting a p53-independent function of *MDM4*.

**Conclusion:** Sensitive detection of CN changes identified amplifications of *ESR1* and *MDM4* as potential drivers of ILC. Functional studies demonstrate that *MDM4* has both p53 dependent and independent functions that warrant further study.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-06-04

Clonal evolution of non-malignant proliferative lesions into breast cancers

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**[Introduction]** Non-malignant proliferative lesions in the breast have been implicated in the development of invasive breast cancer. Previous studies showed that adjacent atypical proliferative lesions and breast cancers shared common genetic alterations, suggesting that these evolved from the same ancestral cell. However, the clonal structure of atypical proliferative lesions and their clonal dynamics during progression to cancer are poorly understood. In this study, we compared genetic profiles (with and without pathogenic germline mutations) of normal mammary ducts, non-malignant proliferative lesions, and cancer tissues from the same patients to illustrate the clonal evolution of cancer from a non-malignant epithelial cell.

**[Methods]** Multiple samples were collected from different proliferative lesions within the cancer-borne breast, including invasive cancers, using micro-dissection from formalin-fixed, paraffin-embedded surgical specimens. Somatic mutations and copy number alterations (CNAs) were then evaluated by whole exome sequencing.

**[Results]** We analyzed a total of 34 samples from 5 premenopausal females carrying estrogen receptor-positive cancers, where the samples were obtained from normal ducts (N = 6), non-atypical (N = 1) and atypical (N = 8) proliferative lesions, and non-invasive (N = 16) and invasive (N = 3) cancers. The number of somatic mutations per sample ranged from 1 to 276 and increased with disease progression, regardless of the germline mutation status. Two cases with bilateral cancers had a pathogenic germline mutation of either *BRCA2* or *TP53*, where no somatic mutations or CNAs were shared by individual proliferative lesions, suggesting multifocal independent cancerous evolutions. By contrast, in the remaining three unilateral cases, no pathogenic germline mutations were detected, but all proliferative lesions, which were separated by a distance of 7-25 mm, shared one or more driver alterations, such as an *AKT1* mutation (UID: KU01), concurrent 1q gain and 16q loss (der(1;16)) (UID: KU02), and a *GATA3* mutation and der(1;16) (UID: KU03), while harboring private mutations and/or CNAs of their own. The phylogenetic analysis based on the number of shared mutations predicted an early origin of these founder mutations, which frequently predated decades before the onset of cancer.

**[Conclusions]** Our results suggest that early breast cancer development is shaped by the evolution of multiple precancerous clones. These clones are originated from a common ancestor that acquired a founder mutation long before the onset of cancer, followed by branching evolution of multiple clones that acquired additional driver mutations of their own, from which an invasive cancer ultimately develops. In hereditary cases, this process is thought to be substantially promoted multi-focally from within the entire breasts by a germline mutation shared by all mammary cells, frequently resulting in bilateral and/or multifocal breast cancers. Our findings provide unique insight into the early development of breast cancer.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-05

Whole genome sequence analysis of 77 breast cancer patients reveal increased somatic transposable element insertions responsible for early onset of cancer

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More than 50% of the human genome consists of transposable elements (TE). However only certain classes of retrotransposons (ALU, SVA, ERV and L1) are believed to be active. They transpose using an RNA intermediate causing target-site duplications along with a poly-A tail. They can also insert into intronic regions and disrupt normal transcription of protein-coding genes. However, most TEs are repressed using various epigenetic mechanisms such as DNA methylation, histone modifications and RNA silencing. The piRNA pathway also plays an important role in retrotransposon silencing by establishing a repressive chromatin state. In this study, somatic and germline TE insertions in 77 TCGA breast cancer cases were characterized. These studies have revealed that somatic TEs can insert into intronic regions of tumor suppressor genes such as LRP1B, RAD51B and WNK2. Characterization of germline TE insertions, reveal insertions in intronic regions of predisposition genes such as CDH1 and BRCA2. Analysis of germline SNPs in high somatic TE patients reveals the presence of deleterious variants in genes such as NRDE2, RAD51C and DDX3X which are known to regulate TEs using RNA silencing via the piRNA pathway and other DNA-damage response mechanisms. Patients having high somatic TE insertions were found to have lower age of diagnosis suggesting a role in the early onset of cancer (median age of patients with high TE=43.37yrs; low TE=59.77yrs, Wilcoxon p=0.023). Further comparison of somatic TE insertion burden against somatic point-mutation burden revealed an inverse correlation suggesting analysis of TE insertions is indispensable to get the complete mutation profile of breast cancer genomes.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-06

Novel breast cancer mutational signatures

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**Background:** Our understanding of the biological processes that generate somatic mutations in breast cancer has increased markedly over the past five years. Using the catalog of somatic mutations present in cancer genomes, over 30 “mutational signatures” have been produced. While these provide important insights into the processes responsible for somatic mutation, gaps remain, and the etiology of several signatures remains unknown.

**Methods:** We have developed a new method in which the specific nucleotide change (e.g., C>T), the codon that each mutation falls in (e.g., GCT), the position in the codon (e.g., 2), and the nucleotides immediately 5' and 3' of the mutation (e.g., 5': C; 3': G) are all considered. The summary of these mutation characteristics forms a mutational profile for each tissue sample. Putting multiple samples' profiles together forms a sparse matrix with the number of samples as rows and the mutation characteristics as columns. Nonsmooth nonnegative matrix factorization was then applied to enable the discovery of intrinsic patterns in this sparse matrix.

**Results:** Using somatic mutations identified in 1017 breast cancer tissues from The Cancer Genome Atlas (TCGA), we have identified four mutational signatures. Signature A correlates with the well-defined APOBEC signatures and signature B with the “aging” signature, which is the result 5-methylcytosine hydrolysis. Signature C and signature D are potentially new signatures. Signature D is enriched with C:G>A:T mutations; these mostly occur in the middle position of codons, and are enriched with GG(CC) either 5' or 3' of the mutation's sequence context. G>T mutations are known to occur as a consequence of oxidative damage that is not repaired. Guanines are vulnerable as they have the highest vertical oxidation potential of the nucleobases. The 5' guanines in GG sites are especially reactive. We hypothesize that Signature D results from oxidative mutagenesis. When correlated with clinical phenotypes, the basal subtype is clearly enriched for tumors with the Signature D mutation pattern (exposure level is in 169 basal tumors and in 797 non-basal tumors,  $p < 0.01$ ), suggesting an etiologic link with basal-like breast cancer. G>T somatic mutations in breast cancer mainly take place during cell replication rather than during transcription. In the normal breast, epithelial cell replication occurs during the luteal phase of the menstrual cycle and during pregnancy, primarily under the direction of progesterone (P). P binds to its receptor (PR) in a subpopulation of PR positive cells where it initiates the transcription of genes including *RANKL*, with resultant paracrine stimulation (through RANK), of the NF- $\kappa$ B signaling pathway in neighboring cells. The four *RANKL* genes (*TOP2A*, *MKI67*, *PBK*, *CDK1*), defined by Nolan et al., are all positively associated with the signature D (P-values < 0.05), suggesting that this type of mutagenesis is associated with *RANKL* pathway upregulation.

**Conclusions:** We have identified a potentially new somatic mutational signature, which we have designated as Signature D, which appears to result from exposure of DNA to oxidative stress during replication. It is associated with the basal subtype of breast cancer as well as *RANKL*- NF- $\kappa$ B pathway upregulation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-07

Recurrent but not pathognomonic fusion genes in mucinous carcinomas of the breast

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**Introduction:** Mucinous carcinoma of the breast (MCB) is a rare histologic subtype of estrogen receptor (ER)-positive invasive carcinoma, and is characterized by tumor cells floating in pools of mucin. Despite their characteristic histology, MCBs are heterogeneous at the genetic level and no driver genetic alterations have been identified. Notably, MCBs lack the common genetic alterations found in ER-positive invasive ductal carcinomas (i.e. 16q losses, 1q gains and *PIK3CA* mutations). Fusion genes have been reported in breast cancer, including highly recurrent or pathognomonic fusions genes, such as the *ETV6-NTRK3* and *MYB/MYBL1* rearrangements in secretory carcinoma and adenoid cystic carcinoma, respectively. In this study we sought to define whether MCBs would be underpinned by a pathognomonic fusion gene.

**Materials and methods:** Seven pure mucinous A (hypocellular), seven pure mucinous B (hypercellular), and the mucinous component of a mixed MCB were microdissected, and subjected to RNA extraction followed by RNA-sequencing for fusion gene discovery. Read pairs supporting chimeric transcripts were identified using INTEGRATE, FusionCatcher and STARfusion. The Bayesian driver probability of the candidate fusion genes was annotated using OncoFuse. In-frame fusion gene candidates with a high driver probability were validated using orthogonal methods (RT-PCR and Sanger sequencing).

**Results:** Our analysis identified fusion genes in 47% (7/15) of the MCBs analyzed (29% (2/7) of type A MCBs, 57% (4/7) type B MCBs and in the mucinous component of one mixed MCB). The *OAZ1-CSNK1G2* and *RFC4-LPP* fusion genes were identified in 20% (3/15) and 13% (2/15) of the cases, respectively. The *OAZ1-CSNK1G2* chimeric transcript results in the truncation of the kinase domain of *CSNK1G2*, which represses ER transactivation. The *RFC4-LPP* chimeric transcript leads to the fusion of exons 1-3 of *RFC4* and exons 5-11 of *LPP*, where the LIM domains of *LPP* are conserved. *LPP* is a known partner of fusion genes identified in mesenchymal tumors and reported to mediate TGF- $\beta$  induced breast oncogenesis via its LIM domain. Additional validated, potentially pathogenic fusion genes identified in MCBs involved kinases, phosphatases or regulators of tyrosine kinase receptor signaling, such as *IRAK3-PPM1H* (n=1), *GIGYF2-GFRA3* (n=1) and *PHF20-FAM217B* (n=1).

**Conclusions:** MCBs harbor fusion genes in almost half of the cases with two fusions being recurrent, involving primarily genes encoding kinases, phosphatases or receptor tyrosine kinase signaling regulators. Nevertheless, due to the lack of recurrence of a specific fusion gene, this special histologic type of breast cancer is unlikely to be underpinned by a highly recurrent/pathognomonic pathogenic fusion gene.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-08

Mucinous and neuroendocrine breast cancers: A spectrum of genetically-related lesions distinct from common forms of estrogen receptor-positive breast cancers

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**Introduction:** Mucinous (MBCs) and neuroendocrine breast carcinomas (NBCs) are special histologic types of estrogen receptor (ER)-positive breast cancers. Together, MBCs and NBCs account for approximately 5% of invasive breast cancers. NBCs are defined by the expression of neuroendocrine markers (i. e., chromogranin and/ or synaptophysin), whereas MBCs consist of nests of cancer cells floating in pools of extracellular mucin and can be either hypocellular/type A (MBC-A) or hypercellular/type B (MBC-B). MBC-B may display neuroendocrine differentiation. We compared the repertoire of somatic genetic alterations in MBCs-A and MBCs-B, and in NBCs, and evaluated whether they differ from ER-positive/ HER2-negative invasive ductal carcinomas of no special type (IDC-NSTs).

**Materials and methods:** Reanalysis of the targeted capture or whole exome sequencing data of 22 MBCs (12 MBCs-A and 10 MBCs-B), and of 15 NBCs was performed. The BAM files were retrieved and somatic mutations were detected with MuTect, indels with Strelka, VarScan2, Scalpel and Lancet, and copy number alterations using FACETS. The mutational repertoire of MBCs-A and MBCs-B was compared to that of NBCs, and of ER-positive/ HER2-negative IDC-NSTs from The Cancer Genome Atlas (TCGA) breast cancer study (n=310).

**Results:** The most frequently mutated genes in MBCs-A were *SF3B1* and *FRG1B* (17%, each); in MBCs-B were *GATA3* and *KMT2C* (30%, each), and in NBCs were *FOXA1* and *TBX3* (20%, each) and *KMT2C* (20%). No significant differences were observed in single gene comparisons between MBCs-A, MBCs-B and NBCs (Fisher's exact tests, p>0.05). When compared with ER-positive/HER2-negative IDC-NSTs from TCGA, NBCs harbored a higher frequency of mutations targeting *FOXA1* (20% vs 2.9%, Fisher's exact test, p<0.05) and *TBX3* (20% vs 2.9%, Fisher's exact test, p<0.05), and a lower frequency of *TP53* mutations (0% vs 23.9%; Fisher's exact test, p<0.05). We observed a lower frequency of *PIK3CA* mutations in both MBCs-A (8.3% vs 40%, Fisher's exact test, p<0.05) and MBCs-B (0% vs 40%; Fisher's exact test, p<0.01) compared to ER-positive/HER2-negative IDC-NSTs. Although not statistically significant, *PIK3CA* mutations were numerically less frequent in NBCs than in ER+/HER2-IDC-NSTs (13.3% vs 40%; Fisher's exact test, p>0.05). Concurrent 1q gains and 16q losses, the hallmark copy number alterations of ER-positive/HER2-negative breast cancer were found in 47% of the ER-positive/HER2-negative IDC-NSTs from TCGA, but only present in 20% of NBCs and in none of the MBCs-A or MBCs-B.

**Conclusion:** Type A MBCs, type B MBCs and NBCs harbor similar patterns of genetic alterations, including a low frequency of *PIK3CA* mutations and of concurrent 1q gains/ 16q deletions. Taken together, our data suggest that both MBCs and the vast majority of NBCs constitute a spectrum of histologically and genomically related subtypes, distinct from ER-positive/HER2-negative IDC-NSTs.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-09

BRCA mutations and not type 1 tandem duplicator phenotypes are associated with pathological complete response in patients with triple negative breast cancer undergoing neoadjuvant carboplatin/nab-paclitaxel

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**Background.** We recently described six distinct genomic configurations characterized by large numbers of distributed somatic tandem duplications (TDs) known as Tandem Duplicator Phenotypes (TDPs). Different TDPs feature TDs of different span sizes, and are enriched in TNBC, ovarian, and uterine cancers. Type 1 TDPs (i.e. groups 1, 1/2mix and 1/3mix) feature short span TDs (~11Kb in size), invariably show abrogation of BRCA1 (via mutation or methylation) and of TP53, and affect ~40% of TNBCs. We had observed, in limited *in vitro* and preclinical PDX models, that TDP status correlates with platinum sensitivity (1). Here, we assess TDP status across a cohort of 42 TNBC patients (pts) undergoing neoadjuvant carboplatin and NAB-paclitaxel to test the hypothesis that type 1 TDP status may be predictive of optimal response to platinum-based therapy.

**Methods.** 42 pts with TNBC were enrolled in a phase II study of neoadjuvant carboplatin/nab-paclitaxel at the City of Hope National Medical Center (NCT01525966). Pathological complete response (pCR) was achieved in 50% of pts (21/42). WGS was performed using standard Illumina protocols. Structural variants were called using Crest, Delly and BreakDancer, and high confidence breakpoints were selected when called by at least two tools and by requiring split-read support. TDP status was ascertained as recently described (2). *BRCA1* methylation was determined by methylation-specific PCR.

**Results.** 45% of the tumors classified as TDP (19/42). Consistent with our previous observation, the vast majority were type 1 TDPs with short span TDs (n=17) and were strongly associated with BRCA1 mutation or methylation (16/17, P= 1.4E-8). However, there was no correlation between TDP status and pCR (OR=1.1, NS). In a more detailed analysis, we found that *BRCA1* mutation correlated with pCR rate (6/7 pCR, P=0.01), whereas promoter methylation did not (4/11 pCR, NS). Moreover, both pts with mutant *BRCA2* achieved pCR. Thus, as a group, pts with *BRCA1/2* mutations (but not *BRCA1* methylation) were more likely to achieve pCR than those with wild type *BRCA1/2* (OR=11.9, P=1.7E-2). Results were unchanged when using RCB 0 and 1 vs. RCB 2 and 3 as the response criteria.

**Conclusions.** This study confirmed that reduction of BRCA1 activity via either mutation or methylation robustly associates with type 1 TDPs in TNBC. However, TDP status did not predict good response, suggesting the separation of BRCA effects on genomic instability and platinum sensitivity. This indicates that genomic signature assessments, such as TDP and HRD, may not be sufficient in predicting pCR in TNBC. Importantly, we found that *BRCA1/2* mutated TNBC pts were more likely to experience pCR (8/9) compared with pts with either *BRCA1* methylation (4/11) or wild type *BRCA1/2* (8/21). The exact genetic underpinnings of response in non-BRCA pts are currently under investigation.

## References.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-10

Multiscale modeling of omics data for precision breast cancer treatment

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**Introduction:** The vast majority of cancer patients continue to receive treatments that are minimally informed by omics data. In the case of breast cancer, only ER and HER2 are routinely used for treatment selection. There is a particular need for personalized treatment in individuals with primary and secondary drug resistance or aggressive breast cancers. Emerging bioinformatics and statistical methods have made a fundamental impact on cancer research. However, challenges remain with regard to patient-centric data analysis and providing genomic data guidance to oncologists. There exists a large number of FDA approved anti-neoplastic drugs used to treat cancers other than breast and the development of innovative informatics methods and algorithms to repurpose those drugs should benefit breast cancer patients.

**Methods and Results:** We have developed precision care systems (such as PANOPLY and CORPUS) to identify personalized therapies for an individual patient and to deliver genomic reports in a standard, searchable format so that a researcher or an oncologist can quickly navigate through molecular data and obtain prioritized drugs and targets. The PANOPLY (**P**recision **c**ancer **g**enomics **r**eport: single sample inventory) algorithm applies machine learning and topology-based network analysis methods to integrate multi-omics profiles and clinical data; individual-specific molecular alterations are identified and compared with a set of matched-controls having similar clinical data. Since there is a lack of a “gold standard” dataset to test such algorithms, we simulated 500 case-control sets and evaluated drug predictions across multiple simulation scenarios. We applied the PANOPLY algorithm to The Cancer Genome Atlas (TCGA) breast cancer cohort, which consists of multi-omics data and clinical data. In addition, PANOPLY was also applied to an in-house neoadjuvant breast cancer study (BEAUTY) that consists of multi-omics data, clinical data, and patient-derived xenografts (PDXs). In the TCGA breast cancer study we obtained survival data to determine the cases and matched-controls; and in the BEAUTY, we used pathologic complete response (pCR) as an outcome to determine responders and non-responders. Recurrent targetable alterations were not enriched in patients without pCR in the BEAUTY study. We have applied the PANOPLY to non-responder patients to identify individual specific alterations, dysregulated networks, drug targets, and drugs for each patient and stored them as case reports in CORPUS (**C**omputational **O**ncology **R**eports and **P**recision therapy **U**tics), a web-based repository that allows clinicians to review genomic reports. Using comprehensive “omic” data derived from a triple negative breast cancer patient who had pre and post-neoadjuvant chemotherapy PDXs, PANOPLY prioritized the PARP inhibitors as the top class of drug. Using the PDX models available from this patient, we tested olaparib and confirmed the *in vivo* antitumor activity (more effective than vehicle with a p-value < 0.05 in the PDXs). Further studies to confirm PANOPLY findings are currently underway.

**Conclusions:** In summary, the PANOPLY and CORPUS systems incorporate molecular data together with clinical data to provide genomic reports with proposed drug targets to advance or enable precision breast cancer care.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-11

Time-course DNA and RNA profiling of tumors from intra-patient cross-over trial of sequential use of aromatase inhibitors

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**Background.** The NEO-LET-EXE trial examines the neoadjuvant use of sequential administration of the aromatase inhibitor letrozole (Femar / Femara) and the aromatase inactivator exemestane (Aromasin). Although both drugs nearly completely inhibit aromatase, resistance to both is developed with time. However, when used sequentially, in some patients after switching to the alternative drug and progressing on the first choice, new responses may appear. The mechanism behind this clinical observation is currently not known. The solution may lead to a novel strategy to re-sensitize tumors to hormonal treatment. Prior studies have examined genomics at the four month time point, but not at both two months and four months.

**Material.** Postmenopausal patients with estrogen receptor (ER) positive (>50%), HER-2 negative locally advanced breast cancer may be enrolled. Age: 18+ (no upper limit). Present accrual and target accrual: 49 out of planned 100 patients have been enrolled so far. The last patient is expected to enter the trial in Q4 2019.

**Study design.** In the neoadjuvant, randomized, open-label, intra-patient cross-over trial NEO-LET-EXE biopsies are taken before treatment, after two months on one aromatase inhibitor and swap to the other aromatase inhibitor, and at surgery at four months.

**Results.** In order to explain the phenomenon of a lack of cross-resistance between steroidal and non-steroidal aromatase inhibitors we profiled biopsies at three time points per patient by whole exome and whole transcriptome sequencing from FFPE from 25 patients. A total of 56 DNA whole exomes and 41 RNA seq transcriptomes were generated from FFPE samples available. When grouping both arms together, mutational burden decreased at two months, while clonality of mutations increased, providing evidence of selection. At four months, mutational burden increased from the two month timepoint. In particular, PIK3CA somatic variants present at the first time point were not detected at two months. However, these were detected again at significant variant allele fractions at four months after switch of treatment. The majority of gene expression changes happen in the initial two months, with fewer changes between two and four months. Instead, significant changes in alternative splicing at two months and four months were observed, for example for FGFR1, which does not experience a large fold change in expression between these two points. Our preliminary results show significant DNA and RNA changes in the first two months of aromatase inhibition leading to fewer, more clonal variants. Comparison of the four month to two month time point shows fewer RNA changes than the prior two months and an increase in the number of somatic variants compared to the two month timepoint.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-12

Genetic heterogeneity of DCIS is a predictor of invasive cancer

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**Background:** Heterogeneity is a hallmark of human cancers that is apparent both between and within individual tumors.

Intra-tumor heterogeneity provides the genetic fuel for natural selection in clonal evolution and cancer progression. Tumors with high levels of genetic heterogeneity are hypothesized to be more likely to progress to invasion and metastasis

**Methods:** We measured the mutational loads from separate areas of pure DCIS and compared this to genetic heterogeneity in DCIS lesions that co-exist with invasive cancer, as a surrogate for progression. Cases of pure DCIS and DCIS diagnosed concurrent with invasive cancer were identified. Two areas of DCIS from each case a minimum of 0.8 cm apart and control tissues were macro-dissected and the DNA extracted from FFPE samples. To analyze the data, we developed new bioinformatics methods that allowed analysis of small amounts of degraded DNA extracted from FFPE samples across multiple regions. Our bioinformatics pipeline was optimized on a series of 28 independent technical replicates of the same DNA sample sequenced twice, as training tools to find the best filtering parameters.

**Results:** Whole exome sequencing was performed on two geospatially separated blocks for each case (41 pure DCIS and 30 DCIS adjacent to invasive disease). Minimum coverage for inclusion in this study was 40X over at least 50% of the exome. We used the ratio of private mutations (only in 1 area) to public (found in both areas) mutations as a measure of intra-tumor heterogeneity. Overall mutational load of DCIS was not a significant predictor to progression; however notably, DCIS adjacent to invasive disease patients had a higher ratio of private/public mutations (heterogeneity) in coding domains (Mann-Whitney  $p=0.016$ ). Functional analysis of mutated coding genes (DAVID 6.8) shows a statistically significant enrichment in signal transduction, olfactory receptors (G-protein-coupled receptors) and cell-matrix interactions in both tumor types, after FDR correction. DCIS adjacent to invasive disease had an enrichment of mutated genes involved in additional cellular functions such as microtubule activity (fold enrichment =7.6, FDR=0.002), protein-protein interactions (fold enrichment =3.65, FDR=5.11E-04) and extracellular matrix remodeling (fold enrichment =8.3, FDR=0.02).

**Conclusion:** We present an approach to measure clonal heterogeneity using a bulk sequencing strategy applied to geospatially distinct foci of DCIS. Our findings suggest that functional heterogeneity may play an important evolutionary role as a driver for invasive progression.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-13

Whole exome sequencing of HER2+ metastatic breast cancer (MBC) patients (pts) with extraordinary durable complete responses (ExdCR) to trastuzumab (T)

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**Background:** Trastuzumab (T) has shown clinical efficacy in early-stage and MBC. However, within 1-year 40-50% develop resistance to T. The exact mechanism of the development of T resistance is not completely understood. Anecdotal observations suggest that a small fraction of patients with HER2+ MBC may be "extraordinary durable complete responders (ExdCR)". Indeed, we previously reported that 9% of MBC achieve dCR following T and chemotherapy. Understanding the genomic mechanisms underlying exceptional dCR to T may improve patient selection and treatment rationale to identify HER2+ MBC pts who are more likely to achieve dCR following T treatment.

**Methods:** Genomic DNA was extracted from paraffin embedded formalin fixed (FFPE) tissue. Whole exome sequencing (WES) on primary tumours from 9 MBC ExdCR > 60 mo (5 matched T:N) and 6 non-responders (NR) or PR < 6 mo (3 matched T:N). Tumours were analysed for single nucleotide variants (SNVs) point mutations, insertions/deletions (indels), copy number alterations (CNA), and tumour mutational burden. Detailed clinicopathologic data was collected for each patient and linked to the genomic information.

**Results:** WES of matched tumour:normal samples revealed differences in SNVs and indels between the ExdCR pts compared to NR. Mutations in TP53 were found in 2/5 ExdCR pts and in 0/3 NR. Initial analysis of CNA revealed that HER2 is significantly more amplified in ExdCR pts compared to NR, and this was also shown by IHC and FISH.

**Conclusions:** We present a genomic landscape of extraordinary durable complete responders compared to non-responders using WES. High variability exists in mutation profile of ExdCR pts with few overlapping genes. Further analysis into clinically relevant genomic and molecular alterations will be performed to potential aid in patient selection and choice of therapy, and novel drug targets.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-06-14

Whole exome analysis of extreme long-term survivors with metastatic breast cancer

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**Background:** Metastatic breast cancer (MBC) is incurable, yet some extreme long-term survivors (ELTS) live for a decade or more. Mechanisms underlying extreme survival are incompletely known, yet are important to improve prognosis and treatment. Extreme survival also provides an opportunity for cancer cells to evolve over decades. To evaluate the genomic characteristics of ELTS, we identified a cohort representative of the extreme tail of the metastatic survival curve and performed genomic analysis of blood and matched metastatic tumor.

**Methods:** We identified a cohort of ELTS at our institution in an IRB-approved study. Eligible subjects had MBC and had lived a  $\geq 10$  years from the original breast cancer (BC) diagnosis with hormone receptor (HR)-positive disease or  $\geq 5$  years if HR-negative. Those with available archived tumor tissue were enrolled for genomic analyses. Archived samples were obtained from metastatic sites obtained late in the clinical course. Whole exome sequencing (WES) was performed on matched blood and tumor. Mutational profiles were qualitatively compared with publicly available reference somatic alterations in primary BC (Ctrl; cbiportal.org). Frequency of mutations is reported for known BC drivers. In an exploratory analysis to identify novel genes, the frequency of mutations in ELTS vs. Ctrl was compared for 15,659 well-covered genes. These were further evaluated for the ratio of non-synonymous to synonymous mutations, which is indicative of selective pressure.

**Results:** 53 ELTS were identified; 13 had available archived tumor tissue and consented. The median survival from original BC diagnosis was 22 years, which corresponds to 99.8<sup>th</sup> percentile for survival, benchmarked to a cohort of MBC patients identified from the Surveillance, Epidemiology, and End Results-Medicare linked database. The 13 subjects had lived with metastatic disease for a median of 9 years (range 4-23). One subject had HR-/HER2+ BC and had lived 16 years with MBC. Three additional subjects had HR+/HER2+ BC with survival ranging from 19-25 years from original BC diagnosis. The remaining 9 subjects had HR+/HER2- BC with survival of 13-40 years from original diagnosis. Matched tumor/somatic WES achieved a mean tumor read depth of  $\sim 600\times$  and 96% coverage. Somatic mutations revealed near-expected rates of known driver alterations in commonly mutated genes such as *PIK3CA*, *TP53*, and *CDH1* (Table). No mutations were found in *PTEN* or *ESR1* in ELTS. Novel gene candidates were identified as having a high prevalence of mutations in this cohort including Androgen Receptor (10 of 13), *MUC4* (12 of 13) *MUC12* (10 of 13), and *HRNR* (12/13). Pathway analysis is ongoing.

**Conclusions:** ETLS tumors obtained late in the clinical course have mutations in known cancer drivers near rates expected from primary BC. However, we have identified additional genes with an increased number of mutations either related to distinct genetic features of these cancers, or due to accumulated mutations that occur over the extended time for tumor evolution.

Frequency of driver mutations in control and extreme long-long term survivors

Gene	Ctrl freq	ELTS freq	Gene	Ctrl freq	ELTS freq
<i>PIK3CA</i>	0.35	0.38	<i>MAP2K4</i>	0.02	0.38
<i>TP53</i>	0.35	0.23	<i>NF1</i>	0.04	0.15
<i>GATA3</i>	0.12	0.08	<i>CBFB</i>	0.03	0.15
<i>CDH1</i>	0.10	0.23	<i>ERBB2</i>	0.03	0.08

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-15

Young breast cancer patients demonstrate worse survival associated with aggressive oncogene expression but not with mutation load, tumor heterogeneity or pro-tumor immune cell infiltrations

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**INTRODUCTION:** Young breast cancer patients have more aggressive subtypes and higher mortality rates. This study investigates the biologic, immunologic, and oncogenic differences between Young ( $\leq 40$  yo) and Non-Young ( $> 40$  yo) patients with breast cancer.

**MATERIALS/METHODS:** The Cancer Genome Atlas (TCGA; n=1095) and the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC; n=1894) were used for analysis. Gene Set Enrichment Analysis (GSEA) was performed on breast cancer patients in TCGA. We calculated mutation load using both TCGA and METABRIC. We also calculated the Cytolytic Activity Score (CYT), Mutant-Allele Tumor Heterogeneity (MATH), T-Cell Receptor (TCR)-Richness, and Ki67 mRNA expression in TCGA.

**RESULTS:** There were 97 and 116 Young patients and 994 and 1788 Non-Young patients in the TCGA and METABRIC databases respectively. Young patients had a lower DFS ( $p=0.012$ ) in TCGA. Young patients had a lower DSS ( $p<0.001$ ) in METABRIC. There were less Stage I (13.5% vs 17.3%) and II (54.2% vs 58.3%) patients and more Stage III (31.2% vs 22.4%) patients in the Young group. There were more basal-like subtypes in the Young in TCGA (17.8% vs 16.1%) and METABRIC (28.4% vs 9.3%). Mutation load in TCGA was lower in the Young ( $p=0.030$ ), but not significantly different in the METABRIC database. MATH, which reflects tumor heterogeneity, was not significantly different between the groups. These results were unexpected since Young patients have a higher proportion of basal-like subtype which is known to be rich in mutations and more immunogenic. In TCGA, Young patients were found to have higher amounts of activated dendritic cells ( $p=0.049$ ). In METABRIC, Young patients had higher amounts of Plasma cells ( $p=0.016$ ), CD4 memory-activated T-cells ( $p<0.001$ ), NK resting cells ( $p=0.015$ ), and M1 Macrophages ( $p=0.002$ ). We also found that regulatory T-cells ( $p=0.029$ ), activated NK cells ( $p=0.016$ ), M2 Macrophages ( $p<0.001$ ), and resting Mast cells ( $p=0.006$ ) were lower in the Young. This unexpectedly showed that anti-tumor immune cells were more enriched in Young patients. Indeed, the CYT, which reflects tumor killing activity, and TCR-Richness, which reflects T-cell function, were both significantly higher in Young patients ( $p=0.034$ ,  $p=0.004$ , respectively), which was opposite from what we expected due to its biological aggressiveness. GSEA was then used to analyze the TCGA database to clarify gene sets that are enriched in Young patients. Of the 50 Hallmark gene sets analyzed, 4 gene sets were found to be enriched in Young patients; G2M Checkpoint ( $p=0.002$ ), Hallmark MYC Targets V1 ( $p=0.004$ ), HALLMARK E2F Targets ( $p=0.035$ ), and Hallmark Unfolded Protein Response ( $p=0.038$ ). Ki67 which reflects cell proliferation was significantly higher in Young vs Non-Young patients ( $p=0.004$ ).

**CONCLUSIONS:** Both TCGA and METABRIC cohorts demonstrated that Young patients have more basal-like subtype and significantly worse survival. Our results support the notion that Young patients have more aggressive cancer not because of mutations, tumor heterogeneity or immune cell infiltrations, but because of aggressive oncogene expressions.

# 2018 San Antonio Breast Cancer Symposium ®

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The pattern of alpha- and beta- adrenergic receptor expression impacts breast cancer outcome

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**BACKGROUND.** Chronic stress promotes myriad of genomic changes collectively termed conserved transcriptional response to adversity (CTRA), contributing to a pro-tumorigenic and immunosuppressive tumor microenvironment (TME). Adrenergic stimulation is one mechanism of CTRA, and adrenergic receptor (AR) modulators are currently repurposed in cancer trials. However, the impact of AR expression on TME and overall survival outcome (OS) in breast cancer (BC) remains unclear. We asked whether AR expression in tumor samples predicts prognosis in BC patients (pts) and whether it correlated to expression in normal cells.

**METHODS.** Public RNA expression data accessed from The Cancer Genome Atlas (TCGA), and fed to deconvolutional algorithm CIBERSORT, estimating 22 immune cell proportions. Clinical and OS data were accessed from XENA. Differential gene expression obtained for 115 CTRA genes known to correlate with stress. Cytolytic activity (CY) appended from Rooney et al.

**RESULTS.** 1,211 pts had clinical and genomic data, including 114 pts with normal breast (BN) samples. When compared to BC, BN samples were enriched for *ARG1*, *PTGS2*, *VCAM1*, *CSF1*, as well as all ARs (*ADR A1A*, *A1B*, *A1D*, *A2A*, *A2B*, *A2C*, *B1*, *B2*, *B3*). There was significant correlation between BC and BN samples in *A2A*, *B1*, *B2*, *IFN-γ*, *PTGS2* (Spearman ρ -0.2, -0.27, -0.2, 0.28, 0.29, P<0.01). On survival analysis, worse OS was associated with higher expression of *A1B* and *A2C* (HR 1.1[1-1.22], 1.1[1-1.17]), while higher *B1* predicted better OS (HR 0.86[0.79-0.93]). OS impact persisted after quantile separation (HR for higher to lower quantiles of *A1B*, *A2C* and *B1* were 1.47[1.1-2], 1.38[1.01-1.9], 0.69[0.49-0.95]). Co-expression of *A1B* and *A2C* predicted significantly worse OS than either alone (HR 1.53[1.1-2.2]). Results persisted after adjusting for age. For TME analysis between quantiles, higher *A1B* and *A2C* expression correlated with higher regulatory T (Treg) cells (OR 1.42[1.1-1.86]), fewer resting and activated dendritic cells (DCs) and memory CD4+ cells, and lower CY (OR 0.81[0.64-0.9]). In comparison, higher *B1* correlated with higher tumor infiltrating lymphocytes (TILs), M1 macrophages (M1), M1/M2 ratio (OR 1.45[1.14-1.84], 3.64[1.03-12.8], 1.86[1.46-2.36]), lower M2 and Treg (0.42[0.33-0.53], 0.65[0.49-0.85]), and higher CY (OR 1.89[1.49-2.38]). CY also correlated with *IFN-γ*, *MMP9* and *CSF1* (Spearman ρ 0.75, 0.59, 0.3 p<0.001). Higher M2 and lower M1/M2 ratio were independently associated with a poorer OS, persisting after control for *B1* (HR 1.78[1.27-2.47], 1.5[1.08-2.08]). T-cell exhaustion (Tex) genes *CD274*, *PDCD1*, *CTLA4*, *IDO1*, *LAG3* and *HAVCR2* were all lower in ADR-α (OR for *A1B* was 0.69, 0.73, 0.68, 0.87, 0.61, 0.69) and higher in ADR-β (OR for *B1* was 1.46, 1.32, 1.29, 1.42, 1.26, 1.4).

**CONCLUSIONS.** AR genes were similarly expressed across normal and tumor samples from BC pts. Pts with higher ADR-α expression had worse OS (higher Treg, lower CY) while higher ADR-β expression pts had better OS (higher TILs, M1, M1/M2, lower Treg, M2). Tex genes were higher in ADR-β, likely due to higher TILs. These findings illustrate the potential impact of chronic stress on TME and clinical outcome, potentially helping to discern pts who can benefit most from AR modulation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-17

Unlocking the transcriptomic potential of formalin-fixed paraffin embedded breast cancer tissues for high-throughput genomic analysis

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**Background:** Transcriptomic analyses of clinical samples can help improve our understanding of disease aetiology, drug effectiveness, assign molecular subtypes and derive prognostic signatures for clinical decision-making. The success of early microarray studies relied heavily on sample quality and predominantly fresh frozen (FF) tissues to generate reliably robust data. The emergence of next-generation microarray and sequencing-based technologies from formalin-fixed paraffin-embedded (FFPE) tissues provides an opportunity to study archival clinical tissues with long-term follow-up. Here we assess 9 technologies, which vary in resolution, cost and RNA requirements, with matched FF and FFPE tissues from the same patient.

**Methods:** Sequential tumour biopsies were taken pre-treatment and on-treatment (at 14-days and 3-months) from 11 postmenopausal patients with oestrogen receptor positive breast cancer treated with 3 months of neoadjuvant letrozole. Half of each sample was snap frozen in liquid nitrogen and half was FFPE, RNA was extracted from both. Transcriptomic analyses were performed using 9 technologies: Illumina Beadarray, Affymetrix U133A, Affymetrix Clariom S, NanoString nCounter, AmpliSeq Transcriptome, Lexogen QuantSeq and IonXpress RNAseq, Tempo-Seq BioSpyder and Qiagen UPX3'.

**Results:** Success rates for generating robust expression profiles from FFPE tissues were 100% all except the Illumina BeadChip (22%) and AmpliSeq Transcriptome (83%) , which varied by the age of tissue. With the total number and position of probes/primers/counts varying widely between approaches, in total 7305 genes were represented across all of the whole-genome technologies tested.

Clear batch effects were evident when comparing data from FF and FFPE tissues and when comparing between different technologies. Standard batch correction approaches such as XPN and ComBat minimised technical bias effect and increased the correlations between matched samples (FF and FFPE) to  $R > 0.9$ , irrespective of the technology used.

When analysed by multi-dimensional scaling following batch correction, samples clustered by treatment time-point. When ranked by expression of 60 proliferation genes, reported by us to change with letrozole treatment, samples ordered again by time-point, consistent with our previous findings, and paired samples clustered together.

## **Conclusions:**

- Robust gene expression profiles can be reliably generated from FFPE tissues and are comparable to those derived from FF tissue using established transcriptomic approaches.
- A range of new technologies are available for the study of FFPE tissues; these vary in cost, resolution and RNA requirements to fit the user's needs.
- Gene expression data from biologically similar studies, generated using different technologies, can be reliably integrated for robust meta-analysis, subject to appropriate batch correction analysis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-18

Comprehensive genomic profiling of carcinosarcomas of the breast

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## **Background:**

Carcinosarcomas of the breast (BCSC) are exceptionally rare and the underlying genomic drivers are still being elucidated. Comprehensive genomic profiling (CGP) determines the tumor mutation burden (TMB) and identifies all four classes of genomic alterations (GA) that have potential to direct personalized treatment strategies.

## **Methods:**

CGP by hybridization capture of exons from up to 315 cancer-related genes and select introns of 28 genes commonly rearranged in cancer was applied to  $\geq 50$ ng of DNA extracted from 9 consecutive BCSC and sequenced to high, uniform median coverage ( $>500\times$ ). Tumor mutational burden (TMB) was determined on 1.1 Mbp of sequenced DNA and microsatellite instability (MSI) was determined by principal components analysis of optimized loci.

## **Results:**

The 9 BCSC patients had a median age of 57 yrs (range 49-78 yrs). CGP was performed on the primary BCSC in 4 cases and on metastasis biopsies in 5 cases (4 lung and 1 lymph node). The mean GA/tumor was 6.6 and clinically relevant GA (CRGA)/tumor was 1.3. The most frequent non-CRGA were in *TP53* (89%), *MYC* (56%) and *LYN* (40%). The most frequent CRGA were in *PIK3CA* (33%), and *NF1*, *BRCA1*, *PTEN*, *RICTOR*, *FGFR1*, *AKT2* and *STK11* (all at 11%). The median TMB for all BCSC was 2.4 mut/Mb with 1 (11%) tumor with a TMB  $> 20$  mut/Mb and 8 BCSC (88%) with TMB  $< 5$  mut/Mb. Five of 5 BCSC (100%) that were available for MSI status testing were microsatellite stable.

## **Conclusions:**

On CGP, BCSC feature a high frequency of GA, but only a modest frequency of CRGA and high TMB. However, when the CRGA and TMB positive cases are combined (77.8% overall in this series), the opportunity for personalized targeted and immunotherapies are significant. Thus, further investigation of precision therapies for BCSC in the clinical trial setting appear warranted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-19

MUC17 and PCNX1 as mediators of chemotherapy response in breast cancer

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Breast cancer is a heterogeneous disease and accumulating evidence suggests that treatment failure may be driven by intra-tumour heterogeneity (ITH). Utilising the current protocol for neoadjuvant (pre-surgery) chemotherapy (NAC) provides the opportunity to study molecular genetic changes between pre- and post-therapy by assessing pre-therapy biopsies and post-therapy surgical resections.

Whole exome sequencing was performed on matched pre- and post-treatment cancer cells from 6 patients with oestrogen receptor positive breast cancers that showed partial responses to the chemotherapeutic epirubicin. Data analysis was performed to determine differences in genetic aberrations between pre- and post-NAC, and in particular to identify evidence of consistent selection by therapy of aberrations that therefore may define chemotherapy resistance or sensitivity.

There were extensive differences in the range of genetic aberrations between pre- and post-NAC. 48 genes were identified for further study based on evidence of mutations conferring a selective advantage or disadvantage during chemotherapeutic response. The relevance of these was screened using siRNA knock-down and assessment of response to chemotherapeutic drug using cell viability assays in vitro. Two genes were taken forward. Potential loss-of-function mutations in the MUC17 gene were selected against during therapy in patients, and in accordance with this MUC17 knock-down was associated with increased sensitivity in vitro. Potential loss-of-function mutations in the PCNX1 gene were selected for during therapy in patients, and in accordance with this PCNX1 knock-down was associated with resistance. Further work was done to investigate mechanisms by which these genes modify cellular chemotherapy response, by examining drug loading and ABC transporter expression levels. Data indicate that both genes impact on drug loading, potentially through modulating ABC transporter activities. Using available transcriptomic datasets, expression of neither gene correlated with breast cancer outcomes in mixed cohorts that received a wide-range of therapies, however, analysis of correlations between protein expression and outcomes specifically after chemotherapy are on-going. We conclude that MUC17 and PCNX1 are potential markers of response to chemotherapy in breast cancer, and therapeutic modulation of their activities could enhance chemotherapy responses.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-06-20

Druggable genomic landscape of primary and metastatic breast cancers

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**Background:** Several studies (e.g. The Pan-Cancer Atlas) have examined the druggable genomic landscape of breast cancers and other malignancies using The Cancer Genome Atlas (TCGA). However, TCGA is representative of newly-diagnosed, treatment-naïve breast cancers, whereas genomic profiling is typically used to inform therapy for metastatic and/or pre-treated breast cancers. Here, we characterize the druggable genomic landscape of breast cancers using AACR Genomics Evidence Neoplasia Information Exchange (GENIE), a multi-institutional pragmatic cancer genomics registry.

**Methods:** We obtained mutation, copy-number amplification (CNA), and fusion data from GENIE, which contains de-identified genomic records from 4506 breast carcinoma and carcinoma-in-situ (CIS) tumors. We used Clinical Interpretation of Variants in Cancer (CIViC) to label the potential druggability of these mutations, CNAs, and fusions. Further, we plan to assess variations in the druggable genomic landscape of breast tumors as a function of tumor characteristics (e.g. histology, tumor mutation burden), patient characteristics (age, sex, ethnicity), and genomics assay characteristics (e.g. PCR vs. hybridization capture).

**Results:** In GENIE, we identified 19932 mutations, 9334 copy number amplifications (CNA), and 987 fusions in 4506 breast tumors. CNAs suggesting potential druggability were relatively common (e.g. *ERBB2* amplifications in 9.9% of tumors), whereas fusions suggesting potential druggability were relatively rare (e.g. *NTRK* fusions in 0.1% of tumors). Mutations suggesting potential druggability were identified in *ERBB2* (3.5% of tumors) and mismatch repair genes [*MLH1*, *MSH2*, *MSH3*, *MSH6*, *PMS1*, *PMS2*] (3.7% of tumors).

**Conclusion:** GENIE can be used to infer druggability across a cohort of breast tumors likely to be considered for approved systemic therapy at tertiary academic centers. This may inform use of genomic assays and/or design of clinical trials for patients with breast tumors.

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## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-07-01

Towards a therapeutically relevant subtyping scheme for triple-negative breast cancer (TNBC), profiling results from A Randomized, TNBC Enrolling trial to confirm Molecular profiling Improves Survival (ARTEMIS)

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Triple-negative breast cancer is a highly diverse group of cancers, with poor prognosis, and currently, there are no targeted drugs available in the clinic. In TNBC around 50% percent of the patients respond to chemotherapy, while, the other 50% percent relapse with poor prognosis. There is a need to understand better the targetable mechanisms driving TNBC via integrative analysis of gene-expression, copy-number, and mutational data.

Samples from 220 triple-negative breast cancer (TNBC) pts treated with NACT were prioritized for transcriptomic and genomic profiling. Non-negative matrix factorization was used on array-based profiling to identify six robust (ARTEMIS) subtypes. Comparing ARTEMIS subtypes with Vanderbilt subtypes, revealed significant overlap with 4/6 clusters while identifying two new clusters. Logistic regression on ssGSEA scores vs. subtypes revealed several pathways, selectively enriched specific subtypes. CL1/IM (Immune subtype), was enriched in INF $\gamma$  and INF $\alpha$ , while CL2 (MYC/mTOR), showed enrichment of several proliferation-related pathways. In addition, LAR and M (Mesenchymal) pts formed overlapping clusters, using either method. Two new subtypes did not associate significantly with any of the previous subtypes. The majority of the tumors from the Vanderbilt BL2 and MSL were reclassified into a CL5 (ANGIO) cluster, which was enriched in angiogenesis geneset, including targetable genes like VEGF and FGFR. Also, an MYO (CL3) subtype was identified, with myogenesis-related genes. Of note, TIL (tumor infiltrating lymphocytes) and LAR quantification using IHC were associated with respective ARTEMIS subtypes. Finally, the IM subtype was significantly associated with higher rates of RCB 0-I and the M (CL4) subtype was associated with higher rates of RCB II-III, irrespective of the neoadjuvant treatment regimen.

ARTEMIS subtypes are a novel classification system for TNBC that is focused on therapeutic translation. Further, we show a possibility to classify previously un-classified (UNS) tumors, which will be validated using additional cohorts (TCGA/METABRIC).

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-07-02

Metaplastic breast carcinomas and uterine carcinosarcomas are histologically and genetically related

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**Introduction:** Metaplastic breast carcinomas (MBCs) and uterine carcinosarcomas (UCSs) are histologically similar, being often characterized by an admixture of adenocarcinoma areas with areas displaying sarcomatoid differentiation. We sought to investigate whether their histologic similarities would be paralleled by similar patterns of genetic alterations, and to determine whether the different histologic components of MBCs and UCSs would be clonally related.

**Methods:** Whole exome sequencing (WES) data from 35 MBCs previously analyzed by our group and 57 UCSs from The Cancer Genome Atlas (TCGA) study were reanalyzed. Somatic single nucleotide variants were detected with MuTect and indels with Strelka, VarScan2, Scalpel and Lancet. Copy number alterations were inferred using FACETS and functional annotation of the non-synonymous somatic mutations, amplifications or homozygous deletions was performed. We further microdissected the histologically distinct components of 11 MBCs and six UCSs and subjected each component to WES. Clonal decomposition was performed using PyClone.

**Results:** The most frequent somatic mutations identified in MBCs were *TP53* (69%), *PIK3CA* (29%), *FAT3* (26%) and *PTEN* (14%), whereas the most frequently mutated genes in UCSs were *TP53* (84%), *FBXW7* (35%), *PIK3CA* (29%), *PTEN* (15%) and *PPP2R1A* (15%). MBCs displayed a significantly higher frequency of mutations targeting *FAT3* (26% vs 4%,  $P<0.01$ ), *FAT1* (11% vs 0%,  $P<0.05$ ) and *CHERP* (11% vs 0%,  $P<0.05$ ) than UCSs. UCSs more frequently harbored mutations affecting *FBXW7* (35% vs 0%;  $P<0.01$ ) and *PPP2R1A* (15% vs 0%,  $P<0.05$ ) than MBCs. MBCs and UCSs displayed similar copy number alteration profiles, with frequent gains/ amplification of 8q, 3q and 1q, and losses of 8p. Pathway analysis based on the genes targeted by somatic genetic alterations revealed that both MBCs and UCSs were underpinned by genetic alterations resulting in activation of similar pathways, including PI3K, p53, Wnt and Notch signaling. Analysis of the separate components of MBCs and UCSs revealed that the histologically distinct components of MBCs and UCSs are clonally-related, with a median of 71% (range 26%-93%) and 78% (range 30%-93%) of somatic mutations being shared by the distinct components in MBCs and UCSs, respectively. In MBCs, clonal *TP53*, *NOTCH3*, *KMT2D*, *FAT4* and *PIK3CA* mutations and several copy number alterations were shared by the histologically distinct components. Mutations private to each of the histologically distinct components included *PIK3R1*, *CHERP* and *MAPK14* mutations. The carcinomatous and sarcomatous components of UCSs shared clonal *TP53*, *PIK3CA*, *CDKN2A*, *ITGB7* and *FGFR2* mutations. Private *KMT2B* mutations were identified in the UCS carcinomatous components. PyClone analysis revealed that the clonally-related histologically distinct components of each case harbored intra-component genetic heterogeneity coupled with parallel evolution.

**Conclusions:** Our findings support the contention that UCSs constitute the uterine counterpart of MBCs due to their similar histology and patterns of genetic alterations affecting the same signaling pathways (i.e. TP53, PI3K, Wnt and Notch). In each MBC and UCS analyzed here, the histologically distinct components were found to be clonally related.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-07-04

Molecular characterization of mucinous breast cancers

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## Introduction

Mucinous carcinoma, a special histological subtype of breast cancer (BC) which accounts for ~2% of all invasive breast tumors, is characterized by the presence of extracellular mucin, typically expresses the estrogen receptor (ER), and lacks *HER2* amplification. The majority of patients with mucinous BC are older at diagnosis, rarely present axillary lymph node metastases, and are associated with a better prognosis as compared to invasive ductal cancer patients (IDC, formally referred to as “breast cancer of no special type”). So far, it is unknown what is driving the mucinous phenotype of these tumors. Here we interrogated the genomics, transcriptomics, immune infiltration and epigenetics profiles of these tumors, using a retrospective institutional series and a publicly available dataset.

## Patients and methods

After central pathology review and DNA extraction, a total of 31 pure mucinous cases from Institut J. Bordet Biobank were included (referred to as IJB cohort). Tumor infiltrating lymphocytes (TILs) were assessed on hematoxylin and eosin (H&E). Low pass whole genome sequencing was conducted to assess ploidy and to detect copy number aberrations (CNAs). A second cohort was analyzed from a publicly available dataset (referred to as BRCA560 cohort), with available centrally reviewed histology subtyping and DNA methylation profiles (Nik-Zainal *et al.* Nature 2016). 207 ER+ primary BC, 14 of which were mucinous, had HumanMethylation450K methylation profiles. RNAseq was available for a subset of 145 cancers, 13 of which were mucinous. DNA methylation data was processed in R using the minfi package. Beta-values were normalized (preprocessQuantile) and dmpFinder was used to identify differentially methylated positions.

## Results

In the IJB cohort, whole genome sequencing revealed that all but two tumors were diploid. The most frequently deleted cancer genes were *RB1* (38,1%), *CDH1* (23,8%), *BRCA2* (38,1%), *TP53* (23,8%), *MAP2K4* (23,8%), *EGFR* (28,6%) and *PGR* (23,8%). In terms of amplifications, only *ZNF217* (19,4%) and *FGFR1/ZNF703* (9,5%) were observed. These mucinous tumors generally displayed low TIL levels (median=5%). In the BRCA560 cohort, there was no significant difference in the clinico-pathological features of ER+ IDC vs mucinous subtypes. At the genomic level, we identified a lower frequency of *PIK3CA* mutations as compared to ER+ IDC from the same cohort. At the DNA methylation level, we identified 8013 differentially methylated CpGs (q-val < .05) between IDC and mucinous tumors. The top differentially methylated CpG mapped to *MUC2*, an extracellular mucin. *MUC2* was significantly demethylated in mucinous tumors as compared to IDC (q-val < .001). There was a negative association between methylation level of this CpG and *MUC2* expression ( $\rho = -0.27$ ,  $p < .001$ ). Finally, *MUC2* expression was significantly increased in mucinous tumors as compared to IDC ( $p < .001$ ).

## Conclusion

To the best of our knowledge, this study is the first to report hypomethylation of *MUC2* in mucinous carcinoma of the breast, as a possible mechanism of extracellular production of mucin in these tumors. Furthermore, it highlights the minor immune infiltration of these cancers, possibly prevented by the mucin, and sheds light on the few genomic alterations present in these overall stable cancer genomes.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-07-05

Refined classification of triple negative breast cancers (TNBC) in women of african descent using the PAM50 NanoString platform

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**Introduction:** Wide-scale genomic studies of African and African American breast tumors are limited. In order to identify the most aggressive forms of breast cancer and associated molecular pathways within women of African descent we used the PAM50 NanoString platform to reclassify Nigerian (NG) and African American (AA) tumors previously subtyped by IHC.

**Methods:** Our study includes formalin-fixed, paraffin embedded (FFPE) tumors from 295 NG, 92 AA, and 74 Caucasian American (CA) breast cancer patients. These tumors were verified by two independent pathologists at our institution. RNAs were isolated from our FFPE tumors using the Roche High Pure Paraffin Kit (Roche) and assayed on NanoString nCounter. PAM50 intrinsic subtyping of 50 genes and gene-expression of an additional 60 cancer related genes were performed on NanoString's Digital Analyzer. The data was evaluated using Microsoft Excel and R statistical software. All study samples were previously annotated and subtyped by the ER/PR/HER2 IHC classifier.

**Results:** Within our NG cohort, the most prevalent subtype was basal-like (36%), followed by luminal A (24%), HER2-enriched (18%), normal-like (13%), and luminal B (9%). In our AA cohort, luminal A was the most prevalent subtype (40%) followed by basal-like (36%), luminal B (12%), normal-like (9%), and HER2-enriched (3%). Similarly, in our CA cohort, luminal A was the most prevalent subtype (54%) followed by basal-like (24%), luminal B (9%), normal-like (7%), and HER2-enriched (5%). Both NG and AA cohort have significantly more basal-like tumors compared to the CA cohort. We verified our subtype calls through ER/PR/HER2 IHC classifier and observed that 90% of our calls were concordant with IHC, suggesting that the NanoString platform can correctly classify our samples. Analysis of 110 genes across the NG cohort showed that approximately 20% of our NG samples highly expressed a majority of these 110 genes. These samples were classified as "normal-like" by the PAM50 classifier. We reconfirmed the morphology of these tumors to ensure this data was not due to technical error, suggesting these "normal-like" NG tumors may represent a unique class of tumors unclassifiable by the current PAM50 classifier. Lastly, risk of recurrence (ROR) scores based off of the sample's subtype and proliferation scores across all cohorts were examined. Basal-like tumors in the CA had the highest proportion of high ROR scores (ROR >50), followed by AA, and NG. However, luminal A tumors from the NG cohort had a greater proportion of tumors with a medium ROR score (20<ROR<50) compared to luminal A tumors from CA cohort, which predominantly have low ROR scores (ROR<20).

**Conclusion:** Gene expression results of our PAM50 classifier and additional 60 cancer-related genes suggest a potential unique subtype existing in the NG population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-07-06

Evidence for tumor heterogeneity and clonal evolution during invasive progression in breast cancer

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**Purpose:** Intratumoral heterogeneity is well recognized to be an important driver of treatment resistance and metastasis. We undertook this N of three study to measure the degree of heterogeneity in three large preinvasive lesions, all with invasive components to determine the relationship between tumor heterogeneity, spatial distribution, clonal evolution, and invasive progression.

**Methods:** We identified patients A, B, C with extensive DCIS measuring 7.5 cm, 6 cm, and 7 cm associated with 0.3 cm, 3.8cm, and 3.4 cm of an invasive component and 0, 7 and 1 positive lymph node, respectively. We sequenced the tumor sample for Case A from 32 unique blocks with precise geospatial localization; invasive cancer was identified in 3 of 32 blocks. Case B had 26 blocks sequenced with invasive cancer in 13 of 26 blocks. Case C had 23 blocks sequenced with invasive in 11 of 23 blocks. For germline reference, we sequenced DNA from an uninvolved tissue from each case. NGS libraries were made from FFPE derived DNA (20-40ng) for full exome sequencing. Variant calling was performed by GATK HaplotypeCaller, Platypus and Mutect. Identified somatic mutations were annotated with Oncotator and pathway enrichment analysis was performed with Bioconductor. To investigate the clonal evolution and progression history, phylogenetic trees were constructed in R and sub-clonal analysis was performed with Treeomics.

**Results:** The sequence data was analyzed with Platypus, MuTect and GATK HaplotypeCaller. The somatic mutation sites were concatenated into one sequence for each sample. Both neighbor-joining trees and maximum parsimony trees were built for each case. Phylogenetic analysis and sub-clonal analysis support the multi-clonal invasion model of invasive cells, in which invasive cancer can evolve from multiple clades, either early or late in the evolutionary history, independently. Dense sampling allowed reconstruction of the temporal order of mutations that accumulated in the cell lineage of the invasive cancers. Furthermore, phylogeny and sub-clone spatial analysis revealed that distant regions may be closely genetically related and showed a weak spatial sub-clone clustering pattern, which is consistent with the predictions of Big Bang model. For driver genes, we find that except for SETD2 in Case B, the majority of driver gene mutations are sub-clonal. Somatic mutations on ATP-binding cassette (ABC) transporter pathway was found in all cases.

**Conclusions:** Extensive sampling and sequencing of tumors yields important insights about tumor heterogeneity and tumor progression of DCIS to invasive cancer. Variable invasive propensity was identified, with foci of invasion were geospatially associated with preinvasive regions of progressively higher mutational load.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-07-08

Multi-omic predictor of rapid and late relapse in primary triple negative breast cancer

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**Background:** Triple-negative breast cancer (TNBC) is a heterogeneous disease. Clinically, we observe three distinct TNBC outcomes: 1) rapid relapse (rrTNBC) characterized by aggressive drug resistant disease; 2) late relapse (lrTNBC) characterized by indolent or treatment responsive disease; and 3) no relapse (NoRTNBC). We hypothesized that distinct clinical and genomic features of primary tumors define rapid versus late relapse in TNBC.

**Approach:** Using three publicly-available datasets (METABRIC, TCGA, and a prior gene expression meta-analysis), we identified 455 patients diagnosed with primary TNBC with adequate follow-up to be characterized as rrTNBC (relapse or death within 2 years of diagnosis), lrTNBC (relapse or death more than 2 years after diagnosis), or NoRTNBC (no relapse/death with at least 5 years follow-up). We compiled basic clinical (n=455 patients) and primary tumor multi-omic data, including whole transcriptome (n=455), whole genome copy number (n=317), and mutation data for 171 cancer-related genes (n=317). We evaluated intrinsic subtypes (PAM50, TNBCtype), 125 gene expression signatures, CIBERSORT immune subsets, copy number, and mutation frequency.

**Results:** We first evaluated patients with relapse (rrTNBC+lrTNBC) vs. NoRTNBC. There was no significant difference in age, grade, stage at diagnosis, or PAM50 or TNBC subtype proportion between relapse and NoRTNBC. Among 125 expression signatures, five immune signatures were significantly higher in NoRTNBCs (FDR p<0.05) suggesting increased immune activity in patients who do not relapse. Using CIBERSORT inferred immune subsets, anti-tumor CD8 T-cell, M1 macrophage, and gamma-delta T-cell subsets were all highly correlated to these immune signatures (all Pearson's r >= 0.3, all p<1.2e-8). Among genomic features, patients who relapsed were significantly more likely to harbor a mutation in *PIK3CA* (Fisher exact FDR p=0.02) but there was no significant difference in tumor mutation burden or percent genome altered (Student's t-test p=0.83 and p=0.99, respectively). We then evaluated primary TNBC genomic data in patients who ultimately developed rapid vs. late relapse. Patients with rrTNBC were more likely to be higher stage (p<0.0001) while lrTNBC were more likely to be non-basal PAM50 subtype (p=0.03). Among 11 significantly altered gene expression signatures (FDR p<0.05), 6 estrogen/luminal signatures were significantly higher in lrTNBC. Mutations in *DNAH11* and *PIK3CA* were more common in lrTNBC (Fisher exact FDR p=0.04 and p=0.05, respectively) but there were no significant differences in tumor mutation burden or copy number burden (Student's t-test p=0.13 and p=0.45, respectively). Using 317 cases with full genomic data divided into training and validation datasets, we will report a comparison of machine learning models for predicting relapse versus no relapse and rapid versus late relapse.

**Conclusions:** Primary TNBC tumors destined for rapid, late, or no relapse reflect distinct genomic features. Anti-tumor immune signatures and subsets are enriched in patients who do not relapse yet no difference in mutational or copy number burden. Relative to rapid relapse TNBCs, late relapse TNBCs are enriched for non-basal tumors, estrogen/luminal expression signatures, and mutations in *DNAH11* and *PIK3CA*.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-07-09

Tumour clonality in paired invasive breast carcinomas

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**Background:** Multiple invasive breast tumours may represent either independent primary tumours or clonal recurrences of the first tumour, where the same progenitor cell gives rise to all of the detected tumours. Consequently, the driver events for the progenitor cell need to have been identical in early tumour development. Molecular classification of tumour clonality is not currently evaluated in multiple invasive breast carcinomas, despite evidence suggesting common clonal origins. Furthermore, there is no consensus about which type of biological data (*e.g.* copy number, mutation, histology) and especially which statistical method is most suitable to distinguish clonal recurrences from independent primary tumours.

**Methods:** Thirty-seven invasive breast tumour pairs were stratified by laterality (bilateral vs. ipsilateral) and the time interval between the diagnoses of the first and second tumours (synchronous vs. metachronous). Both tumours from the same patient were analysed by integrating clinical characteristics ( $n = 37$ ), DNA copy number ( $n = 37$ ), DNA methylation ( $n = 8$ ), gene expression microarray ( $n = 7$ ), RNA sequencing ( $n = 3$ ), and SNP genotyping data ( $n = 3$ ). Different statistical methods, *e.g.* the diagnostic similarity index (SI), distance measure, shared segment analysis *etc.*, were used to classify the tumours from the same patient as clonally related recurrences or independent primary tumours.

**Results:** The SI applied on DNA copy numbers derived from aCGH (array comparative genomic hybridization) data was determined as the strongest indicator of clonal relatedness as it showed the highest concordance with all other methods. The distance measure was the most conservative method and the shared segment analysis most liberal. Concordant evidence for tumour clonality was found in 46% (17/37) of the patients. Notably, no significant association was found between the clinical characteristics and molecular tumour features.

**Conclusions:** A more accurate classification of clonal relatedness between multiple breast tumours may help to mitigate treatment failure and relapse by integrating tumour-associated molecular features, clinical parameters, and statistical methods. In cases of extremely similar or different tumour pairs, the results showed consistency regardless of the method used. The SI can be easily integrated into clinical routine using FFPE samples to obtain copy number data. However, clinical guidelines with exact thresholds need to be defined to standardize clonality testing in a routine diagnostic setting.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-07-10

Triple negative breast cancer tumors subtyping by means of integrated transcriptome and proteome analyses

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**Background:** Heterogeneity and lack of targeted therapies represent the two main impediments to precision treatment of triple-negative breast cancer (TNBC) and therefore, molecular subtyping and identification of therapeutic pathways are required to optimize medical care. The aim of the present work was to confirm unsupervised analysis of TNBC transcriptomic data by means of proteomics.

**Methods:** Transcriptome and proteome of 83 TNBC macrodissected tumors were screened in parallel. These patients were described in a previous study [Jézéquel, et al., Breast Cancer research (2015) 17, 43]. Transcriptome screening was performed using Affymetrix Human Genome U133 Plus 2.0 Arrays (Affymetrix®, Santa Clara, CA). Proteome profiling was performed by means of iTRAQ-OFFGEL-LC-MS/MS approach [Campone, et al, Mol Cell Proteomics (2015) 14, 2936-2946].

**Results:** Unsupervised analysis of transcriptomic data identified three molecular clusters within TNBC: one molecular apocrine (C1: 20%) and two basal-like-enriched (C2: 47% and C3: 33%). C2 presented pro-tumorigenic immune response and C3 exhibited adaptive immune response.

iTRAQ-OFFGEL-LC-MS/MS screening identified 366 out of 1,929 unique proteins, which were quantified in at least 70% of TNBC tumors and therefore could be used for analysis. Principal component analysis (PCA) with projection of 83 TNBC onto the first principal plane showed inhomogeneous distribution: one largest group (n = 77) and two outlier groups composed of three tumors, which have been eliminated for the rest of the work. In order to look for the existence of a partition of 77 TNBC cohort based on proteomics data, we performed clustering analysis using fuzzy clustering. Gap statistic was used to estimate the optimal number of clusters. This number was equal to one, whatever the metric. PCA and estimation of the number of clusters results lead us to conclude that iTRAQ-OFFGEL-LC-MS/MS data could not be used alone to subtype our 77 TNBC cohort most probably due to insufficient information content of proteomics matrix.

TNBC cluster assignment, based on transcriptomics was applied to these tumors. Sixty-two out 366 ANOVA analyses were significant between the three clusters (p < 0.05). Twenty-two differentially expressed proteins between C1, C2 and C3 belonged to biological categories, which characterized these TNBC clusters

Table 1. Proteins found differentially expressed between TNBC cluster defined by means of transcriptomics

Proteins	Biological categories	Clusters
K2C7, K2C8, K1C18, K1C19	Luminal	C1
FAS, UGDH	Androgen induced (molecular apocrine)	
LDHB	Basal-like	C2
PLMN, POSTN, FLNB, TENA, PLOD3, FSCN1, SERPH, FINC	Invasion, extracellular matrix	
MOES	Basal-like	C3
STAT1, SYWC, AMPL, SAMH1	Interferon pathway	
IGKC, IGHM	Immunoglobulines	

. Gene Ontology enrichment analysis based on the set of proteins highly expressed in C2 compared to C1 and C3 (n = 21) displayed enrichment in genes coding for protein involved in extracellular matrix, wound response and RNA splicing.

**Conclusion:** Although iTRAQ-OFFGEL-LC-MS/MS screening did not contain enough information for cluster identification, 22



proteins, which were differentially expressed between the three clusters corroborate transcriptomic subtyping of TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-07-12

Identification of luminal A-like subgroup among ER+/PR+/HER2+ breast cancers and its clinical implications

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**Background** Triple positive breast cancers (TPBCs), i.e. estrogen receptor-positive (ER+)/ progesterone receptor-positive (PR+)/ human epidermal growth factor receptor 2-positive (HER2+) breast cancers, constitute a therapeutic challenge due to the functional crosstalk between the hormone receptors and HER2 pathways. The intrinsic molecular subtyping of TPBCs has rarely been studied and may have implications for the prognostic evaluation and therapeutic decision-making.

**Methods** Our study included four cohorts of patients with TPBC. The first one consisted of 82 patients from The Cancer Genome Atlas (TCGA). The second and third ones were from two publicly available microarray datasets (GSE2603 and GSE2109) and included 37 and 30 patients respectively. The forth one comprised 165 patients from Fudan University Shanghai Cancer Center. First, we examined the PAM50 intrinsic subtypes of TPBCs in the first three cohorts. Then, we tried to find several differentially expressed genes (DEGs) between luminal A and the other subtypes. In cohort 1, we identified DEGs using LIMMA. In cohort 2 and 3, we further filtered and validated them using Wilcoxon's rank sum test. We also performed Receiver operating characteristic analyses to evaluate the accuracy of candidate DEGs in identifying TPBCs of luminal A subtype and determined the top 3 DEGs according to the area under the curve. Finally, in cohort 4, we detected the expression of these 3 genes by immunohistochemical (IHC) staining of tissue sections, defined a group of luminal A-like TPBCs and examined the prognosis and effect of adjuvant trastuzumab for them.

**Results** The distribution of PAM50 intrinsic subtypes of TPBCs was shown as follows.

PAM50 intrinsic subtypes of TPBCs

PAM50 intrinsic subtype	Cohort 1	Cohort 2	Cohort 3
	N (%)	N (%)	N (%)
Luminal A	43 (52.4)	15 (40.5)	13 (43.3)
Luminal B	28 (34.1)	17 (45.9)	12 (40.0)
HER2-enriched	9 (11.0)	2 (5.4)	3 (10.0)
Normal-like	1 (1.2)	2 (5.4)	1 (3.3)
Basal-like	1 (1.2)	1 (2.7)	1 (3.3)

The three genes that exhibited the highest accuracy in identifying TPBCs of luminal A subtype were STC2, BCL2 (highly expressed in luminal A subtype) and MKI67 (lowly expressed in luminal A subtype). In cohort 4, we defined a group of luminal A-like TPBCs as TPBCs with low expression of MKI67 as well as high expression of STC2 and/or BCL2. Compared with patients with non-luminal A-like TPBC (n = 110), those with luminal A-like TPBC (n = 55) had better disease-free survival (DFS) in both univariate (Log-rank  $P = 0.029$ ) and multivariate analyses (hazard ratio = 0.25,  $P = 0.025$ ). In the group with non-luminal A-like TPBCs, patients treated with trastuzumab (n = 67) showed better DFS than those not treated with it (n = 43) (Log-rank  $P = 0.019$ ), while in the group with luminal A-like TPBCs, there is no difference in DFS between patients treated with trastuzumab (n = 22) and those not treated with it (n = 33) (Log-rank  $P = 0.993$ ).

**Conclusions** TPBCs are heterogeneous in terms of intrinsic molecular subtype. Evaluating the expression of STC2, BCL2 and MKI67 by IHC staining can help us to conveniently identify a group of luminal A-like TPBCs. Patients with this group of TPBCs have relatively good prognosis and may only gain limited benefit from adjuvant trastuzumab.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-01

Characteristics, outcomes and prognostic factors of luminal androgen receptor (LAR) triple-negative breast cancer (TNBC)

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**Background:** The LAR subtype is a genomically distinct subset of TNBC. Using a large cohort of non-metastatic TNBC patients (pts) with long term follow-up, we sought to further characterize the clinicopathologic features and outcomes of LAR vs non-LAR TNBC.

**Methods:** From a cohort of 9982 women with surgically-treated non-metastatic breast cancer, 605 met criteria for TNBC (ER/PR<1% and HER2-negative) by central pathology. RNA extracted from 304 FFPE tumor specimens using the HighPure RNA extraction kit was subjected to TruSeq RNA Access library preparation and sequencing on a HiSeq2500. Adequate RNA was available for 283 pts. Tumors were classified as LAR or non-LAR using a shrunken centroid model, CABAL (Clustering Among Basal and Luminal androgen receptor). In addition to previously described analyses [Leon-Ferre et al, Breast Cancer Res Treat 2017], immunohistochemical (IHC) androgen receptor (AR) staining was performed and the impact of various parameters on invasive disease-free survival (IDFS) and overall survival (OS) was assessed using Cox proportional hazards models.

**Results:** 58 (20%) tumors were classified as LAR and 225 (80%) as non-LAR. Compared to non-LAR, LAR pts were older (mean age 65 vs 54) and more often postmenopausal (79%vs53%), both  $p=0.01$ . Apocrine histology was more common among LAR tumors (21%vs0%), which were also lower grade (grade3: 69%vs95%) and had lower Ki-67 (Ki-67>15%: 64%vs82%), all  $p<0.01$ . Additionally, LAR tumors had lower median stromal tumor infiltrating lymphocytes (TILs, 20%vs25%) and were less frequently lymphocyte-predominant [ $\geq 50\%$  stromal or intratumoral TILs (19%vs32%)], although neither reached statistical significance. AR IHC was available for 223 of 283 tumors. Median AR IHC score in LAR was 65% (range 0-100%) vs 0% (range 0-90%) in non-LAR. T/N stage, surgery type, and receipt of adjuvant chemotherapy (AdjCT) or radiotherapy were similar between LAR and non-LAR. LAR pts had shorter IDFS and OS compared to non-LAR (5.6 vs 11.8 yrs and 10.8 vs 20.8 yrs, respectively), although this did not reach statistical significance. Test of proportional hazard assumption was not significant for IDFS or OS ( $p = 0.30$  and  $0.09$ ). IDFS estimates were numerically higher in LAR vs non-LAR (80.2%vs70.5%,  $p = 0.92$ ) at 3yrs post-diagnosis; whereas the opposite was true (40.9%vs55.6%,  $p = 0.07$ ) after 10yrs. OS estimates at 3 and 5yrs were similar between LAR and non-LAR, but at 10yrs OS was inferior in LAR (40.9%vs66.4%,  $p = 0.24$ ). In a univariate analysis including both LAR and non-LAR, older age, higher N stage, lower TILs and absence of AdjCT were associated with poorer IDFS and OS. In a multivariate analysis, higher N stage and absence of AdjCT remained associated with both poorer IDFS and OS; while lower stromal TILs were associated with poorer IDFS ( $p=0.01$ ), and with a trend towards poorer OS ( $p=0.07$ ).

**Conclusions:** LAR TNBCs occurred in older women, were lower grade, and had lower TIL density than nonLAR tumors. While significant differences in IDFS or OS were not demonstrated, LAR pts exhibited a numerically lower risk of a disease event at 3yrs, but higher risk by 10yrs compared to nonLAR pts. In the entire cohort, higher N stage, absence of AdjCT and lower TILs were independently associated with poorer outcomes.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-08-03

Dissecting the heterogeneity of metaplastic breast cancer: A morphological, immunohistochemical and genomic analysis of a large cohort

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Although rare, Metaplastic Breast Carcinomas (MBC) account for significant global breast cancer mortality. This subgroup is extremely heterogeneous and by definition exhibits metaplastic change to squamous and/or mesenchymal elements, including but not limited to spindle, squamous, chondroid, osseous and rhabdomyoid elements. The WHO working group recognizes that the current classification is inadequate and in the interim, has suggested a purely descriptive classification. The mixed epithelial-mesenchymal morphology has led to speculation that MBC represent 'stem cell tumours'; in support of this, MBC have been shown to have a CD44<sup>+</sup>/CD24<sup>-/low</sup> phenotype. Clinically, patients present with tumours that are larger (higher stage), have increased likelihood of distant metastases at presentation and overall, have a reduced 5-year survival rate compared to Invasive Carcinoma-NST. Hence, this is a unique subtype with poor outcome but without a robust classification or understanding of the biology to aid clinical management. We present a detailed morphological, immunohistochemical and genomic analysis of a large series of MBC (n=347), as amassed through the Asia-Pacific MBC consortium. We consider our morphological dissection using the WHO subtyping guidelines and show that an increasing number of phenotypes in a mixed MBC (classified as WHO\_1) significantly associates with a poor prognosis. Immunohistochemical analysis showed that a pure spindle (WHO\_5) is significantly less likely to express vimentin, CK5/6, CK14, and CK19 than a mixed WHO\_1 with spindle features. Similarly, a WHO\_1 with chondroid features is less likely to express EGFR than WHO\_1 with chondroid features and rhabdoid or osseous differentiation. Across the cohort, positivity for the AE1/3 antibody and a lack of EGFR expression both significantly associate with a better outcome. We report no significant association between patient age at diagnosis and breast cancer specific survival, nor between age and specific WHO MBC subtypes. We report a significant association between WHO\_1 types and increasing tumour grade, and also between tumour size and grade, with tumour size being a highly significant prognostic indicator in this cohort. Our exome sequencing confirms a significant enrichment for *TP53* and *PTEN* mutations in MBC, and intriguingly for concurrent mutations of *TP53*, *PTEN* and *PIK3CA*. A novel enrichment for *NF1* mutations is also presented. In summary, we provide a thorough assessment of a large cohort of MBC, including morphology, survival, IHC and exome sequencing, and present our analysis contextualized by the WHO guidelines, extending the existing knowledge base of this rare tumour type.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-04

Transient state change, but not permanent subtype change, after HER2-targeted therapy for HER2-positive breast cancer

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**Background:** In CALGB 40601 (Alliance, NCT00770809), a neoadjuvant phase III trial of paclitaxel and trastuzumab with or without lapatinib for 12 weeks for patients with HER2-positive breast cancer, 33% of pretreatment tumors were Luminal A subtype, however, 69% of post-treatment samples with residual disease were Luminal A subtype. In addition, 71% of Luminal B (12/17) and 67% of HER2-Enriched (6/9) tumors changed into Luminal A, while 80% of Luminal A (20/24) remained Luminal A (Carey et al. J Clin Oncol. 2016). It is not known whether this shift to Luminal A was transient or permanent.

**Methods:** We selected matched pairs of pre- and post-treatment 40601 samples with tumor purity >10% based upon DNA analyses to ensure all samples contained tumor. PAM50 intrinsic subtyping was applied to the 40601 samples gene expression data using a two-step normalization process based on The Genome Cancer Atlas, and PAM50 training set. In addition, a HER2-enriched expression subtype patient-derived xenograft (PDX) tumor called WHIM35, was studied and was either untreated (n=10), or treated with lapatinib at 220 mg/kg for 1 week (wk) (n=5), for 2 wks (n=8), or for 3 wks (n=4). We also treated WHIM35 tumors with lapatinib for 2 wks (on) and then removed lapatinib for 1 wk (off) (n=6), or for 2 wks on and 2-4 wk off (n=6), and finally for 3 wks on, and 1 wk off (n=3). PAM50 intrinsic subtyping was applied to the PDX gene expression data and subtype assessed as well as a genomic-based proliferation score. ANOVA p-values were calculated by comparing median values across all gene signature or correlation scores.

**Results:** We found 10 pairs of 40601 samples that kept their tumor purity values, however, their subtype changed to Luminal A after treatment (i.e., in the residual disease), and in these cases no minor tumor subclone became a dominant clone in the post treatment sample. Pretreatment subtypes were 6 Luminal B, 3 Luminal A, and 1 HER2-enriched. The tumor purity values did not change after the treatments, but correlation to Luminal A was significantly higher (p=0.01), while correlation to HER2-enriched (p=0.004) and proliferation signature scores (p=0.003) were significantly lower in the post-treatment samples. Among the WHIM35 PDX tumors, one sample changed its subtype from HER2-enriched to Luminal A after the lapatinib treatment and the rest remained HER2-enriched, suggesting environmental differences between patient samples and the PDX model. However, correlation to Luminal A was significantly higher in all lapatinib treated WHIM35 samples (p=8.3e-12), and notably went back to the initial low levels just one week after removing lapatinib. Likewise, correlation to HER2-enriched (p=1.2e-10) and proliferation signature scores (p=6.2e-12) also got lower while treated with lapatinib, but went back to the initial levels after cessation of treatment.

**Conclusions:** Our findings suggest that the apparent subtype change during HER2-targeting therapy is not permanent, but is more likely a transient state change from a HER2-enriched subtype into a more Luminal A-like state. When we plan additional treatment strategies using residual disease phenotypes, it may not be clear what is the true subtype of the sample due to this inherent plasticity.

# 2018 San Antonio Breast Cancer Symposium ®

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High APOBEC3C-H gene expression in tumor associates with better survival in breast cancer

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## Background

APOBEC3 (A3) enzymes are strong mutagenic factors. A3B has been well described as an active mutator in breast cancer, whereas the roles of the other APOBEC3s (A3A, C-H) are unclear. While mutations may directly drive cancer progression, they can indirectly suppress cancer growth by generating neoantigens. To elucidate this, we comprehensively analyzed all APOBEC3s for their association with mutations and immune activity in breast cancer.

## Methods

RNA seq.-based gene expression data for 1091 primary carcinomas and 113 adjacent normal tissues was from TCGA. Patients were divided into high and low groups by top and bottom gene expression tertiles. Tumor immune features like cytolytic activity, T cell receptor (TCR) diversity, and cell fractions were quantified from gene expression data. Data for some of these features, mutation-related aspects, and survival outcomes were obtained from the Pan-Cancer Atlas. Gene expression data for 55 breast cancer cell-lines was from Cancer Cell Line Encyclopedia. Cox regression and Spearman methods were respectively used for survival and correlation analyses. Welch's t test was used for group comparison.  $P < 0.05$  was deemed significant. Hallmark gene-sets were used for enrichment analysis with recommended 25% FDR.

## Results

A3B and A3C together represented most (91%) of A3 gene expression in breast cancer cell-lines. In TCGA patients, expression of only A3B was increased by 4.5x in tumors compared to normal tissue, whereas levels for other A3 genes were unchanged. Surprisingly, tumor A3B or A3A levels had no significant association with overall (OS) or disease-specific survival (DSS), whereas for each of A3C-H, higher expression was significantly associated with improved OS (hazard ratios of 0.45-0.66) or DSS (0.43-0.61). The prognostic benefit of high A3C-H expression was also seen in survival analyses of two meta-datasets of microarray-based gene expression (KMPlot and SurvExpress). A3A and A3B levels correlated with both mutation burden and neoantigen load (Spearman  $\rho = 0.28-0.34$ ), which respectively were 2.0-2.9x higher in high compared to low expressors. But there was no association of expression with mutation burden or neoantigen load for A3C-H. On the other hand, A3C-H levels correlated positively with tumor leukocyte fraction ( $\rho = 0.29-0.70$ ) and its lymphocyte subset ( $\rho = 0.20-0.50$ ), whereas the correlation was poor for A3B ( $\rho = 0.10$  &  $-0.01$  respectively). Expression of genes of immune function like interferon response and complement activation was enriched in high A3C-H expressors. It was not so for A3B, for which enrichment was instead observed for cell proliferation. Both CD4 and CD8 T cells were significantly more (2.3-4.0x & 2.1-5.4x resp.), and TCR diversity significantly higher (1.3-2.1x) in A3C-H high expressors. Concordantly, for each of A3C-H, expression correlated with tumor immune cytolytic activity ( $\rho = 0.31-0.79$ ), which was increased 3.1-7.9x in high compared to low expressors.

## Conclusions

These findings suggest that in spite of A3C-H being known as DNA mutators, an increase in their expression confers a survival benefit in breast cancer. Their increased expression likely reflects a heightened anti-cancer immune response, and may be useful for disease prognosis and monitoring immunotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

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A novel integrated, clinical-pathologic and genomic classification method segregates early from late relapsing invasive ductal luminal breast cancers (IDLBC) of the METABRIC study

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## Background and Objectives

Endocrine therapy is one of the most effective anti-cancer treatments for HR-positive breast cancers. However, in a significant proportion of patients the disease will relapse irrespective of the treatment modality (SERM, SERD, AI). Despite the progress made with multigene signatures for molecular classification of luminal breast cancers, predicting sensitivity towards endocrine modalities remains challenging. With the help of invasive ductal luminal breast cancers (IDLBC) from the METABRIC study we have developed an integrated clinical-pathologic and genomic classification method, identifying three distinct groups (IDLBC1, -2 and -3) [1, 2]. The 3 groups differ by DNA damage load, predominant lesion type, characteristic lesion patterns and oncogenic driver mechanisms [1, 3]. Here we present characteristic gene expression profiles of IDLBCs and discuss their biologic and clinical implications.

## Methods

Gene expression profiles based on cDNA microarray data of 1.104 IDLBCs on the METABRIC study were examined (EGAS00000000083) [3]. To identify differentially expressed genes across IDLBC1-3 groups (52%, 23%, 25%), we compiled a gene list (N=2034) from 34 gene signatures reported to be predictive of ER, PR, GATA3, FOXA1 and/or AR signalling. A multiclass significance analysis of microarrays with 100 iterations and a false discovery rate of 0.05 was used to extract differentially expressed genes.

## Results

Of 2034 steroid receptor signalling associated genes 36 exhibited differential expression in the IDLBC1/2 and -3 groups. The IDLBC1/2 specific set included 2 ER-associated genes (WLS, SPARCL1), 1 FOXA1-associated gene (NTN4) and 2 PR-associated genes (ZBTB16, CRY2). The IDLBC3 overexpressed set included 31 genes, amongst them regulators of mitotic fidelity, as well as effectors of centromere and kinetochore functioning. Eight of these genes have been reported as ER-associated (POLR2H, ECE2, SCL7A5, KIF11, FAM83D, GPSM2, MASTL, POLE2). Additional IDLBC3 hallmarks included high proliferation activity, defect apoptosis, epithelial-mesenchymal-transformation (EMT), BRCA-ness, multiple-drug resistance (MDR) and early relapse. The estimated relapse-free-survival rate at 5 years in the IDLBC3 group was 77% [95%CI 72; 82], significantly lower than in IDLBC1/2 tumours (91% [95%CI 89; 93]) and comparable to triple negative IDLBCs on the METABRIC study (70% [95%CI: 65; 76]).

## Conclusions

We have shown that our integrated classification method has the potential to segregate early relapsing luminal breast cancers (IDLBC3) from tumours with favourable prognosis (IDLBC1/2). The data presented here and in the abstract by Demanse et al [4] provide also an intuitive hypothesis why IDLBC3 tumours are sensitive towards Letrozole but not Tamoxifen. Prospective clinical studies will be required to validate this hypothesis and to demonstrate the value of the IDLBC classification approach.

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# 2018 San Antonio Breast Cancer Symposium ®

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Distinct biological signatures describe differences in BRCA mutated subgroups

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**Background:** BRCA mutated (BRCA+) breast cancers are expected to have increased activation of Homologous Recombination Deficiency (HRD) and altered DNA damage repair pathways when compared to BRCA wildtype (BRCA-). To better understand differences in these populations, biological patterns and immune responses to BRCA+ breast cancers were evaluated. The primary aim of our study was to use novel gene expression tools to assess early stage breast cancers with and without germline BRCA mutations, and within distinct BRCA+ subgroups.

**Methods:** We identified 124 early stage untreated breast cancers with and without BRCA mutations (n = 62 and 62, respectively). Our BRCA- group was matched by hormone receptor (HR) status, age, and stage to the BRCA+ group. The NanoString Breast Cancer 360 panel was applied to RNA isolated from 80 breast tumors (BRCA+ = 39; BRCA- = 41). The BRCA+ group had a BRCA1+ subgroup (n=17) and a BRCA2+ subgroup (n=22).

**Results:** There was a significant increase in two BC360 signatures in both the BRCA1+ and BRCA2+ tumors compared with the BRCA- population: Prosigna™ Risk of Recurrence (ROR) score [BRCA1+: HR: 1.142 (95% CI 1.019, 1.279), p=0.02; BRCA2+: HR: 1.321 (95% CI 1.190, 1.466), p<0.001] and HRD [BRCA1+: HR: 3.576 (95% CI 2.174, 5.880), p=0.02; BRCA2+: HR: 1.801 (95% CI 1.142, 2.840), p<0.001]. BRCA1+ tumors had lower expression of ESR1 [p=0.03], PGR [p=0.02], ER signaling [p<0.001], and differentiation [p=0.005]; while BRCA2+ tumors had lower expression of stroma markers [p=0.02] and inflammatory chemokines [p=0.001]. The two BRCA+ subgroups had distinct molecular subtype correlation trends that were highly significant. BRCA1+ tumors were positively associated with a basal subtype [p<0.001], whereas this association was not significant for BRCA2+ tumors. BRCA2+ tumors were associated with an increase in luminal B subtype [p=0.05]. All BRCA+ tumors had a decrease in luminal A subtype correlation [BRCA1+: p<0.001; BRCA2+: p=0.002]. In addition to the BC360 signatures, a differential analysis of all genes in the BC360 panel revealed more single gene differences in BRCA2+ than BRCA1+ tumors when compared to BRCA- tumors.

**Conclusions:** In early stage BRCA+ breast cancer, tumors have higher ROR and increased HRD signature scores compared to BRCA- tumors. Furthermore, BRCA1+ and BRCA2+ tumors have both signature and single gene expression differences when compared to BRCA- tumors, indicating distinct subgroup-related biology. The greater correlation of BRCA1+ tumors with basal-like biology and BRCA2+ tumors with aggressive hormonal biology confirms these trends. Distinctions in hormone receptor signaling, DNA-damage pathways, and microenvironment/inflammatory features between BRCA1 and BRCA2 associated cancers suggest a need for different prevention and therapeutic strategies for each of these breast cancer subtypes. The unique biological patterns identified here should be further evaluated as predictive or prognostic tools that could be translated into clinical care for early stage BRCA+ patients.



## 2018 San Antonio Breast Cancer Symposium ®

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SOX11 is a potential prognostic marker of high-risk breast ductal carcinoma *in situ*

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Ductal carcinoma *in situ* (DCIS) comprises 20-25% of screen-detected breast cancers and, like invasive ductal carcinoma (IDC), is heterogenous in terms of the underlying biology, presentation, and outcome. While there are limited potential biomarkers of outcome for DCIS, estrogen receptor (ER)- positive, progesterone receptor (PR)- positive, and HER2- negative DCIS appears to have a better prognosis compared to ER- negative, PR- negative, and HER2- positive DCIS. The aim of this study was to identify additional clinically relevant markers to stratify DCIS according to risk of relapse or progression to invasive disease. In order to determine the driver genes involved in DCIS evolution, we utilized transcriptional data sets (GSE788, GSE16873), containing data from both normal mammary glands (NMG) and DCIS. Upon performing class comparison (NMG vs DCIS), we identified 297 over-expressed genes and 187 under-expressed genes. The over-expressed genes represented mitotic and proliferative features annotated as mitotic spindle and condensed chromosomes, while the under-expressed genes were associated with loss of epithelial features annotated as epithelial cell differentiation and development. The 484 differentially expressed genes were further correlated with recurrence events using Kessler's breast cancer data set to identify genes contributing to the aggressive features across IDC and subsequently associated with DCIS. Genes correlating with recurrence events were selected. Of the 484 genes, 99 genes were found to be significantly associated with recurrence events of IDC (with  $P < 0.003$ ). Among these 99 genes, component genes of the Oncotype DCIS score and genes reported as relevant to DCIS biology were included for Nanostring transcriptomic analysis. The final number of genes-of-interest were 58, including 5 housekeeping genes. 40 DCIS lesions and 8 NMG tissue were macro- dissected from formalin- fix paraffin- embedded blocks (FFPE) and extracted transcripts were subjected for Nanostring analysis. Gene expression data was clustered in an unsupervised manner using R software. Two sample clusters were identified: an ER/PR- negative cluster and an ER/PR- positive cluster. Over-expression of transcription factor SOX11, along with HER2, was exclusively seen in the ER/PR- negative cluster. This cluster was further categorized into HER2-low/SOX11+ and HER2-high/SOX11+ groups. These RNA expression findings are undergoing confirmation by immunohistochemistry (IHC) of the FFPE tumor sections. An independent series of 15 DCIS cases that have recurred as DCIS or progressed to IDC were analyzed by IHC, revealing SOX11 expression only present in cases displaying a high proportion of HER2+ expression. SOX11 is exclusively expressed in ER/PR-negative DCIS and is a candidate clinical marker for recurrence of DCIS or progression to IDC.

# 2018 San Antonio Breast Cancer Symposium ®

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The 21-gene recurrence score and chemotherapy use in triple negative breast cancer (TNBC) and HER2 positive breast cancer: A National Cancer Database study

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## Background

The 21-gene (Oncotype DX) Recurrence Score (RS) is a multi-gene expression assay that is both prognostic and predictive of adjuvant chemotherapy (AdjCT) benefit in estrogen receptor (ER) positive/HER2 negative breast cancer (BC). Use of the RS in TNBC and HER2+ BC is not recommended by national guidelines. Using the National Cancer Database (NCDB), we sought to evaluate whether oncologists use the RS in these subtypes. Additionally, we assessed the prognostic effects of the RS in node negative patients (pts) who did or did not receive adjuvant chemotherapy.

## Methods

Pts with TN and HER2+ BC diagnosed from 2010-2015 in the NCDB were analyzed. Pts with neoadjuvant therapy or stage IV were excluded. Cases with RS testing were classified as low (RS 0-17), intermediate (RS 18-30) or high (RS 31+). Analysis was performed using multivariable logistic regression; overall survival (OS) was analyzed using the Kaplan-Meier method with log-rank tests.

## Results

142,330 pts were evaluable: 64,830 TNBC and 77,500 HER2+ (21,768 ER-/HER2+ and 55,732 ER+/HER2+). In these subtypes, RS was performed in 5,369 (3.8%) pts as follows: 1,479 (2.3%) TNBC, 185 (0.8%) ER-/HER2+, and 3,705 (6.6%) ER+/HER2+. Given the small ER-/HER2+ cohort, we focused on TNBC and ER+/HER2+. Within these subtypes, factors associated with RS testing included lower grade, smaller pathologic tumor size, pathologic N0 status, white race, age 60-69, and ILC or IMC histology (each  $p < 0.005$ ).

Of the TNBC pts tested, the RS distribution was: low 16.3%, intermediate 13.1% and high 70.6%, while for ER+/HER2+ it was low 30.5%, intermediate 30.7% and high 38.8% ( $p < 0.001$ ).

AdjCT was less frequently recommended in pts with low RS; however, this varied by tumor subtype with AdjCT recommended for 66.8% of TNBC with low RS and 37.9% of ER+/HER2+ with low RS. For intermediate RS, rates of AdjCT recommendation were 74.3% and 73.7%, and for high RS were 94.0% and 93.2% for TNBC and ER+/HER2+ respectively.

In a multivariable analysis among pts with low RS, factors associated with AdjCT recommendation included younger age, larger pathologic tumor size, node positive disease, and higher grade.

In AdjCT untreated TNBC, 5 yr OS did not differ for low RS (96.5%) vs intermediate RS (95.2%,  $p = 0.82$ ). In contrast OS was significantly worse for high RS (76.7%) than the other two groups, each  $p \leq 0.04$ .

In AdjCT untreated ER+/HER2+, 5 yr OS was significantly better for low RS (96.7%) vs intermediate RS (92.5%,  $p = 0.03$ ) and vs high RS (92.1%,  $p = 0.003$ ), with no difference between intermediate RS vs high RS ( $p = 0.32$ ).

Evaluating pts who did receive AdjCT, there was no significant difference in 5 yr OS for either subtype according to RS ( $p = 0.46$  and  $p = 0.83$ ).

Interestingly, in patients with low RS, 5 yr OS was similar with or without AdjCT in both TNBC (89.5% vs 96.5%,  $p = 0.25$ ) and ER+/HER2+ (97.6% vs 96.7%,  $p = 0.47$ ).

## Conclusions

RS testing is being conducted in a small fraction of pts with TN and HER2+ BC with lower clinical risk features. The observation that RS is prognostic for survival in AdjCT untreated patients is hypothesis generating, and suggests that further evaluation of the RS and other multigene assays in ER negative and HER2+ BC is warranted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-10

A unique coding and non-coding benign breast transcriptome in post-menopausal ER+ breast cancer

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**Background:** Differences in ER+ and ER- breast cancer tumor biology are well-documented, but little is known about the field of background benign breast in which those cancers arise. We evaluated the transcriptome of benign breast tissues from women with concurrent ipsilateral ER+ and ER- breast cancers (BC) to characterize coding and non-coding RNA profiles. This pilot study provides insight into the transcriptomic landscapes of benign breast tissues in patients with BC, and the microenvironment of the at-risk benign tissue.

**Methods:** With institutional approval, cryobanked breast tissues from patients with concurrent ipsilateral ER+ BC (benign <sup>ER+BC</sup>, N=14) or ER- BC (benign <sup>ER-BC</sup>, N=10), were selected for benign tissues with similar epithelial:stromal ratios and grouped into pre (preM) and post-menopausal (PM) groups. Following RNA sequencing (Illumina TruSeq Stranded mRNA kit & Illumina HiSeq 4000), reads were processed (MAP-Seq v3.0.0) and aligned (STAR aligner; hg38). Differential expression (DE) analysis (edgeR 2.6.2) identified DE genes from normalized RPKM reads (absolute log2 fold change (FC) > 1 and false discovery rate (FDR) < 0.10), corrected for intra-group biases. Over-representation analysis [Ingenuity pathway analysis (IPA), Ingenuity® Systems] and gene set enrichment analysis [(GSEA), GeneTrail 2.0] identified significantly-enriched pathways.

**Results:** In the PM group, there were 144 DE transcripts between benign <sup>ER+BC</sup> and benign <sup>ER-BC</sup>, including coding RNAs (40%), antisense RNAs (35%) and lncRNAs (7%). In contrast, the preM group had no significantly DE genes between benign <sup>ER+BC</sup> and benign <sup>ER-BC</sup>. In the PM DE coding gene set, the top DE transcripts in benign <sup>ER+BC</sup> included many genes implicated in BC development<sup>#</sup> or ER+ BC progression (\*) (e.g. up-regulated: *KAAG1\**, *DNAJB7/HSP40\**, *TMEM151B*, *ZBTB32*<sup>#</sup> p < 0.001 ; down-regulated: *CPB1\**, *FOS*, *TPPP3*<sup>#</sup>, *CLEC3B*<sup>#</sup> p < 0.001). Top canonical pathways altered in benign <sup>ER+BC</sup> included MAPK, PI3K, and acute phase response pathways (p<0.05). GSEA of the entire gene set (N=15,223; ranked in order of 144 DE genes) identified 72 altered pathways (P < 0.005); those with the highest normalization enrichment scores (NES) (> 0.4) functionally grouped as immune function-related (T cell function and antigen presentation). Depleted pathways with NES > 0.4, (N=6) functionally grouped into proteasome-related, fatty acid biosynthesis and mitochondrial energy metabolism. Among the non-coding DE gene set, notably, the entire DE antisense RNA gene set (N=51 transcripts) was up-regulated in benign <sup>ER+BC</sup> compared to benign <sup>ER-BC</sup> (P< 0.001) with a subset (N=11) showing marked up-regulation (> 4 log2FC). Among the DE antisense RNAs, 70% have reported roles in carcinogenesis or BC progression (e.g. *KRT7-AS*, *NAV2-AS2*, *CCDC144NL-AS1*, *RP11-66B24.4*, *HSA-MIR4454*).

**Conclusion:** The benign breast transcriptome differs between postmenopausal women with ER+ vs ER- BC, with distinctive coding and non-coding RNA signatures. In postmenopausal women with ER+ BC, benign breast expresses a unique antisense RNA set and is enriched in genes implicated in BC development or progression. These data provide insight into at-risk benign breast and facilitate identification of potential biomarkers of carcinogenesis.

# 2018 San Antonio Breast Cancer Symposium ®

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Multi-omics profiling reveals distinct molecular features in young and elderly triple negative breast cancer

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## Purpose

Age at breast cancer diagnosis not only predicts clinical outcome, but also indicates distinct molecular features thus we can choose the most appropriate treatment strategies. Yet little is known about the molecular profile of young and elderly triple negative breast cancers (TNBCs).

## Methods

Clinical, genomic and transcriptome features of young (<40 year-olds) and elderly (≥65 year-olds) TNBC patients were studied in a cohort of 473 TNBCs from Fudan University Shanghai Cancer Center.

## Results

In this study, 50, 354 and 69 patients were <40, 40–64 and ≥65 years of age, respectively. Young TNBCs had more relapse or metastasis within the first 2 years after surgery ( $P=0.036$ ) which was also significant in the basal-like subgroup ( $P=0.004$ ), while elderly TNBCs were more likely to be luminal androgen receptor (LAR) subtype (46%) harboring frequent PIK3CA and KMT2C/KMT2D somatic mutations, with more fibrosis or mesenchymal-like (MES) subtype (in the basal-like subgroup) and featured by significantly lower Ki-67 index. Gene set enrichment analyses revealed that young TNBCs showed elevated expression of genes involved in cell cycle, nucleotide metabolism and DNA damage repair. In further discussion on nucleotide metabolism, TYMS, a crucial gene encoding thymidylate synthase while is also the target of fluorouracil and capecitabine, were identified to be enriched in young TNBCs independent of molecular subtype in both our cohort (adjusted  $P<0.001$ ) and METABRIC (adjusted  $P=0.027$ ). We next studied DNA damage features and found that while TNBCs of different age groups had comparable somatic mutation load, their mutations had distinct generation mechanism that homologous recombination deficiency (HRD) related signature and Aging related signature tend to be enriched in younger and elder patients, respectively. We also observed higher germline BRCA1 mutation rate in young TNBCs (23%). Interestingly, while germline BRCA2 mutation rate was comparable among the groups, copy number (CN) loss of Chr13q13 (with BRCA2 in the 'peak') was almost exclusively found in young patients (adjusted  $P<0.05$ ). We also found enriched CN loss at Chr15q13 (with FAN1 in the 'peak') and CN amplification at Chr1p34 (with KDM4A in the 'peak') in young patients. These two events significantly affected the expression levels of FAN1 and KDM4A, respectively, and were both corrected with genomic based HRD indexes.

## Conclusions

TNBCs of different age had distinct clinical and molecular features. We should pay attention to that nearly half of the TNBCs diagnosed at 65 years-old or later were not basal-like cancers but a special group with positive AR staining. Taking together with the higher fibrosis/MES proportion in elderly TNBC, we should reconsider the benefit of specific treatment strategies (like neoadjuvant chemotherapy) in these patients. The young TNBCs were characterized by activated cell cycle, elevated nucleotide metabolism (especially TYMS expression and corresponding pyrimidine metabolism) and enhanced DNA damage (especially HRD). These molecular features supported the aggressive phenotypes of young TNBC, while also provide us with potential therapeutic strategies.

# 2018 San Antonio Breast Cancer Symposium ®

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PIK3CA mutations in breast cancer: Mutational landscape and clinical implications in ER+/HER2- subtype

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**Introduction:** PIK3CA mutation is one of the most frequent genomic alterations in breast cancer. We evaluated PIK3CA mutational status including spatial and temporal heterogeneity, clinical characteristics and prognostic impact focused on ER+/HER2- subtype.

**Methods:** We performed targeted ultra-deep sequencing (CancerSCAN™) of breast cancer tissue in a prospective cohort. Burden of disease was assessed by metabolic tumor volume(MTV) in 18F-FDG-PET scan. Association with clinical characteristics or survival were tested in ER+/HER2- subtype, using Chi square test or Kaplan-Meier method.

**Results:** PIK3CA analyses were performed in 1274 breast cancer specimens from 1091 patients. 957 patients had early breast cancer. PIK3CA alterations were found in 397 patients(36.3%), and frequency of PIK3CA mutation was significantly lower in triple negative breast cancer(19.0%), compared with 40.4% in ER+/HER2-, 40.9% in ER+/HER2+, and 45.2% in ER-/HER2+ subtype( $p < 0.0001$ ). 158 patients had more than two biopsies. Among 92 patients with second biopsy within one month, 11%(10/92) had spatial heterogeneity of PIK3CA mutation. After neoadjuvant chemotherapy, 10%(3/30) of patients had change of PIK3CA mutational status. Serial biopsy at time of recurrence revealed loss or gain of PIK3CA mutation in 10 out of 59 patients (17%). In ER+/HER2- subtype, PIK3CA had a trend toward longer distant disease free survival without statistical significance. In patients with stage IV ER+/HER2- disease, PIK3CA hotspot mutations were associated with significant longer overall survival(OS) (71.0 vs. 37.8 months,  $p = 0.048$ ) and better progression free survival(PFS) at 1<sup>st</sup> line palliative treatment (37.7 vs. 9.4 months,  $p = 0.0004$ ). Frequency of symptomatic recurrence, recurrence as oligometastases, and specific metastatic sites were not associated with PIK3CA mutational status, except that bone metastases at first distant metastases was less prevalent in patients with PIK3CA hotspot mutations(35.6% vs. 53.8% in PIK3CA wt,  $p = 0.048$ ). Metabolic tumor volume(MTV) at time of first distant metastases was not associated with presence of PIK3CA mutation.

**Conclusion:** We observed variations in PIK3CA mutational status in more than 10% of patients with >1 repeated biopsy. In stage IV ER+/HER2- disease, PIK3CA hotspot mutation seemed to be associated with longer PFS and OS, however metabolic tumor burden was not associated with PIK3CA alterations.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-13

Exploring the role of ctDNA in triple negative breast cancer

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**BACKGROUND:** Previously published work shows that triple negative (TNBC) is a heterogeneous disease with varying levels of genomic instability, where higher genomic instability is associated with poorer prognosis. Subgroups of TNBC patients with distinct patterns of genome aberrations may indicate pathologies in specific genome maintenance/repair processes. Circulating tumor DNA (ctDNA) as assessed by next generation sequencing (NGS) is a relatively non-invasive test that may provide prognostic and predictive information.

**AIM OF STUDY:** To analyze genomic alterations with serial plasma samples using NGS methods of ctDNA analysis and determine the utility for actionability and disease burden monitoring. We shall also determine whether TNBC subgroups differ in their ctDNA profiles. Shallow whole genome sequencing vs targeted capture at depth will be contrasted to determine sensitivity for relapse detection.

**METHODS:** Enrollment of a planned cohort of TNBC patients (N=300) with any stage, at diagnosis (dx) or within 2 years of dx, or at relapse of disease with ongoing plasma sampling every 3 -6 months. Patient age, stage, grade, type of chemotherapy, date of relapse and date of last followup are collected. Tumor tissue (FFPE), saliva for germline mutations and serial blood draws for ctDNA are analyzed with two NGS sequencing methods: (i) a high sensitivity small hotspot gene panel (33 genes, 170 hotspots), directed purely at actionable findings (ii) capture sequencing directed at multiple regions of the genome or shallow whole genome sequencing.

**RESULTS:** Preliminary analysis in 20 patient cases using the targeted hotspot panel. . Median followup 151 days. Two cases had plasma drawn at time of relapsed disease and 1 at the time of de novo metastatic disease; 12 had plasma samples drawn prior to neoadjuvant chemotherapy (clinical T1/T2N1, T3/T4Nany), and 5 had plasma draws after primary surgery (pathologic T1N0, T2N0). Of the neoadjuvant cases, 5 (42%) had a pathologic complete response (pCR); 4 with ctDNA mutations and 1 without. Six (58%) neoadjuvant cases did not achieve a pCR; 3 with ctDNA mutations, 3 without. One patient is awaiting surgery. Twelve (60%) cases had mutations in TP53, one case had 2 different TP53 mutations (no pCR) and one case had 3 mutations: TP53, PIK3CA, KRAS (achieved pCR). Of the cases treated with curative intent, with short followup (FU), there have been no relapses including the case of the sample containing 3 mutations.

**CONCLUSION:** TP53 mutations may be a marker of higher genomic alteration burden and may have prognostic value in patients with newly diagnosed, non-metastatic TNBC with longer FU. Ongoing analysis of serial plasma samples and FFPE analysis may provide further insight into the prognostic value of ctDNA. Full genome sequencing may be needed identify other mutations that have prognostic and/or predictive value. We have accrued over 200 patients with samples being analyzed and plan to present an interim analysis of the cohort at SABCS 2018.

# 2018 San Antonio Breast Cancer Symposium ®

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Quantifying intrinsic subtype admixture in luminal A breast cancer and its relationship to clinical outcomes

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**Background/Objectives:** PAM50 gene profiling assigns each cancer to a single intrinsic subtype. However, individual cancers vary in their adherence to a prototype, and some may exhibit expression patterns that indicate intra-tumor admixture of multiple subtypes. Our objective was to develop admixture metrics from PAM50 gene expression profiles in order to stratify Luminal A cases according to their degree of subtype admixture, and then relate such admixture to clinical and molecular variables.

**Methods:** We re-constructed scaled, normalized PAM50 profiles for 1,980 cases (674 LumA) in the METABRIC cohort and for each case we computed its Mahalanobis (M-) distance from its assigned centroid and its M-distance from all other centroids. We used t-SNE plots to visualize overlaps in subtype clustering. With Normal-like cases excluded, Median Distance Criteria (MDC) classified a case as Pure if it was located within the 50<sup>th</sup> percentile of the LumA centroid and >50<sup>th</sup> percentile from any other centroid. Distance Ratio Criteria (DRC) was computed as the ratio of M-distances from the LumA centroid to the nearest non-assigned centroid; cases were grouped by DRC tertile. Pure and admixed LumA cases were compared on clinical, molecular and survival traits. TCGA LumA cases (n=509) were used for independent validation.

**Results:** Compared to admixed cases in METABRIC, pure ones by MDC had younger age at diagnosis, smaller tumor size, lower grade and lower stage. Comparisons of the highest (T3, most admixed) to lowest tertile (T1) for DRC revealed even stronger associations. Admixed cases, by both metrics, were more likely to show HER2 gain, high proliferation by *AURKA* expression, higher PAM50 Risk of Recurrence scores, more frequent *TP53* mutation, and less frequent mutation of *PIK3CA* and *CBFB*. Similar results were observed in the TCGA validation cohort. LumA-LumB confusion was predominant, but other combinations with LumA were also present. Degree of admixture was associated with overall survival in both cohorts, as was disease-free survival in TCGA, independent of age, grade and stage. (See table for adjusted hazard ratios).

**Conclusions:** Luminal A breast cancers subgrouped based on PAM50 subtype purity support the hypothesis that admixed cases have worse clinical features and survival. Future analyses will explore more extensive genomic metrics for admixture and their spatial significance within a single tumor.

Multivariable hazard ratios for overall (OS) and disease-free survival (DFS) for Luminal A breast cancer, according to degree of subtype admixture. METABRIC and TCGA cohorts.

	Distance ratio tertile						
	T1 (pure)		T2		T3		P trend
	HRadj	95% CI	HRadj	95% CI	HRadj	95% CI	
METABRIC - OS*	1.00	-	1.20	0.91-1.59	1.42	1.07-1.88	<0.001
TCGA - OS^	1.00	-	1.34	0.69-2.62	1.90	1.02-3.54	0.04
TCGA - DFS^	1.00	-	1.14	0.48-2.74	2.85	1.39-5.85	0.002

\* Adjusted for age, tumor size, grade and stage; ^ adjusted for age, size and stage only

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-15

Proteomic profile of PAM50 intermediate risk early breast cancers

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## Background

Breast cancer is a heterogeneous disease with a wide range of outcomes that are not fully predicted by routine clinical and pathologic features. The risk of recurrence of hormone receptor positive early-stage breast cancer, the most frequent tumor subtype, increases continuously over time. Genomic-based signatures have been developed to categorize patients according to their risk of recurrence and guide therapeutic decisions in this tumor subtype. However, the main genomic-based assays used in clinical practice classify up to 40% of the patients in an intermediate risk group. Many interrogations remain about the optimal strategy in this group. The aim of this study was to refine the molecular characterization of the intermediate risk group and determine the proportion of shared features with the low or high-risk groups using a mass spectrometry based proteomic approach.

## Methods

Tumors with available routine PAM50 assay (PROSIGNA) results were selected from a cohort of breast cancer patients treated at Oscar Lambret Cancer Center (France). Fifteen tumors evenly split between PAM50 low, intermediate and high-risk groups were analyzed to determine the proteomic profiles of both cancer cells and stroma using MALDI mass spectrometry imaging combined with microproteomics, a spatially resolved proteomic technology.

## Results

PAM50-Intermediate risk tumors had a distinctive proteomic profile compared to low and high-risk tumors. Heterogeneous nuclear ribonucleoproteins, 4-aminobutyrate aminotransferase, and pleckstrin homology-like domain family A member 1 are discriminating proteins between intermediate risk and low risk tumors. Differences were observed in expression of integrin beta-1, DNA replication licensing factors, splicing factors and interleukin enhancer-binding factor 2 between intermediate and high-risk tumors. Proteomic profiles of stroma according to tumor risk groups also showed differential protein expressions mainly between intermediate and high-risk groups. Breast cancer markers such as nuclear mitotic apparatus protein 1 (NUMA1), C-1-tetrahydrofolate synthase (MTHFD1), cystatin-C (CST3), and T-cell immune regulator 1 (TCIRG1) were identified in high-risk tumors. Specific protein profiles were identified in stroma versus tumor. Immunoglobulin kappa chain, IGHG1, IGHM, IGHM, MMP2, ORM1 & ORM2, podocan, asprorin, immunoglobulin superfamily containing leucine-rich repeat protein (ISLR) were detected in stroma. By contrast, squamous cell carcinoma antigen recognized by T-cells 3 (SART3), shootin-1, mitotic checkpoint protein BUB3, XRCC5 & XRCC6, membrane-associated progesterone receptor component 2 (PGRMC) and hepatocyte growth factor-regulated tyrosine kinase substrate (HGS) were specifically detected in tumor. Further analyses on an expanded cohort of patients will be presented.

## Conclusion

MALDI mass spectrometry proteomics reveal distinctive tumor and microenvironment profiles in PAM50 intermediate risk early breast cancers.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-16

Age's importance in early breast cancer: Oncotype Dx results in patients  $\leq 40$  years

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## Background

The 21-gene recurrence score (RS) predicts the benefit of adjuvant chemotherapy (CT) in ER-positive HER2-negative breast cancer (BC) and has been validated in population where women under 40 are underrepresented. Young BC pts are more likely to receive adjuvant chemotherapy (CT) in addition to endocrine therapy (ET). Our objective was to assess the RS results in young ( $\leq 40$  yo) vs older ( $> 40$  yo) pts and evaluate the impact of age on clinical decision-making according to RS categories.

## Methods

We retrospectively reviewed electronic medical files of all patients with early stage hormone receptor BC for whom RS was available between 2007 and 2017 in 3 specialized cancer centers. We used the Mann-Whitney and Chi-squared tests to assess differences between age group. Similarly, we evaluated the association between age groups and treatment, within each ODX category. To determine if age was associated with CT use in the low risk category, a logistic regression model was constructed.

## Results

A total of 551 pts were included, 53 (9.6%)  $\leq 40$  yo and 498 (90.4%)  $> 40$  yo. No statistical differences were found between the younger and older groups in T ( $p=0.874$ ), N ( $p=0.794$ ), stage ( $p=0.188$ ), or grade ( $p=0.791$ ). Young patients underwent radical surgery more frequently than their older counterparts (41.5 vs 25.7%,  $p=0.014$ ). Statistically significant differences were also observed in ER mean, which was lower in the younger group (80 vs 90%,  $p<0.001$ ). The median RS result was significantly higher in the younger group (19 vs 16,  $p=0.009$ ). Also, high-risk recurrence score category was significantly more frequent in the younger group (22.6 vs 9.2%,  $p=0.009$ ). In the intermediate-risk category there were no differences in the proportion of patients who received CT according to age groups ( $p=0.484$ ). In the low-risk category, 28.0% of patients  $\leq 40$  years vs 11.3% of patients  $> 40$  years received CT ( $p=0.037$ ).

## Conclusions

Our results indicate that RS tends to be higher in patients with BC  $\leq 40$  yo and that the frequency of high-risk RS is significantly higher in the younger group, suggesting biological differences between groups. 28% of young patients with low-risk RS from our cohort are overtreated. Based on these results, it should be considered to develop a test adjusted to the age of the patients.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-08-17

## Evaluation of Oncotype DX testing and subsequent treatment choices in the Latin American setting

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**Background:** The gene expression profiling assay OncotypeDx (ODx) prognosticates the risk of estrogen receptor positive (ER+) breast cancer (BC) recurrence and assesses the likely benefit from adjuvant chemotherapy in addition to endocrine therapy. Numerous clinical utility studies have shown that acknowledging the RS impacts on clinical decision making, leading to a decrease in chemotherapy (CT) use. However, the cost of the assay limits its widespread use, especially in low and middle-income countries. Our objective was to determine the patterns of use of ODx, its results and the subsequent treatment choices in a large Latin American cohort.

**Methods:** We retrospectively reviewed the electronic medical records of patients with early-stage ER+ BC for whom ODx recurrence score (RS) was available. Patients were diagnosed and treated at 3 specialized Peruvian cancer centers between 2007 and 2017. Descriptive results for numeric variables were presented as means with standard deviation (SD) or medians with interquartile range (IQR), depending on their distributions; otherwise, we expressed the qualitative variables as numbers with percentages. We evaluated the association between ODx RS category and treatment using the Chi-squared test.

**Results:** A total of 551 patients were included. Patients had a mean age of  $56.2 \pm 11.9$  (SD) (range: 26-89). 9.6% (n=53) of patients were  $\leq 40$  years old. The size of the tumors ranged from 0.1 cm to 7.2 cm (median = 1.5 cm; IQR 1.0-2.2cm). 36 (6.5%) patients had tumors  $\leq 0.5$ cm and 7 (1%) had tumors  $> 5$ cm. A minority of patients had lymph node involvement (5.8%, n=32). ODx was ordered in 55 cases (10%) of lobular carcinoma and in 23 cases (4%) of favorable histology tumors (19 mucinous, 4 tubular). Most tumors exhibited an intermediate histological grade (71.6%, n=386). Ki67 was available in 58.8% patients (n= 324), with a median Ki67 of 20 (IQR 10-30). Using commercial cutoffs RS was distributed as follows: low (0–17) = 316 (57.4%), intermediate (18–30) = 177 (32.1%), and high ( $\geq 31$ ) = 58 (10.5%). In general, 57.5% (n=317) of patients received endocrine therapy (ET) as their only systemic treatment and 42.5% (n=234), also received CT (ET + CT). In the low-risk category, 87.3% (n=276) of patients received ET and 12.7% (n=40), ET + CT. Within the intermediate-risk category, most patients received ET + CT (77.4%, n=137). Only one patient in the high-risk category did not receive CT. There was a significant association between the RS group and treatment choice ( $p < 0.001$ )

## Impact of ODx RS results on treatment recommendations

	Oncotype risk categories						
	Low		Intermediate		High		p value
Treatment	n	%	n	%	n	%	<0.001
Endocrine therapy	276	87.3	40	22.6	1	1.7	
Chemotherapy + Endocrine therapy	40	12.7	137	77.4	57	98.3	

**Conclusion:** ODx significantly influenced treatment decisions in our cohort, however an overutilization of CT was found in low-risk patients. Further data analysis is needed to explain the higher than expected use of CT. Also, there is room for improvement in the selection of cases that undergo ODx testing.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-18

Clinicopathological characteristics associated with intermediate and high-risk ODx RS

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**Background:** The gene expression profiling assay OncotypeDx (ODx) prognosticates the risk of estrogen receptor positive (ER+) breast cancer (BC) recurrence and assesses the likely benefit from adjuvant chemotherapy in addition to endocrine therapy. There have been several attempts to develop algorithms that provide similar outcome prediction to the ODx assay with the use of routine clinicopathological characteristics. These models appear to predict high-risk ODx RS but are unable to reliably rule out the presence of patients with intermediate-risk disease. Our objective was to identify the clinicopathological factors associated with intermediate and high-risk categories.

**Methods:** We retrospectively reviewed the electronic medical records of patients with early-stage ER+ BC for whom ODx recurrence score (RS) was available. Patients were diagnosed and treated at 3 specialized cancer centers between 2010 and 2017. Two multinomial logistic regression models (crude and adjusted) were constructed to assess the association between clinicopathological characteristics and ODx RS as a categorical variable. The adjusted model included the following variables: ODx RS, age, tumor size, node status, grade, lymphovascular invasion and hormonal receptors. The reported association measure was the relative prevalence ratio (RPR) with its respective 95%CI.

**Results:** A total of 551 patients were included. Patients had a mean age of  $56.2 \pm 11.9$  (SD) (range: 26-89). 9.6% (n=53) of patients were  $\leq 40$  years old. The size of the tumors ranged from 0.1 cm to 7.2 cm (median = 1.5 cm; IQR 1.0-2.2cm). A minority of patients had lymph node involvement (5.8%, n=32). By subtype, carcinomas were mostly ductal (83.5%, n=460), followed by lobular (10.0%, n=55) and mucinous (3.5%, n=19). The majority of tumor exhibited an intermediate histological grade (71.6%, n=386). Ki 67 was available in 58.8% patients (n= 324), with a median Ki67 of 20 (IQR 10-30). In the adjusted multinomial logistic regression model, factors associated with ODx intermediate-risk category were grade 3 (RPR=4.78; 95%CI: 2.01-11.39) and having either ER or PR  $< 50$  (RPR=2.80; 95%CI: 1.83-4.27). Factors associated with ODx high-risk category were grade 3 (RPR=15.89; 95%CI: 3.23-78.19), having either ER or PR  $< 50$  (RPR=4.58; 95%CI: 2.37-8.87), age  $\leq 40$  (RPR=2.96; 95%CI: 1.20-7.29) and T2-3 (RPR=2.20; 95%CI: 1.13-4.32).

**Conclusion:** Grade 3, ER o PR  $< 50$ , age  $\leq 40$  years and T2-3 are clinicopathological characteristics strongly associated with high-risk ODx RS. The associations with intermediate-risk ODx RS are weaker. The way these factors could be integrated into a clinicopathologic risk prediction model to identify high-risk patients needs further analysis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-19

Proteomic tracking of breast cancer metastasis progression

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## Background

Metastases from breast cancers have not yet reached curability mainly because of the continuous evolution of cancer cells leading to treatment resistance and tumor progression. Despite the identification of the main genomic drivers of breast cancer resistance, and the development of specific targeted therapies, resistance has not been eradicated. A deeper understanding of tumor biology underlying treatment escape and tumor progression is necessary to find new targets. The aim of this study was to determine whether mass spectrometry based imaging and microproteomics are able to capture changes in protein expression and pathways occurring during metastasis progression.

## Methods

Ten tumor samples from five progressing metastatic breast cancer patients treated at Oscar Lambret Cancer Center (France) were selected based on the availability of paired metastasis biopsy performed at two time points during the evolution of the disease. The proteomic profiles of paired tumor samples were analyzed using MALDI mass spectrometry imaging combined with microproteomics, a spatially resolved proteomic technology.

## Results

Comparison of paired samples showed gain and loss of proteins associated with tumor progression. However, few were shared between patients. The pathways and biological processes mainly represented during tumor progression, and which were found in at least two patients, were those involved in the negative regulation of leukocyte mediated cytotoxicity, the metabolism of carboxylic acid, peptides and nucleosides, in response to stress, oxidation-reduction process, endocytosis, catabolic processes, actin regulation, and extracellular matrix organization. During metastasis evolution, few shared proteins were identified in at least 3 patients such as SRPX2, CILP1, collagen alpha-2(V) chain, Ras-related C3 botulinum toxin substrate 1, filamin-C, PDZ and LIM domain protein 2 and chloride intracellular channel protein 4. The main proteins lost during progression and found in at least 2 patients were cell surface glycoprotein MUC18, collagen alpha-2(IV) chain, polyadenylate-binding protein 2, and latent-transforming growth factor beta-binding protein 4 (LTAGP4). Results from an expanded cohort will be presented at the meeting.

## Conclusion

MALDI mass spectrometry proteomics identified private and shared changes in proteins and biological processes associated with breast cancer metastasis progression.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-20

Clinical next-generation sequencing analysis in ER-positive HER2-negative metastatic breast cancer patients: Mutation frequency & clinical correlations

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**Background:** Clinical next-generation sequencing (NGS) has opened new perspectives on genome-driven therapy for metastatic breast cancer (MBC) through the identification of recurrent driver mutations. However, therapeutic relevance of the detection of these mutations with a potential impact on disease outcome and treatment resistance remains unclear.

**Patients and Methods:** A monocentric retrospective study was performed to investigate mutation frequency and disease outcome in 86 metastatic breast cancers patients treated in UZ-Leuven and tested for the presence of mutations in representative formalin-fixed, paraffin-embedded tumor tissue using a routine diagnostic panel of 26 cancer genes (TruSight Tumor 26, Illumina, mean coverage 500X). It mainly concerned metastatic lesions (96.5%). Out of 86 patients, 63 had hormone receptor (HR) positive/ HER2-negative disease; 8 were HER2-positive and 15 triple negative as determined by immunohistochemistry (IHC)/Fluorescence in situ hybridization (FISH). The 63 ER-positive/ HER2-negative cases were selected for further investigation. Single-nucleotide variants and insertions/deletions were reported.

**Results:** Overall, mutations (> 5% allelic frequency) were found in 60.3% of the cases. As expected, mutations in *PIK3CA* and *TP53* were being most frequently encountered (35% and 19% respectively); variants in *AKT1*, *KRAS* and *PTEN* were less common (5%, 3% and 2% respectively). Focusing on ER-positive/HER2-negative cases, 13 out of 63 had a single *PIK3CA* mutation, 6 had a single *TP53* mutation and 21 cases had more than 1 mutation. In 23 out of 63 cases, no potentially actionable mutation could be identified using the 26 cancer gene panel. Interestingly, we found a clinically relevant and statistically significant difference in median progression-free survival between patients harboring a *TP53* mutation only (19,8 months (m), range 12.1 – 27.4) and those harboring a *PIK3CA* mutation only (84m, range 7.4 – 215.1) or patients without any detected mutation (45,3m, range 5.8 – 225.8). Similarly, overall survival was significantly worse for *TP53* mutated cases compared with patients with a *PIK3CA* or no mutation at all. Finally, a brief comparison of MBC-therapies used in these different subgroups showed the interesting finding that none of 7 *PIK3CA*-mutated tumors treated with fulvestrant monotherapy showed treatment response.

**Conclusion:** This small retrospective analysis showed that clinical sequencing using a small targeted NGS panel reveals mutations in 60.3% of MBC patients, with 40% being targetable. Besides predictive implications, detection of these mutations could also have major prognostic implications on distant metastasis free survival and overall survival.

# 2018 San Antonio Breast Cancer Symposium ®

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Clinical Impact of HER-3 among patients with breast cancer – Molecular classification

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Receptors of HER family play an important role in breast cancer. Dimerization of HER2 with other members of HER (HER3) is the major driving mechanism for growth and survival of tumor cells. Numerous studies show that overexpression of HER3 gene correlates with bad prognosis. However, other studies show contrarily overexpression of HER3 as a positive prognostic factor. HER3 may provide resistance against certain therapeutics focusing on EGFR or HER2 receptor. The interesting fact is that the expression of HER3 might serve in immunotherapy as a marker among TNBC. It is suggested it participates not only on the cell survival and proliferation, but also on the regulation of expression of PD-L1.

The aim of the study was to monitor ERBB3 gene amplification (ZytoLight® SPEC ERBB3 / CEN12 Dual Color Probe) and IHC expression of the HER-3 (HER-3 / c-erbB-3 RMaB) in a cohort of 40 patients (20 HER-2 positive patients, 20 TNBC patients). Activation of tumor infiltrating lymphocytes (TIL) CD8 (clone C8 / 144B) CD4 (Clone 4B12) (Dako), expression of PD-L1 (Anti-PD-L1, Clone28-8, Dako) and TP53 mutation. Assess their clinical impact in relation to the HER-3 receptor.

20 patients were with a median age of 49.4 years (14 postmenopausal [46.7%] and 16 premenopausal [53.3%]) with detected HER-2 FISH amplification (histologically 18 patients with HER-2 subtype which means without hormone receptor positivity and 12 patients with luminal B subtype and thus hormone receptor positive). All patients were without evidence of distal metastases (6 in stage I, 13 in stage II and 11 in stage III).

20 patients subtype of TNBC were with a median age of 33,6/33,2 years (10/10 no relapse/relapse). All patients were without evidence of distal metastases (2/2 in stage I, 5/5 in stage II and 3/3 in stage III). Median follow-up (months) 59,8/23,3; median time to relapse (months) NR/15,9; median overall survival (months) NR/30,2.

The results demonstrated the amplification / expression of CERB3 / HER-3 in 2/20 (a group of TNBC patients), and 13/20 (a group of HER-2 positive patients). Expression of PD-L1 was demonstrated in 3/20 HER-2 positive and 2/20 TNBC. Expression of HER-3 correlated in 100% with PD-L1 expression. There was a strong correlation between positive expression of HER-3 and PD-L1 ( $p = <0,001$ ). The TP53 mutation was found in 5 patients with HER-2 carcinoma and 6 TNBC and was always a HER-3-free patient (correlation was not found.) Activated antitumor immunity TIL (CD8 + positivity) in more than 15% (group TNBC) / 45% (group HER-2) of tumor and concurrently no expression (CD4-). There was a strong correlation between positive expression of HER-3 and activation of TIL ( $p = <0,001$ ) in the HER-2 positive group.

However, the view of the HER-3 receptor will be far more complex, and it appears that overexpression of this receptor will have both negative and positive prognostic significance. Attention should be focused on the entire signaling pathway, including activation of the immune system. The significance of the current mutation of TP53 and HER-3 expression has not been demonstrated.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-08-22

Oncotype Dx recurrence score risk groups according to Ki67, a predictor to be considered

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## Background

The gene expression profiling assay OncotypeDx (ODx) prognosticates the risk of estrogen receptor positive (ER+) breast cancer (BC) recurrence and assesses the likely benefit from adjuvant chemotherapy in addition to endocrine therapy. There have been several attempts to develop algorithms that provide similar outcome prediction to the ODx assay with the use of routine clinicopathological characteristics. Ki67 is frequently incorporated into these assessments, although there is no standard cut-off for its use.

## Methods

We retrospectively reviewed the electronic medical records of 330 patients with early stage ER+ BC for whom ODx recurrence score (RS) was available. Patients were diagnosed and treated at two specialized cancer centers between 2014 and 2017.

Our objective was to determine the ki67's median differences between ODx risk groups.

We used Spearman rho for the correlation between Ki67 and ODx score and used Kruskal-Wallis test for compare medians, pairwise comparison for the intergroup relations.

## Results

Mean age at diagnosis was 57.42 years (range 28-89). Mean tumor diameter was 15.67 mm. 78.9% were intermediate histologic grade and 9.7% patients had lymph node involvement. Median expression of ER and PR were 90% (5-100) and 70% (0-100), respectively. We assessed the correlation between Ki67 and ODx score, with a pearson  $r:0.31$ ,  $p<0.001$ . The data showed a directly proportional trend between Ki67 and ODx score.

Median Ki67 was 20 (1-100). According to ODX RS, 61.5% of tumors were low risk, 30.3% were intermediate risk and, 8.2% were high risk. Median Ki67 within each category group is as follows: low: 15 (IQR:15), intermediate: 20 (IQR:18) and high: 40 (IQR:35), with a statistically significant difference between medians ( $p<0.001$ ). In the Pairwise comparison intergroup the data showed: Low-Intermediate ( $p<0.05$ ), Low-High ( $p<0.001$ ), Intermediate-High ( $p<0.001$ ).

## Conclusions

The data showed directly proportional trend between Ki67 and ODx score. In our population there is a statistically significant difference between Ki67 medians according to ODx risk groups.

# 2018 San Antonio Breast Cancer Symposium ®

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Prosigna assay for treatment decisions in early breast cancer: A single center, decision impact study

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**Background:** Therapeutic decisions in early breast cancer (EBC) are based on clinical and pathological features, which are subject to intra- and inter-observer variability. Hence, in the era of precision medicine, there is growing need for predictive biomarkers. The Prosigna assay utilizes Prediction Analysis of Microarray, a test based on the analysis of 50 intrinsic subtype-linked gene clusters. This single center decision impact study aimed to evaluate the effect of Prosigna test results on physicians' adjuvant treatment choices.

**Methods:** Between September 2017 and February 2018, FFPE tumor samples from 53 newly diagnosed, postmenopausal, hormone receptor-positive, HER2-negative EBC (T1-T2; pN0-N1a) patients were analyzed. Pre-test clinical judgments and Prosigna test results were compared.

**Results:** Mean age was 59 (42-77). Invasive ductal carcinoma (79.2%), grade 2 (52.8%) and T1c-N0 tumors (43.4%) represented the majority. Before the Prosigna test, 65.4% of the patients were classified as luminal A and 34.6% as luminal B. Of the pre-test risk groups, 40.4% were low-risk, 40.4% were intermediate risk and 19.2% were high risk. Prosigna assay grouped 50% of patients as luminal A, 44.2% as luminal B, 3.8% as basal type and 1.9% as HER2-expressing. Post-test ROR score-based groups were distributed as 25% low-risk, 40.4% intermediate risk and 34.6% high risk. There was a statistically significant correlation between clinically defined and molecularly assessed intrinsic BC subtypes (kappa:0.334, p=0.007). Similarly, pre-test and post-test recurrence risk groups were correlated (kappa:0.397, p=0.001). Before the Prosigna test, endocrine treatment was physicians' primary choice in 20 patients (39.2%), chemotherapy was recommended to 31 patients (60.8%). Overall, the Prosigna assay led to a change in choice of treatment for one patient (2%)

Table 1. Impact of Prosigna Results on Final Treatment Decision

	Prosigna low risk N=13 (%24.5)	Prosigna intermediate risk N=21 (%39.6)	Prosigna high risk N=18 (%34)	Total N=51 (%100)
Treatment choice before Prosigna				
CT+HT	0	12	17	29 (56.9)
HT only	12	7	1	20 (39.2)
CT offered, not accepted by the patient	0	2	0	2 (3.9)
Treatment choice after Prosigna				
CT+HT	0	12	18	30 (58.8)
HT only	12	7	0	19 (37.3)
CT offered, not accepted by the patient	0	2	0	2 (3.9)
Change in treatment choice				
HT to CT	0	0	1	100 (0)
CT to HT	0	0	0	0 (0)

CT: Chemotherapy HT: Hormonotherapy

. There was 40.4% discordance between pre- and post-test recurrence risk groups. In addition, intrinsic subtypes were 34.6%



discordant, which is largely driven by the reclassification of pre-test luminal A tumors into Prosigna luminal B group.

**Conclusions:** Although conventional risk assessment methods are relatively inexpensive with shorter turnaround times, their accuracy for risk assessment and value for risk reduction are suboptimal. According to our results, Prosigna assay was found as a relevant tool for clinical decision-making process. In cases where there is a discrepancy between the clinical assessment results and the Prosigna assay, tumor boards may guide treatment recommendations. Long term follow-up of these patients will elucidate the potential benefits of using multigene molecular tests as biomarkers for EBC treatment.

## 2018 San Antonio Breast Cancer Symposium ®

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### Mutational characterization of HER2-positive breast cancer

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**Background:** Studies of mutational analysis have been widely reported in various global series of breast cancer. However, in the era of different phenotypes of breast cancer, these analyses should be more specific in order to find differences in each phenotype that could predict response to treatment. Particularly HER2 positive is a special subtype with different connotations that make the analysis of its genotype very interesting.

**Methods:** Cohorts of 47 consecutive samples of HER2 positive breast cancer, treated in our institution were analyzed. It was conducted a mutational study with NGS technique of the most common genes involved in breast cancer.

**Results:** 10 patients were metastatic at diagnostic, 17 with negative estrogen receptor expression, the median value of Ki67 was 45 (20-95) and the median initial tumor size was 33.5 mm (18-111). Immunohistochemical expression of HER2 was +3 in 31 tumors and +2 in 15 with a median ratio by FISH of 3,2 (1,18-8,3). We also analyzed the expression of HER by PCR of mRNA in 35 samples with a median expression of 2,49 (0,26-33,15). 38 patients underwent surgical excision of the tumor with a pathologic complete response observed in 19.

We found a total of 38 significant mutations and the genes with more frequent mutations were MCPH1 (100%), HNF1 (98%), p53 (96%), somatic BRCA1 (62%), TSC1 (30%), PIK3CA (23%), ATM (21%), ALK (15%) and somatic BRCA2 (13%). Although the aim of the study was to find the most frequent mutations in this subtype, we have also analyzed possible correlations of those with pathological response or with more common variables, finding no significance in any of them.

**Conclusions:** In our series, we found a high percentage of mutations in MCPH1, HNF1 and p53 genes with almost 100% of mutations in p53 that are also limited in a very specific area. Also interesting is the 62% of mutations found in BRCA1 and 13% in BRCA2 that could allow more specific treatments in this subgroup. Although the study did not have the power to assess the predictive factor of these mutations, it is very suggestive that in the 7 patients with BRCA mutations, 6 achieved a complete pathological response.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-09-01

MYC dysregulates mitotic spindle function in triple-negative breast cancer creating a dependency on TPX2

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Tumors that overexpress the MYC oncogene, including most receptor triple-negative breast cancers, frequently demonstrate aneuploidy, numerical chromosome alterations associated with highly aggressive cancers. Aneuploidy is also associated with rapid tumor evolution and poor patient outcome. We identify that MYC overexpression induces reversible defects in microtubule nucleation and mitotic spindle assembly, in TNBCs and other epithelial cells, promoting chromosome segregation defects, micronuclei and chromosomal instability (CIN). High TPX2 expression is permissive for mitotic spindle assembly and chromosome segregation in cells with MYC overexpression; whereas TPX2 depletion blocks mitotic progression, induces cell death and prevents tumor growth. Attenuating MYC expression reverses mitotic defects, even in established breast tumor cell lines, implicating an ongoing role for high MYC in the persistence of CIN in cancers. Our studies implicate the MYC oncogene as a regulator of spindle assembly and identify a new MYC-TPX2 synthetic-lethal interaction in TNBC that could represent a future therapeutic strategy in MYC-overexpressing cancers. Moreover, our studies suggest that blocking MYC activity can attenuate the emergence of CIN and tumor evolution.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-09-02

CRISPR-Cas9 mediated *BRCA1* mutation in primary cells: Mutation efficiency and effects

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**Background:** Germline mutations in Breast Cancer Associated (*BRCA*) 1 or 2 genes confer an increased risk of the development of breast and ovarian cancer. Germline mutation is followed by somatic loss of heterozygosity (LOH) resulting in biallelic inactivation. *BRCA1* is involved in multiple homeostatic functions including control of chromatin organization, gene transcription, protein stability and cell division. Recent studies have demonstrated heterogeneity in LOH within and between premalignant and malignant breast tissues of *BRCA1* mutation carriers. We hypothesize that LOH does not have a unitary effect on phenotype but differs by the function that is abrogated.

**Methods:** To test our hypothesis, we adopted CRISPR-Cas9 gene editing technology. The guide RNAs for targeting the exon sequence in the RING finger, nuclear export signal (NES), nuclear localization signal (NLS) and *BRCA1* C Terminus (BRCT) domain/motif of *BRCA1* were designed and synthesized. MCF10A cells were transfected with a complex of guide RNA and Cas9 protein (RNP) to cause in/del mutation. The mutation was analyzed by both T7E1 assay, and an innovative and more precise method developed in our lab that utilizes linked nucleic acids (LNA) and qPCR. Proliferation and apoptosis assays were performed using the transfected cells. Organoids prepared from *BRCA1* mutation carriers also were transfected with RNPs and the mutation burden determined.

**Results:** Since single cell clones of the transfected MCF10A cells could not be selected and expanded, a pool of transfected cells was used for the analyses. T7E1 assay and qPCR analysis using LNAs demonstrated the presence of the mutations. A standard curve was created to enable the calculation of the mutation burden. IncuCyte analysis revealed increased proliferation and apoptosis, induced by irradiation, in cells with the mutation in Exon 10, where the extent of increase varied from 11% to 48% depending on the degree of mutation. In contrast, cells with the mutation in Exon 5 displayed diminished proliferation with no change in apoptosis. That mutations in exon10 and 5 have distinct biological effects when compared to the mutations in other exons is intriguing, and modification of binding proteins will be investigated. Organoids generated from *BRCA1* mutation carriers (primary and nonmalignant cells) were able to be successfully transfected using the NEON electroporation system. Mutations were introduced by the CRISPR-Cas9 system and their extent quantified by our LNA-mediated qPCR method.

**Conclusions:** CRISPR-Cas9-mediated gene editing of *BRCA1* in MCF10A resulted in a change in the proliferation rate and the extent of apoptosis that is dependent on the location of the de novo mutation within the gene. The development of a novel method, LNA-mediated qPCR, provides quantitative information regarding the mutations that may be used to correlate mutation burden with biological functional change. Successful establishment of this *BRCA1* tumorigenesis model has provided us with a method to test other putative tumor suppressors.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-09-03

RelB facilitates cell migration and invasion in breast cancer via MMP1 upregulation

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**Background:** RelB is a subunit of the nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling family and appears aberrant expression in breast cancer. The aim of the present study was to further investigate the role of RelB in breast cancer and whether RelB might be a therapeutic target.

**Methods:** 120 cases of breast cancer patients were collected from the Jiangsu Province Hospital during 2015-2017.

Immunohistochemistry (IHC) was performed to detect RelB expression in breast cancer tissues and para-cancer tissues. The relationship between RelB expression and clinical pathologic parameters were investigated. Detection the expression of RelB in 4 kinds of human breast cancer cell lines (MCF-7, T47D, MDA-MB-231, BT-549) by Western blot and RT-qPCR. Knockout of RelB in TNBC cell lines (BT-549 and MDA-MB-231) was using the CRISPR/Cas9 genome editing system. CCK-8 and flow cytometry were performed to observe the proliferation, cycle and apoptosis of MDA-MB-231 and BT-549 after knockout of RelB. Transwell and wound healing experiments were performed to observe cell invasion and migration ability. RT-PCR and Western blotting were performed to detect the expression of mRNA and protein of EMT marker and MMP1 respectively. The luciferase reporter gene was performed to detect whether RelB binding with MMP1 promoter region. In vivo efficacy was tested using the xenograft model and lung metastasis model.

**Results:** RelB was highly expressed in triple-negative breast cancer (TNBC) tissues compared with that in adjacent tissues. RelB levels were remarkably associated with the pTNM stage ( $p=0.01981$ ) and ER expression ( $p=0.4689$ ). RelB expressions in TNBC cell lines were higher than that of hormone receptor positive MCF-7 cells. In vitro studies showed that RelB deletion promoted apoptosis and suppressed breast cancer cell survival, migration and invasion by decreasing snail, vimentin and MMP1. RelB deletion inhibited cell cycle progression and G1/S transition by upregulating p21 and p27 expression. In vivo studies showed that 231-RelB-KO cells completely impaired tumor formation in xenograft models and lung metastasis model in nude mice.

**Conclusion:** RelB is aberrant constitutive expressed in clinical specimens and cell lines of breast cancer, especially in TNBC. RelB regulates malignant phenotypes of breast cancer, including cell survival, migration and invasion, as well as apoptosis and the cell cycle. Moreover, RelB can induce MMP1, which facilitates the invasion of tumor. Collectively, our findings highlight that RelB might serve as a therapeutic target for triple negative breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Possible role of p53/Mieap-regulated mitochondrial quality control as a tumor suppressor in human breast cancer

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**[Background]** In sporadic breast cancers, the most important gene is *p53*, given that it is a tumor suppressor gene and mutated in greater than 50% of human cancers. According to previous reports, *p53* is also mutated in approximately 20–40% of breast cancers. Recent data from the cancer genome atlas (TCGA) revealed that 37% of breast cancer specimens had alterations in *p53*—particularly, 72% in (human epidermal growth factor) HER2-rich and 80% of basal-like breast cancer cases—indicating that it is a critical driver of tumor development even in breast cancer. *p53* is clinically very important not only because of its high mutation rate but also because mutation is associated with more aggressive disease and worse overall survival.

Mitochondria-eating protein (Mieap) is a p53-target gene that plays an important role in mitochondrial quality control. Mieap has been reported to have a critical role in tumorsuppression of colorectal cancer. Here, we investigated the role of Mieap as a tumorsuppressor in breast cancer. **[Material and methods]** We overexpressed Mieap using the constructed adenovirus in breast cancer cell lines such as MCF-7, SK-BR-3, and MDA-MB-231 cells. The percentages of cells in different cell cycle phases (subG1, G1, S, and G2/M) were determined using FACS analysis and also caspase activities (Caspase, 3/7, 9) were measured. Cleaved PARP, which is a marker of cells undergoing apoptosis, was detected by western blot. For *in vivo* experiments, we examined the expression of Mieap using surgical specimens (invasive ductal carcinomas (IDCs): 75, ductal carcinoma in situ (DCIS): 27, fibroadenomas (FAs): 18) by immunohistochemistry. Next, we performed methylation-specific PCR (MSP) for *Mieap*, *NIX*, and *BNIP3* promoters and p53-mutation search using 46 samples that were cryopreserved, among 75 IDC cases used for immunohistochemistry. These studies were approved by the central ethics committee of Gifu University. **[Results]** The

enforced-expression of exogenous Mieap in breast cancer cells induced caspase-dependent apoptosis, with activation of both caspase-3/7 and caspase-9. Immunohistochemistry revealed endogenous Mieap in the cytoplasm in 24/75 (32%) invasive ductal carcinomas (IDCs), 15/27 (55.6%) cases of ductal carcinoma *in situ* (DCIS), and 16/18 (88.9%) fibroadenomas (FAs) (IDC vs DCIS;  $p = 0.0389$ , DCIS vs FA;  $p = 0.0234$ , IDC vs FA;  $p < 0.0001$ ). In IDCs, the *Mieap* promoter was methylated in 6/46 (13%) cases whereas p53 was mutated in 6/46 (13%) cases. Therefore, the p53/Mieap-regulated mitochondrial quality control pathway was inactivated in 12/46 IDCs (26.1%). Interestingly, all of the tumors derived from the 12 patients with the *Mieap*-promoter methylation or *p53* mutation pathologically exhibited more aggressive and malignant phenotype of breast cancers, resulting in significantly shorter disease-free survival (DFS) ( $p = 0.021$ ). **[Conclusion]** These results indicate that p53/Mieap-regulated mitochondrial quality control has a critical role in tumor suppression of breast cancer, possibly in part, through mitochondrial apoptotic pathway.

## 2018 San Antonio Breast Cancer Symposium ®

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CXCL1 chemokine stimulates migration and invasion in ER-negative breast cancer through activation of the ERK/MMP-2/9 signaling axis

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Chemokine (C-X-C motif) ligand 1 (CXCL1), a member of the CXC chemokine family, has been reported to be a critical factor in inflammatory diseases and tumor progression. However, its functions and molecular mechanism in estrogen receptor  $\alpha$  (ER) negative breast cancer (BC) has little been known. In this study, we discovered that CXCL1 is overexpression in ER-negative breast cancer tissues and cell lines compared with ER-positive patient tissues and cell lines. Treatment with recombinant human CXCL1 protein promotes ER-negative BC cell migration and invasion in dose-dependent manner and stimulates the activation of p-ERK1/2, but not p-STAT3 or p-AKT. Whereas, knockdown of CXCL1 in BC cells attenuates these effects. Moreover, CXCL1 induces the expression of MMP2/9 via ERK1/2 pathway. The blockage of ERK by its antagonists (U0126) can abolish the effect of CXCL1 on MMP2/9 expression. Furthermore, immunohistochemical (IHC) analysis reveals a strong positive correlation between CXCL1 and p-ERK1/2 expressions in BC tissues. In conclusion, our study illustrates that CXCL1 is highly expressed in ER-negative BC and stimulates cell migration and invasion through ERK/MMP2/9 pathway, which may serve as a potential therapeutic target in ER-negative breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-09-07

The role of MLK4 amplification in progression of triple negative breast cancer

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Mixed Lineage Kinase 4 (MLK4) is a serine/threonine kinase that plays a role in a variety of cellular processes, including migration, apoptosis and proliferation. It belongs to the family of MAP3 kinases, which regulate the activity of specific MAPKs (mitogen-activated protein kinases). MLK4 is the least described member of the family and it was proved to act as a functional MAP3 kinase that activates the JNK, ERK and NFκB pathways. The rationale of this project is based on the recent data showing gene amplification and mRNA upregulation of MLK4 in invasive breast carcinoma at the frequency of 23%. Here, we performed a series of experiments aiming to characterize the role of MLK4 in breast cancer development and progression. We started from identification of breast cancer cell lines that express high endogenous level of MLK4. Since clinical data indicated that MLK4 expression was higher in triple-negative breast cancer (TNBC), comparing to other breast cancer subtypes, we decided to further study the role of MLK4 in TNBC. We have generated cell lines with doxycycline-inducible knock-down of MLK4, HCC1806 and HCC1599, and we found that MLK4 depletion resulted in reduced cell proliferation. We also observed that the knock-down of MLK4 in these conditions caused the reduction in size of the spheroids. Moreover, we noticed that when MLK4 is depleted in HCC1806 cells, the migration and invasion was significantly impaired. Lastly, our results of immunohistochemistry in samples from breast cancer patients showed that higher levels of MLK4 in TNBC samples significantly correlated with the occurrence of lymph node metastasis. Currently, we are investigating molecular pathways such as JNK, MEK/ERK and NF-κB, that might be affected by knock-down of MLK4. Collectively, our findings indicate that high levels of MLK4 promote aggressive phenotype of breast cancer cells, by increasing their migratory and invasive potential.



# 2018 San Antonio Breast Cancer Symposium ®

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Single-cell profiling identifies hypoxic carcinoma cells as source of an immunosuppressive VEGFA metagene

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## **Background:**

We have previously shown that expression of the IL8/VEGFA-metagene eliminates the good prognostic effect of TILs in TNBC (PMID 21978456, 28750120). We also showed that the VEGFA metagene predicted response to neoadjuvant bevacizumab in the GeparQuinto trial (Karn 2017 SABCS #851166). The main cellular sources of the transcripts that comprise the VEGFA metagene are unknown since mRNA profiling of bulk biopsies contains signals from different cell types.

## **Methods:**

Individual genes that comprise the VEGFA metagene were measured in bulk tissue- and single cell-RNA-Seq from breast cancer subtypes and normal cells on different platforms (Affymetrix n=4915, Agilent n=597, Illumina n=2433, RNA-Seq n=1215, Exome Capture RNA-Seq n=226, HTG-Seq n=243, sc-RNA-Seq n=24710). For blinded, orthogonal validation we performed immunohistochemistry. Effect of neoadjuvant chemotherapy with or without bevacizumab was studied by RNA-Seq and IHC on samples from GeparQuinto trial. SWOG S0800 (GSE114403), PROMIX (GSE87455), and GeparSixto trials were used for validation. TCGA was mined for mutations and somatic CNA. RNA-Seq from GeparNuevo was used for correlation with checkpoint inhibitor treatment.

## **Results:**

We identified a stable core of six genes (VEGFA, ANGPTL4, ADM, NDRG1, DDIT4, CSTB) in different cohorts. Strong expression of this signature was mainly restricted to TNBC subtype and associated with poor prognosis within this subgroup. Single cell RNA-Seq of breast epithelial cells from 4 reduction mammoplasties and 4 TNBC revealed that these genes are coexpressed in individual epithelial cells and not associated with endothelial cells. In line with their presumed functions in cellular stress and hypoxia, immunohistochemistry revealed strong para-necrotic expression in TNBC. Moreover, high gene expression in TNBC was associated with mutations in DNA damage control pathways, somatic copy number alterations, and lower TILs. While chemotherapy led to downregulation, bevacizumab increased expression. In multivariate analysis, high pretreatment values predict pCR to both bevacizumab and chemotherapy (OR 2.40, P=0.006), which may be explained by sensitivity of tumors which are already under cellular stress. On the other hand, expression of the VEGFA metagene seems to create an immunosuppressive environment that counteracts the positive prognostic effect of TILs. In pre-treatment biopsies from the GeparNuevo checkpoint inhibitor trial we found a negative correlation of VEGFA metagene expression with the amount of the recently identified tissue-resident memory T cell subset (CD8<sub>TRM</sub>, PMID 29942092; P=0.002), while the subsequent increase of CD8<sub>TRM</sub> during treatment was larger in tumors with high VEGFA (P=0.019).

## **Conclusions:**

Perinecrotic carcinoma cells under stress from hypoxia and or chromosomal instability are the source of the VEGFA metagene signature. Its predictive value in TNBC suggests estimating and reporting the amount of necrosis in the pathology report may be helpful in predicting response to preoperative chemotherapy, and could be used as stratification factor in clinical trials. The signature indicates an immunosuppressive environment and should be further studied in the context of immune therapies in combinations with anti-angiogenic treatment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-02

Identifying breast cancer molecular phenotypes to predict response in a modern treatment landscape: Lessons from ~1000 patients across 10 arms of the I-SPY 2 TRIAL

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**Background:** The explosion in new treatment options targeting immune checkpoints, HER signaling, DNA repair deficiency, AKT, and other pathways calls for updated breast cancer subtypes beyond HR and HER2 status to predict which patients will respond to which treatments. Here we leverage the I-SPY 2 TRIAL biomarker program over the past 8 years across 10 treatment arms to elucidate a minimal set of biomarkers that may improve response prediction in a modern treatment context, and to investigate which new patient phenotypes are identified by these response-predictive biomarkers.

**Methods:** 986 patients were considered in this analysis. Treatments included paclitaxel alone (or with trastuzumab (H) in HER2+) or combined with investigational agents: veliparib/carboplatin (VC); neratinib; MK2206; ganitumab; ganetespib; AMG386; TDM1/pertuzumab (P); H/P; and pembrolizumab (Pembro). 24 prospectively defined, mechanism-of-action and pathway-based expression and phospho-protein signatures/biomarkers assayed from pre-treatment biopsies were previously found to be predictive in a particular agent/arm in pre-specified analysis. Here we evaluate these biomarkers in all patients. We assessed association between each biomarker and response in the population as a whole and within each arm and HR/HER2 subtype using a logistic model. To identify optimal dichotomizing thresholds for select biomarkers, 2-fold cross-validation was repeated 500 times. Our analysis is exploratory and does not adjust for multiplicities.

**Results:** Our initial set of 24 predictive biomarkers reflects DNA repair deficiency (n=2), immune activation (n=7), ER signaling (n=2), HER2 signaling (n=4), proliferation (n=2), phospho-activation of AKT/mTOR (n=2), and ANG/TIE2 (n=1) pathways, among others. Biomarkers reflecting similar biology are correlated and cluster together. We make use of this correlation structure to reduce the dimensionality of the biomarker set to five predictive signals: proliferation, DNA repair deficiency (DRD), immune-engaged (Immune+), luminal/ER (lum), and HER2-activated. These biomarkers, when dichotomized, identify patient groups with differential predicted sensitivities to I-SPY 2 agents and are present at different proportions within receptor subtypes. For instance, in the HER2- subset, Immune+/DRD+ patients are predicted sensitive to both VC and Pembro, and account for 39% of TN, but only 12% of HR+HER2-. On the other end of the spectrum, only 17% of TN are Immune-/DRD-, compared to the majority (56%) of HR+HER2-. There are also subsets of patients positive for only one marker. For the HER2+ subset, 67% are HER2-activated+, and 25% lum+; of these HER2-activated+ patients are more likely to be Immune+ (44%), vs 23% in lum+. HER2-activated+/Immune+ patients have higher predicted sensitivity to HER2-targeted agents than lum+ or Immune- patients. In all, these molecular phenotypes predict sensitivity to one or more I-SPY 2 investigational agents for 75% of the ~ 1000 patients.

**Conclusion:** Molecular phenotypes reflecting proliferation, immune engagement, HER2-activation, luminal/ER-signaling, and DNA repair deficiency may provide a roadmap to guide treatment prioritization for emerging therapeutics.

# 2018 San Antonio Breast Cancer Symposium ®

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Different pCR rates according PAM50 defined subtypes in HER2 positive early breast cancer treated with neoadjuvant pertuzumab and trastuzumab

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## BACKGROUND

We aim to compare the benefit of adding pertuzumab (P) to the standard neoadjuvant treatment with trastuzumab (T) in patients (pt) with early immunohistochemically (IHC) defined HER2+ breast cancer (BC) in the different intrinsic molecular subtypes defined by PAM50 gene expression analysis.

## METHODS

Two hundred forty-four pt. with IHC HER2 positive BC, stage I-IIIC, diagnosed in 8 Spanish hospitals were consecutively treated with neoadjuvant chemotherapy (NAC) plus antitargeted HER2 therapy. Cohort A (n=128) received NAC+T and Cohort B (n=116) received NAC+T+P. All the patients were classified into intrinsic molecular subtypes based on the PAM50® signature made in the diagnostic biopsies. Rate of pathologic complete response in breast and axilla (pCR) in the different PAM50 subtypes was compared by ChiSquared and Fisher test. A multivariate logistic regression model was used to analyze the potential effects of the covariates over the pCR rates.

## RESULTS

Characteristics of the patients and intrinsic molecular subtypes are shown in

Clinicopathologic and treatment characteristics of patients

	Trastuzumab+NCT ( groupA, n128)	Trastuzumab+pertuzumab+NCT ( groupB, n116)
Median age years (range)	52 (29-83)	50 (30-77)
Menopausal Status	n (%)	n (%)
Pre-menopausal	54 (42)	54 (47)
Post-menopausal	49 (38)	49 (42)
Missing	25 (20)	13 (11)
Histological grade	n (%)	n (%)
Grade 1	2 (2)	3 (2)
Grade 2	58 (45)	30 (26)
Grade 3	43 (34)	44 (38)
Grade unknown	25 (19)	39 (34)
Ki 67	n (%)	n (%)
<14%	11 (9)	10 (9)
15-50%	58 (45)	80 (69)
>50%	24 (19)	23 (20)
Unknown	35 (27)	3 (2)
Hormone receptor status	n (%)	n (%)

Positive	89 (70)	71 (61)
Negative	39 (30)	45 (39)
Tumor stage	n (%)	n (%)
T1	23 (18)	13 (11)
T2	75 (59)	64 (55)
T3	12 (9)	26 (22)
T4	10 (8)	12 (11)
Tx	8 (6)	1 (1)
Clinical node status	n (%)	n (%)
Negative	54 (42)	73 (63)
Positive	74 (58)	43 (37)
Chemotherapy	n (%)	n (%)
Taxanes	9 (7)	16 (14)
Taxanes and anthracyclines	119 (93)	100 (86)
PAM50 Subtypes	n (%)	n (%)
HER2E	88 (69)	64 (55)
Luminal A	16 (12)	17 (15)
Luminal B	19 (15)	21 (18)
Basal like	2 (2)	14 (12)
Normal like	3 (2)	0

NCT:neoadjuvant chemotherapy

. The overall pCR rate was significantly higher in cohort B vs A (61% vs 39%, $p=0.0009$ ). The pCR rate in the HER2E was 50% for T and 75% for T+P ( $p=0.003$ ) and 11% for T and 42% for T+P ( $p=0.004$ ) in Luminal subtype. In the multivariate analysis, the improvement pCR rates was highly associated with type of treatment (cohort) ( $p=0.0015$ ,OR:2.44) and were not related to clinicopathologic covariates (tumor stage, hystological grade, HR) ( $p>0.05$ ). These results were confirmed for the HER2enriched subtype ( $p=0.00398$ ,OR:2.94) and even more strongly for Luminals ( $p=0.0026$ ,OR:13.41).

#### CONCLUSIONS:

- 1.The highest pCR was reached by the PAM50HER2E patients treated with T+P.
- 2.In Luminal subtype the improvement of pCR is strongly associated with the used of P and this association is independent of clinical covariates.

# 2018 San Antonio Breast Cancer Symposium ®

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The fully validated NSABP/NRG 8-gene signature which predicted the degree of benefit in the adjuvant setting (B-31 and NCCTG N9831) associates with pCR in the neoadjuvant setting in NSABP clinical trial FB-7

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**Background:** We previously described a predictive signature for trastuzumab benefit which was validated in the adjuvant setting in an independent cohort within NSABP B-31 (the 8-gene signature) (Pogue-Geile et al JNCI, 2013) and in Alliance/NCCTG N9831 (SABCs 2017). The 8-gene signature subtyped B-31 patients into three trastuzumab benefit groups: high HR=0.27, intermediate HR=0.56 and no benefit HR=1.56 based on disease free survival. The 8 gene signature was also predictive of trastuzumab benefit in N9831. HRs were 0.47,  $P<0.001$ , 0.6,  $P=0.02$ , and 1.54,  $P=0.375$  in the predicted-high, -intermediate and -no benefit groups, respectively based on recurrence free survival (SABCS 2017). The interaction P-value was significant at 0.019 in adjusted Cox models. The RFS at 10 years for trastuzumab-treated pts was 83%, 83% and 72% in the high, intermediate and no benefit groups, respectively. Now we have tested the association of the 8-gene signature groups with pCR in FB-7 which was a 3 arm neoadjuvant study testing the pCR rate of HER2+ breast cancer patients treated with paclitaxel in combination with trastuzumab (T) or neratinib (N) or the combination (T + N).

**Methods:** RNA-Seq data from FB-7 pretreatment biopsies was used to predict the trastuzumab benefit groups (high, intermediate, and no) for each patient's tumor using the 8 gene signature using methods and cut-offs as previously described (Pogue-Geile et al 2013). The pCR rates (percentages) were tested for treatment interaction with a chi-square test.

**Results:** The pCR rates were 75%, 53%, and 22%, in the high (N=12), intermediate (N=32) and no benefit groups (N=9), respectively, when analyzed without regard to treatment arm. The pCR rates for the no benefit group and the high benefit groups were significantly different ( $p=0.030$ ) and there was a significant treatment interaction with the 8-gene benefit group ( $\text{intp}=0.0081$ ). The *predicted* low and intermediate groups were combined to test whether the 8 gene signature could identify a group of patients whose pCR rates might improve by adding N to T, and referred to it as the low benefit group. This was necessary due to the small numbers of patients in each group. The pCR rate in the low benefit group was higher in patients treated with T+N (9/15, 60%) than in the T arm (6/11, 45%) but these differences were not significant.

**Conclusions:** This is the first test of the 8-gene signature in the neoadjuvant setting and interpretations of these data should be interpreted cautiously due to the small numbers. However, if these results were validated in another neoadjuvant trial then the 8 gene signature could provide a rationale for selecting patients who would be appropriate for the addition of neratinib or other TKIs to trastuzumab and chemotherapy.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-05

*BRCA1/2* alterations are present at significant rates across breast cancer subtypes and are associated with a high genome-wide loss of heterozygosity signature

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## Background

The recent approval of PARP inhibitor, olaparib, in HER2-negative breast cancer expands the therapeutic options for patients with germline *BRCA1/2* alterations. The role of somatic *BRCA* alteration as a predictive biomarker in breast cancer is currently unclear. NCCN guidelines call for germline testing in all young patients (<46 yrs) and patients with triple negative breast cancer (TNBC) (< 61 yrs) with a personal history of breast cancer. Here we examined the landscape of *BRCA* mutations to assess whether additional populations may have potential benefit from PARP inhibitors.

## Methods

Hybrid-capture based comprehensive genomic profiling of 395 cancer-related genes using the FoundationOne assay (Foundation Medicine, MA) was performed on 12,508 breast carcinomas. Somatic/germline/zygosity status for *BRCA1/2* variants was analyzed as described in Sun, 2018 (PMID: 29415044). High genome-wide loss-of-heterozygosity (gLOH) was classified as  $\geq 16\%$  LOH. Subgroups were analyzed on histological {Invasive Ductal Carcinoma (IDC), Invasive Lobular Carcinoma (ILC)} and molecular subtypes [ER-positive (ER+), HER2-amplified (HER2+), TNBC], patient age [ $\leq 45$ , 46-60, 61+], and gender.

## Results

Consistent with previous reports, the frequency of *BRCA1/2* alterations was highest in young patients (<46; 15%), TNBC (10%), and male breast cancer (14%); *BRCA1/2* alterations were also identified in HER2+, ILC, and ER+ tumors (5.3%, 6.5%, 8.3%). Overall, *BRCA1* was more frequently mutated in TNBC and young patients (67%), whereas *BRCA2* was more frequently mutated in ER+, HER2+, ILC, and male breast cancer (63%, 57%, 79%, 100%).

The fraction of somatic *BRCA* mutations (*sBRCA*) was 38%, with the lowest fraction of *sBRCA* in TNBC and young patients (30%, 30% overall, 36%, 42% for *BRCA1*) and highest in HER2+, ILC, and older patients (46%, 52%, 51%); the absolute frequency of *sBRCA* is approximately 3.5%. In tumors with *ESR1* mutations, we detected concurrent *BRCA* mutation in 6.4% (85/1319), with 48% predicted somatic.

Almost all tumors with *BRCA* mutations had biallelic inactivation, with LOH of the second allele irrespective of predicted germline status (90% of *gBRCA* and 86% of *sBRCA* under LOH). gLOH score was used as a phenotypic measure of homologous recombination deficiency (HRD): patients harboring biallelic *BRCA* alterations had elevated rates of gLOH with 89% of *gBRCA* and 84% of *sBRCA* tumors harboring a high gLOH score, vs 47% with heterozygous *BRCA*, 34% with no *BRCA* alteration, and 80% for patients with *BRCA* deletion.

## Conclusions

Thirty-eight percent of deleterious *BRCA* alterations in breast are predicted somatic, including 36% of *BRCA1* alterations in TNBC. Germline testing would miss these alterations even though they are frequently under LOH and are associated with high gLOH, a biomarker with predictive value in ovarian cancer. While patients with ER+ and HER2+ tumors have low rates of *gBRCA* alterations, the overall *BRCA* mutation rate, including somatic alterations, is appreciable at 8.3% and 5.3%. Our findings demonstrate that *sBRCA* alterations are associated with a comparable HRD phenotype to *gBRCA* alterations and suggests that PARP inhibitors may have potential value for a wider range of breast cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-06

Expression-based immune signatures as predictors of neoadjuvant targeted-/chemo-therapy response: Experience from the I-SPY 2 TRIAL of ~1000 patients across 10 therapies

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**Background:** Expression-based signatures have been shown to predict neoadjuvant therapy response; but further studies are needed to deconvolve the contribution of different immune cell types. The I-SPY 2 TRIAL is a standing neoadjuvant platform trial which evaluates experimental agents/combinations when added to standard chemotherapy. In this study, we compared published T/B cell-related signatures at 3 different levels of resolution as predictors of response in the I-SPY 2 TRIAL: (1) a combined T/B-cell co-expression module, correlated with general lymphocytic infiltrate, (2) individual T-cell and a B-cell specific signatures derived from purified immune cells and refined using tumor expression, and (3) 9 T cell subpopulation-specific signatures, including a CD8+ T resident memory phenotype (TRM) and a CD8+ T effector memory subset (TEM), generated from microdroplet-based single cell (sc) RNA sequencing of over 6000 tumor associated CD3+ T cells.

**Methods:** Expression data from 989 I-SPY 2 patients randomized to one of 9 possible experimental arms or the standard chemotherapy control were available for analysis. Pre-treatment biopsies were assayed using Agilent gene expression arrays. All I-SPY 2 biomarker analyses follow a pre-specified analysis plan. We used logistic modeling to assess each signature as a predictor of pCR within each arm (likelihood ratio test  $p < 0.05$ ). This analysis is also performed adjusting for HR/HER2 status, and within receptor subsets. Our sample size for each arm is small; and our statistics are descriptive rather than inferential. Our analysis is exploratory and does not adjust for multiplicities of other biomarkers outside this study.

**Results:** In the population as a whole, immune signatures predict response across multiple classes of agents (8/10 arms), including the checkpoint inhibitor Pembrolizumab (Pembro). However, the cell-type and subpopulation-specific signatures most predictive of response vary by subtype and agent. For instance, the T/B-cell co-expression module associates with response to Pembro and the Angiopoetin-1/-2 inhibitor AMG-386 in both HR-HER2- and HR+ERHER2- subtypes. However, in the HR-HER2- subtype, the T-cell signature and the sc-derived CD8-TRM signature are most predictive; whereas in the HR+HER2- subtype, it is the B-cell, CD8-TRM and a novel CD4 signature that are most strongly associated with response. In the HER2+ subtype, the T/B-cell module and B-cell signature is associated with response to the AKT-inhibitor MK2206. Interestingly, among the sc-derived signatures, it is the CD8-TEM and multiple CD4 population-specific signatures, rather than CD8-TRM, that associate with response.

**Conclusion:** Our exploratory study suggests that immune signatures are associated with response to multiple I-SPY 2 experimental agents and implicates different immune cell types as response-predictive within breast cancer subtypes. Single cell sequencing derived population specific signatures may help further de-convolute how different immune cell types contribute to therapy responsiveness.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-07

A 3-gene DNA methylation signature fails to predict response to bevacizumab in metastatic breast cancer patients treated within the TANIA phase III trial

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**Background:** Biomarkers predicting response to bevacizumab containing therapy in metastatic breast cancer (MBC) are of urgent need. In a retrospective single-institution analysis we have previously shown that a 3-gene methylation signature (*MLH1*, *POLK* and *TMBIM6*) could discriminate between responders and non-responders to a bevacizumab-based therapy in two independent cohorts of patients with MBC with an AUC of 0.94 and 0.86, respectively (Gampenrieder SP et al. *Theranostics*. 2018. 8(8):2278-2288). Here, we present the validation of these findings within the prospective phase III trial TANIA (Vrdoljak E et al. *Ann Oncol*. 2016. 27(11):2046-52) randomizing 494 patients with HER2-negative MBC to chemotherapy plus bevacizumab or chemotherapy alone for two consecutive treatment lines (second- and third-line). All patients had already received bevacizumab-containing therapy in the first-line setting.

**Patients and methods:** DNA isolated from archival FFPE tumor samples was available from 200 patients consenting to optional translational research within the TANIA trial. Out of these, 176 samples were collected prior to first-line bevacizumab therapy and were analyzed retrospectively. Sufficient DNA for methylation analysis was available from 124 patients: 64 treated with chemotherapy plus bevacizumab and 60 treated with chemotherapy alone. All samples were isolated from the primary tumor. Quantitative methylation analysis was performed by pyrosequencing on the PyroMark Q24 Advanced System (Qiagen). PFS and OS analyses were performed in both study arms comparing “predicted responders” (PRED\_R) versus “predicted non-responders” (PRED\_NR) based either on median dichotomization or according to the cutoffs for individual CpG and the combined 3-CpG methylation logistic regression model.

**Results:** Out of the 124 evaluable patients, 32 (25.8%) were classified as PRED\_R and 92 as PRED\_NR by the 3-gene methylation signature. PRED\_R did not have a significantly different second-line PFS (HR 0.95, 95%CI 0.57-1.57; P = 0.84) or OS (HR 0.91, 95%CI 0.51-1.60; P = 0.73) when treated in the bevacizumab-containing study arm compared to PRED\_NR. In addition, PRED\_R did not show a longer PFS when treated with bevacizumab compared to PRED\_R treated with chemotherapy alone (HR 0.95, 95%CI 0.59-1.54; P = 0.83). Furthermore, there was no difference in third-line PFS and the combination of second- and third-line PFS between PRED\_R and PRED-NR in the bevacizumab arm. In the control arm, PRED\_NR showed a statistically significant shorter PFS compared to PRED\_R (HR 0.50, 95%CI 0.22-0.77; P = 0.006), but not OS (HR 0.95, 95%CI 0.51-1.77; P = 0.86).

**Conclusion:** Our 3-gene methylation signature was not confirmed as predictive biomarker for bevacizumab efficacy in metastatic breast cancer.

(This research project was partially supported by ROCHE Austria GmbH)



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-08

Markers of response to CDK4 & 6 inhibition from neoMONARCH: A phase II neoadjuvant study of abemaciclib in postmenopausal women with hormone receptor positive, HER2 negative breast cancer

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**Background:** Combination treatments of endocrine therapy (ET) with CDK4 & 6 inhibitors have improved outcomes in patients with HR+ advanced breast cancer, both as initial therapy and after progression on ET. Abemaciclib is a selective inhibitor of CDK4 & 6 approved on a continuous dosing schedule for the treatment of HR+, HER2- MBC patients (pts), alone or in combination with ET. However, biomarkers that predict benefit from this class of agents remain elusive. We previously reported in the phase II neoadjuvant neoMONARCH study (NCT02441946), after 2 weeks of treatment, abemaciclib, alone or in combination with anastrozole (ANZ), led to a significantly higher rate of complete cell cycle arrest (CCCA, defined as Ki67  $\leq 2.7\%$ ) compared to ANZ alone in early stage HR+, HER2- breast cancer (Martin et al. SABCS 2017). As an exploratory aim of this trial, we evaluated the gene expression analyses in order to determine markers of sensitivity and resistance to therapy.

**Methods:** Serial biopsies were collected at 3 time points: Baseline (BL) - prior to treatment, Early – after 2 weeks of therapy with abemaciclib, ANZ, or abemaciclib+ANZ, and Late – after 2 weeks of initial therapy followed by 14 weeks of abemaciclib+ANZ. RNA was extracted from FFPE tumor biopsies at each timepoint and subjected to a Cell Cycle Associated Gene (CCAG) expression panel using the Modaplex® platform and whole transcriptome RNA sequencing. Ki67 was measured at each time point by immunohistochemistry (IHC). Tumors were categorized by the post-treatment Ki67 expression as either sensitive (Ki67  $\leq 2.7$ ) or resistant (Ki67  $\geq 7.4$ ), based upon the IMPACT and POETIC studies. Additionally, tumors intrinsically resistant/sensitive to therapy were also identified.

**Results:** ANZ-treated tumors that did not achieve CCCA at 2 weeks (N= 8) displayed higher expression of the cell cycle associated genes *FOXM1*, *E2F1*, *TOPO2A*, and *RRM2*. The addition of abemaciclib to ANZ decreased gene expression in a majority of the tumors (N=5, 62.5%). Tumors intrinsically resistant to treatment with abemaciclib+ANZ displayed persistently elevated levels of cell cycle associated genes compared to sensitive tumors. Finally, gene expression signature of Rb loss-of-function (Rbsig) and RB1 gene expression levels were associated with sensitivity to abemaciclib.

**Conclusion:** On-treatment Ki67 indicated treatment sensitivity and correlated with cell cycle associated gene expression in sensitive and resistant tumors. These exploratory analyses suggest that gene expression analyses may identify genomic markers for abemaciclib and ET treatment sensitivity and may help inform in which tumors to use abemaciclib.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-09

Relationship between tumor infiltrating lymphocytes (TILs) and response to pembrolizumab (Pembro)+chemotherapy (Chemo) as neoadjuvant treatment (NAT) for triple-negative breast cancer (TNBC): phase Ib KEYNOTE-173 trial

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**Background:** Increasing quantities of stromal TILs (sTILs) are associated with higher pathologic complete response (pCR) rates with conventional chemo in early-stage TNBC. We evaluated the association between sTILs and PD-L1 expression with response to pembro+chemo as NAT for TNBC in the KEYNOTE-173 trial (NCT02622074).

**Methods:** sTILs were quantified using light microscopy of H&E-stained slides from pretreatment and on-treatment (during first 3 weeks of pembro monotherapy) tumor biopsies by a pathologist blind to response data. Pretreatment PD-L1 expression was assessed using the PD-L1 IHC 22C3 pharmDx assay and reported as combined positive score (CPS). Endpoints were pCR rate by ypT0 ypN0 and ypT0/Tis ypN0 and objective response rate (ORR; RECIST v1.1) after the first 4 cycles of NAT (taxane±carboplatin+pembro) by MRI. sTILs and PD-L1 CPS were evaluated as continuous variables. Association between sTILs and PD-L1 CPS with response was assessed using logistic regression and area under the receiver operating curve (AUROC) analyses, with a 1-sided alpha level of 0.10. Correlation between PD-L1 and sTILs was assessed by Spearman's rank correlation coefficient. Multivariate analysis included sTILs (pretreatment and on-treatment) and PD-L1 CPS. Likelihood ratio tests were used to evaluate the added value of factors in predicting pCR rate.

**Results:** Of 60 total pts, 34 had tumors evaluated for pretreatment sTILs, 52 for PD-L1 CPS, and 33 for both sTILs and CPS. On-treatment sTILs were evaluated in 31 pts. Overall pCR rates were 56.7% and 60% by ypT0 ypN0 and ypT0/Tis ypN0, respectively; ORR was 78.3%. In pts evaluated for sTILs and CPS (individually), pCR rates and ORR were comparable with overall pCR rates and ORR. There was a significant correlation between pretreatment sTILs and PD-L1 CPS ( $p=0.65$ ,  $P<0.001$ ). Higher pretreatment sTILs were significantly associated with response: ypT0 ypN0  $P=0.011$ ; ypT0/Tis ypN0  $P=0.006$ ; ORR  $P=0.061$ . On-treatment sTILs were also significantly associated with response: ypT0 ypN0  $P=0.061$ ; ypT0/Tis ypN0  $P=0.041$ ; ORR  $P=0.031$ . Pretreatment PD-L1 CPS was significantly associated with response: ypT0 ypN0  $P=0.073$ ; ypT0/Tis ypN0  $P=0.030$ ; and ORR  $P=0.021$ . AUROC of pretreatment sTIL association with pCR was numerically higher than with on-treatment sTILs and PD-L1 CPS (0.69 vs 0.61 vs 0.56 for ypT0ypN0 and 0.72 vs 0.67 vs 0.62 for ypT0/Tis ypN0). Responders had higher median pretreatment sTIL levels vs nonresponders: 45% [10, 75] vs 10% [5, 20] for pCR rate by ypT0 ypN0 and 52.5% [10, 73.8] vs 10% [5, 20] for pCR rate by ypT0/Tis ypN0; 25% [5, 70] vs 10% [6.3, 27.5] for ORR. In multivariate analysis, only pretreatment sTILs were significant for both pCR endpoints (ypT0 ypN0  $P=0.031$ ; ypT0/Tis ypN0  $P=0.034$ ). Likelihood ratio tests demonstrated that for both pCR endpoints, PD-L1 CPS ( $P=0.683/P=0.422$ ) and on-treatment sTILs ( $P=0.984/P=0.568$ ) did not add significantly more value to pretreatment sTILs when predicting pCR.

**Conclusions:** Higher quantities of pretreatment sTILs and PD-L1 CPS and on-treatment sTILs were significantly associated with higher pCR rates and ORR in primary TNBC treated with pembro and NAT.

# 2018 San Antonio Breast Cancer Symposium ®

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DYRK2 is a novel therapeutic target in ER negative breast cancer

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## Background

Dual specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2) belongs to a family of CMGC kinases that function as modulators of different downstream pathways that allow cells to cope with hypoxia, DNA damage and various stress signals. Additionally, DYRK2 has been implicated in various human cancers with both pro- and anti-tumour roles, which are probably cancer type- and cell type-dependent. Furthermore, studies show that DYRK2 is involved in epithelial-mesenchymal transition, hence suggesting a role in tumour metastasis. The current study investigates the prognostic role of DYRK2 in breast cancer and investigates its potential as a novel therapeutic target.

## Methods

Immunohistochemistry was employed to investigate if nuclear expression of DYRK2 was associated with clinical outcome measures in a cohort of 715 patients. Expression was determined using the weighted histoscore method. Antibody specificity was confirmed in paraffin embedded cell pellets +/- DYRK2 silencing. Cell counts in parental and CRISPR-mediated DYRK2 knocked-out MDA-MB-468 and MDA-MB-231 cells (ER, PR, HER2, AR negative) were measured using Alamar Blue; NSG<sup>TM</sup> mice (n=8) were injected subcutaneously with MDA-MDB-231 with or without DYRK2 depletion to assess tumour growth *in vivo*.

## Results

In a cohort of 715 patients, median follow-up was 160 months with 155 breast cancer deaths and 135 deaths due to other causes. The majority of patients were over 50 years of age (71%), had ductal carcinoma (88%), tumours <20mm in size (56%) and node negative disease (57%). 489 patients had ER positive disease, 226 had ER negative disease and of these 148 had TN (triple-negative) disease. DYRK2 expression was observed in the cell cytoplasm and nucleus and ranged from 3 to 200 weighted histoscore units (WHS) and ROC analysis was used to determine cut-offs, tumours with a cytoplasmic and nuclear WHS <145 were classified as low expression and tumours with a cytoplasmic and nuclear WHS >145 were classified as high expression. In the full cohort (p=0.087) and ER negative (p=0.066) cohort DYRK2 was not associated with cancer specific survival. However in TN disease high DYRK2 expression was associated with cancer specific survival (p=0.012, mean survival 145 months versus 107 months). This was potentiated in patients with ER, PR, HER2, AR negative disease (p=0.005, mean survival 166 months versus 100 months) and independent in multivariate analysis with age, histological tumour type, tumour size tumour grad, nodal status, ki67 index, chemotherapy, radiotherapy and recurrence (p=0.13, HR 3.920). Following this observation, patients with ER, AR negative disease were investigated and again high DYRK2 expression was associated with cancer specific survival (p=0.0003, mean survival 163 months versus 86 months) and was independent when combined in multivariate analysis (p=0.001, HR 4.154). To investigate if DYRK2 was a potential target in TN breast cancer, the effect of silencing DYRK2 was investigated. CRISPR-mediated DYRK2 depletion impeded cell proliferation in TN cell-lines and markedly reduced tumour burden in mouse MDA-MDB-231 xenografts (p<0.0001).

## Conclusions

Our studies indicate that DYRK2 is indeed a potential therapeutic target for patients with TN breast cancer or ER, AR negative breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-11

Clinical implications of 63-gene signature associated with response to PARP inhibition in triple-negative and luminal B breast cancer

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**Background:** Two recent randomized phase 3 trials have demonstrated that treatment with PARP inhibitors results in an improvement in progression-free survival (PFS) in metastatic, BRCA-mutant, HER2-negative breast cancer patients. We have previously identified a pathway-enriched 63-gene expression signature predictive of response to olaparib in seven patient-derived xenograft breast tumors, with a high overall accuracy of 86%. We found that the prevalence of our gene signature was 45% in a cohort of triple-negative breast cancer (TNBC) patients. We wanted to better understand if there were correlations between our 63-gene signature and other known prognostic markers and to determine the prognostic significance of our mutational gene signature in different PAM50 breast cancer subtypes.

**Methods:** We used a publicly available dataset from the NCI GDC Data Portal of TNBC patients (n = 82) to undertake clinico-pathological correlations with our 63-gene expression signature. We correlated the presence or absence of the signature with age, tumor size, lymph node status, and stage using chi<sup>2</sup> analysis, in addition to overall survival (OS) and PFS with STATA SE. We also correlated our gene signature with known TNBC subtypes from TNBCtype. Using the METABRIC cohort (n = 2509), we looked at the mutational frequency of our gene set in cBioPortal in different breast cancer subtypes and determined the prognostic value in each subtype.

**Results:** We did not find any statistically significant correlations between the 63-gene expression signature and age, tumor size, lymph node status, or stage amongst the 82 TNBC patients. All TNBC subtypes including 2 basal-like, immunomodulatory, low androgen receptor, 2 mesenchymal-based, and unspecified were identified in both gene-signature predicted sensitive and resistant groups, but there were no statistically significant differences between groups. The median follow-up of the TNBC cohort was 24 months, and no statistically significant associations were identified with OS or PFS. In the METABRIC cohort, the mutational frequency of any of the 63 genes for the following subgroups was identified: basal (n = 209), 85.2%; HER2+ (n = 224), 68.8%; claudin-low (n = 218), 48.2%; luminal B (n=475), 25.1%; and luminal A (n=700), 12.7%. The median follow-up of the METABRIC cohort was 127 months. We found that patients with a mutation in any of the 63 genes demonstrated a poorer overall survival, 122.8 months, in comparison to patients without any mutation, 164.6 months (P = 0.0002). In particular, luminal B patients with a mutation in any of these genes demonstrated a poorer overall survival, 90.0 months, in comparison to patients without a mutation, 132.1 months (P = 0.009). No statistically significant difference in overall survival was observed for patients with or without any mutation amongst the luminal A subtype (P = 0.26).

**Conclusion:** We found that patients with a mutation from our 63-gene set demonstrated a worse prognosis in comparison to patients without a mutation amongst the luminal B subtype. This is suggestive that there may be a role for our 63-gene signature to select patients amongst the luminal B subtype who may benefit from PARP inhibition.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-12

*ERBB2* copy number analysis of invasive breast carcinoma using digital droplet PCR and targeted next-generation sequencing: A focus on 'non-classical' HER2 FISH groups using the 2018 ASCO/CAP HER2 testing guideline

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**Background:** Non-classical HER2 FISH results were recently reclassified in the 2018 HER2 guidelines update, and concurrent IHC testing was recommended as part of additional workup to determine the final HER2 status in these groups. In this study, we explored the genomic landscape of HER2 FISH groups using digital droplet PCR (ddPCR) and targeted next-generation sequencing (NGS) on invasive breast carcinomas.

**Methods:** Fifty-one clinical samples with HER2 FISH and IHC results were included in our analysis and classified into FISH groups based on the updated 2018 ASCO/CAP HER2 testing guideline: (i) Group 1A with ratio  $\geq 2$  and signals/cell  $\geq 6$ , (ii) Group 1B with ratio  $\geq 2$  and signals/cell  $\geq 4$  and  $< 6$ , (iii) Group 2 with ratio  $\geq 2$  and signals/cell  $< 4$ , (iv) Group 3 with ratio  $< 2$  and signals/cell  $\geq 6$ , (v) Group 4 with ratio  $< 2$  and signals/cell  $\geq 4$  and  $< 6$ , and (vi) Group 5 with ratio  $< 2$  and signals/cell  $< 4$ . Formalin-fixed paraffin-embedded samples were analyzed using two ddPCR assays each targeting an exon in the *ERBB2* tyrosine kinase domain (exon 19 and 21, respectively) and a 130-gene NGS-based assay. For ddPCR, *ERBB2* amplification status was determined from ddPCR ratios by using a recently published algorithm (Otsuji et al. 2017). For targeted NGS, *ERBB2* amplification was called when copy number gains were detected in the majority of exons in *ERBB2* ( $> 50\%$  of exons).

**Results:** Mean ddPCR ratios varied amongst the different FISH groups ( $P < 0.0001$ ). As expected, patients with Group 1A had the highest mean ddPCR ratios compared to those with other FISH findings ( $P < 0.0001$ ). Furthermore, there was a correlation between *ERBB2* ddPCR ratios and HER2 FISH ratios ( $\rho_{e19} = 0.4435$ ,  $P = 0.001$  and  $\rho_{e21} = 0.4644$ ,  $P = 0.0006$ ). Using ddPCR, *ERBB2* amplifications were detected in all classically amplified Group 1A cases (5/5) and in none of the classically non-amplified Group 5 cases (0/12). Interestingly, ddPCR assays called *ERBB2* amplification in four cases with non-classical results: one in Group 2 (1/6), two in Group 3 (2/6), and one in Group 4 (1/17), including two cases in Groups 3 and 4 which also showed concomitant HER2 overexpression by IHC (3+). Similarly, targeted NGS revealed *ERBB2* amplification in all Group 1A cases (5/5) and in none of the Group 5 cases (0/12). Furthermore, NGS detected amplification in three non-classical cases: one in Group 1B (1/5), one in Group 3 (1/6), and one in Group 4 (1/17), including one case in Group 1B which was not called amplified by ddPCR. Notably, the three cases with amplification by NGS were the only three cases in the non-classical groups with HER2 overexpression by IHC. Overall, there was a strong concordance between *ERBB2* amplification status by ddPCR/NGS and HER2 overexpression by IHC ( $\kappa_{e19} = 0.79$ ,  $\kappa_{e21} = 0.92$ ,  $\kappa_{\text{NGS}} = 1.0$ ).

**Conclusion:** *ERBB2* amplification using ddPCR and NGS is correlated with HER2 overexpression in both classical and non-classical FISH groups, thus providing genomic evidence to support the recent recommendation for concurrent IHC testing in cases with unusual FISH results. Our findings also highlight a potential role of ddPCR and targeted NGS in the workup of challenging HER2 cases.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-13

Dual targeting of androgen receptor and IKK alpha is a potential therapeutic strategy for triple negative breast cancer

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## Background

There are limited treatments for triple negative breast cancer (TNBC) patients and an unmet need for targeted approaches in these patients. In the last 4-5 years, the prevalence of high androgen receptor (AR) expression in TNBCs has been noted in up to 50% of tumors suggesting it has clinical relevance. The non-canonical NF-kB pathway is also upregulated in this patient group and it has been reported that there is crosstalk between these pathways. Therefore the aim of this study was to examine the expression of IKK $\alpha$  and AR in breast cancer tissue samples, to assess if combining these markers increased prognostic power.

## Methods

Immunohistochemistry was performed on tissue microarray of 410 patients to assess proteins level of IKK alpha and AR. Protein expression levels were assessed using the weighted histoscore (WHS) method. The median was employed as the cut off for IKK alpha and 1% as cut off for AR. Expression was analyzed for associations with cancer-specific survival (CSS) and recurrence-free survival (RFS).

## Results

In a cohort of 370 breast cancers neither AR nor IKK alpha alone or combined were associated with CSS or RFS. Stratifying patients by ER status did not impact CSS or RFS. However, in TNBC patients (n=82) high expression of AR was associated with shorter CSS (HR 2.55 95 CI 1.61-5.59, p=0.013). To assess if combining AR and IKK alpha increased prognostic power, AR and IKK alpha were combined into a single score: 0= low expression of both or high expression of one and 1= high expression of both. In the full cohort or when stratified by ER status the score was not associated with CSS or RFS, however in TNBC the combined score potentiated the effect observed with AR alone, (HR 1.68 95 CI 1.20-2.33, p=0.001). Patient CSS was stratified from 11.5 years to 4.6 years and was independently associated with CSS when compared with common clinicopathological factors (HR 1.56 95CI 1.11-2.21, p=0.011). In addition, the combined score was associated with decrease radiotherapy use (p=0.032), increased recurrence rate (p=0.014), decreased cytotoxic T cells (p=0.007), B cells (p=0.043) and macrophages (p=0.037).

## Conclusions

A combined AR and IKK alpha score is an independent prognostic classification for patients with TNBC. Patients with high expression of both AR and IKK alpha a significantly reduced survival and were immune cell cold. This study suggests that this patient group will not benefit from immunotherapy but dual targeting with anti-androgens and IKK alpha selective inhibitors could offer a novel therapeutic strategy for this patient group.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-14

LIV-1 expression in primary breast cancers in the I-SPY 2 TRIAL

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**Background:** LIV-1 is an estrogen-inducible gene that has been implicated in epidermal-to-mesenchymal transition (EMT) in preclinical models of progression and metastasis. Its expression is associated with node-positivity in breast cancer; and has been detected in a variety of cancer types, including estrogen receptor positive breast cancers. SGN-LIV1A is a novel antibody drug conjugate targeting LIV-1 that is currently being evaluated in the I-SPY 2 TRIAL. In this pilot study, we evaluated LIV-1 levels by IHC within HR/HER2/MammaPrint (MP) defined subtypes among patients screening for the I-SPY 2 TRIAL and its correlation to microarray assessed LIV-1 expression levels.

**Method:** In a pilot study, LIV-1 IHC staining was performed by Quest Diagnostics on the pre-treatment samples of 38 patients screening for the I-SPY 2 TRIAL. Pre-treatment expression data generated on a custom Agilent 44K platform was also available. We summarized the LIV-1 H-Scores and percent (%) positivity across the population and within HR/HER2/MP subtypes; and we assessed the Pearson correlation between LIV-1 H-Score and LIV-1 gene expression levels. In addition, we compared the pre-treatment LIV-1 expression levels within HR/HER2/MP subtypes across I-SPY 2 TRIAL patients from completed arms and their relevant controls (n=989) using ANOVA and post-hoc Tukey tests. Our statistics are descriptive rather than inferential; and does not take into account multiplicities of other biomarkers outside of this study.

**Results:** Of the 38 patients evaluated, 37 have LIV-1 %-positivity > 0; and 18 (47%) have 100% LIV1 positivity. The median LIV-1 H-Score is 200; and 89% of patients (34/38) have moderate/high LIV-1 staining (with H-Score ≥ 100). Of the 34 patients who proceeded onto the trial (and have known HR/HER2/MP status), 9 are triple negative, 19 are HR+HER2-, and 6 are HER2+. Due to our small sample size, we did not further subset the triple negative and HER2+ cases; but within the HR+HER2- patients, 10 are MP1 compared to 9 who are MP2 class. LIV1 H-Score appears highest within the HR+HER2-MP1 cases (median: 290), followed by the HER2+ (median: 216), then the HR+HER2-/MP2 (median: 155), and the TN (median: 120) subtype. LIV1 H-score is significantly correlated with LIV-1 mRNA expression levels (Rp=0.79, p<0.0001). Consistent with these observations, LIV-1 pre-treatment expression levels are significantly higher in the HR+HER2-MP1 group relative to all other HR/HER2/MP defined subtypes (Tukey HSD p < 0.0001) across the I-SPY 2 TRIAL population. The HR+HER2+MP1 group also have high LIV-1 expression levels.

**Conclusion:** Our result suggest that although LIV-1 expression differs by subtype, it is expressed at a moderate/high level in the majority of patients. The good correlation between IHC and array-based LIV-1 expression levels enables us to leverage the entire existing I-SPY 2 dataset and confirm the high rates of LIV-1 expression across the I-SPY 2 population. Further studies to evaluate LIV-1 expression as a biomarker of response to LIV-1 targeting therapies for the neoadjuvant treatment of breast cancer are warranted and ongoing in I-SPY 2.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-15

Evaluating contribution of hyperactive c-Met and ErbB signaling to tumor progression in mouse breast tumor xenografts: An *in vivo* study of c-Met and ErbB targeted therapies

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**Background:** Hyperactive c-Met signaling, including cross-talk between c-Met and ErbB family receptors, is suspected of contributing to tumor progression in a variety of cancer types. Clinical trials evaluating c-Met inhibitors alone and in combination with other targeted therapies, have produced mostly negative results in patients with amplified c-Met. This suggests other biological factors, such as c-Met and ErbB signaling activity, may be important to measure when identifying patients eligible for c-Met therapies. To measure the c-Met and ErbB signaling activity of a patient's live tumor cells, a new assay using an impedance biosensor, the CELx multi-pathway signaling function (CELx MP) test, was developed. The CELx MP test measures a patient's *ex vivo* live tumor cell response in real-time to specific ErbB and c-Met agonists to diagnose breast cancer tumors with hyperactive HER1, HER2, HER3, HER4, and c-MET signaling activity. In this study, to further elucidate the role of c-Met signaling and its potential involvement with ErbB signaling as a cancer driver, we studied *in vivo* response to a c-Met inhibitor (tepotinib), an EGFR inhibitor (erlotinib), a pan-HER inhibitor (neratinib), and a combination of these therapies using breast tumor xenograft models.

**Methods:** HCC1954, a HER2+ cell line with hyperactive c-Met and EGFR signaling and normal HER3 and HER2-driven signaling, according to the CELx MP test, was studied. Sixty 4-5-week-old female NSG mice were injected with two million cells. Mice were randomly assigned to either a control group that received Captisol or one of five treatment groups that received either neratinib, tepotinib, erlotinib, erlotinib and tepotinib, or neratinib and tepotinib for 17 days.

**Results:** The most effective treatment was the combination of neratinib plus tepotinib, where the average tumor size reduction relative to the control group was 71% ( $p=0.0003$ ). The average tumor size in the neratinib plus tepotinib treated group was 37% smaller ( $p=0.049$ ) than the neratinib treated group, and 67% smaller ( $p=0.0026$ ) than the tepotinib treated group. In the erlotinib plus tepotinib treatment group, the average tumor size was 51% smaller than the control group tumors, but the difference was not statistically significant ( $p=0.11$ ). No significant difference in tumor size was found between the control group and the erlotinib or tepotinib treated groups.

**Conclusions:** The results demonstrate that hyperactive and coincident c-Met and EGFR signaling contributes to the progression of certain breast cancers. This breast cancer sub-type is more responsive to treatment with a c-Met inhibitor plus a pan-HER inhibitor than a c-Met inhibitor plus an EGFR inhibitor or any of the single agents studied. This suggests that when hyperactive c-Met signaling and a hyperactive ErbB family member is present in a patient's tumor, each of the ErbB pathways as well as the c-Met pathway must be inhibited to treat the tumor most effectively. These findings provide strong evidence that HER2-negative breast cancer patients with coincident hyperactive c-MET and ErbB signaling may respond to treatment with a combination of pan-HER and c-Met inhibitors.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-16

Predicting pathological complete response by the combination of microRNAs in patients with HER2-positive primary breast cancer who received neoadjuvant combination therapy of trastuzumab, lapatinib and paclitaxel: Results from JBCRG-16 (NeoLath) study

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[Background] JBCRG-16 (NeoLath) study is a five-arm study to evaluate the efficacy and safety of lapatinib and trastuzumab (6 weeks) followed by lapatinib and trastuzumab plus weekly paclitaxel (12 weeks) with/without prolongation of anti-HER2 therapy prior to chemotherapy (18 vs. 6 weeks), and with/without endocrine therapy in patients with HER2+ and/or estrogen receptor (ER)+ disease. The primary endpoint was pathological complete response (pCR) rate and pCR rate was 47.9% (Masuda N, et al. Breast Cancer, 2018). It is recently reported that microRNAs (miRNAs) are stably present in serum and potentially useful in the diagnosis and evaluation of treatment of cancer. We performed exploratory analysis of detecting pCR by comprehensive analysis of serum miRNAs.

[Materials and Methods] Serum samples were obtained from study participants who received neoadjuvant systemic therapy with trastuzumab, lapatinib and paclitaxel. Before profiling of miRNAs, the overall serum samples were randomly divided in two sets, namely the training set and the testing set with pCR or non-pCR. Pathological complete response (pCR) was defined as the absence of residual invasive cancer of the resected breast specimen and all sampled regional lymph nodes. Total RNA was extracted from a 300 µl serum sample using 3D-Gene® RNA extraction reagent from a liquid sample kit. A comprehensive quantitative expression analysis of miRNA was performed using the by DNA chip 3D-Gene®, which was designed to detect 2565 miRNA sequences registered in miRBase release 21 (<http://www.mirbase.org/>). The expression level of miRNAs were normalized by internal control (miR-2861, miR-149-3p and miR-4463). Clinicopathological data was retrieved from trial data.

[Results] A total of 112 samples were obtained. Seventy were used in the training set and others were used in the testing set. Median age was 54 years (range 26-70). Sixty-five (58%) patients were pre-menopausal. ER was positive in 59 patients (52.7%). Fourteen (12.5%) were T1c, 78 (69.6%) were T2 and 20 (17.9%) were T3. Fifty-seven (50.9%) patients were node-positive. Fifty-nine (52.7%) patients achieved pCR. The formula with the combination of three miRNAs (miR-A, miR-B, miR-C) was found to be able to predict pCR. This set had a sensitivity of 62.5%, specificity of 86.7% and accuracy of 71.8% in the testing cohort. Area under curve of receiver operating characteristic curve was 0.753.

[Conclusion] The combination of three miRNAs has potential to predict pCR in patients who received neoadjuvant combination therapy of trastuzumab, lapatinib and paclitaxel in HER2-positive primary breast cancer. The further analysis of changing expression of miRNAs during neoadjuvant therapy is underway and further results will be presented in the symposium.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-17

mRNA expression of HER2 by PCR as a strong predictor of pathologic response to neoadjuvant antiHER2 therapy

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**Background:** Pathologic response to neoadjuvant therapy is the most important prognostic factor so new agents should aim to improve it. HER2 expression in breast cancer provides prognostic and predictive information of response to antiHER2 therapy. The quantification of HER2 expression by mRNA is one of the most potent markers of cellular dependence to HER2 pathway.

**Methods:** From a series of 160 patients with early or locally advanced HER2 positive breast cancer who received neoadjuvant treatment with chemotherapy and antiHER2 drugs, mRNA expression of HER2 was analyzed in 69 specimens and it was correlated to pathologic complete response (pCR).

**Results:** Median age was of 52.5 years (29-86), tumor size of 33mm (10-111), 39 (53%) patients had nodal involvement. Median ki67 expression was of 45% (17-80), 28 (38%) tumors were estrogen receptor (ER) positive and 46 (62%) negative. We also analyze the expression of tumor-infiltrating-lymphocytes (TILs) by immunohistochemistry in 43 samples because their expression is also a strong predictive of pathologic response. A total of 31/69 patients (45%) achieved a pCR with a median of mRNA expression of 7,55 (3,8 – 11) in comparison with 2.03 (1,2 -2,8) in patients without pCR (p: 0,002). In the univariable analysis, variables correlated to pCR were estrogen receptor=0 (OR 0,186 IC 95% 0,063-0,549, p: 0,002), mRNA (OR 5,689 IC 95% 1,854-17,452, p: 0,002) and TILs (OR 15,167 IC 95% 3,223-71,38, p: 0,001) and in the multivariable analysis, TILs and mRNA expression were found to be independent predictive factors for pCR. The quantification of HER2 by mRNA obtained an impressive response specially in ER negative patients with a rate of pCR of 100% in samples of mRNA expression upper of 3,6 and ER negative. However, patients with mRNA expression lower than 3,6 with a pCR rate of 32%, could reach to an 80% rate if they had high TILs expression.

**Conclusions:** Quantification of HER2 expression by mRNA is a strong predictive factor of pCR (OR 5,689); achieving a 100% pCR in ER negative tumors. TILs are also a predictive factor for pCR (OR 15,167) and its combination with mRNA expression could improve pCR rates; thus, patients with mRNA expression lower than 3,6, if they had a higher TILs expression could reach to an 80% pCR.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-18

Elucidating the role of functional signal transduction pathway activity in sensitivity and response of triple negative breast cancer to PI3K inhibition

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## Introduction

The PI3K signaling pathway is frequently active in triple negative breast cancer (TNBC), but response to PI3K inhibition is still poorly understood. To gain insights in therapy response and resistance, it is important not only to assess genetic alterations of these tumors, but also their phenotypic characteristics. Furthermore, not only is the functional status of the PI3K pathway important to know, but also other signal transduction pathways that may drive tumor growth, as they may explain primary or acquired therapy resistance.

## Material & Method

We analyzed 17 patient derived xenograft (PDX) models of TNBC with varying response to buparlisib treatment. Long-term response (~30 days) was assessed by comparing growth rates between buparlisib and vehicle treated PDXs. For characterization, mRNA expression analysis was performed on tumor material post 3 days of vehicle or buparlisib treatment of each PDX model. Functional pathway activity was determined using computational Bayesian networks that look at mRNA levels of pathway target genes resulting from activation (Verhaegh et al, Cancer Res 2014), amongst others for the FOXO-PI3K (van Ooijen et al, Am J Pathol, in press), ER, AR, Hedgehog, TGFbeta and Wnt pathways. These computational networks were calibrated on samples with known pathway activity, and biologically validated on various healthy and diseased cell and tissue types.

## Results

On an initial set of 6 PDX models, pathway activity clearly varied between different TNBC PDXs, and between vehicle and buparlisib treatment. ER pathway activity was low in all samples, as expected in TNBC. Two PDX models with the most growth reduction by buparlisib showed high PI3K activity, of which one based on low FOXO activity and the other on oxidative stress. The former, best responding PDX showed a clear reduction in PI3K activity (restoring FOXO activity), when comparing 3 days buparlisib to vehicle treatment. Of the two PDX models with least growth reduction, one had low PI3K activity, while the other one, carrying a PIK3CA mutation, did show high PI3K activity (low FOXO), but this remained after treatment. Other differences in pathway activity that were found, included slightly elevated AR and Wnt activity in one PDX with good response, and somewhat higher TGFbeta activity in four PDXs (good, medium and poor response). Analysis of the remaining 11 PDXs is ongoing, including other signal transduction pathways, to investigate the variation in pathway activity across the entire panel and to shed more light on the differences in tumor biology between the PDX models.

## Conclusion

Our computational Bayesian networks measured differences in functional activity of signal transduction pathways across a collection of TNBC PDX models, which may be expected due to the large variation between TNBC tumors. Functional PI3K activity was related to growth inhibition by buparlisib treatment, and reduction of PI3K pathway activity was observed upon treatment in responding PDX models. Other pathways also showed variation across PDXs. Further clinical evaluation of our signal transduction pathway activity measurement is ongoing. RT-qPCR based analysis is available, optimized for FFPE tissue and small samples.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-19

Significance of baseline neutrophil-to-lymphocyte ratio for progression-free survival of patients with HER2-positive locally advanced and metastatic breast cancer treated with trastuzumab emtansine

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**Purpose** The prognosis of human epidermal growth factor receptor 2 (HER2)-positive locally advanced or metastatic breast cancers (MBCs) has dramatically improved due to the introduction of trastuzumab, pertuzumab, and trastuzumab emtansine (T-DM1). The efficacy of T-DM1 is prolonged for some patients; however, the predictive factors remain unknown. There is a report that T-DM1 induced antitumor immunity in patients treated with neoadjuvant therapy, with tumor infiltrating lymphocytes (TILs) increasing after the administration of T-DM1. Based on these observations, the benefits of T-DM1 for prognosis may be mediated by an immune reaction against breast cancers, at least in part. As an indicator of cancer immunity, in addition to TILs, the neutrophil-to-lymphocyte ratio (NLR) has been established in early breast cancers. In the present study, we investigated the usefulness of the NLR for treatment efficacy of T-DM1 in HER2-positive MBCs. **Methods** Fifty-three advanced or metastatic breast cancers treated with T-DM1 were retrospectively recruited from three institutes. The NLR in the peripheral blood was measured at baseline (just before the start of T-DM1) and after one cycle (just before the start of cycle 2). The cutoff value of the NLR was set at 2.56 (median value) and progression-free survival (PFS) and overall survival (OS) according to NLR levels were evaluated. **Results** The PFS of patients with NLR-low at baseline ( $NLR < 2.56$ ;  $n=26$ ; median, not reached) was significantly better than that of patients with NLR-high ( $NLR \geq 2.56$ ;  $n=27$ ; median, 4.13 months; hazard ratio [HR], 0.226; 95% confidence interval [CI], 0.112-0.493;  $p=0.0001$ ). There was a significant association between improved OS and a low NLR (HR, 0.384; 95% CI, 0.170-0.910;  $p=0.0296$ ). In the subgroup analysis, patients with NLR-low consistently had improved PFS compared to those with NLR-high irrespective of the number of prior chemotherapy regimens, prior trastuzumab use, visceral metastasis, estrogen receptor status, and HER2 immunohistochemical staining score. According to univariable analysis of each clinical and biological factor for PFS, the NLR-low group was solely and significantly associated with favorable PFS compared with the NLR-high group (HR, 0.226; 95% CI, 0.112-0.493;  $p=0.0001$ ). The NLR at baseline was significantly decreased ( $p=0.0010$ ) and lymphocyte count was significantly increased after one cycle treatment ( $p=0.0005$ ). Interestingly, the PFS of patients whose NLR was high at baseline but changed to low after one cycle ( $n=12$ ; median PFS, 6.47 months) was better than that of patients with a consistently high NLR ( $n=14$ ; median PFS, 3.27 months). **Conclusion and Discussion** A low baseline NLR was found to be significantly associated with improved PFS for patients treated with T-DM1. Interestingly, lymphocyte count was significantly increased in patients in the NLR-low group but not in the NLR-high group after one cycle treatment. Although detailed mechanisms remain unknown, the treatment efficacy of T-DM1 may be partly mediated by immunoreaction on the basis of present data. A low baseline NLR appears to be beneficial for treatment with T-DM1 in HER2-positive breast cancers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-20

Sub-group of HER2- breast cancer patients with hyperactive and co-involved c-Met and ErbB pathways identified: Functional signal profiling test identifies patient group that may benefit from c-Met and pan-HER combination therapy

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**Background:** Biological factors other than c-Met status, such as c-Met and ErbB signaling activity, may be important to measure when identifying patients eligible for c-Met therapies. A new assay using an impedance biosensor was developed to measure c-Met and ErbB signaling activity of live tumor cells. The CELx Multi-Pathway Signaling Function (CELx MP) Test measures an individual patient's *ex vivo* live tumor cell response in real-time to specific ErbB and c-Met agonists and antagonists to diagnose breast tumors with hyperactive HER1, HER2, HER3, and c-MET signaling. This study set out to: 1) determine the prevalence of hyperactive c-Met and ErbB family signaling amongst HER2- breast cancer patients; and 2) characterize potential cross-talk between c-Met and ErbB pathways.

**Methods:** For the prevalence study, fresh breast tumor specimens were obtained from 74 HER2- breast cancer patients. The amount of HER1, HER2, HER3, and c-Met for each specimen was determined using FACS. Real-time live cell response to specific ErbB and c-Met agonists (NRG1b, EGF, or HGF) alone and in combination, with or without ErbB and c-Met antagonists (2C4, a HER2 mAb dimerization inhibitor, tepotinib, a c-Met TKI, or neratinib, a pan-HER TKI) was measured using an xCELLigence RTCA impedance biosensor. From these responses, HER1, HER3, and c-Met signaling initiated by their respective agonists was quantified. The net amount of HER2 participation in EGF and NRG signaling was also quantified. Signaling activity above a previously determined cutpoint was used to identify abnormal levels of HER1, HER2, HER3 and c-Met signaling activity. For the cross-talk study, three primary HER2- breast cancer specimens with hyperactive c-Met and ErbB signaling were obtained. Response to HGF, EGF, and NRG1 alone, with or without tepotinib, was measured for these specimens using an impedance biosensor.

**Results:** The FACS analysis found all 74 tumor samples had normally expressed amounts of HER1, HER2, HER3, and c-Met. Of these samples, 20 of 74 (27.0%; 95% CI=18%-38%) had hyperactive c-Met signaling coincident with hyperactive signaling from at least one ErbB pathway. In each patient sample, neratinib combined with tepotinib inhibited virtually all signaling activity initiated with a combination EGF, NRG1 and HGF. In the cross-talk analysis of the three tumor samples, signaling response to EGF or NRG1 when combined with a c-Met antagonist was 9%-98% higher than signaling response measured with EGF or NRG1 alone.

**Conclusions:** This test found a significant sub-set of HER2- breast cancer patients with coincidental hyperactive c-Met and ErbB signaling tumors that respond *ex vivo* to a combination of pan-HER and c-Met TKI's. The unexpected increase in EGF and NRG1 signaling in the presence of a c-Met antagonist provides strong evidence that c-Met and ErbB signaling is co-involved and may explain why a c-Met TKI is not an effective antagonistic when c-Met is hyperactive for this patient sub-set. A clinical trial to evaluate treatment response of this patient sub-set to combined c-Met and pan-HER inhibitors is warranted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-21

MARCKS phosphorylation contributes to the aggressive behavior in inflammatory breast cancer

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**Background:** Inflammatory breast cancer (IBC) is the most aggressive form of locally-advanced breast cancer. Despite multimodality treatment, the 5-year survival remains around 50%. Identification of new therapeutic targets is crucial. We previously reported MARCKS mRNA and protein overexpression in IBC in the largest transcriptomics and immunohistochemistry (IHC) study reported to date (1,2). Here, we evaluated phospho-MARCKS (p-MARCKS) protein expression in IBC and non-IBC clinical samples. **Patients and methods:** Using IHC analysis, we retrospectively evaluated the p-MARCKS protein expression in a series of 502 tumors (133 IBC and 369 non-IBC), from Tunisian and French patients. All samples were pre-therapeutic tumor samples. We searched for correlations between p-MARCKS expression and clinopathological features. **Results:** Using 1% of stained tumor cells as positivity cut-off, we demonstrated that p-MARCKS expression was more frequently positive in IBC than in non-IBC ( $p=2.8E-11$ ). The percentage of p-MARCKS-positive cases was 47% in IBC versus 14% in non-IBC, and was not different between the Tunisian and French IBC samples (48 versus 46%,  $p=1$ ). Correlations were found between p-MARCKS expression and young age of patients in IBC vs nIBC ( $1.73E-06$ ) and high grade ( $p=4.62E-02$ ). *In multivariate analysis* integrating all significant variables, phospho-MARCKS protein expression remained associated with the IBC phenotype ( $p=4.7E-05$ ). **Conclusion:** Phospho-MARCKS overexpression might in part explain the poor prognosis of IBC. As an oncogene associated with unfavorable prognostic factors, phospho-MARCKS might represent a new potential therapeutic target in IBC. **Key word:** Phospho-MARCKS, expression, immunohistochemistry, inflammatory breast cancer, unfavorable prognosis features.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-22

BluePrint molecular subtyping versus HER2 assessment by immunohistochemistry and FISH in the real-world diagnostic setting

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**Background:** Both the Aphinity and ExteNET trials for anti-HER2 targeted agents were challenged in showing significant benefit of added HER2-targeted treatment. The findings suggested biological heterogeneity in HER2+ cancers, not entirely identified by IHC/FISH, requiring more nuanced biomarkers to clearly identify patient subsets who may derive benefit from new HER2-targeted agents. We have previously shown that BluePrint (80-gene) molecular subtyping reclassifies nearly half of HER2+/ER+ patients to Luminal-type with differential neoadjuvant treatment response (Whitworth, Ann Surg Oncol 2014; Whitworth, ASCO 2018). Here, we evaluated the reclassification rate in the real-world diagnostic setting. **Methods:** Physicians regularly provide pathology reports to Agendia, Inc for samples which are processed for MammaPrint (70-gene signature) and BluePrint molecular assays as part of routine diagnostic care. For this analysis, 4986 sequentially available pathology reports (submitted between October 2016 to October 2017) were reviewed; HER2 and ER IHC results were captured. The molecular subtype was compared to IHC/FISH status. **Results:** HER2 IHC/FISH results were available for 1568 samples. Of those, 85% (1330/1568) were HER2-nonamplified, 10% (153/1568) were HER2-equivocal, and 5% (85/1568) were HER2-amplified by IHC/FISH.

HER2 IHC/FISH vs BluePrint Subtype

HER2 IHC/FISH Status	HER2-type		Luminal-type		Basal-type		Total
	IHC ER-	IHC ER+	IHC ER-	IHC ER+	IHC ER-	IHC ER+	
Nonamplified (ER-Unknown, n=28)	1	1	8	1203	53	36	1330
Equivocal (ER-Unknown, n=6)			2	131	10	4	153
Amplified (ER-Unknown, n=2)	1	12		66	4		85
Total	2	13	10	1400	67	40	1568

Of the HER2-nonamplified tumors, BluePrint reclassified 0.1% (2/1330) as HER2-type. Of the HER2-equivocal tumors, none were HER2-type by BluePrint; 91% (139/153) were Luminal-type and 9% (14/153) were Basal-type. Of the HER2-amplified tumors, 15% (13/85) were dominant HER2-type, 79% (67/85) were dominant Luminal-type, and 6% (5/85) were Basal-type.

**Conclusions:** In this set of tumors identified as HER2-amplified by IHC/FISH, BluePrint reclassified 85% of tumors to non-HER2 molecular subtypes, mostly Luminal-type for ER-positive tumors and Basal-type for ER-negative tumors. Moreover, BluePrint gave clarity where IHC/FISH could not, classifying all HER2-equivocal tumors to non-HER2 subtypes. Additional therapeutic options should be explored for HER2+/ER+ BluePrint Luminal-type patients who have observed much lower pCR rates versus BluePrint HER2-type patients (12% vs. 51%, respectively; Lee, AACR 2018).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-23

Changes in the expression of HER2 and other genes in HER2-positive metastatic breast cancer induced by treatment with ado-trastuzumab emtansine and/or pertuzumab/trastuzumab

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**Background:** Although tremendous progress has been achieved with targeted therapy for HER2-positive (HER2+) metastatic breast cancer, most advanced tumors eventually develop resistance. Improving our understanding of mechanisms of resistance to anti-HER2 therapy is needed to develop new therapeutic approaches. The purpose of this study was to identify the mechanisms of resistance to treatment with ado-trastuzumab emtansine (T-DM1) and/or taxane/pertuzumab/trastuzumab (TPH).

**Methods:** In our preclinical analysis, HER2+ cell lines resistant to treatment with T-DM1 (n=5), and pertuzumab/trastuzumab (n=3) were generated. HER2 expression in the original and resistant cell lines was compared using Western blot, and HER2 gene amplification was compared in them using fluorescence in situ hybridization (FISH) and a Droplet Digital Polymerase Chain Reaction HER2 copy-number-validation assay. In our clinical analysis, nine patients with HER2+ metastatic breast cancer who had progressed on T-DM1 and/or TPH were enrolled. Patients underwent biopsies following treatment with T-DM1 and/or TPH. Targeted next-generation sequencing was performed using the FoundationOne® assay (Foundation Medicine, Inc.) to identify gene alterations. Also, the HER2 expression before and after the therapy was compared using immunohistochemistry and/or FISH.

**Results:** In preclinical analysis, HER2 expression/amplification by Western blot and gene copy-number analysis was significantly decreased in T-DM1-resistant cell lines (four of five cell lines;  $P < 0.01$ ) but not in pertuzumab/trastuzumab-resistant cell lines (none of three cell lines). In clinical analysis, the patients' median age was 54 years (range, 45-77 years), and five patients (56%) were ER+. Five patients (56%) received first-line anti-HER2 therapy, and four patients (44%) received two lines of anti-HER2 therapy prior to enrollment. We observed loss of HER2 expression in four of nine patients (44%) after undergoing anti-HER2 therapy. After receiving TPH, one of eight patients (13%) lost HER2 positivity according to FISH. In contrast, after T-DM1, three of four tested patients (75%) lost HER2 amplification by FISH. As for next-generation sequencing, we analyzed seven samples: three after treatment with TPH and four after treatment with T-DM1. In four of these samples (57%), we observed loss of HER2 amplification: one after treatment with TPH and three after treatment with T-DM1. TP53 mutations were seen in all patients. Additionally, we observed TOP2A and MCL1 amplification in two patients with ERBB2 amplification and AKT1 amplification in one patient with ERBB2 amplification loss.

**Conclusions:** We show for the first time that T-DM1-resistant breast cancer cells lose HER2 expression and amplification. Additionally, we observed loss of HER2 expression in patient samples following treatment with HER2 targeted therapy. Further study of resistant tumor samples is required to understand the impact of HER2 loss on outcomes. For the time being, repeating biopsy analysis of a metastatic site after treatment with T-DM1 to determine the HER2 expression status is reasonable, and it may increase the efficacy of future anti-HER2 therapy.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-24

Predicting pathological complete response (pCR) to neoadjuvant trastuzumab in patients with breast cancer using HER2 mass spectrometry

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**Background:** Around 40% of HER2-positive (HER2+) breast cancer patients who receive trastuzumab-based neoadjuvant therapy (TNT) achieve pCR (the FDA-recommended outcome measure in this setting). The ability to predict which patients will respond to TNT could support decisions regarding selection neoadjuvant agents or combinations. We previously quantified tumor expression of HER2 with mass spectrometry in archived biopsies of breast cancer patients who received adjuvant trastuzumab; HER2 levels > 2200 amol/ug of tumor protein were associated with superior disease-free and overall survival. We hypothesized that this HER2 protein cutoff would predict pCR in a HER2+ neoadjuvant population treated with TNT.

**Methods:** Formalin-fixed, paraffin-embedded tumor biopsies from patients with breast cancer were microdissected and solubilized for proteomic profiling with multiplexed mass spectrometry. Patients were dichotomized using the pre-specified HER2 cutoff of 2200 amol/ug. Relationships between pre-treatment HER2 expression and pCR following TNT were assessed with Fisher's exact test. Results: Of 65 evaluable patients, 18 achieved pCR (overall pCR rate 28%). In patients with HER2 expression above the 2200 cutoff (n = 30), the pCR rate was approximately 3 times higher than that of lower HER2 expressors (n = 35) (43% vs. 14%; [odds ratio = 4.47; [95% CI: 1.23-18.94],  $p = 0.013$ ). Among the lower HER2 expressors, 86% did not achieve pCR. HER3 protein was co-expressed in 29 of 65 patient tumors including 50% of non-pCR samples.

**Conclusions:** In patients diagnosed as HER2+ using standard methods, a quantitative HER2 cutoff identified a subset of low HER2 expressors in which 86% did not achieve pCR on TNT. Quantitative proteomic testing may be more useful than standard HER2 assessment methods in predicting response to TNT. Multiplexed proteomic analysis of HER2 and HER3 co-expression could potentially inform treatment decisions regarding dual therapy with pertuzumab.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-25

Clinicopathologic factors associated with pCR to neoadjuvant anti-HER2-directed chemotherapy

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**Background:** While the use of neoadjuvant therapy has become the standard of care in certain locally advanced breast cancers, questions remain regarding the optimal chemotherapeutic regimen as well as biologic and patient-specific predictors of pathologic complete response. In this study, we examined patient and tumor-specific characteristics associated with increased prediction of pathologic complete response (pCR) to neoadjuvant anti-HER2-directed chemotherapy.

**Methods:** 204 patients who received neoadjuvant anti-HER2-directed chemotherapy at our institution from 2006-2016 were included in this retrospective study. Univariate analyses were performed to analyze the relationships of multiple clinical and pathologic features to pCR rate. Multivariate analysis was also performed to evaluate the relative impact of specific pathologic characteristics on pCR rate.

**Results:** Among 204 patients with HER2 overexpressing breast cancers treated with neoadjuvant chemotherapy, 52.7% achieved pCR. Pathologic complete response was positively associated with high tumor grade (61.0% grade 3 in pCR group vs 39.0% in non-pCR group,  $P=0.23$ ) and high Ki67 index (mean Ki67 59.1 in pCR group vs 47.4 in non-pCR;  $P=0.015$ ). It was also associated with HER2 IHC 3+ (57.1% in pCR group vs 42.9% in non-pCR,  $p=0.029$ ), HER2 copy number (mean copy number 16.8 in pCR group vs 12.4 in non-pCR,  $p=0.004$ ), and HER2/CEP 17 ratio (mean ratio 6.42 in pCR group vs 5.17 in non-pCR;  $P=0.046$ ). Rates of HER2 FISH positivity were equal in the pCR and non-pCR groups (50.8% vs 49.2%,  $p=0.062$ ). Multivariate analysis demonstrated that higher Ki67 index and higher HER2/CEP17 ratio are significant predictors of pCR after adjusting for other covariates (odds ratio for Ki67 1.03 (1.06-1.49),  $p=0.002$ ; for HER2/CEP17 ratio OR 1.26 (1.06-1.49),  $p=0.009$ ).

**Conclusions:** In HER2 positive breast cancers, a higher Ki67 index as well as higher HER2/CEP17 ratios are associated with an increased pCR rate and may be useful as predictors of response prior to neoadjuvant therapy. Our results also demonstrate HER2 IHC to be a stronger predictor than HER2 FISH of response to upfront therapy. Larger studies would be useful as this association, if confirmed, may have relevance to clinical practice.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-10-26

Predicting benefit from HER2-targeted therapies in patients with ER+/HER2+ breast cancer

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**Background:** ER+/HER2+ accounts for up to 10% of all breast cancers (BCs) and most are treated with endocrine therapy (ET) after surgery to reduce the recurrence risk. We developed and validated an immunohistochemistry (IHC) based test (EA2Clin) that incorporates baseline IL6ST, clinical variables and on-treatment measurement of MCM4. Responders (Rs) and non-responders (NRs) to ET are identified and it accurately estimates recurrence-free survival (RFS) and BC-specific overall survival (BCSS). The aim was to determine if EA2Clin could accurately predict ER+/HER2+ patients likely to benefit from ET and to determine if it can identify those for whom HER2-targeted therapies are required.

**Methods:** 3 cohorts were studied:

A: 32 post-menopausal women (PMW) with large ER+/HER2+ BC treated with neoadjuvant (3-6 months) then adjuvant letrozole. 5 also received adjuvant chemotherapy plus Herceptin. Neoadjuvant clinical response was assessed by changes in tumour volume. Tumour core biopsies were taken at 0, 14 days and 3 months. Gene expression analysis using Illumina HT12 whole-genome beadarrays was performed on a subset (n=17) where fresh tissue was available.

B: 13 PMW with ER+/HER2+ BC who were treated by surgery without neoadjuvant therapy. RNA was extracted from excision tissues and analysed using whole-genome Affymetrix U133A microarrays.

C: 15 PMW with ER+/HER2+ BC treated with 2-weeks of pre-operative letrozole (n=7) or anastrozole (n=8). All received adjuvant letrozole. Tissues were collected at pre-treatment and at surgery. None received Herceptin or chemotherapy.

All patients were followed-up after surgery (median follow-up = 6.4 years).

**Results:** In cohort A, half (16/32) of the patients responded to ET with tumour volume reductions of >70% with neoadjuvant treatment. Innate resistance was apparent in 3 patients with continued tumour growth on ET, whereas 13 patients developed resistance after a period of response. EA2Clin predicted neoadjuvant response with a 92% accuracy. There was increased expression of phospho-AKT and phospho-ERK in NRs, not seen in Rs. Half (8/16) of the NR cancers expressed phospho-ER; but was not seen in any responsive cancer. Gene expression analysis in 17 patients showed increased MAPK and PI3K pathway activity in the 9 NR compared with the 8 R tumours. These results were recapitulated in cohort B where MAPK and PI3K activity were associated with low levels of IL6ST.

In the 16/32 patients who responded well to neoadjuvant ET the actuarial recurrence rate was 0% at 5 and 10 years. The rate of recurrence in the NR was 30% at both 5 and 10 years. Of the 5 patients who received chemotherapy plus Herceptin, none recurred despite a poor response to neoadjuvant letrozole (median length to last follow-up was 6.1 years). Initial data suggest that in cohort B EA2Clin identifies a group of ER+/HER2+ cancers that can be managed by ET alone.

**Conclusions:**

- The EA2Clin test identifies ER+/HER2+ BCs who respond well to ET alone and those with a poor clinical response who have higher risk of recurrence.
- NR to ET have increased expression of PI3K and MAPK pathways, consistent with active HER2 signalling.
- There is potential role for EA2Clin in selecting ER+/HER2+ patients that require and benefit from HER2-targeted therapies.

# 2018 San Antonio Breast Cancer Symposium ®

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Profiling of metastatic carcinomas to the breast for the biomarkers of immuno-oncology therapy

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**Introduction:** Breast metastases from non-mammary carcinomas are very rare and the molecular characteristics of these metastases have not been studied. Due to the uniqueness of this type of metastases, we evaluated whether there was any molecular or immunologic similarities among the patients with metastases to the breast.

**Patients and Methods:** Fifty-one patients with metastatic carcinomas to the breast (49 female and 2 male) were identified in the files of Caris Life Sciences (Phoenix, AZ). Average age at presentation was 57 years (range, 20-90 years). Immunohistochemistry for PD-L1 was performed using SP142 (Ventana) or 22c3 (DAKO) antibodies; 592 genes sequencing (n=47), DNA microsatellite instability (MSI) (n=6) and total mutational burden (TMB) (n=14) were obtained from a next generation sequencing platform (Illumina).

**Results:** Three most common primary sites were lung (19, 37%), ovary (15, 29%) and fallopian tubes/peritoneum (7, 14%). Ten metastases (20%) demonstrated neuroendocrine differentiation. When 1% cutoff was applied, tumor cells PD-L1 expression was detected in 10 cases (20%), and immune cells expression of PD-L1 in 19 patients (37%). Three cases (6%) had PD-L1 staining in both cancer and infiltrating immune cells. 50% of the cases exhibited mutations in *TP53* gene, while other mutated genes followed the pathways typically seen in their primary sites (e.g. *VHL* in renal carcinomas, *BRCA1* in fallopian tube carcinoma). Total mutational burden was high ( $\geq 10$  mutations/Mb) in five out of 14 successfully analyzed cases (36%) and no case (0%) exhibited high microsatellite instability. Interestingly, estrogen receptor status was positive in 13/49 patients (26.5%) including 12 adenocarcinomas originating from ovary, Fallopian tube or peritoneum and one duodenal neuroendocrine. No tumor was HER2 positive.

**Conclusions:** Metastases to the breast proved to be heterogeneous but there were molecular, endocrine and immunologic findings that yielded information that could be used to make therapeutic decisions which including the use of new immunotherapeutic strategies.

# 2018 San Antonio Breast Cancer Symposium ®

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The impact of HER2-directed targeted therapy on HER2-positive DCIS of the breast

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**Purpose/Objectives:** In invasive breast cancer, HER2 is a well-established negative prognostic factor. However, its significance on the prognosis of ductal carcinoma in situ (DCIS) of the breast is unclear. As a result, the impact of adding HER2-directed therapy to HER2-positive DCIS is unknown and is currently the subject of ongoing clinical trials. In this study, we aim to determine the impact of HER2 status on DCIS patient outcomes as well as the possible impact of HER2-directed targeted therapy on survival outcomes for HER2-positive DCIS patients.

**Materials/Methods:** The National Cancer Data Base (NCDB) was used to retrieve patients with biopsy-proven DCIS diagnosed from 2004-2015. Only patients with known estrogen receptor (ER) status, progesterone receptor (PR) status, and HER2 status were included in the analysis. Patients were divided into two groups based on the adjuvant therapy they received: systemic therapy (assumed to be HER2-directed targeted therapy) or no systemic therapy. Statistics included multivariable logistic regression to determine factors predictive of receiving systemic therapy, Kaplan-Meier analysis to evaluate overall survival (OS), and Cox proportional hazards modeling to determine variables associated with OS.

**Results:** Altogether, 1927 patients met inclusion criteria; 430 (22.3%) received HER2-directed targeted therapy, while 1497 (77.7%) did not. Patients who received HER2-directed targeted therapy were likely more likely to be ER-negative. Patients who received HER2-directed targeted therapy had a higher 5-year OS compared to patients that did not (97.7% vs. 95.8%,  $p = 0.043$ ). This survival benefit remained on multivariate analysis. Factors associated with worse OS on multivariate analysis included Charlson-Deyo Comorbidity Score  $\geq 2$  and no receipt of hormonal therapy.

**Conclusions:** In the largest study to date evaluating HER2-positive DCIS patients, the receipt of HER2-directed targeted therapy was associated with an improvement in OS. The results of currently ongoing clinical trials are needed to confirm this finding.

## 2018 San Antonio Breast Cancer Symposium ®

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tRNA-derived fragments as novel predictive biomarkers for trastuzumab-resistant breast cancer

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**Background:** Resistance to trastuzumab remains a common challenge to HER-2 positive breast cancer. Up until now, the underlying mechanism of trastuzumab resistance is still unclear. tRNA-derived small non-coding RNAs, a new class of small non-coding RNA (sncRNAs), have been observed to play an important role in cancer progression. However, the relationship between tRNA-derived fragments and trastuzumab resistance is still unknown.

**Methods:** We detected the levels of tRNA-derived fragments expression in normal breast epithelial cell lines, trastuzumab-sensitive and -resistant breast cancer cell lines using high-throughput sequencing. qRT-PCR was conducted to validate the differentially expressed fragments in serums from trastuzumab-sensitive and -resistant patients. A receiver operating characteristic (ROC) curve analysis was performed to evaluate the power of specific tRNA-derived fragments. Progression-free survival (PFS) was analyzed using Cox-regression.

**Results:** Our sequence results showed that tRNA-derived fragments were differentially expressed in the HBL-100, SKBR3, and JIMT-1 cell lines. tRF-30-JZOYJE22RR33 and tRF-27-ZDXPHO53KSN were found significantly upregulated in trastuzumab-resistant patients compared to sensitive individuals, and the ROC analysis showed that tRF-30-JZOYJE22RR33 and tRF-27-ZDXPHO53KSN were correlated with trastuzumab resistance. In a multivariate analysis, higher levels of tRF-30-JZOYJE22RR33 and tRF-27-ZDXPHO53KSN expression were associated with significantly shorter PFS in patients with metastatic HER-2 positive breast cancer.

**Conclusion:** Our results suggest that tRF-30-JZOYJE22RR33 and tRF-27-ZDXPHO53KSN play important roles in trastuzumab resistance. Patients with high levels of tRF-30-JZOYJE22RR33 and tRF-27-ZDXPHO53KSN expression benefitted less from trastuzumab-based therapy than those that express lower-levels of these molecules. tRF-30-JZOYJE22RR33 and tRF-27-ZDXPHO53KSN may be potential biomarkers and intervention targets in the clinical treatment of trastuzumab-resistant breast cancer.

**Publication Number: P3-11-01**

**Conclusion:** The associations of pre, post-NAC TIL levels with response to treatment and DFS differ between BC subtypes and may deviate from linearity. The characterization of immune subpopulations may improve our understanding of the complex interactions between pre- or post-NAC setting, BC subtype, response to treatment and prognosis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-02

Breast cancer-specific mortality (BCSM) in patients (pts) with node-negative (N0) and node-positive (N+) breast cancer (BC) guided by the 21-gene assay: A SEER-genomic population-based study

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**Introduction:** The 21-gene Breast Recurrence Score® (RS) in the randomized NSABP B-20, SWOG S8814, and TAILORx studies predicted chemotherapy (CT) benefit for pts with N0 and N+ disease. Endocrine therapy was not inferior to chemoendocrine therapy in 6,711 randomized TAILORx pts with RS 11-25 and N0 disease. We characterized BCSM for the TAILORx-defined RS groups (0-10, 11-15, 16-20, 21-25, and 26-100) in the large population-based SEER study of pts treated based on RS results. **Methods:** RS results were provided electronically to SEER registries per their linkage methods (Petkov npj Breast Cancer 2016). Eligible pts were diagnosed Jan 2004 - Dec 2014 with N0 and N+(N1mic, 1-3 positive nodes[N1]), HR+, HER2-negative BC, and had no prior malignancy or multiple tumors, with follow-up information through Dec 2015. BCSM estimates by reported CT use yes vs. no/unknown were computed, and must be interpreted cautiously given lack of randomization. **Results:** There were 80,605 pts with RS results; 70,087 with N0 disease, 4,336 with N1mic, and 6,182 with N1. Median follow-up was 49 months, with 20,151 pts followed >76 months. 1,020 pts had experienced breast cancer death. There was a significant positive association between higher RS results and increased BCSM (p<0.001) without and with adjustment for nodal status, age, tumor size, and grade. Reported CT use increased with increasing RS result (Table). 9-y BCSM was <4% without CT for pts with RS 0-25 and N0 disease and for pts with RS 0-20 and N1 disease. For RS 26-100, 9-y BCSM was lower with CT use than without (Table). Similar results were seen in the 4,336 pts with N1mic disease. Pts treated with CT for every RS group, as expected, tended to have higher risk features (age, tumor size and grade), and multivariable models and adjustment by propensity scores will be presented to allow for more definitive conclusions.

Table

		N0; CT Use No (N=55726)	N0; CT Use Yes (N=14361)	N1; CT Use No (N=3810)	N1; CT Use Yes (N=2372)
RS 0-10	n	13982	389	1005	283
	9-y BCSM	1.4% (1.0%, 2.1%)	2.1% (0.7%, 5.9%)	2.2% (1.0%, 4.8%)	1.4% (0.4%, 4.6%)
RS 11-15	n	16758	1066	1193	453
	9-y BCSM	2.0% (1.5%, 2.6%)	2.7% (1.3%, 5.4%)	1.5% (0.8%, 3.1%)	4.4% (1.4%, 13.6%)
RS 16-20	n	14484	2719	992	587
	9-y BCSM	2.2% (1.7%, 2.8%)	1.9% (1.1%, 3.0%)	3.8% (1.6%, 8.5%)	4.2% (1.6%, 10.9%)
RS 21-25	n	6704	3544	397	431
	9-y BCSM	3.9% (3.0%, 5.0%)	3.4% (2.6%, 4.6%)	7.1% (4.1%, 12.1%)	5.7% (2.9%, 11.2%)
RS 26-100	n	3798	6643	223	618
	9-y BCSM	8.8% (7.4%, 10.4%)	7.0% (5.9%, 8.4%)	15.2% (8.7%, 25.9%)	10.8% (7.2%, 16.2%)

**Conclusion:** In both N0 and N+ disease (up to 3 positive nodes), low RS results identify more than 70% of BC patients with excellent long-term outcomes and no apparent CT benefit, and high RS results (26-100) identifies an important minority of



patients where CT reduces BCSM. Real-world evidence from SEER reconfirms that the 21-gene assay is prognostic and strongly suggests it is predictive of CT benefit, irrespective of nodal status.

# 2018 San Antonio Breast Cancer Symposium ®

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Validation of CIN4 in the DBCG 89D clinical cohort

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**Background:** Chromosome instability (CIN) in solid tumours is associated with poor prognosis and results in numerical and structural chromosomal aberrations. Our group previously have developed the CIN signatures and have demonstrated the CIN signatures as prognostic biomarkers in breast cancer cohorts. Furthermore, our work in the BR9601 and MA.5 clinical cohorts CIN4 provided level IIB evidence that CIN4 was predictive of anthracycline sensitivity. An analysis of the DBCG 89D clinical trial was now performed to validate the role of CIN gene expression signatures as a marker of anthracycline sensitivity.

**Methods:** RNA was extracted from patients in DBCG 89D clinical trial analysed through NanoString technology. The prognostic and predictive values of the signatures on distant relapse-free survival (DRFS) were explored using Cox proportional hazard models. Multivariate models included menopausal status, tumour size, nodal status, ER and Her2 status, histological type and grade, and treatment regimen.

**Results:** All of the 594 samples available from the DBCG 89D we successfully analysed. CIN25 and CIN70 gene expression signatures did not associate with any of the clinicopathological characteristics tested. In addition, CIN25 and CIN70 were not prognostic or predictive of distant relapse free or breast cancer specific survival in this clinical cohort. Low CIN4 score was associated with ER negativity ( $p=0.02$ ), HER2 normal expression ( $p<0.05$ ).

**Conclusion:** In this study we demonstrated that CIN4 was associated with aggressive disease. We were however in DBCG 89D unable to validate the predictive value of CIN4 concerning anthracycline sensitivity.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-04

A model to predict high-risk Oncotype DX scores as defined by the TailorX trial: A report from the National Cancer Data Base

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**Background:** Results from the TailorX clinical trial demonstrated a survival benefit of chemotherapy in those with high-risk (>25) Oncotype DX scores as well as in some patients ≤50yo with intermediate (16-25) scores. The objective of this study was to develop a model that could predict a high-risk Oncotype DX score based on tumor features alone.

**Methods:** From 2010-2015, 84,549 breast cancer patients with Oncotype DX scores were selected from the National Cancer Data Base. Seven pathologic variables including age, estrogen and progesterone receptor status, histologic subtype, lymphovascular invasion (LVI), grade, and tumor size were used to predict high-risk (>25) Oncotype DX scores using logistic regression. A similar analysis was performed on women ≤50yo to predict low (<15) and intermediate (16-25) scores. Nomograms were created for models using bootstrap estimation method of the model coefficients. Cutoffs with at least 80% positive predictive value (PPV) were chosen to classify patients into high or low-risk Oncotype DX score groups. Accuracy of these predictions were developed in a training set and validated in a testing set.

**Results:** For patients >50yo, 6,658 (15.1%) of patients had high-risk Oncotype DX scores. The model yielded a moderately strong C-index of 0.80 for Oncotype DX score of >25. For women ≤50yo, 2,044 (13.5%) were high-risk, 5,760 (38.1%) were intermediate-risk and 7,316 (48.4%) were low-risk. The C-index for women ≤50yo was 0.81 for prediction of Oncotype DX score of >25. C-indexes for intermediate and low risk scores were not strong enough to use for prediction (0.54 and 0.67). Estrogen receptor status, progesterone receptor status and grade were the strongest independent predictors of high-risk Oncotype DX scores in women >50yo and ≤50yo. Age was not a good predictor of high-risk scores in women >50yo. When our nomogram was used in the training set, the PPV of a high-risk Oncotype score was 80% with a negative predictive value (NPV) of 87%, sensitivity of 19% and specificity of 99%. In the testing set, PPV was 81%, with a NPV of 87%, sensitivity of 19% and specificity of 99%.

**Conclusion:** A model incorporating tumor factors can predict a high-risk Oncotype DX score as defined by the recent TailorX trial in all age groups. The model is of limited value in predicting intermediate-risk Oncotype DX scores in women of age ≤50. In resource-constrained healthcare systems, such a model can help identify high risk patients who would benefit from adjuvant chemotherapy without incurring the cost of an Oncotype DX test.

# 2018 San Antonio Breast Cancer Symposium ®

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Predictive gene signatures of adjuvant capecitabine benefit in triple negative breast cancer in the FinXX trial

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**Background:** Recent studies have demonstrated a benefit of adjuvant capecitabine in early stage breast cancer, particularly in patients with residual disease after neoadjuvant chemotherapy. Subset analyses suggest that patients with triple negative breast cancer (TNBC) may be more likely to benefit with capecitabine in this setting. However, more precise biomarkers to predict which patients are most likely to benefit from capecitabine are needed.

**Methods:** The NanoString Breast Cancer 360™ (BC360) panel was used to quantify mRNA expression in FFPE tissue samples from patients with TNBC in the FinXX trial. Gene signature scores were analyzed using prespecified algorithms developed by NanoString. 30 additional custom genes related to capecitabine metabolism and function were added. Patients in FinXX trial were randomized to receive either 3 cycles of docetaxel followed by 3 cycles of cyclophosphamide, epirubicin, and fluorouracil (T+CEF) or 3 cycles of docetaxel plus capecitabine followed by 3 cycles of cyclophosphamide, epirubicin, and capecitabine (TX+CEX). Cox proportional hazard ratio (HR) was used to determine the association of each gene signature with recurrence free survival (RFS).

**Results:** A total of 111 samples from patients with TNBC in the FinXX trial were available for gene expression analysis. 57 patients were treated with T+CEF and 54 patients were treated with TX+CEX. The median age was 52 years and median follow up was 10.2 years. Consistent with the previous analysis of the FinXX trial, patients with TNBC had nonsignificant but favorable RFS with capecitabine (HR 0.60, 95% CI 0.27-1.3, p 0.2). Among 39 individual genes and metagene signatures generated with the BC360 panel, there were 4 gene signatures significantly associated with improved RFS favoring an addition of capecitabine with TX+CEX compared to T+CEF. These were the cytotoxic cell signature (HR 0.37, 95%CI 0.15-0.92, p 0.03), endothelial signature (HR 0.18, 95%CI 0.04-0.83, p 0.03), mast cell signature (HR 0.43, 95%CI 0.21-0.88, p 0.02), and PDL2 gene (HR 0.29, 95%CI 0.09-0.99, p 0.05). PAM50 intrinsic subtype was not predictive of capecitabine benefit within this TNBC subset. Moreover, we identified additional individual 12 genes that were significantly associated with capecitabine benefit (p-FDR<0.05). Among these genes, high expression of CES1, which encodes an enzyme that activates capecitabine, was significantly associated with improved RFS when treated with capecitabine (HR 0.71, 95%CI 0.32-1.61 with p-interaction=0.04).

**Conclusion:** Our analyses demonstrated potential predictive individual genes and metagene signatures that may be used to identify TNBC patients who are more likely to benefit from adjuvant capecitabine. Interestingly, several gene signatures related to immune response and genes related to capecitabine activation were associated with improved outcome in TNBC patients treated with capecitabine in the FinXX trial. While these findings are compatible with basic science reports, future studies are needed to validate the significance of these gene signatures as predictive biomarkers for capecitabine benefit.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-06

HRD (homologous recombination deficiency) score and its clinicopathological features in neoadjuvant chemotherapy treated sporadic breast cancers

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**Purpose** Homologous Recombination Deficiency (HRD) score has been developed to evaluate the DNA double-strand break repair function. BRCA1/2 germline mutation-associated breast cancers are known to show a high HRD score and a high sensitivity to platinum-based chemotherapy as well as PARP inhibitor. HRD can be theoretically induced by not only the dysfunction of BRA1/2 but also the dysfunction of the other molecules involved in homologous recombination, and, actually, high HRD score is reported to be seen in a significant proportion of sporadic breast tumors without BRCA1/2 mutation. The aim of the present study was to elucidate the clinicopathological characteristics of sporadic breast tumors with high HRD score as well as their sensitivity to sequential paclitaxel and FEC chemotherapy (P-FEC).

**Methods** Tumor tissues obtained by Mammotome from 132 sporadic breast cancer patients (stage II-III) before neoadjuvant chemotherapy with P-FEC were subjected to assay for HRD score using Oncoscan CNV kit®. HRD score was a simple sum of NtAI, LOH, and LST (cutoff, 42), and were also subjected to the gene expression analysis using the Affymetrix microarray (U133 plus 2.0). BRCA1 promoter methylation was assayed by methylation specific real-time PCR.

**Result** Of the 132 breast tumors, 32% showed a high HRD score which were significantly associated with high histological grade ( $P=0.001$ ), negative progesterone receptor ( $P=0.023$ ) and high Ki67 index ( $P=0.004$ ). Triple negative breast cancer (TNBC)( $n=19$ ) showed a significantly ( $P<0.001$ ) higher HRD score than the other subtypes (HR+/HER2-( $n=73$ ), HR+/HER2+( $n=12$ ), HR-/HER2+( $n=18$ )). BRCA1 promoter methylation was significantly associated with a high HRD score. There was no significant correlation between HRD score and pCR to P-FEC when all tumors were considered but tumors with a high HRD score showed a significantly ( $P=0.020$ ) lower pCR rate when only TNBC were considered. In TNBC, majority of tumors showed a high HRD score in five subtypes (BL1, BL2, IM, M, MSL) but no tumor showed a high HRD score in LAR (luminal androgen receptor) subtype.

**Conclusion** Approximately one third of sporadic breast tumors show a high HRD score, indicating the presence of homologous recombination dysfunction, and they are most often seen in TNBC with BRCA1 promoter methylation and never in LAR subtype. Interestingly, breast tumors with a high HRD score were less sensitive to P-FEC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-07

Exploratory biomarker analysis from a phase II, multicenter, randomized trial of eribulin plus gemcitabine(EG) versus paclitaxel plus gemcitabine(PG) as first-line chemotherapy for human epidermal growth factor receptor 2 (HER2)- negative metastatic breast cancer(MBC): Korean cancer study group trial (KCSG BR13-11)

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**Introduction :** A phase II, multicenter, randomized clinical trial of the comparison between eribulin plus gemcitabine (EG) and paclitaxel plus gemcitabine (PG) as first-line chemotherapy for patients with human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer (MBC) found EG was less neurotoxic, but had similar efficacy of PG. In this study, we performed exploratory biomarker analysis of the impact of genetic alterations on the efficacy according to EG and PG chemotherapy.

**Methods :** This biomarker study was conducted using tumor samples from 40patients. When tissue collection was possible after disease progression, we performed paired sample analysis. Tumor DNA and RNA were extracted from formalin-fixed, paraffin-embedded tissues. To perform targeted deep sequencing, we used CancerScan™, a 375 cancer gene panel. And we performed an nCounter expression assay for gene expression analysis using 730 PanCancer panel and 730 Immune panel.

**Results:** In total, we obtained 44 tissue samples from 40 patients. Twenty two patients were assigned in EG arm and 18 patients were in PG arm. Thirty-eight were at baseline and six after disease progression. Gene expression assay were performed in 44 tissue samples but only 31 samples were possible to be targeted deep sequencing.

We performed differently expressed gene(DEG) analysis for detecting the association between level of gene expression and disease progression. In this analysis, high expression of CCNE1, TGFB4 and BAMBI and low expression of DDB2, CD14 and SHC3 were associated with disease progression among 730 PanCancer panel genes ( $p<0.05$ , respectively). In terms of immune panel genes, most of immune related genes were highly expressed in a group without disease progression compared with that with disease progression. Only 2 genes, C8G and CD24 were highly expressed in a group with disease progression. Paired sample analysis showed that expression levels of THBS4 and CD27 decreased after disease progression while those of CCNE2 and FGFR4 increased.

In targeted deep sequencing, FAT3 (42.3%) was most frequently mutated gene followed by PKHD1, PIK3CA and TP53. Among mutated genes, EWSR1 mutation and upstream mutation of ETV1 were associated with disease progression, respectively ( $p<0.05$ , respectively). In mutation signature analysis, signature 1 (S, age related), S3(homologous recombination deficiency, HRD), S6 (mismatch repair, MMR), S20(MMR) and S21(microsatellite instability, MSI) were enriched in this population. Mutation signature 3 related to short disease free survival ( $p=0.0026$ ).

**Conclusion:** In gene expression analysis, high expression of TGF-B signaling pathway related genes was associated with disease progression while high expression of immune related genes were related to prolonged disease free survival. In mutation analysis, EWSR1 and ETV1 mutations indicated short disease free interval and HRD mutation signature was also related to poor prognosis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-08

Sperm associated antigen 5 (SPAG5) predicts pathological complete response (pCR) and distant relapse risk to HER2 targeting agents and anthracycline based chemotherapy in HER2 positive (HER2+) breast cancer (BC)

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## Background:

Previously we found that SPAG5 gene was amplified/gained in 20-35% of HER2+BC. Herein, we investigated the prognostic and predictive significance of SPAG5 (mRNA, protein) expression in 1726 HER+BC patients with median follow-up >5 years.

## Methods:

Analysis of SPAG5 mRNA (cDNA array expression) and protein (immunohistochemistry) and their association with distant relapse risk (DRR) were determined in 446 and 642 cases of HER+2 early stage BC in which 36% and 40% of them had received adjuvant Herceptin (H) + Anthracycline based (AC) + Taxane (T); respectively. In 33% and 31% of SPAG5 mRNA cohort and 21% and 38% of SPAG5 protein cohort had received adjuvant (Adj) AC+T or chemotherapy (CT) naïve; respectively. The association between SPAG5 expression (mRNA, protein) and both pCR and DRR after receiving neoadjuvant chemotherapy (Neo-Adj-CT) were evaluated in 476 and 162 patients with HER2+ locally advanced BC; respectively. Neo-Adj AC+T+HER2 targeting (Herceptin, Herceptin+ Lapatinib or Lapatinib) and AC+/-T has been prescribed to 51% and 49% of mRNA cohort whereas the 45% and 55% of the protein expression cohort has received Neo-Adj AC+T+H and AC+/-T; respectively.

## Findings:

In patients with SPAG5 mRNA overexpression (+;> median), those who had received AC+/-T Neo-Adj-CT alone achieved similar pCR to those who had received AC+T+HER2 targeting Neo-Adj (38% vs., 37%; OR (95% CI): 1.0 (0.6–1.6), p=0.923) in either ER- (46% vs., 52%; OR (95% CI): 1.3 (0.5–3.0), p=0.58) or ER+ subgroups (25% vs., 26%; OR (95% CI): 1.1 (0.4–3.4), p=0.88). Whereas in patients with low SPAG5 mRNA (-), those who had received AC+T+HER2 targeting Neo-Adj had achieved 2.5 fold increased in pCR compared to those who received AC+/-T alone (47% vs., 26%; OR (95% CI): 2.5 (1.4–4.4), p=0.001) in either ER- (60% vs., 31%; OR (95% CI): 3.4 (1.7–6.7), p<0.001) or ER+ subgroups (42% vs., 16%; OR (95% CI): 4.0 (1.2–12.9), p=0.018). Similarly in patients with SPAG5- protein expression; receiving AC+T+HER2 targeting Neo-Adj was associated with higher pCR compared to AC+/-T (21% vs., 4%; OR (95% CI): 6.6 (1.4–32.6), p=0.01). Whereas receiving AC+/-T was associated with similar pCR to AC+T+HER2 targeting Neo-Adj in patients with SPAG5+ protein (49% vs., 53%; OR (95% CI): 1.2 (0.5–3.2), p=0.702). Receiving AC+T+HER2 targeting Neo-Adj was associated with lower DRR compared to AC+/-T [HR (95% CI): 0.82 (0.08-0.97); p=0.045] in patients with SPAG5- protein expression but not in those with SPAG5+ protein [HR (95% CI): 1.08 (0.34-3.36); p=0.901]. Similarly, receiving Adj Herceptin+AC+T was associated with lower DRR compared to AC+/-T alone in those with SPAG5- (mRNA, protein) expression [HR (95% CI): 0.48 (0.25-0.93); p=0.029 and 0.82 (0.08-0.97); p=0.045; respectively] but not in those with high SPAG5+ (mRNA, protein) [HR (95% CI): 1.04 (0.48-2.26); p=0.924 and 1.00 (0.82-1.22); p=1.00; respectively].

**Conclusion:** SPAG5 expression could help in selecting patients who would benefit from both HER2-targeting agents and or AC-CT. Therefore patients with SPAG5- expression could avoid unnecessary AC-CT whereas those with SPAG5+ could receive a shorter Herceptin course.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-09

On-treatment biomarkers improve prediction of response to neoadjuvant chemotherapy in breast cancer

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## Background

Neoadjuvant chemotherapy is increasingly used to treat breast cancer. Patient matched samples taken before and on-treatment can potentially provide valuable prognostic information from changes in gene expression. This study aims to identify whether on-treatment changes in gene expression in chemotherapy-treated cohorts can improve accuracy of response and outcome prediction over existing prognostic tests.

## Methods

A total of 97 samples from a cohort of 50 neoadjuvant chemotherapy-treated primary breast cancer patients (aged 29-76 at diagnosis, Allred status 47:53% +/-, Her2 status 80:20% +/-, mixed grade and menopausal status) taken pre-treatment, at 2 weeks on-treatment, mid-therapy and at resection were sequenced with Ion Ampliseq transcriptome yielding expression values for 12,635 genes. Differential expression analysis was performed across response groups (16 Responders, 34 Non-Responders) as defined by Pathological Complete Response and over treatment time to identify significantly differentially expressed genes indicative of response status. The resulting gene lists were used for pathway enrichment analysis to find common up/down regulated pathways unique to each response class. Feature selection was performed with pairwise ranked products (FDR > 0.05) and the results used in a random forest model to generate a binary classifier. Results were validated in a similar, contemporary dataset from the I-SPY 1 Trial (221 evaluable patients, 36 with matching samples).

## Results

On-treatment samples saw significant changes in genes commonly associated with proliferation (PCNA, AURKA, MKI67) regardless of response class. An on-treatment marker for response was identified (AAGAB), which resulted in a testing accuracy of 100% and a validation accuracy of 78% in the I-SPY 1 Trial. AAGAB was predictive of long term survival ( $p = 0.048$  testing,  $p = 0.0031$  validation) in both chemotherapy cohorts at the same expression level as defined for treatment response. The single gene on-treatment biomarker, AAGAB proves more performant than established prognostic tests, Mamaprint (Edinburgh NEO trial, pre-treatment 61%, on-treatment 63%. I-SPY 1 trial, pre-treatment 60%, on-treatment 66%) and Pam50 RORS (NEO trial pre-treatment 50%, on treatment 58%, I-SPY 1 trial pre-treatment 56%, on-treatment 64%) AAGAB was not predictive of response or survival in endocrine therapy treated cohorts.

## Conclusion

Changes in gene expression of on-treatment chemotherapy breast cancer resulted in the identification of a novel gene marker that was as effective in predicting prognostic status as established prognostic tests. These results support the use of on-treatment testing in breast cancer to improve the accuracy of tumour response prediction.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-10

Prosigna prognostic signature in pN1mi, estrogen receptor-positive breast cancer: the pN categorization impacts the BC risk stratification

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## Background

Several validated molecular subtyping tests for breast cancer based on gene expression profiling are now routinely used in clinical practice. The Prosigna® breast cancer (BC) prognostic gene signature assay identifies a gene-expression profile that permits the classification of tumors into subtypes and gives a score for the risk of recurrence (ROR) at 10 years. The assay uses the 50-gene expression profile, weighted together with clinical variables, to generate a risk category and numerical score. The score is reported on a 0-100 scale, for hormone receptor positive BC. Prosigna test results classified tumors according to intrinsic subtypes (Lum A, Lum B, HER2-enriched, basal-like) and ROR risk groups (low risk, 0-40; intermediate risk, 41-60; and high risk, 61-100).

For node-positive patients, the weight given to the node status is similar when considering pN1mi ([0.2-2mm]) or pN1 (>2mm, 1-3 positive lymph nodes). Our aim was to characterize the Prosigna test classification of the pN1mi subgroup in the French national registry for molecular signatures in ER+ BC.

## Methods

Since 2018, four French laboratories using Prosigna test in clinical practice retrospectively implement a national registry for molecular prognostic signatures in ER+ BC. We analyzed the pN1mi subgroup with regards to their clinico-pathological characteristics, Prosigna test results and ROR risk score. Using the definition formula of the prosigna algorithm, we could calculate a hypothetical score if the pN1mi has been considered as pN0 and redefine risk categories for pN1mi breast cancers.

## Results

The database included 886 tests performed in routine practice, including 91 (10.3%) pN1mi. The pN1mi BC were ER+ HER2- (88/91, 96.7%), invasive carcinoma of no special type in 81/91 (89%), with a median tumor size of 23 mm (range 12-60). Among these, the median ROR score was 44, and 51/91 tumors (56%) were classified as LumA tumors, 34/91 (41%) as LumB, two as HER2-enriched and one as basal-like. Interestingly, the probability of distant recurrence was 26% if pN1mi BC were considered as N+ but lowered to 10% if considered as pN0. Among the different molecular subtypes, the change from pN1 to pN0 has different weight: in Lum A tumors, the switch in the probability of distant recurrences was 13 to 6% according to pN categorization of the pN1mi BC, and the median ROR score moved from 27 to 6 with 15/51 (29%) of Lum A tumors considered as high risk if pN1 but only 1/51 if pN0; 31/51 (61%) versus 14/51 (27.5%) were in the intermediate risk group, and only 5/51 (10%) versus 34 (67%) in the low risk group. For LumB tumors, the switch in the probability of distant recurrences was 29% to 17% according to pN categorization and the median ROR score moved from 60 to 15 with 36/37 (97.3%) of Lum B tumors considered as high risk if pN1 but only 13/37 (35%) if pN0; 1/37 versus 20/37 (54%) were in the intermediate risk group, and none of the 37 LumB versus 1 in the low risk group.

## Conclusion

With regards to Prosigna test in pN1mi BC - and the persistent debate as to whether they should bear the same weight as pN1 pN0 tumors - categorization in ROR risk group is likely to be impacted by the node factor weight, and more importantly among Lum B tumors.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-11

Prediction of treatment response to neoadjuvant chemotherapy in breast cancer by subtype using tumor-infiltrating lymphocytes: Study of a large Mexican cohort

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## **Background/aim:**

Tumor-infiltrating lymphocytes (TILs) are emerging as biomarkers mediating tumor response to treatments. Studies have provided evidence that the level of TILs has prognostic value in breast cancer. The level of TILs has been associated with treatment outcome and pathologic response in patients undergoing neoadjuvant chemotherapy. In this study, we analyzed TILs before neoadjuvant therapy and if they correlate with pathological response to treatment

## **Patients and methods**

We retrospectively analyzed the specimen slides of patients with Breast Cancer from the Centro Oncologico Estatal ISSEMyM treated with Neoadjuvant Chemotherapy (NAC) during 2007–2014. We identified 410 patients fulfilling the inclusion criteria of this study. The histological sections had already been evaluated by hematoxylin and eosin slides. They were reassessed by our pathologist for the percentage of intratumoral and stromal TILs. The correlation with pathological response of the tumor after neoadjuvant therapy was also studied in these patients. The proportions of TILs were categorized into high and low groups using a cut-off value of 10%

## **Resultados**

The mean age was 51.4 years. The most frequent type of breast cancer histology was invasive ductal breast carcinoma in 369 (90%) patients, 34 (8.3%) lobular and 1.7% other. The BC subtypes among the 410 patients who received NAC were TNBC in 82 (20%), HER2BC in 70 (17.1%), and HRBC in 258 (62.9%) patients. Treatment response was pCR in 164 (40%) and non-pCR in 245 (60%) patients. Based on subtype, pCR was achieved in 43 (52 %) patients with TNBC, 35 (50.0%) with HER2BC, and 87 (33%) with HRBC. There were 191 (46.5%) patients in the high-TIL group and 219 (53.5%) patients in the low-TIL group. Patients were divided into high-TIL and low-TIL groups, and the clinicopathological characteristics of each group were examined. High TILs that had a pathologically complete response were found in 56 % of patients, and low TILs were found in 44 %. There was a significant correlation between TILs and pathological response in patients with neoadjuvant chemotherapy ( $p = 0.04$ ).

## **Conclusion**

TILs may be a biomarker for predicting treatment response to NAC in patients with Breast Cancer. Studies assessing outcomes and therapeutic efficacies should consider.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-12

**Biomarker phase neo-DDRD trial: Predicting response to neoadjuvant chemotherapy (NAC) in early breast cancer using the DNA damage repair deficiency (DDRD) assay**

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**Background:** The DDRD assay was developed to prospectively identify a molecular subtype of cancers defined by DNA damage activated immune signalling. This 44-gene assay has been validated to predict response to anthracycline-cyclophosphamide chemotherapy in both the adjuvant and neoadjuvant settings in breast cancer. The biomarker phase Neo-DDRD trial assessed the clinical utility of this assay prospectively in patients receiving neoadjuvant chemotherapy (NAC).

**Methods:** 48 patients receiving NAC for early (T1-2, N0-1) or locally advanced non-metastatic breast cancer were recruited from March 2014 – Sept 2017. Chemotherapy regimen was selected according to local standard-of-care guidelines (FEC “100” in N0, FEC-D in N1, FEC-DH in HER2 +ve, with the addition of pertuzumab when approved). The DDRD score was applied to RNA expression data obtained from pre-treatment FFPE core biopsies using a custom cDNA microarray (Breast Cancer DSA™). Response to NAC was assessed on resection specimens following definitive surgical treatment by pathologists blinded to the DDRD scores, using the Residual Cancer Burden (RCB) assessment tool.

**Results:** Patients with a positive DDRD assay call had an odds ratio for pathological complete response (pCR) or minimal residual disease (RCB0/RCB1) of 4.64 (95%CI 1.31-16.42; p = 0.0174). Patients with RCB0/RCB1 had significantly higher DDRD assay score than those with class RCB2/RCB3 responses (p = 0.0011; unpaired t-test). Tumour subtype and DDRD call are summarised in Table 1.

Table 1: tumour subtype distribution and DDRD assay result

Subtype	DDRD+	DDRD-	Total
Triple negative	7	6	13
HER2+ ER-	5	2	7
HER2+ ER+	6	6	12
ER+ HER2-	8	8	16

In total, 29.2% of patients had a pathological complete response (pCR) (RCB0). Tumor infiltrating lymphocytes (TILs) were assessed on baseline pre-treatment samples. Increasing DDRD assay score was not significantly associated with increasing stromal TILs (p=0.1403; Pearson correlation). In assessing correlation between stromal TILs and response to chemotherapy, there was no significant difference found across RCB categories (p=0.45; one way ANOVA).

**Conclusions:** Preliminary results from the Neo-DDRD biomarker phase trial support the ability of the DDRD assay to predict response to neoadjuvant chemotherapy. The DDRD assay was confirmed to be a better predictor of neoadjuvant chemotherapy response than the presence of TILs. Translational work from this trial is ongoing: serial tissue and plasma samples collected at treatment mid-point and surgical resection will be used to study changes in RNA expression profiles in both DDRD assay positive and negative during chemotherapy, as well as changes in tumour immune infiltrates, peripheral chemokines and circulating tumour DNA profiles. Based on the results from this pilot study, we propose a biomarker-driven phase II trial in the neoadjuvant setting using the DDRD assay to stratify patients for treatment.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-13

On-treatment biomarkers can improve prediction of response to neoadjuvant chemotherapy in breast cancer

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**Purpose:** Neo-Adjuvant chemotherapy treatment is increasingly being used in breast cancer to preoperatively shrink tumour volumes and facilitate surgical plans. These datasets however, are still scarce, making it difficult to assess the relative value of multiple time point biopsies compared to diagnostic only sampling. This study aims to identify sequential samplings intrinsic value.

**Method:** A total of 97 samples from a cohort of 50 neoadjuvant chemotherapy treated primary breast cancer patients (aged 29-76 at diagnosis, Allred status 47:53% +/-, Her2 status 80:20% +/-, mixed grade and menopausal status) taken pre- treatment, at 2 weeks on-treatment, mid chemotherapy and at resection were sequenced with Ion Ampliseq transcriptome yielding expression values for 12,635 genes. Differential expression analysis was performed across response groups (16 Responders, 34 Non-Responders) as defined by Pathological Complete Response and over treatment time to identify significantly differentially expressed genes, pathways and markers indicative of response status.

**Results:** An on-treatment marker for response was identified (AAGAB), which resulted in a testing accuracy of 100% and a validation accuracy of 78% in the I-SPY 1 Trial. AAGAB was predictive of long term survival ( $p = 0.048$  testing,  $p = 0.031$  validation) in both chemotherapy cohorts at the same expression level as defined for treatment response. The single gene on-treatment biomarker, AAGAB proves more performant than established prognostic tests, Mammprint (Edinburgh NEO trial, pre-treatment 61%, on-treatment 63%. I-SPY 1 trial, pre-treatment 60%, on-treatment 66%) and Pam50 RORS (neo trial pre-treatment 50%, on treatment 58%, Magbanua trial pre-treatment 56%, on-treatment 64%)

**Conclusion:** Changes in gene expression of on-treatment chemotherapy breast cancer resulted in the identification of a novel gene marker that was as effective in predicting prognostic status as established prognostic tests. These results support the use of on-treatment testing in breast cancer to improve the accuracy of tumour response prediction.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-14

Circulating lymphocytes and pathologic complete response rate among patients with early stage triple negative breast cancer treated with neoadjuvant chemotherapy

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**Background:** Chemotherapy can affect circulating immune cells. Low lymphocyte counts have been associated with worse prognosis in many cancers and inferior responses to immune checkpoint therapy. The effects of neoadjuvant chemotherapy on the immune system and its association with clinical outcomes in breast cancer is not well described.

**Methods:** A database was constructed of patients diagnosed with early stage triple negative breast cancer (TNBC) treated with neoadjuvant chemotherapy. Clinicopathologic information was extracted from the local electronic tumor registry or by chart review. Circulating lymphocyte and monocyte counts were assessed at the time of diagnosis, after neoadjuvant chemotherapy, and prior to surgery (all values in K/cu mm). These were correlated with clinicopathologic data, pathologic complete response (pCR) rates, and disease free survival (DFS) using rank sum test and Spearman correlation, t-test and log rank test, respectively. **Results:** From 2000-2015, 426 patients with breast cancer treated with neoadjuvant chemotherapy were sequentially identified by an institutional electronic database. After excluding those who did not actually receive neoadjuvant therapy, were a subtype other than TNBC, and had missing receptor status or blood counts, 95 patients met eligibility for analysis. The median age of patients was 50 (range 26-79); 63 (66%) patients were treated with anthracyclines plus taxanes, 29 (31%) platinum-based chemotherapy, 2 (2%) with only anthracyclines, and 1 (1%) with only taxanes; 32 (34%) patients achieved a pCR; and 33 (35%) patients had recurrence events. Median follow up time was 47 months (range 13-123). No significant associations were found between pCR and changes in lymphocyte or monocyte count (mean lymphocyte reduction 0.74 in those with no-pCR versus 0.60 in those with pCR,  $p=0.30$ ; mean monocyte reduction 0.0 in those with no-pCR versus 0.016 in those with pCR,  $p=0.78$ ). There was no correlation between changes in lymphocytes or monocytes with DFS. Baseline lymphocytes or monocytes also did not correlate with pCR or DFS. Notably, there was a correlation between monocytes after neoadjuvant chemotherapy and pCR (mean monocyte level was 0.56 in those with no-pCR versus 0.46 in those with pCR,  $p=0.049$ ) and DFS (median DFS in highest monocyte quartile 30 months versus in lowest quartile 107 months,  $p=0.022$ ).

**Conclusions:** Our results suggest that transient lymphopenia from chemotherapy is not associated with clinical outcomes. However, we observed lower absolute circulating monocyte counts after neoadjuvant chemotherapy were associated with better clinical outcomes. Further research assessing the role of circulating immune cells is required to understand the effects of neoadjuvant chemotherapy on clinical outcomes, which may help inform immunotherapy-based strategies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-15

Development of pan-cancer transcriptional signatures that predict chemosensitivity

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**Background:** Despite the increasing understanding of the molecular characteristics of cancer, chemotherapy success rates remain low for many cancer types. Studies have attempted to identify patient and tumor characteristics that predict sensitivity or resistance to different types of conventional chemotherapies, yet a concise model that predicts chemosensitivity based on gene expression signatures across cancer types remains to be formulated. We attempted to generate a pan-cancer chemosensitivity predictive model using publicly available data from multiple sources. Such a model may increase the likelihood of identifying the type of chemotherapy most likely to succeed for a given patient based on the gene expression signature of their tumor.

**Methods:** Data used to build the predictive model were obtained from the Genomics of Drug Sensitivity in Cancer (GDSC) database, consisting of gene expression profiles from 962 cancer cell lines via RNA sequencing (RNA-seq) and drug sensitivity profiles reported as  $\ln(\text{IC}_{50})$ . Predictive gene signatures were generated using a cross-validated generalized linear model (leave one out cross-validation) using elasticnet penalization parameters. Models were generated for each individual drug tested by the GDSC cohort, as well as different classes of chemotherapeutics (platinum agents, topoisomerase inhibitors, mustard agents, antibiotics and anti-fungals, anti-metabolites, and taxanes). Accuracy of the models was determined using normalized mean square error (nRMSE). Models were then validated using publicly available data from Cancer Cell Line Encyclopedia (CCLE), NCI-60, and the Patient-Derived Xenograft (PDX) Clinical Trial (PCT) database. Models were further validated using human tumor datasets available via the Gene Expression Omnibus (GEO). As the training data used to generate the models were from RNA-seq, and some of the testing and validation data were generated using microarray technology, feature-specific quantile normalization was used to enable cross-platform analyses.

**Results:** For most single-drug gene signatures, accuracy measured by nRMSE ranged from 0.10-0.20, which suggests that for any given model the root mean squared error is 10-20% of the range of actual  $\ln(\text{IC}_{50})$  in the tested data. Chemotherapy class-level models yielded slightly less accuracy, with nRMSE ranging from 0.15-0.25 for most classes. When considering how well the models predicted chemosensitivity within cancer types, accuracy was improved in some cancer types (e.g., lung cancer and head and neck cancer), with more heterogeneous cancer types (e.g., breast cancer) giving less accuracy.

**Conclusions:** Our results show that the models generated can predict chemosensitivity across cancer types with clinical useful levels of accuracy, with some cancer types resulting in a high rate of accuracy across several classes of chemotherapy. The inclusion of future datasets, particularly from those cancer types in which chemosensitivity has been difficult to predict, may provide opportunities to strengthen model accuracy as well as decrease the numbers of genes needed to assess chemosensitivity.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-11-16

Immunosuppressive profiles in liquid biopsy predict response to neoadjuvant chemotherapy in triple negative breast cancer

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## BACKGROUND

Despite the recent advances in triple negative breast cancer (TNBC) stratification, TNBC is still a highly heterogeneous subtype that clusters distinct molecular and genetic alterations with diverse responses to neoadjuvant chemotherapy (NAC). Molecular profiling after NAC has unraveled a group of actionable targets in residual TNBC that supports the implementation of targeted therapies in a personalized manner. However, unresponsive or poor NAC responders after first line treatment are committed to poorer outcome. Therefore, early identification of poor NAC responders is essential to select patients that may benefit from alternative therapies, including initial tumor resection before chemotherapy.

## MATERIAL AND METHODS

We have conducted a study on 37 non-metastatic TNBC patients at La Fe Hospital that were homogeneously treated with anthracycline and cyclophosphamide followed by paclitaxel. Tissue and blood-derived biopsies were obtained at diagnosis. Metabolites and miRNA exosomes in plasma were analyzed by ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometric and by miRNA 3.0 arrays (Affymetrix) respectively. Immunosuppressive subpopulations of cells were quantified by flow cytometry with specific markers. IDO1 *in situ* expression was assessed by immunohistochemistry on the tissue biopsies.

## RESULTS

In order to identify blood-derived liquid biopsy NAC predictor biomarkers in TNBC we have studied blood circulating cells and molecules known for their immunomodulation capacity and found that eMDSC and a profile of tryptophan-derived metabolites predict NAC response. In addition, we identified a circulating exosome miRNA profile that identifies poor NAC responders. Interestingly, this profile of miRNAs target pathways involved in the immune response. IDO1 expression in the tumor inversely correlated with circulating tryptophan levels and directly associated with eMDSC. We also observed a trend correlating IDO1 expression levels with poorer response.

## CONCLUSIONS

Our results strongly support the role of immunosuppression in TNBC poor responders and establish an easy and non-invasive tool for the early identification of poor NAC responders, opening the possibility to use alternative strategies.

## Acknowledgements:

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# 2018 San Antonio Breast Cancer Symposium ®

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A novel biodynamic imaging assay predicts success or failure of neoadjuvant chemotherapy in breast cancer patients

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**Background:** Use of neoadjuvant chemotherapy (NAC) in breast cancer patients has increased significantly over the past decade. The clinical benefits of NAC, including potential to downstage disease, facilitation of breast conserving surgery and use of pathologic response as a prognostic marker, are well established. However, with multiple regimens approved and recommended for NAC, choosing the optimal therapy for individual patients remains a challenge. Biodynamic imaging (BI), a novel technology that captures cellular motility in living tissue via Doppler spectroscopy, could be used ex-vivo to prospectively evaluate the efficacy of systemic therapies in patient tumor samples prior to treatment. This study aimed to determine whether BI could accurately predict likelihood of response to NAC in breast cancer patients.

**Methods:** Fresh core biopsies were obtained from 84 patients prospectively enrolled in an IRB-approved clinical trial at 5 institutions between 1/5/17 and 8/3/2018. Patient tumor tissue was collected at time of routine diagnostic biopsy and sent to a central laboratory where it was divided into intact tumor fragments measuring approximately 1mm in diameter. Fragments were placed into 96 well plates and imaged using the BI assay (Onco4D™), while being challenged by various cytotoxic agents for up to 20 hours. Cellular characteristics and motility signatures were evaluated and compared to pathologic NAC response established upon surgical resection (mastectomy or lumpectomy).

**Results:** At the time of this analysis, centrally-confirmed pathologic response data were available for 17 patients treated with doxorubicin/cyclophosphamide  $\pm$  taxane (AC). Pathologic outcomes are pending for an additional 8 AC patients. The remainder of the 84 patients initially enrolled in the study either did not receive NAC (n=10), have not yet selected a course of therapy (n=12), or received NAC regimens other than AC (n=37). Of the 17 currently evaluable AC-treated patients, 4 had triple negative (TN) disease, 12 were hormone receptor positive, and 1 hormone receptor negative patient showed equivocal HER2 results. Two of the TN patients were known to harbor pathogenic BRCA1 mutations and received carboplatin in addition to AC. Seven of 17 patients (40%) displayed resistance to AC (2 with progressive and 5 with stable disease) while 10 experienced objective response (8 partial and 2 complete response). A multilinear regression model using 10 BI markers accurately classified 16 of 17 patients (94%) while producing 1 false prediction of partial response for a patient with stable disease clinically (R-squared=0.9994, p<.0001). The positive predictive and negative predictive values of BI to AC response were 100% and 91%, respectively.

Table 1. Performance Characteristics

		Clinical		
	Outcome	Response	Non-Response	Total
Classifier	Response	10	1	11
	Non-Response	0	6	6
	Total	10	7	17

**Conclusion:** BI was able to accurately predict patient response to neoadjuvant AC, demonstrating the potential for the platform to support personalized patient therapy. This clinical trial is ongoing and will report out results for TC (docetaxel/cyclophosphamide), TCHP (docetaxel/carboplatin/trastuzumab + pertuzumab), and additional AC patients as outcome data are accrued.



# 2018 San Antonio Breast Cancer Symposium ®

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Risk of ischemic heart disease after adjuvant radiotherapy for breast cancer

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**Background:** Adjuvant radiotherapy (RT) for breast cancer (BC) substantially reduces BC mortality and loco-regional recurrences, but incidental radiation exposure to the heart is associated with ischemic heart disease (IHD). We examined the incidence of IHD in a large population-based cohort of women with BC.

**Patients and methods:**

The Breast Cancer DataBase Sweden (BCBase) cohort includes all women diagnosed with BC in three of Sweden's six health care regions from 1992-2012 with five age-matched controls without a history of BC for each BC case. A total of 60217 women with BC were included in the BC cohort, and 300791 women without BC in the comparison cohort. Through linkage with a number of population-based registries, information concerning comorbidity, socioeconomic status, and incidence of IHD was obtained. Cox proportional hazards regression analyses were performed to estimate risk of IHD for women with BC compared to the comparison cohort, and for women with left-sided BC compared to right-sided BC. The analyses were adjusted for previous IHD, comorbidity, and socioeconomic status. The BC cohort was stratified by RT, endocrine therapy, and chemotherapy.

**Results:**

The median follow-up time was 8.1 years. The risk of IHD was significantly lower for the BC cohort compared to the comparison cohort, with a hazard ratio (HR) of 0.91 (95% CI 0.88-0.95). The HR's for IHD was even lower in women with BC selected for adjuvant treatment with RT, endocrine therapy or chemotherapy. When women with left-sided BC were compared to right-sided BC an increased HR for IHD of 1.09 (95% CI 1.01-1.17) was seen for the whole cohort, and of 1.18 (95% CI 1.06-1.31) in women receiving RT. When RT was stratified for pathological nodal involvement, a HR of 1.22 (95% CI 0.98-1.51) for women with 1 to 3 pathological lymph nodes was seen, and of 1.72 (95% CI 1.19-2.48) for women with more than 4 pathological lymph nodes, probably reflecting more extensive RT. When RT was combined with other adjuvant treatments, a HR for IHD of 1.24 (95% CI 1.09-1.42) was seen for endocrine therapy, of 1.28 (95% CI 0.98-1.67) for chemotherapy, and of 1.35 (95% CI 0.95-1.92) for endocrine therapy and chemotherapy combined in left-sided BC compared to right-sided BC, suggesting an additive effect to RT on the risk of IHD.

**Conclusion:**

The results show a persisting increase in risk of IHD in left-sided RT with contemporary radiation techniques and radiation targets. The increase in risk of IHD in women with left-sided RT seen when endocrine therapy and chemotherapy were added to RT suggests an additive effect on the risk of radiation-induced IHD. Long-term side effects of adjuvant treatment have to be taken into consideration in RT planning to ensure health and quality of life for BC survivors. The results are an incentive to conduct further research concerning dose constraints to the coronary arteries, and of implementation of RT techniques that can lower cardiac radiation doses. Selection of patients to active treatment, and a healthier lifestyle in BC survivors may explain the findings of lower risk of IHD in the BC cohort compared to the comparison cohort.

# 2018 San Antonio Breast Cancer Symposium ®

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Intraoperative radiotherapy outcomes in early-stage breast cancer: A study in elderly Canadian women

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**Objective.** Breast-conserving therapy with external beam radiotherapy (EBRT) is currently the standard of care for women with early breast cancer. Our aim was to determine if early-stage breast cancers treated with lumpectomy and primary intraoperative radiotherapy (IORT) have comparable local recurrence rates. This is the first study examining the Canadian experience with IORT.

**Methods.** Patients who underwent breast-conserving therapy with pre-pathology IORT between 2007- 2017 were retrospectively identified. The primary outcome measure was ipsilateral breast tumor recurrence (IBTR). A time to event analysis was performed; Kaplan-Meier estimates report the fraction of patients living free of recurrence. Secondary outcomes included acute and chronic wound complications.

**Results.** 106 patients with a median age of 70 (IQR 65-75) were included. Median follow-up was 33 months. The majority of patients had screen-detected (94.3%), estrogen-receptor positive (96.2%), HER2neu negative (93.4%), invasive ductal carcinomas (92.5%). Only 50 (47.6%) were prescribed adjuvant endocrine blockade. IBTR occurred in 5 (4.7%) patients. Five and ten-year local recurrence-free rates were 0.95 and 0.81, respectively. The superficial skin infection rate was 9.4%. Acute symptomatic seromas occurred in 23 (21.7%), while only 10 (9.4%) persisted chronically.

**Conclusion.** In this cohort of Canadian post-menopausal women treated with breast-conserving surgery and IORT, the IBTR approached 5%. Despite selection of low-risk patients, the local recurrence rate is higher than what is reported in the literature with EBRT. The low rates of prescribed adjuvant systemic therapy may have contributed to this outcome.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-03

Analysis of radiation dose in the gap region between the supraclavicular target volume to the internal mammary target volume in women receiving regional nodal irradiation

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**Purpose:** Consensus guidelines for regional nodal irradiation (RNI)/postmastectomy radiation (PMRT) clinical target volumes (CTV) have slight variations amongst leading national organizations. In the US, the Radiation Therapy Oncology Group (RTOG) defines the caudal edge of the supraclavicular (SCV) CTV as the junction of the brachiocephalic and axillary vessels while the internal mammary nodal (IMN) CTV starts at the superior aspect of the medial first rib. This leaves an anatomical gap between the two target volumes. The European Society of Radiation Oncology (ESTRO) does not recommend leaving a gap between the SCV CTV and IMN CTV. We set to analyze radiation dose and patterns of failure in this region.

**Materials and Methods:** We identified consecutive patients treated with RNI/PMRT at our institution from 2013-2016. Patients with metastatic or recurrent disease were excluded. All patients received 50 Gy/25 fractions to the breast/chestwall+regional nodes (including IMN PTV) +/- boost to the lumpectomy cavity/mastectomy scar using 3D conformal radiotherapy (3DCRT) or intensity modulated radiation therapy (IMRT). We retrospectively contoured the vessels from one slice below the caudal border of the SCV PTV contour to one slice cranial to the first IMN PTV contour. We calculated the mean dose and the relative V40Gy, V45Gy, and V47.5Gy of the gap region. A gap failure was defined as a first recurrence in this region with or without simultaneous loco-regional recurrence (LRR) or distant metastases (DM). We used the cumulative incidence method to calculate the gap recurrence rate with DM, LRR, and death, as competing risks.

**Results:** 230 patients were included with median age 52 years, predominantly stage III disease (60%), and most treated with preoperative (51%) or postoperative (41%) systemic therapy. Breast cancer subtype was ER+/HER2- in 138 patients, triple negative in 44 patients, and HER2+ in 48 patients. The median (IQR) mean dose, V40Gy, V45Gy, and V47.5 Gy in the gap region were: 20.3 Gy (14.8-26.2 Gy), 6% (1.3%-20.0%), 0.6% (0%-7.0%), and 0% (0%-1.3%). The mean dose to the gap region was slightly higher in patients treated with IMRT (N=68) compared to 3DCRT (N=162): 25.3 Gy (SD 7.5 Gy) vs. 19.5 Gy (SD 8.0 Gy),  $p < 0.0001$ . With median follow-up of 32 months, there were 2 recurrences in the gap region, both of which occurred with simultaneous distant metastases. No patients had isolated recurrences in the gap region. The 3-year cumulative incidence of recurrence in the gap region was 0.8%. The predominant pattern of failure was DM (N=31) with a 3-year rate of 14.4% followed by LRR (N=6, 4 with simultaneous distant metastases) with a 3-year rate of 3.1%.

**Conclusion:** In a clinical practice in which we routinely contour and treat the IMN PTV and SCV PTV with a gap region between those two volumes, we found that the mean radiation dose to this region is low, at about 50% or less compared to the prescription dose. Despite this, recurrences in this region are exceedingly uncommon and have not yet occurred in the absence of simultaneous DM. While the follow-up is limited, these data support the current guidelines of not routinely targeting this region.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-04

Analysis of radiation dose to the shoulder by treatment technique and correlation with patient reported outcomes in patients receiving regional nodal irradiation

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**Background:** Shoulder/arm morbidity is a late complication of breast cancer treatment. Postmastectomy radiation therapy (PMRT)/regional nodal irradiation (RNI) increases dose to the muscles and soft tissues of the shoulder and upper neck and back. Most patients are treated with 3D conformal radiation therapy (3DCRT) or Intensity modulated radiation therapy (IMRT). Here, we set to analyze the impact of 3DCRT vs. IMRT on radiation dose to the shoulder, and to retrospectively explore the relationship of treatment technique on long term patient-reported outcomes in the subset of patient who had completed the quick Disabilities of the Arm, Shoulder, and Hand (q-DASH) questionnaire.

**Materials/Methods:** We identified consecutive patients in our department treated with PMRT/RNI for curative intent from 2013-2016. We excluded patients treated for recurrent disease, those with metastatic disease, and those with unresected disease in the supraclavicular (SCV) fossa and/or axillary apex requiring a radiation boost to that area. We contoured the shoulder as all of the muscles/soft tissue/bone from 2 cm above the ipsilateral SCV planning target volume (PTV) to the cranial aspect of the breast or chestwall PTV. No planning constraints were set for the shoulder since this was retrospectively contoured. We used the dose volume histogram to determine the volume of shoulder receiving at least 5 Gy, 10 Gy,...,50 Gy (V5-V50, respectively). We identified patients that completed a q-DASH questionnaire  $\geq 6$  months from the end of PMRT/RNI. Descriptive statistics were used to summarize the shoulder dose and q-DASH values. Differences between groups were assessed by the t-test or chi-square test with  $p < 0.05$  considered significant.

**Results:** We found 237 patients treated with PMRT/RNI with median age of 52 y (IQR 44-60 y), 75% treated with mastectomy, 85% had axillary lymph node dissection (ALND), median of 18 nodes removed (IQR 12-26). All patients received 50 Gy/25 fractions. A total of 68 patients (28.7%) were treated with IMRT. IMRT significantly reduced the V20-V50 to the shoulder vs. 3DCRT (e.g., V45Gy=21.7 mL vs. 208.4 mL,  $p < 0.0001$ ). Of the 237 patients, 66 had completed a q-DASH at least 6 months from the end of radiation therapy (median, 14.5 months). Patients that completed the q-DASH vs. not were similar in age ( $p = 0.29$ ), number of nodes removed ( $p = 0.17$ ), use of ALND ( $p = 0.13$ ), use of chemotherapy ( $p = 0.49$ ) and use of mastectomy ( $p = 0.22$ ). The median (IQR) and mean (SD) q-DASH were 20.5 (6.8-38.6) and 24.3 (20.2) for all patients; 20.5 (9.1-38.6) and 24.1 (19.4) for the 53 mastectomy patients; 18.2 (4.5-45.5) and 25.2 (24.2) for the 13 lumpectomy patients. Most patients (N=49) were treated with 3DCRT. Compared to patients treated with 3DCRT, IMRT patients had a trend towards lower q-DASH mean scores: 16.9 vs. 26.9,  $p = 0.077$ .

**Conclusion:** In summary, we found that IMRT reduces radiation dose to the shoulder and is associated with a trend towards reduced q-DASH scores at least 6 months after PMRT/RNI in a subset of our cohort. These results support prospective evaluation of IMRT as a technique to reduce shoulder morbidity in breast cancer patients receiving PMRT/RNI.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-05

Fractionation patterns in adjuvant breast radiotherapy in Ontario, Canada from 2009 to 2015

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**Purpose:** To report the patterns of use of hypofractionated radiotherapy (HFRT) ( $\leq 16$  fractions) in breast cancer and ductal carcinoma in situ (DCIS) patients in Ontario, Canada from 2009 to 2015 and identify factors related to HFRT use.

**Methods:** A retrospective cohort study of Ontario women diagnosed with breast cancer or DCIS followed by adjuvant breast or chest wall radiation (RT) from 2009 to 2015 was conducted using data from the Institute for Clinical Evaluative Sciences (ICES). Logistic regression models were used to identify factors associated with HFRT use. Physician was included as a random effect. To calculate the potential amount of time that could be saved if all patients were to receive 16 fractions of HFRT, the number of extra RT treatments after the 16<sup>th</sup> visit was multiplied by the median amount of time spent to treat one patient at our cancer centre (8.76 minutes). This time was found with Sunnybrook Health Sciences Centre data collected from October 2017 to March 2018 (n=523) and represents the amount of time from when a patient enters the RT unit for treatment setup, until the RT beam is turned off.

**Results:** A total of 42,072 patients were included. Most patients were aged between 50 and 69 years old (56.7%) with stage I or II breast cancer (74.6%) and had breast conserving surgery (BCS) (76.9%). Half previously received chemotherapy and 7.9% were DCIS patients. Use of sequential boost, simultaneous boost, and regional nodal RT was 17.2%, 3.1%, and 31.7% respectively. Institutional variation in HFRT use ranged from 27.5% to 71.6%. HFRT use has increased in all patient populations over the study period. HFRT use was more common in breast cancer and DCIS patients with BCS than in chest wall and nodal RT.

Trends in the use of HFRT (%) from 2009 to 2015 for DCIS and breast cancer patients in Ontario.

Year	Stage I-IV + BCS + Breast RT	Stage I-IV + BCS + Breast + Nodal RT	Stage I-IV + Mastectomy + Breast RT	Stage I-IV + Mastectomy + Breast + Nodal RT	DCIS + BCS
2009	62.6	10.3	29.0	9.5	45.2
2010	62.0	6.3	23.7	6.9	51.2
2011	62.2	4.1	21.3	4.7	52.7
2012	63.7	9.6	21.7	11.4	52.0
2013	65.1	12.6	29.3	15.0	57.3
2014	69.3	7.8	35.4	15.1	64.5
2015	73.7	11.8	39.2	17.5	73.4

BCS: breast conserving surgery, RT: radiation treatment

Simultaneous boost (OR=0.09), nodal RT (OR=0.08), previous chemotherapy (OR=0.7), stage 0, II, and III breast cancer (OR=0.06 relative to stage I), were correlated with less HFRT use. Older age, later year of diagnosis, sequential boost (OR=0.09), BCS and no surgery (OR=1.2, 1.5 relative to mastectomy) were correlated with higher HFRT use. Institution was significantly correlated to HFRT use. The variance estimate for the physician random effect was 0.33 (p<0.0001). For breast cancer patients with BCS and breast RT specifically, 62,396 extra visits occurred from 2009 to 2015, corresponding to ~9100 hours of treatment if all patients received HFRT. For the entire patient population, a total of 190,726 extra visits occurred or ~27,900 hours.

**Conclusions:** HFRT use in Ontario has increased over time for all patient populations, and reflects the current evidence supporting HFRT in different patient populations, with lower HFRT use seen in chest wall and nodal RT and higher HFRT use in early-stage breast cancer patients with BCS.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-06

**TOxicities of Locoregional Radiotherapy Associated with Bevacizumab in patients with non-metastatic breast cancer (TOLERAB): Final long-term evaluation**

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**Background and Purpose:** Recent phase 3 clinical trials have evaluated the addition of bevacizumab (B) to standard chemotherapy in the treatment of patients with non-metastatic breast cancer. But few data are available about the tolerance of B with locoregional radiation therapy (RT). The objective was to evaluate the 5 years late toxicities of the concurrent B and RT in non-metastatic breast cancer.

**Material and methods:** This is a multicenter prospective study including non-metastatic breast cancer patients enrolled in phase 3 clinical trials evaluating B with concurrent RT (BEATRICE, BETH, BEVERLY 1, BERVERLY 2) versus RT alone. All patients received neo-adjuvant or adjuvant chemotherapy and normo-fractionated breast or chest wall RT, with or without regional lymph nodes RT. B was administrated as an equivalent of 5 mg/kg every week for 1 year. The safety profile (using the Common Terminology Criteria for Adverse Events version 3.0) was evaluated at 1, 3 and 5 years after the completion of radiotherapy.

**Results:** From October 2007 to January 2012, 151 patients totally included. Median follow-up was 60 months (36-84) and 5 years late toxicities were available for 104 patients (46 with B and RT, 58 with RT alone). Median age was 51 (22-81). 61% of patients received regional lymph nodes RT. The majority of tumor was triple negative (65.6%), tumor size <2cm (50%) and nodal status negative (63.8%). Median total dose of B was 15000 mg (13200 – 18550) and median duration was 11.2 months (11-12.6). No grade ≥3 toxicity was observed. Only 16 patients had grade 1-2 toxicities (8 treated with B and RT, 8 with RT alone): n=4 (3.8%) had grade 1 pain, n=5 (4.8%) had grade 1-2 fibrosis, n=1 (1%) had grade 2 telangiectasia and n=5 (4.8%) had grade 1-2 lymphoedema. No significant difference between the 2 arms was observed. One patient of 46 evaluated had Left Ventricular Ejection Fraction inferior to 50%. At 5 years, overall survival was 93.8%, disease free-survival 89% and locoregional free-survival 93.1 %.

**Conclusion:** Concurrent B and locoregional RT provides acceptable 5-years toxicities in patients with non-metastatic breast cancer. No grade ≥3 toxicity was observed.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-07

Conservative breast reconstruction: Outcomes of 146 consecutive cases of prepectoral, subcutaneous implant-based breast reconstruction in a single-Institution series

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**Aims.** To evaluate acute and late toxicity-related factors among breast cancer (BC) patients who underwent prepectoral breast reconstruction (BR).

**Methods.** We performed a retrospective analysis of BC patients who underwent therapeutic or prophylactic mastectomy from October 2012 to May 2016 at our Center. We recorded individual patient-related features (i.e. age, body mass index [BMI], smoke-history, comorbidity, BRCA-carrier), BC-related treatments characteristics (i.e. axillary surgery, adjuvant radiotherapy [RT], adjuvant chemotherapy, primary systemic therapy [PST], endocrine therapy, and use of trastuzumab). Toxicity profile was evaluated in terms of complications related to BR; we recorded acute and late toxicity data and prosthesis/implant explant rate.

**Results.** We analyzed 146 consecutive BC patients treated with subcutaneous BR, 117 therapeutic and 29 prophylactic mastectomies. Thirty-seven patients received postmastectomy RT. Significant factors related to acute toxicity were: previous RT (34.5% [RT] vs 8.5% [no RT];  $p=0.001$ ), BMI (31.3% [BMI  $\geq 25$ ] vs 8.8% [ $<25$ ];  $p=0.003$ ), previous breast surgery (22.2% [surgery] vs 8.7% [no surgery];  $p=0.027$ ), and diabetes (100% [diabetes] vs 11.9% [no diabetes];  $p=0.002$ ). Factors significantly correlated to implant/prosthesis explant were: current or previous smoking exposition (13.8% [smokers] vs 2.6% [non-smokers];  $p=0.029$ ) and PST (18.8% [PST] vs 3.5% [no PST];  $p=0.022$ ); axillary lymph node dissection (ALND) was significantly related to late toxicity (5.7% [ALND] vs 0%;  $p=0.04$ ). At a 3-year median follow up, three deaths, five locoregional recurrences (LRR), and fourteen distant metastasis (DM) occurred among 117 patients treated by therapeutic mastectomy. Overall survival was 78.1%, LRR free-survival was 95%, and DM free-survival was 71.6%. Postmastectomy RT was not significantly related to acute, late toxicity, and explant occurrence.

**Conclusions.** In our experience prepectoral subcutaneous implant-based BR is a safe and effective approach, with low rates of acute toxicity. Major risk factors were evidenced for patients previously treated with RT or surgery, and in case of diabetes or BMI  $\geq 25$ ; postmastectomy RT seems not to be related to higher rate of toxicity. ALND seems to be the only factor significantly related to late toxicity. PST, and smoking exposition were significantly associated with higher rate of implant/prosthesis explant. However, further investigations and mature follow-up are warranted to confirm these encouraging results.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-08

The role of neo-bioscore staging system in guiding the optimal strategies for regional nodes irradiation following primary systemic treatment in breast cancer patients with cN1 and ypN0-1

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**Purpose:** Following primary systemic treatment (PST), the optimal strategies for regional nodal irradiation (RNI) are currently under active investigation, especially in patients with pretreatment cN1 and posttreatment ypN0-1. The Neo-Bioscore staging system has showed promising prospect in assessing prognosis after PST. In this analysis, we evaluate the role of Neo-Bioscore staging system in guiding RNI following PST in patients with cN1 and ypN0-1.

**Methods and Materials:** Continuous women with cN1 pretreatment and who received PST with ypN0 or ypN1 posttreatment between 2009 and 2014 were retrospectively reviewed. According to the report by Mittendorf et al, the Neo-Bioscore staging system is on the basis of pretreatment clinical stage, posttreatment pathologic stage, estrogen receptor (ER) status, HER2 status and grade stage, and assigned and summed points for each factor as shown in

## Point Assignments for the Neo-Bioscore Staging Systems

Factors	Points
Clinical stage	
I	0
IIA	0
IIB	1
IIIA	1
IIIB	2
IIIC	2
Pathologic stage	
0	0
I	0
IIA	1
IIB	1
IIIA	1
IIIB	1
IIIC	2
Tumor Biomarker	
ER negative	1
HER2 negative	1
Grade 3	1

. A pathologic complete response (pCR) was defined as no invasive disease in the breast or regional lymph nodes after surgery.

The curves for survival were generated using the Kaplan-Meier method and compared using the Log-rank test.

**Results:** One hundred and sixty-three patients were enrolled in this study, of them 18 patients received breast conserving surgery.

Of the 163 patients, 119 (73%) received RNI. At surgery, 36 patients (22.1%) achieved pCR, while 89 patients (54.6%) achieved ypN0. The median follow-up was 59.4 months (rang: 16-106). In the whole cohort, RNI was associated with non-significant improved outcomes, with a 5-year locoregional recurrence free survival (LRRFS) rate of 98.6% vs. 95.7% (P=0.393), a regional



recurrence free survival (RRFS) rate of 98% vs. 97.7% ( $P=0.865$ ), a 5-year distant metastasis free survival (DMFS) rate of 91.6% vs. 83.4% ( $P=0.052$ ), a 5-year any first recurrence free survival (RFS) rate of 90.9% vs. 87.4% ( $P=0.43$ ), and a 5-year overall survival (OS) rate of 98% vs. 91.9% ( $P=0.097$ ) in the RNI and non-RNI group, respectively. In the subgroup of patients with Neo-Bioscore score of 1 to 3, RNI significantly increased the 5-year DMFS rate of 97% vs. 76.9% ( $P=0.002$ ), 5-year RFS rate of 95.5% vs. 76.9% ( $P=0.007$ ) and 5-year OS rate of 100% vs. 89.2% ( $P=0.005$ ). However, no significant difference in outcomes was found between RNI and non-RNI group in patients with Neo-Bioscore score of 4 to 6. Among patients who not achieved pCR and those with ypN1, RNI significantly increased DMFS (both  $P<0.05$ ).

Conclusions: Not all patients with cN1 and ypN0-1 could significantly benefit from RNI following PST, those with low Neo-Bioscore score were likely to benefit more.

# 2018 San Antonio Breast Cancer Symposium ®

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Dosimetric analysis of the pattern of local recurrence in breast cancer patients undergoing breast reconstruction and post-mastectomy radiotherapy

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## Background

This study aims to identify spatial and dosimetric patterns of breast cancer relapse in a mono-institutional large series of patients treated with mastectomy and various forms of breast reconstruction.

## Material and Methods

We retrospectively reviewed 196 patients with Stage II-III breast cancer treated with modified radical mastectomy between 1995 and 2016 at the Radiotherapy Department of Careggi Hospital, Florence. All patients performed Skin-sparing mastectomy or nipple-sparing mastectomy with immediate or delayed breast reconstruction and subsequently received post-mastectomy radiotherapy (PMRT). Systemic therapy was prescribed as per local and international guidelines both in neoadjuvant and adjuvant setting. All patients were treated with 3DCRT technique. Diagnostic imaging (e.g. CT, MRI) obtained at recurrence were registered with the original planning computed tomography (pCT) for the dosimetric analysis. Recurrence gross tumor volume (rGTV) were delineated and co-registered with pCT. All rGTV were compared dosimetrically to planned dose and spatially with planning target volumes. Locoregional recurrence (LR) were divided in three categories relative to the high dose region, 95% of prescription dose (D95%). We defined “in field LR” those with more than 90% of their volume within D95% region, “marginal LR” when recurrence volume was between 20 and 90% within D95% and “outfield LR” those with less than 20% of their volume D95%.

## Results

The median age was 49 years (range, 26 - 83 years). 163 women (83 %) were classified as stage III; 118 women (60 %) had more than three positive axillary nodes. The majority of lesions were estrogen receptor positive (75 %), grade 3 (52%), with the presence of LVI (60 %). Adjuvant RT at chest wall were performed in all patients, most of whom underwent a RT of chest wall +infra- supraclavicular nodes (71%). Prescribed RT dose was 50 Gy in 25 fractions. With a median follow-up of 60 months (range, 12-240 months), 22 (11%) patients experienced a locoregional relapse: 10 patients in the chest wall; 7 in ipsilateral axillary region +/- chest wall; 3 in internal mammary nodes +/- chest wall; one locoregional relapse was observed in the supraclavicular nodes. Most of relapses were G3 (75%) with documented LVI (79%). The topographic analysis of the local failure patterns showed: thirteen (59 %) were “in-field LR”; 9 (41%) were “out-field LR”. For the spatial analysis, all relapses on the chest wall were observed above the breast tissue expander or mammary prosthesis. Free time from recurrence disease was 30 months (range 5-86 months).

## Conclusion

This study suggests that chest wall recurrences are rare after PMRT and are related to biologic aggressiveness of the disease than to inadequate irradiation of target volumes. Prospective studies are warranted to evaluate the relationship between treatment volumes and patterns of recurrences in order to refine new delineation guidelines for women undergoing PMRT and breast reconstruction.

# 2018 San Antonio Breast Cancer Symposium ®

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Utilization patterns and temporal trends of internal mammary nodal irradiation (IMNI) at a tertiary cancer centre

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**Purpose:** Despite data from multiple randomized trials, the role and uptake of internal mammary nodal irradiation (IMNI) is variable. This study was designed to quantify the rates and determinants of IMNI at a tertiary cancer centre.

**Methods:** Treatment records of consecutively treated breast cancer (BC) patients receiving adjuvant locoregional (LR) radiotherapy (RT) from January 1, 2012 to October 31, 2017 was studied. LR-RT and use of IMNI as a function of clinicopathological factors, use of deep inspiratory breath-hold (DIBH) and dosimetric parameters were retrieved. Patients were divided into two groups: Group 1 received LR-RT that included the IMN's, supraclavicular (SCLV) ± axillary regions, Group 2 received LR-RT directed only to the SCLV ± axillary areas. For the purpose of utilization analysis and temporal trends, early (2012-2015) and late (2016-2017) cohorts were examined based on the year of RT delivery. To determine if the use of IMNI was dependent on BC risk, we defined 3 risk categories: 1) pT1/2, N0; 2) pT1/2, N1; and 3) pT3/4, N2/3 disease. Differences between the risk categories and groups were evaluated using chi-square/ Fisher's and Mann Whitney test for categorical and continuous variables, respectively. Univariable and multivariable logistic regression analysis was done to determine factors associated with the receipt of IMNI.

**Results:** A total of 1566 patients met eligibility (Group 1=376; Group 2=1190). Of these patients, the percentage receiving LR-RT remained constant (17%) over the study period but the proportion of patients receiving IMNI increased significantly each year ( $p<0.0001$ ), and was higher in the late vs. early treatment cohort (55% vs 8%,  $p<0.0001$ ). On univariable analysis, younger age, LVI positivity, medial/central location, increasing stage, PR negativity, mastectomy, axillary dissection, receipt of chemotherapy and increasing number of positive nodes had higher odds of receiving IMNI. Radiation oncologists with  $<$  or  $\geq 5$  years of practice was predictive of IMNI (31.3% vs 20.5%,  $p<0.0001$ ), staff having  $< 5$  years in practice being more likely to recommend IMNI. The distribution of patients in the different risk categories was similar between Groups 1 and 2 ( $p=0.097$ ), and identified that the majority of patients receiving IMNI were in risk category 2 (83%). Further comparison of risk categories suggested that the odds of receiving IMNI was lower in risk category 3 vs. category 1 ( $p=0.033$ ). On multivariable analysis, decreasing age ( $p<0.001$ ), medial quadrant ( $p=0.0026$ ), PR negative ( $p=0.0011$ ), mastectomy ( $p=0.0055$ ), increasing nodal positivity ( $p<0.0001$ ) and late RT cohort ( $p=0.001$ ) had increased odds of IMNI. Overall use of DIBH was significantly higher in those receiving IMNI (45% vs 26%,  $p<0.0001$ ). Mean heart (2.2 vs 1.7 Gy,  $p<0.0001$ ) and total lung doses (7.8 vs 6.6 Gy,  $p<0.0001$ ) were also significantly higher with IMNI.

**Conclusion:** There was a significant increase in utilization of IMNI from 2012 to 2017. Younger age, medial location, PR negativity and increasing number of positive nodes predicted for receipt of IMNI. Staff with  $< 5$  years in practice were more likely to recommend LR-RT that included the IMNs. The use of DIBH significantly increased with IMNI and allowed for acceptable dosimetric constraints.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-11

Prophylactic application of SkinSafe film strips to the inframammary fold prevents moist desquamation during irradiation for breast cancer in a prospective trial

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**Purpose:** To investigate the effect of prophylactic use of SafeSkin films strips applied to the inframammary fold during whole breast or reconstructed breast irradiation to avoid moist desquamation to the inframammary fold.

**Materials and Methods:** Between November 15, 2016 and April 17, 2018, a total of 30 patients were recruited, with 29 patients contributing to analysis, and one patient with bilateral breast cancer. Areas of the breast treatment field not covered by the film were treated with physician's choice of cream, (80% Jean's Cream, 13% Aquafor, and 7% with no topical therapy). The treatment field was photographed weekly. SkinSafe strips were reapplied as needed, but were not applied if erythema was already observed in the inframammary fold. Severity of skin reaction was assessed using the RTOG scale.

**Results:** There was only one treatment interruption, against medical advice, due to axillary discomfort. One patient refused further trial participation after the first day. No short or long term side effects of film use were noted. Inframammary desquamation developed in only one patient, who tried to replicate the increased comfort of her inframammary tape with adhesive bandages when her tape came off. There was no moist desquamation under the SkinSafe film in the inframammary field in any patient. Axillary moist desquamation, where no film, but rather physician's choice of topical cream was applied, remained at 35%. Areas of skin adjacent to the SkinSafe film showed desquamation in 9.7% of patients, 0.5-1 cm in maximal diameter, all of which had resolved at time of completion.

**Conclusions:** Prophylactic use of SkinSafe film strips applied to the inframammary fold substantially reduces or eliminates the risk of inframammary desquamation during breast radiation.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-12

**Impact of regional nodal irradiation for breast cancer patients with supraclavicular and/or internal mammary lymph node involvement: A multicenter, retrospective study (KROG 16-14)**

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**Purpose:** To evaluate the treatment outcomes of radiotherapy (RT) for breast cancer with ipsilateral supraclavicular (SCL) and/or internal mammary (IMN) lymph node involvement.

**Methods:** A total of 353 patients from 11 institutions were included. One hundred and thirty-six patients had SCL involvement, 148 had IMN involvement, and 69 had both. All patients received neoadjuvant systemic therapy followed by breast conserving surgery or mastectomy, and postoperative RT to whole breast/chest wall. As for regional lymph node irradiation, SCL RT was given to 344 patients, and IMN RT to 236 patients. The median RT dose was 50.4 Gy.

**Results:** The median follow-up duration was 61 months (range, 7-173). In-field progression was present in SCL (n=20) and/or IMN (n=7). The 5-year disease-free survival (DFS) and overall survival rates were 57.8% and 75.1%, respectively. On multivariate analysis, both SCL/IMN involvement, number of axillary lymph node  $\geq 4$ , triple negative subtype, and mastectomy were significant adverse prognosticators for DFS ( $p = 0.022$ ,  $0.001$ ,  $0.001$ , and  $0.004$ , respectively). Regarding the impact of regional nodal irradiation, SCL RT dose  $\geq 54$  Gy was not associated with DFS (5-yr rate, 52.9% vs. 50.9%,  $p = 0.696$ ) in SCL-involved patients, and the receipt of IMN RT was not associated with DFS (5-yr rate, 56.1% vs. 78.1%,  $p = 0.099$ ) in IMN-involved patients.

**Conclusion:** Neoadjuvant chemotherapy followed by surgery and postoperative RT achieved an acceptable in-field regional control rate in patients with SCL and/or IMN involvement. However, a higher RT dose to SCL or IMN RT was not associated with the improved DFS in these patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-13

Comparison of accelerated partial breast irradiation (APBI) with whole breast irradiation (WBI) using 3D conformal external beam radiation therapy (3D CRT)

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## **Purpose:**

To compare accelerated partial breast irradiation with whole breast irradiation in post breast conservation surgery (BCS) women with breast cancer.

## **Material and methods:**

Women >35 years of age with invasive or noninvasive breast cancer ≤4 cm treated by BCS were randomized to 3D CRT APBI (34 Gy in 10 fractions given twice daily) or WBI (40 Gy in 16 fractions given once daily ± boost irradiation). The primary outcome was ipsilateral breast tumour recurrence and important secondary outcomes were adverse cosmetic outcome (fair or poor on a HARVARD/NSABP/RTOG breast cosmesis grading scale) and toxicity using the RTOG scores and LENT SOMA scale. Radiation toxicities and cosmesis was assessed directly by radiation oncologist during radiotherapy and in follow up. Patient and tumour characteristics, locoregional recurrence and distant metastases rates were compared using Fisher's exact tests. All statistical tests were two sided p values less than 0.05 were considered statistically significant.

## **Results:**

Between June 2011 and December 2015, 133 women were randomized to 3D CRT APBI or WBI. Patient characteristics were balanced between two arms

Table 1. Patient and tumor characteristics

Characteristics	APBI(n=65)	WBI(n=68)	p-value
Mean age(range)	50(36-75)(36-75)	50(37-67)(37-67)	0.13
T- Stage			
T1	34(52)	37(54)	
T2	28(43)	28(41)	0.96
T3	3(5)	3(4)	
N stage			
N0	57(88)	59(87)	
N1	7(11)	28(41)	1.00
N2	1(1)	1(1)	
Grade			
1	14(22)	12(18)	
2	37(56)	40(59)	0.5
3	14(22)	16(23)	
Surgical margins			
Negative	60(92)	61(90)	0.79
Positive	5(8)	7(10)	
LVI			
No	56(86)	55(81)	0.49
Yes	9(14)	13(19)	
ER			

Positive	42(65)	45(66)	
Negative	19(29)	21(31)	1.00
Unknown	5(6)	2(3)	
PR			
Positive	38(58)	39(57)	
Negative	23(35)	25(37)	1.0
Unknown	4(7)	4(6)	
Her2-neu			
Positive	7(11)	6(9)	0.78
Chemotherapy			
Yes	35(54)	52(76)	0.78
No	30(46)	16(24)	
Hormone therapy			
Yes	49(75)	49(72)	0.78
No	16(25)	19(28)	
Trastuzumab			
Yes	3(43)	3(50)	1.00
No	4(57)	3(50)	

. There was an increased rate of acute grade 2 dermatitis in WBI arm( $p=0.33$ ). Median follow up was 60 months(range 9-84 months). Grades 1 and 2 late radiation toxicities were higher in the WBI arm compared to the APBI arm; pigmentation, fibrosis and breast shrinkage were significantly more in the WBI arm. Grades 3 and 4 late toxicity was not seen in any of the treatment arms. Adverse cosmesis at last follow up was significantly higher in patients treated with WBI, 32% as compared to 6% with APBI ( $p<0.001$ ). Local recurrence with APBI was 3% as compared to 1.5% with WBI and distant metastasis rate was high in WBI arm as compared with APBI, 5.9% vs 3% respectively; both not statistically significant.

#### **Conclusion:**

In women with BCS, APBI was associated with better cosmetic outcome and late radiation toxicities compared to WBI. Local recurrence and distant metastasis was comparable in both the arms.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-14

Incidence of radiation induced sarcoma attributable to radiotherapy in adults: A population-based study of the SEER cancer registry across 17 primary tumor sites

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**Background:** More than half of cancer patients will receive radiotherapy during the treatment of their cancer; however, a rare and fatal treatment complication is radiation induced sarcoma (RIS). Notably, a large percentage of RIS patients are breast cancer survivors. Previous studies have investigated the incidence of all secondary cancers due to radiotherapy but have not noted the incidence of RIS in a contemporary population-based cohort in the US. To evaluate the relative risk of RIS, we examined data from the Surveillance, Epidemiology, and End Results (SEER) database. We hypothesized that breast cancer would have a higher incidence of RIS compared to 17 other primary cancer sites.

**Methods:** This was a retrospective cohort study that examined SEER registries from 1973 and 2013. We included patients aged 18 years or older who were diagnosed with a primary cancer. We excluded patients with missing information on initial radiotherapy treatment or stage and those who died less than two years from the date of their primary cancer diagnosis. RIS was defined as those who developed a secondary sarcoma near the site of their original malignancy and after a 24-month latency period.

**Results:** Our study included 1,745,867 patients with an average age of 60 years and mean follow up time of 9.2 years. Breast cancer comprised the largest number with 693,701 patients of which 161 (0.02%) had a secondary sarcoma. Prostate cancer comprised the second largest group with 594,271 patients of which 75 (0.01%) had a secondary sarcoma. Of the 359 patients with secondary sarcomas, 242 (67.4%) had RIS. Breast cancer had the highest number of RIS patients at 126 (52.1%) compared to all combined non-breast cancer sites at 116. Interestingly, 172 (71.1%) RIS patients were females, which correlated with the attributable RIS cases from breast and uterine cancer. The relative risk of RIS in breast cancer versus 17 other primary cancer sites was 1.21 (CI: 1.01-1.45,  $p < 0.03$ , adjusted for age at primary diagnosis, gender, and latency).

**Conclusions:** This study confirms the low incidence of RIS in breast cancer, which has been demonstrated to be around 0.03% - 0.2% in the literature. Our results suggest that breast cancer portends a somewhat higher risk for RIS compared to 17 primary solid malignancies. Although some of the risk is attributable to the high incidence rate of breast cancer, breast cancer patients had a higher relative risk of RIS than other solid tumors after adjusting for covariates. Female gender may be associated with an increased risk for RIS compared to males. Further investigations should be performed to confirm these epidemiological findings given that radiation fields, doses, intention to cure, margin status and systemic therapy are not recorded in SEER. Nonetheless, this study is hypothesis generating in that the majority of RIS cases in solid tumors are attributable to breast cancer in this large population-based series.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-16

Five year outcomes of a prospective study of proton radiotherapy for breast cancer regional nodal irradiation

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**Purpose:** Through improved nodal coverage and decreased dose to the heart and lung, proton therapy (PT) may improve the therapeutic ratio for treatment of breast cancer requiring regional nodal irradiation (RNI). The purpose of this study is to report 5 year disease control and toxicity.

**Methods:** From May 2012 to February 2014, 18 women (stage IIA-IIIB) prospectively enrolled on a pilot study. Median age was 52 years (range, 42-73), with equal division between breast-conserving therapy (BCT) and mastectomy and right and left-sided cancers. Median number of positive nodes among the 16 node-positive patients was 2 (range, 1-14). Five patients had  $\geq 10$  nodes positive on axillary dissection (N3a). Treatment targets (CTVs for breast/chest wall, supraclavicular, axillary, internal mammary nodes (IMNs)) and organs at risk were delineated on CT scans. Double scatter PT alone was used for 10 patients (9 post-mastectomy, 1 after BCT) and combined proton-photon in 8 (all BCT). Toxicity was prospectively recorded using CTCAE v4.0.

**Results:** Median follow-up was 4 years (range, 0.3 – 6). 5 year overall survival was 82% and locoregional control was 100%. 5 year distant metastases-free survival was 82%.

No grade 4+ toxicity developed. Four patients developed grade 3 cellulitis, which was the only grade 3 toxicity. One patient had a reconstructive failure associated with a post-surgical cellulitis. A right-sided patient was diagnosed with CHF within 2 months of completion of PT, following diagnosis of a pulmonary embolus 1 month prior. She had an elevated BNP (1101) prior to PT following adriamycin-based chemotherapy. Mean heart dose was 0 Gy and cardiac V5 0%. Atrial fibrillation developed in two patients. One patient developed grade 2 pneumonitis (she received concurrent chemotherapy). One patient, with a history of rib fractures prior to PT, developed an ipsilateral rib fracture 7 months after PT.

**Conclusion:** In a population of women with locally advanced breast cancer, PT for RNI has proven feasible after either mastectomy (with or without reconstruction) or BCT with excellent locoregional control. PT allows for highly conformal radiation delivery without compromise of target coverage or excess exposure of normal tissue. Documentation of the ultimate benefits of PT in this population, freedom from radiation induced cardiac disease and breast cancer recurrence will require prospective study of a larger numbers of patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-17

Intraoperative radiotherapy (IORT) after neoadjuvant chemotherapy in patients with breast cancer– Experience in the Cancer Institute of Lima-Peru

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## Background

Intraoperative radiotherapy (IORT) is a treatment technique that allows the surgical bed to be irradiated in the same surgical act after breast-conserving surgery, in some cases as a single treatment and in others as a boost complemented with external radiotherapy. There are studies that have shown their non-inferiority with respect to conventional external radiotherapy, taking advantage of administration time, costs and side effects.

## Methods

In this study, the IORT (INTRABEAM) was used as treatment in patients who received neoadjuvant treatment with chemotherapy who had a good response and met the criteria to be included (no tumor or tumors less than 3 cm, unifocal tumors, no skin involvement after treatment).

Out of a total of 255 cases of IORT performed in recent years, 42 were studied, those who received neoadjuvant chemotherapy from April 2014 to April 2018 and who completed the treatment.

## Results

The average age was 47.9 years (29-72), 59.5% were between 40-49 years old and 11.4% were under 40 years old. 61.9% were stage II and 38.1% stage III. The diameter of the most used applicator was between 3-3.5 cm (56.8%) and the average irradiation time was 20 mins (12-46 mins). All received external RT. 67.6% received on average 25 sessions and 32.4% received 15 sessions. There were 64.2% luminal (luminal A 7.1%, luminal B 38.1%, luminal B HER2 (+) 19%), 7.1% with ER/PR(-) HER2(+) and 28.6% ER/PR(-) HER2(-). 38% patients had complete pathological response in the primary tumor. Of the 42 patients treated, 37 had a follow-up of more than 6 months. 3 (7.1%) had recurrence, 2 local and 1 local and distant (EC IIIC). Disease-free survival at 12, 24 and 36 months was 97.2%, 90.5% and 90.5% and the overall survival was 100%, 100% and 92.3% respectively.

## Clinical Stages / Immunophenotypes

Clinical Stages	N (%)	Immunophenotype	N (%)
IIA	12(28.6)	Luminal A	3(7.1)
IIB	14(33.3)	Luminal B	16(38.1)
IIIA	7(16.7)	Luminal B HER2(+)	8(19.0)
IIIB	8(19.0)	ER/PR(-) HER2(+)	3(7.1)
IIIC	1(2.4)	ER/PR(-)HER2(-)	12(28.6)

## Disease Free Survival

Months	12	24	36
DFS	97.2%	90.5%	90.5%

## **Conclusion**

The IORT (INTRABEAM) used as a boost in patients with breast cancer and neoadjuvant treatment who complete complementary external RT, is a safe procedure, with low recurrence rate, it is easy to perform with a good tolerance, shorter time of treatment and with fewer side effects.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-18

Comparison of three different topical agents on prevention of acute radiodermatitis during breast cancer radiotherapy

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**Aim:** To compare the effectiveness for radiodermatitis prophylaxis of Fusidic acid and Betamethasone valerate containing cream, pure vaselin and emollient dermocosmetic cream during radiotherapy for breast cancer.

**Method:** We prescribed one of the 3 different creams per patient respectively at the start date of breast cancer radiotherapy (conventional radiotherapy: CRT, 50 Gy in 2 Gy/fraction plus a 10 Gy boost for a total of 60 Gy; or hypofractionated radiotherapy: HRT, 40 Gy in 2.67 Gy/fraction plus a 10 Gy Boost for a total of 50 Gy) at our clinic and instructed them to use the cream daily after each fraction throughout the whole treatment. Weekly photographs of the thorax, including the treated breast anteriorly and laterally, were taken with the onset of treatment, where a gray card setting for color balance standardization was performed. The weekly skin changes were recorded prospectively and assessed retrospectively according to the RTOG radiodermatitis side effect scale. A total of 64 cases have been enrolled in the study till now and 54 out of 58 cases who completed their radiotherapy were evaluated for the current analysis (photographs flawed in three, one refused her photographs to be included in the study). The photographs were processed for color matching with the gray card set using the Adobe Reader Photoshop CC software via the red color histogram and the pre- & post-radiotherapy skin redness were objectively compared via the histogram. The data were analyzed using SPSS, version 23.

**Results:** The treatment was conventional in 25 patients and hypofractionated in 29 patients. Grade 2 radiodermatitis was seen in 37% of the cases at the end of treatment. The color scale final grade 2 radiodermatitis rate was 29.6% (16 cases), consistent with the clinical findings. The cream used was emollient dermocosmetic in 17 (31.5%, CRT 10, HRT 7), fusidic acid and betamethasone valerate in 19 (35.2%, CRT 13, HRT 6), and pure vaseline in 18 (33.3%, CRT 6, HRT 12) cases. There were 4 grade 1 RD in first week, 15 grade 1 RD in second week, 29 grade 1 and 1 grade 2 RD in the third week. The grade 2 RD rate increased to 16.7% (n: 9) in the fourth week. As lower red density values in the red scale representing increased erythema, the pre-radiotherapy breast median value of 230 (range: 166-256) decreased to a median value of 209.5 (range: 114-252) at the end of radiotherapy with a median change of 14%, not significantly different with creams (p=0.06) or with fractionation (p>0.05).

**Conclusion:** Grade 2 acute radiodermatitis was less commonly encountered with prophylactic creams in our cohort while no significant difference could be defined between creams and fractionation used. Digital follow up of the protocol patients is currently continuing to be documented for future reporting.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-20

The use of new delineation tool “MIRADA”, step-by-step development and first results of its use in early and locally advanced breast cancer patients

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**Objectives:** To describe the practical procedure of implementation and optimization of delineation using “Mirada” software, as well as evaluation of the automatic segmentation for the daily practice of lymph nodes (LN) and OARs (organs at risk) in early as well as locally advanced stage breast cancer patients.

**Methods:** Forty patients' CT scans in treatment position were selected and re-contoured according to the ESTRO guidelines. The atlas of dataset was then created for automatic delineation. Thirty patients with breast/chest wall and lymph nodes regions irradiated were recruited for evaluation. With the same treatment position, the CT scan images were acquired and then contoured by the MIRADA system automatically as well as by the radiation oncologist manually (as the reference). The Conformity Index (CI) was used to evaluate the concordance between both of them.

**Results:** The mean time for manual contour was  $24.1 \pm 5.1$  mins and  $26.4 \pm 2.8$  mins for the LN and the OARs resp. All the volumes of interest were contoured using the software (including corrections) in 30 minutes, which reduced the time of delineation of target volumes and OAR by about 40%. Of the 30 cases evaluated, the mean CI of 5 principal OARs showed  $\geq 0.8$ . While the automatic contour of LN was less satisfactory with mean CI of  $0.43 \pm 0.1$  (0.23-0.52).

**Conclusions:** For the breast cancer patients, the studied software permitted to save time for delineation with acceptable OAR contours. The improvement of LN regions contour is needed. More cases and further evaluation are needed for the system to realize its routine use.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-12-21

Development of peptide-based targeted  $\alpha$ -therapy for triple negative breast cancer

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Triple-negative breast cancer (TNBC) is associated with a poorer prognosis, including a higher potential to metastasize and a decrease in the 5-year survival rate as compared to other breast cancer subtypes (77% vs. 93%). The poor prognosis is partially due to the lack of targeted treatments for TNBC, highlighting the need to develop targeted therapies for TNBC. Alpha-emitting radionuclides, such as Actinium-225, provide high linear energy transfer (LET) radiation. Clinical and pre-clinical studies have shown that targeted alpha-therapy is a highly potent treatment for metastatic cancer. Peptides and peptidomimetics are minimally immunogenic and have rapid tissue and tumor penetration, and rapid clearance from non-target tissues, providing an attractive platform for targeted therapeutic agent. The RGD peptide has been shown to have a high affinity for  $\alpha_v\beta_3$ , which has been implicated in TNBC. The purpose of these studies is to investigate the potential of modified RGD peptide scaffolds to deliver <sup>225</sup>Ac to triple negative breast cancer cells for targeted alpha therapy.

## Methods

DOTA-cyclo-RGD(fK) and DOTA-cyclo-RGD(fK) dimer were labeled with Indium-111, a SPECT imaging surrogate for <sup>225</sup>Ac, and Actinium-225. The resulting labeled agents were evaluated *in vitro*, including binding affinity assays (<sup>111</sup>In) and colony formation assays (<sup>225</sup>Ac) that are underway using the MDA-MB-231 cell line. Biodistribution experiments were performed in athymic nude mice bearing MDA-MB-231 tumors to compare the distribution and pharmacokinetics of the <sup>111</sup>In-labeled RGD peptide scaffolds *in vivo*. Alpha camera imaging evaluated the microscale distribution of <sup>225</sup>Ac-DOTA-cyclo-RGD(fK) in non-tumor bearing mice.

## Results

The saturation binding assay of <sup>111</sup>In-labeled DOTA-cyclo-RGD(fK) and DOTA-cyclo-RGD(fK) dimer demonstrated selective binding and nanomolar affinity for  $\alpha_v\beta_3$ . Selective targeting of  $\alpha_v\beta_3$  was further demonstrated *in vivo* for both <sup>111</sup>In-labeled RGD agents. The <sup>111</sup>In-labeled DOTA-cyclo-RGD(fK) demonstrated significantly higher tumor uptake at 30 minutes compared to the dimer ( $5.3 \pm 2.9$  vs.  $3.0 \pm 1.4$  %ID/g). The <sup>111</sup>In-DOTA-cyclo-RGD(fK) dimer was better retained in the tumor over 6 hours with significantly higher uptake at the 6-hour time point ( $1.2 \pm 0.4$  vs.  $2.8 \pm 1.1$  %ID/g). Furthermore, the dimer had significantly higher uptake in the kidney and liver over a 6-hour window as compared to <sup>111</sup>In-DOTA-cyclo-RGD(fK). However, both <sup>111</sup>In-labeled RGD agents demonstrated rapid clearance from normal organs. Alpha camera images of the <sup>225</sup>Ac-labeled dimer supported the rapid clearance from the normal tissues, with the majority of the agents being cleared within the first 90 minutes.

## Conclusion

Modified RGD peptides were successfully labeled with Indium-111 and Actinium-225. The resulting agents were shown to successfully target  $\alpha_v\beta_3$  selectively both *in vitro* and *in vivo*. These studies provide encouraging data to support the development and optimization of the RGD platform for  $\alpha_v\beta_3$ -targeted alpha therapy to treat triple negative breast cancer. Future works will investigate the therapeutic efficacy, macro- and microscale dosimetry, and toxicity of the developed <sup>225</sup>Ac-labeled  $\alpha_v\beta_3$ -targeted agents as well as explore the optimization of the RGD scaffold for improved pharmacokinetics with <sup>225</sup>Ac.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-12-22

Targeted intraoperative radiotherapy (TARGIT IORT) during breast conserving surgery for early stage breast cancer in patients with breast augmentation with implants

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**Background:** Targeted intraoperative radiotherapy (TARGIT) has become a standard option during breast conserving surgery for selected cases of early breast cancer and over 20,000 patients have been treated in over 300 centers around the world. Although a growing number of patients are presenting with implant breast augmentation, no data has been published regarding the safety of TARGIT with implants in situ. TARGIT IORT as a replacement for whole breast irradiation is an important issue in this context because of the high rates of capsular fibrosis following EBRT in such patients.

**Methods:** We are reporting a case series of 12 patients who received TARGIT during breast conserving surgery for early breast cancer, had undergone breast augmentation with implants before and wanted their implants to stay in situ. Patients were informed that no published data existed and decided on this approach on an individual basis. 3 patients received additional EBRT after TARGIT IORT because of the presence of EIC or LVI. TARGIT IORT was performed using Intrabeam - 50 kV – X-rays delivering 20 Gy prescribed at the surface of the tumor bed during the initial lumpectomy procedure.

**Results:** Patient characteristics are given in table 1. Follow-up varied from 78 months to 3 months. 11 patients presented with invasive breast cancer, 1 patient with DCIS. There were no procedure related complications and none of the patients have needed their implant removed. 1/12 patients (ID 7) was diagnosed with a local recurrence in a distant quadrant after 36 months of follow-up. In 11/12 patients no breast-cancer-related events occurred.

## Patient characteristics

ID	ER/PR/HER2	Grade	Sentinel Nodes	Tumor Size (mm)	Distance Implant to Tumor (mm)	EBRT after IORT	Follow up time (months)
1	pos/pos/neg	2	pN1mi (sn)	9	5	No	62
2	pos/neg/neg	2	pN0 (sn)	19	13	Yes	54
3	pos/pos/neg	3	pN0 (sn)	0.8	5	Yes	32
4	pos/pos/neg	2	pN0 (sn)	6	11	No	15
5	pos/pos/neg	3	pN0 (sn)	7	1	Yes	14
6	pos/pos/neg	1	pN0 (sn)	5	15	No	11
7	pos/pos/neg	2	pN0 (sn)	7	not reported	No	37
8	pos/pos/na	1 (DCIS)	N/A	8	not reported	No	78
9	pos/pos/neg	2	pN0 (sn)	15	not reported	No	15
10	pos/neg/neg	1	pN0 (sn)	14	4	No	44
11	pos/pos/neg	2	pN0 (sn)	9	1	No	11
12	pos/pos/neg	2	pN0 (sn)	7	5	No	3

Table 1

**Conclusion:** This series of patients with TARGIT during breast conserving surgery for early breast cancer after breast

augmentation with implants in situ revealed no safety concerns. Our case series gives some confidence in discussing this option with suitable patients. To expand this series, we are gathering details about other cases from the whole TARGIT group worldwide.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-23

Utilization and survival benefit of radiation therapy among hormone receptor positive breast cancer patients with recurrence score from Oncotype DX testing

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**Background:** Radiation therapy (RT) improves survival of breast cancer (BC) patients receiving the lumpectomy. Oncotype DX, a 21-gene Recurrence Score (RS) assay, has been validated to predict the risk of recurrence and chemotherapy benefit for hormone receptor positive (HR+) BC without metastatic lymph nodes (PN0). With increasing use of Oncotype DX, it is unclear if the utilization and survival benefit of RT vary by RS in clinical practice. This study aimed to 1) examine if the RS from Oncotype DX testing influences RT utilization among HR+ BC patients with PN0 who received lumpectomy; and 2) investigate if RT is associated with better cause-specific survival (CSS) and overall survival (OS) among patients stratified by the low, intermediate, and high RS.

**Methods:** Data from Genomic Health Inc., the sole Oncotype DX testing provider in the U.S., was linked with routinely collected data from 17 SEER registries. Women who were diagnosed with PN0 and HR+ BC in 2004-2015, received the lumpectomy, and had RS from Oncotype DX testing were included. Patients who had multiple tumors, received RT before or within surgery, or had less than 2 months follow-up were excluded. Patients diagnosed in 2004-2014 and followed through the end of 2015 were included in the survival analysis. RS was categorized into low (<18), intermediate (18-30), and high (>30). RT was categorized into yes, no or unknown. Multivariable logistic regression was applied to examine the association between RS and RT utilization. To compare survival differences, patients receiving RT and patients having no or unknown RT were matched on propensity score, which was calculated based on diagnosis year, age, race, marital status, tumor size, grade, number of lymph nodes examined, chemotherapy, and participating state. Stratified Cox proportional hazards models were used to compare CSS and OS between two matched groups. Proportional hazard assumption was evaluated.

**Results:** Out of 48,615 patients, 56.8% had low, 36.1% had intermediate, and 7.1% had high RS; 84.5% received RT (86.6%, 82.8%, and 76.4% in low, intermediate, and high RS patients, respectively;  $P < 0.0001$ ). After adjusting for covariates, patients with intermediate (odds ratio [OR] 0.75; 95% CI 0.71-0.79;  $P < 0.0001$ ) and high RS (OR 0.53; 95% CI 0.47-0.59;  $P < 0.0001$ ) were less likely to receive RT than patients with low RS. Among patients with low or intermediate RS, having no or unknown RT was associated with worse CSS (low RS: hazard ratio [HR] 6.00, 95% CI 1.77-20.37,  $P = 0.004$ ; intermediate RS: HR 2.12, 95% CI 1.19-3.77,  $P = 0.01$ ) and OS (low RS: HR 2.29, 95% CI 1.56-3.35,  $P < 0.0001$ ; intermediate RS: HR 2.22, 95% CI: 1.54-3.21,  $P < 0.0001$ ). RT utilization was not significantly associated with CSS or OS among patients with high RS.

**Conclusions:** Among HR+, PN0 BC patients receiving the lumpectomy, lower RS was associated with higher RT utilization. RT was associated with better CSS and OS among patients with low or intermediate RS. Among patients with high RS, no association between RT and survival was observed.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-24

Tumor-secreted predictive biomarkers of response to radiotherapy in breast cancer

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**Background:** In breast cancer (BC), radiotherapy (RT) is used adjuvantly to prevent recurrence and also in the palliative setting. Clinical signs of RT response are often not apparent for several weeks post-treatment and we currently lack tools to predict or monitor tumor response to RT early during treatment. The aim was to identify tumor-secreted biomarkers whose release reflects response to RT, which could be monitored during treatment in the blood or intratumorally by an implantable biosensor, currently under development within the Implantable Microsystems for Personalised Anti-Cancer Therapy (IMPACT) program.

**Methods:** A series of experiments assessed the effect of different radiation doses (2-10Gy) on 3 human BC cell lines – MDA-MB-231 (ER-), MCF-7 (ER+) and HBL-100 (ER-) –, 1 canine breast cancer and 2 sheep lung cancer lines. Culture media was collected from each dose experiment at a range of post-radiation time-points (1-24 hours). Proteins were isolated from collected media for secretome mass spectrometry (MS) analysis. A subset of treatment/time conditions were repeated in the same BC cell lines and radioresistant (RR) derivatives from which RNA was extracted and analysed using Lexogen QuantSeq for whole-genome transcriptomics. In-lab candidate biomarker validation was carried out using immunohistochemistry (IHC), immunofluorescence (IF) and western blotting (WB) using validated antibodies. Levels of candidate biomarkers were also assessed in normal and untreated BC tissues using IHC. ELISA-based methods are currently under investigation for detection of the lead candidate biomarkers in the blood of large animal cancer models treated with RT.

**Results:** Biomarker discovery using the MS data revealed 4 promising candidates: EIF3G, SEC24C, YBX3 and TK1. These are released from BC and animal cancer cells sensitive to radiation in a dose-dependent manner 24 hours after treatment. Analysis of the transcriptomic data showed an 8-fold higher expression of the genes encoding the 4 candidates in the radio-sensitive parental cell lines compared to the RR cell lines. IF and WB confirmed lower intracellular expression of the 4 proteins in RR cells compared to the parental lines. WB of collected culture media confirmed release of each of the 4 candidates 24 hours after a 2Gy dose of radiation in only the parental lines. GAPDH was not found in these media samples, demonstrating that protein release was not due to cell lysis.

## **Conclusions:**

- We have identified 4 promising biomarkers which are released from cancer cells sensitive to RT and not released from RR derivatives.
- All 4 candidates are released 24 hours after a 2Gy radiation dose, which fits with the current clinical dosing schedule where radiation is administered at 24 hour intervals. Ongoing work will elucidate if these biomarkers can be reliably detected in blood or intratumorally using implantable biosensors.
- There are currently no validated predictive tools to monitor RT response during treatment. If successfully validated, these biomarkers could have a clinical role in personalising RT dosing schedules and durations for solid tumors in the neoadjuvant and palliative setting, thus optimising treatment and preventing the administration of ineffective RT and its associated side effects.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-12-25

Dosimetric impact of patient rotation during prone breast radiotherapy

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## Purpose

Prone positioning has been used as a viable alternative to conventional supine position for patients receiving breast radiation therapy. However, little research has been done exploring the axial rotation of patients toward the treated breast when “sinking” into the opening of the breast board and its potentially negative effects on dosimetric outcomes, which may include increased heart and lung dose. The physician may need to move the posterior border away from the chest wall to reduce heart and lung dose.

## Methodology

49 consecutive female patients with left sided early stage breast cancer treated at University of California Davis Medical Center were assessed from 2015 to 2018 (age range: 42-84 years, median age: 62 years). All patients underwent prone whole breast therapy with conventional external beam radiation therapy (EBRT) at doses of 50 Gy (n = 12) or hypofractionated at 42.56 Gy (n = 37). Treatment plans and dose volumes were retrospectively analyzed for each patient. Standard tangents were designed for each patient using clinical landmarks of the midaxillary line and midsternal line, which were then compared to the delivered tangent beams. The angle created between a vertical line centered on center sternum and a line drawn from center sternum to center spinal cord served to define degree of axial rotation. Breast depth was defined by the longest horizontal length from outer rib to edge of breast on sagittal view. Patients were divided into subgroups by degree of rotation and absolute breast depth. A two tailed paired Student's t-test was used for analysis.

## Results

Overall mean heart and lung dose were 82.2 cGy and 50.43 cGy for the entire cohort, respectively. For standard tangents, patients with degree of rotation < 5 degrees in the prone position (n = 23) had significant lung sparing as compared to patients with degree of rotation > 5 cm (n = 26) (mean lung dose: 61.8 cGy vs 129.6 cGy, p = 0.00329). This was also seen for cardiac sparing (mean heart dose: 105.9 cGy vs 183.9 cGy, p = 0.000235). Even with reduction of posterior border for treatment delivery, there remained a significant increase in mean heart and lung dose with increased rotation (p = 0.038, p = 0.046). Although not statistically significant, for patients with > 5 degrees of rotation there was a trend toward increased reduction of the posterior border of the tangent (13 mm vs. 7.5 mm, p = 0.13). A significant predictor of increased rotation was breast depth > 10 cm (p = 0.01). Patients with absolute breast depth > 10 cm (n = 23) in the prone position had significant lung sparing as compared to patients with absolute breast depth < 10 cm (n = 26) (mean lung dose: 58.6 cGy vs 40.8 cGy, p = 0.042).

## Conclusion

To our knowledge, this is the first dosimetric comparison of prone breast therapy exploring the degree of patient roll into the prone-breast setup cavity. This study demonstrates a significant increase in mean lung and heart dose when patient rotation is greater than 5 degrees. Given this, the posterior border may have to be reduced to prevent a higher than intended dose to the heart and lung. Proper attention during simulation is important to allow for optimal dose distribution and special attention should be paid to women with smaller breast size.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-01

Application of supine MRI-based 3D printing breast surgical guide for precision breast-conserving surgery

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## Background

If the size of the tumor is large, neoadjuvant systemic therapy (NST) is performed to reduce the size of the tumor and to conserve the breast. It is known that magnetic resonance imaging is more accurate than mammography (MMG) or ultrasonography (USG) in determining the area of residual cancer in breast-conserving surgery (BSG) after NST. However, there are some problems when performing BCS using MRI. Because the posture of MRI test is different from the posture at surgery, it is difficult to accurately mark the area of the tumor observed in MRI. Neoadjuvant systemic therapy reduces tumor size and often makes it difficult to detect the original tumor area on preoperative MRI. Even if the tumor is not visible in the image, the cancer cells may remain, so it is important to accurately indicate the extent of the initial tumor and remove it. Until now, however, there has been no way to accurately mark past breast tumors in the breast. We have developed a breast surgical guide (BSG) that can mark a range of tumor directly on the breast using three-dimensional printing technology based on supine MRI. This study analyzed the results of patients who underwent BCS using a 3D printing breast surgical guide (3D-BSG) based on supine MRI.

## Methods

This trial was designed as a prospective single-institution cohort study. Our study protocol was approved by the Institutional Review Board of Asan Medical Center, Seoul, Korea (IRB No. 2016-1237). Patients who were expected to undergo BCS after NST were enrolled in this study and supine MRI was performed before and after NST. From MRI images, morphological shapes of breasts and tumors were modeled. The prepared digital model was saved in stereolithography file format and then exported to a 3D printer. 3D-BSG is designed to be able to mark the skin and attach the dye injecting column to mark the around the tumor. The breast tissue was removed with blue dye on the basis of the border. To obtain tumor free margin, intraoperative frozen sections were identified in several cavities and re-excision was performed if tumor positive.

## Results

Between January 2016 and May 2017, 50 patients were enrolled in the study. BCS was applied to 40 patients, except for those who were rejected or mastectomy. Complete remission was observed in 15 patients after NST. Four patients had tumor positive on resection margins on frozen biopsy during operation, two with IDC and two with DCIS. Re-excision was performed in these patients and tumor negative margin was confirmed in all patients in the final pathology results. The median size of the long axis of the tumor was 1.7 cm (range, 0.5 to 4.5 cm) and the median size of the long axis of the removed breast tissue was 5.1 cm (range, 2.3 to 8.1 cm). The distance between tumor and resection margin was 1.2 cm (range, 0.1 to 4.8 cm)..

## Conclusions

In BCS, the application of the supine MRI based 3D-BSG showed low rates of positive margins. Unlike conventional localization techniques, application of 3D-BSG does not cause pain to the patient, has no radiation exposure, and has no time required for the localization procedure, so it will be helpful for patients in BCS in the future.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-13-02

Breast conserving therapy after neoadjuvant systemic therapy in patients with T3 breast cancer is feasible

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## Background

An important advantage of neoadjuvant systemic therapy (NST) in breast cancer patients is down-sizing of the primary tumor. However, many patients with T3 tumors are treated with mastectomy regardless of response to NST. In this study, we evaluated predictive characteristics for positive margins and local control in T3 breast cancer patients who underwent breast-conserving therapy (BCT) after NST.

## Methods

This single institution study included all clinical T3 breast cancer patients (determined by contrast-enhanced magnetic resonance imaging [MRI]) who underwent breast conserving surgery (BCS) after NST between 2000-2015. Clinical T3 was defined as a breast tumor >50mm on MRI pre-NST. Patient, tumor and treatment characteristics were recorded, as well as response on MRI and final pathology. The local recurrence probability was estimated with the Kaplan-Meier method. Predictive characteristics for positive margins in patients undergoing BCS were analyzed using Fishers exact test.

## Results

In total, 115 T3 patients were identified. Patient, tumor and MRI findings are presenting in the table. Median tumor size was 60 mm on MRI pre-NST (range 51-120 mm) and 4 mm after NST (range 0-58 mm). Overall pathologic complete response was 19%: 5% in HR+/HER2- patients, 32% in HR-/HER2+ patients and 40% in TN patients. After initial BCS, 73 patients had negative margins (63.5%), 18 focally positive margins (15.7%) and 24 more than focally positive margins (20.9%). Patients with HR+/HER2- tumors (52%) were more likely to have positive margins than patients with HR-/HER2+ and TN tumors (21% and 19%,  $p=0.002$ ). In addition, positive margins rate was higher in patients with lobular carcinoma compared to patients with ductal carcinoma (57 vs 32%,  $p=0.031$ ). Presence of non-mass enhancement on pre-NST MRI was predictive for positive margins (52% in patients with and 25% in patients without non-mass enhancement,  $p=0.003$ ). Of patient with positive margins, 15 underwent radiotherapy with boost, 6 underwent re-excision and 21 underwent mastectomy. Finally, 94/115 patients were treated with BCT (82%). Of these patients, two had a local recurrence after a median follow-up of 6.5 years (6-year local recurrence probability 2.6% (95%-CI 0-7%).

## Conclusion

In this series, BCT after NST was successful in 82% of patients with T3 breast cancer and local control in this group was excellent. The positive margin rate after BCS was higher in patients with HR+ tumors, lobular carcinoma and tumors with non-mass enhancement on MRI pre-NST. BCT should always be considered in T3 cancers after NST.

Characteristic	Total (n=115)	Positive margins (focally+ >focally), n=42	(%)	p-value
Histology				0.031
Ductal	92	29	(32)	
Lobular	23	13	(57)	
Subtype				0.002
HR+/HER2-	61	32	(52)	
HER2+	34	7	(21)	
TN	20	3	(15)	
MRI morphology of mass pre-NST				0.948
Unifocal	28	8	(29)	
Multifocal	40	12	(33)	

Multicentric	13	4	(31)	
Only non-mass enhancement	34			
MRI non-mass enhancement before NST				0.003
Absent	65	16	(25)	
Present	50	26	(52)	
MRI response after NST				0.952
rCR	52	18	(35)	
non-rCR	62	21	(38)	
Missing	1			

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-03

Risk factors for reoperation following breast-conserving surgery integrated into pre- and postoperative models with high accuracy

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## **Purpose:**

Breast-conserving surgery (BCS) is today applied in 60-70% of patients with invasive and *in situ* cancer and tumor size of 3 cm or less. However, a significant fraction of patients (10-40%) who undergo BCS require a reoperation due to incomplete excision of the tumor despite the evolvement of oncoplastic surgical techniques. We aimed to define risk factors for non-radical BCS due to positive margins in patients with *in situ* and invasive breast cancer operated with BCS. A specific focus was to identify preoperatively available characteristics which could be informative for the surgeon and patients when planning the primary choice of breast surgery.

## **Methods:**

Patients with final diagnosis of *in situ* or invasive cancer who underwent BCS 2015-2016 at Skåne University Hospital in Malmö were included in the study. Patients undergoing neoadjuvant therapy were excluded. Data was extracted from mammography/ultrasound files, type of surgery from clinical files and pre- and postoperative pathological diagnosis and radicality from pathological files according to a prespecified protocol. The definition of radicality was according to international consensus (ie no tumor on ink for invasive cancer and 2 mm for *in situ* cancer). Uni- and multivariable logistic regression analysis were executed separately for pre- and postoperatively available parameters in the SPSS software to calculate odds ratios (OR) for predictors of non-radicality. Separate models for pre- and postoperative characteristics were defined based on data derived in 2015, 2016 was used as a validation set. Accuracy of the models were presented as AUC-values. *P*-values of  $\leq 0.05$  were considered significant.

## **Results:**

202 patients diagnosed in 2015 were included of which 43 (21.3%) patients were considered non-radically operated, whereas 231 patients diagnosed in 2016 were eligible and had a reoperation rate of 14.3%. Multivariable analysis included determinants from the univariate analysis and clinically relevant variables with the following results for the preoperative model: mammographic size per mm: OR 1.06 (95% CI: 1.01-1.11),  $p=0.014$ , invasive lobular cancer on core needle biopsy vs not: OR 7.3 (95% CI: 1.8-29.4),  $P=0.005$ , DCIS diagnosis on core needle biopsy vs not OR 3.60 (95% CI: 1.00-13.02)  $p=0.051$ , benign core needle biopsy vs not OR 6.3 (95% CI: 0.9-46.7),  $p=0.070$ , oncoplastic surgery and presence of calcifications had  $p$ -values  $> 0.1$ . In the postoperative model pure DCIS in the specimen and total extent were significant predictive factors in the multivariate analysis. The preoperative model predicting non-radicality had an AUC of 0.81 and the postoperative model had an AUC of 0.86 in the test set (year 2015), the AUC in the validation set (year 2016) was 0.80 for the preoperative model and 0.81 for the postoperative model.

## **Conclusion:**

Important preoperative prediction factors for non-radicality following BCS derived from core needle biopsies and mammograms have shown promising results and may be helpful when planning the primary choice of breast surgery. An extended study with larger cohort size is desirable.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-04

18F-FDG micro-PET/CT for intraoperative margin assessment in breast conserving surgery using: A proof-of-concept study

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Positive surgical margins represent a high risk for adverse clinical outcome in breast conserving surgery (BCS). Therefore, the goal of BCS is to avoid positive margins and hence avoid reoperation. Unfortunately, most studies currently assess the rate of positive resection margins at 20%. This is in part due to the lack of a time- and cost-effective method for intraoperative margin assessment, which would enable the prediction of positive margins during the initial surgery. We propose to address this problem by performing intraoperative high-resolution <sup>18</sup>F-fluoro-deoxyglucose (FDG) positron emission tomography (PET) with X-ray computed tomography (CT). This method relies on the high sensitivity of FDG-PET for detecting metabolically active tumor tissue, and the delineation of the anatomical margins of the specimen using CT. In this proof-of-concept study we assess the feasibility of this technique.

Twenty patients with breast cancer that were eligible to undergo BCS were enrolled in the study after providing informed consent. The study was approved by the Ethics Committee of Ghent University Hospital. Prior to surgery each patient was administered 4 MBq/kg of FDG. Surgery was performed 2-4 hours after tracer administration. Following surgical excision the breast specimen was oriented with sutures and micro-PET/CT images were obtained using the MOLECUBES β-CUBE (PET) and X-CUBE (CT). The scan time was 10 minutes on PET and 3 minutes on CT. The specimen was then sent for histopathological assessment. Micro-PET/CT images were analyzed using an automated algorithm. Briefly, this algorithm defined the contour of the tumor as the region with high FDG uptake and the contour of the specimen based on the CT image. The margin status of a specimen was positive if the distance between the contour of the tumor and specimen was 0 mm. Images were also analyzed postoperatively by two surgeons blinded to the histopathological and algorithm analysis results. The sensitivity and specificity of the proposed method were then calculated by comparing to the histopathological results, which is the gold standard for margin status assessment.

In all samples a region with high FDG uptake was visualized, which corresponded to the tumor on histopathological. In one specific case a small satellite lesion with high FDG uptake, 3 mm in diameter, was detected on the micro-PET images at a distance from the main tumor. Histopathological confirmed that this previously undetected lesion was a second invasive carcinoma. For margin status, a sensitivity of 75% and specificity of 75% were obtained using automated algorithm analysis. Sensitivity and specificity obtained based on surgeons' analysis was 62,5% and 75% for surgeon A and 87,5% and 91,7% for surgeon B respectively. Taking into account the intra-operative micro-PET results could theoretically have reduced the reoperation rate by 75%.

This proof-of-concept study demonstrates that high-resolution intraoperative FDG-PET/CT is a promising technique for intraoperative margin assessment in BCS that could allow to reduce re-excision rate. This technique achieves both sufficient sensitivity and specificity with minimal disruption of intraoperative workflow.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-05

Comparing conventional breast conserving surgery with the minimally invasive approach technique to treat early breast cancer - a retrospective case control study

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**Background/Objective:** The objective of the study was to compare the oncological safety and aesthetical results between the minimally invasive technique and the conventional breast conserving surgery. Breast conserving surgery was developed to avoid mastectomy and has become the standard of care in early stage breast cancer. Patient concerns with aesthetics have led to the development of oncoplastic surgical approaches. It has been demonstrated that the aesthetic success in breast cancer surgical treatment leads to improved sexual and social recovery. In patients that have no desire or no need for associated mammoplasty, minimally invasive treatments allow the maintenance of the breast pre-surgical appearance. The minimally invasive technique is an oncoplastic surgery aimed to remove both the breast tumor and the sentinel lymph node through one incision, thus providing better aesthetic results than the conventional breast conservative two incision technique.

**Methods:** We retrospectively evaluated 2 cohorts of 60 consecutive early breast cancer patients ( invasive breast cancer measuring no more than 25mm and clinically axillary negative lymph nodes ) operated by either conventional breast conserving surgery (N=26) or one incision surgery (N=34). We selected patients that have no desire or no need for associated mammoplasty. We compared the mammary volume tissue removed; surgical time; number of dissected lymph nodes; surgical complications such as seroma, infection, and dehiscence of the surgical wound; and deformities, retractions, and subsequent aesthetic sequelae.

**Results:** In the minimally invasive technique group the breast volume removed was significantly lower than in the conventional surgery technique group as well as was the surgical time and the number of dissected lymph nodes.

## Demographics and surgical results

	Minimally invasive surgery ( n=34)	Conventional surgery (n=26)	P value
Age			0.241
Medium	53.9(11.4)	57.4(11.3)	
Range	33 - 76	34 - 85	
Disease Stage			0.482
I	30(88.2)	21(80.8)	
II	4(11.8)	5(19.2)	
Incision			>0.99
Axilla	1 (3.1)	0 (0.0)	
Periareolar	21(65.6)	17(65.4)	
Sulcus	10(31.3)	9(34.6)	
Breast dissected volume			<0.001
Medium	16.3(8.5; 26.7)	42.4(14.4; 112.2)	
Range	2 - 90	5 - 270	
Dissected lymph nodes			<0.001
Medium (IIQ)	2(1-5)	4(1-13)	
Range	1 - 18	1 - 31	
Surgical time (min)			0.010
Medium	130(105; 170)	180(110; 240)	
Range	30 - 220	50 - 275	

No cases required enlargement of the margins and aesthetical results were better in the minimally invasive technique with only incision group.

Conclusions: The minimally invasive approach to treat early breast cancer was shown to be similar to the conventional breast conserving surgery in terms of oncologic outcomes but providing better cosmetic result.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-06

Breast conserving surgery by periareolar mammoplasty – Surgical and oncological outcomes

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## Background

Periareolar mammoplasty is a volume displacement oncoplastic technique for patients with small to medium sized breasts. This approach provides good access for a safe excision for both central and peripheral lesions, especially for those in the upper half of the breast. Cosmetically it allows good reshaping of the breast, and contralateral symmetrisation is rarely required. This technique has the potential to replace the standard wide local excision for breast cancer. There is scarcity of data about the results of this procedure in the literature. We present a single surgeon experience with this technique in terms of surgical and oncological outcomes.

## Methods

Retrospective review of patients' records from October 2013 to December 2017 was performed. The patients' demographics, tumour characteristics and the early oncological outcomes were studied. The postoperative complications and rate of symmetrisation were also studied.

## Results

- There were 110 patients in this study period. The median age was 60 years (range 36 – 82 years) and screen detected tumours accounted for 66% (72 patients) of cases.
- Neoadjuvant therapy was given in 14 patients (endocrine therapy – 12, chemotherapy – 1, dual targeted therapy – 1).
- Most patients had the lesion in the upper half of the breast (upper outer-71, upper inner-33).
- The average size of the tumour was 18.9mm (range 1.8 – 70mm) and the average weight of the excised specimen was 47.2gm (range 11-190gms). Invasive carcinoma was seen in 94 patients (85%) of which 86 patients had invasive ductal carcinoma, 4 had invasive lobular carcinoma and 4 had special type. Pure DCIS was present in 16 patients (15%). Most patients had grade 2 cancers (45%). DCIS was present with invasive carcinoma in 55% of cases.
- Thirteen patients had positive margins (<1mm) and DCIS coexisted with invasive carcinoma in 7 of 13 patients. Four patients with pure DCIS had positive margins.
- Of the 13 patients with positive margins: 5 required margin re-excision, 6 had completion mastectomy and 2 received only radiotherapy.
- The median follow-up was 25 months (range 3 – 53 months).

Seven patients developed complications which included 3 hematomas (2 requiring evacuation) and 4 wound infection which were treated with antibiotics. Only two patients required contralateral symmetrisation and no local recurrences were encountered in this study period.

## Conclusion

Periareolar mammoplasty is a robust, easily adaptable and reproducible oncoplastic technique allowing for safe wide local excision of breast cancer. The margin re-excision, postoperative complication and recurrence rates in this study are acceptable and low compared to standard wide local excision. This technique gives excellent access, and allows better breast reshaping resulting in pleasing aesthetic outcome. Contralateral symmetrisation is rarely required with this technique.

# 2018 San Antonio Breast Cancer Symposium ®

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Is radiofrequency ablation better than lumpectomy for margin status in breast cancer? Results of a randomized clinical trial

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**PURPOSE:** To study the safety and efficacy of ultrasound-guided percutaneous radiofrequency ablation (RFA) as local treatment for breast cancer and to intraoperatively evaluate the margin status after RFA in comparison with lumpectomy.

**MATERIAL and METHODS:** Preliminary in vitro RF ablation experimentation with two mastectomy specimens was performed to test the electrode, practice the ultrasound technique and evaluate the macroscopic and microscopic effects of RF. Then, a prospective, randomized open-label phase II clinical trial (NCT02281812) was conducted in a single institution from 2013-2017. Forty subjects, mean age 64 (range 46-86), with ductal infiltrating carcinoma of the breast  $\leq 2$  cm were randomly assigned to RFA plus lumpectomy or lumpectomy alone. Margin status, tumor cell viability (TCV) after RFA (by nicotinamide adenine dinucleotide (NADH) and Cytokeratin 18 (CK18) staining), adverse events and local recurrences were evaluated by univariable and multivariable analyses (SPSS statistical software).

**RESULTS:** In the experimental design with mastectomies, the only procedural complication was a skin burn at the entrance site of the electrodes. We learned that the tip of the electrodes should cut cross the tumor by at least 10mm.

The clinical trial includes two groups: study group (n=20) and control group (n=20). NADH and CK18 staining demonstrated absence of TCV after RFA with at least one of the two techniques. The percentage of intraoperatively affected surgical margins was higher in the control group although local adverse effects after surgery was higher in the RFA treatment arm. Three study subjects presented local infection (two had partial irradiation of the breast) and none in the control group.

## Outcomes

	RFA group (n = 20)	Control group (n=20)	p value
Specimen weight (median, gr)	42 (24-80)	27 (11-60)	0.004
Specimen volume (median, ml)	369 (259-847)	201 (100-602)	0.004
Positive margin (intraoperative)	4/20 (20%)	11/20 (55%)	0.022
Pathological size (median, mm)	11.5 (5-20)	10.5 (6-16)	0.07
Local Adverse effect	8/20 (40%)	1/20 (5%)	0.01
Breast Inflammation	5/20 (25%)	1/20 (5%)	0.182
Breast Infection	3/20 (15%)	0/20 (0%)	0.23

RFA: radiofrequency ablation. n=number of subjects

Median follow up was 25 months (range 1–83). No recurrence or second surgery was required during the study period.

**CONCLUSION:** RFA seems effective in the cases considered and could be more accurate than lumpectomy in terms of obtaining more free margins. Surgical excision associated with RFA leads to a higher amount of local adverse effects, especially if combined with partial irradiation of the breast. RFA could be considered as a less invasive treatment in tumors smaller than 20 mm; however, this warrants further investigation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-08

Usefulness of periareolar zigzag incision in oncoplastic breast-conserving surgery

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## Background

Breast-conserving surgery (BCS) has been performed as a standard procedure because the addition of radiation therapy after breast-conserving surgery in early breast cancer patients is similar to that of mastectomy. The surgical methods for breast cancer have been developed in recent years, and the cosmetic aspect has become important. Several incisions are used to reduce the scar to the incision site during BCS. The periareolar incision is often used when the small tumor relatively close to the nipple. However, periareolar incision does not have sufficient field of sight, which can result in difficult or impossible operations if the tumor is large or far from the nipple. In plastic surgery, various methods such as zigzag incisions have been recommended to achieve satisfactory esthetic results. The periareolar zigzag incision has the advantage of a not only good surgical field but also contributed to better surgical scars. The purpose of this study was to evaluate the oncological safety of procedures by studying the status of the surgical margins of the excised tumor specimen and reduces the need for further surgery

## Methods

From January 2016 to November 2017, we conducted a survey of female patients who underwent BCS using zigzag incision. Patients with exclusion criteria were excluded from this study if they had a Patients with bilateral breast cancer or neoadjuvant chemotherapy was included in the study. Intraoperative frozen section margin was evaluated in all patients and additional resection was performed when the positive margin was present. Final margin status was determined by examination of the permanent paraffin-embedded sections. In patients with invasive breast cancer or relatively large carcinoma in situ, axillary surgery was performed according to the presence of axillary metastasis. Patients characteristics, tumor characteristics, operative time, size of the specimen and the distance from the tumor to nipple were evaluated

## Results

393 patients were enrolled in the study, including 9 patients with bilateral breast cancer, and a total of 402 cases of BCS surgery were analyzed. Thirty-five patients received neoadjuvant chemotherapy.

The median age of the patients was 51 (range: 25-84 years), the median time of operation was 72 minutes in patients who did not undergo axillary surgery or sentinel node biopsy only, and 83 minutes in patients who underwent axillary node dissection or supraclavicular node dissection. The median tumor size was 1.6 cm (range: 0-8.8 cm), median tumor distance from the nipple was 3.0 cm (range: 0.4-8.1 cm), mean excised specimen sized was 5.0 cm (range: 0.9-15.0 cm). Frozen biopsy of the resection margin during surgery revealed tumor-positive in 69 (17.2%) cases and re-excision was performed. All patients were discharged with no sign of infection or skin necrosis.

## Conclusions

The periareolar zigzag incision technique has good cosmetic results and provides a sufficient surgical field, which can be useful for removing relatively large tumors or tumors far from the nipple.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-09

Increased mortality with repeat lumpectomy alone after ipsilateral breast tumor recurrence: A propensity-adjusted, population-based SEER analysis

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**Background:** The benefit of repeat lumpectomy for ipsilateral breast tumor recurrence (IBTR) after breast conserving surgery (BCS) is currently inconclusive. This issue has become even more important as small and isolated recurrent tumors were frequently diagnosed.

**Methods:** IBTR patients with definitive surgery were identified in the Surveillance, Epidemiology, and End Results registry between 1973 and 2013. The effect of different IBTR surgeries on overall and cancer-specific mortality was assessed using risk-adjusted Cox proportional hazard regression modeling and stratified propensity score matching analysis (PSMA).

**Results:** Based on the selection criteria, 5098 patients were recruited. Of those, 4048 (79.4%) women underwent mastectomy and 1050 (20.1%) underwent repeat lumpectomy after IBTR. Patients who received repeat lumpectomy had lower grade (23.7% vs 15% for well-differentiated) and smaller recurrent tumor (47% vs 36.2% for  $\leq 1$  cm) but earlier recurrence (23.9% vs 11.2% for interval times  $< 48$  months) than those who underwent mastectomy. A minority of each group (24.7% of those undergoing repeat lumpectomy and 3% of the mastectomy group) underwent RT after surgery. In multivariable Cox regression analysis, repeat lumpectomy was associated with increased overall mortality (Hazard ratio (HR) = 1.58, 95% CI = 1.353 to 1.844,  $P < 0.001$ ) and cancer-specific mortality (HR = 1.721, 95% CI = 1.345 to 2.202,  $P < 0.001$ ). Similar HRs were derived from the PSMA cohort. However, we found no significant difference in overall mortality for women who underwent repeat lumpectomy followed by RT compared with that for those who underwent mastectomy ( $P = 0.411$ ). Moreover, IBTR patients with small tumors ( $\leq 1$  cm) who underwent repeat lumpectomy with RT rather than without had similar overall and cancer-specific survival rates to those who underwent mastectomy ( $P = 0.189$  and  $P = 0.604$ , respectively).

**Conclusions:** Our investigation suggests that compared with mastectomy, repeat lumpectomy for IBTR is associated with higher overall and cancer-specific mortality under real-world observational conditions. Furthermore, repeat lumpectomy with RT is equivalent to mastectomy with respect to overall mortality and may influence treatment decision making for patients with small IBTR.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-10

Factors impacting surgical option in patients who achieved complete response in the breast after neoadjuvant systemic therapy

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## **Background:**

Neoadjuvant systemic therapy (NST) is the standard treatment for locally advanced breast cancer. Early studies have demonstrated that the rate of breast conservation was significantly increased and NST is now widely applied to operable breast cancer to increase breast conservation rate and to achieve better cosmetic outcome after breast conservative surgery (BCS). For patients who achieved pathologic complete response (pCR), they were perfect candidate for BCS. However, data from prospectively randomized trials have shown that, with the significantly increase of pCR by adding new drugs in the neoadjuvant regimen, BCS rate does not significantly increased. The purpose of current study is to identify factors that have influence on surgical option in patients that have achieved complete response in the breast after NST.

## **Methods:**

From January 2009 to July 2016, 1150 patients with breast cancer received NST in our institute. There were 103 patients achieved complete response (CR) in the breast and were included in current analysis. Medical records were reviewed regarding to individual surgeon, pre-NST clinicopathologic characteristics, and post-NST image findings. Risk factors for mastectomy were assessed by univariate and multivariate analyses.

## **Results:**

Of the 103 patients who achieved CR in the breast, 40 of them (38.8%) received mastectomy. In univariate analysis, physician factor, larger initial tumor size, advanced clinical stages, as well as post-NST image findings including skin change, residual tumor  $\geq 3$  cm, short nipple-tumor distance, and residual axillary lymph node(LN) on ultrasound and segmental/diffuse distribution of malignant calcification on mammography were associated with more mastectomy. In multivariate analysis, physician factor (OR=5.192, CI [1.562 – 17.257], p=0.021), residual axillary LN on echography (OR=4.000, CI [1.552 – 10.319], p=0.004), and segmental/diffuse distribution of malignant calcification on mammography (OR=7.500, CI [1.819 – 30.916], p=0.018) were independently related to mastectomy in patients who achieved CR in the breast after NST.

## **Conclusion:**

Physician factor, residual axillary LN and segmental/diffuse malignant calcifications were risk factors for mastectomy in patients who achieved CR in the breast after NST. In addition to embrace current treatment guidelines of surgical management after NST, our data provide valuable additional information to avoid unnecessary mastectomy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-11

The outcomes of ReFilx soft tissue filler as an immediate reconstruction technique for lumpectomy followed by radiotherapy

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**Introduction:** Lumpectomy for breast cancer can often result in poor cosmetic outcomes which can lead to long-lasting impairment of quality of life. ReFilx is a synthetic porous degradable polyurethane scaffold that has mechanical properties comparable to that of native healthy breast tissue. It has been shown in two previous porcine studies to preserve breast shape and volume over 9 months when inserted into the cavities immediately after lumpectomy. We observed minimal foreign body reaction with good integration of the host tissue while the material degraded over time. In this study, we examined the healing process of lumpectomy treated with radiation. Radiotherapy is currently a standard adjuvant therapy after lumpectomy for breast cancers; and radiation is known to affect the healing process in general. **Objective:** To evaluate ReFilx, as soft tissue fillers for lumpectomy cavities in combination with standard radiotherapy. **Hypothesis:** ReFilx will preserve breast shape and volume by supporting tissue infiltration with minimal inflammation in the presence of clinically relevant radiotherapy. **Methods:** 3 female Yucatan minipigs received lumpectomies carried out using electrocautery to remove normal breast tissue of approximately 2 cm diameter, after which the cavities were filled with (case) or without ReFilx (sham control) (n=3 for each condition, each pig has 12-13 breasts). 6 weeks post-implantation, 2 of the pigs received radiation directed at the right half of their bodies (6 breasts per pig) for a total dose of 42.4 Gy delivered over 15 fractions using a clinical linear accelerator. The delivered doses to target sites and the adjacent tissue were confirmed with optically stimulated luminescent dosimeters (OSLDs). A third pig was maintained as a non-irradiated control. Ultrasound examinations were performed every 3 weeks post-implantation. At week 24 and 36, samples consisted of n=3 for irradiated and non-irradiated ReFilx and sham sites for the half-irradiated pigs, and n=3 for non-irradiated ReFilx and sham sites for the non-irradiated control pig were excised by mastectomy, the tissue samples were fixed in 10% buffered formalin for subsequent histological (H&E, Masson's Trichrome) studies. Potential side effects were monitored by vital signs, pain control, wound checks, ultrasound and blood tests. Necropsies were performed at the conclusion of the study. **Results:** Ultrasound examination indicated no difference between ReFilx's ability to maintain breast shape and volume with or without the presence of radiation treatment, in contrast to the collapse of the sham sites by 24 weeks. Similarly, the histology of irradiated and non-irradiated ReFilx samples showed similar levels of tissue infiltration, inflammatory changes and degradation of ReFilx. There was no significant side effects observed. **Conclusions:** ReFilx's ability to act as a soft tissue filler for breast volume restoration post-lumpectomy does not appear to be significantly impacted by the presence of clinically relevant radiation treatment. Longer-term follow-up is currently in progress for this study. **Acknowledgements:** Connaught Innovation Award (University of Toronto) and Joule Innovation Fund (Canadian Medical Association).



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-13-12

The margin of breast-conserving surgery when ductal carcinoma *in situ* is present within invasive ductal carcinoma

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**Objective:** The presence of ductal carcinoma *in situ* (DCIS) in invasive ductal carcinoma (IDC) may increase the rates of positive margins and re-excision. Literature addressing this association is limited. This study aimed to explore the factors that are associated with positive margins and re-excision, as well as to evaluate the influence of positive margins on the survival of patients with breast cancer containing both invasive and in situ ductal carcinoma.

**Methods:** A retrospective medical chart review of patients diagnosed with DCIS within IDC who underwent BCS at the Revlon/UCLA Breast Center between January 2003 and December 2008 was performed.

**Results:** Of the 488 eligible patients, 267 patients (53.9%) underwent re-excision. The presence of residual disease upon re-excision was the highest in patients who initially had positive margins involved by both DCIS and IDC. In multivariate analysis, calcifications, tumor size, positive lymph nodes, and the presence of extensive intraductal component (EIC) were significantly associated with initial positive margins, with the latter having the highest hazard ratio (HR, 5.5–5.7,  $P = 0.000$ ). Tumor size, molecular subtype, and final margin status were associated with disease-free survival. The final margin and regional lymph node status are significant prognosticators for breast cancer-specific survival.

**Conclusions:** In patients with ductal carcinoma of the breast containing both invasive and in situ components, the rate of initial positive margin after BCS was high and was significantly associated with calcifications, tumor size, regional lymph node status, and the presence of EIC.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-14-01

High-dose vitamin D supplementation for the correction of vitamin D insufficiency in patients undergoing adjuvant chemotherapy for breast cancer, a phase II multicenter study

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**Background:** Vitamin D (VitD) insufficiency affects most of patients with early breast cancer (EBC). Breast cancer treatment may lead to bone loss, due to premature ovarian failure or direct chemotherapy (CT) cytotoxic effects. These increase the risk of skeletal morbidity as compared to women without breast cancer history. We previously published the persistence of VitD insufficiency at the end of adjuvant CT despite an adapted dose supplementation. We report here the safety and efficacy analysis of a phase II trial evaluating a high-dose oral VitD supplementation regimen for correction of VitD insufficiency in insufficient EBC patients treated with adjuvant CT.

**Material and methods:** EBC patients with VitD insufficiency for whom adjuvant CT was planned were eligible for this study. They received one dose of 100,000 IU 25OH-vitamin D every 3 weeks from day 1 of cycle 1 to day 1 of cycle 5, except in case of clinical or biological adverse event related to vitamin D and calcium metabolism, leading to early discontinuation. The primary endpoint was the percentage of serum 25OH-vitamin D level normalization at day 1 of cycle 6 (D1C6). Secondary endpoints were the safety, the Vitamin D and calcium parameters (blood calcium, phosphorus and parathormone [PTH] levels, urinary calcium excretion) at baseline and the description of their evolution during adjuvant CT, as well as the evaluation of the predictive value of these biomarkers and baseline clinical factors on the percentage of VitD normalization at D1C6.

**Results:** Among 45 eligible patients, 44 were evaluable for the primary endpoint. Among them, 21 (47.7%; 95%CI: 33.0-62.8) achieved a 25OH-Vitamin D correction at D1C6. No clinical toxicity linked to the VitD treatment was reported. However, 13 patients (29.5%) presented an asymptomatic grade 1 hypercalciuria, possibly linked to the VitD treatment, without concomitant change in the kidney function, but which lead to the interruption of the high dose oral VitD supplementation in 10 of the 13 patients. VitD normalization rates at 6, 12, 18 and 24 months were 50, 28.9, 80 and 60.9%, respectively. No clinical or biological marker was found to significantly predict the 6-month 25OH vitamin D normalization.

**Conclusions:** A high-dose 25OH-vitamin D regimen allowed a high percentage of serum 25OHD level normalization at D1C6 in EBC patients undergoing adjuvant CT. An asymptomatic increase in urinary calcium excretion was observed in one third of the patients, without clinical consequences. The physiopathology of this urinary calcium increase warrants further evaluation, since it is a classical reason for VitD treatment interruption, leading to a lower rate of correction in this highly requiring clinical setting.

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Upper extremity edema in the at-risk arm among patients receiving PI3K/mTOR/CDK4/6 inhibitors for metastatic breast cancer

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**Background:** Targeted therapies, including mTOR and CDK 4/6 inhibitors, have changed the landscape of management of hormone receptor-positive (HR+) metastatic breast cancer (MBC). These therapies have shown significant improvement in progression-free survival and are generally well-tolerated. In pre-clinical models, modulation of the PI3K/mTOR pathway can impede lymphoangiogenesis resulting in capillary leakage. In this study, we examined the impact of PI3K, mTOR, and CDK 4/6 inhibitors in the development of upper extremity edema (UEE) in the at-risk arm for breast cancer-related lymphedema (BCRL) in patients with MBC.

**Methods:** We conducted a retrospective chart review of patients treated with PI3K/mTOR/CDK4/6 inhibitors for MBC. Clinicopathologic data including age, body-mass index (BMI), specific pathway targeted, treatment duration, and presence of edema were recorded. Characteristics of treatment including surgery type and laterality, nodal surgery, radiation regimen, and tumor subtype were also collected.

**Results:** Among patients with MBC treated with PI3K, mTOR, and/or CDK 4/6 inhibitors (N = 160), the incidence of edema that developed after initiation of the targeted therapy was 11.3% (18/160) for UEE and 31.9% (51/160) for edema in any anatomical location. 50.0% (11/22) of patients treated with a PI3K- $\alpha$  inhibitor, 32.6% (14/43) of patients treated with an mTOR inhibitor, and 33.3% (8/24) of patients treated with a CDK4/6 inhibitor alone developed peripheral edema following initiation of the respective targeted therapy. Further, swelling developed in the at-risk upper extremity after C1D1 in 13.6% (3/22) patients treated with a PI3K- $\alpha$  inhibitor exclusively, 7.0% (3/43) treated with an mTOR inhibitor exclusively, and in 12.5% (3/24) treated with a CDK4/6 inhibitor exclusively. Of the 42 patients treated with a CDK4/6 inhibitor in combination with either an mTOR inhibitor, aromatase inhibitor, or an ER-binding promoter, the incidence of UEE in the at-risk upper extremity after C1D1 was 18.8% (6/32), 0.0% (0/7), and 0.0% (0/3) respectively. In multivariate logistic regression analysis, both therapy with PI3K- $\alpha$  inhibitors (OR: 3.22;  $p$  = 0.049) and a relative decrease in serum albumin after 3 months of treatment (OR: 3.35,  $p$  = 0.024) increased the risk of developing peripheral edema; however, duration of therapy, and nodal surgery were not significant risk factors. Upon stratification of this cohort by number of BCRL-related risk factors, the incidence of BCRL was 18.3%, 39.5%, and 83.3% in women with one, two, or three BCRL-related risk factors, respectively.

**Conclusions:** PI3K, mTOR, and CDK 4/6 inhibitors may influence the development of UEE, which may cause or exacerbate progression of BCRL in at-risk arm among patients with MBC. Further research is needed to prospectively evaluate these novel findings as well as elucidate physiologic and clinical impacts of these therapies on peripheral edema and BCRL. Moreover, it is crucial to understand the role of close monitoring for the development or progression of peripheral edema or BCRL to ensure early detection and treatment, thus potentially minimizing the negative impacts on the quality of life of patients with MBC.

# 2018 San Antonio Breast Cancer Symposium ®

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Changes in vitamin D and calcium metabolism markers in patients undergoing adjuvant chemotherapy for breast cancer

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**Background:** Vitamin D (VitD) insufficiency affects the majority of patients with early breast cancer (EBC). Breast cancer treatment may lead to bone loss, due to premature ovarian failure or direct chemotherapy (CT) cytotoxic effects. These increase the risk of skeletal morbidity compared to women without breast cancer history. However, even if these evidences are well described, the evolution of calcium metabolism under CT is unknown in this population with a high cure rate. We report the evolution of VitD and calcium metabolism markers in patients undergoing adjuvant CT for EBC.

**Material and methods:** We evaluated the VitD and calcium parameters (blood calcium, phosphorus and parathormone [PTH] levels, urinary calcium excretion) in EBC patients treated with 6 cycles of adjuvant CT without high dose calcium and VitD treatment. Variables of interest were recorded at inclusion, then every 3 weeks, at each chemotherapy cycle initiation. Primary endpoint was the occurrence of a hypercalciuria during the course of adjuvant CT (between Day 1, Cycle 1 [D1C1] and Day 1, Cycle 6 [D1C6]).

**Results:** 82 patients were evaluable for the primary endpoint. The median age was 53 years (range 20-71). CT consisted of a sequential anthracyclines and taxane regimen in 96.3% of the cases. Eleven (26.8%) patients received adjuvant trastuzumab. Most patients (66, 80.5%) presented with baseline VitD insufficiency (<30 ng/mL). Median baseline VitD level was 20.65ng/mL (range 2.9-55). Nine patients (8 VitD insufficient, 1 VitD sufficient [calcium only]) received low-dose VitD and/or calcium supplementation during the CT cycles. No baseline clinical parameter was statistically predictive of a VitD baseline insufficiency, while baseline blood calcium level was statistically predictive of a VitD baseline insufficiency ( $p=0.051$ ). 94% of the patients presented with VitD insufficiency at D1C6 (median VitD level 20ng/mL; 9-39). No case of hypercalcemia was recorded. 29 patients (35.4%; 95%CI: 25.6-46.5) developed hypercalciuria between D1C1 and D1C6, none clinically significant. This percentage was not significantly different between VitD insufficient patients and the others (34.8% vs. 37.5%), nor between supplemented and not supplemented patients (37.5% vs. 34.5%). In multivariate analysis, weight and BMI were significantly associated with the occurrence of a hypercalciuria, while a trend was detected for baseline VitD ( $p=0.085$ ) and albumin blood level ( $p=0.072$ ). Baseline PTH level was elevated in 12.7% of the VitD insufficient patients vs. none of the patients with a normal VitD level. These percentages increased to 52.5% and 50% respectively at D1C6.

**Conclusions:** We report here, to our knowledge, the first comprehensive study of the kinetics of VitD and calcium biomarkers during EBC adjuvant CT. This population appears highly VitD insufficient, with a compensatory elevation in blood PTH levels during the course of treatment. Hypercalciuria, while asymptomatic, is a highly prevalent abnormality in this setting, and must not be a limitation for high dose VitD supplementation.

# 2018 San Antonio Breast Cancer Symposium ®

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Prevention of lymphedema in patients undergoing axillary dissection for breast cancer by benzathine penicillin: A non-inferiority randomised controlled trial

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**Background:** Axillary lymph node dissection (ALND) is performed for axillary lymph nodal metastasis. Major complication of ALND is lymphedema. Lymphedema is a morbid condition associated with impaired activities of daily life and deterioration in quality of life (QOL). Olszewski et al (Lymphology. 2005 Jun;38(2):66–80) have shown that low-grade staphylococcal infection is a risk factor for lymphedema. Hence, we embarked upon a randomised controlled trial with non-inferiority hypothesis to evaluate efficacy of injection Benzathine penicillin in preventing breast cancer related lymphedema.

**Methods:** In this 2 group, open label, parallel design randomized controlled trial; patients undergoing ALND for breast cancer were included. Patients were randomized using block randomization to either injection Benzathine penicillin group or in control group. Patients in the penicillin group received injection Benzathine penicillin 1.2 million units as deep intramuscular injection after antibiotic sensitivity testing at intervals of 3 weeks. Primary endpoint was development of lymphedema at 6 months follow up. Analyses were done on an intention to treat basis. Lymphedema was defined as increase in >200ml of volume on water displacement method and or increase in arm circumference of >2cm from pre surgery values (Lancet Oncol. 2013 May 1;14(6):500–15).

**Findings:** Between July 2016 and December 2017, 83 ladies were randomly allocated, 40 in the penicillin group and 43 in control group. At 6 months follow up; a total of 15 (18.07%) patients had lymphedema. Out of them 5 patients were in penicillin group and 10 in the control group. Cellulitis was seen in 2/3<sup>rd</sup> of patients (10 out of 15) having lymphedema. The relative risk of cellulitis in penicillin group was 0.119 with 95% CI of (0.016-0.901) and prevented fraction of 0.881

Comparison of lymphedema and cellulitis in two groups

Outcome		Penicillin group	Control group	RR ( 95% CI)	PF
Lymphedema	Present	05	10	0.538 (0.201-1.437)	0.462
	Absent	35	33		
Cellulitis	Present	01	09	0.119 (0.016-0.901)	0.881
	Absent	39	34		

RR: Relative risk, PF: Prevented fraction, CI: Confidence interval

. Other factors like radiotherapy, type of surgery for primary tumor, extent of axillary dissection and type of chemotherapy were comparable in both groups. None of the patients receiving penicillin have any adverse effect or allergic reaction to the drug during the study period.

**Conclusion:** Penicillin administration in patients undergoing axillary lymph node dissection is safe and significantly reduces risk of lymphedema and cellulitis.

**Novelty:** This is the first randomised trial demonstrating the benefit of long term administration of penicillin in reducing lymphedema and cellulitis.

# 2018 San Antonio Breast Cancer Symposium ®

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Accurately monitoring by specialist teams reduces the time between breast cancer screening and initial surgical treatment

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## Background

Over 266,000 women are newly diagnosed with breast cancer every year in the United States, roughly 21% of women die of the disease annually.[1] Many studies have been conducted to evaluate how delays in diagnosis and treatment can result in adverse patient outcomes. Factors including tumor size and stage, socioeconomic status, race/ethnicity, and hospital size and capabilities have been shown to influence outcomes.

Variation in care delivery and turnaround times can be reduced by accurately examining the patient care path from initial screening mammography to first surgical intervention. We have developed a process to optimize timeliness to initial surgical treatment.

## Methods

The process starts with observing care delivery from diagnostic mammogram, biopsy, pathology tissue processing, diagnosis and lumpectomy or mastectomy.

A team of process and clinical experts (Leica Biosystems) conducted observations to map out current care delivery processes. Standard national metrics for timeliness within the breast care pathway from the National Consortium of Breast Centers' was applied to the process map to understand national averages as well as best practices. Timeliness data on 5,571 patients from over 250 breast centers across the country from the NQMB (National Quality Measures for Breast Centers) database was utilized to review time intervals and intradepartmental hand offs that impact progression through the breast care pathway. Six sigma, black belt specialists conduct analysis of current breast center processes, identifies specific sites for improvement, integrates their recommendations and completes the cycle by repeat monitoring of the entire process.

## Results

Our specialists have reviewed average and ideal overall performance in timeliness to initial surgical treatment. Improvements may be expected from 22%-75% in timeliness to initial surgical treatment.

### Turnaround Time (Days)

	Mean*	Best Practice**
Time from screening to dx mammo	6.0	4.9
Time from dx mammo to bx	5.9	4.1
Time from bx to path report	2.1	1.2
Time from bx to first surgery	22.6	16.9
Total time from screening mammogram to first surgery	36.6	27.1

\* Mean=average number of business days based on NQMB data July-Dec. 2017

\*\* "Best Practice"=The 75th percentile based on data in NQMB database July-Dec. 2017

## Conclusion

A process to impact the timeliness of care between screening mammography and initial surgical treatment has been developed. Standardized and monitored care delivery processes can result in better efficiency in breast cancer patient care delivery from between 22% - 75%. Using a detailed patient care pathway and black belt specialists to analyze processes, we expect to reduce turnaround times and optimize efficiency across the breast cancer patient's care pathway.

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[1] Cancer Facts and Figures 2018; American Cancer Society

## 2018 San Antonio Breast Cancer Symposium ®

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Concordance assessment of IBM Watson for Oncology with MDT in patients with breast cancer

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**Objective:** IBM Watson for Oncology (WFO) is an artificial intelligence cognitive clinical decision support system which provides evidence-based treatment recommendations in cancer. This study aimed to examine the level of agreement (concordance) between treatment recommendations made by WFO and a multidisciplinary tumor (MDT) board for adjuvant therapy in early stage breast cancer and first-line therapy in metastatic breast cancer.

**Methods:** Treatment recommendations were provided by WFO and MDT board for 92 early stage and 40 metastatic breast cancer cases between Jan 2017 and Jan 2018. WFO treatment recommendations were provided in 3 categories: "Recommended", "For Consideration" and "Not Recommended." It was considered concordant if the tumor board recommendations were designated 'recommended' or 'for consideration' by WFO Version 17.10.

**Results:** Overall treatment concordance between WFO and MDT was 76.51%, 73 cases with early stage breast cancer (79.35%) and 28 cases with metastatic breast cancer (70.00%) were concordant. Among all the 132 cases, 49 cases were in 'Recommended' category, 52 cases were in 'for consideration' category, and 26 cases were discordant. Subgroup analysis found that patients with stage I disease or triple-negative breast cancer were the most likely to be concordant. Luminal A type and HER2-positive cases were less likely to be concordant than other types. In early stage breast cancer, based on the low recurrence risk, 11 cases and 2 cases were treated with TC and EC regimen instead of AC-T or CMF regimen recommended by WFO. 4 patients with HER2-positive were discordant by using TCbH or AC-TH regimen instead of AC-TH or CMFH regimen recommended by WFO. The main reason of discordance in metastatic cancer was that CDK4/6 inhibitors had not been approved in China.

**Conclusion:** Treatment recommendations made by WFO and the tumor board showed highly concordant in breast cancer. Cancer stage and subtype had significant influence on concordance, and Watson for Oncology need to be optimized in localization.



# 2018 San Antonio Breast Cancer Symposium ®

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Evaluation of the effects of talazoparib on QT interval prolongation

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**Background:** Talazoparib (TAL), an oral poly ADP-ribose polymerase inhibitor, is under investigation in multiple oncologic clinical trials and has been submitted to the US FDA for use in patients (pts) with germline *BRCA*-mutated, HER2-negative advanced breast cancer.

International Conference on Harmonisation guidance recommends all new drugs be evaluated for effects on cardiac repolarization in a well-controlled clinical study. For drugs for which such evaluation cannot be conducted in healthy volunteers (eg, most anticancer agents), collection of robust corrected QT (QTc) interval data from a dedicated QTc study (hybrid thorough QT/QTc study) in pts is required in the registration dossier. The effect of steady-state (ss) TAL (1 mg once daily) on cardiac repolarization in pts with advanced solid tumors was evaluated in an open-label phase 1 study (NCT03042910).

**Methods:** Continuous 12-lead electrocardiogram (ECG) recordings were collected at baseline (Day -1); time-matched pharmacokinetic (PK) samples and continuous ECG recordings were obtained on Days 1, 2, and 22 (when TAL concentrations achieved ss). On Day -1, pts had continuous 12-lead ECG recording starting at Time 0 (Day 1 dosing time) for 6 hrs. On Days 1 and 22, ECG recording started 45 min before TAL administration and continued for 6 hrs post dose and blood samples for PK were collected before dose and at 1, 2, 4, and 6 hrs post dose. On Day 2, a 30-min ECG recording and a PK sample were obtained before dose at Time 0.

Continuous ECG recordings were submitted to a central laboratory; triplicate 10-sec ECGs were extracted from a 5-min extraction window beginning 15 min before each PK collection time. ECG measurements were reported via blinded manual adjudication process and included PR interval, QT interval, RR interval, and QRS complex. The QT interval was corrected for effect of heart rate using Fridericia's correction (QTcF) and Bazett's correction (QTcB).

The estimate of change from time-matched baseline and its 2-sided 90% confidence interval (CI) was calculated for each nominal time point using PROC MEANS. Additionally, a prespecified PK/pharmacodynamic (PD) model was used to describe the relationship between plasma TAL concentrations ([TAL]) and QTc. The prespecified linear mixed-effects model included [TAL], time (categorical), and treatment with random pt effects on [TAL] and the intercept. If the upper bounds (UB) of 1-sided 95% CIs of time-matched  $\Delta$ QTc for all ECG time points were <20 msec and the UB of 1-sided 95% CIs of the predicted  $\Delta$ QTc at the mean ss maximum [TAL] was <20 msec, the effect of TAL on QTc was not of clinical relevance.

**Results:** 37 of 38 pts enrolled received TAL and were included in the ECG and PK/PD analyses. No pts had a postbaseline absolute maximum QTcF or QTcB  $\geq$ 500 msec or  $\Delta$ QTc  $\geq$ 60 msec. The UB of the 1-sided 95% CI for the time-matched  $\Delta$ QTcF and  $\Delta$ QTcB were <12 msec at all nominal ECG time points. In the PK/PD analysis, the slopes (95% CI) of QTcF-[TAL] and QTcB-[TAL] relationships were -0.14 (-0.78 to 0.50) msec/ng/mL and -0.24 (-0.88 to 0.41) msec/ng/mL, respectively, indicating that TAL did not have a concentration-dependent effect on QTcF or QTcB.

**Conclusion:** TAL does not have a clinically relevant effect on QTc.

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Preclinical and clinical evidence about the use of betablockers for the treatment of triple negative breast cancer: A systematic review

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## Introduction

Triple negative breast cancer (TNBC) is a particularly aggressive subtype of breast cancer (BC) for which very limited therapeutic options are available. Recently, beta-blockers (BB) have been suggested to have favorable effects on the treatment of BC both in preclinical and clinical studies.

## Objective

The aim of this systematic review was to collect evidence from preclinical and clinical studies concerning the scientific evidence for the repurposing of BBs in TNBC treatment.

## Methods

PubMed database was searched for retrieving studies of interest published up to 30/01/2018. All preclinical studies using BC in vitro and in vivo models and assessing the effect of any molecule with sympatholytic or sympathomimetic activity on adrenoceptors were included. Clinical studies concerning BB were considered eligible. Two authors independently reviewed and screened title and abstract of retrieved references. Potentially relevant studies were further assessed through full-texts examination. One author extracted information from preclinical and clinical studies respectively. A second author subsequently reviewed the extracted data. The Newcastle-Ottawa scale was used for the quality assessment of clinical studies.

## Results

A total of 616 study references were initially retrieved. Six additional records were retrieved through snowball search. A total of 62 preclinical studies were included, of which 46 concerned in vitro and in vivo models of TNBC, i.e. cell cultures and/or animal studies (20 in vitro, 9 in vivo, and 17 in vivo/vitro). In vitro studies showed a high expression of  $\beta_2$  adrenoceptors in TNBC cell lines. Propranolol, a non-selective  $\beta_1/\beta_2$  antagonist, was reported to significantly decrease proliferation, migration and invasion of TNBC cells. Similar effects were also reported for carvedilol, a selective  $\beta_2$  antagonist and  $\alpha_1$  antagonist. In vivo studies reported a reduction of metastasis, angiogenesis and tumor growth in animals exposed to propranolol. Clinical studies, reporting evidence from a total of four distinct retrospective observational cohort studies, showed a beneficial effect of BB in TNBC treatment: e.g. study#1: Overall Survival Hazard Ratio (HR)=0.35 (95%CI 0.12-1.00); study#2: metastasis HR=0.32 (95%CI 0.12–0.90); study3# Progression Free Survival: HR=0.52 (95%CI 0.34–0.79); study #4 Relapse Free Survival: HR=0.69 (95%CI 0.35–1.34). The overall quality of the clinical evidence collected was low.

## Conclusion:

Preclinical evidence collected in this systematic review are in line with the results reported in the four clinical studies retrieved, pointing towards a beneficial effect of BB in the treatment of TNBC. However, given the overall low quality of available evidence, no definite conclusion may be drawn. The execution of large scale interventional clinical studies are warranted to shed light on the efficacy/effectiveness of BB in TNBC treatment.

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# 2018 San Antonio Breast Cancer Symposium ®

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Hyperglycemia secondary to everolimus is associated with a prognosis improvement in hormone-sensitive metastatic breast cancer. A retrospective analysis

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## Introduction

The Everolimus and Exemestane combination improves PFS (progression-free survival) in patients with metastatic hormone-sensitive breast cancer pre-treated with aromatase inhibitors (BOLERO-2). However, such combination is associated with an increase in the rate of adverse events compared to monotherapy with Exemestane.

## Patients and Methods

We selected female patients diagnosed in our center from hormone-sensitive metastatic breast cancer who were treated with the combination of Everolimus 10mg plus Exemestane 25mg between January 2012 and June 2018 (n=84). In these patients, we analyzed the number and grade of adverse events, as well as the correlation between each adverse event and the PFS.

## Results

As patient characteristics, median age was 61 years old. 39.3% had bone metastases at the diagnosis of metastatic disease, 14,3% had visceral metastases and 46,4% had visceral and bone metastases. They had received a median of 2 treatments for advanced disease (including chemotherapy or hormonotherapy) previous to Exemestane and Everolimus, and at least 2 months of the combination treatment. Median SLP was 6 months.

AE's in IVO patients (n=84)

	AE's
Hyperglycemia	30 (35.7%) - (0% G3/4)
Hypercholesterolemia	27 (32.1%) - (0% G3/4)
Hypertriglyceridemia	20 (23.8%) - (0% G3/4)
Mucositis	41 (48.8%) - (17% G3/4)
Blood pressure	4 (4.8%) - (0% G3/4)
Neumonitis	10 (11,9%) - (40% G3/4)
Fatigue	37 (44%) - (19% G3/4)
Rash	16 (19%) - (0% G3/4)

Once we had collected the frequency of AE, we tried to see if there was a correlation between the apparition of any AE and an impact in PFS.

	PFS (months)	PFS (months)	
AE	YES	NO	P-VALUE
Hyperglycemia	12,71 (8,7-15,8)	7,52 (5,4-8,2)	0,008
Hypercholesterolemia	10,4 (6,9-13,8)	8,2 (6,3-10,1)	0,18

Hypertriglyceridemia	7,3 (4,9-9,8)	9,3 (7,2-11,4)	0,323
Mucositis	10,3 (7,6-12,94)	7,5 (5,4-9,5)	0,064
Neumonitis	11,8 (7,3-16,3)	8,3 (6,5-10,2)	0,291
Rash	11,6 (7,6-15,6)	8,2 (6,3-10,02)	0,124

In the univariate analysis, we see a statistically significant correlation between the presence of hyperglycemia and an improvement in PFS.

In the multivariate analysis using all AE's, impact of hyperglycemia in SLP remained statistically significant (SLP for variable Hyperglycemia has a p-value of 0,014 and 0,531 Exp(B)

### **Conclusions**

In our experience, mucositis is the most frequent AE in patients treated with the combination, followed by fatigue and hyperglycemia. Moreover, it seems to be a correlation between the apparition of hyperglycemia and better PFS. It's well known that mTORc1 (target of Everolimus) has an important role in glycolysis: maybe the development of hyperglycemia is an indirect data from a better target inhibition. Analysis between other biological and tumor factors and their impact on survival is ongoing.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-14-10

Intralesional steroid injection: A novel method to treat the symptoms of idiopathic granulomatous mastitis

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**Background:** Idiopathic granulomatous mastitis (IGM) is a chronic, inflammatory breast condition of benign nature that can clinically mimic breast cancer. Patients frequently present with a large, painful breast mass with associated inflammatory changes of the skin, and possible ulceration or fistula of the breast. Attempted methods for symptomatic relief include surgical excision and medical therapies including oral corticosteroids. Due to the success of treating dermatologic conditions with intralesional steroid injections, it was hypothesized that injecting the subdermal lesions of IGM may benefit patients with this disease. The use of intralesional steroid injections for the treatment of IGM has not been previously described in the literature.

**Methods:** Retrospective chart review was performed on a series of four patients with IGM who received intralesional steroid injections between August 2017 and April 2018. Patients were selected for treatment with injections based on their subjective report of painful breast lesions, lesion characteristics including size and depth, and the patient's desire to stop oral steroid therapy due to side effects despite active or residual disease. Data were collected on demographics, initial physical examination findings, prior and current treatments, characteristics of disease, details of injections performed, objective and subjective response to treatment, and recurrence.

**Results:** All patients presented with breast pain and either a mass, swelling, or hardness of the breast. Each patient received between one and three injection treatments, with one to four lesions treated in each session. Patients demonstrated improvement in subjective and objective symptoms after 87.5% of injection sessions (7 out of 8) by the subsequent follow-up visit (21-34 days). During the study period, three patients experienced resolution of at least one breast lesion within about 2 months (31-139 days, mean 68 days) without recurrence. One patient had four breast lesions that completely resolved (35-217 days, mean 88 days), but three of them recurred (63-217 days, mean 149 days).

**Conclusion:** In a small group of patients with idiopathic granulomatous mastitis, intralesional steroid injections were associated with an improvement in both subjective symptomatic relief and objective breast lesion characteristics in most cases. This treatment was associated with a good rate of lesion resolution and a low short-term recurrence rate.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-15-01

Metastatic breast cancer: A retrospective study of clinical trials *versus* standard therapy

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**Background:** Breast cancer is a leading cause of death in women. Metastatic breast cancer (MBC) was the leading cause of death among the 41,000 patients with breast cancer who died this year. The number of available clinical trials for breast cancer patients has dramatically increased over the last two decades, yet recruitment to trials remains low.

To better understand the characteristics of women with MBC who agree to participate in clinical trials (CT) as compared to those who do not, control (C), we:

1. Compared the characteristics (age, race, tumor characteristics, and disease course) of women with MBC in the groups CT and C.
2. Compared the outcomes of women with MBC (metastatic survival), who enrolled in a clinical trial vs those who did not.
3. Noted the predictors for poor MBC survival overall

**Methods:** Patients with MBC, from the year 2000 to 2017, were analysed retrospectively from an established metastatic database at our institution. Characteristics and outcomes were compared for patients enrolled in clinical trials (CT) with patients who were not enrolled in any trials (C). Characteristics included race, receptor status (ER/Her2), site of initial metastasis, presence of visceral and/ or CNS metastasis, number of chemotherapy and metastatic hormonal therapy cycles. Comparison of groups utilized the Chi-square test for proportions and Student's T test for means. Univariable and multivariable associations with survival were analysed using Cox regression.

**Results:** Of the 660 patients, 249 enrolled in clinical trial (CT) and 411 served as controls (C). Demographic analysis showed that the age at first metastasis was identical in both the groups ( $52.8 \pm 11.4$  in CT and  $53.8 \pm 11.2$  in C). Racial distribution was predominantly Caucasian,  $n = 92\%$  (CT) vs  $n = 89\%$  (C), with African Americans forming 4.5% ( $n = 11$ ) and 8.3% ( $n = 34$ ) in the respective non-Caucasians in the CT and C groups ( $p = 0.13$ ). No significant differences were noted between CT and C groups in the proportion of CNS metastasis, receptor status (ER/ Her2), initial site of metastasis (visceral/ non-visceral), number of cycles of chemotherapy or a diagnosis of *de novo* metastatic disease. The proportion of patients with visceral metastases was higher in the CT patients (82.3%) vs. (71.3%) ( $p < 0.012$ ). Mortality was noted to be higher in the CT group (85.9%), when compared to C (73.2%), measured over the study duration.

Survival from the diagnosis of metastatic disease was not significantly different between CT and C patients. Worse survival outcome overall was noted in patients with triple negative disease (HR 1.7,  $p < 0.0001$ ), presence of visceral metastases (HR 1.6, 2.0 and 1.9 for 1, 2 and 3+ visceral metastases respectively ( $p < 0.0001$ ) and CNS metastases (HR 1.5,  $p < 0.0001$ ) in the CT group.

**Conclusion:** No significant demographic differences were identified between the patients enrolled in CT vs C. Higher mortality was noted in the CT group over the study duration of 17 years. The CT group had a higher number of patients with visceral metastases, but lower CNS metastases as expected for clinical trial enrolment. Although no survival difference was identified based on trial enrolment, worse outcomes were seen in patients with triple negative disease, presence of visceral or CNS metastases.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P3-15-02

Latin American participation in practice-changing breast cancer trials in the last 25 years

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**Introduction:** Latin America (LATAM) is among the so-called emerging regions for conducting clinical trials. Complex not-harmonized regulatory frameworks and lengthy approval timelines (among other factors) present challenges for increased LATAM trial participation.

**Objective:** to assess LATAM contribution to clinical trials, we conducted a descriptive analysis of the region participation in practice-changing breast cancer (BC) trials.

**Methodology:** we defined practice-changing trial as any one that supported FDA approval of a new drug or a new indication for a previously approved drug; we excluded trials that only led to approval of changes in regimen/doses. Through the FDA website we searched all drugs approved for BC treatment between January 1992 and December 2017. For each FDA-approved drug we identified applicable practice-changing trials in the latest package insert. We analyzed each applicable published article for data of interest: drug approval year, indication, participating countries, number of sites per country, trial start year of enrollment, authors per country, among others. If all these data was not available in the article we searched for it in clinicaltrials.gov. If all data was neither in the article nor clinicaltrials.gov, the trial was excluded from our analysis.

**Results:** 31 trials that led to the approval of 17 drugs were included in our analysis. LATAM participated in 21 trials (67.7%), mean number of LATAM countries per trial = 3.3 and mean number of sites per trial = 13.5. The region participated in 90% of (neo)adjuvant and in 57% of metastatic trials. Additional data is in Table 1. As an average, LATAM contributed with 4.9% of all trial sites. Argentina, Brazil and Mexico had 84% of LATAM sites, Peru and Colombia 8%, and the remaining 8% were distributed among 15 LATAM countries. The 5-year periods with highest number of trials in the region was 2001-05 and 2006-10 (Table 2). Fifteen LATAM authors (87% from Argentina and Brazil) from a total of 530 authors (2.8%) were identified in the primary publication, none of them as first or last author.

**Conclusion:** over the last 25 years the number of practice-changing BC trials conducted in LATAM has increased since 1990s, remained stable from 2001-10 and recently decreased. LATAM participated in the majority of (neo)adjuvant trials; longer time for enrollment and duration of these trials could explain this finding since these would allow for inclusion of regions with longer regulatory timelines. Region's contribution in terms of countries, sites and authors is minor. Disparities within LATAM countries are remarkable and, as expected, the 3 largest countries are the key contributors. A comparison with other emerging regions will be presented.

LATAM participation in practice- changing BC trials

Characteristics	Total	LATAM
Number of trials	31	21
Mean number of sites/trial	188.5	13.5
Indication		
(Neo) adjuvant trials	10	9
Metastatic trials	21	12
Drug Type		
Endocrine therapy	10	5
Chemotherapy	6	4
Anti HER2	10	9
CDK4/6 inhibitors	4	2
Other	1	1

LATAM participation per 5-year period

Period (per year of trial enrollment start)	Number of LATAM trials	LATAM sites per trial
1986-1990	0	0
1991-1995	2	10.5
1996-2000	4	14.3
2001-2005	6	14.7
2006-2010	6	14.7
2011-2015	3	12.0



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-16-01

Responding at patient's time of need: Scaling rapid access to evidence-based treatment plans

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**Background:** Cancer patients worldwide feel intense anxiety, often racing to start treatments at non expert centers. Further, imbalanced oncologist to patient ratios (~1600: 1.8 M in India, ~23,000: 15M in USA), impedes access to expertise. We study the impact of rapid evidence based expert treatment plans in relieving patient anxiety. Navya, a health services technology, generates personalized treatment plans that maps within NCCN Resource Stratified Guidelines [SABCS 2017]. This is vetted on mobile by oncologists at tertiary centers like TMC NCG to provide expert opinion reports to patients. Since 2015, ~19,457 patients from 57 countries have reached out for an online opinion. On the ground, 78% of patients received evidence based treatments recommended by Navya [ASCO 2017].

**Methods:** To assess impact of timeliness, a prospective series of patients (from Sep '17 to April '18) were asked: "Were you relieved to receive expert opinion report in [x] days?" "Does it matter to you to receive expert opinion report in 1 day?" To assess time savings, preliminary reports with treatment options from NCCN and TMC NCG guidelines were shared with patients who matched all guidelines criteria. Subsequently, expert opinion reports were shared as usual.

**Results:** 543/701 patients responded to phone follow-up. 97% [ $\pm$  3.2] were relieved to receive expert opinion reports in 1-2 days (103/106) vs 83% [ $\pm$  3.8 ] for 3+ days (365/437). Of those not relieved by 3+ day turnaround, 83% stated that it would matter to receive expert opinion reports in 1 day (60/72). The first 300 preliminary reports shared in median time of 3.37 hours, resulted in 90% time savings vs expert opinion reports. On 10% of the preliminary reports, experts added information such as de-/escalating therapy (18/31), and additional diagnostic tests (6/31).

**Conclusions:** Navya relieves patient anxieties by responding at the time of need with evidence based treatment plans. Scaling such health services technologies to patients worldwide is feasible.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P3-16-02

Voice of cancer patient: Analysis of breast cancer patients' experience with PARP inhibitors

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## **Background:**

Many breast and ovarian cancer patients have germline or somatic mutations in BRCA 1&2 genes. These proteins are important for repairing double-strand DNA breaks by homologous recombinational repair. In patients who have mutations in these genes, PARP is the major alternative for repairing single-strand DNA breaks. PARP inhibitors (PARPi) inhibit PARP, thereby causing cell death by accumulation of damaged DNA in cells. Many PARPi, including Olaparib, have been approved and used in treatment of metastatic ovarian cancer patients with BRCA 1&2 mutation.

Recently, Olaparib was also approved by the FDA for treatment of metastatic breast cancer patients with germline BRCA 1&2 mutation, and many other PARPi are in clinical trial. In this study we analyzed breast cancer patients' awareness, use and experience with PARPi s.

Many patients share their experiences on online forums which contain millions of freely shared messages. These can be used to analyze patient concerns and experiences. However, this data is unstructured and difficult to analyze. We used our automated system VoCP, that uses techniques from Big Data Science and Artificial Intelligence (deep learning, topic modeling, information retrieval, and natural language processing) to analyze these messages.

## **Methods:**

We collected 15.13 million unique messages by 987,189 users from 37 unrestricted cancer forums that provide clinically relevant information. We built custom ontologies for breast cancer, various PARPi, chemotherapy and side effects, and then used our automated system VoCP to extract relevant information from these messages.

## **Results:**

We found 1,536 breast cancer patients discussing PARPi. 459 patients mentioned use of PARPi whereas 706 patients shared the information about PARPi and 196 inquired about them. 176 patients mentioned that they were planning to use PARPi.

76 patients using PARPi mentioned having BRCA 1 or 2 mutation and 1 patient mentioned CHEK 2 mutation. 91 patients mentioned having triple negative cancer.

212 patients mentioned being treated on clinical trial and 10 mentioned being off trial. 162 patients mentioned use of chemotherapy with PARPi and 40 mentioned use of PARPi as single agent.

Specific PARPi: 47 mentioned Olaparib, 104 mentioned Valiparib, & Talazoparib, 6 rucaparib and 4 Niraparib. Most patient just mentioned "PARP inhibitor." Some patients mentioned iniparib on clinical trial.

Side effects were reported by 60 patients. These include:

- Nausea: 14
- Fatigue: 15
- GI side effect: 7
- Thrombocytopenia: 5
- Anemia: 2
- Neutropenia: 2
- Neuropathy: 5
- Insomnia: 2

99 patients mentioned PARPi were "effective," 21 mentioned they were "somewhat effective" and 36 mentioned they were "ineffective."

144 patient expressed positive sentiments, 30 patients expressed negative sentiments and 16 patients expressed neutral sentiment for PARPi.

## **Conclusion:**

- There is increasing awareness and curiosity for PARPi in breast cancer patients as more patients are being tested for BRCA and other mutations.
- Among the users, PARPi are generally associated with low toxicity and positive sentiments.

- VoCP reliably provides meaningful insights from the patient's point of view; it also gives insight into unmet needs where more resources and research should be focused.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-01

Molecular residual disease detection with circulating tumor DNA analysis predicts relapse in patients with early stage breast cancer

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**Background.** Detection of circulating tumor DNA (ctDNA) after treatment of early stage breast cancer may identify molecular residual disease. In a prior proof-of-principle study we demonstrated that detection of ctDNA predicted relapse with high accuracy (Garcia-Murillas *et al* Science Trans Med 2015). We conducted an independent, prospective, multi-centre validation study.

**Methods.** In this validation study, a cohort of 170 early stage breast cancer patients were recruited from five hospitals into two prospective sample collection studies. Patients were scheduled to receive standard chemotherapy, surgery +/- radiotherapy, adjuvant endocrine therapy and HER2 antibodies as appropriate. Plasma samples were collected for ctDNA analysis at baseline, post-surgery, three monthly for the first year of follow-up, and six monthly thereafter and shipped to a central lab for processing. Using previously established criteria, tumor was sequenced to identify somatic mutations that were tracked by digital PCR in DNA extracted from 4mls of plasma at all available time points. Buffy coat DNA was analysed at all time-points to control for clonal haematopoiesis of indeterminate potential (CHIP) detection. The primary endpoint was to compare invasive disease free survival between patients with and without detection of ctDNA after treatment. A combined analysis of this validation study, and the prior proof-of-principle study, was also conducted to analyse secondary endpoints.

**Results.** After tumor sequencing, 101 patients from the validation study had at least one mutation to track. At median 35.5 months follow-up, ctDNA was detected in plasma of 15.8% (16/101) patients. Detection of ctDNA strongly predicted relapse, hazard ratio 24.5 (95% CI 6.5 to 93.2, P<0.001 time-dependent Cox model), and was predictive of relapse in all tumor subtypes.

In the combined analysis (N=144), lead-time between ctDNA detection and relapse was 10.7 months (95% CI 7.7-17.0). Six patients had a clinical relapse that was not detected by ctDNA prior to relapse. These patients had a distinct pattern of oligo-metastatic relapse, 3 patients with brain-only metastases (P=0.0068), 1 ovarian oligo-metastasis and 2 local disease recurrence. The level of ctDNA in baseline plasma, prior to treatment, was associated with tumor subtype, highest in triple negative breast cancer (P=0.0036).

**Conclusion.** Detection of ctDNA after treatment is associated with a high risk of future relapse in early-stage breast cancer. Prospective studies are required to assess the potential of molecular residual disease detection to guide adjuvant therapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-02

Early detection of residual breast cancer through a robust, scalable and personalized analysis of circulating tumour DNA (ctDNA) antedates overt metastatic recurrence

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## Background:

Many breast cancer patients relapse after primary treatment but there are no reliable tests to detect distant metastases before they become overt. Here we show earlier identification of recurring patients through a scalable personalised ctDNA analysis. The method is applicable to all patients, and not limited to hot-spot mutations typically detected by gene panels.

## Methods:

Forty-nine non-metastatic breast cancer patients were recruited following surgery and adjuvant therapy. Plasma samples (n=208) were serially collected semi-annually. Using the analytically validated Signatera™ workflow, we determined mutational signatures from primary tumour whole exome data and designed personalised assays targeting 16 variants with high sensitivity by ultra-deep sequencing (average >100,000X). The patient-specific assay was used to detect the presence of the mutational signature in the plasma.

## Results:

In 16 of 18 (89%) clinically-relapsing patients, ctDNA was detected ahead of metastatic relapse being diagnosed by clinical examination, radiological and biochemical (CA15-3) measurements, and remained ctDNA-positive through follow-up. Of the 2 patients not detected by ctDNA, one had a small local recurrence only (now resected) and the other had three primary tumours. None of the 31 non-relapsing patients were ctDNA-positive at any time point (n=142). Metastatic relapse was predicted by Signatera with high accuracy and a lead time of up to 2 years (median=9.5 months).

## Conclusions:

The use of a scalable patient-specific ctDNA-based validated workflow detects breast cancer recurrence ahead of clinical detection. Accurate and earlier prediction by ctDNA analysis could provide a means of monitoring breast cancer patients in need of second-line salvage adjuvant therapy in order to prevent overt life-threatening metastatic progression.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-04

ESR1 mutation in cell free DNA (cfDNA) is associated with significantly increased circulating tumor cell (CTC)-clusters and progress in stage III/IV breast cancer after systemic treatments

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**Introduction:** CTCs play a critical role in the process of tumor metastasis, and a portion of CTCs may form clusters that contain two or more CTCs bound together which were reported to have up to 50-fold of potential of forming distant metastasis in breast cancer (MBC) as compared to individual CTCs. However, molecular and genomic characterization of CTCs cluster remain largely unknown. Here we report a highly significant correlation between *ESR1* mutation in cfDNA, CTCs count and CTC-cluster, which may help to understand MBC metastasis and predict treatment benefit, especially for metastatic or recurrent disease.

**Methods:** A total of 80 whole blood samples (7.5ml/each) were collected from 80 patients with stage III/IV BCa after informed consent under IRB-approved trial at the RHLCCC at Northwestern University before and after systemic therapies. Among these 80 patients, 41 patients received chemotherapy and 23 patients received endocrine therapy, among which 20 patients received combo treatments (16 plus Palpociclib, 1 plus Ribociclib, 2 plus Everolimus, and 1 plus trastuzumab). CTC enrichment and enumeration were performed in CELLTRACKS ANALYZERII® System (Menarini) by using CTC Kit Meanwhile, we detected the *ESR1* hotspot mutations (Y537S and D538G) in plasma cfDNA from all 80 patients by Droplet digital PCR (ddPCR) assay using the QX200 ddPCR System (Bio-Rad). cfDNA was isolated from 2 mL of plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen) and the MAF was analyzed using QuantaSoft software (Bio-Rad). Database of CTCs and *ESR1* mutation was linked with clinical database. Kruskal-Wallis test was used for statistics.

**Results:** Of the 80 samples analyzed, there were 57 samples without *ESR1* mutations (Group 1), and 23 samples that had *ESR1* mutations (8 Y537S mutations and 23 D538G mutations, Group 2). CTC positive ( $\geq 5$ ) were detected in 13/57 samples (Group 1) and 15/23 samples, and the average amounts of CTCs were 21.77 CTCs/each sample and 59.86 CTCs/each sample in Group 1 and Group 2 respectively. There was a significant association between *ESR1* mutations and high level of CTCs ( $P=0.000088$ ). More important, CTC-clusters were found in 3 samples in Group 1 (5.26%) and in 5 samples in Group 2 (21.74%) respectively. There was a significant correlation between *ESR1* mutations and CTC-clusters ( $P=0.026$ ). Furthermore, there were 18/57 patients in group 1 and 5/23 in group 2 receiving chemotherapy. Moreover, 26/57 in group 1 and 15/23 in group 2 that received chemotherapy. Our results also confirmed that both endocrine therapy and chemotherapy benefited more patients without *ESR1* mutations in compared with patients with *ESR1* mutations ( $P<0.05$ ).

**Conclusion:** We first elucidated the association between *ESR1* mutations in ctDNA and CTC-cluster in MBC patients, and provides new insights on the molecular mechanisms associated with the metastasis process. In addition with the highly significant association between ctDNA *ESR1* mutations and endocrine resistance we describe a new association allowing to expand the prognostic and predictive role of both tests enabling monitoring the metastatic prognosis and endocrine resistance for clinical decision-making.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-05

Cell free DNA analysis identifies actionable *ERBB2* amplifications in patients with HER2 equivocal breast cancer

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## **Background:**

Determination of *ERBB2* (HER2) expression or amplification informs eligibility of HER2-targeted therapies. ASCO and NCCN guidelines recommend evaluation of HER2 status on primary invasive breast cancers and on a metastatic site if stage IV, where possible, as treatment is based on the status of the metastasis. Reassessment of HER2 status should also be considered in patients with disease recurrence as initially HER2-negative tumors may acquire HER2 amplification at progression. HER2 status can be complicated by equivocal results from *in situ* hybridization (ISH) and/or immunohistochemistry (IHC). Clarification requires reflex testing on the same tissue specimen or repeat testing on a new specimen, however some patients' tissue status remains equivocal. Furthermore, metastases to bone, lung, or brain may be difficult to re-biopsy or of low DNA quality. Rapid and non-invasive blood-based cell-free DNA (cfDNA) NGS may facilitate identification of HER2 targetable disease in advanced breast cancer.

## **Methods:**

We assessed the frequency of *ERBB2* amplification detectable by a blood-based cell-free DNA (cfDNA) assay among patients with metastatic breast cancer with equivocal HER2 results in tissue. cfDNA samples were ordered as part of routine clinical care using an assay validated for the detection of copy number amplification in *ERBB2* (tests run between 03/2014-04/2017 by Guardant Health, Redwood City, CA). Submitted pathology reports were reviewed for HER2 status which was categorized as positive, negative, or equivocal based on the interpretation issued by the reading pathologist at the time the test was ordered. Patients were included if they had an equivocal result on IHC and/or ISH unless both assays were performed on the same specimen and one provided a definitive negative or positive HER2 result. Additionally, 4 patients with equivocal IHC or ISH results were excluded as biopsy of another tumor site revealed a positive HER2 result around the same time as the equivocal test. For the 349 patients with multiple cfDNA samples, the earliest pathology report was referenced.

## **Results:**

Tissue HER2 status was available for 1,853 unique patients (98.8% female, median age at testing was 58y, range 26-91y). 141 patients (7.6%) had equivocal HER2 results in tissue; 99 by IHC alone, 14 by ISH alone, and 28 were equivocal by both assays. Among these, 126 patients (89.4%) had at least one sample with ctDNA detected. 12/126 (9.5%) had amplification of *ERBB2* detected in at least one cfDNA sample. Samples were drawn a median of 267 days after tissue collection (range 4 days – 11.5 years). Frequency of *ERBB2* amplification was similar regardless of time between tissue and blood collection but was higher among patients with ISH results alone (4/14, 36.4%) compared to those with IHC alone (6/89, 6.7%) or both assays (6/26, 7.6%;  $p=0.006$ ).

## **Conclusion:**

cfDNA testing identifies a significant number of patients with HER2-targetable advanced breast cancer whose tissue was HER2 equivocal. cfDNA testing may supplement tissue-based methods to help clarify HER2 status in metastatic disease as well as identify patients who may acquire HER2 amplification subsequent to their initial biopsy.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-06

Comparison of tumor genotyping and cell-free circulating tumor DNA sequencing in metastatic breast cancer patients and their utility in the selection of matched therapy

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**Background:** Oncogenic mutations are potential targets for therapeutic intervention in metastatic breast cancer (MBC). While tumor genotyping (TG) has been viewed as the gold standard for identifying oncogenic mutations, cell-free circulating tumor DNA (cfDNA) is emerging as an alternate technique. We previously reported the selection of matched therapy targeted to an actionable mutation based on **either** TG or cfDNA testing (Vidula N, ASCO, 2018). Therefore, we are now comparing TG and cfDNA results in MBC patients undergoing **both** tests to examine their relative utility in the selection of matched therapy.

**Methods:** Patients with MBC at an academic institution who underwent both TG (Next Generation Sequencing/NGS, institutional platform, 104 gene assay) and cfDNA testing (NGS/Guardant360, 73 gene assay) between 1/2016-10/2017 were identified. A chart review was conducted to identify tumor subtype, demographics, treatment, TG and cfDNA results, and clinical outcomes. The relative utility of these tests in the selection of matched therapy was determined, and linked with clinical outcomes (progression-free survival and overall survival).

**Results:** Thirty patients who underwent both TG and cfDNA testing were identified. The median age was 60 years, the majority (97%) had hormone receptor (HR) positive/HER2 negative disease, and most patients had recurrent disease (83.3%) at MBC diagnosis. The median number of therapies prior to obtaining either test was 1 (cfDNA range 0-9, TG range 0-8). The majority had simultaneous cfDNA and tumor genotyping testing (83.3%) versus sequential testing (16.7%). Twenty-four (80%) patients had actionable mutations detected by cfDNA compared to 19 (63.3%) patients with actionable mutations detected by TG. The median number of actionable mutations detected by cfDNA was 2 (range 0-11) compared with a median of 1 (range 0-4) detected by TG. Failure of TG occurred in 2 of 30 patients (6.7%) but no test failures were seen with cfDNA. Eleven of 30 patients (36.7%) had  $\geq 1$  concordant mutation via cfDNA and TG. Altogether, 12 out of 30 (40%) patients received matched therapy, 5 of which were based on cfDNA actionable mutations alone (ESR1, ERBB2, CCND1, and PIK3CA), and 7 based on cfDNA and TG results (ESR1, PIK3CA, STK11, and BRCA). Twelve of 24 (50%) patients with actionable cfDNA mutations went on to receive matched therapy compared with 7 of 19 (36.8%) patients with actionable TG results. Matched therapies included SERDs, inhibitors of CDK 4/6, PI3K, mTOR, HER2 directed therapy, and DNA damaging chemotherapy. The impact of matched therapy on survival outcomes will be presented at the meeting.

**Conclusions:** In patients undergoing both TG and cfDNA testing, both tests identify a significant cohort of HR+ MBC patients with actionable mutations, with greater detection of actionable mutations by cfDNA. Greater application of matched therapy occurred via cfDNA, which independently informed the selection of matched therapies. Further research is needed to prospectively evaluate the clinical utility of blood based genotyping assays versus TG for patients with MBC.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-07

A comprehensive liquid biopsy in patients undergoing neoadjuvant therapy

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**Background:** Precision medicine is revolutionizing breast cancer (BC) care. Comprehensive liquid biopsies are a tool for personalized care in patients with locally advanced breast cancer (LABC). Identifying robust biomarkers as part of a comprehensive liquid biopsy to predict response to treatment is of immense clinical interest.

**Methods:** After obtaining IRB approval, serial blood samples were collected from patients with LABC undergoing neoadjuvant therapy. Paired biopsies were collected prior to treatment and were sent to Foundation Medicine for next-generation sequencing (NGS). We used a sized-base microfilter technology to capture circulating tumor cells (CTCs) and circulating cancer associated fibroblasts (cCAFs). Patients with one or more CTCs or cCAFs were deemed positive for these tests. Additionally, in collaboration with Foundation Medicine, we extracted circulating tumor DNA (ctDNA) and we analyzed it using the FoundationACT platform. Patients with a detectable genomic alteration in their plasma were considered as having a positive ctDNA test. Our primary objective is to determine if a comprehensive liquid biopsy can serve as a prognostic marker of pathologic complete response (pCR).

**Results:** For this analysis we describe our findings in the initial blood draw of the first 18 patients enrolled. The mean age is 54 years (38-70). All patients who had their tumors sequenced had a detectable mutation. Consistent with the findings of others, we found TP53 mutations to be the most prevalent at 83.3%. We found that 44% of patients had ctDNA, 68.4% had cCAFs and 78.9% had CTCs. Many patients also had clusters of cells, consisting of one cell type, or co-clusters, consisting of both. 38.9% had CTC clusters, 16.7% had cCAF clusters and 16.7% had co-clusters (CTCs and cCAFs together). Some patients with CTCs did not have cCAFs and vice versa. The number of CTCs and cCAFS did not correlate with stage of disease or receptor status.

**Conclusions:** We describe a comprehensive liquid biopsy combining a sized-based microfilter technology for CTC and cCAFs identification and the FoundationACT platform for ctDNA analysis is feasible and these biomarkers can be detected in patients with LABC prior to the initiation of neoadjuvant therapy. Our study is accruing rapidly, and we will update our results with the longitudinal collection and the prognostic value of a comprehensive liquid biopsy at the time of the meeting.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-08

Characterization of circulating tumor free DNA (ctDNA) obtained from patients with metastatic breast carcinoma (MBC) undergoing systemic therapies using comprehensive genomic profiling

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**Introduction:** Therapeutic benefit from individual treatments in patients with MBC is limited to small subsets of patients and of short duration due to tumor heterogeneity. Novel molecular diagnostics including ctDNA has been shown to predict response or resistance and survival. However, the frequency of detection of actionable mutations using ctDNA is variable based upon tumor related factors and diagnostic platform sensitivity (e.g. ddPCR or NGS). We evaluated a novel NGS technology in the ability of detecting driver and clonal genomic abnormalities in samples from MBC patients. Moreover, we wanted to compare the new technology to another state-of-the-art, commercially available diagnostic ctDNA testing with similar sensitivity to demonstrate both are able to detect genomic abnormalities in MBC.

**Methods:** This study included 30 samples from 15 patients with stage III/IV BCa treated at NMH (2016-2017) and who received standard systemic treatments based on disease subtypes longitudinally characterized for ctDNA before or 3 months after systemic therapies respectively. ctDNA from clinical plasma samples was first analyzed using PredicinePLUS, a NGS-based assay (Predicine Inc) with a 180-gene panel for genomic alterations mutations. The results were then independently analyzed with Guardant360<sup>TM</sup> (Guardant Health), a 73-gene panel. Matched pairs variations between Guardant360<sup>TM</sup> and Predicine was compared by Wilcoxon signed-ranks test. The prognostic impact of ctDNA was tested through Cox regression.

**Results:** Genomic Alterations (SNVs, Indels and copy number variations) were detected on 43 genes by PredicinePLUS assay. All samples (100%) demonstrated at least 1 somatic alterations. There were 75 mutations detected within 29 genes, and the variant frequency of mutated genes ranges from 0.11% to 68.56%. Median variant frequency was around 3.42%. Key cancer related genes including *TP53*, *ESR1*, *PIK3CA*, *PTEN* and *BRCA1*, are frequently mutated. Copy number variation were detected on 18 genes, among which 15 genes showed copy number gain, including *MYC*, *PIK3CA*, *CCND1*, and 3 genes (*ATM*, *BRCA1* and *CDKN2A*) with copy number loss. There were no significant difference of % ctDNA ( $P=0.3967$ ) and number of variations ( $P=0.5$ ) between results of Predicine and Guardant360<sup>TM</sup>, neither to the comparison of main detected alterations (*BRCA1*, *ESR1*, *MYC*, *PIK3CA* and *TP53*) with Guardant360<sup>TM</sup> and Predicine ( $P=1$ ). Furthermore, results from Predicine indicated that there is correlation with treatment response and benefit. A significant decrease on variations in %ctDNA levels ( $P=0.028$ ) and variations in the number of genomic variants ( $P=0.028$ ) after systemic therapies, was associated with longer overall survival.

**Conclusions:** Our study describes a novel diagnostic platform with the ability to identify ctDNA mutation and copy number variations in patients with MBC receiving systemic therapy. We also confirm that when comparing ctDNA using NGS platforms with similar sensitivity, the results are robust and reproducible which indicates that these technologies can be reliably and routinely used as non-invasive method for monitoring response to systemic therapies and predict the prognosis in MBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-09

Combined analysis of tissue and blood biopsies by NGS in patients with advanced breast cancer identifies targetable molecular alterations

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**Background:** Targeted-next generation sequencing (t-NGS) analysis in tissue and peripheral blood is increasingly performed in patients with advanced cancer. Liquid biopsy is a non-invasive method that allows to understand the molecular changes occurring in the tumor in real-time, derived from intratumor heterogeneity and/or therapeutic pressure. In the present work we sought to elucidate the ability of liquid biopsy to detect mutations related with acquired resistance.

**Methods:** 190 formalin-fixed paraffin-embedded (FFPE) samples from primary and metastatic tumors of patients with advanced breast cancer (ABC) were analyzed by OncoDEEP® (1000X depth of coverage of 75 gene alterations including point mutations, insertions/deletions, gene fusions and copy number variations), and comparison between targeted-NGS results from matched primary/metastatic tumors (FFPE) and cell-free DNA (cfDNA) recovery from plasma analyzed by OncoSTRAT&GO® in 34 patients with ABC (193 genes with 1000x and 40 genes with 10000x depth coverage for FFPE and plasma, respectively).

**Results:** Of the 190 FFPEs analyzed by OncoDEEP®, 41% were triple negative breast cancer (TNBC), 49% hormone receptor positive (HR+) and 11% were HER2+. Mutations in TP53 (44.2%), PIK3CA (41.1%) and ERBB2 (9.47%) were the most frequent. As expected, PIK3CA and TP53 mutations were significantly higher in HR+ and TNBC tumors, respectively (PIK3CA: 53.8% for HR+ vs 28.6% and 30% for TNBC and HER2+, respectively; TP53 68.8% for TNBC vs 26.9 and 30% in HR+ and HER2+, respectively; in both cases p<0.001). Interestingly, the alteration c.1459-7C>T in JAK1 (unknown significance) was detected in 8% of patients (9.7% and 6.5% in HR+ and TNBC, respectively). Also, in contrast to other reports, high frequencies of APC alterations were found exclusively in HR+ tumors (previously reported as germline mutations). From the OncoSTRAT&GO® analysis (tumor tissue and cfDNA), ESR1 and FGFR mutations in HR+ ABC were significantly more present in cfDNA (9/20 mutations found [45%]) compared to tumor tissue analysis (18/93 mutations found [19.3%]) (p=0.02), and in three patients, mutations in ESR1 and FGFR were only detected in cfDNA. Finally, 11/34 cases (32.3%) showed gene mutations only in cfDNA with predominant alterations in MAPK signaling pathway (54.5%), TP53 (36.4%) and ESR1 (18.2%), the latest as previously described.

**Conclusions:** JAK1 and APC mutations need further evaluation to establish their clinical significance in ABC. The combination of tumor tissue and cfDNA t-NGS analyses into clinical routine enables detailed and comprehensive evaluation of tumor heterogeneity under therapeutic pressure; in our cohort, known mutations for acquired resistance to endocrine therapy (ESR1 and FGFR2) were better detected by cfDNA compared to tumor tissue (45% vs 19.3%) in HR+ ABC patients, increasing the probability to identify early drug resistance to prioritize patients' selection into clinical trials with targeted agents.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-10

The analysis of cell-free DNA and circulating tumor cells from one blood tube might empower treatment decisions in metastatic breast cancer patients

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**Background:** The detection and characterization of circulating tumor cells (CTCs) as one of the analytes in liquid biopsy has been considered as surrogate marker to improve treatment decisions in metastatic breast cancer (MBC). In addition, cell-free tumor DNA (ctDNA) released by tumor cells and harboring tumor-associated variants is further discussed to give additional information for therapeutic options. Thus, CTC and ctDNA analysis from the same blood tube is desired. To test usability of plasma, generated after CTC isolation from whole blood for ctDNA analysis, we analyzed ctDNA from 42 hormone receptor-positive/HER2-negative MBC patients (pts) for the detection of tumor-associated variants (plasma isolated straight from whole blood) and compared the results for similarities and differences of the detected variants in a subgroup of these pts to those, obtained from plasma generated after CTC selection (taken from a separate tube).

**Methods:** 4 ml plasma of all MBC pts and 4 ml plasma obtained after immunomagnetic isolation of CTCs from 2x5ml blood [AdnaTest EMT-2/Stem Cell Select (n=17pts) followed by multimarker qPCR] were used for the analysis of cell-free DNA (cfDNA) applying the QIAamp MinElute ccfDNA Kit. A total of 30ng - 60ng cfDNA was applied for library construction using the QIAseq Targeted DNA Panel for Illumina with integrated unique molecular identifiers. Sequencing was executed on the NextSeq® 500 platform (Illumina, US). Data were analyzed using the QIAseq Targeted Sequencing Data Analysis Portal, the Biomedical Genomics Workbench and the Ingenuity Variant Analysis. All materials used were manufactured by QIAGEN, Germany.

**Results:** In the total cohort of 42 pts, most variants of all analyzed genes were detected in the MUC16 gene (31.2%). ERBB2, EGFR and AR (androgen receptor) also showed high numbers of variants (11.6%, 11.0% and 8.9%, respectively) with a majority detected pathogenic variants (47.7%) in AR. 92% of all detected variants showed an allele frequency of <5% and some of the detected MUC16, ERBB2 and AR mutations significantly correlated with overall survival. Comparing the plasma results from a separate blood draw with the results from plasma samples after CTC selection in a subgroup of 17/42 pts, no significant difference was found for cfDNA concentration but variability within the cohort. Whereas the variant comparison of ctDNA isolated from both plasma sources showed great concordance, additional variants (around 15%) were exclusively found in one of the two matched samples. Interestingly, in the variant population exclusively found in ctDNA isolated after CTC isolation, the relative amount of pathogenic variants was increased compared to the variant fraction only found in ctDNA from plasma of a separate blood tube. Results obtained for frequently overexpressed CTC transcripts in this subgroup included genes involved in the PI3K signaling pathway as well as ERBB2 and ERBB3 in about 30% of the pts.

**Conclusion:** We here present a feasible workflow for CTC and ctDNA evaluation for expression and mutation analysis from the same blood sample. These data emphasize that the use of different liquid biopsy analytes can empower treatment decisions of MBC pts in the future.

## 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-01-11

Genomic alterations of cell-free DNA in early breast cancer patients with recurrence

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Cell-free DNA (cfDNA), as a non-invasive strategy, provides substantial benefit to overcome tumor heterogeneity. Surveillance of recurrence after standard treatment in early breast cancer (BC) using cfDNA, enables to detect minimal residual disease (MRD), also to identify genomic alterations driving recurrences. We aimed to assess the role of cfDNA in detecting MRD by investigating genomic alterations of 1) primary, recurrent tumor and 2) cfDNA at time of recurrence using deep targeted sequencing. Fifty-four early BC patients were enrolled prospectively between 2014 and 2017 at time of recurrence. Median disease free interval was 28.5 months (range 6.2-49.8). 62.7% (32/51) were hormone receptor (HR) positive (28 HRpos/HER2neg, 4 HRpos/HER2pos), 11.8% (6/51) were HRneg/HER2pos and 25.5% (13/51) were triple negative BCs. 59.3% (32/54) patients developed loco-regional recurrence (15 local recurrence only, 13 regional only, 4 with both) and distant metastasis was observed among 40.7% (22/54) patients. Cell-free DNA was extracted from 5cc blood at time of recurrence. Deep targeted sequencing was performed using customized NGS panel –encompassing 426 cancer-related target coding region, 242 fusion and amplification-related region- of cfDNA and FFPE (formalin fixed paraffin embedded) tumor samples archived from surgical resection or biopsy. Deep targeted sequencing data was successfully performed in 72.1% (31/43) plasma samples and sequencing yield was significantly lower when stored for more than 2yrs (46.2% vs 83.3%).

Mutations of cfDNA and tumor (primary, recurrent) were analyzed. Mean sequencing depth of cfDNA and FFPE were x425.7 and x777.6 respectively. Median number of pathogenic mutations found in primary tumor, cfDNA and recurrent tumor were 27 (range 12-99), 25 (range 8-85) and 9 (range 0-23). Among mutations found in primary tumor, 27.4% were shared mutations (range 8.1%-72.7%) with recurrent tumor and 26.1% were shared mutations (range 4.7%-69.2%) observed in cfDNA sample. Among mutations found in recurrent tumor, 40.9% were observed in cfDNA (range 17.7-87.5%). In primary tumor, median number of mutations with allelic fraction (MAF) > 10% were 12 (range 4-21) and at least one mutation was found in cfDNA at time of recurrence. Among mutations with MAF > 10%, 59.4% and 69.1% were found in cfDNA and recurrent tumor. Known oncogenic mutations of *PIK3CA*, *TP53*, *GATA3*, *AKT1*, *ESR1*, *RELN*, *ERBB2*, *ERBB3*, *BRCA1* mutation were found. *PIK3CA* gene (p.H1047R) was found in two cases both in primary tumor and cfDNA at recurrence (MAF 11.4% vs 5.3% and 12.3% vs 15.4%) suggesting *de novo* driver mutation. One patient developed regional recurrence during adjuvant aromatase inhibitor with *ESR1* V392I mutation in both cfDNA and recurrent tumor (MAF 48.1 and 54.5%), while another patient's recurrent tumor during aromatase inhibitor harbored *ESR1* D538G mutation exclusively in recurrent tumor with MAF < 1%. Both patients had no *ESR1* hotspot mutation in primary tumor.

Our data showed sequencing yield of 83.3% in plasma samples within 2yr. Pathogenic mutations in primary tumor, especially when MAF > 10%, half of them was observed in cfDNA at time of recurrence. *ESR1* mutation should be included in cfDNA surveillance for patients undergoing endocrine therapy even absent in primary tumor.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-12

Low peripheral blood CD4/CD8 ratio at the time of surgery is a negative long-term prognostic factor in women with early stage breast cancer

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It is hypothesized that cancer prognosis may be related to the functional status of the immune system. We examined the correlation between peripheral blood CD4/CD8 ratio measured at the time of surgery and clinical outcome in patients diagnosed with early stage breast cancer.

## **Patient and Methods**

Peripheral blood from 57 treatment-naïve early breast cancer patients, not eligible for neoadjuvant chemotherapy, was collected on the day of definitive surgery. CD4+ and CD8+ T cells were enumerated using flow cytometry and the ratio between the two immune cell populations was calculated. Cox regression analyses were performed to determine the relationship between CD4/CD8 ratio vs. distant disease-free survival (DRFS), breast cancer-specific survival (BCSS) and overall survival (OS). The median follow-up times were 10.1 years (range: 0.4-17.5) and 15.0 (range: 1.0-18.5) for DRFS and BCSS/OS, respectively.

## **Results**

The patients' mean age at diagnosis was 54 years old (range: 31-78). 82% were hormone receptor-positive, 21% HER2-positive, and 61% node-negative. The median CD4/CD8 ratio was 2; and a ratio  $\leq 2$  was considered low. CD4/CD8 ratio was not associated with any of the clinicopathologic variable examined. Multivariate analysis using a survival model that adjusted for potential confounding factors (age, tumor size, grade, stage, hormone receptor, HER2, lymph-node status) revealed that patients with low CD4/CD8 ratio have statistically significant increased risk of distant recurrence (DRFS HR 5.3, Wald  $p=0.0381$ ) and death (OS HR 3.8 Wald  $p=0.0271$ ).

## **Conclusions**

Immune dysfunction at the time surgery is correlated with long-term increased risk for metastatic recurrence and death. Larger clinical studies are warranted to confirm the results of this study.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-13

Molecular characterization of circulating tumor DNA in Chinese metastatic breast cancer (mBC) patients

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**Background:** Circulating tumor DNA (ctDNA) have been increasingly used as a minimal invasive test to detect genomic alterations of cancer patients.

**Methods:** We performed a retrospective analysis of blood samples from mBC patients (pts) collected at baseline, on-treatment, and disease progression. A highly sensitive, plasma-derived ctDNA-based NGS assay (PredicinePLUS, a comprehensive 180 key cancer gene panel) was used to detect somatic mutations and copy number variations in ctDNA with an in-house propriety algorithm.

**Results:** In this study, we evaluated 162 blood samples from 109 mBC pts who underwent systematic therapy at Beijing Cancer Hospital with approval from Research Ethics Committee. 129 samples from 92 pts passed NGS quality check, and were included in the analysis. Among these pts, the percent of HR+, HER2+ and Triple-Negative pts is 36.8%, 29.5% and 31.5%, respectively. About 86% samples contain somatic mutations and/or copy number variations. Collectively, 372 somatic mutations (SNV and Indels) were detected on 76 genes, among which *TP53* (44.2%), *PIK3CA* (25.3%), *BRCA2*(10.5%) and *ATM* (10.5%) were frequently altered with frequency varying across different subtypes. ESR1 hotspot mutations ((D538G and Y537S/N/C) were also detected in a subset of HR+ patients. Copy number gain or loss was detected on 32 genes, including amplification of *ERBB2*, *PIK3CA*, *FGFR1* and deletion of *CDKN2A*, *ATM*, *RB1* etc. Importantly, *ERBB2* copy number amplifications were only detected in HER2 IHC positive cases for base-line samples, leading to 68% sensitivity and 100% specificity. Interestingly, ctDNA yield increased as disease progressed, suggesting ctDNA yield may serve as a potential biomarker for predicting treatment response and monitoring disease progression. Additional genomic alterations that change dynamically along with the course of treatment or associate with drug response and/or resistance were also identified.

**Conclusions:** This study demonstrated ctDNA-based genomic analysis was highly sensitive and specific in detecting various genomic alterations, consistent with other published studies. It also suggests that HER2 copy number amplification could be robustly assessed in a non-invasive manner.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-14

Association between interleukin 2 (IL-2) and circulating tumor DNA (ctDNA) is a novel biomarker for patients with metastatic breast cancer (BCa) after systemic therapies

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**Introduction:** The detection and monitoring of ctDNA in metastatic breast cancer showed ability to predict treatment resistance and outcome. But the mechanisms has been a challenge to clinicians. Immune escape and immune tolerance has also been reported to cause BCa progress. Herein, we report a novel finding of the association between plasma IL-2 and the ctDNA in advanced BCa patients who received the systemic therapies, and it is potential utilization in clinic.

**Methods:** This study enrolled 43 patients with stage III/IV BCa at the Northwestern Memorial Hospital (2016-2017) that had longitudinally detection of ctDNA and circulating tumor cells (CTCs) before (baseline, BL) or 3 months after (first evaluation, FE) systemic therapies respectively. Duplicate whole blood samples (7.5ml/each) were collected in EDTA tubes from these patients. Plasma ctDNA was analyzed using the Guardant360 NGS-based assay (Guardant Health) and CTC enrichment and enumeration were performed in FDA approved semi-automated fluorescence CELLTRACKS ANALYZERII® System (Menarini Silicon Biosystems) by using CELLSEARCH® CXC Kit (Menarini). ELISA (Fisher) for IL-2 was performed by using patients' plasma. Database of IL-2, ctDNA and CTCs was generated and linked with clinical database. Kruskal-Wallis test was used for statistics. We previously reported cut-off of 5.7 was used to dichotomize the prognostic value of ctDNA percentage (%ctDNA) in 2018 ASCO. Matched pairs variations between IL2 levels at BL and at FE were tested through Wilcoxon signed-ranks test. Associations between %ctDNA and IL2 levels were explored through Kruskal-Wallis test. The prognostic impact of IL2 was tested through Cox regression.

**Results:** CTCs  $\geq 5$  were found in 23 patients at BL and 21 patients in FE respectively. There were 12 patients that had increase CTCs, and 31 patients with similar or less CTCs FE after systemic therapies. Decreased in CTCs was associated with increased IL-2 ( $P=0.004$ ). The FE analysis showed that IL-2 dropped significantly in patients with CTC stably  $\geq 5$  (from 95.84pg to 79.46pg) after therapies ( $P<0.001$ ). Furthermore, baseline IL-2 levels were significantly higher in patients with % ctDNA levels  $\geq 5.7$  (97.15pg) compared to patients with %ctDNA levels  $<5.7$  (68.64pg) ( $P=0.0027$ ). No other associations were highlighted in respect to age or number of ctDNA alterations. There was no significant variations between BL and FE levels of IL2 were observed according to BCa subtypes nor in respect to baseline %ctDNA  $\geq 5.7$  or CTCs  $\geq 5$ . Compared with low level of BL IL-2 ( $<78.3$ pg) group, high level of BL IL-2 ( $\geq 78.3$ pg) had a significant negative impact on overall survival (OS) ( $P=0.037$ ) in univariate analysis.

**Conclusions:** Our findings indicated that aggressive BCa with high level ctDNA mutation are associated with high level of IL-2 and immune response in patients with advanced disease. In addition we confirm a reverse correlation between change of IL-2 and change of CTCs potentially indication of immune escape. In summary, the study shows a dynamic relation between IL-2 level and tumor burden (ctDNA) and immune escape (CTCs) suggesting another potential biomarker to monitor interaction between tumor and immune environment.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-15

The prevalence of estrogen receptor-1 mutation in advanced breast cancer: The estrogen receptor one study (EROS)

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## Background

Estrogen receptor alpha (ESR $\alpha$ ), encoded by the estrogen receptor-1 (ESR1) gene, is expressed in approximately 70% of all breast cancers, and hormonal therapy represents a major treatment modality in ESR $\alpha$ -positive cancers. The most commonly used endocrine therapies inhibit ESR activity by either targeting the ESR protein itself or depriving the receptor of its ligand. Endocrine therapy has become the mainstay of prevention and treatment of ESR+ breast cancers in all stages of the disease. Furthermore, ESR status might be a strong predictor of response to endocrine therapy. About 20% of patients who present with early disease will develop resistance manifested as recurrences either during or after adjuvant endocrine treatments. While in metastatic breast cancer (MBC) patients, the resistance rate could be as high as 30%. Recent studies unveiled that these ESR1 mutations lead to constitutive activity of the ESR, meaning that the receptor is active in absence of its ligand estrogen conferring resistance against endocrine therapy.

**Purpose:** The goal of the present study is to determine the frequency rate of ESR1 mutations in hormone-sensitive advanced breast cancer by using digital droplet PCR (ddPCR) technique.

## Materials and Methods

This retrospective study was conducted in the Multidisciplinary Breast Clinic of the Antwerp University Hospital. The seven most common ESR1 mutations (c.1138G>C (p. (E380Q)), c.1610A>G (p.(Y537C)), c.1613A>G (p.(p.D538G)), c.1607T>G (p.(L536R)), c.1387T>C (p.(S463R)), c.16410A>C (p.(Y537S)), c.609T>A (p.(Y537N))) were assessed in available baseline plasma samples of women with hormone sensitive progressive breast cancer. Inclusion criteria for study participation were: female, age above 18 years, breast cancer, positive ESR expression, 5 years endocrine therapy of the primary disease, disease progression under endocrine therapy. ESR mutations were analyzed in cell-free DNA (cfDNA) by using ddPCR.

## Results

In EROS study, ESR1 mutations were successfully examined in cfDNA from 21 patients with advanced breast cancer. In the current study, we reported positive ESR1 mutation in 19% of patients (4/21; 95% CI, 5%-42%). The test sensitivity was lower than the targeted value <0.1% in 29% of patients (6/21). No significant statistical difference in baseline clinical characteristics was observed in patients with wild-type and mutant ESR ( $p>0.05$ ). All the patients had received AI with a variable period of good response. Adjuvant endocrine therapy for primary disease was Tamoxifen (TAM) for 57% of patients (12 of 21) of whom 8 patients had received aromatase inhibitor (AI) after two years, while 43% of patients (9 of 21) had received AI as first line adjuvant hormonal therapy. However, there was no sufficient number of samples to formally analyze the clinical impact of ESR mutation on the type of endocrine therapy.

## Conclusion

ESR1 mutation analysis should be considered in hormone-sensitive MBC patients to improve the therapeutic strategies in controlling ESR signaling before the occurrence of wide spread disease metastasis.

**Key words:** Estrogen receptor, Mutation, breast cancer, hormonal therapy, and metastasis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-16

Detection of plasma tumor DNA (ptDNA) in patients with hormone receptor-positive HER2-negative (HR+HER2-) early breast cancer (EBC) in clinical remission

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**Background:** Detection of ptDNA in patients with HR+HER2- EBC in clinical remission may impact recommendations for type and duration of adjuvant endocrine therapy. A sensitive technique to identify tumor mutations in plasma is BEAMing digital PCR. The frequency and timing of detectable mutations in plasma of patients in clinical remission from HR+HER2- EBC are unknown.

**Methods:** We screened a prospective institutional repository for patients that met inclusion criteria. Eligible patients must have been enrolled to the repository between 12/1/2008 (repository start) and 12/31/2016, had HR+HER2- EBC, received follow-up at Johns Hopkins with appointment scheduled between 3/1/2017 and 12/31/2017, completed curative surgery at least 6 months prior to this appointment, been recommended or initiated adjuvant endocrine therapy, and been in clinical remission. Appropriate patients were approached for a current blood sample during their follow-up appointment in 2017. Blood was analyzed using a BEAMing digital PCR platform (Sysmex Inostics OncoBEAM™) for *AKT1*, *PIK3CA*, and *ESR1* mutations.

**Results:** We identified 67 eligible patients and collected blood from 60. Most patients had relatively low risk disease including 40 patients (67%) with stage I disease, and only 21 patients (35%) received chemotherapy. Patients were evenly divided between receiving tamoxifen or an aromatase inhibitor, and some patients switched from one to the other. The majority of patients (68%) had surgery between 1 and 5 years prior to the current blood draw. Detailed patient characteristics are provided in Table 1. Two out of the 60 patients had detectable ptDNA, both with stage IIA disease. One patient had a mutation in the *ESR1* ligand-binding domain P535H 9 months after surgery and while taking adjuvant tamoxifen for 7 months. Sanger sequencing of primary tumor tissue did not reveal this mutation. Another patient had a mutation in *PIK3CA* exon 9 E542K 9.5 years after surgery and after taking adjuvant tamoxifen for at least 7 years. Amplifying this locus in DNA from primary tumor tissue was unsuccessful; further analysis using droplet digital PCR (ddPCR) is planned.

**Conclusions:** Detection of ptDNA was feasible in a relatively low-risk group of patients with HR+HER2- EBC in clinical remission. Sampling a larger number of patients is needed to gain more understanding of the frequency and timing of detectable ptDNA. Next steps should also focus on determining the natural history of detectable ptDNA in patients with HR+HER2 EBC in clinical remission which may impact adjuvant treatment recommendations.

**Funding sources:** Komen SAC110053, P30 CA06973, Breast Cancer Research Foundation

Table 1: Characteristics of included patients

	N (%)
Total patients	60
Age at diagnosis, median(range)	57 (30-77)
Female	59 (98)
Caucasian	54 (90)
Postmenopausal at diagnosis	36 (60)
Tumor size <2 cm	42 (70)
Node negative	45 (75)
Invasive ductal histology	44 (73)
Received adjuvant chemotherapy	21 (35)
Type of adjuvant endocrine therapy	

Tamoxifen	25 (42)
Aromatase inhibitor	26 (43)
Tamoxifen and AI	7 (12)
None	2 (3)
Time after surgery	
6 months to <1 year	6 (10)
1 year to <5 years	41 (68)
5 years to <10 years	13 (22)

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-17

Aggressive subgroups of metastatic triple-negative breast cancer: Inflammatory breast cancer and young patients in the Dana-Farber cell-free DNA cohort

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**Background:** Relative to other metastatic breast cancer subtypes, metastatic triple-negative breast cancer (mTNBC) has a shorter duration of response to therapy and worse overall survival. Within mTNBCs, there is a prevailing belief that inflammatory breast cancer and young women tend to have among the most aggressive phenotypes. We investigated clinical and cell-free DNA (cfDNA) characteristics of inflammatory-mTNBC and young-mTNBC. We hypothesized that inflammatory-mTNBC may have distinct clinical and cfDNA characteristics, offering potential novel biomarker and therapeutic strategies.

**Methods:** 164 patients from the Dana-Farber metastatic triple-negative cell-free DNA cohort (Stover DG, et al J Clin Oncol 2018) were included in this secondary analysis. Patients were stratified into three groups: 1) inflammatory breast cancer ('IBC'); 2) non-IBC patients aged 45 years (yr) or younger at primary diagnosis ('non-IBC young'); and 3) non-IBC patients over age 45 yr at diagnosis. For each subset population, we evaluated clinicopathologic characteristics, sites of metastasis, survival outcomes, and cfDNA 'tumor fraction' – the fraction of DNA in circulation derived from tumor. Those patients with adequate cfDNA tumor content for high confidence copy number calls (n=101) were included in an analysis of copy number alterations.

**Results:** Among 164 patients with metastatic TNBC, 13.4% (22/164) had IBC, 37.8% (62/164) were non-IBC young, and 48.8% (80/164) were non-IBC and over 45 yr. Race and primary receptor status were similar. IBC patients were diagnosed at a higher stage (Chi-square p=0.0009) while non-IBC young patients were significantly more likely to harbor a BRCA mutation (Chi-square p=0.03). Analysis of metastatic sites revealed that IBC patients had significantly greater frequency of ipsilateral and contralateral breast chest wall recurrences (p=0.04 and p=0.046, respectively) while non-IBC young patients had the most frequent lung metastases (p=0.002). There were no significant differences in frequency of bone, brain, or liver metastases. cfDNA analyses showed that cfDNA 'tumor fraction' was highest in non-IBC young patients (ANOVA p=0.03 for maximum tumor fraction). Median overall survival from metastatic diagnosis was 22.9 months. IBC and non-IBC young patients had a worse prognosis relative to non-IBC patients over 45 yr (hazard ratio IBC=1.97, 95% CI 1.09-3.57; HR non-IBC young=1.60 95% CI 1.07-2.41; log-rank p=0.023). By subgroup, median overall survival from metastatic diagnosis for IBC was 15.2 months, non-IBC young 21.2 months, and non-IBC over 45 yr 31.2 months. Analyses of genome-wide copy number alterations from cell-free DNA will be presented.

**Conclusions:** Among metastatic TNBCs, IBC patients and non-IBC young patients have a significantly worse overall survival compared with non-IBC patients over 45 yr of age. Young patients have more frequent lung metastases and higher 'tumor fraction' of cfDNA. Confirmation of the reported findings is limited due to cohort size and may reflect referral bias.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-18

Correlation between circulating tumor DNA (ctDNA) alterations and circulating tumor cells (CTC) uncovers new mechanisms of metastasis for patients with metastatic breast carcinoma (MBC)

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**Introduction:** Novel molecular diagnostics including CTCs and ctDNA have been proved to predict disease metastasis and survival. However, the frequency of detection of actionable mutations using CTCs and ctDNA is variable based upon tumor related factors and diagnostic platform sensitivity. Herein, we evaluated a novel NGS technology in the ability of detecting driver and clonal genomic abnormalities in samples from MBC patients, and compared ctDNA alterations with CTCs and CTC-cluster. This study demonstrated several novel correlation between some specific ctDNA alterations and CTCs or CTCs related biomarkers, which opened new insight on mechanisms of metastasis for MBC.

**Methods:** This study included 52 samples from 26 patients with stage III/IV BCa treated at NMH (2016-2017) and who received standard systemic treatments based on disease subtypes. Whole blood samples (7.5ml/each) were used for CTC enrichment and enumeration in FDA approved CELLTRACKS ANALYZERII® System (Menarini). ctDNA from clinical plasma samples was analyzed by using PredicinePLUS, a NGS-based assay (Predicine Inc) with a 180-gene panel for genomic alterations mutations. Results of CTCs and ctDNA alterations were linked to clinical database. Matched pairs variations between CTCs and ctDNA alterations was compared by Wilcoxon signed-ranks test and Kruskal-Wallis test.

**Results:** Genomic Alterations (SNVs, Indels and copy number variations) were detected on 52 genes by PredicinePLUS assay. All samples (100%) demonstrated at least 1 somatic alterations. There were 75 mutations detected within 29 genes, and the variant frequency of mutated genes ranges from 0.11% to 68.56%. Increased CTCs were highly significantly correlated with genomic alterations in the genes (wild type vs alterations) including GATA3 (8vs 37), ESR1 ( 2.5 vs 41.3), CDH1 (3.5 vs 50.5) and CCND1 (4 vs 120) (P<0.01). Decreased CTCs were correlated with alterations of CDKN2A (20.5 vs 0) (P=0.025). CTC-cluster appear associated predominantly with alterations of CDH1 (P=0.0018), CCND1 (P=0.008) and BRCA1 (P=0.04). Furthermore, in HER positive CTCs group, ERBB2 mutations caused increased CTCs in compared with ERBB2 wild type (0 vs 5), when CCND1, CDKN2A, GATA3 and TP53 alterations were associated with increase of HER2 negative CTCs.

**Conclusions:** By using the novel diagnostic platform with the ability to identify ctDNA mutation and copy number variation, this study demonstrated several novel genes alterations which were highly correlated with CTCs, CTC-cluster and HER2. Some genes (CCND1 and CDH1) got involved into the changes on both CTCs and CTC-cluster, when some genes (CCND1, CDKN2A, ESR1 and GATA3) were related with change of CTCs and HER2 expression. Correlation of CTCs and ctDNA can be reliably and routinely used as non-invasive method for monitoring disease metastasis and predict the prognosis in MBC in clinic.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-01-19

Liquid biopsy and re-biopsy: Tracking mutational trajectories in HER2<sup>+</sup> breast cancer patients undergoing T-DM1 treatment

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**Background:** The antibody-drug conjugate Trastuzumab emtansine (T-DM1, Kadcyla®) is standard of care in HER2<sup>+</sup> breast cancer patients on clinical progression after Trastuzumab/Pertuzumab and taxanes. Despite considerable clinical benefits, most patients rapidly develop progressive disease due to adaptive resistance, the molecular bases of which remain largely unknown and/or controversial. Next generation sequencing (NGS) and digital PCR (dPCR) applied to serial biological samples obtained through liquid biopsy (LB) and re-biopsy (RB) offer a unique opportunity to intercept mutational trajectories and uncover molecular patterns linked to primary as well as adaptive resistance.

**Materials and methods:** Tumor tissues (n=14), either from primary or metastatic lesions, and plasma samples (n=99) were collected, upon informed consent, from 9 breast cancer patients undergoing T-DM1 administration. Tissue (tDNA) and circulating tumor DNA (ctDNA) were extracted by the QIAmp DNA FFPE and CNA kits (Qiagen), respectively, and analyzed by ultra-deep sequencing and dPCR (IonTorrent S5 and QuantStudio 3D, LifeTechnologies) with commercial 400-gene panel and custom-designed dPCR assays. Genomic data were correlated with clinical imaging (CT/PET).

**Results:** Six out of 9 (66.7%) patients experienced progression within 1 year of treatment (mean 192±97 days), whereas the remaining 3 were stable at the last follow up (> 400 days). No correlation was found between outcome and HER2, ER or PR status in the latest available (prior to T-DM1) archival tissue, in which NGS revealed several pre-existing mutations, including some associated with resistance to ERBB2 blockade. LB analysis detected increases in both baseline and *de novo* occurring aberrations in 5/6 (83.4%) relapsing patients. As compared to clinical imaging, progression disease was anticipated by an average lead time of 1.9 months (range 0.7-2.8). Surprisingly, the sixth relapsing patient underwent rapid progression (3 months) in spite of decreased PIK3CA p.E545K in blood, further confirmed in the re-biopsy, thus suggesting heterogeneous response to T-DM1 across multiple cancer cell populations. Of note, we observed progressive accumulation of ERBB2 p.L755S (associated with Lapatinib resistance) in multiple biopsies of serial metastatic foci from a patient over 18 years of multimodal ERBB2 blockade. However, a single administration of T-DM1 resulted in ultra-fast (within few weeks) clearance of ERBB2 p.L755S ctDNA, and stabilization of two distinct 'bystander' TP53 ctDNAs (p.R273H and p.S241T).

**Conclusions:** Non-invasive LB monitoring of a small cohort of T-DM1-treated patients provides proof of principle of intersecting mutational trajectories, anticipation and classification of resistance, as well as *de novo* appearance/clearance of resistance mutations. Thus, LB and RB may hint at disease evolution and successive lines of medical treatment.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-20

Circulating tumor DNA as a predictive biomarker of response to palbociclib-fulvestrant in patients with estrogen receptor-positive, HER2-negative metastatic breast cancer

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## Background:

Following the PALOMA-3 study results, the combination of palbociclib, a cdk4/6 inhibitor, with fulvestrant has become a standard therapy in women with estrogen receptor-positive (ER+) HER2-negative (HER2-) metastatic breast cancer (MBC) whose disease progressed under hormone therapy. Palbociclib increased the progression-free survival (PFS) in all patient subgroups, and no predictive biomarker of palbociclib efficacy has been validated so far. In that context, we evaluated whether early changes of circulating tumor DNA (ctDNA) levels are associated with the efficacy of palbociclib plus fulvestrant.

## Methods:

After providing written informed consent, ER+ HER2- MBC patients were included in a prospective observational cohort ("ALCINA", NCT02866149) prior to the initiation of palbociclib plus fulvestrant. Tumor response was assessed by a radiological evaluation (RECIST v1.1) every 3 months. Fresh plasma samples were collected at baseline, before the initiation of treatment, at day 15, at day 30 and at disease progression. For each patient, DNA from archived tumor tissue was subjected to targeted NGS to identify a driver mutation. In patients with identified driver mutation, circulating cell-free tumor DNA extracted from plasma was subjected to digital droplet PCR (ddPCR). Ratios of ctDNA levels (J15/baseline and J30/baseline) were correlated with prospectively registered patient characteristics and outcomes. For the current analysis, "responders" were defined as patients who experienced a PFS longer than 3 months.

## Results:

61 MBC patients have been included and 26 were eligible for ctDNA mutation detection, after the characterization of single nucleotide variations in *PIK3CA* (N=21 patients), *TP53* (N=3) and *AKT* (N=2). At baseline, 21 patients (81%) had detectable ctDNA levels, with a median level of ctDNA of 381 copies/ml of plasma; There was no significant correlation between ctDNA levels at baseline and PFS (p=0,06).

ctDNA levels decreased under therapy, with 82% and 68% of patients displaying detectable levels of ctDNA at day 15 and day 30. Patients with undetectable ctDNA levels at day 15 and, principally, at 30 were more likely to experience a PFS > 3 month (HR=5,8, 95% IC=1,5-22,5, p=0.004).

We explored ctDNA level ratios (day 15/baseline and day 30/baseline) and found that all patients whom ctDNA increases at day 30 (31%) will not respond to treatment. Moreover, among all patients, ctDNA detection at day 30 is correlated with shorter PFS [HR=2,634 IC95 (1,1 to 6,5)].

At time of tumor progression, all patients presented increased ctDNA levels.

In addition to tumor tissue sequencing, activating ESR1 mutations were found at baseline in 5 patients (19%).

## Conclusion:

Our study suggests that the efficacy of palbociclib and fulvestrant may be monitored by serial analyses of ctDNA levels, before radiological evaluation and that early ctDNA levels and dynamics are prognostics factors for PFS. A large randomized trial, PADA-1 (NCT03079011), is currently testing the utility of real time resistant subclones detection in ctDNA from ER+ HER2- MBC treated with palbociclib and aromatase inhibitor.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-21

Multiplexed targeted digital sequencing of circulating tumor DNA to detect minimal residual disease in early and locally advanced breast cancer

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## Background:

Circulating tumor DNA (ctDNA) analysis holds potential for minimal residual disease (MRD) detection in early stage breast cancer. However, sensitivity for MRD is limited due to low ctDNA levels in early stage patients and limited blood volumes. Loss of input DNA during library preparation, limited multiplexing or low sensitivity of current molecular methods further limit accuracy. To address this gap, we have developed TARgeted DIgital Sequencing (TARDIS), a novel method for simultaneous analysis of multiple patient-specific mutations in plasma DNA.

## Methods:

Using tumor exome sequencing, we identify and prioritize somatic founder mutations, design nested primers and evaluate them for multiplex performance. Using 5-10 ng input plasma DNA, we perform 1) targeted linear pre-amplification to improve downstream molecular conversion, 2) single-stranded adapter ligation to incorporate unique molecular identifiers (UMIs) and 3) targeted PCR to prepare sequencing-ready libraries. The resulting sequencing reads have fixed target-specific ends and variable ligation ends. We utilize fragment size and UMIs to group sequencing reads into read families. To ensure specificity, we require targeted mutations are supported by 2 or more read families.

## Results:

To assess analytical performance, we targeted 8 mutations in cell-free DNA reference samples with 0.25%-2% mutation allele fractions (AFs). Precision across 7-16 replicates at each AF level agreed with expectations of Poisson distribution, demonstrating effective analysis of ~70% of input DNA. At 2%, 1%, 0.5% and 0.25% AFs, variant-level sensitivity was 96.4%, 96.4%, 91.1% and 65.8%, approaching the theoretical limit given input DNA. At 0.25% AF, 3-7 mutations were detected per sample, achieving 100% sample-level sensitivity. In 16 wild-type replicates, no targeted mutations were called (100% specificity). Averaging multiple mutations improved precision in sample-level AF estimates. Mean AFs from 8 mutations for the 2% sample were 2.34%-2.80% (5.8% CV).

In 6 patients with breast cancer treated with neoadjuvant therapy (NAT), we analyzed 8-18 patient-specific mutations (mean 11.8). Before treatment, ctDNA was detected in 5/6 patients at mean AFs of 0.02%-1.19% (mean 0.40%), supported by 2-10 mutations (mean 5.6). Of these 5 patients, 4 had residual disease after NAT and ctDNA was detected pre-operatively or during NAT in 3/4 patients. 1 patient achieved pathological Complete Response and ctDNA was undetectable after NAT.

## Conclusions:

Preliminary results suggest TARDIS enables accurate MRD detection after neoadjuvant therapy in patients with early stage breast cancer. On-going work is expanding this analysis to include additional patients and investigate the clinical validity of peri-operative ctDNA monitoring.

## Summary of clinical results

Patient	Pre-NAT Stage (TNM)	Subtype	No. of Mutations Targeted	Baseline ctDNA (AF%, No. of Mutations)	ctDNA after or during NAT (AF%, No. of Mutations)	Residual Tumor (TNM)
1	T3 N1	ER+ PR+ HER2-	8	+ (0.02%, 2)	-	T2 N1
2	T3 N0	TNBC	12	+ (0.29%, 6)	+ (0.01%, 1)	T1a N0



3	T2 N1	TNBC	18	+ (1.19%, 10)	+ (0.01%, 1)	T1mi N0
4	T3 N1	TNBC	10	+ (0.02%, 3)	+ (0.05%, 3)	T3 N1
5	T2 N0	TNBC	9	+ (0.46%, 7)	-	pathCR
6	T1c N1	TNBC	14	-	-	pathCR

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-01-22

A blood based diagnostic/prognostic for early stage breast cancer

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**Purpose:** Although improvements in mammographic screening, including digital mammography and 3D tomosynthesis, have occurred in recent years, the positive predictive value (PPV) of mammography remains low. Screening mammography has an average PPV for biopsy of 32.6 % (range 22.2-54). Furthermore, for women with abnormally dense breasts, mammography may be hard to interpret or may provide a false negative. Here, we report a blood diagnostic for women with breast lesions that is capable of detecting and discriminating epigenetic biomarkers associated with either invasive or more benign disease. Our approach is based on the premise that circulating lymphocytes undergo epigenetic changes upon exposure to the tumor microenvironment and these changes can be followed to track tumor progression from a non-invasive to an invasive state. We hypothesized that DCIS samples with high risk for progression would maintain a predominant epigenetic signature that would match those observed in invasive disease, while those DCIS samples considered to have low risk or benign disease would resemble controls. To test the utility of these epigenetic biomarkers, we employed a novel diagnostic model which uses a combined machine learning approach integrated with the recombinatorial strategy of a genetic algorithm, to screen a large response space of thousands of CpG sites to identify a small set of CpG sites (< 20) with the highest combined predictive power.

**Methods:** Blood was obtained from women with histologically confirmed DCIS (n=14), or invasive ductal carcinoma (n=10) and women with no evidence of breast lesions on mammography (n=10). PBMCs were isolated using a modified Ficoll-Paque gradient, and DNA was extracted using standard commercial kits. Epigenetic profiling of DNA was performed using a highly sensitive and quantitative analytics platform, which utilizes methylation sensitive restriction endonucleases to detect changes in methylation of CpG sites from standard NGS data. Risk of invasiveness potential was determined based on pathologic criteria. Samples were scored as low risk (Cribriform subtype, no necrosis, low mitotic index) or high risk (Comedo or solid subtype, high mitotic index, necrosis).

**Results:** Non-metric multidimensional scaling ordination analysis of the CpG sites revealed highly distinct methylation patterns between Normal, DCIS and invasive samples. Using a Likelihood Ratio Test with defined ANOVA contrasts, over 14,000 significantly different methylated CpG sites were identified ( $p < 0.05$  after false discovery rate correction). A proprietary machine learning diagnostic model was employed to reduce this high-dimensional variable space to the most effective set of CpG sites. DCIS and invasive samples were discriminated in blinded tests (n=17) with 69% accuracy. Prognostic ability was determined in a blinded study of 10 DCIS samples in which we obtained an accuracy of 90% for risk assessment based on our pathological criteria.

**Conclusions:** Preliminary studies show strong ability of the identified DNA methylation metrics to detect and discriminate invasive and non-invasive breast lesions. The quantitative sensitivity and selectivity of this new diagnostic/prognostic blood test will be determined in larger patient cohorts.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-01-23

Clinical significance of serum PSA in breast cancer patients

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**Background:** Recent preclinical data suggest that estrogen receptor (ER) positive breast cancer (BC), may switch from dependence on ER to androgen receptor (AR) as possible mechanism of resistance to ER-targeted endocrine therapy. AR dependency has also been suggested in a subset of ER-, AR+ BC. Based on these findings, clinical trials testing AR-targeting therapies in BC have been conducted. However, predictive markers for response to this type of therapies remain to be elucidated. PSA is the product of an androgen-responsive gene produced also in BC, and serum PSA (sPSA) could be detected in BC patients by a highly sensitive assay. **Hypothesis:** If sPSA reflects AR dependency of BC, it might be useful as a predictive marker for response to AR-targeting therapy. **Methods:** In this study, we investigated whether tumor-derived sPSA could be detected in BC patient, and if it might reflect tumor biology. In metastatic breast cancer (MBC) patients continuously observed, sPSA was evaluated monthly by CLEIA method (detection sensitivity  $\geq 3$  ng/L). Similarly, sPSA was evaluated at arbitrary points in non-BC control and point analysis BC group enrolled regardless of clinicopathological factor or treatment history. In the observational group, the relationship between change in disease condition and sPSA was analyzed. Next, correlations between sPSA and various clinicopathological factors were analyzed using combined data of point analysis group and initial sPSA value of observation group. In this study, 146 BC (26 observation group and 120 point analysis group) and 99 control were enrolled. **Results:** In the observational analysis, 5 cases showed sPSA change well reflected the disease condition, but not in other 5 cases. In remaining 16 cases, sPSA was undetectable or the observation period was insufficient ( $<4$ m). In the point analysis, sPSA was detected in 28.3% and 28.1% in control and BC respectively. Although in pre-menopausal state, there was no significant difference in sPSA between control and BC ( $4.4 \pm 6.98$  ng/L vs  $3.7 \pm 5.5$  ng/L), in post-menopausal state, sPSA was significantly higher in BC compared with control ( $0.7 \pm 2.5$  ng/L vs  $64.6 \pm 357.4$  ng/L;  $P < 0.05$ ). In analysis limited to post-menopausal BC, sPSA was higher in MBC (de-novo stage 4 and recurrence) compared with non-MBC (Stage 0-3) ( $106.0 \pm 457.2$  ng/L vs  $2.1 \pm 8.5$  ng/L;  $P < 0.05$ ). Similarly, sPSA was higher in low ki-67 ( $<20\%$ ) cases compared with high ki-67 ( $\geq 20\%$ ) ( $105.7 \pm 510.8$  ng/L vs  $30.5 \pm 173.7$  ng/L;  $p < 0.05$ ). There was no significant difference in sPSA due to histological type, ER or HER2 status and nuclear grade. In correlation analysis of quantitative data limited to post-menopausal MBC, sPSA was negatively correlated with Ki-67 ( $rS = -0.35$ ,  $p < 0.05$ ) and positively correlated with treatment line of previous endocrine therapy ( $rS = 0.27$ ,  $p < 0.05$ ). It did not correlate with age, disease free interval, number of metastatic organs or treatment line of previous chemotherapy. **Conclusion:** Our data suggest that sPSA may be tumor-derived at least in post-menopausal MBC and may reflect some kind of tumor biological properties. These all findings justify further studies of the efficacy of sPSA as a predictive marker in AR-targeted therapy.

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S100A11 is a diagnostic marker and promotes cancer progression for luminal breast cancer

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**Background:** S100A11, also known as calgizzarin, is a member of S100 calcium-binding protein family and is upregulated in various cancers including lung cancer, renal cell cancer, ovarian cancer and pancreatic cancer. Many studies have shown that S100A11 is related to cancer progression. However, the function of S100A11 in breast cancer remains largely unknown. In the present study, we aim to investigate the underlying mechanism(s) of S100A11 in cancer cell proliferation, invasion, migration, and evaluate the clinical association with breast cancer.

**Methods:** The mRNA expression level of S100A11 in plasma samples from breast cancer patients and normal control individuals was detected with quantitative real-time PCR. Knockdown of S100A11 using small interfering RNA (siRNA) in luminal breast cancer cells (MCF-7 and T-47D) were used to study the biological function of S100A11 for the progression of breast cancer. Cell proliferation ability was analysed with MTT assay and colony formation assay. Cell invasion ability was analysed with transwell invasion assay. Cell migration ability was detected by wound healing assay. Cell cycle distribution and apoptosis were determined by flow cytometry.

**Results:** The mRNA expression level of S100A11 was significantly increased in the plasma samples obtained from 182 breast cancer patients compared with 115 normal control individuals, and the area under curve was 0.83. In particular, higher expression was associated with luminal breast cancer subtype as well as respective cell lines. S100A11 siRNA effectively inhibited the proliferation, invasion, and migration abilities of MCF-7 and T-47D cells. Knockdown of S100A11 caused cell cycle G1 arrest and induced apoptosis. Silencing of S100A11 decreased the mRNA and protein expression levels of cyclin D1 and NF- $\kappa$ B p50 whereas increased the expression level of E-cadherin in MCF-7 and T-47D cells.

**Conclusion:** These results suggest that S100A11 is upregulated in breast cancer and promoted cell proliferation, invasion and migration in MCF-7 and T-47D cells through the upregulation of NF- $\kappa$ B p50 and cyclin D1. Thus, S100A11 may be a potential diagnostic marker for luminal breast cancer subtype and also a therapeutic target for breast cancer.

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Blood-based DNA methylation as epigenetic biomarkers for non-invasive detection of breast cancer

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**Background:** DNA methylation significantly contributes to all hallmarks of cancer. In particular, promoter hypermethylation has been identified as a potential marker for early detection of cancer, therapy monitoring, assessment of prognosis or prediction of therapy response. Here, we identified and validated breast cancer-specific methylation markers for diagnosis of the disease with high sensitivity and specificity.

**Methods:** 151 bio-banked samples were obtained for a randomized blind study; 65 subjects diagnosed with breast cancer (Stage I to IV), 15 subjects with benign breast disease, 32 subjects diagnosed with other cancer types (11 colorectal, 9 liver and 12 lung cancer), and 39 healthy donor samples. Cell-free DNA was then extracted from the samples, bisulfite converted, and DNA methylation was quantified by using the IvyGene Platform. Finally, the raw data were collected and analyzed to evaluate the test performance.

**Results:** A total of 53 of the 65 breast cancer samples were correctly identified for an overall calculated sensitivity of 89% with little difference between the sensitivity of detecting Stage I to Stage IV breast cancer (range 88% to 94%). The breast cancer subjects were correctly identified with 96% specificity. Additionally, 28 of 32 samples drawn from healthy donor subjects (specificity 95%) and all 15 samples drawn from subjects diagnosed with benign breast disease (specificity 100%) were also correctly identified. Of the samples drawn from subjects with cancer other than breast cancer, over 96% of lung cancer, colorectal cancer and liver cancer samples were correctly identified as not breast cancer.

**Conclusion:** The results demonstrate the high diagnostic potential of ctDNA methylation markers in the blood for the detection of breast cancer. In addition, a quantitative analysis of ctDNA provides an opportunity for non-invasive detection of cancer and it's benefits can be anticipated to improve patient management and healthcare quality.

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Circulating cell-free DNA in serum as a marker for the early detection of tumor recurrence in breast cancer patients

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**Background:** Quantitative estimation of circulating cell-free DNA (cfDNA) isolated from serum by noninvasive procedures can serve as a potential biomarker for the early detection of many cancers. However, a simple, straightforward technique is unavailable to estimate the cfDNA in clinical labs. Moreover, the prognostic value of cfDNA in patients with breast cancer (BrCa) is currently under debate. The aim of this study was to develop a simple yet effective quantitative method for measuring the cfDNA in serum and to eventually investigate the relationship between cfDNA and the occurrence of recurrence in BrCa patients.

**Methods:** A total of 240 patient cases (n=240) were selected and are comprised of different subtypes of breast cancer patients and control individuals. We selected 21 serum samples from patients which showed recurrence after 4-7 years of disease-free survival. For the compare studies, each of the recurrent and non-recurrent serum samples was incubated with the SYBR Green I (2 µM). A standard graph was also made with known DNA concentration to calculate the amount of cfDNA in these recurrent and non-recurrent serum samples. Additionally, a comparative study was also performed with the serum of patients with non-recurrent BrCa versus healthy patients.

**Results:** We develop a simple fluorescent based measuring technique which can easily estimate the cfDNA in one step. SYBR Green binds to DNA, and as a result, the fluorescence of SYBR Green increases substantially. Global Wilcoxon analyses were performed to compare the cfDNA amount between non-recurrent and recurrent patients. There is a significant difference in fluorescent intensities between recurrent patients' samples versus non-recurrent patients which are directly proportional to the cfDNA levels. The amount of cfDNA is higher in recurrent patient (ratio is 1.3 up; p= 0.03; AUC=0.76) compared to similar non-recurrent patients. While we compared the fluorescence data between normal/healthy patients versus non-recurrent is turned out as non-significant (healthy to non-recurrent ratio = 1.03; p= 0.20, AUC=0.61).

**Conclusion:** In this current study, we developed a straightforward one-step technique to measure the amount of cfDNA in serum, which can easily translate into a clinical diagnostic tool. To the best of our knowledge, this is the first report which demonstrates serum cfDNA as an early detection marker for recurrent breast cancer patients. The relatively high level of cfDNA in the serum of recurrent breast cancer patients compared to non-recurrent breast cancer patients indicates an uncovered circulating genetic information which triggers the cancer recurrence pathway to relapse cancer in the near future.

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Analytical validation of an automated digital scoring protocol for Ki67: International multicenter collaboration study

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**Background/Goal:** Ki67 expression has been a valuable prognostic marker in breast cancer, but has not seen broad adoption due to lack of standardization between institutions. Automation could represent a solution. Here we tested 3 automated digital image analysis (DIA) platforms including an open source platform to: (i) Investigate the reproducibility of Ki67 measurement across platforms with supervised classifiers performed by the same operator and by multiple operators. (ii) Compare accuracy of the 3 DIA platforms against outcome (prognostic potential). (iii) Assess inter-laboratory reproducibility of a calibrated DIA tool to evaluate Ki67 in breast cancer among 10 participating labs of the International Ki67 in Breast Cancer Working Group (IKWG).

**Methods:** The Mib-1 antibody (Dako) was used to detect Ki67 (dilution 1:100). HALO (H) (IndicaLabs), QuantCenter (QC) (3DHistech), QuPath (QP) (open-source software) digital image analysis (DIA) platforms were used to evaluate Ki67 expression. As a ground truth, we evaluated Ki67 LI with meticulous manual tissue segmentation using the Spectrum Webscope (SW) (Aperio). Calibration was performed using 30 ER+ breast cancer cases from phase 3 of the IKWG initiative where blocks were centrally cut and stained for Ki67. The inter-laboratory analysis was done with 10 participating laboratories divided into 2 groups where members within the same group were given the same set of images. The outcome cohort consisted of 149 breast cancer cases from the Yale Pathology archives in tissue microarray format. Intra-class correlation coefficient (ICC) was used to measure reproducibility with the pre-specified criterion for success being to exceed 0.80. Kaplan-Meier analysis supported with log-rank test was performed to assess prognostic potential.

**Results:** All 3 DIA platforms showed excellent inter-platform reproducibility (ICC: 0.933, CI: 0.879-0.966). Also, excellent reproducibility was found between all DIA platforms and the reference standard Ki67 values of SW (QP ICC: 0.970, CI: 0.936-0.986; H ICC: 0.968, CI: 0.933-0.985; QC ICC: 0.964, CI: 0.919-0.983). The intra-DIA reproducibility was also excellent for all platforms (QP ICC: 0.992, CI: 0.986-0.996; H ICC: 0.972, CI: 0.924-0.988; QC ICC: 0.978, CI: 0.932-0.991). Comparing each DIA against outcome, the hazard ratios were similar (QP=3.309, H=3.077, QC=3.731). The inter-operator reproducibility was particularly high (ICC: 0.962-0.995). As QP is open source software and also showed the lowest intra-DIA platform variability, we selected the QP platform to investigate inter-laboratory reproducibility among 10 IKWG labs. The different-section ICC across the 10 labs was 0.974 (CI: 0.954 - 0.986). The same-section ICC estimate was 0.984 (CI: 0.971-0.992) for group 1 and 0.978 (CI: 0.956-0.989) for group 2.

**Conclusions:** Our results showed outstanding reproducibility both within and between DIA platforms. We also found the platforms essentially indistinguishable with respect to prediction of breast cancer patient outcome. Automated Ki67 evaluation using a calibrated, open-source DIA platform (QuPath) met the pre-specified criterion of success in the multi-institutional setting. Assessment of clinical utility is planned.

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Outcomes of diagnostic surgical biopsy in a population-based mammographic screening program

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**Background:** Screening mammography allows earlier diagnosis of breast cancer resulting in reduced breast cancer specific mortality, and potentially reduced morbidity from less intense treatment. These benefits may be offset by investigation and treatment of lesions that are detected on screening but which are eventually found to be clearly benign, have uncertain malignant potential, or are malignant but pose no threat to the person during her natural life.

Abnormalities detected on screening mammograms are recalled for further assessment. The outcomes of assessment may be confirmation of the absence suspicious findings. It may be a core biopsy is performed to clarify the nature of a persistently indeterminate or suspicious lesion. In cases when workup has neither diagnosed malignancy nor excluded its possibility, diagnostic surgical biopsy (DSB) is recommended. DSB of a lesion where final pathology is benign is a harm from a screening program. The benefit of DSB where a core needle biopsy (CNB) has shown a high-risk lesion is dependent on identifying more significant pathology than that of the CNB.

Trials are currently recruiting which investigate a non-operative approach for DCIS, yet DSB is still standard of care for less significant/benign pathologies in much of the world. We aimed to determine the outcomes for patients recommended for DSB in a population-based mammographic screening program in order to identify situations in which this may safely be omitted.

**Methods:** Ethics approval was obtained through Melbourne Health. Cases of DSB were reviewed from Northwest BreastScreen and Southern BreastScreen in Melbourne, Australia. Registry data was extracted including all patients where recommendation for DSB was made over a ten-year period (January 2004- December 2013). Patient demographics, imaging characteristics, core biopsy & surgical pathology were reviewed manually from individual BreastScreen files. Data points were entered to an Excell database. We reviewed assessment reports, tabulated indications for DSB, and surgical pathology reports for final histopathology.

**Results:** 1286 patients underwent DSB over the ten-year period. The overall upgrade rate to malignancy on DSB after non-malignant finding on CNB was 21.6% (14% to DCIS and 7.6% to invasive cancer). Atypical ductal hyperplasia (ADH) was the most common pathology identified on CNB generating recommendation for DSB. The overall upgrade rate for ADH was 31%, including 5.5% to invasive cancer, and 25.5% to DCIS. Further analysis of these ADH upgrades to DCIS revealed majority to low grade DCIS.

**Discussion:** A minority of patients with high-risk lesions, detected through screening mammography, were upgraded to malignancy on DSB in this cohort. The psychological and cost-effectiveness implications of this low upgrade rate to significant, life-threatening pathologies warrants further investigation. Future recommendations may involve including lesions of uncertain malignant potential or "high-risk" lesions in prospective observation and/or chemoprevention trials. This series suggests there may not be benefit in earlier diagnosis of the malignancies associated with ADH. Our data may therefore allow identification of groups of women who could be safely managed with less intensive treatment.



# 2018 San Antonio Breast Cancer Symposium ®

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Impact of the 2018 ASCO/CAP HER2 focused update on human epidermal growth factor receptor-2 (HER2) testing in breast cancer: A retrospective review of a single institutional cohort

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**Background:** In the 2013 ASCO/CAP HER2 update, new recommendations for HER2 diagnostic criteria in breast cancer organized in situ hybridization (ISH) results into five categories; group1 (amplified), group 2 (monosomy), group 3 (co-amplified), group 4 (equivocal) and group 5 (non-amplified). Patients falling into groups 2, 3 and 4 were potentially eligible for HER2 targeted therapy, however, there is uncertainty from limited prospective clinical trials that show patients in these uncommon groups would receive the same benefit as group 1. Concern over whether the interpretation criteria should be modified for these uncommon groups led to the recent publication of the 2018 HER2 focused update. This update has modified ISH criteria for groups 2, 3 and 4, recommending the final diagnosis take into consideration both immunohistochemistry (IHC) and ISH results. The publication of this new guideline has prompted us to investigate what impact this would have on our institution.

**Materials & Methods:** A retrospective review of the URM pathologic database revealed 2,281 cases that had undergone HER2 FISH analysis since the 2013 update. IHC for initial HER2 screening was used, followed by reflex testing of all 2+ results and cases with histopathologic discordance. All 2,281 FISH cases were sorted into their 5 HER2 categories based on the 2013 guidelines. The final HER2 diagnosis for groups 2, 3 and 4 were then re-determined after applying the new criteria presented in the 2018 focused update.

**Results:** The results from the 2,281 HER2 FISH cases are shown in Tables 1 and 2. The results for group 1 and group 5 cases remained the same. However, for ISH cases in groups 2, 3 and 4, there were alterations in the final HER2 results. All 25 monosomy cases, originally interpreted as HER2 positive by the 2013 guidelines, were now considered HER2 negative. All group 3 (co-amplified) cases remained positive (due to IHC 2+ results). The largest change was that the original 199 equivocal cases, based on the 2013 guidelines, became split into 198 HER2 negative (99.5%) and 1 HER2 positive (0.5%).

**Conclusion:** Comparison of the HER2 FISH cases between 2013 and 2018 revealed differences in the final HER2 status. Using the 2018 guideline, 13.72% (313 cases) of the 2281 cases were HER2 positive in contrast to 14.77% (337 cases) from 2013. While that is only a difference of 1.05%, the reclassification of 198 of 199 equivocal cases to negative indicates that 9.82% of the final HER2 results for the entire patient cohort was affected. With 266,000 new cases of breast cancer annually, the final HER2 status of approximately 26,000 patients and their potential eligibility for targeted-therapy would change. Further study of the clinical significance of these changes is warranted.

## Final ISH HER2 Status (2013)

Amplified	Monosomy	Co-Amplified	Equivocal	Non-Amplified
272 cases (11.93%)	25 cases (1.10%)	40 cases (1.75%)	199 cases (8.72%)	1,745 cases (76.50%)

## Final ISH & IHC HER2 Status (2018)

HER2+			HER2-		
Amplified	Co-Amplified	Equivocal+	Non-Amplified	Monosomy	Equivocal-
272 cases (11.93%)	40 cases (1.75%)	1 case (0.04%)	1,745 cases (76.50%)	25 cases (1.10%)	198 cases (8.68%)
313 cases (13.72%)			1,968 cases (86.28%)		



# 2018 San Antonio Breast Cancer Symposium ®

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Tumor-infiltrating lymphocytes in invasive lobular breast cancer identify a poor prognostic sub-group

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## Background:

Tumor infiltrating lymphocytes (TILs) are associated with an improved outcome in triple negative and HER2 breast cancers. Conversely, a recent pioneer study showed that TILs in infiltrating lobular carcinoma (ILC) were associated with a worse prognosis. We aimed at assessing the prognostic impact of TILs in a large cohort of primary surgically treated ILC with a long follow-up in a single institution.

## Methods:

We retrospectively reviewed 459 ILC treated at the Curie Institute between 2005 and 2008. Clinico-pathological patients' characteristics were collected and all archived tumor slides were centrally reviewed for TILs quantification. We analyzed stromal TILs scored as a continuous variable. Cox analyses were performed for relapse free survival (RFS) and for overall survival (OS), and chi-square and Fisher test were performed to evaluate the correlation between TILs and other clinico-pathological variables. For statistical analyses, presence or absence of TILs was considered.

## Results:

Patients had a mean age of 60.3 years and a median follow-up of 8.8 years, during which 74 local and/or distant relapse events occurred, among which 37 deaths due to the disease. The presence of TILs were significantly associated with greater tumor size (pT), positive nodal status (pN), molecular class (HER2 amplified), and specific morphological nuclear features such as high nuclear grade, multinucleation, macronucleoli and high Nottingham Prognostic Index class. Presence of intra-tumoral lymphocytes is significantly associated with a worse RFS (HR: 2.07 p=0.003) and OS (HR: 4.58 p<0.001) in a multivariate analysis, independently of chemotherapy.

## Relapse Free Survival

TILs	% (IC 95%)	% (IC 95%)
No	95.3 (92.5-98.2)	89.0 (84.3-94.0)
Yes	89.3 (85.1-93.6)	74.0 (67.5-81.1)

## Overall Survival

	OS at 5 years	OS at 10 years
TILs	% (IC 95%)	% (IC 95%)
No	99.5 (97.8-100)	95.8 (92.3-99.4)
Yes	95.3 (92.3-98.1)	83.5 (77.8-89.7)

**Conclusion:**

TILs are an independent prognostic factor associated with a poor outcome in ILC that are easy to assess on diagnostic biopsies. In order to elucidate the role of TILs in ILC, we will further characterize the immune infiltrate and integrate these results with DNA, RNA and protein analyses of the same carcinomas, in order to gain insight into their molecular features and to facilitate the identification of new therapeutic strategies for these ILC with TILs.

# 2018 San Antonio Breast Cancer Symposium ®

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Apocrine morphology and LAR molecular subtype predict prognosis of TNBC patients with residual disease after neoadjuvant chemotherapy

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**Background:** TNBC molecular subtype classification updated by Lehmann et al. includes 4 subtypes: basal-like 1 and 2 (BL1), (BL2), mesenchymal (M), and luminal androgen receptor (LAR), and as a modifier of these subtypes, an Immunomodulatory (IM) gene expression signature. However, molecular subtypes have not been linked to morphological features of TNBC. Apocrine carcinoma has been proposed as a TNBC category that expresses androgen receptor. LAR-subtype TNBC has a poor response to neoadjuvant systemic therapy (NST). We hypothesized that defining the apocrine-featured TNBC by morphology and molecular subtype predict the prognosis of patients with residual disease after NST. **Methods:** We created the Pan-Pacific TNBC Consortium dataset, which contains paired samples of matched pre and post-NST TNBC tumors from 4 institutions. All patients received NST and didn't have a pathological complete response (pCR). Three pathologists examined hematoxylin and eosin-stained slides of 86 pre-NST samples and determined (1) the presence of apocrine differentiation, (2) the level of tumor-infiltrating lymphocytes (TILs), (3) the histological grade (HG), and (4) the rate of necrosis. These morphological features were compared among the subtypes. For a sample to be considered apocrine positive, apocrine differentiation had to be identified by 2 or more pathologists. Fisher's exact test was used to test the association of subtypes and morphological features. The log-rank test was used to compare disease-free survival (DFS). **Results:** Twelve of 24 (50%) apocrine-positive tumor samples were LAR subtype, and 12 of 17 (70%) LAR-subtype tumor samples exhibited apocrine differentiation. The other subtypes showed following: BL1, 11/44 (25%); BL2, 0/7 (0%); M, 1/10 (10%); unclassified, 0/8 (0%). The median follow-up time was 22 months. In all populations, 2-year DFS rates were higher in patients with apocrine-positive tumors than in those whose tumors did not exhibit apocrine differentiation ( $P = .027$ ; 2-year DFS, 85% vs 54%). The LAR subtype was also associated with lower HG, although LAR tumors had a similar prognosis to the other subtypes. In the combined analysis of subtypes and apocrine differentiation, patients with apocrine-positive LAR tumors had a higher 2-year DFS rate than did those with apocrine-negative LAR tumors ( $P = .044$ ; 2-year DFS, 88% vs. 30%). However, patients with apocrine-positive BL1 tumors had no better DFS than did those with apocrine-negative BL1 tumors ( $P = .133$ ). TIL levels and the presence of the IM signature were positively associated ( $P = .01$ ), and apocrine differentiation positivity tended to be negatively associated with TIL level ( $P = .06$ ). Neither TIL level nor IM signature was associated with survival. **Conclusion:** Apocrine differentiation was associated with the LAR subtype of TNBC and better prognosis in patients who did not have a pCR. The LAR subtype alone did not predict DFS; however, LAR tumors with apocrine differentiation had a better prognosis than did LAR tumors without apocrine differentiation. Using a combination of morphologic and genomic testing may be helpful in determining the prognosis of patients with apocrine-positive TNBC tumors who have residual disease after NST.

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Inter- and intra-laboratory variation in grading of ductal carcinoma *in situ* of the breast: A nationwide study in the Netherlands

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## **Background:**

A considerable part of ductal carcinoma *in situ* (DCIS) lesions may never progress into invasive breast cancer. Yet, standard treatment consists of surgical excision. Trials aim to identify a subgroup of low-risk DCIS that, under active surveillance, can safely forgo surgical treatment. These low-risk subgroups are in all trials solely based on histologic grade, which highlights the importance of accurate, consistent, and reproducible grading by pathologists. To improve standardization, we aimed to gain insight into laboratory-specific variation of DCIS grading in daily clinical practice.

## **Materials and methods:**

All synoptic pathology reports of pure DCIS resection specimens between 2013-2016 were retrieved from PALGA, the nationwide Dutch Pathology Registry. Absolute differences in proportions of grade I-III between laboratories (inter-laboratory) were visualized using funnel plots, in which the proportions per laboratory are plotted against the number of DCIS per laboratory, with the national proportions per grade as target. Multivariable analysis to correct for case mix was performed by logistic regression, providing odds ratios (ORs) and 95% confidence intervals (CI) for high-grade (III) versus low-grade (I-II) DCIS per laboratory. Absolute differences of proportions of grade I-III between pathologists within laboratories (intra-laboratory) were also analyzed. Lastly, an online questionnaire was sent to all pathology laboratories in the Netherlands to identify how pathologists determine histologic grade in daily clinical practice.

## **Results:**

In total 4,952 DCIS cases from 36 laboratories were included, of which 12.5% were reported as grade I (range 6.1-24.4%), 39.5% as grade II (18.2-57.6%), and 48.0% as grade III (30.2-72.7%). After correction for case mix, 14 laboratories (38.9%) reported a significantly lower (n=4) or higher (n=10) proportion of high-grade DCIS than the reference laboratory. Adjusted ORs (95% CI) ranged from 0.52 (0.31-0.87) to 3.83 (1.42-10.39). Intra-laboratory analysis also showed significant differences between pathologists within 25% of the laboratories. The results of our questionnaire showed that pathologists mentioned numerous different guidelines as a reference for the histologic grading of DCIS, while 20.3% of them even stated that they grade DCIS (partially) based on intuition.

## **Conclusion:**

We observed substantial inter- and intra-laboratory variation in the histologic grading of DCIS, which was not explained by differences in case mix. Therefore, there is an urgent need for nationwide standardization of grading practices, especially since the future management of DCIS may alter significantly depending on histologic grade.

# 2018 San Antonio Breast Cancer Symposium ®

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Comparison of breast cancer molecular subtyping by Immunohistochemistry and by BluePrint® next generation RNA sequencing-based test at University Hospitals Leuven and Curie Institute Paris

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## Background

MammaPrint® (MP) and BluePrint® (BP) are microarray-based tests with MP being prognostic for distant recurrence and BP enabling stratification into breast cancer molecular subtypes (Luminal, HER2, Basal-type). Recently, a CE marked MP and BP targeted RNA Next Generation Sequencing (NGS)-based kit was developed at Agendia and validated at University Hospitals Leuven and Curie Institute Paris. Here we compare breast cancer molecular subtype stratification defined by immunohistochemistry (IHC) and by MP and BP NGS- and microarray- based tests.

## Patients and Methods

In this study, 124 primary operable invasive breast cancer patients were included at University Hospitals Leuven and at Curie Institute (n=80 Leuven; n=44 Curie) with the following histological subtypes: ductal-NOS (n=100), lobular (n=16), mucinous (n=3), tubular (n=2), others (n=3). Patients with bilateral breast cancer or with >3 positive lymph nodes were excluded. Surrogate breast cancer subtypes based on IHC were defined as follows: luminal if ≥10% estrogen receptor (ER) expression; triple negative if <10% ER and progesterone receptor (PR) expression and HER2 stained negative by IHC and/or FISH; HER2+ if HER2 receptor stained positive (2+ or 3+) by IHC and/or FISH. Luminal subtypes were further stratified into Luminal A-like (HER2 negative, Ki-67<14%, PR≥20%) and Luminal B-like (HER2 negative or positive, Ki-67 ≥14%, PR<20%). When Ki-67% was not available, tumors with grade 1 or 2 were classified as Luminal A-like and with grade 3 as Luminal B-like. IHC subtypes were compared to the BP NGS and microarray molecular subtypes (Luminal-, HER2- and Basal-type). To further stratify BP luminal type tumors, MP test was used as follows: Luminal A (BP Luminal and MP low risk) and Luminal B (BP Luminal and MP high risk).

## Results

Concordance between IHC and MP/BP NGS subtyping was 75.0% (93/124),

## IHC vs. MP/BP NGS molecular subtyping (n=124)

IHC	MP/BP NGS				
	Luminal A	Luminal B	HER2-positive	Basal	Total
Luminal A-like	46	9	0	0	55
Luminal B-like, HER2-negative	16	21	0	0	37
Luminal B-like, HER2-positive	5	6	5	0	16
HER2-positive	0	0	3	0	3
Triple negative	0	1	0	12	13
Total	67	37	8	12	124

while concordance between MP/BP on NGS and microarray was 89.5% (111/124).

#### Microarray vs MP/BP NGS molecular subtyping (n=124)

	MP/BP NGS				
Microarray	Luminal A	Luminal B	HER2 positive	Basal	Total
Luminal A	60	4	0	0	<b>64</b>
Luminal B	7	31	0	0	<b>38</b>
HER2-positive	0	2	8	0	<b>10</b>
Basal	0	0	0	12	<b>12</b>
Total	<b>67</b>	<b>37</b>	<b>8</b>	<b>12</b>	124

MP/BP NGS subtyping identified more low risk Luminal A tumors compared to IHC (54.0%, (67/124) vs 44.3% (55/124)). Notably, concordance was excellent for triple-negative and, to less extent for HER2 driven tumors (Luminal B-like-HER2 positive and HER2+).

#### IHC vs. MP/BP NGS molecular subtyping (n=124)

	MP/BP NGS				
IHC	Luminal A	Luminal B	HER2-positive	Basal	Total
Luminal A-like	46	9	0	0	<b>55</b>
Luminal B-like, HER2-negative	16	21	0	0	<b>37</b>
Luminal B-like, HER2-positive	5	6	5	0	<b>16</b>
HER2-positive	0	0	3	0	<b>3</b>
Triple negative	0	1	0	12	<b>13</b>
Total	<b>67</b>	<b>37</b>	<b>8</b>	<b>12</b>	124

#### Conclusion

This study shows a discordance of 25.0% between IHC and BP/MP NGS subtyping. This is in line with previous findings where IHC was compared to molecular subtyping based on microarray (Viale 2017, Whitworth 2014) underlining the complementarity of genomic testing in early stage breast cancer. Moreover, we observed a high concordance between NGS and microarray molecular subtyping, which suggests a successful translation of the MP/BP microarray test to a MP/BP NGS test.



# 2018 San Antonio Breast Cancer Symposium ®

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Statistical modeling of influential variables affecting HER2-positivity in breast cancer: Final analyses from two large, multicenter, noninterventional studies in Germany

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## **Background:**

While HER2 testing in breast cancer (BC) has been routine for over a decade, testing quality remains a challenge. Currently, HER2-positivity rate is the only recommended quality indicator. However, the large, observational, prospective NIU HER2 study in Germany quantified the impact of patient- and tumor-related characteristics such as histologic grade, hormone receptor (HR) status, histologic subtype, age, and nodal status on HER2-positivity, indicating that these factors need to be considered when evaluating HER2-positivity as a measure of testing quality (Rüschoff *et al. Mod Pathol* 2017). We now report the final analyses from the multicenter EPI HER2 BC study (ML29763, NCT02666261) in Germany where we compared NIU and EPI study data, and aimed to validate the NIU study model.

## **Methods:**

Data from eligible patients with invasive BC were collected (HER2 test result; patient- and tumor-related factors) and variables influencing HER2-positivity identified and compared between studies. The NIU study model was validated and its predictive power determined using newly collected data from the EPI study, with cutoff and variable coefficients from the previous NIU analysis. Additional promising variables were explored, and their relative influence investigated, using multiple stepwise logistic regression.

## **Results:**

In total, 14,729 (EPI) and 15,281 (NIU) BC samples were analyzed. Distributions of the main variables were comparable; overall HER2-positivity rates were 13.47% (EPI) and 14.24% (NIU). Fitting the NIU study model to EPI study data demonstrated that all five covariates from the NIU study analyses significantly affected HER2-positivity ( $p < 0.01$ ); the influence for each covariate differed only slightly between studies (in EPI, histologic grade had most influence followed by histologic subtype, HR status, nodal status, and age). Prediction profiles were used to visualize the relationship between the model-predicted probability of HER2-positivity and the five identified covariates, which showed good comparability between studies. The receiver operating characteristics area under the curve (ROC AUC) of the NIU model used to predict HER2-positivity in the EPI study data was close to that of the model fitted to the NIU data, thus successfully validating the NIU model. To further improve the model, the categorical HR status was replaced by estrogen receptor (ER) and progesterone receptor (PgR) expression. Inclusion of ER and PgR as continuous variables improved the predictive strength of the model (ROC AUC = 0.74; sensitivity = 0.76; specificity = 0.63). Based on this improved model, PgR status had the highest influence on HER2-positivity, followed by histologic grade, histologic subtype, nodal status, ER status, and age.

## **Conclusions:**

Results from our analyses confirm the statistically and clinically significant influence of patient- and tumor-related factors on HER2-positivity, and highlight the necessity to integrate these factors into the quality control assessment of HER2 testing. Implementation of this model in routine practice may assist in addressing issues with interlaboratory variation, and help to identify centers with HER2 testing problems more accurately.

# 2018 San Antonio Breast Cancer Symposium ®

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Clinical and histopathologic characteristics of breast cancer in very young patients

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**Background:** While breast cancer risk increases with age, about 7% of breast cancer cases in the US are diagnosed in women <40 years of age. Evidence suggests that young breast cancer patients tend to have more aggressive disease subtypes, less favorable tumor biomarker profiles, higher risk of relapse, and poorer survival. However, there is no universally accepted definition of "young patients," and most studies have focused on premenopausal women or those <40 years of age, with some studies using age <35. To date, very little is known on the clinical and histopathologic characteristics of breast cancer in very young women, namely those ≤30 years of age. In this study, we describe such features in a cohort of very young women treated for breast cancer at our academic institution.

**Methods:** Our patient population consisted of all cases of invasive breast carcinoma at Northwestern Memorial Hospital in women <40 years of age at surgery between January 1, 2009, and December 31, 2015. Very young was defined as having an age ≤30. Histopathologic features including tumor size, grade, histologic type, presence of lymphovascular invasion (LVI), lymph node status, tumor markers (ER, PR, HER2) and Ki-67 proliferation rate were recorded. Cases in which specific histopathologic features could not be determined from available materials were excluded from the corresponding analysis. The use of neoadjuvant chemotherapy, type of surgery, and the use of prophylactic surgery on the contralateral side were also recorded and analyzed.

**Results:** A total of 301 invasive breast carcinoma cases were identified (age range 18-39). 40 cases of very young (age ≤30) women with breast cancer (13%) were identified. Nearly all very young patients were diagnosed with tumors of ductal histology (39/40, 98%). Just under half of these patients (19/40, 48%) had a T1 (<2 cm), 16/40 (40%) had a T2 (2-5 cm), and 2/40 (5%) had a T3 (>5 cm) tumor at the time of surgery. The majority of these carcinomas were grade 3 (25/39, 64%), 14/39 (36%) were grade 2, and none were grade 1 tumors. Over half of the tumors were highly proliferative with a high Ki-67 count of >20% (17/31, 55%). ER was expressed in 34/40 (85%), PR in 25/40 (63%) and HER2 in 9/40 (23%). LVI was present in half of the cases (20/40, 50%), and positive lymph nodes were identified in over a third (15/40, 38%) of the cases. Neoadjuvant chemotherapy was used in 9/40 (23%). Three patients (8%) had no residual carcinoma at the time of surgery post chemotherapy. Most of these very young patients chose a mastectomy (28/40, 70%), and 19/40 (48%) also underwent a prophylactic mastectomy on the contralateral side.

**Conclusions:** Our study is one of the largest to date to describe the characteristics of breast carcinomas in very young women (age ≤30). Poor pathologic features such as high tumor grade, high proliferation rate, and presence of LVI were seen in half or more of these very young patients. A high rate of positive lymph nodes was also seen. Mastectomy, often with contralateral prophylactic surgery, was the procedure of choice. Further studies evaluating the molecular characteristics of these tumors and the prevalence of underlying genetic mutations are under way in this unique population of very young breast cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

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Are fine-needle aspiration (FNA)-derived cell blocks a useful tissue sample surrogate for testing conventional biomarkers and PD-L1 in breast cancer?

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**Background** The diagnosis of breast cancer (BC) is based on clinical examination in combination with imaging and confirmed by pathological assessment of core needle biopsy (CNB) or fine needle aspiration (FNA). The biological profile of the lesion is needed to define prognosis and guide therapy. Given the importance of an early and minimally invasive diagnosis, we aimed to verify whether the biological features detected in FNA-derived cytological material reflect the biological characteristics of surgical specimens.

**Methods** We used immunohistochemistry and fluorescence *in situ* hybridization (FISH) to study a panel of biomarkers (ER, PgR, Ki67 and HER2 in 93 patients, programmed death-ligand 1 (PD-L1) in 20 patients) in FNA-derived cell blocks of BC, comparing the results with those obtained on the histological specimens. Immunostaining was performed with the Ventana Benchmark XT system and the Ultraview DAB Detection Kit (Ventana Medical Systems). Confirm anti-ER (clone SP1, Ventana), Confirm Anti-PgR (clone 1E2, Ventana) and Ki67 (clone Mib-1, Dako, Carpinteria, CA, US) antibodies were used. Ventana PD-L1 (SP263) assay (Ventana Medical Systems) was used for PD-L1 immunostaining. HER2 status was analyzed by FISH using PathVysion kit (Abbott Molecular, Abbott Park, Illinois, IL, USA).

**Results** Median immunopositive values of ER, PgR Ki67, and PD-L1 were similar in cell blocks and surgical samples. Concordance for ER and PgR between FNA and histological samples was 98% and 84%, respectively. With regard to Ki67 and HER2 status, concordance between the two specimen types was 90% and 96%, respectively. PD-L1 expression analyzed in FNA-derived samples was 100% concordant with that of surgical specimens. Tumor subtype classification for triple-negative and HER2-positive tumors in FNA samples was always concordant with the subtype determined in surgical material.

Table 1. Concordance of tumor subtype classification between FNAB-derived and histological samples.

Overall series (n=93)	87%
Luminal A (n=26)	81%
Luminal B (n=47)	86%
HER2-positive (n=6)	100%
Triple-negative (n=14)	100%

**Conclusions** We showed that biological marker determination in FNA-derived cell blocks is feasible and provides useful information and comparable results with those obtained by histological evaluation. Given the low cost of the procedure and its minimal impact on patients, that cytological samples could be used as an alternative to tissue samples for early BC biomarker evaluation to facilitate the planning of tailored neoadjuvant therapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Computer vision detects morphological correlates of HER2 positive breast cancer in H&E stained histological images

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**Background/Objectives:** The determination of HER2-positivity by IHC or FISH is critical for identifying patients most likely to benefit from anti-HER2 therapy. However, these methods do not always provide an accurate indication of HER2 overactivity, which can occur without gene amplification or overexpression of the HER2 protein. Our study objective was to determine if a deep learning convolutional neural network (CNN) could be trained, using IHC HER2 staining, to learn a morphological signature for HER2 positivity in H&E stained slides.

**Methods:** For training, we used H&E images (whole slides scanned at 40x) from 10 HER+ patients (IHC 3+) and 15 HER2- patients (IHC 0 or 1+) along with their adjacent HER2 IHC images. We first annotated non-cancer regions in H&E images. We then identified tumor regions in HER2 IHC that were positive (intense/complete circumferential stain) or negative (no or weak stain). We ignored any regions with equivocal IHC response as well as whole slides of IHC 2+ patients. For rigorous testing, slides from a separate set of 7 HER2+ and 19 HER2- patients were used. Digitized slides and expert consensus IHC HER2 status for each patient were provided as part of an international HER2 IHC scoring competition organized by the University of Warwick.

The computer vision pipeline comprised four-stages. First, we color-normalized the H&E images to reduce unwanted color variation between slides. Second, a pre-trained neural network (NN1) marked all nuclear centroids to make it easier for subsequent stages to focus on nuclear morphology and inter-nuclear spatial arrangements. Third, a neural network (NN2) trained on sub-images of size 100x100 centered at nuclear centroids classified nuclei as non-cancer vs. cancer. Fourth, a final neural network (NN3) sub-classified cancer nuclei into HER2+ or HER-. In H&E images of held-out test patients, the percent of cancer nuclei scored as HER2+ was analyzed.

**Results:** NN2 had a classification accuracy exceeding 97% on a validation set of 25,000 nuclei set aside from training patients, while NN3 had a validation accuracy of 88% on 7,500 test nuclei. On the set aside test patients, among the seven HER2+ patients an average of 49.8% cancer nuclei were scored HER2+, while among the 19 HER2- test patients the corresponding proportion was only 24.7% ( $p < 0.01$ ). The AUC of binary classification of test patients into HER2+/- based on percent of HER2+ nuclei was 0.815. Upon closer inspection, the H&E morphology of some of the misclassified HER- patients showed visual similarity to the correctly classified HER2- patients and vice versa.

**Conclusions:** Even when morphological patterns associated with cancer subtypes are too subtle for humans to reliably detect, H&E stained slides analyzed by CNNs may be able to geographically map a HER2 signature. It is unclear if misclassification with respect to IHC status is a reflection of morphological confusion or discordance between genomic subtype and IHC response. By training multiple neural networks to detect morphological signatures corresponding to different molecular subtypes of breast cancer, we may be able to detect and study intra-tumor heterogeneity in a cost effective and/or complementary way compared to multi-region sequencing.

# 2018 San Antonio Breast Cancer Symposium ®

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Comparison of RT-qPCR with consensus immunohistochemistry by three pathologists for ER, PR, HER2 and Ki-67 in Chinese breast cancer patients

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## Background

During the diagnostic work-up of breast carcinomas, immunohistochemistry (IHC) is the currently used method for assessing the expression of estrogen- (ER) and progesterone-receptors (PR), human epidermal growth factor receptor 2 (HER2) as well as of Ki-67 as a marker of tumor cell proliferation. In this study, we analyzed the concordance of these four breast cancer biomarkers between the RT-qPCR- and IHC-based (evaluated by three independent pathologists) determinations.

## Methods

The expression of ER/*ESR1*, PR/*PGR*, HER2/*ERBB2* and Ki-67/*MKI67* was determined in 269 FFPE breast cancer samples with tumor content >20% from Chinese patients. For IHC, the samples were freshly cut, stained and assessed by three independent pathologists using the same scoring methods in a blinded fashion (positivity defined as: ER/PR  $\geq 1\%$ , HER2 >2+ and Ki-67  $\geq 20\%$ ). Measurement of the markers on the mRNA level was done on total RNA extracts prepared from whole tissue sections from the same FFPE blocks using the CE-marked RT-qPCR based IVD MammaTyper® on a Cobas® z480 qPCR cycler. IHC assessments of the three pathologists were compared to each other with regard to concordance of positive/negative results. Subsequently, agreement of RT-qPCR and IHC results for each marker and in samples in which the three pathologists had a consensus positive/negative IHC result was determined. Furthermore, we compared the MammaTyper® assessments from a subset of whole FFPE sections to data obtained from paired samples enriched for invasive carcinoma via macrodissection.

## Results

From the 269 samples, 256 were available for final analysis. When excluding cases with discordant IHC callings between the three pathologists (6.0% for ER; 7.4% PR; 4.1% Her2; 17.1% Ki-67) the concordance to the RT-qPCR determination and consensus IHC-based analysis displayed an excellent agreement for ER (OPA: 95.4%, PPA: 97.5%, NPA: 91.5%, Kappa: 0.897), PR (OPA: 91.1%, PPA: 89.6%, NPA: 93.1%, Kappa: 0.820) and HER2 (OPA: 97.1%, PPA: 91.9%, NPA: 100.0%, Kappa: 0.936). For cancer *MKI67* mRNA and Ki-67 protein expression, a lower but still good concordance was found (OPA: 90.1%, PPA: 91.8%, NPA: 83.3%, Kappa: 0.707). In addition, we could demonstrate an excellent agreement of quantitative RT-qPCR measurements between whole surface and paired tumor-enriched samples in 99 Chinese breast cancer patients with  $R^2$  of 0.927 for ER, 0.926 for PR, 0.923 for HER2 and 0.908 for KI67. Even under highly standardized IHC scoring conditions, the discordance rates in the RT-qPCR marker callings with 0.0% for *ESR1*, 5.0% for *PGR*, 3.0% for *ERBB2*, 13.1% for *MKI67* were lower than disagreements by three pathologists on the identical slide.

## Conclusion

Standardized determination of the breast cancer biomarkers ER, PR, HER2 and Ki-67 on the mRNA level shows high concordance to a consensus IHC determined by three experienced pathologists indicating that RT-qPCR may be a valid alternative for determining the four breast cancer biomarkers. In line with previous research we could show on a large set of samples that macrodissection is not required for reliable assessment of the four breast cancer markers in clinical FFPE samples.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-02-13

Inter- and intra-laboratory variation in grading of invasive breast cancer: A nationwide study in the Netherlands

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## Background

Histologic grading is one of the best established prognostic factors in invasive breast cancer (IBC) and is used to guide patient management. Yet, the level of inter- and intra-observer agreement does not reach high enough clinical standards. To improve standardization, we aimed to gain insight into laboratory-specific variation in histologic grading.

## Methods

All synoptic pathology reports of IBC resection specimens between 2013-2016 were retrieved from PALGA, the nationwide Dutch Pathology Registry. All lesions were graded according to the modified Bloom and Richardson guideline, which combines assessment of cell morphology (nuclear polymorphism), measurement of differentiation (tubular differentiation) and assessment of proliferation (mitotic count). Absolute differences in proportions of grade I-III and the three components of grading between laboratories were compared to the national distributions. For logistic regression analyses, grade was dichotomized into high- and low-grade by two alternative definitions for high-grade IBC (either solely grade III or grade II-III). Multivariable logistic regression, to correct for case mix (age, tumor size, type of surgery, histologic subtype, ER/PR-receptor status, HER2-receptor status), provided two laboratory-specific odds ratios (ORs) and 95% confidence intervals (CI) for high versus low-grade IBC compared to the reference laboratory.

## Findings

In total 33,792 IBC cases from 39 laboratories were included, of which 28.1% were reported as grade I (range 16.3-43.3%), 47.6% as grade II (range 38.4-57.8%), and 24.3% as grade III (range 15.5-34.3%). More than half of the laboratories (22/39) showed proportions outside the 95% confidence limits of the national proportion for both grade I and grade III, followed by 41.0% of the laboratories for grade II. After case mix correction, 20 laboratories (51.3%) showed at least one significantly higher or lower OR than the reference laboratory. Four laboratories (10.3%) showed significantly deviant ORs on both analyses. Most variation between laboratories was observed for nuclear polymorphism. Significant grading differences were also observed between pathologists within 62.5% of the laboratories that could be analyzed. Overall, the indication for adjuvant chemotherapy was dependent on histologic grade in 29.9% of patients.

## Conclusion

We observed substantial inter- and intra-laboratory variation in the histologic grading of IBC, which may likely influence treatment decisions and subsequently patient outcome as the indication for chemotherapy depends on histologic grade in almost every one in three patients.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-02-14

Digitalizing immunohistochemistry analysis (IHC) with quantitative dot blot analysis (QDB) by absolute quantification of biomarkers in FFPE specimens

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**Purpose:** The feasibility of Quantitative Dot Blot (QDB) method for absolute quantification of diagnostic biomarkers was investigated here using diagnostic antibodies for biomarkers in breast cancer tissues, including 4B5 and EP3 for Her2, MIB1 for Ki67. SP2 for Estrogen Receptor and SP1 for progesterone receptor (PR) in more than 300 FFPE specimens. The specificity and accuracy of this method, and the inter-relationship among biomarkers were explored by the absolute expression levels of these biomarkers.

**Methods:** FFPE specimens of 332 breast cancer patients were provided by local hospital, tissue slices of 2X15 um was de-paraffined and total protein was extracted for QDB analysis, with each sample measured in triplicate in three independent experiments. Purified protein standards were included to convert the measurement into absolute levels. IHC and FISH results from local hospital were used to validate the sensitivity and specificity of QDB method.

**Results:** The results show high consistency with intra-cv less than 7% and inter-cv less than 14%. With Her2, 99.5% concordance was achieved with IHC method, and 92 to 94% concordance with FISH analysis. Both Her2 level and Ki67 levels were significantly increased in patients at grade III based on Nottingham histologic scores.

**Conclusion:** The QDB method was used here to achieve digitalization of biomarkers in breast cancer tissue with existing diagnostic antibodies for IHC analysis. The method is simple, objective, consistent, comprehensive and suitable for high throughput analysis. We expect this method to be extended to other diagnostic IHCs with existing diagnostic antibodies, and the easy adoption of this method in clinical practice should have instant impact on pathological practice.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-02-15

The effect of the 2013 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines in HER2 positivity rates among women with breast cancer in SEER registries

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**Introduction:** The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) revised the HER2 testing guidelines in breast cancer (BC) which was published in December 2013, practically becoming effective in 2014. Since then, many studies reported a significant increase in HER2 positivity rate in BC. Although HER2 has been routinely evaluated in BC for over a decade, HER2 status was not included in SEER registries until 2010. The aim of this study is to look at the effect of the 2013 ASCO/CAP guidelines on HER2 status in the SEER database that captures approximately 28% of all cancers in the US.

**Material and Methods.** Using data from 18 SEER cancer registries between the years of 2010-2015 when HER2 status for breast cancers was available, women with invasive BC were identified and their HER2 status and race was recorded. The Chi-square test was used to test the significance between positive proportions before and after the new ASCO/CAP guidelines were effective. Logistic regression was used to test if the changes in positivity rate were the same among different races. All calculations were made after excluding cases with unknown/missing HER2 status.

**Results:** We identified 376,278 women diagnosed with invasive BC between the years 2010-2015. HER2 positivity before 2014 (2010-2013) was 14.4% compared to 15.6% after 2013 (2014-2015) ( $p < 0.001$ ). HER2 positivity increased in all races after 2013. There was no significant difference in the increase of HER2 positivity between whites and blacks after the new guidelines went into effect. Unknown/missing HER2 status continually decreased over the study period from 7.4% in 2010 to 5.3% in 2015. Overall unknown/missing HER2 was significantly different among races: 6.5% in whites; 7.1% in blacks; and 6.2% in other races ( $p < 0.01$ ).

**Conclusion:** Revised ASCO/CAP HER2 guidelines significantly increased HER2 positivity rates in SEER registries from 14.4% to 15.6% after implementation started in 2014. HER2 positivity increased in all races after 2013. Unknown/missing HER2 status is significantly different among different races. Blacks have highest unknown/ missing HER2 status in SEER registries.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-03-01

Pathway level complementarity of germline and somatic events in breast cancer

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**Background:** Progression from a normal cell state to cancer requires multiple genomic hits in key regulatory pathways. In the case of hereditary cancer syndromes, some of these hits occur in the germline, but additional somatic mutations are required for malignant transformation. We hypothesize that this paradigm could be extended to sporadic cancers as well. What somatic mutation function as a cancer driver event may be determined by the constellation of germline variants a person is born with. We propose that even rare, non-recurrent, high functional impact germline variants in genes involved in cancer-related pathways could influence the biological impact of somatic mutations in other cancer-related genes. The goal of the current analysis was to examine associations between pathway alterations caused by high functional impact germline variants or somatic mutations in the “hallmarks of cancer” pathways in breast cancer.

**Methods:** We obtained germline DNA sequencing and copy number variation (CNV) data from the breast cancer TCGA cohort. After population clustering with the HapMap cohort, we selected a homogeneous group of 796 patients of Western European ancestry and downloaded the matching somatic mutations (SNVs and INDELs) that were available for 750 cases, that comprise the current study population. Germline CNVs were classified as recurrent or rare losses or gains. Potentially pathogenic germline variants (SNPs) were obtained from the PanCancer Atlas project. All germline or somatic mutations were mapped at the gene level to the 50 Cancer Hallmarks pathway collection. We designated a pathway mutated if at least 1 gene had a germline or a somatic mutation. Complementarity between pathway alterations by germline and somatic events were evaluated using the Fisher exact test adjusted for multiple comparisons.

**Results:** At the germline level, 2,057 genes were affected by CNVs (mean 30, range 3-151 genes/patient), and a total of 43 genes carried germline pathogenic SNPs that affected 13.8% of the patients. At the somatic level, we detected 40,881 high functional impact mutations (mean 54.3, range 1-3889 mutations/patient) in 13,080 genes (mean 50.8, range 1-3166 genes/patient). The 50 Cancer Hallmark pathways contained 4386 genes (mean 146.5, range 32-200 genes/pathway), and were mutated in the majority of the patients (85% germline, 93% somatic). Several pathways, such as HEME\_METABOLISM, INTERFERON\_ALPHA\_RESPONSE, and KRAS\_SIGNALING, were frequently affected by germline alterations, while the somatic mutations were most frequently involved in the COMPLEMENT, E2F\_TARGET, and UV\_RESPONSE\_UP. Interaction analysis revealed co-occurrence between MYC\_TARGETS\_V1 (germline) and UV\_RESPONSE\_DN (somatic) or MTORC1\_SIGNALING (somatic) ( $p < 0.01$ ), and TNFA\_SIGNALING\_VIA\_NFKB (germline) and IL6\_JAK\_STAT3\_SIGNALING (germline) with E2F\_TARGETS (somatic) ( $p < 0.01$ ). We also observed an exclusive relationship between germline alterations in BILE\_ACID\_METABOLISM and somatic mutations in COMPLEMENT pathway ( $p < 0.01$ ).

**Conclusions:** Our results highlight the importance of pathway-level analysis of germline alterations in breast cancer, which might help to understand the interrelationship between germline and somatic alterations in breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-03-02

Casting a wide net: Finding actionable results in non-breast cancer (BC) genes on multi-gene panel testing (MGPT) in a BC cohort

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**Background:** MGPT for hereditary cancer syndromes allows for concurrent analysis of genes associated with many different cancer types. This may lead to the identification of unexpected mutations in genes with no BC link. The objective of this study was to examine the landscape of pathogenic mutations in a BC cohort who underwent MGPT, to assess if there was clinical suspicion for identified mutations and if the results would affect subjects' medical management.

**Methods:** Retrospective review of subjects with BC seen at a single institution who underwent MGPT from 1/1/15- 5/31/18 was conducted. MGPT was defined as testing of more than the 9 genes associated with BC (*ATM*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *PALB2*, *PTEN*, *STK11*, *TP53*). Deidentified pedigrees were analyzed by genetic counselors to determine whether there was clinical suspicion of the presence of the mutations using national testing guidelines or clinical diagnostic criteria.

**Results:** Among 3044 subjects, 365 (12%) were found to have one pathogenic mutation in at least one cancer susceptibility gene. Subjects with mutations in *APC* I307K, moderate-penetrance BC genes (*NBN*, *RAD50*, *BARD1*), and *MUTYH* were excluded from further analysis. We identified 52 pathogenic mutations in genes not typically associated with risk for BC in 51 (2%) subjects (table 1). There was clinical suspicion for the identified mutation in 17 (33%).

Table 1: Non-BC gene mutation landscape

	Number of Mutations	Clinical Suspicion (%)
<b>Lynch syndrome</b>	11	7 (64%)
MLH1	1	0
MSH2	2	1
MSH6	3	2
PMS2	5	4
<b>Ovarian</b>	18	1 (6%)
BRIP1*	11	1
RAD51C	4	0
RAD51D	3	0
<b>SHDx</b>	6	2 (33%)
SDHA*	3	0
SDHC*	3	2
<b>Other</b>	15	6 (40%)
FH	1	0
HOXB13*	3	2
MITF	3	2
NF1	4	2
VHL	4	0
<b>CDKN2A</b>	2	1 (50%)
<b>Total</b>	52	17 (33%)

\*Contains individuals that also have a mutation in a BC susceptibility gene

**Conclusion:** Of 3044 BC patients who underwent MGPT, 2% were found to have a pathogenic gene mutation that would have been missed by a smaller BC gene panel. Medical or surgical management would be affected by the MGPT result in 86% of subjects. Only 6% of subjects with genetic risk for ovarian cancer had a family history of this disease. The single *FH* and 3 of 4 *VHL* mutations are only associated with disease in the biallelic state; these findings do not affect the subjects' care, but have implications for reproductive risk. The *HOXB13* mutations were found in female subjects only, but would have implications for their male relatives. *NF1* mutations are associated with BC risk, but were included in this analysis due to a historically distinct clinical phenotype. Only 50% of *NF1*+ subjects had a clinical diagnosis or family history of *NF1*. In all cases, cascade testing was offered to at-risk family members, allowing for cancer and reproductive risk stratification and management. This study demonstrates how comprehensive MGPT can provide a more complete and personalized cancer risk assessment for BC patients and their families.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-03-03

Germline potentially pathogenic variants in breast cancer intrinsic molecular subtypes are not associated with somatic TMB

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**Background:** Breast cancer (BC) is a heterogeneous disease. It is estimated that 5 to 10% of all BC to have a germline genetic predisposition. A 50-gene assay (PAM50) identifies 5 intrinsic molecular subtypes (IMS): Luminal A, Luminal B, HER2-enriched, Basal-like, and Normal-like. Basal-like breast cancers are enriched for BRCA1/2 germline mutations. Deleterious mutations in BRCA1/2 or other DNA-damage repair (DDR) genes may increase tumor mutational burden (TMB), a biomarker for response to checkpoint inhibition therapy. We sought to determine the spectrum of germline mutations in molecular BC subtypes (IMS), and their relation to somatic TMB. **Methods:** We performed a retrospective analysis of data from NantHealth database. RNAseq was used to classify breast tumors into IMS. Germline variants within putative driver genes (COSMIC v.76) were detected in analysis of 181 whole-genomes and 89 whole-exomes sequenced using Illumina chemistry. Classification of germline variants into potentially pathogenic variants (pPv) was determined using ClinVar database annotation. Patients were categorized as TMB-high by thresholding on >200 non-synonymous exonic somatic mutations as was previously reported. **Results:** A total of 270 BC patients with comprehensive omics profiling (germline DNAseq, somatic DNAseq, and somatic RNAseq) were available for this analysis. The mean age ( $\pm$ SD) was 56.4 ( $\pm$  12.5) years (range 20.8-86.5). Forty-six patients (17.0%) were classified TMB-high. The IMS distribution was 40.7% Luminal A, 31.5% Luminal B, 5.9% HER2-enriched, 21.5% Basal-like, and 0.37% Normal-like. Over 200 unique germline variants were detected of which 98 were pPv according to ClinVar annotation. These pPv spanned 21 genes, 7 of which are directly related to DDR. One hundred and four patients had  $\geq 1$  pPv (78 had only 1 pPv, and 26 had >1 pPv). The most common pPv were *APC* (5.9%), *BRCA2* (5.2%), *TSC2* (4.4%), *BRCA1* (3.7%), *SDHB* (3.3%), *SDHD* (3.3%), *TSC1* (3.0%), *PMS2* (3.0%), *MUTYH* (2.6%), *MSH2* (1.5%) and *MSH6* (1.5%). *BRCA1* and especially *BRCA2* pPv were mostly seen in the basal-like patients. Luminal B had distinctly more germline pPv in *PMS2*, *BRCA1* & *BRCA2* than Luminal A. TMB-high patients were not significantly enriched for germline pPv (OR 0.73, p=0.41), even when limited to pPv in DDR genes (OR 0.69, p=0.52). TMB-high patients were present in all 4 major IMS types; Her2-enriched 37.5%, Luminal B 23.5%, Basal-like 17.2%, and Luminal A 9.1%. **Conclusion:** We identified differential distribution of germline pPv in BC IMS. Of the pPv found, *APC* was the most commonly detected pPv across subtypes, while BRCA1/2 pPv were clustered in Basal-like subtype, and *PMS2* in Luminal B subtype. 17% of all patients had a pPv within at least one DDR gene, that potentially may benefit from targeted therapy. Despite IMS types having distinct germline pPv profiles especially in DDR genes, there was no association with subsequent somatic TMB. This suggests that either 1. somatic events are the primary drivers of TMB, or 2. that germline variants with either unknown or benign significance need to be revisited. Future analysis in a larger demographically well-annotated dataset (commercial data, ExAC, other) or via functional studies should be considered.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-03-04

Genetic testing contributes significantly to improved identification of women eligible for increased breast cancer screening compared to the Tyrer-Cuzick risk model

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**Background:** Increased screening, including breast MRI, is recommended for women carrying pathogenic variants (PVs) in certain breast cancer risk genes, as well as women with an estimated remaining lifetime risk of breast cancer >20% based on risk models such as Tyrer-Cuzick (TC). We determined the extent to which genetic testing identifies women as candidates for increased screening who would not have been flagged using the TC model.

**Methods:** We evaluated 100,318 women who underwent clinical genetic testing for suspicion of hereditary breast cancer risk between June 2017 and June 2018. Testing with a 28 gene pan-cancer panel included 5 established breast cancer risk genes for which there are National Comprehensive Cancer Network (NCCN) recommendations for increased screening including breast MRI (*BRCA1*, *BRCA2*, *ATM*, *CHEK2*, *PALB2*). A remaining lifetime breast cancer risk estimate was calculated using TC V 7.02, unless the woman was ineligible due to a breast cancer diagnosis, age over 85, or if the sample was submitted without any of the necessary TC data. Risk estimates were calculated entering the *BRCA1/2* status as unknown.

**Results:** 4,640 (4.6%) women were found to carry a PV in one of the five genes, with 57.0% of the PVs in *BRCA1* or *BRCA2*, and 43.0% in *ATM*, *CHEK2* or *PALB2*. Among the women with a PV, 1,479 (31.9%) had a diagnosis of breast cancer, and 407 (8.8%) were ineligible for TC for another reason. A TC risk estimate was calculated for 2,754 women, of whom 41.5% did not meet the 20% threshold using TC. Table 1 shows the breakdown for each gene by age at the time of testing. Women with PVs in *BRCA1* were the least likely to have a TC score <20% and those with PVs in *BRCA2* and *ATM* were the most likely to fall below the 20% threshold. Remaining lifetime risk declined with age in all groups.

[table1]

Age at time of testing	<i>BRCA1</i> (N=1215)	<i>BRCA2</i> (N=1432)	<i>ATM</i> (N=609)	<i>CHEK2</i> (N=971)	<i>PALB2</i> (N=413)
<20	14.8%	11.1%	0.0%	33.3%	
20-29	22.0%	27.3%	29.5%	28.4%	19.1%
30-39	28.7%	38.1%	30.3%	36.4%	22.6%
40-49	38.4%	48.8%	47.7%	42.5%	50.0%
50-59	49.4%	62.7%	64.3%	52.3%	44.4%
60-69	87.5%	78.8%	71.9%	77.8%	71.4%
70-79	--	100.0%	100.0%	100.0%	100.0%
80+	100.0%	--	--	--	--
All ages	33.6%	45.7%	46.3%	42.6%	37.4%

**Table 1. Percentage of women carrying PVs in breast cancer risk genes with a remaining lifetime risk of breast cancer risk estimated at <20% by Tyrer-Cuzick.**

**Conclusions:** Among the women included in this analysis, the TC model failed to identify approximately 40% of women eligible for high risk breast cancer screening due to PVs in breast cancer risk genes. This demonstrates that risk models based on clinical factors alone will fail to identify a significant fraction of women who are candidates for modified medical management.

Additionally, it may be desirable to re-evaluate setting thresholds based on remaining lifetime risk, since this dramatically reduces the likelihood that high-risk women will be flagged for increased screening at older ages, when their immediate risk of a breast cancer diagnosis is likely to be highest.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-03-05

Multi institutional effort for contributing with the clinical classification of variant of uncertain significance in BRCA1 and BRCA2 genes

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*BRCA* variants of unknown significance (VUS) occur in approximately 10% of genetic testing. The clinical interpretation of these VUS is a crucial step in the reporting and counseling process. The likely pathogenic, uncertain, and likely benign categories include some level of uncertainty as to whether the variant is related to the disease. Much of the data regarding variants is proprietary or unpublished, presenting a barrier to reclassifying inconclusive results. Therefore, the aim of this study was to compile and compare VUS in *BRCA1/2* in Hispanic patients with breast cancer at 4 collaborating international institutions: UT MD Anderson Cancer Center (USA), AC Camargo Cancer Center (Brazil), Barretos Cancer Hospital (Brazil) and Albert Einstein Hospital (Brazil).

Each institution queried for patients with *BRCA1/2* testing. Missense variants either not reported in ClinVar database or those classified as “conflicting interpretations of pathogenicity” were compiled in this study. All VUS were assessed by four computational prediction software (SIFT, Polyphen2, CADD and REVEL) and classified in five categories according to the risk of pathogenicity. Clinical variables (age, tumor type and tumor immunophenotype) were also assessed where available.

A total of 327 supposedly unrelated breast cancer patients and of *BRCA1/2* missense variants of unknown clinical significance were identified (87 of *BRCA1* and 242 of *BRCA2*). Here, we detected 271 different VUS (73 in *BRCA1* and 198 in *BRCA2*), which were classified in five classes of estimated pathogenicity (very high, high, medium, low and very low risk of pathogenicity), according to the number of *in silico* programs classifying the variant as probably damaging. In *BRCA1*, 48% of variants and 29% of *BRCA2* were classified as very high/high risk. The age of tumor onset (n = 252) did not differ between the stratified risk categories for both genes. We evaluated the occurrence of the same VUS in more than one institution and identified 4 variants of *BRCA1* that had carriers in two institutions; and 10 and 2 variants of *BRCA2* with carriers in 2 and 3 institutions, respectively. From the 16 VUS identified in more than one institution, three variants were classified as very high or high risk of pathogenicity, one in *BRCA1* (p.A1789T) and two in *BRCA2* (p.P94S and p.R2784Q). Interestingly, the p.R2784Q was identified in AC Camargo and Barretos Hospitals and a different variant in this same amino acid was identified in one patient from MD Anderson (p.R2784L). In both genes, we identified a significant enrichment of variants occurring in known functional domains in the very high/high risk categories, when compared to the other categories.

The information that has been gained in this study will aid to the classification of VUS by co-segregation analysis of probands and their family members. The definition of pathogenicity will be important in regards to their personalized risk management options (increased surveillance, preventive surgery or normal screening). This is one of the global approaches to identify women at increased breast and ovarian cancer risk via *BRCA* mutation classification.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-03-06

Limitations of direct-to-consumer genetic screening for HBOC: False negatives, false positives and everything in between

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## Background

Genetic screening of unaffected individuals for hereditary breast and ovarian cancer (HBOC) risk is a growing opportunity for personalized preventive medicine. The FDA recently authorized a direct-to-consumer (DTC) test to report on 3 *BRCA1/2* variants, commonly found in individuals of Ashkenazi Jewish (AJ) heritage, out of more than 1000 known. Here we define the probability that DTC genetic screening for the 3 *BRCA1/2* AJ founder variants would falsely reassure individuals of AJ and non-AJ ancestry of low risk for hereditary cancer syndromes (HCS). We also assess the frequency of false positive results reported by third parties from raw genotypes, which are routinely provided to clients who submit them for cancer risk analysis.

## Methods

We analyzed de-identified data on three cohorts: 1) An indication-based cohort of 119,328 patients referred by healthcare providers for HBOC genetic testing due to personal or family history, 2) a screening cohort of 5,170 patients without personal or family history who had *BRCA1/2* testing as a health screen, and 3) a confirmation cohort of 102 patients referred for clinical confirmatory testing instigated by positive DTC results from third party analysis of raw data.

## Results

In the indication-based cohort 12,846 patients had a mutation in any HCS-associated gene: 4,733 (37%) were in *BRCA1/2*, of which 12% were one of the 3 AJ founder mutations. Ethnicity impacted AJ founder mutation frequency: 81% of AJ patients with any *BRCA1/2* mutation had one of the 3 founder mutations, but only 6% of non-AJ patients. Overall clinical false-negative rate for the 3 AJ founder mutations in *BRCA1/2* carriers was 88%; rates were 19% and 94% among AJ and non-AJ individuals, respectively. In the screening cohort, where 2.6% were AJ, 40 patients had a P/LP mutation in *BRCA1/2*; 12.5% were an AJ founder mutation. The clinical false-negative rate of 88.5% for any *BRCA1/2* mutations in the screening cohort is similar to the indication-based cohort. Finally, in the confirmation cohort, among patients told of a positive DTC screening result, our analyses indicated 50% (52/102) were analytic false positives.

## Conclusions

This study highlights the limitations of screening restricted to a few of the many genetic variants associated with HBOC risk. We found a greater rate of non-founder *BRCA1/2* mutations in AJ patients than previously reported, and found a concerning rate of clinical false negatives with screening limited to the AJ founder variants. Although increasing access to genetic information is vital, the limitations of current DTC genetic screening, despite warnings from the FDA, may not be well understood by consumers and should be used with caution. Patients screened for HBOC on a platform limited to the AJ founder variants and supplemented by analysis of raw genotyping data, should receive confirmatory testing, regardless of having either a positive or negative result, a recommendation which the DTC companies and third party analysts of raw data ostensibly support. These results suggest that all HCS screening should include the support of a qualified clinician to assess the potential limitations and implement appropriate clinical management recommendations for patients and their family members.

# 2018 San Antonio Breast Cancer Symposium ®

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Analysis of hereditary cancer syndromes by using a panel of genes: Novel and multiple pathogenic mutations

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## BACKGROUND

Hereditary cancer predisposition syndromes are believed to be responsible for approximately 5-10% of all diagnosed cancer cases. In the past, single genes analysis of certain high risk genes was used for the determination of the genetic cause of cancer heritability in certain families. The selection of genes was mainly based on the family history of the individuals analyzed and included only highly associated genes (e.g. the *BRCA1* and *BRCA2* genes for families with breast cancer history. Nowadays though, the application of Next Generation Sequencing (NGS) technology has facilitated multigene panel analysis and is widely used in clinical practice, for the identification of individuals with cancer predisposition gene mutations.

## AIM

The aim of this study was to investigate the extent and nature of mutations in 36 genes implicated in hereditary cancer predisposition in individuals referred for testing in our lab.

## MATERIALS & METHODS

In total, 1197 individuals were referred for testing in our lab in the past four years from Greece, Romania and Turkey. The analysis of genes involved in hereditary cancer predisposition was performed using two NGS approaches. The first 451 individuals were analyzed using an amplicon based sequencing method (26 gene panel), while the following 746 individuals were analyzed using a capture based method (33 gene panel). Genomic DNA was enriched for targeted regions of 36 genes involved in hereditary predisposition to cancer included in both versions of the panel (*APC*, *BMPR1A*, *BRCA1*, *BRCA2*, *CDH1*, *CDK4*, *CDKN2A*, *EPCAM*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *PALB2*, *PMS2*, *PTEN*, *RET*, *SMAD4*, *STK11*, *TP53*, *VHL*, *ATM*, *BRIP1*, *CHEK2*, *NBN*, *RAD51C*, *RAD51D*, *BARD1*, *BLM*, *CHEK1*, *ABRAXAS1* (*FAM175A*), *MRE11* (*MRE11A*), *NF1*, *RAD50*, *RAD51B*, *XRCC2*). Sequencing was carried out using the Illumina NGS technology. Reads were aligned to the reference sequence (GRCh37), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript. The presence of large genomic rearrangements was investigated by computational analysis of NGS results and the use of MLPA for 13 genes. All clinically significant observations were confirmed by orthogonal technologies.

## RESULTS

In total, a pathogenic mutation was identified in 259 of the 1197 individuals (21.6%) analyzed while a VUS was identified in 35.7% of the cases. Clinically significant mutations were identified in 29 of the genes analyzed. Concerning the mutation distribution among individuals with positive findings, 44.7% of them were located in *BRCA1/2* genes whereas 20.9%, 19.9%, and 14.5% in high, moderate and low risk genes respectively. In addition to *BRCA1* and *BRCA2* genes other highly mutated genes were *CHEK2* (10.6%), *PALB2* (7.1%), *MUTYH* (7.1%) and *ATM* (4.3%). Of note is that 25 of the 259 positive individuals (9.7%) carried clinically significant mutations in two different genes and 5.8% had a large genomic rearrangement (LGR).

## CONCLUSIONS

Our results support the clinical significance of analysis of a panel of genes involved in hereditary cancer predisposition. In our cohort, analysis of this panel allowed for the identification of 8.3% additional pathogenic variants in moderate/low risk genes,



enabling personalized management of these individuals.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-03-08

Analyzing the clinical actionability of germline CYP2D6 polymorphism in Chinese breast cancer population

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**Background:** Tamoxifen is effective for endocrine-responsive breast cancer as adjuvant therapy. CYP2D6 enzyme metabolizes tamoxifen to clinically active metabolites, while CYP2D6 polymorphisms may adversely affect tamoxifen efficacy by some retrospective clinical evidence. This study was carried out to estimate genotype frequencies of common variants of CYP2D6 in Chinese population. The relationship between prescription of selective estrogen receptor modulators (SERMs) and CYP2D6 polymorphism was also analyzed.

**Methods:** This was a retrospective research of early-stage patients who underwent surgical treatment at Fudan University Shanghai Cancer Center with ER+ and/or PR+ breast cancer. Genomic DNA was extracted from peripheral blood, which was used for genotyping CYP2D6\*10 (C100T) single-nucleotide polymorphisms by polymerase chain reaction-based methods.

**Results:** A total of 312 patients with primary breast cancer were identified. More than 90.0% patients were in premenopausal status. The allele frequency of CYP2D6\*10 in the Chinese population was 54.3%. The genotype frequencies of CYP2D6\*10 were 20.5%, 50.3%, 29.2%, for wild-type, heterozygous and homozygous type respectively. We also found this SNP had no significant correlation with clinical characteristics. 145 patients were continuing endocrine treatment in first 5 years. 34.5% patients received CYP2D6 polymorphism test before the prescription of SERMs. The results significantly effected the choice of SERMs. Only 6.2% homozygous type patients took tamoxifen, 45.5% heterozygous patients chose tamoxifen, while 75.0% for wild type. 65.5% patients received CYP2D6 polymorphism test during the treatment of tamoxifen. 63.9% homozygous type patients switched to Toremifene, while 18.9% heterozygous patients changed the endocrine treatment.

**Conclusions:** The results showed that the frequency of CYP2D6\*10 allele was high and nearly 30% Chinese breast cancer population were intermediate metabolizer for tamoxifen. The cyp2d6 polymorphism would influence prescription of SERM in premenopausal breast cancer patients. Patients with homozygous types should take other endocrine treatment instead of tamoxifen, which need more evidence of prospective clinical trials.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-03-09

Genetic and hereditary factors in breast cancer: Experience in a mastology service on analysis of genetic tests in high risk women

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**Background:** Breast cancer is the most prevalent cancer in women in Brazil. The estimate by 2018 is 59,700 new cases. Of these, 10 to 15% are hereditary, caused by mutations in susceptibility genes that increase the risk of disease onset. These types of mutations can occur in several genes, each associated with different risks of developing the pathology. In hereditary breast-ovarian syndrome, the most frequently altered genes are BRCA1 and BRCA2. Mutations of these genes are associated with a 70-85% risk of developing breast cancer during life, but we know that there are other types of genetic alterations at risk of developing breast, ovarian, or other cancers - many are of uncertain significance and others, less known, appear to have high pathogenic potential. Thus, individuals identified with hereditary risk for developing breast or ovarian cancer should have intensive follow-up and genetic counseling.

**Materials and methods:** A retrospective study was carried out between 2015 and 2018. The charts of each of the patients submitted to genetic counseling with a total of fifteen women were analyzed. Eleven of these, performed genetic tests to evaluate mutations in BRCA1 and 2, and four patients extended panels. Subsequently, a statistical analysis of the data was performed and the results were analyzed thanks to the informed consent that the patients signed.

**Results:** Among the fifteen cases the age ranged from 27-68 years. Fifteen patients were analyzed, twelve with diagnosis of breast cancer and three without. In the eleven women who tested for BRCA1/2, 27% had mutations for these types of genes and three pathogenic variants of the genes. In the four who did the extended test, three had mutations in six types of genes, two with mutations of uncertain significance(VUS) and one pathogenic variant of the BLM gene. The indications for the exams were: 1)breast cancer patients: five cases with triple negative carcinomas and age less than 60 years-41.66%; 2)four with family history-33.33%; 3)three only by age-25%. In those without breast cancer the indications were family history. The majority of cases were requested for triple negative neoplasia, in which two cases presented mutation(40%) and three cases without mutation(60%), the two mutations in the BRCA1 and BRCA2 genes, one in each of them and none in VUS . In the four cases with a family history, two had mutations and two did not(50%) - two in BRCA 2 and one in VUS. In the three cases without cancer was found mutation in only one of them and with important family history was found mutation in one case(BRCA2).

**Conclusions:** Mutations in BRCA1/2 genes are associated with susceptibility to develop breast-ovarian cancer. Currently these mutations are responsible for only a minority of familial cases and we also find variants of uncertain and pathogenic behavior. Intensive research to identify other types of genetic mutations that could be responsible for a significant percentage of breast cancer in BRCA1/2 negative families, as well as assessing their importance, appear to be necessary. As already published by other authors, although our casuistry is small, we also call attention to the presence of mutations in young patients with triple negative breast carcinomas.

## 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-03-10

Identifying germline APOBEC3B deletion using hereditary cancer panel in Korean patients with operable breast cancer

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**Background:** APOBEC3B is a cytosine deaminase implicated in host immune defense to virus and mutagenesis in cancer. Germline APOBEC3B deletion is known as risk factors for breast cancer with hypermutation and immune activation from previous database-based studies. This study was aimed to evaluate the incidence of germline APOBEC3B deletion in Korean patients with operable breast cancer.

**Method:** The copy number variants of germline APOBEC3B deletion was analyzed from leukocyte DNA of 103 breast cancer patients whose bloods were collected in 2009 for pharmacogenomic study at Seoul National University Bundang Hospital. Hybrid-capture based next-generation sequencing panel targeting 53 hereditary cancer genes were used. We also measured tumor infiltrating lymphocytes (TILs) and programmed cell death-ligand 1 (PD-L1) expression in tumor or immune cell with a rabbit monoclonal antibody (E1L3N).

**Results:** Median age of breast cancer diagnosis was 46 (25-72). In APOBEC3B deletion analysis, 10 (9.7%), 36 (35.0%), and 57 (55.3%) patients were identified as two-copy deletion (A3B<sup>del/del</sup>), one-copy deletion (A3B<sup>del/wt</sup>) and no deletion (A3B<sup>wt/wt</sup>), respectively. In non-APOBEC3B analysis, 9 (8.7%) patients were identified as pathogenic variant: RAD51D(n=1), GJB2(n=1), BRCA1(n=1), BRCA2 (n=2), ATM(n=1), USH2A(n=1), RET(n=1), BARD1(n=1). We observed no significant association between germline APOBEC3B deletion with any clinicopathologic features of breast cancer such as age, family history of cancer, and bilateral breast cancer. Triple-negative subtype was associated with A3B<sup>wt/wt</sup> Tumors (35.1% in A3B<sup>wt/wt</sup> vs. 5.6% in A3B<sup>del/wt</sup> vs. 20% in A3B<sup>del/del</sup>; P=0.018). After a median follow-up time of 92.8 months, APOBEC3B deletion was not predictive of recurrence or survival. In patients with sufficient tumor samples for the assessment of TIL (n=63) and PD-1 (n=71), A3B<sup>del/del</sup> tumor was associated with higher TILs (>10%) than other tumor types (6/7 patients in A3B<sup>del/del</sup> vs. 13/24 in A3B<sup>del/wt</sup> vs. 15/32 in A3B<sup>wt/wt</sup>; Fisher's exact test in A3B<sup>del/del</sup>, P=0.029).

Germline APOBEC3B deletion and TILs (n=63)

	TIL (0-10%)	TIL (>10%)	Total
A3B(wt/wt)	17 (53.1%)	15 (46.9%)	32
A3B(del/wt)	11 (45.8%)	13 (54.2%)	24
A3B(del/del)	1 (14.3%)	6 (85.7%)	7

However, PD-L1 expression was not associated with APOBEC3B deletion status (1/7 patients >1% PD-L1 in A3B<sup>del/del</sup> vs. 4/26 in A3B<sup>del/wt</sup> vs. 8/38 in A3B<sup>wt/wt</sup>; P=0.901).

**Conclusion:** We identified germline APOBEC3B deletion in 9.7% of Korean patients with operable breast cancer. The relationship between A3B<sup>del/del</sup> tumor and high TILs suggests that these tumors might be potential candidates for future immunotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-04-01

The landscape of somatic genetic alterations in breast cancers from *CHEK2* germline mutation carriers

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**Introduction:** Checkpoint kinase 2 (CHEK2) is a tumor suppressor gene, which regulates cell cycle in response to DNA damage response. Selected *CHEK2* germline mutations have been shown to confer an increased risk of breast cancer development. Multiple founder mutations in *CHEK2* have been identified, and meta analyses have shown that CHEK2 truncating variants confer a higher breast cancer risk than missense variants. Here, we assessed the phenotype and repertoire of genetic alterations of breast cancers from 33 patients with *CHEK2* pathogenic germline variants.

**Materials and methods:** We performed targeted capture massively parallel sequencing ( $\geq 410$  genes) of tumor and normal samples from 13 patients with *CHEK2* pathogenic germline variants, and retrieved whole exome sequencing (WES) data (BAM files) of tumor and normal samples from 20 patients with *CHEK2* germline pathogenic variants included in the TCGA breast cancer study. In addition, we retrieved WES data of BRCA1, BRCA2 and ATM associated breast cancers from TCGA and Weigelt et al. (JNCI 2018). Somatic mutations, copy number alterations, mutational signatures and large-scale transitions (LSTs) were defined using state-of-the-art bioinformatics algorithms.

**Results:** Of the 33 *CHEK2*-associated breast cancers included in this study, 21 had missense and 12 had loss-of-function (LoF) germline mutations, and 81% were ER-positive and 12% HER2-positive. CHEK2-associated breast cancers statistically significantly less frequently displayed an ER-negative/HER2-negative phenotype (0%) than BRCA1- (80%) or BRCA2-associated (33%) breast cancers (BRCA1,  $p < 0.0001$  for both comparisons), but were similar to ATM-associated breast cancers. Biallelic inactivation of *CHEK2* through loss of heterozygosity (LOH) of the wild-type allele was present in 17 of 33 samples (52%). LOH of the *CHEK2* wild-type allele was significantly more frequent in tumors with LOF mutations than in those with missense mutations (78% vs 36%, respectively;  $p = 0.0394$ ). *PIK3CA* (36%) and *GATA3* (33%) were the two most recurrently mutated genes in these samples. *TP53* somatic mutations were detected in five cases, four of which harbored missense *CHEK2* germline mutations. Unlike BRCA1- and BRCA2-associated breast cancers, but akin to ATM-associated breast cancers, *CHEK2*-associated breast cancers lacked the mutational signature associated with homologous recombination (HR) DNA repair defects (i.e. signature 3) and only five cases displayed high LST scores.

**Conclusion:** CHEK2-associated breast cancers are phenotypically and genetically distinct from BRCA1- and BRCA2-associated breast cancers, but similar to ATM-associated breast cancers. Akin to ATM-associated breast cancers, CHEK2-associated breast cancers are preferentially ER-positive, lack genomics features consistent with defective HR, and have a repertoire of somatic genetic alterations similar to those of non-BRCA1/2 ER-positive breast cancers. Our results suggest that either *CHEK2* germline mutations contribute to an increased risk of breast cancer independently of the HR DNA repair defects or that the mutational signatures caused by *CHEK2* pathogenic germline mutations differ from those caused by pathogenic germline mutations affecting *bona fide* HR-related genes (e.g. *BRCA1*, *BRCA2* and *PALB2*).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-04-02

Identifying ERBB-2 activating mutations (mts) in HER2 negative tumors for clinical trials – Impact of institute-wide genomic testing and trial matching on trial enrollment in clinical practice

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## Introduction

Tailored treatment trials with biomarker-driven hypotheses are becoming an important strategy in drug development. Umbrella, basket and enrichment trials with eligibility predicated upon results of tumor sequencing are increasingly common. Several institutional and commercial genomic assays have been developed. However, the value of broad-based testing in recruiting patients (pts) to molecular-based clinical trials designed for small subgroups has not been fully evaluated and has been challenging to assess in a real-world setting. We evaluated the likelihood of trial enrollment based upon an institute-wide genomic test.

## Methods

Since 2013, all pts with metastatic breast cancer (MBC) seen at least once at Dana-Farber Cancer Institute have been offered the option of tumor sequencing using multiplexed copy number variation (CNV) and mts detection across the full coding regions of a total of 447 cancer genes and 191 regions across 60 genes for rearrangement detection (Oncopanel; OP). For our primary analysis, we selected the ongoing multi-center phase II trial (NCT01670877) activated at our site on Sep 30, 2013, evaluating neratinib in ERBB-2 mutated pts, as the study provided a clear delineation of eligible mts, and timing of slot availability was retrievable retrospectively over an extended time frame. Our primary aim was to describe the proportion of pts with a qualifying ERBB-2 mt detected by OP who enrolled on the selected trial. Secondary objectives included median time from OP result to trial registration and description of ERBB-2 mts spectrum within each subtype. Associations were calculated by Fisher's test.

## Results

We identified a total of 1,046 pts with HER-2 negative MBC and who had OP results between Sep 1, 2013 and Jun 1, 2017. A total of 43 pts (4.1%) were found to have ERBB-2 mts. Of these, 20 (1.9%) had activating eligible mts. The proportion of these pts who enrolled in the trial was 30% (6/20). Of the remaining 14 pts, 5 screen-failed and 2 were enrolled with known ERBB-2 mt through other testing modalities. Seven of 20 (35%) molecularly eligible pts were not approached (3 pts lost to follow-up, 3 enrolled in other clinical trials and 1 pt chose standard treatment). The median time from OP result to trial enrollment was 85 days (34-554). A significantly higher frequency of ERBB2 activating mts was found in ER+ compared to ER- primary tumors (2.5% vs. 0.3%,  $p=0.036$ ), and in lobular tumors compared with ductal (5.5% vs. 1.25%,  $p=0.003$ ). Frequency of eligible mts in primary tumors were similar to metastatic site (1.9% and 1.8%, respectively  $p=1.0$ )

## Discussion

In this cohort, activating ERBB-2 mts were present in 20 of 1046 (1.9%) pts tested. Although over half of pts with eligible mts on OP testing were approached for NCT01670877, only 0.5% of the total tested population were enrolled (6/1046). Our data illustrate the substantial challenges in screening and enrolling to trials of rare subsets, even within a large academic institution, and point to the need for creative and novel approaches to leverage pts and community- and academic-based providers to more effectively support the success of such studies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-04-03

PIK3CA mutations in breast tumor specimens in a cohort (n=791) of a multicentric study and associations to known prognostic factors and survival data

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## Introduction:

The PI3K/AKT/mTOR signalling pathway plays an important role in cellular processes like proliferation, apoptosis, survival and adhesion of tumor cells. It is one of the most deregulated pathways in cancer.

In up to 30% of breast cancers, dysregulation of this pathway is reported, resulting from mutations in the PIK3CA gene that encodes the catalytic subunit (p110a). Hotspots of mutations, comprising 86 % of all observed PIK3CA-mutations, were described in exon 9 (helical domain, E542K, G > A; E545K, G > A) and exon 20 (kinase domain, H1047R, A > G). Prevalence and the prognostic impact of the PIK3CA mutations as well as the predictive value with regard to endocrine therapy are controversially discussed.

In this study we describe the prevalence of the three most frequent PIK3CA mutations in a consecutive cohort of breast cancer patients and its association to tumor characteristics, to known prognostic factors and survival data.

## Methods:

The cohort consists of 1,047 patients who were newly diagnosed for non-metastatic breast cancer in one of 6 German breast centres from 2009 to 2011 and who were registered within the prospective PiA-study (Prognostic assessment in routine Application, NCT 01592825). DNA of 806 fresh frozen tumors were available for analyzation by qPCR (exon 9: C 763 and C760; exon 20: C 775).

Associations between the PIK3CA mutation status and clinical, pathological parameters were evaluated using binary logistic regression model. Survival probabilities were estimated by Kaplan-Meier-method, Log-Rank-Test and Breslow-Test. Recurrence free interval (RFI) was defined according to STEEP criteria.

## Results:

Mutation status for the three most common PIK3CA mutations was available for 791 tumors. The mutation rate was 29.2%. Only two tumors harbored two mutations (C 765, C 763). Tumors with a PIK3CA mutation were significantly more frequent in HR positive tumors (32%; p=0,001), in HER2 negative tumors (31%, p=0,010) and in tumors with low or intermediate histological grade (39%; 32% resp., each pTumors with a PIK3CA mutation showed a slightly better recurrence-free interval (93.6%, CI 93.2-94.01 vs. 90.3%, CI 89.9-90.7). We found a prognostic impact on RFI after 5 ys F/U in patients with HR-negative tumors (93% vs 71%) and those with TNBC (100% vs. 70%).

## Conclusion:

PIK3CA mutations occur frequently in breast cancer tumors. We found a prognostic impact only in patients with HR-negative and TNBC tumors.

This data adds important information to the heterogeneous results of other previously published patient cohorts. In summary in our study, tumors that harbour a mutation of the PIK3CA-gene, were associated to prognostically more favorable factors and a better recurrence-free intervall.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-04-04

The mutational profile of inflammatory breast cancer reveals a higher mutational burden leading to MAPK activation and chromatin remodeling

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**Introduction.** Inflammatory breast cancer (IBC) is an aggressive form of locally advanced breast cancer with increased metastatic potential. In the past, we have identified a gene expression profile that characterizes IBC, suggesting that a specific molecular biology underpins this devastating disease. Here, we explore the hypothesis that the molecular portrait of IBC is a reflection of underlying genomic alterations.

**Materials and Methods.** Mutation and copy number variation (CNV) profiles for 663 genes were assembled from 2,352 publicly available primary tumor samples (subtype distribution: 1,520 HR+, 355 HER2+, 414 TNBC and 190 unassigned) including 127 profiles from patients with IBC. Gene-wise differences in the frequency of genomic aberrations between patients with and without IBC, stratified per subtype, were investigated using Chi-square testing with adjustment for multiple comparisons. Genomic perturbation differences of pathways and processes, represented by KEGG or Gene Ontology gene sets, were evaluated by collapsing mutation and CNV profiles across all genes associated with the respective gene sets. Finally, mutational signature (MS) profiles were calculated and compared between patients with and without IBC.

**Results.** Seventy-six genes showed evidence of more extensive genomic alterations in samples from patients with IBC as compared to those without IBC (i.e. false discovery rate < 10%), whereas only 3 genes reveal the opposite pattern. Genes mutated in more than 15% (range 16.2% - 63.5%) of the IBC samples include: AXIN1, ERBB2, ERBB3, CBL, CTNNB1, CYP2D6, FGFR1, INSR, KIT, KMT2A, LRP1B, MYC, PBRM1, SACS, SMAD4, TP53 and ZNF217. Analysis of MS profiles revealed differences for signature 1 (i.e. age-related deamination of 5-methylcytosine), 2 (i.e. APOBEC3 activity), 3 (i.e. defective homologous recombination), 11 (i.e. alkylating agents), 20 (i.e. DNA mismatch repair) and 24 (i.e. aflatoxin), of which MS 11 and 24 are more active in IBC. When evaluating the same panel of genes in TNBC only, 28 genes were retained, suggesting data are confounded by the subtype distribution. Pathway analysis revealed genomic perturbation of MAPK signaling and chromatin organizational processes in respectively 55% and 74% of TN IBCs.

**Discussion.** These data suggest that IBC is characterized by an extensive mutational burden that results, amongst others in the activation of MAPK signaling as well as chromatin remodeling. The analysis of MS profiles does not provide a clear biological explanation for the increased frequency of genomic alterations in IBC, with APOBEC3 activity, defective homologous recombination, defective DNA mismatch repair and age-related deamination of 5-methylcytosine all being more prominent in IBC samples. Notably, the lower frequency of age-related C>T transitions is in line with younger age at diagnosis typical for patients with IBC.



# 2018 San Antonio Breast Cancer Symposium ®

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Spatial heterogeneity revealed by genomic profiles comparing 60 matched primary breast tumors and metastatic lymph nodes

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**Background:** Genomic profiling in primary breast tumors has been extensively performed and reveals the heterogeneous complexity in mutational landscape of the disease. However, little is known about differential mutation spectrums in synchronous breast tumors and matched metastasis in axillary lymph nodes. To characterize the spatial heterogeneity and identify potential biological mechanisms involved in the lymph node metastasis, we determined the genomic mutational profiles in synchronous primary lesion (PL) and metastatic lymph nodes (MLN) by using ultra-deep targeted sequencing.

**Methods:** Targeted deep sequencing was performed using a panel including 520 cancer-related genes and spanning 1.6MB of human genome. The mutational profiles were compared between the matched PL and MLN samples from 60 of treatment-naïve patients with invasive breast cancer and axillary lymph node metastasis. KEGG enrichment analysis was further performed between specific and shared mutations in either PL or MLN samples. The relationship between spatial heterogeneity and clinical characteristics was also explored.

**Results:** The cohort had a median age of 45 (ranging from 28-67), with a majority (86.7%) of them diagnosed with infiltrating ductal carcinoma. In this 60-paired cohort, 961 genomic aberrations were identified in 242 genes, including 405 single nucleotide variants (SNVs), 66 insertions or deletions (INDELs), 482 copy-number amplifications (CNAs), and 8 translocations. Although 584 (60.8%) events were shared in PL and MLN samples, 226 (23.5%) mutations spanning 51 genes and 151 (15.7%) mutations involving 46 genes were specific PL and MLN samples, respectively. In addition, 7 of patients (11.7%), all of whom are hormonal receptor (HR) positive, harbored completely similar mutation spectrum between PL and MLN. Twenty-one patients (35%) had PL specific mutations, but had no MLN specific mutations. In contrast, 6 patients (10%) only had MLN specific mutations and the remaining 26 patients (43.3%) had both PL and MLN specific mutations. Interestingly, the Ki67-based proliferation index in 7 of patients with completely similar mutation spectrum were significantly lower than other patients with differential mutational landscapes between PL and MLN ( $p=0.019$ ). Furthermore, KEGG pathway enrichment analysis revealed that deregulation in PI3K/AKT and Ras signaling pathway were enriched in both PL and MLN samples. More importantly, we found that aberrant activation of Proteoglycans pathway and JAK-STAT signaling, as well as aberrant HIF-1 pathway, was specifically occurred in the MLN samples, suggesting crucial roles for these signaling pathways in the involvement of lymph node metastasis.

**Conclusions:** Our study revealed genomic heterogeneity between primary tumors and lymph nodes, and identified mutations as well as pathways that are potentially relevant to lymph node metastasis, emphasizing that such spatial heterogeneity may contribute to the evolution of the disease and result in differential responses of subsequent treatments. *This study was supported by funding from National Natural Science Foundation of China (Grant No. 81602645) and Guangdong Provincial Natural Science Foundation (Grant No. 2016A030313768).*

# 2018 San Antonio Breast Cancer Symposium ®

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Study of the mutational landscape of normal and pregnant breast to predict pregnancy-associated breast cancer risk

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**Background:** Amongst the various risk factors for breast cancer (BC), the molecular basis which may explain the correlation between age at first full-term and breast cancer risks is still understudied. Epidemiology studies indicate that an early first full-term pregnancy (before 25 years of age) confers a significant level of protection towards the development of post-menopausal BC compared to the risk in nulliparous or late-parous women. On the other hand, any pregnancy relates to a higher risk in developing cancer during or within one year of pregnancy (pregnancy-associated breast cancer, PABC). Thus, the relation between age of pregnancy and breast cancer risk may be too difficult to explain using only epidemiology data.

**Aims of the study:** With our research study, we aim to study a cohort of 60 normal breast samples of nulliparous and age-matched early- and late-parous women collected from Komen Tissue Bank, University of Indiana, and to create for the first time a mathematical model of cell clone expansion in the normal breast growth. This will allow us to determine how the rates of both cancer drivers, passenger mutations and genetic variations are affected by pregnancy. We then aim to translate this in cancer tissues, and to determine how the rate of the same mutations in both pregnancy and non pregnancy-associated cancers (post-menopausal). At the same time, we intend to create a mouse model which will be used to further validate our model, where driver mutations will be induced in the mammary epithelium of pregnant mice of different ages. This will allow us to test our model of growth of a mutated clone in a pregnancy environment, and to determine what are the molecular changes in the pregnant mammary gland which can trigger a different BC risk in the early-parous cohort.

**Results:** To examine the mutational landscape in the normal parous and nulliparous women, we extracted DNA from laser-capture microdissected epithelium and the stroma, the latter of which will be used to eliminate germ line mutations. We are currently analysing the results from Whole Genome Sequencing at 30x 100pe on a MGISEQ2000 platform on a first set of samples (two nulliparous samples and two age-matched parous samples from both early and late pregnancy). Our procedure for processing and analysis of this data follows the Broad Institute's "GATK Best Practice Guidelines" for use of next generation sequencing (NGS) data. Based on the collected data, we plan to continue with targeted sequencing or whole genome sequencing on the remaining samples.

**Conclusions:** Our study will provide novel information on which areas of the genome are mostly mutated or altered in the normal breast, and will indicate how mutated cells, including mutations in driver genes for breast cancer, and genetic alterations change in the context of pregnancy. With the mathematical model of clone growth/extinction, we intend to explain how different ages of pregnancy can significantly alter the clone composition in the normal breast and result in a different probability of developing breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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High-throughput barcode screening elucidates the functional characteristics and mutual relevance of *PIK3CA* and *PIK3R1* somatic mutations in Chinese patients with breast cancer

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## Background

Deregulation of the phosphoinositide 3-kinase (PI3K) signaling pathway is essential to malignant cellular processes of breast cancer, including proliferation and drug response. Oncogenic somatic mutations of the PI3K pathway are pervasive in breast cancer. However, identification of impactful mutations and determination of their relevance to major components of this pathway remains difficult. This study was conducted to identify the landscape of somatic mutations in the PI3K pathway in a Chinese population. Notably, we developed a recombination-based mutation barcoding (ReMB) library which enables a high-throughput mutation-phenotype screens for vulnerable mutations that contribute to the cancer development and drug resistance.

## Methods

We collected 149 breast cancer specimens in a Chinese population and performed Ion Torrent Amplicon Sequencing for the key genes in PI3K/AKT pathway: *PIK3CA*, *PIK3R1*, *AKT1*, *AKT2*, *AKT3*, *PTEN*, *PDK1* at 1000× coverage. To discriminate between 'driver' and 'passenger' mutations, we developed a recombination-based mutation barcoding (ReMB) library that contained the novel identified mutations and that reported in TCGA and COSMIC databases in *PIK3CA* and *PIK3R1* genes, each mutation tagging with a specific barcode to identify their potential oncogenic and drug-resistant characteristics. The unique barcode representing each mutation was detected using Illumina Miseq sequencing following proliferation and drug response selection (doxorubicin and BKM-120) assays to screen the functional mutations.

## Results

We identified that mutations in *PIK3CA* (44%), *PIK3R1* (17%), *AKT3* (15%) and *PTEN* (12%) were prevalent and diverse in Chinese patients with a high proportion of tumours harboring multiple mutations, especially *PIK3CA* plus *PIK3R1* mutations (9.0%). With ReMB screening, we found 11 non-synonymous impactful mutations in *PIK3CA*; these included eight proliferation-driving mutations, nine doxorubicin-resistant mutations, and eight BKM120-resistant mutations. The highest-ranking *PIK3CA* mutations include the deleterious mutations E542K, E545K and H1047R/L as well as mutations of unknown significance, including E39K, N345I, E453K, and G1049R. We also identified six non-synonymous impactful mutations in *PIK3R1*, including five proliferation-driving mutations, six doxorubicin-resistant mutations, and five BKM120-resistant mutations. The *PIK3R1* impactful mutations include E160D, Q329L, N564D and K674R. Most impactful mutations in *PIK3CA* and *PIK3R1* occurred at residues lying at the interfaces between p110 and p85, or between the functional domains within p110. These *PIK3CA* and *PIK3R1* impactful mutations exhibit a mutually exclusive pattern, leading to oncogenesis and hyperactivity of PI3K pathway. Additionally, impactful mutations in *PIK3CA* are tightly associated with hormone receptor positivity.

## Conclusion

This study identified the landscape of somatic mutations in the PI3K pathway in Chinese breast cancer patients. A novel developed ReMB screening platform allows the rapid identification of impactful *PIK3CA* and *PIK3R1* mutations in breast cancer and has important implications for PI3K-targeted therapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-04-08

Genomic profiling of 304 treatment-naïve Chinese breast cancer patients: A comparison of Chinese and TCGA cohorts

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**Background:** The complexity of BC at the clinical, morphological and molecular level has been well recognized. Molecular profiling, which reveals the intrinsic biology among subtypes, has significantly advanced the management of this disease. However, previous studies have provided very limited molecular data on Chinese breast cancer patients.

**Methods:** We performed targeted sequencing using a panel consisting of 36 BC related genes to interrogate the genomic landscape of 304 consecutive treatment-naïve Chinese BC patients and compared our results to the TCGA data set.

**Results:** Comparing to TCGA, our cohort had significantly fewer patients with triple negative breast cancer (8.2% vs 15.5%  $p=0.002$ ). The most prominent genomic difference was our cohort had significantly higher *TP53* mutation frequency in HR+/HER2- and HR+/HER2+ groups. The composition of *TP53* mutations also differed significantly between two cohorts in HR+/HER2- group, with TCGA cohort having missense mutation as the predominant mutation; whereas, in our cohort, nonsense and frameshift mutations were predominant. We classified the most populated and diverse group of HR+/HER2- cancer into 4 subgroups based on molecular signature. The clinical significance of this proposed classification was confirmed by differences in overall survival using data from the TCGA.

**Conclusions:** We identified distinctive genomic patterns associated with Chinese breast cancer patients compared to TCGA data, suggesting the importance of mutation-based stratification according to ethnic status. To the best of our knowledge, this is one of the largest study of Chinese BC patients that interrogated the spectrum of mutational events and correlated these molecular signatures with clinical outcomes.

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# 2018 San Antonio Breast Cancer Symposium ®

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Comparing tumor mutation burden detection between whole exome and target enrichment sequencing among Taiwanese breast cancers

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**Purpose:** The ability to detect tumor mutation burden (TMB) is increasingly demanding and has become a pre-requisite for breast cancer personalized therapies, especially for those interrogating immune-modulating agents. The optimized quantification method determining TMB, however, remains inconclusive, and the current study compared tumor mutation yields among Taiwanese breast cancers, either with whole exome or target enrichment sequencing.

**Methods:** Sporadic breast cancers were prospectively recruited. Target-enrichment sequencing was performed with Illumina Solexa<sup>TM</sup> technology with read length of 150 paired ends and was analyzed with Agilent SureCall<sup>TM</sup>. There were 56 targets comprising 990 regions with a total regional size of 173.999 kbp. For whole exome sequencing, SureSelect<sup>TM</sup> Human All Exome V6r2 was adopted. The minimum coverage was set to 20 and the minimum alternative reads was set to 10 to enhance sequencing reliability. Samtools version 1.2 was utilized as variant caller for both whole exome and targeted sequencing. Novel and synonymous variants, as well as those located outside coding regions were removed. TMB was calculated as the number of somatic mutations per megabase (mb).

**Results:** A total of 61 and 52 Taiwanese breast cancers underwent target enrichment and whole exome sequencing, respectively. The number of somatic mutations ranged from 36 to 292 (median: 205), equivalent to 207~1678 (median: 1178) mutations/mb for targeted sequencing. On the other hand, there were 9947 to 10981 somatic mutations (median: 10727) when the whole exome was sequenced, resulting in TMB between 333 and 366 (median: 358). TMB for ER+/HER-, ER+/HER2+, ER-/HER2+, and ER-/HER2- was 1322 (n=31), 713 (n=11), 793 (n=10), and 1230 (n=9) for targeted sequencing and was 358 (n=29), 358 (n=12), 354 (n=5), and 357 (n=6) for whole exome sequencing. The most frequent nonsense mutations were *NOTCH1* (S255\*), *RET* (Q87\*), *KRAS* (G57\*), *JAK3* (Y267\*), and *SMO* (Y61\*), while *MAP2K1* (G148C), *FNACA* (G175V), *BSG* (H254P), *ABL1* (T57P), and *NOTCH1* (H416P) were the most common missense mutations.

**Discussion and conclusions:** High-throughput parallel massive sequencing can identify large numbers of variants, dependent both on the size of the sequenced regions and the variant caller algorithm utilized. Although Samtools identified more variants against reference than other methods, there should be no differential between whole exome and targeted sequencing. The number of somatic mutations, as well as the variability in TMB, was much higher in target enrichment approach than whole exome sequencing, highlighting that different TMB threshold should be established before wide clinical applications. Uneven distributions of “hotspot” mutation regions across genome as well as hundred-fold of sequencing length of whole exome versus targeted sequencing may contribute to such discrepancy.

## 2018 San Antonio Breast Cancer Symposium ®

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Genomic profiling of multifocal breast cancer reveals inter-lesion heterogeneity

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**Introduction:** Multifocal breast cancers are common, and tend to show more aggressive clinical features than unifocal breast cancers. While each foci of multifocal breast cancers with similar histology shares the same hormone and ERBB2 receptor status in most cases, substantial genomic differences among lesions have been reported. We aimed to investigate the potential genomic differences between multifocal breast cancer lesions.

**Materials and methods:** Twenty-one patients with multifocal breast cancer documented in the resection specimen were included. We selected two lesions with the same histology from each of these 21 patients. Capture-based targeted next generation sequencing was performed using a cancer gene panel consisting of 170 genes for single nucleotide variants (SNV) and small insertions/deletions (Indel), and copy number alterations.

**Results:** The most frequent mutation was *TP53* (38.1%), followed by *PIK3CA* (28.6%). Pathogenic mutations (SNV and Indel) were detected in 13 of 21 patients, of whom 11 shared oncogenic variants in the two lesions. The remaining two patients had different mutation results in *TP53* and *PIK3CA*, respectively. Genomic heterogeneity of copy number alteration was observed in 6 (28.6%) of 21 patients, including difference of *FGFR1* status in two patients and difference of *FGFR2* status in one patient.

**Conclusion:** Despite similar histologic features of multifocal tumors, genomic inter-lesion heterogeneity was identified in about one-fourth of patients. The spatial genomic heterogeneity in multifocal breast cancers needs to be considered in representative sampling and molecular tests for personalized medicine.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-04-11

A small amount of primary breast cancer shows high tumor mutation burden that may benefit from immune checkpoint inhibitor therapy

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**Background:** Targeted therapy using immune checkpoint inhibitors (ICIs) is a major breakthrough in cancer treatment in the last decade. ICIs like PD1 or PD-L1 antibodies have been shown to be quite effective in cancer like melanoma. However, in most other tumor types including breast cancer, the situation is not as optimistic. Only a small percentage of those patients respond to ICIs therapy. This highlights the importance of identifying biomarkers to predict which patients may benefit from such treatment. Tumor Mutation Burden (TMB) has been shown to be a sensitive marker for ICI treatment. This study is to investigate whether TMB could be used as a biomarker for breast cancer treatment.

**Methods:** We reviewed next generation sequencing studies of breast cancer. Two such studies with raw data provided were included in our analysis. One study entitled METABRIC performed targeted sequencing of 173 cancer-related genes in around 2500 primary breast cancer tissues. The other study was from TCGA breast cancer project, which performed Whole Exome Sequencing (WES) of around 1000 primary breast cancer samples. Mutation data were downloaded from public data deposit. The number of mutations per sample was calculated. TMB was calculated by divide the coverage in million base pair from that of the total mutation counts.

**Results:** In METABRIC study, 17272 mutations were identified in 2369 samples, with a median of 7 mutations per sample (95% CI: 6 ~ 7). The median TMB of METABRIC dataset was 5.8 SNVs/Mb (95% CI: 5 ~ 5.8). Totally 30 out 2369 (1.3%) samples had a TMB equal or large than 20 SNVs/Mb. In another cohort from TCGA breast cancer study using WES technology, 90172 mutations were identified in 977 samples, with a median of 44 mutations per sample (95% CI: 39 ~ 50). The median TMB was 1 SNVs/Mb (95% CI: 0.9 ~ 1.1). Totally 13 out 977 (1.3%) samples had a TMB equal or large than 20 SNVs/Mb.

**Conclusions:** Breast cancer shows middle to low mutation burden compared to other cancer types. Around 1.3% of breast cancer has quite high TMB of at least 20 SNVs/Mb, which may be qualified for immune checkpoint inhibitors therapy. Our study indicates that TMB may be incorporated as a standard test for late stage breast cancer patients in the clinical practice.

**Keywords:** Breast cancer, Tumor Mutation Burden, Whole exome sequencing, Targeted sequencing, Immune checkpoint

## 2018 San Antonio Breast Cancer Symposium ®

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TZAP mutation leads to poor prognosis of patients with breast cancer

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The zinc finger protein ZBTB48 is a telomere-associated factor that has been renamed as telomeric zinc finger-associated protein (TZAP). It binds preferentially to long telomeres, competing with telomeric repeat factors 1 and 2. However, its expression in cancers has not been investigated. In the present study, we analyzed the TZAP mutation in 128 breast carcinomas (BCs). In addition, its association with telomere length was investigated. The TZAP mutation (c.1272G>A, L424L) was found in 7.8% (10/128) of the BCs and was associated with the N0 stage ( $p = 0.035$ ). BCs with the TZAP mutation had longer telomeres than those without this mutation ( $2.62 \pm 1.71$  vs.  $0.98 \pm 0.37$ ,  $p = 0.009$ ). Telomere elongation was shown in 29.7% (38/128) of BCs and was significantly higher in luminal A and triple negative BCs ( $p < 0.001$ ). Other characteristics were not associated with TZAP mutations and telomere length. Survival analysis showed that the TZAP mutation resulted in a poorer overall survival (52.8 vs. 87.3 months,  $\chi^2 = 4.37$ ,  $P = 0.037$ ). However, telomeres did not have any prognostic value for BCs ( $P = 0.908$ ). These results suggest that the TZAP mutation is a possible prognostic marker in BC.



# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-05-01

Limb-Bud-and-Heart (LBH), a novel WNT effector in promoting basal-like breast cancer

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**BACKGROUND:** Clinically aggressive basal-like breast cancers (BLBC) disproportionally contribute to cancer deaths, but lack effective treatment options due to absence of expression of key therapeutic targets (i.e. ER, PR, HER2). New evidence suggests BLBC may originate from luminal breast epithelial cells through luminal-to-basal cell lineage conversion and dedifferentiation. However, the factors that reprogram cell fate and differentiation states during BLBC development remain poorly understood. Our laboratory has identified a novel WNT/ $\beta$ -Catenin target transcriptional regulator, Limb-Bud-and-Heart (LBH), and shown LBH is majorly over expressed in aggressive BLBC harboring WNT hyperactivation. We previously showed in genetic mouse models *in vivo* that LBH is a key mammary stem cell and basal cell lineage regulator essential for normal mammary gland development. Using crosses between *MMTV-Wnt1* transgenic mice and conditional LBH knockout mice, we tested if LBH is a critical effector of WNT-driven basaloid breast cancer, and whether its inhibition may prevent and/or attenuate BLBC formation and progression.

**RESULTS:** In our *in vivo* mouse model, LBH inactivation in the basal mammary epithelium using a Keratin 14/K14-Cre deleter strain significantly attenuated *MMTV-Wnt1*-induced mammary gland hyperplasia and led to a delay in tumor onset. Surprisingly, tumor burden and tumor volumes were not changed, indicating that LBH is not required for WNT-driven tumorigenesis. However, LBH-deficient *MMTV-Wnt1*-transgenic tumors exhibited pronounced histopathological differences compared to LBH WT *MMTV-Wnt1*-transgenic tumors. Whereas *MMTV-Wnt1+;K14<sup>Cre</sup>Lbh* WT tumors were highly vascularized, disorganized, with mixed basal and luminal cell identity; tumors from *MMTV-Wnt1+;K14<sup>Cre</sup>Lbh* KO mice were more organized, differentiated, and predominantly luminal Keratin 8 positive. Since attenuation of mammary gland hyperplasia and tumor onset in this model suggests that LBH deficiency may negatively affect WNT-induced stem cell expansion, we next investigated the LBH effects on the tumor stem cell compartment. *MMTV-Wnt1+;K14<sup>Cre</sup>Lbh* KO mice were crossed with two different stem cell reporter lines: *Lgr5-eGFP* mice, which specifically mark WNT-responsive epithelial stem cells; and *SHIP-GFP* reporter mice, which mark activated, proliferating mammary stem cells but not quiescent or slow-cycling stem cells. These studies are ongoing and the latest findings will be discussed.

**CONCLUSION:** Our data indicate that LBH is an essential cell fate regulator downstream of oncogenic WNT signaling that maintains basal lineage identity in basaloid breast cancers. LBH inhibition may be a potential novel strategy to treat basal subtype triple negative breast cancer patients through differentiation therapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Immunomonitoring of triple negative breast cancer patients undergoing neoadjuvant therapy with durvalumab - Results from the prospectively randomized GeparNuevo trial

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**Background:** The GeparNuevo trial is a randomized, double-blind, multi-center phase II trial of neoadjuvant therapy in patients with early-stage triple negative breast cancer (TNBC) investigating the role of durvalumab, an anti-PD-L1 antibody, which blocks PD-L1 binding to PD1 and CD80, in addition to standard anthracycline/taxane based chemotherapy (Loibl S et al. ASCO 2018).

**Methods:** In order to determine possible predictive and / or prognostic biomarkers, blood samples were taken before and during the different treatment phases (3 or 4 time points) and evaluated by multicolor flow cytometry by monitoring the absolute cell counts of T cells, B cells and NK cells as well as the frequency, composition and functionality of different immune cell populations using a panel of 35 distinct antibodies.

**Results:** Overall 174 patients were randomized into the GeparNuevo study. 117 patients participated in the window phase of this study and 49 patients provided evaluable material for flow cytometric analyses for material at the above specified time points. Evaluation of the absolute cell count in the whole blood prior and after therapy highlighted a mixed behavior of the total leukocytes. Overall, there was a statistically significant reduction in the lymphocyte count, particularly during the last phase of the treatment (ECdd +/- durvalumab). Further dissection into the different immune populations highlighted an almost complete loss of B cells, which in some patients was also accompanied by a reduction of NK cells, mainly of the CD16<sup>+</sup> subset. The loss of CD4<sup>+</sup> and CD8<sup>+</sup> T cells was less pronounced resulting overall in an enhancement of their percentages within the total lymphocytes. In addition, the different populations have also been evaluated for the expression of PD-L1 activation and exhaustion markers. A pre-specified analysis clearly demonstrated a specific effect of durvalumab during the window phase with mean decreases of 21.7% and 15.0 % in PDL1<sup>+</sup> lymphocytes in the CD4<sup>+</sup> and CD8<sup>+</sup> subgroups, respectively (P<0.001). Currently, all data generated are statistically analyzed focusing on their clinical significance in relation to the treatment received and the pathological complete remission (pCR) of these patients.

**Conclusion:** Using this approach we hope to identify biomarkers, which will allow a better selection of TNBC patients undergoing specific immunotherapies. Final data will be presented at the meeting.

The trial and this translational research project were funded by AstraZeneca and Celgene, Germany.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-06-02

Chemoimmunotherapy with cyclophosphamide plus a toll-like receptor 9 (TLR9) agonist eradicates triple negative breast cancer in a murine model

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**Background.** Toll-like receptors (TLR) recognize conserved molecular patterns expressed by microbes and together with other molecular sensors serve as a first line of defense, inducing soluble and cellular mediators of innate immunity and initiating key steps of an adaptive immune response. The use of TLR agonists for therapeutic purposes relies on the ability of these compounds to induce, at least partially, some of the immune events that occur during natural infections. For example, synthetic CpG-containing oligodeoxynucleotides (ODN) act as TLR9 agonists, mimicking stimulation of the immune system by bacterial or viral DNA. CpG ODNs are being developed for cancer immunotherapy based on their capacity to stimulate innate and adaptive anti-tumor responses. In this study, we examined the efficacy of intratumorally administered CpG-ODN 1826 alone or in combination with immunomodulatory antibodies (anti-PD-1, anti-OX40, anti-CTLA4) or chemotherapy (cyclophosphamide, paclitaxel) in the syngeneic mouse 4T1 breast tumor model.

**Methods.** Tumors were implanted orthotopically in the mammary fat pads on the left and right flanks while only one tumor was injected with CpG-ODN 1826. Therapy began 12-14 days post tumor injection when tumors were 8-10 mm in diameter. CpG-ODN 1826 (100 ug) was administered intratumorally, while immunomodulatory antibodies (anti-PD-1 [200 ug], anti-CTLA4 [100 ug], anti-OX40 [400 ug]) and chemotherapies (cyclophosphamide [150 mg/kg], paclitaxel [10 mg/kg]) were administered intraperitoneally. Tumor volume was monitored on both flanks to assess direct and abscopal/systemic anti-tumor activity. Tumor tissue obtained during the treatment regimen was used to evaluate therapy-induced changes in the immune microenvironment.

**Results.** CpG-ODN 1826, administered intratumorally over 5 consecutive days induced complete regressions in ~50% of the treated tumors, but only delayed growth in the distant lesions. The immunomodulatory antibodies had little effect on their own and did not add to the therapeutic efficacy of CpG-ODN 1826. The best therapeutic efficacy was obtained with a combination of weekly cyclophosphamide + CpG-ODN 1826 resulting in complete regression of both the CpG injected tumors and the contralateral tumors in 100% of the mice.

**Conclusions.** These data support the clinical investigation of the combination of a TLR9 agonist (CpG-ODN) with cyclophosphamide in women with breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-06-03

Unravelling triple-negative breast cancer tumor microenvironment heterogeneity using an integrative multiomic analysis

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**Introduction:** Triple negative breast cancer (TNBC) constitute 10-20% of all breast cancers and is associated with a worse prognosis and limited treatment options. Recent trials evaluating immune checkpoint blockade in TNBC demonstrated encouraging results for a subset of patients. TNBC is highly heterogeneous and its tumour microenvironment (TME) has been recognized as a critical determinant of its behavior and clinical outcome. Genome-wide gene expression profiling analyses have already improved our understanding of the complexity of this disease and have defined 6 different molecular subtypes namely Basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL) and luminal androgen receptor (LAR), exhibiting distinct biological and clinical characteristic.

In this study, we aim to dissect the molecular diversity of the TME and more specifically to assess the immune landscape according to TNBC molecular subtypes.

**Methods:** A cohort of 485 TNBC patient with publicly available data (RNA-Seq and Illumina HT-12 v3) from the METABRIC and the TCGA consortia were used in the gene expression analysis. Gene signatures reflecting different features or cellular components (immune, stromal, angiogenesis, lymphangiogenesis, hypoxia, metabolism) of the TME were used to evaluate multiple biological processes known to contribute to tumorigenesis. A compendium of 17 immune specific gene signatures and T cell localisation classification were used to evaluate the immune composition and spatial pattern of immune infiltrates. All parameters were compared using a logistic regression model to evaluate their relative contribution according to each molecular subtype.

**Results:** Our analyses demonstrated that each molecular subtype exhibits different TME profiles, as well as specific immune composition and localisation. IM tumors were associated with the highest expression of immune-related gene signatures, enriched with adaptive immune cells and with a fully inflamed spatial pattern. MSL tumors were mostly associated with the expression of Lymphangiogenesis and Stromal TME signatures. They also exhibited some immune activity through the expression of immune gene signatures capturing innate immune and adaptive immunosuppressive cells. This subtype was mainly associated with margin restricted and to some extent with fully inflamed spatial pattern. BL1 tumors were associated with the expression of Metabolism TME signatures, along with fully inflamed and stroma restricted spatial pattern. To a lesser extent, this subtype was also associated with activated DC and CD4 Tem cells. LAR and M tumors exhibited an immune cold phenotype. They were associated with Stromal and Metabolism TME signatures, enriched in margin restricted spatial pattern and negatively associated with every immune cells.

**Conclusions:** Our results demonstrate for the first time the huge heterogeneity that characterizes the TME of TNBCs. Identification of specific TME profiles could help to design more rationale and appropriate synergistic therapeutic combinations targeting TME elements in this high-risk disease.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-06-04

Targeting neo-epitopes from *PIK3CA* and *p53* mutations for immunotherapy of breast cancer

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**Introduction:** Preclinical data suggests that priming T cell immunity to mutated or overexpressed proteins can induce tumor rejection, and may potentiate the effects of checkpoint blockade. *PIK3CA* and *p53* are the most commonly mutated genes in breast cancer, and are enriched in high-grade and metastatic tumors. Here, we evaluated the immunogenicity of neo-epitopes derived from common *PIK3CA* and *p53* mutations.

**Methods:** We applied a custom informatics pipeline, EpitopeHunter, to predict neo-epitopes (8-11mers) from twelve *PIK3CA* and ten *p53* mutations restricted to thirty-nine MHC class I alleles. We selected high affinity neo-epitopes (IEDB score < 500) with predicted poor binding of the matched wild type epitope. We used the predicted peptides to stimulate PBMC from healthy donors in vitro, and measured T cell immunity by interferon- $\gamma$  ELISPOT. We compared the T cell specificity of mutant vs wild type epitopes. A positive response to an epitope was defined as a response that met both of the following criteria: 1) net number of spots (after subtracting background) was >5 spots/50,000 cells and 2) net number of spots exceeded the background spots plus two SDs.

**Results:** In total, 1,824 *PIK3CA* and 1,520 *p53* derived peptide sequences were generated. Of these, 42 *PIK3CA* (2%) and 45 *p53* (3%) peptides were predicted to bind to the HLA class I molecules included in our study. Over 90% of the neo-epitopes were 9- and 10-mers, and the predicted neo-epitopes varied across mutations (range 2-10 per mutation). *PIK3CA*<sup>H1047L</sup> and *p53*<sup>R248W</sup> had the highest number of potential binding neo-epitopes (n=10 each). We successfully generated T cell lines specific for A\*0201 *PIK3CA*<sup>H1047L</sup>, A\*1101 *PIK3CA*<sup>H1047L</sup>, A\*1101 *PIK3CA*<sup>E542K</sup> and A\*0201 *p53*<sup>R248W</sup>.

**Conclusions:** Common mutations of *PIK3CA* and *p53* can lead to the generation of potential HLA class I restricted neo-epitopes. We identified four immunogenic neo-epitopes, which may serve as candidates for targeted immunotherapy in breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-06-05

PanCancer profiling reveals population difference in breast cancer immune microenvironment

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**BACKGROUND.** Breast Cancer (BC) outcome in patients (pts) of African ancestry (AA) is worse than pts of European ancestry (EA) despite accounting for socioeconomic status and access. AA have higher hormone receptor negative (HR-) and Triple Negative (TNBC) tumors, subtypes associated with stronger presence of tumor infiltrating lymphocytes (TILs). We hypothesize that BC Immune Microenvironment (IME) composition differs by ancestry, and describe IME from two independent datasets.

**METHODS.** Transcriptome data from the Cancer Genome Atlas (TCGA) (Group 1, Gp1) were used to estimate 22 IME cell types in BC samples by CIBERSORT. Clinical and overall survival (OS) data were accessed from XENA. Gp2 tissue samples were obtained from Women's Circle of Health study and Pathology Resource Network at Roswell Park Comprehensive Cancer Center and processed using NanoString™ PanCancer Immune Profiling panel, consisting of 770 immunity-related genes describing 24 IME cell types. Immune Dysfunction and Exclusion (TIDE) scores were derived from an algorithm by Jiang et al.

**RESULTS.** Gp1 consisted of 183 AA and 752 EA, with median age older in EA (54.5 vs 59). On CIBERSORT IME analysis by race, AA had higher IME infiltrates including macrophages (Mp), dendritic cells (DC) and TILs; notably T regulatory (Treg) and T Follicular Helper (Tfh) cells. The ratios of Tregs and Tfh to total TILs were also elevated. When stratified by subtypes, AAs with TNBC/Basal-like BC had higher Tregs and Tfh cells. CD8+ cells were higher in HR+ and high-grade AA pts only. CD4+/total T-cells was higher in AA across all subtypes, and predicted worse OS (HR 3.15[1.07-9.2]). Gp2 had 190 AA and 177 EA with comparable median age at diagnosis (53 versus 54) and tumor grade. By subtype, TNBC had significantly higher total TILs, CD45+, CD8+, exhausted CD8+, Treg, cytotoxic T cells, B, natural killer (NK), activated NK, DC and Mp; yet significantly lower mast cells and neutrophils (p <0.01). CD8+/Exhausted CD8+ and CD8+/Treg ratios were lower in TNBC and higher-grade tumors, and lowest in HR- grade III. Most of immune pathways were enriched in HR- tumors, with only exception being cell cycle genes being remarkably enriched in HR+ tissues (p <0.01). TIDE demonstrated high immune dysfunction in HR- and high exclusion in HR+ tumors. When compared to EA, AA had more TILs, including B, cytotoxic T-cells, exhausted CD8+, NK, activated NK and Tregs (p <0.01). Neutrophils, Mp and CD8+ were higher in EA. EA also had significantly higher ratio of immune cell types to total TILs across cytotoxic, exhausted CD8+ and Tregs, as well as persistent higher neutrophils, Mp and CD8+ ratios. CD8+/Treg ratio was higher in EA. Consistent with Gp1; CD4+/total T-cell ratio was higher in AA across all subtypes.

**CONCLUSION.** IME differed significantly by HR, grade and ancestry. Aggressive BC demonstrated stronger overall immune response but dysfunctional IME phenotype (higher Treg, lower granulocytes and mast cells ratios). AA had more TILs across all subtypes, but lower ratios of activator (CD8+, Cytotoxic) to suppressor TILs (Treg, exhausted CD8+), demonstrating immune tolerance and immune-desert model, exception being persistently high fraction of CD4+ ratio predicting worse OS.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-06-06

Comparison of the immunological and clinical effect of personalized peptide vaccination for patients with breast cancer

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**Background:** Selected therapeutic personalized peptide vaccines (PPV) were effective for boosting anticancer immune response that was associated with the clinical outcome as a prognostic factor for metastatic recurrent breast cancer (mrBC) <sup>1-2</sup>. In this study, we investigated the immunological and clinical effect of PPV as the prophylactic cancer vaccine for non-recurrent but high-risk BC (nrhrBC) patients (pts), and we compared it's features to those of the mrBC pts who had active cancers or became resistant to the standard therapies(TR-mrBC). **Methods: Material and Patient eligibility criteria:** The peptides were selected from the 31 PPVs according to the results of HLA typing and peptide-specific IgG titers. Pts with a histological diagnosis of BC and their HLA-A molecules should be each of -A2, A3, A11, A24, A26, A31 or A33. The clinical protocols were approved by the institutional review board. (UMIN000003081and 00000184400000). **Treatment schedule:** A maximum of 4 peptides was administrated as weekly for initial four vaccinations and as biweekly for further inoculations. The concomitant standard endocrine therapy and the chemo-endocrine therapy were available for nrhrBC pts after finishing the standard adjuvant chemotherapy, and for mrBC pts concurrently. **Immune and clinical response assessment:** Specific T-cell responses, IgG titers and cytokines were evaluated using by interferon (IFN)- $\gamma$  ELISPOT, Luminex assay and ELISA system in every 6-8 vaccinations. Toxicity, clinical response and correlation with the immune responses were investigated. **Results:** 16 pts with nrhrBC, 41 pts with mrBC and 79 pts with TR-mrBC received median 18, 16 and 14 vaccines, respectively. After PPV therapies, peptide-specific IgG and CTLs increased significantly in a total of 47 (77%) and 37(60%) in nrhrBC pts, 102 (63%) and 98 (61%) in mrBC pts, and 150(53%) and 100 (42%) in TR-mrBC pts. Pts experienced Grade 1-3 skin reaction at injection site, no other grade 3 or 4 SAEs were associated with PPV but with the disease progression or combination therapy. The median time to progression (TTP) and overall survival (OS) were not reached in nrhrBC pts, 7.8 and 29 months in mrBC pts, and were 7.5 and 15.9 months in TR-mrBC pts, respectively. The peptide specific CTL response was correlated significantly with OS in nrhrBC pts and the IgG levels were associated with the better OS in either non TR-mrBC pts or TR-mrBC pts. High levels of IL-6, GM-CSF, IFN-g, IL-2 receptor, BAFF were associated with worse prognosis for pts with TR-mrBC. And high levels of GM-CSF and BAFF were associated with worse prognosis for pts with nrhrBC and mrBC, respectively. In contrast, High levels of IL-2 were associated with the better prognosis for pts with mrBC. **Conclusion:** This study indicated that immunological features of these three groups were different from each other with most potent PPV-induced immune boosting for nrhrBC pts. Pts with mrBC who had lower immune-suppressive cytokine levels had the better prognosis. These results suggested the PPV therapy could be effective for postoperative prophylactic vaccination in patients with nrhrBC. **References:** 1. Takahashi R, Toh U, et al. Breast Cancer Res. 2014; 2. Toh U, Okabe M, et al. THE BREAST 2015.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-06-07

Activity of nivolumab alone or in combination with targeted therapies in a humanized BLT-mouse model of human breast cancer

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**Background:** Recent advances in the field of cancer immunotherapy have increased demand for reliable preclinical models to inform patient selection and rational drug combination strategies. The development of the bone marrow-liver-thymus (BLT) mouse may provide the opportunity to study the complex interactions of human tumor and host immune systems *in vivo*. Other models are limited by the rapid onset of graft versus host disease (GVHD) and a lack of orderly maturation and trafficking of human T and B cells. In BLT mice, implantation of human fetal liver and thymus fragments beneath the kidney capsule of NSG (NOD/SCID/IL-2R<sub>γ</sub><sup>-/-</sup>) mice followed by engraftment of matched *ex vivo* expanded CD34<sup>+</sup> cells supports the production of an almost complete human immune system. In this study, we used this model to assess the efficacy of the anti-PD-1 therapeutic antibody, nivolumab, in combination with targeted therapeutics in specific breast cancer sub-types.

**Materials and Methods:** For triple negative breast cancer (TNBC), the activity of nivolumab was assessed in combination with the PARP1/2 inhibitor, talazoparib, in humanized BLT mice. Xenografts were established by subcutaneous injection of 5.0x10<sup>6</sup>MDA-231 (TNBC) cells. Mice (n=5) were randomized into treatment groups as follows; 1) Vehicle control (PBS), 2) nivolumab (10mg/kg QW), 3) talazoparib (0.33mg/kg Q5/2D) and 4) nivolumab+talazoparib. After 21 days of treatment, tumor tissue, serum and PBMCs were collected for biomarker analysis.

**Results:** Successful reconstitution of mature human T and B cells was confirmed in BLT mice 12-weeks post engraftment of donor tissue and CD34<sup>+</sup> hematopoietic stem cells. MDA-231 cells injected subcutaneously into the flank of these mice formed palpable tumors (150-200mm<sup>3</sup>) within 9 days of injection. For vehicle control treated mice, tumors grew (2.5-fold) throughout the 21-day study. Single agent nivolumab induced significant tumor growth inhibition (TGI) relative to vehicle control treated mice at Day 21. Single agent talazoparib also induced comparable levels of TGI as did the combination of nivolumab plus talazoparib. Nivolumab treated mice continued to gain weight throughout the study without overt signs of toxicity. Reversible weight loss was observed in the talazoparib and combination treated arms. Overt signs of GVHD were not observed in any of these animals. Preliminary tissue analysis identified high levels of cell surface PD-L1 protein in control treated MDA-231 xenografts. Further analysis of the treated tumors will provide valuable insight into the mechanism of action of this class of molecule. We are establishing xenograft models of hormone receptor (ER+) positive breast cancer to measure the activity of nivolumab+/-CDK4/6-inhibition in humanized BLT mice and these data will also be presented.

**Discussion:** The data presented here highlight the potential of the PD-1 antibody nivolumab to have activity in TNBC. Furthermore, these findings illustrate the potential of the humanized BLT-mouse to model responses to immune check-point in the preclinical setting. Expanded use of this model may help to identify response biomarkers and inform design of combination therapies using immune oncology molecules and approved targeted therapies.



# 2018 San Antonio Breast Cancer Symposium ®

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The spatial localization of immune cells predicts prognosis and response to therapy in inflammatory breast cancer

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## Introduction

The mechanisms contributing to the aggressive biology of inflammatory breast cancer (IBC) are still under investigation. Our lab reported a 79-gene signature that is shaped by specific immune response programs and discriminates between IBC and non-IBC (nIBC). Furthermore, the presence of cytotoxic CD8+ immune cells is associated with a better prognosis in proliferative subtypes of breast cancer. However, not only the presence of CD8+ cells, but also the interaction with other immune cells plays a role in the functional immune response. In this study we assessed the spatial associations between immune cells in IBC.

## Methodology

Affymetrix gene expression data of 105 IBC patients were analyzed using CIBERSORT and xCell modules to narrow down the number of stainings for the immunophenotyping. To analyze the composition of the immune infiltrate, we used five validated antibodies: CD79 $\alpha$  (B-cell lineage), CD8 (cytotoxic T-cells), FOXP3 (Tregs), CD163 (Tumor associated macrophages, TAMs) and the SP142 PDL1 antibody.

A standard H&E stained section was used to mark the tumor area on pretreatment biopsy sections. Subsequently, 5 slides were stained according to a validated protocol, scanned and evaluated using VISIOPHARM® software that makes virtual multiplexing possible after the alignment of the scanned images. Using both point pattern analysis and the Morisita–Horn index (MHI), developed for ecological studies, we assessed the co-localization of the different types of immune cells. Currently, we report the result of our validation cohort (30 patient samples).

## Results

Most of our IBC patients presented with a hormone receptor positive carcinoma (64.7%). Almost a quarter of the patients (23.3%) with initially localized disease achieved complete pathological response (pCR) after neo-adjuvant chemotherapy (NACT). For every staining we report the median relative marker area (RMA), MHI for colocalization with CD8 applying a square tessellation of 100  $\mu$ m and the number of cells in a radius of 30  $\mu$ m around CD8+ cells (direct cell-cell contact) in table 1.

	RMA	MHI	# X+ cells (30 $\mu$ m)
CD8	0.33 %		
CD163	0.12 %	0.721	3.13
CD79 $\alpha$	0.04 %	0.652	2.52
FOXP3	0.01 %	0.701	1.37
PDL1		0.746	0.70

**Table 1: Median spatial properties.**

The RMA of CD8 predicted pCR after NACT (RMA= 1.07% pCR vs 0.35% no pCR,  $P=0.04$ ), but was not prognostic for OS ( $P=0.445$ ). PDL1 positivity predicted neither pCR nor OS in this cohort. However, OS of patients with more PDL1+ cells ( $> 0.703$ ) in close contact with the CD8+ cells was significantly shorter (5y OS: 50% vs 68%,  $P=0.03$ ).

Interestingly, the colocalization of CD8+ cells with TAMs (MHI= 0.69 no pCR vs. 0.75 pCR,  $P=0.04$ ) and CD79 $\alpha$ + B-cells (MHI= 0.63 no pCR vs. 0.69 pCR,  $P=0.04$ ) was also associated with pCR after NACT, while the number of CD163+ or CD79 $\alpha$ + cells was not.

## Conclusion

In this study we described the dynamic interplay between cancer and immune cells. In a validation cohort of 30 patient samples

we showed that the colocalization of TAMs or B-cells with cytotoxic T cells was associated with pCR after NACT. Furthermore, patients with more PDL1+ cells around CD8+ cells ( $r = 30 \mu\text{m}$ ) had a worse prognosis while solely the number of PDL1+ or CD8+ cells was not prognostic. By December we will present data on 179 IBC patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-06-10

Immune related gene expression to explore immune escape in primary to metastatic breast cancer transition

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**Background:** Targeting programmed cell death receptor I pathway (anti-PD-1/PD-L1) demonstrated objective clinical response rates in a subgroup of patients with metastatic breast cancer with overall response rates of up to 20%. However, the vast majority of breast cancer patients is not responding to immune checkpoint blockade. With regard to the diverse tumor biology of breast cancer and recent data, immune evasion is likely mediated by various, coincident mechanisms. Consequently, our objective was to identify patterns of immune responses during the transition of primary to metastatic breast cancer.

**Methods:** We performed comprehensive immunohistochemistry on both tumor and immune cells in two independent, matched cohorts of 67 primary and metastatic breast cancer patients. Further, the analysis was integrated with mRNA expression levels (RT-qPCR) of key regulatory and effector function immune genes and clinical as well as histopathologic parameters.

**Results:** Both, immunohistochemistry and mRNA gene expression profiling of immune genes revealed immunological ignorance, defined as an immunological “cold” tumor by particularly low infiltration of CD8<sup>+</sup> T cells and corresponding immune gene and effector function, as a key feature of primary to metastatic breast cancer transition. However, CD8<sup>+</sup> T cell infiltration and effector function (Granzyme B, Interferon- $\gamma$ ) were found to be an independent prognostic marker for PFS and OS. Unsupervised hierarchical clustering and regression analysis identified distinct immune response types according to T-cell infiltration, interferon- $\gamma$  signalling (IFNG), immunosuppression (IDO, TGF- $\beta$ , FOXP3) and humoral immune responses (Immunoglobulin  $\kappa$  constant; IGKC). Expression of programmed death receptor 1 ligand (PD-L1), and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) were linked to CD8<sup>+</sup> T cells and associated with a better prognosis only in triple negative breast cancer patients. Importantly, induction of CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration as well as humoral immune responses (IGKC, CXCL13) in matched pairs during progression from primary to metastatic disease, were strongly associated with improved distant disease free and overall survival.

**Conclusion:** Our findings demonstrate a switch to a less immunoreactive environment in metastatic compared to matched, primary samples of breast cancer patients. Nevertheless, induction of T-cell mediated immunity during tumor evolution, observed across all biologic subtypes, warrants further research with the overall goal to convert immunologically ignorant breast cancers and to stratify future immunotherapies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-06-11

Cryoablation of murine mammary tumors induce robust immune response

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**Background:** Breast cancer is traditionally not considered as a highly immunogenic disease.

Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer with reported high genomic instability and high mutation rate, indicating the possible presence of neoantigens. Cryoablation, the destruction of cells by ultra-low temperatures, can release these neoantigens and induce a tumor specific immune response.

We hypothesized these neoantigens might be sufficient to trigger a robust immune response to prevent and/or reduce spread and relapse of TNBC.

In this pilot study we cryoablated orthotopical 4T1 tumors in immune competent Balb/c mice and compared the results to surgery to evaluate 1) possible induction of immune responses and 2) effects on metastases formation.

**Methods:** We used 4T1 mammary carcinoma cells to initiate tumor growth in the mammary fatpad. Tumors were treated by cryoablation, cryoablation followed by surgery (cryo-surgery), or surgery alone. Tumor growth was followed and allowed to reach 3-4mm in largest dimension. Animals were euthanized 7 days post-treatment and tissues were collected to assess cytokine levels and presence of dissociated 4T1 cells. Single-cell suspensions of tumor, tumor-draining lymph node [TDLN], and spleen were tested for secretion of mouse Th1/Th2 cytokines using a bead array and measured by flow cytometry. Possible metastatic spread was assessed by a clonogenic assay using cells from venous blood, lung, and brain. Cell suspensions were seeded in growth medium supplemented with the selection agent 6-thioguanine, allowing only resistant 4T1 cells to form colonies.

**Results:** Cryoablation transformed tumors into a gelatinous mass surrounded by a fibrotic capsule, as typically seen in the clinic. Frozen sections of tumors revealed a necrotic core and infiltrating lymphocytes in the microenvironment. These animals displayed robust increases of Th1 and Th2 cytokines in both spleen and TDLN compared to animals with cryo-surgery treatment. TDLN of animals with surgically excised tumors secreted only IL-2. Circulating tumor cells were found in animals prior to treatment, while no 4T1 colonies formed from cell suspensions of lung and brain tissue [N=8]. At end-point, the surgery alone group had more 4T1 foci formed from lung and brain [mean foci /animal = 6.25 and 0.75, respectively; N=6] than the other two groups. Two animals in this group progressed and were euthanized early due to numerous lung metastases. The cryoablated group had the lowest number of foci formed in the lung and brain [2.25 and 0 respectively; N=8], and all animals were healthy at the predetermined end-point. Mean foci formation in the cryo-surgery group [N=7] was in-between the two other groups and one animal was euthanized early due to metastatic burden 5 days after surgery.

**Conclusion:** Cryoablation of TNBC can induce stimulatory immune responses in vivo. These immune responses might explain why animals treated with cryoablation, though having circulating tumor cells at the time of treatment, exhibited fewer micro metastatic growths compared to surgery alone and the cryo-surgery combination. On-going experiments aim to identify long-term effects of cryoablation on the formation of metastatic foci and growth.

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Biological characteristics and prognostic value of Tfh-like cells in the tumor tissue of breast cancer

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**Objective** T follicular helper (Tfh) cells formed in germinal center are a distinct subpopulation of CD4<sup>+</sup> T cell, with high levels of CXCR5, ICOS, PD-1, Bcl-6 and CXCL13 as their characteristic markers. Secreting IL-21 and helping humoral response are their key features. They have been identified in different types of tumors. However, it still remains unclear in breast cancer. Researches of them may provide new clues to the diagnosis and treatment of breast cancer, especially for the potential immunotherapies. This study was performed to explore the existence and a possible role of Tfh cells in breast cancer. **Method** Freshly resected invasive breast cancer tissue from Fudan University Shanghai Cancer Center (FUSCC) were collected, with 27 samples for identifying the presence, phenotype and cytokine-producing capacity of Tfh-like cells and 386 for exploring the correlation between Tfh-like cells and clinicopathological characteristics in breast cancer during 03/2015 to 05/2017. Of all those 386 samples, patients were divided into Luminal A (N=61), Luminal B (HER2-) (N=138), Luminal B (HER2+) (N=72), HER2+ (non-luminal) (N=57) and triple negative (N=58) based on St Gallen International Expert Consensus. Data was analyzed based on the result from Multiparameter Flow Cytometry. The correlation between Tfh-like cells and clinicopathological characteristics in breast cancer was examined with t test or one way ANOVA with Tukey's multiple comparisons test. **Results** A subpopulation of PD1<sup>hi</sup>CD4<sup>+</sup> T cells in tumor tissues of breast cancer was identified as Tfh-like cells, with the specific expression of Bcl-6 and CXCL13. Phenotypes of those cells were checked by flow cytometry. They were of a high level of ICOS but negative for CXCR5, suggesting that they were atypical Tfh cells. They had high levels of activated molecules such as CD38, CD71, CD95 and HLADR, showing that they were highly activated. They had high levels of suppressive markers as Tim3, TIGIT, and LAG3, indicating they could have regulatory functions. In addition, Tfh-like cells specifically secreted cytokine IL-21 with stimulation. Data showed that high grade (N=166; mean±SEM, 17.62±0.87) compared with low grade (N=220; mean±SEM, 12.41±0.46) or high Ki-67 level (N=300; mean±SEM, 15.38±0.58) with low Ki-67 level (N=86; mean±SEM, 12.10±0.67), and negative ER expression (N=115; mean±SEM, 17.47±1.05) compared with positive ER expression (N=271; mean±SEM, 13.46±0.50) or negative PR expression (N=177; mean±SEM, 16.87±0.83) compared with positive PR expression (N=209; mean±SEM, 12.77±0.50) was associated with higher frequency of Tfh-like cells (P<0.01 to all). Patients with triple negative had higher frequency of Tfh-like cells than those with Luminal A (difference, 6.83; P=0.0006) and Luminal B (HER2-) (difference, 4.61; P=0.0124). Patients with HER2+ (non-luminal) had higher frequency of Tfh-like cells than those with Luminal A (difference, 5.34; P=0.0146). These results indicate that higher frequency of Tfh-like cells could have negative prognostic influence. **Conclusion** Our study defined a PD1<sup>hi</sup>Bcl6<sup>+</sup>CXCL13<sup>+</sup>CD4<sup>+</sup> T cell subpopulation, which specifically secretes cytokine IL-21 as Tfh-like cells. Higher frequency of Tfh-like cells is more likely to be seen in patients with poor prognosis.

# 2018 San Antonio Breast Cancer Symposium ®

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Programmed death-1 and programmed death-ligand 1 expression in sporadic breast cancer compared to *BRCA* germline mutation related breast cancer and male breast cancer

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**Introduction** Programmed death 1 (PD-1) and its ligand PD-L1 seem to have a prognostic and predictive role in a variety of cancer types, especially the ones with a high mutational load and immunogenic profile. Despite that breast cancer is not considered as immunogenic, recent studies suggested that PD-1 and PD-L1 do have a prognostic and/or predictive value in breast cancer, which varies between molecular subtypes. However, the role of PD-1 and PD-L1 in *BRCA* germline mutation related breast cancer and male breast cancer has not yet been examined. We therefore evaluated the expression of PD-1 and PD-L1 in sporadic, *BRCA* related and male breast cancer.

**Methods** Tissue microarrays were constructed of formalin-fixed paraffin-embedded resection material from 257 sporadic, 132 *BRCA* related and 175 male breast cancer patients and stained for PD-1 and PD-L1 by immunohistochemistry. Expression in *BRCA* related and male breast cancer was compared to the sporadic group and correlations with clinicopathological features were tested.

**Results** PD-1 and/or PD-L1 expression was available for 245 sporadic, 145 male and 103 *BRCA* related breast cancers. PD-1 expression in the *BRCA* group was significantly higher than in the sporadic group (79.0% vs 64.1%), this in contradictory to the male group, where PD-1 was significantly less often expressed than in the sporadic group (46.9% vs. 64.1%). For PD-L1 expression no significant differences were seen between the groups. In subgroup analysis PD-1 expression was higher in grade 3 tumors compared to the lower grade tumors, which was significant in sporadic and male breast cancer. For PD-L1 a significantly higher expression was observed in grade 3 tumors in *BRCA* related breast cancer, while a significantly lower expression in grade 3 tumors was seen in the sporadic subgroup. No differences were observed looking at PD-(L)1 expression and molecular subtype.

Subgroup analysis for PD-1 and PD-L1 expression and tumor grade

	Grade	PD-1 negative, No. (%)	PD-1 positive, No. (%)	P-value	PD-L1 negative, No. (%)	PD-L1 positive, No. (%)	P-value
<b>Sporadic</b>	1	16 (39.0)	25 (61.0)		20 (52.6)	18 (47.4)	
	2	37 (43.5)	48 (56.5)	<b>0.037</b>	40 (50.0)	40 (50.0)	<b>0.036</b>
	3	30 (26.5)	83 (73.5)		68 (68.0)	32 (32.0)	
<b>BRCA</b>	1	-	1 (100)		-	1 (100)	
	2	8 (34.8)	15 (65.2)	0.186	20 (87.0)	3 (13.0)	<b>0.011</b>
	3	13 (17.3)	62 (82.7)		46 (61.3)	29 (38.7)	
<b>Male</b>	1	20 (64.5)	11 (35.5)		20 (64.5)	11 (35.5)	
	2	28 (58.3)	20 (41.7)	<b>0.037</b>	33 (66.0)	17 (34.0)	0.213
	3	24 (39.3)	37 (60.7)		31 (50.8)	30 (49.2)	

**Conclusion** PD-1 expression is higher in *BRCA* related breast cancer compared to sporadic breast cancer, but lower in male breast cancer. Additionally, PD-1 expression is more frequent in grade 3 tumors. Patients with *BRCA* related breast cancer might therefore respond better to PD-1 inhibitors than patients with sporadic breast cancer, this in contrast to men with breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Single-cell RNA sequencing to delineate changes in tumor microenvironment induced by immunotherapy

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**Introduction:** Improved understanding of the interplay between the immune system and cancer would lead to more adequate response prediction to immune-checkpoint blockade (ICB) treatment. Furthermore, insight into immune interactions would allow identifying biomarkers for response to ICB. Single-cell RNA sequencing has emerged as a powerful technology to characterize heterogeneity in a large population of cells and opens up opportunities to predict response to therapy.

**Purpose:** Tracking the effect of a single dose of Pembrolizumab on the tumor microenvironment through single-cell RNA sequencing.

**Methods:** Single-cell RNA sequencing was performed on tumor tissue of one patient before and 10 days after a 200 mg dose of Pembrolizumab (Keytruda®). Changes in cell (sub)populations were analyzed. Fresh tumor material was obtained from a core needle biopsy at diagnosis and from the resection specimen. Single cell suspensions were converted to barcoded scRNA-seq libraries with the Chromium Single Cell 3' kit with 10X Genomics platform, aiming for an estimated 5,000 cells per library. The libraries were sequenced using HiSeq400. Expression matrices were generated using CellRanger and analyzed by Seurat package. Dimensionality reduction using principle component analysis was applied to identify major cell types and their subtypes.

**Results:** The analyzed tumor was a grade 3 invasive ductal adenocarcinoma, hormone receptor negative and HER2+ positive, pT2N0M0. Tumor infiltrating lymphocyte (TIL) count was 30% on core biopsy and 50% on resection specimen. We sequenced a total of 9867 transcriptomes at single cell resolution before and after Pembrolizumab treatment, consisting of 5808 and 4049 cells, respectively. Major cell types of the tumor microenvironment were identified by leveraging single-cell transcriptomics analysis. We observed a sharp decrease in cancer cells after treatment (74% versus 25%), which was accompanied by an increase of tumor infiltrating T cells (18% versus 50%). The residual cancer cells after immunotherapy showed a higher expression of the major histocompatibility complex (MHC), MHC-II in particular. In addition, we found enrichment of B-cells and endothelial cells and a downregulation of fibroblasts and myeloid cells. Furthermore, we analyzed subtypes of each cell type. We found a marked increase in cytotoxic CD4 (6% versus 18%) and cytotoxic CD8 cells (4% versus 12%) in the T cell population. These cytotoxic CD8 cells clearly expressed higher PD-1 after immunotherapy. A decrease in CD4 Tregs, naïve CD4 and intermediate CD8 cells was observed and the B cell enrichment after treatment was mainly driven by the increase of follicular B-cells. The increase of endothelial cells was driven by capillary tumor endothelial but not tip cell population, suggesting vessel normalization rather than neo-vascularization.

**Conclusion:** Single-cell RNA sequencing provides a powerful tool in detecting changes in the tumor microenvironment induced by immunotherapy, and thus offers new opportunities to predict response to immunotherapy. Thirty-three additional patient samples will be analyzed in the near future, with a special focus on T- and B- cell receptor repertoire.

## 2018 San Antonio Breast Cancer Symposium ®

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Role of B lymphocytes and B cell-produced IL-27 in breast cancer progression and drug resistance

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How host immune elements, particularly B cells, are hijacked to promote breast cancer (BCa) tumor progression and acquisition of resistance to current endocrine therapies remain largely unclear. Prompted by our recent findings that B cells are a major source of cytokine IL-27 (the heterodimer of IL-27p28 and EBI3), which regulates many immune functions as both a pro-inflammatory and anti-inflammatory cytokine, as well as the high expression of IL-27 receptor genes in the breast, elevated circulating IL-27 in BCa patients and the association of increased *IL-27p28* expression with reduced survival in BCa patients overall and ER<sup>+</sup> BCa patients after endocrine therapies, here we hypothesize that B cells promote BCa tumor progression and drug resistance and do so by producing IL-27. In C57 mice transplanted with ERa<sup>+</sup> E0771 syngeneic medullary breast adenocarcinoma cells, deficiency in B cells in mMT mice resulted in virtually abolished tumor growth, resulting from significantly high tumor cell death. Remarkably, in residual tumors developed in mMT mice after transplantation with a higher load of E0771 cells, B cells, which were absent in the blood and lymph nodes, were accumulated within tumors – at an even higher frequency than that in wildtype C57 mice – and expressed IL-27. Further, like class-switching B cells that respond to IL-27 produced by neighboring B cells to express an IFN $\gamma$ -induced gene signature in the secondary lymphoid organ during the antibody response, a distinct population of tumor-infiltrating B cells expressed high levels of PD-L1, which could be induced by IL-27 in many immune cell types, including B cells, and several cancer cell types. Finally, IL-27 promoted the growth MCF-7 human BCa cells in the presence of tamoxifen *in vitro*, consistent with a role of B cell-produced IL-27 in the acquisition of drug resistance by BCa. These, together with the delayed tumor growth in mixed bone marrow chimeric mice with B cell-specific deficiency in IL-27 production, outline a potential role of B cell-produced IL-27 in BCa progression and drug resistance through both BCa cell-intrinsic mechanisms and induction of PD-L1<sup>+</sup> B regulatory cells that participate in the immune checkpoint in BCa.



# 2018 San Antonio Breast Cancer Symposium ®

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Analysis of tumor infiltrating lymphocytes in three age categories of luminal B-like breast cancer patients and its correlation with lymph node involvement and systemic immunosenescence/frailty markers

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**BACKGROUND** Immunosenescence, the age-related decrease in immune competence, which is characterized by decreased adaptive immunity and increased low-grade inflammation. It may lead to altered tumor immunity. However, immunosenescence markers have not been correlated yet with tumor infiltrating leukocytes (TILs) nor with clinical frailty.

**METHODS** This is the first study that investigates how age affects the relation between systemic immunity and tumor microenvironment in early luminal B-like breast cancer (BC) in function of age. Luminal B-like BC was defined as grade II-III, ER+, HER2- on core biopsies at inclusion. Three distinct age categories of BC patients were chosen: 35-45 years (y) (N=15), 55-65y (N=19), ≥70y (N=31). Stromal TILs (sTILs) % were assessed according to published guidelines, on representative tumor sections. Further characterization of the TILs by immunohistochemistry, using the antibodies against CD3, CD4, CD8, CD5, CD20, CD68 and FOXP3, is currently ongoing. Immunosenescence was evaluated by looking at eleven inflammatory plasma cytokines and chemokines, circulating insulin-like growth factor-1 (IGF-1), T-cell *p16<sup>INK4a</sup>* expression, PBMC subset profiles and expression levels of twenty immune-related microRNAs. In the old age category, geriatric assessment was performed.

**RESULTS** With increasing age, sTILs % significantly decreased, concomitant with significantly increased plasma levels of several inflammatory cytokines (IL-1 $\alpha$ , IL-6) and chemokines (IP-10, IL-8, MCP-1), intermediate monocytes, as well as T-cell *p16<sup>INK4a</sup>* expression. Significant age-related decrease was seen for plasma IGF-1, naïve CD8+ T-cells and CD8+ T-cells expressing CD27 and/or CD28. Four immune-related microRNAs showed significantly different expression levels between the age groups: miR-18a decreased with age, miR-155 increased with age, miR-19b and miR-20a peaked in the middle group. As expected, various correlations exist between the different blood immunosenescence markers. The % of sTILs showed weak negative correlations with IL-6, IL-8, IL-1 $\alpha$ , MCP-1 and the % of regulatory T-cells. Additionally the % of sTILs and several markers of immunosenescence (MCP-1, miR-20a, miR-155, intermediate monocytes) correlated with components of the geriatric assessment (activities of daily living (ADL), mini nutritional assessment (MNA), mini-mental state examination (MMSE)) and with the Leuven oncogeriatric frailty score (LOFS) in the oldest group. Conversely, lymph node involvement was not associated with the % of sTILs nor with any blood aging biomarker.

**CONCLUSION** sTILs % and several blood immunosenescence markers significantly differ between young and older luminal B-like BC patients. Some of these markers correlated with markers of clinical frailty as well. These findings suggest that interactions between tumor cells and immune/inflammatory cells differ with age and therefore applicable immune biomarkers and approaches for immunotherapy may vary depending on patients' age.

# 2018 San Antonio Breast Cancer Symposium ®

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Investigating trastuzumab-induced myeloid cell alterations for improving combination therapy in HER2+ breast cancer

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**Introduction:** The purpose of this study is to identify temporal changes in the identity and cellularity of myeloid cells infiltrating human epidermal growth factor receptor type 2 positive (HER2+) breast cancer following trastuzumab therapy. Trastuzumab is a targeted therapy used in combination with cytotoxic therapies to treat HER2+ breast cancer. Previous data shows that in HER2+ tumors, trastuzumab increases vascular maturation and decreases hypoxia, both of which can sensitize tumors to cytotoxic therapies. Preliminary immunofluorescent data reveals a significant increase in the amount of myeloid cell infiltrates in trastuzumab treated tumors during the same temporal window as vascular and hypoxia alterations. The balance of myeloid cells in the tumor microenvironment (TME) can impact neovascularization, hypoxia, and tumor progression. Thus, we hypothesize that quantifying immune modulation following trastuzumab treatment will identify mechanistic properties of trastuzumab induced vascular alterations in the TME. We present results identifying an increase in various myeloid infiltrates between control and trastuzumab treated tumors in HER2+ breast cancer.

**Experimental Design:** BT474 HER2+ breast cancer cells were implanted subcutaneously into athymic nude mice. After reaching 250 mm<sup>3</sup>, tumors were treated with trastuzumab (10 mg/kg) or saline for one week and were excised for analysis on days 0, 4, and 7. Each tumor was cut at the largest cross-section; half was fixed in 10% neutral buffered formalin for immunohistochemistry staining of TME markers (pimonidazole, CD11c, F4/80, CD31, and alpha-SMA), and half was digested for single cell analysis using flow cytometry. Data was analyzed with FlowJo software. Distinct populations of myeloid cells have been quantified in day 4 control (n = 6) and trastuzumab treated (n = 4) tumors. A non-parametric Wilcoxon rank sum test was used to determine statistical differences.

**Results and Discussion:** Flow analysis revealed a significant ( $p < 0.05$ ) increase of macrophages in tumors treated with trastuzumab for 4 days compared to control tumors. Spatial immunofluorescent data confirms these findings showing a significant increase in macrophage populations, indicated with increased CD11c+ and F4/80+ co-staining in day 4 treated tumors compared to control ( $p < 0.01$ ). Additionally, flow cytometry results show a significant ( $p < 0.05$ ) increase in neutrophils in day 4 trastuzumab treated tumors compared to control tumors. Ongoing studies are evaluating spatial variations in myeloid infiltration (CD11c, F4/80), hypoxia (pimonidazole), and vascular maturation index (ratio of alpha-smooth muscle actin to total CD31 stained vessel count) through immunohistochemistry as well as comparing myeloid infiltration in trastuzumab-treated and control mice on day 7.

**Conclusion:** Quantifying trastuzumab induced immune-modulation will provide mechanistic insight into how trastuzumab alters the TME and improves tumor sensitivity to treatment. This information will be of great importance to identifying optimal windows to administer combination therapies as well as in the development of novel immunotherapies in breast cancer.

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The effect of relieving adenosine-mediated immunosuppression on trastuzumab-mediated antibody-dependent cell-mediated cytotoxicity (T-ADCC) against HER2+ breast cancer cell lines

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**Introduction:** Trastuzumab (T) is a monoclonal antibody therapy used in the treatment of HER2+ breast cancer. T inhibits HER2 intracellular signalling and is capable of engaging the immune system through ADCC. Adenosine is an important negative regulator of the immune response through its interaction with the A2A receptor (A2AR, ADORA2A). Relieving adenosine-mediated immunosuppression by inhibiting A2AR may improve NK cell-mediated T-ADCC against HER2+ breast cancer cells. In addition, we have previously shown that SKBR3 cells resistant to the EGFR/HER2 tyrosine kinase inhibitor (TKI) lapatinib are less sensitive to T-ADCC and showed increased A2AR protein levels. This study examines the effects of inhibiting A2AR signalling on NK cell-mediated T-ADCC against treatment naïve HER2+ breast cancer cell lines HCC1954 and SKBR3 and lapatinib and afatinib (irreversible pan-HER-family TKI)-resistant sublines of HCC1954 and SKBR3.

**Methods:** HER2+ breast cancer cell lines SKBR3 and HCC1954 were exposed to afatinib (150nM) or lapatinib (1µM) for 6 months to generate TKI-resistant SKBR3-A and HCC1954-L cell lines. Acid-phosphatase-based proliferation assays were used to confirm resistance to TKI treatment. Western blotting was used to examine A2AR and HER2 protein levels in cell lines. NK cells were isolated from healthy volunteer whole blood by MACSxpress isolation kits. Immune cell-mediated cytotoxicity was determined at a 1:1 (NK cell: TC) ratio over 12 hours using a flow cytometry-based method. Direct cytotoxicity and T-ADCC were determined +/- A2AR agonist CGS21680 (1 µM) and/or A2AR antagonist preladenant (100 nM) for all cell lines. Experiments were carried out three times with three separate volunteer samples with representative results presented.

**Results:** HCC1954-L cells were 5.3-fold resistance to lapatinib (IC<sub>50</sub> 1.65 µM +/- 0.22 µM) vs. HCC1954 (IC<sub>50</sub> 0.31 µM +/- 0.15 µM). SKBR3-A cells were 33-fold resistant to afatinib (IC<sub>50</sub> 0.28 µM +/- 0.006 nM) vs. the parental SKBR3 cell line (IC<sub>50</sub> 0.009 µM +/- 0.006 µM). SKBR3 and HCC1954 expressed detectable protein levels of A2AR. A2AR and HER2 levels were not significantly changed between parental and resistant cell lines. Levels of direct cytotoxicity and T-ADCC elicited by NK cells were higher against SKBR3-A (p=0.002) and HCC1954-L cells (p=0.0004) than parental cell lines. The A2AR agonist CGS21680 alone had inconsistent effects on direct cytotoxicity and T-ADCC in all cell lines tested. The addition of A2AR antagonist preladenant to CGS21680, but not preladenant alone, increased T-ADCC against the parental HCC1954 cells by 12.7 +/- 3.4% and parental SKBR3 cells by 9.5 +/- 3.6%. T-ADCC levels in the targeted therapy-resistant HCC1954-L and SKBR3-A cell lines were not impacted by the CGS21680/preladenant combination.

**Conclusions:** A HER2-targeted therapy resistance phenotype is associated with increased T-ADCC in the models tested. Inhibition of activated A2AR can increase T-ADCC elicited by NK cells against treatment naïve HER2+ breast cancer cell lines but not TKI-resistant sublines. Further work is warranted to examine the impact of targeting A2AR in HER2+ breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

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Delivering tumour antigens survivin and mucin-1 on virus-like particles for breast cancer immunotherapy

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Breast cancer is the most frequently diagnosed cancer in women worldwide. Although there are a variety of treatment options available, breast cancer is a difficult disease to treat and many patients experience recurrence following treatment. We have previously shown that tumour antigens delivered on virus-like particles (VLP) induce a targeted anti-cancer immune response. In this study we investigated whether combining the two tumour antigens survivin and mucin 1 as vaccine targets can induce a superior anti-cancer immune response for breast cancer immunotherapy. VLP were designed to recombinantly express the murine survivin epitope. Following expression of Survivin-VLP, aberrantly glycosylated mucin 1 (MUC1) peptide was conjugated onto the Survivin-VLP using intracellular cleavable bis-arylhydrazine linking strategy. Western Blot analysis and electron microscopy confirmed Survivin-VLP expression and UV absorption confirmed conjugation of the MUC1 peptide to the Survivin-VLP. C57mg.MUC1 breast cancer cells were injected into the mammary fat pad of C57Bl/6 and MUC1 transgenic mice. Once tumours were palpable, mice were vaccinated with the Survivin-MUC1-VLP and controls of DPBS, VLP without antigen or VLP delivering either survivin or MUC1 antigen. Tumour growth and mouse survival were monitored for 80 days. Mice vaccinated with Survivin-MUC1-VLP delivering both antigens showed enhanced survival (D78) compared to mice vaccinated with VLP delivering only one of the antigens (D66) and controls (D53). Delivery of two tumour antigens induced an enhanced anti-tumour immune response compared to delivery of single tumour antigens. The induction of multiple immune responses against different tumour antigens may apply additional evolutionary pressure upon tumours, prolonging the ability for tumours to develop resistance, or escape through the proliferation of a resistant subpopulation. The use of VLP for the delivery of multiple antigens and adjuvants represents a promising approach to improve cancer immunotherapy for breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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MEK inhibitor cobimetinib induces immunogenic cell death and immune-modulatory effects in triple negative breast cancer

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## Background

Triple-negative breast cancer (TNBC) has been associated with a robust tumor immune infiltrate. Tumor-infiltrating lymphocytes (TILs) in TNBC have been demonstrated a prognostic value. The mitogen-activated protein kinase (MAPK) signaling pathway have been shown to regulate the immune response with the production of immunomodulatory cytokines, such as TNF $\alpha$ , interleukin (IL)-1, IL-10, and IL-12. Clinical studies have shown that the high expression level of Extracellular signal-related kinase (ERK), a member of the MAPK pathway, correlates with shorter survival in TNBC patients. Accordingly, ERK is a potential target for anti-tumor and cancer immunotherapy. In this study, we aimed to investigate a MEK inhibitor cobimetinib and elucidate whether the MEK/ERK pathway is implicated in immunogenic cell death (ICD) and/or other immunomodulatory effects.

## Methods

Mouse TNBC cell line 4T1 was treated with cobimetinib. The cell viability and cell apoptosis were determined by MTT assay and flow cytometric analysis. Cobimetinib-induced damage-associated molecular patterns (DAMPs), such as cell-surface translocation of calreticulin (CRT), extracellular release of ATP, and increase in high-mobility group box protein B1 (HMGB1) release from dying tumor cells, were examined by immunoblotting and flow cytometric analysis. Further, the molecular mechanisms involving cobimetinib-induced cell death were examined by Immunoblotting. Last but not least, establishment of 4T1 animal model in immunocompetent and immunodeficient mice was conducted to investigate the efficacy of cobimetinib in restoration of immunosurveillance and cancer metastasis *in vivo*.

## Results

The results showed that cobimetinib impaired cell proliferation and induced cell apoptosis in a dose-dependent manner in 4T1 cells. Importantly, cobimetinib treated 4T1 cells elicited ICD, by increasing HMGB1 and ATP release, and by promoting membrane exposure of CRT. The molecular mechanisms involved in cobimetinib-induced DAMPs were partially through the downregulation of p-ERK expression. Moreover, cobimetinib induced processing of procaspase-3 and -8 in 4T1 cells, thereby resulting in caspase activation. In addition, 4T1 tumor bearing immunocompetent mice treated with cobimetinib showed reduced size of primary tumors, fewer lung metastases and increased survival. In addition, we noticed that the tumor-suppressive effects of cobimetinib were much stronger in immunocompetent mice than in immunodeficient mice, evident by distinct mice survival in drug-treated mice. Flow cytometric analysis of murine splenocytes also revealed that cobimetinib treatment increased total number of CD8<sup>+</sup> T cells, dendritic cell maturation, and suppressed the number of Myeloid-derived suppressor cells (MDSCs) in immunocompetent mice.

## Conclusions

Our findings suggested that the MEK/ERK inhibitor cobimetinib induces ICD *in vitro* and exerts additional immune-modulatory effects in immunocompetent TNBC mice model. Our study highlights the possibility for the use of cobimetinib as an ICD inducer and immunomodulator for therapeutic intervention in TNBC.

## Keywords

Cobimetinib, immunogenic cell death, MAPK, ERK phosphorylation, immune regulation

# 2018 San Antonio Breast Cancer Symposium ®

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Transcription factor T-bet and PD-L1 expression in tumor microenvironment of triple-negative breast cancer

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**Background:** Many analyzes regarding immunotherapies using checkpoint blockade has made it clear that tumor infiltrating lymphocytes (TILs) plays an important role in treating cancers with high levels of somatic mutations such as triple-negative breast cancer (TNBC). We reported the relationship between TILs and PD-L1 expression, and revealed that high-TILs/positive-PD-L1 expression population in TNBC was associated with better prognosis (*Oncotarget* 2017). However, its molecular mechanism is still unclear. Meanwhile, T-box transcription factor 21 (T-bet) which regulates effector T-cells activation is derived by stimulation of T-cell receptor and IL-12. Activated T-cells work as antitumor lymphocytes by enhancing the production of cytokines such as INF $\gamma$ . We focused on T-bet and examined the function of activated T-cells.

**Patients and Methods:** This study included 242 patients with primary TNBC who underwent resection without neoadjuvant chemotherapy at our three hospitals between January 2004 and December 2014. The immunohistochemistry scoring for CD8 and T-bet expression on TILs was defined as  $\geq 30$  per 0.00625mm<sup>2</sup>. PD-L1 positivity was defined as  $\geq 1\%$  of tumor cells staining positive for PD-L1.

**Results:** Of the 242 TNBC, CD8 on TILs was expressed as positive in 127 (52.5%) tumors, T-bet on TILs was expressed as positive in 67 (27.7%) tumors, and PD-L1 expression on tumor cells was expressed as positive in 99 (40.9%) tumors. T-bet expression was significantly correlated with CD8 expression ( $P < 0.0001$ ) and PD-L1 expression ( $P = 0.0004$ ). There was no significant difference in recurrence free survival (RFS) and overall survival (OS) regardless of CD8 or PD-L1 expression level. Meanwhile, the patients with T-bet-positive tumors had a longer OS, compared to those with T-bet-negative tumors ( $P = 0.13$  in RFS and  $P = 0.047$  in OS). The multivariate analysis revealed that T-bet expression on TILs was an independent and positive prognostic factor for OS (HR = 0.5, 95%CI 0.1-0.9,  $P = 0.035$ ).

**Conclusion:** OS was significantly longer among patients with high T-bet expressing TNBC. These results may validate the significance of T-bet as a biomarker for various immunotherapies in TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

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Feasibility of syngeneic mice models of breast cancer for research of immune checkpoint blockades

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**Background:** With the increasing success of immune checkpoint blockades for cancer treatment, we increasingly need well-characterized preclinical models. Syngeneic mice models (with a fully competent immune system) have advantages that they are easily established and cost less, though they do not reflect genetic complexity of human tumors. We evaluated feasibility of syngeneic mice models of breast cancer by analyzing efficacy of immune checkpoint blockade and dynamic change of tumor immune microenvironment.

**Methods:** We used syngeneic mice model of JC, 4T1, and EMT6 cells, which are all murine triple negative breast cancer in BALB/c mice. At the time when subcutaneous tumors reach at 50~100mm<sup>3</sup>, each mice models were divided into 2 groups for treatment versus no-treatment control. In the treatment group, mice version of anti-PD-1 antibody was intraperitoneally injected (q 3 days, x 6). Anti-tumor efficacy was monitored by measuring tumor volume. 'Tumor response' was defined as a case with tumor volume less than that of control group by a standard error at a determined time point. Immune microenvironment was evaluated by measuring serum cytokines (IL-2, IL-6, IL-10, IFN $\gamma$ , and TNF $\alpha$ ) with legendplex and immune cells (CD3, CD4, CD8, CD56, and FOXP3) of peripheral blood with FACS before injection of PD-1 blockade, after 1<sup>st</sup> injection, and when euthanized.

Tumor-infiltrating immune cells were evaluated with FACS, when euthanized.

**Results:** The tumor response rate to PD-1 blockade was highest in the 4T1 model (54.5%, 6/11) compared to JC model (40%, 4/10) or EMT6 model (36.4%, 4/11). Bleeding 3 times and tumor obtainment when euthanized in each mouse were feasible for profiling of cytokines and immune cells. Although before treatment with PD-1 blockade, CD3+T cells in peripheral blood were slightly lower in 4T1 model (18.3 $\pm$ 8.1%) than JC model (24.6 $\pm$ 4.7%) or EMT6 model (27.9 $\pm$ 6.3%), after injection of one dose of PD-1 blockade, CD3+T cells increased 1.5 times in 4T1 model (18.3% to 27.3%), whereas those CD3+T cells decreased slightly in JC model and EMT6 model. Dynamic changes were not observed in other subsets of peripheral immune cells in all 3 models. Serum TNF $\alpha$  (with statistical significance) and IFN $\gamma$  (with borderline significance) were higher in responders than in non-responders or no-treatment control.

**Conclusions:** Syngeneic mice models of breast cancer were feasible to investigate immune checkpoint blockades and monitor dynamic change of immune microenvironment. In this regard, such models may be used to evaluate immune checkpoint blockade-based combination therapy as well.

# 2018 San Antonio Breast Cancer Symposium ®

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Microenvironment heterogeneity of triple-negative breast cancer reveals distinct immune escape mechanisms and potential driver events

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**Background** The microenvironment phenotypes strongly affect the immunotherapeutic strategies for triple-negative breast cancer (TNBC). Although the multi-omics profile of TNBC has been comprehensively characterized, few studies have focused on the microenvironment phenotypes of TNBC.

**Methods** With multi-omics data for the largest single-center TNBC cohort (n=386), we first established a TNBC-specific microenvironment cell signature. We further used single sample gene set enrichment analysis to calculate the relative number of microenvironment cell subsets in each sample. Then, we performed k-means clustering to classify the TNBC microenvironment phenotypes into heterogeneous clusters. Furthermore, we systematically analyzed the extrinsic and intrinsic immune escape mechanisms of different TNBC microenvironment clusters. In addition, we explored genomic alterations that might decrease immune infiltration in certain TNBC microenvironment clusters.

**Results** We classified the TNBC microenvironment phenotypes into three heterogeneous clusters. Cluster 1 (type 1 “cold tumor”) had low microenvironment cells infiltration. Cluster 2 (type 2 “cold tumor”) was characterized by resting innate immune cells, fibroblasts and endothelial cells infiltration. Cluster 3 (“hot tumor”) was featured by adaptive immune cells infiltration. Analysis of immune escape mechanism revealed that an incapability to attract innate immune cells (resulting in failure of adaptive immunity) led to immune escape of cluster 1. The chemotaxis but inactivation of innate immunity (also leading to failure of adaptive immunity) and low tumor antigen burden resulted in immune escape of cluster 2. High expression of immune checkpoint molecules contributed to immune escape of cluster 3. In addition, we found that tumor infiltrating lymphocytes (TILs) were positively correlated with immune checkpoint molecules expression, while mutation load was negatively correlated with those indicators in triple-negative breast cancer. Analysis of enrichment pathways, mutations and somatic copy number variations between the “cold tumor” and “hot tumor” clusters revealed that amplification of MYC and activation of MYC-related pathways might decrease the immune infiltration of cluster 1. Mutations in PI3K-AKT pathway members and activation of fibroblasts-related pathways might decrease the immune infiltration of cluster 2.

**Conclusion** Utilizing the largest single-center TNBC cohort with multi-omics data, our study first revealed the heterogeneity of the TNBC microenvironment, with translational significance both clinically and biologically. First, we identified a subtype of “hot tumor” in TNBC (cluster 3), for which immune checkpoint blockers (ICBs) might be effective. TILs and immune checkpoint molecules expression but not mutation load might predict the efficacy of ICBs. Second, we presumed some genomic alterations that might drive “cold tumor” formation in TNBC. Our study represents a step toward personalized immunotherapy for TNBC patients.

**Key Words** triple-negative breast cancer, multi-omics, microenvironment heterogeneity, immune escape



# 2018 San Antonio Breast Cancer Symposium ®

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B7-H3 and B7-H4 expression in ductal carcinoma in situ of the breast: Associations with clinicopathologic features and T-cell infiltration

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**Background:** With the clinical success of immune check point blockades in treating malignant tumors, there are intense investigations in identifying new pathways to activate immune system by targeting immunoregulatory molecules. B7-H3 and B7-H4 play an inhibitory role in T cell function by limiting proliferation and cytokine production. Although several studies have investigated the expression of B7-H3 and B7-H4 in invasive breast cancers, the information of the B7-H3 and B7-H4 molecules in ductal carcinoma in situ (DCIS) remains uncertain.

The present work was undertaken to evaluate the expression of B7-H3 and B7-H4 in DCIS and its association with clinicopathological features in patients with DCIS. In addition, the association of B7-H3 and B7-H4 expression with the T-cell infiltration was also assessed to investigate its roles in the regulation of tumor immune surveillance.

**Materials and methods:** B7-H3 and B7-H4 expression was examined in 8 pairs of DCIS tissues and matched normal adjacent tissues at mRNA and protein levels by RNAscope in situ hybridization (ISH) and immunohistochemistry. Immunohistochemical staining of B7-H3 and B7-H4 was done in 79 DCIS samples with known hormone receptor (HR) and human epidermal growth factor 2 (HER2) expression using tissue microarray. In addition, immunohistochemical staining was also performed for the T cell lineage markers CD3 and CD8 in DCIS.

**Results:** RNAscope ISH and immunohistochemistry of B7-H3 and B7-H4 confirmed their increases in DCIS tissues compared with their corresponding normal tissues. B7-H3 and B7-H4 mRNA and protein expression appeared to be concentrated mainly in the DCIS carcinoma cells. High B7-H3 and B7-H4 expression was observed in 58 (73.4%) and 62 (78.5%) cases with DCIS, respectively. High B7-H3 expression was significantly associated with high nuclear grade and presence of comedo-type necrosis ( $P < 0.05$  and  $P < 0.05$ , respectively). B7-H3 expression was higher in the HR-/HER2+ subtypes than in the HR+/HER2- subtype ( $P < 0.05$ ). B7-H3 and B7-H4 expression tended to be associated with low stromal tumor infiltrating lymphocytes density and was negatively related to the CD3+ and CD8+ T cell infiltrates density.

**Conclusions:** These results suggest that B7-H3 and B7-H4 may play an important role in immune surveillance mechanisms of DCIS and may be useful targets for immune-based therapy to alter or prevent DCIS progression.

# 2018 San Antonio Breast Cancer Symposium ®

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High expressions of CXCR4 and CCR5 are associated with poor prognosis in triple negative breast cancer resistant to neoadjuvant chemotherapy

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**Background:** Novel systemic therapies are under investigation for patients with triple negative breast cancer who are resistant to neoadjuvant chemotherapy or having a partial response to neoadjuvant chemotherapy. Immune check point inhibition or blocking other immunologic markers such as chemokine receptors might be alternative pathways for these chemotherapy-resistant patients. Therefore, we investigated various immune check point receptor expressions along with different chemokine receptors.

**Method:**

Expressions of immunological markers were examined immunohistochemically by staining archival tissue of mastectomy specimen (n=56) using specific monoclonal antibodies for PDL-1 (Ventana SP263 clone kit), CXCR4, CXCR5, CCR5, CCR7, CD73, and CD155. PDL-1 positivity was defined as membranous staining  $\geq 1\%$  in either tumor and /or stromal lymphocytes, whereas positivity of chemokine receptors and CD155 and CD73 were considered as cytoplasmic staining  $\geq 50\%$  and  $\geq 5\%$ , and  $\geq 1\%$ , respectively.

**Results:** Median age was 47 (24-76) years. Of those, 31 were clinically T3-4 (55%), whereas almost all of them were N1-3 (96.4%) before neoadjuvant chemotherapy. All patients received anthracyclines&paclitaxel containing regimens, whereas 3 patients received additional carboplatin before definitive surgery.

Of those, 30 patients (58%) were detected to be positive for PDL1 in tumor site and intratumoral lymphocytes, whereas CXCR5 (41.1%, 23/56) , CCR5 (48.2%, 27/56), CCR7 (41.1%, 23/56), CD155 (30/56, 58%) and CD73 (44.6%, 25/56) were found to be highly expressed in tumors. However, 12.5% of patients have shown high expression of CXCR4. Patients with CXCR5, CCR5, CCR7, CD73, CD155 positivity were more likely found to give a worse chemotherapy response as measured by “Residual Cancer Burden Index”.

Table 1. Associations between MD Anderson Residual Cancer Burden Index and Immunological Biomarker Expressions

Tumor characteristics	Class I	Class 2&3	P-value
CXCR4-negative	8.3%	91.7%	0.999
CXCR4-positive	0%	100%	
CXCR5-negative	12.5%	87.5%	0.131
CXCR5-positive	0%	100%	
CCR5-negative	14.3%	85.7%	0.111
CCR5-positive	0%	100%	
CCR7-negative	12.5%	87.5%	0.131
CCR7-positive	0%	100%	
CD73-negative	13.3%	86.7	0.117
CD73-positive	0%	100%	
CD155-negative	16%	84%	0.037
CD155-positive	0%	100%	
PDL1-negative	4.8	95.2	0.999
PDL1-positive	3.3	96.7%	

Median follow-up time was 36 months (7-177). Five-year disease-free survival (DFS) and disease specific survival (DSS) rates were found to be decreased in patients with CXCR4 (DFS: negative, 55% vs positive, 23%;  $p=0.079$  and DSS: negative, 52% vs positive, 23%;  $p=0.034$ ) and CCR5 positivity (DFS: negative, 63.5% vs positive, 26%;  $p=0.037$  and DSS: negative, 61% vs positive, 25%;  $p=0.047$ ), while no significant difference could be found in DFS and DSS rates in regards to PDL1, CXCR5, CCR7, CD73 and CD155 positivity.

**Conclusion:** These results demonstrate that PDL-1, CD73, CD155 and chemokine receptors are highly expressed in patients with partial response to neoadjuvant chemotherapy. Furthermore, expressions of CXCR4 and CCR5 were found to be associated with poor prognosis in this cohort with triple negative breast cancer resistant to chemotherapy that would justify an additional chemokine receptor inhibitor therapy.

## 2018 San Antonio Breast Cancer Symposium ®

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LncRNA NKILA promotes tumour immune evasion by sensitizing tumour-specific T cells to activation-induced cell death

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Activation induced cell death (AICD) of T lymphocytes is critical to maintain T-cell homeostasis, which is adopted by malignant tumors to convey immune evasion by eliminating tumor-reactive cytotoxic T cells. In this study, we demonstrated excessive apoptosis of tumor antigen-specific CTLs in breast and lung cancers. However, the mechanism involved in AICD of tumor-specific T cells remains obscure. Here, we demonstrated that NF- $\kappa$ B activity in tumor-specific T cells is high at the early phase of CTL activation induced by breast tumor antigens, but is suppressed at the later phase. This results in massive apoptosis of tumor-specific CTLs challenged by tumor cells. Interestingly, NKILA, an NF $\kappa$ B interacting lncRNA, sensitizes CTLs to AICD by inhibiting NF- $\kappa$ B activities after their activation, leading to tumor immune evasion. *In vivo*, administering CTLs with NKILA silencing into immunocompromised mice with breast cancer patient derived xenografts (PDXs) effectively inhibits PDX growth by increasing CTL infiltration. Clinically, NKILA was overexpressed in the tumor specific CTLs of breast and lung cancers, which was associated with less CTL infiltration in the tumors and shorter patient survival. Our findings present the first evidence that AICD in tumor-specific CTLs is crucial to cancer immune evasion, and targeting NKILA in CTLs emerges as a novel anti-tumor immunotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Doxorubicin induces cellular senescence in human breast cancer cells and sensitizes them to cytotoxic T-lymphocytes

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**Background** “Cellular senescence” is a state in which cells undergo irreversible cell cycle arrest in response to various cellular stresses. Senescence is induced in not only normal cells but also cancer cells when anti-cancer agents trigger DNA damage. Recent studies have revealed additional feature of senescent cells: increased secretion of various secretory proteins, such as inflammatory cytokines, chemokines, growth factors, and MMPs. This newly recognized senescent phenotype, termed senescence-associated secretory phenotype (SASP), reportedly contribute to tumor recurrence and promotion. Alternatively, senescent cancer cells could be good targets in anti-cancer immunotherapy because they are cell cycle-arrested.

**Objective** In this study, we determined whether “cellular senescence” could be induced by a chemotherapeutic drug doxorubicin (DXR) and whether senescent cancer cells might increase their susceptibility to cytotoxic T-lymphocytes using human breast cancer cells.

**Methods and Results** A triple-negative (negative for ER, PR, and HER2) human breast cancer cell line (MDA-MB-231) was used. This cell line was treated with DXR for 2 days and examined for their appearance microscopically. The DXR treatment (500 nM) decreased their proliferating ability and increased their cell size. Colony formation assay revealed that cancer cells significantly decreased the number of colonies even with lower doses (3 nM) of DXR. In immunoblot assay, the DXR treatment increased the protein expression of p21, which inhibits cell cycle. In a flow cytometric assay after staining with SPIDER-β-gal, the DXR treatment (500 nM) increased the expression of β-gal in MDA-MB-231 cells. The induction of SA-β-gal in DXR-treated cancer cells were also confirmed by confocal imaging. In addition, the DXR treatment (500 nM) for 2 days beforehand increased their subsequent ability to produce IL-6 and IL-8. Although the DXR treatment (500 nM) decreased the expression of epidermal growth factor receptor (EGFR) on cancer cells, this treatment sensitized them to anti-EGFR chimeric antigen receptor (CAR) T-lymphocytes in apoptosis assay.

**Conclusion** These results suggest that a chemotherapeutic drug DXR can render MDA-MB-231 cells to be senescent and increase their sensitivity to antigen-specific cytotoxic T-lymphocytes. These findings may provide a rationale of combination of chemotherapy and T cell-based anti-cancer immunotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-06-29

Prospective immune-profiling of locally advanced and metastatic breast cancer patients

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**Background:** Breast cancer is still not curable with a substantial resistance rate in all subgroups. Alterations in immunological mechanisms are assumed to play a role in pathophysiology and potential efficiency of immunotherapy approaches. Understanding the immunological changes in these patients may have major implications as predictive biomarkers for disease progression. The aim of our study was to determine immune subsets and functions in patient blood between treatment naïve locally advanced and metastatic breast cancer at diagnosis and to compare it with multiple time points during and after different treatments.

**Subjects and Methods:** The immunological profile of 25 stage II-III patients who were candidates for neoadjuvant treatment and 27 stage IV treatment naïve patients in two comprehensive oncology clinics (Marmara University and Medeniyet University, Istanbul, Turkey) were analyzed. Age-sex-matched healthy samples (n=26) were collected from volunteers. Peripheral blood mononuclear cells (PBMC) isolated from blood samples were frozen. PBMC were thawed and stained using multi parameter antibodies for immune profiling using flow cytometry in Jackson Laboratory, Farmington, CT.

**Results:** Differences between T cell subsets among patients (metastatic and locally advanced group separately) and healthy controls were assessed. We found significant differences (all p values <0.01) in inflammatory and regulatory T cell subsets both among the two patient groups (metastatic vs locally advanced untreated) and vs healthy controls, at first time point blood samples: 1) Increase in memory CD4+ T cells (CD45RO+) proportions in both metastatic and locally advanced groups (2) Increase in central and effector CD8+ memory (CD45RO+ or CD45RO-CCR7-) T cells only in metastatic group compared to healthy and locally advanced group, 3) Increase regulatory T cells (Tregs) only in locally advanced group compared to healthy and metastatic patients, 4) Perturbations in proinflammatory Th17 cells in both patient groups compared to healthy controls. More extensive immune profiling of these groups and comparison of different time points during- and post-treatment and correlation with clinical data will be presented.

**Conclusions:** Our results reveal significant differences in potential T cell activation and regulation in locally advanced and metastatic breast cancer patients, suggesting complex immune response at different disease stages. These findings have implications for as predictive indicators for disease progression for development of future immunotherapy strategies.

## 2018 San Antonio Breast Cancer Symposium ®

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Adipose PD-L1 modulates checkpoint blockade immunotherapy efficacy in breast cancer

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Programmed death-ligand 1 (PD-L1) and its receptor programmed cell death protein 1 (PD-1) play important roles in modulating antitumor immune response and are targeted by checkpoint blockade immunotherapy. While PD-L1 expression in both tumor and host cells is associated with antitumor therapeutic efficacy, the exact contribution of PD-L1 in various tissue and cell compartments to antitumor immune response remains to be elucidated. Here we show that PD-L1 expression is markedly elevated in human and mouse mature adipocytes compared to their preadipocyte counterparts. When co-cultured with mouse splenocytes *in vitro*, adipocytes prevent anti-PD-L1 antibody from activating CD8<sup>+</sup>T cells. Genetic ablation of adipose PD-L1 obliterates the inhibitory effect of adipocytes on anti-PD-L1 antibody. Conversely, enforced PD-L1 expression in preadipocytes confers the antibody-inhibitory activity. GW9662, a pharmacologic inhibitor of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) in adipogenesis, selectively reduces PD-L1 expression in mouse adipose tissue. The same PPAR $\gamma$  antagonist also enhances the antitumor efficacy of checkpoint blockade antibodies for treating multiple mammary tumors. Our findings provide a previously unappreciated approach to bolster anticancer immunotherapy efficacy and suggest a mechanism for the role of adipose tissue in breast cancer progression.

## 2018 San Antonio Breast Cancer Symposium ®

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Investigating the activity of ESR1 allosteric compounds in ER+ breast cancer

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Breast cancer (BCa) is the most frequently diagnosed cancer in women worldwide. Approximately 80% of breast cancer is estrogen receptor positive (ER+) with 74% demonstrating high expression of estrogen receptor alpha ( $\alpha$ ). Thus, current endocrine therapies such as selective estrogen receptor degraders (i.e.- Fulvestrant) or selective estrogen receptor modulators (i.e.-Tamoxifen) are involved modulation of ER signaling. However, most patients develop resistance to these drugs, and disease progression is common resulting in metastatic disease. Therefore, targeting alternative sites on the surface of ER has been proposed as an effective therapy to directly block its activity. In the present study, we investigated putative AF2 compounds that can bind to AF2 allosteric sites and inhibit complex formation and transcription of targetgenes. In the present study, we characterized a series of allosteric compounds using breast cancer cellular assays. MCF7 parental cells were treated with a series of putative allosteric compounds in a seven day CTG cell proliferation assay, and the compound ERX-11 was found to exhibit anti-proliferative activity. Genomic RNA was isolated after overnight treatment with ERX-11 to investigate compounds treatment on the expression of key ER pathway transcription factors via real time PCR. The degradation effect on nuclear ER and cytoplasmic ER after treatment with ERX-11 were assessed via western blots. We successfully characterized a series of putative allosteric compounds with ERX-11 being the only compound that exhibited anti-proliferative activity in both MCF7 parental and LSZ resistant cells. ERX-11 inhibited the activation of ER transcription gene and degradation of nuclear ER. These findings show promising effects of ERX-11 to block the AF2 sites on the binding domain of ER $\alpha$  with potential therapeutics for ER+ breast cancer.



# 2018 San Antonio Breast Cancer Symposium ®

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A small molecule inhibitor (ERX-41) induces endoplasmic reticulum stress in triple negative breast cancer

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**Background:** Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer and represents a disproportional share of the breast cancer mortality, primarily due to a lack of targeted therapies. There is a major unmet need for rationally designed novel therapies that can extend survival of patients with TNBC. TNBCs are characterized by a high basal level of endoplasmic reticulum stress, due to high protein turnover and need for proliferation. Recent studies revealed the role of several members of the Nuclear Receptor (NR) superfamily as molecular drivers in TNBC, including the androgen receptor (AR), glucocorticoid receptor (GR) and the orphan NR tailless (TLX).

**Methods:** Recently, using peptidomimetics, we have developed small molecules that specifically target and block interactions of multiple coregulators with oncogenic NRs. We performed a screen of our 500+ compound peptidomimetic library derived from our ERX-11 oligobenzamide (that was rationally designed to target ER $\alpha$ ) for anti-proliferative activity in TNBC cell lines. Identified leads were then validated in multiple TNBC cell lines. *In vitro* activity was tested using Cell titer glo, MTT, matrigel invasion, and apoptosis assays. Mechanistic studies were conducted using Western blot, reporter gene assays, CRISPR/Cas9 KO and RNA-seq analysis. Xenograft, patient derived xenograft (PDX), patient derived explant (PDE) and xenograft derived explant (XDE) TNBC models were used for preclinical evaluation and toxicity.

**Results:** We have identified a first-in-class drug (ERX-41) that has potent activity (IC<sub>50</sub> = 50-250nM) against all six molecular subtypes of TNBC. Systematic evaluation using CRISPR/Cas9 KO screen and overexpression screen comprising 48 NRs identified TLX as a preferred target of ERX-41. Analyses of primary breast tumors revealed TLX was highly expressed in TNBC. Further, TLX was amplified in nearly 50% of TNBC xenografts (cbioportal.org). Modelling, mechanistic and biochemical studies showed that ERX-41 interact with TLX and selectively blocks its interactions with coregulators. Gene expression analyses revealed both significant reduction of TLX-activated genes (CCND1, WNT7A) and significant activation of TLX-repressed genes (p21) upon treatment with ERX-41 in TNBC models. Gene ontology pathway analyses of RNA-seq data in TNBC cells showed that ERX-41 treatment positively correlated with apoptosis. Our ultrastructural studies indicated that ERX-41 enhances endoplasmic reticulum stress in TNBC inducing autophagic flux and subsequent apoptosis. ERX-41 has significant potency against multiple TNBC xenografts and PDXs *in vivo*, PDEs and XDEs *ex vivo*, indicating its potential for clinical translation. Pharmacologically, ERX-41 exhibited high oral bioavailability and associated with minimal toxicity upon oral gavage for up to 120 days in animal studies.

**Conclusions:** We believe that the ability of ERX-41 to block NR signaling and target a critical molecular vulnerability in TNBC and its ability to enhance endoplasmic reticulum stress in TNBC, will revolutionize the therapeutic landscape of TNBC. ERX-41 is oral bioavailable, potent against multiple TNBC molecular subtypes, and is associated with minimal systemic side effects. (supported by NIH grant RO1 CA223828-01)

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-07-03

Immunomodulation of triple negative breast cancer by caffeic acid phenethyl ester (CAPE)

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**Background:** CAPE is the major active component of propolis, a widely available, safe, honeybee natural product with anti-inflammatory, antioxidant, and antitumor properties. We have previously shown diverse effects of CAPE in breast cancer. We postulated that CAPE may be useful in primary or secondary prevention for triple-negative breast cancers (TNBC) and evaluated this in a *Trp53* null mammary chimera model in which the tumor spectrum is shifted to TNBC when the host is irradiated (Nguyen, 2011). Bioinformatics species comparisons show that TNBC from this model exhibits a spectrum of tumors that is molecularly similar to that seen in human TNBC (Nguyen, 2013).

**Methods:** The model consists of *Trp53* null mammary epithelium transplanted to cleared mammary glands of wildtype mice. Mice irradiated with 1 Gy received transplants 3 days later and were placed on a CAPE or a control diet at 1 month post transplantation that was maintained throughout the experiment. Tumor development was monitored by palpation for up to 18 months. Tumors were immunostained for estrogen receptor (ER) status and ER negative tumors were selected from all the groups for RNA sequencing. TNBC molecular subtypes were determined for control (n=5) and CAPE treated (n=9) samples using methods described by Lehman, 2011. Gene expression signatures consisting of 270 genes was analyzed for functional enrichment to identify patterns of signaling, as well as additional tumor characteristics, in each cohort of tumors.

**Results:** Host irradiation significantly accelerated tumor growth rate compared to the control sham irradiated mice. CAPE treatment blocked this effect, but did not affect the control tumor growth rate. Resected tumors in CAPE treated mice recurred at significantly longer intervals (40 days in control group versus 90 days with CAPE) and much less frequently than tumors from mice on a control diet. Mean expression analysis showed that CAPE induces a distinct gene expression pattern in ER negative tumors from irradiated mice. As previously described (Illa-Bohaca, 2014), ER negative tumors from sham irradiated mice on a control diet were enriched in immune response genes. These genes were suppressed in tumors arising in irradiated host, but this effect of host irradiation was abrogated in mice on the CAPE diet. While untreated tumors were classified into the Basal-like 2 (BL2) or Mesenchymal TNBC molecular subtypes, we observed an increase in subtype diversity after CAPE treatment with tumors being classified as Immunomodulatory and Luminal Androgen Receptor (LAR) in addition to BL2 and Mesenchymal subtypes. Consistent with these data, functional enrichment analyses of RNA sequencing data identified increased immune signaling as well as upregulation of a number of signaling pathways including TGF $\beta$ , HGF, and EGFR in treated samples.

**Conclusion:** These findings support the potential use of CAPE to modify the aggressive behavior of TNBC, which may be due to effects on the immune system in which CAPE acts to re-establish anti-tumor immunity. The finding that CAPE treatment shifts the tumor spectrum to Immunomodulatory subtypes suggests that it may be useful both for women at high risk for TNBC and to prevent or delay TNBC breast cancer recurrence, perhaps in combination with immunotherapy.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-07-05

A novel, first in class Notch transcriptional inhibitor, CB-103 has activity on luminal breast cancer stem cells in combination with fulvestrant

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The Notch signaling pathway plays a central role in cellular differentiation, growth and stem cell maintenance. Expression and activation of Notch pathway receptors and ligands have differential outcomes depending on the tissue, localization and cell type. When Notch pathway is aberrantly activated by genetic lesions, it can be a major driver for Notch-dependent cancers and can cause resistance to standard of care treatment. We and others have shown that in Estrogen Receptor (ER)-positive breast cancers, estrogen deprivation caused by endocrine therapy results in Notch1 and Notch4 activation. In turn, Notch1 stimulated ER-dependent transcription in the absence of estrogen, causing endocrine resistance. Combinations of Notch inhibitors and endocrine therapy are effective in preclinical models of ER-positive breast cancer and have shown promising signals in early clinical trials.

Cellestia's lead development candidate CB-103 is a small molecule, first-in-class, oral pan-Notch inhibitor. CB-103 selectively blocks Notch pathway activation-related gene transcription through binding to a Notch specific protein in the transcription factor complex. The blockade occurs by protein-protein interaction inhibition with a binding site critical for the assembly of the Notch transcription complex. This is a unique mode of action, which allows blocking Notch signaling regardless of the genetic lesions which have activated the pathway.

We have performed mammosphere assays to test the potency and efficacy of this compound on stem cell ability to form sphere. We used two different doses of CB-103; either alone or in combination with a fixed dose of Fulvestrant (30nM), a SERD, in our mammosphere assays. Two different ER<sup>+</sup> luminal, endocrine resistant cell lines were tested and compared with their parental controls. From our data, it's apparent that there is a synergistic effect when using CB-103 in combination with Fulvestrant. It's also evident that the efficacy of CB-103 is maximal maximizes at the lowest concentration tested in our assays. The combination was effective in 3 out of 4 models. However, the effect of CB-103 on MCF7-TAM<sup>R</sup> either as a single agent or in combination was not statistically significant.

Cellestia has received regulatory approval to start clinical development with CB-103 in a first-in-human study Phase I – IIa study investigating safety (Ph I) and preliminary single agent efficacy (Ph IIa) of CB-103 in patients with advanced solid cancers and haematological malignancies. Our data support the notion of testing this agent in ER-positive breast cancer in combination with SERDs.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-07-06

Cyclin dependent kinase 7 (CDK7) inhibition with THZ1 induces mitotic failure and increases genomic instability in triple negative breast cancer

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Triple negative breast cancer (TNBC) is highly proliferative and genomically unstable, making these tumors particularly sensitive to anti-proliferative chemotherapies. While efficacious, these drugs induce dose limiting toxicities. Agents that can selectively target proliferation within cancer cells without inducing systemic toxicity should greatly improve patient outcomes from TNBC. In this regard, we have found that TNBC cells are particularly vulnerable to suppression of the transcriptional regulator, LIN9, that controls cell cycle progression. *LIN9* mRNA is overexpressed in 66% of TNBCs and is correlated with worse patient outcomes. Moreover, suppression of LIN9 expression induces multi-nucleation, micronucleation, mitotic catastrophe, and cell death and/or senescence. While transcription factors are generally considered “undruggable,” *LIN9* expression can be pharmacologically suppressed by blocking the activity of cyclin dependent kinase 7 (CDK7) with the selective, covalent CDK7 inhibitor, THZ1. CDK7 inhibitors inactivate RNA polymerase 2 and destabilize the super-enhancer mediated expression of oncogenes. Treatment of three TNBC cell lines (MDA-MB-231, MDA-MB-468, and HCC38) with THZ1 induces G2/M arrest and phenocopies genetic silencing of LIN9. Use of live cell imaging revealed that THZ1 increases the duration of mitosis and also leads to mitosis-associated cell death. Together, these data reveal that CDK7 inhibitors primarily inhibit TNBC growth by causing mitotic dysfunction and potentiating genomic instability by inducing micro- and multi-nucleation. In addition, they suggest that suppressing LIN9 expression by inhibiting CDK7 may lead to a selective approach for targeting proliferation of TNBC and improving patient outcomes from this disease.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-07-07

Triple targeted combination therapy with tucatinib, palbociclib and fulvestrant is active in hormone receptor and HER2-positive human breast tumor cell lines

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Hormone receptor and HER2-positive (HR+/HER2+) breast cancer displays increased resistance to anti-hormonal and HER2-targeted agents. HER2 and estrogen receptor signaling converge at cyclin D1 and CDK4/6 complex, resulting in the increased cyclin D1 expression and accelerated progression of the cell cycle. Therefore, triple blockade of the estrogen receptor, HER2 and CDK4/6 is reasonable as a novel approach to treatment of HR+/HER2+ disease. Here for the first time we report high activity of novel combination therapy with HER2-targeted small molecule inhibitor tucatinib, CDK4/6 inhibitor palbociclib, and selective estrogen receptor blocker fulvestrant in human breast tumor cell lines.

**Methods:** HR+/HER2+ human breast tumor cell lines BT474, MDA-MB-361 and UACC-812 were cultured in standard conditions. The subclones of BT474 and MDA-MB-361 resistant to tucatinib and palbociclib were generated by culturing cells in the increasing concentrations of inhibitors over 6 months. Cell survival assays were performed with Cell Titer Glow (Promega, Madison, WI) after 72 hrs of treatment with vehicle, single agents tucatinib, palbociclib and fulvestrant, and with dual and triple combinations. Tucatinib was provided by Seattle Genetics (Seattle, WA), palbociclib and fulvestrant were purchased from Selleckchem (Houston, TX). Protein lysates for western blot were prepared after 24, 48 or 72 hrs of drug treatment at IC70 using RIPA lysis buffer (Thermo Fisher, Waltham, MA) containing protease/phosphatase inhibitors (Roche Diagnostic, Indianapolis, IN). Signals were quantified with NIH ImageJ Imaging Analysis Software or Odyssey Imager software (Li-Cor Bioscience, Lincoln, NE) and normalized to vinculin or alpha-tubulin.

**Results:** Dual combinations of tucatinib with fulvestrant and tucatinib with palbociclib were synergistic in all three HR+/HER2+ cell lines. Addition of a constant dose of fulvestrant to a combination of tucatinib and palbociclib further improved tumor cell killing in all three breast tumor cell lines tested. Combination of tucatinib and palbociclib resulted in greater suppression of phospho-AKT and total and phospho-CDK2 comparing to either agent alone. Treatment with single agents tucatinib and palbociclib lead to a significant increase of cyclin E expression at 48 and 72hrs after treatment, however, this compensatory mechanism of cell cycle progression was not observed after treatment with tucatinib and palbociclib combination. Experiments with the resistant BT474 and MDA-MB-361 subclones showed that cross-treatment of cells resistant to palbociclib with tucatinib and vice versa suppressed cell growth and decreased expression of phospho-ERK1/2, phospho-AKT, phospho-CDK2 and cyclin E.

**Conclusions:** In HR+/HER2+ human breast tumor cell lines combination therapy with tucatinib, palbociclib and fulvestrant demonstrated significant synergy, potentially acting through suppression of phospho-AKT and cyclin E pathways, therefore suggesting a promising novel approach to treatment of HR+/HER2+ breast cancer. Clinical testing of tucatinib, palbociclib and anti-hormonal therapy in patients with HR+/HER2+ metastatic breast cancer is ongoing (phase Ib/II clinical trial NCT03054363).

# 2018 San Antonio Breast Cancer Symposium ®

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Budesonide and loperamide do not impact the cytotoxicity of neratinib or HER2-directed monoclonal antibodies in HER2+ breast cancer cell lines

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**Background:** Neratinib is an irreversible pan-HER tyrosine kinase inhibitor with demonstrated clinical activity in HER2+ and HER2-mutated breast cancers. The main toxicity of neratinib is diarrhea, which is common in the absence of prophylaxis. Preclinical models suggest that neratinib-associated diarrhea may involve inflammatory, bile acid malabsorption and secretory factors. The phase II CONTROL study is currently investigating the prophylactic efficacy of the opioid receptor antagonist loperamide in combination with budesonide (a corticosteroid used for inflammatory gastrointestinal conditions) or colestipol (bile acid sequestrant) on neratinib-associated diarrhea in early-stage HER2+ breast cancer (NCT02400476). This *in vitro* study examines the impact of loperamide and budesonide on the anti-proliferative activity of neratinib or trastuzumab and pertuzumab in HER2+ or HER2-low breast cancer cell lines.

**Methods:** HER2+ breast cancer cell lines SKBR3 (estrogen receptor [ER]–), BT474 (ER+), HCC1569 (ER–) and HER2-low, pertuzumab-sensitive MDA-MB-175-VII (ER+) breast cancer cells were investigated using a 5-day acid phosphatase-based proliferation assay to determine the concentrations required to inhibit growth by 50% (IC<sub>50</sub>). Fixed ratios of drugs were utilised in combination assays to generate Combination Index (CI) values (Calculusyn®) where available. Clinically relevant levels of neratinib, trastuzumab and pertuzumab were utilised in all experiments. Physiologically relevant levels of budesonide (~4.2 nM) and loperamide (~2.5 nM) were exceeded to provide IC<sub>50</sub> values for these compounds.

**Results:** All cell lines tested had neratinib IC<sub>50</sub> values in the nM range (Table). Trastuzumab and the trastuzumab/pertuzumab combination did not exceed 50% inhibition in the HER2+ cell lines. In the HER2+ breast cancer cell lines tested, loperamide had no impact on neratinib activity in BT474, enhanced neratinib activity in SKBR3, and the combination of loperamide and neratinib proved synergistic in HCC1569 (CI = 0.77 +/- 0.2). Budesonide produced strong synergism in combination with neratinib in SKBR3 (CI = 0.27 +/- 0.03), had no impact on neratinib activity in BT474 and improved response to neratinib in HCC1569. Loperamide and budesonide improved the activity of trastuzumab and pertuzumab in all three HER2+ models tested, and had no impact on pertuzumab activity in MDA-MB-175-VII. Interestingly, neratinib proved synergistic in combination with pertuzumab in MDA-MB-175-VII (CI = 0.75 +/- 0.5 nM).

**Table.** Anti-proliferative effects of agents tested

Breast cancer cell line	Neratinib IC <sub>50</sub> , nM	Loperamide IC <sub>50</sub> , nM	Budesonide IC <sub>50</sub> , nM	Pertuzumab IC <sub>50</sub> , nM	Trastuzumab (% inhibition, 2.5µg/ml)	Trastuzumab/Pertuzumab (% inhibition, 2.5µg/ml)
SKBR3	2.8 +/- 0.4	7.7 +/- 0.5	2.7 +/- 0.2	NA	26.3 +/- 1.3	NA
BT474	1.4 +/- 0.1	2.6 +/- 0.2	7 +/- 0.6	NA	40.1 +/- 4.3	NA
HCC1569	17.3 +/- 0.7	9.3 +/- 1.7	28.7 +/- 0.5	No effect	No effect	26.1 +/- 2.8
MDA-MB-175-VII	3 +/- 0.3	> 20	> 20	1.2 +/- 0.2	NA	NA

NA = not acquired.

**Conclusions:** Our preclinical results suggest that budesonide and loperamide do not antagonise the anti-proliferative activity of neratinib or HER2-directed monoclonal antibodies in HER2+ breast cancer cell lines.

## 2018 San Antonio Breast Cancer Symposium ®

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Role played by autophagy in breast cancer models exposed to new PI3K/AKT inhibitors, GDC-0068 and GDC-0032

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Abundant preclinical evidences indicate that stress-induced autophagy in tumor cells is predominantly cytoprotective and that inhibition of autophagy can enhance tumor cell death by diverse anticancer therapies. A major negative regulator of autophagy is the mammalian target of rapamycin (mTOR), activated downstream of PI3K/AKT pathway. mTOR inhibitors, including rapamycin, have been shown to induce autophagy in tumor cells, while the combination of PI3K-AKT/mTOR and autophagy inhibitors shown synergistic effect on increased apoptosis and reduced autophagy (Takeuchi H. 2005). This project aimed to characterize the role of autophagy in Breast Cancer models exposed to new potent Genentech PI3K/AKT inhibitors GDC0068 (Ipatasertib) and GDC0032 (Taselisib) currently in phase III clinical trials on TNBC and ER+ patients. However, the efficacy of PI3K/AKT inhibitors may be limited by resistance mechanisms that result in minimal cell death in tumor cells. In order to investigate the role of autophagy as possible mechanisms of resistance, Ipatasertib and Taselisib have been evaluated in breast cancer cell lines characterized by different receptors profile: TNBC, HER2/c-erb-2 and luminal A cell lines. Our results showed that both drugs are able to induce G1/S cell cycle block and increase of autophagy signaling measured by p62 level and LC3 II/LC3 I ratio and by the percentage of cells exhibiting LC3 positive puncta. In addition, apoptosis increase was also evaluated by measuring positive annexin V staining, by flow cytometry technique, and apoptosis markers, such as PARP and cleaved caspase 3, by immunoblot assay. Furthermore, the supplement of pharmacologic inhibitors of autophagy, such as hydroxychloroquine, were able to reduce cell viability evaluated by both short- and long-term assays in cell lines exposed to Ipatasertib and Taselisib. 3D breast cancer models are ongoing to confirm the synergic effect of PI3K/AKT inhibitors and hydroxychloroquine, in order to provide a new therapeutic combinatorial approach potentially translatable to patients.

## 2018 San Antonio Breast Cancer Symposium ®

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Epithelial-mesenchymal transition sensitizes breast cancer cells to paraptosis-mediated cell death via the fungus-derived sesterpenoid, Ophiobolin A

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The epithelial-mesenchymal transition (EMT) enables the dissociation of cancer cells from the primary tumor by facilitating tolerance to lack of cell-adhesion, decreasing cellular division and increasing motility of individual cells, which leads to an invasive phenotype that links EMT to metastasis. Furthermore, EMT results in the acquisition of stem-cell markers and an increased ability to initiate tumor growth, supporting the concept that EMT may contribute to the development of a small, persistent sub-population of the tumor called cancer stem cells (CSCs). Cells that have undergone EMT have characteristically suppressed cell cycles, making them resistant to commonly used chemotherapies that target DNA replication or microtubule dynamics, processes essential to replicating cells. Nonspecific treatments can also rely on inducing apoptotic cell death; however, recent debates challenge the efficacy of apoptosis in solid tumors, citing high rates of acquired resistance. Utilizing a compound that induces alternative cell death, namely paraptosis, becomes attractive when these other treatments fail. Relying on an activated gene expression program, paraptosis results in the swelling of the mitochondria and endoplasmic reticulum and Apaf-1-independent alternative caspase-9 activity. Treating with paraptosis-inducing compounds such as Ophiobolin A (OpA) specifically targets otherwise-insensitive CSC and EMT cells to re-sensitize bulk tumor populations to chemotherapies. We describe EMT as a key driver of enhanced sensitivity to paraptosis-induced cell death following short-term treatment with OpA or other paraptosis-inducing compounds. Further, paraptosis selectively eliminates the CSC sub-population by reducing stem cell activity and highlights the potential of this pathway in breast cancer treatment.



# 2018 San Antonio Breast Cancer Symposium ®

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Is the 8<sup>th</sup> edition of the breast cancer TNM staging system an improvement over the 7<sup>th</sup> edition?

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**Background:** The revised 8<sup>th</sup> edition of the TNM staging system was published on January 25, 2018 (<https://cancerstaging.org/references-tools/deskreferences/Pages/Breast-Cancer-Staging.aspx>). The new prognostic staging system (PrSS) was developed using the outcomes of 334,243 patients from the National Cancer Database (NCDB) with a median follow-up of 3.5 years after treatment in 2010-2012. The PrSS was created by clustering patients by outcome into the eight sub-stages of the 7<sup>th</sup> edition TNM anatomic staging system (AnSS) using more than 169 combinations of stage, ER, PR, H2, grade and molecular test results. This study aimed to assess the prognostic performance of the PrSS in an independent cohort with longer follow-up.

**Methods:** The Breast Cancer Unit Outcomes database was used to identify patients treated without neoadjuvant systemic therapy in 2005-2014, when use of anti-Her2 therapies was routine. Ten-year breast cancer-specific survival (BCSS) was determined using the Kaplan-Meier method. Using age, ER, PR, H2, grade, chemotherapy, type of surgery and radiotherapy variables for the AnSS and the same variables minus ER, PR, H2, and grade, which are built into the PrSS, Harrell's c-indices were calculated to compare the predictive performance of the AnSS and PrSS models (higher quality models have higher c-indices).

**Results:** The study cohort had 19,581 patients with a median follow-up of 7.3 years. Relative to the AnSS, 5.2% (1009/19,581) had higher prognostic stages and 36.5% (7,149/19,581) had lower prognostic stages. The c-indices were 0.83 for the AnSS and 0.81 for the PrSS. Overlapping 10-year BCSS confidence intervals (CI) were observed for stages IIIB and IIIC, which contained few patients in both staging systems. There were also overlapping CIs for two other anatomic stages: IA & IB and four other prognostic stages: IB & IIA and IIB & IIIA.

## Comparison of Anatomic and Prognostic TNM Staging

Stages	Number of Patients		10-year BCSS	
	Anatomic	Prognostic	Anatomic (%) (CI)	Prognostic (%) (CI)
IA	9,195	11,987	96.4 (95.8, 96.9)	95.9 (95.4, 96.4)
IB	608	3,015	95.1 (92.2, 96.9)	86.7 (84.9, 88.3)
IIA	4,840	2,038	89.9 (88.7, 91.0)	85.4 (83.4, 87.1)
IIB	2,495	869	83.9 (81.9, 85.7)	73.9 (70.0, 77.4)
IIIA	1,549	789	75.0 (72.0, 77.6)	70.8 (66.7, 74.5)
IIIB	94	357	54.8 (40.3, 67.2)	57.1 (50.4, 63.2)
IIIC	539	265	53.0 (47.6, 58.1)	50.8 (43.7, 57.4)
IV	261	261	23.8 (17.6, 30.5)	23.8 (17.6, 30.5)

**Conclusions:** For 10-year BCSS, the c-index was higher for the AnSS than for the PrSS. In addition, more statistically distinct stages were observed for the AnSS than the PrSS. The increased complexity of the PrSS did not appear to provide better prognostication than the AnSS in our large series with longer follow-up than the NCDB.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-02

Importance of adverse events during endocrine treatment for the prediction of late distant recurrences

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**Background:** During endocrine treatment of breast cancer, the occurrence of symptoms related to oestrogen depletion are important predictor of treatment efficacy. We have previously shown that appearance of vasomotor and joint related symptoms are a useful biomarker for a greater response to endocrine treatment (Cuzick *et al.*, Lancet, 2008). Furthermore a prediction model including clinicopathological parameters (CTS5) has shown to be a good prognostic tool for the prediction of late distant recurrences (DR) (Dowsett *et al.*, JCO, 2018). Here, we assessed whether the occurrence of endocrine related adverse events were predictive of late DR in the ATAC trial.

**Methods:** The ATAC trial (N=4735) of postmenopausal women with estrogen receptor positive breast cancer treated with 5 years' tamoxifen or anastrozole was used for this analysis. Women who reported symptoms (hot flashes, joint symptoms, gynaecological symptoms) at any time during the trial were compared to those not reporting these symptoms for late DR. Time to late DR, defined beginning at 5 years after randomization, was the primary endpoint. Hazard ratios (HR) and corresponding 95% CIs were estimated by Cox proportional hazards regression models.

**Results:** 2937 women (62%) who were recurrence free after 5 years, reported either hot flashes, joint symptoms, or gynaecological symptoms during the active treatment period. Women who reported joint symptoms (adjusted for CTS5: HR=0.74 (0.59-0.94)), or gynaecological symptoms (adjusted for CTS5: HR=0.68 (0.47-0.97)) had significantly fewer late DR compared to those not reporting these events during the active 5 years' treatment period. Those who reported any symptom during the treatment period had an overall 34% lower risk of a late DR (univariate: HR=0.66 (0.53-0.83), P<0.001; adjusted for CTS5: HR=0.75 (0.60-0.93), P=0.009). The 5-10 year DR risk for women who reported any symptoms was 7.4% (6.4-8.5) compared to 11.0% (9.3-12.8) for those without symptoms. Women who reported any symptoms and who were randomized to either anastrozole (HR=0.67 (0.48-0.92)) or tamoxifen (HR=0.65 (0.48-0.88)) had significantly fewer late DR compared to those not reporting these symptoms. Women with symptoms on tamoxifen did have better 10-year DR risk compared to those without symptoms on anastrozole (8.1% (6.7-9.8) vs. 9.8% (7.7-12.4)).

**Conclusions:** This retrospective analysis of the ATAC trial showed that occurrences of endocrine related symptoms during the treatment period are associated with the risk of developing a late DR, irrespective of treatment allocation. Larger effects were found for joint or gynaecological symptoms and remained significant after adjustment for clinical parameters. These findings might help clinicians and patients in their decision making process about extended endocrine therapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Looking forward to the TNM 9<sup>th</sup> edition: Is it time to stage the different breast cancer subtypes as distinct diseases?

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**Background:** For clinical purposes, four subtypes of breast cancer: Luminal A (LumA), Luminal B (LumB), Her2-positive (H2P) and Triple-Negative (TN) are commonly recognized. This study investigated staging them independently as different diseases. The hypothesis was that the anatomic staging system of the 8<sup>th</sup> TNM edition would have good prognostic performance within each breast cancer subtype, as defined by estrogen receptor (ER), progesterone receptor (PR), Her2 amplification (H2) and grade.

**Methods:** Using the Breast Cancer Outcomes Unit database, we identified patients treated without neoadjuvant therapy between 2005 and 2009, when use of anti-Her2 therapy (AH2T) was routine for H2+ disease. We approximated the four subtypes of breast cancer described in Table 48.2 of the revised January 25, 2018 version of the TNM 8<sup>th</sup> edition

(<https://cancerstaging.org/references-tools/deskreferences/Pages/Breast-Cancer-Staging.aspx>) as follows: LumA ER3+ AND PR3+ AND H2- AND (G1-2); LumB All Non-LumA (ER+ OR PR+) AND H2-; H2P All H2+; and TN ER- AND PR- AND H2-. Breast cancer-specific survival (BCSS) was determined for anatomic stages I-IV within each subtype by the Kaplan-Meier method. The predominant usage of hormone therapy (HT), chemotherapy (ChT) and AH2T was compared by subtype for patients < 70 years, who were generally eligible for ChT.

**Results:** The median follow-up for the 8,640 patients was 10.0 years. The numbers of patients within each subtype were: LumA = 2,288, LumB = 4,097, H2P = 1,374, and TN = 881. The predominant systemic therapies used by subtype were: LumA: HT = 60.2% and HT+ChT = 29.8%; LumB: HT = 45.9% and HT+ChT = 42.1%; H2P: HT+ChT+AH2 = 40.1% and ChT+AH2 = 35.0%; and TN: ChT = 79.1%. The confidence intervals for BCSS in stages I, II, III, and IV were distinct for the four subtypes, as shown in Table 1.

BCSS by Stage for Clinical Breast Cancer Subtypes

1-year BCSS	LumA (%) (CI)	LumB (%) (CI)	H2P (%) (CI)	TN (%) (CI)
Stage I	98.1 (97.0, 98.8)	96.5 (95.5, 97.2)	95.4 (92.9, 97.0)	90.4 (86.5, 93.2)
Stage II	93.4 (91.1, 95.1)	86.5 (84.6, 88.3)	87.2 (84.1, 89.7)	81.4 (77.3, 84.9)
Stage III	79.9 (71.8, 85.9)	66.1 (61.5, 70.3)	74.0 (68.1, 78.9)	58.0 (48.3, 66.5)
Stage IV	42.9 (17.7, 66.0)	16.9 (8.1, 28.6)	33.1 (18.6, 48.3)	0.0 (0.0, 0.0)

**Conclusions:** The anatomic staging system provided reliable BCSS prognostication within breast cancer subtypes. Individualizing treatment using anatomic staging within breast cancer subtypes, would permit decisions about the volume of radiotherapy and the need for intensification of systemic therapy to be made using the familiar and time-tested risk metric of disease extent. In the future, as breast cancer subtyping becomes more sophisticated, prognostication using anatomic staging within these distinct diseases should become increasingly accurate.

# 2018 San Antonio Breast Cancer Symposium ®

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PD-L1 expression and prognosis in triple negative breast cancer (TNBC): An analysis of 265 patients (pts) treated with standard therapy for stage I-III disease

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**Background:** Targeting the PD-L1/PD-1 axis has proved to be effective in various cancers, including promising data for metastatic TNBC pts. The evaluation of PD-L1 expression is limited by the lack of standardized methods. Here we sought to evaluate the prognostic role of PD-L1 expression in a large cohort of patients with non-metastatic TNBC treated with standard therapy.

**Methods:**

Consecutive patients diagnosed with stage I-III TNBC (ER and PgR <10%, HER2 0/1+ or ISH non amplified) between May 2012 and December 2015 and treated at the Istituto Oncologico Veneto of Padova were included. All patients received treatment with surgery, chemotherapy (neoadjuvant or adjuvant) and radiotherapy (when indicated).

For each case, one FFPE tumor slide was stained for PD-L1 with the PD-L1 IHC 73-10 Research Use Only assay developed by Agilent Technologies and one slide was stained for cytokeratins with MNF116 (to distinguish MNF116+ tumor cells from MNF116-stromal cells). Digital slides were evaluated by a specifically-developed Visiopharm® software application. Following alignment of the PD-L1 and MNF116 digital slides, the software analyzed PD-L1 expression on tumor cells (% of positively stained tumor cells/total tumor cells) and stromal cells (% of positively stained stromal cells/total stromal cells). Disease-free survival (DFS) was calculated from diagnosis to relapse or death. In survival analyses, PD-L1 was evaluated as continuous and categorical variable.

**Results:**

265 TNBC pts were evaluated. Median PD-L1 was 2.6% (Q1-Q3 0%-18.6%) on tumor cells and 5.2% (Q1-Q3 0.2%-25.4%) on stromal cells. PD-L1 levels on tumor and stromal cells were positively correlated (spearman's 0.938,  $p < 0.001$ ). For further analyses, PD-L1 on stromal cells was considered. Higher PD-L1 was associated with age  $\leq 50$  yrs ( $p = 0.011$ ), Grade 3 ( $p = 0.003$ ) and Ki67  $\geq 30\%$  ( $p = 0.005$ ). Lower PD-L1 was observed in lobular and apocrine tumors ( $p = 0.001$ ). Cox model for DFS showed HR=0.99 (95%CI 0.97-1.00,  $p = 0.059$ ) for every 1% PD-L1 increment.

3-yrs DFS was 86% for pts with PD-L1 >20% (n=88, 29%) vs 75% for pts with PD-L1  $\leq 20\%$  (n=177, 71%): HR 0.52, 95%CI 0.28-0.97,  $p = 0.039$ . PD-L1 at 20% cut-off maintained prognostic value in multivariate model including stage (HR 0.48, 95%CI 0.25-0.89,  $p = 0.021$ ).

Of the 265 pts included, 108 received neoadjuvant chemotherapy (NACT). Of the 78 pts with residual disease after NACT, 61 had pre- and post-NACT samples evaluable for PD-L1. PD-L1 increased from pre- to post-NACT: median 2.7% (Q1-Q3 0%-26.9%) vs 20.1% (Q1-Q3 5.9%-41.4%),  $p < 0.001$ . Pts with PD-L1 >20% post-NACT showed improved DFS: 3-yrs DFS 68% vs 43% (HR 0.44, 95%CI 0.20-0.96,  $p = 0.039$ ), whereas PD-L1 pre-NACT did not show significant association with DFS in this subgroup (HR 0.47, 95%CI 0.23-1.40,  $p = 0.218$ ).

**Conclusions:**

PD-L1 expression evaluated with a software-assisted method was prognostic for stage I-III TNBC pts treated with standard therapy. The significant increase of PD-L1 on residual disease post-NACT supports the rationale to evaluate the efficacy of anti-PD-L1 drugs in this high-risk population.

# 2018 San Antonio Breast Cancer Symposium ®

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Prediction of distant recurrence using EndoPredict among women with ER-positive, HER2-negative breast cancer with a maximum follow-up of 16 years

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**Background:** EndoPredict has been previously validated as a prognostic test in women with ER-positive, HER2-negative disease who received endocrine therapy only as part of the ABCSG6 and -8 trials. Here, we further evaluate the prognostic value of EndoPredict in this cohort with longer-term follow-up and compare 10-year distant recurrence (DR) and 5-15 years late recurrence according to nodal status.

**Methods:** This analysis included 1702 patients with ER-positive, HER2-negative disease who received endocrine therapy only. Prognostic value of EPclin score and EPclin risk category (high, low) on the risk of distant recurrence adjusted for patient and disease characteristics was evaluated using multivariable Cox proportional hazard models. Kaplan-Meier estimators were used to estimate DR according EPclin class and were compared using log rank test. Analyses were performed for the overall cohort, by nodal status, and for patients who were distant recurrence free at year 5 (late recurrence).

**Results:** The median follow-up was 9.6 years (range 0-16.6), an increase of 4.2 years over previous reports. Reanalysis with longer follow-up confirms that EPclin is a significant predictor of DR after adjusting for clinical factors, regardless of nodal status (Table 1). Overall, 62.6% of patients had low risk EPclin scores and 10-year DR was significantly improved relative to those with high risk scores ( $p < 0.0001$ ; Table 2). When nodal status was considered, 77.8% of node negative tumors and 34.9% of node positive (1-3 PLN) tumors had low risk EPclin scores. Regardless of nodal status, DR was significantly improved for those with low versus high risk EPclin scores (Table 2). Similar results were observed for the patients who were DR free at year 5 (5-15 year follow-up) (Table 2).

**Conclusion:** This reanalysis of previous EndoPredict data with a longer follow-up confirms that EPclin can identify a large group of patients at low risk of distant recurrence after 10 years who might be sufficiently treated with 5 years adjuvant endocrine therapy only, independent of nodal status. Replication of these results for the late distant recurrence between years 5-15 also shows that EPclin scores may also be informative in selecting patients who may safely forgo extended endocrine therapy.

Table 1. Multivariate analysis

Cohort	0-15 year DR		5-15 year DR	
	HR*	p-value	HR*	p-value
All patients	2.55	<0.0001	2.56	<0.0001
Node negative	1.68	0.0035	2.01	0.0088
1-3 positive nodes	2.68	<0.0001	3.43	0.0004

\*Hazard Ratio (HR) per unit score after adjusting for age, tumor grade, Ki67, ER, PR, and treatment

Table 2. DR according to EPclin score

		Low risk EPclin		High risk EPclin		
Cohort	HR*	%	0-10 year DR (95% CI)	%	0-10 year DR (95% CI)	Log-rank p-value
All patients N=1702	4.77	62.6	0.96 (0.94, 0.97)	37.4	0.80 (0.77, 0.84)	<0.0001
Node negative N=1165	3.47	77.8	0.96 (0.94, 0.97)	22.2	0.87 (0.83, 0.92)	<0.0001
1-3 positive nodes N=453	3.65	34.9	0.96 (0.92, 0.99)	65.1	0.81 (0.76, 0.87)	0.0003
Cohort	HR*	%	5-15 year DR (95% CI)	%	5-15 year DR (95% CI)	Log-rank p-value
All patients N=1386	4.52	64.7	0.96 (0.93, 0.98)	35.3	0.84 (0.79, 0.90)	<0.0001
Node negative N=975	3.76	78.3	0.97 (0.95, 0.99)	21.7	0.85 (0.75, 0.96)	<0.0001
1-3 positive nodes N=362	3.00	36.5	0.87 (0.72, 1.00)	63.5	0.84 (0.77, 0.92)	0.0337

\*HR for EPclin high risk versus low risk

# 2018 San Antonio Breast Cancer Symposium ®

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Impact of molecular subtypes on long-term outcomes in triple-negative breast cancer (TNBC) patients treated with adjuvant AC chemotherapy on SWOG S9313

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**Introduction:** TNBC is heterogeneous disease with several molecularly defined subtypes (Lehman et al), each of which may be predictive of response to chemotherapy. TNBC molecular subtypes are associated with varied pathological responses to neoadjuvant chemotherapy. However, subtype specific long-term outcomes for TNBC patients treated with uniform adjuvant chemotherapy are not known.

**Aims:** To characterize long-term outcomes of TNBC molecular subtypes (TNBCtypes) in patients treated with adjuvant doxorubicin (A) and cyclophosphamide (C) on S9313

**Methods:** SWOG 9313 accrued 3,125 women with early stage breast cancer to two alternative dose schedules of AC with no difference in outcomes between the two arms (J Clin Oncol 2007). From this trial we identified 425 (14%) patients with centrally determined TNBC for whom tissue was available. Microarray profiling was performed on genomic RNA extracted from pre-treatment FFPE tissue. A 101-gene expression model which has shown to reproduce the classification provided by the original 2188-gene algorithm (Ring et al) was applied to the microarray profiling to generate the following TNBCtypes–Basal-Like 1 (BL1), Basal-Like 2 (BL2), Mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR). Immunomodulatory +/- (IM) status was assigned independent of the subtypes. Sequencing of BRCA1/2 from tumor DNA was also performed. The subtypes were tested for prognostic effect on DFS and OS using Cox regression model with adjustment for nodal status.

**Results:** For 425 TNBC patients, the median age was 45 years, 33% were node-positive and 10-year DFS and OS = 66.3% and 74.1%, respectively. A total of 381/424 (89.7%) cases could be classified into TNBCtypes with distribution as follows: BL1=24%, BL2=8%, M=24%, MSL=11%, LAR=9%, unclassified (UNL) =24%. No association between TNBCtypes and race or nodal status was noted. Compared to other subtypes LAR subtype was associated with older age at diagnosis (median age 53 vs 45, p<0.001). Overall 24% of samples were IM+ and 25% demonstrated deleterious tBRCA1/2 mutation. DFS, tBRCA1/2 mutation and IM+ status distribution across different subtypes are provided in the table. All subtypes except for LAR demonstrated a drop in hazard function for recurrence after 5 years.

	5 year DFS (%)	10 year DFS (%)	DFS HR (95% CI), p value	Deleterious tBRCA1/2 mutation	IM+ status
<b>BL1</b>	84.5%	77.5%	1	41%	60%
<b>BL2</b>	81.3%	70.5%	1.59 (0.81-3.13) p = 0.18	16%	12%
<b>M</b>	69.2%	61.2%	2.06 (1.25-3.40) p = 0.005	28%	0%
<b>MSL</b>	54.8%	50.0%	2.38 (1.33-4.28) p = 0.004	18%	7%
<b>LAR</b>	74.3%	53.8%	2.24 (1.22-4.14) p = 0.01	12%	8%
<b>UNL</b>	76.4%	71.8%	1.36 (0.80-2.33) p = 0.26	20%	30%

**Conclusions:** In the presence of adjuvant AC, TNBC molecular subtypes have varied prognosis, with BL1 subtype demonstrating the best prognosis and MSL and LAR subtypes demonstrating the worst prognosis. LAR subtype is associated with older age at

diagnosis and continued elevated hazard function for recurrence after year 5. *tBRCA1/2* mutations are distributed across all subtypes with the highest prevalence in BL1 and M subtypes. IM+ status was infrequently noted in non-BL1 subtypes. These findings underscore TNBC heterogeneity and the need to account for this heterogeneity in prospective clinical trials.



## 2018 San Antonio Breast Cancer Symposium ®

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Discordance between surrogated intrinsic subtypes defined by immunohistochemistry compared with PAM50 in ER positive / HER2 negative early breast cancer. Analysis of value of the status of the progesterone receptor and Ki67

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**Introduction:** Classification by intrinsic subtypes by gene expression profiles of early-stage breast cancer (EBC) provides information of prognostic value and constitutes a tool to help in making therapeutic decisions. Several authors have proposed surrogated classifications based on immunohistochemistry results (IHC) in order to facilitate a classification with identical prognostic and predictive value. However, there is evidence that suggests a lack of correlation between these classifications. The aim of this study was to evaluate the correlation between classification by intrinsic subtypes in patients with EBC ER+/HER2neg, obtained by PAM50 and the surrogated classification proposed by St. Gallen 2013.

**Methods:** Samples from 12 centers from the spanish region of Castilla y León were analyzed by PAM50 (nCounter™ / Nanostring) at the University Hospital of Salamanca. The results obtained were compared with the surrogate classification of St.Gallen'13 from local reports. Tumors of patients pre and post-menopausal tests T1-2, N0-N1mi, grade I-II that met criteria for inclusion of the regional evaluation program through Prosigna™ were included.

**Results:** Between August'15 and December'17, 264 samples were analyzed. All patients were classified by IHC as lum-A or lum-B. In total 113 cases were reclassified by PAM50 (43%). The change of LumA by IHC to Lum-B by PAM50 was 18%, while Lum-B by IHC to Lum-A by PAM50 was 58% (n = 89). In those cases considered Lum-B by IHC based only in a value of Ki67>14% (n = 93), 54% was reclassified to Lum-A. Conversely, when low expression (negative or <20%) of Progesterone Receptor (PR) was used as the single criterion of Lum-B by IHC (n = 44), PAM50 reclassified 33 cases as Lum-A (75%). Applying the Kappa test to analyze the concordance between the 2 tests, a coefficient of 0.203 (low agreement) was obtained, statistically significant (0.000).

**Conclusion:** The surrogated classification by IHC of intrinsic subtypes in EBC ER+/HER2neg shows a low concordance with PAM50 analysis, and cannot be considered adequate. In particular, the presence of negative or <20% PR as the only criterion seems the least appropriate and should not be recommended for a surrogated classification of a tumor as Lum-B, overestimating the real risk of numerous patients. PAM50 allowed the reclassification in more than 40% of cases, especially csaes considered Lum-B by IHC.

# 2018 San Antonio Breast Cancer Symposium ®

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Genomic progression, detected by circulating tumor DNA (ctDNA) sequencing, as an early predictor of disease progression in metastatic breast cancer (MBC)

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**Background:** The availability of multiple therapies has transformed the landscape of MBC, but also brought the challenge of selecting the right therapy for an individual patient. Furthermore, in patients with Hormone Receptor positive (HR+) breast cancer who have bone metastases only it may be difficult to assess effectiveness of therapy via imaging. Peripheral ctDNA detection and analysis by next-generation sequencing (NGS) has gained popularity in cancer diagnosis and therapeutics due to its relative noninvasiveness, ease of use, and high sensitivity. Here, we explore the utility of ctDNA change as a predictor for disease progression in MBC. We hypothesized that genomic progression is a harbinger of subsequent radiologic progression in patients with MBC.

**Methods:** We analyzed change from pre-treatment (baseline) to on-treatment ctDNA mutant allele fraction (MAF) among patients with MBC. Patients receiving standard-of-care therapies or investigational agents on clinical trials at our institution were included. All patients were followed from the date of baseline test until death or data cutoff (6/20/2018). All peripheral blood specimens were collected and analyzed between 1/7/2016 and 3/1/2018 via NGS (Guardant360®). Peripheral blood specimens were sequenced prior to initiation of a new therapeutic regimen (baseline) and subsequently at least once while on-treatment, on average 4-12 weeks later. All patients had a follow-up CT scan of chest, abdomen and pelvis 2-4 weeks after the on-treatment NGS. A priori, we defined genomic progression as increase in ctDNA total MAF of at least 20% from baseline. We utilized Cox regression analysis to identify whether genomic progression was a predictor of radiologic progression, adjusting for common prognostic variables.

**Results:** All patients (N= 77) were female, predominantly White (83.1%), and median age was 57 (range 32 to 77). Fifty one out of 77 patients (66.2%) were ER+, 5 HER2+, and 9 had triple negative breast cancer. The median MAF at baseline was 2.2% (range 0% - 61.7%). Common genomic alterations in ctDNA included PIK3CA, TP53, ESR1, AKT1, NF1. 27 out of 77 (35%) patients showed disease progression on the first subsequent CT scan, while 59 out of 77 (76.6%) progressed during the follow up time. We found that an increase in ctDNA MAF of at least 20% was a strong predictor of disease progression (HR =2.46, CI [1.14-5.32], p=0.02), compared to those who had a MAF increase of less than 20% or a decrease in total MAF. In multi-variable analysis, adjusting for age, number of prior therapies, type of therapy, and visceral metastases, increase in ctDNA remained a significant predictor for subsequent disease progression (HR =3.84, CI [1.63-9.07], p=0.002). Subset results in patients with bone metastases only, and relative comparison of ctDNA with standard tumor markers will be presented at the meeting.

**Conclusions:** Genomic progression, identified by an increase in ctDNA MAF, is potentially an early predictor of subsequent disease progression in patients with MBC. Further research is needed to prospectively evaluate the clinical utility of ctDNA change as a surrogate marker in guiding treatment decision-making for patients with MBC.

# 2018 San Antonio Breast Cancer Symposium ®

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DNA methylation markers predict recurrence-free interval in triple-negative breast cancer

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**BACKGROUND.** Chemotherapy remains the treatment mainstay for triple-negative breast cancer (TNBC). Nevertheless, randomized trials have shown that not all TNBC require it, nor does it benefit all patients that receive it. Molecular tools to risk-stratify TNBC are currently lacking. In light of the importance of epigenetic processes modulating gene expression, we performed an array-based genome-wide DNA methylation search in well-documented institutional and clinical trial cohorts of TNBC for markers that can distinguish breast cancers with a favorable natural history from those with a high risk of recurrence. **METHODS.** We performed an array-based genome-wide DNA methylation survey of well-documented institutional and clinical trial cohorts of TNBC and conducted molecular marker discovery on institutional TNBCs (115 patient samples; 53 recurrences) treated by locoregional therapy (LRT) alone. The identified hypermethylated gene signatures were then tested in a TNBC cohort (50 patient samples; 16 recurrences) from the no chemotherapy arms of IBCSG trials VIII and IX, and in a separate combined cohort of TNBCs (131 patient samples; 33 recurrences) treated with chemotherapy from an institutional repository and from IBCSG trials VIII and IX. Cross platform validation was conducted using quantitative multiplexed methylation specific PCR (QM-MSP) on hypermethylated markers in samples from both the Discovery Set and IBCSG LRT Test Set.

**RESULTS.** We identified methylation signatures in the discovery cohort consisting of 100 or 30 CpG probes that discriminated patients who remained recurrence-free from those with recurrent disease. These signatures were then tested in the IBCSG no chemotherapy cohort, and we found that hypermethylation was associated with shorter recurrence-free interval (RFI). A significant association of both 100 CpG ( $P < 0.0001$ ) and 30 CpG ( $P = 0.0021$ ) signatures with shorter RFI was found in the combined institutional and IBCSG chemotherapy cohort. We observed an enrichment of methylation probes residing on chromosome 19, particularly within 19q13.41-43, that significantly correlated with RFI following chemotherapy. QM-MSP results reflected that of the methylation array [Spearman correlation coefficient of  $r = 0.495$  ( $P = 0.0009$ )] indicating that the relationship between high methylation and short RFI is detectable independent of analytical platform. We also observed enrichment for Chromosome 19-specific probes within the 100 and 30 probe sets. While only 5% of all CpG markers are located within Chr19, 15% of the 100 CpG set, 37% of the 30 CpG set, and 47% of the 17 CpGs that are statistically significantly correlated with RFI in the chemotherapy group reside on the Chr19, mostly within 19q13.41-43.

**CONCLUSIONS.** Methylation markers may be of prognostic importance in TNBC and our findings should be validated in additional clinical trial cohorts.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-10

MammaPrint identifies 46% of patients, age  $\leq 50$  years with oncotype RS 18-30, as low risk and safe to forgo chemotherapy

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**Background:** The PROMIS trial (NCT01617954) previously showed that an OncotypeDx (ODx) Intermediate Recurrence Score (RS 18-30) led to uncertainty in prescribing chemotherapy (CT), especially in the middle of the intermediate range from RS 21-26 where an equal number of patients were recommended to receive and forego CT (Tsai, JAMA Oncology 2018). Forty-seven percent (3183/6711) of randomized TAILORx patients were classified as RS 18-25 and are well represented in PROMIS. These patients with RS 18-25 may still lack definitive CT recommendation following TAILORx, reflexing to age and menopausal status to make a decision. Here, we re-evaluate PROMIS using the subgroup analyses adopted by TAILORx. **Methods:** MammaPrint (MP) risk of recurrence was determined for ODx intermediate patients by standard diagnostic testing (Agendia, Irvine, CA). Clinical risk was assessed using the MINDACT, modified Adjuvant Online! algorithm (Cardoso, NEJM 2016). The MP high and low risk classification, and patient and tumor characteristics were re-evaluated and subdivided by RS 18-25 vs. RS 26-30. **Results:** The 840 eligible patients in PROMIS were classified as 61.3% (515/840) clinically low risk and 37.0% (311/840) clinically high risk (including 84 lymph node positive patients). Half (342/684) of all patients with an RS 18-25 and 20.5% (32/156) patients with RS 26-30 were MP low risk.

## MammaPrint Risk by RS and Age

MammaPrint Risk	RS 18-25			RS 26-30			Grand
Classification	$\leq 50$ yrs	$> 50$ yrs	All Ages	$\leq 50$ yrs	$> 50$ yrs	All Ages	Total
High Risk	74	268	342	23	101	124	466
Low Risk	80	262	342	4	28	32	374
All	154	530	684	27	129	156	840

There was no significant difference in the distribution of MP risk in women age  $\leq 50$  yrs vs.  $> 50$  years (Yates chi-square  $P=0.62$ ); MP classified 46.4% (84/181) patients age  $\leq 50$  yrs and 44.0% (290/659) patients age  $> 50$  yrs as low risk. In the clinically-low risk subset of 515 patients, there was also no significant difference in the distribution of MP risk by age (Yates chi-square  $P=0.89$ ); MP classified 48.3% (56/116) patients age  $\leq 50$  yrs and 49.6% (198/399) patients age  $> 50$  yrs as low risk. **Conclusions:** In light of TAILORx and uncertain CT benefit in women  $\leq 50$  yrs, MammaPrint provides a definitive high or low risk answer and identifies 46% of these women who may safely forego CT based on MINDACT data. An analysis of young patients in the MINDACT trial showed that MP low risk patients age  $< 45$  yrs and 45-55 yrs had very good 5-yr DMFS of 95-98%, in both clinically low and high risk groups (Alders, SABCS 2017).

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First prospective outcome data for the clinico-molecular test Endopredict® in hormone receptor positive, HER2-negative early breast cancer in clinical routine

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**Background:** The EndoPredict test is a clinico-molecular test that has been validated to predict the likelihood of distant metastases and late relapse in patients (pts) with hormone receptor positive (HR+), HER2-negative (HER2-) early breast cancer and up to three positive lymph nodes. However, so far prospective outcome results of pts in whom decision on use of adjuvant chemotherapy (CTX) was based on Endopredict has not been available. Here we present three year outcome data of pts, whose adjuvant systemic therapy recommendation was based on EndoPredict test result.

**Methods:** Pts with HR+, HER2- early breast cancer with 0-3 positive lymph nodes were enrolled at a single institution (Interdisciplinary Breast Center of Klinikum rechts der Isar, Munich, Germany). The Endopredict test was carried out on all tumor samples. Demographic, clinical and pathological data as well as EPclin risk class were assessed for each patient at baseline. Therapy recommendations were given during an interdisciplinary tumor board. Pts were evaluated for treatment compliance, local recurrence, distant metastases and survival (cut-off date of last follow up: July 31st 2017). Censored time-to-event outcomes were analysed by cox proportional hazards models. Additional estimates of the event-free-survival were given by the Kaplan-Meier method. Hypothesis testing was conducted on two-sided exploratory 5% significance levels.

**Results:** A total of 373 consecutive pts were enrolled between March 2012 and March 2015. Median age was 59.9 (range: 29.1-88.9) years. In 39% of pts tumor size was >2cm, 24% of pts were node positive, 16% had G3 tumors, in 21% of pts ki67 was ≥25% and in 13% progesterone receptor was <10%. The EndoPredict test allocated 238 pts (63.8%) in the low-risk group and 135 pts (36.2%) in the high-risk group. 128 of the 373 pts were recommended to undergo adjuvant CTX in addition to endocrine therapy. 92 (72%) of these pts were compliant having received standard of care chemotherapy. After 41.6 months median follow up, 3-year disease free survival (DFS) and distant metastases free survival (DMFS) in the EPclin low risk group was 96.6% (95%CI 94.2-99.1) and 99.6% (95%CI 98.7-100) versus 94.9% (95%CI 90.9-99.0) and 97.6% (95%CI 95.0-100) in the EPclin high risk group. With a hazard ratio (HR) of 2.05 (95%CI 0.85-4.96; p= 0.110) risk for any disease recurrence or death in EPclin high risk patients was two fold higher in comparison with EPclin low risk patients. Patients with EPclin high risk were at significant higher risk of experiencing distant metastases than patients with EPclin low risk (HR 5.18; 95%CI 1.04-25.74; p=0.0443). EPclin high risk patients who actually underwent recommended adjuvant CTX had a 3-year-DFS of 96.3% (95%CI 92.2-100) and were at lower risk for death or recurrence than those EPclin high risk patients without CTX (3-year-DFS: 91.5% (95%CI 82.7-100); HR 0.32; 95%CI 0.10-1.05; p=0.061).

**Conclusion:** These first prospective outcome results show, that EPclin in clinical routine is a valid clinico-molecular marker to predict DFS and guide decision of adjuvant CTX use in HR+, HER2- early breast cancer pts with 0-3 positive lymph nodes.

# 2018 San Antonio Breast Cancer Symposium ®

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Comparative analyses of the prognostic value of oncotype and mammaprint using the National Cancer Database

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**INTRODUCTION:** Majority of the approximately 40,610 deaths from metastatic breast cancer in the US each year occur in women with hormone receptor positive breast cancer who recur after treatment for early stage disease. Genomic analysis is increasingly used to personalize breast cancer treatment for women with early breast cancer resulting in AJCC 8 modification of TNM staging. The 70-gene Mammaprint was developed using both ER- and ER+ breast tissue samples, while the 21-gene Oncotype DX (ODX) assay was developed using only ER+ breast tissue. Previous studies found that the two genomic assays gave discordant testing results.

**OBJECTIVE:** To compare the performance of Mammaprint and Oncotype DX in assigning prognosis in early stage hormone receptor positive breast cancer.

**METHOD:** A retrospective cohort of women diagnosed with early stage, hormone receptor positive breast cancer who received ODX or Mammaprint was established using the National Cancer Data Base (NCDB), 2010-2014. Using the propensity score matching method, we defined two groups of patients with similar clinical and demographic characteristics; one group received ODX and another received Mammaprint. The groups were matched by clinicopathologic and demographic factors. We examined the association between ODX or Mammaprint and overall survival using Log-rank test and Cox models in the two groups separately. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated as strength of association. The prognostic values were evaluated using c-index (i.e. area under ROC curve).

**RESULTS:** Of 320,276 eligible patients with breast cancer, 41.5% received ODX and 1.3% received Mammaprint testing. The use of ODX increased from 34.3% in 2010 to 45.2% in 2014, while the use of Mammaprint increased from 0.5% in 2010 to 2.0% in 2014. After propensity score matching, we identified 3319 patients who received ODX and matched to 3319 patients who received Mammaprint. Compared to patients with a low risk Mammaprint score (n=1915), patients with a high risk Mammaprint score (n=1404) had 4.53-fold increased risk of dying (95% CI 2.79-7.36). The c-index for Mammaprint was 0.683. Relative to patients with a low ODX recurrence score (n=1927), the HR for intermediate ODX score was 1.23 (95% CI 0.76-1.98) and the HR for high ODX score was 3.62 (95% CI 2.21-5.94). The c-index for ODX was 0.601. In patients with ODX testing, 28.2% received chemotherapy. In patients with Mammaprint testing, 42.4% received chemotherapy. Based on MINDACT's modification of Adjuvant!Online, 49.2% patients were assigned to the clinical high risk group, including 22.3% to the clinical high risk/genomic low risk (C-high/G-low) subgroup and 26.9% to the C-high/G-high subgroup. The percentage of patients receiving chemotherapy with C-low/G-low, C-low/G-high, C-high/G-low, and C-high/G-high were 4.3%, 70.4%, 32.2%, and 84.9%, respectively.

**CONCLUSION:** The findings from our preliminary study suggest that Mammaprint may achieve better separation of high risk from low risk patients. However, it is possible that having more genes in multigene assays would better capture the heterogeneity of hormone receptor positive breast cancer and guide choice of optimal systemic therapy to reduce risk of metastases.

# 2018 San Antonio Breast Cancer Symposium ®

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Prognostic significance of CD8+ tumor-infiltrating lymphocytes (TILs) in patients with early breast cancer (EBC) treated with dose-dense sequential adjuvant chemotherapy (dds-CT). An observational study (ACTRN12616001043426)

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**Background - aim:** Information on the prognostic role of cytotoxic CD8+ T cells in the era of modern adjuvant CT is limited. The primary objective of the present report is to assess the prognostic impact of CD8+ cells in patients with intermediate or high-risk EBC (T1-3N1-2M0) treated with dds-CT. Secondary endpoints are safety, disease-free survival (DFS) and overall survival (OS).

**Patients and Methods:** Patients (N=1,000) were treated with 4 cycles of Epirubicin, 75mg/m<sup>2</sup>, and Cyclophosphamide, 600mg/m<sup>2</sup> every 2 weeks followed by 4 cycles of Docetaxel (D), 100mg/m<sup>2</sup> every 3 weeks with G-CSF support in all cycles. Trastuzumab was initiated concurrently with D and continued for a total of 1 year. Hormonal and radiation therapy were given post CT, as indicated. Formalin-fixed paraffin-embedded tumors were available for 642 patients (64.2%) and were centrally assessed for immunohistochemical subtypes (IHC4; N=526), stromal TILs density by morphology (N=636), as well as stromal and intratumoral cytotoxic CD8+ T cell numbers (N=554). TILs and CD8+ were assessed as continuous variables for associations and as 10% increments for outcome.

**Results:** In total, 901/1,000 pts (90.1%) completed 8 cycles of CT. Severe (grade III-IV) toxicities included neutropenia (5.6%), leucopenia (3.6%), lymphopenia (2.1%), hand-foot syndrome (2.1%), and hepatotoxicity (1.8%). Febrile neutropenia occurred in 1.6% of the patients. The 5-year DFS and OS rates were 89.5% and 93.1%, respectively. Luminal A tumors were classified in 26.2%, Luminal B in 35.2%, luminal HER2 in 9.5%; HER2-enriched in 7.2%; and, triple-negative (TNBC) in 21.9% of informative patients. Among subtypes, stromal TILs density was higher in HER2-enriched and TNBC (p<0.001); intratumoral CD8+ values were higher in TNBC (p<0.001); and, stromal CD8+ were higher in HER2-enriched (p=0.034). In all patients, TILs density and intratumoral CD8+ cell numbers were not associated with DFS and OS, while increased stromal CD8+ were marginally associated with prolonged DFS (HR=0.98, 95%CI 0.96-1.00, p=0.066). Adjusted for histological grade, menopausal, ER/PgR and nodal status, higher stromal CD8+ were associated with prolonged DFS (HR=0.98, 95% CI 0.96-1.00, p=0.043). In TNBC, higher stromal TILs density conferred prolonged DFS (HR=0.97, 95%CI 0.94-0.99, p=0.029), which retained its prognostic significance in multivariate analysis (HR=0.97, 95% CI 0.94-1.00, p=0.049).

**Conclusions:** In this study, dds-CT was well tolerated and active in patients with EBC. We confirm the presence of morphologically assessed higher TILs density, and of higher cytotoxic CD8+ T cell numbers in hormone receptor negative EBC, as well as the favorable prognostic impact of higher stromal TILs density in TNBC. In comparison to stromal TILs density, higher stromal CD8+ may confer favorable prognosis irrespectively of EBC subtype. Stromal CD8+ seems to be a marker worth further standardizing for reporting on immune cell infiltrates in EBC.

## 2018 San Antonio Breast Cancer Symposium ®

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Invasive micropapillary carcinoma had no difference in prognosis compared with invasive ductal carcinoma: A propensity-matched analysis

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**Background:** Invasive micropapillary carcinoma (IMPC) is a rare histopathological variant of breast carcinoma and usually performs poor clinical characteristics, such as high tendency of lymph nodes metastases. But whether it has worse prognosis than invasive ductal carcinoma (IDC) is still controversial nowadays. We conducted this retrospective study to figure out the prognostic difference between IMPC and IDC, then guide therapy of IMPC ultimately.

**Methods:** In this study, we analyzed 327 cases of IMPC patients and 4979 cases of IDC who underwent primary resection in our institution during 2008 to 2012. By using propensity score matching, two groups were matched at a ratio of 1:1 by age, tumor size, nodal status, hormone and HER2 status to demonstrate the difference of prognosis assessed by Kaplan-Meier estimates and Cox regression analysis.

**Result:** After a mean follow-up of 52 months, we established the IMPC group and figured out 324 IDC patients from the control group by propensity score matching (3 IMPC patients were canceled because of data missing). The result of survival analysis indicated that women diagnosed with IMPC had no significant reduced overall survival (OS) ( $p = 0.752$ ) and disease-free survival (DFS) ( $p = 0.578$ ) compared with women with IDC. Multivariate Cox regression analysis revealed that IMPC was not found as an independent prognostic factor for DFS (hazard ratio [HR] = 0.858; 95% confidential interval [CI], 0.419-1.757) or OS (HR = 0.720; 95%CI, 0.353-1.469).

**Conclusion:** The consequence of survival analysis manifested that there was no statistically significant difference between 2 groups, and elucidated proactive or radical clinical therapy was unnecessary.



# 2018 San Antonio Breast Cancer Symposium ®

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Locoregional recurrence in invasive breast cancer and association with tumor infiltrating leukocyte (TIL) presence

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## **Background:**

The presence of TILs has been correlated with clinical outcomes and response to therapy in breast cancer. However, evaluation of TILs in breast cancer has largely been based on pathologic examination of tumor samples. Here, we report the relationship between invasive breast cancer locoregional recurrence (LRR) and the presence of TILs estimated by transcriptomic analysis with the deconvolution algorithm CIBERSORT.

## **Methods:**

Patients were identified from an IRB-approved prospective tissue collection protocol at one academic institution and two community hospitals. 526 primary breast tumor samples were identified and gene expression profiling was assessed with high density Affymetrix microarray chips. Proportions of 22 different TIL types in samples were inferred based on the CIBERSORT algorithm, which uses gene expression data to estimate TIL presence. TIL presence was determined by dichotomization at the level of the first quartile among all samples (>Q1=TIL presence). Patient characteristics and clinical outcomes were obtained by chart review. Time to event analysis was performed using Kaplan Meier (KM) estimates and the log-rank test. Associations between patient factors, tumor factors, TIL presence, and LRR were explored with univariable (UVA) and multivariable (MVA) analyses. Factors significant on UVA ( $p < 0.10$ ) were included on MVA.  $P < 0.05$  was considered statistically significant on MVA.

## **Results:**

526 women with invasive breast cancer and available genomic profiling were retrospectively identified for analysis. Median age at diagnosis was 58 years. 70% of tumors were Stage I-II. 69% were luminal subtypes and 17% were triple negative. 37% received mastectomy, 25% received mastectomy + radiation, and 32% received breast conserving therapy. 64% received chemotherapy, and 62% received hormonal therapy. Median follow-up was 74.4 months. There were 61 LRRs. We found significant differences in time to LRR when comparing presence vs. no presence of resting memory CD4+ T-cells (RMCD4+) ( $p = 0.01$ ), activated natural killer cells (ANK) ( $p = 0.003$ ), and neutrophils (PMNs) ( $p = 0.03$ ). On UVA, factors associated with LRR were patient age at diagnosis ( $p = 0.009$ ), pathologic T stage ( $p = 0.045$ ), Estrogen receptor status ( $p = 0.03$ ), biologic subtype ( $p = 0.01$ ), lymphovascular invasion (LVI) ( $p = 0.018$ ), positive margins ( $p < 0.0001$ ), receipt of hormonal therapy (0.014), and presence of tumor infiltrating RMCD4+ ( $p = 0.012$ ), ANK ( $p = 0.0004$ ), and PMNs ( $p = 0.033$ ). On MVA, factors remaining significant were LVI (HR 2.16 CI 1.13-4.13,  $p = 0.011$ ), positive margins (HR 4.36 CI 1.57-12.11,  $p = 0.018$ ), receipt of hormonal therapy (HR 0.31 CI 0.12-0.77,  $p = 0.042$ ), and presence of RMCD4+ (HR 0.48 CI 0.26-0.88,  $p = 0.017$ ), ANK (HR 0.43 CI 0.23-0.83,  $p = 0.012$ ), and PMNs (HR 2.15 CI 1.02-4.53,  $p = 0.043$ ).

## **Conclusion:**

In this study of 526 women with invasive breast cancer, we identified that enrichment of certain TILs is associated with LRR. These results suggest genomic-based assays of TIL presence may be useful to predict LRR in invasive breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Validation of the AJCC eighth edition prognostic stage compared with the anatomic stage for breast cancer with a Japanese single-institutional cohort

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**Background:** The American Joint Committee for Cancer (AJCC) 8th edition cancer staging system for breast cancer incorporated biologic factors in addition to the 7th edition anatomic stage. We analyzed how the new AJCC 8th edition prognostic stage refined its stratification compared with the anatomic stage.

**Methods:** We reviewed the data of 4,134 patients with stage I to III breast cancer who underwent surgery at Tokyo Metropolitan Komagome Hospital between 2000 and 2016. The anatomic stage and prognostic stage were re-staged according to the AJCC 8th edition staging manual. Patients who received neoadjuvant chemotherapy or had bilateral breast cancer and those with unknown clinicopathologic factors were excluded. The 21-gene Oncotype DX breast recurrence score was not used for staging in this study.

**Results:** A total of 2,469 patients with a median follow-up of 4.7 years (range 0.1-15.5 years) were identified. According to the anatomic stage, there were 1,259 patients of stage IA, 132 of IB, 591 of IIA, 206 of IIB, 130 of IIIA, 14 of IIIB and 73 of IIIC. According to the prognostic stage, there were 1,610 patients of stage IA, 331 of IB, 236 of IIA, 73 of IIB, 85 of IIIA, 43 of IIIB and 27 of IIIC. Sixty-four patients (2.6%) could not be assigned using the new staging system for the presence of micrometastases in lymph nodes with tumors larger than 2 cm. The 5-year disease-free survival (DFS) rates according to the anatomic stage were 97.4% for stage IA, 97.1% for IB, 95.8% for IIA, 86.5% for IIB, 77.9% for IIIA, 49.2% for IIIB and 54.9% for IIIC. According to the prognostic stage, the 5-year DFS rates were 97.9% for stage IA, 92.9% for IB, 91.2% for IIA, 79.8% for IIB, 67.4% for IIIA, 53.3% for IIIB and 38.7% for IIIC. Compared with the AJCC anatomic stage, the prognostic stage was increased in 148 patients (6.2%) and decreased in 808 patients (32.8%). For those in whom the stage changed, the change was by one stage up or down in 463 (19.3%), by 2 stages up or down in 401 (16.7%) and by 3 stages up or down in 92 (3.8%). Of the 1,842 patients with hormone receptor (HR)-positive and human epidermal growth factor 2 (HER2)-negative (HR+/HER2-) disease, 40.5% (745/1842) of cases were downstaged, and 0.7% (1/1842) were upstaged.

**Discussion:** The AJCC 8th edition prognostic staging system provided more refined stratification than the anatomic stage. In the Japanese cohort, the proportion of the downstaging rate was higher than the upstaging rate, and the prognostic evaluation of HR+ patients in particular was improved.

# 2018 San Antonio Breast Cancer Symposium ®

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Expression score (Escore) for the prediction of likelihood of recurrence of DCIS

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**Background:** Ductal carcinoma in situ (DCIS) accounts for at least 20% of breast cancers. Factors associated with recurrence of DCIS or progression to invasive carcinoma are not well delineated. The goals of the current study were to profile the epithelial cells using the GE Cell DIVE™ immuno-fluorescent based analyses. This was coupled with semi-automated algorithms to characterize the inter-relationships between cell populations and likelihood of recurrence.

**Patients and Methods:** A TMA-based (total 8 TMAs) cohort of cases of DCIS with and without recurrence was obtained from Oxford University. Recurrence in this cohort was defined as ipsilateral DCIS, ipsilateral invasive, contralateral invasive and metastatic. Analysis for 31 epithelial markers (HER4, CK56, ABCG2, PTEN, S6, CKAE1, PR, ER, NaKATPase, CK19, ALDH1, CK PCK26, cMET, CD44v6, HER2, CDCP1, p53, CK15, COX2, VEGFR2, ABCb1, HTF9C, CD10, MRP4, CEACAM5, EGFR, p21, MRP5, SLC7A5, Ki67, DAPI) was performed on a single FFPE TMA section containing cases of DCIS. Briefly, FFPE sections from TMAs containing DCIS were sequentially (cyclically) stained for the markers. Each cycle entailed staining with 2-3 markers followed by imaging, dye inactivation, and re-staining. DAPI was used for nuclear demarcation and for registration of the images, while S6, pan-cadherin, Na+K+ATPase and pan-cytokeratin were used for epithelial segmentation. K-means clustering followed regression analysis was performed to identify inter-relationships between markers and association with likelihood of recurrence. Log-rank analysis was performed and the relapse-free survival data depicted using Kaplan Meier plots. Escore was developed by logistic regression model, classification model on recurrence

**Results:** Filtering of the expression analysis by the quality, specificity, compartment localization and fields entirely composed of DCIS, in addition to availability of clinical data resulted final analysis of 31 markers in 67 cases. Correlation analyses were performed on each of the markers to identify markers that were significantly correlated in univariate analysis. K-means cluster analysis was performed using a set of 4 markers (ER, HER2, SLC7A5 and cMET) to identify 6 clusters. High cMET (cluster 1; low HER2 and SLC7A5) and High ER (low cMET, HER2, SLC7A5; Cluster 5) were associated with low risk of recurrence (p values 0.014 and <0.0001). In contrast, Cluster 2 (High HER2, high SLC7A5, low ER) and Cluster 3 (High HER2, low ER, SLC7A5 and cMET) were associated with increased risk of recurrence (P values 0.038 and 0.076). A regression analysis based algorithm was developed using these markers to calculate a numerical score which could predict likelihood of recurrence. As depicted in the KM plots, the HR for recurrence increases significantly (P-value 2.4E-05; p=0.02 with LOOCV) with increase in expression score (Escore).

**Conclusions:** We describe the development of an Escore using expression 4 markers to predict likelihood of recurrence. Additional ongoing studies will seek to validate the utility of the Escore in predicting likelihood of recurrence of DCIS and development of invasive carcinomas and comparison with other scoring systems.

# 2018 San Antonio Breast Cancer Symposium ®

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Tumor size still impacted prognosis in breast cancer with extensive nodal involvement

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## Background and purpose

Because of screening and early diagnosis, the stage of breast cancer patients has been brought forward earlier when originally diagnosed. Nevertheless, 10-15% breast cancer patients were diagnosed with extensive nodal involvement in US. Although the size of the tumor and the nodal status are the most important prognostic factors, it is believed that nodal status outperformed the tumor size as a prognostic factor. Particularly, when patients have a nodal stage higher than N2 (more than 9 positive lymph nodes), it is well accepted that tumor size lost its prognostic value. Even in the newest AJCC staging system, which included molecular subtype as an important prognostic factor, T1-3N2 patients were still categorized as the same population. So were T1-4N3 ones. So this study aims to investigate the prognostic value of tumor stage(T stage) in patients with extensive nodal involvement and the survival comparison of T4NxM0 and TxN3M0.

## Patients and methods

Breast Cancer female patients with 9 or more positive lymph nodes or with T4 tumors were identified in the SEER registry between 2010-2015. The effect of T stage on BCSS (breast cancer specific survival)and the survival comparison of T4NxM0 and TxN3M0 using Kaplan-Meier survival curve method and risk adjusted Cox proportional hazard regression modeling were assessed.

## Results

Overall, 21697 women with N2-3 tumors were included from 284073 patients. T stage, Nodal stage (N stage), ER, PR, HER2 and grade were all independent prognostic factors ( $p < 0.001$ ). HR of ER/PR/HER2/grade/N stage were 0.662 (0.595-0.738), 0.488 (0.438-0.543), 0.541 (0.489-0.598) ,1.534 (1.293-1.418) and 1.551 (1.435-1.676). Notably, HER2 positivity was correlated with better BCSS, possibly due to the widely adoption of anti-HER2 therapy. Using T1 as reference, HR of T2/T3/T4 were 1.363 (1.200-1.548), 2.092 (1.824-2.399) and 3.497 (3.045-4.017), respectively

multivariate survival analysis by Cox proportional hazard regression modeling

	P value	HR	95%CI	
			lower limit	upper limit
T	<0.001			
T2 vs. T1	<0.001	1.363	1.200	1.548
T3 vs. T1	<0.001	2.092	1.824	2.399
T4 vs. T1	<0.001	3.497	3.045	4.017
N	<0.001	1.551	1.435	1.676
Grade	<0.001	1.354	1.293	1.418
ER	<0.001	.662	.595	.738
PR	<0.001	.488	.438	.543
HER2	<0.001	.541	.489	.598

HR: Hazard Ratio; CI: Confidence Interval

.And the same results held true when subgroup analysis according to N stage were conducted. In the two subgroups, that was T1-3N2 and T1-4N3 women, T Stage was also a significant negative prognostic factor independent of ER/PR/HER2/grade. Moreover, 8328 women staged as T4 with different nodal status were also identified from the whole database. When we compared T4Nx with TxN3 head to head, it was found that T4 tumors had worse outcomes than N3 ones, independent of other prognostic factors. If molecular subtype was included in subgroup analysis, we found that the survival could not be distinguished

between T4 and N3 only in TNBC.

**Conclusions:**

In patients with extensive nodal status, tumor stage is still a prognostic factor, independent of other factors such as ER/PR/HER2/grade. As to patients with T4Nx or TxN3 tumors, T4 tumors have worse outcomes than N3 ones, independent of other prognostic factors. AJCC staging system might be slightly modified due to these outcomes.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-19

Progression-free survival or time to progression in comparative clinical trials of metastatic breast cancer as a potential surrogate for overall survival: A systematic review of 49 trials focusing on breast cancer subtype

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**Background:** Overall survival (OS) is the established endpoint to evaluate the effects of drug treatment in comparative clinical trials of metastatic breast cancer. But assessing OS requires long follow-up periods and large sample size, which raise costs and create long delays in the drug approval process. Progression-free survival (PFS) or time to progression (TTP) is considered as a surrogate for OS and is often used as an alternative to OS. In some cancers the two endpoints are highly correlated, but in others they are not. Furthermore, the effect of breast cancer (BC) subtypes on the surrogacy of PFS/TTP for OS has not been completely defined.

**Method:** A systematic literature review of randomized control trials was conducted to identify studies that reported both the hazard ratio (HR) of PFS/TTP and OS for BC subtypes (i.e. estrogen receptor (ER) positive, HER2 positive, and triple negative (TN)). The correlation between the HR of PFS/TTP and OS was evaluated using weighted Spearman's rank correlation.

**Results:** A total of 49 trials (34 phase III trials and 15 phase II trials) were selected for analysis. Among these trials, there were 8 comparison trials between one chemotherapy and another chemotherapy regimen, 18 comparison trials between chemotherapy and chemotherapy plus molecularly-targeted therapy, 9 comparison trials between one endocrine therapy and another endocrine therapy, and 5 comparison trials between endocrine therapy and endocrine therapy plus molecularly-targeted therapy. There were 17 trials reporting the HR of PFS/TTP and OS for ER positive, 16 trials for HER2 positive, and 9 trials for TN BC. Weighted Spearman's rank correlation revealed that coefficient between the HR of PFS/TTP and OS was 0.721 ( $p < .0001$ ) for all trials, 0.873 ( $p < .0001$ ) for ER positive, 0.642 ( $p = 0.0055$ ) for HER2 positive, and 0.615 ( $p = 0.078$ ) for TN BC.

**Conclusion:** There was a strong correlation between the HR of PFS/TTP and OS for ER positive BC, and a weak correlation between the HR of PFS/TTP and OS for HER2 positive and TN BC. The validity of using PFS/TTP as an OS surrogate marker was shown for metastatic BC, especially for ER positive BC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-20

Pre-analytical effects of FFPE extraction methods on targeted and whole transcriptome sequencing assays for endocrine sensitivity in metastatic breast cancer

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**Background:** The clinical management of patients with metastatic HR-positive breast cancer is often uncertain due to decreased sensitivity to anti-estrogen therapy over time. Recently, we developed a targeted RNAseq based 18-transcript SET ER/PR assay of endocrine sensitivity from biopsies of metastatic cancer. In this work we assess the effect of pre-analytical factors, specifically RNA extraction methods for FFPE tissue samples, on the reliability of the targeted RNAseq assay.

**Methods:** FFPE blocks and matched fresh frozen (FF) sections from 12 tumors were collected at MD Anderson Cancer Center. RNA from FFPE slides was extracted in duplicate using three kits (Norgen, Qiagen, Roche), and RNAseq libraries from all samples were prepared using Kapa Total RNAseq kit. Targeted RNA libraries were prepared using droplet-based PCR (RainDance), and also by transcriptome-wide RNAseq for comparison. Reads were mapped to genomic sequence using STAR and expression was quantified using RSEM. Expression data were normalized based on expression of 10 reference genes. The effect of FFPE RNA extraction kit on the reliability of the SET index was assessed using linear mixed effects model (LME) analysis, and agreement with FF was assessed using the concordance correlation coefficient (CCC).

**Results:** Analysis of the whole transcriptome RNAseq data confirmed minimal 3'-end transcript bias from FFPE samples, irrespective of transcript size or FFPE kit. All 18 genes included in the SET index had high overall concordance between FFPE and FF (median CCC percentile=98.8, range 57.2-99.9 for Norgen; similar for the other two kits) and relatively consistent bias across genes, as estimated by the random effects of the LME model. Furthermore, compared to random 18-gene indices, concordance in the SET index values between FF and FFPE was higher than 99.8% of the random samples, verifying the analytical reliability of the selected genes. For the targeted RNAseq assay, RNA from FFPE extracted with the Norgen kit showed the highest concordance compared to FF (CCC=0.956, 95%CI 0.871-0.985). In general, the analytical variation of SET from FFPE samples was greater than that from FF (1.71-2.71 fold greater), with the lowest variation associated with the Norgen kit. The SET index values from targeted RNAseq for both FF and FFPE samples were consistently lower compared to transcriptome-wide RNAseq but were highly correlated, with the Norgen kit having the highest correlation between targeted and transcriptome-wide RNAseq ( $\rho=0.915$ ).

**Conclusions:** All three FFPE RNA extraction kits have excellent analytical performance compared to FF samples. The Norgen kit may be marginally better yielding higher concordance with FF and lower analytical variation between replicates. All genes in the SET ER/PR showed very good analytical performance in comparison to random indices and individual genes. Targeted gene RNA sequencing appears very promising as a platform for clinical deployment of quantitative assays, showing only a small (fixable) bias compared to RNAseq.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-21

Gene expression profiling – a decision impact analysis – Decision dependency on OncotypeDX and EndoPredict as a function of oncological work experience

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**Background:** Estimating distant recurrence risk in women with estrogen receptor-positive, human epidermal growth factor receptor 2 (HER2)-negative early breast cancer is still challenging. Oncotype DX and EndoPredict are two competing, gene expression-based tests predicting the likelihood of recurrent disease. We analyzed the difference in oncological decision making with and without the knowledge of gene expression tests.

**Methods:** We performed a retrospective, analysis including n = 192 patients diagnosed with G2, HR+, Her2- breast cancer between 2011 and 2015 at the Municipal Breast Cancer Centre Cologne, Germany. All 192 patients received an evaluation by OncotypeDX or EndoPredict. An oncological tumor board (TB) with knowledge of these results served as baseline (control group). This baseline was compared to the treatment decision (adjuvant chemotherapy Yes vs. No) reached by oncologists with different experience levels (less than 5 years, between 5 and 15 years and more than 15 years) who were not provided the OncotypeDX or EndoPredict scores. All clinicians had access to clinical as well to histopathological data only.

**Results:** Within the EndoPredict group no significant decrease between overall TB decision (adjuvant chemotherapy Yes) 48.1% vs. 15+ years = 39.2%, 5-15 years = 39.2% and <5 years = 50.6% group could be shown. Endopredict seemed to overestimate the clinical risk as judged by experienced oncologists. Within the OncotypeDX cohort we were able to find a significant decrease between overall TB decision (chemotherapy Yes) 41.6% vs. 15+ years = 42.5%, 5-15 years = 50.4% and <5 years = 55.6% group (p<0.05). In addition, inexperience led to a significant and numerically greater increase in chemotherapy recommendation. An exploratory subgroup analysis showed significant differences in TB vs oncologist decision for Ki67 >14%, tumor sizes larger than pT2, pN1 and postmenopausal patients for all experience levels.

**Conclusions:** Overall, results for the EndoPredict group were inconclusive. A significant reduction of chemotherapy recommendation was shown for all experience levels in the Oncotype subgroup however, with a maximum reduction of 14.2%. A subgroup analysis showed that differences in decision making were most likely for patients with a Ki67 >14%, tumor sizes larger than pT2, pN1 and postmenopausal patients. Since these are the patients where the question of pro/contra chemotherapy is most important, it is the opinion of this study group that gene expression testing is especially pertinent for these patients.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-22

Impact of application of AJCC 8<sup>th</sup> edition on survival rate of the breast cancer

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## Background

The AJCC 8 edition has changed much in comparison with the 7 edition. In addition to TNM stage, biologic marker (ER, PR, HER2), Histologic grade and multigene assays (oncotype Dx.) should be considered for staging. and it has been applied since January 1, 2018.

patients were recategorized and analyzed in order to know if this more complex classification helps to predict the real prognosis of the patients,

## Method

We review patients who were diagnosed and treated as breast cancer at Konyang Universty Hospital. we studied retrospectively 582 patients who were followed up and were able to review.

Stage was classified according to AJCC 7th edition and AJCC 8th edition. survival rate of each stage were analyzed in both editions.

## Result

Mean follow up period was 68.6 months. Total 582 patients were included. There was no change in the stage in 257 patients. In 195 patients, the stage was elevated and in 130 patients, the stage was changed down. When classified as AJCC 7<sup>th</sup> edition, the 5year-survival rate was 95.9% in stage I, 97.9% in stage II, 93.1% in stage III and 89.9% in stage IV. The survival rate of patients in stage I was lower than that of stage II. However, when the AJCC 8<sup>th</sup> edition was applied, the 5- year survival rate was 97.9% in stage I, 96.9% in stage II, 92.2% in stage III, and 89.9% in stage IV. In 8<sup>th</sup> edition, the patients in lower stage has higher survival rate.

## Conclusion

The prediction of survival rate by stage was more accurate and the difference in survival rate of each stages was more clearly distinguished when The AJCC 8<sup>th</sup> edition was applied than AJCC 7th edition. AJCC 8th edition was reliable and useful for prediction of prognosis of breast cancer patient.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-23

EndoPredict prognostic signature in pN1mi, estrogen receptor-positive breast cancer: analysis of the French national registry for molecular signatures

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## Background

The EndoPredict (EP) test has been developed and validated for assessing recurrence risk in patients with estrogen receptor (ER)-positive HER2-negative breast cancer (BC). This test is based on the expression of 12 genes (molecular score), combined with clinicopathological criteria (*i.e.* tumor size and nodal status) (EPclin risk score). For node-positive patients, the weight given to the node status in the EPclin score is similar when considering pN1mi (0.2-2mm) or pN1 (>2mm, 1-3 positive lymph nodes). Our aim was to characterize the EPclin classification of the pN1mi subgroup in the French national registry for molecular signatures in ER+ BC.

## Methods

Since April 2016, nine French laboratories using EP test in clinical practice prospectively implement a national registry for molecular prognostic signatures in ER+ BC. We analyzed the pN1mi subgroup with regards to their clinico-pathological characteristics, molecular EP score and EPclin risk score. Using the definition formula of the EPclin score [ $EP_{clin} = 0.35t + 0.64n + 0.28EP$ , with  $n=1$  for pN0, 2 for pN1mi/pN1, 3 for pN2, 4 for pN3], we could calculate a hypothetical EPclin score if the pN1mi had been considered as pN0 [ $n$  factor=1].

## Results

By the end of 2017, the database included 1246 EP tests performed in routine practice, including 67 (5.4%) pN1mi. The pN1mi BC were ER+ HER2- (67/67, 100%), invasive carcinoma of no special type in 52/67 (78%), with a median tumor size of 18 mm (range 7-45; pT1c in 40/67). Among these, 22 were classified as EPclin low and 45 as EPclin high, with a median EP score of 6 (range 2-14), a median EPclin score of 3.70 (range 3-6), and a median relapse risk at 10-year of 15% (range 5-73). Most interestingly, 23/67 (34%; *i.e.* 1.8% of all EP tests performed) pN1mi BC displayed an EPclin score comprised between 3.4 and 4, just above the cut-off value of 3.3: these cases (and only these cases) would have been classified in a different risk category (*i.e.* EPclin low risk) if the node status would have been considered as negative.

## Conclusion

With regards to EndoPredict test in pN1mi BC - and the persistent debate as to whether they should bear the same weight as node-positive (pN1) or negative (pN0) tumors - categorization in the EPclin class is not likely to be impacted by the node factor weight in the vast majority (>65%) of the pN1mi cases and >98% of all patients tested with EP. Only cases with an EPclin between 3.4 and 4 might be impacted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-24

Prognostic significance of preoperative neutrophil-to-lymphocyte ratio in postmenopausal breast cancer patients

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**Background:** Several studies have shown the association between high neutrophil-to-lymphocyte ratio (NLR) and poor prognosis in various cancer types, including breast cancer. On the other hand, menopausal status, which is an important consideration in breast cancer treatment, has been shown to be associated with changes in NLR levels. The purpose of the present study was to clarify the prognostic significance of preoperative NLR, especially in postmenopausal breast cancer patients.

**Patients and methods:** Between October 2003 and December 2014, 1868 women underwent surgery without neoadjuvant systemic therapy for primary breast cancer in our department. The optimal cutoff value of preoperative NLR was determined using receiver operating characteristic curve analysis. The patients were divided into two groups based on the NLR cutoff value of 3.34: High NLR (NLR value  $\geq 3.34$ ) and Low NLR (NLR value  $< 3.34$ ). Correlations between clinicopathological characteristics and preoperative NLR were analyzed, and relapse-free survival (RFS) and overall survival (OS) were estimated.

**Results:** Among the 1868 patients, 286 (15.3%) and 1582 (84.7%) patients were classified into High NLR and Low NLR, respectively. Although the patients in High NLR was younger ( $p=0.0023$ ), there were no significant correlations between NLR and all other prognostic factors, such as tumor size, lymph node status, and histological grade. High NLR was associated with tendency to shorter RFS ( $p=0.0583$ ) and shorter OS ( $p=0.0303$ ). In postmenopausal patients ( $n=1177$ ), significant correlations were observed between High NLR and shorter RFS and OS ( $p=0.0002$  and  $p=0.0011$ ). However, NLR was not correlated with RFS and OS in premenopausal patients ( $n=691$ ). Although the postmenopausal patients with relapsed cancer had higher NLR levels than those without relapse ( $p=0.0252$ ), NLR levels were not correlated with relapse occurrence in premenopausal patients. Both univariate ( $p=0.0009$ ) and multivariate ( $p=0.0015$ ) analyses revealed that High NLR as well as larger tumor size, lymph node metastases, and higher histological grade was significantly associated with relapse in postmenopausal patients. Moreover, in postmenopausal patients, subgroup analysis according to cancer subtype revealed that High NLR was correlated with shorter RFS only in patients with hormone receptor (HR)-positive/human epidermal growth factor receptor 2 (HER2)-negative breast cancer ( $p=0.0009$ ), in those without adjuvant chemotherapy ( $p=0.0034$ ) but not in those with adjuvant chemotherapy.

**Conclusion:** Elevated preoperative NLR is an independent predictor of poor prognosis in postmenopausal but not premenopausal patients with breast cancer. In postmenopausal patients, adjuvant chemotherapy may improve prognosis in patients with HR-positive/HER2-negative breast cancer, especially in those with elevated preoperative NLR.

# 2018 San Antonio Breast Cancer Symposium ®

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Decentralized beta testing of MammaPrint and Blueprint NGS kit at University Hospitals Leuven and Curie Institute Paris

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## Background

Many countries restrict patient material exchange to central diagnostic laboratories abroad, limiting access to assays like MammaPrint® (MP) and Blueprint® (BP). Both assays are microarray-based, with MP being prognostic for distant recurrence and BP for molecular subtyping of breast cancer (Luminal-, HER2-, and Basal-type). To increase accessibility, decentralization is required with Next Generation Sequencing (NGS) being the preferred testing platform given that most diagnostic laboratories have the technology in place. The aim of this beta testing study is to validate a previously developed and centrally validated MP and BP NGS kit for RNA samples in two large tertiary academic hospitals in Europe.

## Patients and Methods

Patients with early breast cancer diagnosed at the Multidisciplinary Breast Center at University Hospitals Leuven and Curie Institute Paris were prospectively included between September 2017 and January 2018. Patients with bilateral breast cancer or presenting with more than 3 positive lymph nodes were excluded. Only patients with invasive ductal and invasive lobular carcinoma were included. Twenty tissue sections were cut from formalin-fixed, paraffin-embedded (FFPE) blocks; 10 tissue sections were analyzed at the local site using the MP and BP NGS kit, and 10 tissue sections were analyzed at Agendia using the same kit and procedure, as well as with the golden standard method (gene expression microarrays). Targeted RNA sequencing of the 70 MP and 80 BP signature genes was performed on Illumina MiSeq instruments. The raw NGS data generated at the local test sites was sent through a secure file transfer protocol server to Agendia for interpretation and comparison with microarray and NGS performed in the Agendia laboratories. We aimed for a minimum concordance rate between MP and BP outcome of 90% between each local site and Agendia's centralized site.

## Results

In this study, 116 early breast cancer patients were included (73 from University Hospitals Leuven and 43 from Curie Institute). Out of these patients, 52% were MP Low Risk and 48% MP High Risk according to microarray. The patients had a BP luminal, HER2 or basal subtype in respectively 83%, 9% and 8%. Concordance between MP microarray obtained from Agendia and MP NGS obtained from the local sites was 91.4%. Concordance between MP High and Low Risk classification between NGS Leuven *versus* NGS Agendia was 92.1% and between NGS Curie *versus* NGS Agendia 95.3%. For BP subtype outcomes, the results from microarray *versus* NGS for all patients combined from both local sites gave a 98.3% concordance and NGS Agendia *versus* NGS from each local site gave a 100% concordance.

## Conclusion

The MP and BP NGS kit was successfully validated in a decentralized setting, showing high concordance between results obtained at three different sites. There was a clear benefit of having well-trained NGS experienced diagnostic technical teams. The MP and BP NGS kit the first FFPE targeted RNA sequencing based multigene signature for breast cancer care, will provide a high and equal standard of MP and BP gene expression testing for breast cancer in a decentralized setting.

# 2018 San Antonio Breast Cancer Symposium ®

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Validation of different prognostic scores in breast cancer patients with brain metastases of the BMBC registry (GBG-79)

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## Background

The incidence of brain metastases from breast cancer is increasing and treatment is a major challenge. Different scores were developed to estimate the prognosis of patients with brain metastases by objective criteria. Sperduto et al. established the disease-specific breast-graded prognostic assessment (GPA) score in 400 breast cancer patients which includes age, performance status and tumor subtype. It has been described that the Breast- GPA best identifies patients with bad prognosis (Laakmann et al. 2016). Aim of this analysis was to validate the Breast-GPA in a large cohort of breast cancer patients with brain metastases.

## Materials and methods

Data of 613 breast cancer patients from the Brain Metastases in Breast Cancer (BMBC) registry treated in 80 different clinical German institutions between 2000 and 2016 was analyzed.

## Results

135 patients (22%) had a triple-negative, 199 (32%) a luminal-like und 279 (46%) a HER2-positive primary breast cancer. At diagnosis of brain metastases most patients had a good Karnofsky performance status (100%: 14.4%, 80-90%: 44.7%, 60-70%: 28.9%, 40-50%: 9.0%, 10-30%: 3.1%). Median age of patients was 56 years (22-90 years).

Median survival in the overall cohort was 7.1 months (95% CI 6.2-7.8 months).

Distribution of GPA scores was: 0-1: 11.9% (n=73), 1,5-2: 22.3% (n=137), 2,5-3: 47.8% (n=293), 3,5-4: 18% (n=110).

Median overall survival within the GPA-subgroups varied between 2.4 (CI95% 2.0 - 3.4); 4.8 (3.6 - 6.9); 9.2 (7.2 - 11.3) and 12.3 (8.9 - 18.0) months and, thus, was significantly shorter compared with the times published by Sperduto et al. (3.4; 7.7; 15.1 und 25.3 months).

## Conclusions

The breast-GPA is easy to calculate in clinical routine, allows to assess patients` prognosis objectively and can facilitate making therapeutic decisions. However, absolute survival may differ from the originally published cohort in other settings.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-27

The 21-gene Recurrence® (RS) Score assay in estrogen receptor positive node negative breast cancer: Real-world chemotherapy usage and patient characteristics within the intermediate and high-risk RS category

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## Background:

The Oncotype Dx, a 21-gene recurrence score (RS) assay, has been validated as a prognostic tool in early-stage, hormone receptor-positive, HER2-negative breast cancer. A RS of  $\geq 31$  is predictive for chemotherapy benefit. However, it has not been clearly established whether more intensive chemotherapy regimens for these patients provide further benefit and whether higher RS stratifications ( $\geq 41$ ) influence treatment decisions.

## Methods:

From the prospective British Columbia (BC) Breast Cancer Outcomes Unit database, we identified patients with N0 disease who received Oncotype Dx testing from May 2010 to December 2016. Patients with previous or synchronous breast cancer, and patients treated with neoadjuvant chemotherapy were excluded. Groups were defined that had an Oncotype Dx RS of 31-40 and  $\geq 41$ . Demographic characteristics and type of chemotherapy received were collected. Additional subgroups were defined for patients who had a RS of 21-25 and who were  $\leq 50$  years old and  $> 50$  years old.

## Results:

We identified 1,202 patients who received Oncotype Dx testing over the time period studied, with 14.8% (n=178) having a RS of  $\geq 31$ . Among these high-risk patients, the median age was 58 (range 34-79), 90% received hormonal therapy and 85% received chemotherapy. In this cohort, 46% received docetaxel and cyclophosphamide for 4 cycles and 28% received 3rd generation chemotherapy. The use of 3rd generation chemotherapy in patients with a RS of  $\geq 41$  was significantly higher than in patients with RS between 31-40 (39% vs 22%,  $p = 0.006$ ). Among patients who had a RS of 21-25 and who were  $\leq 50$  years old (n = 49), 53% received chemotherapy. Of patients who had a RS of 21-25 and who were  $> 50$  years old (n = 127), 16% received chemotherapy.

## Conclusions:

Among patients with a RS  $\geq 31$ , decisions regarding chemotherapy usage were heterogeneous with docetaxel and cyclophosphamide for 4 cycles being the most commonly used regimen. However, in those with a RS  $\geq 41$ , 3rd generation chemotherapy was preferred. Patients with a RS between 21-25 and who were  $\leq 50$  years old received more chemotherapy than patients who were  $> 50$  years old.

	RS 31-40 (n=116)	RS $\geq 41$ (n=62)	RS $\geq 31$ (n=178)
Median age	58.0 (range, 36-79)	57.5 (range 34-78)	58.0 (range 34-79)
Pre-menopausal	28.4%	29.0%	28.7%
Hormonal therapy	93.1%	83.9%	89.9%
Chemotherapy	86.2%	82.3%	84.8%
DCx4 (1)	54.3% (n=63/116) Median age 59.0 (range, 36 – 78)	30.6% (n=19/62) Median age 64.0 (range, 42 – 78)	46.1% (n=82/178) Median age 59.5 (range, 36 – 78)
3rd generation chemo (2)	21.6% (n=25/116) Median age 56.0 (range, 39 – 79)	38.7% (n=24/62) Median age 52.0 (range, 34 – 76)	27.5% (n=49/178) Median age 54.0 (range, 34 – 79)
Other chemo	10.3% (n=12/116) Median age 57.5 (range, 52 – 78)	12.9% (n=8/62) Median age 64.0 (range, 42 – 72)	11.2% (n=20/178) Median age 58.5 (range, 42 – 78)

(1) Docetaxel and cyclophosphamide, 4 cycles (2) Anthracycline and Taxane containing regimens, 6 cycles or 8 cycles

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-28

Clinical risk prediction models for breast cancer: A review of models developed between 2010 and 2018

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## Background:

Clinical prediction models provide insight in the probability of a specified event happening based on the personal characteristics of the patient. Predicted probabilities support physicians in tailoring clinical decisions to the patients needs. Throughout the past decades, the amount of developed prediction models has grown, yet the application of these models in daily medical practice falls behind. It is currently uncertain how many prediction models exist to support decision making in breast cancer care and exactly which decisions may be supported using prediction models. This study aimed to identify all developed prediction models on breast cancer care and to assess the clinical applicability of the models in the target breast cancer population.

## Methods:

A literature search was performed to identify developed risk prediction models published from January 2010 up to June 2018. Models predicting breast cancer related events were included. Identified models were assessed on the reported transparency and reproducibility and incorporated in the online platform for prediction models: Evidencio. Clinical applicability of the models was assessed using a digital implementation of the Dutch breast cancer guideline called Oncoguide. Models were assigned to location in the guideline where they may support clinical decisions.

## Results:

A total of 91 studies describing the development of 142 prediction models were identified. Thorough assessment showed that 31 models were reported in full having the description of all the necessary parameters to reproduce the underlying statistical formula and were incorporated in Evidencio.com. For 95 models the formula could be derived through the presented nomogram, table, or online calculator. The remaining 16 models were lacking information to construct any method to predict the outcome of an individual patient and could therefore not be used in practice.

## Conclusion:

A total of 142 prediction models were developed between January 2010 and June 2018. The overall quality of reporting was poor as 111 models were not described transparently. All identified models were assigned to the location in the guideline of which the model may support clinical decision making. Further assessment is necessary on the clinical impact and validity of the models in the Dutch population before implementing them in the guideline.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-29

Lymphatic invasion is an independent risk factor in patients with small node-negative luminal breast cancer

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## [Background]

In patients with node-negative (N0), hormone receptor-positive, human epidermal growth factor receptor (HER2) -negative (luminal) breast cancer, the impact of lymphatic invasion (ly) on the prognosis remains to be clarified.

## [Methods]

Among 3,158 patients with primary breast cancers who underwent surgery in our institute from January 2007 to December 2009, we analyzed 1027 N0 luminal invasive breast cancers without preoperative systemic therapy. The luminal breast cancer was defined as hormone receptor-positive (ER of  $\geq 10\%$  or PgR of  $\geq 10\%$ ) and HER2-negative (immunohistochemistry: 0, 1+ or FISH: ratio  $< 2.0$ ) cancer in the postoperative pathological specimen. ly was defined as positive when cancer cell nests were detected within the lymph duct in the whole specimen. N0 was confirmed pathologically by the sentinel lymph node biopsy in all the patients. The Fisher's exact test was used for comparison between different categories. The distant recurrence rate (DRR) was analyzed using the Kaplan-Meier method and the log-rank test. For multivariate analysis, Cox's regression analysis was performed.

## [Results]

The median follow-up period was 103.8 months (range: 5.6-128.8). Recurrence with distant metastasis occurred in 26 patients (2.5%). There were 5 (0.7%) deaths related to breast cancer. ly was detected in 240 patients (23.4%). In the ly-positive group, the tumor size was larger ( $p = 0.007$ ), and the nuclear grade (NG) was higher ( $p < 0.001$ ) than in the ly-negative group. Postoperative endocrine therapy ( $p < 0.001$ ) and postoperative chemotherapy ( $p < 0.001$ ) were more frequently employed for patients with ly-positive tumor. The univariate analysis showed that ly positivity ( $p < 0.001$ ), large tumor size ( $p < 0.001$ ), high NG ( $p < 0.001$ ), PgR negativity ( $p = 0.002$ ) and the history of adjuvant chemotherapy ( $p < 0.001$ ) were associated with high DRR. In the multivariate analysis, large tumor size ( $p = 0.007$ ) and PgR negativity ( $p = 0.015$ ) remained significant. Although positive ly had a risk ratio of 2.2, it was not an independent risk factor. When restricted to T1 tumor ( $n = 899$ ), the aforementioned factors still showed prognostic value in the univariate analysis, among which ly positivity ( $p = 0.004$ ) remained significant together with PgR negativity ( $p = 0.047$ ) in the multivariate analysis. The 8-year DRR was very favorable (0.8%) in patients with ly-negative T1N0 tumor while it was modest (6.6%) in patients with ly-positive T1N0 tumor ( $p < 0.001$ ). Only 1.3% of the patients had received adjuvant chemotherapy in the ly-negative group while 27% of the patients had in the ly-positive group.

## [Conclusion]

Lymphatic invasion was associated with higher DRR although it was not independent in the multivariate analysis among patients with N0 luminal breast cancer. When restricted to patients with T1N0 luminal breast cancer, the presence of ly was independently associated with higher risk of distant recurrence. It suggests that the assessment of ly is clinically more relevant when considering treatment options for small luminal breast cancer.



# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-08-30

Prognostication of immune related gene expression in patients with triple negative breast cancer

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**Introduction:** To date, the role of immunotherapy with check point inhibitors and/or vaccines in the treatment of breast cancer (BC) is still debating, and the main focus of immunotherapy in BC is on triple negative subtype as a target population in many ongoing clinical trials. Translational research into identifying predictive and prognostic immune biomarkers is of particular clinical relevance, but, there are currently no definite prognostic and predictive immune biomarkers in BC, especially in triple negative breast cancer(TNBC). We investigated the expression profiles of immune genes in patients with TNBC to identify the prognostic value of immune genes in search of clinical implications.

**Methods :** We investigated expression profiles of 770 pan-cancer immune related genes using the nCounter mRNA expression assay (NanoString®) from paraffin-embedded tumor tissues in 200 patients diagnosed as TNBC who received curative surgery at Samsung Medical Center from 2000 to 2004. We analyzed the relationship between stage adjusted level of gene expressions and patients' survival outcomes using Cox regression model.

**Results:** Of 770 genes, 186 genes were selected from univariate analysis with clinical stage adjustment. In multivariate analysis using Cox regression, expressions of CD1B, CD45, CD53, CT45A1, GTF3C1, IL11RA, IL1RN, LRRN3, MAPK1, NEFL, PRKCE, SPACA3 and RANKL were associated with distant recurrence free survival ( $p < 0.05$ , respectively). Among these 13 genes, expression of MAPK1, NEFL, CD45, SPACA3 and RANKL were correlated with favorable outcome in terms of distant recurrence free survival ( $p < 0.05$ , respectively). In terms of overall survival, C3, IL1RL1, IL1RN, IL7 and PRKCE were associated with poor prognosis ( $p < 0.05$ , respectively) and expression of SAA1 CXCL9 and RANKL resulted in favorable outcome ( $p < 0.05$ , respectively)

Table 1

Parameter	Parameter Estimate	Standard Error	p-value	Hazard Ratio	95% Confidence Interval
(a) distant recurrence free survival					
Stage	2.48735	0.68057	0.0003	12.029	3.169, 45.661
CD1B	1.14191	0.2753	<.0001	3.133	1.826, 5.374
CD53	1.53165	0.34851	<.0001	4.626	2.336, 9.159
CT45A1	0.42611	0.13421	0.0015	1.531	1.177, 1.992
GTF3C1	1.19311	0.57972	0.0396	3.297	1.059, 10.271
IL11RA	1.67112	0.46175	0.0003	5.318	2.151, 13.146
IL1RN	0.98028	0.24657	<.0001	2.665	1.644, 4.321
LRRN3	1.42417	0.28742	<.0001	4.154	2.365, 7.297
MAPK1	-0.54274	0.25824	0.0356	0.581	0.35, 0.964
NEFL	-1.1217	0.33561	0.0008	0.326	0.169, 0.629
PRKCE	2.37834	0.49659	<.0001	10.787	4.076, 28.549
CD45	-2.73678	0.43154	<.0001	0.065	0.028, 0.151
SPACA3	-0.74593	0.27227	0.0061	0.474	0.278, 0.809
RANKL	-1.28892	0.2976	<.0001	0.276	0.154, 0.494
(b) overall survival					
Stage	1.35928	0.49781	0.0063	3.893	1.468, 10.329
C3	0.32983	0.15035	0.0283	1.391	1.036, 1.867

CXCL9	-0.37919	0.10068	0.0002	0.684	0.562, 0.834
IL1RL1	0.67936	0.26294	0.0098	1.973	1.178, 3.303
IL1RN	0.43713	0.17237	0.0112	1.548	1.104, 2.171
IL7	0.50728	0.20625	0.0139	1.661	1.109, 2.488
PRKCE	0.83534	0.27291	0.0022	2.306	1.35, 3.936
SAA1	-0.56425	0.13449	<.0001	0.569	0.437, 0.74
RANKL	-0.60499	0.23451	0.0099	0.546	0.345, 0.865

**Conclusion:** High expression of IL1RN, PRKCE were associated with short distant recurrence free survival and overall survival in patients with TNBCs who received curative surgery. In contrast, RANKL expression resulted in prolonged distant recurrence free survival and overall survival.

# 2018 San Antonio Breast Cancer Symposium ®

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Derivation and validation of a novel prediction model in breast phyllodes tumors after surgery

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## **Aim**

This study aimed to develop a nomogram based on clinicopathological features to evaluate the recurrence probability of breast phyllodes tumors following surgery. The criteria for atypia, mitoses, overgrowth, and surgical margin (AMOS) were also validated.

## **Method**

Data from 334 patients with breast phyllodes tumors, who underwent surgical treatment at Sun Yat-sen Memorial Hospital from January 2005 to December 2014, were used to develop a prediction model. Additionally, data of 36 patients from Peking University Shenzhen Hospital and data of 140 patients from Sun Yat-sen University Cancer Center during the same period were used to validate the model. The medical records and tumor slides were retrospectively reviewed. The log-rank and Cox regression tests were used to develop a clinical prediction model of breast phyllodes tumors as well as validating the AMOS criteria. All statistical analyses were performed using R and STATA.

## **Results**

Of all 334 patients included in the study, 224 had benign, 91 had borderline, and 19 had malignant tumors. The local and distant recurrence rate was 17.7%. The 1-, 3-, and 5-year cumulative recurrence-free survival was 98.5%, 97.9%, and 96.8%, respectively. Surgical margin, mitoses, and tumor border were identified as independent risk factors for breast phyllodes tumors. A nomogram was developed based on these three variables. The C-index of internal and external validation was 0.71 and 0.67, respectively. The area under the curve of AMOS criteria was 0.59.

## **Conclusions**

The present study model presented a more concise and objective variables to evaluate the recurrence-free survival of patients after surgery compared with that using the AMOS criteria, which is more appropriate for clinical practice and also allows for a more accurate prediction.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-33

The significance of Oncotype DX recurrence score in  $T_{1-2}N_1M_0$  ER positive HER2 negative breast cancer: An analysis combined with the prognostic stage in the updated AJCC 8<sup>th</sup> edition

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**Purpose:** Oncotype DX recurrence score (Oncotype DX RS) is applied in the prognostic stage in  $T_{1-2}N_0M_0$  ER+/HER2<sup>-</sup> breast cancer. But its significance is unclear in cases with  $T_{1-2}N_1M_0$  ER+/HER2<sup>-</sup> disease. Our study was to evaluate the prognostic significance of Oncotype DX RS in combination with the prognostic stage in the updated AJCC 8<sup>th</sup> edition. **Methods:** The Surveillance, Epidemiology, and End Results (SEER) database was searched to identify invasive ductal breast cancer cases in  $T_{1-2}N_1M_0$  stage with ER positive HER2 negative status and Oncotype DX RS diagnosed between 2004 and 2012. Patients with unknown histologic grade or PR status, no or unknown surgery performed, or less than 6 months of follow up were excluded. Patients with RS 0-10, 11-25, >25 were categorized into low risk, midrange risk and high risk groups respectively. Comparisons of the distribution of RS groups among prognostic stages were performed using Pearson's chi-square. Breast cancer-specific survival (BCSS) and overall survival (OS) were estimated using the Kaplan-Meier method and compared across RS groups using the log-rank statistic. Cox models were fitted to compare the association between RS groups, prognostic stages and BCSS or OS after adjusting for other characteristics. **Results:** Altogether 4059 cases were enrolled, which were categorized into prognostic stage IA-III A (only 357 cases in stage IIB and 35 cases in stage IIIA). More than 60% cases in prognostic stage IA-IIA had their RS in midrange risk group. There were decreasing proportion of low risk RS group and increasing proportion of high risk RS group with the ascending of prognostic stages ( $P<0.001$ ). The median follow up was 59 months. There were significant differences in BCSS and OS among RS groups (log-rank  $P<0.001$ ). In the subgroup analysis, there were also significant differences in BCSS and OS among RS groups in prognostic stages IA-IIA. In the multivariate Cox analysis, the RS group was an independent prognostic factor for BCSS (midrange risk vs. low risk, HR=3.647, 95% CI: 1.124-11.837,  $P=0.031$ ; high risk vs. low risk, HR=15.372, 95% CI: 4.600-51.374,  $P<0.001$ ) and OS (midrange risk vs. low risk, HR=1.718, 95% CI: 1.039-2.840,  $P=0.035$ ; high risk vs. low risk, HR=4.225, 95% CI: 2.462-7.251,  $P<0.001$ ) along with the prognostic stage. **Conclusions:** Oncotype DX RS has prognostic significance in  $T_{1-2}N_1M_0$  ER+/HER2<sup>-</sup> disease. Further prospective research is warranted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-34

MammaTyper® – An in vitro quantitative local gene expression test as a predictor of OncotypeDX®- and EndoPredict®-results

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## **Background:**

Risk stratification in early-stage breast cancer patients still remains a clinical challenge. It is well known that expression of ER/PR, HER2 and Ki-67 are valuable prognostic and predictive markers. According to the commonly used St. Gallen classification (2013), breast cancer treatment decisions can be based on four different subtypes (Luminal A-like, Luminal B-like [HER2+/-], HER2 + [non-luminal] and Basal-like).

Immunohistochemistry is predominantly used to determine the receptor status, although semi-quantitative analysis shows significant inter-observer variability.

Several quantitative gene expression tests – such as Oncotype DX® and EndoPredict® – are available as diagnostic tools but their use is often limited due to financial considerations.

MammaTyper® has been shown to be a precise and reproducible biomarker determination tool to investigate the expression of ER/PR, HER2 and Ki-67 and may prove to be a cost efficient quantitative diagnostic tool.

## **Methods:**

This study tested ESR-1, PGR, MKI67 and ERBB2 mRNA-levels by RT-qPCR in 100 FFPT-samples using MammaTyper® kit. The test identified breast cancer subgroups according to St. Gallen classification. RT-qPCR results were correlated to IHC-test results via  $\chi^2$ -Test and to risk groups of Oncotype DX®- (n=55) and EndoPredict®-testing (n=45). Oncotype®-Recurrence Score levels were separated into low (RS <18), intermediate (RS 18-30) and high risk (RS ≥ 31). EndoPredict® EPScore® and EPclin Risk Score® cut off levels defining high and low risk levels were 7.0, respectively 3.3. Linear regression model was performed investigating the prediction of EPscore® and Recurrence Score® via MammaTyper® mRNA-results.

## **Results:**

IHC-testing resulted in 43 Luminal A-like samples (43%) and 57 Luminal B-like [HER2 negative] (57%) tumors. MammaTyper®-based classification showed significant correlation to IHC-based classification ( $\chi^2=12,68$ ; p=0,005).

EndoPredict®-data showed low-risk levels in 36 patients (80%) and high-risk status in 9 cases (20%). Correlation to mRNA-based St. Gallen-subtypes approved significance ( $\chi^2=17,32$ , p<0,001). In linear regression model only MKI67-mRNA showed independent correlation to EPscore® (p<0,001; R<sup>2</sup> 0,290)

A total of 34 patients (61,8%) showed low risk, 13 (26,3%) intermediate risk (RS 18-30) and 8 (14,5%) high-risk recurrence score levels (RS≥31) in OncotypeDX®. MammaTyper® results correlated to OncotypeDX® risk-levels ( $\chi^2=27,98$ ; p<0,001). In linear regression model mRNA-levels of ESR-1, PGR and MKI67 were significant predictors of OncotypeDX® RS (p-values: p=0,003, p<0,001, p=0,033, R<sup>2</sup>= 0,602)

## **Conclusion:**

This study shows mRNA-testing is a valid marker to identify breast cancer subtypes. Additionally, MammaTyper® results are significantly correlated to Oncotype DX®- and EndoPredict®-results, indicating it might be an efficient as well as cost effective diagnostic instrument. However a large prospective multicenter study should be performed to prove its diagnostic validity in predicting the most appropriate and effective treatment in early-stage breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-08-35

Young age at diagnosis is associated with worse prognosis in the luminal A breast cancer subtype. A retrospective institutional cohort study

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**Background:** Breast cancer (BCA) presents with distinct molecular subtypes, each associated with different patterns of relapse, drug sensitivity, and prognosis. Although age at diagnosis is a recognized independent prognostic risk factor, its relative importance among molecular subtypes is not well documented. The aim of this study was to evaluate the prognostic role of age at diagnosis among BCA patients of different immunohistochemical subtypes (LuminalA, LuminalB, Her2, Triple-negative).

**Methods:** We conducted a retrospective study of women with invasive BCA undergoing surgery at the Johns Hopkins Hospital from January 2000 - December 2016, excluding patients presenting with stage IV breast cancer. Patients were stratified into three age groups:  $\leq 40$ , 41-60, and  $> 60$  years, and multivariable analysis was performed using Cox regression. To explore age-related differences in gene expression, we identified differentially expressed genes (DEG) between age groups among BCA subtypes in the TCGA dataset. Finally, we identified key driver genes within DEG using a weighted gene co-expression network analysis.

**Results:** Our cohort included 3,524 patients with a median follow-up of 85.1 months. LuminalA breast cancer patients had significantly lower 5-year Disease Free Survival (DFS) and Distant Metastasis-Free Survival (DMFS) in the  $\leq 40$  year age group compared to the 40-60 year age group (HR=2.69, 95%CI: 1.72 - 4.23 and HR=2.95, 95%CI: 1.78 - 4.90, respectively), while the other molecular subtypes showed no significant association of DFS or DMFS with age. Age was a stronger predictor of 5- year DFS and 5-year DMFS than tumor grade or proliferative index (Ki67) in LuminalA BCA patients, but not other subtypes.

Gene expression data were obtained from 1097 BCA TCGA patients, divided into two groups ( $\leq 40$ y, n=36;  $> 40$ y, n=455). We identified 374 DEG between  $\leq 40$ y and  $> 40$ y LuminalA BCA subsets. The DEG were enriched in 7 pathways, and the WGCNA analysis identified two modules of co-expressed genes. No age group-specific DEG were identified in non-LuminalA subtypes.

**Conclusion:** Age at diagnosis may be an important prognostic factor in LuminalA BCA and may improve risk stratification and personalized therapy. Prospective studies are needed to further evaluate the prognostic value of age in this subset of BCA patients.

# 2018 San Antonio Breast Cancer Symposium ®

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The DCIS score predicts risk of local recurrence risk after breast-conserving surgery more accurately than ER plus HER2

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**Introduction:** Improved individual prediction of 10 yr local recurrence (LR) risk following breast-conserving surgery (BCS) for ductal carcinoma in situ (DCIS) is needed to identify women at low risk, for whom radiotherapy (RT) may be omitted. We hypothesized that LR prediction that includes the Oncotype DCIS score (DS) would be more accurate, and would identify more women with very low LR risks compared to models that include estrogen receptor (ER) plus HER2 without the DS.

**Methods:** Three predictive models of LR (clinicopathological factors (CPFs) alone; CPFs+ER+HER2; CPFs+DS) were developed and compared in 1,102 cases of DCIS for whom complete covariate and outcome data were available. CPFs included age at diagnosis, lesion size, nuclear grade, comedonecrosis, multifocality, and resection margin width. Categorizations of discrete variables and transformations of continuous variables were examined in Cox models; two-way interactions and interactions with time were assessed. Internal validation was performed by bootstrapping. Individual predicted 10-yr LR risks after treatment with BCS alone were computed from covariate values, estimated regression parameters and the estimated baseline survival function. Model performance was assessed by c-statistics and calibration plots.

**Results:** 863/1,102 (78.3%) women were age  $\geq 50$  years at diagnosis. Lesion size was  $\leq 10$  mm in 555/1,102 (50.4%). Nuclear grade was low or moderate in 62.4%. Comedonecrosis was present in 22.1%. Multifocality was observed in 25.2%. Post-BCS RT was received by 54.4%. Mean DS = 37.49 (sd 23.29). DS risk category = low in 611/1,102 (55.4%). ER = positive in 1,025 /1,102 (93.0%) cases. HER2 overexpression = positive in 212/1,102 (19.2%), equivocal in 95 / 1,102 (8.6%) and negative in 795 / 1,102 (72.1%) cases. Adjusting for all CPFs, the hazard ratios (HR) for LR per 50-unit increase in DS = 2.00 (95% CI 1.42, 2.83), for ER positive = 0.58 (95% CI 0.36, 0.95) and for HER2 positive = 0.73 (95% CI 0.41, 1.30). The strongest prediction model incorporated CPFs+DS. C-statistics for CPFs+DS, CPFs+ER+HER2, or CPFs alone models were 0.7025, 0.6879, and 0.6825. The CPFs+DS model was better calibrated at predicting low ( $\leq 10\%$ ) individual 10-yr LR risks after BCS alone than models incorporating CPF+ER+HER2 or CPFs alone, evidenced by c-statistics and plots of observed by predicted risks. Specifically, among women age  $\geq 50$  with no adverse CPFs, the CPFs+DS model identified the greatest proportion of women (62.3%) with predicted 10-year LR risk  $\leq 10\%$  without RT, compared to the CPFs+ER+HER2 (50.9%) or CPFs alone (46.5%) models. When applying the prediction equations to similar women as those in the cohort who were treated with RT, the CPFs+DS model again identified the greatest proportion of women (44.4%) with a low predicted 10-yr LR risk without RT (for whom RT could have been omitted) compared to the CPFs+ER+HER2 model (39.4%) and the CPFs alone model (32.3%).

**Conclusion:** Individual prediction of LR risk that incorporates the DCIS score plus clinicopathological factors is more accurate than prediction models based on ER plus HER2, and identifies a higher proportion of women with a low predicted risk of LR after BCS alone, for whom radiotherapy may be omitted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-09-02

Validation of iPrevent using the prospective family study cohort (ProF-SC)

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**Background:** iPrevent (<https://www.petermac.org/iprevent>) provides women with highly-tailored risk management information after first estimating their breast cancer (BC) risk using the established risk prediction models, IBIS and BOADICEA. iPrevent has an internal switching algorithm that governs which model is used for each woman, depending on her risk factor data (i.e. LCIS/atypical hyperplasia status, BRCA status, and cancer family history). This study assessed the calibration and discriminatory accuracy of the 10-year BC risk estimates provided by iPrevent. **Methods:** Subjects were 16,574 women in the ProF-SC, aged 18-70 years and without BC or bilateral mastectomy at recruitment. After 10 years follow-up, 655 women (4%) were diagnosed with invasive BC. A “batch mode” for iPrevent is not available, so the iPrevent-assigned cumulative 10-year invasive BC risks were calculated by entering self-reported risk factors at cohort entry into either the IBIS (10,169 women) or BOADICEA (6,405 women) software packages (according to the iPrevent switching algorithm). To assess calibration, the mean iPrevent-assigned risk was compared with the mean 10-year observed invasive BC incidence, using a chi-squared goodness-of-fit statistic for the whole cohort, and by quartiles of risk. To evaluate discriminatory accuracy, the overall area under the receiver operating characteristic curve (AUC) for the development of invasive BC within 10 years was computed. Data were censored at date of invasive or in situ BC diagnosis, bilateral mastectomy, death, loss to follow-up, or at 10 years of follow-up. **Results:** For the whole cohort, iPrevent assigned risk was well-calibrated – 690 expected BCs (E) 655 observed (O) (E/O=1.05, 95% CI: 0.98-1.14), although for women in the highest risk quartile, i.e.  $\geq 6\%$  10-year risk, E/O=1.19, 95% CI: 1.07-1.32. The AUC was 0.70, 95% CI: 0.68-0.72. **Conclusions:** iPrevent is well calibrated overall and has good discriminatory accuracy for predicting 10-year BC risk, thus justifying its clinical use.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-09-03

Simulated outcomes of personalized versus guideline-based breast cancer screening

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**Introduction:** Personalized screening, or screening tailored to individual breast cancer risk, is being studied as an improvement on the current practice of guideline-based screening. WISDOM (Women Informed to Screen Depending on Measures of Risk) is an ongoing randomized trial comparing personalized to annual screening. To project the efficacy, safety, and cost of personalized screening on a population level, we constructed a simulation model comparing personalized to guideline-based strategies across the outcomes of advanced (Stage IIB+) cancers, false positives, biopsies, and cost.

**Methods:** Our simulated cohort consisted of 100,000 women aged 40-74 with demographic and risk factor distributions based on the U.S. screening population. We modeled the WISDOM approach to personalized screening where recommendations are based on the results of panel-based mutation testing and 5-year risk estimates from a clinical risk model modified by a polygenic risk score containing 76 genetic variants (SNPs). Simulated women were randomly assigned a clinical and genetic risk profile, which were integrated to generate a 5-year risk estimate. This was then used to assign a starting and stopping age, frequency, and modality (MRI vs. mammogram) of screening. We compared the aggregate outcomes over a 1-year time window between personalized screening and 3 strategies based on U.S. professional society guidelines (**Table**).

**Results:** There was no statistically significant difference in advanced cancers between screening strategies (**Table**). However, the biennial, hybrid, and personalized strategies resulted in fewer false positives and biopsies compared to annual screening, and at lower cost. Though aggregate outcomes between the hybrid and personalized strategies were similar, the average 5-year risk of women assigned to annual screening under the personalized strategy was higher than that of the hybrid strategy, 1.7% vs. 1.2%. Similarly, the average 5-year risk of women assigned to biennial screening was lower under the personalized strategy, 1.1% vs. 1.9%.

**Conclusion:** Our simulations show that personalized screening results in a similar incidence of advanced cancers as annual screening while reducing false positives, biopsies, and cost. Compared to other guideline-based strategies, personalized screening better allocates screening resources by identifying higher-risk women for more intensive screening, and lower-risk women for less intensive screening.

Descriptions and simulated outcomes of four screening strategies

Strategy	Starting age, years	Stopping age, years	Frequency	Stage IIB+ cancers, RR <sup>1</sup> (95% CI) <sup>2</sup>	False positives, RR <sup>1</sup> (95% CI) <sup>2</sup>	Biopsies, RR <sup>1</sup> (95% CI) <sup>2</sup>	Cost, millions USD per 100,000 women
<b>Annual</b> (American College of Obstetricians and Gynecologists)	40	74	Annual	ref	ref	ref	\$22.1
<b>Biennial</b> (U.S. Preventive Services Task Force)	50	74	Biennial	1.13 (0.98-1.28)	0.44 (0.33-0.56)	0.46 (0.35-0.58)	\$8.4
<b>Hybrid</b> (American Cancer Society)	45	Per life expectancy	45-55: Annual 55+: Biennial	1.09 (0.95-1.25)	0.58 (0.49-0.67)	0.64 (0.56-0.73)	\$14.7
<b>Personalized</b> (WISDOM)	40-50 <sup>3</sup>	Per life expectancy	Annual or Biennial <sup>4</sup>	1.01 (0.89-1.12)	0.55 (0.46-0.65)	0.56 (0.47-0.65)	\$14.1

<sup>1</sup>relative risk; <sup>2</sup>95% confidence interval; <sup>3</sup>start when 5-year risk > 1.3%; <sup>4</sup>annual if age 40-49 + dense breasts or top 2.5th percentile of 5-year risk

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-09-04

Associations between clinical factors in v7.02 of the Tyrer-Cuzick model and a SNP-based residual risk score

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**Background:** Genome-wide association studies (GWAS) have identified common variants, primarily single-nucleotide polymorphisms (SNPs), that individually confer modest risk but together explain a significant proportion of genetic breast cancer (BC) predisposition. GWAS have also demonstrated that SNPs cannot replace family history evaluation: familial BC assessment captures a large magnitude of risk information that is not captured by SNPs. Thus, improved BC risk stratification may be achieved by combining family history assessment with SNP markers. However, to avoid double-counting shared risk information, familial and/or SNP-based risks must be adjusted for confounding.

Additional clinical and biological factors that contribute to BC risk are included in version 7.02 of the Tyrer-Cuzick model. These include height; weight; BMI; age of menarche; parity and age of first childbirth; menopausal status and age of onset; and use of hormonal replacement therapy (HRT). Confounding of SNPs with these factors is not well understood. Here we present an analysis of associations between an 86-SNP Residual Risk Score (RRS) and factors included in version 7.02 of the Tyrer-Cuzick model.

**Methods:** De-identified clinical records and genotypes were collected from a consecutive series of patients referred for hereditary cancer testing with a multigene panel. Study subjects included unaffected women age 18-84 who reported European ancestry and tested negative for mutations in 11 genes associated with BC (*BRCA1*, *BRCA2*, *TP53*, *PTEN*, *STK11*, *CDH1*, *PALB2*, *CHEK2*, *ATM*, *NBN*, *BARD1*).

For each risk factor, we constructed a univariate linear regression model with RRS as the dependent variable and the clinical factor as the independent variable. From these models, we examined regression coefficients, p-values based on F-statistics, and Pearson correlation coefficients. Scatterplots and boxplots were used to visually assess associations. All analyses were conducted using R version 3.4.4. P-values were reported as two-sided with no corrections for multiple testing.

**Results:** 5,489 patients met the study selection criteria. The median age at hereditary cancer testing was 42 years. Nearly one third (33.1%) of women reported a BC diagnosis in a first degree relative.

The RRS was significantly associated with familial BC ( $p < 10^{-08}$ ). We observed marginal evidence of association between the RRS and HRT use ( $p = 0.04$ ). However, this association would not survive a multiple testing correction, and was not significant after multivariate adjustment for family cancer history. We found no evidence for association of the RRS with height, weight, BMI, menopausal stage, age of menarche, age of menopause, duration of menarche, parity, age of first live birth, HRT type, or HRT length of use.

**Conclusions:** The RRS is largely independent from the non-familial risk factors in version 7.02 of the Tyrer-Cuzick model, but is significantly associated with BC family history. Risk assessment based on Tyrer-Cuzick and SNPs must be adjusted for confounding to avoid double-counting familial risk.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-09-06

Analyzing the changes in the HR+/HER2- metastatic breast cancer (mBC) landscape since the arrival of CDK4/6 inhibitors with machine learning and visual analytics

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**BACKGROUND:** The real-world impact of CDK4/6 inhibitor use on HR+/HER2- mBC treatment sequencing, treatment response, and therapeutic utilization is limited. This study sought to describe treatment sequencing and response pre- and post-FDA approval of CDK4/6 inhibitors by conducting a traditional observational study approach and supplementing with a machine-learning methodology.

**METHODS:** Female patients  $\geq 18$  yrs were identified in the MarketScan Commercial and Medicare Supplement databases with continuous enrollment for at least 12 months pre-index,  $\geq 2$  medical claims for a BC diagnosis and  $\geq 2$  medical claims for metastatic disease (earliest=index date), and who had no treatment at any time with HER2 targeted therapy. Patients were excluded if they had received CDK4/6 or everolimus treatment prior to index. Treatment was stratified by line of therapy pre- and post- first CDK4/6 approval (February 3, 2015). We identified treatment patterns with standard distributions and visual analytics. Response to therapy and utilization was analyzed with prediction models and IBM Watson® machine learning population comparison models.

**RESULTS:** A total of 19,558 patients were eligible for the study with a mean age at diagnosis of mBC of 62 (SD 13). Prior to first CDK4/6 inhibitor approval, anastrozole and letrozole monotherapies were most likely to be identified as both first and second line treatment. Following approval of the first CDK4/6 inhibitor in 2015, CDK4/6 inhibitors were observed as a first line treatment in 25% of patients, and as second line treatment in 24% of patients. Of patients diagnosed following the first CDK4/6 inhibitor approval, 44% of patients were exposed to endocrine therapy and 10% were exposed to chemotherapy in the pre-index period. Patients receiving CDK4/6 inhibitors in combination with endocrine therapy as first line treatment were observed to have a longer progression free survival time than patients receiving endocrine monotherapy. Visual analytics demonstrate a large variation in treatment sequencing, especially after first line therapy. Machine learning and prediction models identified a strong secular bias in the use of CDK4/6 and a signal for improved treatment response in patients with no exposure to endocrine therapies in the pre-index period.

**CONCLUSIONS:** The mBC treatment landscape has changed significantly with the introduction of CDK4/6 inhibitors, which may be expected to impact long-term outcomes. Visual analytics and machine learning approaches can improve clinical insight. These approaches can help with identifying patient and clinical characteristics that predict response and utilization as well as appropriate treatment selections across lines of therapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-09-07

Higher risk of metachronous contralateral breast cancer in patients with invasive lobular breast cancer

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**Background:** Risk of metachronous contralateral breast cancer (CBC) is an important health issue in primary breast cancer (PBC) survivors. Individualized CBC risk prediction can help identify patients at high or low risk who may or may not benefit from additional surveillance and treatment. For lobular PBC, inconsistent results have been reported on an association with increased risk of CBC. We investigated CBC risk in lobular versus ductal PBC using a large nationwide dataset and taking into account age at PBC and (neo-)adjuvant systemic therapy effects.

**Patients and Methods:** We selected women diagnosed between 2003 and 2010 with early invasive lobular, lobular mixed with other types, or ductal PBC from the Netherlands Cancer Registry. Categorical and continuous characteristics between the 3 groups were compared using the chi-square statistics and the Kruskal-Wallis test, respectively. Competing risk analyses were applied to determine CBC incidence. Multivariable subdistribution hazard ratios (SHRs) were adjusted for primary tumor stage, age at PBC diagnosis, radiotherapy, (neo-)adjuvant chemotherapy and endocrine therapy.

**Results:** We selected 74,373 women aged >18 years with lobular (n=8,903), lobular mixed (n=3,512), and ductal (n=62,230) PBC. Women with lobular PBC were older at diagnosis than women with lobular mixed or ductal PBC (61 vs. 58 vs. 58 years, respectively), more often had ER-positive PBC (95.7% vs. 94.1% vs. 79.6%) and were more often systemically treated with only endocrine therapy (30.7% vs. 24.9% vs. 19.7%), while less often treated with only chemotherapy (4.2% vs. 5.3% vs. 15.4%). Ten-year cumulative CBC incidences in women with lobular, lobular mixed or ductal PBC were 3.2%, 3.6% and 2.8% when treated with systemic therapy (chemotherapy and/or endocrine therapy) and 6.6%, 7.7% and 5.6% without systemic therapy, respectively. Multivariable SHRs were 1.19 (95% CI: 1.05-1.34) for lobular and 1.39 (95% CI: 1.17-1.65) for lobular mixed versus ductal PBC; for women <50 years, risk differences were larger: 1.60 (95% CI: 1.26-2.05) and 1.43 (95% CI: 1.00-2.06), respectively.

**Conclusion:** Lobular histology and lobular mixed histology are independent risk factors for CBC development, and should be considered as prognostic factors when refining CBC risk prediction models.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-09-08

Shared-patient physician networks and their impact on the uptake of genomic testing in early-stage breast cancer

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**Background:** Oncotype DX (ODX) or 21-gene recurrence score genomic testing is used to stratify risk and determine appropriate treatment in women with early-stage breast cancer (BC). Diffusion of ODX by way of physician networks has not been studied.

**Objective:** To determine the association between physician network connections, defined by shared patients, and the use of ODX testing.

**Methods:** SEER-Medicare claims from 2008-2012 were used to identify a cohort of woman with a diagnosis of BC from registry/ICD codes, continuously enrolled in Medicare fee-for-service Part A and B one year prior to and one year following diagnosis. We identify receipt of ODX from the associated CPT code, claim reimbursement, and performing NPI. To look at the influence of network connections on ODX use, we split the study into two time periods: early adoption from 2008-2009, and late from 2010-2012. Medical oncologists with a BC-related claim in the cohort above, and any rendered BC-related service are considered 'connected' if they shared two or more BC patients. Analyses describe these connections and explore the association between connectedness to an early adopting medical oncologist and ODX use in parallel physician and patient-level analyses using generalized linear mixed models with a hospital referral region-specific random effect. Models control for physician and patient-level characteristics where applicable.

**Results:** 24,463 women met study criteria; 12,874 were diagnosed with BC in the early adoption time period (1,790 received ODX) and 11,589 were diagnosed in the late period (2,334 received ODX). 2,073 medical oncologists treated these patients from 2008-2009. The mean number of BC patients treated per medical oncologist was 86.8 during the early adoption period, and medical oncologists had a median number of peer connections of 11 (IQR: 7-18). Early adopting medical oncologists had higher numbers of peer connections and higher average patient counts than non-early adopters. A higher percentage of female medical oncologists were early adopters (39%) than male medical oncologists (33%) ( $p < 0.02$ ). Among non-early adopting oncologists, peer connection to at least two early adopting providers in 2008-2009 is associated with a 3.2 (95% CI: 2.0-4.9) times increase in the odds of ordering ODX in 2010-2012 after adjustment for physician gender and time in practice. In patient-level models with controls for physician and patient characteristics, seeing a medical oncologist with connections to at least two early adopting physicians is associated with a 1.6 times (95% CI: 1.1-2.2) increase in the odds of receiving ODX testing in 2010-2012.

**Conclusions:** We observe a positive adjusted association between connectedness to an early-adopting physician and ODX prescribing/use in both physician-level and patient-level analyses. These results suggest that provider networks may help diffuse new technologies, and that BC genomic testing is likely to be an area of shared practices between providers. Efforts to increase testing, where appropriate, may benefit from a range of peer-to-peer connection strategies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-09-09

Association between socioeconomic factors at diagnosis and survival in non-metastatic breast cancer: A population-based study

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**Background:** Breast cancer is a severe public health problem for women worldwide. Race disparities and regional disparities are documented regarding incidence, mortality, and survival of breast cancer patients. However, the associations between socioeconomic status and survival outcomes of breast cancer remain unclear and require a comprehensive large-scale investigation of specific socioeconomic factors. Furthermore, no model has included both histological and socioeconomic factors together to predict survival of breast cancer. In this study, we sought to develop nomograms to predict overall survival (OS) and breast cancer-specific survival (BCSS) with consideration of socioeconomic factors for non-metastasis breast cancer.

**Methods:** We included a total of 274,108 female patients, diagnosed with malignant breast cancer between 2007 and 2014 from the Surveillance, Epidemiology, and End Results (SEER) database. Socioeconomic factors involving marital status, insurance status, residence, median household income, poverty rate, unemployment rate and education level were included in the analysis. OS and BCSS were evaluated with log-rank tests and Kaplan-Meier estimates. We identified and integrated significant prognostic factors for OS and BCSS using univariate and multivariate Cox regression analysis to construct nomograms. Calibration plots and concordance indexes were used to evaluate the accuracy and discrimination of the models.

**Results:** Among different age subgroups, insured patients were more likely to have better survival than uninsured patients or patients with Medicaid ( $P < 0.001$ ), and especially for patients who were aged 18 to 35 years old at diagnosis, uninsured patients associated with poor BCSS than Medicaid patients ( $P < 0.05$ ). Through multivariate analysis, we found non-Hispanic black patients experienced worst survival compared with the White and other races ( $P < 0.001$ ). Interestingly, married (vs. single vs. separated/divorced/widowed;  $P < 0.001$ ) and insured (vs. Medicaid vs. uninsured;  $P < 0.001$ ) patients had a better prognosis. Living in the non-metro area increased the risk of death (hazard ratio [HR], 1.084,  $P < 0.05$ ). Furthermore, living in counties with higher median household income ( $>US \$72,800$ ) had favorable impacts on OS (HR 0.843,  $P < 0.001$ ). Four and five socioeconomic factors were involved in constructing the nomograms for 3 years-, 5 years- and 7 years- OS and BCSS, respectively. The C-indexes of the final nomograms were higher than those of the TNM staging system for predicting OS (0.776 vs 0.678;  $P < 0.001$ ) and BCSS (0.842 vs 0.776;  $P < 0.001$ ), respectively. The performance of the nomograms for predicting OS was significantly lower when excluding the socioeconomic factors ( $P < 0.001$ ).

**Conclusion:** Some certain socioeconomic factors (i.e., marital status, insurance status, median household income, and residence) play essential roles in predicting survival of non-metastasis breast cancer. We constructed and validated nomograms including socioeconomic factors to provide more comprehensive and realistic survival estimation. Besides, these findings may highlight the importance of developing health-related policies and the necessity of targeted social support-based interventions for those high-risk patients.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-01

Association of bilateral oophorectomy with sarcopenia and sarcopenic obesity in a diverse, nationally representative sample of U.S. women

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**Background:** Sarcopenia (low muscle mass) and sarcopenic obesity (SO; low muscle mass with high body fat) have been linked to physical disability, poor quality of life, and mortality in older adults. We have previously demonstrated an association between early bilateral oophorectomy and increased body fat. Studies have also indicated that declines in circulating levels of estrogen and dehydroepiandrosterone during natural menopause are associated with loss of muscle mass. Therefore, we sought to evaluate the association between bilateral oophorectomy, sarcopenia, and SO among cancer-free women in the general population. We hypothesized that an abrupt decline in estrogen due to surgical menopause would be associated with sarcopenia and SO.

**Methods:** The study population included cancer-free women aged 35-70 years who underwent whole body dual-energy x-ray absorptiometry (DXA) scans as part of the U.S. National Health and Nutrition Examination Survey 1999-2006 (N=3,764). Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for sarcopenia and SO among women who had previously undergone hysterectomy and bilateral oophorectomy (n=552) compared to women with intact uterus and ovaries (n=3,212). Models accounted for complex survey design and were adjusted for age, race, education, weight at DXA scan, weight at age 25, physical activity, smoking, alcohol use, oral contraceptive use, and parity.

**Results:** The median age at DXA scan was 48 years (interquartile range, 41-56 years) and the median time since oophorectomy was 12 years (5-21 years). About 72.7% of women were non-Hispanic white, 10.8% were non-Hispanic black, 5.8% were Mexican American, and 10.7% other. Women who underwent oophorectomy had two-fold higher odds of sarcopenia (OR, 1.81 [95% CI, 1.12-2.95]) and SO (1.95 [1.17-3.25]) as compared to women with intact uterus and ovaries. The effect was stronger among women who underwent oophorectomy at age <45 (sarcopenia: 2.38 [1.44-3.95]; SO: 2.32 [1.30-4.13]) as compared to women with intact uterus and ovaries, but no difference was observed among women who underwent oophorectomy at age ≥45 (sarcopenia: 1.08 [0.45, 2.58], SO: 1.40 [0.60, 3.28]; sarcopenia:  $p_{\text{interaction}}=0.031$ , SO:  $p_{\text{interaction}}=0.045$ ). Of note, even women with normal BMI (18.5-24.9 kg/m<sup>2</sup>) at DXA scan who underwent oophorectomy at age <45 years had significantly higher odds of sarcopenia (2.37 [1.15, 4.87]) and SO (2.89 [1.23, 6.75]) as compared to women with normal BMI and intact uterus and ovaries.

**Conclusion:** Women who undergo oophorectomy at a young age have an elevated risk of sarcopenia and SO even while maintaining normal weight. This novel finding, if confirmed in prospective studies, suggests that monitoring young women undergoing oophorectomy for sarcopenia and SO may improve long-term health outcomes.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-02

Impact of raloxifene adherence in breast cancer risk

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**BACKGROUND:** Raloxifene is a selective estrogen receptor modulator that has demonstrated to reduce breast cancer risk and reduce the incidence of vertebral fractures. Based on these effects, raloxifene is used as a risk reduction agent and to prevent osteoporosis in postmenopausal women. Little is known about the raloxifene adherence rates or the relationship between adherence and breast cancer incidence.

**METHODS:** Women 60 years or older without a breast cancer history were identified in the MarketScan database (2008-2015). We identified women who received raloxifene by searching for prescription claims. Proportion of days covered (PDC) were calculated, adherence was defined as a PDC  $\geq 80\%$  in the first year after initial prescription claim. We identified factors associated with adherence. ICD-9 codes were used to identify incident cases of invasive breast cancer and cumulative incidence rates were calculated. A multivariable Cox model with propensity score method (matching variables included year of claim, age, comorbidities and family history of breast cancer) was used to evaluate the association between raloxifene adherence and breast cancer risk. All statistical tests were two-sided.

**RESULTS:** A total of 16,179 women were included in the analysis. We identified that during the first year of treatment 6,716 (40.2%) women had a PDC  $\geq 80\%$  and thus were considered to be adherent. Factors associated with increased adherence included the use of generic drug, mail order of 90 days supply and family history of breast cancer (all  $p < 0.001$ ). Using propensity score matching, the 5 year-cumulative incidence of invasive breast cancer was 1.5% among those not adherent and 0.9% among those adherent to raloxifene ( $p = 0.01$ ). Similarly, the 9 year-cumulative rates were 3.6% and 1.9% respectively ( $p = 0.01$ ). After adjusting for potential confounders, patients that were adherent to raloxifene during the first year of treatment had a lower risk of invasive breast cancer compared to those that were non-adherent (HR=0.64; 95%CI 0.04-0.9).

**CONCLUSIONS:** Among women 60 years of age or older receiving raloxifene, adherence to therapy was associated with lower risk of invasive breast cancer. Efforts to ensure adherence and compliance are crucial so patients can receive full benefit from this therapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-03

Tobacco exposure and breast cancer

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**Background:** Smoking is a known risk factor for various types of cancer, and breast cancer patients who smoke are known to have higher breast cancer mortality. However, few studies have found an association between smoking and breast cancer incidence or tumor biology. The Athena Breast Health Network distributes an intake questionnaire at the UCSF and UCSD breast care centers which can be used to investigate links between tobacco exposure and the characteristics of incident breast cancer.

**Methods:** Intake questionnaires were distributed to all new patients at the UCSF and UCSD breast care centers from December 2012 to May 2018. Patients who completed the questionnaire with a known diagnosis of breast cancer were compared to those without in a case-control study. Breast cancer diagnoses were determined by ICD9 diagnosis codes from the patients' medical records. The association of smoking and breast cancer prevalence and biology was analyzed using generalized linear models and Fisher tests in R.

**Results:** Of the 7727 patients who completed the Athena intake questionnaire at UCSF and UCSD, 5499 consented to have their data used for research. A first analysis was conducted on 4175 UCSF patients alone: 2186 of the UCSF patients who had completed the questionnaire had a documented breast cancer diagnosis, vs 1989 with no known diagnosis at the time of this analysis. 1096 of the 4175 UCSF patients reported having ever smoked, including 73 who had accrued 30 or more pack years. Complete pathology data was available for 1120 cancer patients. Controlling for age, more patients with invasive breast cancer reported having ever smoked, with an odd's ratio (OR) of 2.32 ( $p = .0043$ ). By including DCIS, the OR drops slightly to 2.26 ( $p = .0058$ ). Taking alcohol consumption into account as a confounder lowered the OR to 2.19 ( $p = .0454$ ). Overall, the risk of breast cancer increases with each additional pack year (OR = 1.08,  $p = .0211$ ), independent of age. There are no significant differences in tumor biology for any smoking group.

**Conclusions:** A history of smoking is associated with an increased risk of developing breast cancer and is directly related to cumulative pack years exposure. This association should be further validated in cohort studies.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-04

Assessment of clinical trial participation on breast cancer survival

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## **Background**

Accredited cancer treatment centers offer clinical trials for patients since it is universally accepted that participation in these trials is advantageous.

## **Purpose**

The purpose of this study was to assess whether clinical trial participation was associated with a survival advantage.

## **Methods**

We identified 308,291 cases of first primary female invasive breast cancer from the California Cancer Registry 2000-2015. Participation in a clinical trial was documented. Clinical trials were defined as National Cancer Institute (NCI) sponsored studies such as National Surgical Adjuvant Breast and Bowel Project (NSABP) and Southwest Oncology Group (SWOG); pharmaceutical trials; and local investigator initiated studies.

The distribution of age, race/ethnicity, socioeconomic status (SES), and stage between clinical trial participants and non-participants was compared using contingency tables and the  $\chi^2$  Test.

Kaplan-Meier Survival Analysis and the Log Rank test were used to compare differences in breast cancer specific survival between participants and non-participants. Cox Regression Analysis was used to estimate the risk of mortality between participants and non-participants after adjusting for age, race/ethnicity, SES, grade, and treatment. Analyses were conducted separately for each stage. Hazard ratios (HR) and 95% confidence intervals (CIs) were reported.

## **Results**

There were 3,517 (1.1%) patients who participated in a clinical trial. Almost 50% of participants were enrolled in an NCI trial, 38% in a local/investigator initiated study, and 13% were in a trial sponsored by a pharmaceutical company. Trial participation was highest for patients less than 45 years of age (21.9%) and lowest in patients 70 years of age and older (12.3%). Most participants were in stage 2 (44.6%). Participation increased with increasing SES. Only 8.5% of participants were in the lowest SES whereas 35.4% were in the highest SES.

Unadjusted survival analysis indicated that there was no survival advantage for stage 1 patients ( $\chi^2 = 0.41$ ,  $p=0.52$ ) whereas study participants in stages 2, 3, and 4 had statistically significantly better survival. Adjusted hazard ratios indicated that stage 2 patients participating in a trial had a 23% reduced risk of mortality (HR=0.77; CI: 0.65 - 0.94). For patients in stage 4, the risk of mortality was reduced by 36% (HR=0.64; CI: 0.47 - 0.87). There was no risk reduction for patients in stages 1 and 3.

## **Conclusion**

Only a small percent of patients enroll in breast cancer clinical trials and participation is correlated with age and SES. There is a survival advantage for participation in a clinical trial for stages 2 and 4 patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-05

High risk for cardiovascular disease in postmenopausal breast cancer survivors

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**Background:** The majority of women diagnosed with breast cancer are considered to have a good prognosis and can expect to survive the disease. Cardiovascular risk in postmenopausal women treated for breast cancer is higher than in women without cancer.

**Objective:** To evaluate cardiovascular risk factors in postmenopausal breast cancer survivors, in comparison to postmenopausal women without breast cancer.

**Methods:** In this cross-sectional study, 96 postmenopausal breast cancer survivors were compared to 192 postmenopausal women (controls), aged 45 to 75 years. The main group included women with amenorrhea > 12 months, aged  $\geq 45$  years, with a histological diagnosis of breast cancer, without metastatic disease and without established cardiovascular disease (CVD). The control group consisted of women with amenorrhea > 12 months, aged  $\geq 45$  years, without breast cancer and CVD. Groups were matched by age, time since menopause, and body mass index (BMI) in a ratio of 1 case to 2 controls, according to sample calculation, with a minimum of 92 breast cancer survivors. Clinical and anthropometric data (BMI and waist circumference) were collected by interview and physical examination. For biochemical analysis, total cholesterol, HDL, LDL, triglycerides, glucose and insulin levels were measured. Women presenting with three or more of the following criteria were diagnosed with metabolic syndrome (MetS): waist circumference (WC) > 88 cm; TG  $\geq 150$  mg/dL; HDL cholesterol < 50 mg/dL; blood pressure  $\geq 130/85$  mmHg; glucose  $\geq 100$  mg/dL. For measurement of plasma HSP 60 and 70 concentrations, immunoassays were used (ELISA test). Atherosclerotic disease was determined by intima-media thickness (IMT > 1 mm) of the carotid arteries and / or by the presence of atheromatous plaque, assessed by carotid artery ultrasound (scanner duplex). For statistical analysis, Student's t-test, Gamma Distribution (asymmetric variables), Chi-Square Test and Logistic Regression (odds ratio-OR) were used.

**Results:** Breast cancer survivors had high mean systolic and diastolic blood pressure ( $p < 0.001$ ), and mean blood triglycerides and glucose, above desirable levels ( $p < 0.05$ ). Breast cancer patients had higher HSP60 levels and lower HSP70 levels than controls ( $p < 0.05$ ). Atheromatous plaque occurred more frequently in breast cancer survivors than in controls (19.8% vs. 9.4% respectively) ( $p < 0.05$ ). Analysis of risk adjusted for age, time since menopause and BMI, showed that women treated for breast cancer have a significantly higher risk of MetS (OR = 4.21, 95% CI 2.28-7.76), atheromatous plaque (OR = 2.61, 95% CI 1.19-5.72), diabetes (OR=4.42; 95%CI 1.86- 10.49), hypertriglyceridemia (OR = 2.32, 95% CI 1.33-4.0) and increased waist circumference (OR = 11.22, 95% CI 4.0 - 31.65) than women without breast cancer.

**Conclusion:** Postmenopausal breast cancer survivors are at higher risk for metabolic syndrome, diabetes, atherosclerotic disease, hypertriglyceridemia and abdominal obesity (major risk factors for cardiovascular disease), in comparison to postmenopausal women without breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-06

Factors impacting the accuracy of self-reported breast procedures among women with and without breast cancer

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**Background:** Clinical/epidemiologic observational studies frequently rely on participants' recall for information about breast procedures. However, there is limited data on the accuracy of self-reported breast procedures. To address this knowledge gap and inform future study design and collection and interpretation of similar data, we investigated the impact of type, diagnosis, age, time, and other patient characteristics on the accuracy of self-report in a prospective cohort.

**Methods:** All benign breast biopsies, lumpectomies, and mastectomies for breast cancer treatment among women enrolled in the BOSS Cohort, a prospective study of women and men with a familial risk of breast/ovarian cancer, were identified. Study staff obtained pathology reports for 93% of women from self-reported breast procedure locations. For this analysis, we focused on 577 women who had at least one ascertained pathology report, and who self-reported at least one breast procedure at baseline. We estimated the percentage of self-reports (95% confidence interval (CI)) with matching pathology report within 6 months ( $\pm$  6 months), and agreement between self-reported procedures and pathology-confirmed diagnoses (normal/benign vs. atypical hyperplasia vs. LCIS, and DCIS vs. invasive cancer) with the Kappa statistic. We also examined predictors of an accurate biopsy self-report, including age at baseline, personal and family history of breast cancer, educational attainment, and time between biopsy and baseline, using logistic regression models.

**Results:** At baseline, 158 women reported having at least one benign biopsy, 193 women reported having a lumpectomy for cancer treatment, and 174 women reported having a mastectomy for cancer treatment. The median time between biopsy, lumpectomy, mastectomy, and baseline was 9 years, 2 years, and 2 years, respectively. Fifty-seven percent (95% CI: 49-64.5%) of benign biopsy self-reports, 90.7% (95% CI: 85.6-94.1%) of lumpectomy self-reports, and 85.1% (95% CI: 78.9-89.7%) of mastectomy self-reports had a matching pathology report within 6 months. Further diagnostic agreement was moderate for biopsies, lumpectomies, and mastectomies with Kappa statistics of 0.65, 0.66, 0.65, respectively. Age at baseline (p-interaction = 0.01) and time (p-interaction = 0.03) were independent and joint predictors of accurate biopsy self-reports. Women less than 49 years old had the largest reduction in odds of having an accurate self-report (26%) for every additional year between biopsy and baseline [adjusted odds ratio = 0.74 (95% CI: 0.63-0.88)]. Similarly, women with a biopsy within 4 years prior to baseline had a 10% reduction in the odds of having an accurate self-report with increasing age [adjusted odds ratio = 0.9 (95% CI: 0.84-0.97)].

**Conclusions:** In this highly-educated cohort, the overall accuracy of self-report of benign biopsies was only modest, and the accuracy of self-report of lumpectomies and mastectomies was lower than expected. This study suggests that age at baseline and time between procedure and baseline are important predictors of accuracy of self-report and should be considered when utilizing self-reported information. Furthermore, where possible, prospective collection of breast procedure data should be prioritized.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-10-07

Breastfeeding experience among breast cancer patients in the modern era

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**Introduction:** In recent years, the uptake of breastfeeding has become more common as it is regarded as healthy and beneficial for both mother and infant. The process of parturition and lactation plays a role in the normal differentiation and development of breast tissue, and multiparity has been associated with a decreased risk for breast cancer. The current study sought to describe the breastfeeding experience of a modern cohort of women with newly diagnosed BC, and to examine the clinicopathologic characteristics of their disease.

**Methods:** A retrospective review of our institutional Breast Cancer Database from 2009-2017 was performed to identify women with at least one full term pregnancy (FTP). Clinicopathologic and demographic information was recorded, including breastfeeding experience and cumulative duration of nursing. Women were grouped by self-reported breastfeeding experience and duration of breastfeeding for analysis. Pearson's chi-square tests were performed.

**Results:** Of 1919 patients, 1053 (54.9%) reporting breastfeeding. Breastfeeding increased from a low of 30.4% among women with first FTP (FFTP) in the 1950's to 84.6% with FFTP in the 2010's. There were no significant differences between those who did and did not breast feed with regards to race, family history, BRCA status, pathologic stage, grade, tumor histology, lymphovascular invasion (LVI), multifocality, tumor size or receptor status. When stratified by duration of breastfeeding, the most striking finding was that women who breastfed for >12 months were more likely to have tumors associated with LVI (p = 0.028).

Table – Breastfeeding Experience Among Parous Women with Breast Cancer

Variable	No Breastfeeding (n=866)	Breastfeeding (n=1053)	p-value
<b>Race</b>			0.432
White	648 (74.8%)	767 (72.8%)	
Black	73 (8.5%)	112 (10.6%)	
Asian	78 (9%)	102 (9.7%)	
Hispanic	64 (7.4%)	67 (6.4%)	
Other	3 (0.3%)	5 (0.5%)	
<b>Family history</b>	272 (31.4%)	311 (29.6%)	0.397
<b>BRCA 1,2 positive</b>	23 (2.7%)	42 (4.0%)	0.108
<b>Final Pathology Stage</b>			0.224
0	190 (21.9%)	222 (21.1%)	
I	426 (49.2%)	507 (48.1%)	
II	197 (22.8%)	229 (21.7%)	
III	40 (4.6%)	63 (6.0%)	
IV	1 (0.1%)	3 (0.3%)	
No residual (neoadjuvant)	12 (1.4%)	29 (34.1%)	
<b>Invasive Grade</b>			0.127
Low	92 (14.1%)	120 (15.3%)	
Intermediate	371 (56.7%)	398 (50.6%)	
High	191 (29.2%)	268 (34.1%)	
<b>Histology</b>			0.130
DCIS	189 (21.8%)	223 (21.2%)	

IDC	531 (61.3%)	688 (65.3%)	
ILC	113 (13.1%)	99 (9.4%)	
Other	33 (3.8%)	43 (4.1%)	
<b>LVI</b>	127 (14.7%)	174 (16.5%)	
<b>Multifocality</b>	147 (17%)	183 (17.4%)	
<b>Median tumor size (cm; range)</b>	1.4 (0-9.5)	1.3 (0-12.5)	0.489
<b>Estrogen Receptor</b>			0.206
Positive	726 (84.7%)	861 (82.6%)	
Negative	131 (15.3%)	182 (17.4%)	
<b>Progesterone Receptor</b>			0.275
Positive	621 (72.5%)	732 (70.2%)	
Negative	236 (27.5%)	311 (29.8%)	
<b>HER2/neu Receptor</b>			0.068
Positive	78 (12%)	121 (15.4%)	
Negative	571 (88%)	667 (84.6%)	

**Conclusions:** Breastfeeding experience was not generally associated with significant differences in tumor or patient characteristics. However, breastfeeding for longer than 12 months was associated with LVI. It is possible that changes in the breast tissue that occur during the process of pregnancy and prolonged lactation may influence future tumor development. These findings are hypothesis generating and suggest that the relationship of prolonged breastfeeding and breast cancer development should be investigated further.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-10-08

Family history of breast cancer and mammographic density in premenopausal women

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**Introduction:** Mammographic density and family history of breast cancer (FHBC) are independent risk factors for breast cancer. Women with dense breasts have a 4-6-fold increased risk of breast cancer and women with FHBC have a 1.5-3-fold increased risk of breast cancer. However, there is little data on the associations of FHBC with mammographic density, especially in premenopausal women. To address this, we investigated the associations of FHBC in first-degree relatives with mammographic density in premenopausal women.

**Methods:** We used data from 375 cancer-free premenopausal women who were during routine screening mammography at Washington University in St. Louis in 2016. We used Volpara to measure volumetric measures of density including volumetric percent density, dense volume, and non-dense volume. We collected data on first-degree relatives (mother, sister) and numbers of first degree relatives (0, 1, ≥2) with a positive FHBC. We used multivariable linear regression model, adjusted for age, BMI, parity, race, age at menarche, and alcohol consumption, to determine the associations of FHBC and log-transformed volumetric percent density, dense volume, and non-dense volume. Beta coefficients ( $\beta$ ) were evaluated and back transformed for easier interpretation.

**Results:** The mean age of participants was 47.5 years (range=32-58). The mean BMI was 30.8 kg/m<sup>2</sup> (range =17.9-63.1). Volumetric percent density was 25% (p-value<0.001) higher in women who had a FHBC compared with women who had no FHBC

Table 1. Associations of family history of breast cancer (FHBC) and volumetric percent density (VPD), and dense volume (DV) in 375 premenopausal women\*.

FHBC	N	VPD (%)		DV (cm <sup>3</sup> )	
		Beta (95% CI)	P-value	Beta (95% CI)	P-value
No	275	Ref		Ref	
Yes	87	1.25 (1.12,1.41)	<0.001	1.16 (1.04,1.29)	0.007
Unknown	13	1.00 (0.76,1.30)	0.99	0.74 (0.58,0.95)	0.017

\*Adjusted for age, BMI, parity, race, age at menarche, and alcohol consumption.

. The associations appeared confined to women with a positive family history in mothers but not those with a positive family history in sisters. Volumetric percent density was 31% higher (p-value<0.001) among women with a positive FHBC in mothers compared to those without a positive FHBC in mothers, but 8% higher (p-value=0.45) among women with a positive FHBC in sisters compared to those without a positive FHBC in sisters. The associations of FHBC with dense volume were similar to those of volumetric percent density in all the analyses. For instance, dense volume was 16% (p-value=0.006) higher in women who had a FHBC compared with women who had no FHBC.

**Conclusions:** Premenopausal women with a first-degree FHBC have higher volumetric percent density. Our findings could help identify high-risk women who may benefit from targeted screening.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-09

The impact of Charlson comorbidity index on survival of triple negative breast cancer

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## **Background**

Patients with cancer and comorbidities have been found to have poorer survival since they may be unable to receive or complete treatments. It is not clear if comorbid conditions impact survival of patients in all stages of disease and all ER/PR/HER2 subtypes.

## **Purpose**

The purpose of this study was to determine how the Charlson Comorbidity Index (CCI) affected the risk of mortality of triple negative breast cancer (TNBC) within each stage of disease.

## **Methods**

We accessed 22,503 cases of TNBC and documented CCI from the California Cancer Registry 2000-2015. The CCI is a weighted index based on the presence of certain comorbid conditions twelve months prior through six months following the cancer diagnosis and weighted by the severity of those conditions. A score of 0 is interpreted as no significant comorbidity burden and scores of 3 or more are generally interpreted as a high comorbidity burden.

Kaplan-Meier Survival Analysis and the Log Rank test were used to compare differences in breast cancer specific survival between patients with a CCI of 1, 2 or higher (2+) versus a CCI of 0. Cox Regression Analysis was used to estimate risk of mortality of CCI after adjusting for age, race/ethnicity, socioeconomic status, grade, and treatment. Analyses were conducted separately for each stage. Hazard ratios and 95% confidence intervals (CIs) were reported.

## **Results**

There 16,664 (74.1%) cases with a CCI of 0; 3,915 (17.4%) with a CCI of 1; 1,055 (4.7%) had a CCI of 2, and the remaining 869 (3.9%) cases of TNBC had a CCI of 3 or higher.

Unadjusted survival analysis indicated that for stages 2 and 3, there was increased survival with decreasing CCI score. For stages 1 and 4, survival was only better for patients with a CCI of 0 versus a CCI of 2+.

Cox regression analysis indicated that the CCI made no difference in risk of mortality for patients with stage 1 disease. Patients with a CCI of 2+ had an increased risk of mortality when compared with a CCI of 0 for stage 2 (HR = 1.48, CI: 1.25-1.77), stage 3 (HR=1.25, CI: 1.03-1.52) and stage 4 (HR = 1.53; CI: 1.22-1.92).

## **Conclusion**

Comorbidity as measured by the CCI does not increase the risk of mortality for patients with stage 1 TNBC and only increases risk of mortality in higher stages for patients with a CCI score of 2 or higher.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-10-10

Assessment of short inter-pregnancy interval in breast cancer diagnosed during pregnancy

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**Background:** The relationship between pregnancy and breast cancer (BC) risk is not fully understood. Most of the literature has described this interaction in terms of the age at first pregnancy and the number of full-term pregnancies. During the prospective accrual of the “Joven & Fuerte” young women with BC program in Mexico, we identified patients with pregnancy-associated BC (PABC) that experienced a short inter-pregnancy interval (SIPI) (<18 months between a live birth and the beginning of a following pregnancy). To our knowledge, there are no descriptions of the interaction between SIPI and PABC.

**Objective:** We aim to assess the occurrence of SIPI in patients with PABC, as well as describe their clinical features at presentation.

**Results:** We included patients accrued in the “Joven & Fuerte” program from August 2014 and June 2018 at three Mexican cancer centers. A total of 375 patients ≤40 years-old with newly-diagnosed BC were assessed. 37 patients were diagnosed with PABC (10%), 21 during pregnancy and 16 after pregnancy. 6/37 (16%) patients with PABC experienced a SIPI, all diagnosed during pregnancy (28% of 21). The main clinicopathological features are described in the table 1.

Table 1. Clinicopathological features of patients with PABC and SIPI

Case	Age at BC diagnosis (years)	IPI (months)	Pregnancy trimester at diagnosis	Stage	BC subtype
1	31	18	3rd	IIB	Not available
2	32	6	2nd	IIIA	HR neg, HER2 pos
3	37	14	3rd	IIA	HR neg, HER2 pos
4	36	15	2nd	IV	HR pos, HER2 neg
5	31	4	2nd	IIIA	HR pos, HER2 neg
6	31	4	2nd	IIIC	Not available

**Conclusion:** According to the phenomena seen in this cohort of young BC patients, we hypothesize that SIPI may increase the incidence and/or modify the clinical outcomes of PABC. The evidence from clinical and laboratory data suggest that the mechanisms that could alter the interaction include, but are not limited to, the prolongation of the exposure to high concentrations of estrogens or their genotoxic metabolites, interaction with the effect of progesterone and other pregnancy-associated hormones on breast tissue, and immunologic factors. To date, to our knowledge there is no evidence about the effect of SIPI in PABC. Thus, research in this area should be encouraged, particularly in vulnerable women in limited-resource settings, where SIPI is, unfortunately, a common occurrence, and might represent a risk factor for BC/PABC.

# 2018 San Antonio Breast Cancer Symposium ®

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Pregnancy-associated breast cancer in a contemporary cohort of newly diagnosed women

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**Introduction:** Pregnancy-associated breast cancer (PABC) refers to breast cancer (BC) diagnosed during pregnancy, lactation, or in the postpartum period. There is evidence that PABC is associated with a poorer prognosis, and that the development of the disease is influenced by the unique hormonal milieu of pregnancy. The purpose of this study was to investigate the clinicopathologic characteristics associated with PABC in a contemporary cohort of women with newly diagnosed BC.

**Methods:** Our institutional Breast Cancer Database was queried for women diagnosed with breast cancer between 2010-17 who had at least one full term pregnancy (FTP). Variables of interest included patient demographics and clinical and tumor characteristics. PABC was defined as breast cancer diagnosed within 24 months of delivery. Statistical analyses included Pearson's chi-square and logistic regression.

**Results:** Out of a total of 1934 women, 42 (2.2%) had PABC. Median follow up in the total cohort was 4.5 years. After adjusting for age at diagnosis, PABC was associated with older age at first FTP, ethnic minority status, BRCA mutation carriers, presentation with a palpable mass, higher histologic grade, and ER-negative and triple negative receptor status. Variables that were not significantly different between PABC and non-PABC cases included tumor histology, multifocality, presence of lymphovascular invasion, and family history of breast cancer.

Table: Selected Characteristics of Women with PABC

Variable	Non-PABC (n=1892)	PABC (n=42)	P-value*
<b>Age at first full term pregnancy</b>			<0.001
<35 years	1610 (85%)	28 (66.7%)	
≥35 years	277 (15%)	14 (33.3%)	
<b>Race</b>			0.001
White	1397 (73.8%)	23 (54.8%)	
Black	181 (9.6%)	8 (19%)	
Asian	175 (9.2%)	10 (23.8%)	
Hispanic	131 (6.9%)	1 (2.4%)	
Other	8 (0.4%)	0 (0%)	
<b>BRCA 1,2 Positive</b>	56 (3%)	9 (21.4%)	<0.001
<b>Method of Presentation</b>			0.002
Breast Exam	579 (30.6%)	30 (71.4%)	
Mammography	1137 (60.1%)	10 (23.8%)	
Ultrasound	87 (1.6%)	2 (4.8%)	
MRI	67 (3.5%)	0 (0%)	
Other	22 (1.2%)	0 (0%)	
<b>Invasive Grade</b>			0.014
Low	213 (15%)	0 (0%)	
Intermediate	763 (53.8%)	12 (37.5%)	
High	442 (31.2%)	20 (62.5%)	
<b>Estrogen Receptor</b>			0.034
Positive	1572 (83.9%)	29 (69%)	

Negative	301 (16.1%)	13 (31%)	
<b>Triple Negative</b>	135 (7.1%)	7 (16.7%)	0.041

\*P-values are age-adjusted.

**Conclusions:** The association of PABC with ethnic minority status in our cohort is interesting and may be reflected in the increased proportion of triple negative breast cancers in the PABC group. In our contemporary cohort, PABC was associated with older age at first FTP. As more women delay childbearing, risk for PABC may increase. Our findings suggest that women who become pregnant at older ages should be followed carefully during pregnancy and in the postpartum period, especially if they are BRCA mutation carriers. The optimal approach for monitoring older women during pregnancy and the postpartum period is unclear. Clinical breast exam may play an important role, especially for those women known to be at increased risk for breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-12

Gestational breast cancer in Singapore women

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## Background

Gestational breast cancer (GBC), diagnosed during pregnancy or in the first postpartum year, is expected to rise in incidence due to increasing maternal age. GBC poses unique challenges in diagnosis and treatment as clinicians seek to provide optimal treatment for these young patients without compromising neonatal outcomes. We present our retrospective findings from the first study on gestational breast cancer in Singapore.

## Methods

We performed a retrospective study on 88 patients with gestational breast cancer diagnosed from April 2003 to April 2017 at three centres in Singapore. Demographic details, tumour histopathological characteristics, stage, treatment and outcomes data was collected and analysed.

## Results

Eighty-eight patients were diagnosed with GBC at a median age of 35.9 years (26-43 years). Fifty (56.8%) were diagnosed intrapartum and 38 (43.2%) were diagnosed postpartum. Seventeen (19.8%) had a family history of breast cancer but none had proven BRCA mutation. Seventeen patients (19.3%) presented with stage 1, 36 (40.9%) with stage 2, 25 (28.4%) with stage 3, 5 (5.7%) with stage 4 disease. Seventeen patients underwent termination of pregnancy (27.9% of patients diagnosed during pregnancy). Seventeen (19.3%) of patients received neoadjuvant chemotherapy, 8 of whom received it during pregnancy. Forty-eight (54.5%) received adjuvant chemotherapy, 4 of whom received it during pregnancy. Two patients received palliative chemotherapy during pregnancy. Aside from one case of hearing impairment, there was no other documented neonatal complication for patients who received intrapartum chemotherapy.

The 5-year and 10-year overall survival (OS) was 80% and 66%. According to the Singapore National Cancer Registry, the 5-year OS for women younger than 44 years of age was 88.9%, therefore suggesting a lower OS in patients with GBC. The 5-year and 10 year disease-free survival (DFS) of our patients was 73% and 60% respectively. Diagnosis of cancer postpartum conferred a higher risk (hazard ratio (95%CI) 1.86 (0.55, 6.28) of mortality compared to those diagnosed intrapartum. Univariate Cox proportional hazard regression model showed that nodal positivity and clinical stage were significantly associated with DFS while only clinical stage was significantly associated with OS. Race was found to affect survival with Malay patients having a lower DFS compared to Chinese patients.

## Conclusion

GBC patients in Singapore have a lower survival rate. Diagnosis of cancer postpartum confers a higher risk of mortality. This might be due to a delay in diagnosis and treatment. Malay patients have a lower survival compared to Chinese patients. Studies into genetic and social factors might shed light on how ethnicity affects survival of these patients.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-10-13

Screening and risk reducing surgeries for patients at high risk for breast and ovarian cancer at an integrated care setting

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**Background:** Current US guidelines recommend more intensive screening and preventive strategies for patients with a known pathogenic germline mutation or a high lifetime risk based on breast cancer risk prediction models. The American Cancer Society, for example, recommends that yearly mammogram alternating with MRI screening should be considered as early as 30 years old in women with a lifetime breast cancer risk of > 20%. Furthermore, NCCN recommends that BRCA1/2 mutation carriers consider additional risk-reducing strategies, including bilateral mastectomy, bilateral oophorectomy at age 35, or post-child bearing, and hormonal chemoprevention. It is unclear what the uptake of screening and risk reducing strategies are after an HRBROC assessment and recommendations by Genetics at an integrated care setting.

**Methods:** We retrospectively studied female patients diagnosed as high risk for breast and ovarian cancer (HRBOC) (regardless of prior cancer) and/or tested for *BRCA* mutations by a genetic counselor or physician geneticist at Kaiser Permanente Mid-Atlantic States (KPMAS) Genetics clinics between 2005-2016. We identified cancer diagnoses, mammogram and MRI screening, mastectomies and oophorectomies with ICD diagnosis or CPT procedure codes during the study period. We defined prophylactic mastectomy or prophylactic oophorectomy as occurring either 180 days prior to, or without a diagnosis of breast or ovarian cancer respectively. We assessed screening with a mammogram or MRI, post Genetics visit for patients 30-75yrs old at time of HRBOC dx, and had no breast cancer prior to, or within 180 days, of the Genetics visit.

**Results:** Our cohort included 813 women with a HRBOC diagnosis, with a median 51 yrs of age at diagnosis, 45% White, 38% Black and 15% other race. Since genetics services for cancer at KPMAS were established recently, 98% of visits occurred after 1-1-2013. Table 1 shows the distribution of cancer diagnoses and surgeries pre and post Genetics visit. 249 Breast cancer-free patients were screened post Genetic visit between mid-2013-2016: 159 (64%) had at least 1 screening test, 68 had two to three and only 3 women had four to six screening tests. The median time to the latest screen after HRBOC dx was 14.4 mo, (range 2 days to 39 mo). Post-Genetics screening frequency did not differ by race.

**Conclusion:** The majority of women visited Genetics after a diagnosis of breast or ovarian cancer. Patients received prophylactic oophorectomies more often than prophylactic mastectomies. Screening in cancer free patients with a HRBOC diagnosis appears to be limited to 1-3 screens post diagnostic visit. Our findings suggest that earlier detection of patients at high risk for breast and ovarian cancer and closer monitoring is needed.

Table 1. Cancer diagnoses and Surgeries Pre and Post Genetics Visit for HRBOC (2004-2016) (N=813)

	pre-Genetics	post-Genetics	Total
Breast Cancer diagnosed	513	14	527
Post cancer Mastectomy	319	8	327
Prophylactic Mastectomy	3	1	4
Ovarian Cancer diagnosed	55	2	57
Post cancer oophorectomy	40	3	43
Prophylactic oophorectomy	63	26	89

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-14

Impact of route of administration of estradiol (oral vs. transdermal) on genotoxic estrogens concentrations in girls with ovarian failure due to Turner syndrome: Potential implications for breast cancer prevention

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**Objective:** The established link between estrogen and breast cancer occurs via both estrogen receptor (ER)-mediated and non ER-mediated mechanisms. The term *genotoxic estrogens* describes mutagenic metabolites, including estrogen catechols and quinones, which have been linked to breast carcinogenesis in post-menopausal women. Recent data showed that childhood obesity is associated with significantly higher levels of genotoxic estrogens in the blood compared with lean children, raising the possibility of a potential pathogenic roles of these metabolites in breast cancer starting even prior to the onset of puberty (Maura et al: J Clin Endocrinol Metab. 100:2322, 2015). A finding of higher levels of genotoxic estrogens associated with oral estradiol may have breast cancer prevention implications. We hence aimed to assess if the route of administration of 17 $\beta$  estradiol (E<sub>2</sub>) affects the accumulation of genotoxic estrogen metabolites in a model of ovarian failure in young girls with Turner Syndrome.

**Methods:** Stored plasma were used from 40 adolescents with Turner's who participated in a previous 12 months randomized controlled trial of the metabolic impact of E<sub>2</sub> orally (2mg/d) vs. transdermally (100 $\mu$ g/d). The doses of oral and transdermal E<sub>2</sub> were determined to result in similar plasma levels of unconjugated E<sub>2</sub>. Previously we had reported that despite the similar plasma levels of unconjugated E<sub>2</sub>, the oral E<sub>2</sub> administration route was associated with higher levels of biologically active estrogen activity than the transdermal route (Torres-Santiago L et al: J Clin Endocrinol Metab. 98:2716, 2013). In this study, we measured 12 estrogen metabolites (conjugated and unconjugated) using a highly sensitive LCMSMS assay. Results from 48 normally menstruating adolescents were used for comparison.

**Results:** After treatment, least square mean (SE) total (conjugated plus unconjugated) E<sub>2</sub> and estrone (E<sub>1</sub>) concentrations were higher in the oral vs. transdermal group (p<0.0001), as were catechol-estrogens 4-OH-E<sub>2</sub> (149 vs. 28 (49) pmol/L), 2-OH-E<sub>2</sub> (300 vs 76 (52)), 4-OH-E<sub>1</sub> (450 vs 105 (113)), 2-OH-E<sub>1</sub> (304 vs 740 (684)) and 16 $\alpha$ -OH-E<sub>1</sub> (3007 vs 157 (534)) (<0.001 between groups). Levels were much closer to controls in the transdermal group.

**Conclusions:** Common feminizing doses of oral estradiol for 12 months result in greater accumulation of unphysiologic, genotoxic estrogens than transdermal estradiol, expanding concerns about oral estrogens' first hepatic passage contributing to the accumulation of these metabolites. These metabolites have the potential for inducing breast cancer in post-menopausal women. These results suggest the potential benefit of preferential use of transdermal versus oral estrogens as replacement and possibly contraceptive options, in the prevention of breast cancer. Further studies to assess long-term risks of these metabolites in women taking different forms of estrogen replacement are needed.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-15

Obesity influences the character of the breast cancer in postmenopausal women in Japan

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**Background:** We know Estrogen Receptor (ER) positive and/or Progesterone Receptor (PgR) positive tumors tend to develop more frequently in obese individuals in postmenopausal women. However, the average body size of a Japanese woman is relatively smaller than that of a Caucasian woman. There are few reports about the influence of the obesity to the character of the Japanese breast cancer women and the tendency is not clear. The aim of this study is to clarify whether the tendency is the same even in Japanese breast cancer women. Furthermore, we investigate whether HER2 expression (HER2) and Ki-67 index (Ki-67) have some relations with the height (HT), the weight (WT) and the body mass index (BMI) in Japanese breast cancer women.

**Methods:** HT and WT of 279 Japanese women with breast cancer in Kitamura hospital were evaluated. The mean HT and WT of them are 153.6cm and 54.9kg. Subsequently, the women's BMI ( $WT/(HT)^2$ ) was calculated. The mean BMI was 23.3. The ER and PgR were stained for immunohistochemical (IHC) analysis. Regardless of the intensity, stained cases were defined as positive. HER2 was divided into HER2 negative (IHC score 0, +1 and/or FISH negative) or positive (IHC score +3 and/or FISH positive). Ki-67 was determined by staining with MIB-1 antibody, and the cutoff value was decided on 20%, and divided into two groups of more than 20% (Higher) and less than 20% (Lower). Then, we examined the relationship of HT, WT and BMI with ER, PgR, HER2 and Ki-67 in postmenopausal and premenopausal women.

**Result:** In postmenopausal women, WT and BMI were significantly higher in ER positive ( $p=0.0230$ ,  $p=0.0129$ ). WT and BMI were also significantly higher in PgR positive ( $p=0.0049$ ,  $p=0.0294$ ). There was no significant difference between HER2 positive and Her2 negative, and between Ki-67 Higher and Ki-67 Lower either. In premenopausal women, no significant association was observed in all items.

**Conclusion:** In postmenopausal women, HT did not have the significant difference between ER (and PgR) positive and ER (and PgR) negative. However, ER (and PgR) positive were significantly higher in WT and BMI. It was thought that the obesity influences the character of the breast cancer in postmenopausal women. Compared to less obese postmenopausal Japanese women with breast cancer, more obese postmenopausal Japanese women have a propensity for developing hormone sensitive tumors.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-16

Neoadjuvant chemotherapy infusion in the arm ipsilateral to breast cancer increases the risk of lymphedema

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**Background:** Neoadjuvant chemotherapy (NAC) has been administered to clinically axillary positive breast cancer (BC) patients. Current guidelines still recommend axillary lymphadenectomy (AL) in patients with persistent positive axillary lymph node disease. We aimed to evaluate the association of NAC and/or adjuvant chemotherapy (CT) infusion in the ipsilateral upper limb (IA) with AL and the occurrence of lymphedema (LE) secondary to BC treatment. **Methods:** A prospective cohort study of 683 women subjected to AL and treated with NAC and/or adjuvant CT for BC. The patients were evaluated before treatment, immediate and every 6 months after surgery. Cumulative incidence and population attributable risks of LE were calculated. **Results:** 8-year cumulative incidence of LE was 33.1%. NAC and CT infusion and infusion of >2 cycles into the IA respectively increased by 1.68, 1.67 and 1.78 times the risk of LE respectively (all  $P<0.01$ ). LE could be avoided in 9.4% of cases if the CT infusion had not been administered in the IA. **Conclusions:** 33.1% of women developed LE. The risk of LE was increased among women who received CT in the IA. Avoidance of NAC or adjuvant CT in the IA could prevent 9% of the LE cases observed in this population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-10-17

Plasma metabolomic signatures associated with long-term breast cancer risk in the SU.VI.MAX prospective cohort

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**Purpose:** Breast cancer is a major cause of death in occidental women. Mechanisms involved in its etiology remain misunderstood. Metabolomics is a powerful tool which may help elucidating novel biological pathways and identify new biomarkers in order to predict breast cancer well before symptoms appear. The aim of this study was to investigate whether untargeted metabolomic signatures from blood draws of healthy women could contribute to better understand and predict the long-term risk of developing breast cancer.

**Methods:** A nested case-control study was conducted within the SU.VI.MAX prospective cohort (13 years of follow-up) to analyze baseline plasma samples of 211 incident breast cancer cases and 211 matched controls by LC-MS mass spectrometry. Multivariable conditional logistic regression models were computed.

**Results:** 83 ions were significantly associated (corrected-pvalue <0.05) with breast cancer risk. Notably, we observed that a lower plasma level of O-succinyl-homoserine and higher plasma levels of valine/norvaline, glutamine/isoglutamine, 5-aminovaleric acid, phenylalanine, tryptophan,  $\gamma$ -glutamyl-threonine, ATBC, 2-amino-cyanobutanoic acid and pregnene-triol sulfate were associated with an increased risk of developing breast cancer during follow-up. Corrected-pvalues ranged from 0.009 (OR=1.43[1.14-1.78] for phenylalanine and OR=1.45[1.15-1.83] for valine/norvaline) to 0.03 (OR=1.28[1.03-1.58] for 2-amino-cyano-butanoic acid).

**Conclusion:** Several pre-diagnostic plasmatic metabolites are strongly associated with long-term breast cancer risk. If confirmed in other independent cohort studies, these results could help to identify healthy women at higher risk of developing breast cancer in the subsequent decade and to propose a better understanding of the complex mechanisms involved in its etiology.

**Trial registration:** SU.VI.MAX, clinicaltrials.gov NCT00272428. Registered 3 January 2006

**Keywords:** Metabolomics, breast cancer, mass spectrometry, plasma, prospective study

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-10-18

Patterns of failure in a predominately black, inner city cohort of triple negative breast cancer patients at a single institution

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**Introduction:** Triple negative breast cancer (TNBC) accounts for 12-17% of breast cancer (BC) in the US, but behaves much more aggressively. It occurs more commonly in younger, black women and death within two years of diagnosis is more common in this subset of BC compared to hormone receptor positive BC. At the University of Maryland Greenebaum Comprehensive Cancer Center, we see a higher proportion of TNBC and present our comprehensive evaluation of the patterns of failure in women with TNBC treated at our urban breast center.

**Materials/Methods:** A retrospective review of TNBC patients treated from 2005-2017 identified 198 patients with Stage I (33%), Stage II (47%), Stage III (16%) and Stage IV (4%) TNBC. The patients were all female, median age of 54 years (range 22-86 years), 64% black, 40% married, 7% BRCA mutated, and 3% HIV positive. Tumor characteristics revealed 93% infiltrating ductal carcinoma, 68% grade 3, and 18% with lymphovascular space invasion. Self-palpation of the lesion occurred in 76% of women, and the lesion was in the upper outer quadrant 62% of the time. Thirty percent of pts had neoadjuvant and 67% adjuvant chemotherapy. Ninety-eight percent of pts underwent surgical resection, 55% had lumpectomy and 61% sentinel lymph node biopsy. Adjuvant radiation was given in 56% of patients with a median dose of 60 Gy (range 16-70 Gy). Chi-square testing was used to compare variables, while logistic regression with Kaplan-Meier estimate was used to calculate overall survival (OS) and freedom from recurrence (FFR).

**Results:** With a median follow up of 45 months, 33 (17%) documented failures occurred. At time of first documented failure, 30% were local (L), 6% regional (R), 22% distant (D), 6% combination of L/R, 12% combination of L/R/D, 9% L/D, and 15% R/D, with a total combined failure pattern in 42% of pts. There was no significant difference in failure patterns between white and black pts ( $p=0.50$ , Table 1). The 2 and 5 year OS was 88% and 80%, respectively. Median survival was not reached in our cohort. The 2 and 5-year FFR was 90% and 84%, respectively with a median time to any failure of 16 months after initiation of therapy and median OS of 29 months for these pts.

**Conclusion:** Our work shows that with modern BC therapies treatment outcomes for pts with TNBC are improved and 84% are free of disease at 5 yrs after the initial diagnosis. The patterns of failure in TNBC are complex, did not vary by race, and showed the largest proportion of our pts (58%) failing in distant and locoregional sites simultaneously, while an additional 30% of pts fail locally only. These failure patterns did not differ significantly based on race. Future efforts will identify pts most at risk for treatment failure for consideration of treatment intensification, as salvage options are limited when treatment failure occurs.

## Comparison of Failure Patterns Between White and Black Patients

Failure Pattern	White (n,%)	Black (n,%)	p-value
Local only	2 (22.5)	8 (33)	p=0.496
Regional only	1 (11)	1 (4)	
Distant only	2 (22.5)	5 (21)	
Local and Regional	0 (0)	2 (8)	
Local and Distant	0 (0)	3 (13)	
Regional and Distant	3 (33)	2 (8)	
Local, Regional and Distant	1 (11)	3 (13)	

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-11-01

A simple intervention for long-term relief of chronic post mastectomy pain

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**Background:** Post-mastectomy pain syndrome (PMPS) is a common and often debilitating condition. One common cause likely results from injury to the T4 and T5 sensory nerves during breast surgery, with resulting neuroma formation. It manifests as a pain syndrome diagnosed by “trigger points” that reproduce exquisite pain upon palpation. Pain specialists have found a combination of corticosteroids and local anaesthetic given through perineural infiltration, at other sites, effective in alleviating these neuromas or trigger points. Utilizing this principle, we initiated a quality improvement project to treat PMPS. This perineural injection led to remarkable, long-lasting relief of the first few patients, we therefore continued treating patients with clinical symptoms suggestive of a neuroma. We report on long-term pain relief after trigger point injections (TPI) for women with PMPS.

**Methods:** An observational cohort study of women with PMPS and clinical evidence of neuroma was undertaken. Patients were examined by breast surgeons at a single institution. We injected a 2mL mixture of equal parts 0.5% bupivacaine and 4 mg/mL dexamethasone into each trigger point. Demographics, type of breast and axillary surgery, duration of pain, history of surgical complications, adjuvant radiotherapy, number of injections required, location of trigger points and dates of injection were obtained from the electronic medical record. Patients were surveyed via telephone interview for long-term resolution of pain. Descriptive statistics are reported, univariate and bivariate analyses were conducted using Stata 12 (College Station, TX).

**Results:** We identified 89 trigger points on 61 breasts in 53 patients with PMPS. Patient age ranged from 30-92 years. Mean number of surgeries prior to injection was 2.2 (range 1-8). In this cohort, we found mastectomy was the most frequent surgical procedure preceding the development of a neuroma (41 breasts), followed by reduction mammoplasty with or without concurrent partial mastectomy (16 breasts), and least frequently lumpectomy alone (4 breasts). The time from the onset of neuropathic pain to the first trigger point injection varied from as early as 1 week post-operatively to 132 months (mean 22.2 months). Effectiveness of the TPI was assessed by physical examination immediately (1-3 minutes) after the injection, then with telephone interview (at  $\geq 3$  months post TPI). All 53 patients had long-term follow-up data ( $\geq 3$  months). Long-term relief was achieved in 84 of 89 trigger points (94.4%) or 54 of 61 breasts (88.5%). Trigger point injections were well tolerated by all patients and no complications were reported.

**Discussion:** Perineural infiltration with bupivacaine and dexamethasone is a safe, simple, and effective treatment option for PMPS with an associated trigger point. Our data suggest this significant problem can easily be resolved in an outpatient setting. All breast specialists should inquire about the presence of symptoms consistent with PMPS and understand the value of intervention to eliminate neuropathic pain. This technique should be added to the armamentarium of all surgeons who perform breast surgery.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-11-02

Subjective and objective assessment of efficacy of frozen gloves and socks to prevent nab-paclitaxel-induced peripheral neuropathy in patients with breast cancer

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**Background:** Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent side-effect of taxanes which play a central role in the treatment of breast cancer. CIPN can negatively influence long-term quality of life, warranting the development of effective prevention strategies. This study investigates the efficacy of frozen gloves and socks (FGS) in reducing the incidence and severity of nab-paclitaxel-induced peripheral neuropathy. Endpoints were evaluated using both clinician and patient reports.

**Methods:** This is a multicenter phase II single arm trial study of the effects of FGS for advanced or metastatic breast cancer patients receiving nab-paclitaxel (260 mg/m<sup>2</sup>) every 3 weeks. Patients wore FGS on their diseased side hand and foot for 60 min during infusion. The other side acted as the untreated control. CIPN was assessed using Patient Neurotoxicity Questionnaire (PNQ), PRO-CTCAE and CTCAE at baseline and every cycle of nab-paclitaxel. The primary endpoint was the incidence of CIPN assessed by PNQ (grade C or higher) after receipt of up to 4 cycles of nab-paclitaxel.

**Results:** Between September 2012 and January 2015, 50 patients from 16 sites were enrolled in this study. Of 50 patients, 27 (54%) received at least 4 cycles of nab-paclitaxel. There was a trend for the incidence of CIPN assessed by PNQ and PRO-CTCAE to be lower in the intervention side than in the control side, although this difference was not statistically significant. The incidence of CIPN assessed by CTCAE was significantly lower in the treated hand (Table).

**Conclusions:** Among breast cancer patients who received nab-paclitaxel, FGS produced favorable effects as detected by reduced clinician-reported CTCAE grades for CIPN, although the study did not detect differences in self-reported symptoms of CIPN using PRO-CTCAE or PNQ. Clinical trial information: UMIN000007907.

Difference according to the evaluation method of CIPN

			Hands (%)			Feet (%)	
		Intervention	Control	p*	Intervention	Control	p*
Patient-Reporting CIPN	PNQ (grade C or higher)	12	19	0.34	16	16	0.63
Patient-Reporting CIPN	PRO-CTCAE						
	Severity ≥ Moderate	13	18	0.08	15	16	0.56
	Interference ≥ Somewhat	7	10	0.32	8	10	0.32
Clinician-Grading CIPN	CTCAE (≥ Grade II)	15	19	0.03	14	13	1.0

\*McNemar's test

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-11-04

Palliative care and older women with advanced breast cancer in Mexico

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**Background:** Mexico is an upper-middle income country, as other developing regions, there is an accelerated aging of the population that will double the absolute number of new breast cancer (BC) cases by 2035. Unfortunately, the incidence of stage III and IV in women older than 65 years old represented 45% of new cases (higher than high-income regions); also, 21% had diabetes, 41.1% hypertension and 71% overweight/obese. These factors make our older adults patients with BC a high-risk population of recurrence and dead from disease.

**Objective:** Describe the characteristics of older women with BC that received palliative care in a multidisciplinary setting.

**Patients and Methods:** A cross-sectional, retrospective, descriptive of 178 patients with advanced breast cancer, older than 65 years from our data based, that were referred to palliative service during 2010-2016 at National Cancer Institute, Mexico. Statistical analysis was done with STATA V12.0. We described clinical, pathological and sociodemographic characteristics of this older women with advanced BC and analyzed the risk factors that influence dead. Approval from IRB was obtained.

**Results:** The median age was 75 years old (range 69-82), 71(41%) was widows, 51(29%) had none education, 93(52%) had elementary school. Highlights that 93 (52%) of the patients evaluated had any income source. Diabetes was present in 43(24%), hypertension in 81 (46%) and cardiopathy in 17(10%) of cases. ECOG-2 were 58 (33%) patients, ECOG-3 was in 47 (26%) patients and ECOG-4 was reported in 19 (11%). None patient had geriatric assessment. Affected metastasis sites were bone 90 (51%), lung 66 (37%), central nervous system 31 (17%), liver 27 (15%). 69 (62%) patients had hormonal receptor positive, 17 (15%) triple negative, 12 (11%) HER2positive and 13(12%) of the cases were triple positive, 41 (74%) patients had Ki-67 higher than 50%. Polypharmacy was identified in 77 (43%) of the patients. The median survival after the admission in the palliative service was 2 months (IQR 0-10). After a logistic regression univariate Ki-67>20% (OR 10.25), triple negative (OR 1.5), HER2 positive (OR 2.3), influence negative survival.

**Conclusions:** Management of BC in the elderly is complex. Our data show that we have highly vulnerable population. Additionally, we found an unfortunate late reference to palliative care services that limited the impact of the multidisciplinary approach. We need to identify the barriers that influence this delay. Health care providers have a challenge in early reference of older women with advanced breast cancer patients to the palliative care and need to think in integrate to the multidisciplinary team a geriatrician with oncology perspective.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-11-05

Opioid prescriptions in the military health system breast cancer population, FY2007 – FY2014

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**Purpose:** Opioid use among individuals with breast cancer is a safety concern because of adverse events including misuse, overdose, and death. To date, there are two published U.S. population-based studies of opioid use among breast cancer patients, both which address persistent opioid use after surgery. Here, we examine rates, amount, and cost of opioids prescribed to breast cancer patients over an 8-year period.

**Methods:** We used Military Health System (MHS) outpatient, inpatient, and pharmacy claims data to select beneficiaries with a primary diagnoses of breast cancer, fiscal years (FY) 2007- 2014. Logistic regression models were used to identify predictors of having received  $\geq 1$  opioid prescription within a year, and having received  $> 4$  filled opioid prescriptions within a year. Simple linear regression and multiple regression were used to examine trends and predictors of reimbursed opioid cost.

**Results:** Among 25,500 non-elderly breast cancer patients treated in the MHS each year, most were age 55-64, located in the southern U.S., and treated in civilian facilities only. On average per year, 75% had surgery, 90% radiation therapy, 19% chemotherapy, and 8% hospice care. Annual rates of receiving  $\geq 1$  opiate prescription were stable across 8 years, 48.1% (FY2007) to 50.5% (FY2012 and FY2013). The annual number of opioid prescriptions per patient was also stable, 4.26 (FY2014) to 4.39 (FY2007). Average reimbursed cost of opioids steadily increased over time, \$135 (FY2007) to \$260 (FY2014) ( $p < 0.001$ ). Based on regression models, the strongest predictors ( $p < 0.001$ ) of having received  $\geq 1$  opioid prescription were (in order of statistical significance): surgery, chemotherapy, civilian care only or mixed use civilian and military care, number of physical comorbid conditions, hospice use, and depression. The strongest predictors ( $p < 0.001$ ) of  $> 4$  opioid prescriptions per year were: depression, hospice use, number of physical comorbidities, and chemotherapy, followed by anxiety disorder, alcohol use disorder, and drug use disorder. The strongest predictors ( $p < 0.001$ ) of high reimbursed opioid cost were: drug use disorder, hospice use, depression, civilian care only or mixed use civilian and military care, alcohol use disorder, number of physical comorbid conditions, receipt of chemotherapy, fiscal year, and anxiety disorder.

**Discussion:** Half of individuals with a primary breast cancer diagnosis receive  $\geq 1$  opioid prescription per year. Four overlapping constructs predict opioid prescriptions and cost: treatment modalities, physical and mental health comorbidities, severity of illness, and system of care. Increased cost over time coincides with current U.S. population reports suggesting rising daily opioid dosage. Despite limitations with claims data, the results suggest that opioid use among breast cancer patients need to be monitored and accompanied by documented clinical management plans. We recommend that oncology providers implement risk reduction strategies including screening for history of substance use and mental health comorbidities, and implementing guidelines specific for cancer patients such as those found in the Veterans Administration/Department of Defense Clinical Practice Guidelines for Opioid Therapy for Chronic Pain, v. 3.0.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-12-01

Factors associated with accepting chemotherapy despite the risk of fertility loss in Latin American breast cancer patients (LACOG 0414 FERTILITY study)

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## BACKGROUND

Breast cancer (BC) is the leading cause of cancer-related morbidity in young women in Latin America. For young patients, chemotherapy induced ovarian failure is a relevant issue, as evidence suggests that young patients with BC experience greater psychological distress and anxiety due to concern for future fertility. Nevertheless, reports from Europe estimated that 95% of patients accepted chemotherapy despite infertility risk. In this study we aim to evaluate the factors associated to the probability of accepting (neo)adjuvant chemotherapy for stage I-III BC despite the risk of fertility loss in young BC patients in Latin America.

## METHODS

Prospective cohort study conducted in 8 sites from Brazil, Mexico, Cuba and Peru. We included pre-menopausal women from 18 to 40 years-old, with stage I-III BC and indication of (neo)adjuvant chemotherapy. Before chemotherapy administration, patients answered a short, previously pilot-tested questionnaire comprising 8 questions related to pregnancy history and fertility loss awareness. Association between explanatory variables and the outcome variables was assessed using logistic regression.

## RESULTS

Overall, 301 patients completed the fertility questionnaire, 48.5% (N=146) from Mexico, 34.9% (N=105) from Peru, 10% (N=30) from Brazil and 6.6% (N=20) from Cuba. Median age at diagnosis of BC was 34 years old, 84% completed second or higher degree of education, 61% were married and 71.4% had children prior to cancer diagnosis. Clinical stage was I in 10.6%, II in 55% and III in 32.4%. Overall, 85% of patients agreed to undergo chemotherapy despite the risk of fertility loss, 51.3% of patients would like to have children in the future and 32% did not want to have children due to BC diagnosis. 66.3% of patients knew that chemotherapy could reduce their fertility, however 85% would agree to receive it if they know about the risks. Factors associated with higher probability of accepting chemotherapy in univariate analysis were stage II and III vs. I (OR 1.39 and 2.38 respectively, *P* .0023), single marital status (OR 10.65, *P* .0235), previous children (OR 3.18, *P* .0251) and unwillingness of having more children in the future (OR 5.0, *P* .0138). However, in the multivariate analysis, the only factor associated with accepting chemotherapy despite the risk of fertility loss was being single (OR 10.8 95% CI 1.4 – 83.6; *P* .023). Also, most patients (75%) would consider the use of chemotherapy in case of more than 20% improvement in cure rates, and 36.2% even if the maximum risk of fertility loss was from 76 to 100%.

## DISCUSSION

In our study, we identified a lower probability of accepting chemotherapy due to infertility risk than previous reported in the literature from Europe, despite the younger age of patients and worse stage at diagnosis. Moreover, most patients desired a larger benefit of (neo)adjuvant chemotherapy than usually seen in clinical trials in order to accept chemotherapy. Access to fertility preservation strategies is a need for young BC patients in Latin America.

**TRIAL REGISTRY:** NCT02862990

**KEYWORDS:** Breast Neoplasms; Fertility Preservation; Antineoplastic Agents; Treatment Adherence and Compliance



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-12-02

Age-related distress in 3352 breast cancer patients

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**Purpose:** Age-related biopsychosocial distress in breast cancer patients has been poorly studied and understood. The breast cancer experience may be different based on chronological age, which may be a surrogate marker of biologic, psychological, social, and functional age. This study reports patient-reported biopsychosocial problem-related distress in breast cancer patients.

**Methods:** All new patients seen at the City of Hope breast cancer clinic undergo a validated comprehensive biopsychosocial screening prior to their first visit with a medical/surgical oncologist. This touchscreen driven technology queries patients on their physical symptoms, psychosocial concerns, informational and resource needs, interests in clinical trials, and other breast cancer specific concerns. This IRB approved study was conducted in 3,352 patients evaluated from 2009 to 2017. Screening occurred immediately prior to meeting with the physician so that the information could be integrated into the clinical encounter.

**Results:** The age-related groups included 268 Adolescent and young adult (AYA) patients ages 18-39, 2,244 middle aged adults 40-64 years, and 840 older adults ages 65+ years. Regardless of age, four of the top seven highly distressing problems were the same: *worry about the future*, *side effects of treatment*, *sleep* and *fatigue*. AYA patients and middle aged adults, but not older adults, identified *finances* and *being anxious or fearful* among their top five causes of distress. Middle aged adults and older adults, but not AYA patients, identified *physical pain* among their top seven causes of distress. Interestingly, both AYA and older adults, but not middle aged adults, identified getting information about *complementary and alternative practices* as a top source of distress. Although a serious problem across all age categories, *thoughts of ending one's life* and *seriously considering taking one's life* were the least common problems identified.

**Conclusions:** In this series with 3,352 patients, biopsychosocial concerns raised with a diagnosis of breast cancer were similar regardless of age. However, patients under the age of 65 may worry more about finances and patients over the age of 40 may worry more about physical symptoms such as pain. Both AYA patients and older adults cited distress learning about complementary and alternative practices, suggesting a need for providers to address this, especially in these patient populations. Thoughts of ending one's own life were uncommon, which is relevant in a state with the End of Life Option Act.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-12-03

Prospective study: Impact of adjuvant chemotherapy with anthracyclines and taxanes in cognitive skills

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## Introduction

Adjuvant chemotherapy is one of the outstanding treatments in the treatment of breast cancer. Many of its side effects are well known, while others are under study, as cognitive impairment.

One/eight women have breast cancer, which often affects a young population. Cognitive disorders produced by chemotherapy were studied with controversial results.

It seems convenient to study whether chemotherapy produces a cognitive impairment in a homogeneous group of patients receiving adjuvant chemotherapy with concomitant anthracycline cyclophosphamide followed by weekly paclitaxel (AC-T) in a prospective design.

## Methods

Included patients had early-stage breast cancer (Stage I-II) and received AC-T (Anthracycline 60 mg/sqm - cyclophosphamide 600 mg/sqm and sequential weekly paclitaxel 80 mg/sqm) following surgery and before radiation therapy, as indicated. Her 2 positive patients also received adjuvant Trastuzumab therapy.

The cognitive deficit was estimated through the Addenbrooke's Cognitive Examination test, taken the day of the beginning of treatment, and at 3, 6 and 12 months later.

Beck Depression Inventory Test was used to assess depression. It was taken at the beginning of treatment and at 12 months later.

Control group was built by healthy people, which were examined with the same test just once.

## Results

From October 2016 to April 2014, 108 patients were included in chemotherapy group and 50 in a healthy control group. 51 patients completed 12 months follow up examination.

Significant differences were found between patients and control group in attention, concentration, verbal fluency, language, visuospatial abilities and memory parameters.

These parameters were found impaired at the beginning of the treatment, having a favorable evolution at twelve months, with a complete resolution.

Orientation did not suffer significant changes during all study.

27/108 patients were found to be depressed at the beginning of treatment, with a favorable evolution of this parameter at twelve months.

Control group did not suffer from any cognitive deficit or depression.

## Conclusions

In this prospective study, patients suffered cognitive deterioration at the beginning of treatment, which was recovered at the twelve-month follow-up.

Given the prevalence of depression in the chemotherapy group patients at the beginning of treatment versus the control group, this is probably the cause of the cognitive disorder.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-12-04

Use of complementary and integrative medicine therapies in Chilean patients with breast cancer. Experience of a private center: prevalence and characteristics of patients

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**Background:** During the last decade several studies have been reported that the use of complementary and integrative medicine (CIM), defined as health care methods generated outside of standard Western, or conventional clinical practices has been increasing among breast cancer patients in developed countries. However, there is little information about the use of CIM in developing countries. Chile currently has no published statistics on the use of CIM in breast cancer.

**Methods:** We conducted a cross-sectional survey between March and June 2017 in breast cancer patients treated in Clínica Alemana de Santiago to determine the prevalence of CIM use and the types of preferred CIM. We also analyzed associations between CIM and several variables including sociodemographic variables, the reasons that motivated the use of CIM and the subjective benefits that patients attributed to CIM. Statistical analysis included two tailed t-test for continuous variables, Fischer's exact test for categorical variables and logistic regression for association between CIM use and other variables.

**Results:** 288 patients answered the survey. Among them, 98.9% were women, mostly between 41 and 50 years (40.4%). 44.9% (n = 129) reported using CIM. No association was found between the use of CIM and sociodemographic variables (sex, age, education, income). Most used CIM types were: vitamins / minerals (50.4%), herbs (48.8%), special diets (42.6%), meditation/prayers (37.2%). The reasons for CIM use were "to do everything possible" (72%), "improve immunity" (67.8%), "reduce side effects of treatment" (32.7%), recommendation by family member or friend (32.7%). Regarding CIM habits, 50.8% of patients reported having started CIM use at the time of diagnosis and only 51.6% reported about CIM use to their attending physician. Most patients reported benefits associated to CAM use (61%) but no differences were found in reported quality of life.

**Conclusions:** Great amount of the patients reported using CIM and half of them mentioned that they shared this information with their attending physician. It is necessary incorporate to medical routines questions regarding CIM use and to educate the patients regarding the safe use of CIM and the possible interactions of these approaches with conventional clinical practices.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-12-05

First implementation of the International Consortium of Health Outcomes Measurement standard for breast cancer at a major German university hospital using a web-based tool to measure patient reported outcomes

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## Purpose:

Collecting patient reported outcome (PRO) data in a systematic way enables an objective evaluation of treatments and its related outcomes. By using the disease specific questionnaires developed by the International Consortium of Health Outcome Measurement (ICHOM) this allows for comparison between physicians, hospitals and even different countries.

## Methods

In November 2016 we implemented a web-based system to collect PRO data at the breast center at Charité University hospital using the ICHOM data set. All new patients who are seen at the breast center are enrolled and are answering a predefined set of questions using a tablet computer. Once they start their treatment at Charité automated emails are sent to the patient at predefined treatment points. Those emails contain a web-based link through which they can access their questionnaires.

## Results

Until now we have enrolled 834 patients and initiated 2470 questionnaires. 9.44% of patients were under 40 years of age, 49.7% between 40 and 60, 39.6% between 60-80 and 1.3% over the age of 80 years. The average return rate of questionnaires is 72% without any additional intervention. When asked about preference regarding paper versus online 7.9% of the patients 50 to 60 years of age would prefer paper, 18% in the 60-70 years of age group and 21.2 % in the age group over 70 years.

## Conclusion

Measuring PRO in breast cancer patients in an automated electronic version is possible across all age ranges while simultaneously achieving a high return rate.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P4-12-06

Quality of life, fatigue, and subjective cognitive functioning immediately and 6 months after adjuvant chemotherapy in breast cancer patients

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## Background

The aim of this study was to examine quality of life (QoL), fatigue, and subjective cognitive functioning (SCF) after chemotherapy in breast cancer (BC) patients.

## Methods

BC patients were recruited before chemotherapy (3 FEC+3 Tax, BC-Chemo) and compared with disease-specific controls (i.e., BC patients not receiving chemotherapy, BC-Controls) and healthy controls (HC) matched for age and education.

All participants completed questionnaires assessing QoL (using the *Functional Assessment of Cancer Therapy – General Population*), fatigue (using the *Functional Assessment of Chronic Illness Therapy-Fatigue*), and SCF (with the *Cognitive Failure Questionnaire*) at recruitment and 6 months later (i.e., one month after the end of chemotherapy). Follow-up data (1 year after recruitment) were available for BC-Chemo and HC only.

Difference scores were computed for each participant (i.e., score after 6 months/ 1 year minus score at baseline) and transformed when necessary so that negative scores would reflect decline over time (i.e., decreased QOL, increased fatigue, and increased cognitive complaints). These difference scores were analysed using independent t-tests (BC-Chemo vs BC-Controls on one hand, and BC-Chemo vs HC on the other hand).

## Results

Eighteen BC- Chemo, 19 BC-Controls, and 20 HC completed the study (mean age = 49, 58, and 44 years, resp.,  $p = 0.001$ , BC-Controls being significantly older).

Statistical analyses showed that, after chemotherapy, BC- Chemo patients showed significantly lower QoL, more fatigue, and declined SCF than both groups of controls (see Table 1).

However, 6 months later, no statistically significant differences were found between BC- Chemo and HC with respect to QoL and fatigue. Only SCF remained significantly lower in BC- Chemo.

Table 1. Mean Scores (standard deviations) for Quality of Life (QoL), Fatigue, and Subjective Cognitive Functioning (SCF) in Breast Cancer Patients treated with Chemotherapy (BC-Chemo) or not (BC-Controls) and Healthy Controls 6 months and 1 Year After Baseline

	BC-Chemo	BC-Controls	Healthy Controls
QoL (FACT-GP)			
6 months after baseline	-5.8 (8)	1.1 (8.7)*	-0.3 (4.7)*
1 year after baseline	-5.3 (11.8)		0.2 (9.4)
Fatigue (FACIT-F)			
6 months after baseline	-6.1 (11.1)	0 (7.3)*	-0.1 (3.8)*
1 year after baseline	-3.6 (10.3)		-2.1 (6.5)
SCF (CFQ)			
6 months after baseline	-7.4 (9.4)	0.3 (10.9)*	1 (5)*
1 year after baseline	-10.4 (13)		1.2 (6)*

\* $p < 0.05$  (two-tailed independent t-tests vs BC-Chemo). Note. FACT-GP: Functional Assessment of Cancer Therapy – General Population (higher scores = greater QoL). FACIT-F: Functional Assessment of Chronic Illness Therapy-Fatigue Scale (higher

scores = low fatigue). CFQ: Cognitive Failure Questionnaire (higher scores = greater subjective cognitive problems). All difference scores reflect decline over time.

## **Conclusions**

Shortly after chemotherapy (i.e., 6 months after baseline), breast cancer patients experienced diminished QoL and increased fatigue compared to both disease-specific and healthy controls, but these differences were no longer significant 6 months later. In contrast, group differences in SCF were significant at both timepoints, suggesting long-lasting cognitive decline for patients receiving chemotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-12-07

Patients' preferences for postmenopausal hormone receptor-positive (HR+), human epidermal growth factor receptor 2 negative (HER2-) advanced breast cancer treatments in Japan

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**Background:** The objective of this study was to identify factors that affect preferences for treatment of breast cancer (BC) patients in Japan and understand their relative importance. Also this study explored whether patients' sociodemographic and clinical characteristics would affect patient preference in choice of treatment. **Methods:** A questionnaire for discrete choice experiment (DCE) was developed with five attributes, progression free survival (PFS), incidence of diarrhea (IOD), frequency of diarrhea (FOD) which represents the increase in the number of stools per day than usual, duration of diarrhea (DOD) and route and frequency of administration (RFA) referring MONARCH 2, a global phase III study for abemaciclib plus fulvestrant versus placebo plus fulvestrant in patients with HR+/HER2- advanced BC. Each questionnaire was composed of nine choice sets and each choice set contained those five attributes with different levels. Postmenopausal and HR+ BC patients in Japan who recruited from a patient panel were asked to choose one treatment alternative in each choice set. Conditional logit model was used to identify relative preferences of each attribute. The preference weights were evaluated with  $\beta$ coefficient and standard error. In addition, conditional logit model including patient-specific covariates, such as patient characteristics (age, employment status, age of children and marital status) and clinical characteristics (experience of relapse or metastasis and with/without hormone sensitivity), was used to identify factors that affect patient preference in choice of treatment. **Results:** Of 302 respondents recruited, 258 had valid responses and the rest had inappropriate answers for the validity testing choice set. The mean age (SD) was 56.7(6.7), 47.7% had paid employment, median duration since diagnosis was 5.1 years and 98.1% had experienced hormonal therapy. According to the absolute magnitude of coefficients, when the FOD is 6, the order of attributes' relative importance was the following: PFS, DOD, FOD, IOD, RFA. However when the FOD becomes 9, FOD was the most important attribute for patients. All tested attributes were statistically significant ( $p < .0001$ ) on their preference in choice of treatment. When patient-specific covariates were included in the model, the patients who have had experience of relapse or metastasis showed the strongest preference for the longest PFS of 16 months and the patients who were 45 to 59 years old showed the weakest preference for the highest FOD of 9. **Conclusions:** Postmenopausal and HR+ BC patients in Japan showed preference for treatments which can extend PFS even with the potentiality of Grade 2 diarrhea by the grading of Common Terminology Criteria for Adverse Events v4.0. Prevention of diarrhea to make it Grade 2 or lower may maintain patients' motivation for the treatment which can extend PFS. This study also showed that patients' sociodemographic and clinical characteristics tend to affect patients' treatment choices. It will be important to choose treatments with considering patients' characteristics such as their life style, age and therapeutic experience.

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Patients' and surgeons' experiences after failed breast reconstruction: A qualitative study

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**Background:** Breast cancer reconstructive surgery is supposed to contribute to patients' body image and quality of life. However, complications after autologous breast reconstruction (A-BR) and implant-based breast reconstruction (I-BR) do occur in approximately 40% of the women, and may even lead to complete failure in rare cases. This study explores both patients' and surgeons' experiences after failed breast reconstruction. Increased knowledge in this field could help to improve not only the care for women with failed breast cancer reconstructive surgery but may also help to guide professionals in dealing with such failures.

**Methods:** Patients with reconstructive failure form a large multicenter cohort study and participating plastic surgeons were invited to participate in this study. A topic list consisting of ten topics served as a general outline of a semi-structured interview on their experiences with the reconstructive failure, lasting about one hour. The interview data were transcribed and after that analyzed according to the principles of grounded theory by two researchers independently of each other. Data were coded in NVivo software. Next, data were discussed in a larger team, thereby moving back and forth between data and emerging theory.

**Results:** Fourteen patients with a failed I-BR, four patients with failed A-BR and four plastic surgeons participated in this study. Three main categories emerged from the data: personal experiences, the motivation for a redo of the failed reconstructive surgery and patient-doctor communication. With regard to personal experiences, a main patient category was the importance of being (appreciated as) a person as a whole, in his/her specific context (versus focusing on reconstructive technique). This in contrast to the surgeons, for whom it did matter whether the failure concerned an I-BR or A-BR. The latter was perceived as more intense. Some took it as a personal failure, leading to insecurity and feelings of regret towards the patient. This was especially true if they felt a strong bond with that particular patient. With regard to motivation for a redo of the failed reconstructive surgery, both patients and surgeons emphasized the importance of shared decision making. Nevertheless, patients seemed to remain more ambivalent than surgeons about pursuing additional reconstruction after failure. Finally, regarding patient-doctor communication, we found that surgeons were more distant or reflective on these matters, whereas patients expressed their experiences in a more emotional way. Patients expressed a desire to be seen and treated as an unique individual. Experiencing trustfulness, sincerity and empathy from the surgeon in discussing the failure were highly valued.

**Discussion:** Implementing the results of this study in clinical practice may facilitate coping with the distressing or even traumatic event of failed breast reconstruction, in both patients and surgeons.



# 2018 San Antonio Breast Cancer Symposium ®

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Endocrine treatment for hormone receptor-positive advanced breast cancer patients with endocrine-sensitive or endocrine-resistant disease: A systematic review and network meta-analysis

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**Background:** For patients with hormone receptor-positive (HR+) advanced breast cancer (ABC), the choice of endocrine treatment should be based on the best available evidence. However, no direct head-to-head comparison of all tested regimens is currently available. This network meta-analysis aims at providing detailed inference-based data to guide decision making on endocrine therapy for HR+ ABC patients in two different clinical scenarios: endocrine-sensitive and endocrine-resistant ABC, as defined by the ABC guidelines criteria.

**Methods:** Phase II/III randomized clinical trials published or presented up to June 15, 2018 and comparing two or more different endocrine treatments (with or without associated targeted therapies) for HR+, HER2-negative ABC patients were included, irrespective of treatment line. Relative treatment effects were pooled as hazard ratios (HRs) and modeled in a network meta-analysis using a Bayesian framework with fixed-effects models in order to infer the best approach. The main outcomes were progression-free survival (PFS) and overall survival (OS). Four separate analyses were carried out: PFS and OS networks in both endocrine-sensitive and endocrine-resistant populations.

**Results:** A total of 26 trials were included in this meta-analysis (N=11330 patients). In the endocrine-sensitive setting, 12 trials were included in the PFS network (N=5200 patients; 10 different regimens) and 3 trials in the OS network (N=1295; 3 regimens). In terms of PFS, the combination of Fulvestrant 500 mg (F500)+CDK4/6 inhibitors (CDKi) had a 80% probability of being the most effective treatment, followed by aromatase inhibitor (AI)+CDKi (17% chance of being the best approach). The HR for the comparison between F500+CDKi vs. AI+CDKi was 0.82 (95% credibility interval [CrI] 0.54-1.25). Regarding OS, the AI+CDKi approach had a 63% chance of being the most effective regimen, followed by F500 (36% likelihood) – the comparison between the two regimens yielded a HR of 0.93 (95% CrI 0.61-1.40). In the endocrine-resistant setting, 18 trials were included in the PFS network (N=6130; 20 regimens) and 7 in the OS network (N=2701; 10 regimens). For PFS, F500+CDKi had a 59% probability of being the most effective treatment, followed by F500+Everolimus (11% likelihood). The HR of the comparison between F500+CDKi vs. F500+Everolimus was 0.84 (95% CrI 0.57-1.25). In terms of OS, F500+FGFR inhibitors had a 29% chance of being the best treatment option, followed by AI+everolimus (24% likelihood) – the HR for the comparison of F500+FGFR inhibitors vs. AI+everolimus was 1.07 (95% CrI 0.36-3.18).

**Conclusion:** This network meta-analysis provides for the first time a comparison of all tested treatment options for HR+ ABC patients in both endocrine-sensitive and endocrine-resistant settings. In terms of PFS, F500+CDKi appears to be the best treatment option for both disease settings. As for OS, AI+CDKi is possibly the best choice for endocrine-sensitive ABC patients. Concerning endocrine-resistant disease, F500+FGFR inhibitors may be an effective regimen which should be further studied, while AI+everolimus remains the best available option in this setting.

## 2018 San Antonio Breast Cancer Symposium ®

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Selective androgen receptor modulator RAD140 inhibits the growth of endocrine-resistant breast cancer models with defined genetic backgrounds

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Estrogen receptor  $\alpha$ -positive (ER+) breast cancer is routinely treated with therapies targeting the ER axis. However, *de novo* and acquired resistance to the standard-of-care treatment occur in a significant subset of patients. Multiple mechanisms have been proposed for the resistance, among which genetic alterations to the coding gene of ER $\alpha$ , *ESR1*, have been extensively studied. Notably, *ESR1* hotspot mutations within the ligand binding domain (LBD), novel fusion proteins consisting of the N-terminal domain of ER and the C-terminal domain of a partner gene, and *ESR1* gene amplification have been found to be enriched in endocrine-resistant, metastatic breast cancers. Together these ER gene alterations present new challenges in the management of ER+ breast cancer and call for the development of new agents to supplement the current standard-of-care. Around 90% of the ER+ breast cancer cases are also androgen receptor (AR) positive (AR+). Mounting preclinical evidence has demonstrated that AR agonists suppress AR and ER positive (AR/ER+) breast cancer cell growth, in line with clinical activity of androgens. We recently reported the oral selective androgen receptor modulator (SARM) RAD140 is a potent, tissue-selective AR agonist in breast cancer cells which significantly inhibited the growth of AR/ER+ patient-derived xenograft (PDX) models, partly via inhibiting *ESR1* gene expression and ER signaling. It also elicited enhanced tumor growth inhibition when combined with a CDK4/6 inhibitor.

Here we further examine the activity of RAD140 in AR/ER+ breast cancer models that are endocrine-independent with a spectrum of *ESR1* genetic alterations. In PDX models harboring *ESR1* amplification or fusion, RAD140 inhibited tumor growth to a greater degree than fulvestrant, a standard-of-care selective ER degrader (SERD). These results are consistent with the clinical history of the donor patients whose diseases relapsed from or progressed rapidly on fulvestrant. In PDX models with *ESR1* Y537S mutation, RAD140 showed anti-tumor activity comparable to that of fulvestrant. Notably, RAD140 treatment also led to substantial reduction of proliferation and colony formation in cell line models recapitulating resistance to the combination of estrogen deprivation and CDK4/6 inhibition.

In summary, RAD140 showed marked anti-tumor activity in AR/ER+, endocrine-resistant breast cancer models with defined genetic background. Importantly, in models with *ESR1* amplification and fusion, the AR-targeting RAD140 exhibited more profound inhibitory activity compared with fulvestrant. In models with an *ESR1* mutation, the efficacy of RAD140 and fulvestrant was comparable. These results lend support to a clinical hypothesis that AR/ER+, endocrine-resistant tumors with these genetic backgrounds may benefit from AR agonist-based treatment. RAD140 is currently being evaluated in hormone receptor positive (HR+) breast cancer patients (NCT03088527).

# 2018 San Antonio Breast Cancer Symposium ®

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Elacestrant (RAD1901) demonstrates anti-tumor activity in models resistant to CDK4/6 inhibitors

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**Background:** Approximately 75% of all breast cancers diagnosed are estrogen receptor-positive (ER+) and currently approved endocrine therapies rely heavily on blocking of the ER signaling pathway. In recent years, the combination of an endocrine agent with other targeted agents have been evaluated to address endocrine resistance and improve progression-free survival (PFS). Recently, it was demonstrated that the addition of a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor to an endocrine agent roughly doubles PFS, leading to the approval and use of certain CDK4/6 inhibitors in combination with either aromatase inhibitors in the first-line metastatic setting or in combination with the selective estrogen receptor degrader (SERD), fulvestrant, in the second-line metastatic setting. While combining CDK4/6 inhibitors and endocrine therapy can result in significantly increased PFS, patients eventually progressed on these combinations, and to date, there is no cure for patients with advanced metastatic ER+ breast cancer. Given the increased use of CDK4/6 inhibitors in the ER+ breast cancer treatment paradigm, it will be important to understand how treatment resistance to CDK4/6 inhibitors manifests in order to optimize therapeutic strategies to target this patient population. We have previously described elacestrant (RAD1901), a novel and orally bioavailable SERD, as an inhibitor of ER+ breast cancer growth in *in vitro* models and in *in vivo* patient-derived xenograft (PDX) models. Importantly, elacestrant inhibited the growth of PDX models that were derived from heavily pretreated patients, models harboring mutations in ESR1, and models insensitive to standard of care endocrine therapies. Given these results, we hypothesized that elacestrant would have anti-tumor activity in a CDK4/6 inhibitor-resistant setting. Herein, we describe elacestrant activity in multiple *in vitro* and *in vivo* models of CDK4/6 inhibitor resistance in both wild-type and mutant ESR1 backgrounds.

**Methods:** *In vitro* models of estrogen-independent ER+ breast cancer, harboring either wild-type or mutant ER, were exposed to increasing concentrations of approved CDK4/6 inhibitors: palbociclib, ribociclib, or abemaciclib. ER expression/signaling, changes in cell cycle mediators, and the effects of elacestrant and other SERDs were examined in these representative models.

**Results:** Despite prolonged exposure to CDK4/6 inhibitors, the resistant cell lines retained ER, ER signaling, and importantly, ER-driven proliferation. Elacestrant induced dose-dependent growth inhibition in CDK4/6 inhibitor-resistant cells, and this effect was independent of the CDK4/6 inhibitor used to generate resistance. Elacestrant also demonstrated *in vivo* tumor growth inhibition of CDK4/6 inhibitor-resistant ER+ PDX models.

**Conclusions:** Our preclinical data demonstrate that elacestrant is a SERD that can inhibit tumor growth in a CDK4/6 inhibitor-resistant setting and provides rationale for examining elacestrant in patients that have progressed on a combination of endocrine therapy with a CDK4/6 inhibitor.

# 2018 San Antonio Breast Cancer Symposium ®

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Time-to-treatment change for high-dose fulvestrant (HD-FUL) as first-line therapy in HR+ve, HER2-ve advanced pre-treated breast cancer (ABC) patients (pts): The real-world data from the GIM-13 AMBRA study

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## **Background**

In Real World Studies (RWS), Progression Free Survival (PFS) could be not reliable as compared to randomized clinical trials (RCTs), due to the non-predefined times of disease evaluation. A potential surrogate of PFS for RWS could be TTC. We previously compared TTC of two different regimens (1st-line: Paclitaxel+Bevacizumab; any-line: Nab-Paclitaxel ) with the TTP results of the same regimens obtained in RCTs (Cazzaniga et al. J Clin Oncol 36, e13081, 2018; Mustacchi et al. J Clin Oncol 36, e13078, 2018), demonstrating that TTC is a valid surrogate end point of PFS in RWS. Aim of the present analysis is to describe pts' characteristics and clinical outcome in terms of TTC in a population of endocrine-pretreated pts who received FUL as 1<sup>st</sup>-line therapy in a real-life setting.

## **Patients and Methods**

We used data of the HR+ pts of the AMBRA study, a longitudinal cohort study, describing the choice of first and subsequent lines of treatment in HER2-ve MBC pts, treated with HD-FUL in 1<sup>st</sup>-line therapy to validate TTC for this population. Time parameters were estimated by the product-limit method, with failed observations censored to '1'. All statistical tests were two sided at the conventional 5% significance level. Analyses were carried out using NCSS® statistical software (V 12, Hintze J, Kaysville, UT, USA). The study had the approval of the Ethics Committees of the participating centers.

## **Results**

So far AMBRA Gim-13 study enrolled 878 patients, 866 with known molecular subtype: overall, Luminal Tumors are 740 (85.45%). First Line HD-FUL was used in 91 patients (12.3%, median age at diagnosis was 57.8 years (35-82.2), median DFI 71.3 months (95%CI: 60.6-85.2); 36.5% of the pts relapsed while on 5-years adjuvant treatment. Fifty-four patients (59.3 %) had received adjuvant chemotherapy, 96.7% adjuvant endocrine therapy (Tamoxifen 13.6%; Tamoxifen followed by Aromatase Inhibitors 36.3%; AI 48.8%; in 7 cases in association with LHRH Analogs). Median follow up time from 1st Line start was 21.7 months (0 - 93.3). Bone and visceral metastases were present in 61 (67%) and 20 patients (21.9%) respectively. Median PFS of HD-FUL was 12.3 months (95%CI 8.7-13.93) in the whole population, 12.8 (range 2.9-47.7) and 9.16 (range 3-70) months in patients with bone and visceral disease, respectively. Median TTC was 11.2 months (95%CI 8.18-13.1). No difference with PFS was found (p= 0.92).

## **Conclusion**

Median PFS observed in the GIM-13 AMBRA study is lower than that reported in FALCON (PFS: 16.6 months) and similar to the CONFIRM one (PFS: 5.6 months) trials. However, TTC and PFS are similar (p=0.92), so far confirming that the accuracy of data recording is quite correct. The present analysis shows that TTC is a reliable surrogate of PFS in RWS.

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Evolving treatment patterns in hormone receptor-positive, HER2-negative metastatic breast cancer

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**Background:** The last several years have seen the approval of multiple targeted agents for use alone or combined with standard endocrine therapies (ET) in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> line hormone receptor positive (HR+), HER2 negative (HER2-) metastatic breast cancer (MBC) settings. Due to these new approvals, standards of care for treatment are evolving.

**Methods:** Prescribing preferences (PPrefs) of 592 U.S.-based medical oncologists were studied using a validated, proprietary, case-based market research tool (Challenging Cases®). Data were acquired using blinded, audience-response iPad technology at 8 live and virtual events during 2016-2018.

Two core hypothetical cases were presented: 1<sup>st</sup> line MBC and recurrent (REC) MBC. For each core case the following variables were introduced: time from completion of adjuvant (adj) therapy (Tx) to REC disease, site of metastases [visceral (VIS) vs non-visceral (N-VIS)], and age for the 1<sup>st</sup> line case, and type of metastases and line of Tx for the recurrent case. Rx choices for which there are published phase 3 data were offered, as well as a category for “other.”

**Results:**

Table 1. Preferred Rx for recurrent disease during or after 5 yrs of adjuvant non-steroidal aromatase inhibitor (NSAI)\*

Scenario	Exemestane (EXE) + Everolimus (EVE)	NSAI + CDK inhibitor (CDKi)	Fulvestrant (FUL) + CDKi	Chemotherapy (CT)	Endocrine therapy (ET)	Other
REC 18 months (mos) into adj AI Tx, VIS) and N-VIS mets, Age 63	5%	25%	63%	3%	4%	0%
REC post 5 yrs adj AI and 2 yr treatment free interval (TFI), N-VIS mets, Age 67	4%	49%	34%	1%	12%	1%
REC post 5 yrs adj AI and 2 yr TFI, N-VIS mets, Age 85	3%	34%	19%	0%	42%	1%

\* May not equal 100% due to rounding.

Table 2. Preferred Rx for 2nd or 3rd recurrence\*

Scenario	EXE + EVE	NSAI + CDKi	FUL + CDKi	CT	ET	Abemaciclib	Other
REC post 5 yrs adj AI and 2 yr TFI, N-VIS mets, age 67 --> 12 mos 1st line Tx --> N-VIS mets	31%	12%	38%	6%	12%	1%	1%
REC post 5 yrs adj AI and 2 yr TFI, N-VIS mets, age 67 --> 12 mos 1st line NSAI + palbociclib Tx --> N-VIS mets	38%	1%	33%	9%	15%	0%	3%
REC 12 mos into adj AI, age 61, N-VIS mets --> 12 mos Tx 1st line --> N-VIS mets	21%	9%	53%	9%	6%	0%	1%
REC post 5 yrs adj AI and 2 yr TFI, N-VIS mets, age 67 --> 12 mos 1st line Tx, N-VIS mets --> 6 mos 2nd line Tx --> VIS and N-VIS mets	23%	1%	9%	59%	5%	2%	1%

\* May not equal 100% due to rounding.

In the setting of a patient (pt), age 63 with early REC on an adj AI, the most preferred strategy was FUL + a CDKi. In a pt, age 67, who completed 5 years of an adj AI with a TFI of 2 years, the most common preference was NSAI + a CDKi. In an older pt (age 85) who completed 5 years of an adj AI and TFI of 2 years, the most common choice of TX was single agent ET. For a pt with N-VIS mets treated in the 2<sup>nd</sup> line setting, FUL + a CDKi was the most preferred strategy. In a 2<sup>nd</sup> line pt with REC at 12 mos on adj Tx and after 12 mos of 1<sup>st</sup> line Tx, FUL + a CDKi and EXE + EVE were the most preferred therapies. In a 3<sup>rd</sup> line pt, with VIS mets CT was the preferred choice.

**Conclusion:** Treatment patterns in HR+ HER2- MBC are evolving with the approval of several new agents and emerging data. Age, time to relapse, line of Tx, and type of mets may be some of the key factors that determine PPrefs in HR+, HER2- MBC.

# 2018 San Antonio Breast Cancer Symposium ®

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Real-world experience using exemestane and everolimus in patients with hormone receptor positive/HER2 negative breast cancer with and without prior CDK4/6 inhibitor exposure

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**Background:** For patients with metastatic hormone receptor (HR)-positive, HER2-negative breast cancer who progress on a non-steroidal aromatase inhibitor (NSAI), exemestane plus everolimus (EE) has been shown to prolong progression-free survival in comparison to exemestane alone. In the current era, many patients are now receiving a CDK4/6 inhibitor with first-line NSAI therapy. There is limited data describing the utilization and effectiveness of treatments following hormonal therapy - CDK4/6 inhibitor combinations, including EE. The aim of this study was to describe the real-world clinical experience and outcomes associated with EE amongst patients with and without prior CDK4/6 inhibitor exposure treated in our provincial jurisdiction.

**Methods:** All patients prescribed EE from January 1, 2016 through May 10, 2018 were obtained from the Alberta Health Services CancerControl Breast Data Mart (BDM). Patients with HER2+ disease, had received <1 cycle of EE or who had been on a placebo controlled trial of hormonal therapy +/- CDK4/6 inhibitor prior to EE were excluded. Review of the electronic medical record was undertaken to obtain detailed information on lines of treatment prior to EE, EE dosing and reason for EE discontinuation. The cohort was described and analyzed in total and by prior CDK4/6 inhibitor exposure. Time on treatment (TOT) was defined as start of EE to last dose or June 11, 2018 (censoring for data extraction) and was calculated using the Kaplan Meier method. The log rank test was used to compare TOT.

**Results:** There were 110 patients extracted and 88 eligible for analysis (3 excluded for HER2+ disease, 14 for receipt of <1 cycle EE and 5 for participation on a placebo controlled trial of hormonal therapy +/- CDK4/6 inhibitor prior to EE. Median age 62.4 years (range 32.1-86.6 years). EE was administered first line in 12.5%, second line in 46.6% and third or greater line in 40.9%. Median time from start of first line therapy to start of EE was 19.3 months (range 0.3-72.1 months). Visceral metastases at start of EE in 62.5%. EE mean starting dose 7.3 mg (SD 2.5 mg) and mean last dose recorded 7.0 mg (SD 2.7 mg). At time of data extraction, 69 patients had stopped EE, 68.1% for progression and 31.9% for toxicity or other reason. Twenty patients had hormonal therapy + CDK4/6 inhibitor prior to EE. In the first line setting, 10 patients had letrozole and palbociclib. In the second line setting or greater, 5 patients had letrozole + palbociclib, 1 patient had tamoxifen + palbociclib and 4 patients had fulvestrant + palbociclib. Median time on hormonal therapy + CDK4/6 inhibitor was 12.0 months (range 4.0-20.9 months). Those with hormonal therapy + CDK4/6 inhibitor were more likely to have visceral metastases (p=0.02). Median time on treatment for the CDK4/6 exposed vs naïve groups was similar (5.8 vs 5.3 months, p=0.952). Median overall survival not yet reached.

**Conclusion:** In a cohort of patients who have progressed on hormonal therapy + CDK4/6 inhibitor within 2 years, subsequent EE is a clinically meaningful treatment option in the setting of HR-positive/HER2-negative metastatic breast cancer. TOT was similar for CDK4/6 inhibitor exposed and naïve patients.

# 2018 San Antonio Breast Cancer Symposium ®

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**SEQUERPLUS:** A multicenter real practice observational study investigating the endocrine-based (E) therapies sequential approach in hormonal receptor positive (HR+) HER2 negative (-) metastatic breast cancer (MBC)

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**Background:** Despite the sequential E therapy is recognized as the preferred approach for HR+/HER2- MBC, no data from clinical trials support the choice between the different sequential strategies.

**Methods:** In this retrospective study descriptive statistics are reported using the median (Interquartile range, IQR) or frequency. Progression Free Survival (PFS) curves were estimated with the Kaplan-Meier method and compared with the log-rank test. Analysis were performed by SPSS version 21.0 (SPSS Inc., Chicago, IL).

**Results:** From January 2006 to December 2017, 240 patients (pts) with HR+/HER2- MBC receiving at least two consecutive E therapies as first approach were selected from 12 Italian cancer centers. The median age at the time of metastasis onset was 63.5 (IQR: 55-72.5) years; 184 (76.7%) pts were in menopausal status; 38 (16%) had de novo stage IV disease and the remaining 202 (84%) had recurrent BC with a median time of 78 months (5-396 months). At the beginning of MBC diagnosis, 148 (62%) pts had a single site of distant disease, 108 (45%) of whom had bone only disease and 45 (18.8%) presented visceral involvement too. The aromatase inhibitor (AI) was chosen as I-line therapy in 146 (60.9%) pts, followed by Fulvestrant (F) in 62 (25.8%) pts; the alternative I-line options were everolimus-exemestane (Eve-Exe), tamoxifene (T), Palbociclib (P)+AI and F+AI in 13 (5.4%), 14 (5.8%), 1 (0.4%) and 4 (1.7%) pts, respectively. The most favourite II-line option resulted F for 111 (46.2%) pts while the Eve-Exe combination was chosen in 70 (29.2%) pts, AI in 30 (12.5%) pts; T, AI+F, P+F and antiprogesterone were administered in 4 (1.7%), 4 (1.7%), 19 (7.9%) and 2 (0.8%) pts, respectively. For I and II-line, the AI followed by F (40%) and F followed by Eve-Exe (18%) were the most common sequential therapeutic approaches; the several alternative options were scanty used (in less than 10%). The median Progression-Free Survival (PFS) from first and second-line E therapies resulted 15.7 (95% CI 13.3-18.1) and 10.3 months (95% CI 8.7-11.9), respectively. Among 194 pts with disease progression after second-line E therapy, 87 (44.8%) received further E therapies with a median PFS 9.4 months (95% CI 7.9-10.9). The remaining 70 (29.2%) pts was treated with palliative chemotherapy. Interestingly, the median Overall Survival (OS) was even longer for pts receiving more lines of E therapies compared to the group with earlier introduction of chemotherapy (204.3 vs 92.8; p=0.007).

Finally, in the subgroup analyses a longer PFS benefit was observed in pts with disease recurrence over 12 months from initial diagnosis (38.1 vs 30.3 months p=0.04) and limited sites of disease involvement at the time of MBC diagnosis (37.6 vs 28.3 months, p=0.03)

**Conclusions:** The sequential use in first and second-line setting of E therapies for HR+/HER2- MBC improves median PFS up to 32.3 months. According to real practice experience the optimal sequences could be AIs followed by F and F followed by Eve-Exe. A role for these compounds should be redefined in the light of recently introduction of CDK 4/6 inhibitors in combination with AIs or F for the first or later lines.



# 2018 San Antonio Breast Cancer Symposium ®

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Time to treatment discontinuation of second-line fulvestrant monotherapy for HR+/HER2- metastatic breast cancer in the real-world setting

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**Background:** Current treatment guidelines recommend sequential hormone therapy for patients with hormone receptor-positive (HR+) metastatic breast cancer (mBC) who are not in visceral crisis or who are not refractory to endocrine treatment. Second-line (2L) fulvestrant monotherapy is one option for patients with HR+ mBC that is human epidermal growth factor receptor 2 negative (HER2-) who progress on first-line (1L) treatment that may have included palbociclib. Using real-world data, we evaluated the differences in time to treatment discontinuation (TTD) of 2L fulvestrant monotherapy between groups of patients with HR+/HER2- mBC who did and did not receive 1L palbociclib.

**Methods:** Using a Flatiron Health EHR-derived database, we examined TTD of 2L fulvestrant monotherapy among 188 patients who progressed on 1L endocrine therapy. Eligibility criteria included a confirmed diagnosis of HR+/HER2- mBC between 1 Jan 2015 and 31 Oct 2017 and treatment with single-agent fulvestrant in the 2L metastatic setting. 2L fulvestrant monotherapy treatment was considered discontinued if (1) third-line (3L) treatment was initiated, (2) death occurred within 60 days of the last administration of 2L fulvestrant monotherapy, or (3) the duration between last administration of 2L fulvestrant monotherapy and last visit date was  $\geq 60$  days. Patients who did not meet this definition were censored. TTD was defined as the time from the first to the last administration of 2L fulvestrant monotherapy, the day before the 3L start date, death date, or the end of study (30 Apr 2018), whichever came first. TTD was estimated by Kaplan-Meier methods and stratified by previous use of 1L palbociclib. Log-rank test was used to test for differences.

**Results:** Overall, the median TTD of 2L fulvestrant monotherapy was 114 days (95% CI: 102, 127). For patients who previously received 1L palbociclib, the median TTD of 2L fulvestrant monotherapy was 102 days (95% CI: 85, 119) compared with 127 days (95% CI: 112, 141) for those who did not receive 1L palbociclib (log-rank  $P = 0.006$ ). Patients who received 2L fulvestrant monotherapy and who had been previously treated with 1L palbociclib ( $n = 88$ ) were more likely to be White (72.7% vs. 68.0%), younger (mean: 65.0 vs. 72.1 years), treated in an academic institution (9.1% vs. 4.0%), and experience longer median duration of 1L treatment (290.5 days vs. 211.0 days) compared with patients receiving 2L fulvestrant monotherapy who did not previously receive 1L palbociclib ( $n = 100$ ).

**Conclusions:** In general, half of patients on 2L fulvestrant monotherapy discontinued treatment in  $< 4$  months. Patients who previously received 1L palbociclib had shorter TTD of 2L fulvestrant monotherapy. Our data identify a potential area of unmet medical need for patients with HR+/HER2- mBC who are treated with fulvestrant monotherapy in the 2L setting and who previously received 1L palbociclib. Further investigation is warranted to examine whether observed results are driven by endocrine resistance or by confounding, selection, or channeling effects of new drugs.

# 2018 San Antonio Breast Cancer Symposium ®

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Sequential second line endocrine therapy is still an effective strategy for postmenopausal ER+ and HER2- advanced breast cancer with low sensitivity to initial endocrine therapy

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**Background:** It is unclear how to define responsiveness to endocrine therapy (ET) during the clinical course of advanced breast cancer (ABC), especially in evaluation of the effect of sequential ET. **Objective:** The goal of the study was to evaluate the efficacy of second line treatment of physician's choice (2nd-line TPC) for estrogen receptor-positive (ER+) and HER2-negative postmenopausal ABC with very low or low sensitivity to initial ET. **Methods:** A multicenter prospective observational cohort study was performed for 2nd-line TPCs. ABC with low sensitivity to initial ET was defined as recurrence within 5 years (yrs) during adjuvant ET or progression within 9 months (mo.) of initial ET. Similarly, ABC with very low sensitivity to initial ET was defined as recurrence within 2 yrs during adjuvant ET or progression within 3 mo. of initial ET. The expected clinical benefit rate (CBR: defined as patients who achieved CR, PR or SD for 24 weeks) was 50%. The null hypothesis of a CBR of 30% was tested with a one-sided  $\alpha$  of 5%. 90% confidence intervals (CIs) were calculated for hypothesis tests. Results: A total of 56 patients (pts) were enrolled, but 7 were ineligible and one discontinued before starting the protocol treatment. The median age was 66 yrs (range: 41-88) and the median BMI was 23.4 kg/m<sup>2</sup> (16.4-31.9). All pts were ER+ and 80% were PgR+. Most of pts had a baseline PS of 0 or 1, 90% had invasive ductal carcinoma, and 10% had invasive lobular carcinoma. Postoperative recurrence was detected in 84% and these pts had a median duration of adjuvant ET of 30.5 mo. (5.3-58.9). De novo stage IV ABC was present in 16%, with a median duration of first-line ET of 5 mo. (2.3-10.8). Adjuvant chemotherapy including anthracycline- and/or a taxane-containing regimen was administered in 58% (29/49). As adjuvant ET before initial recurrence, 34 pts received non-steroidal aromatase inhibitors (AIs) (88.0%), 1 received a steroidal AI (2.3%), and 3 received a selective estrogen receptor modulator (SERM). As first line ET in de novo stage IV, 7 pts (14%) were treated with AIs or a SERM (1 case). 2nd-line TPCs were also used, with 40 pts receiving fulvestrant (82%), 5 receiving SERMs (10%), 3 receiving a mTOR inhibitor plus a steroidal AI (6%), and one patient receiving an AI alone. The overall CBR was 44.9% (90% CI: 34.6-57.6, p=0.009), and CBR was similar across following subgroups (PgR+: n=39, 51.3%, 90% CI: 39.6-65.2, p=0.0016; very low sensitivity group: n=17, 58.8%, 90% CI: 42.0-78.8, p=0.003; non-visceral metastases: n=25, 40%, 90% CI: 34.1-65.9, p=0.0175). However, there were not statistically significant CBR in PgR- (n=10, 20.0%, 90% CI: 8.73-50.7, p=0.617), fulvestrant subgroup (n=40, 40.0 %, 90% CI: 29.2-54.2, p=0.063), low sensitive group (n=32, 37.5%, 90% CI: 26.0-53.6, p=0.1326), and visceral metastases (n=24, 48%, 90%CI: 28.2-60.3 p=0.072). The median PFS was 7.1 mo. (95% CI: 5.6-10.6). **Conclusion:** This study shows that 2nd line ETs was effective and might be a valid option in the sequence of treatments for postmenopausal women with ABC with low sensitivity to initial ET. It was suggested that PgR and visceral metastasis were significant predictive factors for CBR.

# 2018 San Antonio Breast Cancer Symposium ®

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The outcome of lung metastasis treated with fulvestrant is superior to that of liver metastasis for metastatic breast cancer

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**Background:** Endocrine therapy is the preferred option in patients presenting with hormone receptor (HR)-positive metastatic breast cancer (MBC). While visceral metastasis is a negative prognostic factor, few studies have distinguished between the prognosis of different visceral sites.

**Patients and methods:** 505 HR-positive MBC patients administered with fulvestrant at Fudan University Shanghai Cancer Center during a 6-year period were enrolled, 398 patients receiving fulvestrant 500mg were included in final analysis. Logistic regression models were used to identify prognostic factors associated with progression-free survival (PFS). Kaplan-Meier analysis was utilized to compare PFS of lung and liver metastases.

**Results:** Median follow-up time was 26 months. 233 patients presented with baseline visceral metastases, including 138 lung<sup>w/o</sup> liver metastases (lung metastasis without liver involvement), 51 liver<sup>w/o</sup> lung metastases (liver metastasis without lung involvement), and 41 with both lung and liver metastases. Median PFS was 6.8 months (5.6 months for visceral metastases, 9.2 months for non-visceral metastases,  $P = 0.028$ ). Lung<sup>w/o</sup> liver metastases had longer median PFS compared to liver<sup>w/o</sup> lung metastases or both lung and liver metastases (9.6 months, 3.7 months and 3.2 months, respectively,  $P < 0.001$ ). In addition, patients with liver metastases experienced a significantly worse PFS when compared with those without liver involvement (3.7 versus 9.2 months,  $P < 0.001$ ). In multivariate analyses, PFS benefits of fulvestrant were observed in patients with longer disease-free interval, absence of liver metastases, and no previous chemotherapy for MBC.

Table 1. Univariate and multivariate analysis of progression-free-survival by prespecified stratification factors.

Variables	N		Univariate			Multivariate	
		Median <sup>a</sup>	95% CI	P	HR	95% CI	P
<b>Menopausal status</b>							
Premenopausal	84	11.0	6.4-15.7		-	-	-
Postmenopausal	314	5.9	4.7-7.1	0.052	-	-	-
<b>Disease-free interval</b>							
> 5 y	181	8.6	5.8-11.5		1		
≤ 5 y	171	4.8	3.6-6.0	<b>0.003</b>	1.42	1.01-1.99	<b>0.043</b>
<b>PgR status</b>							
Positive	285	6.9	5.3-8.6		-	-	-
Negative + UK	113	5.5	4.6-6.3	0.107	-	-	-
<b>Bone-only metastasis</b>							
Yes	61	10.9	2.7-19.0		1		
No	337	5.8	4.8-6.8	<b>0.002</b>	1.69	0.91-2.80	0.06
<b>Metastatic sites</b>							
Non-visceral	165	9.2	6.7-11.7		1		
Lung <sup>w/o</sup> liver	138	9.6	5.3-13.9	0.860	-	-	-
Liver	92	3.7	2.9-4.5	<b>&lt;0.001</b>	1.51	1.05-2.18	<b>0.027</b>
<b>ET naïve</b>							
Yes	32	26.8	NE-59.3		1		
No	366	6.0	4.9-7.2	<b>0.01</b>	2.12	0.99-4.54	0.052

<b>Prior ET for metastatic disease</b>							
0	145	11.0	5.5-16.6		1		
≥1	253	5.6	4.9-6.3	<b>0.002</b>	0.91	0.62-1.34	0.645
<b>Sensitivity to prior ET</b>							
Primary resistance	71	4.0	2.9-5.0		-	-	-
Secondary resistance	295	7.0	5.6-8.3	0.05	-	-	-
<b>Prior chemotherapy for metastatic disease</b>							
0	203	9.9	6.5-13.2		1		
≥1	195	4.7	3.9-5.5	<b>&lt;0.001</b>	1.93	1.32-2.82	<b>0.001</b>

Abbreviations: PgR, progesterone receptor; UK, unknown; HR, hazard ratio; 95% CI, 95% confidence interval; ET, endocrine therapy; NE, not estimable. Lungw/o liver, lung metastasis without liver involvement. aMedian PFS in months. P < 0.05 was considered significant, significant values was presented in bold.

**Conclusion:** Patients with lung<sup>w/o liver</sup> metastases benefit as well as those with non-visceral metastases from fulvestrant. Visceral metastases should distinguish between liver or lung when treating HR-positive/HER2-negative MBC with endocrine therapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Meta-analyses of visceral versus non-visceral metastases treated by SERM, AI & SERD agents as 1st line endocrine therapy (ET) for HR+ breast cancer (BC)

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**Introduction:** There is continuing debate whether efficacy of ET is different in non-visceral metastases (nVM) than VM. Recently fulvestrant 500mg, has been reported to have greater efficacy than an aromatase inhibitor (AI), anastrozole, particularly in nVM – implying efficacy may be both site and agent dependent. Absence of significant overall survival (OS) difference in PALOMA 1 & 3 has increased interest in site of disease, especially given the OS advantage for fulvestrant 500mg monotherapy.

**Patients & Methods:** Individual patient level data was obtained from 7 randomised controlled trials (RCTs) involving SERM, AI & SERD used as 1st Line ET in known HR+ BC were used in this meta-analysis (MA). Five were Phase 3 double-blind, placebo controlled RCTs. Details of the studies, type of ET and patient numbers are shown in Table. All were rigorously assessed for clinical benefit rate (CBR), progression free survival (PFS), duration of clinical benefit (DoCB) and OS.: Details of the studies, types of ET and patient numbers are shown in the Table. A two stage MA IPD meta-analysis was used to analyse these outcomes CBR, PFS, OS & DoCB. Peto method for pooled odds ratios was used to calculate p values and CI for CBR, Yusuf Peto method was used to calculate p-values and CI for PFS, OS, and DoCB. Random effect for trial was included when Tarone's test for heterogeneity was significant, otherwise fixed effect models were generated.

## Results:

Outcome data is present for each study and then summarised under SERM, AI, SERD and 'all Ets combined'. Odds Ratios (Ors) & Hazard Ratios (HRs) for VM versus nVM by endocrine agent are shown in the Table

ET	Study	No. of Pats.	HR+ Pats.	CBR	PFS	OS	DoCB
SERM		(n)	(n)	OR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
Tam	EORTC	189	178	1.41	0.85	0.77	0.95
Tam	0027	328	144	1.33	0.98	1.05	1.26
Tam	0030	182	162	2.80	0.59	0.44	0.79
Tam	0025	274	209	1.10	0.78	0.73	0.78
	subtotal	973	693	1.53** (1.11-2.10)	0.79** (0.67-0.94)	0.70* (0.52-0.94)	0.92 (0.72-1.18)
AI							
Exe	EORTC	182	168	0.84	1.11	0.73	1.02
Ana	0027	340	154	3.85	0.55	0.36	1.06
Ana	0030	171	151	0.97	0.88	1.14	0.82
Ana	FALCON	232	232	1.07	0.98	0.83	1.05
Ana	FIRST	103	103	0.97	0.54	0.51	0.53
	Subtotal	1028	808	1.28 (0.73-2.22)	0.80 (0.60-1.06)	0.66* (0.45-0.95)	0.92 (0.74-1.14)
SERD							
Ful 500	CONFIRM	162	162	2.94	0.63	0.56	1.15
Ful 500	FALCON	230	230	2.71	0.41	0.45	0.61
Ful 500	FIRST	102	102	4.11	0.58	0.44	0.55
	Subtotal	494	494	3.06*** (2.00-4.06)	0.56*** (0.45-0.70)	0.50*** (0.39-0.65)	0.71* (0.53-0.91)

Total	2495	1998	1.66*** (1.37-2.02)	0.73*** (0.62-0.86)	0.61*** (0.53-0.71)	0.87* (0.75-1.00)
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CBR-Clinical Benefit Rate (n)= number of patients, pvalues p<0.05\*, p<0.01\*\*, p<0.001\*\*\*

**Conclusions:** This is the largest reported individual patient MA for nVM versus VM in patients with known HR+ advanced BC and clinical outcomes (CBR, DoCB, PFS & OS) approved to regulatory standards.

1) nVM had significantly better clinical outcomes compared to VM when treated by anti-estrogen receptor blocking agents (SERM & SERD) but not when treated by AIs, which have a fundamentally different mechanism of action.

2) SERD (Fulvestrant 500mg) significantly increased all four clinical outcomes. For nVM compared to VM, fulvestrant put more patients into CB, kept them in remission (DoCB) for longer, resulting in 44% reduction in disease progression and a 50% reduction in death.

3) Site of metastases (ie nVM or VM) is one of the factors to consider when selecting patients for endocrine mono or combination ET (plus CDK4/6i) in the 1st Line setting.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-13-12

Estetrol for treatment of advanced ER<sup>+</sup> breast cancer

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**Introduction:** Currently, a multi-center, open-label, phase I/IIA, dose-escalation study with the fetal estrogen estetrol (E4) is ongoing in Germany in postmenopausal patients with advanced breast cancer (ABCE4 study). The objective of the study is to assess safety and tolerability of different doses of E4 (range 20-60 mg per day). In addition initial anti-tumor response will be determined.

**Study design:** Patients are treated for 12 weeks with E4; 4 weeks in Phase I and thereafter 8 weeks in Phase IIA. Phase I of the study will follow the traditional 3 + 3 design to determine the optimal dose in patients with advanced breast cancer. Patients are treated in cohorts of three all receiving the same dose. Occurrence of dose limiting toxicity (DLT) at completion of phase I (4 weeks treatment) will determine escalation to the next higher dose in the study. After completion of Phase I, patients will continue treatment for 8 weeks to assess preliminary anti-tumor response in Phase IIA. Treatment may continue beyond 12 weeks based on evaluation of the patient and her treating physician.

**Results:** Phase I of the first treatment cohort with 20 mg E4 per day has been completed. A total of six postmenopausal women with advanced breast cancer has been included in the first cohort. One patient withdrew consent before treatment with E4 started. She was replaced. Two patients discontinued the study before completion of Phase I for reasons other than DLTs. These patients were unevaluable for toxicity and were also replaced. Three patients completed Phase I. One patient discontinued the study during Phase IIA due to disease progression after 9,5 weeks of E4 treatment. One patient completed both the Phase I and IIA part of the study. She had stable disease at study completion and wanted to continue E4 treatment because of improved well-being. Tumor assessment after 24 weeks of E4 treatment showed again stable disease. One patient is presently in Phase IIA of the study.

None of the patients experienced a DLT. The 20 mg E4 dose was well tolerated by all patients. In total 17 adverse events were reported. Adverse events were mainly of mild or moderate intensity. Five of 17 events fulfilled criteria of seriousness; none of these events were considered to be related to the E4 treatment. Four events were considered to be possibly related to the E4 treatment. These events were increased endometrial thickness, dry skin, pruritus and fatigue, all of mild intensity. A remarkable finding was that three of the five patients treated with E4 reported to the investigator to "feel better" than before the start of E4 treatment. This "feeling better" was described by one of the patients as: "feeling less down and exhausted; instead feeling much more optimistic, powerful and positive when taking E4".

So far anti-tumor response could be estimated in one patient. This patient, who started the study with progressive disease, had stable disease as shown by tumor assessments after 12 weeks and 24 weeks of E4 treatment.

**Conclusion:** Based on these results, we conclude that a daily dose of 20 mg E4 is well tolerated. The majority of patients experienced favorable subjective effects on wellbeing. The data obtained with the 20 mg dose E4 allow dose escalation to the next higher dose of 40 mg E4 per day.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-13-13

Meta-analyses of visceral versus non-visceral metastases treated by AI & SERD agents as 2<sup>nd</sup> line endocrine therapy (ET) for HR+ breast cancer (BC)

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There is a prevailing belief that ET for HR+ advanced BC is not as effective in patients with visceral metastases (VM) compared to non-visceral metastases (nVM), particularly with later lines of ET. Recently fulvestrant 500mg (Ful 500), has been reported to have greater efficacy in nVM compared to i) VM treated by Ful 500 but also compared to ii) nVM treated by Ful 250 (2<sup>nd</sup> line) and iii) nVM treated by aromatase inhibitor (AI), anastrozole (1<sup>st</sup> Line) – implying both site and agent related efficacy. Absence of significant overall survival (OS) difference in PALOMA 3 (2<sup>nd</sup> line) has increased the debate regarding when to add CDK 4/6is to ET, especially given the OS advantage for Ful 500 monotherapy in the 1<sup>st</sup> & 2<sup>nd</sup> line settings.

**Patients & Methods:** Anonymised, individual patient level data was obtained from randomised controlled trials (RCTs) involving AI & SERD used as mono-therapy in 2<sup>nd</sup> or 3<sup>rd</sup> Line setting in known HR+ BC. All the trials were Phase 3 double-blind, placebo RCTs. All were rigorously assessed for clinical benefit (CB), progression free survival (PFS), duration of CB (DoCB) and OS. Details of the studies, types of ET and patient numbers are shown in the Table.

**Results:** Outcome data is presented for each study and then summarised under AI, SERD (Ful 250 or 500) and 'all Ets combined'. Odds ratios (Ors) & hazard ratios (HRs) for VM versus nVM by endocrine agents are shown in the Table.

Agent	Study	Total Pats. (n)	HR+ Pats. (n)	CBR OR (95%Cis)	PFS HR (95%Cis)	OS HR (95%Cis)	DoCB HR (95%Cis)
AI							
Exe	0020	230	183	1.18	1.44	1.27	1.50
Exe	0021	193	168	1.15	1.95	1.83	2.12
Ana	EFFECT	340	336	0.94	1.52	1.20	1.10
Ana	SOFEA	249	249	1.29	1.18	1.05	1.41
	subtotal	763	687	1.11 (0.84-1.48)	1.47*** (1.22-1.79)	1.21* (1.01-1.45)	1.43** (1.10-1.86)
SERD							
Ful 250	0020	219	160	1.79	1.70	1.40	1.23
Ful 250	0021	204	177	1.28	1.81	1.32	2.06
Ful 250	EFFECT	351	345	0.79	1.40	1.31	1.03
Ful 250	SOFEA	231	231	0.70	1.17	1.24	2.22
Ful 250	CONFIRM	152	152	1.13	1.07	1.51	0.84
	Subtotal	926	834	1.05 (0.75-1.45)	1.39*** (1.16-1.67)	1.34*** (1.14-1.57)	1.36 (0.93-1.98)
SERD							
Ful 500	CONFIRM	144	144	2.24 (1.12-4.48)	1.30 (0.90-1.87)	1.33 (1.14-1.57)	0.97 (0.55-1.66)
All ETs	Total	1833	1665	1.13 (0.92-1.39)	1.42*** (1.26-1.59)	1.28*** (1.14-1.44)	1.35** (1.09-1.66)

[Pats=Patients; (n)=number; CBR-Clinical Benefit Rate; p-values pMedian PFS (months) for nVM for AI, SERD250, SERD500 & â– all Ets combinedâ– were 5.4, 5.5, 11.0 & 5.5 respectively: for VM they were 2.9, 3.5, 5.5 & 3.2 respectively.



Median OS (months) for nVM for AI, SERD250, SERD500 & all Ets combined was 24.2, 26.0, 35.4 & 25.4 respectively: for VM the figures were 22.8, 20.8, 26.4 & 22.0 respectively. **Conclusions:** 1) In the 2nd line HR+ setting AI & Ful 250 both significantly increased PFS & OS in nVM versus VM. Longer PFS appears due to longer duration of control (DoCB) than increasing the number of patients responding (CBR).

2) Median OS for nVM ranged from 24 to 35 months versus 20.8-26.4 months for VM: for the majority of patients the 2nd line ET setting is not immediately life threatening and ET is therefore an option to consider.

3) These data on site of disease (nVM vs VM) contribute to the selection of which patients should receive endocrine mono- and which endocrine combination therapy (ie plus mTORi or CDK4/6i) in the second line setting.

# 2018 San Antonio Breast Cancer Symposium ®

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“Real world” characteristics, treatment patterns and outcomes of patients with hormone receptor positive (HR+), human epidermal growth factor 2 negative (HER2-) metastatic breast cancer (MBC)

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**OBJECTIVES:** Outcomes for patients with MBC vary according to disease phenotype and treatment history. We present UK real-world patient characteristics, treatment patterns and outcomes for patients with HR+/HER2- BC treated at a single cancer center.

**METHODS:** A retrospective review of health records including coded data, unstructured text and clinical review of patients treated from January 2012 to March 2018 identified females  $\geq 18$  years with metastatic or locally advanced HR+/HER2- BC. Those enrolled in clinical trials, with operable local recurrence as only disease site, incomplete treatment records or significant secondary malignancy were excluded. Patient characteristics, systemic, local (radiotherapy/surgery) and supportive treatments, health care resource use (HRU) and overall survival (OS) are presented. OS was estimated using the Kaplan-Meier method, censoring patients alive at study end.

**RESULTS:** 253 patients meeting study inclusion criteria were identified (median age 67, IQR 56,76; 84% post-menopausal), of whom 47 (19%) had locally advanced disease (T4 and/or N3), 75 (30%) had MBC at initial presentation and 131 (52%) had metastatic disease at first recurrence. Among patients with MBC at initial presentation, all received systemic treatment following diagnosis, including chemotherapy (35%), endocrine (93%) and targeted therapy (i.e. everolimus 7%). Among those recurring with MBC, 97% subsequently received systemic treatment, including chemotherapy (50%), endocrine (93%) and targeted therapy (27%). For patients recurring with MBC, the most common first line therapies (LoT) were letrozole (20%), exemestane (single agent, 15%), anastrozole (14%), everolimus (with exemestane, 11%) and paclitaxel (9%). For patients diagnosed with MBC, the most common first LoT were letrozole (47%), anastrozole (23%) and EC (11%). At second LoT, the most commonly used regimen for patients recurring with MBC was fulvestrant (13%) and for patients with MBC at initial diagnosis, it was exemestane (19%). Median OS for patients recurring with MBC was estimated to be 2.11 years (IQR 1.80,3.05), compared with 2.65 years (IQR 2.24,2.95) for those with metastatic disease at initial diagnosis. Median available follow-up time for the whole cohort was 2.85 years (IQR 1.42,4.82).

**CONCLUSIONS:** Patients with overt metastatic disease at presentation comprise a substantial proportion of those treated for MBC. With the follow-up time available, estimated median OS for patients with metastatic disease at initial presentation appears somewhat better than for those recurring with metastatic disease. Real world analysis demonstrates diverse treatment pathways for patients with HR+, HER2- MBC, reflecting the individualized care they receive.

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Estrogen levels in premenopausal patients (pts) with hormone-receptor positive (HR+) early breast cancer (BC) receiving adjuvant triptorelin (Trip) plus exemestane (E) or tamoxifen (T) in the SOFT trial: SOFT-EST substudy final analysis

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**Background:** Optimal endocrine therapy for premenopausal pts with early HR+ BC may depend on complete estrogen suppression with GnRH analog, which is crucial when using concurrent aromatase inhibitors (AIs). SOFT-EST is a prospective substudy of the phase 3 SOFT trial aiming to describe estradiol (E2), estrone (E1) and estrone sulphate (E1S) during the first 4 years (y) of monthly Trip+E/T and to assess if there were suboptimally estrogen suppressed (SES) pts in the E+Trip group. Secondary objectives included associations of baseline (BL) factors with SES, early SES with later SES, and SES with disease-free survival (DFS; exploratory objective).

**Methods:** Patients from select centers who consented and enrolled in SOFT, selected Trip as ovarian function suppression method, and were randomized to E+Trip or T+Trip were eligible for SOFT-EST until the accrual goal (120 pts: 90 E+Trip; 30 T+Trip). Prem status for SOFT eligibility was based on local E2. Blood sampling timepoints were 0, 3, 6, 12, 18, 24, 36 & 48 months (m) until Trip stopped. Serum estrogens were measured centrally by high specificity/sensitivity GC/MSMS and were not available during the study. For 4y analyses, SES was defined as E2 levels >2.72 pg/mL in  $\geq 2$  post-BL samples (E2 levels *not consistent with postmenopausal (PM) status on AIs* [Smith IE, JCO 2006]), or vaginal bleeding >3m after Trip start, or pregnancy. We explored 2 additional cutoffs: >10 pg/mL (*clearly inconsistent with PM status on AIs*) and >20 pg/mL (*inconsistent with GnRH analog-related PM status*). The analysis is intention-to-treat based on E/T assignment; as-treated analyses are forthcoming.

**Results:** From Mar 2009 to Jan 2011, 109 pts (E/T=83/26) started Trip and had  $\geq 2$  samples drawn. In pts assigned E+Trip, median reductions from BL in E1, E2 and E1S were >95% at all timepoints and significantly lower than in T+Trip. Post-BL E2 geometric mean ranged 0.8-1.3 pg/mL in E+Trip and 16.5-18.3 pg/mL in T+Trip. 21 (25%), 11 (13%) and 6 (7%) pts assigned to E+Trip had E2>2.72, >10, and >20 pg/mL in  $\geq 2$  post BL samples or vaginal bleeding (n=3), respectively. Early SES [ $\geq 1$  E2 value >2.72 pg/mL or vaginal bleeding in the firsty] predicted later SES [ $\geq 1$  E2 value >2.72 or vaginal bleeding thereafter (n=1); p<0.001]. BL factors related to SES were higher E2, lower FSH and lower LH values (p=0.02, p<0.01, p<0.01 respectively). 12m FSH levels were not related to SES. In pts assigned E+Trip, after 6y median follow-up, DFS events were seen in 0 of 21 pts with SES vs 5 of 62 pts without SES.

**Conclusions:** Most pts on E+Trip had a profound E2 drop consistent with postmenopausal status on AI, but >20% assigned to E+Trip had  $\geq 2$  E2 values >2.72 pg/mL and 4% had vaginal bleeding, with those having higher E2, lower FSH/LH at BL being at higher risk. SES at 12m predicted subsequent SES. Few DFS events limit the ability to assess clinical relevance of SES with disease outcomes.

BL characteristics	N-109
Prior chemo	60 (55%)
Amenorrhea	39 (36%)
Age <35y	8 (7%)
	Median (range)
Age, y	44 (25-53)
BMI, kg/m <sup>2</sup>	24 (22-28)
Estrogen (pg/mL)	
E2	52 (7-119)

E1	41 (24-70)
E1S	894 (304-1320)
FSH/LH (IU/L)	
FSH	15 (7-47)
LH	11 (6-26)

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Analysis of DRFI and OS of HR-positive, HER2-negative breast cancer patients treated on the BIG1-98 study, classified according to a novel, integrated, clinical-pathologic and genomic classification method

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## Background and Objectives

HR<sup>pos</sup>, HER2<sup>neg</sup> early breast cancer is molecularly heterogeneous. Classifications that integrate genetic, genomic and clinical-pathologic data (nodal status, tumour size, tumour differentiation, Ki-67 status, PR expression) are lacking. In a previous study we have defined three different molecular groups of invasive ductal luminal breast cancer (IDLC) using clinical prognostic variables, gene expression, copy number variation and mutation data from the METABRIC series [1, 2] and showed that IDLC groups (IDLC1, -2, -3) had distinct prognostic outcomes [3, 4]. The 3 groups differ by DNA damage load, predominant lesion type, characteristic lesion patterns, oncogenic driver mechanisms and time to first recurrence. Here we evaluated the prognostic and predictive value of these groups on patients treated on the BIG1-98 study.

## Methods

We used our predefined subgroups (IDLC1, -2, -3) to classify patients in the BIG1-98 set [5, 6]. A weighted Cox proportional hazards regression model was used to assess the association between IDLC subtype, treatment on OS and DRFI endpoints in the BIG1-98 study. Weighted chi-squared tests were used for categorical variables and weighted t-tests for mutational burden. All statistical computations were carried out in R v.3.2.3.

## Results

Five hundred thirty eight breast cancers (ductal 75%, lobular 17%, 8% other) were included in this study. The DNA damage burden of IDLC1 or -2 type tumours was significantly lower compared to IDLC3 (mean lesion numbers: 9, 11, 18.5). IDLC1 type tumours (48%) harboured mainly oncogenic point mutations (predominantly PIK3CA 51%, MAP3K1 27%, NCOR1 22%, FANCD2 20%). IDLC2 (34%) and IDLC3 type tumours (18%) were both mutated (PIK3CA 53% vs 38%, GATA3 12% vs 30%, TP53 10% vs 37%, CDH1 20% vs 8%), 8p11-12 (IDLC3 38% vs IDLC2 21% or IDLC1 5%, p value < 0.001) and/or 11q13-14 amplified (IDLC3 46% vs IDLC2 28% or IDLC1 2%, p value < 0.001). Consistent with the higher DNA damage load patients with IDLC3 type tumours had significantly shorter distant recurrence free intervals (DRFI) (HR (95% CI) 2.35 [1.99; 2.78] p-value= <2e-16) and significantly shorter OS compared to IDLC1 or -2 (HR (95% CI) 2.55 [2.2; 2.96] p-value= <2e-16). Patients with IDLC1 or -2 type tumours seemed to have the same survival regardless of hormonal treatment assignment. However, the IDLC3 group derived greater magnitude of benefit from Letrozole compared to Tamoxifen (HR (95% CI): DRFI 0.85 [0.66; 1.09] vs. 0.53 [0.35; 0.79] interaction p-value=0.03, OS 0.98 [0.8; 1.21] vs 0.34 [0.25; 0.47], interaction p-value=2.5e-09).

## Conclusions

We have validated our integrated clinical-pathologic and genomic classification method on an independent dataset of HR<sup>pos</sup>, HER2<sup>neg</sup> early stage breast cancer. Our classification also suggests differing magnitude of benefit for adjuvant Letrozole benefit. Prospective clinical studies will be required to validate and corroborate this novel classification approach.

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# 2018 San Antonio Breast Cancer Symposium ®

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Influence of competing risks of death on the interpretation of adjuvant endocrine therapy trials for breast cancer

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**Background:** Early stage, hormone sensitive breast cancer is associated generally with a good prognosis, with only a minority of patients expected to die of breast cancer. Death from causes other than breast cancer can dilute the patients at risk of breast cancer events and result in over-estimation of risk of recurrence and consequently the benefit from breast cancer therapy, a so-called immortal time bias. The MA.17R trial (Goss et al 2016) evaluated the role of extending adjuvant treatment with letrozole from 5 to 10 years. Here we determine the effect of analyzing the MA.17R trial using methods accounting for competing risks.

**Methods:** We compared conventional and competing risk methods for disease-free survival (DFS) and for distant recurrence-free survival (DRFS). In Kaplan-Meier analyses death from any cause was considered an event while cumulative incidence functions (CIFs) assumed death without recurrence to be a competing risk. The complement of the survival function (one minus the survival function) was used to estimate incidence of the primary event of interest. This was compared to estimates obtained using CIFs accounting for the occurrence of competing events.

**Results:** Non-breast cancer death was the most common event defining DFS and DRFS. Over the course of follow-up, there was increasing discrepancy between the risk of disease recurrence measured using Kaplan-Meier and CIF. Among letrozole treated patients the estimated distant recurrence at 5 years of follow-up was 5.4% using CIF and 9.6% using Kaplan-Meier. At 10 years of follow-up, the estimated distant recurrence was 8.4% using CIF and 20.0% using Kaplan-Meier. Similar results were observed for the placebo group (8.5% vs 12.1% at 5 years and 14.8% vs 27.3% at 10 years), and in patients with baseline cardiovascular disease (see Table). Benefit from letrozole on DFS and DRFS was greater when accounting for competing risk (hazard ratio [HR] for DFS 0.66, 95%CI 0.48-0.90; DRFS HR 0.75, 0.50-1.14) compared to the conventional method (DFS HR 0.79, 0.62-0.99; DRFS HR 0.91, 0.70-1.18). In women with baseline cardiovascular risk, the benefits of extended adjuvant letrozole when considering competing risk (DFS HR 0.38, 0.16-0.89; DRFS HR 0.46, 0.16-1.35) were also greater than those observed in the conventional analysis (DFS HR 0.55, 0.32-0.93; DRFS HR 0.59, 0.33-1.04). Treatment with extended letrozole did not influence non-breast cancer death in women who died with disease recurrence (HR 1.06, 0.74 -1.50) or in those with competing risk or censored from the analysis (HR 1.05, 0.73 -1.49).

**Conclusion:** Over the course of follow-up, estimates of DFS and DRFS differ increasingly if measured using Kaplan-Meier or CIF, with CIF estimates of risk being substantially lower. Using a competing risk model, the reduction in distant recurrence at 8 years with extended letrozole is less than 1%. Additional competing risk analyses of the MA.17 (Goss 2006) and MA.27 (Goss 2013) trials are ongoing.

Cumulative incidence of disease recurrence in patients with baseline cardiovascular risk

Time (years)	CIF (%)	1-KM (%)
Letrozole		
1	1.5	1.5
3	4.4	6.5
5	5.8	13.8
Placebo		
1	3.5	3.5
3	8.3	11.8
5	12.5	20.3



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Time course changes in serum FSH, estradiol, and menstruation restoration in premenopausal patients with breast cancer taking adjuvant tamoxifen after completing chemotherapy: A report from the ASTRRA study

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## BACKGROUND

Assessment of restoration of ovarian function after chemotherapy is critical with respect to the initiation of different types of endocrine treatment in young high risk breast cancer patients

## METHODS

In total, 1289 women who remained premenopausal or resumed premenopausal status after chemotherapy were randomized to receive 5 years of tamoxifen or 5 years of tamoxifen plus 2 years of ovarian suppression. The patients who did not resume menstruation were followed up for 2 years with tamoxifen treatment after finishing chemotherapy. Prospectively collected consecutive post-chemotherapy hormone and menstruation data were available for 705 breast cancer patients who enrolled tamoxifen-only treatment group or did not resume menstruation during follow up. This analysis evaluated the proportion of patients with pre-menopausal FSH levels (<30 mIU/ml), E2 levels (<sup>3</sup>40 pg/ml), and menstruation at any time point during treatment with tamoxifen.

## RESULTS

During 5 years of tamoxifen treatment after chemotherapy for premenopausal breast cancer patients, 62% of patients resumed menstruation. Menstruation returned in 92% of patients under 35 years old but only in 31% of patients over 45 years old. Ovarian function, defined by serum FSH and E2 levels, resumed in 94% and 65% of patients, respectively, over 5 years. Most patients achieved ovarian function restoration during the first 2 years after chemotherapy, with 47.1% resuming menstruation and 86.2% and 50.3% achieving pre-menopausal FSH and E2 levels, respectively, in the first 2 years. Clinical factors related to menstruation restoration were younger age (HR = 6.38, 95% CI 1.33-3.47), 6 month hormone profile after chemotherapy (FSH<30: HR=1.67, 95% CI 1.28-2.17; E2 >40: HR=2.96, 95% CI 2.25-3.89), and anthracycline without taxane chemotherapy (HR=1.63, 95% CI 1.25-2.13).

## CONCLUSIONS

During 5 years of tamoxifen treatment after chemotherapy, half of patients experienced menstruation restoration, including most very young patients under 35 years. The majority of patients experienced menstruation restoration in the first 2 years of tamoxifen treatment.



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Confirmation of the TAILORx 21-gene expression trial using a real world observational database

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**Background:** The TAILORx study (NCT00310180)(TRx) has demonstrated the efficacy of endocrine therapy alone in early stage, lymph node negative, hormone receptor positive, her2neu oncogene negative breast cancer harboring an intermediate recurrence score (RS) on a 21-gene profile (OncotypeDx), obviating the need for adjuvant chemotherapy in a large subset of women. The study randomized and followed 6711 patients (pts) and required 9 years to reach its conclusion endpoints. The availability of the electronic health record (EHR) permits automated reviews, facilitating more rapid “real world” hypothesis testing (but not a replacement for randomized clinical trials), especially when there are clear variations in common practice patterns. However physician bias in treatment selection needs to be considered.

**Methods:** A retrospective review of the Cota Observational Cancer database, drawn from EHRs, of female pts with breast cancer who were 18 to 75 years of age; had hormone-receptor–positive, human epidermal growth factor receptor 2 (HER2)–negative, axillary node–negative breast cancer harboring an OncotypeDx RS 11-25 receiving adjuvant therapy following surgical resection of a 11-50 mm primary tumor (similar to TRx eligibility).

**Results:** 1009 pts from 23 cancer centers (107 oncologists) were identified, 850 (84.2%) received adjuvant endocrine therapy alone (E) and 159 (15.8%) received adjuvant chemoendocrine therapy (CE) as part of standard care (no randomization). 285 pts were age  $\leq 50$  yrs (E:218, CE:67) and 601 pts has RS 16-25 (E:453, CE:148). Treatment selection was imbalanced with oncologists more likely to utilize CE in younger pts (median age E: 59 yrs, CE: 53 yrs;  $p < 0.01$ ), larger tumors (median tumor size E: 16mm, CE: 20mm;  $p < 0.001$ ) and higher RS (median RS E: 16, CE: 21;  $p < 0.001$ ). With a median follow-up for survival since diagnosis of 3.7 years, the Kaplan-Meier estimated 5 yr overall survival rates were 98.9% with E and 97.8% with CE ( $p = 0.23$ ); the corresponding 5-yr OS in TRx were E: 98% and C: 98.1%. With a median 1.7 years follow-up for recurrence, 19 pts have suffered a disease distant or local recurrence (E: 13, CE: 6) yielding a 5-year recurrence-free survival of E: 95.2% and CE: 91% ( $p = 0.05$ ); the corresponding TRx result was E: 96.9% and CE: 97%. The 5-yr invasive disease-free survival (IDFS = death, local/distant, second primary) with 32 events was E: 92.7% and CE: 81.9% ( $p = 0.05$ ); corresponding TRx E: 92.8 % and CE: 93.1%. Given the imbalance in treatment allocations, a multivariate analysis was performed, with older age ( $< 0.001$ ), CE choice ( $< 0.006$ ) and larger tumor size ( $p < 0.05$ ) remaining significant, but not increased RS ( $p = 0.16$ ) for 5-year IDFS. Among women age  $\leq 50$  with RS 16-25 (E: 118; CE: 60) the 5-yr IDFS was E: 95% and CE: 94%; the corresponding RS 16-20 TRx E: 92% and CE: 94.7% and RS 21-25 E: 86.3% and CE: 92.1%.

**Conclusions:** Using a real world data source, endocrine therapy alone appears to yield excellent 5-yr survival rates among pts with 21-gene RS 11-25 similar to the TAILORx trial. Treatment selection bias (with perceived higher risk pts allocated to CE) and shorter median follow-up limits full confirmation by this dataset.

# 2018 San Antonio Breast Cancer Symposium ®

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A study of adverse vascular effects of adjuvant therapy with aromatase inhibitors in women with breast cancer

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**Background:** There is a debate concerning risk of cardiovascular disease (CVD) from adjuvant therapy with aromatase inhibitors (AI). Randomized trials have indicated higher CV risk and one observational study higher risk of myocardial infarction, whereas other studies have pointed out no elevated risk. The first morphological sign of developing atherosclerosis is a thickening of the arterial intima layer, whereas in the longer perspective the media layer gets thinner - a process not captured when using conventional Carotid intima-media-thickness (CIMT). We therefore used high resolution ultrasound (HRU), to separately estimate the intima and media layers and calculate the I/M thickness ratio. A thicker intima and higher I/M thickness ratio are signs of adverse vascular effects. This principle of estimating vascular aging can strongly distinguish 70-yr old subjects with and w/o prevalent CVD, yielding C-values up to 0.90 in ROC-analysis.

**Patients and Methods:** We included 150 postmenopausal breast cancer women of whom 50 were treated with AI during at least 3 years, 50 with at least 3 years of tamoxifen and 50 were without endocrine therapy. Patients were investigated with non-invasive HRU (22MHz) of the common carotid artery (CCA). Non-parametric statistical methods were used, including Bootstrap quantile regression to obtain 95% confidence intervals (CI) and adjustment for potential confounders: age, smoking, use of statins, HbA1c and previous HRT.

**Results:** Of 150 included women, 132 remained having a technically acceptable ultra sound scans and no AI therapy before tamoxifen therapy; AI (#48); tamoxifen (#40) and no anti-hormone therapy (#44). Corresponding median ages were 66, 65 and 65 yrs and median treatment durations were 54 (AI) and 58 (tamoxifen) months.

AI therapy, compared to tamoxifen, showed a significantly thicker CCA-intima layer,  $p = 0.031$ . However, mean values of both CCA-intima thickness and I/M thickness ratio were very similar in the tamoxifen and the no anti-estrogen group; Thus, AI compared to the other two groups combined, had a significantly thicker intima layer ( $p = 0.009$ ) and higher I/M ratio ( $p = 0.047$ ), both indicating adverse vascular effects from AI therapy. After adjustment for potential confounders, AI therapy still showed a thicker CCA intima layer of 0.015 mm (95% CI .003, .027);  $p = 0.016$ .

In contrast, corresponding analysis using conventional Carotid intima-media thickness (CIMT), revealed no significant group differences or association to AI treatment duration.

**Conclusion:** More than three years of adjuvant therapy with aromatase inhibitors, compared to women having no anti-estrogen therapy or tamoxifen, was associated with signs of adverse vascular effects; thicker intima layer and higher intima/media ratio of the common carotid artery. After adjustment, the thicker intima layer remained significant. These findings are in accordance with reports of increased risk of cardiovascular events following adjuvant therapy with aromatase inhibitors.

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Neoadjuvant exemestane or exemestane plus docetaxel and cyclophosphamide tailored by clinicopathological response to 8–12 weeks' exemestane exposure for ER+/HER2– postmenopausal breast cancer patients

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**Aim** To investigate the efficacy and safety of initial neoadjuvant endocrine therapy with exemestane (EXE) alone followed by tailored treatment: continued EXE monotherapy for responders or EXE plus docetaxel–cyclophosphamide (TC) combination therapy for non-responders.

**Methods** This open-label phase II study enrolled postmenopausal patients with primary invasive estrogen receptor (ER)-positive, HER2-negative, stage I–IIIA (T1c–T3 N0–2 M0) breast cancer and Ki67 index  $\leq 30\%$ . Patients first received EXE 25 mg/day for 12 weeks. Based on clinical response and change in Ki67 index, responders were defined as patients who achieved complete response (CR), partial response (PR) with Ki67 index  $\leq 5\%$  after treatment, or stable disease (SD) with Ki67 index  $\leq 5\%$  both before and after treatment. Non-responders were defined as patients with PR and Ki67 index  $> 5\%$  after treatment, or SD and Ki67 index  $> 5\%$  before or after treatment. For the subsequent 12 weeks, responders continued EXE monotherapy (continued EXE group) and non-responders received EXE plus 4 cycles of TC (docetaxel 75 mg/m<sup>2</sup> and cyclophosphamide 600 mg/m<sup>2</sup> every 3 weeks) (EXE+TC group). Primary endpoint was clinical response (CR and PR) at week 24.

**Results** A total of 58 patients (median age 60 years, range 53–67 years) were enrolled between December 2010 and May 2016. Five patients discontinued treatment in the initial 12-week EXE monotherapy period; therefore 53 received the subsequent treatment. After 8–12 weeks of initial EXE monotherapy, 15 patients were classified as responders (8 with PR and Ki67 index  $\leq 5\%$  after treatment, 7 with SD and Ki67 index  $\leq 5\%$  before and after treatment) and 38 as non-responders (5 with PR and Ki67 index  $> 5\%$  after treatment, 33 with SD and Ki67 index  $> 5\%$  before or after treatment). Clinical response rates at weeks 12 and 24 were 71% (10/14, 95%CI 41.9–91.6%) and 57% (8/14, 95%CI 28.9–82.3%), respectively, in the continued EXE group, and 16% (4/25, 95%CI 4.5–36.1%) and 56% (14/25, 95%CI 34.9–75.6%), respectively, in the EXE+TC group. At week 24, no significant difference was found in median Ki67 index between the continued EXE and EXE+TC groups (1.4% and 2.0%, respectively). The proportion of patients with preoperative endocrine prognostic index (PEPI) 0 was higher in the continued EXE than in the EXE+TC group (60% vs 29%), but not significantly so ( $P=0.058$ , Fisher's exact test).

The breast-conserving surgery rate was 93% and 56% (continued EXE and EXE+TC groups, respectively).

Adverse events (AEs)  $\geq$  grade 3 were reported in 40% (21/53) of patients (continued EXE group 8%, 1/15; EXE+TC group 53%, 20/38). The most common AEs were leukopenia (37%, 14/38), neutropenia (32%, 12/38), and febrile neutropenia (16%, 6/38) during chemotherapy (EXE+TC group).

**Conclusion** Tailored treatment maintained the favorable clinical response to EXE alone in responders and improved subsequent clinical response in non-responders. EXE+TC was associated with higher incidence of hematological AEs, but these were manageable. The results show the effectiveness of tailored neoadjuvant endocrine and chemoendocrine therapy in postmenopausal ER-positive breast cancer patients. (JBCRG-11TC; UMIN000004752)

# 2018 San Antonio Breast Cancer Symposium ®

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Serum concentrations of tamoxifen and Z-endoxifen may predict sexual dysfunction in the 2<sup>nd</sup> year of adjuvant endocrine treatment

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**Background and rationale:** Side effects of adjuvant treatment with tamoxifen (tam) may impair Quality of Life (QoL) and have been suggested as an independent variable for discontinuation of tam<sup>1</sup>. There are large inter-patient variabilities in prevalence and severance of side effects among tam users. Therefore, there is a need for biological markers that can predict side effects. A potential biological predictor is the serum concentrations of tam and/or its metabolites. In this prospective observational study we have analyzed serum concentrations of tam and 9 metabolites over 3 years. Patients Reported Outcome Measures (PROM) were obtained to elucidate possible associations between side effects, adherence and tam metabolism.

**Methods:** Breast cancer patients using adjuvant tam (20mg/d) were recruited through the Prospective Breast Cancer Biobank project between 2011 and 2016. Inclusion criteria were ER positive status,  $\geq 6$  months tam use, tumor size of  $\geq 0.1$  cm and being able to read and write Norwegian. Concentration levels of tam and metabolites in serum were analyzed by LC-MS/MS<sup>2</sup> and adherence data were collected through the Norwegian prescription database. PROM-data comprised of validated questioners reporting side effects of endocrine treatment and QoL. Statistical analyses comprised non-parametric tests, logistic regression, chi square tests and the Benjamin-Hochberg procedure to correct for multiple testing.

**Results:** Associations between metabolite concentrations and side effects were run as a cross sectional analysis (N=149) and separate analysis of each year of follow-up with 85, 77 and 65 patients at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> year respectively. We found that 78 % of patients reported side effects, 66 % reported mood swings, 21 % reported severe hot flushes and 71 % reported decreased libido. When analyzing years separately, we found that on the 2<sup>nd</sup> year patients experiencing vaginal dryness had significantly higher levels of tamoxifen ( $P=0.032$ , after correction for multiple testing and adjustment for clinical relevant variables) compared to patients not experiencing vaginal dryness. Also, on year 2 the patients in the lower quartile of Z-endoxifen ( $\leq 17.9$  nM) had significantly lower libido ( $p=0.015$ ) compared to patients with Z-endoxifen levels  $>17.9$  nM after adjustment for clinical relevant variables and correction for multiple testing. Analyses regarding adherence are not complete and more results will be presented in the poster.

**Discussion:** Our data indicates that high serum concentrations of tam and low concentrations of Z-endoxifen are associated with vaginal dryness and sexual dysfunction. Patients reporting “very low libido” had the highest levels of tam, suggesting that slow metabolic conversion and accumulation of tam may contribute to sexual dysfunction. Our results were only significant in the second year of follow-up, possibly because patients wait to resume sexual activity after diagnosis, chemo and surgery. After receiving advice (i.e. lubricants), the symptoms are often reduced in the subsequent follow-up (3<sup>rd</sup> year). In conclusion, our results indicate that monitoring tam serum concentrations may be used to predict side effects.

<sup>1</sup> Owusu C. et al. JCO. 2008

<sup>2</sup> Helland T. et al. BCR. 2017

## 2018 San Antonio Breast Cancer Symposium ®

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A nationwide data on the cardiovascular protective effect of tamoxifen and aromatase inhibitor in postmenopausal women with breast cancer

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A large proportion of breast cancer patients receive hormonal therapy as their adjuvant treatment options. For postmenopausal women, the initial choice for the hormonal therapy is aromatase inhibitor (AI), and tamoxifen (TM) is reserved for women experiencing severe side effects against AI or having low bone density. An important but unresolved clinical question regarding the use of AI in postmenopausal women is the safety of AI regarding the risk cardiovascular events. Studies have shown inconsistent results over the cardiovascular safety of AI and TM. In this study, we investigated the risk of developing cardiovascular and cerebrovascular events in women with breast cancer who receive hormonal therapy using AI, TM, or both. To this end, we used the National Health Insurance Sharing Service in Korea which is provided by National Health Insurance Service. The database provides anonymized insurance data for research purposes after the approval of the review committee. In the database, we identified 47,569 women with the age older than 55 who were diagnosed with breast cancer. Patients were classified as no hormonal treatment group (n=18,807), AI group (n=19,584), TM group (n=7,081), or Switch group (n=2,097). The Switch group was defined as the women with history of both AI and TM prescriptions. During the studied period, a total of 2,032 cardiovascular or cerebrovascular events (CVE) were recorded.

Overall, the women prescribed with TM had significantly less hazard ratio for developing CVE when compared to the women who did not receive any hormonal treatment (HR 0.809 95% C.I. 0.706-0.928). However, this protective effect of tamoxifen was not observed in either AI or Switch group (HR 0.917 95% C.I. 0.833-1.010, and HR 0.856 95% C.I. 0.695-1.053, respectively). The protective effect of TM was also similar in women older than 60 (HR 0.808 95% C.I. 0.696-0.938). The cardiovascular and cerebrovascular protective effects of tamoxifen was also substantial in high risk women defined by their family history of cardiovascular diseases and the diagnosis of hypertension or diabetes.

Our results suggest that the use of TM is associated with a substantial protective effect against developing cardiovascular or cerebrovascular events in women with breast cancer. However, the protective effect was not observed for women receiving AI. Our data suggest the potential tailored approach in hormonal treatment in breast cancer patients who are at high risk of cardiovascular or cerebrovascular events.

# 2018 San Antonio Breast Cancer Symposium ®

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No impact of osteoporosis or bisphosphonate use for osteoporosis on breast cancer outcome: A sub-study of the DATA trial

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## **Background:**

The phase III DATA study (NCT00301457) investigates the efficacy of 6 versus 3 years of adjuvant anastrozole after an initial 2-3 year treatment with tamoxifen in postmenopausal women with breast cancer. A reduced bone mineral density (BMD) is associated with a lower risk of breast cancer. Bisphosphonates as adjuvant therapy in postmenopausal patients have been shown to prevent (distant) breast cancer recurrences. In this planned side-study of the DATA trial we assessed the relationship between osteoporosis and distant recurrence free survival (DRFS), and evaluated the effect of bisphosphonate use for reduced BMD in general on DRFS.

## **Methods:**

Decisions on BMD measurements and bisphosphonate use in the DATA study were left to the treating physician. We registered all BMD measurements and start of bisphosphonate-use. BMD was measured by a dual-energy x-ray absorptiometry (DEXA) scan of the lumbar spine/hip. We used a landmark of 3 years beyond the start of anastrozole, to create 3 different groups by BMD values based on DEXA scans made before the landmark and before a DRFS event (normal T score  $>-1.0$ , osteopenia T score  $<-1.0$ , osteoporosis T score  $<-2.5$ ). Kaplan Meier methodology was used for analyzing the DRFS for these groups. The events ending a period of DRFS were defined according to the STEEP criteria. For analyzing the relationship between bisphosphonate use and DRFS, overall bisphosphonate use was integrated as a time dependent covariate.

## **Results:**

Of the 1860 DATA patients, 1142 (65.5% in the 6-year arm and 62.9% in the 3-year arm) had a DEXA scan within the first 3 years after randomization. A normal BMD was diagnosed in 436 (38.2%) patients, osteopenia in 565 (49.5%), and osteoporosis in 141 (12.3%). Of the latter group, 112 (80.9%) used bisphosphonates. In the total study population (n=1860), bisphosphonates were overall used over time before a DRFS event in 226 patients (24.2%) in the 6-year arm and 201 patients (21.7%) in the 3-year arm. During the study, only 46 patients used bisphosphonates without a diagnosis of osteopenia/osteoporosis.

After a median follow up of 5.0 years from the landmark (interquartile range 4.3-5.7), osteoporosis (n=141) did not have a significant impact on DRFS when compared with the group without osteoporosis (normal/osteopenia (n=1001)) (HR 1.19, p=0.24 in the 6-year arm and HR=0.79, P=0.56 in the 3-year arm, P interaction=0.45). Neither when compared with only the group with a normal BMD (n=436) (HR=1.15, p=0.72 in the 6-year arm and HR=0.67, p=0.34 in the 3-year arm, P interaction=0.35).

Within the group with osteoporosis, bisphosphonate use did not lead to a better DRFS (HR=1.47, P=0.72 in the 6-year arm and HR 0.65, P=0.61 in the 3-year arm, P interaction=0.55). Bisphosphonate use in general was also not associated with DRFS (HR 1.31, P=0.13 in the 6-year arm and HR 0.88, P=0.52 in the 3-year arm, P interaction=0.14).

## **Conclusion:**

In this DATA sub-study, we observed no association between the presence of osteoporosis before the landmark of 3 years after the start of anastrozole and DRFS. However, as most patients with early osteoporosis also were treated with bisphosphonates this may have influenced the results. At the conference additional analyses will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

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Outcomes in hormone receptor positive, invasive lobular cancer in the era of endocrine monotherapy

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**Background:** Invasive lobular carcinoma (ILC) accounts for up to 15% of all breast cancers and is clinically and biologically distinct from invasive ductal carcinoma (IDC). Despite that, women with early stage ILC are often treated similarly to IDC. However, several retrospective studies suggest that patients (pts) with ILC may not derive survival benefit from the addition of chemotherapy to endocrine therapy relative to pts with IDC. The purpose of our study was to compare outcomes of pts with ILC treated with chemotherapy with those who received endocrine monotherapy.

**Methods:** A retrospective review of pts with ILC or pleomorphic lobular carcinoma treated at the Ohio State University James Cancer Center from 2004-2014 was performed. Clinico-pathologic characteristics, treatment summary and clinical outcomes were collected. Distant disease-free survival (DDFS) was defined as time from diagnosis to the first distant metastases or death and overall survival (OS) was the time from diagnosis to death or last known follow up. DDFS and OS curves were created using Kaplan-Meier methods and compared using log-rank tests. Cox proportional hazard models were used to calculate univariate and multi variable hazard ratios (HR) for OS and DDFS.

**Results:** We identified 379 pts with early stage ILC (stage I: 43% (162/379), stage II: 34% (127/379), stage III: 22% (84/379), unknown: 1% (6/379)). The majority of pts were post-menopausal (79%), Caucasian (92%) and ER+/PR+ (87%) and HER2 negative (96%). One hundred seventy six pts (46%) received chemotherapy and 189 (50%) pts received endocrine therapy alone. Pts who received chemotherapy had stage II or III disease, positive lymph nodes and grade 2 or 3 tumors; while pts who received endocrine monotherapy had stage I disease, negative lymph nodes and grade 1 or 2 tumors. We found a 51% decrease in chemotherapy (from 63% to 31%) and an increase in endocrine monotherapy use (from 34% to 65%) between 2004-2010 and 2011-2014. One hundred thirty two pts were evaluated with Oncotype DX, of which 76% (100/132) were node negative with the majority having a low recurrence score (low: 64%; intermediate: 33%; high: 3%). The use of Oncotype DX increased from 21.1% in 2004-2010 to 47.9% in 2011-2014. We found that 112 of 149 pts with at least 5 years follow up (75.2%) successfully completed five or more years of endocrine therapy.

Univariate cox models showed worse DDFS HRs for type of therapy and node status (HR: 2.36, p=0.005, HR: 4.16, p<0.001, respectively). However, the HR for therapy was no longer significant when adjusting for age, grade, and nodal involvement (HR = 1.19, 95% CI: 0.56 – 2.52, p=0.646). Nodal involvement remained significant (HR= 3.56, 95% CI: 1.64-7.70, p=0.001) after adjusting for therapy, age and grade. No significant difference in OS was found between endocrine monotherapy and chemotherapy (p=0.426, log-rank test).

**Conclusion:** We found no difference in DDFS between endocrine monotherapy and chemotherapy after adjusting for age, grade, and nodal involvement in pts with early stage ILC. This supports the hypothesis that ILC may not derive a significant benefit from the addition of chemotherapy. We need more prospective clinical trials considering histology to better understand how best to treat ILC.

# 2018 San Antonio Breast Cancer Symposium ®

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Tamoxifen induced ovarian hyperstimulation during hormonal therapy for breast cancer

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## Introduction

Adjuvant endocrine therapy is an integral component of care for endocrine-dependent breast cancer.

To date, international consensus statements recommend tamoxifen (20 mg/day) for five years as the standard adjuvant endocrine therapy for premenopausal women. Tamoxifen is a potent inducer of ovarian function and consequent hyper-estrogenism in premenopausal women. However, the incidence rate and risk factors associated with this phenomenon were not clarified.

## Methods

Among consecutive patients who were operated under diagnosis of breast cancer from March 2012 to December 2016 in Chung-Ang university hospital, patients who received post-operative tamoxifen therapy for endocrine-dependent breast cancer (stage 0-III) at age under 60 were selected and retrospectively analysed. Serial data on serum estradiol and follicular stimulating hormone (FSH) were collected. When the serum concentration of estradiol was higher than 400 pg/mL, which exceeds the normal estradiol production by a single preovulatory follicle, we classified them as tamoxifen induced ovarian hyperstimulation group. Clinicopathologic factors were analyzed between ovarian hyperstimulation group and non-hyperstimulation group by  $\chi^2$  and student t-test.

## Results

Among 205 patients, 19 patients (9.3%) showed high values of serum estradiol during tamoxifen therapy. They showed 44 times of high estradiol level during follow up period. The serum concentrations of estradiol and FSH were 1047.97638.8 pg/mL and 11.57.3 mIU/mL, respectively. The mean duration from the start of the single administration of tamoxifen to the initial detection of a high concentration of estradiol was 666.4±433.1 days.

Univariate and multivariate analysis between ovarian hyperstimulation and non-hyperstimulation groups showed younger age (<40 years) and only endocrine therapy without chemotherapy were related to higher prevalence of ovarian hyperstimulation significantly. ( $p < 0.001$ ,  $p = 0.031$  each) Pathologic stages and progesterone receptor expressions on breast tumor were not related to manifestation of ovarian hyperstimulation.

## Conclusions

The incidence rate and occurrence time of ovarian hyperstimulation associated with adjuvant tamoxifen treatment in breast cancer patients under age 60 were 9.3% and around 2-year after treatment with tamoxifen. Young age under 40 years old and endocrine treatment without chemotherapy were risk factors predicting occurrence of ovarian hyperstimulation during tamoxifen treatment. It should be noted that tamoxifen is a potent inducer of ovarian function and close monitoring of the endocrine parameters during treatment with tamoxifen would be essential.



# 2018 San Antonio Breast Cancer Symposium ®

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Clinicopathological features and endocrine therapy mode of ER low expression (1%-9%) breast cancer patients in China

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**Purpose:** Since 2010, ASCO/CAP recommended that ER be considered positive if  $\geq 1\%$  tumor cells with positive nuclear staining by immunohistochemistry (IHC) in breast cancer. ASCO/CAP also recommended considering endocrine therapy (ET) in ER positive patients. However, most breast cancers are either ER negative or ER strongly positive ( $\geq 10\%$ ), and tumors with low ER (1-9%) expression are rare. Up to now, the effect of ET is controversial for these patients with ER 1-9% and unfortunately, we know little about the clinical information of this subgroup. In this study, we analyzed the clinicopathological characteristics and ET mode of patients with low ER expression. We sought to figure out whether the ASCO/CAP guidelines affect clinical ET decision in China and Which features are important considerations for doctors to choose ET. We also evaluated the efficacy of ET in these patients. **Methods:** Patients diagnosed stage I-III primary invasive breast cancer with ER low expression (1-9%) between January 2008 and December 2016 were retrospectively identified from six hospitals in China. **Result:** 457 patients (2.7%) had low expression of ER (1-9%) of 17216 patients. Mean age at diagnosis was 49 years. 288 patients (49.9%) were younger than 50 years old. 254 patients (55.6%) had stage II disease and 37 patients (8.1%) had lymphovascular invasion (LVI). 260 patients (56.9%) were HER2 positive; 408 patients had PR negative or low expression; 327 patients' (71.6%) Ki-67 status were  $> 20\%$ . 388 patients (85%) received chemotherapy. Of those 388 patients, 90% patients received anthracycline combined with taxol chemotherapy regimens. 170 patients (37.2%) received ET. Before 2010, only 25.7% patients with low ER expression received ET. The proportion of ET increased after the 2010 ASCO/CAP guideline was published. In 2013, 50% patients received ET. The rate of ET was totally different in six hospitals. 55% patients received ET in the hospital with highest rate, while only 4% patients received ET in the hospital with lowest rate. Using the univariate logistic regression analysis of ET, ER expression, PR expression, Ki-67 status and LVI were associated with the rate of ET. But after adjustment for other covariates, only ER level was significantly associated with the rate of ET. Compared to patients with ER $<5\%$  tumors, patients with ER $\geq 5\%$  tumors had a significantly higher probability of ET rate (OR, 2.882; 95% CI: 1.928-4.308;  $P < 0.001$ ). Median follow time was 30 months. The 5-year RFS rate was 85%. Younger age and positive lymph nodes were associated with worse RFS. Survival rate did not differ significantly between patients with or without ET (without ER vs with ET: OR, 0.870; 95% CI: 0.508-1.448;  $p=0.61$ ). **Conclusions:** 2010 ACSO/CAP recommendation indeed result in an increase of ET rate for patients with 1%-9% ER positive. But these patients do not appear to benefit from ET. Prospective studies are needed for these patients and we need more accurate way to evaluate ER levels, which associate with endocrine response.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-14-15

Prospective study analyzing value of breast Density change predicting ENdocrine therapy response in postmenopausal women taking adjuvant ARomatase inhibitor [DEAR study] (interim analysis)

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**Objective :** To evaluate the value of breast density change of mammography and breast MRI as a predictive marker for a response to postoperative anti-hormone therapy by targeting ER-positive postmenopausal breast cancer patient

**Methods :** Density change of mammography, breast MRI density being taken just before start of anti-hormone therapy, mammography being performed after 6 months, 1 year, 2 years thereafter and breast MRI being performed 1 year after start of therapy will be measured by volpara and 3D-MR method. Molecular profile including ER expression level that has a relation with response rate to anti-hormone therapy will be analyzed and outcome will be evaluated based on disease free survival and overall survival.

Recurrence rate of each group was estimated based on the data of the patients in breast center of Seoul National University Hospital, 2006-2011, who underwent surgery of ER-positive breast cancer. Among 1065 persons, 7.5% (80/1065) showed recurrence rate and among these, recurrence rate of patients who took AI was 6.9% (12/175). Among these, based on MDR 5% cutoff, 1.6% vs 9.8% was represented. By designating recurrence rate as 1.5%, 9.5% and assuming dropout rate by refusal to clinical test as 10%, registration goal was set at total 411 persons based on each 137, 274 persons per each group.

**Results :** (this is interim analysis)

From 2012, total 156 patients are enrolled, among them, 32 patients were eliminated (affirmative consent, switched to Tamoxifen, recurrence and etc). From now total 124 patients are on-going to this study. Compare with Non AI group, breast density change of AI group is much decreased from base line study and it is statistically significant. (1 year follow up – base line, 2 year follow up– base line ; -12.2%, - 18.6% vs - 7.6%, -15.3% P-value 0.002, 0.009 respectively) Only one patient was relapsed within 5 year and there were no death. Psychological anxiety, medication compliance and side effects analysis were done. Psychological anxiety about disease and medication were improved as time goes by ( $p < 0.001$ ). But medication compliance and side effects of AI were worsen. ( $p = 0.178, 0.015$  respectively) Other topics will analyze. (DFS and OS, etc.)

**Discussion :**

70% of breast cancer is ER-positive breast cancer. Endocrine therapy (ET) has been clarified as an effective target therapy in large scale, prospective randomized trial and up to the present, it has been settled down as a standard therapeutic method of ER-positive breast cancer. As a result of 20 years' follow-up after intake of AI (aromatase inhibitor) and 20 years' follow-up after intake of tamoxifen, recurrence was represented as 2-2.5% and at present, clear mechanism of such resistance and predictive biomarker have not been clarified. Due to this resistance, all the ER-positive breast cancer patients are forced to receive anti-hormone therapy for 5 years or 10 years.

According to the taking AI, breast density is significantly decreased compare Non AI group. Of course need more follow up data and analysis, but we can confirm a meaning of endocrine responsiveness of breast density change being measured after anti-hormone therapy as predictive surrogate.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-15-01

Acquired *ESR1* mutation and persistent expression of estrogen regulated genes in ER+ breast cancers on long-term neoadjuvant treatment with aromatase inhibitors

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**Background:** Aromatase inhibitors (AI), which block the conversion of androgen to estrogen, are the mainstay therapy for estrogen receptor-positive (ER+) breast cancer (BC). However, many patients relapse.

**Purpose:** To identify molecular alterations associated with long-term neoadjuvant AI therapy of ER+ BC.

**Methods:** We retrospectively identified 137 patients diagnosed with ER+ BC and treated with neoadjuvant AI for at least 1 month at the Royal Marsden Hospital (2003–2016). Paired pre- (diagnosis) and post-AI (surgery) biopsies with >40% invasive cell areas were available from 87 ER+ BC patients. In all samples, we evaluated ER, PR and Ki67 immunostaining, *ESR1* hot-spot mutations by droplet digital PCR and expression of 801 genes associated with BC and response to endocrine therapy by NanoString.

**Results:** Mean time on neoadjuvant treatment was 26 weeks (range 5.6–92.3). Cell proliferation remained suppressed in most tumours indicating little evidence for acquired resistance: 56/87 (64%) showed residual Ki67 (Ki67r) <2.7% (complete cell cycle arrest) and 14/87 (11.5%) had Ki67r>10%. This was paralleled by reduced expression of proliferation genes, ER (immunostaining and gene expression) and estrogen-regulated genes (ERG) at surgery compared with the diagnostic samples (all p<0.001). There was a weak positive correlation between AI duration and Ki67r and less reduction in proliferation genes, *ESR1* and ERGs expression (p<0.05; r=0.26-0.34). Pathway analysis revealed inhibition of cell cycle (e.g. reduced expression of several cyclins), E2F targets and estrogen response (e.g. reduced expression of ER downstream targets such as *FOXM1*). CDK genes showed a variable response: *CDK1* and *CDK2* decreasing, *CDK4* increasing and no change in *CDK6*. Most notably, 6 surgical samples showed *ESR1* mutations: one of these cases had the mutation at diagnosis. All 5 acquired mutations were detected in patients treated for >6 months, giving a prevalence in this cohort of 5/34 (15%). Tumours with *ESR1* mutations showed less suppression of ERGs (p=0.002) and proliferation (p=0.039) and increased *ESR1* (p=0.016) expression at surgery compared with tumours without mutation. Pathway analysis confirmed lack of inhibition of estrogen response (FDR>5%) and less inhibition of cell cycle [enrichment score: -0.49 vs -0.78] and E2F targets (-0.47 vs -0.8) in tumours with mutation. Tumours without *ESR1* mutation but with Ki67r>10% also showed reduced ERGs response (p=0.006) compared to tumours with Ki67r<2.7%. Additionally, these tumours showed relative activation of cell cycle, estrogen response, E2F targets pathways and mTORC1 signalling (FDR<1%).

**Conclusion:** Overall most tumours showed no evidence for the emergence of resistant disease after neoadjuvant AI therapy even after many months of treatment. However, we detected an enrichment of *ESR1* mutations (15% of cases) after long-term treatment as a putative driver of ERG expression and proliferation and thus reduced AI response. Therefore, mutant ER appears to be associated with ligand-independent ERG activity supporting the clinical validity of dual blockade with a selective ER down-regulator combined with a CDK4/6 inhibitor targeting the RB/E2F axis in this scenario.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-15-02

TILs variations, proliferative response and PEPI scores in patients with luminal breast cancer receiving neoadjuvant letrozole-palbociclib or chemotherapy: An extended analysis of the NEOPAL trial

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## Background

The role of chemotherapy in early luminal breast cancer remains challenged. The NEOPAL trial (NCT 02400567; Cottu et al, ESMO 2017 LBA09) compared sequential chemotherapy (CT) and letrozole-palbociclib (LP) as neoadjuvant treatment in PAM50 defined high-risk luminal breast cancer patients, showing that LP might be as efficient as CT with regard to breast conserving surgery and pathological response. We report here extended exploratory pathological results, focusing on tumor infiltrating lymphocytes (TILs), proliferative response and preoperative endocrine prognostic index (PEPI) scores.

## Material and Methods

Tumor blocks from baseline biopsy and surgical specimens were available for centralized review from the 106 randomized patients (53 in each arm). TILs quantification, Ki67 staining and counting, and ER quantification were performed according to standard methods. Residual proliferative cancer burden (RPCB) and PEPI scores were computed according to published algorithms. Wilcoxon rank sum test and Mann Whitney test were used to compare paired and unpaired data. The chi-square and Fisher exact tests were used for categorical variables.

## Results

Overall, median TILs count did not differ between LP and CT patients, both at baseline ( $p=0.37$ ) and at the end of treatment ( $p=0.42$ ). Median TILs count climbed from 5% (0-60) to 10% (1-60) in the LP arm ( $p=0.0026$ ) and from 2% (0-30) to 10% (0-60) in the CT arm ( $p=0.0023$ ). Median Ki67 dropped sharply in both arms, from 30% (1-80) to 1% (0-30) in the LP arm ( $p=1.10e-8$ ) and from 30% (2-80) to 5% (0-30) in the CT arm ( $p=3.10e-9$ ). Decrease in the Ki67 geometric mean was as sharp. Of note, while baseline Ki67 was similar in both arms ( $p=0.315$ ), decrease in the LP arm was significantly more profound than in the CT arm ( $p=0.00075$ ). Pathological response according to RPCB were as follows, in the LP and CT arm, respectively: class 0: 9.6%/10.2%; class I: 84.6%/73.5%; class II: 5.8%/16.3%. The relapse free survival PEPI scores were as follow in the LP and CT arm, respectively: class I: 13.5%/16.3%; class II: 59.6%/46.9%; class III: 28.9%/36.8% ( $p=0.504$ ). Breast cancer specific survival PEPI scores were as follow in the LP and CT arm, respectively: class I: 18.9%/8.2%; class II: 54.7%/40.8%; class III: 26.4%/51%. These results were significantly better in the LP arm ( $p=0.027$ ). There was no correlation between final TILs quantification and the RPCB or PEPI scores.

## Conclusions

In this prospective multicenter study with centralized pathological review, neoadjuvant letrozole-palbociclib combination generates impressive proliferative and endocrine specific response features. It compared well with chemotherapy. The LP combination also significantly increased lymphocytic infiltration. Its clinical significance and utility remain to be elucidated, but it potentially adds new prognostic and theranostic information.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-15-03

Concurrent neoadjuvant chemotherapy and estrogen deprivation in patients with estrogen receptor-positive, human epidermal growth factor receptor 2-negative breast cancer (CBCSG-036): A randomized, controlled, multicenter trial

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**Background** Limited studies existed to demonstrate clinical benefit from co-administration of neoadjuvant chemotherapy (NCT) and endocrine therapy by estrogen deprivation. Therefore, we conducted a clinical trial to investigate the efficacy of concurrent neoadjuvant chemotherapy (NCT) and estrogen deprivation in patients with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer.

**Methods** In this open-label, multicenter, randomized controlled trial, eligible patients with stage IIB–IIIC, ER-positive, HER2-negative breast cancer (having indications for chemotherapy) were enrolled and randomly assigned to receive NCT with or without estrogen deprivation (an aromatase inhibitor with or without gonadotropin-releasing hormone-analogue according to menopausal status). The primary endpoint was the objective response rate (ORR, including complete response and partial response). The secondary endpoints included Ki67 proliferation marker changes, pathologic complete response, pathological response rate, progression-free survival (PFS), and safety. All analyses were by intention-to-treat principle.

**Results** From 2013 to 2017, 249 patients were randomly assigned to two groups, with 125 patients in the neoadjuvant chemo-endocrine therapy (NCET) and 124 patients in the NCT group. In the intention-to-treat analysis, the ORR was significantly higher in the NCET group than that in the NCT group (84.8% vs 72.6%,  $P=0.019$ ; odds ratio=2.11, 95% confidence interval 1.13–3.95,  $P=0.020$ ). The efficacy of concurrent estrogen deprivation was more prominent in tumors with higher Ki67 (Ki67 >20%), with ORR of 91.1% in NCET group vs 68.7% in NCT group ( $P=0.001$ ). Although there is no significant difference of PFS between the two groups ( $P=0.188$ ), patients with higher Ki67 at baseline might get more PFS benefit from concurrent NCT and estrogen deprivation (2-year PFS: 91.5% in NCET group vs 76.5% in NCT group,  $P=0.058$ ). Adding endocrine agents to NCT was well tolerated and did not result in significant differences in adverse events (grade 3 or 4 toxicity) between two groups.

**Conclusions** Addition of estrogen deprivation to NCT improves the clinical response in patients with ER-positive, HER2-negative breast cancer, especially for those with higher Ki67. Although early survival analysis indicates that patients with higher Ki67 might get more PFS benefit from concurrent NCT and estrogen deprivation, longer follow-up will be necessary to fully evaluate survival benefit.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-15-04

Neoadjuvant endocrine therapy for ER+ DCIS can lead to disease regression and allows BCS in up to a third of patients with disease >40mm at diagnosis

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**Background:** The role of neoadjuvant endocrine therapy (NET) for ER+ DCIS is an area of evolving study. It may allow down-sizing prior to surgery, converting DCIS requiring mastectomy to disease suitable for breast conservation surgery (BCS). Here we report the results from the first European single-institution series of its type.

**Methods:** Data were prospectively collected from patients diagnosed with ER+ DCIS and treated with NET prior to surgery, at a single unit between 2009 -2015. The size of the tumour on initial imaging (mammography) was compared to the size of the tumour on final imaging and pathology using RECIST criteria to determine disease progression. Blocks from initial core biopsy and final pathology are being interrogated by immunohistochemistry and DNA and RNA comparisons.

**Results:** 42 patients diagnosed with ER+ DCIS received NET with median age at diagnosis of 63y (range 37-94y). 7/42 premenopausal women were treated with tamoxifen, 35/42 post-menopausal women were treated with letrozole. 36/42 (85.7%) patients underwent surgery with 18/36 (50%) requiring mastectomy and 18/36 (50%) treated by BCS. 3/18 (16.7%) of the BCS patients required re-excision for positive margins. The median time to operation was 72d (range 15-308d). In total 12/42 (28.6%) had invasive disease on final pathology. 2/36 (5.6%) patients had a pathological complete response (PCR), 14/36 (38.9%) had a partial response (PR), 17/36 (47.2%) had stable disease and 3/36 (8.3%) had larger disease on pathology than imaging; this is a common feature of many lower grade DCIS lesions.

26/42 (61.9%) patients initially had DCIS >40mm (largest 240mm) and yet 9/26 (34.6%) of these patients still underwent successful BCS.

There was a significant correlation between length of endocrine therapy and reduction in size of disease. Immunohistochemical and molecular analyses are ongoing.

## **Conclusions:**

- NET is an effective treatment for ER+ DCIS. It reduces the rate of re-excision to 16.7% in this series - substantially lower than the national (UK) figures for DCIS at 30%.
- It produces path CRs (5.6%) and high response rates that relate to the duration of treatment.
- This unique study shows that the optimal duration of NET is of the order of 6 months, achieving high rates of conversion for mastectomy to BCS.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-15-05

Biomarkers of response to neoadjuvant endocrine therapy with anastrozole (Ana) alone or in combination with fulvestrant (Ful) in ER-positive (ER+) HER2-negative (HER2-) breast cancer (PACT01 trial)

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**Background:** In recent years, several clinical trials showed that fulvestrant (Ful), alone or in combination with an aromatase inhibitor (AI), is more effective than an AI alone. PACT01 is a randomized neoadjuvant trial of Anastrozole (Ana) alone or in combination with Ful in ER+/HER2- breast cancer.

**Methods:** Patients with newly diagnosed ER+/HER2- breast cancers, 2 cm or larger in size, were randomized to 16 weeks of Ana (1 mg orally every day) alone or in combination with Ful (500mg IM days 1, 15, 29, and every 28 days thereafter) for 16 weeks. Patients then proceeded to surgery. Tumor tissue was collected at baseline, day 28 (D28), and at the time of surgery. Primary endpoint was the reduction of Ki67 in tumor tissue between baseline and D28. Baseline and D28 samples were stained for ER, PR, HER2, and Ki67. ER and PR were scored for intensity and percentage (H-score), HER2 was scored for intensity of membrane staining; and Ki67 was scored as percentage. Data were summarized descriptively. Changes in biomarkers from baseline to D28 were calculated and compared by Wilcoxon signed rank test.

**Results:** PACT01 trial enrolled 72 patients. Three of them did not start treatment. Baseline samples were collected from the remaining 69 patients, and D28 samples from 60 patients (5 refused, 2 withdrew, 1 lost to follow up, 1 unknown). Samples from 18 patients had no tumor (5 at baseline, 9 at D28, 4 at both). Of the 42 patients with paired samples, 20 received Ana and 22 received Ana+Ful. All cases except one were centrally confirmed to be ER+, and all were HER2-. Table 1 summarizes median expression of Ki67, ER, and PR. Both treatment regimens led to a significant reduction in Ki67 between baseline and D28. However, Ana+Ful did not reduce Ki67 more effectively than Ana alone. Ki67 was reduced to <10% in 60% of the Ana arm and 68% of the Ana+Ful, which was not statistically significant. PR was similarly reduced in both treatment arms. ER was significantly reduced at D28 in the Ana+Ful arm (p=0.0004) but not in the Ana alone arm. Safety profile of both treatment arms was consistent with package insert and published studies.

Median expression of Ki67, ER and PR in Anastrozole and Anastrozole + Fulvestrant Arms at Baseline and Day 28

ARM	Timepoint	N	Ki67 (%)	ER H-score	PR H-score
Ana	Baseline	20	24.8	182.5	100.3
	Day 28	20	5.6	*170.0	25.0
Ana + Flu	Baseline	22	25.6	198.1	20.5
	Day 28	22	5.1	*117.5	0.0

\* p=0.0004. Other comparisons were not statistically significant

**Conclusions:** In this small neoadjuvant trial, the addition of Ful to Ana did not increase Ki67 suppression at D28. This may be due to untreated primary tumors being exquisitely sensitive to Ana and that fulvestrant may not add to it. It is also possible that the effect of Ful may be noted later in the course of treatment. Further biomarker data on tissue collected at the end of treatment will be presented at the meeting.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-15-06

Results of a randomized double blind trial of neoadjuvant anastrozole plus placebo vs anastrozole plus saracatinib for ER+ postmenopausal breast cancer

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Antiestrogen mediated cell cycle arrest requires the CDK inhibitor, p27. Src kinase mediates p27 loss and antiestrogen resistance in ER+ breast cancer lines in vitro. In ER+ xenografts, the Src inhibitor, saracatinib, restored antiestrogen responses in resistant tumors. This led to a Phase I/randomized double-blind Phase II trial to test effects of saracatinib with anastrozole for ER+ and/or PR+ postmenopausal breast cancer.

Phase I accrued 12 subjects and showed 175 mg po saracatinib is safely given with 1 mg po daily anastrozole with good PK. In Phase II, postmenopausal women with new ER+ and/or PR+, HER2- breast cancers  $\geq 2$  cm were randomized 2:1 to either neoadjuvant anastrozole with saracatinib or anastrozole/placebo over 6 months. Response was assayed by clinical 2D measurements each cycle and by MRI pre-study, at 10 weeks and prior to surgery. The Phase II primary endpoint was to test if tumor volume decrease (from 2D clinical measures) with dual therapy (dual) exceeded that of monotherapy (mono) by  $>20\%$ . Secondary endpoints included tumor response by 3D MRI measures and by RECIST, PK and toxicity, and molecular predictors of drug efficacy in pre-/ post-therapy tumors. Of 58 subjects, 15% were Black, 5% Asian and 79% White. 61% were Hispanic. Dual therapy was well tolerated, with the following grade 1 toxicities: flu-like syndrome 20%, non-pruritic rash 48% (17% for mono), self-limited diarrhea in 55% (33% mono). Transaminasemia with dual therapy was 52.5% and 17% with mono. 6/59 stopped dual due to drug related AEs: 2 had gr 3 hepatitis, one gr 3 anemia, 3 had grade 3 urticarial rash. Dual Rx increased mean anastrozole levels to 50 ng/ml vs 38 ng/ml for mono (T test  $p= 5.45201 \times 10^{-5}$ ). Mean saracatinib level, 269 ng/ml, was similar to prior studies. All of 50 evaluable subjects showed clinical and MRI tumor responses. 17% in both groups progressed, usually after 16 weeks. Mean tumor vol (calculated from 2D clinic measures) declined more rapidly (by 63% in dual vs 55% in mono at 8 weeks), but both groups showed an 89% mean tumor volume decrease by 20 weeks. Clinical RECIST showed size reductions of 61% in dual and 62% after monotherapy. Tumor volumes based on 3D MRI show a non-significant trend to greater response to dual therapy, with mean volume decreased by 64% vs 45% at the end of dual vs monotherapy. RECIST response by MRI also showed a trend to greater % decrease tumor size post treatment by 34% vs 25% in dual vs mono. Thus, clinical volumetric assessment of response to this neoadjuvant endocrine therapy may overestimate response compared to volumes calculated by MRI, while RECIST may underestimate it. Pathologic responses based on initial and residual tumor burden calculated from initial and final tumor volumes and % cellularity in biopsy and surgical specimens will be presented.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-16-01

A randomized, double-blind, placebo-controlled trial of testosterone (T) for aromatase inhibitor-induced arthralgias (AIA) in postmenopausal women: Alliance A221102

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**Background:** Aromatase inhibitors are a mainstay hormone receptor-positive breast cancer treatment. AIA occur in up to 50% of patients (pts), adversely affecting quality of life and treatment compliance. A small phase II clinical trial of oral testosterone undecylenate appeared to improve AIA over placebo (P), with no significant androgenic side effects. The current study was performed to confirm these findings.

**Methods:** This randomized P-controlled trial enrolled postmenopausal women on adjuvant anastrozole or letrozole and experiencing moderate-to-severe AIA ( $\geq 5$  on 0-10 scale). Pts were initially randomized to receive a subcutaneous pellet containing T 120 mg + anastrozole 8 mg (T+Alpellet) or P at the end of the first week on study (after obtaining baseline hot flash data) and at 3 months (mo). Due to slow accrual, the protocol was amended to change the route of delivery to topical T or P applied to the skin once daily for 6 mo. Baseline and monthly questionnaires were administered, including: Modified Brief Pain Inventory for aromatase arthralgia (BPI-AIA), profile of mood states (POMS), the menopause specific quality of life questionnaire (MENQOL), a hot flash diary, the hot flash related daily interference scale (HFRDIS) and a symptom experience questionnaire. The primary endpoint was intra-patient change in joint pain at 3 mo, compared using a two-sample t-test.

**Results:** 227 pts were accrued between 9/1/2013-11/29/2017. 55 pts were randomized prior to the protocol amendment and received T+Alpellet or P. Baseline characteristics were balanced between arms, with the exceptions of median weight, BMI, hemoglobin (all higher in T arm), and breast tenderness, dissatisfaction with personal life/depression, and skin changes (all higher in P arm). Compared to baseline, there were no significant differences between T and P in average pain or joint stiffness at 3 (p=0.483) or 6 mo (p=0.573). Average pain was significantly lower each month compared to baseline, irrespective of treatment arm. There were no significant differences in any other items evaluated by BPI-AIA, POMS, MENQOL, hot flash diary or HFRDIS. Similarly, there were no substantial differences in toxicity. A subset analysis of the 55 pts randomized to receive T+Alpellet or P identified significant reductions in average pain scores with T+Alpellet during the first month (p=0.038), but not thereafter. T+Alpellet pts had significantly more reduction in reported % of baseline hot flash frequency (p=0.034) and score (p=0.031), nausea (p=0.019), fatigue (p=0.042), mood swings (p=0.026), hand/feet swelling (p=0.009), stress urinary incontinence (p=0.039) and changes in appearance, texture or tone of their skin (p=0.0083), than pts on P.

**Conclusions:** Overall, T did not improve AIA or menopausal symptoms compared to P. While there was significant improvement in AIA over the study period, T did not facilitate this process. However, T+Alpellet was associated with improvement in short-term AIA and several menopausal symptoms compared to P, suggesting that subcutaneous T combined with anastrozole may be superior to transdermal T alone.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-16-02

Fertility preservation before neoadjuvant or adjuvant chemotherapy for breast cancer: Final results of PRESAGE trial

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**Background:** Breast cancer is the most frequent form of cancer in young women. For these patients, breast cancer is generally more aggressive and chemotherapy is more often needed. Chemotherapy is commonly associated with amenorrhea and a decrease of ovarian reserve depending on the patient's age, agents and dose. Embryo, oocyte and ovarian tissue cryopreservation are the three options to preserve fertility. Embryo and oocyte cryopreservation require controlled ovarian stimulation (COS). The use of COS is associated with an increase of estradiol levels. It led to develop protocols using Tamoxifen or Letrozole combined with FSH to protect patients of the potential deleterious effects of the COS. PRESAGE is the first French prospective multicenter feasibility study about fertility preservation by COS combined with Tamoxifen and oocyte +/- embryo cryopreservation before neoadjuvant (NAC) or adjuvant (AC) chemotherapy for breast cancer.

**Material and method:** Prospective multicenter study for patients of less than 40 years, with a breast cancer, for whom a treatment of NAC or AC is indicated and who wish to preserve their fertility. The main objective was to evaluate the feasibility of a COS associating Tamoxifen with FSH followed by an oocyte +/- embryo cryopreservation. The secondary objectives were to evaluate the average deadline prior to the beginning of the chemotherapy and the impact of the type of COS (depending on the phase of the menstrual cycle, conventional-start or random-start COS protocol) on the number and the quality of oocytes harvested. Statistical analysis was performed using SAS statistical software version 9.4 (SAS Institute, Cary, NC).

**Results:** 101 patients were included between February 2014 and July 2017 and 97 patients were eligible for statistical analysis. Mean age of the patients was 31,5 +/- 4 years, the half of them was nulliparous (53/97) and 23,7 % (23/97) were single. They presented mainly SBR II or III (91/96, 94,8 %) lesions, ER + (66/96, 68,7 %). 38 patients benefited from a NAC and 59 of an AC. We have found a significant shorter care (time between the first oncologist's consultation and the beginning of the chemotherapy) according to the type of chemotherapy: 29,7 +/- 15,6 days in NAC group vs 45,2 +/- 21,5 days in AC group (p=0,003) with the same duration of ovarian stimulation in the two groups (10,5 +/- 2 days).

The success rate of the COS procedure was 90,7 % (88/97) with no significant difference between the groups according to the type of COS (p = 0.06) or the type of chemotherapy (AC vs. NAC p= 0,3). In the 88 patients who had oocyte retrieval, the number of oocytes harvested per patient was 12,8 +/- 7,8 , the number of oocytes preserved was 9,7 +/- 6,1 and an IVF was performed in 12,5% of patients (11/88) with 5,1 +/- 3,1 embryos obtained. We have found no impact of the type of chemotherapy or the type of COS on the number of oocytes or embryos preserved.

**Conclusion:** with a high success rate (90,7%), our study suggests that COS with Tamoxifen and FSH is feasible before adjuvant or neoadjuvant chemotherapy in breast cancer patients. We also show that COS procedure before neoadjuvant chemotherapy can be realized without increasing the time before introducing chemotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-16-03

Cardiovascular outcomes and long term survival with discontinuation of adjuvant trastuzumab

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**Background:** Trastuzumab (T) induced cardiomyopathy remains a significant limitation to adjuvant HER2 directed therapy. Recent studies have aimed to reduce cardiotoxicity through combination with non-anthracycline (non-A) chemotherapy or shorter treatment duration. However there is limited data regarding cardiac outcomes and long-term survival with early discontinuation of adjuvant T.

**Methods:** An IRB-approved single-institution retrospective analysis was performed for 401 consecutive patients with non-metastatic HER2+ breast cancer treated at the Ohio State University Comprehensive Cancer Center from 2005-2015. Medical records were reviewed for clinicopathologic features, systemic treatment and survival information. Disease Free Survival (DFS) was defined as time from diagnosis to first recurrence (loco-regional or distant recurrence) including second primary breast cancer or death. Overall survival (OS) was defined as time from diagnosis to death or last known follow up. OS and DFS estimates were generated using Kaplan Meier methods and compared using Log-rank tests. Cox proportional hazard models were used to calculate univariate and multivariate hazard ratios for OS and DFS.

**Results:** A total of 371/401 (92.5%) patients received adjuvant T (n= 401, mean age: 59.4 years; stage 1: 120, 30%; stage II: 194, 48%; stage III: 87, 22%; ER+: 235, 58%); among whom 106/371 (28.6%) patients held adjuvant T for any reason.

Table 1- Discontinuation of adjuvant trastuzumab

	Number of patients (%)
Initial treatment	371
Completed therapy with no interruption	265 (71.4)
Interruption of therapy for minimum of 2 weeks	64 (17.2)
Permanently discontinued	42 (11.3)

Median duration of therapy in patients with any interruption with T was 11.3 (0.5-16.9) months and 23/371 (6.9%) received less than 6 months of adjuvant T. Cardiomyopathy (measured as LVEF decline on 2D echocardiogram or MUGA  $\geq$  15 points) was the most common reason for withholding T (66/106, 62.3%). The majority of these patients received a cardiology referral (77/ 106, 72.6%) with a 13 day mean time to evaluation in outpatient clinic. Patients receiving non-A chemotherapy and beta blockers or ACE inhibitors during treatment were significantly less likely to experience cardiomyopathy (A vs non-A: 49/190, 25.8% vs. 16/136, 11.8% p=0.002); (Med vs no Med: 7/148, 4.73% vs 59/184, 32.1%; p<0.001). Log-rank tests indicate a significant worsening in OS and DFS for patients who discontinued T (p=0.021, 0.001 respectively). Multivariate analyses confirmed significant worsening in DFS after adjusting for age, stage, ER , node status, and cardiomyopathy (Adjusted HR: 4.0[2.02 – 7.92], p< 0.001)

**Conclusion:** Discontinuation of adjuvant trastuzumab, most often from cardiomyopathy, is an independent prognostic marker for worse DFS in non-metastatic HER2 positive breast cancer. Non-anthracycline chemotherapy and use of cardio-protective medication is associated with significantly reduced incidence of cardiotoxicity in this population. Future prospective studies should consider optimizing cardiovascular function to avoid interruption in adjuvant HER 2 directed therapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-16-04

Oral ibandronate for osteopenic breast cancer patients receiving adjuvant aromatase inhibitors: secondary 5-year survival outcomes analysis of the single-center phase 2 BONADIUV trial

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**Background.** Several randomized trials demonstrated aromatase inhibitors (AI) superiority in terms of disease-free survival (DFS) compared to tamoxifen treatment for postmenopausal hormone receptor-positive breast cancer (BC) patients. Anyway, AI toxicity profile is a concern due to estrogen suppression. Pivotal trials demonstrated a significant bone mineral density (BMD) loss due to AI, with a consistent 5-year risk of bone fractures, thus impacting on patients' quality of life. Bisphosphonates represent an effective treatment in postmenopausal osteoporosis fractures prevention. However, an adequate patient's selection for adjuvant bisphosphonates treatment during AI endocrine therapy is still a challenge. Final results of BONADIUV trial presented at San Antonio Breast cancer Symposium in 2016 showed that treatment with ibandronate, as compared to placebo, significantly improved BMD change in osteopenic women treated with adjuvant AI, and consistently protected patients' bone loss. We present the secondary 5-year analysis on survival outcomes of the trial.

**Patients and methods.** The BONADIUV trial is a single-blind, randomized, placebo-controlled phase 2 study designed to evaluate the impact of ibandronate treatment on BMD in osteopenic women taking AI. Between January 2011 and May 2014, 171 osteopenic patients (lumbar spine [LS] and/or trochanter  $-1 < T\text{-score} < -2.5$ ), were randomized in a 1:1 ratio to receive either placebo or oral monthly ibandronate (150 mg). Treatment duration was 2 years, with 6-months evaluation. Primary endpoint was the mean BMD difference between the two arms at a 2-year follow up. Secondary analysis on survival outcomes (overall survival [OS] and invasive DFS [iDFS]) have been performed at 5-year median follow-up time. ClinicalTrials.gov identifier: NCT02616744.

**Results.** At the database cutoff time for the present analysis on May 4, 2018, median follow up was 63.3 months (mean 61.2; range 2.7-87.3) for whole series, 64.9 months (range 33.8-84.0) for the placebo arm, and 62.2 months (range 24.2-87.3) for the ibandronate arm. Ten patients in the placebo group and 17 patients in the ibandronate group withdrew the allocated arm before any follow up data collection, and so were excluded from the analysis, performed on 144 patients (72 patients per arm). At the database cutoff time, the OS rate was 97.2% in the placebo group and 100% in the ibandronate arm. We observed four loco-regional relapse (three in the placebo arm, one in the ibandronate arm;  $p=0.33$ ), three distant metastases (none in the placebo arm, three in the ibandronate arm;  $p=0.075$ ), and three contralateral BC (one in the placebo arm, two in the ibandronate arm;  $p=0.65$ ). The number of iDFS events did not differ between groups: four in the placebo group and six in the ibandronate group ( $p=0.56$ ). Up to data cutoff, two deaths have occurred; none in the placebo arm and two in the ibandronate arm ( $p=0.15$ ). The OS rate did not differ between arms.

**Conclusions.** The secondary analysis of survival outcomes showed no difference between arms in terms of OS and iDFS rates. Further large investigations and mature follow-up from the published ones are awaited.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-16-05

Avoidable acute care use associated with nausea and emesis among patients receiving AC, carboplatin, or cisplatin for breast cancer

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**Background:** In order to improve care and reduce costs of cancer treatment, attention has focused on reducing inpatient (IP) and emergency room (ED) utilization. The US Centers for Medicare and Medicaid Services (CMS) recently implemented an oncology outcome measure (OP-35) to assess the quality of care and determine outpatient hospital payment. It assesses 30-day post-chemotherapy rates of IP or ED events deemed “potentially avoidable” by CMS due to association with any of 10 CMS-defined toxicities: anemia, dehydration, diarrhea, fever, nausea, emesis, neutropenia, pain, pneumonia, or sepsis. Although CMS found that 20% of chemotherapy treatment resulted in such events, event rates with highly emetogenic chemotherapy (HEC) are understudied, particularly for breast cancer (bCA) patients treated with anthracycline + cyclophosphamide (AC), carboplatin, or cisplatin.

**Methods:** In a large electronic health record database focused on US integrated delivery networks, HEC courses of therapy were identified from 4Q 2012 to 3Q 2017. IP/ED events within 30 days of chemotherapy administration were considered related to the OP-35 measure if they included a diagnosis code of any of the ten OP-35 toxicities, per CMS' definitions. HEC chemotherapy use, IP/ED events, bCA diagnosis, and toxicities were identified by ICD-9, ICD-10, and procedural codes. Subgroups were evaluated for 3 common HECs: AC, carboplatin (>14 days apart, as a proxy for AUC ≥4), and cisplatin.

**Results:** 4128 courses of HEC in bCA were identified. Of these, 2304 involved AC (median 4 cycles), 1721 involved carboplatin (median 6 cycles), and 103 involved cisplatin (median 4 cycles), with median ages of 55, 59, and 64, respectively. 30-day IP/ED events were seen in 25% of these HEC bCA courses (22%, 30% and 23% for AC, carboplatin, and cisplatin, respectively). The 10 CMS-defined toxicities were associated with an IP/ED event for 73%, 72%, and 76% of events for these AC, carboplatin, and cisplatin courses respectively, confirming that these are principal contributors to 30-day IP/ED use for patients receiving HEC in bCA. The top five that were most commonly associated with IP/ED for bCA for patients receiving AC, carboplatin, or cisplatin were pain 53%/49%/63%, anemia 51%/51%/38%, fever 48%/31%/38%, neutropenia 46%/27%/31%, and nausea/emesis 30%/40%/25%; patients may have had ≥1 toxicity associated with each IP/ED event. The IP/ED rate may be understated because some events, particularly ED, may occur out of the network the EHR data covers.

**Conclusion:** One quarter of patients receiving AC, carboplatin, or cisplatin for bCA visit the ED or are hospitalized within 30 days of at least one of their chemotherapy administrations. Most events involved ≥1 of 10 toxicities deemed avoidable causes of hospitalization by CMS and now tracked as an oncology outcome measure. Relative to patients receiving AC, acute care rates for patients receiving carboplatin were lower for fever and neutropenia and higher for nausea/emesis, perhaps because the study period largely precedes the 2017 addition of carboplatin AUC ≥4 to national guidelines as HEC requiring triplet antiemetic prophylaxis. Toxicity prevention and symptom monitoring are crucial to reduce acute care needs.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-16-06

Incidence of interstitial lung disease in patients with HER2-positive advanced breast cancer treated with everolimus and trastuzumab: A combined analysis of two phase 3 randomized controlled trials

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**Background:** The human epidermal growth factor receptor 2 (HER2) protein is overexpressed in approximately one fourth of breast tumors. Trastuzumab resistance has been demonstrated via aberrant PI3K/AKT/mTOR signaling due to PTEN loss. To circumvent this resistance mechanism, everolimus, an oral mTOR inhibitor, has been employed in treatment of HER2-positive advanced breast cancer (ABC). Lung toxicity due to everolimus is well established and has been reported with trastuzumab. Yet, the incidence of interstitial lung disease (ILD), when everolimus was added to trastuzumab, has never been reported. We performed a systematic review and meta-analysis of randomized controlled trials (RCTs) to determine the incidence of ILD in patients with HER2-positive ABC treated with both everolimus and trastuzumab.

**Methods:** We systematically conducted a comprehensive literature search using MEDLINE, EMBASE databases and meeting abstracts through January 2018. Phase 3 RCTs that mention ILD as an adverse effect were incorporated in the analysis. The primary meta-analytic approach was a fixed effects model using the Mantel-Haenszel (MH) method. It was used to calculate the estimated pooled risk ratio (RR), and risk difference (RD) with 95% confidence interval (CI).

**Results:** A total of 1272 patients with HER-2 positive ABC from two phase 3 RCTs were eligible. Studies compared everolimus + paclitaxel + trastuzumab vs paclitaxel + trastuzumab and everolimus + vinorelbine + trastuzumab vs vinorelbine + trastuzumab. The initial dose of everolimus in BOLERO-1 was 10mg per day and in BOLERO-3, 5mg per day was used. The median relative dose intensity of everolimus was reduced to 0.54 in BOLERO-1 due to toxicity related dose reductions and dose interruptions. The randomization ratio of everolimus to placebo was 2 to 1 in BOLERO-1 and 1 to 1 in BOLERO-3. Everolimus was utilized in trastuzumab-resistant ABC after prior taxane therapy in the BOLERO-3 study (n= 562) and as first-line treatment in the BOLERO-1 study (n= 710). The I<sup>2</sup> statistic for heterogeneity was 0, and the heterogeneity X<sup>2</sup> (Cochran's Q) was 1 (P= 0), suggesting homogeneity among RCT. The incidence of all-grade ILD was 31 (4.122%) in the everolimus group vs 3 (0.577%) in control group and of high-grade ILD was 11 (1.463%) in everolimus arm vs 0 (0%) in the control arm. The pooled RR for all-grade ILD was significant at 7.258 (95% CI: 2.130 – 24.733, p = 0.002) and the absolute RD was 0.035 (95% CI: 0.019 – 0.050, P < 0.001). The pooled RR for high-grade ILD was noted at 7.930 (95% CI: 0.997 – 63.044, p = 0.050) and the absolute RD was 0.014 (95% CI: 0.004 – 0.024, P = 0.004).

**Conclusions:** Approximately 0.46 and 0.61% of patients on trastuzumab alone have been reported to develop ILD in previous studies. Our study showed that the addition of reduced dose of everolimus to trastuzumab, significantly contributed a higher incidence in all grades of ILD with a relative risk of 7.93 for grade 3 and 4 ILD. More randomized trials are required to determine the definitive incidence and actual relation of ILD as well as the optimal dose of everolimus, when combined with trastuzumab or other chemotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

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The CHANCE study: Mechanical skin changes among women with non-metastatic breast cancer receiving chemotherapy and endocrine therapy

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**Background:** The persistent effects on skin hydration and elasticity resulting from cytotoxic and endocrine agents used in early stages of breast cancer are poorly understood. The objective of this preliminary analysis of the CHANCE study is to describe the pattern of persistent biomechanical skin changes in non-metastatic breast cancer patients treated with cytotoxic chemotherapies and/or endocrine therapies.

**Methods:** This is an ongoing single-center, prospective, longitudinal cohort study of non-metastatic breast cancer patients treated with cytotoxic chemotherapies and/or endocrine therapies. Objective skin hydration and elasticity measurements of the forearm were measured using Tewameter® (TM 300; Courage & Khazaka) and Cutometer® (MPA 580; Courage & Khazaka) devices under a controlled ambient environment at baseline and 6 months after chemotherapy completion, or one year after initiation of endocrine therapy.

**Results:** A total of 107 patients were assessed at baseline and follow-up for transepidermal water loss (TEWL) (median age 53, range 26-82) and 106 patients for skin elasticity (median age 53.5, range 26-82). Fifty-three healthy controls were evaluated at baseline with median age 47 (range, 22-73). The mean TEWL at baseline and follow-up among patients were 6.922 g/h/m<sup>2</sup> and 8.521 g/h/m<sup>2</sup>, respectively (p<.0001). Skin firmness (0.420 versus 0.421 mm, p=0.949) and elasticity (77.2% versus 77.4%, p=0.836) did not significantly change during follow-up. When comparing chemotherapy recipients with endocrine therapy recipients, chemotherapy patients had a mildly lower TEWL at follow-up (8.369 versus 8.928 g/h/m<sup>2</sup>, p=.247) but a greater net increase in TEWL (1.687 versus 1.359 g/h/m<sup>2</sup>, p=.5) compared to endocrine patients over the study period.

**Conclusions:** An increase in TEWL was observed in patients receiving cytotoxic and endocrine therapies, suggesting a deterioration of the protective skin barrier possibly attributed to these therapies. No significant changes in skin firmness or elasticity were found in this preliminary analysis. Further studies are needed to elucidate the pathophysiologic mechanisms involved in persistent skin changes after systemic breast cancer therapies.

Objective skin hydration and elasticity in patients receiving breast cancer therapy

	Control (n=53)	Baseline (n=107)	Follow-Up (n=107)	p-value*
TEWL (g/h/m <sup>2</sup> )	9.462	6.922	8.521	<.0001
Cutometer		Baseline (n=106)	Follow-Up (n=106)	
Immediate recovery, R <sub>0</sub> (mm)	0.415	0.420	0.421	0.949
Gross elasticity, R <sub>2</sub> (%)	79.3%	77.2%	77.4%	0.836

\*p-value calculated from baseline and follow-up only

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-16-08

A regional audit of 6-hour monitoring for administration related reactions during the first administration of subcutaneous trastuzumab

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## Introduction

In 2012, Subcutaneous Trastuzumab (TSC) was introduced as an alternative to Intravenous Trastuzumab (TIV) for HER2+ breast cancer. The pivotal HannaH study demonstrated that TSC was non-inferior to TIV, was preferred by patients, and serious administration related reactions (ARRs) were not reported. However, the Summary of Product Characteristics (SPC) advises that patients be observed for ARRs for 6 hours post-first administration (and 2 hours post-subsequent administrations), similar to TIV.

## Aim

To assess the frequency and tolerability of ARRs during the 6-hour observation period post first administration of TSC in patients with HER2+ breast cancer.

## Method:

A retrospective audit of TSC was conducted in Southwest Ireland across five centers from 2014-2016. Patient charts were reviewed to record ARRs reported on the first-administration or at subsequent visit. In addition a subset of patients were interviewed regarding their recollection of ARRs with first or subsequent injections.

## Results:

The study is ongoing having identified 192 patients. These centers have administered 2111 TSC injections in total, associated with 4998 hours of observation as per SPC. From the 385 injections given over the first two TSC administrations, 13 injections (3.4%) were associated with ARRs within 24 hours. Nine patients (2.3%) experienced injection site reactions immediately post injection, one injection site pain (0.3%), and one experienced petechiae on subsequent exposure (0.3%). Three patients experienced pyrexia and dry cough 24 hours post-injection and were hospitalized for respiratory tract infection. There were no reactions experienced between 2 and 6 hours post-first injection. There were no serious ARRs. Telephone interviews are ongoing and these results will be reported.

## Conclusion:

ARRs related to TSC are usually immediate, mild and self-limiting. Observing patients for 6 hours post-first injection and 2 hours post-subsequent injections represents an inefficient use of healthcare resources.



# 2018 San Antonio Breast Cancer Symposium ®

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Effect of simvastatin on cardiac strain in breast cancer patients receiving anthracycline therapy

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**Background:** Cardiac toxicity (CT) is a rare late effect of anthracycline therapy for breast cancer (BC). Statins may attenuate the CT of anthracyclines. Myocardial strain can detect subclinical CT before ejection fraction (EF) declines. Global longitudinal strain (GLS)  $\geq -19\%$  and relative change (Rel $\Delta$ ) in GLS  $\geq 11\%$  predict future decline in EF. We conducted a pilot study to evaluate the effect of simvastatin on GLS in BC patients receiving anthracyclines. **Methods:** We enrolled women with stage I-III BC planning doxorubicin/cyclophosphamide (AC) x 4. Women with heart disease or taking a statin were excluded. Participants were randomized 1:1 to simvastatin 40 mg daily x 24 weeks (wk) + AC or to AC alone. We performed echo with strain 5 times: baseline (BL), pre-AC#2, 1-3 wk after AC#4, 24 wk after AC #1 and 52 wk after AC#1. The primary endpoint was the mean absolute change ( $|\Delta|$ ) in GLS from BL to 1-3 wk after AC#4. Secondary endpoints included Rel $\Delta$  in GLS, feasibility and safety. We used two-sample t-tests to compare mean changes in GLS and Fisher's exact test to compare dichotomized GLS values. The study closed early due to loss of staff. **Results:** Of 31 patients, 15 (48%) received simvastatin+AC. Mean age was 46 years; 71% pre-menopausal, 61% white and 32% black. There were no significant differences in BL cardiovascular risk factors between the arms. After AC, 3 HER2+ patients received trastuzumab. There were no grade 3-4 AEs with simvastatin. Common grade 1-2 AEs included myalgia (20%), elevated AST (27%) and elevated ALT (53%). One patient in the AC arm died from heart failure with low EF 2 months after having a normal echo 1-3 wk after AC#4. The rate of missing echos was 14%. Of 133 completed echos, 124 (93%) were evaluable for GLS. Mean GLS was  $< -19\%$  at all times in the simvastatin+AC arm. Mean GLS was  $< -19\%$  at BL and pre-AC#2 in the AC arm, but  $\geq -19\%$  at post-AC times in the AC arm. Mean EF was  $> 60\%$  at all times in both arms. Among 27 patients evaluable for the primary endpoint, there was no significant difference in mean  $|\Delta|$  in GLS from BL to 1-3 wk after AC#4 between the arms (Simvastatin+AC: 0.42%; AC: 1.11%,  $p=0.57$ ). In addition, there were no differences in the mean  $|\Delta|$  in GLS from BL to any other time between the arms (all  $p>0.1$ ). The proportion of patients with GLS  $< -19\%$  was higher in the simvastatin+AC arm than in the AC arm pre-AC#2 (73% vs 44%), 1-3 wk after AC#4 (67% vs 38%), 24 wk after AC #1 (53% vs 25%) and 52 wk after AC#1 (53% vs 25%) (all  $p>0.05$ ). The proportion of patients with Rel $\Delta$  in GLS  $\geq 11\%$  from BL was lower in the simvastatin+AC arm than in the AC arm pre-AC#2 (13% vs 19%), 1-3 wk after AC#4 (20% vs 44%) and 24 wk after AC#1 (27% vs 31%) (all  $p>0.05$ ). **Conclusion:** Simvastatin did not result in a statistically significant difference in the mean  $|\Delta|$  in GLS from BL to 1-3 wk after AC#4. However, the study was underpowered due to small sample size and there was a suggestion of reduced CT with simvastatin. Co-administration of simvastatin and AC was safe and serial echocardiographic strain monitoring was feasible. Further studies are needed to evaluate the cardioprotective effect of statins on strain in BC patients receiving anthracyclines.

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Bone health in young women with breast cancer

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## Introduction:

There are limited data and consensus regarding bone mineral density (BMD) monitoring, and management of bone loss in younger women with breast cancer (BC). Adjuvant endocrine therapy for estrogen receptor positive (ER+) BC may include ovarian function suppression (OFS) plus use of aromatase inhibitors (AIs) for 5-10 years, both of which contribute to bone loss. The WHO risk prediction tool FRAX does not include BC or AI use as independent risk factors in its calculation thus underestimating risk of fracture. This study aims to evaluate current screening and management of bone health in young women with BC.

## Methods:

A retrospective, IRB-approved chart review was performed in consecutive women  $\leq 40$  with BC diagnosed at Loyola University Chicago Medical Center between 01/01/2015 and 12/13/17. Demographic data, BC treatment, and factors contributing to secondary causes of bone loss were collected through 4/1/18. A descriptive analysis included summary values for all categorical and continuous risk factors.

## Results:

BC  $\leq 40$  yrs was identified in 136 women; 18 were excluded due to missing data. The analysis was performed on 118 patients (pts). Mean pt age was 34.6 yrs (SD 4.7). Stage at diagnosis included: stage 0 = 9 (7.6%), stage 1 = 26 (22%), stage 2 = 44 (37.3%), stage 3 = 23 (19.5%), stage 4 = 7 (5.9%), unknown = 9 (7.6%). Seventy-nine (67%) had ER+ BC; 32 (27.1%) had HER2-positive disease. The majority of pts (101, 85.6%) received chemotherapy in their treatment plan. Menopause was documented in 69 (59.0%) pts. Goserelin was used in 31 pts (44.9%), oophorectomy in 17 (24.6%), both in 5 (7.2%). Tamoxifen was used in 44 (55.7%) ER+ pts; 34 (43.0%) received an AI, and 18 (22.8%) received sequential tamoxifen and AI. 25 Hydroxy-Vitamin D (25 OHD) levels were checked in 61 (51.7%); 43 (70.5%) had levels  $< 30$  ng/ml; 24 (55.8%) received vitamin (vit) D supplementation. There was no difference in the 25 OHD in pre- and post-menopausal women ( $p=0.64$ ). Pts with vit D deficiency had a median BMI of 26.8 vs 23.8 in those with sufficient vit D levels (exact  $p=.049$ ). Secondary diagnoses contributing to low BMD were identified in 14 (11.8%). Dual energy xray absorptiometry (DXA) scans were checked in 23 pts (19.7%), 18 of whom were post-menopausal. At the femur, 0 pts had a z-score (age-matched standard deviation) of  $\leq -2.0$ , 9 pts (39.1%) had a score between 0 to  $-2.0$ . At the lumbar spine, 1 pt (4.3%) had a z-score  $\leq -2.0$ , 9 pts (39.1%) had a z-score between 0 to  $-2.0$ . No T-scores were in the osteoporosis range; 11 pts had T-scores at both femur and lumbar spine in the osteopenia range. The median 10 yr probability of a major osteoporotic fracture (FRAX score) was 1.9% (1.6-2.7%); the median 10 yr probability for hip fracture was 0.1% (0.10-0.20%). There were no differences in FRAX scores between pre- and post-menopausal women. No fractures were reported in the time period studied. Anti-resorptive therapy was used only in patients with metastatic bone disease.

## Conclusions:

25 OHD and DXA scans are not routinely checked in younger women diagnosed with BC. Vit D deficiency and evidence of bone loss is prevalent in those pts who do undergo testing. Further research and guidelines are necessary to address management of bone health in young women with BC to minimize future fracture risk and morbidity.

# 2018 San Antonio Breast Cancer Symposium ®

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A modest proposal for classical massage on chemotherapy-induced peripheral neuropathy in breast cancer patients receiving adjuvant paclitaxel: A randomized controlled trial using electromyography

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**Background:** Oncology professionals face challenges to find out a complete solution in prevention or alleviation of chemotherapy induced peripheral neuropathy (CIPN). Current pharmacological treatments used for CIPN have limited effects and pose major side effects. At this point, management of CIPN by a multidisciplinary team approach is very crucial. Therefore, this randomized controlled study aimed to examine the effect of classical massage on CIPN using objective and subjective measurements in breast cancer patients receiving adjuvant paclitaxel.

**Method:** The study was conducted at Dr. Abdurrahman Yurtaslan Ankara Training and Research Hospital in Turkey between July 2017 and June 2018. The study was approved by a local ethical committee. Eligible patients with breast cancer included those who (a) were 18 years and older; (b) had no documented diagnosis of CIPN; (c) had receiving the first cycle of adjuvant paclitaxel. Exclusion criteria were (a) history of severe psychiatric disorder; (b) history of peripheral neuropathy due to any cause; (c) had lesion on hands or feet; (d) had bleeding or coagulation disorders. Simple randomization method was utilized to allocate patients to an intervention group (n=18) and a control group (n=19). Patients in the intervention group (IG) received totally 12 sessions classical massage, on the days of chemotherapy cycles. The duration of each massage was 30 min, with 20 min for the feet and 10 min for the hands. The CG received only routine care. After completion of paclitaxel regimen no intervention was applied to neither the IG nor the CG, and patients were followed at week 16. Subjective measurement tools included the Self-Leeds Assessment of Neuropathic Symptoms and Sign (S-LANSS); and the European Organization for Research and Treatment of Cancer-Quality of Life Questionnaire Chemotherapy-Induced Peripheral Neuropathy (EORTC QLQ-CIPN20). Electromyography (EMG) was applied as an objective measurement at baseline and week 12 for all patients by the same neurologist. The data were collected at five time points including baseline (T1), week 4 (T2) week 8 (T3), week 12 (T4) and week 16 (T5). To analyze data repeated measures ANOVA and Mann Whitney U tests were used.

**Results:** The S-LANSS scores were significantly lower in the IG when compared the CG ( $p<.05$ ). Motor sub-dimension scores of EORTC QLQ-CIPN20 were significantly lower in favor of the IG compared to the CG. As for sensorial and autonomic sub-dimensions of the EORTC QLQ CIPN20 showed no significant difference. Regarding EMG outcomes, compound muscle action potentials of ulnar nerve and posterior tibial nerve in the IG were significantly higher than the CG at week 12.

**Conclusion:** In this study, classical massage initiated concurrently with adjuvant paclitaxel regimen decreased neuropathic pain severity and prevented motor axonal neuropathy in the IG when compared to the CG. Accordingly, classical massage had promising effects on prevention and alleviation of CIPN. Based on the results, classical massage can be recommended as a preventive complementary option for breast cancer patients receiving adjuvant paclitaxel.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P4-16-12

Does initial cardiac imaging impact clinical outcomes in patients with breast cancer?

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**Background:** Echocardiography (echo) and multigated acquisition (MUGA) scans are the most commonly used modalities to assess cardiac function during breast cancer (BC) treatment. However, a case series of 176 patients with cancer suggests enhanced cardiac care with echo surveillance. We hypothesized that patients with early BC imaged by echo have improved cardiac outcomes compared to those imaged by MUGA.

**Methods:** Consecutive patients with stage I to III breast cancer undergoing pre-treatment echo or MUGA were retrospectively screened from January 2010 to December 2014. Patients participating in clinical trials with mandated imaging and/or cardiac reviews were excluded. Demographics, medical history and clinical events were collected via chart review and electronic health records. All patients had a minimum 1 year of follow-up. The primary outcome was a composite of death, cardiac hospitalization or cardiac emergency room visit.

**Results:** 598 patients were identified as having a baseline echo and 636 had had baseline MUGA. Mean follow-up was 4.5±1.4 years. Patients undergoing MUGA were younger, had more advanced stage of disease and received more anthracycline and trastuzumab (table1). Patients imaged by MUGA had lower cardiac function at baseline compared to echo, LVEF 64% vs. LVEF 65% respectively, P <0.001. Cancer therapy related cardiac dysfunction was similar between groups, 10% vs. 11%, p=0.81. Patients in the echo group were more likely to be seen by cardiology, 7% vs. 3%, p<0.0001, and to be initiated on beta blocker, 4% vs. 1%, p=0.006, or angiotensin converting enzyme inhibitor, 3% vs. 1%, p=0.002. However, there was no difference between groups for the primary outcome, 10% event rate in each group, even after adjustment for age, BC stage, chemotherapy and cardiac medications, hazard ratio 1.04 (CI 0.72-1.49), p=0.842.

**Conclusion:** For patients with early stage BC, the choice of cardiac imaging modality at baseline does not impact adverse cardiac events. However, patients undergoing echo were more likely to be evaluated and managed by cardiology.

Table 1. Baseline Characteristics

	Echo (N=598)	MUGA (N=636)
Age mean	54±10	53±10*
BMI mean	29±6	29±7
Cardiovascular History		
Diabetes	66(11%)	56(9%)
Hypertension	154(26%)	155(24%)
Dyslipidemia	83(14%)	75(12%)
CAD	9(2%)	6(1%)
CHF	7(1%)	4(1%)
Beta Blocker	22(4%)	28(4%)
ACE-Inhibitor	51(9%)	64(10%)
Angiotensin Receptor Blocker	69(12%)	44(7%)*
Cancer History		
Stage*		
Stage I	65(11%)	56(9%)
Stage II	377(63%)	361(57%)
Stage III	155(26%)	219(34%)

Receptor Status			
Triple negative	64(11%)	76(12%)	
HER2 negative, hormone positive	342(58%)	387(61%)	
HER2 positive	192(32%)	173(27%)	
Cancer Therapy			
Chemotherapy (any)	528(88%)	594(93%)*	
Anthracycline	310(52%)	394(62%)*	
Trastuzumab	170(28%)	148(23%)*	
Anthracycline & trastuzumab	6(1%)	19(3%)*	
Hormone therapy	459(77%)	487(77%)	
Radiation (any)	487(81%)	527(83%)	
Radiation left side	237(49%)	259(49%)	
Surgery	597(100%)	633(100%)	
* p<0.05 for comparison between echo and MUGA groups			

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Prospective study of scalp cooling for hair loss prevention in Japanese breast cancer women receiving (neo)adjuvant chemotherapy

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## Introduction

Recently scalp cooling during chemotherapy infusion has been reported to be quite effective to mitigate chemotherapy-induced alopecia. But data in Asian patients are quite limited.

## Patients and methods

Japanese breast cancer female patients who planned to receive (neo)adjuvant chemotherapy were offered to participate in this prospective study of scalp cooling with Paxman Scalp Cooling System for alopecia prevention. The scalp cooling was done 30 minutes prior to and during and 90 minutes after each chemotherapy infusion. Photographs of the head of the participants were taken from 5 directions, namely front, back, both sides, and top, on the day of chemotherapy infusion and 1 month after the last infusion. Two investigators consisting of a physician and a nurse judged the grade of alopecia by looking at the photographs according to the WHO classification. The primary outcomes were the rates of patients with Grade 3 alopecia (defined as hair loss of > 50%) and the rates of patients who used a wig or hat to conceal the hair loss one month after the last infusion of chemotherapy. This paper reports on the former outcome mainly. They were asked to answer a brief questionnaire regarding headache, fatigue, chill etc. shortly after each cooling. They could use the cooling cap for free on the first cycle of chemotherapy. But they were required to purchase it (about 1,130 US\$) for the scalp cooling of the following cycles.

## Results

One hundred forty three patients participated in the study and actually used the cooling cap at least once. The mean and median age of them are 50.6 and 50, respectively (28 - 76). One hundred twenty nine patients completed the planned chemotherapy of 4 to 8 cycles (89 Pts 4 cycles, 1 Pt 6 cycles, 39 Pts 8 cycles). Among them (7 patients were not evaluable), 74 patients (60.7 %) had Grade 3 alopecia 1 month after chemotherapy. In 80 patients who used the scalp cooling system throughout the planned chemotherapy (1 patient was not evaluable), 36 patients (45.6 %) experienced Grade 3 alopecia. On the other hand, among 49 patients who discontinued the cooling mostly after the 1st cycle (6 were not evaluable), 38 (88.3 %) had Grade 3 alopecia. When we restrict them to 33 (5 were not evaluable) who decided to discontinue the cooling by day10 of the first cycle of chemotherapy to exclude the patients who discontinued it because of less effect on alopecia prevention than they expected, 25 (89.3 %) experienced Grade 3 alopecia. Comparing the results of those who completed the cooling and patients who decided to discontinue it by day10 of the first cycle, the rates of Grade 3 alopecia (45.6 % vs. 89.3 %) were statistically significantly different in favor of the former ( $P = 0.0001$ ). Most patients complained of some headache, chill, and pain of the jaw.

## Conclusion

Scalp cooling with Paxman Scalp Cooling System during chemotherapy infusion in Asian women seems as effective for hair loss mitigation as in Caucasian women.

# 2018 San Antonio Breast Cancer Symposium ®

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Meta-analysis of Phase I pharmacokinetic/pharmacodynamic results of proposed biosimilar pegfilgrastim

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## Background

The long-acting granulocyte colony-stimulating factor (G-CSF) pegfilgrastim is widely used to prevent chemotherapy-induced neutropenia (CIN). Biosimilars could potentially improve sustainability of cancer care. Sandoz proposed biosimilar pegfilgrastim is under development and has been evaluated in Phase I and III studies.<sup>1,2</sup> The current meta-analysis uses data from two Phase I studies in healthy volunteers (HVs) comparing pharmacokinetic (PK)/pharmacodynamic (PD) properties of Sandoz proposed biosimilar and EU-reference (Neulasta®) pegfilgrastim.

## Methods

Data from two studies were included: a single-dose, double-blind, parallel-group study (study 1, data on file) and a single-dose, double-blind, crossover study (study 2),<sup>3</sup> both in HVs randomized to receive proposed biosimilar or reference biologic (PK/PD analysis populations: study 1, n=93 per arm; study 2, n=169 per arm). Primary PK and PD parameters were  $AUC_{0-inf}$ ,  $AUC_{0-last}$ ,  $C_{max}$  and  $ANC$   $AUEC_{0-last}$ ,  $ANC$   $E_{max}$ , respectively. For each parameter, geometric mean ratios and confidence intervals (CIs) for treatment comparisons (proposed biosimilar vs reference biologic) from the two studies were combined using meta-analytical techniques with a fixed-effects model. The 90% (PK) or 95% (PD) CIs were calculated and PK/PD biosimilarity was demonstrated if all CIs fell within equivalence margins of 80% to 125%. Non-baseline corrected PD parameters were used.

## Results

The combined CIs of the geometric mean ratios for primary PK and PD parameters were all contained within the predefined equivalence margins. Safety, tolerability and immunogenicity were found to be similar between proposed biosimilar and EU-reference biologic in HVs (data not shown).

[table1]

		Combined geometric mean with 90% (PK)/95% (PD) CI		Combined ratio with 90% (PK)/95% (PD) CI
		Proposed biosimilar	EU-reference	Proposed biosimilar vs EU-reference
PK parameters				
$AUC_{0-last}$ (ng×h/mL)	N	93+169	93+169	
	Geometric mean 90% CI	6823 [6122, 7603]	6034 [5404, 6738]	1.1385 [1.0606, 1.2221]
$AUC_{0-inf}$ (ng×h/mL)	N	92+168 <sup>a</sup>	93+168 <sup>a</sup>	
	Geometric mean 90% CI	6973 [6268, 7757]	6183 [5560, 6876]	1.1335 [1.0570, 1.2156]
$C_{max}$ (ng/mL)	N	93+169	93+169	
	Geometric mean 90% CI	196 [178, 216]	180 [163, 198]	1.0994 [1.0265, 1.1774]
PD parameters				
$AUEC_{0-last}$ (10 <sup>9</sup> ×h/L)	N	93+169	93+169	
	Geometric mean 95% CI	4986 [4855, 5121]	4952 [4816, 5093]	1.0119 [0.9959, 1.0281]
$E_{max}$ (10 <sup>9</sup> /L)	N	93+169	93+169	

	Geometric mean 95% CI	36.4 [35.3, 37.5]	36.2 [35.1, 37.2]	0.9981 [0.9790, 1.0175]
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AUC=area under serum-concentration curve; AUEC=area under effect curve; CI=confidence interval;  $C_{\max}$ =maximum observed serum concentration;  $E_{\max}$ =maximum effect attributable to investigational medicinal product; PD=pharmacodynamic; PK=pharmacokinetic. <sup>a</sup>One subject in study 2 had AUC<sub>0-inf</sub> extrapolated >20% and was excluded from AUC<sub>0-inf</sub> analysis

## Conclusions

This meta-analysis of two Phase I studies supports PK/PD similarity of Sandoz proposed biosimilar to EU-reference pegfilgrastim. Also, no clinically meaningful differences in safety, tolerability and immunogenicity were found. Sandoz proposed biosimilar pegfilgrastim presents as a sustainable option to manage CIN in patients with cancer.

## References

- <sup>1</sup>Blackwell et al. Oncologist 2016;21:789 94
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# 2018 San Antonio Breast Cancer Symposium ®

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The effectiveness of breast ultrasonography in cancer screening: A comparison with mammography

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## Purpose

Mammography(MMG) is a measure of screening that is proven to be effective in lowering death rate in breast cancer validated by randomized control trials. Recently, the number of incidence of breast cancer in Korean women is showing ascending tendency, and also as compared to those in North American and European countries, prevalence age tends to be lower. Since they tend to have much dense breast, MMG alone can hardly be considered as a sufficient measure of breast cancer screening.

The purpose of the study is to study the effectiveness of breast ultrasonography(US) in breast cancer screening in Korean women by investigating the ratio of patients who are diagnosed as malignant in breast US over those who are diagnosed as negative or benign(category 1 and 2) and dense breast(category 0) in MMG.

## Materials and Methods

We conducted the retrograde study over 2776 female subjects who had undergone screening MMG in single center in South Korea during the period between Jan, 1<sup>st</sup> 2010 to Dec, 31<sup>st</sup> 2016. Female patients are categorized according to BI-RAD category. 164 of subjects were previously undergone a breast cancer surgery, which made them eliminated from the list, and 2612 subjects are finally selected.

## Results

Some more 33 subjects are excluded from the list and rest 2579 subject had undergone breast US. Among these, 1133 subjects (43.9%, 1133/2579) correspond to dense breast of BI-RADS category 0, while 303 of them are suspected of category 4 or more

Subject categorization by BI-RAD system

Category 0	dense breast	983	
	dense breast with calcification	94	
	dense breast with probable mass	28	
	others	28	
	total	1133	43.9%
Category 1		663	25.7%
Category 2		251	9.7%
Category 3		229	8.9%
Category 4 or more		303	11.7%
total		2579	100.0%

. The subjects corresponding to category 1 to 3 are 663, 251 and 229 respectively. 307 of 1133 subjects in category 0 are confirmed as having suspicious lesion based on findings of breast US. Among them, 66 subjects are identified as malignant based on biopsy, which accounts for 5.8% (66/1133). 104 subjects in category 1, are confirmed to have lesions based on findings of breast US, where 25 alone were identified as malignant (3.8%, 25/663). Likewise, 10 (4.0%, 10/251) in category 2 and 13 (5.7%, 13/229) in category 3 are identified

Malignancy ratio of each category after biopsy

	total	suspicious lesion on breast US	biopsy		
			benign	malignant	malignancy ratio

Category 0	1133	307	241	66	5.8% (66/1133)
Category 1	663	104	79	25	25 3.8% (25/663)
Category 2	251	41	31	10	4.0% (10/251)
Category 3	229	72	59	13	5.7% (13/229)

## Conclusion

It appears that 43.9% of total 2579 subjects undergone MMG and breast US are confirmed as dense breast in category 0 and 5.8% of them are diagnosed as breast cancer according to breast US and biopsy. Likewise, for those who are considered as either category 1 or 2, which is negative or benign lesion category, 3.8% are diagnosed as breast cancer. As it is appeared in this study, cancer detection can be more effective in parallel measure of MMG and breast US rather than MMG alone, especially for breast cancer screening of Korean female subjects.

# 2018 San Antonio Breast Cancer Symposium ®

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Glandular dose in contrast-enhanced dual-energy mammography

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## INTRODUCTION

Estimation of the Average Glandular Dose (AGD) absorbed by the breast during x-ray based examination is an established part of quality control procedures for breast imaging, both for standard mammography and new techniques as digital breast tomosynthesis (DBT) and contrast enhanced digital mammography (CEDM). It is however fundamental that every optimization program obtains an adequate imaging quality. In our study we evaluated the AGD absorbed by the breast, during CEDM examinations, performed with Dual Energy (DE) technique in our Department.

## MATERIALS AND METHODS

We retrospectively evaluated 37 DE mammograms; we reported and analyzed AGD, Entrance Skin Exposure (ESE), anode/filter combinations, breast thickness, kV (kilovolt), mAs (milliAmpere per second), and compression in daN (decaNewton) values, for each mammography view obtained on high and low energy. In 10 Patients, it was possible to compare DE with a recent standard mammography performed on the same mammography unit.

## RESULTS

AGD values for the entire DE study, in craniocaudal and mediolateral oblique views, resulted between 4.23 mGy and 13.44 mGy; based on the breast thickness (27-79 mm) and on the anode/filter combination. We found out a significantly lower AGD for small breast thicknesses and Mo/Rh and Mo/Cu combination compared to Rh/Rh and Rh/Cu.

In the whole study evaluation, the AGD percentage report due to low and high energy acquisitions resulted between 76.8% and 81.6%, and between 18.4% and 23.2%, respectively.

For the 10 Patients in which was possible a direct comparison with the data obtained with recent standard mammography, resulted a ratio between DE AGD and standard mammography AGD variable from 1.43 to 2.48 (mean value: 2.0), again with lower AGD values obtained with Mo/Rh - Mo/Cu combination and for small breast thicknesses.

AGD ratio between low energy and standard mammography acquisition resulted between 1.23 and 3.31.

## DISCUSSION

CEDM is proving to have all the potential to get a definite role in diagnosis and breast tumor staging, providing a direct correlation between morphologic and functional imaging.

In our study we assessed the dosimetry to determine if an additional x-ray exposure will be a limit to DE use and, if that is the case, in which measure. Data in the literature on DE dosimetry are still sparse and extremely heterogeneous.

In any case, the increase of delivered dose could be justifiable when compared to the great benefits given by this technique in breast cancer early diagnosis and staging, especially in dense breast tissue, in the follow-up of Patients with breast cancer history and in case of MRI incompatibility.

Furthermore, when compared to MRI, CEDM is an easy-access, low-cost, fast and well accepted exam by the patient.

## CONCLUSIONS

Although the risk of induced carcinogenesis associated with x-ray breast modalities is small, dosimetric aspects should be considered both for the risk evaluation and for optimization of acquisition systems; with the advance of the technologies we will be able to have a dose reduction by maintaining high quality standards.

Results obtained from this preliminary study needs to be extended by a larger case study to get a complete evaluation and comprehension of the phenomenon.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-01-03

Comparison of mammography findings between dense and non dense breast in Japanese subjects: The potential limitation of routine mammography

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## Introduction

Recently “Dense breast” has attracted numerous attention because of diagnostic difficulty in mammography among those harboring dense breast, which is far more frequent in Asian than Caucasian women. Therefore, in this study, we retrospectively evaluated the risks of subsequent development of malignancy through comparing the detailed mammographic characteristics between Japanese subjects harboring dense and non dense breasts.

## Methods

We retrospectively examined mammographic findings taken from March 2013 to March 2016 at Nahanishi Clinic, Okinawa, Japan. We stratified its density according to the suggestion of the Japan Central Organization on Quality Assurance of Breast Cancer Screening, which was defined by the proportion of fat area as follows; extremely high dense:10-20%, heterogeneously dense:40-50%, scattered fatty:70-90%, fatty: almost all the breast fat. “Dense breast” includes extremely high and heterogeneous dense. We evaluated the detailed radiological findings of each phenotypes including the characteristics of the mass, calcification and focal asymmetric density(FAD) and architectural distortion. We also compared the rates of subsequent cancer development and sensitivity of detecting cancer between those harboring dense and non dense breasts.

## Results

We reviewed the mammography findings of 7747 Japanese women including 857 with breast cancers. When adjusted for age, the rate of dense breast was significantly associated with age, 88.6% in women in their 20s(vs40s  $p<0.001$  OR3.402), with incremental decrease, 80.4% in 30s( $P<0.001$  OR1.802), 69.5% in 40s, 55.9% in 50s( $P<0.001$  OR0.512), 32.3% in 60s( $P<0.001$  OR 0.108), 19.5% in 70s( $P<0.001$  OR 0.106) and 5.3% in over 80s( $P<0.001$  OR0.024). The rate of malignancies was 9.1% (385) in dense and 13.6% (472) in non dense breasts. We then compared the mammographic findings between dense and non dense breast. Abnormal calcifications were detected more frequent(7.6%vs5.3%  $P<0.001$  OR1.478) but masses less so(16.4%vs23.7%  $P<0.001$  OR 0.632) in dense breast, while no significant differences detected in FADs(4.9%vs4.6%  $P=0.35$  OR1.074) and distortions(1.2%vs1.4%  $P=0.29$  OR=0.859) between dense and non dense breast. The rate of carcinoma was less frequent in dense breast among those associated with mammographic calcification(19%vs27.3%  $P<0.01$  OR0.626) but more frequently in dense breast among those with masses (13%vs19%  $P<0.001$  OR0.628). The rate of carcinoma was not different between dense and non dense breast in those with FADs (21.6%vs20.6%  $P=0.72$  OR1.067) and distortions(71.7%vs74.7%  $P=0.64$  OR0.857). In addition, among 37 breast cancer patients who did not harbor the mammographic findings above (26 dense and 11 non dense breasts), the average mass length was significantly larger in dense (13.6mm) than non-dense breast (9.9mm) ( $P=0.018$  used Welch's t test), respectively.

## Conclusion

Results of our present study did demonstrate that detection of malignancy in those with mammographic dense breast is more difficult. Therefore, in those harboring dense breast in mammography, addition of other modalities such as US could improve the detection of breast carcinoma.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-02-01

The relationship of breast density in mammography and magnetic resonance imaging (MRI) in women with triple negative breast cancer (TNBC)

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## Introduction:

TNBC represent 10%–20% of invasive breast cancers. Previous studies showed that TNBC usually present with benign features on mammography, ultrasound and MRI. However, there is a dearth of information on the relationship of mammographic breast density (MBD), background parenchymal enhancement (BPE) and fibroglandular tissue (FGT) on MRI with TNBC. The purpose of this study was to evaluate the relationship between BD, BPE, and FGT in women with TNBC compared to non-TNBC in a contemporary cohort of women with breast cancer.

## Methods:

The Institutional Breast Cancer Database was queried for women who had invasive breast cancer and underwent mammography and MRI between (2010-2017). Variables of interest included clinical, pathologic, and imaging characteristics. Statistical analyses included Pearson's Chi Square and logistic regression.

## Results:

Of 2224 women, 210 (9%) had TNBC. The median age was 59 years (22-95) and median follow up was 4 years. When we looked at the clinical characteristics of women with TNBC compared to non-TNBC, race, BRCA1,2 status, method of presentation, palpability, histology, grade, and Ki67 were statistically different (Table 1). When we looked at the correlation of MBD, FGT, and BPE for women with TNBC, MBD was correlated with FGT ( $r=0.64$ ) but weakly correlated with BPE ( $r=0.22$ ). We found a significant association of low BPE and TNBC compared to the non-TNBCs ( $p=0.021$ ) (Table 1). In a short period of time, only 8 women with TNBC had a recurrence with no significant association with MBD, BPE, or FGT (Table 1).

**Table 1. Imaging Characteristics among TNBC compared to non-TNBC**

Variable	TNBC (N=210)	%	Non-TNBC (N=2014)	%	P-value
<b>Race</b>					0.001
White	136	65	1533	76	
Black	35	17	174	9	
Hispanic	12	6	115	6	
Asian	23	11	173	9	
Other	4	2	19	1	
<b>BRCA1/2</b>					<0.001
Positive	30	25	42	5	
Negative	89	75	791	95	
<b>Method of Presentation</b>					<0.001
Breast exam	112	54	757	38	
Mammography	77	37	1057	53	
Ultrasound	7	3	110	6	
MRI	12	6	55	3	
<b>Palpable</b>					<0.001
Yes	118	57	833	42	
No	91	44	1152	58	
<b>Histology</b>					<0.001
DCIS with Microinvasion	2	1	38	2	

IDC	196	93	1590	79	
ILC	5	2	269	13	
Invasive Other	7	3	117	6	
<b>Invasive Grade</b>					<0.001
Grade 1	1	1	310	16	
Grade 2	27	14	1118	58	
Grade 3	168	86	501	26	
<b>ER</b>					<0.001
Positive	0	0	1891	94	
Negative	210	100	122	6	
<b>PR</b>					<0.001
Positive	0	0	1600	80	
Negative	210	100	412	21	
<b>Ki67</b>					<0.001
Median (range)	60 (0-99)		10 (0-99)		
<b>Mammographic Density</b>					0.165
Less dense	82	41	78	46	
More dense	119	59	1034	54	
<b>MRI BPE</b>					0.021
Low BPE	70	76	555	64	
High BPE	22	24	312	36	
<b>MRI FGT</b>					0.370
Less dense	47	54	404	49	
More dense	40	46	421	51	

### Conclusions:

In our study population, MBD and FGT did not differ between patients with TNBC compared to non-TNBC. Interestingly, we found a higher proportion of women with lower BPE in the TNBC compared to the non-TNBC group. BPE refers to the amount of enhancing fibroglandular tissue and has been demonstrated to reflect variations in estrogen-mediated vascular permeability. Lower BPE in TNBC may reflect the fact that these tumors are not hormonally sensitive. This may also have implications for radiogenomics, which aims to correlate imaging characteristics with gene expression and genome-related characteristics in tumor biology. Further studies are warranted in looking at these imaging biomarkers and TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-02-02

Improving efficiency of breast MRI utilization by coordinating primary care, breast imaging and surgeons

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**Introduction:** Breast MRI in the workup of a new breast cancer diagnosis is both a valuable and costly imaging study. The decision to obtain a breast MRI is often made by the surgeon who is referred the newly diagnosed breast cancer patient. That is followed by a delay in their management due to scheduling the MRI. Our breast center program leadership developed a protocol to move the time of MRI prior to the surgeon's evaluation while avoiding unnecessary breast MRIs ordered by some well-intentioned primary care providers. Coordinating a breast MRI protocol can optimize timely performance of breast MRI, guiding primary care to become the ordering clinician while avoiding unnecessary breast MRIs.

**Methods:** Recognizing a delay in the journey of newly diagnosed breast cancer patients that have their breast MRI ordered only after the multidisciplinary conference or surgical consultation, we developed a protocol to improve timeliness of care. Guidelines were identified by our breast program leadership when a newly diagnosed breast cancer patient would warrant a breast MRI. Agreed upon indicators included dense breast tissue, invasive lobular breast cancer, patients typically under 50 years old, and vague imaging of primary lesions. When these findings were identified, the radiologist included a statement with the core needle biopsy report. It stated that our breast program leadership identified this patient as benefiting from a breast MRI ordered soon after the positive biopsy. This avoided the issue of self-referral since our breast leadership created the guidelines. The message went to the primary care provider who now ordered the breast MRI prior to conference or surgical consultation. We examined sixty consecutive patients from two time periods, half before and half after institution of the MRI protocol.

**Results:** Prior to this policy, patients who needed breast MRI would obtain the study on average 12 days after our multidisciplinary breast conference (MDC), while after institution of the policy breast MRI was obtained 3 days PRIOR to conference. Before only 43% of necessary breast MRIs were ordered prior to surgical consultation while after the protocol 100% of breast MRIs were ordered PRIOR to surgical consultation. Before the protocol rarely did primary care order breast MRIs. After the protocol primary care providers ordered 80% of all breast MRIs. While ordering more breast MRIs, primary care ordered less unnecessary studies. After the protocol was instituted, inappropriate studies as determined by the MDC decreased from 21% deemed unnecessary to only 10%.

**Conclusions:** Institution of a breast MRI ordering guideline by the breast program leadership with participation of primary care had the benefits of obtaining the breast MRI before the multidisciplinary conference and/or surgical consultation while avoiding unnecessary breast MRI orders. Institution of a breast MRI protocol enhances patient care, eliminates delays in treatment, avoids unnecessary tests, shifts appropriate care to primary care providers and allows initial surgical consultation to have all the data necessary to make definitive decisions. This quality improvement effort via program leadership improved comprehensive care.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-02-03

Testing machine learning algorithms' performance for non-invasively identifying breast cancer molecular subtypes using BI-RADS evaluations

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**Purpose:** Inter- and intra-observer variability adversely affects interpretation of highly advantageous MRI data in breast cancer diagnosis, prognosis and therapeutic decisions. Machine learning (ML) might potentially provide support to the physicians for objective decision strategies. In this work, ML methods using BI-RADS features were tested for non-invasively determining molecular subtypes of breast cancer.

**Methods:** In this IRB approved study, Out of 126 consecutive patients' retrospective data with written consent, 69 patients (mean±std age 48.24±11.62 and range [27-82]) with full histopathological and MRI data were selected. In surgical histopathological data ER+PR+HER2- was classified as luminal HER2(-), ER+PR+HER+ was classified as luminal HER2(+), ER-PR-HER2+ was classified as HER2(enriched) and ER-PR-HER2- was classified as triple negative. The cohort revealed 51 luminal HER2(-), 11 luminal HER2(+), 7 triple negative cases without any HER2(enriched) occurrence. DCE, DW, T2W MRI data were obtained on a 1.5T Magnetom Espree (Siemens, Erlangen, Germany) scanner which were subsequently interpreted in consensus by 2 radiologists of 15 and 10 years of experience.

Age, mass and non-mass properties, non-enhancing BI-RADS findings, ADC values obtained from radiologist drawn ROIS and kinetic curve properties were fed to 22 standard ML algorithms provided in Matlab® (Mathworks, Natick, MA, USA) as predictors for 3 categories. The algorithms were run by cross validating on 50 folds whereby reported classifier accuracy was obtained from each of the observations when in held-out fold.

**Results:** Out of 22 ML algorithms tested Support Vector Machine's Fine Gaussian variant, Ensemble Boosted Trees and Fine K-Nearest Neighbor resulted in 76.8%, 71.0% and 53.6% accuracy with (100%, 18%, 0%), (92%, 9%, 14%) and (67%, 18%, 14%) true positive rates for predicting each histopathological category respectively. Furthermore, PCA based dimension reduction worsened the outcomes indicating high sensitivity to the feature set.

**Conclusion:** ML algorithms demonstrated potential as a decision support system increasing assessment objectivity with an added non-invasiveness advantage. However, overall algorithm performance indicates further studies with larger cohorts and broader feature are necessary for refinements and improving ML methodology in breast cancer imaging.



# 2018 San Antonio Breast Cancer Symposium ®

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Low-dose imaging technique (LITE) MRI: Introduction of a reduced-dosage dynamic contrast enhanced MRI technique in breast imaging

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**Methods and Materials:** Between October 2017 and April 2018, six patients (age range: 18-60) with a total of eight lesions (lesion size range: 0.5-2.0 cm as measured on ultrasound) with imaging features suggestive of a fibroadenoma were imaged. All lesions were ultimately either biopsy-proven or clinically-confirmed to be benign. Each patient underwent an IRB-approved dynamic contrast-enhanced MRI scan utilizing a novel dual-dose injection protocol. Pre-contrast scans including T2-weighted scans and high temporal resolutions scans were obtained. Next, 15% of the contrast was administered with post-contrast imaging including: standard T1 weighted scans and high temporal resolution scans. Approximately 10 minutes later, 85% of the contrast was administered with repeat post-contrast imaging similar to prior. Two radiologists reviewed the low-dose MR images and high-dose MR images to evaluate for: lesion conspicuity, imaging characteristics and enhancement kinetics.

**Results:** In all 8 out of 8 lesions, there was concordance between the low-dose MR images and high-dose MR images in terms of lesion conspicuity and imaging characteristics. While the ratio of the contrast doses administered was roughly 0.18, this was not reflected in the ratios of kinetic parameters. The uptake rate ratio (low-to-high dose) was  $1.30 \pm 0.39$ , upper limit of enhancement had a  $0.31 \pm 0.06$  ratio, and  $0.35 \pm 0.06$  for initial area under the uptake curve. Rates of initial uptake measured with low-dose MRI were uniformly and significantly greater than rates measured by the high-dose MRI. Lesion time-to-enhancement was similar for both doses, with a ratio of  $0.91 \pm 0.06$ . Lesion conspicuity was measured as the ratio of the signal increase in the lesion to the signal increase in the surrounding parenchyma. The average lesion conspicuity over the first minute of enhancement had a low-to-high dose ratio of  $1.87 \pm 0.99$ .

**Conclusion:** This preliminary study demonstrates that LITE MRI has the potential to be diagnostically equivalent to standard DCE MRI in breast imaging. Low-dose imaging technique (LITE) MRI can be a promising alternative to standard-dose breast MRI, particularly with recent concerns related to gadolinium deposition.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-02-05

Correlation of proliferation index markers (Ki-67 and PHH3) with breast MRI apparent diffusion coefficient values

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**Objective:** In recent literature, increasing number of studies have analyzed associations between the MRI findings and histopathological features of breast cancer. Ki-67 index is an important prognostic factor in breast cancer which provides information about the mitotic activity and the growth rate of the tumor. The proliferation index markers like Ki-67 and PHH3 (Phosphohistone H3) play an important role in decision making in the treatment regimes. PHH3 is a relatively new entity which is specific only to the proliferation giving it a unique characteristic because it is not observed at the interphase. PHH3 provides a more sensitive and accurate mitotic index with less interobserver variability when compared with conventional H&E staining, thus emphasizing its potentially increased value in practice. In this study, our aim is to correlate both proliferation index markers (Ki-67 and PHH3) with breast MRI features in cases of invasive breast carcinoma.

**Material and Methods:** In this IRB approved study, out of 126 consecutive patients' retrospective data with written consent, 29 patients (mean±std age 48.86±13.10 and range [28-82]) with full apparent diffusion coefficient (ADC), Ki67 and PHH3 data were selected. In all of these surgical specimens, immunohistochemically administered Ki-67 and PHH3 and immunopositive cells in the digital pathology were counted. MRIs were performed in a 1.5T MR unit (MagnetomEspree with Syngo MR B15 software; Siemens, Erlangen, Germany). Images were evaluated by two radiologists in consensus. Mean ADC values were obtained from radiologist drawn ROIs. The results were analyzed with principle component analysis (PCA) and pairwise covariance analysis. **Results:** Mean ADC value was (mean±std) 0.92±0.18 with range [0.67-1.3] (10-3mm<sup>2</sup>/s), mean Ki67 was 18.62±13.32 with range [5-62] ('%' for Ki-67) and PHH3 27.17±30.81 with a range [1-111] ('10HPF' for PHH3). When PCA was conducted in (ADC, Ki67, PHH3) space, after normalizing each of the variables 45.223% of total variance was explained mainly by the new variable obtained as a linear combination of the normalized values  $-0.0866 \cdot \text{ADC} - 0.9879 \cdot \text{Ki67} - 0.7910 \cdot \text{PHH3}$ , 37.282% by  $-0.9879 \cdot \text{ADC} + 0.1545 \cdot \text{Ki67} - 0.0101 \cdot \text{PHH3}$  and 17.494% by  $0.1283 \cdot \text{ADC} + 0.7806 \cdot \text{Ki67} - 0.6117 \cdot \text{PHH3}$  respectively. The analysis demonstrates that while Ki67 and PHH3 values explain much of the variance (45.223% + 17.494%) and thereby are highly correlated as expected, ADC on its own determines a significant portion (37.282%) of the data as well. Moreover, pairwise covariance analysis resulted in ADC, Ki67 and PHH variance values of 2.170, 1.528 and 2.197 respectively and, covariances of ADC|Ki67 (-0.1169), ADC|PHH3 (0.2048) and Ki67|PHH (1.31).

**Conclusion:** Ki-67 and PHH3 both show good correlation in the assessment of proliferative status of breast cancer. However, breast MRI ADC values cannot be reliably used for the prediction of Ki-67 and PHH3 status of the invasive breast tumors. More studies with larger series are needed to assess the potential of breast MRI to serve as a clinical and/or prognostic factor.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-02-06

High-risk lesions diagnosed at MRI-guided vacuum-assisted breast biopsy: Imaging characteristics, outcome of surgical excision or imaging follow-up

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## PURPOSE

To evaluate imaging characteristics, outcome of surgical excision or imaging follow-up on high-risk lesions diagnosed at MRI-guided vacuum-assisted breast biopsy (MRI-VAB).

## MATERIALS AND METHODS

We retrospectively reviewed 581 lesions, to include benign, high-risk and malignant lesions, undergoing 9-gauge MRI-VAB from January 2015 to April 2018. We collected patient demographics, risk factors and indications, breast MRI BI-RADS descriptors, histopathological diagnosis at MRI-VAB and surgical excision, frequency of upgrade to malignancy and imaging follow-up of high-risk lesions found in this period.

## RESULTS

101 patients with 107/581 (18.4% of all lesions) had high risk lesions at MRI-VAB, including atypical ductal hyperplasia (ADH)(n=19/107, 17.8%), lobular neoplasia (n=35/107, 32.7) including atypical lobular hyperplasia (ALH), lobular carcinoma in situ (LCIS), ALH plus LCIS, papillary lesions (n=44/107, 41.1%), radial scar/complex sclerosing lesion (RS/CSL)(n=8/107, 7.5%) and flat epithelial atypia (FEA)(n=1/107, 1%). 71/107 (66.4%) high risk lesions were excised. 16/71 (22.5%) were upgraded to malignancy (4 invasive cancer, 12 DCIS). The upgrade rate for ADH and lobular neoplasia was 5 /16 (31.3%) and 8/26 (30.8%) respectively. The upgrade rate for RS/CSL was 1/6 (16.7%). Of the 22 papillary lesions excised, 2 (9.1%) demonstrated pathologic atypia and were upgraded to DCIS. The other 20 papillary lesions had no upgrade or atypia. Excised high risk lesions showing upgrade varied from 0.4 to 6 cm in length (average 2.1 cm). Increasing size correlated with tendency towards increased upgrade to malignancy, but there were no other specific imaging features to predict malignancy upgrade.

## CONCLUSION

There were no specific MRI imaging characteristics of high-risk lesions to predict malignancy upgrade. Therefore, surgical excision is recommended for high risk lesions especially ADH or lobular neoplasia. Additionally, non-atypical papillomas did not demonstrate malignancy upgrade in our small study. And further larger studies may demonstrate that these lesions may not need to be surgically excised.

## 2018 San Antonio Breast Cancer Symposium ®

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LIN9 regulation of NEK2 underlies taxol resistance in triple-negative breast cancer

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Currently, there are no targeted strategies to combat triple negative breast cancer, resulting in poor patient survival. TNBC initially respond well to cytotoxic chemotherapies such as paclitaxel, yet resistance and metastatic recurrence are common. Paclitaxel causes defects in mitotic spindle formation and centrosome function, as well as improper chromosome segregation leading to cell death. While being some of the most effective drugs for this disease, taxanes are associated with high toxicity. Thus discovering new therapeutic targets that are selective for TNBC should yield novel approaches for improving patient outcomes. We discovered that *LIN9*, a transcriptional regulator of mitosis, is overexpressed in 66% of TNBC and associated with poor survival. We further found that both *LIN9* mRNA and protein expression are upregulated in paclitaxel-resistant versus sensitive cells and directly correlates with paclitaxel IC<sub>50</sub> values across nine breast cancer cell lines. In MDA-MB-231 and MDA-MB-468 cell lines, enforced overexpression of *LIN9* increases multi- and micronucleation, indicators of chromosomal instability. Conversely, *LIN9* silencing also results in multi- and micronucleation, and supernumerary centrosomes. Most importantly, *LIN9* silencing increases sensitivity to paclitaxel in TNBC cells with intrinsic (BT549) or acquired (MDA-MB-231 and MDA-MB-468) resistance. We have previously reported that treating TNBC cells with Bromodomain and ExtraTerminal protein inhibitors (BETi) reduces *LIN9* expression, thus we determined if BETi could reverse paclitaxel resistance. Treatment with the BETi, JQ1, and paclitaxel caused a greater induction of apoptosis compared to either drug alone. Dual treatment also resulted in a potentiation of abnormal centrosomes, multinucleation, and micronucleation compared to that caused by either BETi or JQ1 alone. To identify the mechanism(s) by which genetic or therapeutic suppression of *LIN9* reverses paclitaxel resistance, we compared the transcriptomes of TNBC cells transiently transfected with non-targeting or *LIN9*-targeted siRNAs in MDA-MB-231 and HCC70 cell lines. We further narrowed the list of candidate *LIN9* targets by identifying genes that were bound by *LIN9* in a published ChIP-Seq dataset from HeLa cells whose expression is also correlated with *LIN9* expression and associated with reduced breast cancer patient survival. Using this approach, we identified NIMA-related Kinase 2 (NEK2), a serine/threonine kinase required for centrosome separation during mitosis as a potential mediator of the effects of *LIN9* suppression. *NEK2* is overexpressed in 47% of basal breast cancers and is associated with poor survival. In addition, *NEK2* is upregulated in paclitaxel-resistant cells and *LIN9* silencing decreases expression of *NEK2*. Silencing *NEK2* expression also restores sensitivity to paclitaxel in resistant cells. Together, these data indicate that increased *LIN9* expression in TNBC promotes paclitaxel resistance by contributing to centrosome dysfunction through upregulation of *NEK2*. They also indicate that targeting *LIN9* expression in addition to paclitaxel treatment may be a viable therapeutic approach for TNBC patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-02

Pre-clinical investigation of PP2A inhibitor LB-100 in overcoming and preventing lapatinib resistance in HER2-positive breast cancer

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**Background:** HER2-positive breast cancer (BC) accounts for approximately 15% of all BC. HER2-targeted therapies, such as trastuzumab and lapatinib, have significantly improved the outcome for these patients. However, HER2-targeted therapy resistance is a common clinical issue. We have previously shown that protein phosphatase 2A (PP2A) plays a role in mediating acquired lapatinib resistance in HER2-positive BC and that response to lapatinib is enhanced *in vitro* by the lab-grade PP2A inhibitor, okadaic acid. The aim of this study was to examine the *in vitro* and *in vivo* efficacy of LB-100, a PP2A inhibitor that has completed phase I clinical testing (NCT01837667), in models of HER2-positive BC with acquired resistance to lapatinib.

**Methods:** HER2-positive SKBR3 and HCC1954 BC cell lines were treated with 250 nM or 1  $\mu$ M lapatinib, respectively, for 6 months to generate lapatinib-resistant SKBR3-L and HCC1954-L cell lines. *In vitro* sensitivity to lapatinib and LB-100 was assessed by 2D acid phosphatase assay. Combination index (CI) values were generated to identify synergistic combinations. Propidium iodide staining was used to determine cell cycle arrest and apoptosis. In order to examine the *in vivo* efficacy of LB-100, HCC1954-L cells were implanted into the mammary fat pad of BALB/c nude mice and treated with vehicle, lapatinib, LB-100, or lapatinib plus LB-100. To examine the prevention of the development of lapatinib resistance, SKBR3 and HCC1954 cells were treated twice weekly with lapatinib, LB-100 or the combination and stained with crystal violet when confluent.

**Results:** SKBR3-L and HCC1954-L cells were resistant to lapatinib at clinically relevant concentrations ( $IC_{50}$  values =  $2.37 \pm 0.58 \mu$ M and  $1.67 \pm 0.34 \mu$ M). This represents a 46- and 5.2-fold decrease in lapatinib sensitivity. LB-100 had a greater anti-proliferative effect in the lapatinib-resistant SKBR3-L and HCC1954-L cell lines compared to their respective parental cell lines ( $IC_{50}$  values =  $2.12 \pm 0.2 \mu$ M v  $5.38 \pm 0.6 \mu$ M, and  $2.31 \pm 0.19 \mu$ M v  $5.32 \pm 0.82 \mu$ M, respectively). LB-100 overcame lapatinib resistance in both models, as lapatinib plus LB-100 was synergistic in both cell lines (CI values =  $0.56 \pm 0.13$  and  $0.68 \pm 0.26$ ). LB-100 caused cell death through the induction of apoptosis in SKBR3-L ( $p = 0.019$ ) and HCC1954-L ( $p = 0.046$ ) and the addition of lapatinib to LB-100 increased apoptotic induction in HCC1954-L cells ( $p = 0.046$ ). Lapatinib plus LB-100 was well tolerated *in vivo*. The HCC1954-L cell line maintained resistance to lapatinib *in vivo* and the combination of lapatinib and LB-100 significantly reduced HCC1954-L tumour volume compared to all other treatment arms ( $p = 0.0006$ ). Interestingly, *in vitro* short-term resistance assays showed that the addition of LB-100 to lapatinib could also block the emergence of lapatinib resistance in both parental SKBR3 and HCC1954 cell lines.

**Conclusions:** This study indicates that LB-100 has *in vitro* and *in vivo* efficacy against lapatinib-resistant HER2-positive BC cell line models and justifies further investigation into its potential to circumvent or prevent lapatinib resistance in HER2-positive BC.

# 2018 San Antonio Breast Cancer Symposium ®

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Polo-like Kinase 1 (PLK1) inhibition synergizes with docetaxel in basal breast cancer cells

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**Background.** Triple negative breast cancer (TNBC) is a heterogeneous disease entity and gene expression analyses have identified molecular subtypes that are refining our understanding of breast cancer biology and enabling development of targeted therapy. The basal-like 2 (BL2) subtype, as described by Lehmann et al, is characterized by overexpression of EGFR, loss of PTEN, and mutations in the TP53 gene and those patients had a 0% pathologic complete response rate following neoadjuvant chemotherapy. Thus, BL2 breast cancers are intrinsically resistant to chemotherapy and patients with this type of breast cancer have a poor overall survival rate. In a recent genome-scale short hairpin RNA (shRNA) screen of breast cancer cells, PLK1 was a frequent and strong hit in the basal-like subtype, inflammatory breast cancer SUM149, indicating its importance for growth and survival of these breast cancer cells. PLK1 regulates progression of cells through the G2 phase of the cell cycle by phosphorylating FOXM1, which then regulates the expression of cyclins and other genes necessary for cells to progress through cell cycle. However, most of the drug companies discontinued clinical development of PLK inhibitors (PLKi) (e.g. GSK461364 GlaxoSmithKline, Volasertib Boehringer Ingelheim) in solid tumors due to elevated toxicity shown in phase I-II studies.

**Methods.** We selected GSK461364 as our PLKi for the in vitro experiments. GSK461364 was combined with docetaxel and cisplatin in a set of TNBC cells including SUM149, SUM159, SUM1315, SUM229, DU4475 and the control MCF10A. We performed cell proliferation assays in order to determine the IC25 (Inhibition Concentration of 25%) and IC50 concentration for each drug across the panel of cell lines. We then examined the influence of drugs on the clonogenic potential of the panel of cell lines, alone and in combination. Synergism or antagonism was determined using the Chou-Talalay method, by determining the combination index (CI) at  $F_a$  0.5 (50% of cell death induced by drug treatment). We used FxCycle™ Stain (Invitrogen) for cell cycle analysis, and CD24/CD44 antibodies (BD Pharmingen™) for stem cell marker analysis. All experiments were carried in triplicate.

**Results.** GSK461364 caused growth inhibition with IC50 varying from 6.1 nmol/L (SUM229) to 56.5 nmol/L (DU4475). In our growth assay, GSK461364 showed synergism with docetaxel in SUM149 (CI 0.70) and SUM159 (CI 0.62), and with cisplatin in SUM149 (CI 0.58), SUM159 (CI 0.85), and SUM229 (CI 0.54). Only GSK461364 in combination with docetaxel decreased the clonogenic potential of SUM149 ( $p < 0.001$ , Interaction Test, 0.001). In SUM149 cells, GSK461364 caused mitotic arrest in phase G2-M with the maximum effect 24 hours after administration of the drug. Moreover, when combined with docetaxel, GSK461364 reduced the relative percentage of CD44<sup>+</sup>/CD24<sup>-dim</sup> population of SUM149.

**Conclusion.** PLKi in combination with chemotherapy shows promising results in a subset of TNBC intrinsically resistant to chemotherapy. GSK461364 significantly decreased the clonogenic potential and stem cell population of SUM149 when combined to docetaxel. In combination with docetaxel, we demonstrated that it is possible to lower the dose of PLKi by 5 folds in order to obtain the same activity.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-03-04

Targeting the tumor microenvironment by CXCR4 inhibition to abrogate trastuzumab resistance in HER2-positive breast cancer

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**Background:** Despite the fact that trastuzumab along with other HER2-targeting drugs has significantly improved the survival of patients with HER2 overexpression breast cancers (HER2+BC), resistance to trastuzumab is a clinical challenge in HER2+BC. Discerning actionable mechanisms of resistance to trastuzumab remains an important unmet need.

We previously reported dysregulation of CXCR4 involved in trastuzumab-resistance, but its causal role and the associated mechanisms remain unknown.

**Methods** We established trastuzumab-resistant (TR) human breast cancer HER2+ cell lines by continuously exposing cells to trastuzumab (20 µg/ml) for at least 6 months. CXCR4 expression was assessed in TR cells or parental cells with Western blot. Quantitative densitometric analysis of the density was performed with AlphaView SA software. Relevant cell phenotypes were measured, including mammosphere formation, *in vitro* antibody-dependent cellular cytotoxicity (ADCC) assay, and cell invasion induced by 10% fetal bovine serum with or without 100 ng/ml stromal cell-derived factor-1α (SDF-1α, CXCL12), the ligand of CXCR4. ANOVA was used to test differences between more than two groups, while the differences between two groups were assessed based on paired *t*-test.

**Results:** To better capture the heterogeneity of HER2+BC, we chose two trastuzumab-sensitive cell lines, BT474 (HER2+/HR+) and SKBR3 (HER2+/HR-) and an intrinsically trastuzumab-resistant cell line, HCC1419 (HER2+/HR-). We found much higher CXCR4 expression levels in cells with intrinsically trastuzumab-resistant cells compared to trastuzumab-sensitive cells. Upregulation of CXCR4 expression was found in each of the acquired TR cell lines compared to their parental cells. Dysregulation of CXCR4 significantly enhanced mammosphere formation and cell invasion ( $P < 0.001$ , respectively). SDF-1α induced cell invasion and clumping. Down-regulation of CXCR4 with shRNA significantly increased trastuzumab induced-antibody-dependent cellular cytotoxicity (2.17 folds of control cells,  $P < 0.01$ ). Targeting CXCR4 with its approved inhibitor AMD3100 significantly decreased mammosphere formation and invasion of HER2+BC with TR ( $P < 0.01$ ;  $P < 0.0001$  respectively).

**Conclusion:** Our results suggest that the SDF-1-CXCR4 axis plays a critical role in resistance to trastuzumab. Targeting CXCR4 signaling may lead to novel combinational therapies to overcome intrinsic or acquired resistance to trastuzumab in advanced HER2+BC, including postulated effects of trastuzumab on signal transduction, differentiation and immune activation.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-05

Model of acquired resistance to the tyrphostin NT157 in hormone receptor-positive breast cancer

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As previously reported, the small-molecule tyrphostin NT157 binds to the type I insulin-like growth factor-1 receptor (IGF-1R) beta subunit (IGF-IR $\beta$ ) resulting in degradation of the insulin receptor substrate (IRS) adaptor proteins and inhibits growth of tamoxifen sensitive and resistant ER+ breast cancer cells (Yang, Y. et al. Horm Canc, in press). To investigate effects related to long-term NT157 exposure on IGF signaling, we incubated T47D parental cells in increasing concentrations of NT157 for 20 months. This prolonged exposure resulted in resistant cells (T47D-NTR). Monolayer growth assays showed parental cells inhibited at 0.7  $\mu$ M of NT157 whereas resistant cells were suppressed by 8  $\mu$ M of NT157. In parental cells, NT157 caused G0-G1 arrest and an increase in the sub-G1 fraction. However, resistant cells did not show these cell cycle changes after exposure to NT157. Furthermore, the antiproliferative effect of prolonged exposure to 96 hours of NT157 was correlated with decreased activation of Akt/S6k signaling axis and reduction of cyclin D1 expression in T47D parental cells. 48 hours treatment with 5  $\mu$ M of NT157 resulted in a decrease of IGF-IR and insulin receptor (InR) expression level at 33% and 39% respectively, along with the downregulation of the adaptor proteins IRS-1/2 in only the parental cell line. In resistant cells, the NT157 treatment induced minimal IGF-1R suppression and a 21% increase of IR expression. Additionally, the T47D-NTR cells-maintained IRS protein levels at 10  $\mu$ M. This absence of IRS protein degradation observed in NT157-resistant cells correlated with the continued expression of estrogen receptor-alpha (ER $\alpha$ ), suggesting functional changes within IGF and ER $\alpha$  complex. IGF ligands (IGF-1 or IGF-2) and estradiol (E2) stimulated growth in both cell lines. When NT157 was removed from T47D-NTR cells, they were not re-sensitized to NT157 nor were they affected by IGF dependent growth in the re-introduction of NT157. These findings suggest that resistance to NT157 is mediated by the maintained expression of the IRS adaptor proteins. The continued responsiveness to estradiol and IGF ligands could be due to either a decrease in the drug's half-life or possible changes in NT157 binding to the beta subunit of IGF-IR in resistant cells.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-06

Overcoming MEK inhibitor resistance in triple-negative breast cancer by targeting myeloid cell leukemia-1 (*MCL1*), an anti-apoptotic protein

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**Background:** Triple-negative breast cancer (TNBC), which affects over 170 000 women worldwide every year, is considered the most arduous to treat subtype of breast cancer. With no targeted therapy, high rates of drug resistance and rapid metastasis, TNBC carries a poor prognosis. The MEK-ERK-MAPK signaling cascade is known to play a role in numerous cancers. Despite the lack of activating Ras/MAPK mutations in breast cancer, transcriptional signatures of this pathway are prevalent in TNBC. Our previous work showed that TNBC patients with tumors overexpressing ERK2 had a lower overall survival rate than did patients with low-ERK2-expressing tumors. MEK inhibitors selumetinib (AZD6244) and pimasertib (AS703026) are active in preclinical models, but not as single agents in the clinic. Using a synthetic lethal siRNA screen, we identified myeloid cell leukemia-1 (*MCL1*) as a potential contributor to selumetinib resistance. Mcl-1 is an anti-apoptotic protein that is highly amplified in numerous human cancers. It is associated with cell immortalization, transformation, and chemoresistance. Patients with TNBC tumors expressing high levels of Mcl-1 have lower overall survival and distant-metastasis-free survival rates. We hypothesized that Mcl-1 promotes MEK inhibitor resistance in TNBC.

**Methods/Results:** To model MEK inhibitor resistance, we established selumetinib- and pimasertib-resistant clones of SUM-149 and MDA-MB-231 TNBC cells by continuous exposure to increasing concentrations of inhibitors over a six month period. We confirmed the onset of MEK resistance by demonstrating that resistant cells, in comparison to the parental cells, exhibited no change in cell proliferation upon treatment with the MEK inhibitors. Resistant cells also displayed more effective cell migration and mammosphere formation than parental cells, suggesting a higher fraction of tumor-initiating cells.

We found Mcl-1 to be highly expressed in 83% (15 of 18) of TNBC cell lines but only 30% (3 of 10) of other breast cancer cell lines. Resistant cells had higher levels of Mcl-1 than did parental cells. To determine whether Mcl-1 is required for MEK sensitivity, we treated parental and resistant cells with either selumetinib or pimasertib together with S63845, a highly specific Mcl-1 inhibitor. The Mcl-1 inhibitor restored MEK sensitivity in both resistant cell lines. After treatment with the Mcl-1 inhibitor, the resistant SUM-149 and MDA-MB-231 cells had similar cell proliferation rates to those of their parental counterparts. Similar studies were done using an siRNA against Mcl-1.

**Conclusion:** Our data demonstrate that Mcl-1 may promote TNBC resistance to MEK inhibitors and that Mcl-1 is a promising target for combination therapy. We will continue to explore the mechanisms of MEK inhibitor resistance by screening for additional genes/pathways involved. Our long-term goal is to design rational combination approaches to counteract the emergence of resistance by using novel molecularly targeted therapeutics.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-07

TIMP-4 as a biomarker and alternative activator of growth and survival in trastuzumab-resistant cells

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**Background:** Within all invasive breast cancers, 20-30% harbor HER2 gene amplification. HER2 activates signaling pathways promoting survival and cell proliferation resulting in an aggressive phenotype. Even with the development of trastuzumab (anti-HER2 therapy) this subtype has one of the worst out-comes of all breast cancers. Primary and acquired resistance to trastuzumab is increasingly recognized as a major obstacle in the clinical management of HER2 disease. Reported mechanisms of resistance involve a truncated HER2 receptor, mutations in the PI3K/AKT pathway, and up-regulation of alternative tyrosine kinase receptors.

In our prospective IRB-approved study of breast cancers, we observed an over-representation of TIMP-4 positive cancers among HER2-positive patients with disease progression while receiving, or shortly after completing, treatment. Secreted TIMP-4 acts through the tetraspanin CD63 on tumor cells to initiate the same signaling pathways as HER2. Based on this observation we *hypothesized* that TIMP-4 provides an alternative mechanism to promote continued growth and spread of HER2 positive tumors in the presence of anti-HER2 therapy and might be used as a predictive biomarker for treatment resistance.

**Methods:** Cell culture experiments using human breast cancer cell lines with HER2 amplification, with or without mutations in PIK3CA and PTEN, were used to assess the effects of anti-HER2 treatment on cell growth and downstream signaling pathways. Cells were grown in the presence or absence of human recombinant TIMP-4 and used to determine the effects on growth, AKT/MAPK activation, apoptosis and response to neutralizing antibodies against HER2 alone or in combination with anti-TIMP4 antibody.

**Results:** Under normal growth conditions, SK-BR-3 cells (HER2 amplified, wild type PI3K and PTEN) demonstrated a significant growth reduction in the presence of anti-HER2, while anti-TIMP4 had no effect. However, in TIMP-4 elevated conditions cells were resistant to anti-HER2 treatment while anti-TIMP4 had a modest effect. Combining the two antibodies reduced the growth significantly. Cells with mutations in either PI3K (MDA-MB-453) or PTEN (ZR-75-1) were insensitive to anti-HER2 treatment under normal growth conditions. In the presence of TIMP-4, cells with PI3K mutation were still insensitive, while cells harboring PTEN mutation responded to anti-HER2 treatment. Cells with either mutation pattern had a modest response to anti-TIMP4 and the combination treatment. Both antibody treatments were associated with reduced activation of growth and survival promoting enzymes as determined by Western blotting.

**Conclusions:** Elevated TIMP-4 levels could contribute to what clinically appears as resistance to trastuzumab. While regular anti-HER2 treatment caused diminished growth among cells with wild type PI3K and PTEN, the presence of high TIMP-4 levels necessitated treatment with both antibodies for the same growth reduction. Tumor cells insensitive to anti-HER2 treatment due to mutations in growth and survival promoting pathways acquired a modest response when treated with both anti-HER2 and anti-TIMP4 antibodies. We are currently evaluating the possible mechanism for the observed conversion in sensitivity.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-08

Eribulin-induced EpCAM expression provides drug resistance in triple-negative breast cancer cells

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Breast cancer is the most common cancer in women in the world. Among breast cancer subtypes, triple-negative breast cancer is aggressive. Because there is no effective therapy for triple-negative breast cancer, development of a novel therapeutic strategy is required.

Eribulin, a derivative of halicondrin B, is an anticancer chemical. Eribulin treatment has been approved in many countries for patients with metastatic or inoperable breast cancer, including triple-negative breast cancer. The known functions of Eribulin are inhibition of microtubule polymerization and induction of the expression of epithelial genes in breast cancer cells. However, the function of Eribulin-induced genes on the therapy for patients with triple-negative breast cancer remains unclear.

EpCAM (Epithelial cell adhesion molecule) is one of epithelial markers and known as a cell-cell adhesion molecule. In addition, EpCAM expression is involved in drug resistance in cancer. We therefore focused on EpCAM expression in Eribulin-treated breast cancer cells.

We used MDA-MB-231 cells that is a commonly used triple-negative breast cancer cell line. We treated cells with 10 nM Eribulin and observed inhibition of cell growth. In the same condition, we found up-regulation of EpCAM expression in both mRNA and protein levels. In mouse xenograft model with MDA-MB-231 cells, EpCAM expression was detected in whole tumor at 96 h after Eribulin administration. We analyzed the effect of other microtubule polymerization inhibitors, nocodazole and colchicine, on EpCAM induction. In the results, there was no up-regulation of EpCAM expression in cells treated with these chemicals, suggesting that EpCAM expression is not induced by inhibition of microtubule polymerization and growth inhibition.

To analyze EpCAM function on Eribulin treatment, we constructed shRNA-mediated EpCAM knockdown system. We introduced EpCAM knockdown in MDA-MB-231 cells and treated with Eribulin. In the results of cell growth assays, the cell number of Eribulin-treated EpCAM-knockdown cells was significantly smaller than that of Eribulin-treated control-shRNA cells, whereas no change was observed between solvent-treated control-shRNA and EpCAM-knockdown groups. In mouse xenograft experiments, EpCAM-knockdown cells showed smaller tumor weights than control-shRNA cells after administration of Eribulin. These results indicate that EpCAM expression provides Eribulin resistance in breast cancer cells.

In conclusion, we found Eribulin-induced EpCAM expression in triple-negative breast cancer cells. The EpCAM expression provides Eribulin resistance both *in vitro* and *in vivo*. These suggest that a combination of Eribulin treatment and EpCAM inhibition may improve therapy for breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-09

LINC00624 binding with SFPQ/MATR3/ NONO protein complexes enhances resistance to neoadjuvant therapy in HER-2 positive breast cancer by inhibiting tumor immune response

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## Background

Neoadjuvant chemotherapy(NAC) has been widely used for its ability to downstage breast cancer. With anti-HER2 therapy, around half of the HER2 positive patients achieve pCR after NAC. However, treatment failure is still a concern and leads to a worse prognosis. New biomarkers and biological mechanisms are waiting to be discovered to tackle this problem.

## Methods

RNA-Seq was performed to identify differently expressed RNAs between pCR and non-pCR group. The IC50 value was measured by CCK-8 assay in HER-2 positive cells (SK-BR-3 and JIMT-1). Quantitative RT-PCR (qRT-PCR) was used to detect the expression levels of LINC00624 and its potential target genes. RNA-seq and GSEA analysis was carried out to determine the target pathways regulated by LINC00624.

## Results

Core needle biopsy tissue from HER-2 positive patients was collected before NAC. LINC00624 was found to be down regulated in pCR group compared with non-pCR group by RNA-seq. High expression of LINC00624 was associated with poor overall survival in TCGA database and our own patients cohort. Overexpression of LINC00624 promoted cell proliferation and colony-forming ability. Meanwhile, the IC50 values of Paclitaxel and Trastuzumab in HER-2 positive breast cells were decreased by LINC00624. Via RNA pull-down assay and mass spectrometry, the SFPQ/MATR3/NONO protein complex, which was reported to be associated with the innate immune system, was found to interact with LINC00624. RNA-seq further revealed that several immunological and inflammatory factors, including 'IFN- $\alpha$  response', 'IFN- $\gamma$  response' and 'TNF- $\alpha$  via NF- $\kappa$ B' were suppressed by LINC00624. IFN downstream molecules were inactivated in LINC00624 overexpressing cells following TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\beta$  and poly (I:C) treatment, which implies the immune inhibition ability of LINC00624 .

## Conclusion

LINC00624 is associated with resistance to NAC in HER-2 positive breast cancer. It binds with the SFPQ/MATR3/NONO complex and desensitizes cell response to chemotherapy and anti-HER2 agents via suppressing immunological and inflammatory effect. This study suggests that LINC00624 could serve as a biomarker to predict therapy response to NAC in HER-2 positive breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-10

Circulating exosomal microRNA profiling to depict mechanisms of chemotherapy resistance among triple negative breast cancer

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**Background:** Chemotherapeutic resistance leads to high mortality among triple negative breast cancer (TNBC) and underlying mechanisms are poorly understood. Exosomes have been new member of liquid biophsy. MicroRNAs (miRNAs), as the most important inclusions in exosomes, making them ideal candidate biomarkers and therapeutical targets.

**Methods:** We isolated exosomes and analyzed exosome-carried miRNAs signatures in several TNBC cells lines sensitive or resistant to adariamycin, docetaxel or cisplatin. The resistance transfer capacity was determined by flow cytometry after sensitive cells incubated 48 hours with exosomes from drug resistant cells. Locked nucleic acid probes and enzyme-labeled fluorescence (LNA-ELF-FISH) was performed to detect exosomal miRNA molecules transfer. Animal mode was constructed to evaluate treatment feasibility using miRNA modified exosomes. Serum exosomes from 40 TNBC stage IV patients underwent chemotherapy before or after progress disease (PD) status were isolated to analyze miRNA profiling for potential biomarkers identification.

**Results:** We successfully isolated and identified exosomes from several drug sensitive and resistant TNBC cell lines and patients. Exosomal miR-222, miR-4443, miR-100, miR-17, miR-210 were found significantly upregulated from chemotherapy resistance cells. Incubation of exosomes from the resistant cells with the sensitive cells resulted in increasing resistant capacity among sensitive cells. Exosomal miRNA molecules transfer were detected using LNA-ELF-FISH. Transfection of synthesized miRNAs competitors into exosomes increased drug sensitivity in vivo. Exosomal miR-222, miR-4443, miR-100, miR-17, miR-210 were also found upregulated significantly from serums of patients after PD status. These five miRNAs were able to differentiate patients with PD status from those with CR or PR status with at least 89% accuracy.

**Conclusion:** Exosomes from chemotherapy resistant TNBC cells could transfer drug resistance to sensitive cells via exosomal miRNAs. A circulating exosomal microRNA profiling was established for potential biomarkers and therapeutical targets identification.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-03-11

Sensitivity to cell cycle inhibitors in taxane resistant breast cancer models

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**Background:** The use of anthracycline and taxane chemotherapy has improved overall and disease-free survival in breast cancer. However these agents have significant toxicity. In addition, breast cancers can acquire or possess intrinsic chemoresistance. It is imperative to identify patients who will benefit most from adjuvant taxane treatment and those with resistant tumours who could be spared unnecessary toxicity.

**Methods:** A panel of *in vitro* derived cell lines models of taxane resistance were generated by serial culture in escalating doses of either paclitaxel or docetaxel until resistance was achieved. Taxane resistant cells were characterised by 2D growth, cell cycle and apoptosis analyses. Genomic profiling using the NanoString® platform was performed to identify differentially expressed genes. The identification of kinases which target the chemoresistant models was achieved through a small molecule kinase inhibitor screen. Effects of selected target kinases on cell proliferation, cell cycle, apoptosis and protein expression were assessed.

## Results:

Resistant cell lines exhibited an IC50 at least 40-fold higher than that of the parental cells and displayed cross-resistance to the non-establishing taxane.

Table 1: Sensitivity of taxane resistant cell lines models to paclitaxel and docetaxel

Cell line model	Paclitaxel (µM)	Docetaxel (µM)
MDA-MB-231 Parent	0.004 ± 0.003	0.002 ± 0.003
MDA-MB-231 PACR	0.184 ± 0.03	0.017 ± 0.02
MDA-MB-231 DOCR	0.414 ± 0.047	0.262 ± 0.058
MCF7 Parent	0.004 ± 0.0005	0.005 ± 0.001
MCF PACR	0.769 ± 0.105	0.07 ± 0.02

Cell cycle analysis revealed taxane treatment failed to induce G2/M arrest in the resistant models. A reduced apoptotic response was demonstrated. Genomic profiling identified pathways associated with the cell cycle as being significantly altered.

Table 2: Gene ontology enrichment analysis of biological process terms significantly over-represented in MDA-MB-231 PACR cell line model

GO Term	P-value	FDR
positive regulation of transcription from RNA polymerase II promoter	1.11E-16	2.44E-13
positive regulation of cell proliferation	9.99E-16	1.10E-12
activation of cysteine-type endopeptidase activity involved in apoptotic process	1.43E-10	6.27E-08
negative regulation of apoptotic process	2.13E-09	5.83E-07
extrinsic apoptotic signaling pathway	8.33E-09	1.62E-06
cell cycle arrest	8.89E-09	1.62E-06
positive regulation of cell migration	2.83E-08	4.42E-06

Dinaciclib, a CDK inhibitor of CDK1, CDK2, CDK5 and CDK9, inhibited taxane resistant cell growth with IC50s comparable to the parental lines. Upon exposure to dinaciclib, cell cycle arrest at G2/M was induced and marked apoptosis demonstrated. A reduction in cyclin B1, PLK1 and pRB was observed by western blotting.

**Conclusion:** In this study we identified candidate resistance-associated pathways which were differentially expressed between *in vitro* derived taxane resistant cell line models and the sensitive parental line. The CDK inhibitor, dinaciclib, demonstrated potent activity against the taxane resistant cell line models. Clinical validation to ascertain the role of dinaciclib as a novel therapeutic in the treatment of chemorefractory breast cancer is required.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-12

Signature-guided biomarker discovery and therapy for trastuzumab-resistant HER2-positive breast cancer

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## **Background/Objective:**

Breast carcinomas with HER2 receptor amplification and overexpression account for approximately 20% of all breast cancers, which also confer more aggressive phenotypes and are associated with poor prognosis. Up to 23% of patients with early-stage HER2-positive (HER2+) breast cancer treated with adjuvant chemotherapy and trastuzumab experience disease recurrence within 10 years, highlighting the importance of identifying which HER2+ patients respond to this treatment and those that do not. Efforts to identify biomarkers predictive of response in the initial breast tumor biopsy to trastuzumab have been complicated by the clinical and biological heterogeneity of HER2+ tumors. Therefore, we aim to develop a trastuzumab-resistant (TRR) signature which could faithfully predict patients' response to this treatment using transcriptome profiles in engineered cell line models. We further investigated the possible mechanisms mediating TRR by bioinformatics and biochemical approaches in order to reveal promising new therapeutic targets TRR HER2+ patients.

## **Methods:**

Publicly available transcriptome profile (GSE15043) of genetically engineered isogenic models of TRR cell lineages in mammary HER2+ BT474 cells (initially trastuzumab sensitive, TRS) are used to generate the TRR signature using Bioconductor in R. Performance of this gene signature was tested on independent in vitro and in vivo transcriptome datasets using Receiver Operating Curve (ROC). Signature scores were determined by calculating the correlations between medium centered gene expression levels within the signature and gene expression levels for that gene within a given sample, following quantile normalization. Three pairs of TRR vs. TRS cell lines were used for accessing their profiles of proliferation, migration, genomic stability and signature scores. Genes of interest in the signature were further perturbed in TRR cells for drug sensitivity assay.

## **Results and Conclusion:**

TRR cell lines have significant more malignant phenotypes in comparison to TRS cell lines, indicated by their shorter doubling time, faster migration and more innate double strand breaks. 43 differentially regulated genes between BT474R (engineered TRR cell line) and BT474 are identified and the area under the curve (AUC) of ROCs are 0.75, 0.88, and 0.84 for microarray data of CCLE, RNA sequencing data of CCLE and patient dataset GSE62327, respectively. In addition, TRR signature is significantly enriched in non-responding cell lines, TRR patients and patients relapsed within 3 years after treatment. TRR cell lines – BT474-C5, T47D and HCC1954 exhibit significant higher TRR signature scores in comparison to TRS cell lines by the 43-gene PCR array. Interestingly, overexpression of one of gene candidates, S100A8, which is also a key cytokine in regulating antibody-dependent cytotoxicity (ADCC), conferred trastuzumab resistance in TRR cell lines. This finding could assist in clinicians' diagnosis and selection of treatments for HER2+ breast cancer with a more accurate risk evaluation and offering promising alternatives such as immunotherapy.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-13

ERVMER34 sensitizes the response of HER2 positive breast cancer to neoadjuvant therapy

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Although anti-HER2/neu antibody therapy combined with chemotherapy has achieved an outstanding effect, many patients with HER2-positive breast cancer still succumb due to therapeutic resistance. Understanding the mechanisms of therapeutic resistance is of vital importance. In this study, core needle biopsy tissue from HER2-positive patients was collected before neoadjuvant chemotherapy. Differentially expressed RNAs between pCR and non-pCR group have been identified through RNA-seq. Here we found that ERVMER34, an endogenous retroviral envelope protein was significantly inhibited in non-pCR group compared to pCR group. High ERVMER34 expression was associated with good overall survival both in TCGA database and our own patients cohort. Further study showed that ERVMER34 could be glycosylated and secreted to cell supernatant. The IC50 of paclitaxel and trastuzumab in HER2 positive breast cancer cells was decreased by ectopic overexpression of ERVMER34. Furthermore, RNA-seq, FACS assay and western blot revealed that ERVMER34 could promote cell apoptosis and sensitize therapy response through inhibiting mTOR pathway in HER2 positive breast cancer cells. Via mass spectrometry, TRIM21, an E3 ligase was found to interact with ERVMER34 and promoted its ubiquitination degradation. Taken together, these data suggest that ERVMER34 may serve as a novel biomarker to predict therapy response in HER2 positive breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-14

Exosome mediated breast cancer drug resistance: Delivery of Hsp70 and related mechanisms

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## Background

Adriamycin is widely used in postoperative adjuvant chemotherapy for breast cancer. Although the majority of patients were responsive to the initial treatment for certain time, the patients eventually developed more aggressive tumor forms that were generally resistant to the chemotherapy. Exosomes shuttle bioactive molecules and lead to the exchange of genetic information and metabolic reprogramming of the recipient cells, resulting in transmitting drug resistance. However, the specific mechanism of breast cancer cell derived exosomes mediating drug resistance remains obscure.

## Methods

The exosomes derived by MCF-7 resistant cells (A/exo) were co-cultured with sensitive MCF-7 cells and were extracted from the supernatant of cell culture and the exosomal Hsp70 was analyzed. Fluorescence labeled A/exo and Hsp70 were used to observe an increase of Hsp70 fluorescence signal after co-incubation. Meanwhile, the cellular glycolysis level and oxidative phosphorylation level were observed by Seahorse XF analyzer. In addition, we measured the cellular activity of lactate dehydrogenase (LDH) and phosphofructokinase (PFK).

## Results

Co-incubation MCF-7 cell with Adriamycin-resistant in MCF-7 derived exosomes (A/exo) was found to enhance Adriamycin resistance in MCF-7. Exosomes were extracted from the supernatant of cell culture and the exosomal Hsp70 was analyzed. Fluorescence labeled A/exo and Hsp70 were used to observe an increase of Hsp70 fluorescence signal after co-incubation, demonstrating that transferring Hsp70 may be one of the mechanisms that A/exo mediates drug resistance. Seahorse XF Analyzer has been used to measure glycolysis and mitochondria oxidative phosphorylation, the results exhibit an increase in glycolysis but a decrease in oxidative phosphorylation in Adriamycin-resistant MCF-7 (MCF-7/Adr) when compared to sensitive MCF-7. While glycolysis level significantly decreased and oxidative phosphorylation level increased after knockout Hsp70 in MCF-7/Adr. Furthermore, we measure activities of lactate dehydrogenase (LDH) and phosphofructokinase (PFK), the results showed that Hsp70 could promote activities of those two glycolysis key enzymes. The results indicated that glycolysis levels in MCF-7/Adr are higher than those in sensitive cells, and mitochondrial damage and increased activity of glycolysis key enzymes may account for this.

## Conclusions

Our results demonstrate that Adriamycin-resistant MCF-7 derived exosomes induce a drug-resistant phenotype in sensitive cells through Hsp70 transfer. Hsp70 could promote Adriamycin resistance in breast cancer through regulating PFK and LDH activities and inducing glycolysis.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-15

Mechanism of activation of PPP1R1B drug-resistance gene in trastuzumab-resistant Her2+ breast cancer

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Trastuzumab (Herceptin) resistance is a persistent challenge in the treatment of metastatic Her2+ breast cancer, with an average relapse time of 1 year after treatment. Human breast cancer cell-lines (SKBr3 and BT474) selected for trastuzumab resistance have a PPP1R1B gene (chr17:39,626,924-39,636,626) that is transcriptionally activated compared to their drug-sensitive counterparts. PPP1R1B codes for two related protein products, Darpp-32 (long) and t-Darpp (short), that are overexpressed in 50% of clinical breast cancer samples in comparison to normal breast tissue; reintroduction of t-Darpp into drug-sensitive cells confers drug resistance. The mechanism controlling overexpression of these transcripts is unknown. To gain insight into this mechanism we used ATAC-seq to map open chromatin areas in trastuzumab-resistant breast cancer cell lines and compared their open chromatin areas to those in the parental trastuzumab-sensitive counterparts. The PPP1R1B gene consists of 7 canonical exons. In each trastuzumab-resistant line there is two significantly increased open chromatin peaks, one within exon 2 and one within exon 3. We are performing deep sequencing (>200 million reads) to identify transcription factor footprints in these peaks. Furthermore, we performed RNA-seq analysis and identified genes that are similarly regulated in the resistant lines in comparison to their drug-sensitive counterparts. In summary, these results shed light on an area of high clinical importance and point to potential drug targets to be developed to overcome trastuzumab resistance in Her2+ breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

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KCNN4 confers multiple chemoresistance in breast cancer

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Multiple chemoresistance is a major unmet clinical obstacle in breast cancer treatment. Here, we uncovered that calcium-activated channel subfamily N member 4 (KCNN4) was an important modulator that limit the cytotoxicity of gemcitabine via a pooled ORF library screen. Importantly, elevating KCNN4 expression enhances the multiple resistance to anti-metabolism chemotherapy drugs, and accelerates cell proliferation. Conversely, silencing or using specific inhibitor, TRAM-34, can counteract its multiple chemoresistance and cell proliferative effects. Mechanically, we found that KCNN4 up-regulated BCL2A1 to suppress cell apoptosis progression via activating RAS-MAPK and PI3K-AKT signaling. In clinical specimens of breast cancer, KCNN4 expression was frequently upregulated and positively correlated with BCL2A1 expression. Moreover, high expression of KCNN4 and BCL2A1 was associated with shorten disease-free survival in studied cohorts. Collectively, our findings showed that KCNN4 was a key modulator in breast tumor progression, implicating that targeting the KCNN4 may serve as a promising therapeutic strategy to overcome multiple chemoresistance in breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-03-17

Sabutoclax, pan-active BCL-2 protein family antagonist, eliminates cancer stem cells through inhibiting PI3K/Akt pathway in breast cancer

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## Introduction/Purpose:

Misregulation of BCL-2 family of proteins renders a survival signal to withstand cytotoxic anticancer drugs and is often found in drug resistant cells. The drug resistance phenotype often accompanies activation of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, as well as an enhancement of cancer stem cell-like (CSC) characteristics. Thus, inhibition of anti-apoptotic BCL-2 family proteins has been proposed as a possible antineoplastic strategy. However, the effects of BCL-2 inhibitors on drug resistant breast cancer have not yet been elucidated. In the present study, the effect of sabutoclax, a pan-active BCL-2 protein family antagonist, on two chemoresistant breast cancer cell lines was assessed.

## Methods:

MTT assays and drug resistance clonogenic assays were performed to evaluate the cell viability in response to drug treatments. Apoptosis was determined by flow cytometry after annexin V staining, by caspase 3/7 and caspase 9 activity assays and the levels of a series of apoptosis-related proteins by real-time PCR and western blot. **The expression levels of AKT and pAKT were assessed by western blot to exam the effect of sabutoclax on the activity of PI3K/AKT pathway.** The expression of breast cancer stem cells (BCSC) related markers (CD44, CD24 and ALDH1) were detected by flow cytometry. Mammosphere formation assays and soft agar colony formation assay were performed to test the effect of sabutoclax on the proportion of stem-like cell population. To validate the results showed in breast cancer cell lines, we also tested the ex vivo effect of sabutoclax on nine fresh human breast tumor samples by IHC. The effect of sabutoclax on tumor growth was also studied in mouse xenografts.

## Results

We found that sabutoclax showed a significant cytotoxic activity on chemoresistant breast cancer cells both in vitro and in vivo. When chemotherapeutic agents were combined with sabutoclax, strong synergistic antiproliferative effects were observed. Inhibition of BCL-2, MCL-1 and BCL-xL leads to caspase-3/7 and caspase-9 activation, sabutoclax modulated Bax, Bim, PUMA and survivin expression. Furthermore, sabutoclax effectively eliminated the CSC subpopulation and reduced sphere formation of drug-resistant cells through down-regulation of the PI3K/AKT signaling pathway. A similar effect was observed in a panel of nine breast tumors ex vivo.

## Conclusions:

Our findings indicate that sabutoclax partially overcomes drug resistance of breast cancer cells by reactivation of apoptosis, mediates by the inhibition of several anti-apoptotic BCL-2 family proteins such as BCL-2, MCL-1, BCL-XL and BFL-1, and, by abolition of PI3K/AKT pathway and crosstalk between AKT and BCL-2 family proteins is indispensable for maintaining stemness and chemoresistance in BCSCs. This suggests a strong rationale to explore the therapeutic strategy of using sabutoclax alone or in combination for chemotherapy-nonresponsive breast cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-04-01

Functional and therapeutic significance of *ESR1* fusions in metastatic ER+ breast cancer

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**Background.** Next-generation sequencing methods have identified several *ESR1* fusion genes in treatment refractory ER+ breast cancer, however detailed functional studies in experimental models are lacking and how they might be targeted remains poorly understood. We recently reported two transcriptionally active, in-frame *ESR1* fusions, ESR1-YAP1 and ESR1-PCDH11X, identified in a small cohort of metastatic ER+ cases, that induce not only pan-endocrine therapy resistance but also metastatic disease progression (Lei et al., Cell Reports, *in press*). Limited characterization of ESR1-DAB2 and ESR1-GYG1, also identified in metastatic ER+ disease from a recent study, suggests these two *ESR1* fusions also drive estrogen-independent gene activation (Hartmaier et al., *Annals of Oncology*, 2018). Here, we functionally characterize ESR1-DAB2 and ESR1-GYG1 along with additional *ESR1* fusions discovered in metastatic ER+ breast tumors to further support a causal role for in-frame *ESR1* fusions in driving endocrine therapy resistance and promoting metastasis-associated biology, and explore therapeutic vulnerabilities induced by *ESR1* fusion gene formation.

**Methods.** RNA-seq identified *ESR1* fusions from treatment refractory, ER+ metastatic breast tumors. In-frame *ESR1* fusion constructs were generated and stably expressed in ER+ breast cancer cell lines: T47D, MCF7, and ZR75-1. Estrogen-independent and fulvestrant-resistant growth was monitored in hormone-deprived stable cell lines. mRNA-qPCR was performed to examine expression of estrogen responsive and epithelial-to-mesenchymal transition (EMT) genes. *In vitro* sensitivity to CDK4/6 inhibition was tested with palbociclib and abemaciclib.

**Results.** In addition to previously described ESR1-YAP1, ESR1-PCDH11X, ESR1-DAB2, and ESR1-GYG1, that follow a pattern retaining the first 6 exons of *ESR1* (ESR1-e6) fused in-frame to C-terminal sequences provided by the partner gene, additional in-frame ESR1-e6 fusions, ESR1-PCMT1, ESR1-ARNT2, and ESR1-ARID1B, all identified in metastatic ER+ samples, were found to follow the same fusion pattern. ESR1-DAB2 and ESR1-GYG1 produced stable *ESR1* fusion proteins in ER+ breast cancer cell lines. In T47D, these two fusions drove estrogen-independent and fulvestrant-resistant growth. In addition, T47D and ZR75-1 models revealed that ESR1-DAB2 drove estrogen-independent expression of estrogen responsive genes and also EMT genes, including *SNAIL1*, suggesting this fusion, like ESR1-YAP1 and ESR1-PCDH11X, could also drive metastasis. Treatment with CDK4/6 inhibitors suppressed growth induced by ESR1-DAB2 and ESR1-GYG1.

**Conclusion.** The majority of in-frame *ESR1* exon 6 fusions found in metastatic ER+ breast are transcriptionally active, drive endocrine therapy resistant proliferation, and induce an EMT-like transcriptional program. The ability to block *ESR1* fusion induced growth with a CDK4/6 inhibitor is clinically significant as *ESR1* fusion gene formation renders ER insensitive to all endocrine therapies that target the ligand binding domain. Furthermore, clinical diagnosis of an active *ESR1* fusion could potentially stratify patients for CDK4/6 inhibitor treatment. This presentation is the most complete description of the role for *ESR1* fusions in endocrine therapy resistance and metastasis described to date.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-02

Progesterone receptor (PR) antagonism by telapristone acetate (TPA): A randomized, placebo-controlled phase IIB pre-surgical window trial in women with stage 0-II breast cancer

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**Background:** In vitro and preclinical data indicate that TPA, a selective PR modulator, has activity against hormone-sensitive early breast cancer. We conducted a pre-surgical window trial of oral TPA in Stage 0-II breast cancer to assess the effect of TPA on suppression of cell proliferation (Ki67), and on differential gene expression in responsive and non-responsive tumors.

**Methods:** We enrolled 70 pre and postmenopausal women into a 1:1 randomized, double-blind, placebo-controlled trial of oral TPA 12mg (Repos Therapeutics Inc.) for 2-10 weeks. The primary endpoint was Ki67 labelling, comparing diagnostic core needle biopsy to post-therapy surgical specimens. Ki67 changes were quantitated by dual immunohistochemistry (Ki67/pan-cytokeratin) and image analysis (Aperio ImageScope and Definiens Tissue Studio®). RNA-sequencing (using RNA extracted from the paraffin blocks) was performed with Illumina TruSeq RNA Coding Access method. Differential gene expression pre-post therapy was assessed, followed by Gene Set Enrichment Analysis for pathway analysis. Ki67 changes from baseline were tested with Paired signed-rank test. For gene expression analysis, p values were calculated by Wald test and adjusted for multiple comparisons by Benjamini-Hochberg method (adjusted p <0.05 and 2-fold gene expression cut-off).

**Results:** Among 61 evaluable women, (29 placebo and 32 TPA) 97% of tumors were ER or PR positive and 91% were ER and PR positive (balanced across arms). A significant 6% decrease in mean %Ki67 was seen in the TPA arm (p= 0.003). When stratified by menopause, the significance held in premenopausal women (n= 22, p= 0.03) but not in postmenopausal women (n=10, p= 0.08). However, a Ki67 decrease (4%) was also observed in placebo group (p = 0.04); this was non-significant after pre- postmenopausal stratification. Overall, differential gene expression analysis showed no significant modulation of genes in either group. Using a pre-specified response parameter (50% relative reduction in Ki67), we identified 12/32 (38%) “responders” in the TPA, and 9/29 (31%) in the placebo arm. In sub-group analysis of these responders, we found 103 genes to be significantly modulated by treatment in the TPA “responders”, but saw no significant change in any gene expression in placebo “responders”. Gene set enrichment analysis for the 103 genes showed that TPA blocked the progression of cell cycle genes (PTTG1, PLK1, UBE2C, HIST1H3F, PSMD3, and etc.) and suppressed PGR and ERBB2 expression. In a pre-planned pooled analysis, these results will be combined with NCT02314156, reported in SABCS abstract 851790.

**Conclusions:** An anti-proliferative (Ki67) signal of TPA was observed in early stage breast cancer patients, but interpretation was limited by placebo group changes. The TPA group demonstrated differential suppression of proliferation-related genes among Ki67 responders, but the placebo group did not. Ongoing analysis will examine signatures related to stemness, metastasis, and immune suppression (potentially better endpoints in trials targeting P signaling). These analyses may help us select the right population and the right biomarkers for future trials.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-03

Molecular characterisation of ER+ breast cancer dormancy and acquired resistance using a clinical model: Potential involvement of epigenetic regulation

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**Background:** The risk of recurrence for oestrogen receptor positive (ER+) breast cancer patients treated with 5 years of adjuvant endocrine therapy persists for many years or even decades following surgery and apparently successful adjuvant therapy. This period of dormancy and acquired resistance is inherently difficult to investigate. Therefore, previous efforts have been limited to *in vitro* or *in vivo* approaches. In this study sequential tumour samples from patients receiving extended neoadjuvant endocrine treatment were characterised as a novel clinical model of ER+ breast cancer dormancy and acquired resistance.

**Methods:** Consecutive tumour samples from 62 patients undergoing extended (4-45 months) neoadjuvant letrozole therapy were subjected to transcriptomic and proteomic analysis, representing pre- (before treatment), early-on (13-120 days) and long-term (>120 days) neoadjuvant letrozole treatment. Patients with at least a 40% initial reduction in tumour size by 4 months of treatment were included. Of these, 42 patients with no subsequent progression were classified as “dormant”, and the remaining 20 patients as “acquired resistant”. Expression analysis was performed by using Illumina BeadChips. R and BioConductor packages were used for analysis. Differentially expressed genes were determined by using paired Rank Products (FDR, 5%).

**Results:** Multidimensional scaling using most variant 500 genes demonstrated that long-term treated dormant samples clustered separately from their matched pre- and early-on samples whereas long-term treated resistant samples were indistinguishable from their pre-treatment counterparts. Therapy-induced changes in resistant tumours were common features of treatment, rather than being specific to resistant phenotype. Comparative analysis of long-term treated dormant and resistant tumours highlighted changes in epigenetics pathways including DNA methylation and histone acetylation. DNA methylation marks 5-methylcytosine and 5-hydroxymethylcytosine were significantly reduced in resistant tumours compared to dormant tissues after extended letrozole treatment. Decrease in 5-hydroxymethylcytosine were significant early-on.

**Conclusions:** This is the first patient-matched gene expression study investigating long-term aromatase inhibitor-induced dormancy and acquired resistance in breast cancer. Dormant tumors exhibit distinct molecular changes under extended treatment whereas acquired resistant tumors are more similar to matched diagnostic samples supporting the molecular concordance between primary tumors and metastases. Global loss of DNA methylation was observed in resistant tumours under extended treatment which can be predicted within first 4 months of therapy. Epigenetic alterations may lead to escape from dormancy and drive acquired resistance in a subset of patients supporting a potential role for therapy targeted at these epigenetic alterations in the management of endocrine resistant breast cancer.

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## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-05

Glucocorticoid receptor activation inhibits proliferation of endoxifen resistant breast cancer cells and resensitizes cells to hormonal therapy

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**Background:** Despite the prevalent treatment options for ER $\alpha$ -positive breast cancer patients, and their initial efficacy for many women, ER $\alpha$ -positive disease still accounts for more breast cancer related deaths than any other subtype. Relapse in these patients is largely due to the development of resistance to anti-estrogen therapies such as tamoxifen. While tamoxifen and its resistance mechanisms have been extensively studied from both the bench and the bedside, relatively little is known about its active metabolite endoxifen. Our group has provided evidence that endoxifen is the most potent and clinically relevant metabolite of tamoxifen, suggesting that its characterization may be crucial to understanding tamoxifen resistance.

**Methods:** We have developed novel endoxifen resistant MCF7 and T47D cell lines through chronic exposure to endoxifen during a period of 12-24 months. Using these models and their respective controls, we compared global gene expression profiles of endoxifen resistant cells to tamoxifen resistant cells and found marked differences between the two models. Additionally, we subjected treatment naïve cells to a genome-wide, CRISPR-mediated knockout screen to identify genes, and their associated pathways, that are likely involved in mediating endoxifen resistance.

**Results:** Analysis of CRISPR guide RNAs enriched or depleted in response to chronic endoxifen treatment revealed that disruption of genes regulated by dexamethasone (Dex), a potent glucocorticoid receptor (GR) agonist, enhanced cells' ability to survive and proliferate in the presence of endoxifen. These data suggest that GR activation may inhibit endoxifen resistance, and that treatment of resistant cells with Dex may restore endoxifen efficacy. Indeed, Dex treatment significantly inhibited the proliferation rates of endoxifen resistant cells by 50-60% with little to no inhibitory effects in endoxifen sensitive models. Further, Dex was shown to synergize with endoxifen in resistant cells to further suppress cell proliferation, implying that Dex treatment could be utilized as an effective therapy for endocrine resistant disease. Conditioned media harvested from cells chronically exposed to Dex also resulted in substantial inhibition of endoxifen resistant cell proliferation rates. To explore potential mechanisms of these effects, we performed RNA-seq on both treatment-naïve and endoxifen resistant cells following Dex treatment. Out of 246 genes significantly regulated by Dex in endoxifen resistant cells, we identified 61 genes that were not differentially regulated in treatment naïve cells. These genes may provide insights into the mechanisms of GR activity specific to endoxifen resistant cells.

**Conclusions:** To our knowledge, we have developed the first models of endoxifen resistance and have demonstrated that global transcriptomic changes that occur during this process are substantially different than those observed in tamoxifen resistant models. We have shown that activation of GR signaling elicits significant growth-inhibitory effects specifically in the setting of endoxifen resistance. These data identify the GR pathway as a potential novel therapeutic target for the treatment of endocrine resistant breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-07

**GDC-9545: A novel ER antagonist and clinical candidate that combines desirable mechanistic and pre-clinical DMPK attributes**

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ER+ breast cancers depend on ER signaling throughout disease progression, including after acquired resistance to existing endocrine agents, providing a rationale for further optimization and development of ER-targeting agents. Fulvestrant is unique amongst currently approved ER ligand therapeutics due to its classification as a full ER antagonist, which is thought to be achieved through degradation of ER $\alpha$  protein. However, the full clinical potential of fulvestrant is believed to be limited by poor physiochemical properties and exposure limitations due to its administration by intramuscular injection.

Strategies to generate orally bioavailable molecules that retain fulvestrant's full antagonist profile but with considerably improved drug-like properties are thus being widely employed to identify next generation ER therapeutics. However, we find that therapeutic candidates that have recently emerged from prospective optimization of ER degradation, including GDC-0810 and GDC-0927, are not mechanistically equivalent. GDC-0810, GDC-0927, and fulvestrant display unique profiles in terms of ER degradation, transcriptional phenotypes and anti-proliferative potential across a panel of ER+ breast cancer cell lines. In HCl-011 (ER.WT) and HCl-013 (ER.Y537S) ER+ patient-derived breast cancer xenograft (PDX) models, GDC-0927 achieves more robust transcriptional suppression of ER than GDC-0810, and also and greater efficacy. Although displaying a more desirable mechanistic profile than GDC-0810, GDC-0927 has more rapid clearance and poor oral bioavailability, leading to a high pill burden and potential exposure limitation.

Here, we describe for the first time GDC-9545, in which the distinct liabilities of GDC-0810, GDC-0927 and fulvestrant are addressed. GDC-9545 is a non-steroidal ER ligand that is highly potent in competing with estradiol for binding and in driving an antagonist conformation within the ER ligand binding domain. Like fulvestrant, and displaying some improvements over GDC-0927, GDC-9545 consistently induces ER turnover and drives deep transcriptional suppression of ER, resulting in robust *in vitro* anti-proliferative activity.

GDC-9545 exhibits reduced metabolism and increased oral bioavailability relative to GDC-0927, resulting in an overall improved oral exposure in multiple species. As a result of both its mechanistic pharmacology and improved oral exposure, GDC-9545 can achieve the same degree of anti-tumor activity as GDC-0927 but at 100-fold lower doses in the HCl-013 PDX model. The *in vivo* efficacy of GDC-9545 in this model is greater than GDC-0810 and fulvestrant at clinically relevant exposures. The highly potent *in vivo* efficacy of GDC-9545 likely arises due to the particular combination of high binding potency, full suppression of ER signaling, and an improved DMPK profile when compared to GDC-0927 and fulvestrant. GDC-9545 is currently being evaluated in Phase 1 clinical trials (ClinicalTrials.gov Identifier: NCT03332797).

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-08

Timing provides context for the paradoxical effects of AMPK activation in ER+ breast cancer: Suppressing growing tumors, but promoting dormant tumor cell survival and recurrence

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Precision oncology requires delivering the right drug to the right patient at the right time, but “time” is rarely studied preclinically before a drug enters a particular clinical setting. Despite showing efficacy against recurrent/metastatic solid tumors, drugs sometimes fail to prevent tumor recurrence when given (neo)adjuvantly. The biology and microenvironments of overt tumors likely differ substantially from dormant cancer cells. The development of preclinical models to investigate clinically dormant disease will increase understanding of residual tumor cell biology and enable the development of therapeutics for rational adjuvant trials. Despite treatment with adjuvant anti-estrogen therapies, ~30% of patients with ER+ breast cancer (BC) experience recurrence. In contrast to other BC subtypes, ER+ relapses occur late, often appearing years to decades after initial diagnosis and treatment. This delay suggests that ER+ BC cells can undergo extended periods of clinical dormancy. We developed novel, clinically relevant xenograft models of dormancy in ER+ BC. Low systemic levels of estrogens in mice can be further suppressed by ovariectomy, mimicking the effects of aromatase inhibitor (AI)-induced estrogen deprivation (ED) therapy. In ovariectomized mice, luciferase-labeled MCF-7, HCC-1428, HCC-1500, and MDA-MB-415 cells, as well as the HCI-017 PDX model, formed palpable orthotopic tumors upon 17 $\beta$ -estradiol supplementation. ED induced rapid tumor regression and decreased bioluminescent signal. However, a small proportion of ER+ BC cells survived ED for >1 year in a clinically dormant, growth-suppressed state. This residual cell population stabilized after ~90 days of ED, as evidenced by stabilization of bioluminescent signal. Transcriptional and immunohistochemical analyses revealed significant upregulation of AMP-activated protein kinase (AMPK)- $\alpha$ -2 levels and activity in clinically dormant tumor cells compared to estrogen-driven or acutely ED xenografts. Dormant tumor cells were dependent upon AMPK activity for survival, as short-term pharmacologic inhibition of AMPK reduced residual bioluminescent signal.

Metformin is an AMPK-activating drug approved for the treatment of diabetes. Metformin is currently being tested as an anti-cancer agent in many clinical trials at various points in the disease progression of diverse cancer types. In our models of clinically dormant ER+ BC, AMPK activation via metformin slowed ED-induced tumor regression, promoted residual tumor cell survival, and caused earlier tumor regrowth. In vitro studies indicated that metformin promotes cell survival during ED by enhancing fatty acid  $\beta$ -oxidation. Conversely, metformin treatment slowed estrogen-driven tumor growth, in agreement with prior observations that metformin slows growth of various tumor subtypes. These findings suggest that AMPK activation may be efficacious against growing tumors, but deleterious when used in combination with drugs that suppress tumor growth and induce regression. More broadly, this work highlights the issue that the time in a disease course needs to be considered when testing potential anti-cancer agents such as AMPK modulators.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-09

Genome-wide copy number analysis of cell-free DNA identified frequent chromosome 6q losses in metastatic breast cancer

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## **Background:**

Endocrine-resistant disease remains a leading cause of metastatic breast cancer (MBC) mortality. The molecular basis for this frustrating resistance are diverse and still largely unexplored. Next-generation sequencing analysis of cell-free DNA (cfDNA) offers minimally invasive genomic profiling of tumor alterations without tumor biopsy. Copy number alterations (CNAs) derived from cfDNA could be used to complement the genomic features in addition to actionable mutations, yet little is known regarding CNAs in endocrine-resistant MBC. We sought to analyze the utility of CNAs to monitor endocrine treatment exclusively via cfDNA and determine if CNAs is associated with progression free survival (PFS) in MBC.

## **Methods:**

In this retrospective cohort study, we identified 61 patients with biopsy-proven MBC at a single tertiary care institution who received prior endocrine therapy in the adjuvant or metastatic setting. We performed low-pass whole-genome sequencing of cfDNA from plasma, and CNAs were analyzed by the Ultrasensitive Chromosomal Aneuploidy Detector assay. PFS was measured from the date of new treatment initiation after cfDNA collection to the date of progression or tumor-specific death. PFS analysis was performed using the Cox proportional hazard model.

## **Results:**

The study population consisted of 61 MBC cases (40 hormone receptor-positive [HR+], 24 HER2+) with a median age of 50 years, and 28 of them had no prior MBC therapies. Median follow-up duration was 15.7 months, and median PFS was 7.5 months.

We determined tumor fraction of cfDNA for 45 out of 61 pts (73.8%). Frequent focal gains were identified, including 8p11.23 (13.1%), 8p11.21 (27.9%), 17q21.1 (36.1%), 20q13.11 (13.1%), 11q13.2-13.4 (26.2%), and 7p11.2 (3.3%), where potential oncogene FGFR1, IKBKB, ERBB2, SGK2, CCND1 and EGFR was located. Copy number estimation of HER2 from cfDNA were highly consistent with immunohistochemistry results ( $P < 0.001$ ) and were completely consistent with fluorescence in situ hybridization results.

Patients with high CNAs was predictive of worse PFS (median, 6.6 vs 10.6 months, hazard ratio (HR)= 2.02, 95%CI 1.01-4.04,  $P = 0.047$ ) and inferior objective response of subsequent systemic treatment (33% vs 58%,  $P = 0.030$ ). Especially, for the HR+ patients, patients with no CNA changes experienced a better PFS (HR=3.76, 95% CI 1.39-10.2,  $P = 0.009$ ). In 14 patients with primary resistance to endocrine treatment, CNAs in 6q related with ESR1 were found in 8 patients (57.1%). Meanwhile, 6 out of 14 patients with acquired resistance had CNAs in 6q (42.9%). Notably, in 40 patients with HR+ MBC, 6q loss was exclusively found in patients with endocrine-treated patients. 14 out of 28 patients with endocrine-resistance had 6q loss (50.0%), which was higher than the ones observed in HR+ MBC patients from the TCGA datasets (31%,  $P = 0.024$ ).

## **Conclusions:**

Here, we present the first large-scale CNA profile of patients with endocrine-resistant MBC to our knowledge. CNAs tracking by cfDNA is reliable and predicts worse response of the following systemic therapy. Of interest, a novel loss region, involving chromosome 6 was identified to be related with endocrine resistance, which may warrant further investigation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-10

The role of DAXX on enrichment of breast cancer stem cells from ER+ breast cancer

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**Background:** Resistance to endocrine therapy (ET; tamoxifen or aromatase inhibitors) is a major cause of mortality for women with estrogen receptor-positive (ER+) breast cancer (BrC). One mechanism of ET-resistance is due to heterogeneity of breast tumors, comprised of differentiated cells plus undifferentiated breast cancer stem cells (BCSCs). Survival of BCSCs requires Notch signaling and thus determining how it is activated in response to ET may elucidate a new target of therapeutic significance. We found that expression of the transcriptional repressor, DAXX, was inversely correlated with Notch inhibition in a human pre-surgical biomarker trial of ER+ BrC patients (ClinTrials.gov NCT00756717). Further, it has been demonstrated that expression of a sumoylation enzyme, UBC9, is dependent on ER $\alpha$  activation, and that it sumoylates DAXX. We hypothesized that targeting ER $\alpha$  by ET depletes UBC9 causing destabilization of the DAXX protein, resulting in Notch activation and enrichment of BCSCs.

**Methods:** A panel of ER+ BrC cells were grown in conditions of 5nM estradiol (E2) or ET conditions of E2 deprivation. Expression levels of NOTCH4, other BCSC-associated genes, UBC9 and DAXX were quantified by real-time PCR (RT-PCR). BCSC survival was assessed by the mammosphere forming assay. To test if ET increases BCSC by destabilizing DAXX, DAXX was depleted or overexpressed by cell transfection. To assess the role of DAXX in tumor initiating potential, an extreme limiting dilution assay (ELDA) was conducted by injection of normal or DAXX-depleted cells into the mammary fat pads of female mice. Nuclear levels of DAXX protein were quantified by cell fractionation and enrichment of DAXX at the promoter regions of BCSC-associated genes was assessed by chromatin immunoprecipitation (ChIP). Protein levels of DAXX following UBC9 depletion were determined by cell fractionation.

**Results:** ET or siRNA depletion of DAXX increased survival of BCSCs, and expression of BCSC-associated genes, including NOTCH4. Conversely, DAXX overexpression under ET-conditions inhibited BCSC-associated gene expression and survival. ELDA demonstrated that cells depleted of DAXX had a higher estimated stem cell frequency compared to DAXX-expressing cells. Cell fractionation indicated that DAXX protein was concentrated in the nucleus, and these levels were decreased by ET treatment. ChIP studies revealed an enrichment of DAXX binding at the promoter regions of BCSC-associated genes (NOTCH4, SOX2, OCT4, NANOG), which was lost following ET. UBC9, but not DAXX mRNA levels, are dependent on activation of ER $\alpha$ , and depletion of UBC9 resulted in a decrease in DAXX levels.

**Conclusions:** These results suggest that E2 activation of ER $\alpha$  increases UBC9 levels, allowing for stabilization of nuclear DAXX in ER+ BrC cells, where it represses expression of BCSC-associated genes. Conversely, ET depletes UBC9, destabilizing DAXX, ultimately relieving repression of BCSC-associated genes and promoting increased BCSC survival. This would potentially result in resistance to ET. Thus, new strategies to stabilize DAXX protein during ET may prevent induction of BCSC gene expression and enrichment of BCSCs, improving therapeutic outcomes in ER+ BrC.

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# 2018 San Antonio Breast Cancer Symposium ®

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Non-canonical, clinical ESR1 mutations promote resistance to antiestrogen therapies

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ESR1 hotspot mutations have been identified in 30-40% of patients with ER+ MBC and promote resistance to aromatase inhibitors (AIs). Identification of these mutations has been aided by the use of plasma DNA for their detection, however many such tests only survey for hotspot mutations. In this study, we examined the prevalence, biologic and clinical significance of mutations in ESR1 that lie outside previously described hotspots (E380Q, Y537, D538G). Using next generation sequencing of tumor DNA from over 4000 patients with breast cancer, we have identified numerous somatic alterations in ESR1. Among the somatic alterations were mutations detected in the transcription activation function-1 (AF-1), DNA binding domain, dimerization interface and C-terminus of ER.

We characterized the functional significance of these non-canonical mutations alongside hotspot mutations, starting with assays of ER driven transcription and deduced several classes of mutations: (1) mutations that weakly promote ligand-independent activity, (2) mutations that led to ligand-independent activity comparable to estradiol stimulation, and (3) mutations that resulted in impaired transcriptional activity. Class 2 mutations remain localized at amino acids 536-538, while the class 1 mutations are observed in various domains of ESR1, including the DNA binding domain and dimerization interface. Several Class 3 mutations were found in proximal to Helix 12, highlighting the critical role of this region.

Clinically, non-canonical mutations were not exclusively observed among patients treated with AI, as there were several mutations from SERM/SERD treated patients. We thus examined the effects of different mutants on their sensitivity to ER antagonists, such as fulvestrant or tamoxifen. The data revealed key differences between the different classes of mutants; with majority of the class 2 mutants exhibiting reduced sensitivity to the antagonists compared to wild type. This also correlated with the relative binding affinities (RBA) of the mutants to fulvestrant and 4-hydroxytamoxifen, in which the RBA of class 2 mutants (Y537S and D538G) were significantly lower than wild type, perhaps accounting for their reduced sensitivities. Nevertheless, it appeared that all mutants could be effectively inhibited either by higher concentrations or more potent ER antagonists, implying a continued ability to distort ER into the antagonist conformation. Interestingly, several Class 1 mutants showed weak agonism in response to specific antagonists raising the possibility of their role in SERM/SERD resistance. Taken together, the data demonstrated that not all ESR1 mutations affect receptor function or respond to antiestrogen therapies similarly. These data also imply the importance of more broad sequencing coverage of ESR1 in the clinic to effectively capture the spectrum of biologically relevant alleles.

# 2018 San Antonio Breast Cancer Symposium ®

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Splicing factor *ESRP1* controls ER-positive breast cancer progression by altering metabolic pathway genes

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**Background** Epithelial Splicing Regulatory Protein (*ESRP1*) is a key splicing factor that regulates Epithelial-to-Mesenchymal Transition (EMT) splicing program. Our previous study demonstrated that high levels of *ESRP1* are associated with poor prognosis in human ER-positive (ER+) breast tumors in an independent manner of EMT process. We next explored the potential mechanisms that contribute to the role *ESRP1* in endocrine therapy-resistant breast cancer.

**Methods** Probe based-Human Transcriptome Array 2.0 (HTA; Applied Biosystems/Thermo Fisher) was performed using RNAs from control and *ESRP1* knockdown cells (LCC2 versus 2C3 *ESRP1* and LCC9 versus 9C2 *ESRP1*) of endocrine resistant breast cancer. Functional enrichment analyses were performed using the DAVID functional annotation tool (<http://david.abcc.ncifcrf.gov/>). To confirm the functional importance of *ESRP1* on regulation of cellular metabolism, we have performed experiments that analyze the metabolic substrate flux in response to *ESRP1* knockdown in resistant cells (The Seahorse XFp Cell Energy Phenotype Assay). Differentially expressed genes were validated using Western blotting assay.

**Results** Transcriptome profiling of *ESRP1* in 2C3 and 9C2 knockdown models revealed differentially expressed genes using HTA 2.0 platform. In LCC2 versus 2C3 *ESRP1* knockdown, expression of 1186 genes (1263 transcripts) have been altered significantly, while 413 genes (432 transcripts) have been significantly regulated in LCC9 versus 9C2 *ESRP1* knockdown with FDR<0.1. Of these significant genes, 34 downregulated and 68 upregulated (102 genes total) were shared by both 2C3 and 9C2 *ESRP1* knockdowns. Using the DAVID Functional Annotation Clustering Tool, we identified the biological processes altered significantly in response to *ESRP1* knockdown. The most significant annotation clusters downregulated in *ESRP1* knockdown consists of fatty acid metabolism/lipid metabolism (*SCD*, *ACACA*, *FASN*, *ACAT2*, *PLCH1*, and *HPGD*), and oxidoreductase processes (*SCD*, *PHGDH*, *FASN*, *DHTKD1* and *HPGD*). We confirmed the altered metabolic function using the Seahorse analyzer. These analyses confirmed that *ESRP1* knockdown altered the glycolysis rate (extracellular acidification rate; ECAR) in both tamoxifen-resistant and fulvestrant-resistant models. In addition, *ESRP1* knockdown decreased the mitochondrial respiration in tamoxifen-resistant cells, but not fulvestrant resistant cells. Using Western blotting, we validated the altered levels of fatty acid synthase (*FASN*) and Stearoyl-CoA desaturase 1 (*SCD1*), key enzymes in fatty acid metabolism. Phosphoglycerate Dehydrogenase (*PHGDH*), a poor prognosis marker in cancers including breast cancer, was also downregulated in response to *ESRP1* knockdown. Taken together, we have demonstrated a novel functional impact of *ESRP1* on the regulation of tumor growth at the functional and molecular level independent of EMT.

**Conclusions** For the first time, we show the role of *ESRP1* in altering the cellular metabolism thereby contributing to tumor growth. The study provides a molecular evidence for the role of altered metabolism in determining adverse prognosis of ER+ breast cancer via the control of *ESRP1*, a splicing factor. Further studies to determine the therapeutic value are underway.

# 2018 San Antonio Breast Cancer Symposium ®

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Tracking ESR1 mutation clonal evolution in breast cancer using *in situ* mutation detection

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**Background:** Approximately 70% of breast cancers (BCs) are estrogen receptor positive (ER+). Not all ER+ cancers respond to endocrine therapy (ET) and many eventually develop acquired resistance. Next-generation sequencing (NGS) has shown ESR1 mutations (ESRM) are present in 10-50% of recurrent/metastatic cancers treated with aromatase inhibitors (AIs). Many of these mutations are located in the ligand-binding domain of ER, so they can lead to constitutive activation. This suggests ESRMs are a major mechanism of acquired resistance to endocrine therapy (ET) and numerous studies have shown a link between ESRMs and reduced sensitivity to 2<sup>nd</sup> line ET. The aim of this project was to investigate the incidence and clonal evolution of common ESRMs in BCs resistant to multiple sequential ETs using NGS, as well as novel PCR and *in situ* mutation detection methods.

**Methods:** We have optimised an allele-specific real-time PCR (rtPCR) assay and an *in situ* mutation detection method (ER-ISMD) for the assessment of ESRMs. Both have been designed to identify a missense gain-of-function D538G mutation with a single nucleotide-resolution in formalin-fixed paraffin-embedded (FFPE) BC tissues.

Two unique cohorts have been studied:

(A) 20 post-menopausal women (PMW) with ER+ BC who acquired resistance to letrozole and were treated with up to 4 subsequent lines of ET. Serial RNA and DNA from 3-5 cancer samples per patient (58 samples from 20 patients) were analysed by Ribo0-RNAseq, DNA exome sequencing, rtPCR and ER-ISMD.

(B) 150 PMW with ER+ BC who developed local (n=79), lymph node (n=59) or distant (n=12) recurrences on 1<sup>st</sup> line adjuvant letrozole, anastrozole or tamoxifen. Of these, 48 patients developed subsequent recurrences on 2<sup>nd</sup> line ET. Tissue samples from each recurrence and matched primary BC were collected.

**Results:** In cohort A, 5/20 patients (20%) had expansion of a D538G ESR1 mutation clone at time of resistance 1<sup>st</sup> line ET (3:letrozole, 1:anastrozole, 1:tamoxifen). The mutant allele frequency (MAF) increased further in the 4 BCs treated with 2<sup>nd</sup> line ET (2:tamoxifen, 2:exemestane) and further still in the 1 BC who received 3<sup>rd</sup> line exemestane. 0/6 patients with ESRM responded to subsequent ET. Allele-specific rtPCR and ER-ISMD have been used to validate these findings and also identified low frequency ESRM clones in the sequential samples prior to the development of clinical resistance, that were not reported by NGS. Both methods have also been applied to screen tissues from patients in cohort B, where ESRMs have also been identified in recurrent samples. Complete analysis is currently ongoing.

## Conclusions:

- ESRMs develop and expand in some BCs as a mechanism for acquired resistance to ET and are associated with a lack of response to subsequent standard ETs.
- Allele-specific rtPCR can detect ESRMs and is more cost-effective and easier to use than NGS for ER mutation analysis.
- Some ESRMs predate clinical resistance.
- ER-ISMD is a novel approach that allows for identification and visualisation of the distribution of mutant clones in morphologically intact FFPE tissue.
- ER-ISMD has the potential to become a clinically useful tool to help direct the use of 2<sup>nd</sup> line ET in routine care.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-15

Enhancing response to estrogen therapy in ER+ breast cancer with novel scheduling and dosing regimens

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Estrogen therapy was historically used as a standard breast cancer treatment from the 1940s until the introduction of tamoxifen in the 1970s. Although a clinical trial directly comparing the synthetic estrogen diethylstilbestrol and tamoxifen in post-menopausal patients demonstrated no significant difference in progression-free survival between the two, tamoxifen showed a more favorable toxicity profile and estrogens fell out of favor. However, 1 in 3 patients eventually recur and develop advanced disease that is resistant to all available anti-estrogens. Estrogen therapy is being resurrected in clinical trials for advanced, heavily pre-treated ER+ breast cancer. These studies demonstrate that ~30% of patients respond to treatment with estrogens. However, little pharmacologic work has been done to optimize the schedule and method of hormone delivery.

Estrogen therapies such as 17 $\beta$ -estradiol (E2) and ethinylestradiol are typically administered orally several times daily. Toxicities have been observed with such regimens, and there is no evidence that the maximally efficacious doses or schedules are being used clinically. We used two *in vivo* tumor models to preclinically define the optimal dosing schedule and method of E2 delivery that maximizes response. WHIM16 patient-derived ER+ breast cancer xenografts (PDX) and C7-2-HI ER+ murine mammary adenocarcinoma allografts both grow in ovariectomized ("estrogen-deprived") mice, and regress in response to E2 treatment. We tested an array of E2 doses and delivery methods [*e.g.*, oral gavage, subcutaneous (s.c.) injection, s.c. pellet, and s.c. osmotic pump] in mice bearing C7-2-HI and WHIM16 tumors to determine optimal methods for inducing maximal and sustained tumor regression. We found that oral dosing is less effective at inducing tumor regression than other methods. However, intermittent high-dose estrogen given as 1 mg of E2 orally every 14 days both prevented tumor growth and did not result in toxicity, suggesting that intermittent dosing should be further examined as a means to increase therapeutic index.

Although estrogen therapy induces clinical response in a subset of patients with ER+ disease, nearly all patients eventually experience disease progression and develop resistance to the therapeutic effects of estrogen. Clinical observations suggest that some cancers that progressed on estrogen therapy are re-sensitized to anti-estrogen treatment. We therefore examined whether resistance to estrogen therapy could be prevented by preemptively cycling estrogen therapy with estrogen deprivation in mice. Discontinuous scheduling of E2 and estrogen deprivation was tested with either 1 or 4 weeks on E2 treatment, followed by estrogen deprivation until tumors re-grew to baseline volume, then E2 treatment was repeated. In the WHIM16 tumor model, the 4-week E2 treatment cycle nearly doubled time to recurrence compared to mice treated continuously with E2. Surprisingly, there was no difference in time to recurrence between groups treated with the 1-week cycle vs. continuous E2. However, tumors that re-grew on continuous E2 were sensitized to estrogen deprivation. These collective findings warrant clinical testing of schedules of alternating estrogen and anti-estrogen therapies.

# 2018 San Antonio Breast Cancer Symposium ®

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Total estrogenic activity during neoadjuvant therapy with letrozole and exemestane – An intra-patient cross-over comparison using the AroER tri-screen

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**Background.** Aromatase inhibitors (AIs), letrozole (Femara<sup>®</sup> / Femara<sup>®</sup>) and exemestane (Aromasin<sup>®</sup>), are widely used anti-hormonal drugs for breast cancer. Both compounds strongly reduce circulating estradiol levels in postmenopausal women. In the setting of metastatic breast cancer, these drugs may be used after another, causing new responses in selected patients by switching to the alternative drug after progressing on the first choice. This well-known "lack of cross resistance" has been recognized for some time and is documented by several clinical trials. However, the precise explanation for this clinical observation is still unknown. The availability of mechanistic information may lead to an improved strategy against hormone-sensitive breast cancer.

**Patients and methods.** NEO-LET-EXE was a neoadjuvant, randomized, open-label, intra-patient cross-over trial. Postmenopausal patients with estrogen receptor (ER) positive ( $\geq 50\%$ ), HER-2 negative, locally advanced breast cancer were enrolled. Sequential blood samples (obtained at baseline, after 2 months and 4 months of treatment) were available from 29 patients. All patients were randomized to sequential treatment starting with either letrozole (2.5 mg o.d.) or exemestane (25 mg o.d) for 2 months followed by another 2 months on the alternative compound. The total estrogenic activities in the collected blood samples were determined using AroER tri-screen assay developed by the Chen laboratory. The assay utilizes MCF-7aro ERE cells which contain both aromatase and ER. The samples were assayed in the presence as well as the absence of letrozole, to estimate relative contributions of estrogen and estrogen+androgen to the activities, respectively.

**Results.** Using the highly sensitive AroER tri-screen assay, estrogenic activity were detected at three time points in all blood samples. Importantly, a significantly higher total estrogenic activity was found during therapy with exemestane compared to letrozole in 23 out of 26 patients. Only in three patients, the activity was higher during therapy with letrozole compared to exemestane. When letrozole was included in the assay, the estrogenic activities in most samples collected during exemestane treatment were further reduced, suggesting that low levels of androgen were present in samples from exemestane treatment. Four samples collected after exemestane treatment and three after letrozole treatment had higher activities than baseline samples when assay was carried out with letrozole.

**Discussion.** Our results suggest the AroER tri-screen to be a very sensitive method to estimate the overall estrogen-mediated activity in human samples. Significant higher levels of estrogenic activity in human serum were observed during exemestane than those during letrozole treatment. Our observations, that additional letrozole could reduce further the estrogen activity in the exemestane-treated samples, demonstrate probably residual aromatase activity during therapy with exemestane alone. In addition to distinguish the effects of exemestane and letrozole, our results also demonstrate that the assay can also potentially detect the effects of estrogenic mimics.

# 2018 San Antonio Breast Cancer Symposium ®

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Local network topology differences between early and late recurrence in ER+ breast cancers

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**Background:** Late recurrence is characteristic of ER+ breast cancers. Despite an apparently effective adjuvant endocrine therapy, many breast cancers recur years after their initial endocrine treatment. Why some tumors recur early (<3 years) and some recur later (>5 years) is poorly understood. If systemic endocrine therapies killed all cells, recurrence would reflect only the appearance of new disease. Thus, we hypothesized that cells that survive and lie dormant may be driven, in part, by altered wiring of their cell death signaling. We, therefore, studied how cell death signaling is differentially wired in primary tumors that will recur early versus those that will recur later.

**Method:** Genes involved in apoptosis, autophagy, ferroptosis, necrosis, and pyroptosis were identified from KEGG to initiate network feature analysis of gene expression data from public and our first in-house gene expression dataset. Data were collected from ER+ breast cancer pre-endocrine treatment samples with up to 20 years follow-up. Publicly available datasets used were GSE6532, GSE2034, GSE7390, GSE17705, GSE12093, and TCGA. We applied our Knowledge-fused Differential Dependency Network (KDDN) analysis tool to the public datasets; KDDN has provided powerful new insights into signaling in breast and other cancers. Common gene-gene interactions (edges) predicted in at least two different datasets were extracted from all KDDN analyses results. To strengthen the relevance of these features, predicted network edges that represent known protein-protein interactions (PPI) were identified from the STRING database, and these edges were noted in the signaling graphs. Final network graphs were constructed using the common edges from all overlaid networks. We conducted IPA analysis on all nodes in the final network and selected those incorporating network hubs. We took a similar approach to our second in-house dataset, which we used for independent testing. Here, patients were included if their tumor exhibited an initial reduction in volume of at least 40% by four months in response to neo-adjuvant Letrozole. Patients were then classified into two groups during follow-up of up to 3.7 years: i) initial tumor size reduction followed by continued response (expected to recur late); ii) initial reduction followed by tumor regrowth (expected to recur early). KDDN analysis was performed on pretreatment samples from these two groups and a network created annotated with PPI information.

**Results:** MAPK8 and CYCS (Molecular Mechanisms of Cancer,  $p=1.58E-52$ ), TNFRSF1A Neuroinflammation Signaling Pathway,  $p=1.26E-54$ ), RELA, and NFKB1 (Colorectal Cancer Metastasis Signaling,  $p=7.94E-35$ ), were identified as hubs. Hubs may be critical signaling components driving the differences between tumors that will become dormant and recur late. Connections between SLC25A6 and SQSTM1 ( $p = 0.008$ ), BIRC2 and GABARAP ( $p = 0.021$ ) in the early group, and AKT3 and IRS2 ( $p = 0.014$ ) in the late group, were shared between the two final networks. With longer follow-up time on the second in-house dataset, we will better define the two groups and identify additional common phenotype specific gene-gene interactions.

## 2018 San Antonio Breast Cancer Symposium ®

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ARV-471, an oral estrogen receptor PROTAC degrader for breast cancer

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ARV-471, an estrogen receptor (ER) alpha PROTAC, is a hetero-bifunctional molecule that facilitates the interactions between estrogen receptor alpha and an intracellular E3 ligase complex, leading to the ubiquitylation and subsequent degradation of estrogen receptors via the proteasome. ARV-471 robustly degrades ER in ER-positive breast cancer cell lines with a half-maximal degradation concentration (DC<sub>50</sub>) of ~ 2 nM. PROTAC-mediated ER degradation decreases the expression of classically-regulated ER-target genes (PR, GREB1, TFF) and inhibits cell proliferation of ER-dependent cell lines (MCF7, T47D). Additionally, ARV-471 degrades clinically-relevant ESR1 variants (Y537S and D538G) and inhibits growth of cell lines expressing those variants. In an immature rat uterotrophic model, ARV-471 degrades rat uterine ER and demonstrates no agonist activity. Daily, oral-administration of single agent ARV-471 (3, 10, and 30 mpk) leads to significant tumor volume regressions of estradiol-dependent MCF7 xenografts and concomitant tumor ER protein reductions of >90% at study termination. Moreover, when a CDK4/6 inhibitor is combined with ARV-471 in the MCF7 model, even more pronounced tumor growth inhibition is observed (~130% TGI), accompanied by significant reductions in ER protein levels. In an ESR1 Y537S, hormone-independent patient-derived xenograft model, ARV-471 at 10 mpk completely inhibited growth and also reduced mutant ER protein levels. Taken together, the preclinical data of ARV-471 supports its continued development as a best-in-class oral ER PROTAC-degrader.

# 2018 San Antonio Breast Cancer Symposium ®

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Preclinical *in vitro* and *in vivo* evaluation of seribantumab, a human epidermal growth factor receptor-3 antibody, combined with standard therapies in hormone receptor-positive and human epidermal growth factor receptor-2-negative breast cancer models

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**Background:** Heregulin (HRG)-induced human epidermal growth factor receptor-3 (HER3) signaling is associated with a poor response to standard-of-care across multiple cancers. Seribantumab is a human monoclonal antibody that targets and downregulates HER3 and blocks HRG from binding to HER3. In a randomized Phase 2 trial in hormone-positive (HR+) and human epidermal growth factor receptor-2-negative (HER2-) metastatic breast cancer (mBC), HRG+ patients appeared to have shortened progression free survival when receiving exemestane monotherapy and benefited from addition of seribantumab to this regimen. Activation of the HRG-HER3 axis has been implicated in mediating resistance to fulvestrant in HR+ HER2- breast cancer cellular models. We investigated the role of HRG-HER3 in mediating resistance to CDK4/6 inhibitors (i.e., palbociclib) alone or in combination with fulvestrant. Additionally, we evaluated *in vitro* and *in vivo* anti-tumor activity of seribantumab with these therapies and elucidated potential mechanisms of action (MoA).

**Methods:** *In vitro* growth inhibition and MoA were investigated primarily in a HR+ HER2- MCF7 cell line by CellTiter-Glo viability assay and cell cycle profiling. *In vivo* anti-tumor activity of seribantumab plus fulvestrant and/or palbociclib was assessed in the orthotopic MCF7 mouse model.

**Results:** HRG desensitized MCF7 cells to *in vitro* anti-tumor effects of fulvestrant and CDK4/6 inhibitors, whereas HRG-HER3 inhibition by seribantumab restored this sensitivity through the inhibition of HRG-mediated retinoblastoma (RB) protein phosphorylation and G1-S progression. CDK4/6 inhibitor treatment resulted in increased HRG mRNA expression suggesting that HRG-HER3 might serve as an escape route from CDK4/6 inhibition. In the *in vivo* MCF7 mouse model, seribantumab potentiated the anti-tumor activity of fulvestrant and CDK4/6 inhibitor, consistent with *in vitro* findings.

**Conclusion:** HRG-HER3 signaling impairs the activity of CDK4/6 inhibitors alone or in combination with fulvestrant in preclinical HR+ HER2- breast cancer models. HER3 blockade by seribantumab restores the drug sensitivity. This study provides rationale for the ongoing SHERBOC study (NCT03241810) testing seribantumab in combination with fulvestrant in postmenopausal women with HRG+ HR+ HER2- mBC who have received prior CDK4/6 inhibitor.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-20

Targeting CDK4/6 and IGF1R or insulin receptor synergistically inhibits growth of endocrine sensitive and endocrine resistant breast cancers

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Estrogen receptor positive (ER+) breast cancers are treated with hormonal therapies such as tamoxifen or aromatase inhibitors. More than 30% of patients with early-stage ER+ breast cancer treated with endocrine therapy will relapse and all patients with metastatic breast cancer expressing ER eventually acquire resistance to hormonal therapy. Endocrine therapy resistant patients therefore require novel therapeutic options. Insulin-like growth factors (IGFs) and insulin signaling via the type I IGF receptor (IGF1R) and insulin receptor (IR) respectively regulate breast cancer biology. Unfortunately, IGF1R targeted therapies failed to show a benefit in prolonging either disease-free or overall survival in clinical trials. In pre-clinical models acquired resistance to tamoxifen results in loss of IGF1R and enhanced sensitivity to IR signaling. Inhibition of mTOR alone relieves the negative feedback loop regulating levels of the adaptor protein IRS-1, which mediates proliferative effects of IGFs and insulin and enhanced phosphorylation of Akt. The ribosomal protein S6 kinase (S6K) phosphorylates IRS-1 on serine residues targeting it for proteasomal degradation, and this negative feedback regulation is important in attenuating IGF and insulin signaling. Cyclin dependent kinases (CDKs) 4 and 6 are required for cell cycle progression. CDK4/6 inhibitors have recently been approved for treatment of ER+, Her2- advanced breast cancers and these such as palbociclib and abemaciclib block phosphorylation of retinoblastoma. IGFs and insulin stimulate cell cycle progression and increase cyclin D1 levels in breast cancers. Therefore, we hypothesized that CDK4/6 inhibition combined with IGF1R or IR targeting, to block mitogenic functions of IGF or insulin signaling, could be a viable therapeutic option in endocrine sensitive and endocrine resistant breast cancer, respectively. Parental MCF-7 and T47D were more sensitive to palbociclib compared to matched cells with acquired resistance to tamoxifen, MCF-7/TamR and T47D/TamR. Palbociclib also blocked IGF-I and insulin stimulated entry into cell cycle leading to G0/G1 arrest in ER+ breast cancer cells. Unlike mTOR inhibitors that upregulated IRS-1 levels leading to increased phosphorylation of Akt through IGF1R/IR, palbociclib did not affect IRS-1 levels and did not enhance phosphorylation of Akt in ER+ breast cancer cells. Combination of palbociclib with an IGF1R inhibitory antibody (huEM164), but not IR antibody (83-7), was better at inhibiting growth of endocrine sensitive MCF-7 and T-47D cells than either drug alone. Further, in a formal synergy study using the method of Chou-Talalay, the combination index of palbociclib and the IGF1R antibody was <1 for MCF-7 parent cells, indicating the two drugs synergistically inhibit growth. Combination of palbociclib with an IR antibody synergistically inhibited the growth of endocrine resistant MCF-7/TamR cells. Our data show that cotargeting CDK4/6 and IGF1R is more effective in endocrine sensitive but cotargeting CDK4/6 and IR is more effective in tamoxifen resistant breast cancer cells. These data indicate that targeting CDK4/6 and IR could be a therapeutic option for patients with endocrine resistant disease.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-21

FGFR4 is a novel druggable target for recurrent ER-positive breast cancers

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## Background

Breast cancer recurrence is a major clinical problem for estrogen receptor positive (ER+) disease, even decades after initial surgery. These long-term recurrences are a challenge for invasive ductal carcinoma (IDC), and are particularly frequent for the histological subtype of invasive lobular carcinoma (ILC). To study the long-term endocrine resistance seen in ILC patients, our lab recently generated six long-term estrogen deprivation (LTED) models of ILC cells and performed RNA-Sequencing to identify differentially expressed genes that ostensibly allow these cells to grow in the absence of estrogen. We overlapped these results with a previously published microarray dataset of tamoxifen-resistant cells, and found that FGFR4 is the most consistently overexpressed gene in the setting of acquired resistance to endocrine therapy in ILC cells. From a recent publication of RNA-Seq from other LTED models, FGFR4 RNA overexpression is also seen in all five IDC cell lines.

## Hypothesis

FGFR4 is an important mediator of acquired endocrine resistance in breast cancer.

## Methods

To study the role of FGFR4 *in vitro*, we used multiple shRNAs and specific small molecule inhibition for growth assays. To study the role of FGFR4 in *de novo* resistance to endocrine therapy, we collected 129 well curated ER+ ILC tumor specimens and performed gene expression analysis on the pre-treatment samples using a custom NanoString panel. To study the role of FGFR4 in acquired resistance, we collected over 50 pairs of primary-metastatic ER+ tumors and performed exon capture based RNA-Sequencing.

## Results

FGFR4 inhibition decreases parental and LTED cell growth in classic 2D conditions and in colony formation assays. The LTED cells, with higher FGFR4 expression, are more sensitive to its inhibition. For the parental cells, combination FGFR4 and ER-targeting drugs results in synergistic decreases in growth. In our database of primary ILC clinical samples, increased expression of FGFR4 is predictive of shorter time to distant recurrence. Among primary-recurrent tumor pairs, FGFR4 is an outlier expression gain in 20/50 (40%), spanning all recurrence sites studied (i.e. local recurrences, and metastases to the brain, bone, ovaries, and GI tract). Finally, in analyzing large cohorts of metastatic tumors, there is a significant enrichment of hotspot FGFR4 mutations in tumors originating in the breast, with >2% of metastatic ILC tumors containing such a mutation.

## Conclusion/Future studies

FGFR4 may play an important role in *de novo* resistance to endocrine therapy in ILC and acquired resistance in both ILC and IDC. Ongoing studies include overexpression of wild-type and FGFR4 hotspot mutations in ILC and IDC cell lines to determine growth and metastatic phenotypes.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-23

Enhancing the activity of a novel estrogen receptor coregulator binding modulator (ERX-11) against ER-positive therapy resistant breast cancer

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**Background:** We had previously reported a novel small molecule, ERX-11, that directly interacts with ER and blocks the interaction between a subset of coregulators with both native and mutant forms of ER. ERX-11 effectively blocks ER oncogenic signaling and has potent anti-proliferative activity against therapy-sensitive and therapy-resistant human breast cancer cells. To enhance the clinical translation of ERX-11, we sought to pursue both lead optimization and evaluate combinations of ERX-11 with other approved agents in breast cancer.

**Methods:** We designed, synthesized and tested 500 derivatives of ERX-11 in multiple models of ER+ breast cancer. We also tested combinations of ERX-11 with multiple agents, including other ER targeting agents, chemotherapies and CDK4/6 inhibitors. We tested the effect of combination therapy using breast cancer cells with acquired resistance (Tamoxifen, Letrozole, Ribociclib resistant) and engineered models that express ER mutations. *In vitro* activity was tested using Cell titer glo, MTT, and apoptosis assays. Mechanistic studies were conducted using Western blot, reporter gene assays and RNA-seq analysis. Xenograft, patient derived xenograft (PDX), patient derived explant (PDE) and xenograft derived explant (XDE) models were used for preclinical evaluation and toxicity.

**Result:** Evaluation of 500 analogs of ERX-11 identified a number of leads with differential activity against ER+ and ER- breast cancer cells, identified several analogs including ERX-144, 208, 296, 315 with nanomolar potency against ER+ and therapy-resistant ER+ breast cancers. Validation of the mechanism of action of these analogs is ongoing. The combination of ERX-11 and palbociclib significantly blocked ER-mediated and ER-coregulators mediated oncogenic signaling and showed potent anti-proliferative activity against both endocrine therapy-sensitive and resistant breast cancer cells. In addition, ERX-11 inhibited ribociclib-resistant ER+ cell proliferation in a dose dependent manner. Mechanistic studies using IP-Mass spectrometry demonstrated that ERX-11 and palbociclib blocks the interaction between larger subset of coregulators with ER in therapy resistant breast cancer models. ERX-11 and palbociclib both exhibited potent anti-proliferative activity against therapy-sensitive and therapy-resistant ER+ve breast cancer cells, in xenograft models and in PDEs. Importantly, combination therapy of ERX-11 and palbociclib synergistically reduced the growth of tamoxifen and letrozole resistant xenograft tumors compared to either drug alone. Mass spec based DIA analyses and RNA-seq studies revealed that combinational treatment uniquely activated p53, unfolded response mediated apoptotic pathways, altered DNA damage response and suppressed E2F and Myc target genes. Biochemical studies confirmed combination therapy significantly altered E2F1, ER and DNA damage response pathways.

**Conclusion:** We have successfully pursued two avenues to improving ERX-11 for clinical translation. We have developed ERX-11 analogs with higher potency against ER+ breast cancer. We have shown that combinational treatment with ERX-11 and palbociclib may overcome endocrine therapy resistance and CDK4/6 inhibitor (ribociclib) resistance.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-24

Molecular stratification of ER+/HER2- breast cancer cell lines to predict sensitivity to targeted agents

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**Background:** Approximately 70% of all breast cancers are estrogen receptor positive (ER+) at diagnosis and are dependent on estrogen signaling for tumour growth and proliferation. Some ER+ breast cancers can be effectively treated with adjuvant endocrine therapies including tamoxifen, but despite favorable improvements in overall survival, resistance to endocrine therapy is common and has been associated with dysregulation of several signaling pathways. These pathways can be targeted with specific inhibitors, many of which are currently under clinical investigation. However currently there is a lack of predictive biomarkers to identify which patients should receive treatment with targeted therapy. The goal of this study was to determine whether alterations in specific signaling pathways can be identified and used to stratify breast cancer cell lines to the most effective experimental treatments.

**Methods/Results:** Fifteen ER+/HER2- cell lines were characterized using a NanoString PAM50-like assay as well as next generation sequencing and were then stratified according to alterations in three key signaling pathways: CCND/CDK, PI3K/AKT/mTOR and FGFR. High-throughput small-molecule screenings were performed to identify the IC50 values of 24 inhibitors across the strata. Variation in inhibitor sensitivity was observed between cell lines based on molecular alterations. Cell lines with a *PIK3CA* mutation in combination with a CDK-pathway alteration were more sensitive to CDK inhibitors (50 to 120nM) than cell lines with alterations in the CDK-pathway alone or *PIK3CA* mutations alone (170nM to >5000nM). In addition, cell lines with the dual alterations demonstrated stronger synergy between CDK and PI3K-pathway inhibitors compared to either alteration alone.

**Conclusions:** The results suggest that stratification according to molecular alterations in specific signaling pathways may predict sensitivity to targeted inhibitors in a panel of ER+/HER2- luminal breast cancer cell lines. Work is ongoing to identify the optimal synergistic inhibitor combinations for each strata. The ultimate goal is to translate this work into a novel personalized medicine approach, using molecular stratification based on a combination of molecular events in a functional pathway as opposed to single genes.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-25

Characterization of FGFR1/2 genetic alterations reveals novel fusions of FGFR1 in Chinese breast cancer

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**Background:** Deregulation of fibroblast growth factor receptor 1 (FGFR1) signalling has been extensively studied in various tumor types, and has been implicated in driving endocrine resistance in breast cancer. Genetic alterations of FGFR1, especially FGFR1 amplification, is one of particularly important mechanisms leading to enhanced FGFR signaling in breast cancer. However, the prevalence of FGFR1/2 genetic variations in Chinese breast cancer patients has not been well explored.

**Methods:** To investigate the characteristics of FGFR1 and FGFR2 genetic variations in Chinese breast cancer, we employed customized next-generation sequencing panel to screen the somatic mutation or copy number variations of FGFR1/2 in primary breast cancer tissues by using two ongoing breast cancer Cohorts, in which we have recruited 289 of early breast cancer patients (EBC Cohort) and 74 of advanced breast cancer patients (ABC Cohort).

**Results:** In EBC Cohort, we found FGFR1 amplification in 9.0% (26/289) patients and FGFR2 amplification in 2.1% (6/289) patients, and also found 3 of somatic FGFR1 mutations (FGFR1 p.W4C; p.E334K; p.V396I ) and 2 of FGFR2 mutations (FGFR2 p.S702L; p.Y779\*). Unlike the comparable prevalence of FGFR2 genetic variations in 2.8% (8/289) of EBC Cohort and 2.7% (2/74; one amplification event and one FGFR2 p.E499D mutation) of ABC Cohort, there were more FGFR1 genetic alterations in ABC Cohort (27%; 20/74 patients; p<0.001), including 19 events of FGFR1 amplification and 1 of FGFR1 c.2186+20G>A intron mutation. More importantly, we identified 5 novel FGFR1 fusion genes in our cohorts, including TACC1-FGFR1, FGFR1-KCNU1, FGFR1-MIR1268A, FGFR1-LZTS1-AS1 and FGFR1-RNF5P1. Although FGFR1-TACC1 fusion gene has been previously reported in breast cancer and TACC1 is fused to the C-terminal of FGFR1 protein leading to aberrant activation of FGFR1, we found TACC1 was fused to the N-terminal of FGFR1 at exon 6 of FGFR1 in our study. In addition, we identified and verified FGFR1-MIR1268A fusion gene at mRNA level using RNA-seq analysis, and further found this fusion gene might result in the truncation of FGFR1.

**Conclusions:** Collectively, we characterized the prevalence of FGFR1/2 genetic alterations in Chinese breast cancer, and identified 5 of novel FGFR1 fusion genes. The potential roles for novel FGFR1 fusion genes in regulating breast cancer cellular biology and in affecting the efficacy of endocrine therapy have been under the investigation in our laboratory, and the result from which will help us better elucidate the molecular mechanism of FGFR1 in driving the resistance of endocrine therapy. This study was supported by funding from National Natural Science Foundation of China (Grant No. 81602645), Guangdong Provincial Natural Science Foundation (Grant No. 2016A030313768) and Research Funds from Guangzhou Science and Technology Bureau (Grant No. 201707010418 and 201804010430).

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-26

Identification of preclinical mechanisms driving acquired resistance to endocrine therapy in estrogen-receptor positive (ER<sup>+</sup>) breast cancer cells

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Estrogen Receptor positive (ER<sup>+</sup>) breast cancer accounts for the majority of breast cancer cases and standard of care for these tumors is treatment with endocrine therapy, including the blockade of estrogen production (i.e. aromatase inhibitors; AIs) as well as the use of antagonists of ER function, i.e. selective estrogen receptor modulators (SERMs, i.e. tamoxifen) and selective estrogen receptor degraders (SERDs, i.e. fulvestrant). Despite the initial dependency of ER<sup>+</sup> breast tumors on estrogen and ER for their survival and proliferation, treatment in the metastatic setting invariably leads to therapeutic resistance. While mechanisms of resistance to AIs include mutations in the estrogen receptor gene ESR1, less is known about mechanisms of resistance to SERMs and SERDs, thus it is essential to further investigate the latter, in order to successfully treat relapsed patients. To pre-clinically model cell-autonomous acquired resistance to these agents, we used T47D, an ER<sup>+</sup> and p53<sup>-</sup> estrogen-responsive cell line treated with increasing concentrations of the SERM/SERD hybrid (SSH) ER-targeting agent GDC-0810 over the period of several months during which individual clones with acquired resistance to GDC-0810 were selected. GDC-0810-resistant clones were cross-resistant to other endocrine agents, including SERMs (tamoxifen) and SERDs (fulvestrant), consistent with general loss of dependency on ER. Surprisingly, the cells also lost sensitivity to palbociclib, the latter likely linked to their loss of one copy of the retinoblastoma (Rb) tumor suppressor gene. Comprehensive genetic and phenotypic characterization of the resistant clones relative to the parental cells revealed multiple mutations and deletions in DNA repair and cell cycle genes, and associated defects in DNA repair and cell cycle checkpoints. Cell cycle, proteomic, and mRNA expression analysis of parental versus resistant clones at baseline and upon DNA damage, identified a distinct cell cycle profile in the GDC-0810-resistant clones, characterized by accumulation of cells in the mitotic phase. A broad chemical screen identified pharmacologic inhibitors of cell cycle regulators and chemotherapeutic drug classes that preferentially target the ER-independent, GDC-0810 resistant clones compared to the parental cells. Our work provides novel insights into mechanisms and biomarkers of acquired resistance to estrogen therapies in ER<sup>+</sup> breast cancer and reveals the acquisition of actionable dependencies that may potentially be exploited in resistant tumors. Furthermore, our studies provide rationale for testing specific chemotherapy regimens upon endocrine resistance accompanied by cell cycle and DNA repair checkpoint dysfunction in ER<sup>+</sup> breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-27

Investigating the incidence of ESR1 gene amplification in breast cancers resistant to multiple endocrine agents

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**Background:** Around 70% of all breast cancers (BCs) are estrogen receptor positive (ER+), but some do not respond to endocrine therapy (ET) and many eventually develop resistance. ESR amplification (ESRA) linked to an increase in ESR1 gene expression is known to occur in some cancers that are endocrine resistant. However, the incidence of ESRA has been the object of debate and its clinical significance remains unclear. This study aimed to investigate the incidence of ESRA in BCs resistant to multiple sequential ETs and optimise a fluorescence *in-situ* hybridisation (FISH) methodology to robustly detect ESRA.

**Methods:** Two unique cohorts have been studied:

(A) 20 post-menopausal women with ER+ BC with acquired resistance to letrozole, subsequently treated with up to 4 different lines of ET. Serial RNA and DNA from 3-5 cancer samples per patient (58 samples from 20 patients) were analysed by Ribo0-RNAseq and DNA exome sequencing;

(B) 18 post-menopausal women who developed ER+ BC recurrences on 1<sup>st</sup> line adjuvant letrozole, then on 2<sup>nd</sup> line tamoxifen and subsequently on 3<sup>rd</sup> line exemestane. Tissues were collected at the time of each surgery.

We have optimised a FISH method to assess ESRA in these tissues.

**Results:** In cohort A, 6/20 patients developed ESR1 gene amplification (ESRA) at some point during treatment. In 5 of these cases, ESRA was only found while on 2<sup>nd</sup> or 3<sup>rd</sup> line exemestane but was not present on acquired resistance to previous letrozole or tamoxifen. 1 patient had ESRA at the time of first recurrence on letrozole.

The FISH method showed concordance with the genomic analysis. This suggests that ESRA may be associated with BCs that are treated with and then become resistant to exemestane.

ESRA is also evident in samples from Cohort B, which includes 18 exemestane resistant cases. The complete analysis is ongoing.

**Conclusions:**

- ESRA can be seen in ER+ recurrent BCs.
- ESRA may be associated with BCs treated with 2<sup>nd</sup> or 3<sup>rd</sup> line exemestane.
- The frequency of ESRA in endocrine and exemestane resistance can now be ascertained using an optimised FISH-based method, which is more cost-effective than alternative genomic and biochemical methods.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-28

A potential role for IL6ST mediating endocrine resistance in breast cancer via interaction with the ER signaling pathway

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## **Background**

Interest in the ubiquitously expressed transmembrane receptor IL6ST (gp130) has developed as it has been identified as a predictive biomarker of endocrine treatment response in breast cancer patients and is included in the 'Endopredict' test. At least seven cytokines (IL-6, OSM, LIF, IL-11, CNTF, IL-27, CT-1) signal via IL6ST. Interleukin-6 (IL-6) can mediate effects via two signaling pathways; classic signaling (through the membrane-bound IL-6 receptor, IL-6R) and trans-signaling (via non-signaling membrane-bound soluble IL-6R, sIL-6R). Both pathways may occur in parallel and activate cells. Crosstalk between the ER signaling pathway and IL6ST through STAT3 has been identified and implicated in the conversion from ER responsiveness to non-responsiveness.

## **Method**

A panel of 3 ERa+ luminal breast cancer cell lines (MCF-7, T47D, ZR-75-1) were chosen to examine IL6ST expression by western blot, gel electrophoresis and qRT-PCR. Proliferation assays (SRB) were carried out to investigate the effects of seven cytokines (IL-6, OSM, LIF, IL-11, CNTF, IL-27, CT-1) on cell growth. The action of both IL-6 and Oncostatin M (OSM) on cell migration and downstream signaling pathways (pSTAT3 and pERK1/2) was studied. The extent of trans-signaling occurrence and interaction between cytokines and estrogen was investigated using proliferation assays.

## **Results**

Three cell lines (MCF-7, T47D, ZR-75-1) were shown to express varying levels of full-length IL6ST, with MCF-7 having the least expression. Gel electrophoresis and qRT-PCR confirmed the presence of all previously described IL6ST soluble forms in the three cell lines. Surprisingly, the growth response to the cytokines was variable across the cell lines. IL6 caused a modest increase in growth in MCF-7 but produced inhibition in ZR-75-1. OSM and LIF stimulated growth in MCF-7, whereas OSM inhibited ZR-75-1 and LIF had no effect. Interestingly, no significant effect on growth was seen in T47D with any of the cytokines except IL-11 which generated a significant growth effect on T47D cells. Both STAT3 and MAPK/ERK pathways were activated to different extents in the three cell lines as a result of OSM and IL-6 activation, whereas cells migration was only stimulated in ZR-75-1. The trans-signaling pathway significantly enhanced the growth inhibition in ZR-75-1. Estrogen eliminated the effect of both IL-6 and OSM on MCF-7 growth, while both cytokines decreased estrogen-induced cell proliferation in ZR-75-1. There was no effect in T47D.

## **Conclusion**

These results indicate:

- The presence of both classic and trans-signaling occurrence in breast cancer cell lines.
- Differential patterns of pSTAT3 and pERK1/2 signaling which could help explain the variation in responses to the cytokines.
- IL6ST full-length form is the most abundant form in all three cell lines under basal conditions.
- The levels and roles of the different IL6ST soluble forms will be further studied after estrogen stimulation.
- The effect of IL6ST silencing in the presence of estrogen and tamoxifen on cell growth and the gene expression currently being examined.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-29

Efficacy and safety of low-dose everolimus in Chinese HR-positive, HER2-negative metastatic breast cancer patients

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**Background:** Everolimus has been testified to be effective among postmenopausal women with hormone receptor-positive human epidermal growth factor receptor-2 negative (HR+/HER2-) metastatic breast cancer (MBC). However, everolimus at dose of 10mg was accompanied by a higher incidence of mTOR-inhibitor class-effect adverse events (AEs) which usually leading to dose reduction or interruption. Additionally, some studies suggested that there was no correlation between dose intensity (5 vs. 10 mg labeled dose) and efficacy. Consequently, we conducted the present study aiming to explore the efficacy and safety of everolimus at dose of 5 mg plus endocrine therapy in Chinese population.

**Methods:** 68 HR+/HER2- MBC patients were included in this exploratory study who received everolimus at the dose of 5mg in Zhejiang Cancer Hospital between September 2014 and September 2017. Progression free survival(PFS) and overall survival(OS) were estimated by the Kaplan- Meier method, and the hazard ratios(HRs) and corresponding 95% confidence intervals(CIs) were estimated using the Cox proportional harzard model. Besides, objective response rate(ORR), clinical benefitrates(CBR) and safety profile were also evaluated.

**Results:** After a median follow up of 14 months, PFS was 5.3 months (95%CI 4.2-6.4), and OS was immature. 16(23.5%) were at the first or second-line, 52(76.5%) were at third-line or later. PFS for the first and second-line is significantly longer than that for the third-line or later (12.9 months vs. 4.6 months,  $P=0.009$ , HR=0.395, 95% CI=0.192-0.811). 11(16.2%) achieved partial response (PR), 42(61.7%) had stable disease (SD), and 15(22.1%) reported progressive disease (PD). The ORR and CBR were 16.2%, 35.2%, respectively. Most common all grade adverse events were stomatitis(26.5%), fatigue(10.0%), infection(11.8%), thrombocytopenia(5.9%), anemia(4.4%), hyperglycemia(4.4%). The most common  $\geq 3$  grade adverse events were stomatitis(4.4%), infection(2.2%) and thrombocytopenia(1.1%).

**Conclusions:** The combination of low-dose everolimus and endocrine therapy was highly effective especially at the earlier line in Chinese population. And the safety profiles were similar to previous studies but the incidences were greatly lower. The combination of low-dose everolimus and endocrine therapy may become a proper option for HR+/HER2- MBC.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-04-30

Developing an immunohistochemistry protocol to detect neurofibromin as an effective, simple, and rapid method to identify NF1-negative breast cancer patients

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**Background:** Neurofibromin is a key tumor suppressor, well-known as a GTPase-Activating-Protein (GAP) to attenuate Ras signaling. It is encoded by the *NF1* gene, so named because its inactivation was first discovered to cause Neurofibromatosis type 1, an autosomal dominant genetic disorder. We have recently reported that nonsense (NS) and frameshift (FS) mutations, but not missense mutations, in *NF1* are associated with a markedly higher risk of relapse and death in early stage ER+ breast cancer after adjuvant tamoxifen monotherapy (Griffith et al. *in press*). Surprisingly, despite being best known as a GAP, no missense mutations inactivating NF1's GAP activity were found in our cohort. We have evidence that these *NF1* NS/FS mutations cause the resulting mRNAs to be degraded, leading to depletion of the entire NF1 protein. In a separate study that was presented here last year, we showed that NF1 is also an ER co-repressor, which partially explains why the loss of the single tumor suppressor NF1 is so detrimental — because this turns on two potent oncogenic pathways. Thus far there is no effective means to assess loss of NF1 protein in cancer. The objective of this project is to identify these aggressive NF1-negative breast cancers by establishing an immunohistochemistry (IHC) protocol with a valid NF1 antibody in order to properly find and treat them in the future.

**Methods:** A monoclonal antibody was raised against the C-terminus of NF1. Immunostaining as well as IHC was performed using a set of breast cancer cell lines with varying degrees of NF1 protein levels, including several NF1 null-like cell lines as negative controls. To assess whether the IHC protocol can be used on patients, NF1+ and NF1-PDXs as well as patient biopsies were examined.

**Results:** We have purified a monoclonal antibody against NF1 (m376). By immunostaining, a strong NF1 signal can be seen in T47D cells, which have four copies of the *NF1* gene. In contrast, there was barely any signal in MDA-MB-175VII cells, which lack detectable NF1 due to *NF1* FS mutations. While NF1 appears mostly cytoplasmic, 10-15% can be seen in the nucleus. Nuclear NF1 levels can be further increased by the nuclear export blocker leptomycin-B, suggesting that NF1 is shuttled in and out of the nucleus. IHC staining confirmed these features of NF1. In addition, a weak nuclear signal can be seen in cancer cells carrying *NF1* FS mutations. Experiments are on-going to assess how to analyze tumor samples for NF1 loss and whether *NF1* FS mutations cause expression of truncated proteins that are nuclear.

**Conclusion:** Our results suggest that the m376 antibody has the potential to be used for IHC, provided that samples known to be NF1+ or NF1- are included as controls. The success of this project will have particular clinical impact in telling us who should not be treated by tamoxifen. Furthermore, we have an approved clinical trial protocol to assess the concept that these NF1- patients should instead be treated by combining a Ras pathway inhibitor and a SERD.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-01

Metabolic enzyme PFKFB4 activates transcriptional coactivator SRC-3 to drive aggressive metastatic breast cancer

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**Background:** Metabolic rewiring is one of the central hallmarks of cancer progression and survival to support anabolic and energetic demands. Tumor cells constantly alter their metabolic state in response to oncogenic stimuli, nutrient availability, and interaction with immune cells however the precise regulation that precedes the metabolic alteration is poorly understood. Here we report a direct interaction of glycolytic enzyme PFKFB4 with transcriptional coregulator SRC-3. PFKFB4 functions as a critical regulator of Warburg effect and our study reveals that upon glucose stimulation PFKFB4 activates SRC-3 driving an invasive-metastatic breast cancer.

**Methods:** Molecular experiments were performed to understand the transcriptional activation of SRC-3 by PFKFB4 enzyme. Chromatin immunoprecipitation and gene expression studies were performed to investigate the functions of PFKFB4/SRC-3 crosstalk on transcriptional regulation. Metabolomics and isotope tracing studies were performed to identify the metabolic adaptations regulated by PFKFB4/SRC-3 in breast tumors. PFKFB4-knockout was established using CRISPR-Cas9 system and functional studies were carried out to define its role in tumor cell proliferation, invasion-migration, and breast to lung metastasis. Human breast tumor samples were evaluated to identify the clinical importance of PFKFB4/SRC-3 crosstalk in patients.

**Results:** Molecular studies revealed that PFKFB4 enzyme phosphorylates SRC-3 at serine 857 (S857) enhancing its transcriptional activity, whereas either suppression of PFKFB4 or ectopic expression of a phosphorylation-deficient SRC-3 mutant S857A (SRC-3<sup>S857A</sup>) significantly abolished SRC-3-mediated transcriptional output ( $p < 0.000001$ ). Functionally, PFKFB4-driven SRC-3 activation drives glucose flux towards the pentose phosphate pathway enabling purine synthesis by transcriptionally upregulating the expression of enzyme transketolase (TKT). Deletion of PFKFB4 by CRISPR-Cas9 system resulted in significantly reduced proliferation ( $p < 0.05$ ) and migration-invasion ( $p < 0.001$ ) compared to wildtype breast tumor cells. Ablation of SRC-3 or PFKFB4 suppressed *in vivo* breast tumor growth and prevents metastasis to the lung from an orthotopic setting ( $p < 0.0001$ ). PFKFB4 and phosphorylated SRC-3 levels are significantly increased in breast tumors ( $p = 0.02$ ), whereas, in patients with the basal subtype, PFKFB4 and SRC-3 drive a common protein signature that correlates with the poor survival of TNBC patients ( $p = 0.03$ ).

**Conclusion:** Our data suggest that the Warburg pathway enzyme PFKFB4 acts as a molecular fulcrum that couples sugar metabolism to transcriptional activation by stimulating SRC-3 to promote aggressive metastatic tumors. It also provides first evidence how Warburg pathway drives aggressive breast tumorigenesis by directly activating powerful oncogene SRC-3. Our work suggests that targeting the PFKFB4–SRC-3 axis may be therapeutically valuable in breast tumors that are notably dependent on glucose metabolism.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-02

Thyroid hormone replacement therapy shortens survival in hormone receptor positive early stage breast cancers

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## Purpose

A majority of breast cancer (BC) patients have clinical or subclinical thyroid disease as compared to a minority of unaffected women. This comorbidity has been recognized for over a century. We hypothesized that thyroid hormone (TH) promotes breast carcinogenesis and growth, and may reduce survival for patients on long term TH replacement therapy (THRT). We sought to validate these findings in a study of BC patients and investigate mechanisms of hormonal interaction using model systems.

## Methods

Over 800 consecutively diagnosed lymph node (LN) negative, mostly chemo-naïve invasive BC patients from 1976 to 1993 at were evaluated. Clinical and outcomes data were derived from medical records (10.2 year median follow up). Primary tumors from surgery (typically mastectomy with node dissection) were analyzed for receptors and biomarkers. Univariate and multivariate statistical methods were used. *In vivo* and *in vitro* studies were performed using immortalized human BC cell lines and patient derived xenograft(PDX) models.

## Results

LN- THRT vs. non-THRT BC patients showed no significant differences in clinical, histologic or prognostic data. THRT was significantly associated with a higher relative risk (RR) of recurrence 2.376 fold ( $p < 0.0001$ ) and disease specific survival (DSS), 2.02 fold ( $p = 0.0183$ ). Subset analyses showed only steroid receptor (SR) positive patients had a deleterious interaction with THRT (mean RFS 68 mo., RR 3.431,  $p < 0.0001$ ), mean disease specific survival (DSS) 81 mo., RR 3.960,  $p = 0.0001$ ) as compared to non-THRT patients (mean RFS 206 mo., mean DSS 223 mo.). Tamoxifen (Tam) treated patients on THRT had an especially poor outcome, DFS  $p = 0.0004$ . Using *in vitro* methods we showed potent induction of cell proliferation by estrogen (E2) and/or thyroid hormone (TH) across a wider range of concentrations in SR+, but not SR- BC ( $P < 0.0001$ ). Fulvestrant (ICI) significantly inhibited these interactions, whereas Tam failed to provide an equivalent antagonistic effect. In some SR+ cells, Tam further increased cell proliferation. *In vivo* studies showed co-localization of ER and THRA were upregulated by co-administration of E2 + TH. Tam further induced co-localization, whereas ICI lowered receptor expression and co-localization. ER+ PDX tumors in mice with continuous E2 showed enhanced tumorigenesis with TH (tumor growth ( $p < 0.0001$ ), tumor weight (mg) ( $p = 0.0052$ ) and Ki67 labeling ( $p = 0.001$ )). Mice on E2 + TH with PDX tumors treated with Tam showed enhanced tumor growth and proliferation ( $p < 0.0001$ ), whereas ICI inhibited and in some cases eliminated tumors. RNA-seq data performed on our ER+ PDX tumors treated with hormone combinations +/- Tam show that E2 + TH potently upregulates numerous cell cycle and hormone regulated genes.

## Conclusion

We show statistically significant interactions between THRT at the time of BC diagnosis, early relapse and shortened survival in SR+ (but not SR-) Stage I BC patients. *In vitro* and *in vivo* BC models confirm these deleterious interactions. Mechanistically we observe co-localization of the cognate receptors and activation of ER, with downstream upregulation of cell cycle and hormone regulated genes. These interactions are blocked by ICI, whereas Tam fails to block and may in fact enhance tumorigenesis.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-05-03

Estrogen receptor beta elicits anti-cancer effects in triple negative breast cancer through suppression of NFκB signaling

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**Background:** Triple Negative Breast Cancer (TNBC) affects approximately 15-20% of BC patients, yet accounts for a disproportionately higher rate of BC morbidity and mortality, in part due to lack of targeted therapies. Using well-validated antibodies, Estrogen Receptor Beta (ERβ) protein has been shown to be expressed in approximately 25% of TNBCs and is associated with improved patient outcomes. Using multiple ERβ +/- TNBC cell lines and PDX models, we have demonstrated that ligand-mediated activation of ERβ by estradiol (E2) decreases cell proliferation, invasion, and migration *in vitro*, as well as primary tumor growth and metastatic spread *in vivo*.

**Methods:** To determine the mechanisms by which ERβ elicits these anti-cancer effects, we elucidated the ERβ transcriptome and cistrome via Microarray and ChIPseq, respectively, in TNBC cells stably expressing ERβ in a doxycycline-inducible manner. We also performed gene expression and luciferase assays to assess the impact of ERβ on NFκB signaling, followed by ChIP-PCR and ChIPseq to assess how ERβ modifies chromatin architecture near NFκB target genes.

**Results:** Pathway analysis of ERβ-regulated genes identified NFκB signaling as one of the most suppressed pathways in response to E2 treatment. Indeed, numerous NFκB target genes were among the most down-regulated genes following E2 treatment but only in the presence of ERβ expression. Chromatin Immunoprecipitation followed by sequencing (ChIPseq) revealed that ERβ primarily associated with estrogen response elements (EREs), but was also enriched around NFκB binding sites following E2 treatment. In fact, 12% of all ERβ binding sites were enriched for NFκB response elements and ERβ was shown to physically associate with NFκB protein. Using an NFκB reporter construct and qPCR, ERβ was shown to block TNFα-mediated induction of NFκB signaling and NFκB target gene expression. Globally, RNAseq identified 200 genes to be significantly regulated by TNFα in TNBC cells, of which 81 were significantly altered in the presence of E2+TNFα. ChIPseq demonstrated that ligand-mediated activation of ERβ significantly diminished an activating histone mark (H3K27Ac) at many of these NFκB target genes while enhancing a repressive mark (H3K27Me3). These modifications are also associated with recruitment of the histone methyltransferase, EZH2, to enhancer elements of these NFκB target genes. Drug-mediated blockade of HDAC and EZH2 activity reversed suppression of NFκB target gene expression by ERβ.

**Conclusions:** Our data suggest that ERβ may elicit its anti-cancer effects in part via formation of a novel co-repressor complex consisting of ERβ, NFκB, and EZH2. These data are in keeping with prior observations of the importance of NFκB signaling as it relates to TNBC cell proliferation and invasion, and that decreased expression of NFκB target genes is associated with improved outcomes in TNBC patients. Currently, a Mayo Breast SPORE prospective study is underway to investigate the role of estradiol in ERβ expressing TNBC and to further evaluate the cross-talk between ERβ and NFκB signaling in TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-04

Myc as a poor prognostic marker for ER+ inflammatory breast cancer (IBC): Quantitative estrogen receptor (ER) expression analysis and gene expression analysis in ER+ IBC vs non-IBC

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## Background

Estrogen receptor-positive (ER+) primary inflammatory breast cancer (IBC) has a poorer prognosis than ER+ primary non-IBC. Our objective was to determine the association between ER positivity and survival outcome in order to elucidate the biological reason that ER+ IBC is more aggressive than non-IBC.

## Methods

We retrospectively determined the relationship between ER expression by immunohistochemistry staining and neoadjuvant chemotherapy response as well as survival outcome for 189 patients with ER+ and HER2-negative (HER2-) IBC and 896 case-matched patients with stage III non-IBC seen at MD Anderson Cancer Center between January 1989 and April 2015. We performed gene expression (GE) analysis for 39 patients with ER+/HER2- IBC and 40 patients with non-IBC to detect genes that are specifically overexpressed in IBC. Logistic regression and Cox proportional hazards model were used to determine the predictive and prognostic value of percentages of cells positive for ER and progesterone receptor (PR) among the patients with ER+/HER2- IBC and non-IBC. Recursive partitioning analysis (RPA) was used to determine the optimal cutoff points for ER% and progesterone receptor (PR) % that maximized differences in survival. The identified cutoff points were tested in an external cohort of 192 ER+/HER2- IBC patients from Institut Paoli-Calmettes in France.

## Results

The median values for ER% for IBC and non-IBC were 85 (range, 1-100) and 90 (range, 1-100), respectively. The logistic regression model demonstrated a lack of a relationship of ER% with pathological complete response rate to neoadjuvant chemotherapy both in IBC (P=0.29) and non-IBC (P=0.14). Expression of ER was significantly associated with distant disease-free survival (DDFS); hazard ratio (HR), 0.56 [95% CI, 0.37-0.83] per 50% increase in ER%; P<0.05). Also, ER% was significantly associated with overall survival (OS) (HR, 0.40 [95% CI, 0.25-0.63] per 50% increase in ER%; P<0.05). RPA showed that 91.5% and 9.0% were the optimal cutoff points for ER% and PR%, respectively, for DDFS and overall survival in IBC patients. However, the cutoff points could not be validated in the French external cohort. In the GE study, 84 genes were detected as significantly distinguishing ER+ IBC from non-IBC. Among the top 15 canonical pathways shown by IPA, the ERK/MAPK signaling pathway, PDGF pathway, insulin receptor signaling pathway, and IL-7 signaling pathway were associated with the ER signaling pathway. MYC upregulation was observed in three of these four pathways. Indeed, ER+/HER- IBC had significantly higher MYC amplification compared to those with non-IBC (P<0.05) and higher MYC level was associated with poor relapse free survival for IBC (HR, 1.85 [95% CI, 1.05-2.70], P<0.05).

## Conclusions

Increased ER positivity was significantly associated with improved survival in ER+/HER- IBC patients. ER+/HER- IBC had several activated pathways with MYC upregulation compared to non-IBC. MYC upregulation was associated with a poor survival outcome for ER+/HER- IBC. The results indicate that MYC is a key gene for understanding the aggressive biological behavior of ER+/HER- IBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-05

Targeting the androgen receptor with seviteronel, a CYP17 lyase and AR inhibitor, in triple negative breast cancer

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**Background:** Androgen receptor (AR) protein is expressed across breast cancer (BC) subtypes, including up to 50% of triple negative breast cancers (TNBC) and it is often maintained in metastases. Our research demonstrated that TNBC cell lines rely on increased AR for anchorage independent survival, mammosphere formation, tumor initiation, and other stem-cell like properties. Further, AR mRNA levels increased in circulating tumor cells in the blood as compared to levels in the primary tumors of mice harboring human patient derived xenografts (PDX). Seviteronel (SEVI), a CYP17 lyase and AR inhibitor that blocks androgen and estrogen biosynthesis and AR activation, is in phase 2 clinical development for men and women with AR+ solid tumors, including advanced BC and has clinical activity in both basal and LAR subtypes (Innocrin data on file).

**Hypothesis:** We hypothesized that targeting both androgen biosynthesis and AR activation with an agent such as SEVI would decrease tumor growth in preclinical models of AR+ TNBC.

**Methods:** Genes regulated by AR under anchorage independent conditions with and without 10 nM dihydrotestosterone (DHT) were identified by AR chromatin immunoprecipitation (ChIP)-seq, RNA-seq, and Ingenuity Pathway Analysis. In vitro activity of SEVI was assayed in AR+ TNBC cell lines (MDA-MB-453 and SUM159). The IC<sub>50</sub> of SEVI was determined via crystal violet. Soft agar colony formation assays and growth on poly-HEMA coated plates measured anchorage independent growth. In vivo, an AR+ TNBC patient derived xenograft (HCI-009, generated by Dr. Alana Welm) was grown in NSG mice given either DHT vs. vehicle control or, in a separate experiment, SEVI (150 mg/kg/day PO) vs. vehicle control.

**Results:** ChIP-seq and RNA-seq in MDA-MB-453 demonstrated that AR chromatin binding and gene regulation increased under anchorage independent conditions in a ligand dependent manner and showed an increase in mTOR signaling, aryl hydrocarbon receptor signaling and metabolism. SEVI inhibited proliferation of AR+ TNBC in a dose-dependent manner and growth on soft agar. In vivo, the AR+ TNBC HCI-009 PDX exhibited a significant increase in tumor volume with DHT indicating AR dependence. SEVI treatment also decreased HCI-009 tumor volume (p=0.0054) and rate of growth (p=0.0146). Nuclear AR protein and classical AR-regulated genes such as *KLK3* and *FKBP5* were increased with DHT in the HCI-009 xenograft tumors and decreased in mice treated with SEVI and were confirmed at the protein level.

**Conclusion:** These results further demonstrate the activity of SEVI, a CYP17 lyase and AR inhibitor, in preclinical models of AR+ TNBC. Use of AR targeting agents may be a rational treatment approach for a subset of patients with AR+ TNBC since such tumors may respond less well to chemotherapy than other TNBC molecular subtypes. We continue to identify markers of AR dependence/responsiveness to AR-targeted therapy.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-06

Progesterone receptor (PR) isoform-specific expansion of breast cancer stem-like cells occurs through a phospho-PR/FOXO1 driven signaling axis

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Luminal breast cancers account for ~75% of cases and are positive for progesterone receptor (PR) and estrogen receptor (ER) expression. PR is a classical ER-target gene and is used as a biomarker of ER activity; however, a growing body of evidence supports the role of PR as an important modifier of ER actions and a key driver of luminal breast cancer progression. Progesterone signaling is mediated by two PR isoforms: full-length PR-B and truncated PR-A, which lacks the N-terminal 164 amino acids. Little is known about PR isoform-specific actions in PR-expressing breast tumors, given that total PR expression is measured clinically. Herein, we sought to identify phenotypic differences in luminal breast cancer cells (T47D) overexpressing PR-A (T47D-YA) or PR-B (T47D-YB). We demonstrate that PR-B expression is required for anchorage-independent colony formation, while PR-A expressing cells fail to proliferate in soft agar. PR-B driven proliferation has been mapped to PR phosphorylation events, which include MAPK or CDK consensus sites such as Ser294. We demonstrate that in contrast to previous reports, PR-A is well phosphorylated at Ser294 in response to progestins (e.g. R5020) using our custom phospho-PR (Ser294) polyclonal antibody. Interestingly, Ser294 phosphorylation of PR-A occurs more rapidly and robustly following hormone treatment compared to PR-B expressing cells. Our findings indicate that PR-A is a dominant driver of stem-like expansion in breast cancer cells. PR-A tumorspheres exhibited enriched ALDH+ and CD44+/CD24- populations compared to PR-B and promoted heightened gene expression of stem-like genes (e.g. *FOXO1*). We demonstrate that the PR target gene and co-activator FOXO1 promotes both PR-A and PR-B phosphorylation at Ser294 and augments tumorsphere formation. Direct inhibition of FOXO1 levels abrogates phospho-PR (Ser294) levels and tumorsphere formation in PR expressing cells. Finally, we show that Ser294 is required for PR-A induced expression of stem-like genes (e.g. *FOXO1*) and stem-like behavior as measured by ALDH+/CD44+ tumorspheres. Taken together, our data reveal unique functions of PR isoforms as modulators of distinct and opposing pathways (i.e. proliferation versus stem-like expansion) in luminal breast cancer models. A clear understanding of PR isoform-specific actions, phosphorylation events, and the role of co-factors such as FOXO1 may lead to novel biomarkers of advanced tumor behavior and reveal new approaches to pharmacologically target PR isoforms in luminal breast cancers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-07

Expression of the cocaine- and amphetamine-regulated transcript (CART) recruits SWI/SNF chromatin remodelling complexes to the estrogen receptor

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## **Introduction**

Cocaine- and amphetamine-regulated transcript (CART) peptides are neuropeptides involved in regulating physiological processes, such as feeding and drug reward. Recent studies have associated high CART expression with worse overall survival in patients with small-bowel carcinoid tumours and estrogen receptor-positive (ER+), lymph node-negative breast cancer. CART was also shown to be associated with poor patient response to tamoxifen, suggesting CART may play a role in conferring tamoxifen resistance.

## **Materials and methods**

We have previously demonstrated that CART can impact the transcriptional activity of ER $\alpha$  through the use of western blotting and qPCR for specific ER $\alpha$  gene targets. RNA sequencing was carried out using a stable CART-inducible cell line model to identify genes which are upregulated/downregulated in cells expressing CART. Further, using our stable CART-inducible cell line model, we performed ER $\alpha$ -Immunoprecipitation followed by in-solution mass spectrometry to identify differentially recruited protein complexes +/- CART expression.

## **Results and discussion**

RNA sequencing revealed 156 significantly downregulated, and 100 significantly upregulated, genes in cells expressing CART ( $p < 0.05$ ). Through mining of publicly available ER $\alpha$  ChIP-seq data sets, both upregulated and downregulated gene sets were found to contain genes which have previously been shown to contain ER $\alpha$  binding events within their promotor regions. Mass spectrometry analysis revealed that the majority of proteins recruited to ER $\alpha$  in the presence of CART were members of the SWI/SNF (BAF) chromatin remodelling complex. The identification of SMARCD1 within this complex was of particular interest to this study, as this protein has previously been reported to be a critical mediator of nuclear receptor function. Further *in silico* analysis demonstrated high expression of *SMARCD1* correlates with poor overall survival (OS) ( $p < 0.00001$ ) and distant metastasis free survival (DMFS) ( $p = 0.00708$ ) in a cohort of ER+ breast cancer patients. Intriguingly, SMARCD1 expression did not correlate with poor OS or DMFS in a cohort of ER- breast cancer patients, suggesting that this negative impact on survival is dependent on ER status.

## **Conclusion**

In conclusion, we suggest that CART expression results in the recruitment of chromatin remodelling complexes to ER $\alpha$  in order to facilitate the regulation of receptor function and this impacts on patient outcome.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-08

Molecular cross-talk between retinoic acid and NOTCH1 signaling pathways: Role in triple negative breast cancer

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Triple Negative Breast Cancer (TNBC) represents 10-20% of all breast cancers and it is characterized by poor prognosis and high recurrence rate. The heterogeneity of the disease and the absence of well-defined molecular targets have so far challenged successful therapeutic strategies. NOTCH1 has been found to act as a driver oncogene in a small subset of TNBC characterized by constitutive activation of the protein, acting as a transcription factor. Preclinical studies support an anti-tumor activity of All-Trans Retinoic Acid (ATRA) in specific subsets of breast cancer.

By screening a large panel of breast cancer cell lines recapitulating the heterogeneity of TNBC we identify a specific subset sensitive to the anti-proliferative activity of ATRA. These cell lines (N-TNBC cell lines) are characterized by a Notch1 intragenic fusion transcript conferring gain of function activity to the protein. Indeed, sequence analysis reveals that the cell lines harbor an interstitial deletion in the NOTCH1 gene encompassing the negative regulatory region (NRR) domain. These cell lines depend on Notch active signaling for their proliferation since their cell growth is impaired by Notch inhibitors ( $\gamma$ -secretase inhibitors, e.g. DAPT, PF-3084014). Proliferation assays reveal that ATRA and  $\gamma$ -secretase inhibitors act synergistically in inhibiting cancer cell growth in N-TNBC cell lines suggesting the existence of a cross talk between the molecular pathways engaged by retinoic acid and NOTCH1.

By using retinoic acid receptors (RARs) agonists and antagonists as well as RAR specific silencing experiments we identify RAR $\alpha$  as the retinoic acid receptor responsible of the anti-proliferation activity of ATRA in N-TNBC cell lines. In particular N-TNBC cell lines respond to RAR $\alpha$  activation by inducing high amounts of the onco-suppressor protein RAR $\beta$ . This feature is unique in ATRA sensitive TNBC cell lines and does not occur in ATRA sensitive luminal cell lines arguing for the existence of a retinoic acid specific mechanism of action in N-TNBC. Since RARs act as transcription factors inside the cells, to gain insights into the molecular pathway at the basis of the observed ATRA/NOTCH1 cross talk, we performed RNAseq analysis of ATRA and/or DAPT treated N-TNBC cells. Gene set enrichment analysis reveal that ATRA is able to directly affect NOTCH1 transduction pathway by modulating the expression of NOTCH1 target genes. In particular, in two out of three N-TNBC cell lines ATRA directly inhibits the NOTCH1 expression at a transcriptional level and its downregulation is increased by ATRA/DAPT combinations. Pathway analysis has allowed the identification of putative molecular hubs responsible for the synergistic effects observed and therefore likely at the basis of the crosstalk between ATRA/NOTCH pathways. These findings are of clinical interest since both the retinoid and NOTCH signaling display crucial physiologic activities and their pleiotropic effects could impinge on the success of therapeutic options based on their pathway modulation.

The newly discovered specificity of ATRA action in the context of NOTCH1 addicted TNBC provides new tools for the identification of patients candidates benefitting from strategies targeting the ATRA/NOTCH axis.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-05-09

Systemic perturbations induced by all-trans retinoic acid in the gene-expression profiles of sixteen breast cancer cell lines characterized by sensitivity and resistance to the anti-proliferative effects of the retinoid

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**Background:** All-trans retinoic acid (ATRA) is a promising agent in the treatment of breast cancer. In view of ATRA-based therapeutic strategies aimed at the personalized treatment of mammary tumors, we recently demonstrated that approximately 70% of estrogen-receptor-positive (ER<sup>+</sup>) breast cancer is sensitive to the anti-proliferative effects of ATRA (1). In contrast only 10-20% of the HER2-positive and triple-negative counterparts respond to the retinoid. On the basis of these data and the available basal gene-expression profiles of breast cancer cell lines and primary tumors, we developed a model consisting of 21 genes (ATRA-21) which correctly predicts ATRA-sensitivity in the context of breast cancer (2).

**Aims and Approach:** The present study is aimed at getting insights into the molecular mechanisms underlying the anti-tumor action of ATRA in the specific subsets of breast cancer identified. In addition, we intend to determine specific genes and gene-networks modulated by ATRA which may represent pharmacological targets for the design of rational combinations between the retinoid and unrelated therapeutic agents to be used in the personalized treatment of breast cancer. A final goal is the identification of potential bio-markers of the anti-tumor response to ATRA to be used in the clinics. To address all these points, we performed deep-sequencing experiments on a panel of sixteen cell lines recapitulating the heterogeneity of the breast cancer phenotype and characterized for their anti-proliferative response to ATRA

Panel of Breast cancer cell lines and characteristics

Cell line	Phenotype	Class	ATRA-score	ATRA-sensitivity
SKBR3	LUMINAL	HER2+	1.0	yes
HCC1500	LUMINAL	ER+	0.7	yes
CAMA1	LUMINAL	ER+	0.7	yes
MDAMB361	LUMINAL	HER2+	0.6	yes
HCC202	LUMINAL	HER2+	0.2	no
MDAMB175VII	LUMINAL	ER+	0.2	no
ZR751	LUMINAL	ER+	0.1	no
HCC1419	LUMINAL	HER2+	0.1	no
HCC1599	BASAL	TN	1.0	yes
MB157	BASAL	TN	0.3	yes
MDAMB157	BASAL	TN	0.2	yes
HS578T	BASAL	TN	0.2	yes
MDAMB231	BASAL	TN	0.0	no
CAL851	BASAL	TN	0.0	no
HCC1187	BASAL	TN	0.0	no
MDAMB436	BASAL	TN	0.0	no

ATRA-score = ATRA sensitivity index, the higher the score the higher is the sensitivity to ATRA. ER<sup>+</sup> = estrogen-receptor-positive cell line. HER2<sup>+</sup> = Her2-positive cell line.

**Results:** We exposed each cell line to ATRA (1 µM) for 24 hours and extracted total RNA which was subjected to deep sequencing. The global gene-expression data were analyzed with a number of complementary bio-informatic tools which resulted



in the identification of approximately 100 genes whose expression is up- or down-regulated specifically in ATRA-sensitive luminal and/or basal cell lines. Pathway and gene-network analysis indicate a strong enrichment in the up-regulation of genes involved in the pathways modulated by interferons. These last results are consistent with the idea that ATRA exerts a strong immuno-modulatory action in breast cancer cells and represents proof of principle for the evaluation of combinations between the retinoid and check-point inhibitors in the treatment of breast cancer.

**References:** 1) Centritto F. et al. EMBO Mol Med. 2015 Jul;7(7):950-7. 2) Bolis M et al.. Ann Oncol. 2017 Mar 1;28(3):611-621.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-10

Estrogen receptor  $\beta$  suppresses metastasis of inflammatory breast cancer by regulating cell cytoskeleton and cytokine signaling

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Inflammatory breast cancer (IBC) is the most lethal form of breast cancer that accounts for about 10% of breast cancer mortality annually in US. Poor prognosis is largely due to the high propensity of IBC tumors to develop distant metastasis that occurs directly from the gland epithelium and through lymphatic invasion in which dermal lymphatics are filled with tumor emboli. Owing to the complex metastatic process, the molecular basis of IBC aggressiveness is poorly understood, and no specific therapeutic target has been identified. Despite the lack of estrogen receptor  $\alpha$  (ER $\alpha$ ) in the majority of IBC tumors, estrogen may still play a role in these cancers through pathways that involve ER $\beta$ . Our tissue staining reveals expression of ER $\beta$  in more than 50% of IBCs that is reproduced in IBC cell lines. Furthermore, analysis of IBC datasets indicates correlation of receptor expression with good prognosis. We studied this association in preclinical models of IBC by knocking out ER $\beta$  in IBC cells. This promotes migration and invasion through cytoskeleton remodeling whereas re-expression of the receptor in knockout cells restores the cytoskeletal structure and migration to the levels of control cells. Consistent with increased migration, deletion of ER $\beta$  activates large gene networks of cell de-differentiation and cytokine synthesis that trigger tumor microenvironment responses to promote the motile phenotype of IBC cells. In contrast, ligands that activate the receptor inhibit signaling that contributes to metastasis in IBC. Analysis of an orthotopic xenograft model shows that IBC tumors lacking ER $\beta$  have higher propensity for metastasis compared with the ER $\beta$ -proficient tumors supporting the anti-metastatic activity of the receptor. Our findings point towards a role of ER $\beta$  in preventing distant metastases by inhibiting dissemination of IBC cells and maintaining the integrity of emboli. This function combined with distinct expression indicates the potential of ER $\beta$  to represent a unique prognostic marker and therapeutic target that can be utilized to repress IBC metastasis and eliminate its associated mortality.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-11

Clinical significance of androgen receptor expression in breast cancer

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## Background

Breast cancer is highly heterogeneous and immunohistochemistry (IHC) is used to determine breast cancer subtypes using estrogen and progesterone receptor (ER and PgR), HER2 and Ki-67. The androgen receptor (AR) is frequently expressed in breast cancer, but evaluation of AR has not been standardized and the oncogenic activity in breast cancer is still unclear. The objectives of this study were to assess the clinical significance of AR expression in breast cancer patients with primary (pretreatment and posttreatment) and recurrent breast cancer in relation to breast cancer subtype.

## Methods

Primary and recurrent breast cancer patients who underwent treatment from March 2017 to May 2018 were enrolled in this study. A total of 591 primary breast cancer cases and 52 recurrent cases were analyzed. Thirty-four primary cases received treatment before surgery. The factors investigated included nodal status, tumor size, nuclear grade, ER/PgR and HER2 status, p53 overexpression, and the Ki-67 index value. The AR expression was evaluated using IHC and the expression was divided into 3 groups; negative, low (<10%) and high (≥10%). Breast cancer subtypes were categorized based on the IHC data derived from ER/PgR, HER2 and Ki-67 (cutoff point: 20%) in invasive tumors.

## Results

The AR expression rates were 69.7%(low: 33.9% and high: 35.8%)in all primary cases. Patients who received treatment before surgery had an AR rate of 38.2% which was significantly different from the untreated cases (p=0.002). In the cases with neoadjuvant chemotherapy, the positive rate significantly decreased after chemotherapy in the cases with non-pCR (pathological complete response). The positive rate of recurrent/metastatic cases was 57.7% (low: 34.6% and high: 23.1%). Higher AR expression significantly correlated with smaller tumor size, positive ER/PgR, lower Ki-67 values and nuclear grade and negative p53 overexpression. The AR expression rate was 72.5% in Luminal A, 73.2% in Luminal B, 80% in Luminal HER2, 56.8% in HER2 enriched and 43.5% in triple negative (TN) cases. Moreover, in the TN tumor cases, AR expression significantly correlated with postmenopausal status and a higher degree of malignancy determined by Ki-67, p53, and nuclear grade. However, there was no significant relationship between these factors and the other subtypes.

## Conclusion

The AR expressions were higher in the primary breast cancer cases than in the pretreated and recurrent cases. The AR expression significantly correlated with a lower degree of malignancy and postmenopausal status only in the TN breast cancer cases. These findings suggest that the TN cases with AR-positive tumors have a more favorable prognosis compared with the cases with AR-negative tumors. However, further studies are needed to determine the predictive and prognostic factors for clinical use.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-12

Progesterone drives ER-positive and triple negative breast cancer cell proliferation through progesterone receptor membrane component 1

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**Introduction:** The role of progesterone and its receptors in breast cancer progression continue to be studied but remain controversial. Progesterone membrane receptors with the ability to regulate kinase signals, mediating breast cancer proliferation have been demonstrated. Increased expression of the Progesterone Receptor Membrane Component 1 (PGRMC1), a heme – binding protein with the ability to interact and stabilize epidermal growth factor receptor (EGFR) is frequently found in breast cancer tissue. Some evidence suggests that progesterone can stimulate and regulate breast cancer cell proliferation. The basis of the signaling mechanisms by which Progesterone exerts its function remains largely unknown. Both the nuclear and membrane progesterone receptors could play a significant role in the development and progression of breast cancer and both could become viable therapeutic options. We, aim to investigate the role of PGRMC1 in progesterone driven breast cancers.

**Materials and Methods:** Human breast tissues were utilized to identify PGRMC1 expression along with a panel of normal and breast cancer cell lines. Two breast cancer cell lines (ZR-75-1 and MDA-MB-468) were selected and treated with progesterone and AG-205 (PGRMC1 inhibitor) at different concentrations to assess optimum dosage. We performed MTS assay, qRT-PCR, Western blot, immunofluorescence, immunohistochemistry and flow cytometry for measuring cell proliferation, apoptosis and key markers involved in these processes. We also performed an in silico analysis to compare the expression of PGRMC1 in various cell lines and breast cancer tissues.

**Results:** Immunohistochemistry demonstrated strong staining for PGRMC1 in human breast cancer tissue compared to normal tissue. Increased PGRMC1 expression was observed specifically in ZR-75-1 and MDA-MB-468 cells by qRT-PCR, western blot and immunofluorescence, these results were validated and compared to microarray-based gene expression analysis of breast cell lines and breast tumor data sets. Progesterone treatment increased cell proliferation in a dose dependent manner while AG-205 decreased cell proliferation in a dose dependent manner in ZR-75-1 and MDA-MB-468. Minimal effects of AG-205 were observed in normal breast epithelial cells. AG-205 also, induced apoptosis in both ZR-75-1 and MDA-MB-468 cell lines. Furthermore, short-term treatment of progesterone increased both mRNA and protein expression of PGRMC1. Key markers of cell proliferation (pAKT, CCND1, pEGFR, pmTOR) and apoptosis (PTEN, Bcl2, Bax, Bim) revealed that PGRMC1 facilitated the proliferative effect of progesterone. Interestingly progesterone increases phosphorylation of EGFR and treatment of AG-205 alters EGFR expression in a dose dependent manner.

**Conclusion:** Our data demonstrates that PGRMC1 plays a prominent role in regulating progesterone driven cell proliferation in both ER-positive and triple negative breast cancer cells. These initial findings uncover the potential of PGRMC1 as a therapeutic target for breast cancers.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-13

Quantitative combinatory indexed ChIP-seq reveals distinct transcriptional complexes containing estrogen receptor and GREB1 at chromatin in breast cancer cells

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*GREB1* is an estrogen-activated gene in estrogen receptor a (ER)-positive breast cancer cells. GREB1 is also required for estrogen-stimulated breast cancer cell growth and its level is highly correlated to ER level in breast cancer cells and tumor samples. In endocrine resistant diseases, GREB1 is often dysregulated. GREB1 has been shown to interact with ER and bind to the same ER binding sites throughout the genome. There is no identified functional domain in GREB1 and it is not completely known how GREB1 exerts its function to regulate the transcription of ER target genes. In order to demonstrate whether GREB1 is present in the ER-containing transcriptional complex at chromatin, we have adapted and developed a quantitative combinatory indexed ChIP-seq assay suitable for dissecting components in a transcriptional cofactor complex in a genome-wide scale. In ER-positive MCF-7 breast cancer cells, we found that almost all GREB1 binding sites are the same sites bound by ER or its *bona fide* coactivator SRC-3. We further found that GREB1 and SRC-3 are both present in the same ER-containing complex at chromatin. Thus, both GREB1 and SRC-3 are integral members of the ER transcriptional complex at chromatin. Moreover, we discovered that only a portion of GREB1 at chromatin is present in the ER complex while the other portion of GREB1 is present in a different complex lacking ER or SRC-3 at the same genomic loci. Thus, two distinct GREB1-containing complexes are identified in equilibrium at chromatin: one contains ER/SRC-3 and the other one lacks ER/SRC-3. Our results suggest a non-traditional role of GREB1 in transcriptional regulation of ER target genes. The method used in our study can be widely applied for probing components of transcriptional complexes at chromatin.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-15

Pregnancy inhibits mammary carcinogenesis by persistently altering the hypothalamic-pituitary axis

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Pregnancy, carried to term at an early age, is probably the best natural protection against breast cancer development. The relative life-long breast cancer risk for women that give birth to their first child before the age of 20 years is approximately half that of nulliparous women. In contrast, if a woman undergoes her first full-term pregnancy after the age of 35, her risk for breast cancer is increased even more than nulliparous women. In the current generation many women are career oriented and have children later in life. Universally the average age at first birth is on the rise. It is critical to understand the underlying mechanism of this protective effect of pregnancy against breast cancer to develop novel prevention strategies to reduce the risk of breast cancer without women having to undergo pregnancy early in life. Earlier, we and others have demonstrated that post-pregnancy there were persistent changes in circulating levels of hormones. In order to understand the significance of these systemic changes we determined alterations in the hypothalamic-pituitary axis in parous rats. In particular, we examined the static and dynamic alterations in the hypothalamic-pituitary axis in response to pregnancy. Seven weeks old female Lewis rats were injected with the chemical carcinogen N-methyl-N-nitrosourea (MNU) at a dose of 50mg/kg body weight intraperitoneally. Two weeks post-carcinogen treatment these rats were housed with a male rat. On the observation of the vaginal plug the male was removed from the cage. Once the rats gave birth they nursed the pups for three weeks and were weaned after that period. Mammary tumorigenesis was monitored through weekly palpation for a period of nine months. A subset of rats at 6, 12 and 24 weeks post-weaning were used to study static and dynamic changes in the level of hormones. We investigated the static alterations in the hypothalamic-pituitary axis in response to pregnancy by measuring the levels of thyrotropin releasing hormone (TRH), growth hormone releasing hormone (GHRH), somatostatin (SS), dopamine (DA), growth hormone (GH) and prolactin (PRL). Next we investigated if the dynamic alterations in the hypothalamic-pituitary axis in response to pregnancy. Control and parous animals were subjected to secretagogue treatments (Growth Hormone Related Peptide 6 for GH and Perphenazine for PRL) and the levels of GH and PRL were measured. We also isolated the pituitary and treated them with the secretagogues and measured the levels of GH and PRL. Our data demonstrated that pregnancy resulted in persistent static and dynamic alterations in circulating levels of hormones. Parous rats response to the secretagogues was severely blunted compared to the control nulliparous rats. The levels of TRH, GHRH, GH and PRL were significantly lowered in parous rats while DA and SS levels were higher in nulliparous rats. As expected mammary carcinogenesis was significantly inhibited in parous rats. Overall, these preliminary results suggest that pregnancy induces persistent changes in hypothalamic-pituitary axis, which results in a lowered hormonal promotion environment resulting in inhibition of mammary carcinogenesis.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-05-16

Role of estrogen receptor alpha acetylation in estrogen-dependent gene regulation in breast cancers

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Estrogens, such as 17 $\beta$ -estradiol (E2), act through estrogen receptor alpha (ER $\alpha$ ), a ligand-regulated transcription factor that binds across the genome to promote enhancer formation and regulate gene expression. ER $\alpha$  is expressed in approximately 70% of breast cancers, where it regulates the transcription of genes involved in mitogenic and inflammatory pathways. We are exploring the the acetylation of ER $\alpha$  on lysines 266 and 268, a modification that enhances the DNA binding and transcriptional activities of ER $\alpha$ . ER $\alpha$  acetylation is catalyzed by the lysine acetyltransferases p300 and CBP in an E2- and steroid receptor coregulator (SRC)-dependent manner. Acetylation-dependent activation of ER $\alpha$  has potential implications in breast cancers associated with enhanced coregulator interactions, such as SRC-3/Amplified in Breast (AIB1) gene amplifications and gain-of-function ER $\alpha$  mutations in endocrine resistant metastatic tumors, such as Y537S and D538G. Increased association of ER $\alpha$  with SRCs and p300/CBP leading to enhanced ER $\alpha$  acetylation may promote gain-of-function ER $\alpha$  activity. Our hypothesis is that acetylation of ER $\alpha$  alters its function by increasing E2-responsive gene transcription and signaling in breast cancers. Our current efforts are focused on investigating the role of ER $\alpha$  acetylation on chromatin binding and accessibility, enhancer activity, and target gene transcription. We are using the ER-positive MCF-7 breast cancer cell line with a knockdown/re-expression strategy with biochemical mimics of acetylated (K266/268Q) or unacetylated (K266/268R) ER $\alpha$ . Genomic ER $\alpha$  binding profiles using chromatin immunoprecipitation-sequencing (ChIP-seq) has defined overlapping, but unique, sets of transcriptional targets and recruitment kinetics for the ER $\alpha$  mutants. Current efforts are focused on defining global transcriptional responses associated with the unique binding activities using precision run-on sequencing (PRO-seq). Ultimately, our goal is to define how acetylation affects the binding of ER $\alpha$  to chromatin and its effect on the transcriptional activity of E2-target genes in breast cancers in the context of gain-of-function ER $\alpha$  mutations and coregulator amplifications. *Supported in part by a postdoctoral fellowship from the Lalor Foundation to YMV, and grants from the NIDDK, NICHD, and CPRIT to WLK, as well as support from the Cecil H. and Ida Green Center for Reproductive Biology Sciences Endowment.*

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-06-01

Distinctive coding and non-coding RNA profiles of pre-menopausal and post-menopausal benign breast

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**Background:** Despite a paucity of data on the benign breast transcriptome, reduction mammoplasty tissue is commonly used as a control for RNA biomarker discovery in breast cancer (BC). We evaluated the transcriptome of benign breast to characterize pre-menopausal and post-menopausal coding and non-coding RNA profiles. These profiles will provide insight into the metabolic landscape of benign breast and inform transcriptomic studies of BC and peri-tumoral microenvironments.

**Methods:** With institutional approval, cryobanked fresh breast tissues from reduction mammoplasties (age 15-62y; follow-up: 4-10 y with no cancer events), with histologically-confirmed benign tissues with similar epithelial: stromal tissues were grouped into 4 age sets (< 30y, N=10; 30-39y, N=11; 40-49y, N=10; ≥50y (clinically post-menopausal), N=10). Following RNA extraction, library preparation (Illumina TruSeq Stranded mRNA kit), and sequencing (Illumina HiSeq 4000), reads were processed (MAP-RSeq v3.0.0) and aligned (STAR aligner; hg38). Differential expression (DE) analysis (edgeR 2.6.2) identified DE genes from normalized RPKM reads (absolute log<sub>2</sub> fold change (FC) > 1 and false discovery rate (FDR) < 0.10), corrected for intra-group biases using medians (absolute FC cut-off of >1.5). Over-representation analysis [Ingenuity pathway analysis (IPA), Ingenuity® Systems] and gene set enrichment analysis [(GSEA), GeneTrail 2.0] identified significantly-enriched pathways.

**Results:** Across the 4 age sets, 561 DE genes were identified. Compared to the post-menopausal (PM) set, the number of DE genes was highest in <30 y set (N= 372) and decreased with increasing age (N= 170, age 30-39 set and N=20, age 40-49 set), generating up-regulated (PM<sup>up</sup>) and down-regulated (PM<sup>down</sup>) PM transcriptomic profiles. The top PM<sup>down</sup> DE genes included RANKL, WNT4, MKI67, extracellular matrix, and lactation-related genes (lactoferrin, MUC4, MUC16, p < 0.01). Top PM<sup>down</sup> canonical pathways were cell cycle-related (*CDK1*, *CCNA2*, *CCNB2*, *ESPL1*, *TOP2A*) (p< 0.001). Top PM<sup>up</sup> genes included those involved in adipogenesis, *NPY1R*, *NPY2R*, unsaturated fatty acid synthesis and eicosanoid signaling (P< 0.001). Top PM<sup>up</sup> canonical pathways included acyl co-A hydrolysis, stearate biosynthesis, acute phase response and RXR signaling (P < 0.001). GSEA of the entire gene set (N=15,466; ranked in order of 561 DE genes) identified 16 significant pathways, functionally grouped as mitochondrial energy metabolism, proteasome, and unsaturated fatty acid biosynthesis and signaling (PPAR pathway). While coding RNAs comprised 85% of DE transcripts, lncRNA comprised ~5%. Top DE lncRNAs and precursor miRNAs included MEG3, a tumor suppressor, LIN00092, miR22 and miR1182 (p < 0.0001).

**Conclusions:** Pre and post-menopausal benign breast tissues have distinctive transcriptomic profiles. PM benign breast has down-regulated proliferation/cell-cycle-related pathways, and up-regulated genes in mitochondrial energy pathways, lipogenesis and inflammatory pathways. Notably, many of the top DE coding and non-coding RNAs in PM benign breast have been implicated in BC progression, highlighting their value in better understanding breast carcinogenesis and the need to characterize their functional roles in normal aging and menopause.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-06-02

Organochlorine pesticide residues in human breast tissue and their relationships with clinical and pathological characteristics of breast cancer

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Agricultural pesticides are abundant environmental contaminants worldwide, prompting interest in studying their possible detrimental health effects. We examined organochlorine residues by quadrant (n = 245) in breast adipose tissues from 51 women with various stages of breast health to determine patterns of bioaccumulation within the breast and to assess relationships with patient clinical characteristics. Three organochlorine residues — 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE), hexachlorobenzene (HCB), and mirex — assayed by high resolution gas chromatography were abundant in breast tissue. *p,p'*-DDE (745 ± 1054 ng/g lipid) was the most prevalent residue, comprising 97.5% of the total chemical burden. Mean levels of *p,p'*-DDE and HCB were significantly correlated ( $P < 0.001$ ) with patient age at mastectomy, and levels of *p,p'*-DDE were correlated ( $P < 0.05$ ) with BMI. Pesticide concentrations did not differ significantly by breast quadrant and were not different in the quadrant(s) where the primary tumor was located compared to other cancer-free quadrants. In invasive cancer patients, organochlorine levels differed significantly based on clinical characteristics of the primary carcinoma, including stage, grade, ER status, and HER2 status, indicating that body burden of organochlorines may influence the development of specific subtypes of breast cancer. Potentially carcinogenic organochlorines were present at high levels within the human breast warranting further research to determine the impact of organochlorines in the etiology of breast cancer.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-01

Computational scoring of tumor infiltrating lymphocytes in triple-negative breast cancer

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**Background:** Stromal Tumor Infiltrating Lymphocytes (sTIL) are an established prognostic feature in triple-negative breast cancer, yet manual assessment or visual estimation of sTILs with conventional light microscopy may be subject to inter-pathologist variability. Recently published guidelines by the International TIL Working Group help address inter-pathologist variability, yet there remains a need for more objective and quantitative computational sTIL scoring.

**Methods:** Our study used 120 triple-negative breast cancer slides (one slide per patient). A deep-learning based image analysis workflow is used to perform segmentation and classification of tissue regions and cells on the digital whole slide image. We used 14 annotated slides to train and validate the deep learning model, and to obtain image segmentation and classification accuracy statistics. Non-training slides were used to evaluate the concordance of manual (m-sTIL) and computationally derived (c-sTIL) scores. To generate data to create the model we manually annotated tissue regions in FFPE H&E stained digital slides, including: tumor, stroma, and necrosis. Initial classification of cell nuclei was performed using a semi-automated image analysis method, and then manually corrected to generate ground truth for tumor, stroma (fibroblasts), and lymphocytes. All annotations were performed by a trained research fellow and reviewed by a board-certified pathologist. Corresponding region and nucleus-level annotations were combined to train and validate a fully-convolutional neural network that jointly classifies tissue regions and cell nuclei. Tissue region segmentation accuracy was assessed by the Dice coefficient to measure degree of overlap between predicted tissue regions and ground truth annotations. Cell classification accuracy was assessed using area under curve (AUC). Two board-certified pathologists independently generated an m-sTIL score for all slides according to clinical guidelines, and discrepancies between pathologists were resolved by consensus. c-sTIL scores were calculated as the percentage of classified stromal areas occupied by nuclei classified as lymphocytic infiltrates.

**Results:** Tissue region segmentation was accurate for both stroma (0.77 Dice) and tumor (0.83 Dice) regions, and accurate overall (0.78 Dice). Cell classification was highly accurate for lymphocytes (0.89 AUC), tumor cells (0.90 AUC), stromal cells (0.78 AUC), and overall (0.89 AUC, micro average). Inter observer spearman correlation between the m-sTIL scores of our two pathologists was 0.66 ( $p < 0.001$ ). By comparison, the correlation between c-sTIL and consensus m-sTIL was higher at 0.73 ( $p < 0.001$ ). Dichotomizing at a threshold sTIL score of 10%, c-sTIL scoring identifies low-sTIL patients with an accuracy of 85%. High- and Low- sTIL score patient groups show clear separation on a Kaplan-Meier curve for both c-sTIL and m-sTIL scoring approaches.

**Conclusions:** Our pipeline quantifies stromal TILs with high concordance with manual pathologist scores, and sheds light on the ability of computational approaches in standardizing diagnostic pathology workflows. Future work will investigate how other computationally driven histology biomarkers can predict outcomes and help prognosticate breast cancer patients.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-02

Normal breast stromal fibroblast interaction with human ER<sup>+</sup> breast cancer cells create an IL1 $\beta$ -enriched environment that suppresses normal breast progenitor proliferation while promoting cancer cell growth

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Previous studies suggest that cancer-induced activation of stromal fibroblasts is an early event in tumour progression by promoting cancer cell proliferation through secretion of various cytokines. However, the nature of breast cancer cell interaction with the normal stromal fibroblasts is not clear and their role in tumour progression remains controversial. In particular, the role of fibroblasts as a major component of tumor niche in supporting ER<sup>+</sup> tumour cell proliferation remains unexplored due to the lack of protocols to maintain and expand primary human ER<sup>+</sup> breast cancer cells *in vivo* and *ex vivo*. In this study, we used a modified patient-derived organoid culture system and demonstrate that constitutively secreted cytokines from normal breast tissue fibroblasts specifically initiate a paracrine signaling mechanism with primary human estrogen receptor positive (ER<sup>+</sup>) breast cancer cells (ER<sup>+</sup>BCC) which results in creation of an IL1 $\beta$ -enriched microenvironment. This paracrine crosstalk is initiated with constitutively secreted cytokines (IL6, IL8, CCL7) by the normal breast fibroblasts that result in PDGF-BB production in ER<sup>+</sup> breast cancer cells but not in the normal breast epithelial cells. We further show that IL1 $\beta$  prevents the proliferation of normal breast epithelial progenitors while at the same time it promotes breast cancer cell proliferation. Moreover, we found that this new paracrine signaling mechanism is shared between normal and activated tumour-associated fibroblasts. Interestingly, we observed that in reconstructed tumour microenvironment in organoid cultures initiated with primary human ER<sup>+</sup>BCC, tumour-associated fibroblasts and tumour-infiltrating immune cells obtained from the same original tumour sample, ER<sup>+</sup>BCC show significantly enhanced proliferation and are less sensitive to anti estrogens such as tamoxifen. However, tamoxifen is more effective in reducing tumour cell proliferation when our newly identified paracrine crosstalk between fibroblasts and ER<sup>+</sup>BCC (IL1 $\beta$  and PDGF-BB) signaling is blocked. Our observations then suggest that ER<sup>+</sup> tumour cells could create a growth-promoting environment without activating stromal fibroblasts. Taken together, our findings suggest that in patients who undergo breast conserving surgeries, normal fibroblasts could be a significant modulator of tumour recurrence by enhancing proliferation of residual breast cancer cells in the tumour-adjacent breast tissue. Finally, our results suggest that patients diagnosed with malignant ER<sup>+</sup> breast cancer could potentially benefit from combination therapy consisting of IL1 $\beta$  Receptor and PDGF Receptor blockers to enhance effectiveness of endocrine therapies such as tamoxifen.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-03

Small molecule inhibition of smoothened in triple negative breast cancer-associated fibroblasts depletes cancer stem cells and sensitizes to cytotoxic chemotherapy in mice and humans

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The cellular and molecular basis of stromal cell recruitment, activation and crosstalk in carcinomas is poorly understood, limiting the development of targeted anti-stromal therapies.

In mouse models of triple negative breast cancer (TNBC), we use single cell genomics to show that Hh ligand produced by neoplastic cells reprograms cancer-associated fibroblast (CAF) gene expression, driving tumor growth and metastasis.

Hh-activated CAFs upregulated expression of FGF5 and deposition of fibrillar collagen, leading to FGFR and FAK activation in adjacent neoplastic cells and the acquisition of a stem-like, drug-resistant phenotype.

Treatment with smoothened inhibitors (SMOi) reversed these phenotypes. Stromal treatment of TNBC patient-derived xenograft (PDX) models with SMOi downregulated the expression of cancer stem cell markers and sensitized tumors to docetaxel, leading to markedly improved survival and reduced metastatic burden.

In the phase I clinical trial EDALINE, 3 patients with metastatic TNBC derived clinical benefit from combination therapy with the SMOi Sonedigib and docetaxel chemotherapy, with one patient experiencing a complete radiological response. Responders also exhibited high baseline FGFR activation and ECM deposition, suggesting a mechanism of action consistent with findings from the animal models.

These studies identify Hh signaling to CAFs as a novel mediator of cancer stem cell plasticity and drug resistance and an exciting new therapeutic target in TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-04

Isolation and characterization of colony forming cells from primary malignant human breast cancer tumours

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Advances in imaging technologies and new therapies have increased the 5-year survival of breast cancer patients to ~80%, but metastatic relapses continue to make this malignancy the leading cause of death in women. Recent data suggest that relapses arise from the activated proliferation of malignant clonogenic cells that are therapy-resistant. However, direct characterization of these malignant clonogenic cells has been hindered due to a lack of methodology to isolate them from primary breast tumours and to maintain them in culture. We now report the development of two robust culture systems that allow primary malignant human breast cancer cells to be propagated *ex vivo* at clonal densities and with retention of their tumorigenic potential as assessed in transplanted immunodeficient mice. Using the first set of culture conditions, we found that colony-forming cells (T-CFCs) could be detected at frequencies of 0.2-0.5% in 14 primary tumor samples and these included 9 ER+, 4 HER2+, and 1 triple negative breast cancers. The colonies generated had one of two distinct types of morphology and cytokeratin expression. Type A colonies stained positively for CK8/18 only and were obtained primarily from ER+ breast cancers. Type B colonies stained for CK14 only and were most frequently present in HER2+ and triple-negative breast cancers. Type A and B T-CFCs could be separately isolated based on their differential expression of CD49f (α6-Integrin) and the Epithelial Cell Adhesion Molecule (EpCAM); T(A)-CFCs being CD49f<sup>+</sup>EpCAM<sup>+</sup> and T(B)-CFCs being CD49f<sup>+</sup>EpCAM<sup>-</sup>. We also identified culture conditions that enable T-CFC expansion. Using this second system, we expanded 14 primary breast cancer tumours up to 5 passages in 2D and 3D organoid cultures. These *ex vivo* expanded breast cancer cells (Exp-BCCs) contained both type A and type B T-CFCs and were detectable at much higher frequency (20-fold) than in the primary tumors. These Exp-BCCs produced ER+, or HER2+, or triple-negative breast cancer tumors in transplanted immunodeficient mice within 7 weeks, indicative of their retained malignant properties. We have also found that T-CFCs are less sensitive to chemotherapies than the non-CFCs in the primary tumours. These systems now offer consistent and powerful methods to isolate and characterize the cells in primary human breast cancers that are implicated as responsible for relapse.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-07-05

Obesity, adipose inflammation, and race in patients with early stage breast cancer

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**Background:** Elevated body mass index (BMI) is associated with increased risk of estrogen receptor-positive postmenopausal breast cancer. Mechanistically, most individuals with elevated BMI have breast white adipose tissue inflammation (WATi) which confers increased breast cancer risk, particularly in those with existing benign breast disease. Individuals with WATi have elevated in-breast expression of aromatase and several systemic changes that increase breast cancer risk, including hyperinsulinemia and higher levels of C-reactive protein. However, women with normal BMI but high levels of body fat are also likely to harbor WATi and are at increased risk of postmenopausal breast cancer. The accuracy of BMI for assessing adiposity and predicting obesity-related disorders, including cancer, varies across race and ethnicity. Whether the association between BMI and WATi varies by race is unknown. Here we aimed to characterize relationships among breast WATi and clinicopathologic features in a racially diverse cohort undergoing mastectomy for breast cancer treatment.

**Methods:** Non-tumorous breast tissue and fasting blood were collected from women undergoing mastectomy for breast cancer treatment or prevention at a single center serving a racially diverse patient population. Breast WATi was detected by the presence of crown-like structures in the breast (CLS-B), which are composed of a dead/dying adipocyte surrounded by CD68+ macrophages. Clinicopathologic data were abstracted from electronic medical records. Associations among categorical variables were examined using Fisher's exact test. Relationships between continuous variables were examined using the Spearman correlation.

**Results:** As of May 18, 2018 62 patients have been accrued; median age 55 (range 32 to 84). Self-reported race distribution was: 36 (58%) Asian, 5 African American (8%), 20 (32%) Caucasian, and 1 (2%) unknown. Breast tissue has been analyzed for WATi in 60 cases thus far. Clinicopathologic features stratified by the presence or absence of breast WATi are presented in

[table1]

Variable	Breast WATi Absent (n=25)	Breast WATi Present (n=35)
Age, years		
Median (range)	51 (32 to 71)	59 (36 to 80)
BMI, kg/m2		
Median (range)	22.5 (18.1 to 35.3)	28.0 (19.2 to 38.9)
BMI Category		
Underweight	1 (4%)	0 (0%)
Normal	16 (64%)	10 (29%)
Overweight	5 (20%)	18 (51%)
Obese	3 (12%)	7 (20%)
Race, n (%)		
Asian	15 (60%)	19 (54%)
African American	2 (8%)	3 (9%)
Caucasian	8 (32%)	12 (34%)
Unknown	0 (0%)	1 (3%)
Menopausal Status, n (%)		
Pre	10 (40%)	12 (34%)

Post	15 (60%)	23 (66%)
Hypertension, n (%)	7 (28%)	16 (46%)
Diabetes mellitus, n (%)	0 (0%)	5 (14%)
Dyslipidemia, n (%)	1 (4%)	11 (31%)

. Breast WAT inflammation was associated with obesity ( $P=0.02$ ) and a trend to association was observed with dyslipidemia ( $P<0.09$ ).

**Conclusions:** Breast adipose inflammation is associated with elevated BMI and possibly metabolic syndrome disorders in a racially diverse population. These findings are consistent with observations from predominantly Caucasian cohorts. Race-specific characteristics will also be examined. Study accrual is ongoing and updated results will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-06

Investigating breast cancer dormancy in response to microenvironment cues with well-defined synthetic extracellular matrices

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Breast cancer recurs in approximately 20% of disease-free patients 5+ years after successful treatment of the original tumor. These late recurrences often occur at metastatic sites, including the bone marrow and lungs, and are hypothesized to arise from disseminated tumor cells (DTCs) that reactivate after a long period dormancy. Interactions between DTCs and the microenvironment at these sites are thought to be important regulators of both breast cancer cell dormancy and reactivation. New approaches are needed to study this complex process and test hypotheses about key cell-microenvironment interactions in dormancy toward improved treatment strategies.

In this work, we investigated the role of cell-matrix and cell-cell interactions on dormancy and activation of breast cancer cells utilizing well-defined materials to mimic the extracellular matrix (ECM) of metastatic sites and selectively introducing metastatic niche cells via dynamic co-culture. We established synthetic ECMs with well-defined properties that mimicked key aspects of the mechanical properties and biochemical content of bone marrow and lung metastatic sites (Young's modulus  $E \sim 0.5-5$  kPa, respectively, and rich in collagen). These synthetic ECMs were constructed with a bioinert, multifunctional poly(ethylene glycol) crosslinked with a cell-degradable peptide and decorated with integrin-binding peptides derived from collagen and laminin, creating three-dimensional (3D) environments for the culture of breast cancer cells and niche cells. We cultured breast cancer cells of different metastatic potential (estrogen receptor positive [ER+, T47Ds] and triple negative [ER-, MDA-MB-231s]) within matrices, with or without co-culture of niche cells, and examined differences in markers of dormancy for up to 6 weeks. Both breast cancer cell types exhibited good viability in 3D culture (> 90%). Different degrees of cell proliferation (metabolic activity, EdU assay, cell/cluster number and volume) were observed over the first 2 weeks in culture, dependent upon matrix composition. Interestingly, ER- MDA-MB-231s were more responsive to differences in the biochemical content of the matrix, with increased elongation and proliferation observed within collagen mimic environments, whereas fewer differences were observed in the responses of the ER+ T47Ds to these same compositions. Further, both cell types exhibited significant decreases in proliferation in response to increased matrix density, mimicking the moduli of bone marrow to lung tissues. Dormant single cells or small cell clusters, reminiscent of micrometastases, were observed over weeks 2-6. Immunostaining and bioinformatics analyses revealed increased expression of autophagy markers for these dormant cells, suggesting a potential mechanism of survival. Additionally, co-culture with specific niche cells, including human mesenchymal stem cells and osteoblasts, was observed to enhance or suppress breast cancer cell proliferation within these well-defined environments. These studies have established a new controlled 3D breast cancer dormancy culture model, with on-going investigations examining cell re-activation for the identification of therapeutics to maintain dormancy and prevent recurrence.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-07

The immune microenvironment of ductal carcinoma *in situ* of the breast

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**Background:** The importance of tumor-infiltrating lymphocytes (TIL) in invasive breast carcinoma for tumor development and therapeutic response is widely accepted. However, the immune microenvironment of breast ductal carcinoma *in situ* (DCIS) has not been fully elucidated. Evasion of immune surveillance is a necessary step in tumor evolution. In DCIS, the tumor cells are relatively protected from the immune system due to an myoepithelial cell layer and basement membrane, and intraductal immune cells are rarely detected. In contrast, in invasive disease, cancer cells and immune cells are often intermingled. Thus, understanding the immune microenvironment of *in situ* to invasive carcinoma transition might be particularly important to identify novel targets for early stage of tumor invasion.

**Aims:** The aim of this study is to evaluate the clinical importance of TILs in DCIS.

**Methods:** TILs were assessed in 133 DCIS samples with or without microinvasive disease according to the proposed method from the International Immuno-Oncology Working Group on Breast Cancer. In addition, the relationship between TILs in DCIS and clinicopathological features was evaluated.

**Results:** TILs are present in most DCIS in varying levels. The median proportion of TILs in DCIS was 14%. Only a minority of DCIS showed >50% TILs, which represented only 12.8% of all cases. High TILs in DCIS was significantly associated with comedo necrosis ( $p<0.0001$ ), high nuclear grade ( $p=0.0030$ ), ER negativity ( $p<0.0001$ ), PR negativity ( $p<0.0001$ ), HER2 positivity ( $p=0.0030$ ). Triple negative DCIS and HER2 positive DCIS had significantly higher level of TILs ( $p=0.0008$ ). No correlation was demonstrated between TILs and recurrence risk.

**Conclusions:** High TILs in DCIS was significantly associated with adverse histopathologic features. Further characterization of immune environment of DCIS may be essential for immunotherapy and breast cancer prevention.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-10

The prognostic significance of estrogen formation as a consequence of aromatase expression in tumor microenvironment

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Estrogen synthesis via aromatase in adipose tissue has an important role in progression of postmenopausal breast cancer. The increased local concentrations of estrogen in breast cancer via aromatase overexpression within the tumor tissue have been demonstrated by some investigators. Although aromatase inhibitor is the standard endocrine therapy for postmenopausal breast cancer patients, it is not uncommon for patients to have poor compliance to the drugs due to their side effects. This research is based on the hypothesis that if aromatase expression is related to prognosis and if therapeutic effect varies depending on the degree of aromatase expression, then this study may be able to suggest a new guideline in terms of choosing between aromatase inhibitors and tamoxifen.

Methods: 154 postmenopausal breast cancer patients who underwent surgery and aromatase inhibitor therapy in Busan Paik Hospital, Inje University from January 2005 to December 2010 were enrolled. Patients with DCIS or stage IV breast cancer were excluded. Patients' clinicopathological data were collected and TMA blocks were created for immunohistochemistry studies to examine aromatase expression.

Results: The recurrence has occurred in 7 patients (6.9%). Stage, tumor size and number of lymph node metastasis were related to increased risk of recurrence ( $p=0.051$ ,  $0.043$ ,  $0.001$ ). The aromatase expression in cancer cells had significant correlation with clinical stage ( $p=0.041$ ). There was also a positive correlation between Ki67 and aromatase expression in cancer tissue ( $p=0.006$ ).

### **Correlation between clinicopathologic factors and aromatase expression (Linear by linear association and Spearman's correlation coefficient test)**

Site of aromatase expression	ER	PR	Ki67	P53	BMI	Stage
In Cancer	0.606	0.592	0.006	0.451	0.956	0.041
In Stroma	0.220	0.471	0.584	0.329	0.367	0.229
In Adipose	0.988	0.265	0.159	0.117	0.770	1.000

However, aromatase expression in cancer, stromal, and adipose tissue had no relationship with recurrence ( $p=0.410$ ,  $0.627$ ,  $0.552$ ).

Conclusions: Aromatase expression in cancer cells was correlated with clinical stage. This implies that aromatase expression might have a role of prognostic marker in addition to role of treatment indicator. There was no direct correlation between aromatase expression and recurrence.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-11

Transcriptome alteration of breast cancer cells in the process of tumor-associated macrophages-promoted metastasis

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**Introduction:** Tumor-associated macrophages (TAMs) play an important role in the process of tumor metastasis. Previous studies have shown that TAMs induce breast cancer cells undergo epithelial-mesenchymal transition (EMT) by secreting CCL18. Reciprocally, the induced mesenchymal-like breast cancer cells activate macrophages into a TAM-like phenotype via secreting the cytokine granulocyte-macrophage colony stimulating factor (GM-CSF), forming a positive feedback loop that is essential to breast cancer metastasis.

**Methods:** To better understand the underlying mechanism that links this positive feedback loop to breast cancer metastasis, a TAMs-induced EMT cell model (MCF-7) was used. RNA-seq was used to compare the transcriptome of MCF-7 cells which were cocultured with TAMs and those with or without monocyte-derived macrophages (MDMs). Results were further verified by qRT-PCR.

**Results:** Among 811 known protein-coding genes with reliable readouts, 42 were significantly changed between groups ( $P < 0.001$ ). Further analysis using KEGG, Reactome and Wikipathways databases found that these 42 genes were enriched in 15 signal pathways ( $P < 0.01$ ). According to their biological function, these pathways can be divided into 5 functional groups: SUMOylation related, virus related, immune related, interferon related and cytokine related. The last 3 groups demonstrated that our model were representative and able to provide some real and useful information, while SUMOylation and virus related signal pathways have never been reported to be involved in TAMs induced breast cancer metastasis.

**Conclusion:** We investigated the transcriptome alteration of MCF-7 cells in the process of TAMs-promoted metastasis. Results from bioinformatic analysis indicated SUMOylation and virus related signal pathways in the process of TAMs induced breast cancer metastasis, which are yet to be confirmed.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-12

Circular RNA NCAPG promotes breast cancer metastasis through acting as the sponge of miR-200s

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## Background

Metastatic breast cancer remains incurable and underlying mechanisms are poorly understood. Tumor-associated macrophages (TAMs) represent a major component of the tumor microenvironment that supports breast cancer metastasis. Circular RNAs are new class of endogenous noncoding RNAs. Unlike linear RNAs terminated with 5'caps and 3'tails, they uniquely have a covalently closed loop structure, making them stable and ideal candidate biomarkers. Here, we identified a circular RNA and explored for the first time how it played a dual role in epithelial to mesenchymal transition (EMT) and macrophage recruitment.

## Methods

We first analyzed TCGA database about whether mesenchymal tumors could be more likely to recruit TAMs. ELISA assay was performed for determining expression level of chemokines (CSF-1, CCL2, CCL5, C5a, VEGFA, IL-34) after cell line MCF-7 finishing EMT. Next, we predicted microRNAs which could regulate CSF-1 expression and inhibit EMT using TargetsCan database. Circular RNA was identified via comparing expression levels between mesenchymal-like and epithelial-like cells using circRNA microarrays. The specific mechanisms were explored via series of molecular biology assays in vitro and in vivo.

## Results

Correlation analysis of relationship between EMT score and expression of all classical chemokines that could recruit macrophage revealed a positive association ( $r^2 > 0.3$ ,  $P < 0.05$ ) among 1079 breast cancer cases. Increased expression level of CSF-1 was found the most significant after EMT. miR-200b and miR-200c that inhibiting breast cancer EMT were confirmed targeting 3'UTR of CSF-1 mRNA and decreasing migration of TAMs. Circular RNA NCAPG (circNCAPG) was found highly expressed in mesenchymal-like cells and could promote breast cancer metastasis in vitro and in vivo. Poor clinical outcome was found in circNCAPG overexpression group among 141 breast cancer cases. CircNCAPG was proven to be located in cytoplasm and found could act as the sponge of miR-200b and miR-200c. Moreover, expression level of CSF-1 and ZEB-1 (also targeted by miR-200s and regulating key process of EMT) was found decreasing significantly after knocking down circNCAPG both in vitro and in vivo, leading to breast cancer metastasis inhibition.

## Conclusion

Mesenchymal tumors have the ability to recruit more TAMs, therefore further promoting breast cancer metastasis. A new molecule: circNCAPG participates in this process via acting as the sponge of miR-200s and regulates CSF-1 and ZEB-1 expression, which warrants further study in clinical settings.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-07-13

Conceptual model of transdisciplinary science - Advocacy collaboration for the physical sciences and oncology: A case study focusing on breast density, biomarker discovery, and emerging therapeutics

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**BACKGROUND:** What happens when you mix the foundations of tissue mechanics with advocacy? In a shared quest for exciting scientific frontiers, Bay Area physical scientists, clinical researchers, and advocates work in dynamic symbiotic relationships to integrate concepts drawn from their respective fields. Focusing on the mechanobiology of tumor progression in breast cancer, researchers and advocates are co-creating system change interventions for revamping convergent research processes.

**METHODS:** As vital catalysts of transdisciplinary innovation, advocates affiliated with the National Cancer Institute (NCI) Physical Sciences and Oncology Network (PS-ON) applied core principles that synergize with the evolving disciplines of Implementation Science (IS) and the Science of Team Science (STS). Diverse methodologies to describe the intersections of physical sciences, breast density, biomarker discovery, emerging therapeutics and advocacy are presented. Additionally, we introduce a theoretical framework and conceptual puzzle illustrating multimethod science advocacy engagement strategies, a typology of contextual factors influencing collaboration, as well as the antecedents, processes, strategic priorities, and overall potential impacts of collaborative transdisciplinary science advocacy exchanges.

**RESULTS:** Through proactive participation in four areas: 1) research and programmatic support, 2) education and outreach, 3) policy and strategy, and 4) representation and advisory, advocates, representing patient/consumer perspectives, worked toward a common set of goals with researchers and clinicians in determining how tumor microenvironments regulate cancer initiation and behavior through interactions among cell types (e.g., initiated cells, activated stromal cells, and components of the extracellular matrix). Applying NCI Office of Advocacy Relations (OAR) and NCI PS-ON Advocacy Working Group goals for strategic innovation, collaborative execution, and ethical codes of conduct, researchers and advocates codeveloped guiding conceptual frameworks based on organizational foundations, systems readiness, leadership commitment to change, and transdisciplinary levers to promote shared governance, bidirectional collaboration, advocacy inclusion, and the prioritization of research addressing questions of importance to patients.

**DISCUSSION:** Embedding advocate patient/consumer evidentiary and experiential insights/perspectives regarding mechanics-directed research priorities and clinical interventions in the early phase of convergent research efforts contributes to our understanding of the important role of the physical organization in cell-to-cell contacts, tissue architecture, tumor microenvironments, and mechanical properties in response to therapy. Notably, catalyzing and leveraging advocate engagement across the research continuum provides novel opportunities for advancing institutional changes, spurring unique training/mentoring exchanges, and fostering innovative research and translational opportunities.

## 2018 San Antonio Breast Cancer Symposium ®

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CCL18 signaling from breast tumor-associated macrophages fosters the activation of fibroblasts into a chemoresistance-inducing phenotype

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Carcinoma-associated fibroblasts (CAFs) are abundant and heterogeneous stromal cells in tumor microenvironment critically involved in cancer progression. Our recent study identified a specific CAFs subset by two cell-surface molecules, CD10 and GPR77. We proved that CD10<sup>+</sup>GPR77<sup>+</sup> CAFs could promote tumor formation and chemoresistance by providing a survival niche for cancer stem cells (CSCs). However, their origin and underlying activating initiation mechanism are unclear. Tumor-associated macrophages (TAMs) are the most abundant immune-related stromal cells and the important source of inflammatory cytokine. Here, we demonstrated that CCL18 secreted by TAMs could induces the activation of normal breast fibroblasts into a CD10<sup>+</sup>GPR77<sup>+</sup> CAFs phenotype, which are not only resistant to chemotherapy themselves, but also can induce chemoresistance in cancer cells by abundantly producing IL-8 and IL-6 and enriching the population of cancer stem cells. In conclusion, our studies have identified an inflammatory signaling network in the interaction of different kind of stroma cells in tumor microenvironment and highlighted its potential values as predictive markers and therapeutic targets to abrogate tumor formation and improve chemotherapeutic efficacy.

## 2018 San Antonio Breast Cancer Symposium ®

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Adipose-derived peptides from breast cancer patients promote the malignancy of breast cancer MCF-7 cells

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Adipose stromal/stem cells (ADSCs) could regulate malignancy behaviors of breast cancer, which was due to adipose-derived cytokines in part, such as leptin and resistin. Recently, peptides have been demonstrated to be potential targets for cancer therapy. It's valuable to analyze the characters of adipose-secreted peptides systematically. In this study, we isolated primary adipocytes from adipose tissue adjacent to breast tumor and breast benign lesions respectively, and then co-cultured breast cancer cells MCF-7 with tumor adjacent adipocytes (TAAs)/breast benign adipocytes (BBAs) separately. Compared to BBAs, TAAs could promote proliferation and migration ability of MCF-7. Then we extracted and purified peptides from supernatant of TAAs and BBAs using ultrafiltration, and the effects of TAAs-derived peptides compound on breast cancer cells appeared accordance with the adipose cells. 100 peptides, which were derived from 90 protein precursors, were found to be differentially secreted between TAAs and BBAs by LC-MS/MS ( $p < 0.05$ ). Gene Ontology (GO) and Pathway analyses appeared that these altered peptides were mainly contributed to cell adhesion, and regulation of Ras-protein signal transduction. These results showed that these differentially secreted peptides could regulate breast cancer cell malignancy, which indicated that they could be potential therapeutic targets and diagnostic bio-marker for breast cancer.

## **2018 San Antonio Breast Cancer Symposium ®**

**Publication Number:** P5-07-16

Role of collagen X in enhancing the metastatic potential of breast cancer cells using a MDA-MB-231 cell line model

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Breast cancer is the second highest cause of cancer related deaths for women in developed countries. Breast cancer patients with distant metastasis at the time of diagnosis have an estimated 5-year relative survival rate of 26% as compared to a 99% survival rate of patients who have localized tumors. Evidence suggests that collagens play a role in enhancing the metastatic capability of breast cancer cells. Short chain collagen, collagen X, is encoded by the collagen type x alpha 1 chain (COL10A1) gene and is normally expressed exclusively by hypertrophic chondrocytes during endochondral ossification. Recently, COL10A1 gene expression has been found to be overexpressed in various tumor types, including breast tumors. It is hypothesized that an increase in COL10A1 expression may play a role in breast cancer metastasis. The goal of our project was to evaluate the role of collagen X in breast cancer metastasis using the MDA-MB-231 breast cancer cell line. Stable cell lines were generated to express either GFP only (MDA-VEC) or GFP tagged COL10A1 (MDA-COL). GFP and COL10A1 transcript and protein levels were examined to confirm overexpression of collagen X and transwell assays were used to determine changes in the invasive capability of the cells. Cells overexpressing collagen X demonstrated a higher rate of invasion suggesting that collagen X may play a role in enhancing the metastatic potential of breast cancer cells. Understanding the role collagen X plays in breast cancer metastasis may provide a mechanism for developing diagnostic and prognostic strategies for identifying patients whose breast cancer is more prone to metastasize.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-08-01

DPYSL3 modulates mitosis, migration and epithelial to mesenchymal transition in claudin-low breast cancer

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Proteogenomics is the field of integrating data from mass spectrometry-based shotgun proteomics, and phosphoproteomics into next-generation RNA and DNA sequencing data analysis pipelines that promises new insights into cancer biology and therapeutic targeting. As well as analyses of clinical samples for disease phenotype association analysis, the application of proteogenomics to model systems also has considerable potential. A Clinical Proteomic Tumor Analysis Consortium (CPTAC) proteogenomic analysis prioritized dihydropyrimidinase-like-3 (DPYSL3) as a multi-level (RNA/Protein/Phosphoprotein) expression outlier specific to the Claudin-Low (CLOW) subset of triple negative breast cancers. A Pubmed informatics tool indicated a paucity of data in the context of breast cancer which further prioritized DPYSL3 for study.

DPYSL3 was identified as a protein that is regulated during neuronal differentiation in the cerebral cortex and in neuronal cell lines and plays a role in regulating neurite outgrowth somehow through an association with vesicles in the growth cone. In addition, DPYSL3 expression has been observed in several malignant tumors, including prostate cancer, pancreatic cancer, gastric cancer and neuroblastoma. DPYSL3 is reported to play a role in cell migration and metastasis suppression in prostate cancer. However, in pancreatic cancer, DPYSL3 is positively associated with liver metastasis and poor outcome.

DPYSL3 knock-down in DPYSL3 (+) CLOW cell lines demonstrated reduced proliferation, yet enhanced motility and increased expression of Epithelial to Mesenchymal Transition (EMT) markers suggesting that DPYSL3 is a multi-functional signaling modulator. Slower proliferation in DPYSL3 (-) CLOW cells was associated with accumulation of multi-nucleated cells indicating a mitotic defect that was associated with a collapse of the vimentin (VIM) microfilament network induced by VIM hyperphosphorylation. On the other hand, DPYSL3 suppressed the expression of EMT regulators TWIST and SNAIL and opposed p21 activated kinase 2 (PAK2) dependent migration, but these EMT regulators in turn induced DPYSL3 expression, suggesting DPYSL3 participates in negative feedback in EMT. Cell migration in DPYSL3 (-) cells correlated with increased phosphorylation of PAK2 on Ser20 and was sensitive to PAK2 siRNA and pharmacological PAK inhibition. Immunoprecipitation and mass spectrometry-based proteomics or western blotting strongly suggests that PAKs interact such that DPYSL3 may function as a direct negative regulator of PAK family kinases. Thus, a PAK inhibitor could potentially mitigate increase migration as an adverse effect of DPYSL3 suppression.

In conclusion, DPYSL3 is a remarkable multifunctional signaling scaffold that should be examined further to provide insights into the stem cell-like state of claudin-low breast cancers, particularly in terms of their cell cycle dependencies, migratory activity and capacity for EMT.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-08-02

PTK6 small molecule inhibitor enhances efficacies of chemotherapy in mesenchymal TNBC

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**Background:** EMT in cancer promotes resistance to chemotherapy and radiotherapy, as well as immune suppression in the tumor microenvironment. EMT is also associated with enhanced tumor dissemination to other organs. EMT is a dynamic cell re-programming process whereby cancer cells lose epithelial markers and acquire mesenchymal markers, enhanced cell migration, and anoikis resistance. EMT is promoted by transcriptional and epigenetic regulators, as well as by signaling pathways. TNBCType and 101-gene model have identified distinct subsets of TNBC that exhibit mesenchymal gene signatures and phenotypes. This particular subset may be associated with chemotherapy resistance and metastatic recurrence in patients with TNBC. We identified protein tyrosine kinase 6 (PTK6) as a promoter of EMT in mesenchymal TNBC through its ability to prevent degradation of SNAIL, a key EMT transcriptional factor. A higher level of SNAIL expression is associated with poor TNBC patient prognosis. We investigated whether SNAIL suppression and EMT reversal by PTK6 small molecule inhibitor treatment enhance efficacy of chemotherapeutic agents that are part of standard of care treatment for patients with TNBC. **Methods:** Mesenchymal TNBC cell lines or organoids generated from TNBC PDX tumors were treated with varying concentrations of PTK6 small molecule inhibitor alone or in combination with chemotherapeutic agents in 3D cell cultures. The cell viability was assessed using 3D CellTiter-Glo or alamar blue. The combination Index was calculated to examine potential synergistic effects (CI<1: synergism, CI=1: additive, CI>1: antagonism). The in vivo combination effects of PTK6 inhibitor and paclitaxel were also assessed in two TNBC PDX models. Gene ontology analysis, targeted RT-PCR gene expression profiling and protein array were performed to identify potential mechanisms for chemosensitization effects of PTK6 inhibitor treatment. **Results:** Pre-treatment with PTK6 inhibitor increases sensitivity to paclitaxel or doxorubicin in 3D matrigel culture of TNBC cell lines, as well as in TNBC PDX organoids. The Combination Index suggested synergies between PTK6 inhibitor and chemotherapy treatment (paclitaxel or doxorubicin). While administration of PTK6 inhibitor or paclitaxel alone only modestly suppressed growth of PDX tumors in vivo, PTK6 inhibitor treatment sensitized tumors to paclitaxel treatment, as evidenced by the dramatic suppression of tumor volume and rate of growth. Gene ontology analysis identified gene sets that are significantly differentially expressed in PTK6 inhibitor-treated TNBC tumors, including extracellular matrix, cell migration, cell cycle and microtubule activity. A targeted RT-PCR profiling and protein array found that PTK6 inhibitor modulates expression of molecules that are associated with chemotherapy resistance and immune regulation; decrease in MMP3, PLEK2, osteopontin, IL-6 and CCL5 and increase in CD40. We are currently investigating whether these changes are dependent on Snail downregulation/EMT reversal caused by PTK6 inhibition. **Conclusion:** PTK6 inhibition may sensitize TNBC to chemotherapy treatment by suppressing Snail and reversing EMT. We will further validate these effects of PTK6 inhibitor treatment with other chemotherapies and immunotherapies.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-08-04

Defining chromatin accessibility profiles of partial and reversible EMT

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The epithelial-to-mesenchymal transition (EMT) reversible cellular reprogramming event, used repeatedly throughout development, is hypothesized to be involved in the cell migration and consequently metastasis that ultimately contributes to most breast cancer-related fatalities. In this process, cuboidal epithelial cells, marked by the presence of tight junction proteins and cell-cell adhesions, lose their apicobasal polarity and acquire a spindle-like morphology and migratory traits. EMT was originally regarded to have only two states, with cells exhibiting either epithelial or mesenchymal phenotypes; however, recently, researchers have demonstrated the existence of a dual epithelial/mesenchymal state, termed hybrid- or partial-EMT. Due to its inherent plasticity, it is believed that EMT and its reversal mesenchymal-to-epithelial transition (MET) is, at least, partly regulated by epigenetic means such as alterations in chromatin structure. Assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) is a novel technique that employs the use of a mutant Tn5 transposase to cleave nucleosome-free DNA regions in a non-biased manner. In this investigation, we induced MCF10A mammary epithelial cells to undergo a short-term (<4 days) or long-term (>4 days) EMT. Addition and withdrawal of exogenous TGF $\beta$ 1 produced partial- or full-EMT and MET conditions which were interrogated by ATAC- and RNA-seq. Hierarchical clustering of ATAC cleavage peaks revealed that pre-EMT and short- and long-term MET conditions demonstrate similar chromatin accessibility profiles with cleavage sites enriched for specific binding motifs. Notably, transcription factors typically not associated with EMT displayed dynamic enrichment in the accessible chromatin at various timepoints in our assay. Correlation with RNA-seq data reveals highly dynamic changes in gene expression suggesting dynamic and reversible use of regulatory programs. Importantly, partial-EMT cells were characterized by unique accessibility patterns, motif enrichment, and gene expression supporting the conclusion that this is not merely an intermediate but a unique state.

# 2018 San Antonio Breast Cancer Symposium ®

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The interplay between stromal density, epithelial mesenchymal transition and chemoresistance in breast cancer

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Epithelial Mesenchymal Transition (EMT) refers to the transition of cells from a more differentiated epithelial phenotype to a less differentiated mesenchymal phenotype, a process that may be triggered by a range of therapeutic interventions including cytotoxic treatment, and which we have previously linked to poor breast cancer (BrCa) outcome after neoadjuvant chemotherapy (NAC)<sup>1</sup>. Mammographic breast density (MBD) represents the white radiographic appearance of epithelial and stromal breast tissue on a mammogram. High MBD in patients being treated for BrCa also associates with chemoresistance, correlating with lower pathological complete response rates (pCR)<sup>2</sup>. Linking these two stimuli, EMT can also be induced by artificial high-density stroma, where it also leads to chemoresistance in vitro<sup>3</sup>.

Here we set out to validate the link between poor outcome after NAC and EMT in a larger validity patient cohort, and to ascertain the molecular drivers through which EMT is triggered in this setting. Further we look to confirm the association of high MBD with poor chemoresponse in the same cohort, and to assess whether this chemoresistance is mediated through EMT with the same drivers.

In a pilot cohort of 50 NAC-treated locally advanced BrCas with a pCR rate of 20%, pre-NAC biopsies and post-NAC surgical specimens were analysed for expression changes in a panel of EMT-related markers across treatment using 230 Nanostring assays. This included the EMT-driving transcription factors TWIST 1 and 2, SNAIL 1, 2 and 3 and ZEB 1 and 2, which were correlated with risk of relapse. Snail-3 showed significantly greater induction in relapsers compared to non-relapsers (OR=1.8, p=0.04) with a borderline significantly greater induction of TWIST-1 (OR=2.4, p=0.08) in relapsers in addition.

In a subsequent 240-patient validation cohort with a pCR rate of 18%, contralateral cranio-caudal view mammograms from the time of diagnosis have been collated and digitized with MBD assessment employing Cumulus software ongoing. Percent breast density will be assessed both as a continuous variable and by quartiles. Immunohistochemistry on pre- and post-operative tissue sections with pan-cytokeratin-vimentin co-staining to identify EMT and staining for SNAIL-3 and TWIST-1 is also in progress. Associations between MBD, EMT before and after chemotherapy, pCR and relapse-free survival will be presented. The role of Snail-3 and TWIST-1 in the interplay between MBD, EMT and outcome is being explored and will be reported.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-08-06

Differential effects of eribulin on key transcription factors snail and slug

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Microtubule targeting agents (MTAs) are a mainstay in the treatment of breast cancer and a growing body of evidence demonstrates that they have non-mitotic effects that contribute to their anticancer actions. Even after decades of clinical use, there is much to still be learned about the mechanisms of action these drugs, and differences among them, for optimal utility. MTAs rapidly alter microtubule dynamics, often within minutes, leading to significant changes in oncogenic cellular signaling. We evaluated the early effects of eribulin on key oncogenic signaling pathways following a 2 h incubation using clinically relevant concentrations and compared the effects to those initiated by other MTAs. These studies led to the identification of novel mechanisms by which MTAs disrupt oncogenic signaling and contribute to the reversal of epithelial to mesenchymal transition (EMT), including unanticipated differences among drugs.

The TGF- $\beta$ -mediated expression of the Snail transcription factor is a key driver pathway of EMT in breast cancer. The effects of eribulin and other MTAs on TGF- $\beta$ -induced, Smad-dependent expression of Snail were evaluated. Our results show that eribulin and vinorelbine, but not paclitaxel or ixabepilone, inhibit the ability of TGF- $\beta$  to promote the transcriptional induction of Snail by impeding the nuclear transport of Smad2/3 proteins in 4 triple-negative breast cancer cell lines. This study begins to explain how microtubule disruption might contribute to the eribulin-mediated EMT reversal observed in vitro, in vivo, and in patients.

Slug is another member of the Snail family of transcription factors that plays a central role in breast cancer EMT. Although Snail and Slug are often grouped together due to functional similarities, it is becoming increasingly clear that Slug has an independent role in regulating stemness and cancer cell survival during partial EMT. In contrast to our findings with Snail, eribulin and vinorelbine, but not paclitaxel or ixabepilone, induced Slug expression independent of TGF- $\beta$  stimulation in a subset of triple-negative breast cancer cell lines. Studies are ongoing to identify the molecular pathways and consequences of eribulin and vinorelbine-induced Slug induction, which might begin to identify biomarkers of patient response to different MTAs. This work highlights the multifaceted nature of MTA-mediated effects on EMT-associated signaling pathways in breast cancer cells and prompts a reevaluation of their differential efficacy in tumors with distinct molecular profiles. These studies are supported by Eisai Inc.

## 2018 San Antonio Breast Cancer Symposium ®

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Study of diphenylamine analogs as inducers of mesenchymal to epithelial transition in breast cancer

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The organization of cell cytoskeleton is altered in events of epithelial to mesenchymal transition (EMT), promotion of cell motility, and cancer metastases. EMT is associated with decreased cell-cell adhesion, downregulation of epithelial markers like E-Cadherin, cytokeratins, and occludins, and upregulation of mesenchymal markers such as N-cadherin, vimentin, and various transcription factors such as slug and ZEB. Epithelial to mesenchymal transition is also a consequence of drug resistance and is responsible for cancer metastases. Triple negative breast cancer is highly aggressive cancer and patients show poor prognosis and disease-free survival due to the lack of targeted therapy. Mitogen activated protein kinase pathway, including extracellular activated kinase ERK1/2 and ERK5, and phosphoinositide 3-kinase (PI3K) pathway are known to alter the cytoskeleton through the downstream activation of oncogenes such as FRA-1 and loss of focal adhesions. Of these pathways, the MEK5-ERK5 pathway is understudied in triple negative breast cancer TNBC, and there are few research tools available to selectively inhibit this pathway. The diphenylamine analogs were derived from the parent molecule Mekinist, a FDA approved MEK1/2 inhibitor for melanoma, and modified to gain selectivity towards MEK5. SC-1-151, a type-III allosteric inhibitor of MEK5 is a dual MEK1/2 (98.6%) and MEK5 (59%) inhibitor; the molecule inhibits cell viability and colony formation, and attenuates tumor growth. SC-1-151 was serendipitously identified as a mesenchymal to epithelial transition activator in TNBC cell line MDA-MB-231. E-cadherin protein expression and cell morphology were examined to study MET after the treatment of MDA-MB-231 cells with different structural analogs of SC-1-151 after treatment for 5 days. The compound was further found to induce E-cadherin expression and epithelial phenotype in tamoxifen resistant estrogen positive MCF-7 cell line that underwent EMT. The compound is identified to promote this activity by targeting at least the ERK-FRA1-ZEB1 axis. Alkyl or N-Methyl piperazine substituents on the amide of ring 1 produced similar result as SC-1-151, and substituting the amide group with acid or ester also induced MET. In contrast, ortho-fluoro, para-iodo functional groups of the arene ring 2, when replaced with a meta-bromo substituent did not induce MET. We aim to test the compounds on EGF treated MDA-MB-468 cells to observe the attenuation of EGF induced EMT. Future studies will be performed to determine the specific protein interactions of the promising compounds.

## 2018 San Antonio Breast Cancer Symposium ®

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Loss of NSrp70 promotes metastasis of triple-negative breast cancer by activating TGF- $\beta$  signaling

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Triple-negative breast cancer (TNBC) is a highly aggressive tumor subtype associated with a poor prognosis. The mechanism involved in TNBC progression remains largely unknown. To date, there are no effective therapeutic targets for this tumor subtype. In this study, by performing quantitative proteomic analyses in highly metastatic and parental breast cancer cell line, we found that nuclear speckle-related protein 70 (NSrp70), a novel serine/arginine (SR)-related protein, was significantly down-regulated in highly metastatic breast cancer cells. Moreover, down-regulation of NSrp70 was also found to promote the migration and invasion of TNBC cells in vitro and in vivo. Mechanistically, loss of NSrp70 increased levels of phosphorylated SMAD3 and TGF- $\beta$ -induced epithelial-mesenchymal transition (EMT). Furthermore, low NSrp70 expression correlated with poor prognosis in breast cancer patients. Basal-like breast cancer patients with high expression of NSrp70 have better prognosis. However, the expression of NSrp70 have no statistical significance in the Luminal A and luminal B breast cancer patients.

### Results

Taken together, our findings reveal that NSrp70 acts as a metastasis suppressor in TNBC by regulating the TGF- $\beta$ /SMAD signaling pathway and NSrp70 may serve as a novel biomarker of prognosis and the response to anti-tumor therapeutics for patients with TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

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Tannic acid inhibited TGFβ-induced phenotype transition and mammosphere formation of breast cancer cells via modulation of NF-κB signaling

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**BACKGROUND:** Cancer stem cells (CSCs) play a crucial role in tumor initiation, metastasis and recurrence, which are therefore regarded as promising therapeutic target. Aberrant NF-κB signaling has been identified in many cancers, and proven to promote CSC formation via several mechanisms, including an induction of epithelial-to-mesenchymal transition (EMT) of cancer cells. Tannic acid (C76H52O46, TA) was reported to inhibit the proliferation of cancer cells, however there was no data regarding the effect of TA on CSCs. The present study investigated the effects of TA on CSC formation, NF-κB signaling, and EMT in breast cancer cells.

**MATERIALS AND METHODS:** The effect of TA on mammosphere formation and the activity of aldehyde dehydrogenase 1 (ALDH1) was investigated in MCF7 cells. IKK phosphorylation, nuclear translocation of p65 and the expression of IκBα and CSCs markers (CD44/CD24, ALDH1) were assessed by western blotting. Role of NF-κB signaling in EMT of MCF7 cells were evaluated using p65 siRNA (sip65) and NF-κB specific inhibitor (PDTC) or IKK inhibitor (Bay11-7082). The effect of TA on tumor growth was also examined in a mouse xenograft model established by subcutaneous implantation of MCF7 cells ( $1.5 \times 10^7$ ) in 6-week-old Balb/c nude mice with intraperitoneal injection of TGFβ (40 ng/kg, 3 times/week). In 4 weeks of intraperitoneal administration of TA (2 mg/kg, twice/week), tumor volume was measured with an evaluation of the alteration of CSC markers and NF-κB signaling.

**RESULTS:** TA (10 μM) inhibited the formation and growth of mammosphere in MCF7 cells expressed as a decrease in mammosphere formation efficiency (MFE) and ALDH1 activity. An activation of NF-κB pathway was observed in MCF7-derived mammosphere shown as an up-regulation of p65, a degradation of IκBα and an increased IL-6. Blocking of NF-κB signaling by sip65, PDTC or Bay11-7082 resulted in a decrease in the MFE, the expression of ALDH1, and an increase in CD44<sup>high</sup>/CD24<sup>low</sup> ratio. TA alleviated the markers of NF-κB activation in MCF7-derived mammosphere. TGFβ (10 ng/mL)-induced EMT, increase in MFE and NF-κB activation was alleviated by TA. In murine xenograft model, tumor volume was decreased by TA with a decrease in CD44 expression and IKK phosphorylation in xenograft.

**CONCLUSIONS:** The present study showed that TA inhibited mammosphere formation in breast cancer cells, which was associated with an alleviation of both NF-κB signaling and EMT. Moreover, TA treatment ameliorated tumor growth in an *in-vivo* mouse xenograft of breast cancer. These data suggest TA may serve as an effective therapeutic agent for breast cancer patients.



# 2018 San Antonio Breast Cancer Symposium ®

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Racial/ethnic differences in *BRCA1/2* and multigene panel testing among young breast cancer patients

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**Background:** Breast cancer (BC) patients diagnosed at age 50 and under are recommended to have germline genetic testing for hereditary BC due to a high likelihood of carrying a pathogenic mutation in a moderate or high penetrance risk gene. Completion of genetic testing among racial/ethnic minorities, particularly multigene panel testing, is understudied. We examined predictors of completion of *BRCA1/2* and multigene panel testing among women with early onset BC and assessed racial/ethnic differences in genetic testing completion and results.

**Methods:** We performed a retrospective cohort study of 1370 BC patients diagnosed at  $\leq 50$  years of age at Columbia University Medical Center (CUMC) from January 2007-December 2016. Data on socio-demographics, clinical factors, and genetic testing completion and results were collected from the medical record. We conducted descriptive statistics and univariate and multivariable logistic regression models.

**Results:** Our study population had a median age of 44 years (range, 19-50); 44% non-Hispanic white, 24% Hispanic, 13% non-Hispanic black, 10% Asian, 9% other; 61% private insurance, 22% Medicaid, 17% other. Nearly half of the women (N=607; 44.3%) had genetic testing performed. In the multivariable regression model, genetic testing completion was less likely with increasing age at diagnosis (odds ratio [OR]=0.93; 95% confidence interval [CI]=0.91-0.95) and stage 0 or 4 BC compared to stage 1 (OR=0.67; 95% CI=0.46-0.97 and OR=0.35; 95% CI=0.19-0.64, respectively). Completion of genetic testing was more likely with a family history of BC (OR=5.55; 95% CI=3.92-7.87). Genetic testing completion did not vary by race/ethnicity or insurance coverage. Across all racial/ethnic groups, the frequency of pathogenic/likely pathogenic variants identified was 13.0% and 10.5% had at least 1 variant of uncertain significance (VUS). The highest VUS frequency was among Asians (21.2%). The percentage of women undergoing genetic testing increased over time from 18.5% in 2007 and reached a peak of 69.3% in 2015. From 2007 to 2016, the percentage of pathogenic/likely pathogenic variants detected increased from 3.4% to 9.1% and the VUS frequency rose from 3.4% to 13.3% with increasing use of panel testing.

Frequency of pathogenic variants and VUS among women  $\leq 50$  years diagnosed with BC at CUMC (2007-2016)

	Pathogenic variants	VUS
Total	81 (5.9%)	74 (5.4%)
BRCA1	44 (3.2%)	10 (0.7%)
BRCA2	21 (1.5%)	10 (0.7%)
ATM	3 (0.2%)	9 (0.6%)
CHEK2	3 (0.2%)	8 (0.5%)

Other variants detected in: APC, BARD1, BRIP1, CDH1, CDKN2A, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PHOX2B, PMS2, POLE, PTEN, RAD50, RAD51C, SDHA, STK11, TP53

**Conclusions and Relevance:** Nearly half of the women with early onset BC had genetic testing. We did not observe disparities in genetic testing by race/ethnicity or insurance coverage. Genetic testing completion, as well as the frequency of pathogenic/likely pathogenic variants and VUS detection, increased over time as panel testing replaced *BRCA1/2* testing. Counseling on the likelihood of obtaining uncertain results should be provided to all patients undergoing hereditary BC genetic testing, particularly to racial/ethnic minorities.

# 2018 San Antonio Breast Cancer Symposium ®

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Clinical and pathological characteristics and screening outcome for secondary cancers of women with breast cancer and Li-Fraumeni syndrome

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**Background:** Germline TP53 mutations predispose to early onset breast cancer in women and are associated with Li-Fraumeni syndrome. Published data on the clinical and pathological characteristics and screening outcome for secondary cancers among women with breast cancer and TP53 mutations is limited. To the best of our knowledge this is the largest cohort of breast cancer associated with Li-Fraumeni syndrome.

**Methods:** Patients with breast cancer and Li-Fraumeni Syndrome were identified from a prospective research database from 2001 to 2017. Patients had genetic counselling and testing at The University of Texas MD Anderson Cancer Center and confirmed to have TP53 mutations associated with Li-Fraumeni syndrome. We reviewed the patient's charts to identify the clinical and pathological characteristics of their breast cancer. Data for secondary cancers are obtained only for patients with breast cancer as their initial cancer diagnosis and who are followed at The MD Anderson's Li-Fraumeni Education and Early Detection (LEAD) clinic which conducts comprehensive cancer screening for these patient's per the NCCN guidelines, including yearly whole body MRIs.

**Results:** Fifty-nine patients confirmed to have Li-Fraumeni syndrome and breast cancer (100% female, median age 30 years). 94% of the patients were pre-menopausal at the time of breast cancer diagnosis and 6% were post-menopausal due to bilateral salpingo-oophorectomy. 61% were diagnosed after abnormal self or clinical breast exam and 26% based on abnormal screening mammography or ultrasound. In terms of the histologic subtype of breast cancer: 69% had invasive ductal carcinoma, 5% mucinous carcinoma, 5% mixed ductal and lobular, 5% sarcoma, 3% phylloides tumor and 13% with missing data. Pathologic stage per the 7th edition of AJCC cancer staging system was as follows: 23% stage I, 26% stage II, 23% stage III, 28% remaining with unknown pathologic stage. Pathologic markers include: 70% with positive estrogen receptor expression, 64% with positive progesterone receptor expression, 57% with HER-2 amplification defined per the ASCO-CAP HER-2 test guidelines and 7% with triple negative disease. Forty three patients were followed at LEAD clinic. Of the 43 patients, 40% (N=17) were diagnosed with 1 primary cancer other than breast cancer, 7% (N=7) with 2 primary cancers other than breast cancer and 44% (N=19) with breast cancer only. Out of the 43 patients, 5 were diagnosed with acute myelogenous leukemia, 4 with leiomyosarcoma, 3 osteosarcoma, 4 with other types of sarcoma, 4 with central nervous cell tumors (astrocytoma or glioblastoma multiforme), 3 with papillary thyroid carcinoma, 1 with pancreatic cancer, 1 with renal cell carcinoma, 1 adrenocortical carcinoma, 1 with uterine cancer, 1 with melanoma and 1 with cervical cancer.

**Conclusion:** This study to our knowledge is the largest cohort of patient's with Li-Fraumeni syndrome and associated breast cancer that is followed in a dedicated clinic for patients with Li Fraumeni Syndrome. This cohort highlights the characteristics of patients with Li-Fraumeni syndrome and associated diagnosis of breast cancer as well as other primary cancers.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-09-03

Expanded panel testing superior to BRCA1/2 and breast cancer panel in patients with breast cancer

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**Background:** The testing of hereditary breast and ovarian cancer (HBOC) patients for *BRCA1/2* only was established years ago to identify patients with clinically actionable variants and limit the economic burden. However, the cost of genetic testing has plummeted, and the number of breast cancer-risk genes with management guidelines has expanded. We created a community-based registry to test all breast cancer patients. A primary objective of this registry included accruing and comparing patients who did and did not meet NCCN guidelines and determining if providing *all* breast cancer patients with comprehensive multi-gene panel testing yields additional clinical value than testing *BRCA1/2* alone.

**Methods:** An IRB-approved multicenter prospective registry was initiated with 20 community-based and academic breast sites, selected to insure geographic and ethnic diversity. Consecutive patients ages 18-90 with current or prior breast cancer were offered testing with an 80-gene panel (Invitae, San Francisco, CA). HIPAA-compliant case report forms collected patient diagnosis, test results, and physician recommendations made after test results.

**Results:** Over 1,000 patients were enrolled and data on 911 have been analyzed to date. Median age of patients is 60.5 (range 22 to 93). 56.0% were recently diagnosed with breast cancer. Of these patients, 50.54% met NCCN criteria, and 49.5% did not. 10.9% had history of a prior non-breast cancer. The pathogenic/likely pathogenic (P/LP) variant rate for patients on a comprehensive 80-gene panel was 8.9%. When restricted to a guidelines-based 11-gene breast cancer panel (*BRCA1/2*, *ATM*, *CDH1*, *CHEK2*, *NBN*, *NF1*, *PTEN*, *STK11*, *TP53*, *PALB2*), 4.9% had P/LP variants; when limited to *BRCA1/2*, 1.6% had P/LP variants. Of all patients with P/LP findings, 93% had variants in cancer-risk genes with established management recommendations (Table 1) and 80% had germline variants conferring eligibility for precision medicine-based cancer treatments, such as PARP inhibitors, through actively enrolling clinical trials.

**Conclusions:** This study demonstrates that comprehensive panel testing of breast cancer patients provides a higher yield of clinically actionable P/LP variants than *BRCA1/2* testing alone. Limited panels may miss clinically relevant P/LP variants, leaving risk for preventable cancers undiscovered and unnecessarily restricting patients' treatment options. These results also suggest that variants in tumor suppressor genes, not previously thought related to breast cancer, may contribute to its etiology. A comprehensive panel strategy reveals untapped clinical utility and can impact breast cancer patient care by informing implementation of precision medicine treatment interventions and guiding long-term medical management and surveillance for patients and their family members.

	Patients	Variants
With breast cancer management guidelines (including variants ATM*, BRCA1*, BRCA2*, CHEK2*, NBN*, NF1, PALB2*, TP53*)	45 (56%)	46 (55%)
With cancer guidelines and clinical management implications (including variants BARD1*, FH, MTF, MSH6*, MUTYH*, PTCH1, RAD50*, RAD51C*, RAD51D*, RB1, RET, VHL)	31 (38%)	33 (39%)
Evidence of actionability accruing (including variants BLM, DIS3L2, RECQL4)	5 (6%)	5 (6%)

Totals	81	84
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\*P/LP variants in these genes confer potential clinical trial eligibility, e.g. NCT02401347.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-04

Impact of premenopausal RRSO on breast cancer risk in *BRCA1/2* mutation carriers: Maximizing bias-reduction

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## **Background:**

To prevent ovarian cancer in *BRCA1/2* mutation carriers, risk reducing salpingo-oophorectomy (RRSO) is recommended around age 40. In women with no prior history of cancer, there are conflicting data regarding the impact of RRSO on breast cancer risk. Our aim was to explore the association between premenopausal RRSO and BC risk by using a methodology that maximally reduced potential biases.

## **Methods:**

Prospective multicenter cohort study of *BRCA1/2* carriers who underwent genetic testing under age 51, had no history of bilateral salpingo-oophorectomy, mastectomy or cancer prior to genetic testing and during the first six months of surveillance (to avoid cancer-induced testing bias and prevalent cancer bias). Observation period started six months after genetic testing (to avoid event-free time bias), and ended at BC or other cancer diagnosis except for non-melanoma skin cancer and cervical cancer in situ, risk reducing mastectomy (RRM), last follow-up or death. We calculated person-years of observation (PYO) starting at age 30 and RRSO was only accounted for when performed before age 51 (considered premenopausal). Cox proportional hazards models with RRSO as a time-dependent covariate (to avoid immortal person time bias) were used to calculate the BC risk reduction. Sensitivity analysis, censoring at age 51, was performed to calculate the impact of RRSO on the premenopausal BC.

## **Results:**

We included 853 (444 *BRCA1* and 409 *BRCA2*) women. Median age was 36.2 (30-50.9) years, 337 (39.5%) women underwent RRSO prior to age 51 with a median age at RRSO of 42.8 (30.5-50.9) and 240 (28.1%) women performed RRM at a median age of 40.7 (30-61.7) years.

After a mean follow-up period of 4.3 years, 96 women (11.3%) were diagnosed with BC (54 *BRCA1* and 42 *BRCA2*). Overall, women who underwent RRSO had a significant reduction in BC risk with hazard ratio (HR) of 0.57 (95% CI= 0.32 to 1; p=0.05); in *BRCA1* carriers we found HR of 0.45 (95% CI= 0.22 to 0.92; p=0.03), while *BRCA2* carriers had HR of 0.77 (95% CI= 0.35 to 1.67; p=0.51).

When follow-up was censored at age 51, the HR estimates remained similar with overall HR of 0.54 (95% CI= 0.29 to 1; p=0.05); *BRCA1* carriers had HR of 0.35 (95% CI= 0.15 to 0.82; p=0.02), while *BRCA2* carriers had HR of 0.88 (95% CI= 0.39 to 1.96; p=0.75).

## **Conclusions:**

This robust bias-reducing analysis in a large prospective cohort supports a role of premenopausal RRSO for BC risk reduction in *BRCA1* carriers. A longer follow-up may be needed to estimate the potential benefit of the intervention in *BRCA2* carriers.

# 2018 San Antonio Breast Cancer Symposium ®

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Hereditary breast cancer beyond BRCA: Clinical and histopathological characteristics in patients with germline CHEK2, ATM, PALB2 and TP53-mutations

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## Background

The introduction of multi-gene panel testing in the diagnosis of hereditary breast and ovarian cancer (HBOC) has led to an important increase in the detection of breast cancer predisposition genes other than BRCA1 and BRCA2.

## Methods

All individuals who underwent HBOC-testing at our institution since the introduction of multi-gene panel testing were included (March 2016-August 2017). In this retrospective analysis, the BRCA Hereditary Cancer MASTR Plus® panel is used (Multiplicom, Belgium), with sequencing of BARD1, BRCA1, BRCA2, BRIP1, RAD51C, RAD51D, TP53, MRE11A, RAD50, NBN, FAM175A, ATM, PALB2, STK11, MEN1, PTEN, CDH1, MUTYH, CHEK2, BLM, XRCC2, EPCAM, MLH1, MSH6, PMS2, MSH2.

In breast cancer patients with a recurrent germline alteration, age and TNM stage at diagnosis, histological subtype, grade of differentiation and molecular surrogate subtype were recorded. Given the low numbers of TP53-carriers diagnosed by HBOC testing, also patients with a germline TP53-mutation diagnosed by targeted sequencing at our institution were included. Statistical analysis were performed with SPSS version 25.

## Results

In 11.9 % of 2806 patients who underwent panel testing, a germline pathogenic alteration was detected. BRCA1 and BRCA2 were the most prevalent alterations, detected in respectively 3.35 and 2.92 % of patients. Germline alterations in CHEK2, ATM, PALB2 and TP53 were detected in respectively 2.5 %, 1.1 %, 0.5 % and 0.1 %. In 1 % of patients, germline alterations were retrieved that only contribute to ovarian cancer risk (BRIP, RAD51C, RAD51D). Germline DNA mismatch repair alterations were detected in 0.39 % of patients.

The median age at onset of breast cancer in patients with germline CHEK2-, ATM-, PALB2- and TP53-mutations was 47, 53, 39 and 33 years respectively. The age of breast cancer diagnosis in patients with germline TP53-alterations was significantly younger compared to patients with CHEK2-mutations ( $p = 0.01$ ), ATM-mutations ( $p = 0.01$ ) and PALB2-mutations ( $p = 0.04$ ). In situ carcinomas were diagnosed in respectively 9 %, 11 % and 11 % of patients with CHEK2-, PALB2- and TP53-mutations. Patients with CHEK2, ATM, PALB2 and TP53-alterations were diagnosed with  $\geq T3$ -tumors in respectively 13 %, 12 %, 33 % and 22 %. Nodal status at diagnosis was negative in 40-60 % in these 4 subgroups. Upfront metastatic disease was diagnosed only in 2/43 CHEK2-carriers. More than half of the breast cancer diagnoses were luminal tumors in CHEK2-, ATM- and PALB2-carriers, while cases with germline TP53-alterations only presented with luminal cancers in 22 % in our series.

## Conclusion

Almost half of the pathogenic mutations detected in HBOC-genes are alterations in genes other than BRCA1 and BRCA2. CHEK2-mutations are by far the most prevalent, followed by ATM, PALB2 and TP53.

The range of the CHEK2- and ATM-population was wider then expected at the lower-age boundary. The age of breast cancer diagnosis in patients with germline TP53-mutations was significantly younger compared to patients with CHEK2-, ATM- and PALB2-mutations. The distribution of the histological subtypes and grade of differentiation was not suggestive of a specific correlation with germline mutation status.

# 2018 San Antonio Breast Cancer Symposium ®

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Underdiagnosis of HBOC in breast cancer patients: Are genetic testing guidelines a tool or an obstacle?

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**Background:** Pathogenic genetic variants are estimated to occur in 10-15% of all breast cancer patients, with BRCA 1/2 accounting for 40-50% of pathogenic/likely pathogenic (P/LP) variants. However, it is estimated that <30% of breast cancer patients harboring a BRCA 1/2 variant have been identified, with the percentage being much less for ~20 other breast cancer associated genes. Reasons for this are multifactorial and include complicated and restrictive testing guidelines developed at a time when the cost of testing was high and guidelines for management were limited. Today, cost has plummeted and there are definitive management guidelines for a broader range of genes. We created a community based Registry to determine the incidence of P/LP variants in breast cancer patients who meet and who do not meet the NCCN 2017 genetic testing criteria.

**Methods:** An IRB-approved multicenter prospective registry was initiated with 20 community and academic sites experienced in cancer genetic testing and counseling.

Eligibility criteria included patients with a breast cancer diagnosis who had not been previously tested. Consecutive patients aged 18-90 were consented and underwent an 80 gene panel test (Invitae –Multi-Cancer Panel). The non-inferiority study was powered to detect a difference in P/LP variant rate of 4 percentage points with statistical significance ( $p < 0.05$ , Fisher's exact test).

HIPAA compliant electronic case report forms collected information on patient diagnosis, test results, and physician recommendations made after test results were received.

**Results:** Over 1000 patients were enrolled and data from 910 subjects analyzed to date. 50.4% met NCCN criteria and 49.5% did not. Median age for the enrolled patients is 60.5 and ranged from 22-93. 56.0% of patients were recently diagnosed with breast cancer. 10.9% of patients had a history of a prior non breast cancer. Overall, 8.9% of patients had a pathogenic variant. 9.6% of patients who met NCCN criteria with test results had a P/LP variant. 8.2% of patients who did not meet criteria had a P/LP variant. The difference of positive cases among the two groups is not statistically significant ( $P = 0.49$ )

4.9% of patients had pathogenic variants if only an 11 gene standard breast cancer panel was considered.

The spectrum of mutated genes varied between the two groups, with some overlap.

## Conclusions:

There was no statistically significant difference in the number of pathogenic/likely pathogenic variants between those patients who met and those who did not meet NCCN guidelines. Expanded panel testing yields more medically actionable P/LP variants than testing BRCA 1/2 alone or breast cancer panels with 11 genes. This study demonstrates that there will be a significant number of patients with P/LP variants are missed if NCCN guidelines are required for genetic testing. Current NCCN guidelines for the genetic testing of breast cancer patients are an obstacle to identifying patients with P/LP variants and should be removed.

Universal BC Genetic Testing Registry

NCCN Criteria (910 patients analyzed)	#/% who have P/LP variants	#/% who do not have P/LP variants
Patients who meet guidelines	44/459 (9.6%)	415/459 (90.4%)
Patients who do not meet guidelines	37/451 (8.2%)	414/451 (91.8%)





# 2018 San Antonio Breast Cancer Symposium ®

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Risk reducing strategy in germline BRCA mutated patients with locally advanced breast cancer. Establishing mastectomy as a preventing procedure of local recurrence

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## Introduction

Neoadjuvant chemotherapy (NAC) is proposed for locally advanced breast cancer (LABC) to increase the breast conservative treatment (BCT). In France, mastectomy is the risk-reducing prophylactic surgical strategy only for pre-symptomatic germline *BRCA*-mutated (g*BRCAm*) patients. On the other hand, BCT is proposed to all patients following NAC based on clinical response, regardless the g*BRCAm* status. The aim of this retrospective study is to evaluate the risk of local recurrence (LR) according to *BRCA* status.

## Patients and methods

Inclusion criteria were: (i) patients treated for unilateral LABC, T2-3, N $\geq$ 0, M0 by NAC, and (ii) patients who underwent germline *BRCA* screening. , using targeted next-generation screening, was carried out either during NAC (rapid process) or after surgery. Deleterious mutations were confirmed using Sanger sequencing before passing on the results to the clinical geneticist. Some g*BRCAm* patients from Olympia study were also included. Patients were followed-up over a long term for overall survival, LR and disease-free survival. Chi-square and Fischer test were used to generate statistical comparison.

## Results

Between 2007 and 2015, 988 women were treated for LABC at our institution. Among them, 151 patients underwent clinical genetic testing for g*BRCAm* based on these criteria: young age at diagnosis or familial history of breast or ovarian cancer or histological characteristics as grade 2/3, *Her2*-3+ or basal like. A total of 122 patients were included in the study; 28 patients had g*BRCAm* status and no mutations were detected in 94 patients (wt*BRCA*). Significant differences between the two groups (g*BRCAm* vs wt*BRCA*) were observed for

Mean age, (36.7 vs 40.1y (p=0.0032) ,

Intrinsic tumor subtypes basal like (64.3% vs 42.5%, p=0.0432)

ER are more often negative (21.4% vs 46.8%, p=0.0165).

Among the 30 patients who underwent *BRCA* screening during NAC and eligible for BCT, 8 of the 9 patients with g*BRCAm* choose mastectomy (88%). Among the 92 patients with screening mutation after breast cancer treatment, 5 of the 19 patients with g*BRCAm* had a mastectomy (28%). In the 28 g*BRCAm* patients, 15 had a BCT and 13 a mastectomy. In the 94 wt*BRCA* patients, 67 had a BCT and 27 a mastectomy. After a follow-up of 4.32 years, we observed 8 relapses, 5 LRs after BCT and 3 contro-lateral relapses. Of the 5 LRs, 3 came from 15 g*BRCAm* with BCT and 2 of the 67 wt*BRCA* (p=0.0403).

## Discussion

In this selected subgroup of patients, g*BRCAm* rate is higher (23%) than the rate based on familial criteria for *BRCA* testing (12%). Regarding the rationale for BCT or mastectomy procedure in LABC and pre-symptomatic g*BRCAm* patients, this study led us to establish mastectomy as risk-reducing strategy in a sole surgery procedure for g*BRCAm* patients. Moreover, 88% g*BRCAm* patients chose mastectomy; the mastectomy rate was lower when the patient was unaware of their *BRCA* status (26%). The LR rate was higher in the g*BRCAm* vs wt*BRCA* with a statistical difference. In LABC patients with high genetic risk, the knowledge of mutation status could influence patients' and surgeons' choice of surgery. In case of g*BRCAm* status, mastectomy is recommended to decrease LR risk.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-08

Germline variants in non-BRCA homologous recombination genes detected in HER2-negative breast cancer patients

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**Background:** PARP inhibitors (PARPi) are FDA approved for a subset of metastatic human epidermal growth factor receptor 2-negative (H2N) breast cancer patients who harbor a germline pathogenic or likely pathogenic variant (PV) in *BRCA1/2*, two of the most well-described breast cancer susceptibility genes in the homologous recombination (HR) pathway. While the NCCN guidelines recommend consideration of *BRCA1/2* testing for patients with H2N disease that are eligible for single-agent therapy, there are currently clinical trials available for women with advanced H2N breast cancer who have PVs in HR genes *beyond BRCA1/2* to investigate outcomes of receiving PARPi. The yield of germline PVs in other HR genes in the H2N population is not well-described.

**Methods:** Clinical histories and test results were reviewed for women with a diagnosis of H2N breast cancer who underwent multi-gene hereditary cancer panel testing that included a minimum of 10 homologous recombination (HR) genes in addition to *BRCA1/2* (*ATM*, *BARD1*, *BRIP1*, *CHEK2*, *FANCC*, *NBN*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*). Those with prior *BRCA1/2* testing were excluded. We assessed the yield of PVs in non-*BRCA1/2* HR genes in the H2N breast cancer population. In addition, we compared the yield of PVs in non-*BRCA1/2* HR genes in a “low risk” population (probands with H2N breast cancer with no reported personal history of ovarian or pancreatic cancer and no reported family history of breast, ovarian, pancreatic, or prostate cancer) to a “high risk” population (probands with H2N breast cancer who also have a personal history of ovarian or pancreatic cancer and/or a reported family history of breast, ovarian, pancreatic, or prostate cancer) via a two-tailed Fisher's exact test.

**Results:** A total of 6179 women with H2N breast cancer were identified. Of these, *BRCA1/2* PVs were identified in 4.8% (299/6179), while 5.7% (351/6179) carried PVs in HR genes other than *BRCA1/2*. These included *CHEK2* (145), *ATM* (62), *PALB2* (59), *BRIP1* (26), *FANCC* (18), *BARD1* (17), *RAD51C* (12), *NBN* (11), *RAD51D* (6), and *PTEN* (2). No statistically significant difference in the likelihood to harbor a PV in one of the 10 non-*BRCA1/2* HR genes was observed between those with a “low risk” presentation (4.8% (53/1096) as compared to those with a “high risk” presentation (5.9% (298/5083)) (p=0.1956).

**Conclusions:** Our findings show that the yield of PVs in HR genes other than *BRCA1/2* is appreciable in the H2N breast cancer population. As such, it may be beneficial to include all HR genes when testing H2N breast cancer patients, regardless of other personal or family history, if and/or when a patient develops metastatic disease.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-09

CHEK2: the third susceptibility BReast CAncer (BC) gene?

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## INTRODUCTION:

Considered a medium penetrance gene, *CHEK2* codes for a kinase that is a key component of the DNA damage-signaling pathway. *CHEK2* pathogenic variants were previously associated with breast and colorectal families and also with Li-Fraumeni phenotypes. Next generation sequencing (NGS) allowed for systematic inclusion of *CHEK2* into gene panels. In here, we characterize the growing subgroup of *CHEK2* BC families identified through our multidisciplinary program.

**METHODS:** Identification and review of *CHEK2* families identified between 01/2000-06/2018 (until 2014 only the c.1100delC was tested (MLPA, MRC Holland); since 2014 NGS methods used were either, TruSight Cancer sequencing panel (Illumina, San Diego, CA, USA) or BRCA MASTR Dx (Multiplicom, Niel, Belgium). Carriers were included in a prospective follow up program.

**RESULTS:** 3646 index pts consented on gene testing. Most hereditary families (HF) were *BRCA1/2* (374) (92%) but among non-*BRCA* HF bigger subgroups were 16 *CHEK2*, 10 *Tp53* and 5 *PALB2* HF. All *CHEK2* index pts were diagnosed with only 3 different pathogenic variants: c.1100delC (9) c.319+2T>A (6) and c.593-1G>T (1 case of the only male BC pt in all *CHEK2* pedigrees).

**Index pts:** mostly (93,8%) to females, with a mean age at first cancer diagnosis of 39 years (yrs) (30-52), 62,5% between 30-39yrs. With the exception of a Non Hodgking's Lymphoma index case, all index pts had BC(93,8%), 68,8% of which were ductal carcinomas and 12,5% of intraductal, all strongly positive for the estrogen receptor. With a mean follow up of 8,26yrs (3-15), secondary cancer cases occurred in 37,5% of index pts (mostly, 12,5%, BC at a mean of 53yrs (41-59).

**Family phenotypes:** data from 98 relatives (53,5% females) revealed diagnoses of BC (31,6%), prostate (8,1%), colorectal (7,1%) cancers. Only 22,2% of family cancers were diagnosed before 50yrs.

**VUS:** Among several complex variants of unknown significance, c.1036C>T;p.Arg346Cys co-segregates in a predominantly male family with 3 prostate, 1 male and 1 female BC.

## DISCUSSION AND CONCLUSIONS:

In the Portuguese population, emerging recurrent pathogenic variants in the *CHEK2* gene, make it the most important non-*BRCA* BC gene so far. Carriers are included in prospective follow up but non-*CHEK2* relatives are a challenge to genetic testing, as well as pedigree review, that questions its classification as a medium penetrance gene (or suggest the role of modifier factors).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-10

Uptake of genetic testing and prevalence of mutations among minority populations: A retrospective cohort study

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**Background:** Hereditary cancer syndromes account for 5-10% of cancers. Limited data exists on the uptake of genetic testing and the prevalence of mutations in minority populations.

**Objective:** To estimate genetic testing uptake and prevalence of mutations in a minority population seeking care in a safety net healthcare system.

**Methods:** We conducted a retrospective cohort study of patients who were assessed at John H Stroger Jr., Hospital of Cook County cancer genetics risk clinic between October 2015 and March 2018 to determine the uptake of genetic testing and the prevalence of genetic mutations. Patients who met National Comprehensive Cancer Network (NCCN) guidelines for genetic testing for hereditary breast and ovarian cancer syndrome (HBOC), Lynch syndrome and familial adenomatous polyposis were included. Data was abstracted through chart review to obtain age, socio-demographics, personal cancer history, primary language, personal and family history of cancer including type of cancer and age at diagnosis, insurance status, genetic testing status and test results. Uptake of testing was calculated as the percentage of patients who underwent testing among those patients who met NCCN criteria for testing. Prevalence of mutations among those patients who underwent testing was determined and compared between different ethnicities using Chi-square test.

**Results:** Of 510 patients offered genetic counseling & testing, 478 (94%) underwent genetic testing. Among those who tested, 34% (n=165) were African American and 45% (n=212) were of Hispanic origin. Prevalence of any mutation was 48%(n=232), Pathogenic mutations were identified in 11% (n=54) of testers and variants of uncertain significance (VUS) were identified in 37% (n=178). Deleterious mutations and VUS rates in patients affected with hereditary cancer syndromes were 13% (n=42) and 39 % (n=132) respectively. Deleterious mutations and VUS rates in women unaffected with cancer were 9% (n=12) and 33 % (n=46) respectively. Overall mutation prevalence did not differ between affected and unaffected patients (p=0.12) or between Black and Hispanic patients (p=0.96). Deleterious mutation rate in Hispanic patients was 15.6% (CI 20.5%-10.7%) and in Black patients was 3.6% (CI 3%-4%). There was a significant difference in the percentage of deleterious mutations between Hispanic and Black patients (p=0.0009) .

**Conclusion:** Genetic testing uptake was high in minority populations. In the Population who were tested prevalence of pathogenic mutations was higher among Hispanics than African Americans.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-11

Direct-to-consumer genetic testing as an alternative model for identifying *BRCA* carriers

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**Background:** Three *BRCA1* and *BRCA2* founder variants (185delAG, 5382insC, and 6174delT) are common in the Ashkenazi Jewish population. Our objective was to use data collected by direct-to-consumer (DTC) genetic testing (23andMe, Inc., Mountain View CA) to determine the frequency of these variants in a generally unselected group of genotyped individuals and to characterize the cohort of individuals carrying one of these variants in terms of self-reported ancestry, genetic ancestry, and personal and family history of cancer.

**Methods:** Individuals were genotyped prior to November 2017 on one of four custom Illumina genotyping arrays, which included the three Ashkenazi founder variants. Ashkenazi Jewish genetic ancestry was calculated by an analysis of local ancestry. Self-reported data from customer surveys on ancestry and personal and family history of cancer was used in the analysis. Inclusion criteria were 23andMe customers who were 18 years or older and consented to participate in research. IRB approval was obtained from Ethical & Independent Review Services.

**Results:** More than 80% of individuals consented to participate in online research. 2,853 carriers of at least one Ashkenazi Jewish founder variant were identified out of 1,980,076 eligible participants. 54% (1,539) were males; 46% (1,314) were females; 86% (2,462) were over 30 years of age. 249 (9%) had no detectable Ashkenazi Jewish genetic ancestry. 1,967/2,853 (69%) provided self-reported ancestry information. 415/1,967 (21%) did not self-report Jewish ancestry but of those, 258 (62%) had at least 1% Ashkenazi Jewish genetic ancestry. Individuals with less than 20% Ashkenazi Jewish genetic ancestry were less than 50% likely to report having Jewish ancestry.

Self-reported vs. genetic ancestry information

	No detectable Ashkenazi Jewish genetic ancestry	>1% detectable Ashkenazi Jewish genetic ancestry	Total
Self reported Jewish ancestry	9 (1%)	1,543 (99%)	1552
Self reported no Jewish ancestry	157 (38%)	258 (62%)	415
Total	166	1801	1967

1,837 participants provided personal cancer history information and 393 provided first-degree family history information. 1,435/1,837 (78%) reported no personal history of breast, ovarian, pancreatic, or prostate cancer. 44% (172/393) reported no first-degree family history of breast, ovarian, pancreatic, or prostate cancer.

**Conclusions:** This is the first description of a large, unselected cohort of *BRCA* carriers identified through DTC genetic testing. 21% of carriers in our cohort did not self-report Jewish ancestry despite more than half of those having detectable Ashkenazi Jewish genetic ancestry. Even though personal or family history was only available for a subset, many did not report suggestive histories of breast, ovarian, prostate, or pancreatic cancer. Although there are limitations to the data set, these findings suggest the need for alternative models for identifying carriers of specified *BRCA* variants who may not otherwise be identified for genetic testing. Additionally, the number of males identified underscores the importance of male carriers in cancer genetic risk assessment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-12

Germline mutation in *TP53* gene in a cohort of 2,561 Chinese high-risk breast cancer patients using multigene panel testing

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**Background:** Li-Fraumeni syndrome (LFS) is a rare autosomal genetic disorder with germline *TP53* mutations. Patients with *TP53* mutations have a higher risk of developing breast cancer than those harboring *BRCA* mutations. Although limited studies have shown that *TP53* mutation carriers are less responsive to low dose radiation and more susceptible to induce new malignancies from radiotherapy. Moreover screening strategies allows early detection of a spectrum of cancers related to *TP53* mutations. From work of *BRCA* mutations where over 40% novel mutations were detected in Chinese cohort, it is important to evaluate the frequency of *TP53* mutation in Chinese to better understand the spectrum to guide appropriate clinical management of these high risk individuals.

**Methods:** *TP53* gene mutation screening was performed on 2,561 high-risk breast cancer patients using multigene panel testing. The patients were accrued by Hong Kong Hereditary and High Risk Breast Cancer Program from March 2007 to May 2018. All detected pathogenic mutations were further validated by bi-directional DNA sequencing and analyzed by our in-house developed bioinformatics pipeline.

**Results:** Sixteen distinct pathogenic or likely pathogenic variants were identified, and 3 of them were de novo *TP53* mutations (18.75%). The mean age of patients who harbored *TP53* mutation was 30.44 years (range 18-44), and 50% of the tumors were bilateral breast cancer. Of sixteen different pathogenic mutations, majority of them were missense mutation (87.5%), and 2 were nonsense mutation (12.5%). Four of the sixteen *TP53* mutation carriers had family history of breast cancer, while others had a family history of lung cancer (43.75%).

**Conclusion:** This study revealed that seven patients were found to harbor *TP53* mutation even when they did not meet the criteria of LFS or LFS-like phenotype, implicated the importance of using multigene panel test for probands and their relatives to offer a comprehensive surveillance programme for *TP53* carriers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-13

Disparities in germline mutation testing: do Medi-Cal and uninsured patients with breast cancer receive genetic testing in a safety net setting?

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**Background:** National Comprehensive Cancer Network guidelines recommend that women with breast cancer diagnosed at age  $\leq 50$  undergo cancer genetic risk assessment and that those age  $\leq 45$  undergo testing for hereditary breast and ovarian cancer (HBOC) syndromes such as BRCA. In prior studies of primarily commercially insured women, 30% of those  $\leq 40$  years of age received BRCA testing (Levy 2011), and 34% of those  $\leq 50$  years were referred for genetic assessment (Stuckey 2016). Our aim was to determine rates and predictors of genetic testing among publicly insured or uninsured women at a safety net medical center.

**Methods:** Women diagnosed with invasive breast cancer or ductal carcinoma in situ from 2010 through 2016 were identified from the institutional tumor registry of a Los Angeles County public hospital. Eligible for this analysis were those at high risk for HBOC syndromes as defined by 1) age  $\leq 50$  at diagnosis or 2) age  $\leq 60$  with triple negative breast cancer. Women were excluded if they received all of their cancer treatment at an outside institution. Medical records were reviewed to determine receipt of genetic testing and test results. Univariate logistic regression was used to evaluate associations between patient characteristics and receipt of testing.

**Results:** 307 women were included in the analysis. 92.5% (284) were age  $\leq 50$  and 54.7% (168) were age  $\leq 45$ . The majority (72.1%) had Medi-Cal or Medicare and 26.1% were uninsured at diagnosis. The racial distribution was 63.8% Hispanic, 19.2% black, 11.1% Asian and 5.5% non-Hispanic white. Overall, 52.1% (160/307) underwent genetic testing and 13.1% (21/160) were found deleterious germline mutations (BRCA1=10, BRCA2=8, BRCA not specified=1, PALB2=1, TP53=1). Among women who underwent testing, 55.0% were tested for BRCA1/2 only and 36.8% received multigene panel testing; specific testing information was unavailable for the remainder. Age, race/ethnicity, and presence of metastatic disease at diagnosis were associated with differences in genetic testing rates, whereas insurance status and year of cancer diagnosis were not (Table 1).

**Conclusions:** In a safety net setting with mostly Medi-Cal-insured and uninsured patients, the overall rate of germline mutation testing in women with breast cancer (52.1%) was at least comparable to that in studies of commercially insured populations. Notably, testing rates in this population were significantly different when stratifying by age, race, and cancer stage. These findings highlight subgroups in this underserved population who warrant additional attention to assessment of their cancer genetic risk.

	Total (N=307)	Number tested	% tested	P value
<b>Age at diagnosis</b>				P<0.001
<30 years	11	8	72.7	
30-39 years	65	45	69.2	
40-49 years	181	95	52.5	
$\geq 50$ years	50	12	24.0	
<b>Race/ethnicity</b>				P=0.011
Non-Hispanic white	17	6	35.3	
Asian	34	19	55.9	
Black	59	21	35.6	
Hispanic	196	114	58.2	
Unknown	1	0	0.0	

<b>Stage at diagnosis</b>				P=0.007
Non-metastatic	285	155	54.4	
Metastatic	22	5	22.7	
<b>Insurance</b>				P=0.594
Uninsured	80	47	58.8	
Medi-Cal/Medicare*	223	111	49.8	
Private insurance	2	1	50.0	
Unknown	2	1	50.0	
<b>Year of diagnosis</b>				P=0.098
2010	42	17	40.5	
2011	47	23	48.9	
2012	46	26	56.5	
2013	60	29	48.3	
2014	39	24	61.5	
2015	39	21	53.8	
2016	34	20	58.8	

\*1 patient had Medicare; the remainder had Medi-Cal



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-14

Prevalence and type of BRCA mutations in young breast cancer patients undergoing genetic cancer risk assessment in two developing countries: Analysis of two cohorts from Romania and Mexico

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**Background:** a previous study found significant clinicopathological differences between young Breast Cancer BrCA patients (pts) from Romania (Ro) and from Mexico (Mx). Here, we provide a molecular and clinical description of pts carrying BRCA mutations of both cohorts.

**Methods:** in this retrospective study, we analyzed 2 cohorts of BRCA1/2 mutations carriers tested in COEI from Mx between 2014-2017 and IOCN from Ro between 2015 to 2016. Ro pts were selected according to NCCN criteria, while Mx pts were selected based on risk evaluation models. NGS analysis and MLPA for BRCA1&2 were performed in all pts. We compared demographic, clinicopathological and molecular data.

**Results:** 65 pts, 21 (32.7%) from Mx and 44(67.7%) from Ro carried a BRCA mutation. 66.7% of Mx pts and 65.9% of Ro pts carried a BRCA1 mutation. We found clinical similarities: Mean age was 44.5y for Mx and 40.59y for Ro pts. IDC was the most frequent type of BrCa in both series (90.5 vs 90.9%). TNBC was seen in 13 Mx vs 27 Ro pts (61.9% vs 61.4%), HR positive was seen in 7 (33.3%) and 12 (27.3%) cases. Grading 3 was more frequently seen in Ro, while grading 2 was mainly noted in MX pts(p=<0.020). BRCA1/2 pathogenic mutations were different between the 2 cohorts and no BRCA 1/2 identical mutation was identified. 15 different mutations in 21 Mx pts, and 16 different mutations in 44 Ro pts were found. Mutations were different between 2 cohorts. Notably for the Ro cohort, 4 founder mutations (c.181T>G, c.3607C>T, c.5266dupC in BRCA1, and c.9928A>G[GPC1] in BRCA2) were found in 31/44 pts, while the Mx cohort, c.5123C>A and del 9-12del ex in BRCA1 were found in 6/21 mutations. Three Large rearrangements (LR) were exclusively seen in the Mx pts (5/21).

**Conclusions** Both cohorts didn't share any mutation, but the clinical features are similar. BRCA1 del 9-12del is a Mexican founder mutation, we found it in 14% of Mx pts, LR have been described more frequently in Latin American Populations, we found it in 30% of the MX cohort, while for Ro cohort four recurrent mutations qualify as founder mutations.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-15

Is BRCA-testing based on risk models still a valid approach in developing countries?

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**Background:** Genetic testing is not affordable in Mexico; average *BRCA1&2* testing is \$900, while average income in Mexico is \$6423 per year. Genetic testing can be an economic burden for public-funded institutions. Current NCCN for genetic counselling and testing are broad to avoid missing patients at risk. Nonetheless, this approach could not be realistic in developing countries, thus we still must select our pts with risk evaluation models. This study describes the features of our BRCA mutation carriers.

**Methods:** New Breast Cancer (BrCa) cases were selected from nov2016 to march2018 according to NCCN criteria for genetic counseling. We gathered demographic, family and medical data. We also calculated the likelihood of carrying a BRCA mutation in each patient with one of two risk models: the Family History Assessment Tool (FHAT) or BRCAPRO. NGS analysis and MLPA for *BRCA1&2* were performed in selected pts according to coverage, and availability of the test. The main target of the study was to compare the features of patients carrying a BRCA mutation (mBRCA) vs non-carriers, and the eligibility of the pts according to the risk models.

**Results:** We offered genetic counseling to 180 pts based on personal or familial BrCa history. Median age was 44y (range 21-71). 18 (10%) pts had bilateral cancer, 2 (1.1%) had Br and ovarian Ca. 160 (88.9%) tumors were DCI, 10 (5.6%) pts didn't have cancer. 63 (35%) were TN, 87 (48.3%) were HR positive. All of them met criteria according to NCCN guidelines, but only 87 (48.3%) pts met criteria according to the risk evaluation models. 15 pts refused genetic testing because of lack of coverage. 63 (35.6%) pts had genetic testing, 21/64 (32.8%) of them were carriers of BRCA1 or BRCA2 mutation. TBNC was more frequent seen in BRCA mutated carriers vs non-carriers ( $p>0.024$ ). Family History (FH) was seen in all but one carrier vs non-carriers ( $p>0.001$ ), and all carriers met criteria by any risk model  $p>0.0001$ . Age was not statistically different between groups ( $p=0.472$ ), but none carrier was younger than 30y.

**Conclusions:** We confirm that the use of risk evaluation models are useful to identify mBRCA carriers, 32.8% in our cohort. Some features such as TNBC with family history were statistically different between carriers vs non-carriers. 23 pts were positive for risk model but were not mBRCA carriers, which underlines the need to offer larger genetic testing. Also, younger patients (21-30y) had more frequently triple positive phenotypes, and all were negative for BRCA testing; a TP53 testing would also be needed. Unfortunately, multi-gene panels are more expensive than BRCA testing.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-16

Multiple mutations in a single lineage: is it time for a shift in predictive testing?

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Germline cancer genetic testing is a critical part of patient care influencing cancer risk reduction, prevention and now treatment decision-making. Single syndrome and mutation testing has been available for twenty years, however massively parallel sequencing technology has made multigene panel tests more readily available and at lower costs. Currently, it remains unclear if there is benefit related to panel testing in cancer families and patients already harboring a pathogenic mutation. We report 5 families found to harbor additional pathogenic mutations identified with multigene panel testing.

One Ashkenazi family was found to harbor both a founder and non-founder BRCA mutation in a single lineage. Four additional families were found to harbor a second mutation in either a novel breast/ovary cancer gene or in a gene related to an established cancer syndrome. All patients were either initially BRCA2 or PALB2 positive and were subsequently found to have a second mutation in either CHEK2, RAD51D, ATM, MUTYH or PMS2. In 2 cases, the secondary mutation either increased frequency and/or decreased start age of colonoscopy screening. Interestingly, 2 of the additional mutations were first reported as variants of uncertain significance later reclassified as likely pathogenic.

In all cases the traditional approach of single site/syndrome analysis for predictive testing would have failed to identify the second pathogenic mutation resulting in an underestimate of cancer risk in negative family members. Notably, the majority of secondary mutations were present within the same lineage as the known familial mutation. In 3 of 5 cases, the finding of a second mutation in already identified mutation carriers led to additional changes in medical management. As multigene panel testing becomes more accessible in both cost and provider acceptance, this case series illustrates the need to strongly consider use of multigene panel testing for relatives undergoing testing for known familial mutations regardless of family history, and ultimately indicating a role for re-testing established mutation carriers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-17

Risk reducing interventions among BRCA 1 and 2 female carriers in Newfoundland and Labrador: A provincial analysis

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## Introduction

Germline mutations in the tumour suppressor genes BRCA 1 and 2 result in a significant increase in cancer predisposition. Female carriers can have up to a 50-70% chance of developing a breast malignancy in their life time and a risk of 20-40% for ovarian cancer. Management options for women with a BRCA mutation include screening with annual mammography and magnetic resonance imaging (MRI), prophylactic surgery and chemoprevention. There is substantial evidence that preventative strategies may reduce the risk of developing breast and ovarian cancer and in some cases improve survival.

Newfoundland and Labrador (NL) is the most easterly province in Canada with a population of 525 983. It is geographically and genetically isolated with the majority of residents from English and Irish ancestry. While no one single founder effect has been identified, geographically distinct mutations for both BRCA 1 and 2 have been described. The objective of this study is to characterize the population of BRCA mutation carriers in NL and to evaluate their uptake of risk reducing interventions.

## Methods

All BRCA 1 and 2 carriers tested through the Provincial Medical Genetics program between 1996 - 2018 were captured. Inclusion criteria for this study were females  $\geq 18$  years of age residing in the province. Demographic, clinical history and information on uptake of risk reducing interventions were abstracted from the electronic medical record. Descriptive statistical analysis was performed.

## Results

One hundred and sixty one women were identified that met inclusion criteria (38% of patients had BRCA1 and 62% had BRCA 2 mutations). Of those unaffected carriers eligible for mammography and MRI screening, only 58% were adherent in the last 18 months. Consultation with a medical or gynecological oncologist increased mammogram screening within the last 18 months to 71% compared with 29% of women who did not see an oncologist. MRI screening increased to 80% for those assessed by oncology versus 20% who did not. For those women who underwent prophylactic surgeries, 27% had bilateral mastectomies with the majority having breast reconstruction (>70%). Fifty two percent of carriers had bilateral salpingo-oophorectomies (BSO) at a median age of 45 years in BRCA 1 and 51 years in BRCA 2.

Fifty-three women had a diagnosis of breast cancer and 8 had ovarian cancer. In this cohort, most underwent genetic testing after their diagnosis of cancer (>80%). Median age at presentation of breast cancer was 44 years versus 54 years for ovarian cancer.

## Conclusion

This study demonstrates that women with BRCA 1 and 2 mutations are not adequately availing of risk reducing interventions for breast and ovarian cancer. Furthermore, most patients with malignancy did not undergo genetic testing until after they were diagnosed with cancer. Patient focused research designed to explore factors which may contribute is planned. Consultation with an oncologist increased the likelihood of adherence to breast cancer screening. This highlights the importance of specialty care for patients with a hereditary predisposition to breast and ovarian cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-18

Multiple cancer susceptible genes sequencing in BRCA-negative breast cancers with high hereditary risk

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**Background** Breast cancer susceptibility is strongly associated with a patient's hereditary background. Comprehensive BRCA mutation screening in our cancer center shows a lower BRCA mutation prevalence (9.1%) in Chinese breast cancer patients with hereditary risk. Multiple cancer susceptible genes sequencing can assist in discovering detrimental germline mutation in BRCA-negative breast cancers.

**Methods** From 2005-2014, BRCA-negative breast cancer patients with any two of the following risk factors were recruited: (1) pathological diagnosis of triple-negative breast cancer, (2) male breast cancer, (3) primary bilateral breast cancers in one individual, regardless of synchrony or asynchrony, (4) early-age onset breast cancer (less than or equal to 40 years of age at diagnosis), or (5) patients with a family history of breast and/or ovarian cancer (at least one first- and/or second-degree relative with breast cancer or ovarian cancer). A total of 384 Chinese subjects were screened by next-generation sequencing for 32 breast cancer associated susceptibility genes (APC, ATM, BARD1, BMPR1A, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MEN1, MET, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PALLD, PMS2, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RET, SMAD4, STK11, TP53, VHL). Mutations were classified as pathogenic/likely-pathogenic if they had a truncating, initiation codon or splice donor/acceptor effect, or if pathogenicity was demonstrated in published literature.

**Results** In total, we acquired 39 patients (10.2%) with pathogenic/likely-pathogenic germline mutations, including one carrying two distinct mutations. Major mutant non-BRCA genes were MUTYH (n=11), PTCH1 (n=7), RET (n=6) and PALB2 (n=5). Other mutant genes included TP53 (n=3), RAD51D (n=2), CHEK2 (n=1), BRIP1 (n=1), CDH1 (n=1), MRE11 (n=1), RAD50 (n=1) and PALLD (n=1). In addition, PTCH1 mutation carriers were found to be associated with the clinical features of triple-negative and early-age onset breast cancer.

**Conclusions** Among BRCA-negative breast cancer patients with high hereditary risk in China, 10.2% carried mutations in breast cancer associated susceptibility genes. MUTYH and PTCH1 had relatively high mutation rates (2.9% and 1.8%). Some clinical features might be associated with germline mutations of particular genes in breast cancer.

**Key words** germline mutation, BRCA-negative, hereditary breast cancer, multiple-gene sequencing

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-19

The complexity of germline panel testing: Cost, access and variant interpretation in an Irish context

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## Objectives

Diagnostic germline genetic testing of single or multiple cancer predisposition genes is increasingly central to the care of women with breast cancer. Testing for mutations in BRCA1/2 has therapeutic relevance in perioperative and metastatic settings. Variants of uncertain significance (VUS) are identified in approximately 5-20% of tests. Single gene testing in the Rep. of Ireland occurs through 3 clinics using laboratories in England. Panel testing is offered through Color Genomics and Myriad Genetics. Color genomics offers a less expansive panel test. Panels have made variant interpretation a common clinical challenge. We investigated interpretation of 10 VUS in 3 laboratories.

## Methods

We selected 10 patients who had VUS reported in BRCA1/2 in NHS laboratories. Further diagnostic testing for mutations in BRCA1/2 was subsequently completed using BRCAAnalysis on stored DNA from these individuals. Patients were re-contacted and offered Color panel testing for mutations in 30 genes. Previous test reports were not provided to either company. A review of all 10 BRCA1/2 VUS was performed on ClinVar database.

## Results

Two VUS in BRCA1 and 8 VUS in BRCA2 were included in the study. Retesting was completed at a median time of 20mths (14-70mths) after the original testing. All 10 individuals underwent BRCAAnalysis testing, only 7 had panel testing (2 did not make contact following calls/ letters, 1 had left Ireland). ClinVar classified 3 variants as benign, 2 likely benign, 2 VUS and 2 pathogenic (1not on ClinVar). All 10 VUS were re-classified by Myriad and Color. One result was deemed 'pathogenic' by Color and 'suspected deleterious' by Myriad (pt 9, table 1). Panel testing identified two additional VUS: an MSH6 and a RAD51.

## Interpretation of testing

	CliniVar	NHS laboratory testing			Myriad	Color Genomics
Pt	Database Review	Gene	exon		BRCAAnalysis	30 gene panel test
1	Benign (2018)	BRCA1	11	c.3708T>G, p.(Asn1236Lys)	No deleterious mutation	MSH6 VUS c.2585T>A (p.Leu862Gln)
2	Benign (2015)	BRCA1	11	c.4910C>T, p.(Pro1637Leu)	No deleterious mutation	No mutations identified
3	Not on ClinVar	BRCA2	11	c.3037A>G, p.(Lys1025Glu)	No deleterious mutation	No mutations identified
4	Pathogenic (2016)	BRCA2	3	c.316+5G>C	Deleterious Mutation	No sample submitted
5	VUS (2016)	BRCA2	4	c.343A>G , p.(Lys115Glu)	No deleterious mutation	No mutations identified
6	Likely benign (2016)	BRCA2	11	c.5635G>A, p.(Glu1879Lys)	No deleterious mutation	RAD51D VUS c.26G>C (p.Cys9Ser)
7	Benign (2017)	BRCA2	11	c.6821G>T, p.(Gly2274Val)	No deleterious mutation	No sample submitted
8	Likely Benign (2018)	BRCA2	15	c.7601C>T, p.(Ala2534Val)	No deleterious mutationv	No mutations identified
9		BRCA2	17			

	Pathogenic (2017)			c.7958T>C, p.(Leu2653Pro)	Suspected deleterious mutation	BRCA2, c.7958T>C, p.(Leu2653Pro) PATHOGENIC
10	VUS (2016)	BRCA2	19	c.8351G>A, p.(Arg2784Gln)	No deleterious mutation	No sample submitted

### Conclusions

All 10 VUS in BRCA1/2 were reclassified with additional testing by two commercial laboratories at a later date. The results of this testing by two different companies are clinically concordant. Panel testing identified 2 additional VUS in potentially clinically relevant genes. Less expansive testing increases access to germline genetic testing but requires responsible interpretation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-20

Improved patient compliance and genetic screening efficiency following implementation of an electronic pedigree survey (PROGENY)

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## *Introduction*

Patients referred for genetic counseling do not consistently complete a family history questionnaires (FHQ), which is required before scheduling a genetic counseling appointment (GCA). A paper survey has traditionally been used to collect this FHQ data. This requires the genetic counselor to manually input the patient's FHQ information into a pedigree generator prior to scheduling their GCA, thereby delaying the genetic screening process. Some genetic counseling centers have switched from using this paper survey to a web-based pedigree-generator, like PROGENY, for collecting patient's FHQ data. No literature to date has evaluated the impact of this change on patient compliance with completing the required FHQ, the genetic screening process, or patient outcomes. This study assesses changes in patient compliance and genetic screening efficiency with the implementation of PROGENY compared to traditional surveying as a means of collecting FHQ data.

## *Hypothesis*

More patients will complete the FHQ and GCA and there will be reduced time between patient referral and completion of the FHQ and GCA with the use of PROGENY compared to the traditional paper survey.

## *Methods*

This is a retrospective chart review of patients referred for genetic counseling six months before and after implementation of PROGENY. Independent t-tests assuming unequal variances were conducted using JMP to compare genetic screening efficiency with and without the use of PROGENY.

## *Results*

This study found that 41% more patients completed the FHQ and 25% more patients completed a GCA when using PROGENY. Further, there was a statistically significant reduction in the time between referral and completion of the FHQ ( $t(311)=9.14$ ,  $p<.0001$ ) as well as the GCA ( $t(280)=3.04$ ,  $p<.0001$ ). Specifically, the average time for patients to complete the FHQ was 84.45 days faster with PROGENY and the time for patients to complete a GCA was 34.83 days faster with PROGENY than without PROGENY.

## *Conclusions*

These results support our hypothesis and suggest that utilizing PROGENY in collecting patient FHQ data correlates with improved patient compliance and genetic screening efficiency. Future work should investigate how quality improvement such as this impacts patient outcomes, such as the timeliness of finding an actionable genetic mutation and detecting cancerous and precancerous lesions. Some limitations of this study include high variability. This is likely due to factors such as appointment cancellations, geneticist availability and workload, and patient age, ethnicity, gender, and socioeconomic status. Future work should assess the impact of these factors on variables such as patient compliance and efficiency of the genetic screening process.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-09-21

Genetics insights into hereditary cancer risk in the Brazilian population

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**Introduction:** At least 5–10% of cancers are associated with germline mutations in oncogenes and tumor suppressor genes. The identification of individuals with these germline mutations and subsequent cancer risk is crucial for preventive and therapeutic options and represents an opportunity for cancer risk reduction. In Brazil, there are limited specialists devoted to hereditary cancer diagnosis and follow up, and as a result the distribution of germline mutations and cancer risk in the Brazilian population is not well understood. Here, we analyze the results of germline genetic testing for hereditary cancer risk in a Brazilian cohort.

**Methods:** We describe the demographics and genetic results of 758 Brazilians who were referred by a physician to receive genetic testing for hereditary cancer risk. The test consisted of next generation sequencing (NGS) based assessment of 19 or 30 genes associated with hereditary cancer risk. The relatedness of individuals was not assessed in this study.

**Results:** In this cohort the overall pathogenic rate was 20.8%. Pathogenic variants were most commonly identified in BRCA1 (23.8%) and BRCA2 (22.0%), followed by MUTYH (11.9%), CHEK2 (10.7%), TP53 (8.9%), PALB2 (5.4%), ATM (3.0%), MLH1 (1.8%), MSH2 (1.8%), PMS2 (1.8%), RAD51D (1.8%), APC (1.2%), BARD1 (1.2%), MITF (1.2%), MSH6 (1.2%), RAD51C (1.2%), BRIP1 (0.6%), and NBN (0.6%). The VUS rate was 25.3%. The average age of the cohort was 47.8 years and positive rate by age group was: 18-24 (29.4%), 25-34 (29.1%), 35-44 (18.1%), 45-54 (21.9%), 55-64 (17.7%), 65 or older (18.8%). Individuals in this study self-reported their ancestry: Unknown (34%), Caucasian (29%), No answer (21%), Multiple Ethnicity (4%), Hispanic (4%), Native American (3%), African (3%), Ashkenazi Jewish (1%), and Asian (0.5%). A total of 435 (57.4%) individuals reported a personal history of cancer. Of the people who did not have a personal history of cancer the overall pathogenic rate was 12.1%. A total of 10 (1.3%) individuals were found to carry two concurrent pathogenic variants in different genes.

**Conclusions:** This is one of the first multi-gene panel studies of hereditary cancer risk conducted in the Brazilian population on individuals with a diverse set of clinical indications; nearly half of the people tested didn't have a personal history of cancer yet there was still a significant rate of mutation in that unaffected group highlighting an opportunity for earlier intervention. BRCA1 and BRCA2 accounted for the majority of genetic alterations, however mutations in TP53 were more frequent than in other populations, which is consistent with the presence of a known Brazilian founder mutation in TP53. The VUS rate of 25.3% was a little higher than the VUS rate (18.4%) we have observed previously when reporting on a largely caucasian cohort, indicating a need to increase testing in diverse population to help resolve variant classification.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-10-01

Using whole genome sequencing and somatic mutation signatures to unravel insight into familial breast cancer aetiology

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Approximately 10-15% of breast cancers are associated with a strong family history of disease. Pathogenic variants in *BRCA1*, *BRCA2* or other moderate to highly penetrant susceptibility genes (e.g. *TP53*, *ATM*, *CHEK2*, *PALB2* and *PTEN*) account for a number of breast cancer families. However, for over 50% of families the underlying genetic contribution to their risk remains unknown (termed here as non-*BRCA1/2*). This has a profound impact for how individuals and their families are managed in the clinic. We applied whole genome sequencing (WGS) to determine whether somatic mutation analysis can reveal insight into the aetiology of familial breast cancer. The full repertoire of somatic mutations was evaluated in 26 *BRCA1*, 22 *BRCA2* and 32 non-*BRCA1/2* tumours; including SNPs, indels, copy number changes and structural rearrangements, and mutational signatures. Genomes were also analysed using the HRD Index and HRDetect, as predictors of homologous recombination deficiency. *BRCA1*, *BRCA2* and non-*BRCA1/2* tumours exhibited a different burden of mutations, a different spectrum of mutational signatures and different telomere length. Based on collective patterns of mutation signatures, tumours were classified as '*BRCA1*-like', '*BRCA2*-like' or 'non-*BRCA1/2*-like' with a 15% rate of tumour re-classification from their original clinical BRCA status. The results demonstrate the power of WGS to differentiate between *BRCA1* and *BRCA2* driven tumours; in the identification of double-pathogenic germline mutation carriers based on the resulting somatic mutation signature; and in the interpretation of *BRCA* unclassified variants. WGS of tumour genomes reveals fascinating insights into tumour aetiology and could compliment current genetic testing of breast cancer families.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-10-02

Development and validation of a polygenic score to predict breast cancer risk in unaffected Hispanic women negative for mutations on a multi-gene hereditary cancer panel

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**Background:** Breast cancer (BC) is the most commonly diagnosed cancer and the leading cause of cancer-related death among Hispanic women in the United States. For women of European ancestry, genome-wide association studies (GWAS) have identified common variants, primarily single-nucleotide polymorphisms (SNPs), that individually confer modest risk but together explain a significant proportion of genetic BC predisposition. For Hispanic women, the genetic contribution of SNPs to BC risk is not well understood. In these studies, we aim to develop and validate a polygenic score to improve risk assessment for Hispanic women who test negative for mutations in known BC susceptibility genes.

**Methods:** Genotypes and clinical histories were collected from consecutive development and validation cohorts of patients referred for hereditary cancer testing. Study subjects include women who report strictly Hispanic or Latin American ancestry, and who test negative for mutations in 11 genes associated with breast cancer (*BRCA1*, *BRCA2*, *TP53*, *PTEN*, *STK11*, *CDH1*, *PALB2*, *CHEK2*, *ATM*, *NBN*, *BARD1*).

Based on a development cohort ascertained through June 2017, we evaluated an 86-SNP Residual Risk Score (RRS) that was previously developed and validated for women of European ancestry. In the same cohort we are developing a Hispanic Residual Risk Score (HRRS) optimized for women of Hispanic ancestry. BC associations of individual SNPs are being established through meta-analysis of the development cohort and published Hispanic studies.

Multivariate logistic regression models were used to evaluate the 86-SNP RRS, and were the primary statistical tool for evaluation of individual SNPs and candidate polygenic scores. All models included personal/family cancer history and age as independent variables. P-values are based on likelihood ratio test statistics, and reported as two-sided. The development and validation studies are being conducted according to a protocol approved by the Quorum Institutional Review Board.

**Results:** The development cohort included 5,454 Hispanic women, 24% of whom reported a personal history of BC. The 86-SNP RRS was significantly associated with a personal history of BC after accounting for personal and family cancer history ( $p < 10^{-19}$ ) with odds ratio per unit standard deviation 1.39 (95% CI = 1.30-1.50). To date, more than 5,000 Hispanic women have been ascertained for inclusion in the validation cohort. Results comparing discriminatory accuracy of the RRS and the HRRS will be presented.

**Conclusions:** The implementation of a clinically validated polygenic score may improve risk assessment and medical management of Hispanic women who test negative for monogenic BC mutations. The HRRS will be validated in an independent study population according to a pre-specified statistical analysis plan.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-10-03

Breast cancer diagnosed in young women  $\leq$  age 35: Importance of germline mutations and cancer subtypes on treatment outcomes

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**Background:** While breast cancer is rare in women  $\leq$  age 35, patients in this age group are more frequently diagnosed with aggressive cancer subtypes and pathologic germline mutations in cancer susceptibility genes than older patients. Higher recurrence and mortality rates in young breast cancer patients may be related to differences in tumor biology, pathologic mutation status, host characteristics or treatment.

**Specific Aims:** The primary purpose of this retrospective study was to evaluate germline mutation status, breast cancer subtypes, and other associated risk factors that may impact recurrence-free and overall survival in patients diagnosed with breast cancer at age  $\leq$  35.

**Methods:** This was a retrospective review of all breast cancer cases diagnosed in women  $\leq$  age 35 (n= 306) at Allina Health facilities from the years of 2000 – 2017. We collected information on germline mutation status, histopathologic tumor characteristics (including grade, hormone receptor status, human epidermal growth factor receptor 2 [HER2] status, molecular subtype), pregnancy-associated cancers (within a year of diagnosis), treatment, and recurrence-free and overall survival in this cohort. Kaplan Meier analyses were conducted for recurrence-free and overall survival by mutation status and molecular subtype. **Findings:** With a mean follow-up of 6.5 years, the overall survival rate was 88.2%, with a recurrence-free survival rate of 85.2%. A total of 69.3% of patients obtained genetic testing, and 26.9% of those patients had high-risk pathologic mutations, particularly breast cancer gene (BRCA)1 and BRCA2. There were no significant differences in recurrence-free or overall survival between patients with high-risk genetic mutations as compared to those with low-risk/no mutations. However, the definitive surgery and chemotherapy use did vary by mutation status; patients with high-risk mutations were more likely to have bilateral mastectomies and receive chemotherapy (p = 0.002 and p = 0.04, respectively). Kaplan Meier analysis showed that survival was impacted by breast cancer molecular subtype; patients with Luminal B cancer had the lowest overall survival followed by HER2 positive and triple negative cancers (p = 0.05). Other factors associated with decreased survival included cancer stage, angiolymphatic invasion, tumor grade, and pregnancy-associated diagnoses (within a year of diagnosis).

**Conclusions:** Our study shows that patients with high-risk genetic mutations (primarily BRCA1 and BRCA2) were more likely to receive chemotherapy and bilateral mastectomies. Patients with high-risk mutations had similar rates of survival and recurrence-free survival compared to patients without mutations in this study despite an increased risk of overall recurrence and subsequent cancer. In spite of their young age at diagnosis, nearly a third of patients in this study did not receive genetic testing; these results may have impacted treatment and outcomes. Patients diagnosed with Luminal B subtype had the lowest overall survival. Pregnancy-associated breast cancer diagnoses were associated with decreased survival in this age cohort.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-10-04

Exome sequencing in high breast and ovarian cancer incidence families which lack detectable mutations in established cancer susceptibility genes

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At our center, a standard 25 gene panel has proved useful for the identification of pathogenic germline mutations in many families with a high penetrance of breast and ovarian cancer, yet there remain several families with very high cancer incidence but from whom these assays have not identified pathogenic alleles. To attempt to define the genetic mechanism of susceptibility in such cases, are conducting germline whole exome sequencing in affected individuals. Here we describe the identification of strong candidate susceptibility variants in two such families. Both variants cause loss-of-function of DNA repair genes not previously implicated in breast cancer, but which share properties with genes with known roles in maintenance of genome integrity. In one individual, with a personal history of ovarian cancer and two independent breast tumors, we identified a novel point mutation in POLD2 which introduces a mutation at a residue which is biochemically conserved in all animal species thus far sequenced. In the second individual, a frameshift mutation was detected in ERCC4. In both cases, the variants found have not been observed in large exome datasets (e.g. ExAC) or reported in ClinVar. Our study highlights the potential utility of whole exome sequencing as a discovery tool in those families with high cancer incidence yet which lack mutations in known hereditary cancer predisposition genes.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-10-05

An association of PALB2 T1100T(3300T>G) polymorphism with breast cancer in the KYRGYZ ethnic group

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**Background:** PALB2 (a partner and localizer of BRCA2) is an important regulator of homologous recombination and a key-component of DNA excision repair. PALB2 was firstly characterized in patients with Fanconi anemia, complementation group N, and then It has been discovered that heterozygosity for loss of function mutations at PALB2 increases risk of breast cancer 2- to 6-fold. Inherited PALB2 mutations associated with increased risks of breast cancer (BC) have been identified in families from many parts of the world. An association of PALB2 with BC has never been tested in the Kyrgyz ethnic group.

**Methods:** This was a case-control study of 201 women of the Kyrgyz ethnic group with a morphologically verified breast cancer (N=99) and 102 controls age-, ethnicity-matched with BC cases. The mean age of the patients was 53 years (24 -74, SE mean = 0.967, STD = 9.81). 63 of 99 BC patients had family history of breast cancer and uterine cancer. The extraction of DNA was carried out from venous blood. The genotyping was performed by using the method of polymerase chain reaction and restriction fragment length polymorphism.

**Results:** In our study PALB2 gene presented with the TT ( 95%) genotype and the heterozygous TG (5%) genotype. We have observed a statistically significant difference between occurrence of both genotypes in two different groups: patients with T1-T2 (primary tumor's size is 2-5cm across) and patients with T3, T4 (primary tumor's size is larger than 5cm across) (according to TNM classification of breast cancer). The genotype TT occurred in 76.5% of BC cases in patients with T1-T2, and in 23.4% of BC cases in patients with T3-T4, whereas the heterozygous genotype TG occurred in 25% of BC cases in patients with T1-T2, and in 75% of BC cases in patients with T3-T4 (p=0.02, z-ratio=2.318).

**Conclusion:** The results of our study suggest that the heterozygous TG genotype of PALB2 T1100T(3300T>G) polymorphism can be associated with primary tumor's size larger than 5cm across (corresponds to T3-T4) and consequently related to advanced stages of BC. The TT genotype can be associated with primary tumor's size 2-5cm across (corresponds to T1-T2), and related to early stages of BC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-01

Pharmacodynamic and circulating tumor DNA evaluation in a phase I study of GDC-0927, a selective estrogen receptor antagonist/ degrader (SERD)

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**Background:** Modulation of estrogen activity and/or synthesis is the mainstay therapeutic strategy in the treatment of ER positive breast cancer. However, despite the effectiveness of available endocrine therapies, many patients ultimately relapse or develop resistance to these agents via estrogen-dependent and estrogen-independent mechanisms, including mutations in *ESR1* affecting the ER ligand binding domain that drive ER-dependent transcription and proliferation in the absence of estrogen. Based on preclinical and clinical data, SERDs are expected to be effective in patients harboring *ESR1* mutations. Biomarker analysis was performed on plasma and tumor samples from the Phase I study of GDC-0927 in metastatic breast cancer (Dickler et al, SABCS 2017) with the goal of evaluating activity in both *ESR1* mutant and wildtype tumors, and to assess ER pathway modulation.

**Methods:** Hotspot mutations in *ESR1*, *PIK3CA*, and *AKT1* were analyzed in baseline, on-treatment and end of treatment plasma derived circulating tumor DNA (ctDNA) using the BEAMing assay in patients treated at multiple dose levels of GDC-0927. A subset of samples was analyzed with Foundation Medicine's next generation sequencing ctDNA assay (FACT), which covers genomic alterations in 62 commonly altered genes. Paired pre- and on-treatment biopsies were collected to assess ER pathway modulation. ER, PR, and Ki67 protein levels were analyzed by immunohistochemistry. Gene expression analysis was performed using Illumina's RNA Access library preparation kit followed by paired-end (2x50b, 50M reads) sequencing on the HiSeq.

**Results:** Baseline and on-treatment plasma samples were available for 40 patients. *ESR1* and *PIK3CA* mutations were observed in 52% and 33% of patient baseline samples, respectively (BEAMing method). Mutant allele frequencies (MAF) generally declined in the first on-treatment samples collected for both *ESR1* (16 out of 21 samples) and *PIK3CA* (7 out of 12 samples). The majority of the reductions were greater than 95% relative to baseline. Increases in *ESR1* MAFs were observed in later time-points and were not associated with any particular *ESR1* mutation. There were six instances for which an *ESR1* mutation was detected in an on-treatment sample that was not detected in the baseline sample, three at L536P and one each at D538G, L536H, and S463P, and four out of six with MAFs close to the limit of detection. The FACT assay also detected alterations in *CDH1*, *NF1*, *PTEN*, and *TP53* in baseline samples. The relationship between MAF changes and clinical benefit to GDC-0927 will be presented. A predefined, experimentally-derived set of ER target genes were evaluated in pre- and on-treatment tumor biopsy pairs from six patients. Four of the six patients showed evidence of suppression in ER pathway activity, one patient treated at the 1000 mg dose level and three at the 1400 mg dose. The degree of pathway suppression was associated with pre-treatment pathway levels and decreases of ER and Ki67 protein levels.

**Conclusions:** We report here evidence of consistent reduction of *ESR1* and *PIK3CA* ctDNA in patients treated with GDC-0927. ER pathway suppression was observed at both the transcript and protein level confirming pharmacodynamic activity of the SERD.

# 2018 San Antonio Breast Cancer Symposium ®

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Recurrent *ESR1* fusions in primary tumors; a promising predictive factor for outcome to first-line endocrine therapy?

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## Introduction:

While fusion genes have been identified and are being utilized as prognostic and predictive markers in various types of cancer, their relevance still needs to be established and verified for breast cancer. Recently, recurrent estrogen receptor alpha (*ESR1*) fusion genes have been identified as putative endocrine resistance markers, but their predictive value for response to endocrine therapy has not yet been independently validated. Here we studied the presence of fusions of *ESR1* exon 2 with exons 1 to 11 of *CCDC170*, resulting in constitutively activated *CCDC170*, of *ESR1* exon 4 with *AKAP12*, a putative tumor-suppressor gene, and *ESR1* exon 1 with *C6orf211/ARMT1*, a methyltransferase and their association with outcome in a large cohort of *ESR1*-positive metastatic breast cancer patients.

## Methods:

Fusion gene mRNA levels were measured in 307 *ESR1*-positive primary tumors by quantitative reverse transcriptase PCR (RT-qPCR). If the RT-qPCR generated a positive Cq value, the expected fusion gene product sizes were validated by MultiNA. All patients in this study were hormone-naïve and all experienced a recurrence and subsequently received 1<sup>st</sup> line endocrine therapy. The association of the presence of *ESR1* fusion genes in the primary tumor with disease-free interval (DFI) before, and progression-free survival (PFS) up to 36 months after start with 1<sup>st</sup> line tamoxifen (n=219) or aromatase inhibitors (n=88), were evaluated.

## Results:

74 patients (24.1%) experienced a disease recurrence within one year after removal of the primary tumor (mean DFI; 34.8 months) and 257 patients (83.7%) progressed on 1<sup>st</sup>-line endocrine therapy within 3 years (mean PFS; 12.5 months). For the tamoxifen cohort, *ESR1-CCDC170* fusion transcripts were found in 84 patients, of which fusions restricted to exon 1, 4, 6, 10 and 11 of *CCDC170* were present in 18 patients who all but one progressed within 3 years (mean PFS 9.1 months). Of note, overall, these 18 patients also had a reduced DFI. Similarly for the 7 patients with *ESR1-AKAP12* fusions and the one patient with an *ESR1-ARMT1* fusion; all these patients progressed within 3 years. But in contrast to the *ESR1-CCDC170* fusion positive patients, these patients had a prolonged DFI (see Table). Similar observations were made for the smaller AI cohort, though with the - with respect to their predictive value - most relevant *ESR1-CCDC170* fusions restricted to exon 4, 5, 6 and 10 of *CCDC170* and here we now also observed a decreased DFI for the 7 patients with *ESR1-AKAP12* and the 3 patients with *ESR1-ARMT1* fusions (see Table).

1st line tamoxifen (n=219)				1st line AI (n=88)			
Fusion	% positive	DFI (months) pos/all	PFS (months) pos/all	Fusion	% positive	DFI (months) pos/all	PFS (months) pos/all
E2-CCDC170 Exon 2 to 1/4/6/10/11	8.2%	19.8/27.9	9.1/12.0	E2-CCDC170 Exon 2 to 4/5/6/10	12.5%	32.4/51.9	10.5/13.8
E2-AKAP12	3.2%	33.1/27.7	11.9/12.0	E2-AKAP12	8.0%	23.6/54.7	10.3/12.0
E2-ARMT1	0.5%	(n=1)	(n=1)	E2-ARMT1	3.4%	30.3/52.7	9.3/12.0

## Conclusion:

Measuring recurrent *ESR1* fusions in primary breast cancer might become a promising tool to identify patients with intrinsic resistance to endocrine therapy or aggressive disease biology. Importantly however, which fusions are relevant appears to depend on the type of endocrine therapy given.



# 2018 San Antonio Breast Cancer Symposium ®

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Measurement of on-treatment proliferation biomarkers in nodal metastasis improves prediction of endocrine therapy response using the EA2Clin test

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**Background:** The majority of patients with early-stage estrogen receptor positive (ER+) breast cancer (BC) are treated with adjuvant endocrine therapy (ET) after surgery to reduce the risk of recurrence. Recently, we have developed and validated an immunohistochemistry (IHC) based assay (EndoAdjuvant2 Clinical; EA2Clin) that measures pre-treatment IL6ST level together with clinical variables and on-treatment MCM4 to assess proliferation. We have previously shown that it can accurately identify responders and non-responders to ET and predicts recurrence-free survival (RFS) and BC-specific overall survival (BCSS). We postulated that measuring on-treatment proliferation in lymph node metastases (LN+) rather in the primary cancer might further improve the accuracy of the test for these patients. The aim was to test and validate this in cohorts of pre- and post-menopausal women (preMW & PMW) treated with preoperative ET (tamoxifen (T), fulvestrant (F), letrozole (L) or anastrozole (A)) and subsequent adjuvant ET.

**Methods:** Cohorts: (1) 137 PMW with ER+ BC, 59 were LN+, treated with neoadjuvant L (median duration 4.8 months, range 1-33), then surgery followed by adjuvant L (n=109) or other ET (n=28); (2) 148 PMW with ER+ BC, 55 were LN+, treated with 2 weeks of preoperative L (n=76) or A (n=72), then surgery followed by adjuvant L (n=69) or T (n=79); (3) 52 preMW with ER+ BC, 24 were LN+, treated with 2 weeks of preoperative T (n=26) or 1x750mg dose of F (n=26), then surgery followed by adjuvant T. All LN+ patients had sentinel node biopsies or clearance. The median follow-up was 6.5 years (cohort 1), 6.3 years (cohort 2) and 10.2 years (cohort 3).

**EA2Clin:** Patients are classified as:

- **Low risk:** ER+ and LN-negative and <2cm or pre-treatment IL6ST 2+/3+ (IHC) and post-treatment MCM4 in the primary has <20% positive nuclear staining.
- **High risk:** ER+ LN+ grade 3 BCs >2cm or pre-treatment IL6ST is 0 or 1+, or IL6ST is 2+ or 3+ and MCM4 in the primary has >10% positive nuclear staining.

**EA2Clin** uses the post-treatment level of MCM4 in the nodes, rather than the primary cancer.

**Results:** In cohort 1, EA2Clin (using primary tumour MCM4) was significantly associated with both RFS (P=0.0003, HR=13.17, 95%CI=5.48-13.61) and BCSS (P=0.005, HR=11.91, 95%CI=8.73-31.42). The 5 and 10 year actuarial recurrence rates were 5%/5% and 48%/64% for the low and high-risk groups respectively.

In the same cohort, using the MCM4 level in the node (EA2Clin) there was an even more significant association with both RFS (P<0.00009, HR=18.16, 95%CI=12.59-19.46) and BCSS (P=0.002, HR=12.93, 95%CI=5.43-25.62). The 5 and 10 year actuarial recurrence rates were 0%/0% and 48%/72% for the low and high-risk groups respectively. Further validation of EA2Clin in cohorts 2 and 3 is underway.

**Discussion:**

- Direct measurement of on-treatment proliferation biomarkers in LN metastases improves prediction of outcomes to ET in women with BC.
- This tests identifies a group of low risk women that are node negative and node positive with a 100% RFS and BCSS.
- This is the most impressive predictive test for patients with ER+ breast cancer yet developed.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-04

Anti-proliferative effect of oral metronomic vinorelbine (mVNB) in PAM50 Luminal/HER2-negative early breast cancer (SOLTI-1501 VENTANA): A randomized, three-arm, window-of-opportunity study

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**BACKGROUND:** The anti-proliferative effect of mVNB alone or in combination with endocrine therapy in patients with hormone receptor-positive/HER2- breast cancer (BC) has been scarcely addressed.

**METHODS:** Postmenopausal women with untreated stage I-III BC were randomized (1:1:1) to receive 3 weeks of letrozole (LTZ) 2.5mg/day, oral mVNB 50mg 3 days/week or the combination. The 1<sup>ary</sup> objective was to evaluate, within PAM50 Luminal A/B disease, if the anti-proliferative effect of mVNB+LTZ was superior to monotherapy. An anti-proliferative effect was defined as the mean relative decrease of the PAM50 11-gene Proliferation Score in each arm. 2<sup>ary</sup> objectives included safety and the comparison of the anti-proliferative effect between arms. An unplanned analysis of stromal tumor infiltrating lymphocytes (sTILs) was performed. PAM50 analyses were performed using the nCounter®-based Breast Cancer 360™ panel. Changes in the expression of 790 genes/signatures tracking multiple biological processes from tumor cells and the microenvironment were evaluated within each arm using paired (surgery vs. baseline) univariate analyses. P-values were corrected for multiple comparisons using false discovery rate (FDR).

**RESULTS:** A total of 61 patients were randomized and 54 paired samples (89%) were analyzed. Main patient characteristics were mean age 67, mean tumor size 1.7 cm, stage I (55.7%) and grade 1-2 (90%). Grade 3 toxicities occurred in 3.3% of cases. Baseline samples were Luminal A (72.3%) or B (27.7%). The anti-proliferative effect of mVNB+LTZ (-73.2%) was superior to both monotherapy arms combined (-49.9%; p=0.001) and mVNB (-19.1%; p<0.001). The anti-proliferative effect of mVNB+LTZ (-73.2%) was higher compared to LTZ (-65.7%) but did not reach statistical significance (p=0.328). Across the mVNB+LTZ, LTZ and VNB arms, 413 (52.3%), 403 (51.0%) and 21 (2.6%) genes/signatures were found differentially expressed (FDR<5%) between baseline and surgery samples. Compared to mVNB+LTZ baseline samples, surgical samples showed higher expression of AP-1 transcription factor subunits FOS and JUN, inflammatory chemokines (e.g. CCL4 and IL6), stromal-related genes (e.g. CAV1 and stroma signature) and immune infiltration (e.g. CD8 T-cell signature) and lower expression of proliferation-related genes (e.g. MKI67 and UBE2C), estrogen receptor-signaling and Risk of Recurrence. Of the 413 genes found differentially expressed in surgical samples compared to baseline samples in the mVNB+LTZ arm, 108 (26.2%) were not found in the LTZ arm. Among them, high expression of LAG3, CD24, CD84 and CCR5. Under the microscope, sTILs (≥10% at week 3) were observed in 6.6% (mVNB), 15% (LTZ) and 26% (mVNB+LTZ) of the cases. In tumors with ≤10% TILs at baseline, an increase in TILs was observed following LTZ (p=0.049) and mVNB+LTZ (p=0.012).

**CONCLUSIONS:** mVNB is well-tolerated and presents antiproliferative activity alone and in combination with LTZ. The increase of activated CD8 T-cells or TILs observed with LTZ+mVNB opens the possibility of studying combinations with immunotherapy. Further investigation comparing these biological results with other metronomic schedules or combinations is warranted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-05

Concordance of genomic alterations with targeted sequencing of circulating tumor DNA (ctDNA) and circulating tumor cells (CTC) in endocrine-resistant metastatic breast cancer

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**Background:** Liquid biopsy has emerged as a valuable strategy for analyzing resistance biomarkers in advanced breast cancer. Differences in genomic alterations between CTC and cfDNA has been reported before and may reflect differences in the cell compartments of origin. Our aim was to determine the mutational concordance between CTC and cfDNA in luminal metastatic breast cancer (MBC) with different levels of endocrine resistance.

**Methods:** We evaluated the mutational spectrum in matched cfDNA and CTC samples of 38 patients with luminal (hormone receptors [HR] positive, HER2 negative) MBC. Using QIAmp Circulating Nucleic Acid Kit, cfDNA was isolated from plasma samples (5 mL), and from CTC samples obtained from plasma (7.5 mL). CTC were isolated by Epcam-based immune-magnetic enrichment and confirmed with AdnaTest Breast Cancer Detect (Epcam, Muc1, HER2). Sequencing of both cfDNA and CTC was performed using the Oncoming Breast cfDNA Assay (150 hotspots, 10 genes). Limit of detection range was 0.01-0.15%, with 92.1% cases equal or less than 0.1%. Concordance between mutational profile of CTC and cfDNA was analyzed and correlated with endocrine resistance and patient characteristics.

**Results:** Forty-seven patients with HR+ HER2- MBC were included, yielding enough cfDNA for analysis in 38 cases (80%), with isolation of CTC in 8 cases (16.7%). Rate of mutation detection in CTC was 87.5% (7/8), with TP53 mutations in all cases and no cases of ESR1 mutations; two cases of ERBB3 and PIK3CA mutations were found only in CTC, without a correlate in cfDNA. In cfDNA, genetic alterations were observed in 63.4% of cases, with 70.8% of TP53 mutations, 37.5% of PI3KCA mutations and 25% ESR1 mutations (2 D538G, 3 Y537S, 2 V392I). Average number of mutations was 2.28 in CTC and 1.96 in cfDNA, with a range of 1-4 in both cases. Concordance between cfDNA and CTC was low, with only 20% of CTC mutations simultaneously detected in cfDNA. CTC were only detected in cases with primary or secondary endocrine resistance. The percentage of patients with genetic alterations in cfDNA varied according to the degree of endocrine resistance (no resistance, 42.8%; secondary resistance, 65.2%; primary resistance, 75%), although the difference was not statistically significant. An association was found between the rate of detection of mutations in cfDNA and the response status (progression disease: 90.9%; stable disease: 57.1%; objective response: 33.3%) (p=0.04).

**Conclusions:** The low rate of CTC detection in HR positive MBC may limit its utility for guiding therapeutic decisions. However, the information provided by CTC sequencing does not completely overlap with the data provided by cfDNA, and may be complementary to cfDNA in endocrine-resistant MBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-06

Does hormone expression by IHC predict ER pathway activity? An analysis in a metastatic breast cancer patient cohort

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**Background:** Immunohistochemistry (IHC) staining for ER and progesterone (PR) receptors in breast cancer tissue is the current standard for testing for eligibility for hormone targeted therapies. However, these markers are imperfect predictors of response, and ER/PR expression can be heterogeneous, especially in the metastatic setting. Within an adjuvant tamoxifen-treated ER positive patient group functional ER pathway activity was associated with improved patient outcome (Verhaegh et al, Cancer Research 2014). This study investigated how ER/PR staining correlates with ER pathway activity in a cohort of metastatic breast cancers.

**Methods:** Cases of metastatic breast cancer with variable reported ER expression by IHC were selected from the Stanford Pathology Database. Cases were scored by a breast pathologist for percent cellularity of sample, percent and intensity of ER and PR staining, and a qualitative rating of heterogeneity of staining. Digital slides were annotated for areas of cellularity to perform ER pathway activity analysis. Functional ER pathway activity was measured in a quantitative manner using a biologically validated method, based on Bayesian computational model inference of functional pathway activity from RT-qPCR measurements of mRNA levels of target genes of the pathway transcription factor, providing an ER Pathway Activity Score (PAS)(Verhaegh et al, Cancer Research 2014).

**Results:** A total of 64 samples were tested for ER/PR expression as well as for ER pathway analysis. Annotated tumor areas of 57 samples were used for measurement of ER PAS. 61.4% were ER expression high (defined as >50% ER IHC positive, mean 94.0%) and 38.6 % were ER low (defined as <50% ER IHC positive, mean 23.0%). ER high cases had higher mean ER PAS when compared to ER low cases (ER high, mean PAS 47.6, SD 19.2; versus ER low, mean PAS 19.4, SD 11.7; unpaired one-tailed 2-sample t-test  $p < 0.001$ ). Importantly, there was wide variation in ER PAS even in cases with high ER expression levels. PR levels did not correlate with ER PAS. Three cases had clustered areas with different ER expression levels within the tissue slide (heterogeneous cases), resulting in 7 separately analyzed areas; ER pathway activity was separately analyzed in these areas. ER PAS was remarkably similar across areas with variable ER staining, e.g. within one tissue slide, areas with 20% and 90% ER expression had nearly the same ER PAS.

**Discussion:** Grouping cases into high vs low ER IHC staining reveals expected differences in ER PAS. However, nuclear ER expression (IHC) levels may be an imperfect predictor of actual ER pathway activity on an individual case basis. Preliminary results on cases with regional heterogeneity for levels of ER IHC expression suggest that ER PAS is more homogenous than IHC levels. Clinical studies examining value of ER PAS to predict response to hormonal therapies are ongoing.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-07

The significance of androgen receptor co-expression in ER+ metastatic breast cancer patients treated with palbociclib

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**Background:** The PALOMA-1 and PALOMA-3 studies demonstrated a significant progression-free survival (PFS) advantage for palbociclib, a CDK4/6 inhibitor, in combination with letrozole or fulvestrant in the first or second line setting compared to these therapies alone in estrogen receptor (ER)+, HER2-negative metastatic breast cancer (MBC). Recent studies have revealed preliminary efficacy signals for androgen receptor (AR) blockade in MBC, predominately in AR+, triple negative patients. We sought to further evaluate AR expression and its significance in ER+ MBC patients treated with palbociclib early in their metastatic treatment course.

**Methods:** A retrospective review identified 22 patients treated with palbociclib for ER+, HER2- MBC after its FDA approval between January 1st, 2015 and October 1st, 2016 at our center with available pre-treatment tumor samples for analysis. Records were reviewed and clinical characteristics for each patient were analyzed. Archival tumor tissue was tested for AR, phosphorylated retinoblastoma (pRb), CDK6, p16, and CyclinE1 by immunohistochemistry assay for each patient. For AR, nuclear staining >0% was considered positive. For all other IHC studies, intensity of staining  $\geq 2+$  or staining in >10% of cells was considered positive.

**Results:** The median age was 63.5 years (range 34-84); 23% were  $\geq$  age 70. Our cohort was 35% African American, 60% Caucasian, and 5% Asian American. 64% of patients were post-menopausal and 59% had visceral metastases. 45% of patients were on their first line of treatment, 23% second line, and 32% third line. 68% of patients were on an aromatase inhibitor. Median follow up was 18.7 months (95% CI 13.9, 23.3 months). The AR was expressed in 59% of patients; 55% had expression  $\geq 10\%$  and 41% had expression  $\geq 20\%$ . AR+ patients were significantly more likely to experience event-free survival (EFS) (HR 0.26,  $p=0.01$ ), with a median EFS of 18.8 months (AR- median EFS 5.4 months). AR expression was significantly associated with expression of pRb (100% of AR+ patients,  $p=0.02$ ). CDK6, p16, and CyclinE1 expression were not associated with AR expression or EFS.

**Conclusions:** Our data show preliminary evidence of the significance of ER and AR co-expression in ER+, HER2- MBC. ER+, AR+ patients have significantly improved EFS when treated with palbociclib and endocrine therapy as compared with AR-, ER+ patients. There is evidence that AR expression is associated with pRB expression, which may represent a mechanism by which cell cycle inhibition with palbociclib is particularly efficacious in these patients. AR expression rates in ER+, HER2- MBC are significant, and may provide rationale for combining CDK4/6 inhibitors with AR targeting as a subsequent line of targeted therapy in these patients before cytotoxic chemotherapy is initiated. Further studies based on these results are underway.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-08

Genetic landscape of prior and resistance to CDK4/6 inhibition in targeted NGS panel analysis of palbociclib or abemaciclib and endocrine versus placebo and endocrine

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**Background:** CDK4/6 inhibition combined with endocrine therapy is now a standard of care for advanced estrogen receptor (ER) positive breast cancer. Predictive genetic landscape and mechanisms of resistance to CDK4/6 inhibitors have not been described clearly because of limited evidence from clinical samples. We investigated the genetic landscape and mechanisms of resistance to CDK4/6 inhibitors (palbociclib or abemaciclib).

**Methods:** Using driver mutation targeted sequencing, we conducted longitudinal ctDNA analysis in 39 pairs of baseline of primary tumor and end of treatment or monitoring plasma samples. DNA were extracted and the genomic profiles were analyzed based on NGS.

**Results:** In our cohort, the most frequency mutations were PIK3CA (38.5%), TP53(17.9%), CCND1, ZNF703, FGFR1, MAP2K4, RB1, ARID1A, ATRX, DNMT3A. In five patients harboring RB1 mutations prior to CDK4/6 inhibitor, only one case occurred progressing early with 4mos of PFS, and the others were 5.5+, 6.5+, 13.5+, 21+months, it represented different from pre-clinical results that RB1 mutation was associated with resistance to CDK4/6 inhibitor. Interesting, patients with PIK3CA/PTEN/AKT1 mutations (n=15) were likely sensitivity to CDK4/6 inhibitor than those (n=11) with wild type (median PFS: 17 mos vs. 9.5mos, P=0.046). In another cohort of everolimus, new driver mutations emerged in both FGFR1 and ERBB3 at end of everolimus and significantly disappeared in AKT1 mutation. Three patients used palbociclib after progress disease of everolimus, the prognosis were poor with PFS of 1, 6, 9 mos. Evolution of driver gene mutations was uncommon in patients progressing on CDK4/6 inhibitors treatment, but common in patients progressing on everolimus treatment.

**Conclusions:** De novo PIK3CA/PTEN/AKT1 mutations were likely better prognosis biomarkers with the treatment of CDK4/6 inhibitor. RB1 mutation are common and may not contribute to CDK4/6 inhibitor resistance differ from PALOMA3 ctDNA analysis. Our small sample finding indicate it may be a bad strategy for CDK4/6 inhibitor after progress disease with mTOR inhibitor everolimus.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-09

Feasibility of tracking plasma DNA mutation kinetics in estrogen receptor positive metastatic breast cancer using a novel digital PCR amplicon sequencing assay

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Estrogen Receptor 1 (*ESR1*) gene mutations in estrogen receptor positive (ER+) metastatic breast cancer (MBC) are associated with clinical resistance to endocrine therapy. A highly sensitive digital PCR (dPCR) assay was developed to evaluate the baseline abundance and kinetics of hotspot *ESR1* mutations in plasma circulating tumor DNA (ctDNA) from 58 ER+ MBC patients who had progressed on at least one line of prior endocrine therapy. Consistent with prior reports, we demonstrate that detection of hotspot *ESR1* mutations in plasma ctDNA is associated with shorter progression-free survival. However, after patients initiated a new course of systemic therapy, changes in plasma *ESR1* mutation abundance did not correlate with the duration of disease control. To investigate whether mutational heterogeneity may account for this lack of correlation, we developed a customized dPCR multiplexed amplicon next-generation sequencing (NGS) assay to detect plasma ctDNA mutations in the complete coding sequence of *ESR1* and *TP53*, as well as hotspot regions in *PIK3CA*. Assay validation revealed a 79% sensitivity and 100% specificity for detecting plasma ctDNA mutations with a lower limit of detecting a 1.6% mutant allele fraction. The NGS assay revealed non-hotspot *ESR1* mutations in plasma DNA from 14 of 31 (45%) patients. We also observed dynamic changes in plasma ctDNA mutant allele fraction of *PIK3CA* and *TP53* during systemic therapy. Changes in the cumulative mutant allele fraction of *ESR1*, *PIK3CA*, and *TP53* in plasma ctDNA correlated with duration of clinical treatment response in a limited subset of patients (n=8) for whom NGS was performed at two or more time points. Thus, mutational heterogeneity limits the clinical utility of monitoring individual hotspot mutations in *ESR1* over time in ER+ MBC patients. However, a plasma ctDNA NGS assay targeting *ESR1*, *PIK3CA*, and *TP53* may be useful as an early predictor of response to systemic therapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-10

Biomarkers associated with resistance or response to CDK4/6 treatment in patients with metastatic hormone-receptive positive breast cancer

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## **Background:**

CDK4/6 inhibitor (CDKi) drugs are the current standard of care for treatment of first and second-line hormone-receptor positive/HER2 negative (HR+/HER2-) metastatic breast cancers. Numerous research efforts have commenced to understand biomarkers of response and resistance. To date, no biomarker of response has been identified. Treatment induced RB1 mutations were noted as mechanism of resistance to palbociclib and fulvestrant in about 5% of patients treated on PALOMA3 trials, whereas PI3K and ESR1 mutations emerged as potential resistance to the anti-hormonal backbone<sup>1</sup>. Additionally, FGFR1 amplification has been suggested as a resistance pathway to fulvestrant and ribociclib<sup>2</sup>. A better understanding of the molecular landscape of CDK4/6 treatment is needed. Utilizing next-gen sequencing (NGS), chromogenic in situ hybridization (CISH) and immunohistochemistry (IHC) data from HR+/HER2- patients treated at the University of Tennessee West Cancer Center, we attempted to retrospectively identify a molecular signature of resistance or response as measured by PFS.

## **Methods:**

We analyzed 115 breast cancer patients who received CDKi treatment and 30 matched controls not exposed to CDKi and underwent comprehensive molecular profiling (Caris Life Sciences, Phoenix, AZ). A Cox proportional hazards model was built using genetic test as predictors and progression free survival (PFS) time as response. Only alterations with known pathogenic potential were considered aberrant. The R package glmnet was used to perform regularized lasso regression for feature selection on the entire data set. Important features were then used to construct Kaplan-Meier curves and perform a log-rank test for difference in PFS times.

## **Results:**

Here we report the analysis for 2 known pathogenic biomarkers, ESR1 and TP53 based on PFS for patients who test positive versus negative. The median PFS for all patients was 234 days. Patients who harbored ESR1 mutations had reduced PFS of 180 days compared to 237 days. Patients who had P53 mutations had shorter PFS of 201 days compared to 240 days. When both groups with positive mutations were combined, the median PFS was 191 days compared to 276 days for patients without either ESR1 or P53 mutation with a p-value of 0.011. Further analysis using 4 way Kaplan Meier Curves for controls versus treated, altered versus non-altered genes, or PDL-1 expression is ongoing and will be presented at the conference.

## **Conclusion:**

**This data further support, in a real world model, the poor predictive value of ESR1 and P53 mutations on clinical outcome.** Because no testing data was used, additional validation will be necessary to confirm the findings from this analysis.

## **References:**

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-11

A preliminary assessment of knowledge, attitudes, and awareness surrounding ESR1 mutations and biomarker testing amongst medical oncologists

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**Background:** Estrogen receptor gene (ESR1) mutations present in breast cancer patients are associated with resistance to endocrine therapy and worse patient outcomes. Recent findings suggest ESR1 mutations are present in up to 40% of metastatic breast cancer (mBC) patients' tumors. The objective of this study was to evaluate the perceptions of precision medicine and biomarker testing specific to ESR1 mutations among medical oncologists.

**Methods:** Ten 60-minute web-assisted, telephone interviews were conducted with medical oncologists. Each physician was prescreened prior to being interviewed to ensure board certification and practice experience between 2 and 35 years. Each participant spent greater than 30% of their time on direct patient care and managed the treatment of more than 30 different cancer patients per month, with a minimum of 15 breast cancer patients, including at least five who had metastatic breast cancer and at least one patient with an ESR1 mutation.

**Results:** A 10-20% prevalence of ESR1 mutation was estimated by the oncologists. However, the practitioners did accept the possibility of a 40% prevalence. Physician knowledge of ESR1 mutations included the relationship between ESR1 mutations and efficacy of endocrine therapies and its association with poorer outcomes. None of the medical oncologists interviewed were highly satisfied with the existing armamentarium of treatments for patients with an ESR1 mutation.

In general, the interview participants were highly comfortable ordering ESR1 companion diagnostics to test for an ESR1 mutation, pending viable treatment options are available. Nonetheless, most physicians will wait until the patient has progression of disease before ordering a biomarker test. Several areas of unmet need in the mBC arena were offered by the survey participants, including more efficacious hormonal options for later-line therapies, better durability of remission, improved drug tolerability profiles, and lower treatment costs.

**Conclusion:** Most oncologists acknowledged that personalized treatment is beneficial because it allows for better efficacy than a "one size fits all" approach. The study results also suggest that there are few barriers and drawbacks to the use of personalized medicine in the mBC arena, as most physicians expect precision medicine to account for the majority of advances in breast cancer treatments in the foreseeable future.

**Support:** Sermonix Pharmaceuticals

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-11-12

Longitudinal changes in volumetric breast density and fibroglandular volume with endocrine therapy in African American women with estrogen receptor positive breast cancer

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## **Background and Aim:**

Reduction in breast density has been proposed as a biomarker of response to endocrine therapy (ET). The vast majority of current data are derived from white or Asian women. Because baseline breast density is associated with race, it is possible that changes in breast density with treatment may also be affected by race. Our objective was to assess the impact of ET on volumetric breast density (VBD) and fibroglandular volume (FGV) in African American (AA) women with invasive breast cancer.

## **Methods:**

We conducted a retrospective study of AA women diagnosed with estrogen receptor positive invasive breast cancer at our institution from 2009-2013. Mammograms within two years prior to diagnosis and at least 6 months post-diagnosis were utilized for comparing density measurements. Using Volpara automated software, VBD and FGV were measured for the contralateral normal breast by averaging the respective values measured on the craniocaudal and mediolateral oblique views.

## **Results:**

51 women met the inclusion criteria and were confirmed to have received ET. Sixteen women received tamoxifen, 34 received an aromatase inhibitor, and medication data was unavailable in one case. The mean age at diagnosis was 56 years (range 29-72, median 55). 53% of women had stage I disease, 29% had stage II disease, and 18% had stage III disease. The majority of women had ER+ PR+ HER2 - disease (82.4%). 53.0% of women received systemic chemotherapy and all but one woman were treated surgically. Average body mass index (BMI) at diagnosis was 36.5, with data not available for 22 women. The mean time between diagnosis and baseline mammogram was 32 days, and the mean time between follow-up mammogram and baseline mammogram was 401 days. Average BMI at one year follow up was 33.7, with data not available for 19 women. The mean baseline VBD was 7.5% (range 1.9-21.5%, median 6.3%) and the mean follow-up VBD was 6.9% (range 2.0-23.6%, median 5.6%). Fifteen women had a longitudinal increase in VBD. The mean absolute change in VBD was -0.6% (range -3.4% to +9.8%, median 0.7%), with a mean 8.0 percent decrease from baseline to follow-up (range -0.7 to +0.5, median 0.1). The mean baseline FGV was 72.3 cm<sup>3</sup> (range 18.5-208.4, median 65.3) and the mean follow-up FGV was 69.7 cm<sup>3</sup> (range 22.7-197.5, median 60.5). Nineteen women had a longitudinal increase in FGV. The mean absolute reduction in FGV was 2.6 cm<sup>3</sup> (range -53.3 to 49.3, median 4.8), with a mean 0.9 percent decrease from baseline to follow-up (range -111.6 to +53.0, median 5.2).

## **Conclusions:**

We observed an overall decrease in Volpara-calculated VBD and FGV in our cohort of AA women treated with ET. It remains to be determined whether changes in VBD and FGV across serial mammograms may be a biomarker for response to ET in women of all races. Large prospective studies are needed to evaluate the effects of ET on longitudinal changes in VBD and FGV while controlling for confounders such as menopausal status, BMI, and chemotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-01

A novel gene expression signature prognostic for both locoregional and distant failure and predictive for adjuvant radiotherapy

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**Background:** Most patients with early stage breast cancer (BC) are treated with adjuvant radiotherapy (RT) following breast conserving surgery (BCS) to prevent locoregional recurrences (LRR). No predictive tools are currently available to select patients for RT, resulting in considerable over- and under treatment. We aimed to create and validate a gene expression-based classifier to prognosticate for LRR and to stratify patients for treatment with RT.

**Patients and methods:** A 27-gene expression signature was developed using three publicly available early stage BC gene expression datasets where patients were treated with RT and had detailed local recurrence information. The largest of the datasets was used to train the signature, and the other two datasets were used for signature refinement. As age was the strongest clinical factor for the endpoint in the training dataset, it was included in the model, resulting in a final clinical-genomic classifier of 27 genes and age. The classifier was locked before external validation in the SweBCG91-RT trial. This phase III clinical trial included primary tumors from 765 patients and for which gene expression data was available. The trial randomized node-negative BC patients to +/- RT following BCS, with sparse use of adjuvant systemic treatment (9%) and a median follow-up of 14.0 years for LRR in patients free from event. The classifier was validated using Cox regression with LRR as the primary endpoint, and hazard ratios (HRs) were calculated using the raw continuous classifier score (range: 0.5 to 2.5).

**Results:** The novel classifier was highly prognostic for LRR in SweBCG91-RT patients treated with RT (HR=7.5[3.3-16.9],  $p<0.001$ ), and remained prognostic in multivariate analysis (MVA) that included systemic treatment, subtype and grade (HR=7.2[3.1-16.4],  $p<0.001$ ). To a lesser extent, the classifier was also prognostic for LRR in patients not treated with RT (HR=1.9[1.0-3.5],  $p=0.03$ ; MVA HR=1.9[1.0-3.3],  $p=0.05$ ). Patients at high risk of LRR had a smaller effect of RT, and the treatment predictive potential was confirmed by testing for interaction ( $p_{\text{interaction}}=0.008$ ). In patients treated with RT, age and the genomic component of the model were both prognostic for LRR ( $p<0.01$ ) as well as predictive for RT response ( $p_{\text{interaction}}<0.05$ ) and provided independent information ( $p<0.01$ ). The combined classifier has increased performance over its individual components (10-year AUC=0.72, 0.67, 0.65 for the classifier, age, and genomic component, respectively). While the novel signature was prognostic for metastasis (HR=4.3[2.3-7.8],  $p<0.0001$ ), calculated scores from previously published signatures to the metastasis endpoint, including the Oncotype-like score, were not prognostic for LRR.

**Conclusions:** This novel gene expression signature is highly prognostic for LRR, can identify patients at risk of LRR despite RT, and appears to be treatment predictive for adjuvant RT. Furthermore, the current signature is highly prognostic for metastasis. In contrast, calculated scores of previously published signatures modeled for the metastasis endpoint had inferior performance for LRR. These results underscore both the importance of signatures prognostic for LRR and the similarities in the biology of LRR and distant failure.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-12-02

*PTEN* alterations and tumor mutational burden (TMB) as potential predictors of resistance or response to immune checkpoint inhibitors (ICI) in metastatic triple-negative breast cancer (mTNBC)

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**Purpose:** To date no biomarker has been identified that predicts response to ICI in mTNBC. This study aimed to explore if tumor genomic alterations correlate with efficacy of PD-1/PD-L1 inhibition in patients (pts) with mTNBC. **Methods:** Demographic, treatment response, and long-term outcome data were collected on patients with mTNBC treated at Dana-Farber Cancer Institute (DFCI) under several clinical trials incorporating PD-1/PD-L1 inhibitors, given as monotherapy or combined with chemotherapy (CT). Pts included in this analysis had available results of targeted exon sequencing performed using Oncopanel, our institutional gene sequencing panel, on archival tumor tissue. TMB was calculated by determining the number of non-synonymous somatic mutations that occur per megabase of exonic sequence data across all genes on the panel. High TMB was defined as <sup>3</sup>10 mutations/megabase. TMB and gene alterations were correlated with objective response rate (ORR) per RECIST 1.1, progression-free (PFS) and overall survival (OS). **Results:** A total of 50 pts with mTNBC were included in this analysis. At baseline, the median age was 55.9 years (31.8–75.9), 60% had ECOG 0 and 40% had ECOG 1, 72% had visceral metastasis, and 46% had received <sup>3</sup>1 prior lines of systemic therapy in the metastatic setting. While 26% of pts received monotherapy [pembrolizumab (n=7; NCT02447003); atezolizumab (n=6; NCT01375842)], 74% received combination with CT [pembrolizumab plus eribulin (n=31; NCT02513472); atezolizumab plus nab-paclitaxel (n=6; NCT01633970)]. *PTEN* alterations were present in 30% of pts (mutations = 7; one copy number loss = 7; two copy number loss = 1). Median follow-up was 14 months (1–40). Pts with tumors harboring *PTEN* alterations had lower ORR (7% vs 57%; P<0.001), shorter median PFS (2.3 vs 6.3 months; P=0.027), and shorter median OS (8.1 vs 20.1 months; P=0.012) compared to pts without *PTEN* alterations. The median TMB was 6.6 mut/Mb (1.2–50.8), and 23% of pts had a high TMB. While high TMB was not associated with higher ORR (P=0.56), it was associated with better median PFS (16.5 vs 2.4 months; P=0.017), and better median OS (not reached vs 13.5 months; P=0.026). Both *PTEN* status and TMB remained significantly associated with PFS in the multivariable model.

Table 1. Multivariable analysis for PFS

	Hazard ratio	Confidence Interval	p-value
Combination therapy	0.42	0.16 – 1.13	0.009
Visceral metastasis	1.31	0.63 – 2.77	0.46
Previous lines of therapy	1.02	0.09 – 0.70	0.85
ECOG 1	2.1	1.06 – 1.28	0.034
PTEN altered	3.74	1.65 – 8.44	0.002
Hypermutated tumors	0.85	0.75 – 0.97	0.011

Only *PTEN* status remained significantly associated with OS in the multivariable analysis with the same covariables. Ongoing analysis to better understand if these predictors are specific for predicting benefit to immunotherapy and/or a marker of chemotherapy resistance will be presented at the symposium. **Conclusion:** *PTEN* genomic alterations and TMB may impact benefit from PD-1/PD-L1 inhibitors largely administered with chemotherapy in mTNBC. These observations warrant prospective

validation and may inform the importance of stratifying pts according to these characteristics in future randomized studies with ICI.

# 2018 San Antonio Breast Cancer Symposium ®

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Genome copy number entropy as predictor of response for neoadjuvant therapy in early breast cancer

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## Background

Copy Number Alterations (CNAs) represent changes in the copy number of genomic segments of somatic cells due to chromosomal instability. CNAs include gene amplifications or deletions and can be involved in tumorigenesis. We analyzed CNAs data in pre- and post-treatment (ttm) tumors from patients (pts) with early breast cancer (BC) in the neoadjuvant trials GEICAM/2006-03 and GEICAM/2006-14, with the aim to identify CNAs in particular genomic regions (genetic entropy) associated with treatment response.

## Methods

GEICAM/2006-03 (NCT00432172) HER2-negative pts were selectively treated according to clinical subtypes: triple negative (TN) pts were treated with standard anthracycline/taxane -based chemotherapy (AT-CT) +/- carboplatin, while luminal patients were randomized to AT-CT vs. hormone therapy; GEICAM/2006-14 (NCT00841828) HER2+ pts received AT-CT plus anti-HER2 therapy. Shallow-whole genome Illumina sequencing DNA data from 204 paraffin-embedded tumors (100 pre- and 104 post-ttm) were segmented to obtain CNAs and recurrent altered genomic regions were defined. We used Wilcoxon test to analyze the frequency of altered regions and logistic regression analyses to explore their association with tumor response, in terms of pathological complete response (pCR) in breast and axilla. Validation of altered genes associated with therapy response was performed in the microarray gene expression-based Hatzis dataset (GSE25066) from pts receiving neoadjuvant AT-CT (1).

## Results

A total of 672 regions covering the whole genome were identified upon analysis of CNAs data. Regions were categorized according to their alteration status as amplified, normal and lost. Comparative analysis of alterations revealed 11 regions significantly different ( $p < 0.05$ ) in pre- vs post-ttm tumors. Logistic regression analysis showed that in pre-ttm tumors specific alterations of 8 regions localized in 3 different genomic loci (11q12, 16q22 and 21q22) were significantly associated with pCR ( $p < 0.05$ ). Independent analyses of CNAs data with "CGH regions" and "GISTIC2.0" tools confirmed the special relevance of 2 of these 8 regions (#653 and #654), amplified in the locus 21q22.12. This locus contains 20 genes whose expression was tested in Hatzis dataset (1) (GSE25066): the analysis showed that overexpression of 5 of these 20 genes (*CHAF1B*, *CBR1*, *CBR3*, *RCAN1* and *SLC5A3*) turned out to be significantly higher in the cohort of pts who reached pCR, in agreement with our findings. Some of these genes have already been described as proliferation markers (*CHAF1B*) or involved in treatment response (*CBR1*) in BC. Other genes related to BC in this genomic region are the transcription factor *RUNX1* and the Lysine Methyltransferase *SETD4*.

## Conclusions

According to our results, neoadjuvant therapy can modulate genomic aberrations landscape in BC. Our data suggest that amplification of specific genes in the genomic locus (21q22.12) is involved in the neoadjuvant therapy response in early BC.

(1): Hatzis et al., JAMA 2011, 305(18) 1873-81

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-04

A new method of data analysis to derive DNA methylation signatures that stratify risk of recurrence in triple negative breast cancer

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**Background:** Triple negative breast cancer (TNBC) accounts for 10-17% of all breast cancer and is more likely to be of higher histological grade, poorly differentiated, associated with a higher recurrence rate and with decreased overall survival. The clinical course of a TNBC patient remains difficult to predict, as tumors with homogenous morphological characteristics may vary in response to therapy and have divergent outcomes. Therefore, additional analytical methods are needed to better classify TNBC. Our goal is to refine the analysis of methylome datasets to derive reliable molecular signatures that can distinguish TNBC patients with good outcomes who may benefit from less aggressive treatment, from those with poor outcomes who would be candidates for more aggressive treatments.

**Methods:** Our laboratory has conducted and reported, in this meeting, results from analysis of 450k methylation array data on a discovery set of 53 high-risk TNBC cases and 62 low-risk controls treated by locoregional therapy alone, as well as 5 normal breast tissue samples. High-risk cases were defined as patients that relapsed within 0.5 to 6.5 years from the time of diagnosis, while low-risk controls had no relapse and >4 year recurrence-free intervals (RFI). In this work, we devised and applied a novel methylation biomarker discovery program named Hypermethylated Outlier Detector (HOD) that emphasizes the selection of highly methylated markers in cases compared to controls, to find a high-risk signature in the TNBC discovery set. The methylation signature identified by HOD was interrogated in a test set of 50 TNBCs (with 16 recurrences) that did not receive chemotherapy, and in a second test set of 131 TNBCs (with 33 recurrences) that did receive chemotherapy.

**Results:** HOD identified 39 hypermethylated markers (beta >0.20) that could accurately distinguish between the high-risk cases and the low-risk controls in the discovery set of TNBCs (n=115) treated with locoregional therapy alone. In the test set of TNBC (n=50) with no chemotherapy the 39 markers distinguished high from low risk individuals (likelihood ratio test P=0.049). In a second test set of TNBC (n=131) that received chemotherapy the 39 hypermethylated markers again distinguished high from low risk individuals (likelihood ratio test P=0.0043).

**Conclusions:** We have presented evidence that a methylation signature identified by HOD can be used to identify TNBC patients that have a high-risk of relapse regardless of receiving chemotherapy. This methylation signature could potentially be used to inform physician decisions on therapeutic strategies for TNBC patients. This could ultimately lead to less aggressive treatment given to patients possessing a methylation profile consistent with a better prognosis. Conversely, patients with hypermethylation in the 39 markers will likely benefit from a more aggressive course of treatment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-05

Phenotypic discordance between primary and metastatic breast cancer (MBC) in a large scale real-life multicentre French cohort

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**Background:** Therapeutic options at diagnosis for metastatic breast cancers (MBC) differ largely according to cancer phenotype (namely, hormone receptor (HR) and HER2 status). Reported discordance rates between primary tumor and metastasis vary widely in literature, with a median of 18% for estrogen receptor, 31% for progesterone receptor and 10% for HER2. The present study aimed to compare phenotypic profiles between primary and MBC in the real-life setting.

## **Patients (pts) and methods:**

Epidemio-Strategy and Medical Economics (ESME)MBC data platform (NCT03275311) is a French national, multicenter, observational cohort using clinical trials' methodology for data capture, monitoring and quality controls. At the time of analysis, it comprised data of 16703 consecutive newly diagnosed MBC pts (1/01/08-31/12/14) treated in 18 French comprehensive cancer centres. The primary endpoint was the discordance rate of HR and HER2 status between primary tumor and MBC (biopsy of metastatic site done within 6 months of MBC diagnosis). Only patients with both histological reports available were considered. Potential factors associated with phenotype discordance were assessed in a multivariate logistic regression.

**Results:** 2933 out of 16703 (17.6%) had a biopsy in the first 6 months of metastatic disease. HR and/or HER2 status was available in 1677 pts.

## Patient characteristics

	Total population (n=16703)	Pts with primary and MBC phenotype available (n=1677)
Age at metastatic diagnosis Median (range)	61 (19-99)	60 (24-93)
De novo MBC	4507 (27.1%)	221 (13.2%)
Number of metastatic sites Median (range)	1 (1-9)	2 (1-7)
MBC sites		
Brain	1200 (7.2%)	138 (8.2%)
Visceral	7755 (46.4%)	928 (55.3%)
Non-visceral	7748 (46.4%)	611 (36.4%)
Phenotypic profile	N = 2933	N=1677
TNBC	356 (18.5%)	272 (19.3%)
HR+/HER2-	1251 (65.0%)	917 (65.2%)
HR-/HER2+	150 (7.8%)	105 (7.5%)
HR+/HER2+	168 (8.7%)	112 (8%)
Missing	1008	271
Metastatic site sampling	N=2933	N=1677
Bone	692 (24.2%)	419 (25.5%)
Liver	514 (18.0%)	355 (21.6%)



Skin	379 (13.3%)	203 (12.4%)
Node	306 (10.7%)	169 (10.3%)
Lung	258 (9.0%)	168 (10.2%)
Pleura	283 (9.9%)	121 (7.4%)
CNS/CSF*	132 (4.6%)	42 (2.6%)
Other or multiple	296 (10.3%)	165 (10.0%)
Missing	73	35

\* CNS= central nervous system, CSF=cerebro-spinal fluid

The discordance rate between primary and matched MBC was 14.2% (222/1566) for HR: loss of expression in 72.5%, gain in 27.5%. For HER2, the discordance rate was 7.8% (84/1076): 45.2% of losses and 54.8% of gains of expression. The primary HR+/HER2+ subgroup had the highest rate of changes: 53% (49/92) with either a loss of HR (43%), loss of HER2 (43%) or a loss of both (14%). 18% (33/181) of primary triple-negative breast cancer (TNBC) had a phenotypic change with a majority of HR gain (79%). In multivariate analysis, administration of adjuvant chemotherapy +/- targeted therapy was the sole independent predictor of HR status modification (OR: 1.73, 95%CI 1.27-2.36, p=0.001). The presence of a mixed histology was the only predictor of HER2 discordance (OR =2.57, 95%CI 1.19-5.55, p=0.016).

**Conclusion:** Biopsy and phenotype re-evaluation of MBC early in the disease course has a confirmed potential significant therapeutic impact in this large scale real life setting and should be proposed as often as possible.

# 2018 San Antonio Breast Cancer Symposium ®

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Nuclear p-ser394-FOXO3a/FOXO3a ratio is associated with an improved survival and response to therapy in breast cancer

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**Background:** Forkhead box O (FOXO) transcription factors, one of large forkhead family members, control a wide spectrum of carcinogenesis, therapy response and progression in human cancer. Phosphorylation of FOXO3a by ERK1/2 at ser294, ser344, and ser425 induces its nuclear exclusion and sequestration in the cytosol, therefore avoiding FOXO3a transcriptional activity and consequently promoting cell proliferation and tumorigenesis. We aimed to explore the prognostic significance of subcellular p-ser294-FOXO3a distribution patterns in breast cancer.

**Methods:** Immunohistochemistry was used to explore expression patterns of ERK1/2, FOXO3a and p-Ser294-FOXO3a in 216 breast cancer lesions. The obtained information was statistically correlated with clinicopathological parameters including tumor size, lymph node metastasis, tumor stage, histological grade, estrogen receptor  $\alpha$  (ER), progesterone receptor (PR), HER2 and patients' follow-up data. The median follow-up period was 118 months. Kaplan-Meier survival curves were analyzed using with log-rank test and Cox-regression model was used to determine their independent prognosis values.

**Results:** Our results showed that nuclear FOXO3a and p-ser394-FOXO3a expression were progressively increased from normal, early to advanced breast tumor. Nuclear p-ser394-FOXO3a and FOXO3a expression was positively correlated ( $p=0.021$ ). Nevertheless, cytoplasmic ERK expression was inversely correlated with nuclear FOXO3a expression ( $p=0.037$ ), but not its phosphorylation at the ser394 residue. To determine the real impact of p-ser394-FOXO3a, we redefined the ratio of p-ser394-FOXO3a over FOXO3a as p-FOXO3a index. Using  $\chi^2$  analysis, we found nuclear p-FOXO3a index in tumor tissue was positively associated with lymph node metastasis ( $p=0.035$ ) and inversely correlated with recurrence rate ( $p=0.035$ ) in breast cancer patients. The Kaplan-Meier survival curve showed that nuclear p-FOXO3a index, but not cytoplasmic p-FOXO3a index, improved the overall survival rate ( $p=0.037$ ). In addition, those patients who received hormone therapy would have a better survival rate when they had high nuclear p-FOXO3a index ( $p=0.005$ ), suggesting that nuclear p-ser394-FOXO3a expression might be required for the response of hormone therapy. Intriguingly, low nuclear p-FOXO3a index significantly associated with the worse overall survival rate of patients who didn't receive chemotherapy or radiotherapy ( $p=0.049$  and  $p=0.049$ , respectively), implicating that nuclear p-ser394-FOXO3a expression might also modulate the response of chemotherapy and radiotherapy. Multivariate Cox regression analysis demonstrated that the nuclear p-FOXO3a index (HR=0.46, 95% CI=0.22-0.97,  $p=0.040$ ), stage (HR=2.48, 95% CI=1.04-5.96,  $p=0.041$ ), and recurrence (HR=5.69, 95% CI=2.69-12.04,  $p<0.001$ ) were independent prognostic factors for the overall survival rate of breast cancer patients.

**Conclusions:** In conclusion, this study highlights that the phosphorylation of FOXO3a at the ser394 residue may play a crucial role in treatment response of breast cancer. Increased p-FOXO3a index may serve as a prognostic factor for an improved survival rate of breast cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-07

Expression of adipocyte fatty acid binding protein promotes obesity-associated mammary tumor growth

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**Background:** The underlying mechanisms that drive obesity-related breast cancer remain unclear. Adipocyte/macrophage fatty acid binding protein (A-FABP) is linked to obesity and breast cancer, while transforming growth factor (TGF) $\beta$  appears to play a pleiotropic role in breast cancer, suppressing its development but promoting its progression. Whether the proteins work together to drive breast cancer progression is not known. We evaluated the expression of these two markers in matched serum from healthy women and women with breast cancer.

**Hypothesis:** A-FABP and TGF $\beta$  drive breast cancer development and progression.

**Methods:** Serum was collected under an institutional review board approved protocol. A-FABP was measured in serum collected from 275 women (92 with breast cancer and 183 without) and TGF $\beta$  from 245 matched women (92 with breast cancer and 153 without). A-FABP levels were measured using a human A-FABP4 ELISA kit while TGF $\beta$  was measured using human TGF $\beta$  ELISA kit. The Kruskal-Wallis test was used to determine if a difference in marker expression existed between women with and without breast cancer, as well as in women with early vs. more advanced breast cancer. Linear mixed effect models were used to examine the relationship between A-FABP and BMI, as well as between TGF $\beta$  and BMI, controlling for age, menopause status and a diagnosis of breast cancer.

**Results:** A-FABP expression was higher in obese than lean postmenopausal women, both those with breast cancer (mean : 44.9 vs. 25.1 ng/mL,  $p=0.002$ ) and without breast cancer (39.4 vs. 26.9 ng/mL,  $p=0.003$ ). A-FABP expression was also higher in premenopausal obese vs. lean women with cancer (28.9 vs. 12.7 ng/mL,  $p=0.027$ ), but not in premenopausal healthy women. A-FABP expression was higher in postmenopausal obese vs. lean women with early stage (0-2A) breast cancer (45.6 vs. 21.9 ng/mL,  $p=0.013$ ) and was inversely associated with HER-2 expression, though being of borderline significance ( $p=0.060$ ). This was most notable among triple negative vs. ER/PR negative HER2 positive breast cancers. Considering both early and advanced breast cancer, TGF $\beta$  expression trended higher in post- than in pre-menopausal obese women with breast cancer (138.9 vs. 68.7 pg/mL,  $p=0.061$ ), however among premenopausal women with advanced (Stages 2B-3C) disease, TGF $\beta$  expression was 5 fold higher in lean than obese individuals (251.7 vs. 48.2 pg/mL,  $p=0.029$ ) but trended higher in obese vs lean postmenopausal women. TGF $\beta$  and A-FABP were found to be significantly associated ( $p=0.14$ ,  $p=0.024$ ).

**Impact:** Both A-FABP and TGF $\beta$  expression are associated with postmenopausal breast cancer among obese women, and their expression in matched samples is significantly associated.. TGF $\beta$  is associated with premenopausal advanced breast cancer in premenopausal women. TGF $\beta$  is known to induce epithelial mesenchymal transition and may play a role in pregnancy associated breast cancer. Further studies are needed to determine if A-FABP and TGF $\beta$  work together in postmenopausal breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-12-08

CD73 predicts response to neoadjuvant treatment in TNBC patients

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**BACKGROUND** Immune system plays a key role in tumor surveillance and escape. Tumor infiltrating lymphocytes (TILs) and PD-L1 seem to predict clinical outcome and pathological response (pR) after neoadjuvant chemotherapy (NAC) in breast cancer. Recently CD73 has been proposed as prognostic biomarker associated with better disease free survival and overall survival patients affected by triple negative breast cancer (TNBC). CD73 catalyzes the conversion of AMP in adenosine which leads to an immunosuppressive microenvironment. Indeed adenosine inhibits CD8+ lymphocytes and dendritic cells while enhances M2, myeloid suppressor cells and Treg activity.

## MATERIALS AND METHODS

We enrolled 61 pts who had received NAC (EC for 4 cycles followed by Paclitaxel q7 for 12 cycles or q21 for 4 cycles) between January 2013 and June 2017 at Policlinico Umberto I and San Giovanni Addolorata Hospital of Rome. We performed immunohistochemistry for CD20, CD3, CD4, CD8, CD68, N-CAM, PD-L1 (Ventana SP142 clone) and CD-73 in basal paraffin-embedded biopsies. CD73 and PD-L1 expression on tumor cells was evaluated both qualitatively and quantitatively. The percentage of tumor-infiltrating immune cells positive for PD-L1 and CD73 was also recorded. Statistical analysis was performed with Mann Whitney test, univariate, and multivariate logistic regression models.

**RESULTS** We enrolled 61 females (median age: 49 y; range 28-74) affected by TNBC: 59 ductal Ca G3 NST (96.7%), 1 mucinous carcinoma (1.6%), 1 medullary carcinoma (1.6%). The clinical stage before NAC was as follow: 13.1% cT1 (8 pts), 75.4% cT2 (46 pts), 4.9% cT3 (3 pts), 6.6% cT4 (4 pt). 32 pts were cN+ (52.5%). After NAC 29 patients underwent mastectomy (47.5%) and 32 conservative surgery (52.5%). Twenty-three patients (38%) showed pathological complete response (pCR). The median value of expression of CD73 on tumor cells was 40%. In 29 (48%) basal biopsies CD73 expression was under median value. Five out of 61 patients presented PD-L1 expression higher than 25%. Fourteen out of 29 (48%) patients with low level of CD73 expression achieved pathological complete response (pCR), on the contrary only 9 out of 32 (28%) of patients with high levels of CD73 showed pCR ( $p=0.011$ ). Four patients (80%) with high PD-L1 expression achieved pCR ( $p=0.035$ ). At multivariate analysis a significant association was found between pCR and CD73 expression ( $p=0.027$ ) while no association was found with TILs and PD-L1.

**CONCLUSION:** CD73 expression seems to be associated with pCR in TNBC patients. Moreover these preliminary results suggest the possibility of using CD73 inhibitor plus anti-PD-1 in those patients with high expression of CD73.

# 2018 San Antonio Breast Cancer Symposium ®

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The  $\geq 5\%$  cut-off reveals tumor PD-L1 positivity as potential selection biomarker for patient enrollment into the trials evaluating anti-PD-1 or anti-PD-L1 agents in neoadjuvant treatment of triple negative breast cancer

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**Background:** Durable responses of triple negative breast cancer (TNBC) to pembrolizumab (anti-PD-1) or atezolizumab (anti-PD-L1) have been reported in the metastatic setting. Moreover, it is currently being hypothesized that immune checkpoint inhibitors might be more effective in the neoadjuvant setting, due to better preserved anti-tumor immunity in early TN disease. However, biomarkers predictive of response to anti-PD-1 or anti-PD-L1 agents, as well as biomarker-based strategies for testing those drugs in the neoadjuvant setting are still lacking. We evaluated PD-L1 protein expression and the composition of tumor-infiltrating lymphocytes (TILs) in untreated TNBC, in order to get a better insight into the TNBC sub-population(s) which would be suitable for neoadjuvant anti-PD-1 or anti-PD-L1 therapy evaluation. **Methods:** TNBC patients consecutively treated at the Jean Perrin Comprehensive Cancer Centre (Clermont-Ferrand, France), from 01/01/2010 to 12/31/2014, were included. FFPE tumor tissues were assessed for PD-L1 expression by immunohistochemical (IHC) laboratory-developed test (clone 28-8, Abcam), in tumor cells (tPD-L1) and in TILs. Positivity cut-offs evaluated were  $\geq 1\%$ ,  $\geq 5\%$  and  $\geq 10\%$ . The amount CD8+, CD4+, FoxP3+ or PD-1+ TILs was determined by counting those cells, detected by IHC methods, within 5 consecutive HPFs (x400), from tumor invasive front toward tumor center. Clinical disease stage was determined using the TNM system. **Results:** One hundred and two TNBCs were assessed. There were 28.4%, 23.5% or 16.7% tPD-L1-positive cases (cs), for cut-offs  $\geq 1\%$ ,  $\geq 5\%$  or  $\geq 10\%$ , respectively. Similarly, 32.4%, 15.7% or 5.9% of cs were positive for PD-L1 in TILs, using the same cut-offs. With  $\geq 5\%$  as cut-off, positivity for tPD-L1 significantly correlated with the amount of CD8+ ( $p=0.023$ ), FoxP3+ ( $p=0.0036$ ) or PD-1+ TILs ( $p=0.043$ ). The same cut-off, applied to TILs, revealed significant correlations between PD-L1 positivity and the amount of each CD8+, CD4+ or PD-1+ TILs ( $p=0.025$ , 0.039 and 0.0042, respectively). Interestingly, when the  $\geq 5\%$  cut-off was used, tumors of T2 size were most frequently tPD-L1+ (11/41 cs, 26.8%), compared with the T1 (3/35 cs, 8.6%) and T3+T4 (3/18 cases, 16.7%) ( $p=0.04$ ). With regards to TILs, with the  $\geq 5\%$  cut-off, the PD-L1+ cases belonged exclusively to the T1+T2 group (15/76), whereas the T3+T4 group was PD-L1-negative (0/18 cs). Other two cut-offs revealed only occasional correlations. **Conclusion:** This single-center, real-world TNBC cohort contained a high number of smaller tumors (T1-T2). The IHC-based PD-L1 assessment, with  $\geq 5\%$  as the positivity cut-off, revealed that approximately 1/4 of TNBC could be candidates for neoadjuvant anti-PD-1/anti-PD-L1 approaches. Combined with the amount of CD8+ and PD-1+ TILs, tumor PD-L1 positivity might make an easy-to-use composite biomarker for the 1st-level patient selection for PD-1 or PD-L1 inhibitors in neoadjuvant TNBC treatment. The 2<sup>nd</sup> level could exploit, for example, the assessment of mutation burden in tumors with low tPD-L1 or amount of CD8+ or PD-1+ TILs. Such tumors might be more frequent among larger TNBC (T3-T4).

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-10

Circulating progranulin (GP88) level is associated with both response to therapy and progression of disease in metastatic breast cancer patients

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Imaging is the method of choice for monitoring of disease response to therapy during and post-treatment in metastatic breast cancer (MBC) patients. However, imaging has limited sensitivity for real-time monitoring so clinicians use circulating tumor markers such as CA15-3, as surrogates for monitoring changes in disease, albeit with some limitations. Measuring tumor markers associated with the biologically driven tumor processes of development, proliferation and survival would add significantly to current disease information available and would improve the clinical management of this underserved patient population.

Biological studies demonstrated that the 88kDa glycoprotein Progranulin (GP88) is a critical driver of breast cancer (BC) cell proliferation, survival, invasiveness and drug resistance. GP88 is expressed in tumor tissue and not in normal breast tissue and is secreted in the circulation of BC patients. Studies demonstrated that high tumor GP88 expression is prognostic for recurrence and that BC patients had a statistically elevated GP88 serum level compared to healthy controls. We hypothesized that changes in serum GP88 levels correlate with changes in disease state as defined by RECIST 1.1. In this current study, we examined whether GP88 and CA15-3 serum levels were correlated to disease progression and response to therapy. Additionally, we compared the information provided by the GP88 /CA15-3 combination in disease progression or response to therapy.

101 stage 4 MBC patients undergoing Standard of Care therapy were consented and enrolled under an IRB approved protocol at the University of Maryland Greenebaum Comprehensive Cancer Center. Patient demographics together with clinical and disease characteristics were collected as part of the study. Blood samples were drawn from each patient at specific times at follow-up visits during and post-therapy and tested for GP88 using A&G's GP88 enzyme linked immunoassay. Samples were tested for CA15-3 at the University's laboratory. Disease status was monitored and assessed by RECIST1.1 criteria and such assessment entered into the database.

Statistical analyses were carried out using the serum GP88 and CA15-3 results to test each for association with contemporaneous RECIST assessment of disease progression (PD) or response (R). We determined that contemporaneous GP88 is significantly associated with PD (p 0.0101) and R (p 0.0194) while CA-15-3 is associated with PD (p <0.001) but not R (p 0.7316). Further, we used logistic regression to examine for additional information provided by each biomarker by modelling disease (PD or R) as dependent on the log-transformed GP88 and CA15-3 values. We determined that both GP88 and CA15-3 show significance for PD meaning biomarkers adds information while for R only GP88 had high significance meaning GP88 alone is significant and sufficient for R and CA15-3 does not add any value.

We conclude that circulating levels of GP88/PGRN in MBC patients are correlated with both disease progression and response to therapy and that monitoring circulating GP88/PGRN levels would be complementary to CA15-3 determination and imaging to provide valuable insight into real-time monitoring of the disease status of MBC patients.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-12-11

M2 macrophages increase after neoadjuvant HER2 targeted chemotherapy

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**Purpose:** Recent observations suggest a positive association between the presence of tumor-associated myeloid and lymphoid immune cells and clinical responses to HER2 therapies. The specific immune cell composition of HER2+ tumors and the effects of therapy on the tumor immune microenvironment remains poorly understood; limiting efforts to effectively direct immunomodulatory agents in HER2+ breast cancer. We sought to assess the feasibility of a multiplex immunofluorescence strategy to characterize the immune milieu of HER2+ tumors, pre and post treatment. **Methods:** We conducted a feasibility study using the Perkin Elmer OPAL multiplex dye chemistry for immunofluorescence of tumor, myeloid, and lymphoid cells in pretreatment biopsy and surgical resection tissues of 11 patients who received neoadjuvant HER2 antibody with chemotherapy. Two panels of up to 6 antibodies were studied including a predominantly myeloid panel targeting CD80, CD68, CD163, CD206, PD-L1, and cytokeratin; and a 'lymphoid/proliferation' panel targeting FoxP3, cytokeratin, Granzyme B, CD4, CD8, and Ki67. For paired samples, analysis of pre/post differences were analyzed using two tailed, t-test. **Results:** 100% of the pre-treatment biopsy samples yielded high quality immune and tumor cell immunofluorescence profiles for both panels. In contrast, post treatment specimens were more challenging. For the post treatment tissues, ~25% of specimens failed to yield results in at least one panel. As shown in Table 1, the %CD206+ M2 type macrophage population increased between pre and post treatment (p=0.012). This was reflected in a decrease in the M1:M2 ratio and variability in the ratio in favor of M2 (median 0.34 [IQR = 1.01] to 0.11 [IQR=0.10], p=0.0003). In the lymphoid panel, we observed a non-significant reduction in % FoxP3+CD4 T cells with less variability between patients post treatment and a significant decrease in total CD8+ T cells. Further, there was a significant reduction in %Granzyme B positive T cells (median 6.63 to <1%, p=0.02) and non-significant decrease in proliferating (Ki67+) T cells post treatment. PD-L1 expression was low in both pre and post specimens.

Marker	Sample	Min	Median	Max	IQR
%CD206+ Mono/Mac	Pre	1.35	8.71	35.89	11.05
%CD206+ Mono/Mac	Post	25.4	38.97	77.6	21.54
M1/M2 Ratio	Pre	0.11	0.34	2.14	1.01
M1/M2 Ratio	Post	0.03	0.11	0.21	0.1
%Ki67+ T cells	Pre	0.13	7.92	20.04	11.06
%Ki67+ T cells	Post	0	4.56	21.83	6.31
%PD-L1+ Myeloid	Pre	0	1.05	12.06	0.95
%PD-L1+ Myeloid	Post	0	0.55	4.26	1.47
%Granzyme B T cells	Pre	2.92	6.63	33.72	7.2
%Granzyme B T cells	Post	0	0.89	2.48	1.73
%FoxP3+ CD4 +	Pre	0	1.64	56.04	16.44
%FoxP3+ CD4 +	Post	0	1.16	6.82	4.14
#Total CD8+ T cells	Pre	578	8,366	35,426	15,744
#Total CD8+ T cells	Post	12	1,371	7,904	3,658

**Conclusions:** Multiplex immune profiling is a practical approach to characterize the tumor immune microenvironment in biopsy and post treatment specimens. Neoadjuvant HER2 targeted chemotherapy significantly shifts the myeloid population to an M2 (immunosuppressive) phenotype with evidence for a reduction in the number of Ki67 and Granzyme B+ T cells. These preliminary

results suggest immunomodulatory agents that are able to induce or maintain an M1 polarized tumor microenvironment may have utility to enhance long term anti-tumor immunity in HER2 disease.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-12

ROCplot.org: validating predictive biomarkers of response to chemotherapy/hormonal therapy/anti-HER2 therapy using transcriptomic data of 3,150 breast cancer patients

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Prognostic biomarkers are capable to predict survival and predictive biomarkers are capable to predict therapy response. Systemic therapy of breast cancer can include chemotherapy, hormonal therapy, and targeted therapy. Here, we report the initial release of the first online available tool capable to identify gene expression based predictive biomarkers using transcriptomic data of a large set of breast cancer patients.

Published gene expression data of 44 publicly available datasets was integrated with treatment data into a unified database. The classification is based on either author-reported pathological complete response (n=1,775) or relapse-free survival status at five years (n=1,329). Treatment data includes chemotherapy (n=2,108), endocrine therapy (n=971), and anti-HER2 therapy (n=267). The transcriptomic database includes 20,089 unique genes and 54,675 probe sets. Gene expression and therapy response are compared using receiver operating characteristics and Mann-Whitney tests.

We demonstrate the utility of the tool by validating a set of established biomarkers including TP53 for chemotherapy in luminal breast cancer (p=5.2e-20, AUC=0.77), ERBB2 for trastuzumab therapy (p=8.4e-04, AUC=0.629), and PGR for hormonal therapy (p=8.6e-05, AUC=0.7).

The tool is designed to validate and rank new predictive biomarker candidates in real time. By analyzing the selected genes in a large set of independent patients, one can select the most robust candidate and quickly eliminate those which are most likely to fail in a clinical setting. The analysis tool is accessible at [www.rocplot.org/breast](http://www.rocplot.org/breast).

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-13

Changing level of serum heat shock protein 90 alpha as a diagnostic and predictive marker in breast cancer: Diagnosis of early breast cancer and prediction of response to neo-adjuvant and surgical therapy

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To analyze the correlation of the level of serum heat shock protein 90 $\alpha$  (HSP-90 $\alpha$ ) in healthy and breast cancer patients, studying the evaluation of neoadjuvant efficacy and Clinical value of relapse monitoring.

A total of 102 healthy women were selected, 51 cases of breast benign tumor, 423 cases of female breast cancer all diagnosed by pathology, other system malignancies 62 cases. ALL the serum samples HSP-90 $\alpha$  was detected by double-antibody ELISA. The CEA, CA125, CA15-3 were detected by Roche Cobas ECL analyzer. The ROC curve was used to analyze the effectiveness of serum HSP-90 $\alpha$  in the early diagnosis of breast cancer. The dynamic changes of HSP-90 $\alpha$  level before and after treatment were analyzed by Wilcoxon's rank test. Moreover, we also combine the HSP-90 $\alpha$  with CEA, CA125, CA15-3 to evaluate the clinical value of monitoring the recurrence of breast cancer.

The levels of serum HSP-90 $\alpha$  ( $123.49 \pm 105.1$  ng / ml) in breast cancer patients was significantly higher than that in healthy controls ( $40.33 \pm 14.18$  ng / ml), benign breast disease ( $80.15 \pm 103.09$  ng / ml) and carcinoma in situ ( $34.7 \pm (114.31 \pm 146.91$  ng / ml), the difference was statistically significant ( $P < 0.001$ ). According to the ROC curve analysis of patients and healthy subjects, the cut-off value was set as HSP-90=59.7 ng / ml, AUC=0.834, the sensitivity and specificity to diagnosis of breast cancer was 90.3% and 78.6% respectively, the negative predictive value was 93.88%. The levels of serum HSP-90 $\alpha$  was significantly decreased ( $P < 0.05$ ) after tumor resection and the patients who obtain PR from the neoadjuvant chemotherapy. When HSP-90=43.22 ng / ml was set as the cut-off value for diagnosing the recurrence of breast cancer, AUC=0.877, the sensitivity and specificity were 95.7% and 74.5% respectively, and the negative predictive value was 96.2%. At the same time, we find that combined HSP-90 $\alpha$  with CEA, CA125, CA15-3 can improve the accuracy of recurrence prediction.

Serum HSP-90 $\alpha$  has a good clinical diagnostic value in breast cancer and can be used as a recurrence monitoring tool for post-adjuvant treatment patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-14

A pilot study of metabolomics biomarkers in breast cancer tumors treated with neoadjuvant therapy

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## Introduction

Breast cancer is one of the most prevalent cancers in the world. Traditionally, early breast cancer treatment is based on surgery and, after surgery, hormone treatment or chemotherapy. However, the neoadjuvant treatment is increasingly used. Metabolomics is the most recent “omics” which allows quantify metabolites into blood patient samples. Coupled with computational analyses it could be possible to study differential metabolomics patterns and associate them with neoadjuvant response.

## Material and methods

Blood plasma samples from patients with breast cancer treated with neoadjuvant chemotherapy were used to perform metabolomics experiments. One sample before the treatment (basal) and one sample after the chemotherapy (post-treatment) were analyzed and clinical data regarding response (complete response or partial response) was also collected. Metabolomics experiments were performed using liquid chromatography coupled with mass-spectrometry. Bayesian network and class comparison analyses were used to establish differential metabolic patterns between conditions. Additionally, a response prediction model based on logistic regression was build using metabolomics data from basal samples.

## Results and discussion

A network showing the relationships between metabolites was build. Comparing metabolite measurements between complete response and partial response tumors in basal samples, 19 metabolites showed a differential quantification between both types of responses. Moreover, one of these metabolites is linoleic acid, previously described as a biomarker of complete response in neoadjuvant treatment in breast cancer. Using these 19 differential metabolites, a response predictive model was build. According to this model, it is possible to predict response to neoadjuvant treatment based on the amount of one metabolite, still only identified by its mass and charge. On the other hand, comparing basal and post-treatment samples, the network showed differential metabolomics patterns. These differential metabolites could be used as predictive biomarkers of response.

## Conclusion

This study is a proof of concept that using a new “omics” technique such as metabolomics in blood samples, coupled with computational analyses, it is possible to identify differential metabolomics patterns between complete and partial response or basal and post-treatment samples and design predictive models of response. These results could facilitate in the future the implementation of blood-based tests into the clinical routine.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-15

Hepatic-metastasis pattern as a prognostic marker in metastatic breast cancer

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**Background:** Liver is the third most frequent site for breast cancer metastasis. Despite of relatively high frequency of liver metastasis and its poor prognosis (median survival between 14-16 months), little is known about clinical outcome and histologic features or molecular mechanisms. The aim of this study was to identify the prognostic factor of breast cancer with liver metastasis, including the hepatic metastasis pattern by imaging studies and pathologic feature.

**Methods:** We reviewed medical record of breast cancer patients with hepatic metastasis diagnosed between January 2003 and June 2017. We classified spreading pattern of liver metastases detected by computed-tomography scan as following three types: mass-forming (longitudinal diameter of new hepatic lesion > 10mm, well-defined border for minimal 3 consecutive studies by CT scan), nodular (longitudinal diameter of any newly-noted hepatic lesion < 10mm or 10mm, well-defined border for minimal 3 consecutive studies by CT scan) and infiltration pattern (to spread with ill-defined border for minimal 3 consecutive studies by CT scan). The overall survival (OS) was defined as the period from the date of initial diagnosis of liver metastasis to the date of patient's death or last follow-up.

**Results :** 115 of patients with initially metastatic breast cancer, and 42 patients with recurrent breast cancer after curative surgery were enrolled. 13% of total patients presented with only liver metastasis when they are initially diagnosed as the metastatic breast cancer. 19% of total patients presented with nodular or infiltration pattern. Median OS of the patients with mass-forming liver metastasis was 18.1 (95% CI: 6.8-29.5) months and in nodular distribution and diffuse infiltration pattern was 13.4 (95% CI: 7.8-18.9) months (p=0.001). To elucidate the role of hepatic-metastasis pattern as a prognostic marker, we performed the cox-regression analysis. The patients with nodular/infiltrative liver metastasis showed poorer prognosis (hazard ratio: 2.18, 95% CI: 1.23-3.86, p-value: 0.007) than patients with mass-forming liver metastases significantly. Other prognostic marker is the subtype of breast cancer. Either HER2-positive (hazard ratio: 2.88, 95% CI: 1.59-5.23, p-value: 0.001) or triple negative breast cancer patients (hazard ratio: 6.17, 95% CI : 2.57-14.8, p-value<0.001) showed poorer prognosis than hormone-receptor positive subtype.

**Conclusion:** In the present study, the patients with mass-forming pattern of liver metastases hormone-receptor presentation showed favorable outcome in overall survival after liver metastases. Further studies are required for the different hepatic metastatic patterns and their mechanisms in metastatic breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-12-16

Combinatorial risk scores: Personalized multi-omic prediction of disease risk

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Complex traits with heritable and lifestyle components present a challenge for traditional polygenic risk scores. These aggregate disease risk associations from multiple (tens to millions) low effect size SNVs to provide a quantitized group level risk. Typically this results in a binomial distribution of risk with the top and bottom percentiles having relatively clear clinical guidelines and a large number of intermediate patients for whom guidance is unclear.

From previous work on the genetic architecture of breast cancer (<http://bit.ly/2JHLk8q>) it is clear that particular combinations of multiple SNPs (epistatic interaction signatures) are significantly associated with specific levels of disease risk and disease protective effects. We have now extended identification of these signatures to include both genomic and phenotypic factors. We found:

- **3,045 novel risk signatures (combinations of 5-13 SNP variants) that occurred in large numbers of patients and zero controls**
- **5 novel protective disease signatures (combinations of 2-10 SNPs associated with reduced disease risk)**
- **10 genetically non-overlapping patient cohorts, each with a different disease risk**
- **Disease risk signatures that combine genetic and phenotypic factors, such as ethnicity, obesity, drinking and smoking**

We have worked with UK Biobank, CIMBA and BCAC datasets to evaluate the risks/protective effects associated with each of these signatures. From these we are building combinatorial risk scores that better predict total lifetime disease risk, age of onset and therapy response. In the context of an individual patient, their specific SNVs and phenotype data can now be used to construct a personalized combinatorial risk score, which has the potential to enable both better diagnosis and theranostics. New results from these efforts will be presented at SABCS.

GWAS aim to find (single) genetic variant loci associated with specific phenotypes. While GWAS has been useful at identifying disease associated factors, it is known to provide only a limited model for explaining complex diseases as very few loci have significant effect sizes and most diseases are highly polygenic (Boyle, 2017). In addition, GWAS cannot directly include the impact of non-genomic factors such as phenotype, lifestyle and comorbidities that may modulate disease processes and exert significant influence over disease risks. Current detection methods for disease associated combinations of SNPs (epistatic interactions) are able only to find combinations of two or at most three SNPs from a preselected list. Here, we present an alternative to the single-locus limitations of GWAS

Penetrance for Clusters (sets of non-redundant states) validated using different FDRs for BRCA2 dataset

(FDR) False Discovery Rate %	# of Clusters	# Layers	# of SNP Genotypes	# of Cases	Penetrance %
20	404	5-13	2,113	799	50.7
10	235	5-13	1,320	627	39.8
5	157	5, 7-13	868	513	32.6
1	32	7, 10-13	142	221	14.0

Analysis using a False Discovery Rate (FDR) of 5% identified 3,045 states (unique n-SNP combinations) at layers (order) 5 and 7–13 that were found to differentiate breast cancer susceptibility. The penetrance in the cohort depends on the FDR used as shown in Table.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-12-17

Prognostic and predictive value of serum level of vascular endothelial growth factor-A in metastatic breast cancer patients treated with bevacizumab plus paclitaxel

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## Background

Several studies showed that first-line bevacizumab plus chemotherapy for HER2-negative metastatic breast cancer improves progression-free survival and tumor response rate but not overall survival. MERiDiAN trial evaluated plasma vascular endothelial growth factor-A (VEGF-A) prospectively as a predictive biomarker for bevacizumab efficacy in metastatic breast cancer. However, results of this trial do not support using baseline plasma VEGF-A to identify patients benefitting most from bevacizumab. We measured baseline serum VEGF-A level from stored blood samples of metastatic breast cancer patient with treated bevacizumab plus paclitaxel as first-line and later line therapy, and evaluated a correlation between serum VEGF-A level and efficacy of bevacizumab and prognosis of breast cancer patients treated with bevacizumab, retrospectively.

## Patients and methods

We examined blood samples from 57 metastatic breast cancer patients treated with bevacizumab and paclitaxel, after obtaining written informed consent. And, we evaluated a correlation between baseline serum VEGF-A level and time to treatment failure (TTF) and overall survival (OS). We also compared the serum VEGF-A level of response group (CR and PR) and that of non-response group (SD and PD).

## Results

Baseline serum level of VEGF-A ranged from 80 to 2079 pg/ml. Cases of treatment line were as follows: first-line, 22 cases (38.6%); second line, 11 cases (19.3%) and third-line and the later line, 24 cases (42.1%). The cutoff identified by ROC curve analysis that was able to differentiate response group and non-response group in first-line setting was 360pg/ml for serum VEGF-A. And, we separated high serum VEGF-A group and low serum VEGF-A group of patients treated with bevacizumab plus paclitaxel.

In patients treated as first line therapy, median TTF was 4.0 months with high serum VEGF-A group versus 5.0 months with low serum VEGF-A group, and median OS was 12 months with high serum VEGF-A group versus 11 months with low serum VEGF-A group. There were no significant differences in both TTF and OS in first line setting. In patients treated as second line and later line therapy, median TTF was 2.8 months with high serum VEGF-A group versus 7.1 months with low serum VEGF-A group, and median OS was 6.4 months with high serum VEGF-A group versus 12.7 months with low serum VEGF-A group. The prognosis of high serum VEGF-A group was significantly worse than that of low serum group in both TTF and OS.

The serum VEGF-A level of response group was tend to be higher than that of non-response group in first line setting, and was lower in second and later line setting. However, there were no significant differences.

## Conclusion

In this study, serum VEGF-A cannot be a predictor for efficacy of bevacizumab plus paclitaxel as first line therapy for metastatic breast cancer patients. On the other hand, there was a possibility that high serum level of VEGF-A can be a poor prognostic factor in late line therapy setting of bevacizumab.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-01

Two-year breast cancer survival in Sub-Saharan Africa: The multi-country ABC-DO cohort

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**Background:** Breast cancer incidence in sub Saharan Africa is rising steeply and has recently replaced cervical cancer as the most common woman cancer in this region. Despite relatively low breast cancer incidence rates compared to those in the west, the 2012 GLOBOCAN estimates suggest mortality rates higher than those in the west, of 17.4 per 100,000 in all Africa and even up to 20.1 in Western Africa. However, breast cancer survival studies in the region are few, outdated, and often hampered by excessive losses to follow-up. Determinants of survival and progress over time are unmeasured.

**Method:** Using an innovative mHealth approach, the African Breast Cancer – Disparities in Outcomes (ABC-DO) cohort recruited over 2100 women newly diagnosed with breast cancer during 2014-17 in 5 Sub Saharan Africa countries. Women's complete breast cancer journeys are captured via three-monthly Smartphone based mHealth live data collection. Here we present the two year overall survival analysis for Namibia, Uganda and Nigeria.

**Results:** Breast cancer survival rates differ greatly between countries, but were generally low. After two years 129 of 501 women in Namibia, 160 of 429 women in Uganda and 177 of 395 women in Nigeria were deceased. Two-year year survival rates were 74%, 60% and 49% in Namibia, Uganda and Nigeria respectively. Stage at diagnosis had a strong influence on survival. The country adjusted preliminary hazard ratios compared to stage I and II were 3.1 (95% CI: 2.4; 4.0) for stage III and 8.4 (95% CI: 6.4; 11.2) for stage IV. Further, women from a higher compared to low socioeconomic position had lower mortality (preliminary HR: 0.55 (95% CI: 0.43; 0.70)).

**Conclusion:** Taking advantage of mobile technology, the ABC-DO study for the first time provides evidence based insight into the situation of breast cancer patients in Sub Saharan Africa. The real-time survival rates presented here provide alarming evidence that downstaging and public health care interventions are needed in order to prevent breast cancer deaths in this region.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-02

Understanding women's perspectives on breast cancer: Knowledge, intentions, and care-seeking in Tanzania

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**Background:** The rate of increase and absolute burden of breast cancer is higher in less developed countries, with over 1 million projected new cases per year in low- to middle-income countries (LMICs) alone by 2020. As these countries develop clinical programs to combat the increasing incidence, understanding women's perspectives on breast cancer and care-seeking are key to successful implementation.

**Methods:** We conducted a community-based survey using convenience sampling of women aged 30 and older in Mwanza, Tanzania to assess knowledge and understanding of breast cancer, including causes and symptoms, and intentions regarding care-seeking for a breast health issue.

**Results:** Among 1,101 women, with a median age of 38 (IQR: 31-46), 75% of women have heard of cancer. Women self-evaluated their knowledge of cancer from 1 (no knowledge) to 10 (extremely knowledgeable) with a median response of 2 (IQR: 1-4) for women age 30-39 and 3 (IQR: 1-4) for women 40 and older ( $p=0.01$ ). Only 14% felt they knew any signs or symptoms of breast cancer, with no difference by age, but 56% were fairly or very confident they'd notice a change in their own breasts. Overall, 47% said they'd be somewhat likely and 26% said they'd be very likely to seek care if they noticed a change. In fact, 18% reported having a clinical breast exam, 3% a breast ultrasound, and 2% a mammogram. Among 27 women who reported breast-related care, 78% said the reason they sought care was because they'd had a breast exam ( $n=21$ ), 52% because they had education about breast exams ( $n=14$ ), and 48% because they had breast symptoms ( $n=13$ ). Half of the 27 women who sought care ultimately got a breast diagnosis: 5 were cancer whereas 9 were not cancer. All women diagnosed with cancer said they received treatment.

**Conclusions:** The success of efforts to improve early diagnosis, the cornerstone of achieving clinical down-staging and ultimately, reduced mortality depends on women being aware of breast cancer, its causes, signs and symptoms, and being encouraged to seek care for breast concerns. Fortunately, the majority of women said they would seek care if they noticed a change in their breasts but the low levels of cancer knowledge and symptoms of breast cancer highlight the need for community education and awareness campaigns.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-03

The effect of the affordable care act on the initial stage of presentation of breast cancer in a safety-net hospital

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**Background:** There are disparities in breast cancer (BC) diagnosis. Black and Hispanic women have a lower rate of screening mammography while the uninsured are less likely to seek medical care even if a palpable mass is found in the breast. Jackson Memorial Hospital is the Safety-Net Hospital that serves the 2.5 million residents of Miami-Dade County which is the most populous county in the State of Florida. The Affordable Care Act (ACA) was signed into law on March 3, 2010 and the goal was to increase the number of insured individuals. In January 2011, it was mandated that health insurance providers cover the cost of screening mammograms. The decision to expand Medicaid was left to the individual states. Florida did not expand Medicaid coverage. Prior to the implementation of the ACA it was estimated that 25-35% of the residents of Miami-Dade County were uninsured or underinsured. By January 2017, more than 1.3 million Floridians had signed up for health insurance through the ACA. Currently almost one in five Americans receiving health coverage via Healthcare.gov or the Spanish language version, CiudadodeSalud.gov, resides in Florida. South Florida is home to the eight congressional districts with the highest concentrations of ACA enrollees in the US. The top 38 zip codes for ACA sign-ups in Florida are in South Florida.

We postulated that evaluating the number of patients diagnosed, the stage at which they are diagnosed and the type of insurance used for the period of time 6 years before (2007-2012) and 4 years after the implementation of the ACA (2014-2017) should give a picture of the effect of the ACA on breast cancer diagnosis in an underserved population.

**Methods:** A retrospective review of patients diagnosed with invasive and in situ breast cancer at the Breast Diagnostic Center at Jackson Memorial Hospital between 1/1/2007 – 12/31/2017 was approved by the University of Miami IRB. We collected data including: age, stage at diagnosis and type of insurance. In keeping with other similar studies, the year 2013 was excluded since it was a transition period. The trends were compared using ANOVA and chi-squared tests.

**Results:** A total of 2155 patients were analyzed. The absolute number of patients with stage 0 seen every year remained stable (30 per year) throughout the time period. The percentage of patients with Stage 0 increased from 12.5% to 16.02% ( $p=0.049$ ). Both the absolute number and percentage of patients with stage IV decreased, from 15 to 8 patients per year (6.42% to 4.12% ( $p=0.016$ )). The total number of patients diagnosed yearly with breast cancer decreased from 233 to 186 ( $p=0.005$ ). The absolute number and percentage of insured patients increased from 75 to 126 patients per year (23.5% to 38.3% ( $p=0.02$ )).

**Conclusions:** Fewer patients were diagnosed with breast cancer at the safety net hospital in Miami-Dade County after the initiation of the ACA. More patients had insurance and there was a stage shift towards lower stage at presentation. Availability of insurance to residents of Miami-Dade County decreased the burden on the safety net hospital and resulted in the diagnosis of lower stage breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-04

Needle-to-knife wait time and impact on pCR in patients undergoing neoadjuvant chemotherapy for breast cancer

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**Introduction:** In the setting of pre-operative neoadjuvant chemotherapy (NAC) for the treatment of breast cancer, it is important to ensure coordination between medical and surgical oncology. A study by Sanford et al. show that patients receiving surgery >8 weeks after NAC have worse overall survival. As NAC continues to expand its role in breast cancer treatment, a closer look at the maximal acceptable time between NAC and surgery becomes more important. We wished to assess the impact of time between last dose of chemotherapy and surgery on pathological complete response (pCR), disease free survival (DFS), overall survival (OS), and surgical complications.

**Methods:** A cohort study was conducted utilizing the BC Cancer Agency's prospective neoadjuvant database, located in Vancouver, BC. Patients were selected if they had undergone NAC with curative intent for treatment of breast cancer, followed by surgical resection. Patients who received neoadjuvant radiation and/or hormone therapy were excluded. Patients were divided into three groups: those who had surgery <4 weeks from last dose of chemo, 4-8 weeks from last dose, and >8 weeks from last dose. Charts were audited for demographic data, tumour characteristics, and complications from surgery. Data was analyzed using a Chi Squared test to determine any differences in pCR, OS, DFS, and surgical complications, between the three different time intervals.

**Results:** 347 patients were identified and included in this study. The median time to surgery after last dose of chemotherapy was 4.86 weeks (range 0.86-22.86 weeks). The percentage of patients that achieved pCR was 31.3%, 30.5%, and 28.6% in the <4 weeks, 4-8 weeks, and >8 weeks groups respectively (p= NS for all comparisons). There was no difference in pCR observed between the three groups based on receptor status. At the median follow up of 3 years, DFS was 85%, 85.8%, and 85.7% in all three groups. Likewise OS was 95%, 90%, and 89% respectively. The rate of surgical complications are 16%, 23.4%, and 21.4% for the three groups respectively (p=NS).

**Conclusions:** This study demonstrated no difference between receiving surgery <4 weeks, 4-8 weeks, or >8 weeks after last dose of NAC on pCR, survival, or surgical complications. This finding was preserved in all receptor subtypes. This has important implications for resource allocation. This data may also help in counselling and easing patient anxiety in terms of the urgency (or lack thereof) and wait times for surgery. Subsequent studies with larger sample sizes will help to ensure that clinical differences in outcomes are not affected by wait times.

Comparison of outcome measures based on needle to knife time

	< 4 weeks (n=80)	4-8 weeks (n=239)	>8weeks (n=28)	p (<4 weeks vs. < 8 weeks)
pCR	31.3% (25)	30.5% (73)	28.6% (8)	0.791
HR+Her2-	4.5% (1/22)	6.5% (6/92)	0% (0/5)	--
TNBC	31.5% (6/19)	42.1% (24/57)	0% (0/6)	--
Her2+	46.1% (18/39)	48.8% (44/90)	47.1% (8/17)	--
3 year DFS	85%	85.5%	85.7%	0.927
3 year OS	95%	90%	89%	0.290
Surgical Complication	16.3%	23.4%	21.4%	0.536

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-05

Overcoming barriers to achieve a successful breast cancer patient recruitment: Professionals' views

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Accurate recruitment in performing clinical trials is crucial to avoid delays in the availability of potential beneficial treatments for patients. However, optimal strategies to improve it remain elusive and highly variable. The aim of this study was to identify the factors affecting recruitment from a multi-professional approach.

**Methods:** A convergent mixed method study was used, including key informant in-depth interviews (n=13), 5 focus groups (n=40) and a 209-item Likert-scale survey for the measurement of a whole array of variables of interest (i.e. job attitudes, job satisfaction, job stressors and social status). A purposive sample of professionals from thirty-three clinical trial sites in Spain were targeted. Qualitative data were analyzed alongside quantitative data to document patterns and modifiers of recruitment performance. Audio material was recorded, transcribed verbatim and analyzed using the framework method.

**Findings:** Pattern observed in qualitative data was confirmed in quantitative analysis. A total of 32 key barriers to successful patient recruitment were identified and clustered in three categories. The most commonly reported barriers and facilitators regarding a multifactorial approach were: System/organization: Counting on experienced recruiters and having a strong team-workforce awareness (92,3%); automated recruitment software, scientific culture, recruiting philosophy, adequate workload demands (88%); high stimuli and interprofessional trust (84%). However, lack of time devoted to clinical research in favor of assistance demands, excessive workload and bureaucratic tasks were the most salient stressors to fulfill professional research and recruitment responsibilities (54%). Working team conditions: Insufficient workforce support as well as high employee turnover contributed to emotional exhaustion and depersonalization and to a lower personal accomplishment (15%). Interpersonal relations affected not only motivation and performance but professionalism satisfaction with outcomes. Professional recruiter characteristics: Professionals with a high level of expertise and academic specialization (>5 years), greater active involvement in relevant clinical trials just as interprofessional prestige and recognition showed a greater satisfaction. Consequently, they were prompted to intensify their efforts to fulfill recruitment. The more effective clinical research settings were those better aligned with attributes for becoming high recruiters and with better multi-professional articulation. Suggestions to improve recruitment included providing professionals with devoted time for research, setting up collaborative research agreements, building a clinical research network, collaborative tumor registries, clinical trials data sharing resources, multidisciplinary functional units and committees, flexible staffing models and empowering oncologists/managers leadership.

**Conclusions:** This study provided useful understanding of how to maximize patient recruitment at clinical research sites. Strong team-workforce and adequate workload were the most important identified factors for achieving it. Results will be used to inform professionals and stakeholders of the next steps needed and to engage them to fulfill accurate recruitment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-06

Analysis of barriers to clinical trial accrual in an NCI-designated comprehensive cancer center: Results of identifying clinical trial gaps

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**Background:** NCI-designated centers struggle to meet the 10% minimum accrual benchmark even for common malignancies such as breast cancer. The University of New Mexico Comprehensive Cancer Center (UNMCCC) average annual breast cancer trial accrual rate is approximately 11%, despite an average of 20 open breast cancer trials. Many published barriers were perceived to account for UNMCCC's low accrual including suboptimal screening, patients' unwillingness to participate, and disparities arising from socioeconomic and cultural barriers related to the large minority patient population. We sought to identify the true barriers to breast cancer therapeutic trials accrual to strategically formulate targeted solutions.

**Methods:** In 2016, a retrospective study was performed to review all breast cancer patients from UNMCCC's 2014 NCI Data Table 3. Barriers were classified into (a) trial specific, in which no trial was available or the patient was ineligible (b) patient specific, in which an appropriate trial was available but the patient declined participation (c) provider specific, in which screening was not documented.

**Results:** 145 cases were retrospectively evaluated. 6% declined participation, 13% were ineligible and 4% were not screened. Only 11% were enrolled in the 21 open trials.

No trial was available for 66% of patients. Out of this group, the majority of cases (68%) were stage 0/ I/ II (DCIS 12% LCIS 4%, stage I/II node negative, HR+, Her2- 52%), 17% were triple negative and 15% were HER2 positive.

The findings showed that despite the extensive clinical trial menu, the trials did not in fact adequately match the majority of the patient population who largely had early stage disease. This was identified as the main barrier to accrual. This resulted in a change in trial prioritization. Previously, the majority of trials were for advanced disease. From 2016-2018, the priority shifted to opening trials focused on early stage HR+, Her2 negative population. Investigator initiated, pharmaceutical, NCTN trials and symptom control trials for this population were opened.

As of December 2017, Data Table 3 accrual showed that 19% (70/353) new breast cancer patients were enrolled. This represents an increase in accrual from 11% to 19 %. Of these, 28% (20/70) had early stage HR+ Her2 negative disease.

As of 2018, therapeutic trials that include early stage HR+, Her2 -, node negative disease now comprise 30% of the UNMCCC breast cancer trial menu.

**Conclusion:** Detailed analysis of UNMCCC's breast cancer Data Table 3 showed that contrary to prior perceptions, the main accrual barrier was not ineffective screening, patient ineligibility or unwillingness to participate. The main barrier was that the extensive trial menu did not match the patient population. A large gap in the clinical trial menu was identified.

Published barriers to clinical trial accrual may not necessarily hold true for a specific institution. A disease-specific root cause analysis can facilitate development of tailored solutions. A shift in strategy in trial prioritization resulted in an increase in annual breast cancer trial accrual from 11% to 19%.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-07

Tolerance of HER-2 directed therapy for early stage breast cancer in a predominantly Hispanic population with high prevalence of cardiovascular risk factors

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## **Introduction:**

Trastuzumab based therapy is recommended for patients with early stage HER-2 overexpressing breast cancer, because it improves survival. Significant racial disparities exist in the receipt of trastuzumab with minorities being 25% less likely than whites in being treated with it. (Reeder-Hayes K et al, JCO 2016; 34:2003-2009).

Trastuzumab can affect cardiac function. Cardiac complications associated with trastuzumab are influenced by age and pre-existing risk factors including obesity, hypertension and diabetes mellitus. These co-morbidities occur more frequently in medically underserved minority populations and might influence the practitioner's decision regarding withholding the drug. We previously identified a high prevalence of metabolic syndrome and obesity in our predominantly Hispanic, medically underserved patient population (SABCC 2015 abstract P1-09-07)). For this study, we analyzed the echocardiographic data and cardiac complications associated with trastuzumab use in this patient population.

## **Methods:**

All patients diagnosed with early stage (stage 1, 2 or 3) HER 2 positive breast cancer between Jan 1st, 2010 and Jan 1st, 2015 at our institution were identified. Age, race, body mass index, pre-existing cardiovascular risk factors, and antihypertensive medication use was collected. Tumor size, nodal status, ER, PR and HER 2 status was recorded. All echocardiograms obtained were reviewed for ejection fraction changes. Repeated measures one sided ANOVA was used to analyze changes in EF. Hospitalization for cardiac complications was recorded. Early interruption of planned therapy and its reasons were recorded. Study was approved by the institutional IRB.

## **Results:**

Sixty patient were treated with trastuzumab based chemo immunotherapy over the study period. 93% were Hispanic, median age was 61 years (Range 31-83), 40% had hypertension, 35% had dyslipidemia, 35% had glucose intolerance or type II diabetes mellitus and 70% were overweight or obese. 33% were dependent on charity care. 26% had stage 1, 37% had stage 2 and 37% had stage 3 cancer. Docetaxel, carboplatin, trastuzumab (TCH) was the most commonly used regimen (63%) followed by doxorubicin, cyclophosphamide, paclitaxel and transtuzumab (28%).

Ten patients (16.6%) required early discontinuation of cytotoxic chemotherapy. Only 1 patient was unable to complete planned 1 year of trastuzumab due to declining ejection fraction. There were no hospitalizations related to cardiac events during therapy. Trastuzumab based treatment elicited statistically significant changes in LVEF over time,  $F(2,98) = 13.974$ ,  $p < .0005$ , with LVEF decreasing from  $65.4 \pm .844$  prior to therapy to  $64.7 \pm .724$  during 3-6months of therapy and  $62.2 \pm .81$  at the end of therapy. Two patients had a decline in LVEF  $\geq 10\%$ . Of these, 1 resolved at follow up ECHO in 6 weeks.

## **Conclusion**

In a predominantly Hispanic, HER 2+ breast cancer cohort, with a high prevalence of cardiovascular risk factors and limited health care access, we found that the vast majority were able to complete 1 year of trastuzumab without significant cardiac complications. As trastuzumab improves survival, practitioners should adhere to national guidelines regarding its use as much as possible.

# 2018 San Antonio Breast Cancer Symposium ®

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Association of tumor infiltrating lymphocytes and chemotherapy regimen in response to preoperative chemotherapy in underserved patients

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## Introduction

Preoperative chemotherapy can demonstrate an individual's response to the chemotherapeutic regimen by comparing the amount of cancer at presentation to the amount remaining after treatment. Multiple previous studies have demonstrated that the amount of residual cancer, or final pathologic stage, is a better indicator of prognosis than the initial stage at presentation. Tumor infiltrating lymphocytes have been recognized in breast cancer, and when found concentrated in breast cancer specimens, have been associated with a good prognosis. Breast cancer is not only a heterogeneous disease, but also displays varied presentation and behavior in patients of different race/ethnicities.

This study was performed to evaluate factors which predict response to chemotherapy. The effectiveness of different chemotherapeutic regimens, the effect of breast cancer subtype, and tumor infiltrating lymphocytes (TILs) were evaluated in our racial/ethnic minority population.

## Methods

All patients at the safety net institution in Phoenix, AZ who underwent preoperative chemotherapy from 2002 to 2017 and had tissue available for evaluation were included in the study. Response to chemotherapy regimen was recorded. Pathologic complete response (pCR) was defined as no invasive cancer in the breast and lymph nodes in the final pathologic specimen. Breast cancer subtypes were divided based on IHC and FISH testing. Luminal subtypes were classified based on Ki67 (>15%) and/or PR (<20%) for Luminal B. Her2 subtype was defined as Her2 IHC 3+ or Her2 FISH amplified. Triple negative breast cancer (TNBC) was defined as ER and PR (<5%) and Her2 negative. TILs concentration was determined from fixed formalin paraffin embedded (FFPE) core needle biopsy specimens.

## Results

A total of 259 patients were included in the study. The mean age was 45 years. 80% of the population were racial/ethnic minorities. The vast majority (94%) were underinsured or uninsured, with 75% uninsured. The mean clinical tumor size at presentation was 6cm. 52% presented at clinical stage 2 while 48% presented at clinical stage 3. The overall pCR rate was 32%. pCR rate was impacted by breast cancer subtype with TNBC 52% and Her2 38% showing a better response to chemotherapy, while Luminal B was 16% and Luminal A 2% ( $p < 0.05$ ). In TNBC, chemotherapy regimens with anthracycline and docetaxel may have improved efficacy with pCR of 56% ( $p = 0.05$ ). In the subgroup available for TIL evaluation, breast cancer subtype appeared to show similar importance with pathologic complete response rates of TNBC 50%, Her2 44%, Luminal B 12%, and Luminal A 0%. TIL appeared to affect the likelihood of pCR. When TIL were less than 5% the pCR rate was 16% compared to when there were TIL of at least 5% or more the pCR rate was 41% ( $p < 0.05$ ).

## Conclusions

In our racial/ethnic minority population, breast cancer subtype and chemotherapy regimen did affect likelihood of pathologic complete response. Tumor infiltrating lymphocyte concentration as low as 5% may indicate a higher likelihood of pathologic complete response and could be used as an additional factor in the evaluation of patients for preoperative therapy.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-09

Genetic counseling in an emergent country: Disparity in access to genetic cancer risk assessment for breast cancer within the Mexican population

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**Background:** Breast cancer (BC) is the most common malignancy in Mexico. 5-10% of BCs are hereditary. In Mexico the lack of resources, including geneticists/genetic counselors and the cost of genetic testing, are barriers to set up GCRA (Genetic Cancer Risk Assessment) clinics. Besides few public health programs offer GRCA to their beneficiaries; moreover 62% of Mexican population lacks health insurance. Seguro Popular (SP) offers health services to 50 million of low-income Mexicans without health coverage, but SP doesn't cover GRCA nor testing for BC. COEI offers services to two different populations: COEI Beneficiaries (CB) and SP. GCRA and genetic testing are only covered for CB.

**Methods:** This retrospective study compared clinical and demographic data between these cohorts. From Nov 2016 to Dec 2017, all new BC patients (pts) were referred to GRCA according to NCCN guidelines. SP pts were also invited to participate; we provided them voluntary this service. We collected demographic, familial and medical data. Genetic testing was offered only to some CB pts because of our limited budget. Descriptive and nonparametric statistics were used.

**Results:** We compared the data of 74 cases: 51 were CB and 23 SP pts. The average age at diagnosis was 41.8, 18(24.3%) of tumors were triple negative. SP pts tended to be younger, and with more advanced disease than the CB population but without statistical differences. SP group was less educated than the CB pts ( $p > 0.001$ ).

**Conclusions:** Except for education level, no other difference was seen. More research is needed to explore the relationship with the lower degree of education, clinical stage and health care access. Our sample is small, but it reflects the lack of access to GCRA services for low income and less educated pts across the country. GCRA could benefit SP group, to modify habits, start promptly and adapted screening, and have the choice to undergo genetic testing. If we consider the high percent of population who lack of GRCA services, we are missing opportunities to inform, and promote early detection of cancer.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-13-10

Recommendations to improve the lived experience of early stage and metastatic breast cancer patients in Canada

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**Introduction:** In 2017, the Canadian Breast Cancer Network (CBCN) undertook two surveys of Canadians who have experienced a breast cancer diagnosis to better understand the lived experience of patients and what opportunities exist to improve support for patients, survivors and their families and minimize the impact of this disease. There were 278 people diagnosed with early stage breast cancer, defined as stage I, II or III for the purpose of this report, and 180 people living with metastatic breast cancer, or stage IV breast cancer, who responded to these surveys.

**Results:** The survey data shows that while patients feel supported and well cared for in certain aspects there are still significant opportunities for improvement. CBCN has identified five overarching factors that could greatly improve health outcomes and the quality of life of Canadians who experience a breast cancer diagnosis.

**1. Improved Educational Resources:** The quality and availability of patient education has increased over the past couple of decades; however, there are still some patient friendly educational resources including, specific resources for **newly diagnosed metastatic breast cancer patients; decision aids that support breast cancer surgery and post-surgery decisions; navigation of financial resources; treatment timelines and recovery expectations; private insurance navigation and information on dying-well.**

**2. Increased Access to Treatments:** This challenge was specifically identified and vocalized by people living with metastatic breast cancer. Efforts need to continue to **shorten the drug approval process time, increase equitable access to new medications for all Canadians,** and ensure **equitable access for take home oral cancer medications.**

**3. Increased Access to Information:** Information available to patients about their health and treatment has increased; however, there is still information that isn't always communicated to patients that would help them make informed decisions about their health. This includes **information about breast density, palliative care options** and information around **clinical trials.**

**4. Integrated Systemic Supports:** The health care system as a whole is responsible for many of the services and supports that patients need to achieve optimal health and manage their breast cancer; however, these supports can be challenging to navigate and are sometimes lacking. Supports that need to be addressed at a systemic level include **developing survivorship care plans for early stage patients, patient navigation, communication tools to support general practitioners during the diagnosis process, access to psychosocial professionals** and **increased Employment Insurance Sickness Benefits.**

**5. Increased Awareness and Understanding of Metastatic Breast Cancer:** A lack of accurate statistics and overall awareness of metastatic breast cancer makes it challenging to truly understand the impact of this disease and also leaves people living with an incurable form of breast cancer feeling isolated and disconnected. **Accurate statistics** and **increased awareness** would help further the understanding of the impact of this stage of breast cancer and better support those with it.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-11

Impact of public health insurance “Seguro Popular” on breast cancer recurrence at Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Mexico City

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**Introduction.** Public health insurance called Seguro Popular (**SP**) for breast cancer treatment (**BCT**) was instituted in Mexico in 2007. Our third level center was enrolled until 2011. SP covers systemic BCT (WHO 20th model list essential medicines). The aims were: 1) Describe frequency and type of breast cancer recurrence (**BCR**), 2) compare which factors were contributing to recurrence of breast cancer patients (**pts**) before and after the implementation of SP. **Methods.** Pts with diagnosis of breast cancer (**BC**) from 2004 to 2018 were analyzed and pts with BCR whom primary tumor (**PBC**) was treated and followed at INCMNSZ were recruited. They were divided into two groups according to access to SP: pts without Seguro Popular (**non-SP**) and, pts with SP. Xi-square, non-parametric tests and Kaplan-Meier curves (log-rank test) were used. Logistic regression was done for multivariate analysis. A p-value <0.05 was considered significant. **Results.** Between 2004 and 2018, 929 pts with BC were diagnosed; 438 non-SP and 473 SP. Ninety-one (9.7%) had a BCR (loco-regional or distant) during this period. Median follow-up was 125 months. The BCR proportion was higher within non-SP than in SP (74 (16.8%) vs 17 (3.5%) pts) (p=<0.05). Median age at diagnosis was higher in non-SP (p=0.028). Distant relapses were higher in non-SP than in SP (44 (59.4%) vs 8 (47%) pts). St Gallen 2017 Luminal A/B subtypes were most common in non-SP compared to SP where Basal-like subtype was higher (p=0.048). Luminal-A subtype had the best survival among non-SP compared to SP in which HER2 non-luminal had the higher survival (p<0.001). **Conclusions.** Experience with SP at our center shows promising results in the improvement of BCR. Access to adequate systemic management (hormone-blocking and HER2 targeted therapy) has a positive impact on recurrence, disease-free survival, and cancer-specific global survival. A longer follow-up time is required in the SP group (prospective cohort) to confirm these findings, especially in luminal tumors with late recurrence risk. Efforts should be made in the Mexican public health system to secure basic and continuous access to BCT.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-12

Breast cancer in Colombia: A growing challenge for the health care system

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## INTRODUCTION

Colombia has a population of roughly 49 million people of predominantly Mestizo ethnicity. Cancer has become a growing public health problem in Colombia with nearly 71,000 newly diagnosed malignant tumors per year. It is expected that by 2035, 150,000 new cases of cancer will be diagnosed, making Colombia an intermediate country with regards to global cancer incidence according to IARC.

## METHODS

Epidemiological data on breast cancer is scarce and varied due to multiple sources of information. These numbers are obtained thru population-based cancer registries that represent 4 distinct regions of the country. Other data originate from non-governmental institutions and healthcare providers within Colombia. The Colombian National Cancer Institute publishes a Cancer Mortality Atlas annually.

## RESULTS

Local cancer registries have shown increases in breast cancer incidence in Colombia. In 2007, age-standardized incidence rate was 27.8 per 100,000 persons increasing to 49.7 cases per 100,000 persons in 2012. Approximately, 2200 women die every year in Colombia due to breast cancer with rates increasing historically, but now are stabilizing. Advanced breast cancers are most frequently found among women without health insurance, while early breast cancers are usually found among working women and those covered by private health insurance. Early breast cancer screening was made mandatory as public policy in the year 2000. However, only 30% of health care coverage was reported, translating to very low coverage by opportunistic screening programs with only 33% of women having had a mammography. In 2012, a National Cancer Control Plan was planned and implemented. It aims to increase early stage cancer diagnosis, increase biannual screening coverage, and guarantee timely access to diagnosis and treatment. A national health survey in 2015 showed only 48% of women had an annual mammographic screening. Multiple disparities have been found with regards to screening and early diagnosis such as economic strata, health insurance coverage, origin, and accessibility. Specifically, data shows that 23% needed to travel in order to obtain access to mammography. Often it is necessary for some patients to sue healthcare insurance systems to obtain specific health care, causing an increase in time to diagnosis and treatment. In 2016, on average a 90-day period was reported from time of onset of symptoms to suspected diagnosis of breast cancer, while the time to the initiation of treatment was 100 days for chemotherapy and close to 120 days for surgery.

## DISCUSSION

These data serve to impact the landscape of breast cancer and improve patient outcomes in Colombia. While the National Cancer Plan has led to major changes, a big challenge remains related to the delays between suspicion of breast cancer and diagnosis and treatment. Quality of care provided by private and public insurance administrators is also of concern. General practitioners should receive more detailed training in breast cancer detection and management. The healthcare system should provide quality cancer care with urgent improvement in mammography, especially in more rural areas. Widely, more timely and appropriate follow-up is needed.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-13

Non-clinical drivers of variation in preoperative MRI utilization for breast cancer

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**Background:** Preoperative MRI utilization in breast cancer treatment has increased significantly over the past two decades but its use continues to have inter-provider variability and disputed clinical indications. The objective of this study was to evaluate non-clinical factors associated with preoperative breast MRI utilization.

**Methods:** This study utilized claims from the Military Health System Data Repository (MDR) on TRICARE Prime beneficiaries, from fiscal years 2006-2015. TRICARE provides health benefits for Active Duty service members, retirees, and their dependents at both military (direct care with salaried physicians) and civilian (purchased care with fee-for-service physicians) treatment facilities. We studied patients aged 25-64 years old with a breast cancer diagnosis who had undergone mammogram/breast ultrasound alone or with subsequent breast MRI prior to surgery. Patient demographics and treatment characteristics were abstracted from the data. The National Center for Health Statistics (NCHS) urban-rural classification was used to determine the urbanization level of the treatment facility. Adjusted multivariate logistic regression tests were used to identify independent factors associated with preoperative breast MRI utilization.

**Results:** Of the 25,656 identified patients, 64.4% of patients (n=16,511) received preoperative mammogram/breast ultrasound alone while 35.6% of patients (n=9,145) underwent additional MRI after mammographic and/or ultrasound imaging. On multivariable analysis, younger age, presence of two or more comorbidities, active duty or retired beneficiary category, officer rank (surrogate for socioeconomic status), Air Force service branch, metropolitan location, and purchased care were associated with increased likelihood of preoperative MRI utilization. Nonmetropolitan location and Navy service branch were associated with decreased MRI use.

		Odds Ratio	95% Confidence Interval
Age Group (Ref: 55-64 years)	25-34 years	1.85	1.60-2.15
	35-44 years	1.59	1.47-1.72
	45-54 years	1.27	1.19-1.35
Charlson Comorbidity Index (Ref 0-1)	2+	2.47	2.33-2.61
Beneficiary Category (Ref: Dependent)	Active Duty	1.20	1.04-1.38
	Retired	1.23	1.09-1.40
Rank (Ref: Senior Enlisted)	Junior Enlisted	0.93	0.78-1.11
	Junior Officer	1.25	1.14-1.37
	Senior Officer	1.48	1.36-1.60
	Warrant Officer	1.23	1.06-1.42
Service Branch (Ref: Army)	Air Force	1.10	1.03-1.18
	Navy	0.92	0.85-0.99
	Marines	0.95	0.84-1.07
	Coast Guard	1.07	0.89-1.29
Urban-Rural Classification (Ref: Medium Metropolitan)	Large Central Metropolitan	1.80	1.68-1.93
	Large Fringe Metropolitan	1.59	1.47-1.71
	Small Metropolitan	0.65	0.59-0.71
	Micropolitan	0.40	0.34-0.46

	Noncore	0.25	0.18-0.34
Treatment Facility Care Setting (Ref: Direct Care)	Purchased Care	1.60	1.48-1.73

Conclusions: After controlling for expected clinical risk factors, patients were more likely to receive additional MRI when treated at larger metropolitan facilities or through the purchased, fee-for-service system. Both associations may point toward non-clinical incentives to perform MRI in the treatment of breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-14

Marital status is associated with late stage presentation in patients with breast cancer

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**Objective:** To determine whether marital status is associated with late stage presentation for breast cancer patients

**Materials/Methods:** Patients with breast cancer treated with radiation therapy at a single institution from 2012-2017 were identified (n=1060). There were 189 patients with stage 0/DCIS, 442 with stage I, 318 with stage II, 74 with stage III, and 38 with stage IV disease. Patients were categorized by marriage status as single, married, divorced, or widowed. A logistic regression model was run using stage as the outcome to analyze odds ratios for late stage presentation (stages III and IV) based on marital status. The model included age, ethnicity/race, insurance, marital status, and language. Additional interactions between age and ethnicity/race, and ethnicity/race and insurance improved the fit of the model and were also included. All odds ratios were interpreted against the reference subset defined as white, married, English-speaking patients with public insurance.

**Results:** There was a significant association between marital status and stage of presentation, most notably for widowed patients. Widowed patients have 2.04 odds of presenting with late stage disease compared to married patients (95% CI 1.20-3.47). Additionally, single patients present at an earlier stage than widowed patients, and have 0.50 odds of late stage disease compared to widowed patients (95% CI 0.28-0.91). There was a trend towards divorced patients having a higher odds of late stage presentation, however, it was not statistically significant. Divorced patients had 1.33 times the odds of having late stage disease compared to the married patients (95% CI 0.90-1.96), and 1.30 times the odds of having late stage disease compared to the single patients (95% CI 0.82-2.06). However, relative to widowed patients, the divorced patients have lower odds of presenting with late stage disease (OR=0.65, 95% CI 0.35-1.21). Interestingly, there is no statistically significant difference between single and married patients for stage of presentation (OR=1.03, CI 0.75-1.41).

**Conclusion:** Marital status is correlated with late stage presentation, especially for widowed patients. Interestingly single and married patients had almost identical probabilities of presenting with all stages of disease. They had the highest probability of having stage 0/DCIS or stage I disease, and the lowest probability of stage III or stage IV disease. Divorcees had a trend towards late stage presentation, with a smaller probability of stage 0/DCIS and stage I disease, and greater probability for stage III and stage IV disease. The biggest difference was seen for widowed patients who had the smallest probability of stage 0/DCIS or stage I disease, and the greatest probability of having stage III or stage IV disease. Widowed patients had higher odds of presenting with late stage disease than both single patients and married patients. It remains to be explored why this population is more vulnerable to late stage breast cancer presentation than their single, married, and divorced counterparts.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-15

The value of patient navigation in breast cancer being tested in Rio de Janeiro, Brazil

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**OBJECTIVES:** The main objective of this Patient Navigation Program in Rio de Janeiro (PNP Rio), Brazil, is to promote adherence to the "60 Day Law", which states that all patients with cancer within the public system should start treatment within 60 days after diagnosis of cancer. Thus, the objectives are: 1.) to establish feasibility of PNP in this setting; 2.) to identify barriers to compliance the Law of 60 days and 3.) to ensure that at least 70% of recruited breast cancer patients begin treatment within the mandated 60-day period. One report by FEMAMA states that only 30% of breast cancer patients in Rio de Janeiro to initiate treatment within the 60-day mandate.

**METHODS:** From August 2017 to May 2018, one hundred patients aged 33-81 years (mean age 59 years) were recruited for navigation at Rio Image - an advanced breast cancer diagnosis center administered by the state health secretary and located in the capital city of Rio de Janeiro, attending patients from the public system from all 92 municipalities in the state. Patient Navigator (PN), a trained social worker, starts navigation from diagnosis, administering questionnaires to collect: patient population data, dates and information of historical milestones, and patient satisfaction. Patients were followed up by phone, e-mail or text message to identify barriers to initiation of treatment.

**RESULTS:** Patients presented staging 0-I (17%), II-III (78%) and IV (5%). There were two deaths related to breast cancer in this group. All patients reported at least one barrier, ranging from 2 to 12 barriers (M=5). The barriers to compliance with the "Law of 60 days" were: Fear and fatalistic thoughts (99%), Financial problems (79%), Uncoordinated health care (76%), Health professionals ignore the Law of the 60 days (75%), Need to do staging exams again (52%), Concern about communicating with medical staff (52%), Transport (42%), Difficult in obtain surgical risk consultation (12%), Line of surgeries in hospitals (12%), Difficult of insertion in the regulation system in Primary Care(11%), Patient cannot express herself (5%), Social support(4%), Absent of the immunohistochemistry panel (4%), Cognitive problems (3%), Comorbidities (2%). The PNP had 100% patient satisfaction and in 60% of the cases it helped the patients to start treatment within the period established by law.

**CONCLUSIONS:** In summary, PNP Rio generated a positive experience for patients in the public health system because it is an intentional and proactive process of assisting the individual through the cancer system, accessing services and actively overcoming barriers to quality care. The PNP Rio did not achieve the success rate of 70% of compliance with the Law as intended (achieved 60%). However, the barriers that the PN can not overcome such as lack of human resources and medical supplies, were informed to health authorities and hospital administrators. This is an opportunity for discussion of reallocation of funds, focusing on the use of scarce resources in prevention and early treatment rather than late-stage disease. In the Brazilian context, PNP may represent an opportunity to implement existing legislation adequately, and as such, would have great potential for integration at the federal, state, and local health systems.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-16

Exploring the role of social support and adjuvant endocrine therapy use among breast cancer survivors

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**Background:** The side-effects of adjuvant endocrine therapy treatment (AET) are one of the main reasons why women discontinue therapy. Helping women manage the side-effects may improve adherence since only half are adherent for 5-years. Generally, patients experience a decline in social support from family/friends and medical providers when beginning AET because the severity of the side effects are not as physically apparent as those associated with other cancer treatments. Therefore, the objective of this study is to explore the role of social support and identify how it is delivered during the on-going management of AET.

**Methods:** We conducted semi-structured in-depth interviews with breast cancer patients (n=19) who had filled a prescription for AET in the previous 12 months. Women were recruited from Los Angeles, California and Houston, Texas between 2014-2015. Interviews were audio recorded and professionally transcribed. Interview questions were designed to prompt discussion about experiences with AET and examine how sources of support affect the management of AET drugs. We used an integrated approach to develop a code structure, which was applied to interview transcripts using qualitative inductive reasoning to identify major themes and subthemes. The study team met regularly, in an iterative process, to redefine the codebook by adding, removing, and revising codes to capture emerging themes. We grouped social support into four major categories to identify the sources and delivery of support: emotional, informational, instrumental, and appraisal.

**Results:** Patients most commonly described informational support from their medical providers via consultations or phone, during which providers would explain the purpose, benefits, and side-effects of AET in a manner that they could understand. Many patients confided in their health providers when struggling with the side-effects of AET, and those who were well-received expressed trust and confidence in their physician's recommendations. Women also discussed other noteworthy sources of informational support through technology, survivorship groups, family/friends, and religious groups. Emotional support was an equally important type of social support as patients discussed the need for ongoing reassurance, communication, and empathy to help them deal with the stressors of managing their side effects, which they received from family, survivorship groups, and different forms of spirituality and religiosity but could also be delivered by medical providers. Instrumental and appraisal support played a more peripheral role as patients identified different types of organizations and exercise classes that provided them with physical and emotional benefits and that was provided by family/friends and medical providers.

**Conclusion:** We identified all four forms of social support delivered to a group of breast cancer survivors on active AET in formal and informal settings by family/friends, medical providers, and support groups. The social support provided women with educational, physical, and emotional benefits that may play an important role in their continuation of AET.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-17

Comparing characteristics of patients who fill out online surveys before visits with patients who fill out surveys in-clinic with staff assistance at the UCSF breast screening clinic

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**Background:** At the UCSF breast screening clinic, intake surveys are sent to women with upcoming mammogram appointments to obtain their demographic data, comorbidities, and assess breast cancer risk (family history, biopsy history). Many patients complete surveys online **before** their visit. For those who do not, a staff member is present to assist with survey completion on a tablet **in-clinic**.

**Methods:** Data was collected from 10,755 patients from December 2012–May 2018. To assess if different survey modalities capture different demographic groups, we analyzed these submissions, comparing responses completed by patients online **before** visits and **in-clinic** with assistance.

**Results:** On average, 48% of invited patients complete a survey. Of respondents 76% completed surveys **before** visits and 24% completed surveys **in-clinic**. Both methods captured electronic data that was summarized and presented to clinicians for clinical decision support. Compared to the **in-clinic** group, a **before** group patient was more likely to be white, married, and have at least a college education. The **before** group included a smaller proportion of patients who were Black/African American, Hispanic/Latina, and 65 years or older. Furthermore, a greater proportion of the **before** group reported 2 or more comorbidities. The **before** population reported more often having fair or poor health over the preceding 30 days. While these differences were statistically significant, it is important to put some of these results into perspective: while only 24% of survey responses were collected **in-clinic**, 59.1% of all Black/African American responses and 33.5% of all Hispanic/Latina responses were represented in this group.

	<b>Before</b> group (N=7869)	<b>In-clinic</b> group (N=2886)	P value
White	64.43%	55.79%	<0.0001
Black/African American	3.57%	10.91%	<0.0001
Hispanic/Latina	7.96%	10.91%	<0.0001
Aged 65 or older	29.84%	39.54%	<0.0001
Married	66.40%	56.10%	<0.0001
College educated or more	79.93%	66.87%	<0.0001
2 or more diagnosed comorbidities	38.18%	30.32%	<0.0001
Poor or Fair health over last 30 days	10.41%	5.45%	<0.0001

**Conclusions/Future Directions:** 1) Online surveys are completed more often by traditionally well-represented groups. Offering staff supported electronic surveys **in-clinic** improves the total yield and diversity of patients who complete surveys. More research is required to see the impact of income levels. 2) We did not anticipate a greater incidence of fair or poor health over the last 30 days or the higher number of patients reporting 2 or more comorbidities in the **before** group. This could result from the **before** group having better access to health care, and more familiarity with health surveys, but more detailed study is needed. 3) We will investigate further issues of health care trust, familiarity, and access to adjust our clinic practices. As more studies move surveys entirely online, we need to identify and address factors that prevent patients from completing surveys before appointments. Alternative survey modalities must be made available in accessible ways and integrated into routine clinical



practice.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-18

Barriers to adjuvant radiotherapy treatment for breast cancer in a teaching hospital in Brazil

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**BACKGROUND:** Adjuvant treatment of non-metastatic breast cancer (BC) represents an important paradigm of multimodality approach in oncology practice, with an established role for radiotherapy (RT). A delay of adjuvant radiotherapy can lead to poorer results. When chemotherapy (CT) is not indicated, RT should be initiated within 8 weeks after surgery. If CT is administered first, RT should be started within 7 months from surgery, since there is a continuous relation between time to radiotherapy and local recurrence. Brazil's public healthcare system, SUS, faces many challenges caring for cancer patients: inadequate funding, inequitable distribution of resources and services, among others. According to research done by Lins *et al*, around 458 linear accelerators would be necessary to supply the Brazilian public health demand and end the waiting line for radiation therapy. Currently, our healthcare system has 283 machines, which are responsible for more than 70% of our population. Furthermore, patients lack understanding of treatment windows, which is an additional hurdle.

**METHODS:** We randomly selected 122 charts of female BC patients submitted to treatment with curative intent from 2003-2017 in Hospital das Clínicas da UFMG, the biggest teaching hospital of the 3rd largest city in Brazil. Primary endpoint was to determine median time from surgery to adjuvant radiotherapy, and second point was to determine median radiotherapy time.

**RESULTS:** Twenty eight patients were not included in the analysis, 26 due to lack of information in the charts and two for not having received the proposed radiotherapy. Ninety four patients were included: median age was 49 years old (21-90), 21.5% were stage I, 41.9% stage II and 34.4% stage III at diagnosis. Patients received chemotherapy (neo or adjuvant), hormonotherapy, or both, according to oncologists discretion. All patients were submitted to surgery and radiotherapy. Long median times from referral to RT initiation and to radiotherapy completion were identified: 54 days and 97 days, respectively, as well as 7 months from surgery to beginning of RT (1-16) and 9 months from diagnosis to its completion (2-29). Biopsies were performed in 27 different sites and RT in 12.

Interval	Median Time (days)
Biopsy to results	13 (1-77)
Referral to RT initiation	54 (1-238)
Referral to RT completion	97 (43-238)
RT initiation to RT completion	42 (20-80)

Interval	Median Time (months)
Surgery to RT initiation	7 (1-16)
Diagnosis to RT completion	9 (2-29)

**CONCLUSIONS:** This study shows that intervals for completion of adjuvant radiotherapy are well above recommended, mostly due to long delays in initiating radiotherapy. Although in our study all patients were conducted by the same oncology team, the

system is fragmented, making it even more difficult for patients to receive multidisciplinary care and improve prognosis. There is undeniable need for more radiotherapy machines, but since their acquisition depends on costly governmental actions, we need to think about strategies that may allow us to better use the resources already available. We believe that Patient Navigation plays an important role here and were are establishing this program in our institution with Global Cancer Institute support.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-19

Addressing non-adherence for breast cancer screening across ethnicity in southern Arizona

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**Background** In Arizona, female breast cancer has the highest incidence rate and the second highest death rate compared to all other cancers. This ongoing single arm intervention study investigates non-adherence with recommended annual mammography or follow-up breast imaging. Data collected for this study is both retrospective, using the university Electronic Health Record (EHR) system (January 1, 2014 to September 30, 2017), and prospective, implementing a questionnaire during the intervention phase. Potential study participants were identified using EHR and categorized by BI-RADS (Breast Imaging-Reporting and Data System) 0 to 5. With IRB approval from University of Arizona, we designed a questionnaire to measure barriers to adherence and we navigate participants to schedule and attend follow-up appointments. This study's overall specific aims are to increase first time mammography screening by 25% among women in Southern Arizona; increase adherence or repeat screening rate by 20% among women lost to follow-up; establish the framework for a community academic partnership in ethnically diverse areas. Women, age 40 and older who are not compliant with recommended annual mammograms or recommended follow-up screenings after a suspicious finding are eligible to participate in this study. Men and children, as well as women for whom breast imaging is not recommended are excluded from participating in this study. **Results** Patient's age was summarized by mean  $\pm$  standard deviation for continuous variables and frequency and the associated percentage for categorical variables. BI-RADS scores were classified into Negative, Benign, Possible Malignancy and Proven Malignancy and compared between ethnic and racial groups using Fisher's exact test. Of 8823 non-compliant woman over nearly 4 years of data, 0.2% are BI-RADS 4 and 5, 2.2% are BI-RADS 3, 96% are BI-RADS 1 and 2, and 0.3% are BI-RADS 0. The mean age is 61.59 years, with 25% reporting as Hispanic, 66% reporting as non-Hispanic women (NHW), and 10% preferring to receive care in Spanish. Initial data shows only .24% with proven malignancies. Further, the data reveals that Hispanics have a slightly higher rate of possible malignancy (.36%) than NHW (.18%); however, NHW show a slightly higher rate of proven malignancy (.27% compared to .18%, respectively). **Discussion** These data provide valuable information for the direction of this study; in particular, understanding the disparity between Hispanic and NHW malignancies and developing culturally competent interventions and education materials to increase compliance with breast cancer screening recommendations. Further, these data indicate our focus should be on screening compliance for BI-RADS 1 and 2. These data also point to a possible high non-compliance issue. Comparing non-compliance data from other regional clinics will continue to shape this study's direction. The target sample size for this study is 300 participants. We accept a 95% confidence level and a 5% margin of error. Out of 420 recruitment letters mailed, the navigators have reached 152 potential participants by phone and have a 26% study recruitment rate (n=40).

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-13-20

Overall survival of women with breast cancer treated with newly approved targeted drugs in Manitoba: A population based study

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**Background:** Cancer drugs are approved based on the results of clinical trials in selected, rigorously monitored groups of participants. The ultimate goal of new cancer drug approvals is to improve outcomes, preferably overall survival, at population level compared to existing standard of care.

**Methods:** We conducted a population based study using data from the provincial Manitoba cancer registry, Manitoba Health, and electronic patient records. We collected data on patient demographics, toxicity outcomes, and efficacy outcomes including recurrence and Overall Survival (OS) of patients who were treated with the 10 most frequently used new targeted cancer drugs between January 2005 and December 2017 in Manitoba. Patient demographics were reported using descriptive statistics. Toxicity events were reported in frequency, and Kaplan-Meier curves were used for time-to-event outcomes.

**Results:** Four breast cancer drugs – Trastuzumab Emtansine (T-DM1), pertuzumab, fulvestrant, and palbociclib qualified for inclusion. During the timeframe, 71 women were treated with T-DM1, 100 with pertuzumab, 102 with Fulvestrant, and 21 with palbociclib. Patient demographics, disease stage, and line of therapy were comparable to those reported in pivotal trials leading to approval of these drugs. Median OS was 15.82 months for T-DM1, 25.34 months for pertuzumab, 14.65 months for fulvestrant, and not assessable for palbociclib – of note, these were consistently about half the median OS duration reported in pivotal trials leading to approval of the respective drugs. [For example, median OS in pivotal trials was 55.6 months for pertuzumab, 29.9 months for T-DM1, up to 26.4 months for fulvestrant, and 37.5 months (one trial) for palbociclib]. Serious toxicities were observed more frequently than reported in the literature – detailed results will be presented.

**Conclusion:** Despite impressive improvements in outcomes reported in clinical trials leading to approval of new targeted therapies for breast cancer, such agents only yield modest OS at population level which was often worse than even the control groups used in pivotal clinical trials. Given the main aim of new interventions are to improve outcomes at population level, future research should explore causes, and identify solutions to minimize such efficacy-effectiveness gaps. Cautious patient selection, early identification and management of toxicities, and increasing resources available to individual patients may minimize such gaps.

## **2018 San Antonio Breast Cancer Symposium ®**

**Publication Number:** P5-13-21

The European initiative in breast cancer (ECIBC) - A program to reduce disparities in cancer care by accreditation of all breast centers in Europe

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In order to reduce disparities in breast cancer services in Europe, the European Parliament passed resolutions in 2003 and 2006 calling for all women with breast cancer in Europe to be treated by a multidisciplinary team and called on Member States to establish a network of Certified multidisciplinary breast centres.

In the light of these resolutions the European Commission has tasked the Joint Research Centre (JRC) based in Ispra in Italy to set a programme of work from 2014-2020 to update the European Screening Guidelines and produce standards for an accreditation programme for all European Member States.

The methodology is evidence based and the Ibero American Cochrane group has been recruited to examine the evidence for the key questions concerning quality indicators. The whole pathway for breast cancer treatment is being explored from screening to follow up/survivorship, and only indicators supported by evidence are accepted. Indicators have to be relevant, measurable and feasible and currently 50 indicators have been approved using the Delphi system. Many currently used indicators have failed the test of usability due to lack of precision in measurement (eg cosmetic outcomes, margin assessment), or lack of clear definitions (eg counselling, breast care nurse roles, waiting times, MDT working).

A further problem is the different resources applied to breast services across the wide range of economies in European countries (Western v Eastern Europe), which affects the availability of expensive therapies.

The Indicators will form the basis of the accreditation program which will be published in 2019. The detailed evidence for each indicator will be presented in the poster together with the feedback from the 30+ European countries involved.

A pilot of the accreditation program will take place in 2019 to test the feasibility of the audit process in a variety of breast clinic settings.

This project is the most comprehensive attempt to date to produce a robust measurement of breast centers performance based on evidence, and might have global applicability.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-14-01

Advance directives in Korean cancer patients: Preliminary analysis

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“End-of-life” is a difficult conversational topic in East-Asian culture, even among patients and doctors. In 2016, the “Law on Advance Medical Decisions for End-Stage Patients,” with the so-called “Advance directive (AD),” was enacted in South Korea. Unlike other solid cancers, breast cancer patients have a longer period of survival due to many novel treatments. This study aimed to investigate the acceptance rate of issuing AD and potential barriers to its completion in patients with cancer, including breast cancer (BC).

We conducted a cross-sectional descriptive survey of metastatic or recurrent cancer patients visiting Korea University Anam Hospital oncologic department. The primary end point was the rate of completing AD on doctors’ suggestion. Written interview was conducted to understand the perceptions and factors influencing patients’ decision.

The total sample of 77 patients was analyzed in August, 2018 (29 BC and 48 non-BC patients). The median age was 66.0 years, and duration since diagnosis to suggestion of AD was 56.4 months. The mean follow-up time of physicians who suggested AD was 27.1 months. Of 77 patients, 52 (67.5%) agreed to write AD. Of 29 BC patients, 23 (79.3%) agreed to complete AD, while only 29 of 48 (60.4%) non-BC patients did so.

BC patients who agreed to write AD had older median age, shorter duration since cancer diagnosis, and shorter duration of follow up than those who refused [age 61 vs. 59 yrs p 0.34, diagnosis duration 61.30 vs. 70.63 p 0.40, follow-up period 22.67 vs. 24.13 p 0.64]. Non-BC patients who completed AD had younger age, longer duration since diagnosis, and longer follow-up period [age 68 vs. 71 p 0.67, diagnosis duration 33.1 vs. 33.2 p 0.20, follow-up period 18.0 vs. 16.9 p 0.25]. However, all the differences were statistically insignificant due to small sample size.

The survey showed that patients who agreed to issue AD had relatively high education and were more religious [college graduates 34.6% vs. 16%, respondents with a religion 63.4% vs. 56%]. Of the respondents who completed AD, 42 (80.8%) reported a fair understanding of the concept of hospice palliative care. 21 (40.4%) patients reported understanding AD system “well” or “very well”. Most patients got information about the system from conventional social media like TV and newspaper, followed by their physician [33 (42.9%) and 27 (35.1%)]. Decision on the preparation of advanced life care was mainly by the patients themselves, followed by the spouse and children [38(49.3%), 16(20.1%) and 11(14.3%), respectively].

Reason for writing AD was diverse, including “to leave my own will, not guardian’s” (40.4%), “because my doctors recommended it” (36.5%), “to avoid suffering from meaningless treatment” (26.9%), and “to ease the economic burden on the family” (11.5%). The two major reasons for refusal were “needing more discussion with family” (40.0%) and “lack of understanding of the system” (36%).

In this study, we identified that BC patients are more likely to accept AD than other types of cancer patients. Highly educated and religious patients tended to accept AD without hesitation. Better education and information shared through media and conversation with doctors might improve understanding of the AD system in Korean cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-14-02

Time to treatment discontinuation as a pragmatic endpoint: A U.S. Food and Drug Administration pooled analysis of CDK 4/6 inhibitors

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**BACKGROUND:** The primary endpoint in many registration trials is progression-free survival (PFS), defined as the time from randomization until objective tumor progression or death. In the clinical setting, however, PFS can be difficult to measure and explain, especially in patient-friendly language. For real-world evidence (RWE) based studies, time to treatment discontinuation (TTD), defined as time from randomization to discontinuation of the investigational study drug, may be a more practical endpoint. We hypothesize TTD will correlate with PFS and analyzed registration trials from recently FDA approved cyclin dependent kinase 4/6 inhibitors (CDKIs) to test this hypothesis. TTD was defined as time from randomization to discontinuation of the CDKI.

**METHODS:** We pooled data from five phase III randomized, controlled, registration trials of CDKIs with an aromatase inhibitor (AI) in the first-line or fulvestrant in the second-line setting. The CDKI discontinuation dates used to calculate TTD were derived from the patient-level datasets. We calculated patient-level correlation between TTD and PFS. We also summarized median TTD and PFS as well as the number of TTD and PFS events.

**RESULTS:** A total of 3017 patients were included in the pooled analysis (n=1899 treated with CDKI, n=1118 with placebo). The median PFS and TTD and the number of events are shown in Table 1. The patient-level Spearman correlation coefficient of TTD and PFS ranged from 0.87-0.95. The trial-level association was approximately  $R^2=0.23$ .

Table 1: Primary Results

Treatment Arm	N	PFS Events	TTD Events	Median PFS, in mo (95% CI)	Median TTD, in mo (95% CI)	Spearman Correlation Coefficient
CDKI	1899	873	1383	20.7 (19.2-22.2)	14.3 (13.6-14.9)	0.87
Placebo	1118	714	883	11.7 (11.0-13.6)	11.0 (9.7-12.0)	0.95

**CONCLUSION:** Patient-level pooled cross-trial analysis show a relationship between TTD and PFS in registration trials of CDKIs. In all trials, the difference in time between median TTD and median PFS appeared greater in the CDKI arm than the placebo arm. This analysis is limited by the relatively small number of studies included. Further research is needed to determine both whether: 1) TTD can serve as a pragmatic endpoint for analyses of RWE, and 2) is a more meaningful endpoint to patients.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-14-03

Patient perspectives on HER2+ breast cancer recurrence: Results from an online patient survey

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**Background:** The objective of this study was to gather insights from patients with early-stage HER2+ breast cancer regarding fears and knowledge of breast cancer recurrence and approaches to reducing risk of recurrence.

**Methods:** From Nov. 7-30, 2017, patients with stage I-IIIC HER2+ breast cancer were recruited via postings to Breastcancer.org discussion boards to participate in an online survey comprising 17 questions designed to quantify fears and emotions related to recurrence, identify and characterize knowledge gaps, and understand what patients are willing to tolerate to reduce recurrence risk.

**Results:** Of 307 respondents, 87% were aged  $\geq 40$  years and over half were  $\geq 50$  (58%). The majority had completed (48%) or were undergoing (39%) postsurgical treatment. Recurrence was a concern for 93% of patients and was the highest rated concern for 78%; however, most patients (76%) perceived their personal risk of recurrence to be moderate or low. 59% of patients felt they were at least somewhat informed about risk of recurrence; 55% had discussed risk of recurrence with their health care team, with discussions most frequently initiated by the patient (56%) vs the health care team (40%). Oncologists were the primary source of information about risk of recurrence (58%), but patients also frequently sourced information from online content (29%), published research (18%), and Breastcancer.org (15%). Almost all (96%) patients were at least somewhat involved in treatment planning. While 55% of patients would inquire about a new drug if it conferred a 10%-20% reduction in risk of recurrence, 26% required a benefit of  $\geq 50\%$ . For treatments reducing the risk of recurrence, patients were willing to tolerate (in order from most to least willing) hot flashes, fatigue, diarrhea, joint pain, and nausea/vomiting.

**Conclusions:** While patients with HER2+ breast cancer are highly concerned about recurrence, most feel that their personal risk is moderate. Patients are highly engaged in treatment planning, often initiate discussions about risk of recurrence, and supplement their knowledge with their own research. The threshold to motivate inquiry into a new drug to reduce recurrence risk was  $<20\%$  for over half of patients. Patients were also frequently willing to tolerate common adverse events.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-14-04

Patient acceptance of multidisciplinary clinic and an app to communicate with their care team in breast cancer treatment

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**Background:** Interdisciplinary management improves the care of breast and other cancer treatments. Challenges to such coordinated care have been identified by The National Academy of Medicine (NAM): 1-Poor collaboration among siloed physician 2- Poor patient education and involvement in a shared decision making environment. Multi D cancer conferences are a step in the right direction but true multi D clinics(MDC) with patients contemporaneously engaging their care team are rare. We initiated such clinic at our center and collected feedback information from our patients. We then helped design a HIPAA compliant mobile app, The Accord App™, to allow continued discussion centered around that patient's care and potentially including the patient in the discussion. We are reporting feedback from MDC and any available data on the Accord App™ feedback.

**Methods:** All new breast cancer patients attending our MDC were offered a survey seeking feedback regarding the whole clinic experience, their interaction with team members, clinic environment and the duration of this long consultation. Team members included breast surgery, medical and radiation oncology in addition to other services. Surveys were anonymous and non-obligatory. Surveys asked patients to grade different item on a 3 level scale: 1- Needs improvement 2- Met expectations 3- Exceeded expectations. The length of the consultation(LOC) was rated as: 1- Too short 2- Just right 3- Too long. The Accord App™ was then developed as a HIPAA compliant tool that is being offered to patients and their care teams allowing ongoing discussion of patient care, sharing results and responding to patients' queries. Initial feedback from provider and patient users is being collected and will be reported.

**Results:** 67 consecutive surveys were prospectively collected and analyzed. The LOC mean score was 2.01. The other scores ranged from 2.49/3 for the physical environment to 2.87/3 for clarity of surgeon provided information. The overall experience was rated as 2.80/3. Feedback from Accord App™ users is being collected.

**Conclusions:** Current collaboration tools including EMRs and Patient Portals have not resolved many of these teamwork and patient engagement issues reported by NAM. The MDC which frequently took over 2 hours was felt to be "just right" to the vast majority of patients. Our patient mix is quite varied in age groups, levels of literacy, socioeconomic status and ethnicity and we experienced very little variability in that response. Patients rated their experience highly suggesting MDC to be a potentially valuable model for case discussions, cancer care team collaboration and patient engagement in a "shared decision" environment. Initial feedback from the Accord App™ seems promising and will be reported. More rigorous research into MDCs and mHealth tools such as the Accord App™ is warranted to define and potentially expand their role in breast and other cancer care.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-14-05

Use of complementary and alternative medicine in cancer patients receiving chemotherapy in Ireland

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## **Introduction:**

The use of complementary and alternative medicine (CAM) in cancer patients has been documented in major cities across USA, Canada, Europe, Nigeria, and Saudi Arabia. These studies suggest that cancer patients on chemotherapy simultaneously use CAMs. In Ireland, there have been two studies in pediatric cancer centers that have documented the use of CAM. To our knowledge, no study has examined the use of CAM in the adult population in Ireland.

## **Method:**

A cross-sectional survey was conducted at a single adult cancer center over a three-week period. The survey was offered to all oncology and hematology patients attending the medical day unit.

## **Results:**

The survey was completed by 81 patients, 51 of them were females (63%). The majority (93.8%) of the patients in our sample were in the age range of 41-80.

47 (58%) of the patients reported using CAM concurrently with conventional chemotherapy. The average cost of CAM was under €20 per month, but five patients (6.2%) spent over €100 per month. The major reasons for taking CAM were to enhance quality of life (23.5%), improve psychological/emotional wellbeing (17.3%), improve immunity (16%), relieve side effects of cancer (9.9%), relieve side effects of treatment (8.6%), and to directly treat/cure cancer (2.5%). Patients using CAM reported their source of information as healthcare professionals (30.9%), family/friends (19.8%), media (13.6%), and CAM practitioners (2.5%). Out of 81 patients, only 27 (33.3%) discussed the use of CAM with a healthcare professional involved in their care, of which 18.2% asked regarding interactions with the conventional therapy, 18.2% asked regarding CAM effectiveness and the type to use, 16.7% asked advice whether to pursue it, and 15.2% asked regarding safety of CAM. From the 26 patients using CAM who did not discuss with HCP the reasons cited were that they were never asked by the HCP (25.9%), did not think it was important to discuss with the HCP (13.0%), and 61.1% did not specify their reason.

Of the 81 patients, 18 consumed herbal products (13.6% green tea, 8.6% flax seed, 3.7% evening primrose, 2.5% soy supplements), 34 used dietary supplements (28.4% vitamins, 12.3% minerals, 3.7% fish oils), and 21 used other CAMs (8.6% massage, 7.4% meditation/mind-body technique, 7.4% acupuncture, 6.2% reflexology, 2.5% reiki).

## **Conclusion:**

The use of CAM in adult cancer patients has not been well documented in Ireland. As demonstrated from the pilot study, adult cancer patients in Ireland do seek out CAMs when simultaneously receiving chemotherapy, highlighting the importance for physicians to explicitly ask all patients regarding their intentions of CAM in order to provide safe and evidence-based options. This cancer center appeared to not have patients pursuing ayurvedic or Chinese medicine. However, similar conclusions cannot be made for other urban centers with more diverse population mixes with differing cultural experiences and attitudes to CAM.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-14-06

Prioritization of patient reported outcomes by women with metastatic breast cancer

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**Introduction:** An emphasis on patient-centered care has led to a growing interest in collecting patient-reported outcomes (PROs) in the setting of cancer care. Routine collection of actionable PROs has been shown to improve patient satisfaction with care and even prolong survival. However, completion rates of PROs outside of the research setting are low, which may be due to an incomplete understanding of the outcomes patients value most. Prior work has focused primarily on symptom burden, but patients are also affected by disease and treatment across multiple domains (e.g. physical, psychological, social, and financial). To address this knowledge gap, we conducted a qualitative study among women with metastatic breast cancer (MBC) to identify the optimal patient-centered approach to collecting PRO data.

**Methods:** We conducted 1-on-1 interviews with patients who had started a treatment regimen for MBC within the past 6 weeks at the Breast Center at Smilow Cancer Hospital of Yale New Haven Hospital to determine which PROs were most personally relevant. We assessed heterogeneity across patients in their prioritization. Patients were asked which of a list of six PRO domains they would like their provider to have information about and then ranked the domains by order of importance (from most to least important). The following domains were created from the NCCN Distress Thermometer: physical well-being, emotional well-being, treatment burden, functional status, financial concerns, and social well-being. For each ranked domain, patients were asked to rank items within the domain using a card sorting exercise where the number of items ranged from 5 to 15. Patients were then asked where and how often they preferred to report PROs.

**Results:** Ten women with MBC completed the card sorting exercise: mean age was 58 years (+/- 12), 7 were white, 2 African American and 1 Asian; 1 identified as Hispanic. After 10 interviews, it was apparent that no single set of domain rankings was common across patients. Patient prioritization of PRO domains was unique and varied. Selection and prioritization of PRO domains and items within each domain were unique and varied. Five women reported "physical well-being" as the most important domain; treatment burden and emotional well-being were also selected as most important or ranked as highly important. Participants preferred reporting MBC PROs while in the waiting room for all domains except emotional well-being (from home was the preference). However, participants were willing to complete PRO assessment in the waiting room for about ten minutes and at home for twenty minutes.

**Conclusion:** Substantial variation exists in how women with MBC rate the importance of specific PRO domains and items within each domain. Importantly, "physical symptoms" was not the top concern for half of the interviewed patients. This is an important finding, given that previous published studies of patient-reported outcomes have focused on one domain, such as symptoms and side effects or the financial burden of treatment. Our findings support the development of multi-dimensional tools for the collection of PROs. Although toxicity and physical symptoms are of utmost concern, clinicians should not neglect other dimensions of quality of life in women with MBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-14-07

Accuracy of psychosocial assessments in an online surgical decision aid developed for early breast cancer patients with resource and educational constraints

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**Background:** Women with early breast cancer routinely face a choice between breast conservation therapy and mastectomy, and assume agency through shared decision making. However, for women with lower socioeconomic power or education, barriers such as access to understandable information, involvement of family in decision making, and a decreased sense of autonomy inhibits this agency. To better empower this population, a simple to understand, online, self-administered, conjoint analysis based decision aid called “Navya Patient Preference Tool” (PPT) is developed to be used outside the physician encounter. PPT is unique in its incorporation of several psychological scales that assess potential confounders of participation in shared decision making.

**Methodology:** This is a pre-planned analysis of the reliability and validity of the psychological scales used in all three arms of an IRB approved randomized controlled trial to assess PPT. Women with operable node negative breast cancer eligible for BCT or MRM at one of Asia's largest academic tertiary cancer centers were eligible. PPT trial consists of an initial conjoint analysis questionnaire analyzing implicit preferences for breast conservation given to the intervention arms. The following psychological scales were given to all patients regardless of randomization: Autonomy Preference Index (API), Traditional-Egalitarian Gender Roles (TEGR), Caregiving Role, Brief Resiliency Scale (BRS), Appearances Scale, and Decisional Conflict Scale (DCS). Cronbach's alpha as a measure of internal reliability for all scales, and correlations of scores with known demographic trends as a measure of external validity are calculated.

**Results:** Of the 102 patients enrolled, 30 completed PPT in English, 39 in Hindi, and 33 in Marathi, (vernaculars). 69/102 were in middle and lower socioeconomic groups (Kuppuswamy Index). 53/102 had completed less than high school education. Internal reliability of all scales were high, with Cronbach's alpha above 0.7: API 0.74, TEGR 0.78, Caregiving 0.7, BRS 0.7, Appearance 0.84. DCS was highly reliable at 0.91, and is the primary outcome measure for the RCT. Correlations in the dataset met those expected in real world data, suggesting external validity. For e.g., education was inversely correlated with traditional gender roles on TEGR ( $R = -0.4$ ,  $p < 0.01$ ), and positively correlated with resilience on BRS ( $R = 0.228$ ,  $p < 0.05$ ). Individual scale items that are unrealistic were not chosen by any of the 102 respondents (e.g., My doctor should not participate in my medical decisions), substantiating nuanced reading. 85% of patients “Strongly Agreed” on a 1-5 Likert scale that “The survey questions were easy to understand” (mean score 1.18/5. SD 0.4).

**Conclusions:** Women with limited education and low socioeconomic status complete the online, self administered PPT outside of a physician encounter, with high internal reliability and external validity. Decision Aids such as Navya PPT, which account for psychosocial confounders of agency, have the potential to benefit women otherwise marginalized from shared decision making.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-14-08

Psychosocial needs identified by the healthcare team in Latino breast cancer patients

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Psychological consequences in patients with BC. According to the literature, it is pertinent to classify the consequences in: a) physical, (which will be divided according to the type of treatment); b) sexual; c) psychological (that contemplate cognitive and emotional aspects); and d) social (including work or professional conditions, family and couple life).

Methodology. A cross sectional design was used. A questionnaire was sent in electronic format to a group of 3,000 health professionals from different disciplines throughout Mexico who care for breast cancer patients in public and private health services. 256 health professionals responded ( $46 \pm 11.4$  years, (55% female). The highest academic level achieved was subspecialty (46%), specialty (22%), master's degree (14%), undergraduate (11%) and doctorate (7%).

Instrument: The survey consists of 34 items in sections that assess psychosocial aspects, physical symptoms, disability, intensity of symptoms, psychosocial needs of patients, effects on the medical treatment and available mental health services.

Results. Health professionals dedicate ( $31.6 \pm 11.4$ ) hours per week to see patients and attend ( $46.4 \pm 42.2$ ) patients per week. They consider that the presence of emotional problems in patients (69.3% average) can affect the quality of life (66%), adherence to treatment (42%), severity of side effects (40%), mortality (33%), and cause communication problems with family and healthcare team (33 to 39%). Additionally, 64% of patients with cancer have depression and 71% anxiety. Mental health professionals see, in average 61% of patients with cancer problems.

Only 8% of health professionals use a validated instrument to detect emotional distress and 72% do so through questions. Up to 20% of them wait for patients to tell them directly at outpatient clinics or in hospitalization. The main physical symptoms reported that professionals identify in cancer patients are: fatigue (31%), appetite problems (23%), pain (20%), sleep (19%) and nausea (18%).

The main needs to be met in patients with cancer are: information about their diagnosis (66%), emotional support (30%), information about treatment (34%), or prognosis (27%). When they identify mental health problems, health professionals refer their patients to psycho-oncology (55%), psychiatry (37%) or social work (21%). The most frequent disciplines of health professionals were: surgical oncology (24%), oncological gynecology (9%) and only 3% were psychologists. 32% of healthcare professionals had 20 or more years since graduation, or were between 10 and 19 years of graduation (31%), while 38% had 9 or less years since graduation. The main setting was general hospital (33%) or oncological hospital or clinic (32%).

Conclusions. Healthcare professionals who treat patients with BC have a high level of preparedness; However, a third of them had more than 20 years since graduation, which suggests specific needs for updating, especially in the identification of psychosocial issues and timely referral for emotional management. On the other hand, there is a very low prevalence of psychologists specializing in psycho-oncology, who receive the greatest burden of internal referrals from health professionals.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-14-09

Integration of primary care practitioners in a breast multidisciplinary team meeting- A pilot study

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Primary care practitioners are key to integrated patient care, as they often have an established relationship with patient and family, with knowledge of comorbidities and psychosocial factors. They can encourage compliance with cancer treatments and manage transition from acute care through survivorship.

Communication with primary care however has often been unsatisfactory, with information not helpful to that practitioner or not provided in a timely manner.

We recruited a general practitioner (GP) to attend the weekly breast cancer multidisciplinary meeting, with a responsibility to make contact with the primary care practitioner of patients presented prior to the meeting, so that relevant information from a primary care perspective could be taken into consideration for team deliberations. This also meant that the primary doctor was aware the patient was being discussed. Recommendations were fed back to that doctor after the meeting so that information was timely, and, being given by a peer, more useful.

The GP attended 12 consecutive MDTs where 105 patients were discussed. In 55% contact was able to be made with the patient's GP prior to the meeting. This was perceived as not usually changing management plans, but reinforced known medical and social concerns. Feedback to the patients GP was regarded favourably, with reported greater confidence in managing the patient, particularly those with complex needs, as well as improving hospital to community communication in general.

This pilot has shown that it is feasible to include primary care practitioners in a multidisciplinary meeting and improve interdisciplinary communication.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-15-01

The use of 18F-FDG PET/CT as an initial staging procedure for stage II-III breast cancer reduces false positives, costs, and time to treatment: A multicenter value analysis in the I-SPY2 trial

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**Introduction:** Diagnostic metastatic staging imaging (SI) for asymptomatic stage I-II patients (pts) is not routinely recommended, but is warranted in stage II-III pts with high risk biological subtypes, where previous trials have shown up to a 15% rate of de novo metastatic disease. NCCN guidelines endorse CT CAP and bone scan (STD) for stage III pts, but not PET/CT, and PET/CT is not covered in most parts of the country. We present data on the performance and value of PET/CT.

**Methods:** Data were available for 799 high risk clinical stage II-III pts screened for I-SPY2 at UCSF, Uminn, UAB, and Georgetown. Of these, 564 pts ranging in age from 25-81 (median = 48) had complete records that were retrospectively reviewed for SI and potential false positives (FP), defined as incidental findings on SI proven benign by subsequent workup. Economic evaluation was conducted from the payer perspective using the mean national 2018 Medicare Physician Fee Schedule and representative costs from the UCSF billing department. The incremental cost effectiveness ratio (ICER) measured the cost of using PET/CT per percent patient (pt) who avoided a FP.

**Results:** The rate of de novo metastatic disease was 4.8% (38/799), range 3.6-6.4%. Of the 564 pts with complete records, diagnostic SI varied significantly among the four sites ( $p < 0.0001$ ). STD was used for most pts at UAB (92.8%, 141/152) and Georgetown (85.7%, 54/63), while PET/CT was used for most pts at UCSF (86.6%, 226/261) and Uminn (63.6%, 56/88). Chest X-ray was used for 29.5% (26/88) at Uminn. There were significantly more pts with FP in the group that received STD (22.1%, 51/231) vs. PET/CT (11.1%, 33/298) ( $p < 0.05$ ). Mean time between incidental finding on SI to determination of FP was 10.8 days. When controlling for institution, mean time from cancer diagnosis to initiation of neoadjuvant chemotherapy was significantly different between STD (44.3 days) and PET/CT (37.5 days) groups ( $p < 0.05$ ). When aggregating the four sites using mean costs from the 2018 Medicare Physician Fee Schedule, the mean cost/pt was \$1132 for STD vs. \$1477 for PET/CT. The mean increase in price from baseline SI costs due to FP workup was \$216 (23.6%) for STD vs. \$65 (4.6%) for PET/CT. The ICER was \$31 per percent pt who avoided a FP. When analyzing UCSF pts alone using representative reimbursements from Medicare, the mean cost/pt was \$1236 for STD vs. \$1081 for PET/CT; using representative reimbursements from Anthem Blue Cross, the mean cost/pt was \$3080 for STD vs. \$1662 for PET/CT. The ICERs were -\$10 and -\$95 per percent pt who avoided a FP, respectively.

**Conclusion:** As compared to STD metastatic staging workup, PET/CT added value by decreasing FP two-fold. This reduced direct costs of FP workup procedures that took a mean time of 10.8 days to resolve. PET/CT also accelerated treatment start. Reducing the chance of FP workup for metastatic disease is of enormous value to pts. Our data establish the value of PET/CT for staging in our high risk clinical stage II-III trial population and highlight the need for alignment between hospital pricing strategies and payer coverage policies in order to deliver high value care to pts.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-15-02

Evaluating the cost of endocrine therapy vs. radiation therapy alone for low risk hormone positive early stage breast cancer in elderly patients

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**Objective:** Elderly patients with low-risk hormone-positive breast cancer are at risk of over treatment. Avoidance of radiation therapy (RT) in favor of endocrine therapy alone was first heralded as the optimal conservative strategy due to logistical simplicity, low acute sequelae and a reduction of contralateral cancers not seen with RT. However, long-term use of aromatase inhibitors (AI) is not without costs and morbidity, often leading to low compliance and notable late effects. We therefore performed a cost-effectiveness analysis to compare the outcomes and costs between AI for five years without RT versus hypofractionated RT alone without endocrine therapy.

**Materials and Methods:** Using data from available phase III trials and meta-analyses, we constructed a patient-level microsimulation Markov decision model to replicate the comparative outcomes between the strategies above from the societal perspective among 200,000 simulated patients. Five years of anastrozole was compared to a 15-fraction hypofractionated whole breast RT course without boost in a cohort of patients with low-risk disease as defined by CALGB 9343 entry criteria. Noncompliance with AI was modeled from recent population-based data. Relative effectiveness on ipsilateral breast tumor recurrence and contralateral breast cancers were based off the NSABP B-21 trial, adjusted to match the modern outcomes demonstrated in CALGB 9343 and PRIME II with further adjustment for AI over tamoxifen (ATAC, EBCTCG meta-analysis). Indirect costs of travel were accounted for, as were the costs of common and serious side-effects from RT (dermatitis, fibrosis, second malignancy, heart disease) and AI (arthralgia, hot flashes, osteopenia, fracture, thrombosis). A 1-year cycle time and lifetime horizon were used, with all costs adjusted to 2018 US dollars and extracted primarily from Medicare reimbursement data. The primary measure of efficacy was the quality-adjusted life-year (QALY) with age-adjusted utilities extracted from the literature. Half-cycle correction and a 3% discount rate were applied. Probabilistic sensitivity analysis was used to vary all parameters simultaneously.

**Results:** On average, RT was approximately \$3,981 more expensive than endocrine therapy over the lifetime horizon. Under a number of assumptions, RT appeared similar in long-term effectiveness to AI therapy, with a difference of less than 0.03 quality-adjusted life years. Given the low value of the denominator in the incremental cost-effectiveness ratio (ICER), RT did not meet the formally defined \$100,000/QALY threshold. On one-way sensitivity analysis, the ICER was particularly sensitive to the incidence and impact of salvage strategies for recurrence, treatment of contralateral breast cancers, cardiac events and fracture rates.

**Conclusions:** Modeling with the available evidence suggests it is likely that quality-of-life after RT-alone is nearly identical to an AI-alone strategy but associated with a small increase in cost. These results suggest select patients at risk of noncompliance can safely be treated with RT-alone rather than AI alone. Given the relative pros and cons of each strategy, RT-alone should be considered for select elderly low-risk breast patients.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-15-03

Imaging is not indicated in staging of asymptomatic patients with early breast cancer – Are we following current recommendations?

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Breast cancer is the most common malignancy in women with estimated care costs of \$20.50 billion/year by 2020. In 2012, ASCO released the Choosing Wisely Initiative which recommended against the use of routine staging imaging in patients with newly diagnosed early stage breast cancer. We examined physician's adherence rate and factors associated with non-adherence to current guidelines in patients with early stage breast cancer treated within a large urban health care system.

We identified all women with stage I-II breast cancer diagnosed between January 1, 2014 and December 31, 2015 from the Cancer Registry of the Mount Sinai Health System. Patients with history of prior malignancy or symptom-triggered imaging were excluded. Demographic, clinical and treatment related factors were collected. Medical records were reviewed to identify patients who had routine staging scans. Data of initial and follow-up imaging over 1-year period were collected. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated from logistic regression models.

Among 917 breast cancer patients, the median age at diagnosis was 59 years (range 26-98). One hundred seventy one patients (18.6%) had routine staging imaging with a mean number of initial scans of 1.48. Eighty-two patients (48%) had at least 1 subsequent scan in the 1-year follow up (range 1-4 scans/year). PET/CT was the most frequent modality (49%), followed by CT scan (33%). The medical oncologist was the ordering provider in 50.3% of the cases and surgical oncologist in 43.2%. Routine staging scans identified no cases of metastatic disease. False-positive findings were identified in 49.7% and incidental findings in 9.3% of cases. Total cost of imaging in this group was \$3990/patient. Young age (<50 years old), tumor size >2cm, positive lymph nodes, and triple negative disease were associated with presence of routine staging scans on univariate and multivariate analysis (Table 1).

Our study highlights the prevalence of unnecessary staging scans in up to 18.6% of patients with early stage I-II breast cancer. Routine imaging resulted in increased radiation exposure, multiple subsequent imaging, and increased economical burden particularly for those of young age, T2 tumors, positive lymph nodes, and triple negative disease. Further educational efforts are needed to avoid unnecessary scans in patients with early stage breast cancer and improve high-value practices among medical and surgical oncologists.

Factors associated with routine staging scans in early breast cancer

	OR	95% CI	p-value
<b>Univariate Analysis:</b>			
Age < 50	1.69	1.17-2.42	0.005
T2 tumors	4.35	3.01-6.28	<0.0001
Positive lymph nodes	5.07	3.43-7.51	<0.0001
Triple negative disease	2.94	1.86-4.63	<0.0001
<b>Multivariate Analysis:</b>			
Age < 50	1.57	1.03-2.39	0.037
T2 tumors	3.47	2.34 -5.17	<0.0001
Positive lymph nodes	4.04	2.64-6.18	<0.0001
Triple negative disease	2.99	1.76-5.05	<0.0001

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-15-04

Oncotype DX cost effectivity to a Brazilian public hospital

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**Introduction:** The Oncotype DX was associated to 14% of adjuvant chemotherapy administration to Hormonal Receptor positive (HR+) and HER2 negative, T1N0 or T2N0 breast cancer patients at Tailor X trial recently published (RS>25)

**Objective:** To describe the adjuvant chemotherapy administration to a Brazilian public hospital for HR+ HER2 negative, T1N0 or T2N0 breast cancer patients. And the estimate the cost effectivity of Oncotype DX in our low income scenary.

**Materials and Methods:** This retrospective cross-sectional study was conducted at the Oncology Division of the Women's Hospital - CAISM of the State University of Campinas (UNICAMP), Brazil. All patient data were found from the hospital records from 2007 to 2009. It was included T1N0 and T2N0 HR+/HER2 negative breast cancer patients. Patients submitted to neoadjuvant treatment were excluded.

We calculate the final cost of different types of chemotherapy used and the potential impact to oncotype DX introduction in this scenary.

**Results:** It was found 109 patients records. 66% (72/109) had received adjuvant chemotherapy. 35% (38/109) had AC (X6), 29% (32/109) had CMF (X6) and 2% (2/109) had AC-T (X4). The total cost for chemotherapy scheme were; AC (X6) US\$ 346,9; CMF (X6), US\$300,6; ACT (X4), US\$395,9. The total cost of chemotherapy was US\$ 23.596,83 to 72 patients. If we consider 14% (15/109) of adjuvant chemotherapy associate to a Oncotype DX use (Tailor X RS>25), It would reduce adjuvant chemotherapy administration to 15 patients. The chemotherapy cost would be US\$ 4588,27. In our scenary, It could save US\$ 19.008,56. Nevertheless, the Oncotype Dx cost to Brasil is US\$ 3.200,00 for each test. To 109 patients the total cost would be (109 X US\$ 3.200,00) US\$ 348.800,00. Therefore, the total cost for Oncotype DX program plus adjuvant chemotherapy for our patients would be US\$ 348.800,00 + US\$ 4.588,27= US\$ 353.388,27. While in the real situation we had spent US\$ 23.596,83. The total estimate cost would be 15 times more.

**Conclusion:** At the moment, because of the assay high cost and the low cost of the adjuvant chemotherapy to HR+, HER2 negative T1N0 and T2N0, It would be difficult to consider Oncotype DX cost-effective to Brazilian public heath system. Even considering many advantages to spare chemotherapy to this population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-15-05

Long-term quality of life after four common surgical treatment pathways for breast cancer and the effect of complications

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**Background:** Comparing the quality of life (QoL) for all common breast cancer surgery options could support decision-making. However, current evidence generally consists of non-preference-based outcomes for sub-comparisons of options which are unsuitable for the calculation of Quality Adjusted Life Years (QALYs), the preferred outcome in health economics. The preference-based EQ-5D-5L is specifically designed to provide the Q in QALY and could facilitate a (health-care) wide comparison of breast cancer surgery options. This study gives an overview of both non-preference and preference-based QoL after four common surgical breast cancer treatment pathways.

**Method:** Breast cancer patients from a large multicenter observational cohort in the Netherlands were invited for participation in an online survey. QoL was obtained by the EQ-5D-5L, EORTC-QLQ-C30/-BR23 and Breast-Q questionnaires. Patient cohorts based on surgical procedure (breast-conserving surgery (BCS), mastectomy (MAS), autologous breast reconstruction (A-BR) and implant-based breast reconstruction (I-BR) were compared after propensity-weighted adjustment of pretreatment differences.

**Results:** Of all invited patients, 1871 responded, respectively patients treated with BCS (n=615), MAS (n=507), A-BR (n=330), and I-BR (n=419). Table 1 shows QoL outcomes without overlapping 95% CI. Patients reported complications in 47% of the A-BR, 32% of the I-BR, 18% BCS and 22% of the MAS groups. QoL outcomes differed statistically significantly between patients with and without complications for all groups, except the I-BR group.

Table 1: patient-reported quality of life per surgical treatment for propensity-weighted cohorts

			BCS	MAS	A-BR	I-BR
		Estimated sample size	n=434.0	n=386.3	n=178.6	n=295.5
EQ-5D-5L						
	Utility					
		Overall	0.844a	0.805b	0.849a	0.850a
		No complication	0.859a	0.878b	0.847a	0.818a,b
		Complication	0.771a	0.771a,c	0.816b,c	0.861b
Breast-Q						
	Satisfaction with breasts		65.52a	60.65b	71.29c	59.39b
	Satisfaction with outcome				75.75a	66.37b
	Psychosocial well-being		73.77a	66.50b	75.78a	71.60a
	Sexual well-being		62.70a	50.00b	63.33a	56.38c
	Physical well-being: chest		67.39a	73.47b,c	75.81c	72.64b
EORTC-QLQ-C30						
	Physical function		84.53a	82.94a	85.62a,b	87.97b
	Role function		84.35a	80.70b	84.02a,b	86.02a
	Pain		15.41a	18.93b	17.18a,b	15.89a,b
	Financial problems		5.23a	8.22b	12.30b	7.71a,b
EORTC-QLQ-BR23						

	Body image		87.45a	80.49b	82.28b	82.35b
	Sexual functioning		27.90a	27.51a	31.95a,b	33.35b
	Sexual enjoyment		57.03a	54.82a	64.24b	63.80b
	Breast symptoms		13.45a	9.94b	8.79b	10.82a
	Arm symptoms		12.68a	17.12b	18.18b	16.82d

Mean values of the respective outcome scale after propensity score weighting and are derived from an estimator of the average treatment effect on the population. Values in the same row not sharing the same subscript do not show overlapping 95% Confidence Intervals in a pairwise comparison.

**Discussion:** Preference-based QoL outcomes are an essential input for cost-effectiveness analyses, used to justify the use of care in a society progressively confronted with increasing health care expenses. The results from this large multicenter cohort study contribute substantially to the current knowledge in the field of breast cancer reconstructive surgery.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-15-06

Potential Medicare beneficiary out-of-pocket cost reductions through use of biosimilar filgrastim-sndz over reference filgrastim among breast cancer patients: A simulation model analysis

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**Rationale & Objective:** Granulocyte colony-stimulating factors (G-CSFs) are utilized to decrease the incidence of febrile neutropenia (FN) in patients with cancers undergoing chemotherapy treatments. In 2015 biosimilar filgrastim-sndz was the first biosimilar to be approved and launched in the US market. Limited data exists in ascertaining the impact of biosimilars on patient out-of-pocket (OOP) expenditures. The objective of this simulation model was to estimate potential OOP cost savings through use of filgrastim-sndz over reference filgrastim from a Medicare breast cancer patient perspective.

**Methods:** An Excel simulation analysis was conducted among breast cancer patients treated with biosimilar filgrastim-sndz or the branded reference filgrastim (identified through HCPCS codes). Data from the 2016 Medicare Limited Data Set (5% sample of the carrier file) was used to populate the model. The payment calculation worksheet within the Medicare carrier file was used to calculate the average Medicare payment to the provider and the average beneficiary OOP responsibility per claim of either filgrastim-sndz or reference filgrastim. The average OOP reduction per claim for a filgrastim-sndz beneficiary relative to a reference filgrastim beneficiary was multiplied to a hypothetical FN prevalent population of 100,000 beneficiaries (average of 10 claims per beneficiary) to estimate the potential OOP savings.

**Results:** Data for 616 filgrastim-sndz and 1,064 reference filgrastim claims were used to populate the model. The average Medicare allowed charge amount per claim for a filgrastim-sndz beneficiary was \$362.8 versus \$406.9 for a reference filgrastim beneficiary, while corresponding average Medicare payments to the provider were \$284.1 and \$316.9, respectively. On an average, OOP responsibility for a filgrastim-sndz beneficiary was lower compared to a reference filgrastim beneficiary (\$72.9 versus \$82.5) leading to a cost saving per claim of \$9.60. When extrapolated to 100,000 beneficiaries (1,000,000 claims), the overall cost saving was projected to be around \$9.6 million.

**Conclusions:** Our simulation model estimated a potential OOP Medicare breast cancer beneficiary saving of around \$9.6 million, based on a hypothetical population of 100,000 FN beneficiaries, with the use of biosimilar filgrastim-sndz over reference filgrastim. Further real-world analyses are required to evaluate the true cost saving potential from a breast cancer patient perspective with the use of biosimilars over reference biologics.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-15-07

Breast surgery – Is routine 'group & save' required? A quality improvement project

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## Introduction

Breast surgery, especially for malignant disease, is not associated with significant blood loss and therefore rarely requires blood transfusions. Pre-operative protocols in our hospital and in most other centers routinely require a blood group screening process (group and save) for elective breast cancer surgery. The process of group and save (G&S) in our hospital requires two blood samples to be drawn with at least 30 minute interval in-between. It takes at least 40 minutes for the samples to get processed and the cost for each test is £2.12. The test is valid for a period of 5 days after which time it needs to be repeated. This process in busy hospitals is clearly time consuming, results in delays and is inconvenient for patients and medical staff.

The aim of this study was to ascertain the number G&S performed and rate of post-operative blood transfusion for patients undergoing surgery for malignant breast disease. This was to establish if mandatory pre-operative G&S is necessary in all cases, and if the number of tests performed can be reduced. The second part of the study was to analyze the outcome after implementation of changes to reduce the number of G&S tests.

## Method

Retrospective review of patient records who had surgeries for malignant breast disease from December 2015 to November 2016. Exclusion criteria included benign disease, flap reconstruction and breast surgery combined with another major procedure. Following the initial analysis, it was proposed that only the operating surgeon will request the test and that it will not be routinely requested by the pre-op assessment team. A re-audit was done prospectively to study the outcome of change in practice and close the audit loop.

## Results

151 cases were eligible for review. 97.35% (147 patients) had G&S, and the post-operative transfusion rate was 1.32% (2 patients). Following a change in protocol a significant fall in the testing (97.35% to 35.5%) was seen and there were no post-operative blood transfusions in this period.

## Conclusion

This simple yet important audit showed that routine G&S did not contribute to the patient management, increased cost of treatment, caused patient inconvenience and consumed hospital personal time. The changes we implemented significantly reduced the frequency of this test and was cost effective to the trust. This study has now been converted into a Quality Improvement Project and through this we will continue to improve the results further. This project has also stimulated an interest in the General Surgery department, and other units are now considering changing their practice based on our results.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-15-08

Economic Impact of MammaPrint (70-gene signature) in a clinical high risk population: A 10yr Markov model, 6,000-patient retrospective analysis of US claim data

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**Background:** MINDACT provided evidence that MammaPrint (MP) can identify patients (pts) with early-stage breast cancer (ESBC) who can safely forego adjuvant chemotherapy (CT). As a result, 46% of clinicopathologically high-risk pts were spared from unnecessary CT toxicities. This study investigates direct and indirect costs for pts and payers during ESBC treatment regimens. The study objective was to quantify average cost of care and savings in a clinical high-risk group where the value of CT is unclear, and MP demonstrates economic and clinical impact. **Methods:** Risk assessment was based on 6,000 MP tests performed on BCBS members (2016-17). CT claims were retrospectively analyzed utilizing a Blue Cross Blue Shield (BCBS) member database (January-December 2016). Case data was restricted to HR+HER2- ESBC and filtered by authorization for the MP test. All CT claims were including, but not limited to standard of care (SOC) systemic drugs such as cyclophosphamide, docetaxel, paclitaxel and doxorubicin. HCPCS codes were utilized to filter member CT claims. Exclusion criteria: <3 SOC CT claims and previous history of BC. A hybrid decision tree-Markov model was used to estimate cost effectiveness of MP compared to modified Adjuvant Online (mAOL) from a US healthcare payer perspective over a 10yr timeframe. Overall, distant metastasis free survival, and test-utility scores were collected from MINDACT for HR+HER2- pts, and costs derived from the BCBS registry and literature (2016 US dollars). Two scenarios were modelled (1) all women classified as high-risk received CT plus endocrine therapy (ET) while low-risk pts received ET; (2) MP guided treatment was modelled based on the IMPACT study (91% MP Low Risk pts received ET and 83% MP High Risk pts received CT). Outcome measures were quality-adjusted life years (QALYs), and incremental cost-effectiveness ratios (ICERs). **Results:** The majority, 3,540 (59.0%) pts were Low Risk or could safely forgo CT; 2,460 (41.0%) were High Risk. The primary driver for cost differences was whether all services were performed in a physician office (\$4.6M) or an outpatient setting (\$2M). Including provider settings (inpatient, outpatient, emergency), SOC CT regimens and related services the final average amount authorized was \$39,675 per patient. In the first scenario, MP guided treatment was associated with a 0.06 QALY gain (0.05 in scenario 2) compared to mAOL. For cost savings and QALY gain MP was dominant over mAOL in both scenarios. Budget impact analysis estimated cost savings of 88.2- 96.6 million dollars based on BCBS registry data. Given the new cases of BC that are expected to be diagnosed in women in the U.S. annually, pts covered in this population by BCBS (~30%) and MP eligible cohort (~36K pts), projected cost savings are estimated at 529.2-644.3 million dollars per annum for the health insurer. **Conclusions:** CT was associated with significant annual costs from both patient and payer perspectives. As demonstrated in MINDACT, less treatment for some pts is optimal without risk to safety or survival. These findings suggest that MP is cost effective and provides substantial value by sparing pts from toxicities, both therapeutic and financial.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-15-09

Impact of oncotype dx genetic signature used in early breast cancer. Clinical and economic analysis of a 110 patient cohort treated in the Catalan Oncologic Institute (ICO), Spain

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## Introduction

Benefit from adjuvant chemotherapy (CT) is doubtful in a high percentage of patients with early breast cancer. The 21-gene recurrence-score (RS) assay (Oncotype DX, Genomic Health) is one gene-expression assay that provides prognostic and predictive information in hormone-receptor (RH) positive breast cancer. The results of the TAILORx study have confirmed that the majority of patients with tumors RH + and HER2 negative can avoid CT without increasing their risk of relapse. From 2012 to 2015 we used Mammprint (MMP), in our institution and 60% of cases could avoid CT (communicated in SABCS 2015). Since 2017 we use RS for this purpose.

## Primary Objective

To analyze the impact of using RS to change the indication of adjuvant CT.

## Secondary Objectives

To analyze the association between different clinical pathological factors and the RS value, and calculate the difference between the cost of all RS test and the cost in direct expense of the treatment with CT of all patients who could avoid it thanks to the RS

## Material and methods

We analyzed all RS test performed in the three ICO centers during 2017. We sent 112 tumor samples; in 2 samples adequate RNA for RS was not obtained. We compared the adjuvant treatment initially planned according to institutional treatment protocol with the treatment given after RS. We compared the direct economic costs of CT with the costs of the diagnostic test, and performed a logistic regression analysis of some pathological factors and RS value.

## Results

The RS could be determined in 110 of 112 cases, in which there was indication of adjuvant CT. Only 14 patients received CT (12,72%) with the RS value, so CT was avoided in 96 patients (87,28%).

The clinical-pathological characteristics of the series are summarized in the table 1. Of the risk factors analyzed, only grade 3 (p 0.001) and PR <20% (p<0.002) showed a statistically significant relationship with a higher probability of RS> 25. No association was found between age, nodal status, tumor diameter, Ki67, Infiltrating Ductal Carcinoma vs neither Infiltrating Lobular Carcinoma nor Lympho-Vascular invasion.

The cost of the genetic studies was 180000€ (1636€ each). The cost of each CT schedule (EC x 4 followed by paclitaxel x 12) was 7214€ and the total cost of 96 cases 692590€. Direct costs savings estimated from the reduction in CT treatment were 512590€

**Conclusion:** Our series shows that RS avoided unnecessary CT in 87% of cases and was more cost-effective than a previous series with MMP. G3 and RP <20 were the only pathological factors associated with an increased risk of RS> 25.

Table 1. Patients characteristics and clinical-pathological details from the analyzed tumors

Patient characteristics	
Age, mean (range)	53,76 (19 – 75)
≥50y	72 (65.5%)

<50y	38 (34.5%)
<b>Histology</b>	
Infiltrating ductal carcinoma	88 (80%)
Infiltrating lobular carcinoma	20 (18,2%)
Others	2 (1,8%)
<b>TNM</b>	
Tumor diameter (mm), mean (range)	19,25 (1 – 160)
pN0	58 (52,7%)
pN1mic	21 (19%)
pN1	31 (28,3%)
<b>Hormone receptors</b>	
RE 2-100%	110 (100%)
RP <20%	22 (20%)
RP ≥20%	88 (80%)
<b>Ki67 median, mean (range)</b>	20, 21 (2-75)
<b>Lymphovascular invasion</b>	
yes	9 (8%)
no	101 (92%)
<b>Adjuvant treatment</b>	
CT	14 (12,72%)
Hormonotherapy	96 (87,28%)

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-01

Is breast reconstruction safe in women over 70? An analysis of the national surgical quality improvement program (NSQIP) database

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**Background:** Less than 14% of older women undergo post-mastectomy breast reconstruction. A major reason for the low rate is the concern about post-operative complications. A thorough analysis of surgical complications by age group is limited in previous studies. The aim of this study is to determine the surgical complication rates of older women ( $\geq 70$  years old) with breast cancer who underwent breast reconstruction and compare them to younger women (18–69 years old).

**Methods:** Data from the National Surgical Quality Improvement Program (NSQIP) database were used to identify women with carcinoma in situ and invasive breast cancer who underwent delayed or immediate breast reconstruction (2005-2016). The primary outcome was 30-day post-operative surgical complications; the secondary outcome was 30-day mortality. Patient demographics, comorbidities, and 30-day postoperative complications and mortality rates were compared across age groups and each type of reconstruction.

**Results:** Of 42,929 women who underwent breast reconstruction, 2,615 (6.1%) were older women. Although compared to young women, older women were more likely to have medical comorbidities their American Society of Anesthesiologists' (ASA) classification was lower. Tumor histology distribution was similar in both groups. Lymph node surgery and neoadjuvant chemotherapy was significantly less frequent among older women. Compared to young women, older women more frequently underwent immediate breast reconstruction (IBR) [n=2,405 (92%) versus n=33,580 (88.3%),  $p < 0.0001$ ] but less frequently underwent delayed breast reconstruction [n=209 (8%) versus n=4,734 (11.7%),  $p < 0.0001$ ]. Prosthesis-based reconstruction was the most common technique in both age groups. Autologous reconstruction was significantly less common amongst older women than young women [n=517 (19.8%) versus n=10,011 (24.8%),  $p < 0.0001$ ]. Older women experienced higher rates of superficial surgical site infection (SSI) [n=69 (2.6%) versus n=716, (1.8%),  $p = 0.002$ ] and urinary tract infection [n=15 (0.6%) versus n=101 (0.3%)  $p = 0.005$ ]. However, the rates of deep SSI, dehiscence, pneumonia, thromboembolism, renal complications, cardiac events, and sepsis were similar between both groups. Older women had significantly lower rates of events of bleeding requiring transfusion [n=27 (1%) versus n=736 (1.8%),  $p = 0.002$ ] and flap failure [n=2 (0.4%) versus n=210 (2.1%),  $p = 0.006$ ]. Return to the operating room within 30-days was similar between older and young women [n=171 (6.5%) versus n=2,821 (7.0%),  $p = 0.4$ ]. Thirty-day deaths were rare events [older n=3 (0.1%) and young n=10 (0.02%),  $p = 0.05$ ].

**Conclusions:** Overall, 30-day postoperative complications in older women who undergo breast reconstruction are extremely low. Infection rates were slightly higher in the older group however; severe complications such as flap failure, bleeding, reoperation, and death were more common in young women. Age alone did not confer an increased risk of complications after breast reconstruction. Breast reconstruction can be safely offered to older women undergoing breast cancer treatment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-02

Enhance post-lumpectomy breast contour using oncoplastic surgery (OPS) plus a bioabsorbable 3-D tissue implant

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**Introduction:** The appearance after breast surgery has become an important aspect of survivorship. The post lumpectomy/post radiation hollow surgical defect negatively impacts cosmesis and patient satisfaction. Oncoplastic procedures will mobilize surrounding tissues into the lumpectomy cavity but adds no volume to the breast. Use of a bioabsorbable 3-dimensional tissue implant (used for targeting radiation) has the additional benefit of adding volume to the breast and enhances the overall cosmetic appearance. Our experience over 3 years provides serial mammograms from which we may objectively categorize cosmetic contour. We report on our 2 and 3 year serial images of our treated patients compared with baseline.

**Methods:** Between May 2014 and June 2018, during lumpectomy for breast cancer we implanted a 3-D tissue implant marker in 170 patients, often combined with oncoplastic reconstruction and followed by radiation treatment. For long term follow-up we had 37 patients with serial mammograms at 2 or 3 years to assess cosmesis. All patients had interviews, physical exams, and serial mammograms to evaluate their cosmetic appearance. Both physician and patient graded their appearance. We also objectively measured and compared the pre-treatment mammogram and the 2-year and 3-year, post-treatment mammogram for symmetry and size using each breast as its own control. Using the post-treatment mammograms, we compared the relative anterior-posterior (depth) measurement of the quadrant bearing the implant as well as the non-cancer quadrant to the similar locations of the pre-treatment mammogram. Both mammogram positioning and radiation effects would balance. We compared the relative change from baseline in the non-cancer portion of the breast to the change from baseline in the cancer portion of the breast as a percent difference from baseline.

**Results:** Patients were treated with lumpectomy, oncoplastic reconstruction, and placement of a 3-D tissue implant. Three implants were removed due to positive margins. No implants were removed for any other reason. There have been no local recurrences. Overall, radiation oncologists felt the 3-D implant was useful for treatment planning in 85% of patients. Of the 37 consecutive patients who have completed an average of 27.8 months of follow-up, cosmesis was rated as excellent/good by clinicians (96%) and patients (94%). Mammograms taken at 2-3 years were compared with initial images. Whole-breast radiation effect varied among patients. Some had significant shrinkage while others had none. These changes were equal in the non-cancer post-radiation quadrants (86.2% vs 87%) demonstrating maintenance of normal breast contour. Our use of the 3-D implant and oncoplastic tissue advancement maintained the pre-operative contour of the breast after lumpectomy with radiation.

**Conclusions:** Breast cancer surgery and radiation is often complicated by poor cosmesis with retraction and volume loss. Using a combination of oncoplastic surgery combined with a 3-D tissue implant, we found the forward projection and contour of the breast at the lumpectomy site was preserved and patient satisfaction was good to excellent. Further investigation of the long-term cosmetic effects of breast cancer surgery should be encouraged.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-03

The long-term outcomes of the BROWSE multicentre cohort study comparing Strattice™-assisted implant based reconstruction and submuscular reconstruction

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## Introduction

Implant based reconstruction accounts for approximately 85% of reconstructions in the UK and 80% in the US with an increase in use of acellular dermal matrices (ADM) e.g. Strattice™. There is little long-term data on the outcomes of ADM reconstructions and its efficacy. Our aim was to establish the most comprehensive long-term surgical, cost-effectiveness, quality of life and cosmetic outcomes in three large UK reconstructive centres.

## Methods

All women who had undergone immediate implant based breast reconstruction with Strattice™ or a submuscular technique between 2009 and 2015 across three tertiary centres in the UK were invited for prospective clinical (examination and tonometry), cosmetic and quality of life assessment. An eight year retrospective review of case notes, theatre database and implant log was performed.

## Results

601 patients underwent 837 reconstructions. 589 Strattice™-assisted (331 therapeutic, 258 risk reduction) and 248 submuscular (152 therapeutic, 96 risk reduction).

Revision surgery was performed in 43% of Strattice™-assisted reconstructions and 35% of submuscular within the follow-up period ( $p=0.034$ ). Strattice™-assisted reconstructions were revised significantly sooner than submuscular, median time to first revision of 12 months vs. 21 months ( $p<0.0001$ ). There was a significant reduction in the need for revision surgery for capsular contracture in the Strattice™-assisted group (Strattice™  $n=19$ , submuscular  $n=16$ ,  $p=0.04$ ). Revision rates for capsular contracture in those having prior or adjuvant radiotherapy were 33% ( $n=9$ ) in the Strattice™-assisted group and 66% ( $n=2$ ) in the submuscular.

At a median time of 58 months from initial procedure, 10% in the Strattice™-assisted group and 14% in the submuscular had significant capsular contracture (Baker 3/4). At the time of assessment 7% of the Strattice™-assisted group and 17% of the submuscular had already undergone revision surgery for capsular contracture. Therefore, overall there was significantly more capsular contracture in the submuscular group (17% vs. 31%,  $p=0.047$ ). There was no difference between the mean (of the four quadrant readings) breast tonometry reading between the two groups (0 hard – 10 soft). The median reading was 5.3 in the Strattice™-assisted group, 5.4 in the submuscular and 6.6 in native breasts. Those with Baker 1/2 had a median reading of 5.4 compared to 4.8 in those with a Baker 3/4 capsule.

Quality of life was equivalent between the two groups at a median time of 58 months. There was no difference in median Breast Q score for satisfaction with breasts, Strattice™-assisted 62 vs. submuscular 58 or satisfaction with outcome 67 vs. 75. The mean cost of the index reconstructive procedure was less in the Strattice™-assisted group (£3634 vs. £4230) but there were no significant differences in long-term cost.

## Conclusion

Long-term clinical outcomes support the use of Strattice™ in breast reconstruction. It reduces capsular contracture and enables patients to have their surgery in one rather than two procedures. The increased revision rate in the Strattice-assisted group was multi-faceted e.g. patient request to upsize and correction of contouring defects. Strattice™ reduces healthcare cost.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-04

Evaluation of autologous fat grafting local morbidity (fat necrosis and biopsy rates) in breast reconstruction after breast cancer: A retrospective study on 257 patients in Oscar Lambret Center

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**Background.** Autologous fat grafting (AFG) is a widely used procedure in breast reconstruction after breast cancer. Indications are in constant increase but there is a lack of data about global morbidity, especially fat necrosis and management of local complications. The purpose of this study was to evaluate the complications rate in term of abnormal clinical examination or imaging and the proportion of additional explorations.

**Methods.** We retrospectively reviewed the computerized files of consecutive patients who underwent AFG for breast reconstruction after breast cancer or for preventive surgery and aesthetic sequelae after lumpectomy in the Oscar Lambret center between January 2013 and December 2016. Fat grafts were harvested with a fat trap then processed and injected according the Coleman technique. We collected demographics, operative details, local complications, incidence of palpable masses and/or suspicious breast imaging findings leading to additional explorations (breast imaging or biopsy), and locoregional cancer recurrence. Descriptive statistics were generated.

**Results.** Over a 4-year period, 257 women underwent autologous fat grafting for breast reconstruction and aesthetic sequelae after lumpectomy. Their mean age was 50 years [range 28-75], the mean BMI was 25 [range 18-44], 26% (n=66) were smoking and 74% (n=190) underwent radiotherapy. A total of 303 breasts were operated by 270 mastectomies (89%) or 33 lumpectomies (11%). The reconstruction was delayed in 63% (n=171) and the main techniques used were breast implant (44%, n=119) and autologous latissimus dorsi (31%, n=84). The mean number of fat grafting procedures was 1,9 per patient [range 1-7] with a mean volume of 181 mL [range 30-535]. The mean time interval between cancer diagnosis and first fat graft session was 56 months [range 3-285], and the follow-up ranged from 0 to 51 months (mean=16). The prevalence of donor site complications was 6% (n=16) and infections was 2% (n=5). Sixty six (25,6%) patients had a clinically palpable lesion and 54 (21%) underwent additional imagings, mostly by ultrasounds (53 patients, 98%) except the usual follow-up. Twenty one biopsies (8%) were performed and showed 16 benign results (76,2%) and 5 malignant results (23,8%) leading to 6,2% of fat necrosis and 1,9% of locoregional recurrence after AFG in our study. Tobacco (p=0.45), BMI (p=0.95), radiotherapy (p=0.56) and amount of fat grafted (p=0.09) didn't appear to be risk factors for fat necrosis.

**Conclusions.** A good knowledge of local complications by surgeons and radiologists enables to avoid systematic and repeated further imaging explorations. Multicentric, prospective studies with long term follow up and evaluation of patients reported outcomes are needed to evaluate anxiety generated by biopsies and costs generated by repeated imagings.

**Key words:** autologous fat grafting, breast cancer, local morbidity, fat necrosis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-05

Prepectoral immediate implant-based reconstruction using Braxon® acellular dermal matrix – National audit from the United Kingdom

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## Background

Implant based reconstruction is the most common method of reconstruction in the United Kingdom (UK) for women having a mastectomy for breast cancer or as a risk reducing procedure. Prepectoral reconstruction with full implant coverage using an acellular dermal matrix (ADM) – BRAXON - is a relatively new technique. Prepectoral reconstruction has the advantages of a better aesthetic outcome, less postoperative pain, quicker return to normal activities and no postoperative problems with animation. We report on the outcomes of prepectoral immediate breast reconstruction (IBR) using Braxon®ADM from a National audit.

## Methods

A retrospective multi-centre audit of all direct-to-implant reconstructions using Braxon® in the United Kingdom was carried out . The demographic details, treatment details, short-term and long-term outcomes were evaluated. Factors affecting complication rates were analysed.

## Results

Data from 406 Braxon reconstructions in 324 patients across 20 centres in the UK were collated. Mean age of the cohort was 50.48 (SD – 11.11, range – 20-82) years with a mean BMI of 26.05 (SD – 4.87, range – 18-42) kg/m<sup>2</sup>. Demographic and treatment characteristics are given in Table 1.

Patient demographics and treatment details

Characteristic	Number (Percentage)
Number of patients	324
Number of reconstructions	406
Mean Age (years)	50.48
Mean Body Mass Index (kg/m2)	26.05
Smoker / Ex-smoker	30
Indication for surgery	
Therapeutic mastectomy	241 (74.3)
Prophylactic mastectomy	83 (25.6)
Neo-adjuvant chemotherapy	55 (16.9)
Previous radiation therapy	17 (5.24)
Adjuvant radiation therapy	55 (16.9)
Adjuvant chemotherapy	55 (16.9)
Type of mastectomy	
Skin-sparing mastectomy	143 (44.13)
Nipple-sparing mastectomy	143 (44.13)
Skin-reducing mastectomy	37 (11.41)
Management of axilla	
Sentinel node biopsy	207 (63.8)
Axillary nodal clearance	54 (16.6)

No axillary surgery	63 (19.4)
Mean specimen weight (grams)	433.24
Mean implant volume (cc)	374.5
Complications	105 (32)
Implant loss	36/406 (8.87)

The mean follow-up period was 10.94 months (0.3 to 34.8 months). The overall complication rate was 32% with a readmission rate of 16% and an implant loss rate of 9%. Of the factors evaluated for their effect on complication rates, patient age ( $p = 0.005$ ), therapeutic mastectomy ( $p = 0.001$ ), specimen weight ( $p < 0.005$ ) and axillary nodal clearance ( $p = 0.006$ ) were significantly associated with higher complication rate on univariate analysis.

### **Conclusion**

Implant-based prepectoral breast reconstruction with Braxon® has satisfactory short-term and long-term operative outcomes, comparable to the National Mastectomy Audit data from the United Kingdom. Patient-reported outcomes, aesthetic outcomes and post operative pain need to be evaluated. Further studies with larger numbers of patients and longer follow-up have been planned.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-06

Prepectoral versus retropectoral implant-based breast reconstruction - The surgical and radiotherapeutical perspective

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**Background:** Subpectoral implant positioning has been the standard of care in breast reconstruction despite involving disadvantages owing to the detachment of the pectoralis major muscle such as disruption of the muscle function, animation deformities and prolonged postoperative pain. Refined ablative techniques as well as dermal matrices and synthetic mesh products have led to the reintroduction of subcutaneous implant-based breast reconstruction possibly avoiding the negative sequelae of pectoralis disinsertion.

**Objective:** The primary objective of this study was to compare procedure-related complication rates following prepectoral versus retropectoral implant-based breast reconstruction. Furthermore the effect of the implant position on the quality of post-mastectomy radiation therapy (PMRT) was analysed.

**Methods:** All patients who underwent an implant-based breast reconstruction after mastectomy at the Department of Obstetrics and Gynecology of the University Clinic of Vienna within the years 1.1.2013 to 31.12.2017 were included in the study. A retrospective chart review of the patients was conducted assessing parameters regarding the mastectomy, the reconstruction, complications following the reconstructive procedure, patient-associated risk factors and radiation treatment plans. Complication rates were analysed one week, one month and one year after the implant-based reconstructive operation.

**Results:** In total 57 patients (94 breasts) were reconstructed following a prepectoral implant-placement approach, 95 patients (149 breasts) were reconstructed with implants in a retropectoral position. The two patient cohorts did not differ significantly in the occurrence of complications including the following dehiscence, infection, seroma, secondary bleeding, necrosis, fistula, capsular contracture and rippling. No significant differences regarding reinterventions and reoperations including seroma drainage, secondary suture and reoperation following secondary hemorrhage and necrosis could be detected between the two study populations. The two surgical procedures were not associated with a different rate of implant loss.

12 (2 in the cohort of patients with prepectorally placed implants and 10 in the subgroup of patients with subpectorally positioned implants) out of 152 patients needed PMRT for oncological safety. Prepectoral versus retropectoral implant positioning did not affect breast  $D_{\text{mean}}$  or  $D_{90}$ , heart  $D_{\text{max}}$  or  $V_5$  or lung  $V_{20}$  across treatment plans.

**Conclusion:** The study demonstrated no inferior outcome regarding the occurrence of complications, reinterventions, reoperations and implant loss of prepectoral implant-based breast reconstruction compared to retropectoral implant positioning. Therefore, subcutaneous implant placement permits reconstruction of the breast with comparable procedure-related complication rates while avoiding disadvantages associated with the detachment of the pectoral muscle.

Regarding the radiation perspective both prepectoral and retropectoral implant positioning allow for optimal coverage of the chest wall with acceptable doses to the heart and lung.

## 2018 San Antonio Breast Cancer Symposium ®

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Short-term outcomes and QoL following autologous fat transfer in oncoplastic and reconstructive breast surgery

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**Background:** Breast cancer (BC) is with 30% among all cancers the most common cancer among women in Austria. Regarding therapy and management of BC, surgical treatments with resection and reconstruction play a crucial role. Breast-conserving therapy (BCT) represents a common procedure. However, mastectomy is still needed in about 30% of the patients with the diagnosis BC and as prophylactic surgery in indicated cases. Recently, autologous fat transfer (AFT, lipofilling) has been used to improve cosmesis in oncoplastic and reconstructive breast surgery. There is a gap of evidence for the oncological safety as well as the benefit for quality of life (QoL) in patients who had an AFT after the BC or prophylactic surgery.

**Material and methods:** We assessed peri- and postoperative results, oncological outcomes and QoL in 55 patients undergoing AFT after BCT or mastectomy at our department between 2013 and 2018. QoL was assessed with the EORTC QLQ C30 and QLQ-BRECON23 questionnaires.

**Results:** Overall, 55 patients (73 breasts) underwent AFT after BCT or mastectomy. 39 patients had a diagnosis of invasive BC (four bilateral) and six patients a diagnosis of DCIS. 24 mastectomies were done as prophylactic surgery. AFT was done in 22 cases with nipple sparing mastectomy (NSM), in 30 cases with skin sparing mastectomy (SSM), in 9 cases with simple mastectomy ± reconstruction and in 12 cases with BCT. The number of AFT sessions was 1-8 (mean 1.8). The injected fat volume per session was 30-390 ml. There were 10 (14%) complications: 3 hematomas (none needed reintervention), local inflammation requiring antibiotic therapy (n=3), formation of an oil cyst (n=1), and fat necrosis (n=3). In two cases of fat necrosis biopsy was performed to confirm the diagnosis. After a median follow-up of 34 months there was one recurrence (Paget's disease after BC). QoL data will be presented at the meeting.

**Conclusion:** Lipofilling after mastectomy for cancer appears to be oncologically safe. Our complication rate is relatively high, although additional surgical intervention (biopsy) was only necessary in two cases. Patients considering AFT should be informed about additional risk for biopsy, as well as other common complications.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-08

Applications of rib sparing technique in internal mammary vessels exposure of abdominal free flap breast reconstructions

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## **Background:**

Internal mammary vessels (IMVs) are widely used recipient vessels in abdominal free flaps breast reconstructions. Exposure of IMVs usually needs to resect a segment of costal cartilage or rib during the operations. The rib sparing technique is an alternative method with less damage. This study aims to analysis the applicability and advantages of rib sparing technique of IMVs exposure in breast reconstruction.

## **Methods:**

medical records of 215 patients who underwent abdominal free flap reconstruction from November 2006 to December 2017 were analyzed. The factors influencing the choice of vessels and rib sparing were analyzed. The outcomes of rib sparing were assessed. Intercostal space (ICS) width and other related data were measured by the preoperative thin slice chest computed tomography (CT) scan images.

## **Results:**

Among all 215 patients with 218 flaps, 172 flaps used IMVs as the recipient vessels while 13 used thoracodorsal vessels and 33 used subscapular vessels. The proportion of IMVs as recipient vessels showed a rising trend in general and remained over 90% for the last three years in our center. Patients with immediate reconstruction ( $p=0.005$ ) and axillary lymph nodes dissection (ALND) ( $p<0.001$ ) were less likely to use IMVs, both in univariate and multivariate logistic regression analysis. Patients' BMI and radiotherapy history showed no statistically significant differences between the IMVs group and the other vessels group ( $p=0.338$  and  $0.811$ ). The rib sparing rate in IMVs exposure increased yearly and exceeded 40% in 2013, now it maintained more than 60% during the recent 3 years. Additionally, among the patients who received rib sparing IMVs exposure in 2017, the mean ICS width was relatively smaller than that in 2013 (2.54cm V.S 2.93cm,  $p=0.124$ ). Compared with rib resection group, patients with rib sparing were higher ( $163.57 \pm 4.44$  cm vs.  $161.83 \pm 4.30$  cm,  $p=0.047$ ) and with a wider ICS ( $2.65 \pm 0.54$  cm vs.  $2.25 \pm 0.38$  cm,  $p<0.01$ ), while the depth from the surface of the pectoralis major muscle to the IMVs and distance between the parasternal line and IMVs had no difference between the two groups. Rib sparing group has a shorter surgery and hospitalization time, as well as a lower severe complication rate, but the differences were not statistically significant ( $p=0.120$ ,  $0.450$  and  $0.296$ ).

## **Conclusion:**

IMVs were used more frequently as the recipient vessels in abdominal free flap breast reconstructions, especially when axillary operation was not carried out at the same time. Rib sparing technique had the potential to decrease surgery time and hospitalization days, as well as the severe complications rate. It could be used in most of patients received free flap reconstruction when IMVs were used, particularly in higher patients and patients with a wider ICS. Preoperative slice chest CT scan can be used to measure the ICS width to provide suggestions for dealing with the ribs.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-09

Same-day surgery impact on breast reconstruction program in a public healthcare system: An affordable booster

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## Background

Breast cancer represents the most common malignancy in Latin America, and since 2006 it is the cancer type with the highest incidence among Mexican women. Providing multidisciplinary high-quality care for the growing population of patients with breast cancer represents a challenge to low and middle-income countries, which have limited economic resources and limited health staff and facilities.

Since 2007, Seguro Popular insurance program has provided coverage for the treatment of women with breast cancer, including surgery, radiotherapy, chemotherapy, endocrine therapy and Her2-targeted treatments; before this program up to 50% of the Mexican population did not have healthcare coverage, and had to pay out of pocket for cancer care. Unfortunately, due to financial constraints, this does not include other interventions which may be relevant, such as supportive care and reconstructive surgery.

National Cancer Institute of Mexico (INCan) is a part of the Mexican federal government and as such provides care to uninsured individuals with all types of malignancies, including breast cancer. Since the start of Seguro Popular insurance program (2007), INCan has provided oncological care to over 5,000 women with breast cancer.

In 2012, INCan received a grant from the federal government (P017 Reproductive Health and Gender Equality in Health Grant) in order to establish the “Post-Mastectomy Program” (PMP), which aimed providing free patient navigation, supportive care and breast reconstruction for women after mastectomy. Starting on July 2016, derived from PMP, it was possible the setting of a Same-Day Surgery Facility (SDSF) at INCan: two small, fully equipped operation rooms with a small recovery area; and the hiring of 4 nurses and 2 anesthesiologists, adding all this to the one main operation room already set for breast cancer surgery. This allowed an increase in all kind of breast cancer surgery: conservative surgery, mastectomy with sentinel lymph node and breast reconstruction procedures. One of this rooms was assigned to breast reconstructive surgeries.

## Material and Methods

Data were retrospectively collected from a 5 year period, 30 months before SDSF and 30 months after SDSF. Before SDSF an average 66.4 (54-73) of breast reconstruction surgeries were done by six month period, total of 332 breast reconstruction surgeries. After implementation of SDSF an average of 124 (107-138) by six month period, with a total 621 surgeries, which represents an 87% increase in breast reconstruction procedures for the same time period.

## Conclusion

Same day surgery has been proven before to be safe in breast cancer surgery and in breast reconstructive surgery when co-morbidities are accounted for.

This work shows that implementation of Same-Day Surgery could be a tool to increase the offer of breast reconstruction in economically restrained systems, in early or experienced breast reconstruction programs in developing economies, and even in developed ones.

In our experience, this approach achieved an 87% increase in breast reconstruction procedures, in a 30 month period, which allowed us to benefit more women and offer them a better quality of life.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-10

Post-mastectomy radiotherapy following immediate implant based reconstruction: A possible solution to a reconstructive challenge

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**Aims:** The National Mastectomy and Breast Reconstruction Audit report (NMBRA, 2011)<sup>1</sup> revealed that immediate implant-based breast reconstruction (IIBR) was the most common type of primary reconstruction performed in the UK (37%). The main reason given by clinicians for not offering immediate breast reconstruction was the need for adjuvant radiotherapy. Post-mastectomy radiotherapy (PMRT) decreases the rate of local recurrence as well as increase the long-term survival in patients who demonstrate intermediate to high-risk features<sup>2,3</sup> but has been shown to increase the risk of implant complications in IIBR by up to 24% (Berry et al, 2010)<sup>4</sup>. Cordeiro et al (2004)<sup>5</sup> showed the incidence of capsular contracture was 28% higher in the PMRT group compared with non-irradiated patients. Most patients in the UK receive hypofractionated PMRT of 40.05Gy in 15 fractions over 3 weeks based on the UK Standardisation of Breast Radiotherapy (START) trial<sup>6</sup>, which demonstrated that hypofractionated PMRT is as safe and effective as the conventional PMRT of 50Gy in 25 fractions over 5 weeks.

The aim of this study was to determine whether the conventional PMRT of 50Gy in 25 fractions over 5 weeks (2Gy per fraction) was associated with a reduced risk of implant complications in patients undergoing mastectomy with IIBR compared with hypofractionated PMRT regiment of 40.05Gy in 15 fractions over 3 weeks (2.67Gy per fraction).

**Methods:** A single centre retrospective review of data on patients who underwent IIBR followed by PMRT between September 2012 and May 2017 was conducted. Radiotherapy-related complications (surgical site infection, contracture, implant rupture or leakage, wound breakdown) were compared between the two groups of patients receiving conventional and hypofractionated PMRT.

**Results:** Fifty-nine patients underwent IIBR followed by PMRT. Twenty-six patients received hypofractionated PMRT and thirty-three patients received conventional PMRT. Radiotherapy-related complications occurred in 62% of patients in the hypofractionated PMRT group compared with 45% in the conventional PMRT group ( $p = 0.30$ ). The incidence of capsular contracture (31% in vs. 21%,  $p = 0.55$ ) and wound breakdown (23% vs. 15%,  $p = 0.51$ ) was higher in the hypofractionated PMRT group, but surgical site infection (SSI) was more common in the conventional group (4% vs. 6%,  $p = 1.00$ ).

**Discussion:** Possible confounding factors (BMI, smoking status, and adjuvant chemotherapy) were not analysed due to the small sample size and limitations of the retrospective nature of this study. However, our overall rate of SSI is low in comparison with national data from the NMBRA (2011), which states the SSI rate of 25% in patients who underwent breast reconstruction surgery.

**Conclusions:** This study suggests that the rate of radiotherapy-related complications is lower in patients treated with conventional PMRT compared with hypofractionated PMRT, however the sample size is too small to demonstrate statistical significance. Further research is required to evaluate the effectiveness of conventional PMRT as an option to facilitate immediate implant-based reconstruction following mastectomy.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-11

Intraoperative central nipple biopsy in subcutaneous mastectomies - A retrospective analysis of 200 patients

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**Background:** Subcutaneous nipple sparing mastectomies (NSM) are an important tool in modern oncoplastic surgery. Especially when an immediate implant-based reconstruction (IBR) is desired, clean margins are of the utmost importance. Central nipple biopsies during surgery serve two main purposes. Most importantly, it is hypothesized that a general recurrence risk reduction occurs due to elimination of glandular and ductal components within the nipple. In addition, intraoperative pathological evaluation of this biopsy may increase clean margin resection rates. This trial aimed to evaluate complication rates, local recurrence and clean margin resection rates in a head-to-head retrospective manner.

**Methods:** Starting in 2015, intraoperative central nipple biopsy in NSM with IBR was introduced at the Municipal Breast Cancer Centre Cologne, Holweide, Germany. This trial evaluates global complication rates (necrosis, implant loss, seroma and hematoma), long term local recurrence and clean margin status for cohort 1 (n=100) SSM with nipple biopsy vs. cohort 2 (control, n = 100) without nipple biopsy. In case of an involved central nipple biopsy, the nipple areola complex was removed. This analysis is a single center, multi-surgeon, retrospective, head to head analysis. Median follow-up is three years. All IBR procedures used an epipectoral implant pocket.

**Results:** Global complication rates did not differ significantly between both cohorts. There was no increased rate of implant loss and or revision surgery. Within the medium three-year follow-up there was no case of local recurrence within nipple areola complex for both cohorts. An Involved central nipple biopsy was however found in 2% (n=2 /100) of the performed SSM procedures leading to the immediate removal of the nipple areola complex. Surgical time did not increase with central biopsy. A significantly higher rate of re-surgery due to involved margins was shown in the control cohort.

**Conclusions:** This analysis showed that intraoperative evaluation of the ductal components of the nipple is a safe procedure. At least 2% of the patients showed an immediate advantage of this procedure since clean margins were obtained by removing the nipple areola complex during the same surgery. The medium follow-up showed no significant difference in local recurrence. We therefore strongly, recommend a central nipple biopsy for all NSM procedures.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-12

Implementation and outcomes of a microsurgical breast reconstruction program at a public cancer center in Mexico

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**Background:** Breast cancer (BC) is the most common malignancy in Mexico, and although many Mexican women need breast reconstruction, this is not covered by most public insurance schemes. The National Cancer Institute of Mexico (INCan), located in southern Mexico City provides care to uninsured individuals with all types of malignancies, including BC. In 2012, INCan received a grant from the Mexican federal government in order to establish the “*Post-Mastectomy Program*” (PMP), aimed at providing free breast reconstruction to women after mastectomy. Here, we describe the implementation and outcomes of our microsurgical breast reconstruction program.

**Methods:** We retrospectively reviewed medical records of all patients undergoing microsurgical breast reconstruction after mastectomy at INCan between the establishment of the PMP in 01/2013 and until 12/2017. Sociodemographic, clinical and surgical characteristics were collected. We also recorded for the presence of complications directly related to the reconstructive procedures, including local complications (infections, necrosis, thrombosis etc.), flap loss, need for reoperation and hospitalization time. Data were analyzed using descriptive statistics such as means, medians and proportions.

**Results:** 161 microsurgical breast reconstructions were conducted at INCan between 01/2013 and 01/2017, ranging from 23 in 2013 to 41 in 2016. Median patient age was 45 years (y) (21-66), and 57% had < high school education. 2.5% had diabetes, 9.9% hypertension, and 2.5% rheumatologic diseases. Median body mass index was 26.8 (18.2-39), 82.6% were non-smokers and 46.5% (n = 108) had previous abdominal scars. Regarding reasons for mastectomy, 67.7% were due to invasive BC, 11.8% to ductal carcinoma in situ, and 5.6% to BRCA mutations. Of the 109 invasive carcinomas, 21.8% were stage I, 57.3% stage II, and 20% stage III; 21.7% received neoadjuvant chemotherapy. 125 patients underwent immediate reconstruction, of which 89 used unilateral deep inferior epigastric perforator flaps (DIEP), 35 bilateral DIEPs, and 1 other technique. Mean preoperative albumin was 4.2g/dL (SD 0.35), while mean preoperative hemoglobin was 14.2g/dL (SD 1.2). 41.6% of the patients (n = 67) had at least one surgical complication, with the most common being delayed wound healing in 17% and fat necrosis in 14%. 26% of patients had to be reoperated, and flap loss occurred in 13% (n = 21). No differences were noted in the clinical or surgical characteristics of patients with or without flap loss. Median length of stay was 6 days (range 2-17).

**Conclusions:** This is the first detailed description of the outcomes of a microsurgical reconstruction program in a country with limited resources. Developing and implementing such a program is feasible, and may provide access to breast reconstruction to women who would normally be unable to obtain it.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-13

First results of the NOGGO-AWOGyn observational study: Skin and nipple sparing mastectomy and breast reconstruction with an implant and Epiflex®

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Acellular dermal matrices (ADM) were used to cover the implant to reduce implant rotations and to improve esthetic results. Current retrospective data on complication rates are heterogeneous. Prospective data are rare. Primary objective was to evaluate the explantation rate.

11 German sites, with experience in breast reconstruction using ADM, have been selected for participation in this study. Patients were enrolled according to predefined in- and exclusion criteria into two cohorts: group A - primary reconstruction and group B - secondary reconstruction after capsular contracture.

After documentation of baseline characteristics and surgical parameters, endpoints such as rate of seroma, red breast syndrome, infection and explantation were assessed at 1 day, 7 days, 1 month, 3, 6 and 12 months after surgery. In addition, patient- and surgeon-satisfaction were recorded.

Data of 83 patients were analyzed (A: 50, B: 33). In 29 patients Epiflex was used bilaterally, so that it was used 112-times in total. The average age of the patient was 45.1 years (A: 41.5; B: 50.3), the average BMI was 22.2 (A: 22.3; B: 22.1). In total, 17.0 % seromas (A: 19.4 %; B: 12.5 %), 10.7 % red breast syndrome (A: 9.7 %; B: 12.5 %), 4.5 % infections (A: 6.9 %; B: 0 %) and 5.4 % explantations (A: 6.9 %; B: 2.5 %) were observed.

During the average observation period of 8 month, the satisfaction of the patients and surgeons was high at all measured time points. On a scale from (1 - very satisfied to 6 - not satisfied) the satisfaction of patients and surgeons was 2.3 (A: 2.3; B:1.9) and 2.0 (A: 2.2; B: 1.8) respectively.

Our data show, that both patients and surgeons are generally satisfied with the outcome of breast reconstruction using ADM. Complication rates were adequate and commonly lower in secondary reconstructions.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-14

Outcome of lipofilling in patients with a history of breast cancer: A retrospective cohort study

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**Background:** Autologous fat grafting [AFG] is a surgical technique used to correct breast deformities or to reconstruct partial breast deformities after breast conservative treatment [BCT] or mastectomy for breast cancer. However, a strong debate remains upon the topic of safety and the oncological risk after lipofilling. There is a need for more studies assigned with a higher level of evidence.

**Objective:** This retrospective cohort study aimed to provide a strong evidence-based argument concerning the safety of fat grafting in patients who underwent breast cancer surgery in comparison to a group with similar characteristics who did not receive lipofilling.

**Methods:** Two cohorts consisting of women with a history of breast cancer were retrospectively assembled. All patients underwent BCT and/or a mastectomy as breast cancer treatment. An intervention population of patients that had received primary or secondary lipofilling was statistically compared to a control population of patients that had received a breast reconstruction without lipofilling.

**Results:** Fifty patients with a mean age of  $53.6 \pm 9.3$  years at the time of lipofilling were included in the intervention cohort. The control group included 67 patients with a mean age of  $52.8 \pm 8.5$  years at the time of breast reconstruction. Both cohorts showed no significant differences for demographic or oncological data. Mean follow-up time was  $15.0 \pm 15.6$  months in the intervention group and  $45.4 \pm 35.1$  months in the control group. The prevalence of the complications are shown in the table

Prevalence of complications

	Study population	Study population	Control population	Control population	p-value
Local oncological recurrence	0		1	(1.5%)	0.386
Infection	0		5	(7.5%)	0.048
Fat necrosis	15	(30.0%)	9	(13.4%)	0.028
Microcalcifications	3	(6.0%)	3	(4.5%)	0.712
Other	1	(2.0%)	17	(25.4%)	0.086
- hematoma	1	(2.0%)	5	(7.5%)	
- seroma	0		2	(3.0%)	
- capsular contracture	0		1	(1.5%)	
- implant leak	0		1	(1.5%)	
- implant/flap loss	0		6	(9.0%)	
- implant redo	0		1	(1.5%)	
- rash	0		1	(1.5%)	
None	35	(70%)	38	(56.7%)	0.142

. No complications were seen in 35 patients [70%] after lipofilling. Fat necrosis was mostly observed [n=15] but no infections or local oncological recurrences were reported. In the control group a complication-free follow-up occurred in 38 patients [56.7%] after breast reconstruction. One control patient demonstrated a local oncological recurrence. A higher prevalence of infections was observed in the control population [p = 0.048] and the prevalence of fat necrosis was higher after lipofilling [p = 0.028].

**Conclusion:** Lipofilling is a safe technique for breast reconstruction in patients with a history of breast cancer. There is no increase in local oncological recurrence. A side effect is an increase in fat necrosis which can potentially lead to more investigations.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-16-15

3D symmetry of pre-operative breasts in cancer patients

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## INTRODUCTION

Achieving breast symmetry and mitigating ptosis are important aims of cosmetic and reconstructive breast surgery. Breast asymmetry typically occurs when the left and right breasts differ in size, shape, or position. A few studies have associated breast asymmetry to hormonal changes and breast cancer risk. Similarly, changes in breast ptosis have been attributed to age, BMI, breast volume, smoking history, pregnancy, and weight loss. The purpose of this study is to provide normative data and understand factors that may attribute to breast asymmetry and ptosis.

## MATERIAL AND METHODS

The dataset consisted of 87 surface images of women scheduled to undergo mastectomy for the prevention or treatment of breast cancer. Patients were enrolled in an IRB approved research study from 2011 to 2014 at MD Anderson Cancer Center (Houston, TX). Images were obtained using 3dMDTorso (3dMD LLC, Atlanta, GA). Patient demographics were recorded and tumor size was obtained from MD Anderson Breast Cancer Management System, and tumor location data were obtained through a search of the MD Anderson Tumor Registry Database. Patients with a BMI > 41, rare congenital breast abnormalities, radiation therapy, or major breast surgeries were excluded. Breast symmetry was assessed by computing the ratio of the sternal notch (SN) to nipple (N) distance and breast volume ratio of the left and right breasts. Perfect symmetry occurs when the ratio is one. The smaller breast was evaluated over the larger breast so that ratios were less than one. A plastic surgeon rated each breast for ptosis. The correlation between age, BMI, and symmetry parameters were assessed using Pearson's r, Spearman's rho, and Kendall's tau coefficients. A multivariable linear model was used to evaluate the association between the ratios and demographic factors. A multiple ordinal logistic regression analysis was performed for age, BMI, and breast volume against ptosis.

## RESULTS

The average sternal notch to nipple (SN-N) distance was  $24.1 \pm 3.30$  cm and the SN-N ratio was  $0.975 \pm 0.020$ . The average breast volume was  $794 \pm 323$  cc and the breast volume ratio was  $0.914 \pm 0.065$ . When comparing the right and left breasts, for SN-N distances, we found that 58.6% of the patients had a difference of less than 5 mm, 17.3% of the patients had a difference between 5 and 10 mm, and 24.1% of the patients had a difference greater than 10 mm. For volume, 49.4% of the patients had less than 50 cc of difference, 21.5% of the patients had a difference between 50 and 100 cc, and 29.1% of the patients differed by more than 100 cc. Tumor size did not significantly affect breast volume based on the t-test ( $p = 0.181$ ). Symmetry ratios did not show significant correlation with age or BMI. Age and BMI were also not significantly associated with ptosis ( $p > 0.05$ ), but larger breast volumes were associated with increasing degree of ptosis (OR: 1.14; 95% CI: 1.06-1.22;  $p < 0.001$ ).

## CONCLUSION

Our findings indicate that larger breast volumes are associated with increased ptosis grade. Age and BMI did not appear to significantly impact asymmetry or ptosis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-16-16

Reconstructive breast surgery with partially absorbable bi-component Seragyn® BR soft mesh: An outcome analysis

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## Objective:

Synthetic meshes and acellular dermal matrices are increasingly used in implant based breast reconstruction. The objective of this study was to determine the incidence and severity of complications following the implantation of the partially absorbable bi-component soft mesh SERAGYN ® BR and assess risk factors for adverse operative outcomes.

## Methods:

A retrospective clinical study was performed: The SERAGYN ® BR soft mesh was utilized in 148 operations (skin-sparing mastectomy, nipple-sparing mastectomy, breast conserving surgery, secondary reconstruction after mastectomy) in four different institutions in Germany from

June 2012 to February 2014. We analyzed whether the results were affected by tumor morphology (e.g. grading), patient characteristics and co-morbidities, previous surgery or therapies and use of alloplastic materials.

## Results:

The SERAGYN ® BR soft mesh was successfully implanted in 131 of 148 operations. The rate of reconstructive failure was 11.5 %. The most common complication was seroma (25.7 %), followed by hematoma and skin infection (each 14.2%). Wound healing issues were detected in

13.5% cases, secondary wound infections in 10.8%. 83.8% of operations had no severe complications. Independent predictors for reconstructive failure were wound healing issues, nipple- or skin necrosis, wound- or skin infections, a high volume of excised tissue, hematomas, seromas and sentinel lymph node excisions. A higher body mass index was correlated with a higher rate of infection.

## Conclusion:

SERAGYN ® BR mesh can be used successfully in breast reconstructive surgery. Rates of major complications or reconstructive failure are comparable to the use of other synthetic or biological meshes.

## 2018 San Antonio Breast Cancer Symposium ®

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Titanium-coated polypropylene mesh for immediate implant-based breast reconstruction – Our initial experience

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**Background:** The use of titanium-coated polypropylene mesh (TCPM) is an alternative to a-cellular dermal matrix for the implant based breast reconstruction by providing extra implant coverage especially in the lower half. The aim of our study was to analyze short-term outcomes of TCPM based implant breast reconstruction and compare the patient- and procedure-related factors to implant loss and surgical complications.

**Methods:** Between 2013 and 2016, implant breast reconstructions after conservative mastectomies using TCPM was performed in 50 patients (with 58 reconstructions). Complications were divided into major (need for additional surgery), minor (conservative treatment), and implant loss. Univariate analyses were performed to determine the influence of the patient- and procedure-related factors on postoperative complications and implant loss.

**Results:** Fifty patients had therapeutic mastectomies with 8 of them also had contra lateral risk reducing mastectomies. With median follow-up of 17.5 months, four of 50 patients had implant loss. Reasons for implant loss were skin necrosis in 2 cases, infection in 1 case and necrosis with infection in another case. One of these patients had a revised reconstruction one year later. One additional patient required implant replacement because of capsule contracture. No risk factors were observed for patient-associated complications. Univariate analysis revealed an increased risk for implant loss in patients with skin necrosis ( $p < 0.01$ ).

**Conclusions:** This titanium-coated polypropylene mesh shows acceptable complication rates and its use in immediate implant breast reconstruction is safe and effective.

# 2018 San Antonio Breast Cancer Symposium ®

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Oncologic outcomes and reconstruction quality of immediate autologous breast reconstruction in intermediate and locally advanced breast cancer

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**Purpose:** We analyzed oncologic outcomes and reconstruction quality in locally advanced stage breast cancer after performing immediate autologous breast reconstruction (IABR).

**Methods:** From 2007 to 2014, data of patients with stage II-III breast cancer of  $\leq 70$ -years-old who received total mastectomy (TM) from two institutions were extracted. Exclusion criteria were: previous contralateral breast cancer, follow-up loss before adjuvant therapy completion, and artificial reconstruction. Patients were divided into two groups; 1) TM alone and 2) TM+IABR. Overall survival (OS) and loco-regional recurrence free survival (LRRFS) were calculated and minor revision, abnormal volume on CT, and breast height change were observed.

**Results:** Sixty-one of 188 patients received IABR after TM. Neoadjuvant chemotherapy and postoperative radiotherapy was done in 27 and 80 patients, respectively. Fifty-nine percent, 19.2%, and 21.8% of patients were in stage II, IIIA, and IIIB-C, respectively. Stage IIIB-C was the most important prognostic factor for OS and LRRFS. In a median of 56.8 follow-up months, 5-year TM and TM+IABR OS rate were 96.8% and 100% for stage II ( $P=0.324$ ) and 57.6%, 95.5% and 91.7% for stage IIIA ( $P=0.698$ ), and 62.5% for stage IIIB-C ( $P=0.544$ ), respectively. Five-year TM and TM+IABR LRRFS were 98.1% and 95.7% for stage II ( $P=0.998$ ) 91.1% and 100% for stage IIIA ( $P=0.277$ ), and 70.8% and 62.5% for stage IIIB-C ( $P=0.378$ ), respectively. However, two locoregional failures after 5-years were developed in stage IIIB-C of TM+IABR at 71 and 94 months. Minor revisions 3 months of IABR, including two major complications, were done in 49.2%. The reduction of breast height was 21.2% (11/52) and 31.9% (15/47) in about 18 months and about 42 months observations after IABR, respectively. The volume of abnormal imaging was over 10 cc in 27.3% (15/55) on CT of 6 months after IABR. Although minor revisions, abnormal volume on CT, and breast height change were common, they were not related to therapeutic methods, including radiotherapy and tumor stage.

**Conclusions:** In about 5-years follow-up, IABR did not aggravate oncologic outcomes, and adjuvant radiotherapy was not closely related with quality of reconstruction. It must be considered, however, that absolute oncologic outcomes of advanced stage were not sufficient, and minor problems post-IABR were commonly developed.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-17-01

Patient-derived organoid models of inflammatory breast cancer

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**Background:** Inflammatory Breast Cancer (IBC) is an aggressive subtype of breast cancer that is associated with an increased likelihood of early metastasis and a poor prognosis, accounting for approximately 10% of all breast cancer mortality. Currently, there is limited availability of cell line and patient-derived xenograft (PDX) models of IBC. As a result, there is an urgent need for additional model systems to facilitate our understanding of this disease. Organoids have been used to grow breast cancer cells with high efficiency, resulting in long-term cultures with genetic and phenotypic stability; however, the extent to which IBC can be modeled using this system is unknown. We undertook an evaluation of organoid culture technology for growing IBC patient samples, with the goal of generating a model of IBC that more faithfully recapitulates the key features of the disease.

**Methods:** Tumor samples (taken from lumpectomy, mastectomy, PDX, or core biopsy samples), were digested to the organoid level using collagenase, and were grown in three dimensional cultures using a basement membrane extract and a fully-defined organoid medium, as described (Sachs et al. Cell 2018). First, we surveyed non-IBC metastatic breast cancer samples to determine the efficiency of growth of multiple breast cancer subtypes under these conditions. Then, we assessed the efficiency of growth of IBC samples taken after treatment, at the time of mastectomy. Co-culture experiments in which IBC organoids were grown in the presence of human endothelial cells were performed in three dimensional culture using a mixture of type I collagen and basement membrane extract.

**Results:** Our success rate for establishing organoid cultures from breast cancer samples in general was 75% (33 out of 44 samples), and for triple-negative breast cancers, the success rate for establishment of organoid cultures was 100% (14 out of 14 samples). Expansion of ER+ tumor organoids (16 of 26 samples) was primarily restricted by a slow growth rate in culture, as compared to triple-negative tumor organoids. We were able to establish 12 breast cancer organoids from core biopsies of multiple metastatic sites, including brain, liver, bone, breast, and skin. We subsequently were able to establish organoid cultures from five out of five IBC tumor samples. Our IBC organoids preserved histopathologic features of the original tumor, and expressed key markers, such as E-cadherin, that are known to be associated with IBC. In addition, our IBC organoids interacted with human endothelial cells under co-culture conditions designed to model lymphovascular invasion, an essential feature of IBC.

**Conclusion:** We have shown that organoid cultures can be established to propagate IBC tumors with high efficiency, preserve key pathologic features of IBC in vitro, and model interactions between tumor cells and the microenvironment. Thus, organoids are useful complements to existing IBC models, and can be used to identify potential therapeutic vulnerabilities associated with this particularly aggressive breast cancer subtype.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-17-02

Dissecting the biology of inflammatory breast cancer (BC) through cell free DNA and a circulating tumor cells (CTC)-derived signature

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**Background:** The biological characteristics conferring Inflammatory BC's (IBC) distinctive and aggressive clinical features are currently not fully clarified. The aim of this study is to dissect IBC's biology through the integration of DNA and CTC-based circulating biomarkers.

**Methods:** This study retrospectively analyzed 251 Advanced BC (ABC) patients (pts) treated and longitudinally characterized for CTCs and circulating tumor DNA (ctDNA) at Thomas Jefferson University (Philadelphia, PA) and Northwestern University (Chicago, IL). CTCs were enumerated through CellSearch (Menarini Silicon Biosystems), and characterized for HER2 expression using the CellSearch CXC Kit, while ctDNA was analyzed using the Guardant360 NGS assay (Guardant Health) and its percentage (%ctDNA) was classified based on the previously reported cut-off of 5.7% (Gerratana et al 2018). A subset of 117 pts was further characterized for circulating cell-free DNA (ccfDNA) through Qubit® dsDNA HS quantitation Assay (Thermo Fisher Scientific) and quantitative real-time PCR assay for ALU DNA repeats on chromosome 1. Associations between clinical characteristics, CTCs-derived biomarkers and IBC were explored through Fisher's exact test; survival was tested through Cox regression and log-rank test.

**Results:** Of the total 251 pts, 115 were diagnosed with IBC. Among the 117 patients characterized for ccfDNA, 70 had IBC. Median ccfDNA was 1.59 for IBC (IQR 1.02-3.19) and 2.37 for non-IBC (nIBC) (IQR 1.13-3.52),  $P=0.27$ . Consistent results were observed for %ctDNA levels (median value: 2 vs 1.6). The impact on OS of ccfDNA after log transformation was significant for the total population (HR 1.73 95%CI: 1.11-2.69) but not in IBC pts (HR 1.40 95%CI: 0.84-2.34). On the other hand, ctDNA high pts had a significantly worse OS (nIBC: HR 5.34 95%CI: 1.70-18.81  $P=0.004$ ; IBC: HR 4.05 95%CI: 1.91-8.58  $P<0.001$ ). In the ctDNA high subgroup no differences in total number of CTCs were observed between IBC and nIBC, while significantly lower CTCs were observed in ctDNA low IBC pts ( $P=0.0097$ ). The ctDNA low IBC subgroup had a higher incidence of HER2 positive BC ( $P=0.003$ ) and a significantly lower incidence of CTCs clusters ( $P=0.006$ ), HER2 positive CTCs ( $P=0.041$ ). Notably, no associations were observed with stage at baseline, number of metastatic sites, liver, lung and visceral involvement. On the other hand, the ctDNA\_high IBC subgroup was characterized by a lower incidence in liver, bone and visceral involvement ( $P=0.017$ ,  $P=0.014$  and  $P=0.03$  respectively) and a marginally high incidence in soft tissue involvement (0.084). Moreover, IBC diagnosis conferred a significantly worse prognosis only in the ctDNA low subgroup (OS at 12 months nIBC: 100% vs IBC: 70%;  $P=0.049$ ), while no differences were observed in the ctDNA\_high subgroup (OS at 12 months nIBC: 29% vs IBC: 26%;  $P=0.767$ ).

**Conclusion:** ctDNA is able to stratify BC according to aggressiveness independently from the sites and type of metastases, both in the IBC and nIBC subgroups. IBC has a distinctive CTCs/ctDNA-based signature, in particular ctDNA\_low pts have a lower incidence of HER2 positive CTCs and CTC clusters. This signature is probably due to predominant lymphatic metastatic spread and aggressive phenotype.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-17-03

How is inflammatory breast cancer (IBC) different? Integration of clinico-pathological features and circulating tumor cells (CTCs)-based biomarkers for disease and prognostic assessment

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**Background:** Since IBC is rare and burdened by a particularly unfavorable prognosis, biomarkers able to enhance diagnosis and risk assessment are of pivotal importance and a current unmet need. The aim of this study is to integrate standard clinico-pathological features with CTCs-based biomarkers for a more objective and detailed characterization of IBC.

**Methods:** This study analyzed retrospectively 251 Advanced Breast Cancer (BC) patients (pts) longitudinally characterized for CTCs and CTCs-based biomarkers at Thomas Jefferson University (Philadelphia, PA) and Northwestern University (Chicago, IL). CTCs were enumerated through the CellSearch system (Menarini Silicon Biosystems), and characterized for HER2 expression using the CellSearch CXC Kit. Pts were defined as stage IV aggressive based on the previously reported  $\geq 5$  CTCs cut-off (Davis et al. 2018). Associations between clinical features, CTC-derived biomarkers and IBC were tested through uni and multivariate logistic regression. Survival was tested through log-rank test.

**Results:** Within the analyzed cases, 46% were diagnosed with IBC and among them, 38% was stage IV aggressive. CTC clusters (CTC\_CL) were detectable in 12.5% of pts and HER2 positive CTCs (HER2\_CTC) in 29.5%. Notably, IBC patients (pts) had a significantly lower CTC count with respect to non-IBC (median 2.5 vs 0 respectively for non-IBC and IBC;  $P=0.019$ ). BC subtype (HER2 positive BC: OR 2.97; Triple negative BC: OR 2.13), liver and bone involvement (liver: OR 0.46; bone involvement: OR 0.31) were the only significant clinico-pathological features associated with IBC at univariate logistic regression. Interestingly, a marginal significance was observed for soft tissue involvement (OR 1.65, 95%CI 0.95 - 2.87,  $P=0.07$ ). Stage IV aggressive and presence of HER2\_CTC at baseline were moreover inversely associated with IBC. The multivariate model confirmed the significant association between IBC and HER2 positive BC subtype (OR 2.64, 95%CI 1.08 - 6.48,  $P=0.034$ ), absence of bone involvement (OR 0.31, 95%CI 0.14 - 0.68,  $P=0.003$ ) and absence of HER2\_CTC (OR 0.38, 95%CI 0.15 - 0.98,  $P=0.045$ ). The baseline detection of CTC\_CL was a strong predictor of prognosis for OS in IBC pts (median OS (mOS) 7.6 months (mts) vs not reached (NR) respectively for detectable vs non-detectable CTC\_CL;  $P<0.0001$ ), while a trend was observed for HER2\_CTC (mOS 9.9 mts vs NR respectively for detectable vs non-detectable HER2\_CTC;  $P<0.082$ ). Pts negative for CTC\_CL at baseline had higher odds of developing CTC\_CL in later time-points if stage IV aggressive (OR 12.27, 95%CI 2.10 - 71.57,  $P=0.005$ ). Despite no baseline factors were significantly associated with the onset of HER2\_CTC in later time-points, a trend ( $P=0.05$ ) was observed for patients without lymph node involvement (OR: 5) and with bone involvement (OR: 4.3).

**Conclusion:** HER2\_CTC and in particular CTC\_CL are promising prognostic predictors in IBC. Stage IV aggressive IBC pts could benefit from a longitudinal CTCs assessment, being more prone to develop CTC\_CL and therefore at higher risk of rapid disease progression. Probably due to the tropism for soft tissue, IBC is characterized by a lower number of HER2\_CTC.



# 2018 San Antonio Breast Cancer Symposium ®

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Metastatic inflammatory breast cancer: Clinical features and outcomes in the national, multicentric, real-life ESME cohort

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**Background:** Primary inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer. Survival of IBC patients has been improved by multimodal therapy. However 5-year overall survival (OS) still remains close to 50-60%, due to high risk of disseminated disease. Given the low incidence, prognosis of metastatic cases stages is poorly described.

**Methods:** This study aimed to describe OS of IBC (T4d AJCC TNM classification) with upfront or recurrent metastatic disease compared with non-IBC patients in the ESME database (N=16,702 patients). OS was calculated from the diagnosis of metastasis to the date of death (from any cause), or censored to date of latest news. Secondary objectives included progression-free survival (PFS).

**Results:** From 2008 to 2014, 7,465 patients with diagnosis of MBC and known clinical status of their primary tumor (T) were identified, including 582 IBC (T4d) and 6,883 non-IBC. As expected, metastatic IBC was associated with pejorative features compared to non-IBC, with less hormonal receptors-positive tumors (44% vs 65.6%), more HER2-positive (30% vs 18.6%) or triple-negative (25.9% vs 15.8%) cases ( $p<0.001$ ), more frequent upfront M1 stage (53.3% vs 27.7%;  $p<0.001$ ), and shorter median disease-free interval (2.02 years vs. 4.9 years;  $p<0.001$ ). With a median follow-up of 50.2 months (0-104), median OS was 28.4 [95%CI 24-33.8] versus 37.2 months [95%CI 36.1-38.5] in metastatic IBC and non-IBC cases respectively ( $p<0.0001$ , log-rank test). By multivariate Cox model with adjustment for major prognostic factors [including age, disease-free interval, type of relapse, visceral metastases, molecular subtype, grade], OS was significantly shorter in the metastatic IBC group compared with non-IBC group (HR 1.25 [95%CI 1.1-1.4],  $p=0.0002$ ). Of note, survival of metastatic IBC patients improved over the last years: median OS 24 months [95%CI 20-31.9], 29 months [95%CI 21.7-39.9] and 36 months [95%CI 27.9-NE] if diagnosed before 2011, between 2011 and 2012, or after 2012 respectively ( $p=0.003$ ). Such improvement was not observed in non-IBC patients. IBC was associated with shorter median PFS under first line systemic treatment compared with non-IBC (7.2 months [95%CI 6.6-8.3] vs 9.5 months [95%CI 9.1-9.8] respectively,  $p=0.0136$ ). This was maintained in a multivariate Cox model adjusting for same factors as for OS (HR 1.15 [95%CI 1-1.3],  $p=0.0050$ ).

Compared with non-IBC, synchronous metastatic IBC showed worse median OS and PFS (39.9 months [95%CI 34.2-45.3] vs 48.4 months [95%CI 46.3-50.8],  $p=0.0035$ ; 10 months [95%CI 8.8-12.7] vs 14.5 months [95%CI 13.6-15.7],  $p=0.0027$ , respectively. Similar results were obtained in metachronous metastatic cases (20.01 months [95%CI 17.1-21.2] vs 32.8 months [95%CI 31.5-34.3],  $p<0.0001$ ; 5.1 months [95%CI 4.1-6] vs 7.9 months [95%CI 7.6-8.3],  $p<0.0001$ , respectively).

**Conclusion:** In this large national and multicentric study, IBC is a major and independent factor associated with adverse outcome in metastatic setting. Of note, the independent adverse impact on PFS identified in this study may suggest a lower sensitivity of metastatic IBC to available therapeutics. However, results seem to improve in the last years. Detailed analysis according to phenotype will be available.

# 2018 San Antonio Breast Cancer Symposium ®

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Outcome of triple negative inflammatory breast cancer (TNIBC) treated with dose-dense dose intense neoadjuvant chemotherapy (NAC), prognostic impact of post NAC lymphovascular invasion and tumor infiltrating lymphocytes (TIL)

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## Background

Inflammatory breast cancers (IBC) particularly triple negative (TN) subtype have poor prognosis. There are few series reporting IBC outcome according to their immunohistochemical profile. We have already shown the efficiency of dose dense dose intense chemotherapy in triple negative breast cancer (1). We report a series of TNIBC treated with dose dense anthracycline cyclophosphamide followed with taxane and analyzed the correlation between pathological complete response (pCR), pre and post NAC TIL, post NAC LVI and disease free survival (DFS).

## Methods

Between January 2010 and December 2016, all patients with TNIBC seen at breast cancer disease center, St Louis hospital, Paris, France, were treated with neoadjuvant dose dense dose intense Cyclophosphamide (1.2g/m<sup>2</sup> d1) - Epirubicin (75mg/m<sup>2</sup> d1) q2w (SIM regimen) followed with 12 injections of paclitaxel (80 mg/m<sup>2</sup>) qw or 4 injections of docetaxel (100 mg/m<sup>2</sup>) q3w. All patients have histologically proven TN tumors and no evidence of metastases assessed by initial FDG PET Scanner. Mastectomy and axillary clearance was performed after chemotherapy. pCR was defined as no residual invasive tumor in breast and lymph nodes. TIL and lymphovascular invasion were evaluated pre and post NAC by 2 independent anatomopathologists dedicated to breast cancer. Delta TILS was defined as the difference between post chemotherapy and pre chemotherapy TIL.

## Results

Thirty TNIBC pts were treated, 28 underwent surgery and 2 progressed during chemotherapy. Median follow-up was 39 months (8 – 86). 9/30 patients (30%) achieved pCR. Median disease free survival (DFS) was 41 months (2 – 86). Median TIL infiltration at diagnosis was 11% (0-60) and dropped to 1% after chemotherapy (0 – 80). Median delta TIL was - 9% (-50% – +40%). TIL increase after chemotherapy was associated with a decrease of DFS (14 months vs not reached ; p = 0,0009). LVI was present on surgical specimens in 12 cases (12/30, 43%; 12/21 non pCR pts 57 %). Presence of LVI after chemotherapy was significantly associated with a decrease of DFS in the whole population (21 months vs not reached ; p = 0.008) and no significantly among the patients without pCR (23 months vs not reached; p = 0.07).

## Conclusion

To the best of our knowledge, it is the best pCR rate reported in TNIBC (2). We showed in this retrospective series of 30 TNIBC that dose dense dose intense chemotherapy is efficient in this population. Presence of lymphovascular invasion and TIL after neoadjuvant chemotherapy in TNIBC are strong prognostic factors associated with DFS. Systematic determination of post NAC TIL and LIV could be a surrogate to propose adjuvant treatment after NAC in TNIBC.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-17-06

Immune modulation with humanized anti-EGFR antibody panitumumab in an immunocompetent mouse model for inflammatory breast cancer

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**Background:** Inflammatory breast cancer (IBC) is the most lethal and aggressive form of breast cancer and there are no approved targeted therapies specifically for IBC. Targeting the epidermal growth factor receptor (EGFR) pathway is a promising therapeutic target for patients with triple-negative IBC (TN-IBC) with a reported pathological complete response rate of 42% (JAMA Oncology, 2018). The tumor microenvironment (TME) is a critical contributor to the aggressiveness of IBC. Delineating cross-talk between EGFR-targeted therapies and TME components, which define IBC, could inform more efficient combination regimens and novel clinical trial designs for IBC. However, such studies have not been conducted due to the lack of a syngeneic IBC mouse model. Here we report the establishment of an IBC immunocompetent mouse model and the effects of panitumumab (PmAb) on IBC tumor growth and the TME.

**Methods:** TN-IBC cell lines, SUM149 or FC-IBC-02, were mixed with 50% Matrigel and inoculated into mammary fat pads of hu-NSG-SGM3 mice engrafted with hematopoietic stem cells (The Jackson Laboratory). SUM149 tumor growth in hu-NSG-SGM3 mice treated with either IgG2 (isotype control, 4 mg/kg) or PmAb (1 mg/kg and 4 mg/kg) was measured. The percentages of TME components, including human CD4<sup>+</sup> T, CD8<sup>+</sup> T, regulatory T (Tregs), and natural killer (NK) cells, and M1 or M2 macrophages, in the peripheral blood and tumor tissues treated with IgG2 and PmAb for 7 weeks were measured by flow cytometry.

**Results:** Hu-NSG-SGM3 mice supported the growth of TN-IBC SUM149 and FC-IBC-02 xenografts. These humanized mouse models were named SUM149-huSGM3 and FC-IBC-02-huSGM3, respectively. Analysis of the blood cells showed that SUM149-huSGM3 mice display human CD4<sup>+</sup> T, CD8<sup>+</sup> T, Tregs, M1 and M2 macrophages. T cell infiltration and M1 and M2 macrophages were also detected in SUM149-huSGM3 tumors. NK cells were not detected in both peripheral blood and tumors. PmAb treated SUM149-huSGM3 mice had significantly reduced SUM149 tumor growth, compared with mice that received the IgG2 control. PmAb treatment increased the percentage of CD8<sup>+</sup> T cells and reduced the percentage of Tregs in peripheral blood. A similar analysis of tumor infiltrating lymphocytes isolated from each group showed an increase in percent CD8<sup>+</sup> T cells in mice treated with PmAb. There were no significant changes of M1 or M2 macrophages following PmAb treatment. These results suggest that the increase in percentage of CD8<sup>+</sup> T cells in peripheral blood and IBC tumors, and the decrease in percentage of Tregs in peripheral blood may contribute to the therapeutic efficacy of PmAb.

**Conclusion:** We established the first immunocompetent mouse model to study the TME and immune response in IBC, which provides the premise for conducting a diversity of novel preclinical therapeutic studies. The mechanism of how immune responses of TN-IBC xenografts mediates the therapeutic efficacy of PmAb in IBC tumors needs to be further investigated. Our study also suggests that combination therapy with immune checkpoint inhibitors may potentiate the efficacy of anti-EGFR therapy in IBC. The therapeutic efficacy of PmAb and anti-PD-L1 combination in SUM149 humanized mice is in progress.

# 2018 San Antonio Breast Cancer Symposium ®

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Risk of contralateral breast cancer (CBC) in women with ductal carcinoma in situ (DCIS) with and without and synchronous lobular carcinoma in situ (LCIS)

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**Background:** LCIS is considered a risk factor for bilateral breast cancer, but the effect of LCIS diagnosed concurrently with DCIS is not known. We sought to compare CBC and ipsilateral breast tumor recurrence (IBTR) rates in women with DCIS with and without synchronous LCIS treated with breast conserving surgery (BCS).

**Methods:** A prospectively maintained database of DCIS patients undergoing BCS from 2000-2011 was used to identify women with a contralateral breast at risk. Patients with synchronous ipsilateral LCIS found at core needle biopsy or surgical excision were included in the “DCIS + LCIS” group; those with contralateral or bilateral LCIS were excluded. Associations of patient, tumor, and treatment factors with CBC and IBTR were evaluated using logistic regression.

**Results:** Of the 1888 patients identified, 1475 (78%) had DCIS only and 413 (22%) had DCIS with synchronous LCIS. Median follow-up was 7.5 years (range 0-17 years). 305 patients had a subsequent breast event; 216 IBTR and 89 CBC. The 5 and 10-year cumulative incidence of IBTR was similar in both groups: 6.3% and 14.4% for DCIS only, compared with 5.9% and 14.0% for DCIS + LCIS (p = 0.94), respectively. The 5 and 10-year cumulative incidence of CBC was significantly greater in the DCIS + LCIS group: 5.7% and 10.0%, compared with 2.4% and 5.0% for DCIS only (p < 0.001).

Table 1 summarizes uni-and multi-variable analyses of risk factors associated with CBC and IBTR among women with DCIS treated with BCS.

Table 1: Risk factors associated with CBC and IBTR in patients with DCIS treated with BCS

	Univariate Analysis		Multivariate Analysis	
	Hazard Ratio (95% CI)		Hazard Ratio (95% CI)	
	CBC	IBTR	CBC	IBTR
<b>Age (continuous)</b>	1.02 (1.01-1.04) *	0.99 (0.98-1.0)	1.02 (1.0-1.04) *	0.99 (0.97-1.0) *
<b>Presentation</b>				
Radiologic	1	1	1	1
Clinical	0.36 (0.11-1.14)	1.56 (1.05-2.3) *	0.41 (0.13-1.31)	1.67 (1.12-2.47) *
<b>Family history</b>				
Yes	1.32 (0.87-2.01)	1.0 (0.76-1.32)	1.33 (0.87-2.03)	1.03 (0.78-1.35)
<b>Grade</b>				
Low	1	1	1	1
Intermediate/high	1.83 (1.0-3.37)	1.32 (0.93-1.88)	1.74 (0.92-3.29)	1.62 (1.11-2.35) *
<b>Radiation</b>				
Yes	1.16 (0.75-1.8)	0.7 (0.54-0.92) *	1.14 (0.71-1.82)	0.64 (0.48-0.86) *
<b>Endocrine Therapy</b>				
Yes	0.43 (0.23-0.81) *	0.51 (0.35-0.74) *	0.42 (0.22-0.8) *	0.53 (0.36-0.77) *
<b>DCIS group</b>				
DCIS only	1	1	1	1
DCIS + LCIS	2.28 (1.49-3.5) *	0.99 (0.71-1.36)	2.37 (1.54-3.65) *	0.99 (0.71-1.37)

\* p < 0.05

After adjustment for other factors, CBC risk was more than 2-fold higher in the DCIS + LCIS group compared with the DCIS only group (HR 2.37, 95% CI 1.54-3.65,  $p < 0.001$ ). There was no difference in IBTR risk based on presence of synchronous LCIS. Younger age and receipt of endocrine therapy were significantly associated with decreased risk of CBC.

**Conclusions:** LCIS diagnosed concurrently with DCIS is not associated with IBTR, but increases the risk of CBC two-fold. Endocrine therapy should be considered both for the index DCIS and for prevention of subsequent CBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-18-02

A quantitative centrosomal amplification score (CAS) predicts local recurrence in ductal carcinoma in situ

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**Background:** About 60-80% of ductal carcinoma in situ (DCIS) cases are high-grade (HG) DCIS with an elevated risk of local recurrence (LR) even after a lumpectomy. Patients are often under or over treated due to the lack of accurate recurrence risk prediction models. Current prognostic models such as OncotypeDX and Van Nuys Prognostic Index (VNPI) lack consistency and are limited to a specific subset of patients. Here in this study, we show that the extent of centrosome amplification (CA) in a DCIS lesion can predict the risk of LR after lumpectomy. CA refers to presence of supernumerary or large centrosomes and is a characteristic of pre-invasive lesions, and breast tumors, and promotes erroneous mitoses and chromosomal instability.

**Methods:** We have pioneered a semi-automated pipeline that integrates immunofluorescence confocal microscopy with digital image analysis and yields a quantitative Centrosomal Amplification Score (CAS) for each patients' tumor sample by evaluating severity and frequency of centrosomal aberrations therein. To this end, we first immunofluorescently stained centrosomes in formalin fixed paraffin embedded resection samples from DCIS patients (discovery cohort n=133 and a validation cohort n=119) using an antibody against  $\gamma$ -tubulin, and co-stained nuclei with DAPI. Next, we imaged the slides and processed the raw 3D image data using IMARIS Biplane 8.2 3D volume rendering software. Finally, we calculated centrosome numbers and volume in ~250 cells from each patient sample. Using a mathematical algorithm, we generated a composite CAS score for each patient sample by integrating the numerical (CASi) and structural (CASm) aberrations.

**Results:** We found that DCIS patients with recurrence exhibited higher CAS. Intriguingly, higher CAS was also associated with greater risk of developing ipsilateral breast events [Hazard ratio (HR) =7.58 for discovery cohort and HR=5.8 for validation cohort, p<0.0001] which remained significant (HR=8.5 for discovery and HR=3.39, p<0.0001) after accounting for the confounding factors like age, tumor size, comedo necrosis and radiotherapy. Kaplan Meir survival analysis indicated that high CAS was associated with poor recurrence-free survival (RFS) (p<0.001). For the high and low CAS groups, the 5-year risk of recurrence was 87.5% and 12.5% respectively (p<0.001). In our discovery cohort, a head-to-head comparison of the ability of VNPI and CAS to predict recurrence illuminated that CAS was able to stratify the DCIS group in recurrence and recurrence-free group with much higher significance (p<0.0001) than the Van Nuys Prognostic Index (VNPI) (HRs for CAS- 8.8 vs. VNPI 0.959). Finally, the Harrell's concordance index using SAS PROC PHREG tests yielded that the probability of a patient with poorer/lower RFS to be in the high CAS group is 76.2%.

**Conclusion:** Our data compellingly show that CAS quantifies the risk of recurrence in DCIS patients with the highest concordance and provides a novel and innovative tool to tailor their treatment based on their risk profile.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-18-03

A predictive model for local recurrence in patients treated for ductal carcinoma *in situ* of the breast (DCIS)

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**Background:** Ductal carcinoma *in situ* (DCIS) is a heterogeneous precursor, non-invasive breast lesion. There is a lack of specific DCIS molecular predictors of in breast tumour recurrence (IBTR) or progression to invasive breast cancer (IBC) after breast conserving surgery (BCS) +/- radiotherapy (RT). The aim of this was to identify novel biomarkers and combine these with clinical parameters to develop a new model to predict IBTR in patients treated by BCS for DCIS.

**Methods:** A single institution DCIS biomarker discovery study included a case-control matched series of 180 patients (median age 61, range 35-94) treated at the Edinburgh Breast Unit between 2000 and 2010:

- 88 patients with low/intermediate grade DCIS treated with BCS alone; 18 recurred within 10 years.
- 92 patients with high grade DCIS treated by BCS and RT; 22 recurred within 10 years.

Median follow-up was 7.4 years. RNA was extracted from DCIS lesions and whole-genome transcriptomics analysis was performed using Lexogen QuantSeq. Predictive models were generated based upon the most informative genes. Independent validation cohorts are also available and are currently being used for validation.

**Results:** The models developed predict risk of IBTR in patients with low or intermediate grade DCIS treated with BCS alone and high grade DCIS treated with DCIS plus RT. The models were found to be independent of grade and stratify patients into binary groups of high and low risk of recurrence.

A promising model was developed based on the expression of 5 genes combined with tumour diameter  $\leq 15$ mm or  $> 15$ mm.

- In low/intermediate grade DCIS expression levels of a solute carrier family gene, kinetochore associated gene and an immunomodulatory gene are predictive of recurrence.
- In high grade DCIS an additional solute carrier and a glutathione S-transferase related gene are predictive of recurrence.
- In the training sets the models have 96% (high-grade) and 92% (low/intermediate grade) accuracy of prediction of subsequent recurrence and estimates of IBTR-free survival were highly significant in both groups ( $< 0.0001$ ). Validation of the model by RT-PCR and immunohistochemistry is underway in both the training cohort and an independent validation cohort.

**Conclusions:**

- Promising models to predict risk of IBTR in patients treated for DCIS have been developed.
- Novel biomarkers that predict recurrence have been identified using new technologies that may have clinical potential.
- Independent validation is currently underway.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-18-04

HER2 overexpression in ductal carcinoma in situ: A biomarker for risk stratification and therapeutic implication

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## **Background:**

After initial treatment for ductal carcinoma in situ (DCIS), a subsequent clinically significant event (SCSE) such as another diagnosis of DCIS, atypia or invasive breast cancer (IBC), will likely lead to further surgical/medical therapy. Pathologically, DCIS is treated on the basis of estrogen (ER) and progesterone receptor (PR) status with hormonal therapy (HT), ie, tamoxifen (TMX) or aromatase inhibitor, regardless of human epidermal growth factor receptor (HER2) overexpression. Although HER2 is a well-established prognostic marker for IBC, the role of HER2 in DCIS is less appreciated. Recent studies have documented the prognostic value of p16, COX-2 and Ki67 as prognostic biomarkers for locoregional invasive recurrences due to abrogated response to cellular stress (ARCS). Notably, a high-risk population of DCIS patients lacking ER expression but over-expressing HER2, p16 and COX-2 has been recently identified. In the present study, we compared expression levels of HER2 and the 3 ARCS markers in a large cohort of DCIS patients treated with contemporary standard of care and with >5-year follow-up to assess their association with cancer progression and predictive value for developing a SCSE.

## **Methods:**

Formalin-fixed paraffin-embedded tissue sections from 99 patients diagnosed with primary DCIS(1999-2013) were stained for ER, PR and HER2 expression by immunohistochemistry (IHC). If equivocal, HER2 amplification was assessed by Silver In Situ Hybridization. An additional slide was stained for p16, Ki67 and COX-2 using a novel multiplex IHC strategy. Quantification of the 3 ARCS markers' expression in both epithelial and stromal compartments was carried out using a software (Inform<sup>TM</sup>)-guided approach. For expression level comparison of the three markers between HER2+ and HER2- DCIS, Wilcoxon-Mann-Whitney test was used. Fisher's exact or Chi-square test was used for other data analysis.

## **Results:**

Simultaneous increase in epithelial p16, COX-2 and Ki67 expression in DCIS lesions was associated with subsequent IBC progression. HER2+ DCIS was significantly associated with high grade ( $p=0.0014$ ), comedo-necrosis subtype ( $p=0.0022$ ) compared to HER2- lesions. Upregulation of stromal COX-2 and p16 was significantly ( $p=0.008$ ) associated with progression to SCSE in HER2- DCIS. The majority (5/6, 83%) of HER2+ DCIS patients treated with HT developed a SCSE, while only 22% (6/27) of HER2- DCIS patients treated with HT developed a SCSE. COX-2 upregulation was significantly associated with resistance to HT in HER2+ DCIS patients.

## **Conclusion:**

High expression of p16, COX-2 and Ki67 in DCIS lesions can serve as powerful predictive biomarkers for DCIS progression to IBC. Our preliminary data suggest that overexpression of stromal p16 and COX-2 may help predict SCSEs in HER2- DCIS. Additionally, assessment of HER2 expression in DCIS may allow identification of patients who would not benefit from traditional HT as HER2 overexpression may predict TMX resistance. Since our data suggest that TMX resistance in HER2+ DCIS may be linked to upregulation of COX-2, one may propose that COX-2 inhibitors in conjunction with TMX may minimize TMX resistance in HER2+ DCIS. These preliminary data will need to be reproduced in a larger cohort to solidify their significance.



# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P5-18-05

Clinicopathological characteristics and prognosis of young patients with ductal carcinoma in situ

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**Background:** The peak age at diagnosis of breast cancer differs between patients in Asian countries (40 - 50 years), and those in Western countries (60 - 70 years). With the increasing use of screening mammography, the incidence of ductal carcinoma in situ (DCIS) has increased significantly in younger Asian women. Nevertheless, our knowledge of the clinicopathological features and prognosis in young patients with DCIS is relatively limited. We aimed to compare the clinicopathological features of younger patients with that of older patients with DCIS and to evaluate their prognostic factors. **Methods:** A total of 1445 women were diagnosed with DCIS between the years 2005 and 2015. Patients with the past history of breast cancer and managed without surgery were excluded. The young age group included patients <50 years of age, whereas the old age group included patients ≥50 years of age at diagnosis. We compared the clinicopathological characteristics [tumor size, surgery type, estrogen receptor (ER) and progesterone receptor (PgR) status, HER2 status, nuclear grade, margin status, radiotherapy, endocrine therapy, family history of breast cancer, and screening presentation or presentation with symptoms] and prognosis [disease-free survival (DFS), and overall survival (OS)] between the groups. DFS included the following events: contralateral breast cancer, loco-regional, and distant recurrences. DFS and OS were estimated using the Kaplan–Maier method. The risk factors associated with events were estimated using the log-rank test for univariate analysis. *P* values < 0.05 were considered statistically significant. **Result:** Among the 1445 patients diagnosed with DCIS, 1281 were included in this study. The median age at diagnosis was 47 years (range, 22-87 years). The median follow-up time was 72 months (range, 1-162 months). ER and/or PgR status was positive in 1133 patients (88%). HER2 status was positive in 289 patients (23%). Premenopausal status was noted in 867 patients (68%). The median tumor size was 3.0 cm. Of 1281, 202 (18%) patients received endocrine therapy, 846 (66%) received breast conserving surgery, and 724 (86%) received radiation therapy. There were 765 patients (60%) in the young group. Significantly more patients in the young group had low nuclear grades, were ER and/or PgR positive, were HER2 receptor negative, underwent mastectomy, presented with symptoms, and had close/positive margins. Fifty-eight (4.5%) events occurred: 41 (3.2%) contralateral breast cancers, 19 (1.5%) loco-regional recurrences, and one (0.1%) distant metastasis. No death due to breast cancer was reported. On multivariate analysis, the young group (hazard ratio: 2.24, 95% CI: 1.01 - 4.95, *P* = 0.04), and presentation with clinical symptoms (hazard ratio: 2.09, 95% CI: 1.07-4.10, *P* = 0.03) significantly correlated with worse DFS. OS was not significantly different between the groups. **Conclusion:** This was the largest study with young patients with DCIS in the Asian population. We found that age at diagnosis was a significant independent factor associated with DFS. While genetic background also requires consideration, women with DCIS at <50 year of age may require intensive surveillance. This result requires confirmation with longer follow-up.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-18-08

Defining molecular signatures to personalise management of patients with early breast cancer

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## **Background**

A review of breast screening highlighted the need to reduce overdiagnosis. Ductal Carcinoma In-Situ (DCIS) contributes significantly to this overdiagnosis. Epithelial cells in DCIS are as genetically advanced as those in invasive disease, focusing attention on the tumour microenvironment (ME). A key components of the ME in DCIS is the myoepithelial cell(MEC). These cells lie at the interface of the epithelial and stromal compartments, regulating cell function. We previously have identified changes in the MEC that contribute to tumour progression. Here we investigate the functional and clinical significance of a novel change in MEC phenotype: loss of Galectin-7 (Gal-7) expression. Gal-7 is proposed to play a role in apoptosis. We hypothesise that changes in MEC phenotype in DCIS alter the ME towards a pro-invasive phenotype, and hypothesise that loss of Gal-7 modifies the ME, destabilizes the MEC interface and ultimately may lead to loss of the MEC population through apoptosis.

## **Methods**

Gal-7 expression and function was investigated in clinical samples and in-vitro model systems, respectively.

Gal-7 expression was assessed in a series of pure DCIS samples (low risk model) and DCIS with co-existant invasion (high risk model). Tissue sections were stained for Gal-7 and MEC expression scored on a duct-by-duct basis as positive, heterogeneous or negative.

An in-vitro model of normal primary myoepithelial cells isolated from reduction mammoplasty was used to investigate the functional impact of loss of Gal-7. These cells have high endogenous levels of Gal-7. Gal-7 was knocked down using siRNA and apoptosis assessed using cleaved caspase-3. The effect of Gal-7 on MEC layer integrity was assessed using immunofluorescence and adhesion assays.

The global impact of loss of Gal-7 was investigated using RNA sequencing.

## **Results**

In the tissue analysis 1926 DCIS ducts were scored for MEC expression of Gal-7. Significantly more ducts showed loss of Gal-7 in DCIS with co-existant invasion, with pure DCIS showing 388 ducts positive and DCIS with invasion 144 DCIS ducts positive ( $p=0.0014$ ). Pure DCIS and DCIS with invasion had 99 and 646 negative DCIS ducts respectively ( $p=0.0002$ ).

In model systems of primary MEC, knockdown of Gal-7 resulted in increased expression of cleaved caspase-3, suggesting lower levels of Gal-7 increases apoptosis. In functional assays silencing Gal-7 reduces adhesion to both fibronectin and laminin extracellular matrices ( $p$ -value 0.005 and 0.001 respectively)

RNA sequencing indicates silencing Gal-7 increases LOX expression - a key regulator of the collagen matrix of the microenvironment.

## **Conclusion**

Normal MEC strongly express Gal-7. Expression is lost in DCIS, with significantly more frequent loss in DCIS with co-existant invasion, suggesting that loss is associated with a more advanced phenotype. Functional assays indicate that loss of MEC Gal-7 enhances MEC apoptosis, which may be one mechanism by which this interface is lost during progression. Gal-7 negative MEC also show impaired adhesion to matrix proteins and lead to up-regulation of LOX, an enzyme key in promoting tumourigenesis. The incorporation of Gal-7 expression into a risk stratification algorithm has functional evidence and is currently being investigated.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-18-09

## Performance of Oncotype DX DCIS score across diverse ethnic populations

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There is a paucity of data on Oncotype DX DCIS Score and ethnic variation. It has been postulated that there are different molecular features /drivers among different ethnic populations. The Oncotype DX DCIS Score is a genomic test designed to analyze the expression of a group of 12 genes which can quantify 10-year local recurrence risk after surgery. The purpose of this study is to evaluate diverse ethnic patients with DCIS relative to traditional clinical pathologic factors and Oncotype DX DCIS Score

We analyzed consecutive cases of DCIS from 2011-2017 who underwent Oncotype DX DCIS Score testing at a single institution. Eighty-four female patients were divided into 5 groups based on self-reported ethnicity: White (36%), African American (AA) (48%), Asian (8%), Hispanic (4%) and Other (4%). Clinical and traditional pathologic factors were collected including age, nuclear grade (NG), ER/PR status and Oncotype DX DCIS Score. The distribution of NG and Oncotype DX DCIS Score was analyzed across ethnic groups.

The mean age at diagnosis was 63. Overall 99 % of cases were hormone positive. Comparison of White and AA patients revealed a correlation between ethnic group and DCIS Score, with a p value of 0.0087 (Table 1). Similarly, we looked at all five ethnic groups and Oncotype DX DCIS Score and found a p value of 0.022. We evaluated ethnicity with White and AA patients versus NG and obtained a p value of 0.084 (Table 2). In addition, we assessed all five ethnic groups and NG. We obtained a p value of 0.068. No AA patients with DCIS had a high DCIS Score. No patients with low NG DCIS had a high DCIS Score.

Analysis of the three factors (NG, ethnicity and DCIS Score) concurrently showed that they are not independent.

In summary, our study provides valuable data on Oncotype DX DCIS Score and NG across a diverse patient population. These data highlight the importance of incorporating both traditional clinical pathologic factors and DCIS Score molecular testing for making treatment decision across different ethnic patient populations.

Table 1. Comparison of DCIS Score and Ethnic Group

Ethnic Group	Low DCIS Score	Intermediate DCIS Score	High DCIS Score
AA	37	3	0
White	27	0	4

p=0.0087

Table 2. Comparison of Nuclear Grade and Ethnic Group

Ethnic Group	Nuclear Grade 1	Nuclear Grade 2	Nuclear Grade 3
AA	17	19	4
White	13	9	9

p=0.084

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-18-10

BHLHE40-AS1 is an enhancer associated long noncoding RNA critical to breast cancer progression

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Increased emphasis on breast cancer screening has led to a dramatic increase in diagnosis of ductal carcinoma *in situ* (DCIS). DCIS lesions are nonobligate precursors of invasive ductal carcinoma (IDC) and thus, the current standard-of-care is aggressive therapy to prevent invasive and metastatic disease. However, only ~40% of DCIS cases are predicted to progress leading to a current state of overtreatment and overdiagnosis. Thus, there is a critical need to identify functional determinants of progression of DCIS to IDC to allow discrimination between indolent and aggressive breast cancers and refine patient treatment strategies. We propose that long noncoding RNAs (lncRNAs) functionally drive breast cancer progression and their expression can discriminate between innocuous and potentially invasive DCIS.

Using biopsies from women with tandem DCIS and IDC lesions, we identified 35 lncRNAs whose expression can distinguish between pre-invasive and invasive disease. From this candidate list, the lncRNA BHLHE40-AS1 is found enriched in multiple breast cancer progression models, in HER2+ cell lines, and HER2+ patient tumors. Functionally, BHLHE40-AS1 is found to support tumor cell cycle and motility and may serve as a clinically relevant biomarker and therapeutic target in invasive breast cancer.

Further, BHLHE40-AS1 is expressed from a known super-enhancer that preliminary evidence suggests undergoes altered chromatin looping during breast cancer progression. Interestingly, over half of the candidate lncRNAs are associated with enhancer regions. Current studies are investigating super-enhancers, and associated lncRNAs that become acquired during breast cancer progression.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P5-18-11

Prognostic impact and possible pathogenesis of lymph node metastasis in ductal carcinoma in situ of the breast

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**Background:** By definition, ductal carcinoma in situ (DCIS) does not metastasize to the lymph nodes. However, since the introduction of molecular whole-node analysis using the one-step nucleic acid amplification (OSNA) assay for sentinel node (SN) biopsies, the number of DCIS patients with SN metastasis has increased. The clinical management of node-positive DCIS remains controversial because these patients can be treated as different stages based on the pathogenesis: e.g. occult invasive cancer with true nodal metastasis (T1N1) or true DCIS with iatrogenic dissemination of benign or tumor cells into lymph node (TisN0). In this retrospective cohort study, we aimed to elucidate the pathogenesis of nodal metastasis in DCIS and the clinical management of node-positive DCIS.

**Patients and Methods:** Subjects comprised of 427 patients with a routine postoperative diagnosis of DCIS who underwent SN biopsy using the OSNA assay between 2009 and 2012. The cut-off values of the OSNA assay for negative/positive results and micro/macrometastasis were defined at 250 and 5,000 copies/ $\mu$ L of cytokeratin 19 mRNA, respectively. In the SN-positive patients, all paraffin blocks containing the primary tumor were step-sectioned with 0.5-mm intervals until the tissue was exhausted, and all microscopic slides were examined for detecting occult invasions. Afterwards, the patients were classified into three cohorts based on the SN status and occult invasion: (1) no SN metastasis (TisN0), (2) SN metastasis without occult invasion (TisN1), and (3) SN metastasis with occult invasion (T1N1). Tumor characteristics including risk factors of occult invasions (e.g. large size, comedo-type), prognosis, and SN and non-SN status were compared among the three cohorts. The median follow-up time was 73.6 months.

**Results:** Of the 427 patients, 408 (95.6%) were SN-negative and 19 (4.4%) were SN-positive. By examining a total of 1,421 step-sectioned slides, 9 of the 19 SN-positive patients had occult invasions in the primary tumors. Overall, 408 (95.6%), 10 (2.3%), and 9 (2.1%) were classified into the TisN0, TisN1, and T1N1 cohorts, respectively. Either of adjuvant endocrine therapy or chemotherapy was given much more in the TisN1 and T1N1 cohorts than in the TisN0 cohort (80.0% and 88.9% vs. 5.4%). Other tumor characteristics were similar among the three cohorts. Although one patient had distant recurrence in the TisN0 cohort, none had locoregional or distant recurrences in the TisN1 and T1N1 cohorts. Regarding the lymph node status in the TisN1 and T1N1 cohorts, median tumor burdens in the SN are 590 and 310 copies/ $\mu$ L, and 2 (20.0%) and 2 (22.2%) patients had additional non-SN metastasis in the axillary dissection materials, respectively.

**Conclusions:** Tumor characteristics and prognosis were similar among the three cohorts albeit the TisN1 and T1N1 cohorts tended to receive adjuvant systemic therapy. Moreover, the SN and non-SN status were similar between the TisN1 and T1N1 cohorts. Therefore, pathogenesis of nodal metastasis in DCIS cannot uniformly be explained, and tumors with different stages may be mixed in the node-positive DCIS. Thus, considering the favorable prognosis of node-positive DCIS, the clinical management should be determined on a case-by-case basis.

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Effectiveness of Oncotype DX DCIS scoring in a community setting

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The Oncotype DX DCIS Score was developed to assist in determining a low risk for recurrence subgroup of patients who can avoid radiation therapy following breast conserving surgery. We sought to evaluate our experience using the Oncotype DX DCIS Score and to see if a greater number of patients who did not receive radiation therapy were experiencing a local recurrence than the score predicted.

Between February 23, 2011 and November 29, 2017, 145 women were diagnosed with ductal carcinoma in situ (DCIS) without an invasive component. 126 (87%) underwent breast conserving surgery. Thirty-five underwent Oncotype DX DCIS Score, of whom 26 (74%) were low risk, four were intermediate risk (11%), and five were high risk (14%).

The scores ranged from 0 (7 patients) to 100 (1 patient). Of the 26 patients with low risk scores, one chose to undergo mastectomy, three received radiation therapy, 20 chose observation without radiation therapy, and two patients were unknown because they had no further treatment or follow-up at our facility. Two of the four intermediate risk patients underwent radiation therapy and two did not. Four of the five high risk patients underwent radiation therapy, but one did not.

Twenty-two of the 35 patients who underwent Oncotype DX Score met the criteria for size (based on grade) and margins (at least 3 mm). Ten patients had margins that were less than 3 mm but met the size criteria. Two patients did not meet the size criteria but had at least 3 mm margins. One patient did not meet either the size or margin criteria. None of our 20 patients with low risk Oncotype DX DCIS Score and who met both the size and margin criteria recurred.

With the median follow-up of approximately 2-1/2 years, three of the 21 patients (14%) with low or intermediate risk scores who underwent Oncotype DX DCIS Score and did not receive radiation therapy suffered a local recurrence. The predicted average recurrence risk for these patients based on their Oncotype DX DCIS Score was 12%. Two of these patients who recurred had margins less than 3 mm, and one patient met the size and margin criteria, but had an intermediate risk score.

By comparison, five of 61 (8%) of patients who underwent breast conserving surgery and adjuvant radiation therapy had a local recurrence.

Twenty of the 23 (87%) low risk Oncotype DX DCIS Score patients did not receive radiation therapy and overall 20/35 (57%) of the patients undergoing Oncotype DX DCIS Score did not receive radiation therapy.

Although the follow-up is still relatively short, Oncotype DX DCIS Score allows a considerable number of women to avoid adjuvant radiation therapy.

# 2018 San Antonio Breast Cancer Symposium ®

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Immunohistochemical staining and *in vitro* analysis of HER2-positive breast cancer using trastuzumab and pertuzumab to develop an appropriate tracer in image-guided surgery

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**Background:** Pre- and intraoperative visualization of cancer cells using monoclonal antibody-based multimodal tracers can demarcate tumor margins in breast-conserving surgery. We focused on trastuzumab (Tmab) to develop a tracer for HER2-positive breast cancer by conjugation with a fluorescent dye. However, whether Tmab can be used as an imaging tracer for patients who receive Tmab as neoadjuvant therapy (NAD) is unclear, because tumor-cell HER2 could be bound by NAD Tmab at the time of surgery. This study evaluated immunohistochemical (IHC) staining and *in vitro* experiments with Tmab and pertuzumab (Pmab) as primary antibodies to find a suitable tracer. Pmab has a different antigen epitope than Tmab.

**Methods:** We included 43 patients with HER2-positive breast cancer, who were treated between 2010 and 2016. The NAD cohort ( $n=10$ , with 26 lesions) received chemotherapy and Tmab before surgery. The Tmab-naïve cohort ( $n=33$ , with 95 lesions) did not receive chemotherapy or Tmab before surgery. We excluded NAD patients with pathological complete responses. We evaluated the lesions, using IHC with Tmab and Pmab. We also performed flow cytometry and fluorescent microscopic analysis (FMA) using Tmab and Pmab conjugated with a fluorescent agent for the HER2-negative MCF7 and HER2-positive HCC1954 cell lines, which were pre-treated with Tmab to model NAD conditions.

**Results:** IHC with anti-HER2 antibody showed positive staining in all patients. Tmab staining was less intense, but positive Tmab IHC reactions were detected on tumor cell membranes in 77.8% of lesions in the naïve cohort and 70.8% in the NAD cohort. Pmab staining was seen in 46.3% of naïve cohort lesions and 22.2% of NAD lesions. Notably, we observed cytoplasmic staining in 87.8% of Pmab-negative cases in the NAD cohort. Flow cytometry showed less Tmab binding than Pmab binding in HCC1954 cells. After 24 hours' pretreatment incubation with Tmab, FMA showed a clear decrease in Tmab binding from 73.3% (without pretreatment) to 5.7% (after pretreatment), and a smaller decrease in Pmab binding from 72.7% (without pretreatment) to 66.4% (after pretreatment) in HCC1954 cells.

**Conclusions:** Pmab might be a suitable tracer for image-guided surgery after NAD, but identifying a suitable tracer for HER2-positive breast cancer will require further study.

# 2018 San Antonio Breast Cancer Symposium ®

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Machine learning-based structural analysis and oxygen saturation measurement of tumor-associated vessels in breast cancer using a photoacoustic tomography system

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## Introduction

Breast cancer induces angiogenesis, one of the primary factors responsible for tumor progression. Therefore, the ability to visualize angiogenesis at a higher resolution is crucial. Photoacoustic tomography is a noninvasive method of visualizing angiogenesis involving light absorption and ultrasonic wave emission. If the irradiation light wavelength is adjusted for hemoglobin, vascular imaging is possible. Furthermore, using two wavelengths for oxidized and reduced hemoglobin, "S-factor," can be calculated, which nearly corresponds to oxygen saturation. Therefore, photoacoustic imaging allows the assessment of breast lesions from vascular structural and functional viewpoints.

## Objectives

This study aimed to demonstrate the possible utility of photoacoustic tomography for clinical application focusing on the morphologic features and oxygen saturation status of breast tumor-related vessels.

## Methods

For the morphological analysis, we applied a machine learning-based method for automatic vessel extraction, and for the functional analysis we evaluated hemoglobin oxygen saturation calculating signals obtained at two wavelengths. In our system, a 3D ultrasound image was simultaneously acquired as a volume image of a tumor, which helped analyze the positional relationship between the vessels and the tumor.

## Results

On morphological analysis, the fine structure of tumor-related vessels was rendered in high resolution. In our system, the blood vessels branched toward the tumor 2-3 more times more frequently than observed on contrast-enhanced MRI, illustrating a finer level of blood vessels near the tumor on our system than on MRI. Next, we analyzed the six morphologic features of vessels (radius, volume, curvature, contraction, maximum angle and vessel branch number) that are associated with the pathologic condition in neuroscience. We determined that the feature distribution of vessels located close to the tumor differed from that located away from the tumor. For example, vessels near the tumor had higher curvature, which means they are more tortuous than healthy vessels. The difference in the distribution of all six features was statistically significant on the Kolmogorov-Smirnov test.

On functional analysis, S-factor measurement of the healthy human breast demonstrated clearly demarcated arteries and veins. The S-factor of any artery was nearly 100%, while that of the veins inside the breast cancer tended to be a little higher (approximately 5%) compared to that in the healthy part. This tendency of veins was not recognized in benign tumors. This could show arteriovenous shunt in cancer microenvironment. We found low saturation signals emerging in the tumor tissue following bevacizumab-containing chemotherapy, indicating the possibility that our system reveals microenvironment changes.

## Discussion

If our system can identify the structure or oxygen saturation characteristics unique to tumor-associated vasculature, it could contribute to the improved accuracy of breast cancer diagnosis and allow the observation of tumor vessel normalization because of the drug treatment. An earlier grasp of the therapeutic effect could lead to the provision of individualized medicine.



# 2018 San Antonio Breast Cancer Symposium ®

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Exploring the relationship between an *in vitro* model of breast cancer cell mineralisation and the cancer grade specific composition of ex vivo microcalcifications

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**Background:** Microcalcifications resulting from calcium deposition in the mammary gland play a central role in the early detection of breast cancer [1]. However, the relationship between their occurrence in the breast and cancer progression remains poorly understood. Our approach is to use vibrational spectroscopy and imaging, which is non-invasive, non-destructive, label-free and chemically specific, to assess the composition and distribution of the deposits in an *in vitro* cancer cell model of mineralisation [2]. In parallel we will utilise the same methods to measure the biochemical composition of microcalcifications found in breast biopsies from different grades of cancer. The ultimate aim of the study is to link the changes identified during the *in vitro* mineralisation process with the different stages of breast cancer. Vibrational spectroscopic methods can provide incredibly detailed biomolecular fingerprints enabling us to elucidate both the compositional changes with advancing pathology and the spatial distribution of those changes within the calcification and the surrounding tissue.

**Methods:** The breast cancer cell line MDA-MB-231 has the ability to produce mineralisation. This mineralisation was assessed over a 14-day period in the presence of different osteogenic cocktails: one composed of ascorbic acid,  $\beta$ -glycerophosphate ( $\beta$ G) and dexamethasone (Dex), and another one composed of inorganic phosphate (Pi). Fixed cells were analysed using Raman spectroscopy and micro-FTIR imaging at different time points (3, 7, 11 and 14 days). Tissue sections from patients with microcalcifications identified in histopathology will be sectioned to 3 mm and imaged with infrared (Agilent 670 FTIRinterferometer and Focal Plane Array imaging microscope) and Raman (Renishaw inVia) microspectrometers.

**Results:** We observed distinct and specific phosphate peak ( $\text{PO}_4^{3-}$ ) at 960 and 1020  $\text{cm}^{-1}$  in Raman and FTIR spectra, respectively, corresponding to hydroxyapatite crystal and indicating the presence of microcalcification formation. Treatment with Pi induced a faster mineralisation (day 3) compared to cells treated with  $\beta$ G (day 11) and different spectral profiles during this development phase. In addition, there are changes in both the relative DNA and protein concentrations in the cells following 11 days exposure to the osteogenic agents.

It has been shown that the level of carbonate substitution in the calcium hydroxyapatite crystal correlates directly with the pathology of the tissue surrounding the microcalcification. Here we compare the mineral composition found ex vivo versus the in vitro model.

**Conclusion:** It could be possible to link the progressive biophysical changes associated with mineralisation to distinct stages of breast cancer pathology based on protein, lipid and carbonated apatite contents of the mineralised cells.

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# 2018 San Antonio Breast Cancer Symposium ®

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Initial experience of dedicated breast PET imaging of ER+ breast cancers using [F-18]fluoroestradiol

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**Introduction:** Breast cancer is a heterogeneous disease encompassing distinct subtypes with variable treatment response, relapse risk and overall prognosis. The majority of breast cancers are estrogen receptor-positive (ER+). While neoadjuvant endocrine therapy trials have been proposed to better identify therapeutic approaches for ER+ breast cancer, accurate quantification of the ER biomarker is necessary to assess the primary tumor and its likelihood of response to treatment. Dedicated breast positron emission tomography (dbPET) is an emerging technology with a high spatial resolution that enables detection of sub-centimeter lesions and depiction of intratumoral heterogeneity. In this study, we report our initial experience with [F-18]fluoroestradiol (FES) dbPET in assessing ER+ primary breast cancers.

**Materials and Methods:** In an IRB-approved protocol, patients with biopsy-confirmed ER+ breast cancers were imaged with dbPET (MAMMI, OncoVision, Valencia, Spain) as a companion diagnostic tool to standard breast MRI. A dose of 5 mCi of FES was administered and patients were imaged in the prone position at 45 min post-injection. As part of routine clinical care, MR images were reviewed by a certified breast radiologist experienced in breast MRI. DbPET was reviewed by a radiologist specialized in nuclear imaging.

**Results:** Five patients with ER+ breast cancers were imaged. Patient ages ranged from 33 to 64. Two patients with infiltrating lobular carcinomas measuring up to 6.7 cm and 5.3 cm at MRI demonstrated corresponding FES tumor-to-normal maximum standard uptake value ( $SUV_{max}$ ) ratio at 4.81 and 2.49 respectively. A third patient demonstrated multifocal FES uptake corresponding to multifocal invasive ductal carcinoma and ductal carcinoma in situ with disease foci ranging from 9-13 mm. In this patient, the more posterior disease foci seen on MRI were excluded from the field of view of dbPET. One patient demonstrated an absence of FES uptake in her 3.4 cm infiltrating ductal carcinoma, which was due to estrogen receptor blockade from the recent administration of tamoxifen for a fertility preservation procedure. The final patient had metastatic cervical and axillary lymphadenopathy secondary to a breast primary that was occult on mammography and MRI. FES-dbPET also showed no corresponding uptake in the ipsilateral breast, possibly due to the small size of the primary lesion and/or low tumor to background uptake ratio.

**Conclusions:** FES-dbPET imaging has potential as a diagnostic tool that is complementary to MRI in characterizing ER+ primary breast cancers. Limitations include variations of FES uptake in different ER+ breast cancer diseases and exclusion of posterior breast tissue near the chest wall and the axillary regions. However, FES-dbPET has a high potential for clinical utility, especially in measuring response to neoadjuvant endocrine treatment. Further development to improve the dbPET field of view and studies with a larger cohort of ER+ breast cancer patients are therefore warranted.

# 2018 San Antonio Breast Cancer Symposium ®

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Detection of HER2 positive tumor cells using functionalized iron oxide nanoparticles

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**Background:** Iron oxide nanoparticles (NPs) have been used for a variety of *in-vivo* and *ex-vivo* applications within the biomedical sciences. Moreover, when intended for clinical *in-vivo* applications, NPs need to meet rigorous requirements to ensure safety as well as bio-functionality including blood circulation time and specificity for cellular targets. PrecisionMRX® NPs are extensively characterized superparamagnetic NPs composed of a 25nm magnetite cores that are currently employed in a variety of *in-vivo* applications including non-invasive/in vivo diagnosis of cancer, Magnetic Particle Imaging, MRI, and magnetic hyperthermia.

**Objective:** Here we report on the extensive pre-clinical development and functionality of antibody (Herceptin)-conjugated NPs for *in-vivo* and *ex-vivo* detection of HER2+ tumor cells by Magnetic Relaxometry (MRX).

**Results:** We observed: 1) specific binding and detection of HER2 positive tumor cells in-vitro; 2) specific detection of HER2+ tumors in mice; 3) binding and amplitude of magnetic signal to be proportional to the level of HER2 expression in-vitro and in-vivo; 4) the nanoconstruct remains stable in circulation; 5) the particles do not induce a pro-inflammatory response nor activate complement; 6) the particles are biodegradable; and do not induce acute or delayed signs of morbidity in mice.

**Conclusion:** Precision MRX® nanoparticles offer great clinical promise including the *in- vivo* detection of tumor cells by magnetic relaxometry. Given the stability and safety of these NPs, our pre-clinical results support progressing to clinical testing. A first-in human ex-vivo clinical research study design and strategy will be discussed.

# 2018 San Antonio Breast Cancer Symposium ®

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Predictive models for CEUS of the breast: Is it feasible in improved performance of BI-RADS evaluation of critical breast lesions?—Primary analysis from multi-center prospective study in China

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## [Background]

Breast imaging reporting and data system (BI-RADS) is widely used to assess malignant risk of breast lesions but causes high false-positive biopsy.

## [Objective]

To determine whether predictive model for contrast-enhanced ultrasound (CEUS) of the breast can improve the precision of BI-RADS.

## [Methods]

A total of 1034 patients with 1060 solid breast lesions (5.0-39.8mm,  $17.89 \pm 8.65$ mm) classified as BI-RADS 4 or 5 on conventional ultrasound and mammography were evaluated. CEUS was performed before core needle biopsy or surgical resection and a revised BI-RADS classification was assigned based on 6 predictive models for CEUS of malignant and benign breast lesions as follows: malignant predictive models: (1) hyper-enhancement with enlarged size, with or without irregular shape; (2) hyper-centripetal enhancement with perfusion defect, with or without enlarged size; (3) rapid or synchronous wash-in with hyper- or iso-enhancement, present penetrating vessels or crab claw-like pattern, with or without perfusion defect. Benign predictive models: (4) rapid wash-in with hyper-enhancement, clear margin after enhancement without enlarged size; (5) synchronous or slow wash-in with iso-enhancement, and cannot distinguish margin and shape after enhancement; and (6) synchronous or slow wash-in with hypo-enhancement, with equal or smaller size after enhancement. Receiver operating characteristic curve analysis was then conducted to evaluate the diagnostic performance of CEUS-based BI-RADS assignment with pathological examination as reference criteria.

## [Results]

The CEUS-based BI-RADS evaluation classified 287/1060 (27.08%) lesions into category 3, 195 (18.40%), 124 (11.7%) and 144 (13.58%) lesions into categories 4A, 4B and 4C, respectively, and 310 (29.24%) into category 5, compared with 423/1060(39.91%), 348(32.83%), 150(14.15%) and 139(13.11%) in BI-RADS 4A, 4B, 4C. and 5 based on conventional ultrasound and mammography. Selecting CEUS- based BI-RADS category 3 as an appropriate cut-off gave accuracy, sensitivity, specificity, positive and negative predictive values of 69.25%, 98.06%, 49.47%, 58.99% and 96.86%, respectively for the diagnosis of malignant disease. The cancer-to-biopsy yield was 60.16% with CEUS-based BI-RADS 3 selected as the biopsy threshold compared with 43.86% otherwise, while the biopsy rate was only 72.92% compared with 100% otherwise(Figure 2). Overall, only 1.94% of invasive cancers were misdiagnosed as BI-RADS 3 we use nowadays.

## [Conclusion]

This study suggests that evaluation of BI-RADS 4 or 5 breast lesions with CEUS result in reduced biopsy rates and increased cancer-to-biopsy yields.

# 2018 San Antonio Breast Cancer Symposium ®

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Near-infrared spectral tomography (NIRST): A prognostic assessment tool for predicting residual cancer burden (RCB) during neoadjuvant chemotherapy (NAC) in breast cancer (BC)

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**Background:** NIRST, a noninvasive imaging with no ionizing radiation, has been found to be prognostic as a tool to monitor early pathologic response to NAC in BC using biophysical properties of the tumor compared with normal breast tissue. We aim to establish NIRST indicators as early surrogates of treatment response and to evaluate its potential as a predictive tool in treatment decisions.

**Methods:** 27 women with locally advanced BC undergoing NAC were enrolled in this pilot study. NIRST imaging was performed pre-treatment, after cycle 1 and 2, at the mid-point of NAC, and at the conclusion of NAC prior to surgery. Biophysical data including oxy- and deoxy-hemoglobin, water, lipid, and scatter components were obtained at these time points. To minimize inter-subject variability due to breast density and its effects on the NIRST data, statistical analysis was conducted using ratios of obtained biophysical data to pretreatment average of the contralateral normal breast tissue. Residual Cancer Burden (RCB) index was used to evaluate residual disease after treatment with NAC. RCB scores and classes were determined in 24 of the 27 surgical tissue specimens and these were compared to the NIRST data. RCB data for 3 patients were excluded: 2 patients had undergone positive excisional lymph node biopsy prior to NAC and 1 patient had surgery at an outside hospital.

**Results:** Of the 27 patients, 7 had triple negative BC and 13 had HER-2 positive BC. The change in total hemoglobin ( $\Delta\text{Hb-T}$  %) after the first cycle of NAC when compared to the pre-treatment total hemoglobin was determined to be the best predicting factor for RCB (p-value <0.001). The Pearson correlation coefficient was calculated for both RCB class and RCB score (0.7 and 0.6). The significance of the correlation coefficient was evaluated using two-sided t-test and the resulting P-values of 0.006 and 0.001 respectively demonstrate that these correlations are statistically significant.

Summary of the NIRST biophysical data and the correlating RCB

Patient	Age	ER	PR	Her2	RCB Score	HbT - $\Delta$	HbT-pre
1	36	+	-	+	0	-139.93	3.30
2	51	-	-	-	0	-43.53	2.53
3	41	+	+	+	0	-42.28	1.89
4	30	-	-	-	0	-43.06	1.59
5	52	-	-	-	0	-42.54	1.71
6	63	+	+	+	0	-151.88	3.12
7	60	-	-	+	0	-46.47	1.59
8	52	-	-	-	0	-42.72	1.96
9	66	-	-	+	0	-110.02	2.47
10	39	-	-	+	0	-9.09	1.10
11	71	+	-	+	0	-13.33	1.50
12	52	+	+	-	4.121	53.20	1.58
13	62	+	+	-	3.747	5.74	1.53
14	70	-	-	+	1.931	100.52	1.63
15	53	+	+	-	3.444	7.83	1.18
16	41	+	+	+	4.189	29.84	1.51
17	56	+	+	+	4.444	59.00	1.47

18	50	+	+	-	4.008	-41.11	2.18
19	54	-	-	-	3.050	11.11	1.80
20	63	+	+	-	2.900	20.00	1.50
21	49	-	-	-	0.780	26.32	1.90
22	57	+	+	-	1.850	5.88	1.70
23	47	+	+	-	3.600	-7.69	2.60
24	70	-	-	-	3.100	47.06	1.70

**Conclusions:** We have demonstrated a statistically significant correlation between  $\Delta\text{Hb-T \%}$  after the first cycle of NAC and the RCB. These findings suggest the potential of using NIRST as an early assessment tool to evaluate response to NAC in BC patients and warrant further evaluation in a larger study.

# 2018 San Antonio Breast Cancer Symposium ®

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Interpretation schema for optical coherence tomography images in breast tissue

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## Purpose

Optical coherence tomography (OCT) is an attractive technology for surgical imaging because it permits the real-time visualization of microscopic tissue morphology with a handheld probe without the need for exogenous agents, tissue manipulation, ionizing radiation, or histological processing. While initial studies have shown that OCT is an effective margin-evaluation tool for breast conserving surgery (BCS), image interpretation and feature identification have not been directly studied. In this work, breast pathologies were assessed with a handheld OCT probe and the images were compared to histology.

## Methods

Mastectomy and BCS specimens from 26 women were imaged with a handheld OCT probe, and histology slides from the same region were digitally photographed. OCT and histology images from the same region were paired by selecting the best structural matches. Because image characteristics in OCT are akin to those in ultrasound, descriptive OCT image feature terminology similar to that of ultrasound was developed. Each of these characteristics was used to select and describe OCT-histology image matches.

## Results

In total, 2880 OCT images were acquired from 26 breast specimens, and 48 matching OCT-histology image pairs were identified. These matched image pairs illustrate tissue types including adipose tissue, dense fibrosis, fibroadipose tissue, blood vessels, regular and hyperplastic ducts and lobules, cysts, fibroadenoma, IDC, ILC, DCIS, calcifications, and biopsy cavities. Differentiation between pathologies was achieved by considering feature boundaries, interior appearance, posterior shadowing or enhancement, and overall morphologic patterns.

## Conclusions

This is the first work to systematically catalog the features of breast OCT images. The results indicate that OCT can be used to identify important structures and distinguish between benign and malignant breast pathologies.

## 2018 San Antonio Breast Cancer Symposium ®

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Ultrasound assessment of residual disease after neoadjuvant chemotherapy (NACT) in node positive triple negative breast cancer (TNBC)

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**Purpose:** To determine accuracy of preoperative ultrasound after NACT to predict residual disease in triple negative breast cancer (TNBC) patients with confirmed axillary nodal metastasis.

**Methods:** This is an institutional review board approved retrospective study of TNBC patients who received NACT at MD Anderson Cancer Center from January 1999 - June 2015. We identified 327 TNBC patients who had cytologically confirmed breast and nodal disease at baseline evaluation and had preoperative ultrasound evaluation of residual disease. Ultrasound response was divided in three categories: radiologic complete response (rCR) - complete resolution of the malignant mass); near-rCR - no discernible mass, only an isoechoic flat tumoral bed); and residual disease (RD) - a discernible mass is seen. Axillary ultrasound images were evaluated for lymph node size, cortical thickness and residual morphological type after NAC (type I-VI). Ultrasound breast and axillary findings were compared with final surgical pathology.

**Results:** In 89 cases (27%), pCR was achieved. 74% (242/327) were unifocal and 26% (86/327) multifocal. Ultrasound rCR was seen in 11% patients (36/327). Of those, 64% (23/36) showed pCR and 36% (13/36) showed residual disease. Ultrasound near-rCR was seen in 26% (84/327). Of those, pCR was seen 49 % (41/84) and residual disease in 51% (43/84). Residual disease was seen in 63% (207/327), 12% (25/207) showed pCR and 88% (182/207) showed residual disease. Regarding axillary lymph nodes, long axis diameter mean was 1.57 cm for patients with pCR and 1.6 cm for no pCR, short axis diameter mean was 0.67 cm for pCR and 0.87 cm for no pCR. Cortical thickness mean was 2 mm for pCR versus 9 mm for no pCR.

Sensitivity of ultrasound for assessment residual disease (ultrasound was considered positive if either breast ultrasound or axillary ultrasound showed residual disease) was 97%. Specificity is 22.47% with a NPV of 74% and PPV of 77%.

**Conclusion:** Breast and axillary ultrasound performed after NACT showed low specificity but high sensitive to detect residual disease. rCR and near rCR were related with pCR in 64% and 49 % of the cases respectively.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-02-05

Downstream workup after post-treatment mammography in breast conservation therapy: Is there a significant difference between tomosynthesis and 2-dimensional mammograms?

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## Introduction:

Emerging data suggest that tomosynthesis mammograms (TS) are considerably superior to two-dimensional (2D) screening mammograms (2DMG) at reducing false positive biopsies for breast cancer screening. However, very little is reported about the comparative efficacy of the two modalities in the post-treatment setting. We compared the rate of downstream workup up after undergoing post-radiation screening 2DMG and TS following breast conservation therapy at our institution.

## Methods:

Between the years 2011-2017, 712 breast cancer patients (range 31-91 years) were treated with lumpectomy and adjuvant radiotherapy. As per institutional standard, follow up included either screening 2DMG (n=569) or TS(n=143) and reviewed in this IRB-approved study. The primary endpoint for comparison was the rate of further imaging/workup post-treatment. Comparative analysis was conducted via multivariable binomial regression with propensity matching between the 2DMG and TS groups. Patients with clinical suspicion of recurrence otherwise were excluded.

## Results:

The patient cohort in both groups included the following clinical characteristics,: 129 patients with ductal carcinoma in-situ (the remainder were invasive carcinoma; ductal or lobular). A total of 418 patients had T1 lesions, 143 T2, and 22 T3/T4. Eighty-five patients were node positive. Of those, 501 ER+/Her2-, 101 triple negative, and 96 triple positive. Adjuvant radiation included conventional fraction (457) or hypofractionation (153) with boost to the surgical cavity in 523. Accelerated Partial Breast Irradiation (APBI) was delivered in 106 patients. Post treatment scans occurred within 3 months (166), at 3-6 months (256), or 6+ months (281). The aforementioned characteristics were similarly distributed between 2DMG and TS groups, except for slightly more DCIS in the 2D group.

There was a significantly higher proportion of patients that were recommended for immediate downstream workup in the 2D group (40.7%) compared to in the tomo group (16.8%) (HR = 3.40, P < 0.001), leading to 12 biopsies in the 2D group (3 positive) and 4 biopsies in the TS group (0 positive). Upon multivariate analysis, the use of tomo was the lone correlate of reduced downstream workup (p < 0.05), although there was a trend toward significance in patients who were first imaged at a post-treatment interval of 6+ months and in patients not undergoing a radiation boost (P < 0.10).

## Conclusion:

Post-breast conservation follow-up with tomo synthesis mammography resulted in significantly less downstream workup as compared to conventional 2D screening mammography. Further investigation is warranted to unveil the absolute and relative cost-effectiveness between the two modalities.

# 2018 San Antonio Breast Cancer Symposium ®

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Characteristics of lymphocyte-predominant breast cancer in ultrasound images and their application to diagnostic prediction

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## Purpose

Tumor-infiltrating lymphocytes (TILs) is a prognostic factor for breast cancer, however, an accurate and simple evaluation method remains elusive. Therefore, we focused on findings characteristic of lymphocyte-predominant breast cancer (LPBC) in ultrasound (US) images. In this study, the application of preoperative US image assessment to diagnostic prediction of LPBC evaluated from postoperative pathological specimens, was appraised.

## Methods

We evaluated 191 patients with invasive breast cancer between January 2014 and December 2017. All patients were treated by either mastectomy or breast-conserving surgery. Stromal lymphocytes were evaluated on surgical pathological specimens. Breast cancer samples with  $\geq 50\%$  stromal TILs were defined as LPBC. Preoperative US was performed in all cases and images were examined for characteristics indicative of TILs. Scores were given to US images with characteristic TILs and these TILs-US scores were assessed for their application to predict LPBC.

## Results

There were 39 cases of LPBCs and 122 cases of non-LPBCs in surgical pathological specimens. The characteristic US image findings predicting LPBC were shape (more lobulated), internal echo level (weaker) and posterior echoes (stronger). The TILs-US scores were given based on these three ultrasound tissue characterizations. We set TILs-US score cut-offs for predicting LPBC at 4 points (Sensitivity, 0.73; specificity, 0.87; accuracy, 0.83) based on the receiver operating characteristics (ROC) curves (AUC, 0.88). There were significant predictors for LPBC in multivariate logistic analysis (Nuclear Grade (NG): OR3.4,  $p=0.02$ ; ER: 5.7,  $p=0.007$ ; HER-2: OR4.1,  $p=0.04$ ; TILs-US score2: OR14.9,  $p<0.001$ ) in preoperative clinicopathological factor. The sensitivity, specificity and accuracy of NG for predicting LPBC were 0.75, 0.69 and 0.71. Those of ER and HER2 were 0.33, 0.96 and 0.79. Sensitivity, specificity, and accuracy of NG, ER, and HER2 diagnoses were all lower than the TILs-US score, and the TILs-US score showed the best diagnostic ability.

The sensitivity, specificity and accuracy of predicting LPBC

Performance measure	TILs-US score (95%CI)	NG	ER or HER-2	P, TILs-US score vs. NG,	P, TILs-US score vs. ER or HER-2
Sensitivity	0.73 (0.63-0.81)	0.75 (0.64-0.84)	0.327 (0.24-0.39)	0.08	<0.001
specificity	0.87 (0.83-0.90)	0.69 (0.65-0.72)	0.957 (0.93-0.98)	<0.001	<0.001
accuracy	0.83 (0.77-0.88)	0.707 (0.65-0.376)	0.785 (0.74-0.82)	0.004	0.25

## Conclusions

LPBC has characteristic ultrasound tissue characterizations in US images. Thus, TILs-US scores based on US may be applicable to accurate and convenient preoperative diagnosis of LPBC.

# 2018 San Antonio Breast Cancer Symposium ®

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Is real-time rescanning by radiologists necessary for all ACR BI-RADS US category 3 to 5 lesions in the diagnostic setting?

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**Purpose:** To assess the value of radiologists' rescanning before final interpretation for American College of Radiology (ACR) Breast Imaging Reporting and Data System ultrasonography (US) category 3–5 lesions.

**Materials and Methods:** Image data on 1070 patients with 1070 category 3–5 breast lesions with a pathological diagnosis scanned between January 2016–June 2017 were included. Both real-time and static images were acquired for each lesion. The diagnostic performance of assessing static images and rescanning by the radiologist were each calculated. The positive predictive values (PPVs) of each category in the two groups were calculated and compared.

**Results:** The sensitivity, specificity, PPV, and negative predictive value for real-time US were 98.9%, 58.2%, 44.8% and 99.4%, and for static images were 98.9%, 57.1%, 44.1% and 99.3%, respectively. Areas under the curves were not significantly different if final assessment was only dichotomized as negative or positive ( $\geq$  category 3:  $P = .566$ ). Significant differences were observed if more detailed classification was performed (category 3–5 without subcategory: 0.969 vs. 0.955,  $P = .0113$ ; category 3–5 with subcategory 4A–4C: 0.915 vs. 0.855,  $P < .0001$ ). All PPVs of each category for each assessment were within the reference range provided by the ACR in 2013 except subcategory 4B of static image evaluation, which was also significantly higher than that of real-time assessment (54.8% vs. 40.7%,  $P = .037$ ). BI-RADS US of real-time and static images evaluation correlate with pathology are listed in Table 1.

Table1

BI-RADS US category/subcategory	PPV provided by ACR	Real-time US n (%)	Static image US n (%)	P
3	>0 & ≤2%	467	458	1.000
Benign		464(99.4%)	455(99.3%)	
Malignant		3(0.6%)	3(0.7%)	
4A	>2% & ≤10%	288	301	0.257
Benign		271(94.1%)	276(91.7%)	
Malignant		17(5.9%)	25(8.3%)	
4B	>10% & ≤50%	91	135	0.037
Benign		54(59.3%)	61(45.2%)	
Malignant		37(40.7%)	74(54.8%)	
4C	>50% & <95%	50	79	0.473
Benign		5(10.0%)	4(5.1%)	
Malignant		45(90.0%)	75(94.9%)	
5	≥95%	174	97	1.000
Benign		3(1.7%)	1(1.0%)	
Malignant		171(98.3%)	96(99.0%)	

BI-RADS US Breast Imaging Reporting and Data System for Ultrasonography, PPV positive predictive value, ACR American College of Radiology

The overall agreement of the two approaches was moderate ( $\kappa = 0.43$  if lesions were assigned to category/subcategory 3, 4A–4C, 5).

**Conclusion:** Both static image evaluation and real-time assessment had similar diagnostic performance for most lesions; however, the diagnostic performance of static image evaluation for subcategory 4B lesions was lower. Real-time rescanning by the interpreter is strongly recommended for lesions of subcategory 4B after assessing static images. YH and JM contributed equally to this study.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-02-08

SUV max of <sup>18</sup>F FDG-PET/CT: A predictive factor associated with pathologic complete response in luminal HER2–negative breast cancer patients (receiving neoadjuvant chemotherapy)

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**Purpose** Neoadjuvant chemotherapy (NAC) is considered to be the standard of care for locally advanced breast cancer. When pathological complete response (pCR) is obtained with NAC it is a predictor of better outcome and often used as a surrogate for survival. However, response to NAC of luminal type breast cancer is variable and mostly limited. This study investigated the predictive relevance of several clinicopathological factors, including parameters of <sup>18</sup>F FDG-PET/CT, on the pCR to NAC in patients with luminal HER2–negative breast cancer.

**Methods** From 2009 to 2015, 117 hormone receptor-positive, HER2-negative breast cancer patients who were treated with NAC followed by curative surgery at the Kyungpook National University Hospital (Daegu, Korea) were retrospectively analyzed. pCR was defined as the absence of cancer cells in breast and axillary node. <sup>18</sup>F FDG-PET/CT maximum standardized uptake value (SUV max) was measured at baseline. Patients received from 6 to 8 cycles of anthracycline-based and taxane-based NAC.

**Results** The median age of the patients was 48 years (range-29-68 years). 104 patients (88.9%) were PR-positive, and forty-nine (41.9%) patients showed high ki-67 expression at initial diagnosis. After NAC, nine patients (7.7%) achieved pCR and patients who had high initial SUV max ( $\geq 9.09$ ) achieved improved pCR rate compared to low initial SUV max ( $< 9.09$ ) patients (77.8 % vs. 22.2%).

**Conclusion** In luminal HER2-negative breast cancer, <sup>18</sup>F FDG-PET/CT SUV max was useful for predicting pathologic complete response after NAC

**table 1 Characteristics of study population**

Median, range	48 (29-68)
Initial clinical T stage 1 2 3 4	8 (6.8) 83 (70.9) 19 (16.2) 7 (6.0)
Initial clinical N stage 0 1 2 3	10 (8.5) 49 (41.9) 47 (40.2) 11 (9.4)
Initial ER expression Negative (0-2) Weak (3-5) Strong (6-8)	8 (6.8) 14 (12) 95 (81.2)
Initial PR expression Negative (0-2) Weak (3-5) Strong (6-8)	13 (11.1) 20 (17.1) 84 (71.8)
Initial Ki-67 expression <14% $\geq$ 14% Not assessed	55 (47) 49 (41.9) 13 (11.1)
Pathologic T stage 0 I II III	16 (13.7) 50 (42.7) 42 (35.9) 9 (7.7)
Pathologic Stage (AJCC) pCR 1A 1B 2A 2B 3A 3C	9 (7.7) 23 (19.7) 7 (6.0) 27 (23.1) 21 (17.9) 23 (19.7) 7 (6.0)
Histologic grade 1 2 3	22 (18.8) 61 (52.1) 14 (12)
Nuclear Grade 1 2 3	16 (13.7) 9 (7.7) 50 942.7)
Pathologic complete response Yes	9 (7.7)
Recurrence	18 (15.4)
Death	10 (8.5)

**table 2 Association between pathologic complete response and clinicopathologic features**

Factors	Pathologic CR No	Pathologic CR Yes	P-value
All patients	108 (92.3)	9 (7.7)	
Age <40 40-55 56-70	16 (18.2) 54(61.4) 18 (20.5)	2 (22.2) 6 (66.7) 1 (11.1)	0.789
Initial clinical T stage 1 2 3 4	8 (7.4) 76 (70.4) 7 (6.5) 17 (15.7)	0 (0) 7 (77.8) 2 (22.2) 0 (0)	0.676
Initial ER expression Negative (0-2) Weak (3-5) Strong (6-8)	7 (6.5) 12 (11.1) 89 (8.4)	1 (11.1) 2 (22.2) 6 (66.7)	0.504
Initial PR expression Negative (0-2) Weak (3-5) Strong (6-8)	11 (10.2) 20 (18.5) 77 (71.3)	2 (22.2) 0 (0) 7 (77.8)	0.247
Initial Ki-67 expression <14% ≥14%	52 (54.7) 43 (45.3)	3 (33.3) 6 (66.7)	0.301
SUV max (tumor) < 9.09 ≥ 9.09	72 (66.7) 36 (33.3)	2 (22.2) 7 (77.8)	0.012
SUV max (axilla) < 6.08 ≥ 6.08	58 (67.4) 28 (32.6)	3 (42.9) 4 (57.1)	0.228

# 2018 San Antonio Breast Cancer Symposium ®

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Diagnostic performance of dedicated breast PET for the prediction of pathological response after neoadjuvant chemotherapy

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**Background:** Neoadjuvant chemotherapy (NAC) is a standard treatment for operable breast cancer. However, imaging methods for evaluating treatment response have not been established. Previous studies reported that ring-type dedicated breast positron emission tomography (DbPET) detected residual tumors following NAC more accurately than whole-body PET/CT. This study assessed DbPET parameters for predicting pathological complete response (pCR) in patients with breast cancer.

**Patients and Methods:** Among patients with breast cancer who underwent surgery after NAC, 61 were examined using ring-type DbPET before and after NAC. The maximum standardized uptake values (SUVmax) and tumor-to-normal-tissue ratio (TNR) were calculated before and after NAC (pre-SUVmax, pre-TNR, post-SUVmax and post-TNR, respectively). Moreover, the reduction rates ( $\Delta$ SUVmax and  $\Delta$ TNR) were determined. pCR was defined as complete remission of breast cancer.

**Results:** The median patient age was 52 years. Forty patients (65.6%) were estrogen receptor (ER)-positive, whereas 25 patients (41.0%) were HER2-positive. Fifteen patients (24.6%) achieved pCR after NAC. The calculated values for the parameters of DbPET are summarized in Table 1. The most promising parameters for predicting pCR were  $\Delta$ SUVmax (area under the curve [AUC]: 0.506) and post-TNR (AUC: 0.640)

**Table 1. Diagnostic performance of dedicated breast positron-emission tomography for the prediction of pathological complete response after neoadjuvant chemotherapy**

Parameters	pCR Median (IQR)	Non-pCR Median (IQR)	P	AUC (95% CI)
Pre-SUVmax	14.8 (9.2–17.7)	14.4 (10.7–19.8)	0.604	0.454 (0.285–0.624)
Post-SUVmax	1.8(1.5–2.1)	2.0 (1.6–3.4)	0.237	0.603 (0.460–0.746)
$\Delta$ SUVmax (%)	87.32 (81.5–91.8)	88.6(82.8–91.8)	0.954	0.506 (0.329–0.682)
Pre-TNR	8.0 (5.3–9.2)	7.3(5.9–10.2)	0.757	0.472 (0.297–0.647)
Post-TNR	1.7(1.0–1.1)	1.2 (1.0–2.4)	0.098	0.640 (0.506–0.774)
$\Delta$ TNR (%)	87.3 (78.3–89.1)	79.8 (62.7–86.7)	0.113	0.638 (0.472–0.803)

**pCR, pathological complete response; IQR, interquartile range; AUC, area under the curve; CI, confidence interval, SUV, standardized uptake value; TNR, tumor-to-normal-tissue ratio.**

. Although neither of these two parameters reflected the pathological response to NAC in patients with ER-positive disease, post-TNR showed the highest AUC (i.e., AUC: 0.750) for pCR in patients with ER-negative disease. The sensitivity and specificity of post-TNR in the ER-positive group were 85.7% and 39.4%, respectively. In the ER-negative group, these values were 100% and 58.3%, respectively

**Table 2. Diagnostic accuracy of post-TNR according to estrogen receptor status**

	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)	NPV (%)
ER-positive	85.7	39.4	47.5	23.1	92.9
ER-negative	100	58.3	75.0	61.5	100

**TNR, tumor-to-normal-tissue ratio; ER, estrogen receptor; PPV, positive predictive value; NPV, negative predictive value**

[Table 2]

**Conclusion:** In DbPET,  $\Delta$ SUVmax and post-TNR were shown to be promising parameters for predicting pathological response to NAC. Post-TNR provided the highest sensitivity for predicting pCR in patients with ER-negative breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

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Early ultrasound evaluation for prediction of treatment response to neoadjuvant chemotherapy in triple negative breast cancer patients

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**Background:** Triple negative breast cancer (TNBC) is molecularly heterogeneous disease. Genomic profiling to identify the distinct TNBC subtypes is costly with long turnaround time. Early ultrasound after two cycles of neoadjuvant chemotherapy (NAC) has the potential to identify patients who are likely to have pathological complete response. Suspected non-responder patients can undergo comprehensive genetic testing and triaged for specific targeted therapeutic trials.

**Aim:** To determine the value of ultrasound evaluation after two cycles of NAC to predict complete pathologic response in TNBC breast cancer patients.

**Methods:** 98 patients enrolled in “A randomized triple Negative Breast Cancer Enrolling Trial to Confirm Molecular Profiling Improves Survival” (Artemis) at the University of Texas MD Anderson Cancer Center had ultrasound evaluation before treatment and after two cycles of NAC (Adriamycin and Cyclophosphamide). Three-dimensional measurements of the tumor were obtained at baseline and after 2 cycles of the NAC. Change in the tumor volume after 2 cycles of NAC was calculated. Residual cancer Volume (RCB) was calculated based on the final histopathology at surgery. Linear regression analysis evaluated associations between residual cancer burden (RCB) and change in volume of the index tumor.

**Results:** Median tumor size at diagnosis was 3 cm, range 0.6-11.9cm. Median size after two cycles was 2 cm, range 0.6-12.8 cm. RCB 0-I was seen in 55% of patients (54/98). Linear regression analysis demonstrated that of 22 patients with volume reduction >75%, 18 patients (82%) had RCB0-I (95%CI, 61%-93%).

**Conclusion:** Our data suggest that ultrasound exam after 2 cycles of NAC can identify TNBC patients who are unlikely to respond to standard NAC. These non-responder TNBC patients can be triaged for additional genetic testing and subsequent targeted clinical trials. Study on the larger number of patients is currently on the way.

# 2018 San Antonio Breast Cancer Symposium ®

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The TILs-US scores based on ultrasonography can predict lymphocyte-predominant breast cancer before surgery

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## Purpose

Tumor-infiltrating lymphocytes (TILs) has been shown to be useful for predicting outcomes after surgery in breast cancer, and while TILs can be evaluated in preoperative biopsy tissue, heterogeneous distribution of TILs requires examination of all biopsied tissue samples.

We gave scores to preoperative ultrasonography (US) images with characteristics indicative of lymphocyte-predominant breast cancer (LPBC) and attempted to apply these for diagnostic prediction of LPBC. In this study, TILs-US scores based on preoperative US were assessed for their usefulness in predicting LPBC, the diagnosis of which was confirmed with postoperative pathology.

## Methods

We evaluated 161 patients with invasive breast cancer between January 2014 and December 2017. All patients were treated by either mastectomy or breast-conserving surgery. Stromal lymphocytes were evaluated on preoperative biopsy tissues and surgical pathological specimens. Breast cancer samples with  $\geq 50\%$  stromal TILs were defined as pre-LPBC (preoperative biopsy tissues) and LPBC (surgical pathological specimens). TILs-US score was calculated from US before curative surgery. Based on clinicopathological factors including TILs-US scores based on preoperative US and pre-LPBC indicators, determinants useful for prediction of LPBC were examined.

## Results

There were 39 cases of LPBCs and 122 cases of non-LPBCs in surgical pathological specimens. We set TILs-US score cut-offs for predicting LPBC at 4 points based on the receiver operating characteristics (ROC) curves (AUC, 0.88). There were significant predictors for LPBC in multivariate logistic analysis (TILs-US score: OR26.8,  $p<0.001$ ; pre-LPBC: 18.6,  $p=0.002$ ; HER-2: OR9.2,  $p=0.009$ ) in preoperative clinicopathological factor. The sensitivity, specificity and accuracy of TILs-US score for predicting LPBC were 0.74 (0.62-0.84), 0.89 (0.85-0.92) and 0.85 (0.79-0.90). Those of pre-LPBC were 0.51 (0.42-0.55), 0.98 (0.96-1.00) and 0.87 (0.82-0.89), and those of HER2 were 0.28 (0.19-0.36), 0.94 (0.91-0.97) and 0.78 (0.74-0.82), respectively. The sensitivity of TILs-US score for predicting LPBC was significantly greater than those of pre-LPBC ( $p=0.04$ ) and HER2 ( $p<0.001$ ). On the other hand, the specificity of pre-LPBC for predicting LPBC was significantly greater than that of TILs-US score ( $p=0.002$ ).

The sensitivity, specificity and accuracy of predicting LPBC

Performance measure	TILs-US score (95%CI)	Preoperative biopsy	HER-2	P, TILs-US score vs. Preoperative biopsy	P, TILs-US score vs. HER-2
Sensitivity	0.74 (0.62-0.84)	0.51 (0.42-0.55)	0.28 (0.19-0.36)	0.04	<0.001
Specificity	0.89 (0.85-0.92)	0.98 (0.96-1.00)	0.94 (0.91-0.97)	0.002	0.11
Accuracy	0.85 (0.79-0.90)	0.87 (0.82-0.89)	0.78 (0.74-0.82)	0.23	0.11

## Conclusions

TILs-US scores are an important factor that can predict LPBC preoperatively. The TILs-US score has particularly high sensitivity and may be an applicable index in the preoperative evaluation for LPBC.



# 2018 San Antonio Breast Cancer Symposium ®

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Artificial Intelligence over thermal images for radiation-free breast cancer screening

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**Introduction:** Breast cancer is the largest cause of cancer deaths in women today. NIRAMAI has developed a novel solution for detecting early stage breast cancer in women of all age groups. It is low cost, non-contact and portable solution. This radiation-free solution also works on dense breasts and hence is applicable beyond developing countries. The core of the solution is a Computer Aided Diagnostics engine called Thermalytix, which uses Artificial Intelligence algorithms on high resolution thermal images.

In this paper, we present a comparative analysis of Thermalytix solution with Mammography, the standard screening modality, in a retrospective trial that was conducted across 3 reputed cancer hospitals in India.

**Aim:** To compare the sensitivity, specificity, NPV and PPV of Thermalytix with Mammography.

**Methods:** A multisite comparative study was performed on 194 patients across 3 reputed cancer hospitals in India with informed consent from subjects and ethics committee approval of respective hospitals. Every person who was going for a mammography test was made to undergo the non-invasive Thermalytix test prior to mammography examination. As per standard of care in India, all women who had a suspicious lesion in Mammo was sent to Ultrasound and then biopsy. 93 of the 194 subjects enrolled were found to be malignant by this standard procedure (9 of them did not have a biopsy report).

Niramai Thermalytix test gave the automated reports detecting patients with suspected malignancy and those results were compared with ground truth derived from mammography, sono-mammography and biopsy. In a similar manner, standalone mammography observation was compared with the above ground truth to compare the two modalities as individual modalities.

**Results:**

Out of the 194 subjects, 93 positive cases and Niramai accurately detected 91 cases and called out one as boundary case. Sensitivity of Thermalytix was 98% and NPV was 97%, while sensitivity of Mammography was 94% with an NPV of 94%. NIRAMAI Thermalytix detected 4 more malignant patients compared to Mammography. On the other hand, mammography scored over Thermalytix in PPV and Specificity by 10%. These results are tabulated at

Comparing NIRAMAI Thermalytix with Mammography

	NIRAMAI	Mammography
Sensitivity	98%	94%
Specificity	76%	89%
PPV	79%	89%
NPV	97%	94%
Accuracy	87%	91%

Number of subjects = 194 high risk patients

Among the 194 patients, 39 women were found to have dense breasts and 19 with heterogenous dense breasts – on whom ultrasound and elastography was performed by the Radiologist to correlate with mammography results and make the final observations. Niramai test alone was effective in these patients.

The results show that Thermalytix is an emerging, radiation-free diagnostic modality that has comparable accuracy to Mammography. We conclude that NIRAMAI Thermalytix can be complementary modality to mammography as it works well on women of all age groups, including women with dense breasts. Its higher sensitivity and NPV adds to its potential to be the first screening test for early detection of breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Analysis of non-cancerous lesion with high FDG uptake in breast

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## Background

Positron emission tomography (PET) is an imaging method that provides insight into the metabolism of glucose in tissues, and the most widely used tracer is 18F-fluorodeoxyglucose (18F-FDG). Fluorodeoxyglucose (FDG) is transported by glucose transporters, metabolized by the enzyme hexokinase and builds up in cancer cells. So FDG uptake within cells mainly depends on cellular metabolic activity and the number of glucose transporters. However, this mechanism is not specific to cancer cells. FDG can accumulate in inflammatory cells and benign processes, causing false-positive findings. In this study, we analyzed the non-cancerous lesion diagnosed by histology which showed abnormal absorption of FDG in 18F-FDG PET/CT test and demonstrated the image feature of these cases.

## Materials and Methods

We undertook a retrospective review of data from 373 women who had an 18F-FDG PET/CT test and analyzed the false positive results in 48 cases according to final pathological results between January 2016 and April 2018.

## Results

Of 296 patients, who had abnormal FDG absorption, 248 cases were histological diagnosed with breast carcinoma and 48 cases were non-cancerous disease including fibroadenoma, intraductal papilloma, adenomyoepithelioma, purulent inflammation, cyst with ductal ectasia, adenosis, tuberculosis and fibroadenoma with inflammation. We focused on the 48 cases with false positive FDG absorption, and the pathological results and 18F-FDG PET/CT of these patients were showed in Table 1. Fibroadenoma and purulent inflammation accounted for 33.3% and 35.4% of all cases following by intraductal papilloma with the incident of 12.5%. In purulent inflammation cases the max diameter of tumor showed significantly larger than other cases ( $3.2 \pm 1.2$ ,  $P < 0.05$ ). The max diameter of lesion in other diseases showed no significantly differences. 18F-FDG PET/CT results showed that the value of SUVmax in purulent inflammation was significantly higher than that in other diseases ( $13.2 \pm 2.2$ ,  $P < 0.05$ ), and adenomyoepithelioma, cyst with ductal ectasia and adenosis were showed with a lower SUVmax.

Table 1. Histological results of 48 cases with false positive FDG absorption in PET/CT test

Pathological result	Number(ratio%)	Max diameter of tumor (cm,X±SD)	SUVmax (X±SD)
Fibroadenoma	16(33.3%)	1.3±0.3	4.2±0.6
Intraductal papilloma	6(12.5%)	1.0±0.2	4.1±0.5
Adenomyoepithelioma	2	1.1	2.1±0.4
Purulent inflammation	17(35.4%)	3.2±1.2 *	13.2±2.2 *
Cyst with ductal ectasia	1	0.7	1.8
Adenosis	3	0.8±0.2	2.6±0.7
Tuberculosis	1	1.0	6.6
Fibroadenoma with inflammation	2	1.3	4.6

\*:  $P < 0.05$

## Conclusion

In conclusion, despite the low false positive rate, 18F-FDG PET/CT is of great value for preoperative evaluation of breast lesion. In the non-cancerous cases which had a indication of carcinoma in 18F-FDG PET/CT test, the most common diagnosis were fibroadenoma, purulent inflammation and intraductal papilloma. The characteristics of purulent inflammation in 18F-FDG PET/CT image presented a higher SUVmax value with a larger max diameter in length.

# 2018 San Antonio Breast Cancer Symposium ®

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TILs-US score using ultrasonography before chemotherapy predicts the outcome of neoadjuvant treatment in HER2 positive breast cancer

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## Purpose

Tumor-infiltrating lymphocytes (TILs) have been reported to be predictive factors of therapeutic effect in neoadjuvant chemotherapy (NAC). And while TILs can be evaluated in preoperative biopsy tissue, heterogeneous distribution of TILs requires examination of all biopsied tissue samples. We gave scores to preoperative US images with characteristics indicative of LPBC and attempted to apply these for diagnostic prediction of LPBC. In this study, TILs-US score based on US images obtained before chemotherapy was examined as an alternative method to LPBC evaluated on preoperative biopsy tissues (pre-LPBC) for application in the prediction of pathological complete response (pCR).

## Methods

We evaluated 67 patients in triple negative type (n=22), HER-2 positive (n=33), luminal type (n=12) with invasive breast cancer who underwent neoadjuvant chemotherapy between March 2012 and July 2016. Ultrasonography (US) was performed before NAC. All patients were treated by either mastectomy or breast-conserving surgery after NAC. Stromal lymphocytes were evaluated on preoperative biopsy tissues. Breast cancer samples with  $\geq 50\%$  stromal TILs were defined as LPBC. There were 39 cases of LPBCs and 122 cases of non-LPBCs in pathological specimens using a biopsy before NAC. TILs-US score (0~7 point) was calculated from US before NAC. We set TILs-US score cut-offs for predicting LPBC at 4 points based on our previous data. Based on clinicopathological factors including TILs-US scores based on US and LPBC indicators before NAC, determinants useful for prediction of LPBC were examined.

## Results

The surgical pathological findings after NAC indicated pCR and non-pCR in 32 (47.8 %) and 35 (52.2 %) patients. There were significant predictors for pCR in univariate (Clinical N: OR 2.83,  $p=0.043$ ; HER-2: OR 4.80,  $p=0.002$ ; TILs-US score: OR 2.83,  $p=0.04$ ) and multivariate analyses (HER-2: OR 6.42,  $p=0.009$ ) in all breast cancer. There were not significant predictors for pCR in univariate and multivariate analyses in triple negative breast cancer. While, there were significant predictors for pCR in univariate (TILs-US score: OR 17.5,  $p=0.002$ ) and multivariate analyses (TILs-US score: OR 82.8,  $p=0.02$ ) in HER2 positive breast cancer

Multivariate analysis for prediction of pCR in HER2 positive breast cancer

	Multivariate logistic analysis		
Characteristic	OR,	95%CI,	P
Age, <50 v $\geq 50$	12.7,	0.84-191.8,	0.07
Clinical T, T1-2 v T3- T4	16.5,	0.23-1177.2,	0.20
Clinical N, Negative v Positive	1.74,	0.16-19.2,	0.65
Nuclear Grade, 3 v 1-2	2.57,	0.12-55.7,	0.55
ER, Negative v Positive	3.01,	0.16-55.2,	0.46
LPBC, $\geq 50\%$ v <50 %	3.52,	0.22-55.2,	0.37
TILs-US score, $\geq 4$ v <3	82.8,	2.15-3186.8,	0.02

## Conclusions

In patients with HER2 positive breast cancer, TILs-US score can predict the therapeutic effect in neoadjuvant chemotherapy (NAC) and help with the development of appropriate treatment plans.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-02-16

The efficacy of preoperative pathological features to improve diagnosis accuracy in lymph node metastasis by axillary ultrasound

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## Purpose

Recently a sentinel lymph node biopsy has been a part of standard surgical procedure instead of an axillary dissection with a mastectomy for breast cancers. It is necessary to make an accurate diagnosis of lymph node metastasis for deciding surgical procedure either a sentinel lymph node biopsy or an axillary dissection. Preoperative ultrasound is one of widely used tools to make a diagnosis of sentinel lymph node. This study presents results regarding preoperative pathological features to improve diagnosis accuracy of sentinel lymph node by preoperative axillary ultrasound.

## Materials and methods

One doctor performed preoperative ultrasound. He used HITACHI HI VISION Ascendus. Axillary lymph node metastasis was suspected when these findings were observed such as partial thickening of lymph node cortex, loss of lymph nodes' hilum, circular swelling shape change and difference compared to a contralateral axillary lymph node. When we suspected axillary lymph node metastasis, we performed fine needle aspiration cytology except other findings such as CT, MRI or physical appearance showed obvious axillary lymph node metastasis. Preoperative core needle biopsies were performed to determine pathologic types and hormone characters. Clinical cases such that ductal carcinoma in situ, lobular carcinoma and ones with primary systemic treatment (neoadjuvant therapy) were excluded from statistical analysis.

## Results

During a study period between April 2015 and December 2017, altogether 662 patients were participated in this study. 304 cases were analyzed by statistical analysis. 268 cases were diagnosed as negative and 36 case as positive in axillary lymph node metastasis by preoperative ultrasound. In the 268 cases 225 cases were diagnosed as negative by sentinel lymph node biopsy during operations (84.0%) but 43 cases were diagnosed as positive (16.0%). 23% cases included less than 20mm micro metastasis (10/43). To investigate correlation between false negative ratio of axillary lymph node metastasis diagnosed by an ultrasound and preoperative pathological features such as hormone receptor, HER2, Ki-67, nuclear grade and subtypes (Luminal, Her2 and triple negative). The false negative ratio was 17.0% vs 11.1% in hormone receptor (positive vs negative,  $p = 0.434$ ), 14.5% vs 16.0% in HER2 (positive vs negative,  $p = 0.887$ ), 15.4% vs 19.1% in Ki-67 ( $<20\%$  vs  $\geq 20\%$ ,  $p = 0.441$ ), 18.4% vs 9.38% in nuclear grade (1 vs 2 and 3,  $p = 0.997$ ), 11.3% vs 23.8% vs 16.7% vs 16.7% (Luminal A vs Luminal B vs Her2 vs triple negative,  $p > 0.05$ ).

## Conclusion

There were no statistical significant difference between the axillary lymph node metastasis diagnosed by an ultrasound and the preoperative pathological features. However cases categorized in Luminal B by preoperative pathology had relatively high false negative ratio of axillary lymph node metastasis diagnosed by an ultrasound. It is necessary to investigate a way to improve diagnosis accuracy of sentinel lymph node by a preoperative axillary ultrasound.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-02-17

Hand-held impulse-radar detector for breast cancer: development and a pilot study

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**Background:** Microwave breast imaging, which using the difference in the dielectric properties between breast cancer and normal breast tissue, is a painless and non-radiation method. We have created a novel hand-held prototype of breast cancer detector using impulse-radar based imaging system, and conducted a pilot clinical study.

**Methods:** The detector consists of complementary metal-oxide-semiconductor (CMOS) integrated circuits covering the ultrawideband width from 3.1 to 10.6 GHz, which enable the generation and transmission of Gaussian monocycle pulse (GMP) trains and single port eight throw switching matrices (SP8T-SW) for controlling a 4×4 cross-shaped dome antenna array. The size of the detector was 19.1 × 17.7 × 18.8 cm. After evaluation using a breast tumor phantom and the resected breast specimens obtained through mastectomy, we recruited 5 patients with histologically confirmed breast cancers in the clinical study. The detector was placed on the breast with the patient in a supine position. The primary endpoint was a detection rate of breast cancers, and the secondary endpoints were positional accuracy and adverse event. This study was registered with the UMIN Clinical Trials Registry (UMIN000026181).

**Results:** The three-dimensional positions of the tumors in the imaging results using a phantom and resected specimens are consistent with the results of histopathology analysis. In the clinical study, all 5 targeted breast cancers were detected and were visualized at the sites confirmed by other diagnostic modalities. Among 5 tumors, one was not detected via mammography because of heterogeneously dense breast and another was a microinvasive carcinoma of invasive tumor size 0.5 mm. No study-related adverse events occurred.

**Conclusions:** We succeeded in creating a new device of hand-held impulse-radar detector for breast cancer. The detector has sufficient detective capability, is safe for clinical use, and might detect an early stage breast cancer. In the future, we will proceed with the development to clinical application.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-02-18

Hepatic complications of breast cancer

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## **Background**

Breast cancer is the most common malignancy and the leading cause of cancer death in women in the United States. Nearly 30% of women with metastatic breast cancer will have liver metastases, with a median survival from time of 4.23 months from time of metastatic diagnosis. Hepatic involvement of breast cancer is common and can result from metastatic tumor burden or treatment with chemotherapy or radiation. Being able to recognize and diagnose hepatic involvement of breast cancer is of utmost importance, as it has a dramatic effect on patient prognosis and management.

## **Learning Objectives**

To familiarize multidisciplinary clinical team members with the radiographic presentations of hepatic complications of breast cancer in a variety of imaging modalities including computed tomography, dual-energy computed tomography, ultrasound, and MR. This will facilitate accurate and prompt diagnosis and treatment of hepatic involvement of breast cancer.

## **Abstract**

Our educational pictorial essay demonstrates the typical appearance of hepatic metastases on multiple imaging modalities, as well as atypical presentations such as hyperdense, hypervascular, or hypoechoic lesions and pseudoprogression. We will show examples of hepatic metastases presenting as pseudocirrhosis, left lobar atrophy, capsular retraction, as well as sequelae of pseudocirrhosis such as malignant ascites, varices, portal vein thrombosis, and biliary obstruction. We will discuss the radiographic appearance of the liver after being treated with locoregional liver-directed techniques such as surgical resection, ablation, and chemoembolization. Importantly, we will illustrate examples of hepatic complications from systemic therapy including steatohepatitis from tamoxifen, as well as hepatic fibrosis from gemcitabine.

## **Conclusion**

Clinicians must have a thorough understanding of the hepatic complications of cancer to ensure excellent diagnostic accuracy and management.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-02-19

Machine learning based analysis of CT radiomics for the simultaneous indeterminate pulmonary nodules of breast cancer

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**PURPOSE:** To investigate texture features of simultaneous indeterminate pulmonary nodules of breast cancer for predicting their potential metastasis.

**METHODS AND MATERIALS:** 150 patients with simultaneous breast cancer diagnosed by biopsy and pulmonary nodules (diameter: 5-20mm) detected by preoperative CT were enrolled in this study. After surgery and breast cancer treatment, the patients were followed up for at least half a year or longer by CT to observe the changes of lung nodules, thereby inferring the potential of metastasis. We classify pulmonary nodules into two groups: the reduced or enlarged pulmonary nodules were defined as highly metastasis possibility (Group 1), and long-term stable pulmonary nodules were defined as low metastasis possibility (Group 2). In addition, pathologic proven primary lung cancer in this study (Group 3) was compared with Group 1. Therefore, we carried out a comparative analysis of the texture features between the groups, and additional statistical were used three regression testing to extract texture features. Finally, we construct a machine learning classifier and calculate the accuracy of cross-validation.

**RESULTS:** We collected 106 features by the texture analysis(TA). There are 18 features with significant differences between Group 1 and the Group 2( $p<0.05$ ), and 76 features with significant differences in the Group 1 and Group 3 ( $p<0.05$ ). We tried to find key features related to pathology in 106 features using three methods: lasso regression, ridge regression and forward stepwise regression. The accuracy in different regressions respectively is 94.5%,94.5%,89.7% using KNN between Group 1 and Group 2. The accuracy in different regressions respectively is 96.2%(KNN),96.2%(Tree),92.3%(Linear Discriminant)in the Group 1 and Group 3.

**CONCLUDES:** The identified radiomics features have the potential to be used as a biomarker for metastasis prediction of simultaneous indeterminate pulmonary nodules in breast cancer patients, and it may contribute to preoperative treatment and postoperative follow-up planning.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-03-01

Development of patient-derived xenograft tumor model with organ-specific metastatic potential for evaluation of new therapeutics for hormone receptor-positive advanced breast cancer

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**Background:** Breast cancer (BC) is a heterogeneous disease with most common metastatic sites of liver, lung, brain, and bone. Endocrine resistance in hormone receptor-positive (HR+) advanced BC (ABC) cancer is a clinical challenge. *ESR1* mutations are a key mechanism in acquired resistance, primarily occurs after exposure to endocrine therapy such as aromatase inhibitors but also selective estrogen modulators and degraders (i.e. Tamoxifen and Fulvestrant). Circulating tumor cells (CTCs) enumeration is a prognostic biomarker in ABC but the relation between the onset of *ESR1* mutations and CTCs status is still unclear. Aim of this project is to define the clinical behavior of *ESR1* mutated ABC in terms of metastasizing potential, through CTC enumeration and pattern; and to establish *ESR1* mutated HR+ ABC PDX models able to recapitulate these characteristics.

**Methods:** CTCs and circulating tumor DNA (ctDNA) were characterized in 55 HR+ ABC patients. *ESR1* mutations status from 55 patient plasma cell-free DNA were generated using Guardant Next Generation Sequencing. Samples were also examined for numbers of CTCs by CellSearch. Association of *ESR1* mutations with sites of distant organ metastasis and with CTC enumeration was analyzed by Chi square test and Kruskal–Wallis test, respectively. In preclinical model development, six samples of pleural effusion-derived tumor cells from Stage IV HR+ ABC patients were collected to establish HR+ ABC with *ESR1* mutation PDX tumor model and its derived 3D organoid/spheroid cultures

**Results:** *ESR1* mutations were identified in 10 out of 55 patients (4 Y537S variant and 3 D538G variant, 4 other variants, 1 patient with both variants). In 55 patients, 72 visceral vs 27 bone metastatic incidences were observed; the data indicated 9 observed vs 4.5 expected in *ESR1* mutated and 16 observed vs 20.5 expected in wild type (WT) ( $P=0.003$ ) for liver metastasis; 10 observed vs 7.1 expected in *ESR1* mutated and 29 observed vs 31.9 expected in WT ( $P=0.026$ ) for bone metastasis. Further liver metastasis analysis of individual hot spot mutation site indicated 4 observed vs 1.8 expected in Y537S and 21 observed vs 23.2 expected in WT ( $P=0.037$ ); and 3 observed vs 1.4 expected in D538G and 22 observed vs 23.6 expected in wild type ( $P=0.088$ ). The analysis of correlation/distribution between CTCs numbers and *ESR1* mutated suggested CTCs median of 13 (IQR 7-49) in *ESR1* mutated and 0 (IQR 0-4) in WT HR+ patients ( $P=0.0044$ ). Four ABC PDX tumor models were developed in immunodeficient NSG female mice demonstrated by pathology to have highly heterogeneous characteristics and metastatic features of the origin patient tumor, in particular, breast fat pad xenografted PDX tumor can result in metastasis to liver and lung tissue. In addition, two patient 3D tumor organoid/spheroid cultures were successfully established.

**Conclusions:** *ESR1* mutated ABC is associated with more aggressive (Stage IV) clinical behavior demonstrated by association with visceral metastases and CTCs detection. *ESR1*-mutated PDX models recapitulate aggressive features of the disease and can be used for preclinical testing of novel agents in endocrine resistant disease.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-03-02

Budesonide and colesevelam reduce neratinib-induced diarrhea in a rat model

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**Background:** Neratinib (Puma Biotechnology Inc.) is an irreversible pan-HER tyrosine kinase inhibitor approved for use as extended adjuvant therapy in women with early-stage HER2+ breast cancer. Diarrhea is the main adverse event of neratinib; grade 3 events are common in the absence of antidiarrheal prophylaxis (40%) [Chan et al. Lancet Oncol 2016]. To investigate the underlying pathogenic mechanisms and to explore possible targets for its prevention, we developed a reproducible rat model of neratinib-induced diarrhea. Findings from the model indicated that neratinib-induced diarrhea is multifactorial, involving anatomical disruption and mucosal inflammation in the ileum and colon [Secombe et al. Asia Pac J Clin Oncol 2017]. We investigated the effects of budesonide, a locally-acting corticosteroid used for gastrointestinal conditions, and colesevelam, a bile acid sequestrant, on neratinib-associated diarrhea and intestinal changes in this model.

**Methods:** Male albino Wistar rats were randomly allocated to vehicle control (5% DMSO/1% carboxymethyl cellulose), neratinib 50 mg/kg alone or combined with budesonide 1 mg/kg or colesevelam 300 mg/kg by oral gavage for 14 or 28 days. Diarrhea severity was graded daily [0, no diarrhea; 1, mild; 2, moderate; 3, severe]. A tissue injury score was assigned based on validated histological criteria (villus fusion and atrophy, disruption of brush border and enterocytes, crypt losses/architectural disruption, crypt cell disruption, infiltration of polymorphonuclear cells/lymphocytes, dilation of lymphatics/capillaries, edema). Inflammation was assessed using multiplex cytokine/chemokine ELISA. Fecal bile acids were measured in pooled fecal samples over an 8-hour period.

**Results:** Findings at 28 days:

Variable	Control (n=16)	Neratinib (n=16)	Neratinib + budesonide (n=16)	Neratinib + colesevelam (n=16)
<b>Incidence of diarrhea, %</b>				
Grade 1	37.5	0	0	0
Grade 2	12.5	87.5	100	100
Grade 3	0	12.5	0	0
<b>Mean±SEM days with grade 2 diarrhea</b>	0.1±0.1	15.8±2.7	10.0±1.0*	10.0±2.1*
<b>Median (range) tissue injury score</b>				
Distal ileum	0.5 (0–2.0)	4.0 (3.0–5.0)††	3.0 (2.0–4.0)	3.0 (2.0–4.0)†
Proximal colon	2.0 (0–3.0)	5.0 (4.0–6.0)††	3.0 (2.0–5.0)*	3.0 (2.0–5.0)
Distal colon	0.5 (0–2.0)	2.5 (2.0–4.0)††	1.0 (0–2.0)*	1.5 (0–2.0)
<b>Median (range) cytokine levels<sup>a</sup></b>				
<b>Ileum</b>				
IFN-γ	27 (15–48)	49 (39–67)†	43 (29–55)	–
IL-4	63 (15–111)	223 (109–318)	464 (309–559)**	–
IL-10	21 (14–54)	32 (10–84)	60 (52–89)*	–
<b>Colon</b>				
IFN-γ	22 (6–32)	16 (12–22)	16 (6–19)	–
IL-4	147 (88–270)	165 (15–327)	345 (170–433)*	–

IL-10	54 (18–128)	55 (37–96)	47 (43–60)	–
<b>Mean±SEM total fecal bile acids, umol/mg</b>	3.5±0.4	3.9±0.2	–	5.5±0.4††*

<sup>a</sup>ng/ml (IFN- $\gamma$ ); pg/ml (IL-4, IL-10); \*p<0.05, \*\*p<0.005 vs neratinib; †p<0.05, ††p<0.005 vs control; IFN, interferon; IL, interleukin; SEM, standard error of the mean

**Conclusions:** Budesonide and colesevelam reduced duration of neratinib-induced diarrhea and prevented severe diarrhea. Budesonide also reduced histopathological injury and inflammation via preservation of intestinal morphology and upregulation of anti-inflammatory cytokines in the ileum and colon. The phase II CONTROL study (Clinicaltrials.gov NCT02400476) is currently investigating the effects of adding budesonide or colestipol to loperamide prophylaxis in the prevention of neratinib-induced diarrhea and will help to determine the clinical relevance of our observations.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-03-04

Successful development of patient-derived orthotopic xenograft models of brain and lung metastases of human breast cancer

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**Background:** Metastatic breast cancer is the reason that we continue to lose 40,000 women every year in the US. Without appropriate pre-clinical model, success rate of clinical trials continue to suffer. Because syngeneic mouse models utilize murine neoplasm that may not represent human cancer, patient-derived xenografts (PDX) have emerged as a pre-clinical model that maintains human cancer features such as intratumoral heterogeneity. However, there is no established orthotopic PDX model for metastatic breast cancer even though the main cause of death is brain and lung metastasis. Orthotopic brain or lung PDX is expected to reproduce the original tumor microenvironment. We describe our new patient-derived metastasis orthotopic xenograft (PDMOX) mouse models of human breast cancer.

**Methods:** All work was performed in female NSG mice of age 8-12 m. Breast cancer metastatic tumors from brain and lung that had been passaged 3x in mammary fat pads were used. Tumors of 1 mm<sup>3</sup> were implanted orthotopically in two forms: solid piece, or minced tissue with 3 µl Matrigel. Tumor growth was monitored by MRI.

**Results:** Two methods for brain PDMOX were compared. "Manual push" method implanted minced tumor through a frontal bone burr hole into right caudate putamen at 4 mm depth using forceps. "Pipette tip" method utilized either a pipetter for minced tissue or Hamilton syringe for solid tissue to inoculate tumor. One hour post-surgical survival was 37.5% (3/8) after "manual push", and 100% (30/30) after "Pipette tip" method. All tumors engrafted in surviving mice with either method. However, the tumors formed on brain surface and parenchyma invasion was rare after "manual push" method, whereas solid tumor invaded parenchyma by "pipette tip" method. Therefore, it was no surprise to find large variation in tumor growth after "Manual push" (detection time 17±5.0 d, range: 17-26; volume 5.6±21.0 mm<sup>3</sup>, range 2.8-48.7). One mouse developed ptosis, and 2 out of 3 mice that underwent "Manual push" had sudden death. On the other hand, all mice that underwent "Pipette tip" method lived until tumor grew to 125-200 mm<sup>3</sup>, without neurological symptoms. These brain tumors could be passaged with 100% success (9/9). For right lung PDMOX, "thoracotomy" and "non-thoracotomy" methods were compared. "Thoracotomy" method implanted a solid tissue using forceps or 8-0 nylon suture, or injected minced tissue 1 mm below pleura. "Non-thoracotomy" method injected minced tissue using 23G needle. One hour post-surgical survival was 30% (9/30, 8/30) after "thoracotomy" method using forceps or suture, resp. However, survival using suture method could be significantly improved to 97% (29/30) by reducing thoracotomy length (<10 vs. ≥10 mm: t test P = 0.003). Post-operative survival was not affected by age, weight, or operation or anesthesia time. On the other hand, all mice after "non-thoracotomy" method survived, but chest wall implantation occurred in 67% (4/6) when the method was performed using a cell line.

**Conclusion:** By simple modifications of surgical techniques, we could establish orthotopic brain and lung xenografts of breast cancer tumors with almost zero mortality and 100% engraftment. Our novel PDMOX models can be powerful tools for preclinical studies.

# 2018 San Antonio Breast Cancer Symposium ®

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The role and mechanism of active vitamin D mediated by Bmi-1 in inhibiting the allograft growth and bone metastasis of breast cancer

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**Background:** Breast cancer is a common female malignancy and bone is the most common distant metastasis organ, which seriously affects the quality of life of patients. An ongoing clinical study shows that vitamin D deficiency is a common risk factor for bone metastases in breast cancer. However, the mechanism has not been reported.

**Objectives:** 1) To clarify the role of active vitamin D in inhibiting breast cancer cell growth and bone metastasis; 2) To clarify the role of active vitamin D mediated by Bmi-1 in inhibiting the allograft growth and bone metastasis of breast cancer; 3) To reveal the mechanism of action of active vitamin D in inhibiting the occurrence and metastasis of breast cancer.

**Methods:** A murine breast cancer TM40D cells were treated with 1,25(OH)<sub>2</sub>D<sub>3</sub>, the proliferation, migration and invasive ability were examined using CCK-8 assay, scratch test, and transwell assay, respectively. The TM40D cells were transplanted into tibiae of WT or Cyp27b1<sup>-/-</sup> female mice treated with or without exogenous 1,25(OH)<sub>2</sub>D<sub>3</sub>, the phenotype of bone metastases was analyzed using imaging and histopathological methods. A TM40D stably transfected with Bmi-1 knockdown cell line was established. The effects of Bmi-1 knockdown on the proliferation, migration, and invasion of TM40D cells were examined; TM40D cells transfected with an empty vector or a Bmi-1 knockdown were transplanted into the subcutaneous or upper tibial medullary cavity of WT or Cyp27b1<sup>-/-</sup> mice, treated with or without exogenous 1,25(OH)<sub>2</sub>D<sub>3</sub>, and the phenotypic changes of transplanted tumors and bone metastases were analyzed.

**Results:** 1) 1,25(OH)<sub>2</sub>D<sub>3</sub> inhibited the proliferation, migration and invasive ability of TM40D cells in vitro; 2) 1,25(OH)<sub>2</sub>D<sub>3</sub> inhibited the tumor growth and osteolytic bone destruction in vivo; 3) 1,25(OH)<sub>2</sub>D<sub>3</sub> inhibited osteoclastic bone resorption induced by TM40D; 4) 1,25(OH)<sub>2</sub>D<sub>3</sub> can significantly down-regulate the Bmi-1 expression in breast cancer cells in vivo and in vitro; 5) Bmi-1 knockdown inhibited proliferation and promoted senescence by elevating the expression levels of P16, P19, and P53, and inhibited the allograft growth; 6) Bmi-1 knockdown suppressed the proliferation, migration and invasive ability of TM40D cells; 7) Bmi-1 knockdown suppresses osteolytic bone destruction in vivo; 8) Exogenous 1,25(OH)<sub>2</sub>D<sub>3</sub> and Bmi-1 knockdown both upregulated the expression of tumor suppressor genes in vivo.

**Conclusions:** The results indicate active vitamin D can inhibit the occurrence of breast cancer and bone metastasis by down-regulating Bmi-1, activating of P16/Rb and P19/P53 signaling pathways, inhibiting breast cancer cell proliferation and growth in bone tissue, inducing breast cancer cell senescence, and reducing osteolytic bone destruction. The results not only revealed the mechanism of action of active vitamin D in inhibiting the occurrence and metastasis of breast cancer, but also provided experimental and theoretical basis for the clinical application of active vitamin D in the prevention and treatment of breast cancer and its bone metastases.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-03-06

Androgen supplementation in patient derived xenografts in androgen receptor positive breast cancer to increase engraftment and growth rate

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## Background:

The European fund for regional development (EFRE) supported Precision Oncology and Personalized Therapy Prediction (POP) Project is establishing preclinical models to further the development of personalized therapy options. In the subgroup breast cancer the current goal is to increase the growth and engraftment rates of breast cancer patient derived xenografts (PDX) models.

## Methods:

Breast cancer patients of the Department of Gynecology with Breast Center Charité Universitätsmedizin Berlin, Germany are recruited since May 2017. In total 29 tissue samples were collected and included so far.

Treatment naive and treatment refractory patients, triple negative breast cancer (TNBC), hormone receptor positive (HR+) and Her2 positive tumors, primary disease, recurrence or metastasis are sampled. Fresh tumor tissue is extracted via surgery or biopsy. The materials are then implanted into female immunodeficient NOG mice. To establish PDX models for HR+ breast cancer the mice received estrogen supplementation.

To increase engraftment and growth rates androgen receptor (AR) testing and subsequently androgen replacement was started since April 2018.

Up to date, 6 new samples have been collected. One HR+ and two TNBC samples tested also positive for AR. These samples are currently in passage 0 (p0) and are now supplied with androgens to increase engraftment and growth rate. One already established AR+ TNBC PDX is being regrown with androgen supplementation to compare growth rates.

## Results:

Out of the initial 23 tissue samples ten (six HR+ and four TNBC) have been able to be engrafted into PDX mice.

The TNBC PDX models are one in p1, one in p2, one in p3 and one is being tested with systemic therapy. Engraftment time in p1 were between 19 and 97 days. Growth time to passagable size between 21 and 112 days.

The HR+ PDX models are four in p1 and two in p2. Engraftment time in p1 was between 26 and 123 days. Growth time to passagable size has been achieved in 2 HR+ PDX within 17 to 48 days.

The engraftment/growth rates and times of the androgen supplemented PDX models will be presented.

## Conclusion:

Breast cancer grows in humans slowly and this is also the case in the PDX models. To achieve faster growth and higher engraftment rates androgen supplementation in AR+ breast cancer might be an additional enhancing factor.

# 2018 San Antonio Breast Cancer Symposium ®

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Identification and targeting of clinically actionable genes in bone metastases

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**BACKGROUND:** The most common site of cancer metastases from breast is to bone, which occurs in 65-80% of patients resulting in significant comorbidities caused by pathological fractures, pain, hypercalcemia, and nerve compression. The current standard of care involves surgery, radiation, and treatment with bisphosphonates to target osteoclast driven resorption. These modalities all have limitations, complications, and cannot prevent new bone metastases from developing. There is an obvious unmet need for new therapeutic targets and models to treat and study breast cancer related bone metastasis. A large portion of breast cancer research utilizes cell lines and animal models with tissues taken from early stage, primary breast cancers. Furthermore, endpoints such as tumor size reduction and growth in these studies do not always translate to tumor spread. As a result, these study endpoints are not relevant for individuals that are already living with metastatic disease.

**MATERIALS and METHODS:** Potential therapeutic targets involved in metastasis and osteomimicry were identified by performing exome-capture RNA-sequencing (ecRNA-seq) on eleven matched primary breast tumor and bone metastases. Expression gains and losses were identified from the clinically actionable gene set obtained using the Drug Gene Interaction Database (DGBIdB 2.0). A unique organotypic bone model comprised of endothelial cells (EC), osteoblasts (OB), and mesenchymal stem cells (MSC) co-cultured with primary metastatic bone samples from breast cancer patients is being utilized to target these genes of interest.

**RESULTS:** ecRNA-seq of metastatic bone samples revealed expression gains in genes of interest (GOI): EPH Receptor A3 (EPHA3), Protein Tyrosine Phosphatase, Receptor Type D (PTPRD), Patched 1 (PTCH1), and Platelet Derived Growth Factor Receptor Alpha (PDGFRA). The GOI were highly recurrent in patients with endocrine-resistant disease that had developed bone metastases after treatment but were absent in the *de novo* bone metastasis cases where patients had not yet received endocrine therapy. Analysis for expression of these GOI as well as osteomimicry genes are being assessed in our organotypic bone model.

**CONCLUSIONS:** The GOI identified in this study have been previously associated with the growth and progression of cancer. Furthermore, these genes may be regulated by the RB1-E2F pathway, which was also found to be upregulated in these same samples, and previous studies have linked this pathway's ability to either directly or indirectly regulate our GOI expression levels. Targeting *EPHA3*, *PTPRD*, *PTCH1*, and *PDGFRA* in breast cancer recurrences may provide a novel therapeutic approach to treat bone metastases that have developed endocrine resistance in patients. Our organotypic bone scaffold provides a unique model to study the interactions of breast cancer cells with bone cells and to test inhibition of novel target genes. Future development of this model will provide a tool for high-throughput screening of drugs to target bone metastases of breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Evaluation of breast cancer intratumor heterogeneity and its implications to the therapeutic agents with organoids subclones

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**Background:** Breast cancer is the second most common cancer in women, accounting for 15.3% new cancer cases and 6.7% cancer death in 2018. Chemotherapy, target therapy and endocrine therapy are main strategies for breast cancer treatment after surgery to prevent cancer recurrence and metastasis. The choice of these strategies is mainly based on the molecular and pathological markers as well as experience from clinicians. However, due to the heterogeneity of intra-tumor and also between different patients, it is hard for clinicians to make the best therapeutic regimen to target all the cancer sub-population at current stage. Developing more precise and personalized chemotherapy strategy for individual patient would be of great importance. Cancer organoids, established based on *in vitro* 3-D culture techniques, were demonstrated to largely retain the biological characteristics of tumors from patients, and made it possible to assess the responses to various strategies for individual patient. Comparing with other cancer type such as colon cancer, breast cancer organoids study was relatively immature. This study was designed to efficiently establish breast cancer organoids from clinical patients and further test the sensitivity of these organoids to therapeutic agents.

**Methods:** Fresh breast cancer tissues from patients were dissected into small pieces and subjected to enzyme digestion to prepare a single cell suspension. Organoids were established from the single cell suspension in Matrigel and modified PDTO culture medium. Organoid clones derived from various cancer cells were heterogeneous, which were isolated with pipette tips to establish breast cancer organoids subclones. First line chemotherapy drugs such as Docetaxel, Adriamycin, and Fluorouracil, target therapy agents and endocrine therapy drug such as PD-991, Neratinib and Afimoxifene were used to treat each organoid clones. Cell survival rate was measured by CellTiter-Glo Kit and drug sensitivity was assessed by IC<sub>50</sub> value.

**Results:** 23 organoid subclones from 5 patients were successfully established. Drug response (effect) varied to individual organoid subclones from the same patient. Some subclones showed totally resistance while others were sensitive or partial sensitive to indicated therapeutic agents, indicating the heterogeneity of breast cancer and different intra-tumor subpopulation have distinct drug response. The heterogeneity, especially the resistant subclones put forward to explain the acquired drug resistance and relapse in clinical practice.

**Conclusion:** Breast cancer organoids are good *in vitro* models for drug sensitivity screening, which would assist clinicians make better chemotherapy strategy to achieve precise and personalized medication for patients. Moreover, organoid models would also be an ideal platform to help dissect the underlying mechanism of cancer heterogeneity induced drug resistance.



# 2018 San Antonio Breast Cancer Symposium ®

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Anticancer potential evaluation of natural products with breast cancer organoids

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**Background:** Breast cancer is one of the most malignant diseases threatening the health of our society. It is estimated that there will be more than 300,000 new cases of breast cancer in the US in 2018. Currently, surgery combined with chemotherapy is the most common strategy for breast cancer treatment. However, due to the cancer heterogeneity induced drug resistance, continuously developing new anti-cancer drugs is of great importance. Nature compounds extracted from traditional Chinese herbs were demonstrated to have anti-cancer activity through cell based experiments, but lacking suitable pre-clinical models, which could faithfully reflect *in vivo* tumor property, prevents these compounds from further characterizing. Now cancer organoids, as a new *in vitro* 3-D culture technique, were demonstrated to largely retain the biological characteristics of tumors from patients, and potentially served as an ideal platform for drug sensitivity test. Breast cancer organoids study was relatively limited when comparing with other type of cancer such as colon cancer. In this study, we set up system to efficiently establish breast cancer organoids from clinical patients and further test the sensitivity of these organoids to various nature compounds.

**Methods:** We collected fresh breast cancer tissues from 17 patients after surgery and established organoid models. Different dosage of herb derived nature compounds such as Gypenoside, Berberine, Oxymatdne, Sophoridine, Betaine, Chelerythrine Chloride, Harmine, Cantharidin were used to treat each organoid clone. Luminescent cell viability assay was used to indicate tumor survival rate.

**Results:** Organoid were successfully established for all 17 patients. A Short Tandem Repeat (STR) analysis was employed to detect sample contamination and HE(hematoxylin and eosin) stain was used to confirm the histological features. All organoids were resistant or slightly response to Oxymatdne, Sophoridine, Gypenosides, Betaine. For Berberine and Cantharidin, 10 out of 17 and 14 out of 17 organoids showed dose-dependent inhibition efficacy respectively, and the rest showed resistance. For Chelerythrine Chloride and Harmine, only organoids from specific patient showed sensitivity in a dose-dependent manner. Interestingly, different patients had distinct response pattern to these nature compounds, indicating organoids were useful in the anti-cancer lead screening and biomarker identification.

**Conclusion:**Breast cancer organoid, as a wonderful pre-clinical model largely containing tumor *in vivo* property, provides an ideal platform for new drug discovery and inhibitor screening. Moreover, serving as *in vitro* replacements for breast cancer, organoid models make it possible to test drug sensitivity for individual patient to achieve precise and personalize medication.

# 2018 San Antonio Breast Cancer Symposium ®

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A novel cleaved cytoplasmic lncRNA LacRNA interacts with PHB2 and suppresses breast cancer metastasis via repressing MYC targets

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Long noncoding RNAs (lncRNAs) have been implicated in breast cancer metastases through largely unknown mechanisms. In this study, we used microarray analysis to compare lncRNAs expression levels between matched pairs of breast lymph node metastatic tissues and primary tumors. We discovered that lncRNA LINC00478 was substantially downregulated in the metastatic tumor samples. Interestingly, we found that LINC00478 could be cleaved by RNase to simultaneously generates the mature 5' ends of cytoplasmic RNA and 3' ends of nuclear RNA by polyadenylation. We named 5' ends 791-nt RNA as LacRNA (LINC00478-associated cytoplasmic RNA). Over expression of full-length LINC00478 and LacRNA, but not LINC00478 3' RNA, significantly inhibited breast cancer proliferation, invasion and metastasis *in vitro* and *in vivo*. We used CRISPR-dCas9 complex to mediate efficient transcriptional activation of LacRNA at endogenous genomic loci followed by RNA-seq analyses. Gene set enrichment analysis (GSEA) showed that the MYC pathway/targets were prominent gene sets negatively enriched in LacRNA-activated cells. Further study revealed that LacRNA exerted its tumor suppressive activity by directly binding with prohibitin2 (PHB2) to enhance its protein stability, which promoted PHB2 competing with MYC for transcriptionally suppressing the MYC target genes (e.g., *CDC20*, *CDC45*, *CCNA2* and *MAD2L1*). Mechanistically, LacRNA inhibits breast cancer invasion and metastasis by interacting with PHB2 through LacRNA's 1-300nt region. In addition, taking advantage of CRISPR system to knock-out and activate the expression of LacRNA, as well as rescue experiment, we uncovered the positive correlation between LacRNA and PHB2 and their role in suppressing MYC target genes and cancer metastasis. At the same time, LacRNA can attenuated the MYC induced activation of MYC targets through binding with PHB2, indicating that LacRNA plays a central role in the suppression of MYC target genes. We further explored the role of LacRNA in inhibiting lung metastasis by implanting LacRNA-activated LM2 cells into the mammary fat pads of NOD-SCID mice. Luciferase imaging and histological analysis were used to detect lung metastasis and found that LacRNA significantly suppressed lung metastasis. Immunohistochemistry were used to detect the expression of PHB2 and MYC targets in both orthotopic tumors and lung metastasis and verified their correlation *in vivo*. Extensive analyses of clinical data indicated that LacRNA level was substantially downregulated in metastases tumors accompanied by enrichment of MYC targets. The robustness value of LacRNA expression was further verified in two independent patient cohorts, including 530 invasive breast cancer tumors in Fudan University Shanghai Cancer Center (FUSCC) and 819 breast patients' data from TCGA. High LacRNA expression level had a significantly better clinical outcome in both cohorts and represented an independent prognostic predictor for DFS (HR=0.48, *P*=0.006, multivariate analysis) and OS (HR=0.32, *P*=0.009, multivariate analysis) in FUSCC cohort. Collectively, LacRNA functions as a tumor suppressor lncRNA that inhibits breast cancer invasion-metastasis cascade.

# 2018 San Antonio Breast Cancer Symposium ®

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Identification of microRNAs differentially expressed in brain metastasis secondary to breast cancer

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**Background:** Despite sequential improvements in the adjuvant treatment of breast cancer (BC), recurrence and metastasis remains a major clinical problem and in particular, brain metastasis (BCBM). A number of microRNAs (miRNAs) have been linked to the metastatic process in BC, but to date there is limited work on the microRNAs involved in BCBM. The current study aim to identify differentially expressed miRNAs within primary breast cancer who did not recur (BCNR) versus primary BC cases which did recur (BCR) and their matched BCBM cases.

**Methods:** Formalin-fixed paraffin-embedded (FFPE) material was collected of 12 primary BCNRs from the Liverpool tissue bank and of 40 paired primary BCR samples and their matched BCBM from the Walton Research Tissue Bank and RCSI National Breast Cancer Bioresource. miRNA was extracted (Qiagen miRNeasy FFPE kit) and profiled using the NanoString™ nCounter™ miRNA Expression Assay (Human v3 miRNA). The differentially expressed miRNAs between BCNR versus BCR and BCR versus their matched BCBM were identified by significance of microarray analysis (SAM) on the MeV4.9 software. Pathway analysis was performed using the DIANA-mirPath v3.0 software and the Ingenuity Pathway Analysis (IPA) to identify a network of genes/pathways regulated by the differentially expressed miRNAs.

**Results:** 12 BCNR and 30 matched pairs of BCR and BCBM passed the quality control and normalisation processes. Principal component analysis (PCA) performed on 166 miRNAs after QC/normalisation clearly distinguishes the BCNR and the primary BCR from the matched BCBM cases, whereas SAM revealed 58 differentially expressed miRNAs with a 10% FDR (false discovery rate) and an absolute log2 fold-change (FC) >1 between BCNR and BCR and 11 between the matched BCs and BCBMs. Pathway clustering revealed that these differentially expressed miRNAs (10% FDR, log2FC>1) within both BCNR vs BCR and BCR vs BCBM cohorts are highly enriched for genes involved in extracellular matrix (ECM)-receptor interactions, proteoglycans, adherens junctions, TGF-β, p53 and Hippo signalling. IPA identified a network of genes, implicated in the processes of breast cancer invasion and metastasis, regulated by the identified miRNAs, such as, TWIST, MET, TP53, MYC, EZH2, ZEB1, TAGLN and BIRC5. Four of the significantly differentially expressed miRNAs, hsa-miR-132-3p, hsa-miR-199a-5p, hsa-miR-150-5p and hsa-miR-155-5p were present within both cohorts (BCNR vs BCR and BCR vs BCBM) and regulate genes involved in Hippo and TGF-β signalling (DIANA-mirPath v3.0 analysis: p=5.23x10<sup>-08</sup> and p=2.67x10<sup>-07</sup> respectively).

**Conclusion:** The current study, utilising a large cohort of paired BCR and BCBM cases, provides novel insight into the molecular mechanisms and role of miRNAs in BCBM. Four miRNAs (hsa-miR-132-3p, hsa-miR-199a-5p, hsa-miR-150-5p and hsa-miR-155-5p) in particular could be potentially used to identify patients with increased risk of developing brain metastasis and help facilitate the development of specific treatments for BCBM, which to date have proved elusive. The miRNAs identified require further exploration as potential biomarkers as well as novel therapeutic targets.

# 2018 San Antonio Breast Cancer Symposium ®

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Exploring the microRNA landscape of nipple aspirate fluid in the context of mammographic breast density

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## **Background**

High mammographic density is an established risk factor for breast cancer but studies are needed to elucidate the etiological pathways underlying this relationship. The identification of factors (indirectly) regulating the accumulation of fat and connective tissue may help explain why high mammographic density is linked to breast cancer risk. Those factors may provide markers for predicting and even clues for modifying this risk. MicroRNAs could regulate specific cellular processes involved in regulating mammographic density, like fat accumulation, connective tissue density and immune response. Nipple aspirate fluid represents an excellent opportunity for biomarker detection within the breast microenvironment.

## **Materials and Methods**

MicroRNA expression profiling was performed using TaqMan OpenArray Human MicroRNA Plates (ThermoFisher Scientific) in 40 pooled (paired left and right breast) nipple aspirate fluid samples obtained from age-matched women participating in a population-based breast cancer screening program with 'extremely dense' (n=20) and 'almost entirely fatty' breasts (n=20), without further abnormalities on mammography. Breast density was assessed using Volpara software in categories comparable to American College of Radiology (ACR) breast density categories.

## **Results**

25 significantly differentially expressed microRNAs were identified, including hsa-miR-19a-3p (p=0.001; upregulated in 'extremely dense' versus 'almost entirely fatty'), hsa-miR-324-5p (p=0.003; upregulated), hsa-miR-425-5p (p=0.001; upregulated), hsa-miR-660-5p (p=0.004; upregulated), hsa-miR-29b-3p (p=0.011; upregulated) and hsa-miR-187-3p (p=0.024; downregulated). Based on expression profiles of the 8 most significantly dysregulated microRNAs alone, nipple aspirate fluid samples could reliably be classified into 'extremely dense' and 'almost entirely fatty' mammographic density groups (31/40 (78%) samples correctly classified). Several of the dysregulated microRNAs have been associated with regulation of collagen deposition or crosslinking (e.g. miR-29, miR-222 and let-7) and adipocyte differentiation and proliferation (e.g. miR-29, miR-222, miR-155 and let-7), suggesting that these aberrant miRNAs may play a role in the development of breast tissue with high mammographic density. In addition, pathway enrichment analysis demonstrated an enrichment of the 'proteoglycans in cancer' pathway suggesting that proteoglycan dysregulation is intimately linked to microRNA dysregulation.

## **Conclusions**

In summary, microRNA profiling has resulted in a panel of microRNAs that might help explain why high mammographic density is linked to breast cancer risk and may ultimately result in a biomarker panel for predicting this risk, or provide attractive targets for future breast cancer preventive strategies. Further validation in an independent set of nipple aspirate fluid samples from 'extremely dense' and 'almost entirely fatty' categories is currently ongoing.

# 2018 San Antonio Breast Cancer Symposium ®

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NEAT1 enhances drug sensitivity by inhibiting cancer stem-like cells in triple-negative breast cancer

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**Background:** Accumulating evidence showed that long non-coding RNAs (lncRNAs) dysregulation is the hallmark of cancer. Nuclear paraspeckle assembly transcript 1 (NEAT1) has been reported to overexpress in many cancers, and promote cell growth and disease progression. However, the role of NEAT1 on drug sensitivity and stem-cell like property in triple-negative breast cancer is largely unknown.

**Methods:** lncRNA expression profile were compared between breast cancer patients and healthy individuals using lncRNA array. Large scale validation of NEAT1 expression in blood samples were performed by real-time PCR. Triple-negative breast cancer (TNBC) cells, MDA-MB-231 and its cisplatin resistance subline (MDA-MB-231/cis) were used. Stable transfection of cells with NEAT1 knockdown by shRNA, and evaluated single cell clonogenic assay, aldehyde dehydrogenase (ALDH) activity, CD44<sup>+</sup>/CD24<sup>-</sup> and other cancer stem cell (CSC) markers. Drug sensitivity assay, flow cytometry analysis, immunofluorescence staining and xenograft model were used to assess the functional role of NEAT1.

**Results:** Array data showed that NEAT1 was the top upregulated lncRNAs in the plasma of breast cancer patients. Consistent with the array data, validation of larger cohort of patients and healthy individuals (n=369) also demonstrated a higher expression of NEAT1 in breast cancer patients, in particular TNBC subtype. Knockdown of NEAT1 by shRNA sensitized breast cancer cells to cisplatin and taxol treatment. Cell proliferation and colony formation abilities were reduced with S-phase cell cycle arrest in shNEAT1 cells. Flow cytometry analysis revealed an induction of apoptosis with increased cleaved caspase-3. Cells expressing shNEAT1 abrogated ALDH activity, CD44<sup>+</sup>/CD24<sup>-</sup> subpopulation and expression of CSC markers (SOX2, NANOG and OCT4). More importantly, shNEAT1 cells retarded tumor growth in xenograft mice model and reduced CSC markers.

**Conclusion:** Taken together, NEAT1 expression is differentially expressed in breast cancer patients, and particularly higher in patients with TNBC. These findings suggest a potent therapeutic target to improve drug sensitivity in patients with TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

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Epigenetic regulation of ER through miR-18a shows ECM activation and identifies poor prognostic subtype within ER+ve HER2-ve breast cancer

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## Introduction

Although endocrine therapy has improved the survival of patients with hormone receptor (HR) positive breast cancer considerably, patients with weaker HR expression tend to have poorer outcomes. There exists significant intra tumoral heterogeneity of ER expression and a recent study suggested that patients with high intra tumoral heterogeneity of ER had an increased long term risk of recurrence (Lindstrom *et al* JNCI 2018). Multiple reports have supported a role for alterations in microRNA levels having a role in promoting tumor invasion and metastasis. In this study we have explored the epigenetic regulation of ER by microRNA hsa-miR-18a-5p and its role as a prognostic marker in ER positive tumors.

## Methods

123 surgically excised specimens of ER positive primary breast cancers were analyzed. Samples were segregated into high and low ER positive groups using 50% positivity as the cut-off. Relative abundance of hsa-miR-18a-5p in these samples was assessed using a TaqMan qRT-PCR. hsa-miR-18a-5p was over-expressed in MCF7 cells using a synthetic mimic and downstream changes in gene and protein expression were assessed using q-RT PCR, immunofluorescence and western blot. Migratory and proliferative ability was assessed using wound healing and MTT assay respectively. Microarray based global gene expression analysis of hsa-miR-18a-5p over-expressing cells was performed. Disease free survival analysis was done using Kaplan-Meier survival analysis.

## Result

We estimated the relative abundance of hsa-miR-18a-5p in 123 ER positive primary breast cancers and found the distribution of this miRNA to be highest in the lower ER group ( $p < 0.05$ ). hsa-miR-18a-5p also correlated negatively with *ESR1* and *PGR* ( $p < 0.05$ ). To further probe the role of miR-18a-5p in invasion and metastasis, we over-expressed this microRNA using a synthetic mimic in a luminal cell line MCF7. Microarray analysis revealed an increase in the expression of ECM associated genes, and the Cadherin signalling pathway. We observed a decrease in the expression of luminal genes (*PGR*, *TFF1*, *GREB1*;  $p < 0.05$ ), loss of Tff1 and increase in the levels of basal cytokeratin 14 at the protein level in addition to an increase in proliferative rate of upto 35% ( $p < 0.05$ ). Further, we observed a 15 % ( $p < 0.05$ ) increase in the invasive ability by wound healing assay with a significant loss in the levels of E-cadherin protein. In order to study the prognostic importance of hsa-miR-18a-5p, we performed Kaplan-Meier survival analysis and found that stratification of the ER+ve tumor samples by hsa-miR-18a-5p levels produced significant separation of the groups based on disease-free survival (log rank  $p < 0.05$ ). The prognostic value was also validated with multivariate Cox-proportional hazard analysis (Hazard Ratio (HR) of 3.18 (1.07-9.42);  $p = 0.03$ ).

## Conclusion

The results from the over-expression of miR-18a-5p in MCF7 cells support the existence of an epigenetic pathway of repression of the luminal phenotype and increased acquisition of traits associated with basal-like and mesenchymal characteristics. It is possible that the over-expression of miR-18a-5p plays a role in patients with poorer outcomes.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-05-06

Estrogen induced miR-489 acts as a negative feedback to confine uncontrolled estrogen signaling and cell proliferation in breast cancer

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**Estrogen induced miR-489 acts as a negative feedback to confine uncontrolled estrogen signaling and cell proliferation in breast cancer.**

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Approximately 75% of diagnosed breast cancer tumors are Estrogen receptor positive tumors and are associated with better prognosis due to response to hormonal therapies. However, around 40% of patients relapse after hormonal therapies. Identification of novel molecular targets is necessary to combat such resistant tumors. In the current study, using microarray, qRT-PCR, western blot, luciferase reporter assay and immunofluorescence, we found that miR-489 is an estrogen regulated miRNA that negatively regulates estrogen signaling. Depletion of miR-489 using Anti-miR-489 siRNA or CRISPR-Cas9 significantly increased estrogen induced proliferation, colony formation ability and stem like cell population. Loss of miR-489 also induced estrogen independent proliferation. Mechanistically we found that depletion of miR-489 enhanced nuclear localization of estrogen receptor while restoration of miR-489 increased cytosolic ER and inhibited estrogen induced transcription. Furthermore, we found that miR-489 depletion also increases estrogen independent growth through activation of MAPK and PI3K-AKT pathway. We also observed loss of miR-489 in tamoxifen resistance breast cancer cell line and found increased resistance to tamoxifen upon miR-489 inhibition while miR-489 restoration sensitized Tamoxifen resistant cell lines. Clinical analysis of estrogen receptor positive breast cancer patient showed that ER<sup>+</sup> breast cancers with low miR-489 expression represents aggressive cancers with significant reduction in survival time. In summary, these results indicate potential role of miR-489 as a biomarker to predict aggressiveness of ER<sup>+</sup> breast cancer and response to tamoxifen therapy and can potentially be used as a therapeutic agent to treat or sensitize tamoxifen resistant tumors.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-05-07

The effects of microRNA modulation of polo-like kinase 1 in breast cancer cell lines

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**BACKGROUND:** Elucidating a more universal therapeutic target is key to effectively combat the heterogeneity of breast cancer (BrCa). MicroRNAs (miRs) are small noncoding RNAs that are an abundant class of endogenous regulatory molecules, which act by targeting mRNAs for cleavage and/or translational repression. Continuing studies into BrCa genetics show that the various subtypes of BrCa are also affected by miR activity from the regulation of various cancer-related genes. MiR expression can even influence drug response in multiple cancer types. This prompted our investigation into the nuances of how miRs may influence BrCa behavior and response to treatment with targeted therapies. The Polo-like Kinase 1 (PLK1) enzyme plays an important role in the cell cycle, and is considered to be a proto-oncogene. Inhibiting PLK1 has shown promise in reducing tumor volume and promoting tumor cell death in various cancers. Understanding how miRs regulate PLK1 in BrCa will improve our understanding of the PLK1 pathway, and whether this miR-directed regulation affects anti-PLK1 therapy. This knowledge could inform future anti-PLK1 BrCa treatment regimens, resulting in better outcomes for patients.

**METHODS:** The effects of an anti-PLK1 drug, NMS-P937, were tested on triple-negative (TN) (MDA-MB-231, BT549) and luminal (ZR-75-1, CAMA-1) BrCa cell lines. From miRNA target prediction sites (TargetScan, MiRTarBase), a panel of miRs predicted to bind to PLK1 was selected and then assessed using dual-reporter luciferase assays. MiR expression was transiently induced in TN and luminal BrCa cell lines, and the effects were verified using western blotting. Apoptosis was assessed by Annexin V staining in BrCa cell lines transiently transfected with the validated miR and treated with different concentration of NMS-P937 for 48 hrs. To validate the contribution of the miR-PLK1 axis for NMS-P937-induced apoptosis, BrCa cells were transfected with PLK1 siRNA, and the Annexin V staining experiment was repeated.

**RESULTS:** From the panel of miRs tested, miR-183-5p was the only one to demonstrate binding to the PLK1 gene. Western blotting showed that PLK1 expression was downregulated when miR-183-5p is overexpressed. Annexin V staining suggests that miR-183-5p induces apoptosis through PLK1 targeting in BrCa cell lines, and this effect seems to amplify NMS-P937-induced apoptosis in both cell line subtypes. Apoptotic markers assessed by western blot confirm the over-activation of apoptotic signals by miR-183-5p. Future work will continue to focus on determining the effects of miR-183-5p targeting on pathways downstream of PLK1, and utilizing tumor xenografts to elucidate the effects of miR-183-5p on NMS-P937 response in an *in-vivo* model.

**CONCLUSIONS:** MiR-183-5p appears to directly target PLK1 and reduce its level of expression. Additionally, miR-183-5p increases the number of apoptotic BrCa cells when used in combination with NMS-P937, and it is the inhibition of PLK1 specifically that contributes to the increase in the apoptosis of BrCa cells treated with NMS-P937. Taken together, our findings identify a new mechanism of PLK1 modulation through miR. Our next steps will focus on studying these modulatory effects *in vivo*, in an effort to move forward for clinical applications.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-05-08

LINC02273 interacts with hnRNPL and promotes metastasis through directly activating AGR2 in breast cancer

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The prognosis of breast cancer patients with metastasis is still poor even improved by current treatment modalities. Unveiling new biomarkers and molecular mechanisms that underlie metastasis are of vital importance for the treatment of breast cancer. The recent discovery of long noncoding RNAs (lncRNAs) has gained widespread attention. To identify critical lncRNAs that contributed to breast cancer metastasis, we profiled their expression in 5 pairs of primary tumors and lymph nodes metastasis loci by HTA2.0 microarray. LINC02273 is significantly upregulated in metastasis loci and its high expression is associated with poor diseases free survival in a validation set of 254 patients. LINC02273 was mainly located in the cell nucleus. RACE-PCR showed two isoforms and the longest one was the most abundant isoform in breast cancer. Through transwell assay, 3D spheroid invasion assay and mice xenograft metastasis model, we found that LINC02273 promoted breast cancer cell migration, invasion and metastasis. Via mass spectrometry, hnRNPL was found to interact with LINC02273 to enhance its stability, which was further confirmed by Actinomycin D inhibition assay and luciferase reporter assay. Furthermore, ChIRP-seq and ChIP-seq showed that LINC02273 stimulated oncogene AGR2 expression by directly binding to the AGR2 promoter region and increasing H3K27ac modification. Triplex formation assay was performed for verification. We demonstrated that the expression level and oncogenic ability of AGR2 were regulated by hnRNPL through LINC02273. Clinical data and mouse xenograft tumors also revealed the positive correlation of AGR2 and LINC02273. In conclusion, LINC02273, which is stabilized by hnRNPL can promote breast cancer metastasis through upregulation of AGR2 and may serve as a prognostic biomarker for breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-05-09

Exosome microRNA contents are altered during breast cancer progression

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**Background:** The progression of breast cancer involves the transformation of normal mammary epithelial cells to ductal carcinoma in situ (DCIS) and invasive breast cancer (IBC). This process is initiated by genetic alterations and characterized by changes to gene expression programs and microenvironmental alterations. However, the specific drivers of DCIS progression to IBC are not well understood nor has an indicator of progression been identified. Exosomes are small secretory vesicles that can contribute to cancer progression by transferring oncogenic factors, such as microRNAs (miRNAs), to surrounding cells in the tumor microenvironment, and enter the circulation to act at distant sites. miRNAs are short noncoding RNAs that regulate the expression of a target messenger RNA (mRNA). Altered regulation by miRNAs is implicated in cancer progression. In this study, we sought to characterize the exosome miRNAs in the MCF10 isogenic model of breast cancer progression in order to identify potential drivers of breast cancer.

**Methods:** Exosomes were isolated from the conditioned media of the MCF10 isogenic cell line model of breast cancer progression representing the following stages: normal, benign proliferative, carcinoma *in situ*, and invasive carcinoma. RNA was extracted from the exosomes and next generation RNA sequencing was performed. Exosome miRNA expression was validated in breast cancer cell lines and in plasma exosomes collected from a mouse-intraductal transplantation (MIND) model implanted with MCF10DCIS.com (DCIS) cells that can mimic human DCIS progression *in vivo*.

**Results:** Comparisons were made between differentially expressed miRNAs among each condition (fold change >1.5; Kruskal-Wallis  $p < 0.05$ ). Twenty-nine miRNAs were differentially expressed among invasive and DCIS exosomes. The expression of 5 oncogenic miRNAs (miR-30c-5p, -210, -182-5p, -200c-3p, and -200b-3p) were consistently increased, while 2 tumor suppressive miRNAs (miR-423-5p and -92b-3p) were consistently decreased with invasive progression. Exosome miRNA expression was confirmed in breast cancer cell lines and mouse plasma exosomes.

**Conclusion:** This work demonstrates that the microRNA contents of exosomes change upon malignant transformation to invasive breast cancer and indicate that certain exosome microRNAs are consistently up- or down-regulated and may contribute to breast cancer progression.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-05-10

MicroRNA-137 inhibits cancer progression by targeting DEL-1 in triple negative breast cancer cells, MDA-MB-231

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**Background:** This study aimed to investigate the function of microRNA-137 in Del-1 expression in triple negative breast cancer (TNBC) cells and tissues.

**Methods:** The Del-1 mRNA and microRNA levels were measured using a qRT-PCR in breast cancer cells (MDA-MB-231, MCF7, SK-BR3, and T-47D) and tissues from 20 patients with TNBC. The effects of miR-137 on cell proliferation, migration, and invasion were determined using MTT, wound healing, and Matrigel Transwell assays.

**Results:** microRNA-137 (miR-137) levels were remarkably low and Del-1 mRNA expression was higher in MDA-MB-231 cells as compared to other breast cancer cell lines. The luciferase reporter assay revealed that miR-137 binds directly at the 3'-UTR of Del-1 and that Del-1 expression was downregulated by miR-137 mimics and rescued by its inhibitors. Furthermore, miR-137 inhibited the cell proliferation, migration, and invasion of MDA-MB-231 cells. Moreover, among the 30 TNBC specimens, miR-137 was downregulated ( $p < 0.0001$ ) and the level of Del-1 in plasma was significantly elevated as compared to normal controls ( $p < 0.0001$ ).

**Conclusions:** In conclusion, miR-137 regulates Del-1 expression in TNBC via directly binding to the Del-1 gene, and thereby affects cancer progression. This suggests that miR-137 may be a new therapeutic biomarker for patients with TNBC.

**Keywords:** Del-1, triple negative breast cancer, miR-137, biomarker

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-05-11

Mir-4319 suppresses the malignancy of triple-negative breast cancer by regulating self-renewal and tumorigenesis of stem cells

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**Background/Aims** High levels of cancer stem cells (CSCs) in patients with triple-negative breast cancer (TNBC) correlate with risk of poor clinical outcome and possibly contribute to chemoresistance and metastasis in patients with highly malignant TNBC. Aberrant microRNA expression is associated with the dysfunction of self-renewal and proliferation in cancer stem cells, while there is little information about the TNBC-specific microRNAs in regulating CSC ability.

**Methods** Solexa deep sequencing was performed to detect the expression levels of TNBC or non-TNBC stem cells (CSCs) microRNAs. Mammosphere formation assay, qRT-PCR and the xenograft model in nude mice were performed. Bioinformatic analysis and microarray were used to select the target gene, and luciferase reporter assays were used to confirm the binding sites.

**Results** Solexa sequencing data exhibited differential expression of 193 microRNAs between TNBC and non-TNBC stem cells. The gene ontology analysis and pathways analyses showed that genes were involved in the maintenance of stemness. Overexpression of miR-4319 could decrease the tumorsphere-forming efficiency in MDA-MB-231 CSCs and also inhibit tumor initiation and metastasis in vivo. Knockdown of mir-4319 showed the reverse. Overexpression of miR-4319 greatly reduced luciferase activity of E2F2 by almost 50% and when the mismatch mutation was introduced into the region, the inhibition of luciferase activity was almost abolished. Moreover, increased E2F2 could reverse the effect of mir-4319 on the self-renewal in TNBC CSCs.

**Conclusions** Mir-4319 suppresses the malignancy of TNBC by regulating self-renewal and tumorigenesis of stem cells and might be a remarkable prognostic factor or therapeutic target for patients with TNBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-06-01

Analyzing the physical and functional protein interaction landscape of breast cancer

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A key unanswered question in cancer genetics is how different mutations, dispersed across a multitude of genes, elicit similar pathology and patient outcomes. The answer may lie in understanding the molecular networks and protein complexes (i.e. signaling pathways, chromatin architecture, etc) in cancer and mapping mutated genes into the complexes and pathways in which they function. Determining how systematic interaction networks are wired in cancer cells and how different mutations perturb these networks will guide the search for new cancer genes and provide a platform for integrating patient data to make biological and clinical predictions more accurate. The goal of this study is to uncover the comprehensive protein-protein interaction networks and pathways in various breast cancer subtypes to better understand how mutated cancer genes and genomes hijack and re-wire pathways and complexes during the course of breast tumorigenesis.

Here we catalog protein-protein interactions for 40 genes recurrently mutated in breast cancer, using affinity purification and mass spectrometry. To identify co-associated proteins, cDNA clones expressing each protein were tagged with 3xFLAG at either N or C-terminus and introduced into MCF10A (non-tumorigenic “healthy” control), MCF7 (luminal A subtype), and MDA-MB-231 (claudin-low) cells using doxycycline-inducible lentiviral vectors. For proteins with prevalent pathogenic mutations (e.g. PIK3CA-H1047R, BRCA1-C61G), mutant cDNA clones were also analyzed in parallel. Our interaction network reveals subtype and mutation-specific protein-protein interactions, many of which are not previously reported. Given that genes encoding components of a protein complex or a biological pathway often share similar phenotype upon genetic perturbation, we genetically knocked out genes interacting with DNA damage response (DDR) proteins using CRISPR/Cas9, and found multiple novel interacting genes whose knockout results in significant PARPi (olaparib) and/or cisplatin sensitivity. This result not only functionally validates the physical protein interactions, but also demonstrates that our interactome mapping approach can help identify newdruggable vulnerabilities in cancer cells.

We anticipate the breast cancer interactome study will uncover aberrant pathways and protein complexes uniquely operating in breast cancer cells, and thus pinpoint proteins that may potentially serve as distinct biomarkers or therapeutic targets for tumors having the same or similar subtypes and/or genomic mutations.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-07-01

A translational and five-year clinical update in patients treated with neoadjuvant chemotherapy randomized to bevacizumab or control in HER2 negative breast cancer

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**Background:** Bevacizumab added to conventional neoadjuvant chemotherapy increase the proportion of patients achieving a pathological complete response (pCR). Identifying patients responding to antiangiogenic therapy have been challenging. The primary objective of this study was to determine the molecular characteristics and treatment induced changes of the primary tumors with reference to treatment response. Clinical outcome measurements according to treatment were exploratory endpoints. Recent updated clinical results, in addition to extended molecular analyses are presented.

**Methods:** A phase II randomized clinical trial of HER2 negative primary tumors of  $\geq 25$  mm (n=132) was conducted, treated with neoadjuvant chemotherapy (4xFEC100 followed by taxane-based therapy) with or without the addition of bevacizumab. Biopsies were obtained at the time of diagnosis, after 12 weeks of treatment, and after 25 weeks at surgery. The response was evaluated using the criteria for determining the residual cancer burden (RCB). We derived a mean immune score per patient by calculating the average score from the 770 genes in the nCounter PanCancer immune panel to detect an association between immune activity and response to antiangiogenic therapy. In addition, the median five-year follow-up for disease recurrence are reported.

**Results:** The addition of bevacizumab increased the RCB class 0 (pCR) rate in the study population from 12% to 17% and the rate of “good responders” (RCB class 0 and 1) from 24% to 33%, without reaching statistical significance. A pronounced effect of bevacizumab combination therapy was observed in the hormone receptor (HR) positive tumors, where the percentage of patients achieving RCB class 0 increased from 5% to 20% (Fisher's Exact test,  $p=0.02$ ). More HR positive patients achieved a good response and fewer patients were poor responders (RCB class 3) in the combination treatment arm (Wilcoxon,  $p=0.035$ ). Previously, our unsupervised analyses demonstrated an enrichment of immune related genes in pretreatment samples from patients responding to combination therapy. A significantly higher mean immune score ( $p<0.001$ ) was detected among the HR positive patients who received bevacizumab and achieved RCB class 0 after neoadjuvant treatment (n=11, 20%). Five-year follow-up data revealed a total of 21 events in the study population; 9 relapses in patients treated with combination therapy, and 12 relapses in patients treated with chemotherapy only. DFS was not statistically different between the treatment groups (log rank,  $p=0.4257$ ). However, among the patients achieving a good response an improved DFS was observed for those treated with combination therapy (1/22 vs. 5/16, log rank,  $p=0.0254$ ).

**Conclusion:** Among locally advanced HER2-negative HR positive breast cancer patients, the addition of bevacizumab to neoadjuvant chemotherapy increased the rate of good responders and improved the DFS among these patients. An increased primary tumor immune score may predict good response to neoadjuvant antiangiogenic therapy in HR positive disease. Further studies are needed to validate the use of such immune panels for selection of patients most likely to benefit from antiangiogenic therapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-07-02

The CARMA3-Bcl10-MALT1 signalosome mediates pro-angiogenic IL-6 and IL-8 paracrine signaling in GPCR+ breast cancer

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**Background:** The overexpression of selected G-protein coupled receptors (GPCRs) has been linked to the pathogenesis of multiple cancer subtypes. We recently demonstrated that overexpression of two such GPCRs, the angiotensin II receptor type I (AGTR1) and protease-activated receptor type 1 (PAR1), occurs in a substantial fraction of luminal breast cancers and is associated with treatment resistance and poor prognosis. Further, experimental models demonstrate that overexpression of these receptors in breast cancer cell lines promotes aggressive features that include EMT, invasion, migration, and loss of ER expression. In addition to these cell intrinsic effects, we find that GPCR overexpression in breast cancer cells can impact the tumor microenvironment. Specifically, we recently reported that conditioned media from angiotensin II-stimulated AGTR1+ breast cancer cells induces endothelial chemotaxis in vitro and tumor angiogenesis in vivo. We also demonstrated that this pro-angiogenic phenotype requires the CARMA3-Bcl10-MALT1 (CBM) signalosome, a signaling complex that links upstream GPCR stimulation with downstream NF- $\kappa$ B activation. We hypothesized that stimulation of either AGTR1 or PAR1 induces CBM-dependent secretion of NF- $\kappa$ B responsive, pro-angiogenic factors from these GPCR+ breast cancer cells which then exert proangiogenic effects on neighboring endothelial cells through paracrine signaling.

**Methods:** To identify CBM-dependent secreted factors, we evaluated the AGTR1+ BT549 cell line, +/- Bcl10 or MALT1 siRNA knockdown, for expression of 770 genes of significance to solid tumor pathogenesis using the NanoString PanCancer Progression Panel followed by Ingenuity Pathway Analysis (IPA). RT-PCR and ELISA were used to validate hits and determine if the CBM signalosome controls expression of the same genes in the PAR1+ cell lines, MCF7-N55 and MDA-MB-231.

**Results:** We identified IL-6 and IL-8 signaling pathways as the two most significantly downregulated angiogenesis pathways following either Bcl10 or MALT1 knockdown. Using quantitative RT-PCR and ELISA, we confirmed that IL-6 and IL-8 gene expression and protein secretion are significantly induced in response to stimulation of BT549 cells by angiotensin II and MCF7-N55 and MDA-MB-231 cells by TRAP6, a PAR1 agonist. siRNA-mediated MALT1 knockdown in BT549 cells led to a significant reduction of IL-6 and IL-8 gene expression upon angiotensin II stimulation; similarly, CRISPR/Cas9-mediated MALT1-deletion in MCF7-N55 cells resulted in failure of these cells to secrete IL-8 upon TRAP6 stimulation.

**Conclusions:** The GPCR-CBM-cytokine signaling pathway provides a common druggable target to curb pro-angiogenic paracrine signaling in GPCR+ breast cancers. Importantly, the CBM signalosome has also been shown to be required for IL-8 dependent upregulation of VEGF in endothelial cells, indicating that inhibition of the signalosome could exert complementary effects on both cancer cells and endothelial cells to effectively limit the pro-angiogenic phenotype driven by GPCR overexpression. Several small-molecule MALT1 inhibitors are now available and can be tested for their efficacy as angiogenesis inhibitors in the setting of GPCR+ breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-07-03

A live tissue platform allows dynamic measurement of neovascularization and prediction of clinical response in human breast cancer samples, *ex vivo*

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**Background:** Outgrowth of new blood vessels (neovascularization) allows tumors to supply themselves with oxygen and nutrients, and to rapidly metastasize throughout the body. Triple negative breast cancer (TNBC) is particularly susceptible to neovascularization. However, success with anti-angiogenics is highly variable and often patient-specific. This is particularly true as anti-angiogenics are being combined with immunotherapies. Thus, there is a huge unmet need for clinicians to test and predict clinical efficacy of anti-angiogenics at the individual patient level, prior to treatment.

**Methods:** Here, we characterize a patient-autologous, *ex-vivo* tumor model, termed CANscript, as a platform to study the intratumor microvascular density (iMVD) of breast cancer samples (N=15). To profile iMVD we used immunohistochemical (IHC) analysis of CD34, an early biomarker of neovascularization. We then introduced anticancer and anti-angiogenic agents (e.g. Avastin) for 72 hours, and subsequently quantified phenotypic response to drugs by testing viability, cell death, proliferation and morphology. These quantitative data were then fed into a machine learning algorithm that provides a clinical response prediction (M-Score).

**Results:** We determined that *ex-vivo* culture reliably retains baseline heterogeneity of iMVD based on expression of CD34+ nodes per visual field by IHC. Furthermore, we show that anticancer and anti-angiogenic agents will dynamically alter iMVD, *ex-vivo*, in a patient-specific manner. Finally, we show that prediction of clinical response using the 'M-Score' algorithm associates with diminished expression of CD34 per visual field of IHC after drug pressure.

**Summary:** Neovascularization and iMVD are features of aggressive cancers, such as TNBC. CANscript provides a rapid assessment of clinical response to anticancer drugs, many of which induce their antitumor effect by targeting the tumor vasculature. We show that pharmacodynamics of antiangiogenics can be captured during acute *ex-vivo* culture under drug pressure, which associate to clinical response prediction. Therefore, we highlight the ability of CANscript as a platform to predict clinical response to anti-angiogenic drugs, and may therefore be a logical 'testing ground' to predict clinical efficacy of antiangiogenic drugs combined with immunotherapies.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-07-04

Gene expression profiles of angiogenesis related cytokines and cognate receptors predict breast cancer progression and survival

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**Background:** Angiogenesis plays a critical role in breast cancer development, invasion and metastasis, and *VEGF*, *TGFβ1*, and *IL10* genes and their cognate receptors are implicated in cancer behavior, mediating membrane and microenvironment remodeling. Our goal is to assess relationships of expression of these genes in LCM-procured carcinoma cells from primary breast cancers with clinical outcomes to predict risk of recurrence.

**Methods:** Gene expression determined by microarray and qPCR, ER/PR quantified by radio-ligand binding or EIA, features of primary breast cancers and clinical outcomes were performed by univariable and multivariable Cox regressions, Kaplan Meier plots and LASSO with R software v3.2.5. Microarray analyses of ~22,000 genes utilized RNA isolated, purified and amplified from LCM-procured carcinoma cells. Gene expression was validated by qPCR while molecular signatures were externally validated with SurvExpress (Aguirre-Gamboa et al. PLoS One e74250, 2013).

**Results:** Univariable Cox regression of microarray results of cytokines and cognate receptor genes revealed *IL10* expression was significant for prediction of Progression Free (PFS) or Overall Survival (OS) using an adjusted p-value of  $\geq 0.30$  (discovery cut-off). Without regard to ER/PR status, Kaplan Meier plots confirmed *IL10* or *TGFβ1* expression was significant for predicting PFS and OS. Using ER+ cancers, *IL10* or *VEGF* expression was related to PFS while only *IL10R* was related to OS of ER- cancers using univariable Cox regression. Validation by qPCR using tissue curls disclosed independent expression of either *TGFβ1* or *VEGF* predicted PFS and OS while that of *TGFβ1R* was only related to PFS. *IL10R* expression only predicted OS of breast cancer patients. Violin plots of qPCR results indicated elevated expression of each of these six genes in ER+ primaries without regard to outcome. Multivariable Cox regression analyses of expression levels of cytokine-receptor gene pairs revealed that IL10-IL10R co-expression was highly significant for predicting OS. Strongly supportive, backwards selection (R software v3.2.5) utilizing microarray expression levels derived a significant model (molecular signature) consisting of *IL10*, *IL10R* and *VEGFR* genes for predicting OS. External validation of this 3-gene subset was accomplished utilizing patient data collected on SurvExpress. A second clinically relevant signature predicting OS in PR+ breast cancers was detected when *TGFβ1R* expression was included with these 3 genes. Finally, backwards selection of qPCR data derived a significant 3-gene model composed of *IL10R*, *TGFβ1* and *VEGF* for predicting PFS that was undeniably validated by SurvExpress.

**Conclusions:** Using gene expression results derived from microarray analyses of LCM-procured breast carcinoma cells of primary lesions and validated by qPCR, subsets of angiogenesis related genes were identified that predict a patient's risk of recurrence and overall survival. Expression of candidate genes appears to be related to either/both ER or PR protein status of the primary lesion. Collectively, results suggest that expression of certain angiogenesis related genes may serve as biomarkers for assessing prognosis of breast carcinomas thus impacting clinical management.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-08-01

Metformin modulates the senescence-associated secretory phenotype (SASP) in paclitaxel-resistance triple-negative breast cancer cell line

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**Background:** Breast cancer (BC) is a complex disease subdivided into distinct tumor types based on the expression of molecular markers, which might also guide the prediction of patient's outcome and tumor response to therapy. Triple-negative breast cancer (TNBC) is an aggressive form of BC that frequently recurs and leads to cancer-related death. This is due, at least partially, to the absence of specific targets that can be used in the fight against TNBC, narrowing the disease care to conventional chemotherapy based on anthracyclines and taxanes. Chemoresistance is a major cause of tumor relapse, and because it is a multifactorial phenomena, it still challenges clinicians and investigators. Amongst the mechanisms involved in chemo/radio-resistance of tumor cells, there is the acquisition of a senescence-associated secretory phenotype (SASP). Metformin, a safe hypoglycemic drug, has been shown to act in synergy with certain anti-cancer agents, overcoming chemoresistance in various types of tumors. The present work aimed to investigate the use of metformin as a novel treatment strategy for paclitaxel-resistant BC.

**Methods:** MDA231-PR cell line was established from its parental cell MDA-MB-231, using a pulse-selection in clinics. The effects of metformin alone or in combination with conventional drugs on BC cell lines were investigated using the MTT assay for cellular metabolic viability (CMV). Gene and protein expression, as well as secreted proteins were determined in BC cells by real-time RT-PCR, Western blotting and ELISA assay, respectively. Flow cytometry analysis was applied for apoptosis analysis, and SA- $\beta$ -Gal staining was used to investigate cellular senescence.

**Findings:** We characterize SASP in MDA231-PR. Moreover, several pathways related to the acquisition of chemoresistant phenotype by tumor cells were modified in MDA231-PR, including CSC and autophagy phenotypes, MAPK, mTOR and GSK-3 $\beta$ . Furthermore, we showed that metformin inhibits the expression of genes coding for multiple pro-inflammatory cytokines accompanying cellular senescence. Remarkably, metformin reversed the paclitaxel resistance in MDA-MB-231 in the presence of conditioned medium from MDA231-PR.

**Conclusions:** Altogether, our observations suggest that paclitaxel-treated TNBC cells induced SASP, contributing for senescence-related inflammation and stem cell dysfunction. Additionally, our findings support the fact that metformin is a potential agent against TNBC, which remains a major challenge for clinical oncology, especially in tumors refractory to taxanes.

# 2018 San Antonio Breast Cancer Symposium ®

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In primary, non-metastatic breast cancer patients, increased serum levels of RANKL significantly correlate with tumor cell spread to the bone and the occurrence of bone metastasis whereas high levels of its soluble decoy receptor osteoprotegerin predict poor survival

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**Background:** Receptor activator of nuclear factor kappa-B ligand (RANKL) is an essential protein for osteoclast regulation and its activity is controlled by its soluble decoy receptor osteoprotegerin (OPG). RANKL has been associated with benign as well as malignant bone disease and there is increasing evidence that RANKL may also directly affect breast cancer (BC) progression and metastasis to the bone. Here we assessed serum concentrations of RANKL and OPG in 509 patients with primary, non-metastatic BC and correlated the results with clinical parameters including the presence of disseminated tumor cells (DTCs) in the bone marrow (BM), survival and the risk of developing metastatic disease.

**Patients and Methods:** Patients with first diagnosis of BC between Aug 2006 and Dec 2009 were included in our study. BM sampling was performed before surgery in an adjuvant setting and two BM aspirates were analyzed for DTCs using density centrifugation followed by immunocytochemistry applying the pan-cytokeratin antibody A45-B/B3. Blood was collected from each patient and sRANKL and OPG levels in the serum were measured by ELISA (Biomedica, Vienna, Austria). **Results:** Mean serum values for RANKL and OPG were  $0.23 \pm 0.20$  pmol/l and  $4.24 \pm 1.70$  pmol/l, respectively. RANKL levels were significantly lower in women above the age of 60 ( $p < 0.0001$ ) and RANKL/OPG ratios were higher in patients with lymph node involvement ( $p < 0.05$ ). High OPG levels were associated with a higher risk of death from BC (HR 1.94 95%CI 1.23-3.07;  $p = 0.005$ ) and multivariate analyses revealed OPG to be an independent prognostic marker for BC specific survival ( $p = 0.035$ ). DTCs were detected in 207/507 (41%) patients and RANKL levels were 33% higher in DTC-positive patients ( $p < 0.0001$ ). Interestingly, in DTC-negative patients, high RANKL levels were associated with a significantly better BC specific survival compared to low levels (HR 0.524; 95%CI 0.30-0.95;  $p = 0.04$ ). RANKL serum levels were significantly enhanced in patients that developed bone metastases ( $p = 0.01$ ) and patients in the highest quartile of RANKL had a significantly increased risk of developing bone metastases compared to those in the lowest RANKL quartile (HR 4.62, 95%CI 1.49-14.34,  $p = 0.03$ ). **Conclusion:** Increased OPG levels indicated poor survival and high RANKL significantly associated with an increased risk of developing bone metastases while indicating a positive prognostic marker in DTC-negative patients. These findings warrant further investigation as it may provide a rational for novel diagnostic or therapeutic approaches.

# 2018 San Antonio Breast Cancer Symposium ®

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Effects of palbociclib on thymidine kinase-1 (TK1) in hormone receptor positive (HR+) breast cancer cell lines

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**Introduction:** TK1 plays a crucial role in DNA synthesis and is a well-established marker of cell proliferation. We and others have previously described the potential role of TK1 activity (TKa) as predictive biomarker of response to endocrine therapy in HR+/HER2 negative metastatic breast cancer patients. TK1 synthesis is regulated by the E2F pathway, the target pathway of CDK4/6 inhibitors, and TKa has recently been reported as a potential circulating pharmacodynamic marker of CDK4/6 inhibition in breast cancer. However, modulations of TK1 levels and activity during palbociclib treatment and in the development of treatment resistance are unknown. Here, we report how TK1 expression and TKa are modulated in response to palbociclib in a panel of HR+ breast cancer cell lines: both palbociclib-sensitive (PDS) and with acquired resistance to (PDR).

**Material and methods:** We used a panel of 7 PDR HR+ breast cancer models previously developed in our lab via chronic exposure of parental cells (MCF7, T47D, ZR75-1, BT474, MDAMB361 and two MCF7 endocrine resistant derivatives) to escalating doses of palbociclib, from a Starting Treatment Concentration (STC) of 50 nM or 350 nM according to the cell line, up to 1 µM. We analyzed gene expression profiles of PDS cells treated with drug vehicle (DMSO) as a control or palbociclib at STC for 3 days, and PDR cells grown with palbociclib 1 µM. Cell proliferation was assessed by methylene blue assay in MCF7 and BT474 PDS and PDR treated for 3, 6 and 9 days with DMSO, palbociclib STC and 1 µM. In parallel, TKa was measured in cell lysates at 3 days of treatment using the DiviTum™ assay (Biovica, Sweden).

**Results:** Among E2F target genes, gene expression data demonstrated that TK1 was one of the most differentially expressed genes between PDR and PDS treated cells. In PDS cells compared to control, treatment with palbociclib resulted in reduced TK1 expression, with the HER2 positive models (BT474 and MDAMB361) showing the highest reduction. In PDR cells, TK1 expression was higher, but remained slightly inhibited compared to untreated PDS cells. TKa was significantly reduced in PDS cells treated with palbociclib for 3 days compared to vehicle ( $p < 0.05$ ). TKa response to palbociclib was more dramatic in BT474 cells as compared to MCF7. As expected, palbociclib inhibited cell proliferation in PDS models, with a significant reduction observed only after 6 days of treatment, suggesting that TKa may be an early marker of growth inhibition in response to palbociclib. No significant alterations in TKa were observed in PDR cells, at any dose of palbociclib. Similarly, proliferation rate was not affected by palbociclib in PDR cells.

**Conclusions:** TK1 expression and activity are regulated by palbociclib in HR+ breast cancer cell lines, particularly in HER2 positive models. Ongoing studies of TKa in patients treated with palbociclib will assess the role of TKa as a circulating biomarker for predicting and monitoring response to CDK4/6 inhibitors.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-09-03

Association of Caspase 8 polymorphisms with TILs and disease-free survival in primary breast cancer patients

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**BACKGROUND:** The minor allele of two caspase-8 polymorphisms, namely CASP8 -652 6N InsDel and CASP8 Asp302His, were shown to promote survival of T-lymphocytes and were repeatedly associated with reduced breast cancer susceptibility. However, besides some preliminary findings, clinical relevance of these polymorphisms in patients with already existing primary breast cancer has not yet been established. Considering an immunomodulatory and potentially tumor-protective role of these caspase-8 variants, we genotyped 785 primary breast cancer patients and correlated caspase-8 variants with i) disease-free survival (DFS) and ii) the presence of tumor infiltrating lymphocytes (TILs).

**METHODS:** Primary breast cancer samples were collected at the Martin-Luther University, Halle-Wittenberg between 2009 and 2011 as part of the multicenter prospective PiA trial (NCT 01592825). The majority of patients had luminal-like tumors (75.9%), followed by triple negative (10.1%), luminal-Her2-like (9.6%) and Her2-enriched tumors (4.5%). Genotyping was performed by pyrosequencing, TILs status was assessed by hematoxylin and eosin staining.

**RESULTS:** The CASP8 -652 deletion was significantly associated with improved DFS in an allele-dose dependent manner ( $p=0.027$ ). Homozygosity for the -652 6N Del variant was an independent predictor for improved DFS ( $p=0.005$ ). In patients with a 302His/His genotype, there was no event of recurrence during the entire observation time. Combined analysis of diplotypes revealed that both polymorphisms had an influence on DFS ( $p=0.029$ ). Interestingly, patients with the 302His/His variant among the unstratified patient cohort and among the luminal-like subtype alone had tumors with very low lymphocyte infiltration (0-10% TILs in 65% of cases compared to 31% of cases for other genotypes,  $p=0.025$ ).

**CONCLUSION:** In line with previous epidemiological findings, we propose a prognostically favorable role of the CASP8 -652 6N Del and the Asp302His variant in primary breast cancer patients. Moreover, we suggest for the first time a role of the Asp302His variant in immunosurveillance and lymphocyte infiltration of breast cancer. Our findings strongly encourage further analyze of these genetic variants as a biomarker for prognostic and immunotherapeutic considerations.

# 2018 San Antonio Breast Cancer Symposium ®

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Predictive factors for considering to avoid axillar lymphadenectomy in selected node positive breast cancer patients after neoadjuvant chemotherapy

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## Background:

To perform a systematic axillar lymphadenectomy (ALND) in clinical node positive (N+) patients after neoadjuvant chemotherapy (NACT) is currently under discussion. We aimed to study which factors are related to a pathological complete axillar response (ypN0) after NACT in order to select which patients could benefit from a sentinel lymph node biopsy without interfering with survival.

## Material and methods

N+ patients who underwent ALND after NACT between June 2008 and December 2016 were retrospectively analyzed. Clinical features, molecular and histological factors, recurrence and specific mortality rates were compared between patients achieving a complete pathological axillary response vs not (ypN0 vs ypN+).

## Results

345 N+ patients were reviewed. After NACT, 137 (39.6%) become ypN0[CF1], 9 (2.6%) ypN1 mic, 113 (32.7%) ypN1, 60 (17.3%) ypN2 and 22 (6.4%) N3. Univariate analysis results regarding the predictive factors for ypN0 are detailed in [table 1].

Table 1. Predictive factors for ypN0

	YpN0 (n = 137)	YpN+ (n = 208)	p
Age (mean, years)	58.3 ± 13.27	58.59 ± 12.34	0.799
BMI (mean)	27.8±5.49	27.8±5.36	0.973
Dosis of QT (median)(%)			0.575
IIA	6 (31.6)	13 (68.4)	
IIB	71 (39.3)	110 (60.8)	
IIIA	28 (36.8)	48 (63.2)	
IIIB	24 (43.6)	31 (56.4)	
IIIC	7 (58.3)	5 (41.7)	
Radiological image(%)			0.930
Nodule	77 (38.1)	125 (61.9)	
Non-mass distortion	10 (43.5)	13 (56.5)	
Radiological size (median)	32 (0-115)	29 (0-130)	0.246
Suspicious a-LN by US(%)			0.486
1	30 (30.9)	37 (24.3)	
2	5 (5.2)	14 (9.2)	
>2	57 (58.8)	91 (59.9)	
Histological subtype(%)			0.093
Invasive Ductal Carcinoma	133 (40.9)	192 (59.1)	
Invasive Lobular Carcinoma	2 (20)	8 (80)	
Others	2 (22.2)	7 (78.8)	
Nottingham grade(%)			<0.001

G1	1 (6.2)	15 (93.8)	
G2	44 (28.6)	110 (71.4)	
G3	86 (53.4)	75 (46.6)	
Molecular-like subtype(%)			<0.001
Luminal A-like	2 (5.3)	36 (94.7)	
Luminal B-like (Her2 -)	21 (18.1)	95 (81.9)	
Luminal B-like (Her2 +)	40 (63.5)	23 (36.5)	
HER-2 enriched (non luminal)	43 (74.1)	15 (25.9)	
Triple Negative	31 (44.9)	38 (55.1)	
Vascular invasion	19 (42.2)	26 (57.8)	0.889
Clinical Response(%)			<0.001
Complete	61 (75.3)	20 (24.7)	
Partial	69 (31.8)	148 (68.2)	
No response	6 (20.7)	23 (79.3)	
Progression	1 (10)	9 (90)	

Percentage are given per row.

Multivariate analyses showed molecular subtype (TN and Her2+) and clinical response as independent predictors of ypN0 [table 2].

Table 2. Multivariate analysis logistic regression of clinical predictive factors of ypN0.

	OR	95% Confidence Interval	p value
Molecular subtype			
No-luminal vs Luminal	7,748	3,913-15,343	<0,001
Clinical response			
Response vs not response	6,849	1,834-25,571	0,04

OR: Odd ratio. No-luminal includes: luminal B (HER2 +), HER2 Henriched and triple negative. Luminal includes: Luminal A and Luminal B (HER2 -).

After a mean follow-up of 58 months, overall survival was statistically superior in ypN0 vs ypN1 (p= 0.001).

#### Conclusions

A remarkable percentage of N+ became ypN0 after NATC. Molecular subtype and complete clinical response were independent predictive factors of ypN0. We propose to offer the benefit of a targeted axillary procedure in those patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-09-05

**Optical Prediction of Time Interval to Metastasis (OPTIM):** A rapid nondestructive optical assay applied to tissue microarray samples identifying high risk of distant recurrence in the lowest risk groups defined by the TAILORx trial

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Recent reporting of the 9 year follow-up for the TAILORx trial suggests that there may be no benefit with adjuvant chemotherapy for ER +, HER2 -, N(0) breast cancer patients with a Oncotype DX® (ODX) recurrence score (RS) <26. Since endocrine therapy for this group of patients who comply with treatment still results in distant recurrence (rMBC) in 3% and 5% of the ODX low and ODX intermediate risk groups at 9 years, respectively, we are motivated to help find early treatments for these patients by identifying their recurrence risk at diagnosis with improved risk stratification.

**Methods:** Optical Prediction of Time Interval to Metastasis (OPTIM), a novel assay, prognostic for rMBC, is based on an intrinsic optical signature from collagen, derived from the average of point by point ratios of forward to backward (F/B) second harmonic generation (SHG) light scatter that is sensitive to form and structure of fibrillar collagen in the extracellular matrix of archival tissue microarray samples. (Burke et al. BMC Cancer 15 (2015): 929). The 125 patients in this cohort were part of a clinical trial, looking for genomic predictors of rMBC in untreated patients, so we were able to calculate a surrogate 21-gene RT-PCR assay (S-ODX) value based on gene expression data available through NCBI GEO database (Gyorffy et al. Breast Cancer Res Treat (2012) 132:1025). We analyzed these patient's rMBC outcomes using logistic regression and Kaplan-Meier (KM) analysis.

**Results:** OPTIM alone stratified at 2.5X relative risk (RR) between quartiles Q1 and Q4, similar to S-ODX low vs high recurrence score (RS) groups (from TAILORx Trial) with 2.8X RR. Using quartiles of OPTIM vs S-ODX together we stratify patients to recurrence risk (rMBC/at Risk), with an improved risk stratification of 5X RR in the RS<26 low risk groups.

## OPTIM Quartiles vs RS Risk Groups in TAILORx Trial

S-ODX →	High (RS>25)	Intermediate (RS 11-25)	Low (RS <11)	All
OPTIM	↓	↓	↓	↓
Q1	7/9	7/12*	5/10	19/31*
Q2	5/9	5/14	2/8	12/31
Q3	8/12***	1/8	1/11***	10/31
Q4	6/10**	2/17*	0/5**	8/32*
All	26/40***	15/51	8/34***	49/125

Recurrence at 10 years by KM analysis \*p<0.05, \*\*p<0.005, \*\*\*p<.0005

Combining S-ODX with OPTIM, low (L) or high (H) risk by assay, shows that they are independent and complementary. Notably 68%=85/125 are classified L by S-ODX (RS<26) and OPTIM effectively reclassifies H and L, and when combined with S-ODX H identifies 92%=45/49 of all rMBC at 10 years without treatment. Risk stratification improves to 6.8X RR comparing highest risk HH 66.7%=12/18 to lowest risk LL 9.8%=4/41.

## Distant Recurrence Identified by High Risk Group of Each Assay

S-ODX Assay	H	H	L	L
OPTIM Assay	H	L	H	L



<b>rMBC</b> (total=49)	12	14	19	4
<b>At Risk</b> (total n=125)	18	22	44	41

rMBC at 10 yrs. S-ODX RS>25=H, RS

**Conclusion:** OPTIM as an independent prognostic optical bio-marker from collagen in intact tissue. Combination of OPTIM with the Oncotype DX® assay may produce a continuous risk estimator with higher dynamic range than either assay alone and will be the focus of future study, especially in a treated population, to determine if OPTIM might also predict response to treatment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-09-06

BRCAness as a prognostic marker in triple-negative breast cancer patients treated with neoadjuvant chemotherapy: A multicenter retrospective study

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**Background:** Triple negative breast cancer (TNBC) has several subtypes. Identification of markers associated with recurrence and poor prognosis in TNBC patients is critically needed. BRCAness is a set of traits in which BRCA1 dysfunction, arising from gene mutation, methylation or deletion, results in DNA repair deficiency. In the present study we evaluated the clinical significance and prognosis of BRCAness in a multicenter retrospective study.

**Methods:** Ninety-four TNBC patients treated with neoadjuvant chemotherapy (NAC) were enrolled from three hospitals in Japan for this retrospective study. Of these patients, 86 (91.5%) were treated with anthracyclines and taxanes. BRCAness was determined from 94 core needle biopsy (CNB) specimens prior to NAC and 50 surgical specimens without pathological complete response (pCR). The samples were assessed using multiplex ligation-dependent probe amplification (MLPA) and the amplicons were scored. Samples with scores >0.5 were defined as having BRCAness.

**Results:** Of the 94 TNBC patients, 51 (54.3%) had BRCAness in CNB specimens. There were no significant differences in pCR rate or recurrence between the BRCAness and non-BRCAness groups. Among surgical specimens, the BRCAness group (19/50, 38%) had a significantly shorter recurrence-free survival (RFS) and disease-specific survival (DSS) compared with the non-BRCAness group ( $p < 0.01$  and  $p < 0.05$ ). The BRCAness group had independent prognostic factors for recurrence and survival in multivariate regression analysis.

**Conclusions:** The BRCAness of surgical specimens is a useful marker to predict prognosis in TNBC patients after NAC. A clinical trial to assess the clinical impact of platinum-containing drugs with BRCAness is planned.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-09-07

Expression of miR-106b in circulating tumor cells is associated with EMT and prognosis in metastatic breast cancer patients

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## **Abstract**

**Background:** Circulating tumor cells (CTCs) display changes in epithelial-mesenchymal transition (EMT) markers and miRNAs regulate EMT in breast cancer cells. The association between EMT characteristics and miRNA expression in CTCs of metastatic breast cancer (MBC) patients and their clinical implications remain unknown.

**Methods:** CTC-specific miRNAs were screened based on comparison of the miRNA profile between CTC and primary tumor. RT-PCR was used to quantify the expression levels of EMT makers and miRNA candidates. We enrolled 219 MBC patients with CTCs  $\geq 5/7.5\text{mL}$  blood from 2 cohorts and CTCs were detected and enriched by CellSearch. Overall survival (OS) and radiological response were analyzed. CTCs were divided into epithelial- (E-CTCs) and mesenchymal-like CTC (M-CTCs) phenotypes based on a cut-off value derived from suspended breast cancer cells recovered from PBMCs.

**Results:** MiR-106b displayed upregulation in CTCs, with a higher level in M-CTCs than E-CTCs. Patients with E-CTCs showed better OS than those with M-CTCs (HR 1.77, 95% CI 1.14-2.78,  $P=0.012$ ). CTCs from chemo-resistant MBC patients exhibited higher miR-106b. CTC-specific miR-106b was negatively associated with therapy response and OS (HR 1.73, 95% CI 1.06-2.84,  $P=0.029$ ).

**Conclusions:** CTC-specific miR-106b was associated with EMT phenotypes of CTCs and may predict prognosis in MBC patients.

# 2018 San Antonio Breast Cancer Symposium ®

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High circulating levels of Periostin are associated with a poor survival in primary, non-metastatic breast cancer patients

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**Background:** Periostin, also known as osteoblast-specific factor OSF-2, functions as a ligand for integrins to support adhesion and migration of tumor cells which leads to increased cell survival, invasion, angiogenesis and metastasis in different cancer types including breast cancer (BC). Assuming that metastasis requires a dissemination of tumor cells, associated with worse outcome in BC, the aim of this study was to determine the expression of Periostin in blood samples of patients with primary, non-metastatic BC and to correlate the results with clinical parameters including the presence of disseminated tumor cells (DTCs) in the bone marrow (BM), survival and the risk of developing metastatic disease.

**Patients and Methods:** BM and blood sampling were performed before surgery in an adjuvant setting in 509 patients with first diagnosis of BC between Aug 2006 and Dec 2009. Two BM aspirates were analyzed for DTCs using density centrifugation followed by immunocytochemistry applying the pan-cytokeratin antibody A45-B/B3. Blood was collected from each patient and Periostin serum levels were measured by ELISA (Biomedica, Vienna, Austria).

**Results:** Periostin levels were detectable ( $504.8 \pm 178.7$  pmol/l) in all BC patients. There were no significant differences between serum Periostin levels when stratifying according to tumor stage, lymph node involvement or grading. Periostin levels were significantly increased in women above the age of 60 ( $468.6 \pm 166.6$  pmol/l vs.  $540.1 \pm 184.2$  pmol/l;  $p < 0.0001$ ) and significantly enhanced in postmenopausal compared to peri- or premenopausal women ( $p < 0.05$  and  $p < 0.001$ , respectively). No differences were observed between DTC-positive and DTC-negative patients. When separating patients according to high (top 50%) or low (low 50%) Periostin levels, patients with low Periostin levels had a significantly shorter BC specific survival (HR 0.61; 95%CI 0.39-0.96;  $p = 0.03$ ).

**Conclusion:** In summary, while Periostin levels were unchanged in patients with and without DTCs, high levels of Periostin were associated with a poorer BC specific survival. These results warrant further studies on the role of Periostin in BC.

# 2018 San Antonio Breast Cancer Symposium ®

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Analysis of serial circulating tumor cell count during neoadjuvant systemic therapy in breast cancer patients

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**Background:** We aimed to evaluate the clinical implication of circulating tumor cell (CTC) counts in correlation with prognosis and radiologic/pathologic response to therapy in locally advanced breast cancer patients undergoing preoperative systemic therapy.

**Methods:** From Feb 2014 to May 2017, 207 patients without distant metastasis were prospectively enrolled from AMC. CTC counts were analyzed before-during-after the therapy. CTC isolation was performed using a SMART BIOPSY™ SYSTEM Isolation kit (Cytogen, Inc., Seoul, Korea). Recurrence-free and overall survival was analyzed according to CTC counts.

**Result:** The mean follow-up period was 22.46 months and mean age was 46.48 years. One or more CTC was identified in 132 of 203 patients(65.0%) before NST, in 135 of 186 patients(72.0%) during NST and 103 of 171 patients(60.2%) after NST. Initial tumor burden at diagnosis -tumor size, lymph node metastasis- was not correlated with CTC positivity. Overall, CTC count ( $\geq 1$  CTC,  $\geq 2$  CTCs, and  $\geq 5$  CTCs) was not correlated with response to therapy. Using RECIST criteria, 86.5% (179/204) were responders (complete, partial response, CR/PR) and 12.1% (25/204) were non-responders (stable, progressive disease, SD/PD). 14.5% (30/207) showed a pathologic complete response (pCR), yet no association was found between CTC count/changes and radiologic/pathologic response to therapy. Also, CTC count was not correlated with prognosis among the whole population. However, HR+ tumors, CTC detection before NST was significantly associated with treatment response by RECIST criteria (responder vs. non-responder) ( $p=0.003$ ,  $p=0.017$  and  $p=0.023$ , respectively).

**Conclusions:** Our findings support limited value of CTC count for locally advanced breast cancers undergoing neoadjuvant systemic therapy.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P6-09-10

Association of dermal lymphatic involvement and survival in inflammatory breast cancer

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**Purpose:** The pathologic hallmark of inflammatory breast cancer (IBC) is the presence of tumor emboli within the papillary and reticular dermis of the skin, termed dermal lymphatic invasion (DLI). DLI can be confirmed with a skin-punch biopsy in approximately 75% of cases, but its presence is not required for the diagnosis of IBC because of variability of tumor emboli in affected areas. The impact of confirming DLI by skin biopsy on the clinical outcome of IBC is unknown. We hypothesize that the ability to confirm DLI by biopsy is associated with a higher tumor emboli load and consequently a poorer disease prognosis. Therefore, we examined whether documented DLI was associated with poorer overall survival (OS) in IBC.

**Methods:** Clinical characteristics were evaluated among 286 women presenting with IBC between 1999 and 2016 and enrolled in the IRB-approved IBC registry at Dana-Farber Cancer Institute. Kaplan-Meier curves were used to estimate overall survival and the log-rank test to examine survival differences based upon the presence of DLI. Multivariable Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals for associations of DLI and risk of death among women with IBC.

**Results:** A total of 102 deaths occurred in our study population over a median follow-up of 2.5 years (yr) (range 10 days to 16 yr). Compared to IBC without DLI, IBC with confirmed DLI was more likely to be associated with the presence of lymphovascular invasion in breast biopsies (73.1% vs. 37.7%; p-value <0.01) but less likely to be seen with edema of the skin (59.1% vs. 81.1%; p-value=0.02). Documented DLI was not related to the presence of *de novo* metastasis at presentation (11.5% vs. 28.7%; p-value=0.07). OS did not significantly differ among women with IBC based upon the presence of DLI (log-rank test p-value=0.68). In multivariable models, DLI was not independently associated with OS in inflammatory breast cancer, HR(95%CI) = 0.92(0.48-1.78).

**Conclusions:** Our findings suggest that IBC presenting with documented DLI on skin biopsy may vary with regard to clinical characteristics at diagnosis, including lymphovascular invasion within the breast, edema of the skin of the breast, and the presence of *de novo* metastases. These clinical distinctions suggest potential differences in biology of IBC according to the presence or absence of DLI, and the extent of tumor emboli. However, in this study, DLI was not found to be an independent prognostic factor in IBC with respect to OS. Due to the variability in the clinical features of IBC at presentation and inherent complexities in selecting skin biopsy sites, studies to investigate the accuracy of determining DLI based on punch biopsy are necessary to more comprehensively assess the impact of DLI on clinical outcomes in IBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-09-11

NFATC2 suppresses the metastatic cascade in breast cancer patients

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For the patients with breast cancer, the pathological status of axillary lymph nodes is one of the most important predictor of prognosis. In recent years, the application of sentinel lymph node biopsy has provided us with an opportunity for the further study on the mechanism of lymph node metastasis in breast cancer. Through detailed pathological examination of sentinel lymph nodes, we can easily find patients who are in the early stage of lymph node metastasis - that is, the tumor cells from the primary tumor, invading the lymph nodes and successfully colonized, but not yet break through the lymph node microenvironment defense to the subsequent lymph node. It is the most important stage during lymph node metastasis.

In the present study, we selected the pairing samples from breast cancer patients with early stage lymph node metastasis to carry out differential genomics research. A total of 182 genes with significant function and involved in significant signal transduction pathways were obtained. Bioinformatics method was used to screen out the mRNA, microRNA and transcription factor in the process of lymph node metastasis of breast cancer. We investigated the role of activated T cells c2 (NFATC2) in the core transcription factors as a target for further clinical and basic research.

The expression of NFATC2 was detected in the tissue microarray prepared from 200 patients with primary breast cancer. After the median follow-up period of 89.5 months, it showed that the prognosis of patients with NFATC2 overexpression was significantly better than that of the control group ( $p = 0.022$ ). We also measured the expression of NFATC2 in 50 pairs of breast cancer patients with lymph node metastasis. The results showed that the expression of NFATC2 in the primary tumor was significantly higher than that in the matched lymph node, suggesting that in patients with breast cancer, the down-regulate or deficiency of NFATC2 expression may be related with lymph node metastasis. We also attempted to add the expression of NFATC2 as a new parameter in the prediction model of lymph node metastasis based on preoperative clinical and pathological parameters. The area under the ROC curve obtained in the newly established model was 0.767, the expression state of NFATC2 had impact on the performance of the prediction model. Furthermore, when we removed the NFATC2 parameter, it could reduce the area under the ROC curve in the validation group, suggesting that the expression of NFATC2 in the primary tumor may be used as a predictor of the pathological state of axillary lymph nodes.

In vitro and in vivo experiments, we demonstrated that NFATC2 has a significant suppression effect on the proliferation, migration and invasion of breast cancer cells. The potential NFATC2-target genes were determined by RNAseq and ChIPseq. Nine differentially expressed genes were found to be regulated by NFATC2 in the Chip-seq analysis and bound to the promoter region of the gene, whereas GRAMD3 and SRGAP2 in the subsequent validation showed a positive correlation with the expression of NFATC2, suggesting that the two genes are likely to have a positive regulatory relationship with NFATC2.

This study clarifies that NFATC2 may represent a therapeutic target for early metastasis breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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Using multiphoton laser scanning microscopy to assess neoadjuvant therapy outcome in core needle biopsies: A novel methodology

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**Background:** Over-expression of Human Epidermal Growth Factor receptor-2 (HER2) in breast cancer is associated with an aggressive clinical course and poor prognosis. Targeting HER2 over-expression has been shown to be a remarkably effective therapeutic modality in the metastatic, adjuvant and neoadjuvant setting and the pathologic response to neoadjuvant treatment in HER2-positive breast cancer has been shown to be an excellent surrogate for a good outcome. The stromal tumor microenvironment is implicated in fostering tumor growth, facilitating cell migration and ultimately resulting in metastatic disease. Specifically, the collagenous extracellular matrix, which includes fibrillar collagen, has been suggested to play a role in the migration of malignant breast epithelial cells within the surrounding stroma. We have developed a novel methodology which uses an intrinsic optical signature to quantitatively evaluate fibrillar collagen (Burke et al. *BMC Cancer* 15 (2015): 929). Here, we evaluate the ability of this quantitative methodology to predict the pathologic response after neoadjuvant HER2-targeted treatment as assessed by the Residual Cancer Burden score/class (RCB). This quantitative evaluation in **pre-treatment** biopsies is then correlated with the pathologic response to treatment in the post-therapy resection. **Material and Methods:** Clinical pathologic variables for 29 cases of HER2-positive breast cancer that had undergone neoadjuvant chemotherapy plus HER2-targeted therapy were retrieved from the medical record database at URM, including the post-treatment RCB score and ER/PR/HER2 status. Second harmonic generation (SHG) is an intrinsic optical signal produced by fibrillar collagen. To quantify collagen microstructure in the pre-treatment core biopsy, we used SHG imaging to determine the average forward to backward-light scattering ratio (F/B). The F/B ratio is sensitive to structural properties of collagen fibers. **Results:** Logistic regression was used to assess the association between F/B and the binary response variable RCB class (0/1 or 2/3). A likelihood ratio test was used to calculate the p-value to test whether the regression coefficient for F/B was zero (i.e. no effect) in the tumor-stromal interface. The average F/B ratio at the leading edge of the tumor stratified by RCB class is shown in Table 1. When evaluated in the bulk of the tumor tissue, F/B was not correlated with RCB status; however, when evaluated at the leading edge of the tumor stromal interface, F/B was significantly correlated with RCB status (p=0.035).

Table 1: RCB class and average F/B

RCB class (n)	Average F/B $\pm$ SEM
0/1 (19)	16.95 $\pm$ 1.06
2/3 (10)	12.32 $\pm$ 1.84

**Conclusions:** We have previously shown that the measurement of F/B in the primary tumor after resection is an independent prognostic indicator of metastasis-free survival in breast cancer. Our results in the current study furthers these observations and suggests that the evaluations of the microstructure of collagen fibers by F/B measurement from the pre-treatment biopsy, specifically at the leading edge of the tumor-stroma interface, may be useful for predicting pathologic response to trastuzumab-based neoadjuvant therapy. Further studies in a larger patient cohort are warranted.



# 2018 San Antonio Breast Cancer Symposium ®

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Consumption of sugar-containing beverages and cancer risk: Results from the NutriNet-Santé prospective cohort

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## OBJECTIVE

To assess the associations between the consumption of sugar-containing and artificially sweetened beverages and cancer risk.

## DESIGN

Population based prospective cohort study.

## SETTING AND PARTICIPANTS

Overall, 101 257 participants aged  $\geq 18$ y (mean age:  $42.2 \pm 14.4$ y) from the French NutriNet-Santé cohort (2009-2017) were included. Consumptions of sugar-containing and artificially sweetened beverages were assessed using repeated 24h-dietary records, designed to register participants' usual consumption for 3300 different food and beverage items.

## MAIN OUTCOME MEASURES

Associations between beverage consumption and overall, breast, prostate and colorectal cancer risk were assessed by multivariable Cox Proportional Hazard models adjusted for known risk factors.

## RESULTS

A 100mL increase in the consumption of sugar-containing beverages was significantly associated with an increased risk of overall cancer (HR=1.08, 95% confidence interval 1.04 to 1.12,  $P < .0001$ ) and breast cancer (HR=1.11, 95% confidence interval 1.04 to 1.19,  $P < 0.002$ ). The consumption of artificially sweetened beverages was not associated with cancer risk. In sub-analyses, a 100 mL increase in the consumption of 100% fruit juice was significantly associated with an increased risk of overall cancers (HR=1.08, 95% confidence interval 1.02 to 1.15,  $P = 0.01$ ). These associations were strongly mediated by the sugar contained in these beverages. In contrast, weight gain during follow-up did not appear as a strong mediator. Besides, results were similar in overweight and non-overweight participants.

## CONCLUSIONS

In this large prospective study, a 100mL increase in the consumption of sugar-containing beverages in the diet was associated with an 8% significant increase in overall cancer risk and an 11% significant increase in breast cancer risk. 100% fruit juices were also associated with an 8% increased risk of overall cancers. Given the massive consumption of sugar-containing beverages in Western countries, these results suggest that they may represent a key modifiable risk factor for cancer prevention.

# 2018 San Antonio Breast Cancer Symposium ®

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Supplemental vitamin D intake amount needed to reduce breast cancer risk

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**Background:** Numerous studies have linked higher serum 25-hydroxyvitamin D [25(OH)D] concentrations to a lower risk of breast cancer. A recent study by McDonnell et al., which used data pooled from two randomized clinical trials (N=1129, N=2196) and a prospective cohort (N=1713), found that among women aged 55 years and older, those with 25(OH)D concentrations  $\geq 60$  ng/ml had an 80% lower risk of breast cancer than those with concentrations  $< 20$  ng/ml (HR=0.20, P=0.03), adjusting for breast cancer risk factors such as age, BMI, smoking status, and calcium supplement intake. Supplemental vitamin D intake is needed for most women today to achieve 60 ng/ml; however, the intake amount is unclear.

**Objective and Methods:** The objective of this analysis was to estimate the dose-response relationship between supplemental intake of vitamin D and serum 25(OH)D concentration using the pooled data from the McDonnell et al. study (N=5038) in order to provide guidance about dosing requirements to achieve 60 ng/ml. Women in the RCT cohorts participated in a four year, placebo-controlled, population-based trial of vitamin D and calcium supplementation in Nebraska. Vitamin D supplement intake was assessed by bottle weight for study provided supplements and by self-report for non-study vitamin D. Serum 25(OH)D concentrations were measured using radioimmunoassay. Women in the prospective cohort voluntarily participated in a longitudinal study and resided in 57 countries worldwide (91% in the United States or Canada). Supplemental vitamin D intake was assessed by self-report and serum 25(OH)D concentrations were determined by analysis of dried blood spot test kits using liquid chromatography-mass spectroscopy (LC-MS/MS). The relationship between supplemental vitamin D intake and 25(OH)D concentration was fitted to the following equation:  $Y = Y_0 + a(1 - e^{-bx}) + cX$ , where X is daily supplemental vitamin D intake and Y is serum 25(OH)D concentration. This equation includes terms for the zero supplement intake value, hepatic 25-hydroxylation saturation, and zero-order kinetics for 25-hydroxylase.

**Results:** On average, a supplemental dose of 6,400 IU/day of vitamin D produced a 25(OH)D concentration of 60 ng/ml in this population of mostly white women aged 55 years and older. The dose required to ensure that 90% of this population achieved a 25(OH)D concentration of at least 60 ng/ml was approximately 13,000 IU/day. Concentrations above 100 ng/ml were observed in  $< 0.2\%$  of those taking 6,400 IU/day or less and  $< 0.5\%$  of those taking 13,000 IU/day or less. There was a significant amount of inter-individual variability in the response to any given dose. Therefore, serum 25(OH)D testing is recommended to determine the specific intake amount an individual needs to attain a specific 25(OH)D concentration.

**Conclusion:** To reduce breast cancer risk, the dosing recommendation for the general population of women is 6,400 IU/day with personalized dosing adjustments based on serum 25(OH)D testing.

# 2018 San Antonio Breast Cancer Symposium ®

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Field testing of a point-of-care decision support tool for contralateral prophylactic mastectomy

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**Background:** The majority of women undergoing contralateral prophylactic mastectomy (CPM) overestimate their risk of developing a contralateral breast cancer and incorrectly believe the CPM will substantially improve their overall survival. Using the results of our published micro-simulation decision-analytic model (Davies et al. Breast Cancer Res, 2016), we created and tested a CPM decision support tool to provide patients and breast cancer surgeons with individualized estimates of contralateral breast cancer risk and overall mortality.

**Methods:** The CPM decision support tool had four entry parameters from the micro-simulation model (age, family history of breast cancer, estrogen receptor status, and stage), and a visual depiction of outcomes using icon arrays for chances of developing a contralateral breast cancer and overall mortality with and without CPM. A user-centered design strategy was used with input and iterative refinement from stakeholders, i.e., breast cancer surgeons, patient advocates and breast cancer survivors. The tool was field-tested at MD Anderson Cancer Center with 5 breast cancer surgeons each using the tool with 5 breast cancer patients considering CPM (25 total). Patients completed a knowledge survey immediately before and after viewing the tool and the Decisional Conflict Scale (DCS) after viewing the tool. Surgeons completed the System Usability Scale (SUS) and ratings of the acceptability of the tool.

**Results:** The mean age of patients was 58 years. All patients reported the tool was helpful in making a decision about CPM and would recommend it to others. Knowledge of breast cancer and key CPM facts increased from before to after using the tool (64% vs. 75%, respectively,  $P < 0.05$ ). The mean score on the DCS was 10.5 (standard deviation = 14.3) indicating patients were overall sure about the CPM choice. The majority (72%) of patients were unsure of their interest in CPM before viewing the tool. After viewing the tool, 13 (52%) of patients indicated they did not want CPM, 4 (16%) indicated they wanted CPM, and 8 (32%) remained unsure. Surgeons rated the tool as having a positive impact on the decision-making process and SUS scores were highly favorable (mean 93 on 0-100 scale, with 100 indicating highest usability).

**Conclusion:** The CPM decision tool had high overall patient satisfaction and improved knowledge about CPM without affecting decisional conflict. Decision support tools may be used to improve the quality of decision-making about CPM by providing surgeons and their patients with useful individualized information about CPM's impact on relevant clinical outcomes, which may lower the incidence of CPM.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-11-02

Reduced sedentary time intervention for breast cancer survivors: objectively-measured outcomes for activity and metabolism

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Physical activity (PA) promotion and sedentary behavior reduction among cancer survivors is a national priority and the number of PA-based behavioral interventions has expanded considerably in recent years. However, due in part to past limitations related to precise quantitative measurement of sedentary behaviors, there have been relatively few trials focused on reduction of sedentary time among cancer survivors. Additionally, many PA interventions rely on clinic-based coaching, which is both time-intensive and unrealistic for many clinics. The purpose of this study was to investigate the effects of a home-based 6-week reduced sedentary time intervention (RSTI) in breast cancer survivors who had completed primary treatment. Questionnaires, anthropometric measures, fasting blood glucose, lipid profiles and other cancer-relevant biomarkers, and an oral glucose tolerance test to assess dynamic insulin were completed at baseline and post-intervention. Participants wore ActivPALs (on right thigh) for 7 days at baseline and post-intervention. The mailed RSTI included tailored feedback on PA and sedentary habits and provision of personalized visual displays generated from baseline ActivPal data, with specific tips for how to reduce sedentarism through environmental modifications to home and work spaces (example: moving your printer from next to your desk to down the hall). Eligibility criteria included: females ages 20-80; not meeting national exercise guidelines (150 minutes per week); diagnosis of Stage I-III breast cancer treated >6 months and <5 years prior; overweight or obese (BMI >25); no history of diabetes. Sixteen enrolled and 13 completed the study. Average BMI at baseline was 31.9 and all participants' daily step counts were significantly below national recommendations (mean=6190 steps/d, SD: 2086 steps/d). Post-intervention changes from baseline were analyzed using non-parametric statistics. No statistically significant changes in daily bouts of sedentarism, energy expenditure, or total steps were detected, although participants' mean daily steps post-intervention were 6326/d (SD: 2788/d), still well below recommended guidelines. Leptin levels also showed a significant reduction pre/post ( $p<0.01$ ). Results indicate that similar home-based RSTIs are safe, acceptable to survivors, and feasible to implement by cancer center staff. Further research with larger samples is required to establish efficacy and effect sizes for the intervention. A larger intervention dose may be required to see clinically-meaningful changes in sedentarism, daily activity, and metabolism.

## 2018 San Antonio Breast Cancer Symposium ®

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isomiR-140-3p-regulated mevalonic acid pathway as a potential target for prevention of triple negative breast cancer

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**Background:** Prevention of triple negative breast cancer (TNBC) is hampered by lack of knowledge about the drivers of tumorigenesis. **Methods:** In order to identify molecular markers and their downstream networks that can potentially be targeted for TNBC prevention, we analyzed small RNA and RNA sequencing of a cell line model that represents early stages of TNBC development. We identified direct gene targets of an isomiRNA and using cell based and in vivo model systems we demonstrate the utility of targeting downstream pathways for prevention of TNBC. **Results:** These analyses showed that 5'isomiRNA of miR-140-3p (miR-140-3p-1) were deregulated in the normal-to-preneoplastic transition. We also identified novel direct gene targets of miR140-3p-1, HMG-CoA reductase (HMGCR) and HMG-CoA synthase 1(HMGCS1), key enzymes in the cholesterol biosynthesis pathway, and found that these too are deregulated in the normal to preneoplastic transition, resulting in activation of the cholesterol synthesis pathway. Upregulation in the cholesterol pathway creates a metabolic vulnerability that can be targeted with the statin class of inhibitors. Consistent with this hypothesis, we found direct targeting of miR-140-3p-1 and its downstream pathway by fluvastatin inhibits growth of these preneoplastic MCF10.AT1 cells. However, although, fluvastatin inhibited the growth of MCF10.AT1-derived xenografts, histological progression remained unchanged. The cholesterol pathway is highly regulated, and HMGCR enzymatic activity inhibition is known to trigger a feedback response leading to restoration of the pathway. Indeed, we found fluvastatin treatment induced HMGCR transcript levels in the explanted xenografts, with higher transcript induction directly correlated with the degree of histological progression of lesions, indicating an adaptive resistance mechanism that circumvents statin targeting of HMGCR. Therefore, we hypothesized that dual targeting of HMGCR and the feedback loops are necessary to overcome this adaptive resistance. To test this hypothesis, we generated MCF10AT1 cells resistant to fluvastatin. We then treated these resistance AT1 cells with an activator of AMP-activated protein kinase (AMPK), a brake in the cholesterol feedback pathway. AMPK activation by aspirin effectively abrogated the statin-induced upregulation of HMGCR and sensitized these resistant cells to fluvastatin. **Conclusions:** Our preclinical data suggests that the statin treatment induced homeostatic upregulation of the cholesterol pathway is a barrier to effective chemoprevention with statins. However, our results also suggest that this adaptive resistance mechanism can be abrogated by combined treatment with statin and AMPK activators such as aspirin. Clinical studies of this combined regimen should be considered in high risk patients.

# 2018 San Antonio Breast Cancer Symposium ®

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MRI breast cancer screening compared to mammography in women with a familial risk: A multicenter randomized controlled trial

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**Background:** Screening guidelines for women with a family history of breast cancer without a known causative gene mutation differ per country. No randomized controlled trial has been performed to assess the optimal screening strategy for these women.

**Methods:** In twelve centers, 1355 women aged 30–55 years with a cumulative lifetime risk of  $\geq 20\%$  without a *BRCA1/2* mutation were randomized into two arms. From January 2011 until December 2017, women in the MRI-arm received yearly MRI-screening, clinical breast examination (CBE), and mammography every other year; and in the Mx-arm yearly mammography and CBE. Outcomes were number and stage of detected breast cancers, sensitivity, specificity and positive predictive value, and stratified by screening round and by mammographic density.

**Results:** After on average 4.3 screening rounds per woman, in the MRI-arm (N=675) compared to the Mx-arm (N=680) more breast cancers were detected (41 versus 14,  $p < 0.001$ ), invasive cancers were smaller (median size 8 versus 17 mm,  $p = 0.006$ ) and less often node positive (20% versus 71.4%,  $p = 0.019$ ) (Table). In the MRI-arm, sensitivity was slightly higher (95.1% versus 92.9%,  $p = 1$ ), and specificity significantly lower (82% versus 90.1%,  $p < 0.001$ ), compared to the Mx-arm. After two rounds, specificity improved for both modalities (87.1% for MRI; 93.0% for Mx;  $p < 0.001$ ) and no  $\geq T2$  tumors or interval cancers occurred in the MRI-arm. All tumors  $\geq T2$  were in the two highest density categories. MRI detected more small invasive tumors than Mx across all density categories.

**Conclusions:** In real-life practice the MRI-arm detected more, relevantly smaller, and far more often node negative tumors, and also at low density in women with a familial risk for breast cancer.

**Table 1 Characteristics of participating women at baseline and of the detected breast cancers, according to study arm**

Participants	MRI-arm n=675	Mx-arm n=680	MRI-arm vs. Mx-arm p-value
Mean age yr $\pm$ SD	44.6 $\pm$ 6.2	44.7 $\pm$ 6.3	
Premenopausal	512 (76%)	505 (74%)	
Previous Mx $\leq$ 2 yr	536 (79 %)	542 (80%)	
Previous Mx > 2 years ago	23 ( 3%)	29 ( 4%)	
Previous MRI $\leq$ 2 years ago	62 ( 9%)	81 (12%)	
Previous MRI > 2 years ago	91 (14%)	89 (13%)	
BI-RADS density category*			
I (entirely fat)	88 (13%)	92 (14%)	
II (scattered densities)	248 (37%)	229 (34%)	
III (heterogeneously dense)	238 (35%)	243 (36%)	
IV (extremely dense)	98 (15%)	102 (15%)	

Mean age at cancer detection	49,6 ± 7.0	49,8 ± 4,7	0.74
No cancer – no. (%)	634 (94%)	666 (98%)	
Invasive breast cancers – no. (%)	25 (4%)	7 (1%)	<0.001 (noBC/inv BC/DCIS)
DCIS – no. (%)	16 (2%)	7 (1%)	
Median size of invasive cancers	8 mm	17 mm	0.006
T1a/b	15 (60%)	1 (14%)	
T1c	7 (28%)	4 (57%)	0.078 (T1a-b/T1c/≥ T2)
≥ T2	3 (12%)	2 (29%)	
Node pos	5 (20%)	5 (71%)	0.019 (N+/-)
Node negative	20 (80%)	2 (29%)	
DCIS grade 1	5 (31%)	2 (29%)	
DCIS grade 2	8 (50%)	4 (57%)	1 (dcis gr1,2,3)
DCIS grade 3	3 (19%)	1 (14%)	

**\*Determined by radiologists, according to the fourth ACR BI-RADS edition**

# 2018 San Antonio Breast Cancer Symposium ®

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Reduced incidence of breast cancer with testosterone implant therapy: A 10-year cohort study

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**Introduction:** There is evidence that androgens are breast protective and that testosterone (T) therapy treats many symptoms of hormone deficiency in both pre and postmenopausal patients. However, there is a lack of data on the effect of long-term T therapy on the incidence of breast cancer.

**Methods:** A ten-year prospective, IRB approved study (Dayton study) was designed to investigate the incidence of breast cancer (BCA) in women with symptoms of hormone/androgen deficiency who were treated with subcutaneous testosterone (T) implants or, T combined with an aromatase inhibitor, anastrozole, implants (T+A). Breast cancer events during 'active therapy' (within 120 days post implant, i.e, clinically effective/therapeutic T levels), and 'post therapy' (240 days and 1-year post implant) were reported as incidence per 100 000 person-years. Person-days for each participant were calculated from the date of first T pellet insertion up to the date of cancer registration, the date of death, a set number of days post last implant, or the set date of 31 March 2018, whichever came first. Person-years (p-y) were calculated by dividing (total) person-days by 365.25. Bootstrap sampling distributions were constructed to determine if there were important differences in breast cancer incidence rates between our results and the SEER data. Allowing for patient aging and different cancer rates over the period of the study, the range of expected values based on SEER data was calculated from the age composition of our study patients and the published grouped age breast cancer incidence rates for two time periods, SEER 2006-2011 and SEER 2011-2016.

**Results:** 1267 pre (23.2%) and post (76.8%) menopausal women, mean age  $52.1 \pm 8.6$  y, were enrolled in the study March 2008-2013 and were eligible for analysis. As of March 2018 there have been 12 cases of invasive breast cancer diagnosed within one year of last T or T+A pellet implant. The incidence of breast cancer at each specified time frame is listed in Table 1. For comparison, the calculated age matched SEER incidence rate is approximately 271/100 000 p-y.

## Incidence of BCA

Time frame	120 days	240 days	365 days
P-Y	6372	6666	6960
BCA (n)	7	11	12
N/100 000 p-y	110	165	172

Time frame is number of days post last pellet insertion.

Bootstrap results confirm a significant reduction in BCA incidence compared to age specific SEER data.

## Bootstrap results

Time frame	Dayton incidence	Dayton sd	Seer incidence	Seer sd	Ratio corrected	Ratio sd
120 d	110.26	41.72	270.73	3.10	0.41	0.15
240 d	165.33	50.31	270.46	3.01	0.61	0.19
365 d	172.65	49.45	270.33	2.93	0.64	0.18

Bootstrap estimates of Dayton and expected SEER incidence rates (per 100 000 P-Y), their standard deviations (sd), the ratio (R) of the Dayton incidence rate to the SEER and its sd for various time frames.



Conclusion: Long-term therapy with T or T+A subcutaneous implants, used to treat symptoms of hormone deficiency, reduced the incidence of breast cancer by 59% during active therapy ( $p < 0.001$ ), which continued up to one year following the last pellet implant, 36% reduction at 1-year ( $p < 0.001$ ).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-13-03

A phase III, randomized, multi-centre, double-blind, placebo-controlled clinical trial of F-627 in women with breast cancer receiving myelotoxic chemotherapy

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**Purpose:** Neutropenia is a common side effect of chemotherapy. Chemotherapy-induced neutropenia increases a patient's risk of infection and disrupts cancer treatment. F-627, a rhG-CSF-FC dimer, is a once-per-cycle therapy for the preventive management of neutropenia. The objective of this phase 3 multinational study was to evaluate the efficacy and safety of F-627 given as a single fixed dose pre-filled syringe.

**Patients and Methods:** This trial was conducted at 16 study centers in the United States, Ukraine, Russia, and Hungary. A total of 122 women with stage II-IV breast cancer undergoing 4 cycles of myelotoxic TA chemotherapy treatment (docetaxel + doxorubicin) were randomized. Among them, 83 subjects received F-627, 39 subjects received placebo in cycle 1, and all subjects received F 627 in cycles 2, 3, and 4. For efficacy and patient safety, absolute neutrophil count (ANC) post-chemotherapy was closely tracked; electrocardiogram and other laboratory values were also monitored. All AEs were noted and followed to resolution or stabilization. Overall, 118 subjects completed the study and 4 subjects discontinued prematurely due to TEAE including 1 (2.6%) subject from placebo arm and 3 subjects (3.6%) from F-627 arm. All 122 subjects were analyzed for efficacy and safety.

**Results:** F-627 administration significantly reduced the duration of Grade 4 (severe) neutropenia in chemotherapy cycle 1 ( $P < 0.0001$ ), the mean treatment difference was 2.8 days (1.1 days vs 3.9 days). F-627 administration also resulted in lower incidence rate and shorter duration of Grade 4, Grade 3 and Grade 2 neutropenia. Importantly, F-627 treatment significantly reduced the incidence of febrile neutropenia (FN) ( $P < 0.0016$ ). The incidence of FN was 4.8% (F-627) vs 28.2% and 25.6% (placebo) across all cycles and cycle 1 respectively. Subjects from F-627 arm also had a lower rate of antibiotic medication and pain medication use. In this study, F-627 was shown to be safe and well tolerated with no deaths, no injection site reactions and less gastrointestinal AEs (diarrhea, vomiting, stomatitis and gastritis). In cycle 1, the five most common F-627 TEAEs (incidence rate  $> 10\%$  in the F-627 arm and  $\geq 2\%$  higher than placebo arm) were: leukopenia, anemia, thrombocytopenia, nausea and alopecia. Over all cycles, there were 17 SAEs from 15 subjects of which 14 were FN.

**Conclusion:** A single subcutaneous injection of F-627 provided effective neutrophil support to breast cancer patients undergoing high dose chemotherapy, significantly reducing the duration and incidence of severe neutropenia and febrile neutropenia while maintaining a sound safety profile.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-13-04

Estrogen-alone based hormone replacement therapy (HRT) reduces breast cancer (BrCa) incidence and mortality whereas estrogen plus progestin Provera based HRT increases both BrCa incidence and BrCa mortality: A comparative analysis of Women's Health Initiative trials

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**OBJECTIVE:** To quantitate breast cancer incidence (BrCa-I) and mortality (BrCa-M) outcome differences between the two Women's Health Initiative (WHI) HRT trials,<sup>1,2</sup> the ratio of hazards was calculated for estrogen-alone based hormone replacement therapy (E-HRT) vs. placebo (P), and E + progestin Provera (ProgProv) combination HRT vs. P trials.

**METHODS:** Hazard ratios (HR) of BrCa-I and BrCa-M and 95% confidence intervals (CI) were obtained from both WHI HRT trials. Subsequently, to compare BrCa outcomes between E-HRT vs. E + ProgProv, the ratios of HRs between the trials (HR1/HR2) were estimated separately for i. BrCa-I all women, ii. BrCa-I low Gail score (Gail score <1.75\*), and iii. BrCa-M. The 95% CI was derived through logarithmic transformation of the 95% CI originally reported.

## RESULTS:

Outcome Comparison, the two WHI HRT randomized trials. Ratio of Hazards, BrCa Incidence and BrCa mortality

	E-HRT vs. P, <b>HR1</b> (95% CI)	E-HRT + ProgProv vs. P, <b>HR2</b> (95% CI)	<b>HR1/HR2</b> (95% CI)	<b>p</b>
BrCa-I All Woman <sup>1</sup>	0.77 (0.62-0.95)	1.25 (1.07-1.46)	0.62 (0.47-0.80)	0.0004
BrCa-I Low Gail Score* (Gail score <1.75) <sup>1</sup>	0.65 (0.50-0.86)	1.24 (1.01-1.51)	0.53 (0.38-0.74)	0.0002
BrCa-M <sup>2</sup>	0.55 (0.33-0.92)	1.44 (0.97-2.15)	0.38 (0.20-0.75)	0.004

\*Gail score <1.75; HRs calculated from Reference 1, Figure 3

**CONCLUSIONS:** Our calculations show that the different outcomes between the two WHI HRT trials, estimated as ratio of hazards, are highly significant on statistical basis, both for BrCa incidence and for BrCa mortality. These findings highlight the potential carcinogenic impact of ProgProv and the major public health benefits of HRT based on E alone.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-13-05

Association between the *UGT2B17* gene deletion, exemestane metabolites and vasomotor QOL in women participating on the MAP3 prevention trial

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**Background:** The aromatase inhibitor Exemestane (EXE) reduces the risk of breast cancer in postmenopausal women. However, participants have varied responses to EXE treatment in terms of efficacy and toxicity, possibly due to differences in EXE metabolism. One of the main elimination pathways for EXE is through glucuronidation by *UGT2B17*. **Aims:** This project examined the relationship between the *UGT2B17* gene deletion, EXE metabolites and menopause-related quality of life (QOL) in postmenopausal women. **Hypothesis:** Glucuronidation of the main EXE metabolite, 17-dihydroexemestane (17-DHE), is reduced in women with the *UGT2B17* double gene deletion, leading to increased circulating 17-DHE and potential toxicity. **Methods:** This study included 3576 women nested within the CCTG MAP.3 trial, who were allocated to EXE or placebo treatment groups. Genotyping analysis was conducted with baseline blood cell DNA using real-time PCR and allelic discrimination. Women who were homozygous null were considered "exposed". In addition to EXE, EXE metabolites including 17-DHE and glucuronidated 17-DHE (17 DHE-Gluc) were analyzed from serum by UPLC/MS. Ratios of the main metabolites (17-DHE/EXE) and glucuronidated metabolites (17-DHE-Gluc/17-DHE) were standardized, using an autoscaling method. Metabolite levels that were below the detection limit were replaced by "half the detection limit for that metabolite". Women had the outcome if they experienced a clinically meaningful ( $\geq 10\%$ ) worsening in vasomotor QOL from baseline within the first year. Modified Poisson regression models were used to calculate the relative risks for both the (1) *UGT2B17* gene deletion and (2) metabolite ratios and vasomotor QOL. **Results:** Ten percent of participants exhibited the homozygous *UGT2B17* deletion genotype. There was no significant relationship between the *UGT2B17* deletion polymorphism and worsened vasomotor QOL (RR= 1.04, 95% CI: 0.93, 1.17), adjusted for age, race and treatment. Among women with no vasomotor symptoms at baseline but extremely bothersome symptoms at follow-up (incident vasomotor symptoms), there was a suggestive but non-significant protective effect of the *UGT2B17* deletion (RR=0.61, 95% CI: 0.32-1.19). This effect was more extreme in the placebo arm (RR=0.20) than in the EXE arm (RR=0.78; p-interaction=0.17). Among women on EXE, levels of EXE and 17-DHE were not different between *UGT2B17* genotypes, but levels of 17-DHE-Gluc were significantly lower for the *UGT2B17* deletion genotype (p<0.0001). An increasing ratio of 17-DHE-Gluc/17-DHE [per standard deviation (SD) increase] had a borderline protective effect against worsened vasomotor QOL (RR=0.94, p=0.049), adjusted for age and race. In contrast, an increasing ratio of 17-DHE /EXE (per SD increase) was associated with a small but significant increased risk of worsened vasomotor QOL (RR=1.02, p=0.01). The effect observed for the 17-DHE/EXE ratio was stronger for very bothersome incident vasomotor symptoms at follow-up, but this did not reach statistical significance [17-DHE/EXE (per SD increase): RR=1.36, p=0.12]. **Conclusion:** EXE metabolite levels could potentially be used as a biomarker for extreme vasomotor QOL changes in breast cancer chemoprevention settings using EXE.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-13-06

Estrogen-based hormone replacement [HRT] therapy is substantially more effective than tamoxifen in reducing breast cancer mortality and breast cancer case fatality ratio: Emergence of a new paradigm

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**OBJECTIVE:** To compare, in the setting of breast cancer (BrCa) prevention, the impact of estrogen-based hormone replacement therapy (E-HRT) vs. tamoxifen (TAM) on breast cancer mortality (BrCa-M) and breast cancer case fatality ratio (BrCa-CFR), by analyzing data from the Women's Health Initiative Trial 2 (WHI HRT Trial 2, E-HRT vs. placebo [P])<sup>1</sup> and the International Breast Cancer Intervention Study 1 (IBIS-1), TAM vs. P.<sup>2</sup>

**METHODS:** Hazard ratios (HR) and confidence intervals (CI) for BrCa incidence and mortality were extracted from the original WHI HRT Trial 2 and IBIS-1 trials.<sup>1,2</sup> BrCa-CFRs were estimated by dividing the mortality HR by the incidence HR. Subsequently, to compare E-HRT vs. TAM outcomes, the ratios of HRs (HR1/HR2) between the two trials were estimated separately for BrCa-M and BrCa-CFR. The 95% CI was derived through logarithmic transformation of the 95% CI originally reported.

## RESULTS:

Mortality and Case Fatality Outcomes: Impact of E-HRT versus TAM, expressed as ratio of HRs

	E-HRT vs. Placebo, <b>HR1</b>	TAM vs. Placebo, <b>HR2</b>	<b>HR1/HR2</b>	<b>p</b>
Mortality	0.55 (0.33-0.92)	1.19 (0.68-2.10)	0.46 (0.22-0.99)	0.046
Case Fatality	0.70 (0.40-1.20)	1.68 (0.93-3.01)	0.42 (0.18-0.94)	0.040

**CONCLUSIONS:** While acknowledging between-trial comparisons including eligibility differences, E-HRT yields significant reductions in BrCa mortality and case fatality as compared with TAM (54% and 58% respectively). These unexpected breast cancer mortality reductions represent major public health gains, additional to the already known superiority of E-HRT over TAM in terms of skeletal fracture rates and Alzheimer's dementia mortality reduction, and, in women entering menopause, also of cardiac and all-cause mortality reductions.

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## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-14-01

Impact of educational workshops on patient-provider communication: Results from the *Frankly Speaking about Cancer: Metastatic Breast Cancer* educational workshops

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Patient education about metastatic breast cancer can help patients and caregivers in interactions with their health care team and provide tools to deal with the psychosocial effects of the disease. This analysis explores participants' experiences related to the gains from Cancer Support Community's national evidence-based educational program, *Frankly Speaking about Cancer: Metastatic Breast Cancer*. This comprehensive psychosocial education program, created for people diagnosed with metastatic breast cancer and their families, provides information about current treatments, side-effect management, and social and emotional challenges of an advanced breast cancer diagnosis.

Participants from 43 workshops across the country between 2014 and 2017 completed program evaluation surveys on factors including: pre- and post-workshop knowledge and intentions for patient-provider communication post-workshop. In total, 427 individuals attending in-person Frankly Speaking about Cancer: Metastatic Breast Cancer workshops nationwide assessed their experiences and learnings during the educational workshops. Survey questions focused on how participants currently met their informational and assistance needs in regard to metastatic breast cancer and whether the workshop was associated with positive gains. Descriptive analyses and pre-and post-workshop comparisons were conducted to assess workshop outcomes.

71% of participants were cancer patients/survivors; the remainder served in the caregiving capacity and included spouses/partners (11%), family members (10%), and friends (8%). The average age of participants was 47 years old (s.d.= 24 years). Among patients/survivors, 70% received the diagnosis within the last two years; and 65% reported being highly involved in their treatment decisions. The most common treatment side effects included anxiety (10%), pain (10%), depression (10%) and fatigue (15%). The majority of respondents (72% patients/survivors and 81% caregivers) reported experiencing emotional distress due to their/their loved one's cancer. Most workshop participants (83%) reported gaining a high or very high level of knowledge about metastatic breast cancer, which was a significant increase compared with pre-workshop levels ( $\chi^2= 13.2$ ,  $p < .05$ ).

Caregivers were equally as likely as patients/survivors to report that as a result of their participation, they gained knowledge about metastatic breast cancer treatment options, confidence to participate in treatment decision-making with their health care team and to ask questions about side effects of metastatic breast cancer and its treatment. Finally, 73% of cancer patients/survivors and 78% of caregivers reported that as a result of the workshops, they felt better prepared to emotionally cope with their metastatic breast cancer experience.

These findings indicate that educational workshops can play a role in enhancing patients' self-perceived knowledge about metastatic breast cancer and empowering patients and caregivers to become active participants in their treatment decisions. These enhancements can, in turn, support caregivers in their interactions with their health care team and provide tools to manage the psychosocial effects of the disease.

# 2018 San Antonio Breast Cancer Symposium ®

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Facebook Live social media programming: engaging and reaching young breast cancer survivors

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**Background:** Young Survival Coalition (YSC) is a nonprofit organization that provides resources, connections and outreach to young women diagnosed with breast cancer. Educating constituents is a priority to ensure that they receive information they need in a manner that is timely, engaging and convenient. Young women with breast cancer are in a variety of different life stages and have competing demands for their time. Some are in treatment or recuperating from surgery; others are working or pursuing higher education; and some are raising children. It is not feasible to reach all of them at the same time in the same manner.

**Methods:** YSC evaluated various modes of communicating and providing information to its constituents. Convenience to constituents, ease of use for YSC staff and affordability were key considerations. Ultimately, YSC decided to use Facebook Live as its platform via BeLive TV, using a social media strategy to advertise the programming. After the live event, the recorded program is housed on YSC's YouTube channel as enduring educational material.

**Results:** YSC has held 13 Facebook Live events since the beginning of 2018 on a variety of topics ranging from recent legislation to YSC programs to wellness. The data from these "live" events show that although the number of live viewers is not high, the amount of engagement from these posts, as well as the number of video views after the live event are significant. Total video views from January 1st to June 25, 2018 are 17,000. See Table 1.

Table 1: Event Views and Engagement

Program	Peak Live Viewers	Post Engagements	Video Views
Healthy Food Choices	13	59	1252
Career	11	112	1159
Living Fully after Breast Cancer	27	260	4817
YSC Summit FAQs	9	65	683
Meditation Session	8	49	925
Chat with Dietitian	4	23	475
Employment Rights	14	58	836
Resilience	13	58	993
YSC Symposium FAQs	7	95	724
Genetics	17	118	1506
Finances	11	239	1542
Estate Planning	9	46	769
Right to Try Legislation	17	84	1412

In addition, the programs were efficient for staff to plan, coordinate and manage at reasonable cost.

**Conclusion:** Facebook Live is an easy-to-use method of programming that reaches a substantial number of YSC constituents in a manner that is engaging and convenient. Data from these programs provide evidence regarding the viability of this technology as a means of providing extensive education and support.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-14-03

Metastatic breast cancer alliance's patient education and access to trials: Perceptions and actions

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## Background

Those living with metastatic breast cancer (MBC) have distinct and shifting concerns in regard to education and decision making in considering clinical trials as a treatment option. Clinical trials designs, are becoming increasingly complex, and many patients have concerns for biomarker requirements

### Aims/Research Questions

- What is the status of MBCA advocacy members' and partners' digital information, education and access to metastatic breast cancer trials?
- What plans do MBCA members/partners have for the next 6-18 months to educate and inform their constituents for the 2018 rollout of BreastCancerTrials.org's (BCT)Metastatic Trial Search (MTS) and Metastatic Trial Talk (MTT) and other trial matching systems?
- What are the top 5 barriers regarding trial enrollment?
- What are best practices for MBC trial education?

## Research Methodology and Design

A comprehensive analysis was conducted comprising both secondary and primary research to inform these specific aims. Secondary research was conducted using previous capture of MBCA online digital resources and strengthened to include additional research on MBCA members and partners online resources including pages specifically devoted to clinical trials and metastatic clinical trials.

Mixed methods approaches include:

- 1) An assessment of MBCA members' and partners' digital media presence regarding MBC trials using a standardized form and rating system, and an analysis of MBCA members 2017 use of MTS using BCT secondary data;
- 2) Structured, recorded interviews with selected MBCA members/ partners, sharing the results of the assessments and querying them regarding their future plans and perceived barriers; and
- 3) Mixed methods analyses of the interview recordings using DeDoose to assess and articulate key trends and perceptions.

## Statistical Methods

Simple frequency percentages and means were used in the assessment rankings of the MBCA members. DeDoose was used to provide mixed method analyses of the MBCA member and partner interviews.

### Results

Analysis of the MBCA members and partners websites and digital media showed that, increasingly, both groups use the full variety of digital media to educate their constituents regarding MBC clinical trials. The 13 MBCA advocacy members providing online access to BreastCancerTrials' MTS in 2017 provided 97% of the traffic to the MTS trial matching service. System types accessing the MTS widget were 57.3% desktops, 28.6% mobile devices and 14% tablets. Table 1 shows assessment totals of 5 categories of the 13 MBCA advocacy group members' websites using MTS as compared to the 19 MBCA members not using the widget. Table 2 shows the 2017 usage of BCT's MTS with 97% of the page views coming from MBCA members.

**Table 1. Assessment Ratings of MBCA Advocacy Partners on Metastatic Trial Education/Access**

	Awareness of Trials (e.g. explains trial Phases)	Knowledge Sharing	Interest in Metastatic Breast Cancer	Action Potential for clinical trial access or enrollment	Total (0-100)
13 MBCA Advocate Members with MTS widget	22.15	23.00	24.31	24.15	93.62
	13.21	13.95	13.53	11.95	52.16



19 MBCA Members without MTS widget					
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**Table 2. 2017 Use Of Metastatic Trial Search**

<b>BCTâ– s Metastatic Trial Search (MTS) Results</b>	<b>2017</b>
Annual Page views	33,360
Unique Sessions	14,295
Show Trials	8,100
Show Trials by month	675
Average Time on Site (minutes)	3:34
Total Engagement Events	5,006

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-14-04

Improving breast health literacy through an innovative breast cancer awareness campaign using "Know Your Lemons" materials in Malaysia

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**Background:** Breast Cancer (BC) is the most common cancer in Malaysia, which has the worst survival rate in Asia Pacific. Main drivers identified in prior research include women presenting with advanced or late-stage disease, poor adherence to treatment and socio-cultural barriers. Factors accounting for late presentations include lack of awareness and information about basic symptoms of cancer and poor access to early detection and treatment. Breast cancer awareness campaigns have been done but few evaluations conducted. The "Show You Care, Be Aware" campaign was held on the University of Malaya campus from October 8-20, 2017 using "Know Your Lemons" education materials in awareness booths, public talks and forums. KYL visual materials in Malay and English language contain information on the normal breast, 12 BC symptoms, breast self-exam and screening and diagnostic pathways. Evaluation was conducted.

**Aim:** Evaluate improvement in breast health-recognizing symptoms and diagnostic care pathways of breast cancer-after implementing the "Know Your Lemons"(KYL) campaign.

**Methods:** Quasi-experimental research (n=679). KYL materials were forward and backward translated by 2 native bilingual individuals for accurate context and content and fitting the KYL leaflet design; self-designed questionnaire assessed effectiveness of the educational material; educational intervention sessions displayed KYL materials (leaflets, posters, banners) in awareness booths in Faculty of Arts, Science, Medicine, Islamic Studies and University Malaya Medical Centre; discussion on leaflets with Q&A and practical demonstrations of breast self-examination on a dummy were done in booths; questionnaire was administered via face to face interview; data analyzed via SPSS20; descriptive and Wilcoxon matched paired signed rank test was performed to analyze patients pre- and post knowledge at a significance level of <0.05.

**Results:** 72% (n=492) of participants were Malay, 19%(n=119) Chinese and 10%(n=68) Indians and others. Majority of participants were female (94.2%), age 30 or below (61%), single (62.9%), had college or university education (85.7%), some 10% had primary school education. Majority of participants (96.2%) stated language used in the "KYL" poster or leaflets was clear and understandable; 95.3% thought materials were attractive and drew attention; and 89.2% thought materials were acceptable in Malaysian culture. Materials improved knowledge on the process of detecting BC (96.5%). 92.8% participants either agreed or strongly agreed, after viewing the 12 BC symptoms in KYL materials, they felt more confident recognizing symptoms of BC. There was an increase in self-reported knowledge of BC; mean scores before and after exposure to KYL materials were 2.83 versus 4.30 respectively (p <0.001).

**Conclusions:** Health education using KYL materials increases breast cancer awareness and the visuals are acceptable amongst an urban and highly educated community in Malaysia. Developing and implementing a BC awareness program based on KYL materials can enhance awareness and confidence in the Malaysian public towards early detection of breast cancer. Future research in lower educated and rural communities in Malaysia is warranted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-14-05

Co-survivors: A continuing need for education and support

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**Background:** Young Survival Coalition (YSC) is a nonprofit organization that provides resources, connections and outreach to young women diagnosed with breast cancer. Since 2016, YSC has increased its information and support for the family, friends, adult children (18 and older) and spouses/partners of these young women, collectively called “co-survivors.” In 2016, YSC conducted a survey of its co-survivors (CS) to better understand their information and support needs. Previously reported results influenced the creation of a private Facebook group for CS and additional workshops and events at the YSC National Summit. The Summit is an annual conference for young women affected by breast cancer, their loved ones and healthcare providers. **Methods:** In order to evaluate its recent efforts directed to CS and learn more about them, YSC conducted an online follow-up survey that opened in early 2018.

**Results:** Sixty responses were received, of which 34% were spouses, 23% friends, 25% parents, 15% siblings and 3% adult children. These figures represent a higher proportion of respondents who were parents and friends compared to the 2016 survey. Survey respondents were mainly female (65%, compared to 46% female in 2016) and white (86%, compared to 75% in 2016). Most respondents were a CS of a young woman with early stage disease (61%), not in active treatment (43%) and within two to five years after diagnosis (56%). Survey results showed that CS felt isolated frequently (29%) or at times (35%), felt guilty asking for help very much (26%) or to some extent (51%), felt anxious or depressed as a result of being a co-survivor very much (40%) or to some extent (48%) and had sleep difficulties to some extent (52%) or very much (23%). Most respondents were aware of CS programming offered by YSC (44% to some extent; 37.5% very much) although 43% had not participated in any YSC offerings. Of those who had participated, 36% attended the Summit, 30% in the private Facebook group, and 6% in peer-to-peer matching. Thirty-four percent receive YSC's “Just for You” CS newsletter/blog, a new initiative started after the first CS survey, although 19% didn't know it existed. To date, 2208 CS receive this newsletter. Fifty-seven percent would like a CS guidebook and, in commentary, a few suggested a half-day retreat at the Summit.

Similar to the 2016 survey, over two-thirds (78%) preferred to receive important information via an email newsletter with Facebook (54%) as the next highest response. Topics of greatest interest included self-care (62%), managing day-to-day details (47%) and talking with kids (36%).

**Conclusion:** CS of young women diagnosed with breast cancer are a population interested in resources and support. Initiatives started after the 2016 YSC survey are being utilized with continued room for growth. YSC intends to use the results of its research to refine and expand its programmatic offerings to this varied population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-14-06

A patient-centered approach to education: Evaluating and improving radiation treatment education in breast cancer patients

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## Introduction

Breast cancer patients rapidly receive large amounts of information from multiple practitioners during treatment. It is therefore essential that education be presented in an appropriate manner to promote satisfaction and retention of important facts regarding treatment. Educational materials at the Memorial Radiation Oncology Department have not been evaluated by patients, and it is unclear how effectively and efficiently they express information. We hypothesized that satisfaction was already very good, but patients lacked knowledge of key aspects of their radiation treatment. This quality improvement project aimed to achieve a patient comprehension of key treatment-related concepts of 75% by March 2018 via evaluating and improving educational materials.

## Methods

A survey was designed to evaluate patient satisfaction with and comprehension of educational materials. The survey inquired about satisfaction with several specific aspects of their education and tested understanding of concepts that impact treatment. The survey was administered to all breast cancer patients over a three-month period. Those results were used for a PDSA cycle to implement improvements. These improvements included eliminating redundant resources, reemphasizing essential concepts throughout treatment, and consolidating important concepts from the materials into a more accessible one-page summary sheet. Following those changes, the surveys was adapted and administered to every breast cancer patient over another three-month period, which were analyzed to evaluate the effectiveness of and satisfaction with the interventions. Statistical comparisons were performed using Student's t-test and Fisher's exact test.

## Results

Following the interventions, overall satisfaction improved from 97% to 100% ( $p=1.0$ ). However, 27% and 18% of patients still did not remember receiving an informational letter and physician handout, respectively. On knowledge based questions, average percent correct improved from 52% to 78% ( $p<0.001$ ). However, there were areas where comprehension declined following the interventions. Select results of individual survey items are shown below in Table 1:

## Outcomes of Educational Interventions

Category	Initial Result (%) (n=31)	Final Result (%) (n=26)	Change (%)	p-value
Remembered getting pre-treatment letter	74	65	-9	0.566
Remembered getting post-consult physician handout	94	88	-6	0.651
Watched emailed informational video	14	N/A	N/A	N/A
Correctly reported recurrence risk reduction associated with radiation treatment	30	16	-14	0.341
Correctly identified timing of peak skin reaction	47	88	+41	0.002
Correctly identified contraindication of antioxidant use during radiation treatment	29	64	+35	0.015

**Table 1:**

## Conclusions

A patient-centered approach to developing and delivering patient education can lead to better satisfaction and patient

comprehension, thereby improving compliance and encouraging patient engagement, which has a positive impact on outcomes. Although some education remained unmemorable or improperly emphasized following the interventions, overall trends in comprehension validate tailoring educational materials to patient feedback. In the future, the PDSA cycles will be continued with a focus on addressing persistent educational deficits and identifying further avenues to improve patient retention.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-14-07

Impact of multidisciplinary team meetings on the management of patients with breast cancer at Cabrini Hospitals in Melbourne, Australia

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**Introduction:** Multidisciplinary meetings (MDM) allow patient management plans to be discussed in the context of multidisciplinary, expert knowledge and experience. MDMs also provide opportunities for clinical trial recruitment. However, the eventual management plan remains at the discretion of the treating specialist.

We aim to determine 1) The level of impact of MDM on pre-MDM management plans in patients with breast cancer and 2) The rate of implementation of the breast cancer MDM management plans into clinical practice.

## **Methods:**

Patients with a new diagnosis of unilateral, invasive breast cancer presented at the weekly breast cancer MDMs were prospectively and anonymously enrolled into the study. Patients with bilateral breast cancer and/or a history of breast cancer receiving active treatment are excluded. The impact of breast cancer MDMs on their management plan was evaluated, by comparing pre-MDM management plans to MDM recommendations. Recruitment continued until 50 cases are available for final analysis.

Prior to each MDM, study investigators will collect the required information from patients' medical history and histopathology reports to determine a pre-MDM management plan. The MDM recommendations will then be recorded. Any discordance between the pre-MDM management plan and the MDM recommendations will be documented and then scored according to a pre-determined level of impact: low, high or none.

## **Results:**

- 33/50 (66%) cases discussed at MDM showed an impact, with 19/50 (38%) recorded high impact scores and 14/50 (28%) with low impact scores.
- 15/50 (30%) patients had a change in plan after MDM discussion with further investigations recommended, the highest among all modalities (7 with low impact scores, 8 with high impact scores)
- The highest proportion of high impact scores were recorded with recommendations to refer for clinical trial participation (7/8 with high impact scores; 1/8 with low impact score)
- The highest proportion of low impact scores were recorded when recommendations for chemotherapy are made (12/13 with low impact scores; 1 high)
- 11/17(65%) patients with no impact scores recorded were low grade, ER+, node negative breast cancers with recommendations for Endocrine therapy and radiotherapy or Endocrine therapy alone only.
- Three month follow up plans were available for 49/50 patients records, from which 8/49 had a change in management plan. Reasons for change in management plan include the incorporating results of further investigations, consideration of other medical comorbidities, and patient/physician preference.

Number of impact score episodes recorded for each management modality

	High impact episodes	Low impact episodes	No impact episodes
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Surgery	2	3	45
Endocrine therapy	3	0	47
Chemotherapy	1	12	37
Targeted therapy	1	2	47
Radiotherapy	3	9	38
Clinical trial	7	1	42
Further investigations	8	7	35
Further referral	6	1	43

#### MDM plan implementation rate

Implementation	Rate
YES	41/49 (83.7%)
NO - physician/patient preference	5/49 (10.2%)
NO - medical comorbidities	1/49 (2.0%)
NO - additional investigation results	1/49 (2.0%)
NO - other	1/49 (2.0%)

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-14-08

Effects of online resource to support laypersons' understanding of media reports on breast cancer research

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**Rationale:** Breast cancer is the second leading cause of death among women ages 20 to 39. Approximately 7% of women with breast cancer are diagnosed before age 40. Women age 45 or younger with breast cancer or who are at high risk for breast cancer have distinct health risks and different needs from their older counterparts. Breast cancer risk, etiology, treatment, outcomes, and related survivorship and quality of life concerns often vary between younger and older women. Diagnosis at a young age is associated with higher risk of recurrence, second malignancy, mortality, morbidity, and quality of life issues. Young women with or at high risk for breast cancer need clearly presented information based on sound evidence to help them make informed decisions about their specific health needs. To help women better understand media coverage about new research, Facing Our Risk of Cancer Empowered (FORCE) developed XRAYs (eXamining Relevance of Articles to Young Survivors). XRAYs is an online resource that provides brief articles summarizing recent research relevant to young women with or at risk for breast cancer. XRAYs articles rate the quality and relevance of scientific research and the quality of media reporting on that research.

**Objectives:** The objectives of the current project were to:

- 1) conduct an initial test of XRAYs's effectiveness in improving users' knowledge about information covered in media reports
- 2) assess the degree to which XRAYs facilitates awareness of recent research findings
- 3) Obtain feedback regarding XRAYs' utility and appeal
- 4) Use results to inform XRAYs development

**Methods and Results:** An independent evaluator randomly assigned 21 participants to a treatment (read media article plus XRAYs review) or control (read media article only) condition. Each respondent completed multiple choice pre- and post-intervention tests about information contained in one of three media reports (Two respondents took their written surveys with them. Statistical analyses were conducted on data from the remaining respondents). Results demonstrate that both groups improved between pre- and post-test. The treatment group increased knowledge significantly more than the control group.

Table 1. Results of within group comparisons

Group	N	Pre-test Mean, Percentage Correct Responses (s.d.)	Post-test Mean, Percentage Correct Responses (s.d.)	t-score (d.f.)	P
Comparison Group	8	57.5 (12.82)	72.5 (23.75)	-2.05 (7)	n.s.
XRAYs	11	52.73 (20.54)	87.27 (16.18)	-7.29 (10)	<.001

Table 2. Results of between group comparison

Group	N	Mean Change Score (s.d.)	t-score (d.f.)	P
Comparison Group	8	15.00 (20.70)	-2.34 (18)	<.05
XRAYs	11	34.55 (15.72)		



Participants also contributed to one of three focus groups. Focus group results indicate that XRAYs is a valuable resource for identifying relevant recent research findings and for explaining limitations in research methods, relevance, and reporting quality. Results also indicate that it is critical for XRAYs to be brief, use non-technical language, and address the most recent trends in media coverage. FORCE is using focus group feedback to guide decisions about XRAYs content, format, and dissemination. FORCE will collect additional data to confirm test findings and to assess effects of XRAYs on understanding of evidence quality.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-15-01

Military sexual trauma is associated with an increased prevalence of mastectomy versus breast conserving surgery in a population of female veterans with early stage breast cancer

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## Background:

The National Accreditation Program for Breast Centers (NAPBC), in a 2014 statement of standards, endorsed breast conservation surgery (BCS) for women with AJCC stage 0,I, or II breast cancer, with a target of 50% BCS.

Women comprise the fastest growing segment of Veteran's Health Administration (VA) enrollees, and have high rates of trauma exposure including military sexual trauma (MST). The VA conducts universal screening of all Veterans for MST. Implications for medical care, e.g. an increased rate of hysterectomy among survivors of MST, have been reported. While the numbers of breast cancers treated in VA are relatively small compared to non-VA centers, a trend toward decreasing rates of BCS in VA from 2000-2006 has been reported. Reasons for declining rates of BCS within VA, and the lower rates of BCS in the VA compared to the private sector remain unclear. The objective of this study was to identify determinants of mastectomy versus BCS in women Veterans with early stage breast cancer and to examine whether history of MST was associated with choice of mastectomy versus BCS.

## Methods:

As a quality improvement study, we conducted a retrospective review of all early-stage (0,I,II) female breast cancer patients identified in the tumor registry between 2006-2015 at one Northeastern VA to determine rates of mastectomy and BCS. Through chart review, we examined potential determinants of BCS including age, stage, distance from treating VA facility, genetic testing, contralateral prophylactic mastectomy, and reconstructive surgery. History of MST was documented through chart review. Analyses were performed using unpaired t-test for age and distance from treatment facility and Fisher's exact test for significance comparing history of MST between surgical groups.

## Results:

70 women with early stage breast cancer were eligible for BCS. Of these, 39 underwent BCS and 31 underwent mastectomy (55% rate of BCS). Age and distance to treating VA were not significant. Women who underwent mastectomy were on average younger ( $p=0.21$ ) and lived farther away ( $p=0.42$ ) and were more likely to undergo genetic testing. Of the women who underwent genetic testing (10/70), none had mutations. The biggest difference seen between surgical groups was in history of MST, with women in the mastectomy group having more than twice the prevalence of MST, 58%, vs 31% in the BCS group ( $p=0.0154$ )

Patient characteristics by type of surgery performed

	BCS N=39	Mastectomy n=31
Age (mean)	63.5	59.3
Distance from VA	56.7 miles	67.7 miles
Genetic testing (no mutations found)	4 (10%)	6 (19%)
Reconstructive surgery	0	5
Prophylactic contralateral mastectomy	0	11
MST	11(28%)	18(58%)

## Conclusion:

These data suggest that MST is associated with choice of mastectomy over BCS. MST results in poor body image which may impact decisions about breast surgery. Trauma-informed strategies for counseling women about options for surgical management

of early stage breast cancer may be needed to ensure that women with MST and other forms of trauma pursue evidence-based cancer treatment. Further work with a larger cohort is needed to better understand these findings.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-15-02

Study result dissemination preferences of research participants in the Army of Women® program

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## Background:

The Army of Women (AOW) is a breast cancer research program of Dr. Susan Love Research Foundation (DSLRF). This initiative aims to accelerate breast cancer (BC) research by engaging and connecting the public with clinical research. To date, over 382,000 women and men, with and without BC, have registered as members. AOW members have full access to a curated repository of supported research studies and educational resources about the science of BC and the clinical research process. This highly motivated, proactive membership has donated their time, biological samples, and health data to advance studies over the last 10 years. Previous literature has demonstrated that research volunteers are less likely to participate in future studies if not informed of study results. With a membership that has volunteered to participate in over 130 studies, we sought to examine members' interest in receiving study results and preferred dissemination methods.

## Methods:

An adaptive, anonymous, online survey collected data on demographics, motivations, engagement behaviors, and attitudes toward research. Participants (N=1,486 to date) were recruited via email (AOW members only) and social media (AOW members and non-members; Facebook posts and ads). Responses were analyzed to understand participant preferences in study result dissemination and outreach methods. For these analyses, the cohort was limited to AOW members who completed the survey and reported past participation in AOW-supported studies.

## Results:

Of the 1,486 respondents, 527 (mean age 62.7 years, range 30-85) indicated AOW membership and reported past participation in AOW-supported studies. The majority was female (99%) and non-Hispanic white (94%). Over half (65.46%) reported a history of BC.

Of participants, 71% would like to learn about scientific results from studies that included members of the AOW. The preferred dissemination frequency and methods are monthly via email (61%) or social media (26%). Based on participants' reported social media use, dissemination through Facebook (74%) would yield the greatest reach – followed by YouTube (24%), Instagram (22%), LinkedIn (15%), Twitter (14%), and Snapchat (4%).

In addition to updates about study results, 75% of participants want to hear about publications from past AOW-supported studies. Importantly, a majority (84%) of those also want access to a brief overview and lay explanation of the publication. The preferred methods of dissemination for these publications and lay overviews are AOW newsletters (84%), a dedicated menu on the AOW website (51%), social media (29%), and blog posts on the DSLRF website (16%).

## Conclusions:

As in prior literature, clinical research participants in the AOW program indicate a high preference for receiving updates and staying informed about past study results. Participants indicate that they prefer to receive these results through emailed newsletters, websites, and social media. Researchers should consider these low-cost methods as they plan for result dissemination. Our findings support that volunteers want to be informed about results of their participation. This provides a clear opportunity for researchers to keep volunteers engaged and interested in participating in future clinical research.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-15-03

MBC Connect™, an open-access, patient-reported registry of de-identified data from patients living with metastatic breast cancer

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Metastatic breast cancer (MBC) remains an incurable disease and is the cause of nearly all deaths from breast cancer. Several barriers prevent efficient research into various questions about living with MBC. A key unmet need is a national database for MBC patient-reported outcomes, which does not exist anywhere in the world. Furthermore, compartmentalized data prevents broad collaborative efforts. Treatment patterns and responses, survival times, and metastatic patterns are not documented systematically and remain unavailable. Large-scale data extraction is challenging, and expensive, and electronic medical records do not provide information regarding patient experiences. **Methods:** MBC Connect™ was created to help overcome these barriers. MBC Connect, which is sponsored by the Metastatic Breast Cancer Alliance, is a multi-national registry of participant reported information about their experience of MBC. MBC Connect allows MBC patients to voluntarily provide information about their disease, treatment outcomes, and experience of living with their disease so that researchers can gain insight into unmet needs in MBC. MBC Connect has three main goals: 1. Establish an interactive registry of patient-entered, de-identified data for MBC. 2. Create an open-access portal for researchers to study these data. 3. Create a connection between investigators of clinical trials and clinical research studies and registered users who may be interested in clinical trials. **Results:** MBC Connect collects, via the use of a mobile app (on a smartphone or tablet, iOS and Android compatible) or via a website for desktop users, participant consent, general patient characteristics and demographics, disease characteristics, genetics and tumor mutations, treatment history, quality of life data, and clinical trial experience. This information can be provided by patients living with MBC or their caregiver. The data are collected from responses to surveys and via creation of a treatment profile. The data are de-identified and made available on an open-access Researcher portal, allowing them to be used to advance multiple areas of research into MBC, including both medical treatment aspects and quality of life issues. An interactive feature of MBC Connect is that researchers may submit a request for participants to answer additional surveys. Participants may also be notified about clinical trials for which they may be eligible. In addition, participants will regularly receive "Insights," which are engaging bytes of information related to MBC. Insights can offer general information about the disease, upcoming events, and other facts, or they can be personalized for the participant based on the information he or she has entered the registry. **Conclusions:** MBC Connect is a novel platform that aims to accelerate MBC research by providing open access to patient-reported, de-identified data about patients living with MBC. The overarching objective of this technologic initiative is to increase patient engagement with the research community.

# 2018 San Antonio Breast Cancer Symposium ®

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Health-related quality of life in MONARCH 3: Abemaciclib plus an aromatase inhibitor as initial therapy in women with HR+, HER2- advanced breast cancer

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**Background:** In the MONARCH 3 trial, abemaciclib plus an aromatase inhibitor (AI) significantly improved progression free survival and overall response rate with a generally tolerable safety profile compared to placebo plus AI. Here we report patient-reported outcomes (PRO) including health-related quality of life (QoL), functioning, and symptoms.

**Methods:** MONARCH 3 was a double-blind, randomized phase III study of abemaciclib or placebo plus an AI in 493 post-menopausal women with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer with no prior systemic therapy in the advanced setting. Two European Organization for Research and Treatment of Cancer (EORTC) questionnaires were included: Quality of Life Questionnaire (QLQ)-Core 30 (C30) and the EORTC QLQ-Breast 23 (BR23) that were assessed at baseline, every 2 cycles through cycle 19, then every 3 cycles until treatment discontinuation, and at short-term follow up. Higher scores on functional and health status/QoL outcomes indicate higher/better levels of functioning or health; conversely higher scores on symptom outcomes indicate higher/worse levels of symptom burden. Between-arm comparisons of change from baseline were conducted using mixed model methods. Statistical significance was set at 0.05 and clinical meaningfulness was set at  $\geq 10$  points on a 0-100 scale<sup>1</sup>.

**Results:** PRO completion rates were >91% through cycle 19; duration of treatment was longer for abemaciclib plus AI patients (median number of cycles 19 vs.15). Compared to the placebo arm, diarrhea PRO scores in the abemaciclib arm showed a clinically (18.68 points) and statistically significant ( $p < 0.001$ ) increase/worsening. By-cycle analysis showed group mean diarrhea scores returned to near-baseline levels post-therapy. Other symptom PROs showed statistically significant ( $< 0.05$ ) but not clinically meaningful differences; fatigue (4.96;  $p = 0.004$ ), systemic therapy side effects (4.48,  $p < 0.001$ ), appetite loss (4.03;  $p = 0.034$ ), and nausea/vomiting (2.77;  $p = 0.013$ ). These results were consistent with the investigator-reported treatment emergent adverse events (TEAEs). Several non-symptom results were also statistically significant but not clinically meaningful including global health/health status (-4.36;  $p = 0.003$ ), role function (-4.25;  $p = 0.025$ ), social function (-3.41,  $p = 0.047$ ), and body image (-5.11,  $p = 0.009$ ). No statistically significant between-treatment differences were observed for physical, emotional, and cognitive functioning or for symptoms of pain, dyspnea, insomnia, constipation, or financial difficulties.

**Conclusions:** The addition of abemaciclib to an AI resulted in clinically and statistically significant changes in diarrhea without clinically meaningful differences in other symptom scores. Increased GI-related symptoms were consistent with the manageable, reversible AE profile; the highest symptom burden was reported during early visits. No clinically meaningful differences in global health status or functional scores were observed.

**ClinicalTrials.gov:** NCT02246621

## Reference:

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# 2018 San Antonio Breast Cancer Symposium ®

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Fertility preservation in young breast cancer patients: Real life data on 1390 patients treated in the Institut Curie

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**Introduction:** Adverse effects of chemotherapy on fertility are a critical concern for young breast cancer (BC) patients. Fertility preservation (FP) is currently offered to BC patients, though literature data concerning reproductive outcomes are scarce. Also, very few data are available on whether these procedures are associated with delay to treatment, or whether they impact oncologic outcomes. The objective of our study is to evaluate: (i) efficacy of FP procedures in terms of stored material and pregnancy rates, (ii) safety regarding time from BC diagnosis to chemotherapy, and oncologic outcomes in a large real-life cohort of BC patients.

**Methods:** We retrospectively analyzed medical charts of all consecutive patients aged between 18 and 43 diagnosed with invasive BC between 01/01/2011 and 30/09/2017 and treated with chemotherapy at Institut Curie (Paris and Saint Cloud). Baseline factors (antral follicle count (AFC), AMH), details on fertility preservation procedures, and results (number of frozen oocytes and embryos) were retrieved in 3 academic hospitals (Jean Verdier, Antoine Bécclère and Cochin). All medical charts were reviewed in March 2018 to assess time from diagnosis to surgery / chemotherapy, pregnancy outcomes, recurrence and survival. We compared time from first consultation to start of chemotherapy (time diagnosis-to-CT) in case of neoadjuvant chemotherapy (NAC between patients who had or who did not have PF.

**Results:** On 1.390 patients identified, 622 had NAC, 768 had adjuvant CT. Median age at diagnosis was 38.8 y.o. 136 were BRCA mutated.

- 264 patients (19%) underwent a FP procedure: In Vitro Maturation (IVM) (58%, n=154); ovarian stimulation protocol (STIM) (31%, n=82); others (10%, n=28). The mean number of oocytes preserved was 5 [0-36] and was not different between IMV and STIM.
- Delays from diagnosis to CT were not different in patients who had FP than those who did not, neither in patients with NAC (no FP: 24.1 days VS FP: 22.8, p=0.24) nor in patients with adjuvant CT (no FP: 70.6 days VS FP : 66.8, p=0.11).
- 39 patients had at least one pregnancy: 28 spontaneous, 6 without information, and 5 from oocyte/embryo donation. The pregnancy rate was higher in patients in FP group (n=16 ; 6%) than in no FP group (n=23 ; 2%). 3 reused material : 2 without pregnancy and one had a miscarriage.
- About oncologic outcomes, 90 patients underwent relapse (6,4%), and this rate was not significantly different in the 2 groups (n=12, 4,5% VS n=78, 6.9%).
- Patients with BRCA mutation (BRCAm) had lower AMH (2.9 VS 4.1 ng/mL ; p = 0,03) and antral follicle count (17.6 VS 24 ; p = 0.01). However, there was no difference on the stored material, and pregnancy rate was higher than in patients with no mutation or unknown status (7.6 VS 2.6% ; p = 0,01).

**Conclusion:** Pregnancy rate was higher in patients with FP, however majority of pregnancies was spontaneous, and no live birth was observed after material reuse. FP procedures were not associated with delay to treatment. Though bias cannot be excluded, preliminary data do not show an adverse impact of FP on oncologic outcome. Further follow-up is needed.

# 2018 San Antonio Breast Cancer Symposium ®

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The impact of ductal carcinoma in situ of the breast on health services utilization and general health and well-being of women

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**Background:** The impact of DCIS diagnosis on the development of significant anxiety and depression and on the utilization of breast imaging and breast biopsy procedures, which may reflect fears of breast cancer recurrence, is unknown. We established a population cohort of women with DCIS and examined the impact of the DCIS diagnosis on health services utilization (physician, emergency room visits) before and after DCIS, including those related to anxiety or depression, and all breast-related interventions, compared to similar women without DCIS.

**Methods:** From hospital claims we identified a population-based cohort of women with a first diagnosis of DCIS between 2010-2015. Cases with prior history of cancer were excluded. We identified treatment (mastectomy, breast-conserving surgery (BCS) +/- radiation therapy (RT) by linkage of administrative databases. Using DCIS diagnosis date for each case, we matched 5 unaffected controls (with no history of DCIS) by age, mammography history, socioeconomic status, and comorbidity at same date. For all cases and controls we extracted the following from physician billing claims: breast imaging (mammography, ultrasound, MRI), breast biopsies, breast surgery, all physician visits, and all hospital claims (including those with a diagnosis of anxiety or depression), for five years prior to and following the index date. We excluded claims from the year prior to and subsequent to the index date to remove the effect of services utilization surrounding the diagnosis and management of the index DCIS lesion. We then computed the rate of each service per 100 person-years for 5 years before and after the index date. Cases who developed local recurrence (LR) (and their matched controls) were censored 3 months prior to LR date. We used negative binomial regression to test differences in rates of service utilization before and after DCIS diagnosis and between cases and unaffected controls.

**Results:** The cohort includes 4,977 women treated for DCIS were identified, each matched to 5 controls. 911/4,977 (18.3%) of cases were < 50 years old at diagnosis; 1,006/4,977 (20.2%) were treated by BCS alone, 2,995/4,977 (60.2%) by BCS+RT, and 976 (19.6%) by mastectomy. For cases diagnosed with visits with a breast diagnosis code, utilization of breast imaging procedures and the use of breast surgery was significantly greater relative to unaffected controls irrespective of age at diagnosis and treatment (all p values <0.0001). Primary care visits were higher among cases (RR=1.10 (95% CI 1.06, 1.14), p<0.0001), including those with a breast diagnosis code (RR=3.69 (95%CI 3.29, 4.14)). However, there was no increase in relative rates of physician or hospital visits for anxiety or depression (RR 1.13 (95% CI 0.97, 1.32 p=0.11), psychiatry visits (RR 1.07 (95% CI 0.82, 1.40) p=0.6), non-breast surgical procedures (RR 1.10 (95% CI 0.88, 1.37) p=0.4), or emergency department visits (unrelated to breast) in cases with DCIS compared to unaffected controls.

**Conclusion:** DCIS is associated with more visits and procedures related to the breast compared to women without a diagnosis of DCIS but overall health services utilization and visits related to anxiety and depression were not increased.



# 2018 San Antonio Breast Cancer Symposium ®

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A self-administered geriatric assessment tool for Spanish-speaking older women with breast cancer

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**Background:** Almost a quarter of older adults in the United States will identify themselves as Hispanic/Latino by 2060. Our group has previously developed and validated a self-administered geriatric assessment tool which can be used to identify functional, psychological, social and cognitive impairments among older patients with various types of cancer. Among English-speaking older adults, completing this tool using paper/pencil or a tablet takes a median of 15-21 minutes (min), with < 10% needing assistance to answer it (*Hurria, JOP 2016*). However, the utilization of this tool among Spanish-speaking older adults has not been tested. We assessed the feasibility of administering a translated and validated Spanish version of our geriatric assessment tool for older Hispanic women with breast cancer, and identified their preferred format (tablet or paper/pencil).

**Methods:** Spanish-speaking women aged  $\geq 65$  years with a diagnosis of breast cancer completed the geriatric assessment twice on the same day. Patients were randomized into 3 groups: paper/pencil twice; tablet and paper/pencil in random order; and tablet twice. We assessed the proportion of patients requiring assistance to complete the geriatric assessment, the time needed to complete it, and the proportion of patients who thought the geriatric assessment was difficult/very difficult.

**Results:** 140 older women with breast cancer completed the geriatric assessment twice and were evaluable. Mean age was 71.6 years (SD 5.8), 53% had  $\leq 8^{\text{th}}$  grade education, 43% were married, 45% were retired, 32% were homemakers, and 6% were employed. The participants came from 13 different Spanish-speaking countries, although 70% were born in Mexico. For 90%, Spanish was their primary language, and 75% spoke only in Spanish at home. Regarding computer skills, 64% of the patients said they had none. 39% (n = 54) were unable to complete the geriatric assessment on their own; mean time to complete the geriatric assessment was 29 min (range 8-90); and 28% (n = 39) thought the geriatric assessment was difficult/very difficult. The most common reasons for needing assistance were difficulty understanding questions (39%) and visual problems (31%). Patients with  $\leq 8^{\text{th}}$  grade education took longer to complete the geriatric assessment (mean 37.2 vs 29.4 min,  $p < 0.01$ ), and more often needed help completing the assessment (51% vs 19%,  $p < 0.01$ ) than those with  $\geq 9^{\text{th}}$  grade education. 53% of the participants preferred using a tablet to answer the geriatric assessment, while 47% preferred paper/pencil.

**Conclusions:** A substantial proportion of Spanish-speaking older women with breast cancer required assistance to complete our self-administered geriatric assessment tool. This may be a consequence of the low educational level we found among this patient population. Tailoring assessments for diverse populations with particular attention to educational level is needed in multicultural settings.

# 2018 San Antonio Breast Cancer Symposium ®

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Radiation oncologists' views on breast radiation therapy guidelines: Utilizing an online Q&A platform to assess current views on whole breast radiation therapy

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**Introduction:** Adherence to clinical practice guidelines (CPGs) has long been a challenge due to a lack of knowledge about CPGs and the fact that CPGs may not be practical in the context of a particular patient's situation. Poor adherence to the 2011 American Society for Radiation Oncology (ASTRO) evidence-based guideline on whole breast irradiation has been reported, especially for hypofractionated whole breast radiotherapy (HBRT). In 2018, updated ASTRO guidelines were published. We utilized theMednet, an online Q&A platform of over 3,600 US community and academic radiation oncologists (ROs), to assess current views regarding whole breast RT and evaluate relationships between the the strength of guideline recommendation statements and ROs views on treatment choices.

**Methods:** We identified 11 questions previously asked by community ROs on theMednet that were not addressed in the 2011 ASTRO whole breast irradiation guideline. Questions were originally asked between 10/27/2014 and 5/2/2017. These questions were sent to a senior author of the 2018 guidelines to post an updated response on theMednet, citing the guidelines and degree of consensus. A link to view the full guidelines was provided. New answers citing the guidelines were disseminated in 3 biweekly newsletters between 3/16/2018 and 5/1/2018, along with polls to survey radiation oncologists participating in theMednet.

**Results:** A total of 792 ROs responded to polls, of whom 523 were community ROs, 129 were academic ROs, and 93 were residents. Of the 11 questions, 10 pertained to WBRT and 1 pertained to conventional dose fractionation. For each question, the answer choice receiving the majority of the votes aligned with the 2018 guideline. Of the 5 questions which referenced a conditional recommendation in the 2018 guideline, the majority vote ranged from 54% to 87%; whereas, of the 6 questions which referenced a strong recommendation in the 2018 guideline, the majority vote from 62% to 97%. The strongest consensus was for HBRT to be used regardless of histology (97%), followed by molecular subtype (90%), grade (89%), and concurrent use of trastuzumab (87%). The least consensus was for age at which HBRT should be offered, with only 54% of respondents agreeing with the guideline consensus that HBRT should be offered regardless of age. When comparing academic and community ROs, 73% of academic ROs do not specify an age threshold for offering HBRT, compared to 47% of community ROs ( $P=0.0240$ ). Regarding boost dose-fractionation, 83% of academic ROs prefer a 10Gy in 4 fractions while 54% of community ROs prefer 10 Gy in 5 fractions and 41% prefer 10Gy in 4 fractions ( $P=0.1495$ ). Responses were similar between academic and community ROs for all other questions.

**Conclusion:** Utilizing an online platform, theMednet, we were able to assess current views of management of whole breast radiation therapy among participating ROs. The majority of ROs agree with the major recommendations from this guideline, with practice variation greater in areas with weaker underlying evidence.

# 2018 San Antonio Breast Cancer Symposium ®

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Twitter chats: Utilizing an innovative technology platform to offer education and tools to people with breast cancer

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**Background.** The use of social media for health information and peer support has grown significantly over time. According a 2015 study from the Pew Research Center, 65 percent of U.S. adults utilize social media, up from 7 percent a decade earlier. A 2014 study reported 72 percent of adult internet users search online for health information, often focused on specific diseases or treatments, with 16 percent seeking others with similar health concerns. Twitter, a popular social media platform for information- and support-seekers, grew from 54 million monthly active users in 2010 to approximately 241 million in 2013.

To address the growing use of Twitter as an information and support portal, Living Beyond Breast Cancer (LBBC) decided to pilot a Twitter chat in 2014. The goals for the chat were to: (1) increase understanding of a breast cancer concern; (2) connect impacted individuals to one another in a supportive and safe online space; (3) offer practical resources for further exploration; and (4) increase LBBC's reach online among users interested in breast cancer.

**Methods.** LBBC staff researched best practices for Twitter chats. The topic of triple-negative breast cancer (TNBC) was chosen for LBBC's pilot program to coincide with Triple Negative Breast Cancer Awareness Day. The staff developed 7 questions and 10 alternates to deliver over 60 minutes. Using publicly available data from Symplur, hashtags were considered and developed. A panel was recruited of one medical oncologist, two advocates, and two women with TNBC. Panelists were encouraged to prepare Tweets in advance and, during the chat, to use assigned hashtags, correct medical inaccuracies, and attend to emotional concerns among chat participants. LBBC staff developed resource-rich tweets. The program was promoted over social media channels. At the end of the chat, participants were offered an incentivized opportunity to evaluate the program via a URL link to LBBC's existing survey instrument.

**Results.** Using the #TNBCchat hashtag, the March 3, 2014, program and activity the next week produced 234 tweets from 127 contributors, reaching 198,985 accounts. Participants wrote 118 tweet replies and retweeted 291 comments. Questions covered the definition of and treatments for TNBC, differences between metastatic and early-stage disease, unique emotional concerns, and methods to reduce recurrence risk. The three most popular tweets promoted the program, introduced panelists, and shared resources about PARP inhibitors.

Encouraged by the pilot program's reach and results, LBBC has delivered 20 Twitter chats as of June 2018. Topics have included nutrition, parenting, caregiving, and breast reconstruction. Over time, LBBC staff has refined the program model with the goals of increasing engagement, delivering more useful content, and driving users toward the evaluation.

**Conclusions.** Twitter chats allow nonprofit organizations to inexpensively utilize innovative technology to provide educational information to and foster a community of support among people impacted by breast cancer. By utilizing core organizational principles of appropriate program delivery, LBBC delivers practical content that may improve the quality of life of users in distant locations.

# 2018 San Antonio Breast Cancer Symposium ®

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The BREAST-Q: Translation and validation for Mexican population

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**BACKGROUND:** BREAST-Q is a patient-reported outcome (PRO) that has been designed to evaluate perception outcomes among women undergoing different types of breast surgery, the modules include evaluation for mastectomy, breast reconstruction, augmentation, reduction/mastopexy and breast-conserving therapy.

**OBJECTIVES:** Generate a translated version that is conceptually equivalent to the original version and to validate for Mexican population.

**METHODS:** A linguistic and psychometric validation was performed in 494 women. We carry out pre and postoperative test. Reliability and internal consistency were performed by Cronbach's alpha and intraclass correlation coefficient (ICC).

**RESULTS:** The results of patient testing, number of participants, acceptability and reliability are shown in table 1

Summary of BREAST-Q validation analysis

N =494							
Module=n	Mean age(range)	Time to completion Test(minutes) average(range)	Time to completion Retest(minutes) average(range)	Number of items	Number of missing items	Cronbach's Alpha min-max*	Test-Retest ICC min-max*
Mastectomy=160							
Pre=62	48(26-76)	7.8(2-20)	6.4(3-20)	37	0	0.81-0.94	0.72-0.94
Post=98	52(32-78)	13(4-40)	11(4-28)	63	1	0.87-0.97	0.87-0.96
Breast Conserving Therapy=153							
Pre=81	50(21-78)	7(2-17)	5.2(2-13)	32	2	0.88-0.95	0.82-0.95
Post=72	55(37-73)	18.7(5-44)	15.4(6-29)	87	2	0.92-0.98	0.92-0.98
Reconstruction=181							
Pre=65	44(23-64)	7.9(3-22)	6.4(2-20)	42	2	0.90-0.97	0.90-0.97
Post=92	46(24-74)	18.5(8-41)	15.7(5-42)	116	1	0.86-0.99	0.85-0.99
Lattisimus Dorsi=24	45(33-74)	5(1-18)	4(2-10)	19	1	0.95-0.96	0.96-0.96

We report the low and the high value between all sub scales, per questionnaires

. The average scores were in all cases >0.80.

The internal consistency and reproducibility support the reliability of the instrument; all of the scores were acceptable.

**DISCUSSION:** The importance of measurements quality of life in patient whit cancer, become an essential end-point, we need validated tools that help us improve our performance in different methods of treatments.

Is the first validation study of an instrument that measures the impact of surgical treatment on the quality of life of breast cancer patients in Mexico, our results support the equivalent Spanish version for Mexican population. Breast-Q will provide valuable metrics for a surgeon team to document and measure their clinical performance and improve quality of healthcare in our Hispanic patients.

**CONCLUSIONS:** The Mexican Spanish version of tree Modules of Breast-Q is reliable and easy to implement in the population with breast cancer in different scenarios in México with the advantage to measure the quality of life and satisfaction on our population with a locally advanced stage that will help to improve quality of healthcare.

The high acceptability of the questionnaire demonstrate that the version is well accepted for our population so we will include a

significant number of patient in our country; therefore, more hospital centers will be invited to participate for further studies that allow us to evaluate the population in Latin America and thus compare our results.

# 2018 San Antonio Breast Cancer Symposium ®

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Using continuing professional development (CPD) as an investigative tool to improve clinical practice and interdisciplinary team treatment

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## Background:

Continued and rapid evolution of diagnosis and staging methods along with the development of new treatments has changed the decision-making process for cancer patients into a more complex task. In breast cancer, quality measures related to sentinel lymph node biopsy (SLNB), axillary dissection (AD), HER2 testing and use of HER2-targeted therapy, provide parameters for assessing care quality. We assessed the influence of quality improvement education (QIE) on alignment with BC quality indicators.

## Methods:

39 members of the interprofessional team at Inova (an integrated care network of 5 hospitals) participated in the QIE components. For a baseline Inova provided deidentified electronic medical records (EMR) data that included a total of 67 breast cancer patients treated between 12/1/16 – 2/28/17. The patients diagnosed with HER2+ breast cancer records were retrospectively reviewed for adherence to quality measures pertaining to HER2 testing, SLNB, AD, and treatment. Follow-up reviews were completed 6 months after the launch of the online component, which included 142 patients.

## Results:

Patient and disease characteristics were generally similar across the 2 cohorts. At baseline, treatment selection for patients with advanced stage cancer was consistent. The original goal of the activity was to assess adherence to quality measures in breast cancer treatment, in our assessment stage the team discovered potential undersampling of sentinel lymph nodes in patients who underwent neoadjuvant chemotherapy for clinically node-positive, with 3 of 8 patients (37.5%) having only 1 or 2 lymph nodes sampled. The educational programming improved recognition of the risk of false negative rates of sampling under 2 lymph nodes in this setting. In addition, the Inova team recommended that the triage protocol be changed to include the medical oncologist earlier in the treatment decision making process in tandem with the surgeon. In the post-program patient data, 26 patients with clinically N1/N2 disease had neoadjuvant therapy and a SLN biopsy. Of these, 3 (11.5%) had only 1 lymph node sampled, suggesting an increased adherence to recommended sampling.

The educational program also improved participant knowledge of the phase III APHINITY trial efficacy findings, which was consistent with an increase in the use of pertuzumab-containing adjuvant therapy for early breast cancer (use of TCHP, 0% vs 46% in the pre-program and post-program charts, respectively). In addition, participants in the educational programming increased awareness of the need for reflex testing in the event of an equivocal HER2 IHC result. In the post-program follow-up, no cases of equivocal HER2 IHC staining lacked reflex testing with FISH.

Taken together, these findings are consistent with practice change following interventional medical education for incorporating best practices and emerging clinical data in the management of HER2-positive breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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What research questions matters most to patients? Final results of the metastatic breast cancer priority setting partnership

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**Background:** Research is fundamental to the management of cancer, however many studies are primarily researcher or industry led, with minimal input and involvement from the people most affected by the outcomes- the patients and caregivers. The James Lind Alliance (JLA) is a not for profit initiative that brings patients, caregivers and clinicians together in priority setting partnerships (PSPs) to determine key priorities in research. Breast cancer remains the most common cancer among women, with an estimated third of women diagnosed developing metastatic disease. With advances in treatment, women are living longer with metastatic breast cancer (MBC), in some cases many years. **Objectives:** The aims of this study are to utilize the JLA approach to (1) identify the unanswered questions about treatment of MBC from patient and clinical perspectives, and (2) to prioritize those that patients and clinicians agree are the most important. **Methods:** Following the established JLA approach, MBC patients, caregivers, and health professionals were surveyed to elicit their questions pertaining to MBC. Research questions were generated from the survey responses, and following literature review that the questions were currently not completely answered, an interim prioritization survey was conducted to identify a shortlist of questions to take to a final consensus meeting. **Results:** One thousand, one-hundred and ninety-four responses were collected from 668 individuals (49% patients; 13% physicians; 9% caregivers; 4% allied health care professionals; 2% patient organization representatives; 23% other), which were refined into 62 unique unanswered research questions. The interim prioritization survey was completed by 174 individuals, and the top 27 questions were taken to a final meeting where MBC patients, caregivers, and health care professionals prioritized all the questions, and reached consensus on the top 10

List of final top 10 ranked priorities

Rank	Question
1	What biomarkers or intrinsic features of the tumour can be used to identify response to specific treatments and dosing schedules?
2	What is the role of immunotherapy for MBC?
3	How can treatment resistance be delayed and minimized?
4	What causes (i.e. cellular, genomic) breast cancer cells to metastasize, and what changes allow them to penetrate the blood brain barrier?
5	What is the right sequence of therapy in MBC?
6	Does local therapy (radiation or surgery to sites of metastatic disease) improve survival outcomes in MBC?
7	Is continuous treatment with systemic therapy (including HER2-targeted therapy and chemotherapy) better than intermittent?
8	Does early palliative care improve outcomes for MBC patients?
9	What are the best methods of education for patients around treatment options and decision making that can lead to improved patient outcomes?
10	Can safer, more accurate methods, including blood tests of detecting spread of disease (including following curative treatment) be developed?

. **Conclusion:** The top 10 questions cover a wide range of research questions, identified by valuable stakeholders as being priorities. These priorities can be used to fund and inform future MBC research.

## 2018 San Antonio Breast Cancer Symposium ®

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Is pregnancy testing during chemotherapy standardized?

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The initiative to improve awareness about the risks of infertility for premenopausal patients receiving chemotherapy has improved significantly over time. While the risks for infertility are high, there is still a small risk of pregnancy during chemotherapy. The incidence of cancer diagnosed during pregnancy is 0.1-0.2%. The incidence of women who become pregnant while on chemotherapy is less clear but does occur. Amenorrhea commonly occurs during chemotherapy but this does not necessarily correlate with lack of ovarian function. Treating a patient with an unidentified pregnancy is an adverse event which must be avoided given the high risk it poses to the fetus, especially during the first trimester. Pregnancy testing prior to the initiation of chemotherapy is recommended by the NCCN and ASCO. However, recommendations on how to monitor for pregnancy after an initial screen are inconsistent and lack standardization. Formal guidelines and policies are needed to prevent and/or identify pregnancies while on chemotherapy.

We surveyed five breast medical oncologists and six infusion nurses at a busy urban breast center to determine their baseline practices in regards to pregnancy counseling and testing. Of physicians and nurses surveyed, 40% (2/5) and 33% (2/6) respectively have diagnosed a pregnancy while on chemotherapy. When surveyed about counseling patients on the risks of pregnancy, 80% of physicians counsel patients prior to initiation of chemotherapy, but one physician and one infusion nurse said they do not counsel patients about the risk of pregnancy at any time but do discuss the risks of infertility. All physicians surveyed check a pregnancy test prior to the initiation of chemotherapy, but 60% check urine and 40% check serum. Thereafter 60% will check a pregnancy test after initial screen if the patient is concerned and 40% will check intermittently during chemotherapy. Of the nurses surveyed, 33% will check a pregnancy test after initial screen if the patient is concerned and 50% check intermittently during treatment. Half of the infusion nurses said they ask patients if they are concerned they may be pregnant intermittently during chemotherapy and 50% answered that they do not ask again after the initial screen. On subsequent screens the choice of urine or serum hCG testing varies and is not standardized.

These survey results demonstrate that both pregnancy counseling and pregnancy screening practices vary widely even within the same institution. A standardized approach is essential to increase awareness of pregnancy risk while on chemotherapy, improve education of this risk to patients, prevent unwanted pregnancies, and identify pregnancies as soon as possible. We have initiated a quality improvement project to check urine pregnancy tests monthly in all premenopausal patients (age <55) receiving chemotherapy for breast cancer. Based on our findings we will institute a protocol at our institution for uniform teaching on the small risk of pregnancy and uniform pregnancy testing while on chemotherapy.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-02

Trastuzumab deruxtecan (DS-8201a) in subjects with HER2-low expressing breast cancer: Updated results of a large phase 1 study

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**Background:** HER2-targeted therapies have improved survival for advanced HER2-positive breast cancers (BC), but none have been approved for tumors with low levels of HER2 expression (ie, HER2 IHC 1+ or 2+/ISH-negative). Trastuzumab deruxtecan (DS-8201a) is a novel HER2-targeted antibody-drug conjugate with a humanized HER2 antibody attached to a potent topoisomerase I inhibitor payload by a cleavable peptide-based linker, which is designed to have broad antitumor activity in HER2-expressing tumors. It has a drug-to-antibody ratio of 7 to 8, a novel linker that is stable in plasma and that is selectively cleaved by lysosomal cathepsins which are upregulated in cancer cells, and its payload has a short systemic half-life. In 2015, a phase 1 study (NCT02564900) was initiated to evaluate the safety and efficacy of DS-8201a in subjects with advanced HER2-expressing or HER2-mutated solid tumors, including HER2-low expressing BC. In this study, the overall confirmed response rate (ORR) in the evaluable subjects was 49.3% (103/209) (April 2018 data cutoff; Iwata H, et al. ASCO 2018). Expanded results from the HER2-low expressing BC subjects are presented here.

**Methods:** This ongoing phase 1 trial included 2 parts. The dose escalation part served to determine the dose-limiting toxicities, the maximum tolerated dose, and to select the recommended dose for expansion (RDE). The dose expansion part further evaluated the safety, tolerability, and efficacy of the DS-8201a at the RDE (5.4 and 6.4 mg/kg; q3wks) in various advanced HER2-expressing or HER2-mutated solid tumors, including heavily pretreated HER2-low BC (IHC 1+ or 2+, ISH-negative). Enrollment of HER2-low subjects is ongoing.

**Results:** At the cutoff date of 18 April 2018, data from 34 HER2-low BC subjects were collected. The median age was 55.8 (range; 33, 75) years, the median number of prior endocrine therapies was 2, and the median number of prior chemotherapies was 3. In this HER2-low BC population, most patients had hormone receptor (HR)-positive disease (85.3%; 29/34); of which 17.2% (5/29) received prior treatment with a CDK4/6 inhibitor. The confirmed ORR was 50.0% (17/34), the disease control rate was 85.3% (29/34), the median time to response was 2.8 (range; 1.2, 13.8) months, the median duration of response (DOR) was 11.0 months, and the median progression-free survival (PFS) was 12.9 months. In the subgroup with HR-positive disease, the ORR was 55.2% (16/29), the median DOR was 11.0 months, and the median PFS was 13.6 months. After exclusion of 8 HER2-low subjects who received prior HER2-targeted therapy, the ORR was 46.2% (12/26). In the overall study, among the 145 BC subjects who received  $\geq 1$  dose of DS-8201a (5.4 or 6.4 mg/kg), the most frequent grade  $\geq 3$  adverse events included anemia (14.5%), and decreased counts of neutrophils (13.8%) and white blood cells (10.3%). There were 4 fatal cases of interstitial lung disease/pneumonitis in BC subjects, including 2 fatal cases in HER2-low BC subjects.

**Conclusions:** In this study, DS-8201a showed substantial antitumor activity and acceptable safety in heavily pretreated HER2-low BC.

# 2018 San Antonio Breast Cancer Symposium ®

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24 months results from a double-blind, randomized phase III trial comparing the efficacy and safety of neoadjuvant then adjuvant trastuzumab and its biosimilar candidate CT-P6 in HER2 positive early breast cancer (EBC)

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**Background** CT-P6 is a proposed biosimilar to reference trastuzumab (RTZ). A double-blind, randomized, phase III trial showed similar efficacy and safety for CT-P6 and RTZ in HER2 positive EBC (NCT02162667). The primary endpoint, pathological complete response rate was within the predefined margin to demonstrate similarity (Lancet Oncol 2017). Safety and efficacy at 1 year (ESMO 2017), and cardiac toxicity at a median of 19 months (SABCS 2017) were similar between the two treatment groups. Here we report updated disease-free survival (DFS), overall survival (OS) and cardiac toxicity data with a median follow-up of 2 years.

**Methods** A total of 549 patients with HER2 positive EBC were randomized to receive CT-P6 (n=271) or RTZ (n=278) in combination with docetaxel (Cycles 1-4) and 5-fluorouracil, epirubicin and cyclophosphamide (Cycles 5-8). CT-P6 or RTZ was administered at 8 mg/kg (Cycle 1 only) followed by 6 mg/kg every 3 weeks. After surgery, patients received CT-P6 or RTZ monotherapy then entered the follow-up period up to 3 year of last patient enrollment. Time to event analyses were performed using Cox regression and Kaplan-Meier methods.

**Results** A total of 528 patients (259/271 in CT-P6 and 269/278 in RTZ) entered the follow-up period after completing therapy. The median follow-up duration was over 27 months. The number of DFS events (32 [12.4%] in CT-P6 and 26 [10.0%] in RTZ) and OS events (14 [5.2%] in CT-P6 and 12 [4.3%] in RTZ) were comparable in the ITT set. Additionally, DFS and OS were similar between CT-P6 group and RTZ group in both the per-protocol set (PPS) and the ITT set. In the ITT set, the proportion of 2-year DFS (95% CI) was 86% (80% – 90%) in CT-P6 and 90% (85% – 93%) in RTZ. The proportion of 2-year OS was 97% (93% – 98%) in CT-P6 and 98% (96% – 99%) in RTZ. Median DFS and OS have not been reached. After 1-year treatment, no new cases of heart failure were reported during the follow-up period. Left ventricular ejection fraction (LVEF) was similar in both groups (mean LVEF, more than 60%).

Table 1. Summary of Long Term Efficacy Endpoints

	PPS		ITT set	
	CT-P6 (n=248)	Reference Trastuzumab (n=256)	CT-P6 (n=258)	Reference Trastuzumab (n=261)
Proportion of DFS				
1 year (95% CI)	0.95 (0.91 – 0.97)	0.96 (0.93 – 0.98)	0.95 (0.91 – 0.97)	0.96 (0.93 – 0.98)
2 years (95% CI)	0.86 (0.80 – 0.90)	0.90 (0.85 – 0.93)	0.86 (0.80 – 0.90)	0.90 (0.85 – 0.93)
p-value	0.3324		0.3085	
	CT-P6 (n=248)	Reference Trastuzumab (n=256)	CT-P6 (n=271)	Reference Trastuzumab (n=278)
Proportion of OS				
1 year (95% CI)	1.00 (1.00 – 1.00)	1.00 (0.97 – 1.00)	0.99 (0.97 – 1.00)	0.99 (0.97 – 1.00)
2 year (95% CI)	0.97 (0.94 – 0.99)	0.98 (0.96 – 0.99)	0.97 (0.93 – 0.98)	0.98 (0.96 – 0.99)
3 year (95% CI)	0.94 (0.88 – 0.97)	0.93 (0.86 – 0.96)	0.92 (0.86 – 0.96)	0.93 (0.87 – 0.96)
p-value	0.9329		0.5057	

**Conclusions** The efficacy and cardiac toxicity profile between CT-P6 and RTZ in EBC patients were consistent with published

data. Time to event analyses as secondary efficacy endpoints supported the similarity for the two study drugs. CT-P6 was consistently well tolerated with a similar cardiotoxicity profile to that of RTZ through long duration of follow-up.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-04

3-year relapse-free survival of stage II-III HER2-neu positive breast cancer treated with pertuzumab and trastuzumab-containing neoadjuvant therapy compared to trastuzumab-containing therapy

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**Background:** Pertuzumab (P) in combination with trastuzumab (H) based chemotherapy is FDA-approved as a standard neoadjuvant treatment for patients with clinical stage II-III HER2-positive (HER2+) breast cancer (BC). The goal of this study was to evaluate the pathologic complete response (pCR) rate for neoadjuvant HP-containing regimens compared to H-containing regimens and report the 3-year relapse-free survival (RFS) for patients who had a pCR compared to those with residual disease (RD).

**Methods:** All patients with stage II-III non-inflammatory HER2+ BC who received neoadjuvant H-containing or HP-containing therapy and underwent definitive breast and axillary surgery were identified from 2005 to 2016 through an institutional database. Medical records were examined for patient demographics, breast cancer stage, pathology results, surgical outcomes, and treatment details. pCR was defined as ypT0/is, ypN0. RFS was defined as the interval from surgery to date of last followup or death from any cause. Descriptive statistics, Cox proportional hazards, and Kaplan-Meier estimates were used for statistical analysis.

**Results:** Patient characteristics and results by pCR or RD status are shown in the table below. The median age was 51 (22-84) years for the HP group and 50 (21-87) years for the H group. The median follow-up time was 1.9 (0-4.2) years for the HP group and 5.3 (0.1-12) years for the H group. For the HP group, the 3-year RFS was 98% (95% CI: 95, 100) for the pCR group and 90% (95% CI: 83, 97) for the RD group; HR 0.17 (0.04, 0.82), p=0.012. For the H group, the 3-year RFS was 91% (95% CI: 88,94) for the pCR group and 75% (95% CI: 71-79) for the RD group; HR 0.31 (0.22, 0.44), p<0.0001. Among the 520 patients who achieved pCR and the 502 patients who had RD, the effect of HP vs. H was statistically significant (pCR: HR 0.24 (0.06, 1.00); p=0.015) (no pCR: HR 0.46 (0.22, 0.94); p=0.017).

**Conclusion:** Patients who achieve pCR have an improved 3-year RFS compared to patients who have RD. Treatment with HP-containing neoadjuvant regimens is associated with a high 3-year RFS.

Variable		HP (n=215)		H (n=807)	
		pCR n=121	RD n=94	pCR n=399	RD n= 408
Age at Diagnosis	<50	43%	46%	46%	51%
	≥50	57%	54%	54%	49%
Menopausal Status	Premenopausal	46%	50%	53%	57%
	Postmenopausal	54%	50%	47%	43%
Clinical Stage at Diagnosis	IIA	40%	29%	34%	29%
	IIB	29%	31%	23%	28%
	IIIA	14%	15%	17%	16%
	IIIB	0%	5%	5%	9%
	IIIC	17%	20%	21%	18%
Clinical Nodal Status	Node (+)	63%	76%	69%	73%
	Node (-)	37%	24%	31%	27%
Nuclear Grade <sup>1</sup>	II	25%	32%	22%	28%

	III	75%	65%	78%	72%
<b>HR status</b>	HR(+)	52%	74%	52%	67%
	HR(-)	48%	26%	48%	33%
<b>Adjuvant therapy</b>	Trastuzumab	88%	80%	100%	100%
	Trastuzumab and Pertuzumab	3%	5%	0%	0%
	Unknown	9%	15% <sup>2</sup>	0%	0%

<sup>1</sup>1 patient in the HP pCR group had nuclear grade 1; 2 patients in the HP RD group had nuclear grade 1 tumors <sup>2</sup> 2 patients received adjuvant TDM-1 on the NSABP B50 protocol

# 2018 San Antonio Breast Cancer Symposium ®

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Independent validation of a combined biomarker based on the PAM50 HER2-enriched subtype and ERBB2 mRNA levels following HER2 blockade without chemotherapy in the PerELISA phase II trial

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**Background:** A combined biomarker based on HER2-enriched subtype (HER2-E) and ERBB2 mRNA predicts response and survival in HER2+ breast cancer following trastuzumab +/- lapatinib in the absence of chemotherapy (Prat et al. ASCO 2018). Here, we tested the ability of the combined biomarker to predict pathological complete response (pCR) following neoadjuvant trastuzumab, pertuzumab and endocrine therapy.

**Methods:** RNA from 40 baseline tumor samples from the phase II PerELISA trial were evaluated. PerELISA evaluated the efficacy of a de-escalated, chemotherapy-free neoadjuvant regimen based on dual HER2 blockade with trastuzumab and pertuzumab in combination with letrozole in HER2+/hormone receptor-positive breast cancer selected on the basis of Ki67 response after short course letrozole-alone (Guarneri ASCO 2018). Ki67 response was defined by protocol as relative Ki67 reduction  $\geq 20\%$  from baseline at day 14. Gene-expression was measured using the nCounter platform. Intrinsic subtypes and ERBB2 levels were determined by the PAM50 gene expression predictor. A pre-specified ERBB2 cutoff was determined to define ERBB2-high. Univariate and multivariable logistic regression analyses were performed.

**Results:** The proportion of HER2-E disease within the ERBB2-high and ERBB2-low groups was 46.2% (6/13) and 18.5% (5/27), respectively. The discordance rate at the individual level was 30% (12/40). A total of 6 (15%) and 34 (85%) samples were HER2-E/ERBB2-high and others, respectively. The magnitude of Ki67 reduction of the HER2-E/ERBB2-high and others groups was 64.8% and 63.2%, respectively ( $p=0.88$ ). The pCR rate of HER2-E/ERBB2-high was 66.7%. The pCR rate of the others group was 14.7%. The univariate odds ratio between HER2-E/ERBB2-high tumors and the others groups was 11.60 (95% CI 1.66-81.10;  $p=0.014$ ). No other clinical-pathological variable was significantly associated with pCR.

**Conclusion:** The combined HER2-E/ERBB2-high biomarker can identify patients who might be good candidates to receive dual HER2 blockade alone without chemotherapy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-06

Characterization, monitoring and management of interstitial lung disease in patients with metastatic breast cancer: Analysis of data available from multiple studies of DS-8201a, a HER2-targeted antibody drug conjugate with a topoisomerase I inhibitor payload

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**Background:** Several classes of anti-cancer agents including certain immunotherapies, systemic chemotherapies, and targeted therapies including trastuzumab and T-DM1 increase the risk of interstitial lung disease (ILD) and fatal cases have been reported. For DS-8201a, interim efficacy and safety analyses of available data established a final recommended dose of 5.4 mg/kg IV q3wk in advanced HER2-positive breast cancer (BC). Based on preliminary clinical results, ILD was identified as an important risk for DS-8201a. A robust monitoring and management plan was established across all studies and an international, independent ILD adjudication committee (AC) reviews the cases reported as ILD on an ongoing basis.

**Methods:** All subjects (sbj) who received  $\geq 1$  dose of DS-8201a across 7 ongoing studies were included in this analysis. Reported ILD (standardized MedDRA Query terms) included the terms ILD, pneumonitis, and organizing pneumonia. ILD frequencies were calculated based on investigator's assessment and after adjudication. The analysis of potential risk factors associated with ILD is ongoing.

**Results:** As of 21 June 2018, 448 sbj received  $\geq 1$  dose of DS-8201a across multiple tumor types, including BC. Of the 321 sbj with BC, 173 (53.9%) were from Japan, 103 (32.1%) from the US, and 45 (14.0%) from 6 other countries (Spain, South Korea, Taiwan, Belgium, France, and Italy). These sbj received 1 of 7 doses of DS-8201a (0.8 mg/kg: 3 sbjs, 1.6 mg/kg: 1 sbj, 3.2 mg/kg: 3 sbjs, 5.4 mg/kg: 111 sbjs, 6.4 mg/kg: 178 sbj, 7.4 mg/kg: 20 sbj, 8.0 mg/kg: 5 sbj). Overall, 44 cases of potential ILD were reported by the investigators across all tumor types (44/448, 9.8%; Grade  $\geq 3$  10/448, 2.2%). In sbj with BC who received 5.4 mg/kg, any grade and Grade  $\geq 3$  investigator-reported ILD were 7.2% (8/111) and 0.9% (1/111), respectively. The ILD AC assessed 30 of 44 cases; 22 were considered drug-related ILD, 4 were ILD but not drug-related, and 4 were found not to be ILD.

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	All Grades
<b>All tumors, All doses (N=448)</b>						
Investigator-reported	20 (4.5)	14 (3.1)	4 (0.9)	1 (0.2)	5 (1.1)	44 (9.8)
Cases adjudicated	13	8	4	0	5	30
Adjudicated as drug-related ILD	9 (2.0)	6 (1.3)	3 (0.7)	0	4 (0.9)	22 (4.9)
<b>BC, All doses (N=321)</b>						
Investigator-reported	17 (5.3)	11 (3.4)	3 (0.9)	1 (0.3)	4 (1.2)	36 (11.2)
Cases adjudicated	11	8	3	0	4	26
Adjudicated as drug-related ILD	8 (2.5)	6 (1.9)	3 (0.9)	0	4 (1.2)	21 (6.5)
<b>BC, 5.4 mg/kg (N=111)</b>						
Investigator-reported	4 (3.6)	3 (2.7)	0	0	1 (0.9)	8 (7.2)
Cases adjudicated	1	2	0	0	1	4

Adjudicated as drug-related ILD	0	0	0	0	1 (0.9)	1 (0.9)
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n (%), except where noted

For adjudicated drug-related ILD cases, the median time to onset was 159 (range; 46-591) days from the time of first dose.

**Conclusions:** These analyses confirm that ILD is an important identified risk for DS-8201a. Further analyses are ongoing to better understand the potential risk factors associated with the incidence of on-treatment ILD. When ILD is suspected, early diagnosis through appropriate imaging, laboratory tests, and pulmonary consultation as well as prompt management with steroids are recommended.



# 2018 San Antonio Breast Cancer Symposium ®

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Gene signatures and subtype changes during HER2 dual blockade in PAM50 HER2-enriched HER2-positive breast cancer

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**Background:** HER2-positive (HER2+) breast cancer (BC) is composed of 4 molecular subtypes: Luminal A and B, HER2-enriched (HER2-E) and Basal-like. Among them, the HER2-E is highly sensitive to anti-HER2 treatment. However, ~60% of HER2-E tumors do not achieve a pathological complete response (pCR) following neoadjuvant dual HER2 blockade without chemotherapy. Here, we aimed to better understand the molecular changes of the HER2-E subtype during anti-HER2 treatment.

**Methods:** Gene expression was evaluated in 101 patients with HER2-E tumors from the PAMELA neoadjuvant phase II trial (Lancet Oncol 2017). Briefly, women with HER2+ BC were treated with lapatinib and trastuzumab (and hormonal therapy if hormone receptor [HR]-positive) for 18 weeks. The median time between the last dose of treatment and surgery was 35 days (range=213; interquartile range=16). Expression of the PAM50 genes and 6 PAM50 signatures (Luminal A, Luminal B, HER2-E, Basal-like, normal-like and the PAM50 proliferation score) were determined using the nCounter platform at baseline (n=101), after 2 weeks of treatment (n=96) and in residual tumors (non-pCR) at surgery (n=57). Same analyses were done in 2 HER2+/HER2-E cell line models (BT474 [HR+] and SKBR3 [HR-]) following *in vitro* treatment with trastuzumab in combination with lapatinib. Biological changes between 2 time-points were determined by paired t-tests with a false discovery rate (FDR) <5%.

**Results:** After 2 weeks of treatment, 85.7% and 94.6% of the 56 genes/signatures were found differentially expressed (FDR<5%) in HER2-E/HR+ (n=35) and HER2-E/HR- (n=61) tumors, respectively. The two gene lists were highly correlated (correlation coefficient=0.93). Overall, a significant relative increase in Luminal A and normal-like signature scores, and a relative decrease in proliferation, HER2-E and Luminal B signature scores, were observed between baseline and week 2. Interestingly, a PAM50 subtype switch to Luminal A was observed in 31.6% and 4.8% of HER2-E/HR+ and HER2-E/HR- tumors. In BT474 and SKBR3, all genes/signatures were also found differentially expressed (FDR<5%) following 72h of dual HER2 blockade. The *in vitro* findings recapitulated the *in vivo* findings in 80-86% of the genes/signatures. Similar to tumors, a switch to a Luminal A subtype following dual HER2 blockade was observed in BT474 but not in SKBR3. Finally, 92.9% of the 56 genes/signatures were found differentially expressed (FDR<5%) in residual tumors at surgery compared to week 2. Contrary to the findings in the first 2 weeks of treatment, a general rebound effect in gene expression was observed between week 2 and surgery. Similarly, a rebound effect was observed in 60% of the genes/signatures in BT474 after removing anti-HER2 therapy for 72h, leading to a subtype switch from Luminal A back to HER2-E.

**Conclusions:** Dual HER2 blockade in the HER2-E subtype induces large biological changes that lead to a more low-proliferative Luminal A phenotype both in tumors and *in vitro* models, especially in HER2-E/HR+ disease. These phenotypic changes are reversible upon stopping anti-HER2 treatment. This finding supports the use of maintenance anti-HER2 treatment +/- endocrine therapy (if HR+) in advanced HER2+ BC.

# 2018 San Antonio Breast Cancer Symposium ®

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Dynamics of tumor-infiltrating lymphocytes (TILs) during neoadjuvant dual HER2 blockade in HER2-positive (HER2+) breast cancer in the absence of chemotherapy

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**Background:** TILs in HER2+ breast cancer (BC) predict 1) prognosis in early setting, 2) complete pathological response (pCR) following neoadjuvant antiHER2-based therapy and 3) response to trastuzumab and pembrolizumab in the metastatic setting. However, less is known regarding changes in TILs during antiHER2-based treatment.

**Methods:** Stromal TILs were evaluated centrally using H/E slides in tumor samples from the PAMELA (NCT01973660) neoadjuvant phase II trial. Briefly, 151 women with HER2+ BC were treated with lapatinib and trastuzumab, and hormonal therapy if HR positive, for 18 weeks. TIL levels were determined at baseline (n=148), after 2 weeks of treatment (n=134) and at surgery (n=137). Expression of 560 genes, including immune-related genes (e.g. CD8A, CD4, PD1 and PDL1) was measured at the same timepoints (baseline n=151, 2-weeks n=144, surgery n=144) using the nCounter platform. Intrinsic subtyping at baseline was determined using the PAM50 gene expression predictor. Changes in TILs between 2 time-points were determined by paired t-tests. Correlation of TILs with gene expression was assessed by quantitative SAM analysis using a False Discovery Rate <1%. All statistical tests were two-sided and considered significant when p<0.05. All statistical analyses were carried out using the R software.

**Results:** Compared to baseline, a significant increase in TILs was observed at week 2 in HR- (p<0.001) and HER2-enriched (HER2-E) tumors (p=0.001), but not in HR+ (p=0.133) and non-HER2-E tumors (p=0.067). Within HR- and HER2-E tumors, increase in TILs at week 2 from baseline was observed regardless of pathological response at surgery (pCR and HR- [p=0.008]; RD and HR- [p=0.037]; pCR and HER2-E [p=0.010]; RD and HER2-E [p=0.056]). Compared to week 2, a significant decrease in TILs at surgery was observed in HR- (p=0.002) and HER2-E (p=0.003) tumors, but not in HR+ (p=0.616) and non-HER2-E tumors (p=0.578). Within HR- and HER2-E tumors, a significant decrease in TILs between week 2 and surgery was observed in tumors achieving pCR (p=0.004 and p=0.005), while, in tumors not achieving pCR, no significant tendency was observed (26.4% and 33.0% of tumors showed an increase and a decrease of TILs between week 2 and surgery). Nonetheless, the vast majority of residual tumors (non-pCR) at surgery had TILs above ≥5%: 34.3% 5-10%, 21.0% 10-20%, 15.2% 20-40% and 11.4% ≥40%.

Finally, TILs scoring was found highly enriched (FDR<1%) for immune-related genes tracking activated CD8 T-cells (i.e. CD8A, CD3G, LAG3 and PD1). Expression of these immune genes consistently correlated with TIL levels across the 3 time-points.

**Conclusions:** In early HER2+ BC, a general increase in TILs is observed following 2 weeks of dual HER2 blockade. This observation is mostly observed in HR- and HER2-E subtype, but regardless of pathological response at surgery. After 2 weeks of treatment, TILs consistently decrease in patients achieving a pCR, whereas two main patterns of TILs expression are observed in patients with residual disease at surgery. Nonetheless, most residual tumors at surgery are inflamed (i.e. TILs ≥5%) and might be good candidates for clinical trials evaluating adjuvant immune checkpoint inhibitors.

# 2018 San Antonio Breast Cancer Symposium ®

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Event-free survival by ADCC status from a follow-up study comparing SB3 (trastuzumab biosimilar) with reference trastuzumab for HER2 positive breast cancer in neoadjuvant setting

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**Background:** SB3 has been approved by the European Commission as a biosimilar of reference trastuzumab (TRZ). Equivalent breast pathologic complete response (bpCR) rate and comparable event-free survival (EFS) and overall survival between SB3 and TRZ have been reported.<sup>1-3</sup> Upon monitoring quality attributes of TRZ for the development of SB3, a marked downward shift in antibody-dependent cell-mediated cytotoxicity activities (ADCC) was observed in TRZ lots with expiry dates from Aug 2018 to Dec 2019.<sup>4</sup> Some of these lots were used in this study. The objective of this report is to evaluate event-free survival by ADCC status from an additional one-year follow-up study.

**Methods:** Patients with HER2 positive early or locally advanced breast cancer were randomly assigned to receive SB3 or TRZ in neoadjuvant setting concurrently with chemotherapy. Patients then underwent surgery followed by adjuvant SB3 or TRZ. After completion of therapy, patients from selected countries participated in a long-term follow-up study. In TRZ, patients exposed to at least one shifted ADCC lot and those not exposed to shifted ADCC lot during neoadjuvant period were considered as "Exposed" and "Unexposed," respectively. EFS was defined as the time from the date of randomization to the date when the first event occurred. An event was defined as disease recurrence or progression (local, regional, distant or contralateral) or death due to any cause. EFS after additional one-year follow-up was analyzed by ADCC status in the long-term follow-up set (LFS).

**Results:** A total of 367 patients (SB3, N=186; TRZ, N=181) were included in the LFS. Within TRZ, 55 patients were Unexposed and 126 patients were Exposed. At a median follow-up duration of 30.1 months in SB3 and 30.2 months in TRZ from initiation of study treatment, 4.8% patients in SB3, 3.6% in Unexposed and 10.3% in Exposed experienced events. 4.3% patients in SB3, 1.8% in Unexposed and 9.5% in Exposed experienced recurrence after surgery (Table). Two-year EFS rate was 96.7% in SB3, 98.2% in Unexposed and 92.5% in Exposed.

**Conclusion:** A significantly higher proportion of patients experienced events in Exposed compared to Unexposed (HR 0.07, 95% CI 0.01-0.58,  $p$ -value=0.0137). No significant difference in EFS was found between SB3 and Unexposed. Although this study has a relatively short follow-up and has not been powered to evaluate the impact of ADCC on survival, these results suggest a possible correlation between ADCC and clinical efficacy. Further long-term results will follow.

## Summary of Event-free Survival (LFS)

	SB3 N=186	TRZ			EFS Hazard ratio (95% CI), $p$ -value		
		All N=181	Unexposed N=55	Exposed N=126	SB3 vs TRZ All	SB3 vs TRZ Unexposed	TRZ Unexposed vs TRZ Exposed
Patients with event, n (%)	9 (4.8%)	15 (8.3%)	2 (3.6%)	13 (10.3%)	0.49 (0.21, 1.14) $p=0.0975$	1.19 (0.23, 6.18) $p=0.8376$	0.07 (0.01, 0.58) $p=0.0137$
Recurrence after surgery	8 (4.3%)	13 (7.2%)	1 (1.8%)	12 (9.5%)			
Progression before surgery	1 (0.5%)	1 (0.6%)	1 (1.8%)	0 (0.0%)			
Death	0 (0.0%)	1 (0.6%)	0 (0.0%)	1 (0.8%)			

**Reference:**

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-10

Dose justification for DS-8201a, a HER2-targeted antibody-drug conjugate, for HER2-positive breast cancer: Observed clinical data and exposure-response analyses

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## Background

DS-8201a is a HER2-targeting antibody-drug conjugate with a high drug-to-antibody ratio of 7 to 8. A novel cleavable peptide-based linker joins the humanized HER2 antibody to a topoisomerase I inhibitor payload. In an ongoing phase 1 study (J101), DS-8201a was tested at doses of 0.8 to 8.0 mg/kg and tolerated with no predefined dose-limiting toxicities; 5.4 and 6.4 mg/kg doses were recommended for expansion. Subjects with HER2-positive breast cancer (BC) treated at these 2 doses had an overall response rate (ORR) of 54.5% (54/99). The phase 2 DESTINY-BREAST01 trial began enrollment in August 2017 with a dose-finding stage, including 5.4, 6.4, and 7.4 mg/kg. To determine the recommended dose for continued development in HER2-positive BC, a comprehensive analysis of observed data and exposure-response (ER) from both trials was performed.

## Methods

A population-PK (PPK) model was developed using data from all subjects with available concentration data. Individual exposure parameters ( $C_{min}$ ,  $C_{max}$ , AUC) were estimated from the PPK model and used in the ER analyses. ER analyses were conducted using logistic regression or Cox proportional hazard modeling for efficacy (ORR, duration of response, and PFS) and safety (nausea, diarrhea, left ventricular ejection fraction, neutropenia, anemia, thrombocytopenia, dose reductions due to TEAE, discontinuations due to TEAE, and interstitial lung disease [ILD]).

## Results

As of 18 Apr 2018, in J101, there are 111 HER2-positive BC subjects treated at 5.4- or 6.4-mg/kg doses. As of 25 Apr 2018, DESTINY-BREAST01 enrolled 65 HER2-positive BC subjects across 3 doses (5.4, 6.4, and 7.4 mg/kg). Confirmed ORRs in J101 for HER2-positive BC subjects at 5.4 and 6.4 mg/kg were 52.6% (20/38) and 55.7% (34/61), respectively. In J101, AEs Grade  $\geq 3$  were reported in 35.6% (16/45) at 5.4 mg/kg and 50% (33/66) at 6.4 mg/kg. The relationship between DS-8201a intact  $C_{min}$  and ORR was statistically significant ( $P=0.035$ ). There was a trend of improved PFS with higher intact exposures ( $P=0.238$ ).

Statistically significant relationships were observed between exposures and the following safety endpoints based on logistic regression: neutropenia (any grade,  $P=0.003$ ; Grade  $\geq 3$ ,  $P=0.037$ ), anemia (any grade,  $P=0.002$ ; Grade  $\geq 3$ ,  $P<0.001$ ), thrombocytopenia (any grade,  $P=0.021$ ), dose reduction due to AE ( $P=0.003$ ), discontinuations due to AE ( $P=0.035$ ), and ILD/pneumonitis (any grade,  $P=0.017$ ). Additionally, Cox proportional hazards modeling suggested higher risk of ILD with higher intact exposures (any grade,  $P<0.001$ ; Grade  $\geq 2$ ,  $P=0.007$ ).

## Conclusions

In J101, DS-8201a demonstrated an acceptable safety profile and high response rates in HER2-positive BC at both doses. The ER analyses showed a statistically significant relationship between exposures and ORR (with a trend for higher PFS at higher doses), as well as exposures and risk of key adverse events. Considering the predicted benefit/risk profile, 5.4 mg/kg is the recommended dose for continued development of DS-8201a in the DESTINY-BREAST01 trial and in phase 3 clinical trials in HER2-positive BC.

# 2018 San Antonio Breast Cancer Symposium ®

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The small molecule inhibitor of HER2, tucatinib, has potent and highly selective activity in preclinical models of HER2-driven cancer

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**Background:** Pharmacologically targeting the HER2 oncoprotein provides clinical benefit for patients with HER2-amplified cancers. However, a significant number of patients do not respond to the currently approved HER2-targeted therapies, despite carrying the HER2-alteration. Small molecule inhibitors of HER2, that target other receptor tyrosine kinases such as EGFR (i.e. lapatinib), are approved and provide some clinical benefit but are often associated with increased toxicity. Tucatinib (ARRY-380) is an orally available, potent, highly selective small molecule inhibitor of the HER2 kinase. In this study, we assessed the *in vitro* and *in vivo* activity of tucatinib, relative to approved HER2-targeting molecules, in a panel of molecularly characterized breast cancer cell lines.

**Materials and Methods:** The growth inhibitory activity of tucatinib, trastuzumab and lapatinib were evaluated in a panel of 48 breast cancer cell lines molecularly characterized at baseline by genomic (array-CGH) and proteomic (Reverse Phase Protein Array; RPPA) profiling. IC<sub>50</sub> values for tucatinib and lapatinib were determined from direct cell counts using a Cellavista Cell Imaging System. Trastuzumab activity was measured as % inhibition of cell growth at fixed concentrations. *In vivo* efficacy of tucatinib was assessed in cell line xenograft models of HER2+/ER- and HER2+/ER+ breast cancers as a single agent or in combination with targeted therapies for breast cancer.

**Results:** A broad range of IC<sub>50</sub> values (3.2nM to >10µM), was seen for tucatinib with a high degree of selectivity for the HER2-amplified sub-type. High levels of total and phosphorylated HER2 (pHER2) accompanied by high levels of pEGFR and pHER3 enriched for sensitivity to tucatinib, confirming that HER2-driven cancers may be uniquely sensitive to tucatinib. The response profile for lapatinib was less clean, with responses also observed in HER2-low/EGFR-high cell lines. Sensitivity to tucatinib was also observed in HER2-amplified cell lines that were either *de novo* or acquired resistant to trastuzumab. Single agent tucatinib induced tumor regressions in a xenograft model of HER2+/ER+ breast cancer. Tumor regressions were further enhanced by combination with trastuzumab. The combination of tucatinib plus trastuzumab was as efficacious and better tolerated than trastuzumab plus docetaxel or trastuzumab plus pertuzumab plus docetaxel. The triple combination of tucatinib plus hormonal blockade (fulvestrant) and CDK4/6 inhibition (abemaciclib) also induced robust tumor regressions, without significant body weight loss.

**Discussion:** These preclinical data highlight the potential of the HER2-selective small molecule inhibitor, tucatinib, to provide benefit to patients with HER2-amplified cancers. Furthermore, our biomarker analysis of response to tucatinib has identified a HER2-driven signature within the HER2-amplified sub-type that selects for sensitivity to tucatinib. Selecting patients based on this profile may further enrich for individuals most likely to benefit from tucatinib-based therapies.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P6-17-12

Neratinib in combination with trastuzumab is superior to each alone and to pertuzumab plus trastuzumab in HER2-positive *in vivo* breast cancer models

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**Background:** Lapatinib (L) plus trastuzumab (T) alone or with endocrine therapy for HER2+/ER+ tumors but without chemotherapy, yielded complete tumor eradication in xenograft models. In neoadjuvant trials (NCT00548184, 00999804, 01973660), a substantial number of patients achieved pathologic complete response with this same strategy. The irreversible pan-HER inhibitor neratinib (N) has been recently approved by the FDA for early stage HER2+ breast cancer and has shown greater potency compared to L in the preclinical setting. However, the therapeutic efficacy of N in combination with T (N+T) and how it compares to pertuzumab (P) +T (without chemotherapy) has not been well studied.

We hypothesize that dual HER2 inhibition using N+T will be highly efficacious and more effective than P+T due to more complete blockade of the HER pathway. Here, we evaluate the therapeutic efficacy of N, P, and T, either alone or in combination, with a primary focus on comparing N+T vs. P+T in established cell line- and patient-derived xenograft (PDX) models.

**Methods:** Athymic nude and SCID/Beige mice bearing BT474-AZ cell line (ER+/HER2+), and BCM-3963 PDX tumors (ER-/HER2+, wild-type *PIK3CA*), respectively were randomized to vehicle, N (20mg/kg, 5 days/week), T (10mg/kg, twice a week), P (6mg/kg, once a week), N+T, or P+T, with simultaneous estrogen (E2) deprivation (ED) in BT474-AZ model. Treatment response was assessed by biweekly tumor measurements. Study endpoints included time to tumor regression (TTR) and progression (TTP) (tumor halving/doubling over baseline, respectively), and the rate and time of complete response (CR and TCR, respectively). Results were analyzed using survival analysis (Kaplan-Meier estimates) and generalized Wilcoxon tests.

**Results:** In the BT474-AZ model, mice treated with E2+vehicle and ED+vehicle showed steady tumor growth, with a median TTP of 8 and 25 days, respectively. While tumor regression was observed in 100% of mice treated with N, P, T, N+T, and P+T, tumors treated with N+T regressed faster compared to P (p<0.001), T (p=0.004), and P+T (p=0.044). Further, N+T was superior to N (p=0.018) and T (p=0.007) alone in achieving accelerated CR. In the BCM-3963 model, tumors treated with vehicle, T, P, and P+T continued to grow with a median TTP of 11, 16, 19, and 17 days, respectively. In contrast, CR was achieved in 100% of N and N+T treated mice. Importantly, combining N with T accelerated the attainment of CR compared to N alone (p=0.026).

Molecular and pathologic analysis of short-term treated tumors in both models to evaluate alterations in HER signaling, cell proliferation, and apoptosis is ongoing.

Model/Treatment	N of mice	Median TTP (Days)	Median TTR (Days)	Median TCR (Days)	CR (%)
<b>BT474-AZ</b>					
E2+Vehicle	9	8	-	-	0
ED+Vehicle	10	25	-	-	0
ED+N	13	-	2	14	100
ED+T	12	-	5	19	100
ED+P	12	-	18	54	92
ED+N+T	13	-	2	10	100
ED+P+T	14	-	4	14	100
<b>BCM-3963</b>					
Vehicle	15	11	-	-	0
N	15	-	4	17	100

T	14	16	-	-	0
P	13	19	-	-	0
N+T	19	-	6	14	100
P+T	16	17	-	-	0

**Conclusions:** Our findings establish the preclinical efficacy of combining N with T for HER2+ breast cancer and warrant further clinical testing to investigate the efficacy of N+T without chemotherapy in the neoadjuvant setting for patients with HER2+ breast cancer.



# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P6-17-13

ZW49, a HER2 targeted biparatopic antibody drug conjugate for the treatment of HER2 expressing cancers

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**Background:** HER2-targeted therapies have transformed the treatment of patients with HER2-expressing breast and gastric cancers. Despite this, there remains a need for new treatments that are well tolerated and effective not only for cancers with high HER2 expression levels but also those with lower levels of expression. ZW49 is an antibody drug conjugate (ADC) that combines a novel auristatin payload (potent anti-cancer agent) with the unique mechanisms of action of the anti-HER2 biparatopic antibody, ZW25, which binds to the same domains as trastuzumab and pertuzumab. In preclinical studies in cancer cell lines with low to high levels of HER2 expression, ZW25 is associated with increased binding and internalization compared to trastuzumab, while the novel *N*-acyl sulfonamide auristatin payload has demonstrated increased *in vivo* tolerability compared to other microtubule inhibitors. ZW49 therefore has the potential to address unmet medical need across a range of HER2-expressing cancers.

**Methods:** Multiple *in vitro* and *in vivo* experiments were performed to characterize ZW49 as a potential therapeutic candidate. Internalization and cell growth inhibition of ZW49 were evaluated in HER2-expressing cell lines. Anti-tumor activity was assessed in patient-derived xenograft (PDX) tumor models of HER2 low and high expressing breast cancers. Tolerability was assessed in a 6-week repeat-dose non-GLP toxicology study in non-human primates (NHP) with intravenous administration of ZW49 at either 9 mg/kg or 12 mg/kg once every two weeks.

**Results:** *In vitro*, ZW49 was more rapidly internalized into HER2-expressing cells compared to a monospecific trastuzumab-ADC. ZW49 also displayed potent *in vitro* cell growth inhibition in several breast cancer cell lines with a range of HER2 expression. This activity was confirmed in multiple *in vivo* PDX models, including a HER2 IHC 3+ HBCx-13b xenograft where two doses of ZW49 at 3 mg/kg or higher generated tumor regressions, and a HER2 IHC 1+ ST-910 xenograft where a single dose of ZW49 at 6 mg/kg or higher generated regressions. In both models, regressions occurred at exposures that were well tolerated in NHP. In a repeat dose pilot non-GLP study the highest dose tested (12 mg/kg) was considered to be the no observed adverse effect level (NOAEL) and a GLP repeat-dose toxicology study was ongoing at the time of abstract submission.

**Conclusions:** ZW49 is a novel biparatopic HER2-targeted ADC that demonstrated anti-tumor activity in low and high HER2-expressing breast cancer cell lines and PDX models. Notably, tumor regressions were observed at exposure levels that were well tolerated in NHP. These results support the potential of ZW49 as a novel therapeutic agent that may help address unmet medical need in patients with high and low HER2-expressing cancers.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-14

Eribulin, trastuzumab, and pertuzumab as first-line therapy for patients with HER2-positive metastatic breast cancer: A multicenter, collaborative, open-label, phase II clinical trial for the SBCCSG-36 investigators

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**Background:** Docetaxel, trastuzumab, and pertuzumab (DTP) therapy is established first-line therapy for patients with HER2-positive metastatic breast cancer (HER2 + MBC). However, the poor tolerability of docetaxel impedes its long-term administration. The safety of eribulin, trastuzumab, and pertuzumab (ETP) therapy for HER2 + MBC has been confirmed in Japan. We examined the primary endpoint—overall response rate, the secondary endpoints—time to treatment failure, progression-free survival, and overall survival, as well as adverse events (AEs) of ETP therapy. (University Hospital Medical Information Network identifier:000021585)

**Methods:** Eribulin 1.4 mg/m<sup>2</sup>/day iv (days 1 and 8), trastuzumab 8 mg/kg iv over 90 min (initial dose) and 6 mg/kg iv over 30 min (second and subsequent doses), and pertuzumab 840 mg/body over 60 min (initial dose) and 420 mg/body over 30 min (second and subsequent doses) were administered. Cycles consisting of 2 doses of eribulin and 1-week drug holiday were repeated. Patients were treated with trastuzumab and pertuzumab when AEs developed that did not allow medication continuation by reducing the dose of eribulin. Antitumor effect was assessed according to RECIST version 1.1. and toxicities to CTCAE Japanese version 4.0. All patients provided written informed consent before enrollment. The study protocol was approved by the Institutional or Central Ethics Committee, and the study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and local ethical and legal regulations.

**Results:** 25 female patients (median age: 57 years [41-75]) were enrolled from April 18, 2016, through November 22, 2017. Twenty-four had performance status (PS) 0, 1 PS 1, 8 stage 4 breast cancer, and 17 metastatic breast cancer. Anthracycline, taxane, and trastuzumab were administered as neoadjuvant and adjuvant pharmacotherapies to 13, 15, and 14 patients, respectively. Primary tumor was positive for estrogen and progesterone receptors in 12 and 6 patients, respectively. Lung, liver, and bone metastases occurred in 9, 9, and 6 patients, respectively. Three (12%), 17 (68%), 1 (4%), 1 (4%), and 2 (8%) patients showed complete response (CR), partial response (PR), long-term stable disease (LSD; stable disease ≥24 weeks), stable disease (SD; stable disease <24 weeks), and progression disease (PD), respectively; 1 (4%) was unassessable because ETP therapy could not be conducted due to the grade 3 infusion reaction of pertuzumab. Major AEs were neutropenia, anemia, fatigue, peripheral neuropathy, alopecia, and anorexia. The overall response rate (CR+PR) was 80.0% (95% confidence interval 59.3-93.2%). The median follow-up was 10.1 months [3.9-21.5]. Six and 3 patients showed tumor deterioration and died of breast cancer, respectively.

**Conclusions:** Similar to DTP, ETP showed high response rates and good safety as first-line therapy for HER2 + MBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-15

Evaluating preclinical efficacy of anti-HER2 drug combinations using ER+/HER2 mutant models

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## Background

Until recently, HER2 gene amplification was the only mechanism of HER2 activation recognized. However, activating HER2 mutations have been noted in different cancer types. A trials of HER2 mutant breast cancer and the subsequent SUMMIT trial data have shown that monotherapy with the pan-HER drug neratinib as showed clinical efficacy, but with poor response durability. This study therefore investigates the preclinical efficacy of anti HER2 agents alone or in combination with endocrine therapy agents or in combination with CDK4/6 inhibitors using ER+/HER2 mutant cell lines and *ex vivo* HER2 mutant patient derived xenograft (PDX) model to define a more effective treatment approach.

## Methods

ER+ breast cancer cell lines (T47D and MCF7) stably expressing HER2<sup>V777L</sup>, and ER+/HER2 mutant PDX model (HER2<sup>G778\_P780 dup</sup>) were used to examine HER2 signaling and drug responses. Signaling downstream mutant HER2 was examined by immunoblot analysis. Effects of neratinib alone, neratinib + fulvestrant, and neratinib + abemaciclib on cell growth were examined in ER+/HER2 mutant cell lines and in an *ex vivo* HER2<sup>G778\_P780 dup</sup>.

## Results

We found that MCF7/T47D cells expressing HER2<sup>V777L</sup> and HER2<sup>G778\_P780 dup</sup> PDX tumors showed strongly activated autophosphorylation of HER2 and increased expression of CDK4, CDK6, phospho-Rb, and cyclin D1 as compared to MCF7/T47D cells expressing HER2<sup>WT</sup> or ER+/non-HER2<sup>mut</sup> PDX modes, suggesting that HER2 mutations preferentially depend on CDK4/6 signaling for cell growth. Additionally, we showed that activating MCF7 HER2<sup>V777L</sup> cause resistance to endocrine therapy treatment (fulvestrant IC<sub>50</sub> >5μM). Further, we show that neratinib alone is effective at higher concentrations (IC<sub>50</sub> < 2μM) in MCF7/HER2<sup>V777L</sup> cells. We also demonstrate that abemaciclib alone exhibited moderate activity against MCF7 HER2<sup>V777L</sup> cells (IC<sub>50</sub> < 0.4μM) and additional activity in combination with neratinib (IC<sub>50</sub> < 0.06μM) was seen. Moreover, *ex vivo* HER2<sup>G778\_P780 dup</sup> cells are relatively resistant to fulvestrant alone (IC<sub>50</sub> < 0.2μM), neratinib alone (IC<sub>50</sub> < 0.006μM), abemaciclib alone (IC<sub>50</sub> < 0.04μM), and neratinib in combination with abemaciclib (IC<sub>50</sub> < 0.005μM), suggesting that patients harboring ER+/HER2-mutant tumors may benefit from neratinib in combination with abemaciclib.

## Conclusion

These preclinical data suggest that neratinib monotherapy may not be effective to treat ER+/HER2 mutant patients and we propose that simultaneous targeting of both HER2 and the CDK4/6 axis will be required for effective treatment of ER+ breast cancers harboring HER2 activating mutations.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-16

Efficacy and safety of shorter duration of adjuvant trastuzumab for patients with HER2 positive early breast cancer: A meta-analysis of randomized controlled trials

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**Background:** Trastuzumab has been shown to be able to improve disease free survival(DFS) and overall survival(OS) in HER2-positive breast cancer patients. Adjuvant trastuzumab is empirically recommended for 1 year as a standard regimen. However, several studies claimed that shorter duration of adjuvant trastuzumab is non-inferior to 12 months treatment with reduced cardiac toxicities and costs.

**Methods:** PubMed, EMBASE, Cochrane Library, Google scholar Web, ISI Web of Science, BIOSIS and CNKI, and major conference abstracts were searched systematically in June 2018 to identify eligible non-inferiority studies comparing the intervention outcomes of adjuvant trastuzumab in chemotherapy for women with HER-2 positive breast cancer between short-term and 1-year treatments. Hazard-Ratios(HR) and corresponding 95% Confidence Intervals(CI) were calculated to compare OS and DFS of trastuzumab between short-term and long-term treatments. Pooled data of Odds-Ratio was analyzed for cardiac toxicities.

**Results:** 5 articles were finally eligible in the study. Totally, there were 11,376 women with HER-2 positive early breast cancer, with 5,684 in short-term group and 5,692 in the 1-year group. We found a distinct difference of DFS (HR=1.19, 95% CI=1.08-1.30) and OS (HR=1.22, 95% CI=1.07-1.39) between short-term and 12 months trastuzumab in the total analysis, which demonstrated short-term treatment exhibited a worsening trend on DFS and OS. Subgroup analysis was performed based on estrogen receptor (ER) and lymph node status, and no statistical interaction could be found( $p=0.12$ ,  $0.52$ , respectively). The two groups with different duration of trastuzumab treatment displayed statistically significant difference for cardiotoxicities, which favored shorter duration(OR=0.54, 95% CI=0.38-0.77).

**Conclusions:** 1-year adjuvant trastuzumab remains the standard strategy for HER2 positive early breast cancer, however, a concomitant higher risk of associated cardiac adverse effects should not be ignored.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-17

Pertuzumab, trastuzumab, and docetaxel for HER2-positive early or locally advanced breast cancer in the neoadjuvant setting: Efficacy and safety analysis of a randomized phase III study in Asian patients (PEONY)

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## Background

Pertuzumab and trastuzumab (P and H; F. Hoffmann-La Roche Ltd, Basel, CH) bind to distinct HER2 subdomains and have complementary modes of anticancer activity in HER2-positive breast cancer (BC). A global Phase II study (NeoSphere) reported that neoadjuvant treatment with P+H+docetaxel (D) significantly increased breast pathologic complete response (bpCR) vs H+D in patients (pts) with early/locally advanced/inflammatory HER2-positive BC (Gianni et al. *Lancet Oncol* 2012). PEONY (NCT02586025), a randomized, multicenter, double-blind, placebo-controlled, Phase III trial conducted in an Asian population (mainland China, Taiwan, Korea, Thailand), primarily compared the efficacy, safety, and tolerability of P+H+D vs placebo (Pla)+H+D in the neoadjuvant setting. We present data from the primary analysis.

## Methods

Pts with centrally confirmed HER2-positive early (T2–3, N0–1)/locally advanced (T2–3, N2 or N3; T4, any N) BC were randomized 2:1 to 4 cycles of P+H+D or Pla+H+D every 3 weeks, before surgery: P, 840 mg loading/420 mg maintenance doses (or Pla); H, 8 mg/kg loading/6 mg/kg maintenance; D, 75 mg/m<sup>2</sup>. Post-surgery, pts received 3 cycles of fluorouracil, epirubicin, and cyclophosphamide followed by 13 cycles of P+H or Pla+H for up to 1 year (total of 17 HER2-targeted therapy cycles). The primary endpoint was total pCR rate (tpCR; absence of any residual invasive cancer in the breast and lymph nodes [ypT0/is, ypN0]) assessed by independent review committee (IRC) when pts completed surgery with a tpCR assessment. Missing/invalid assessments were considered residual disease.

## Results

A total of 329 pts were randomized: 219 to P, 110 to Pla. Baseline characteristics were well balanced. Most pts had early BC (69.6%) and were from mainland China (79.3%). In the intention-to-treat population, the tpCR rate by IRC was 39.3% in the P arm and 21.8% in the Pla arm; a clinically and statistically significant difference of 17.5% (95% CI 6.9–28.0; p=0.0014). The local pathologist-assessed tpCR rates were 39.3% and 20.9%, respectively. A consistent treatment benefit of P vs Pla was observed in subgroups. Incidences of grade ≥3 adverse events (Aes) were 48.6% in the P arm and 41.8% in the Pla arm. Of the most common grade 3 Aes (≥3% of pts), neutropenia was higher in the P arm (38.1% vs 32.7%). Of the most common any-grade Aes (≥5%), diarrhea was higher in the P arm (38.5% vs 16.4%). No heart failure (New York Heart Association Functional Classification III or IV) or significant left ventricular ejection fraction decline events (≥10 percentage points from baseline and to <50%) were observed during neoadjuvant therapy.

## Conclusions

PEONY met its primary endpoint: P+H+D resulted in a clinically meaningful and statistically significant improvement in the tpCR rate by IRC vs Pla+H+D for the neoadjuvant treatment of HER2-positive early/locally advanced BC in Asian pts. Safety data were in line with the known P safety profile and generally comparable between treatment arms. Results were similar to NeoSphere, and confirm that P+H+D provides superior anticancer activity to H+D alone.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-18

Pathologic complete response (pCR) in locally advanced HER2+ (HER2+) breast cancer (BC) treated with anthracycline-free neoadjuvant therapy

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**Background:** Response to neoadjuvant therapy is a predictor of progression-free and overall survival in HER2+. To decrease treatment associated toxicities in patients with HER2+ breast cancers we utilized a non-anthracycline regimen with pertuzumab (pert), trastuzumab (trast), and nab-paclitaxel (nab). Pre- neoadjuvant therapy biopsies were procured to evaluate possible biological predictors of pathologic complete response (pCR).

**Methods:** Women with locally advanced HER2 positive breast cancers were recruited from our breast cancer clinics. After obtaining informed consent for this IRB-approved trial, patients were treated with 6 cycles of pertuzumab (day 1 every 21 days [d]), and weekly trastuzumab 2 mg/kg with and nab-paclitaxel 100 mg/m<sup>2</sup>. Formalin fixed paraffin embedded (FFPE) or frozen biopsies pre-NT and post-NT were collected, along with blood samples at pre-treatment, and at the end of study for correlative analysis.

**Results:** Accrual is complete, with 42 of the 45 HER2+ patients assessed for pCR rate (3 too early to evaluate). The median age was 54 yrs (range 31-77 years). 12 patients were stage 3, 26 stage 2, and 1 stage 1 patient. The pCR rate was 64.2% (27/42), with 73.7% (14/19) in ER/PR negative patients and 56.5% (13/23) in ER/PR positive patients. The initial primary tumor size was similar for in those who achieved pCR and non-pCR patients (mean 4.1 cm vs 3.2 cm, respectively). Most patients required dose modifications. Grade 3 AEs reported included 6 patients with hypertension, 3 patients with hematological AEs, 3 patients with elevated LFTs, and 2 patients with diarrhea.

**Conclusions:** This anthracycline-free regimen in HER2+ BC can achieve promising pCR response rates, with toxicities well-managed with dose modifications. Results of correlative analysis will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-19

What to do with trastuzumab therapy after achieving radiological complete remission (rCR) in HER2+ metastatic breast cancer (MBC)?

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**Intro** MBC is generally considered incurable, but patients with HER2+ disease treated with trastuzumab do relatively well and some have an exceptional durable response and survive over 10 years. We analyzed the clinical-pathological characteristics associated with long-term survival in patients with HER2+ MBC treated with trastuzumab. In addition, we studied the effect of stopping trastuzumab in case of rCR.

**Methods** We included all patients with HER2+ MBC treated with first- or second-line trastuzumab-based palliative therapy between January 2000 and December 2014 in 8 Dutch hospitals (Netherlands Cancer Institute, Erasmus Medical Center, Albert Schweitzer Hospital, Reinier de Graaf Hospital, Amphia Hospital, St. Antonius Hospital, Ikazia Hospital, Haga Hospital). Patients were identified through the Netherlands Cancer Registry and linkage with the institutes' tumor registries. Data was collected from medical records using case record forms. Primary endpoint was overall survival (OS), defined as first-date of MBC until death due to any cause. Kaplan-Meier survival estimates were calculated and multivariable Cox-regression models used to identify prognostic factors for improved survival. Time to progression (TTP) after achieving rCR for patients who continued and stopped trastuzumab and breast cancer specific survival were secondary outcomes.

**Results** We included 744 patients (median age 53, range 24-87). Median follow-up (FU) was 109 months (range 0-178). Clinical factors associated with improved survival in multivariable analyses were single-organ metastases, ER-positivity, no skin or liver metastases, no prior trastuzumab, local therapy of metastatic disease and achievement of rCR. In line with our first single center analyses<sup>1</sup>, achievement of rCR was the strongest predictor of improved survival (multivariable HR 0.30, 95%CI 0.20-0.46). RCR was observed in 71 patients (10%), of whom 60 had been treated with trastuzumab and chemotherapy, 9 with trastuzumab and hormonal therapy, and 2 with hormonal therapy. In patients with rCR the estimated 10-year OS was 53% versus 7% in patients who did not achieve rCR ( $p < 0.001$ ).

Thirty patients stopped trastuzumab after achieving rCR. Median time between onset of rCR and last gift of trastuzumab in these patients was 6 months (0-132). Twenty-one patients (70%) remain in complete remission after a median FU of 75 months (range 54-90) since onset of rCR. Nine patients experienced disease progression after a median time of 14 months (range 9-62) since last gift of trastuzumab. Of these, 8 patients died due to MBC and one again achieved an ongoing rCR. Out of 39 patients who continued trastuzumab after achieving rCR, 12 are in ongoing remission after a median FU of 71 months (range 51-91). In this group median TTP was 14 months (range 5-23).

**Conclusion** Achieving rCR is strongly associated with long-term survival in patients with HER2+ MBC. Seventy percent of patients who stopped trastuzumab after achieving rCR remained in remission, suggesting this can be an attractive approach in selected patients. External validation of these findings is required, however, as well as additional analyses to characterize the patients -and their tumors- who achieved rCR.

1 Steenbruggen, CancerRes 2017

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-21

Matching the critical function of the biosimilar ABP 980 and trastuzumab: Totality of evidence and scientific justification for extrapolation across trastuzumab indications

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## Background

Approval of biosimilars is based on the totality of evidence (TOE) supporting clinical equivalence between the proposed biosimilar and the reference product (RP). TOE comprises comprehensive studies designed to evaluate structural, functional, pharmacokinetic (PK), and clinical similarity. TOE provides scientific justification for possible extrapolation across indications that share a common mechanism of action. ABP 980 has been shown to be biosimilar to trastuzumab using robust analytical and functional assessments. Results from phase 1 and phase 3 studies have shown PK and clinical similarity to trastuzumab. Here, we extend the similarity assessment using multiple orthogonal assays for the primary mechanism of action.

## Methods

Multiple lots of ABP 980, trastuzumab (US) and trastuzumab (EU) were compared in the biological activity assessment. In addition to the primary assessment, we conducted additional characterization assays assessing human epidermal growth factor receptor 2 (HER2) cell binding, inhibition of HER2 signaling, inhibition of HER2 ligand-independent proliferation in gastric cancer cells, and a synergy study conducted with docetaxel chemotherapy in gastric cancer cells. HER2 cell-based binding assays were performed using SKBR-3 target cells and a competitive inhibition format. Inhibition of AKT phosphorylation was assessed by comparing the effect of test samples on basal pAkt in the BT-474 breast cancer line. Inhibition of HER2 ligand-independent proliferation was assessed using the gastric cell line NCI-N87 and specificity of inhibition was evaluated using MCF-7 cells that do not have amplified HER2 expression. The synergy study was conducted using NCI-N87 gastric cancer cells and docetaxel using the Combinatorix™ method.

## Results

Relative cell binding to HER2 was similar (ABP 980, 93%-112%; trastuzumab [EU], 98%-109%; trastuzumab [US], 96%-102%). Synergy scores (mean ± SD) with docetaxel (in NCI-N87 cells) for ABP 980, trastuzumab (US) and trastuzumab (EU), respectively, were 16.1 ± 1.4, 16.4 ± 1.8, and 15.8 ± 1.3. Functionally, ABP 980 and trastuzumab similarly inhibited HER2 ligand-independent proliferation in NCI-N87 gastric cancer cells, exhibited specificity against MCF7 non-amplified HER2 expressing breast cancer cells, and showed similar inhibition of pAkt phosphorylation in BT-474 breast cancer cells.

## Conclusions

In this study, we show similar activity between ABP 980 and trastuzumab RP, either alone or in combination with docetaxel, in both breast cancer and gastric cancer cell lines. Our results add to the TOE supporting the biosimilarity of ABP 980 and trastuzumab, and the justification for extrapolation across its approved indications.



# 2018 San Antonio Breast Cancer Symposium ®

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Progression free survival (PFS) and overall survival (OS) of patients treated with trastuzumab emtansine (T-DM1) after previous treatment with pertuzumab in patients with advanced breast cancer (NCT02338167)

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## Background

Studies leading to the approval of trastuzumab emtansine (T-DM1) have been conducted without pertuzumab as previous therapy. Therefore data about patient characteristics and the efficacy of T-DM1 after a treatment with pertuzumab is scarce. Aim of this study was to analyze a real world patient cohort of advanced breast cancer (aBC) patients, who were treated with T-DM1 after a treatment containing pertuzumab in the metastatic setting with regard to patient characteristics and progression free survival (PFS).

## Methods

The PRAEGNANT metastatic breast cancer registry (NCT02338167) is a prospective registry for metastatic breast cancer patients with focus on molecular biomarkers. Patients of all therapy lines with any kind of treatment are eligible for this registry. Collected data comprises all relevant patient and tumor characteristics, therapies, adverse events, quality of life, patient reported outcomes, response and survival (PFS/OS). Here we report on the patient characteristics and PFS data for HER2 positive patients treated with T-DM1 after a treatment with pertuzumab. Patients had to be included before or at the beginning of the T-DM1 therapy.

## Results

A total of 58 patients could be identified, who were suitable for the analysis. Of those 34 were treated in the second line, 14 in the third line and 10 in the fourth line or higher. Most of the pertuzumab therapies before T-DM1 were conducted in first line (n=46; 79.3%). Median PFS for all patients was 4.8 months (95% CI: 3.0-7.8 months). Median PFS for patients treated in the 3<sup>rd</sup>line and 4<sup>th</sup>line or higher was 4.2 months(95%CI: 2.3 - NA) and 4.0 months (95%CI: 1.8-NA.), respectively. In patients treated 2<sup>nd</sup>line with T-DM1 PFS was 7.7 months (95%CI: 2.8 – 12.2).

## Conclusion

T-DM1 is effective as 2<sup>nd</sup>and further line therapy following pretreatment with pertuzumab. Overall PFS was about 5 months with 7.7 months as 2<sup>nd</sup>-line therapy. The PFS in higher therapy lines might be shorter. As the sample size of this real world cohort was rather low and analyses have to be considered exploratory, this data need to be confirmed in studies with a larger sample size.

# 2018 San Antonio Breast Cancer Symposium ®

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Randomized phase II study of lapatinib plus vinorelbine versus vinorelbine in patients with HER2 positive metastatic breast cancer progressed after lapatinib and trastuzumab treatment

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## Background

The continuum of anti-HER2 agents is regarded as a standard strategy for HER2 positive metastatic breast cancer patients who had progressed disease with anti-HER2 agent- containing treatments. However, there has been lack of data on which agents should be continued and how long continuous anti-HER2 therapies would be effective. This study was aimed to evaluate the efficacy of lapatinib plus vinorelbine in HER2 positive metastatic breast cancer patients who had progressed on both trastuzumab and lapatinib treatments.

## Methods

A total of 149 patients were randomly assigned to lapatinib with vinorelbine (LV) (n=75; lapatinib, 1000mg daily ; vinorelbine 20mg/m<sup>2</sup> D1,D8 q3w) or vinorelbine alone (V) (n=74; 30mg/m<sup>2</sup> D1,D8 q3w). The stratification factors were followings; 1) visceral metastasis, 2) previous response to lapatinib treatment, CR+PR vs. SD  $\geq$  12 weeks. The primary endpoint was progression free survival (PFS) rate at 18 weeks. The secondary endpoints were objective response rate (ORR), PFS, and overall survival (OS).

## Results :

Both arms were well balanced in various clinical factors. The median number of previous anti-HER2 therapies were 2 (range 2-5). There was no significant difference in PFS rate at 18 weeks between LV and V arms (44.0% vs 36.5%, p=0.44). ORR was 19.7% in LV arm and 16.9% in V arm (p=0.881). PFS and OS did not differ between two arms (LV vs V; median PFS, 16weeks vs 12 weeks, HR= 0.86, 95% CI 0.61-1.22, p=0.41; median OS, 15.0 months vs 18.9 months, HR= 1.07, 95% CI 0.72-1.58, p=0.72). In subgroup analysis, there was no difference in PFS and OS between two arms according to previous response to lapatinib (median PFS, CR+PR vs. SD  $\geq$  12 weeks, 12.1weeks vs.17.4 weeks; HR= 1.242, 95% CI 0.881-1.751, p=0.215; median OS, 14.9 months vs. 19.4 months; HR= 1.179, 95% CI 0.797-1.744, p=0.41). Most common adverse events in both arms were neutropenia which was more often observed in V arm (55% vs 73%, p=0.03). Overall, the profiles of adverse events were similar in both arms and all were manageable.

## Conclusion

Lapatinib plus vinorelbine treatment was tolerable, however, it did not demonstrate the clinical benefits compared to vinorelbine alone in HER2 positive metastatic breast cancer patients after progression on both trastuzumab and lapatinib.

# 2018 San Antonio Breast Cancer Symposium ®

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Use of subcutaneous and intravenous trastuzumab: Real-world experience from three hospitals in Sweden

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## Background

Trastuzumab (H; F. Hoffmann-La Roche Ltd, Basel, CH) reduces recurrence and improves survival in patients (pts) with HER2-positive early breast cancer (EBC). Two formulations which are noninferior in terms of pathologic complete response and serum trough concentration (Ismael et al. *Lancet Oncol* 2012) are available, for intravenous (IV) and subcutaneous (SC) administration. A randomized open-label study, PrefHer, reported compelling pt preference for H SC (Pivot et al. *Lancet Oncol* 2013; *Ann Oncol* 2014), and a prospective observational study reported less societal cost and pt time associated with H SC vs H IV in Sweden (Olofsson et al. *Breast* 2016), which may allow for earlier release of pts from hospital. To assess how this may translate to actual practice, we aimed to describe H SC and H IV use in a real-world setting and investigated a potential switch from one formulation to the other in the real world.

## Methods

This observational, retrospective, longitudinal cohort study evaluated electronic medical records of adult female pts with a first incident diagnosis of EBC (neoadjuvant or adjuvant settings) and first use of H from 01/01/10–04/24/18 in three mid-size hospitals in Sweden (Sundsvall, Eskilstuna, and Kalmar). Data from first H administration (index date) until last recorded visit up to the day before metastasis (if any) were analyzed. Combination anticancer treatment was defined as any drug recorded from index date to index date +28 days. A switch between formulations was defined as any H administration using a formulation different from that given at the index date.

## Results

Four hundred twenty-one pts received initial H (median age 60.2 years, range 23.3–88.5). Of these, 260 (61.8%) started treatment with H SC and 161 (38.2%) with H IV. After H SC was introduced in Nov 2013, use of H SC as the initial formulation increased from 72.7% in 2014 to 100% in 2017 (Table). Both H SC and H IV were mostly initiated with a combination IV chemotherapy e.g., a taxane (65.8% vs 74.5%, respectively). Some combination pertuzumab (F. Hoffmann-La Roche Ltd) was observed (2.3% vs 1.9%). Since 2013, 40 of 63 pts (63.5%) switched from initial H IV to H SC; ten (25.0%) switched back to H IV. Four of 260 pts (1.5%) switched from H SC to H IV; three (75.0%) switched back to H SC.

	H SC n=260	H IV n=161
Calendar year of the index date, n (%/year)		
2010	0	21 (100)
2011	0	26 (100)
2012	0	51 (100)
2013	1 (3.7)	26 (96.3)
2014	48 (72.7)	18 (27.3)
2015	55 (79.7)	14 (20.3)
2016	77 (93.9)	5 (6.1)
2017	72 (100)	0 (0)
2018	7 (100)	0 (0)
Combination anticancer treatment, n (% of all)		
Taxanes	171 (65.8)	120 (74.5)

Anthracyclines	5 (1.9)	1 (0.6)
Pertuzumab	6 (2.3)	3 (1.9)
Other (platinum salts, metabolites, other cytotoxic/cytostatic agents)	11 (4.2)	4 (2.5)

## Conclusion

H SC quickly became the prevailing (practically exclusive) formulation for treatment of pts with HER2-positive EBC. Furthermore, most pts who started on H IV switched to H SC, while a minority switched from H SC to H IV. Most pts also received similar combination IV treatment (e.g., pertuzumab or chemotherapy) regardless of H formulation. Results are in line with pt preference for H SC in the PrefHer study, and the body of evidence that oncology units switch to SC formulations of biologics as an integral part of routine care.

# 2018 San Antonio Breast Cancer Symposium ®

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PAM50 and CGH-array genomic characterization of HER2-equivocal breast cancers defined by the ASCO/CAP2013 recommendations and response to treatment

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## Background

HER2 breast cancer status determines patients' eligibility for targeted therapy. *HER2* level of amplification is associated with a better response to anti-HER2 therapy (Arnould CCR 2007, Singer CCR 2017). Benefit of anti-HER2 therapy for equivocal cases remains debated.

## Objectives

We aimed to better characterize HER2-equivocal breast cancers by immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) according to 2013 ASCO/CAP guidelines using PAM50 gene expression-based molecular subtyping. We then investigated genome-wide copy number alterations of HER2-equivocal cases, assessing agreement between genomic alterations of the chromosome (chr) 17 and molecular subtypes.

## Methods

PAM50 (nCounter assay; Nanostring) was performed on RNA from formalin-fixed paraffin-embedded samples of 40 HER2-equivocal cases. These cases were subsequently analyzed by Agilent 60-mer oligonucleotide microarrays for array-based comparative genomic hybridization (aCGH).

## Results

The 40 HER2-equivocal cases were classified as Luminal B in 16 cases (40%), HER2-Enriched in 14 cases (35%), Luminal A in 9 cases (22.5%) and Basal-like in 1 case (2.5%) using PAM50.

By IHC, 34 cases (85%) were ER+, 24 (60%) were also PgR+, 26 (65%) were grade III and 33 (82.5%) showed a high Ki67  $\geq$  20%.

Using aCGH, 10 cases (25%) presented chr 17q large copy number gain, 10 (25%) showed segmental copy number gains including *HER2*, 9 (22.5%) showed *HER2* amplification, one (2.5%) showed a large copy number loss and 10 cases (25%) didn't show any copy number alteration of the chr 17.

Out of the 14 PAM50 HER2-Enriched cases, only 5 (35.7%) showed *HER2* genomic amplification (**Table 1**). Four *HER2* amplified cases at the genomic level were classified as Luminal B (3 cases, Ki67  $\geq$  20%, ER+, PgR- by IHC) or Luminal A (1 case, Ki67<20%, ER+, PgR>10% by IHC) using PAM50, although these luminal B tumors presented strong correlation with the HER2-Enriched centroid. In total, 13 cases (32.5%) were discordant between molecular classification and genomic alteration status of the chr 17.

Among patients with early stage HER2-equivocal breast cancers (n=37), 2 received neo-adjuvant chemotherapy (5.4%), 25 received adjuvant chemotherapy (67.6%) and 2 received adjuvant trastuzumab (5.4%). With a median follow up of 5.8 years (3.8-6.9), one contralateral recurrence (2.7%), four metastatic recurrences (10.8%) and three deaths were observed (8.1%).

## Conclusion

Using PAM50, the majority of HER2-equivocal cases were classified as Luminal tumors.

At the genomic level, HER2-equivocal cases harbored mostly chr 17 segmental or large copy number gains. These results emphasized the need of HER2 status genomic determination. In line with the new ASCO 2018 recommendations intending to decrease the number of cases considered as HER2 equivocal, we showed that these tumors were mainly *HER2* not amplified, ER positive and grade 3. There is no evidence of benefit of anti-HER2 therapy in these cases.

Table 1

Genomic alterations of chromosome 17	Basal-like	HER2-Enriched	Luminal A	Luminal B	Total
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<b><i>HER2</i> amplified</b>	<b>0</b>	<b>5</b>	<b>1</b>	<b>3</b>	<b>9</b>
<b>Large copy number gain</b>	0	<b>1</b>	3	6	10
<b>Segmental copy number gain</b>	1	<b>5</b>	3	1	10
<b>No alteration</b>	0	<b>3</b>	2	5	10
<b>Large copy number loss</b>	0	<b>0</b>	0	1	1
<b>Total</b>	1	<b>14</b>	9	16	40

# 2018 San Antonio Breast Cancer Symposium ®

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Care 001: multi-center randomized open-label phase II trial of neoadjuvant trastuzumab emtansine (T-DM1) in combination with lapatinib and nab-paclitaxel compared with paclitaxel, trastuzumab and pertuzumab in HER2-neu over-expressed breast cancer patients (TEAL study)

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**Background:** We conducted a multicenter, randomized open-label phase II neoadjuvant study of trastuzumab-emtansine (T-DM1), Lapatinib (L) and Nab Paclitaxel (Nab-P) compared to standard of care (SOC) Paclitaxel (Pac), Trastuzumab (T), and Pertuzumab (P) in patients with HER2 over-expressed breast cancer.

**Methods:** Patients in the experimental arm received a biologic window of targeted therapies alone for 6 weeks (T-DM1 and L) followed by T-DM1 3.0 mg/kg Q3W, L 750mg oral daily and Nab-P 80 mg/m<sup>2</sup> weekly (QW) X 12 weeks. Patients in SOC arm received targeted therapies alone for 6 weeks (T and P) followed by Pac 80mg/m<sup>2</sup>QW, T 2mg/kg QW, and P 420mg Q3W X 12 weeks. The primary objective was to evaluate the proportion of patients with residual cancer burden (RCB) 0 or 1. Key secondary objectives included correlative assessments of PIK3CA mutations, PTEN expression, and HER2 subtypes which are being reported.

**Results:** Thirty of the 33 enrolled patients were evaluable. Patient demographics were well balanced. HER2 subtypes and altered PIK3CA (low PTEN or PIK3CA mutations) pathway were not statistically different between both arms. We have previously reported that all patients achieved RCB 0 & I in the T-DM1, L and Nab-P arm, compared to SOC (100% vs. 62.5%, p 0.0035). In the SOC arm, the 6 week change in tumor size on breast MRI during targeted biologic window treatment is significantly different between the responders and non-responders based on two-sided Wilcoxon rank-sum test (p =0.0065).

Table 1: Breast MRI Tumor Size Standard of Care Arm

Response	N	Mean	Standard Deviation	95% CL Mean		Minimum	Maximum
No	6	-0.1333	0.4457	-0.6011	0.3344	-1.0	0.3
Yes	5	2.5800	1.8833	0.2415	4.9185	0.2	4.9

Sixteen patients total were present in standard of care arm but 5 had incomplete imaging data.

Consistent with literature, among ER positive patients treated with SOC, PTEN low expressers were less likely to respond (0%, 0 of 2) than PTEN high expressers (67%, 2 of 3). In the experimental arm, all patients responded regardless of PTEN. There was only 1 PIK3CA mutation on the experimental arm where all responded.

**Conclusions:** TDM1 plus L and Nab-P therapy was well tolerated with noteworthy responses in all patients, including in PTEN low expressers. Change in tumor size at 6 weeks of biologic therapies was significant between responders and non-responders and can be evaluated as a surrogate for future studies.

# 2018 San Antonio Breast Cancer Symposium ®

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Real world experience of the medical and surgical management of HER2 positive early breast cancer treated with neoadjuvant trastuzumab and pertuzumab via the NHS England cancer drug fund

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**Background:** Studies of neoadjuvant (NA) dual HER2 blockade with trastuzumab (T) and pertuzumab (P) in combination with chemotherapy (CT) for early breast cancer (BC) have reported pathological complete response (pCR) rates of 39 to 62%. These studies also report manageable toxicity with diarrhoea reported in up to 73% of cases. To date no real-world studies have explored the efficacy and toxicity of this treatment. The objective of this study was to describe the medical and surgical management of women treated with neoadjuvant T-P in combination with CT (NAT-P-CT). As well as to determine the efficacy toxicity of NAT-P-CT in the context of a routine UK NHS clinical practice.

**Methods:** Patients with HER2+ BC treated neoadjuvantly with T-P accessed via the NHS England Cancer Drug Fund (CDF) at the Clatterbridge Cancer Centre NHS Foundation Trust between October 2016 and January 2018 were retrospectively identified. Clinico-pathological information, treatment data, nurse led toxicity review and echocardiographic were reviewed. Data lock was 19<sup>th</sup> June 2018.

**Results:** 78 female patients were identified with a median age of 50 years (IQR: 44.4-60.2). **At diagnosis:** median tumour size 30mm (23.0-47.5mm), 62% (48/78) were LN positive & 56% (44/78) ER+. **CT regimens:** 81% (63/78) FEC-DHP of these 30% (19/63) switched to weekly paclitaxel (wP). or nab-paclitaxel; 5% (4/78); AC/EC-DHP; 9% (8/78) TCHP with 13% (1/8) switched to wP. At time of analysis, 88% (69/78) had undergone definitive surgery. **Surgical details:** Breast: 52% (36/69) mastectomy & 48% (33/69) WLE, Axillary management: 51% (35/69) axillary dissection (Ax Dx) & 49% (34/69) sentinel node biopsy (4 performed prior to NA treatment). 91% (32/35) of those undergoing Ax Dx were LN+ at presentation, of these 59% (19/32) had no evidence of axillary involvement at surgery. **pCR rate (ypT0/is, N0) was 46% (32/69) [pCR by HR: ER+ 43% (21/49) & ER- 55% (11/20)].** pCR for 20 patients switched to wP was 60% (12/20). 7% (5/69) achieved pCR in the breast alone (in these LN status ITCx1, micrometsx3 & macrometsx1). Of the 54% (37/69) with residual breast tumour median size was 13mm (1-22mm). **Toxicity Data:** Ejection fraction (EF) did not decline beyond 10% of baseline in any patients. Diarrhoea (any grade) occurred in 74% of cases, and CTCAE grade 3-4 toxicity occurring in >2% of patients: diarrhoea, fatigue, and infection. Updated analysis regarding pCR rate and toxicity, as well as initial outcome data will be presented.

**Conclusion:** These results (1) confirm the efficacy of NA T-P in a real world population; (2) support the use of NA wP; (3) indicate significant proportion of patients axilla are downstaged & (4) reveal diarrhoea rates in keeping with the literature. Currently, NHS England rules do not allow wP to be used routinely in NA setting with T-P this should be reviewed in light of these data and those of the BERENICE study. Measures to identify patients who can avoid axillary dissection as well as to mitigate diarrhoea should be considered.



# 2018 San Antonio Breast Cancer Symposium ®

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Outcomes of real-world use of eribulin plus trastuzumab for HER2-positive metastatic breast cancer

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## Background

Eribulin mesylate is approved for the treatment of metastatic breast cancer (mBC) after two prior chemotherapy regimens including an anthracycline or a taxane in either the metastatic or adjuvant setting. Eribulin in combination with trastuzumab (E+T) has demonstrated tolerability and anti-tumor activity in phase I and II trials but is not FDA-approved for the treatment of HER2-positive mBC. Case series and retrospective research have noted the use of E+T in clinical practice. We sought to describe patient characteristics and long-term outcomes of treatment with E+T for HER2-positive mBC patients treated outside of clinical trials in the US.

## Methods

US-based community oncologists from an open network of over 7,000 oncologists, hematologists, and urologists were invited to participate in identifying HER2-positive mBC patients treated with E+T between 01/01/11 and 12/31/13 outside of clinical trials. Data were collected from 03/18/2016 until 09/01/2016. Providers completed an electronic case report form (CRF) by abstracting data on disease characteristics, treatment patterns, disease response (per provider assessment), adverse events (Aes), and date of death. Duration of treatment and overall survival (OS) were calculated from the initiation of the E+T regimen. The target sample size was 60 patients and patients were selected according to resource available for chart data abstraction.

## Results

Twenty-three providers submitted CRFs for 62 total patients. After data collection, 59 of 62 submitted records were validated for analysis. At mBC diagnosis, 69.4% of patients were ER/PR negative and 42.4% of patient had *de novo* stage IV disease. At initiation of E+T, the median age was 57 years and 81.4% were ECOG-OS 0/1. Mean length of follow-up from the initiation of any therapy was 33.6 months. Twenty-two (37.3%) patients initiated E+T as their first- or second-line of treatment; those remaining were in third-line or greater. At initiation of E+T, 72.8% of patients had prior treatment with trastuzumab in combination with chemotherapy, 25.4% had prior trastuzumab in combination with pertuzumab and chemotherapy, and 16.9% had received TDM-1. Mean duration of E+T treatment was 5.2 months (SD=2.4). A response (complete [CR] or partial [PR]) was recorded by the providers for 64.4% of patients (not independently verified). The most common Aes reported were fatigue (67.8%), weakness (50.8%), decreased appetite (28.8%), decreased hemoglobin (27.1%), peripheral neuropathy (25.4%), and neutropenia (18.6%). At the end of the study period, 34 patients (57.6%) were deceased; the median OS from the initiation of E+T was 23.9 months (95% CI: 17.8-30.4).

## Conclusions

In a small cohort of patients treated with E+T in the community setting, the observed response rate of 64.4% (CR+PR) was comparable to that of a prior phase II trial of E+T which reported an ORR with first-line E+T of 71.2% overall, 77.4% among T-naïve and 61.9% in T-pretreated patients. Further research is warranted to examine the tolerability and efficacy of E+T for metastatic HER2-positive breast cancer patients in different treatment settings.

# 2018 San Antonio Breast Cancer Symposium ®

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Clinical characteristics of prolonged and exceptional responders to HER2 targeted therapy in metastatic breast cancer

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**Background:** The evolution of HER2 targeted therapies continue to improve outcomes for patients with HER2-positive (HER2+) metastatic breast cancer (MBC). Although most patients progress after a relatively short duration, some experience prolonged (PRO) or exceptional (EXC) response to anti-HER2 therapy. However, these are usually described in single case-reports, with larger cohorts being poorly characterized in the literature.

**Objectives:** To evaluate the clinical characteristics of HER2+ MBC patients experiencing prolonged or exceptional response to HER2 targeted antibody (Ab) therapy.

**Methods:** Patients receiving HER2 Ab therapy for HER2+ MBC at Princess Margaret Cancer Centre in Ontario, or in the province of Alberta (AB), Canada were evaluated. HER2 therapies were categorized by treatment era. Dates were selected based on provincial approval(s) of Ab therapies and included: chemotherapy plus trastuzumab (CT) for 2005-2013; and taxane plus trastuzumab-pertuzumab (THP), or trastuzumab-emtansine (TDM1) for 2012-2016. Total time on HER2 therapy (without treatment switch) was recorded regardless of treatment line. Patients were then selected using median duration of response (MDR) seen in respective phase II/III clinical trials and identified as having a PRO (2 x MDR) or EXC (3 x MDR) response. Clinical characteristics (ie: pathology, survival, treatment regimen/duration, sites of metastatic disease) and oncologist/radiologist reported or estimated best response (complete [CR] or partial [PR] response, or stable disease [SD]) was collected. Descriptive statistics, Kaplan-Meier method (survival) and, one-way ANOVA with post-hoc Tukey (comparative) were used.

**Results:** From 2005-2013, 1,830 patients (PMCC, n=651 AB, n=1179) received CT. During 2012-2016, 394 patients (PMCC, n=70; AB, n=324) received THP, and 179 (PMCC, n=40; AB, 139) received TDM1. Selection of PRO (CT=18.2 months [mths]; THP=40.4 mths; TDM1=25.2 mths) and EXC (CT=27.3 mths; THP=60.6 mths; TDM1=37.8 mths) responders using MDR criteria identified 75 (4%) patients as having a PRO (n=20) or EXC (n=55) response to CT. In the THP cohort, 35 (9%) patients had a PRO (n=33) or EXC (n=2) response, whereas in TDM1, 20 (11%) patients had a PRO (n=18) or EXC (n=2) response. Evaluation of best response for historically first-line CT (CR=30%, PR=45%, SD=25%) and current standard of care THP (CR=31.4%, PR=42.9%, SD=25.7%) showed high rates of PR and CR. Lower rates of CR were seen with TDM1 (CR=5%, PR=55%, SD=40%). In the CT cohort, more patients with a CR vs SD were ER/PR-negative ( $p<0.05$ ); also more CT patients with a CR vs PR ( $p<0.05$ ) or CR vs SD ( $p<0.01$ ) were still alive. Median PFS was higher in the CT (PFS=3.0 years, 95%CI: 2.07-3.93;) than TDM1 cohorts (PFS=2.4 years, 95%CI: 1.80 – 2.98), and not reached in the THP cohort.

**Conclusion:** Small numbers of patients experience a PRO or EXC response to HER2 Ab therapies. Patients treated in the first-line setting are more likely to experience a CR. Patients treated with CT having a CR are more likely to be ER/PR-negative and have improved survival. Further studies are ongoing.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-30

Changes in patient, tumor, and treatment characteristics over time in first-line trastuzumab plus taxane (paclitaxel/docetaxel) arms in HER2-positive metastatic breast cancer trials

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## Background:

A retrospective analysis showed improved progression-free survival and consistent underestimation of trastuzumab (H) + taxane (T) efficacy assumptions in first-line (1L) human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer (MBC) trials over time (ISCB 2016). We evaluated changes in baseline and treatment characteristics and the safety profile of H+T in 1L HER2-positive MBC trials conducted during 1995–2015.

## Methods:

Trial-level data on patient, tumor, and treatment characteristics and safety profiles in H+T arms of 15 randomized trials were extracted from published data and clinical study reports. Characteristic and safety profile changes over time were explored by weighted regression; relevance was assessed by F-test.

## Results:

Patient and tumor characteristics were generally stable over time. We observed relevant changes in types of adjuvant therapy used. Serious adverse events (SAEs) and study withdrawals due to AEs decreased over time; rates of treatment discontinuation due to AEs increased.

	No. of trials (patients)	Difference in patients with characteristic per 5 years, % (95% CI)	P value
<b>Baseline characteristics</b>			
Median age	15 (2895)	0.5 (0.1, 0.9)*	.28
ECOG status 0	12 (2472)	0.2 (-1.1, 1.6)	.87
Hormone receptor-positive	11 (2124)	1.3 (-1.1, 3.6)	.61
HER2 IHC 3+	8 (1657)	5.8 (3.0, 8.6)	.08
Visceral disease	10 (1970)	0.5 (-2.5, 3.6)	.86
<b>Adjuvant therapy</b>			
Hormonal therapy	11 (2194)	-10.1 (-12.4, -7.8)	.002
Radiotherapy	9 (2010)	-4.2 (-5.8, -2.6)	.035
HER2-targeted therapy	9 (2176)	20.2 (13.0, 27.4)	.026
Chemotherapy	15 (2893)	-7.0 (-9.2, -4.7)	.008
<b>Safety</b>			
Grade 3+ AE	6 (1342)	-15.4 (-28.4, -2.5)	.30
SAE	7 (1462)	-12.6 (-15.4, -9.8)	.006
Study withdrawal due to AE	8 (1525)	-6.8 (-9.0, -4.5)	.023
Treatment discontinuation due to AE	6 (1370)	11.5 (6.7, 16.3)	.075

\*Years (95% CI). AE, adverse events; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; IHC, immunohistochemistry; SAE, serious adverse events.

## Conclusions:

In 1L HER2-positive MBC trials, the percentage of patients who received adjuvant chemo-, hormonal, and radio-therapy decreased from 1995 to 2015. These changes in treatment patterns suggest a shift towards a higher percentage of patients with *de novo* metastatic vs recurrent disease in 1L MBC trials over time. Adjuvant H use increased following approval in 2005 as expected. While SAEs decreased over time, no firm conclusions on safety management can be made.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-31

Real world evidence of neoadjuvant treatment based on dual blockade with pertuzumab plus trastuzumab for early HER2+ breast cancer: The NeoPETRA study

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**Background:** Evidence from clinical trials supports the neoadjuvant use of pertuzumab and trastuzumab with chemotherapy to improve the pathologic complete response (pCR) in early HER2+ breast cancer (BC), but information on the efficacy of this combination in the clinical practice setting is still limited. In order to widen its real-world data (RWD), we conducted an observational study to determine the pCR rate obtained in routine clinical practice.

**Methods:** This was a multicenter, retrospective, observational study in patients (pts) with early HER2+ BC who received neoadjuvant therapy based on dual blockade with pertuzumab and trastuzumab. All data were retrospectively collected from pts medical charts. The primary study objective was the total pCR rate (ypT0/is ypN0). Secondary objectives included patient characteristics, neoadjuvant treatment patterns with the dual blockade, surgery types, breast pCR rate (ypT0is), and neoadjuvant safety profile.

**Results:** Two hundred and forty-three evaluable pts treated in Spain between November 2013 and September 2017 were enrolled between October 2017 and February 2018 (median age [range] 51.7 [44.7-60.2] years; postmenopausal 47.7%; ECOG 0/1 88.9/10.6%; stage II/III tumors 54.3/28.7%; hormone receptor-positive 62.4%). The most common neoadjuvant chemotherapies used were taxanes+anthracyclines (n=180, 74.1%), platinum-based chemotherapies (n=35, 14.4%), and taxanes (n=27, 11.1%). Response rates (radiologic/clinical assessments) before surgery were: complete response 62.1% (n=151), partial response 33.3% (n=81), stable disease 4.1% (n=10), and non-evaluable 0.4% (n=1). Breast-conserving surgery (tumorectomy/lumpectomy and quadrantectomy) was performed in 57.8% (n=133) pts and not conserving (simple, radical and modified radical mastectomy) in 42.2% (n=97). The total pCR was 66.4% and breast pCR was 67.6%. Total pCR in the breast was achieved by 80.7% (n=67) hormone receptor-negative and 56.3% (n=76) hormone receptor-positive pts (statistically significant differences; p<0.001). According to neoadjuvant treatment, total pCR in the breast was achieved by 71.0% (n=120) taxanes+anthracyclines, 48.6% (n=17) platinum-based chemotherapies, and 59.3% (n=16) taxanes users (statistically significant differences; p=0.028). Sixty-three (35.0%) pts experienced at least one adverse reaction (AR) to pertuzumab/trastuzumab+taxanes+anthracyclines (grade 3 ARs: asthenia 0.6%, diarrhea 2.2%, febrile neutropenia 1.1%, and neutropenia 1.1%; grade 4 AR: neutropenia 0.6%), 3 (8.6%) to pertuzumab/trastuzumab+platinum-based chemotherapies, and 22 (11.1%) to pertuzumab/trastuzumab+taxanes (grade 3 AR: diarrhea 3.7%).

**Conclusions:** This is the first, to the best of our knowledge, multicenter observational study to determine the pCR rate obtained in routine clinical practice in Spain. Results obtained from it have been remarkable: neoadjuvant therapy based on dual blockade with pertuzumab and trastuzumab for HER2+ BC in the routine clinical practice setting enabled the achievement of total pCR rates over 66%, which are even higher than those obtained in previous clinical trials. In addition, this therapy has showed an acceptable safety profile, in line with previous assessments.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-32

Impact of the line of treatment on progression-free survival in patients treated with T-DM1 for metastatic breast cancer

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**Background** Trastuzumab emtansine (T-DM1) is a monoclonal antibody targeting human epidermal growth factor receptor 2 (HER2) receptors, conjugated with a cytotoxic component (a microtubule inhibitor). It is indicated as second-line treatment for HER2-positive metastatic or unresectable locally advanced breast cancer, after progression on trastuzumab and a taxane-based chemotherapy.

In HER2-negative metastatic breast cancer, progression-free survival (PFS) declines with each line of therapy, while patients with HER2-positive disease receive the most lines of chemotherapy and the longest duration for every line. We wished to investigate whether the line of treatment in which T-DM1 is administered has an impact on PFS. We also wished to explore whether prior treatment with capecitabine / lapatinib or pertuzumab had an impact on PFS, as none of the patients included in the registration trial had received these treatments before their inclusion in the trial.

**Methods** This is a multicenter retrospective study performed in 3 Belgian institutions. All patients received T-DM1 for HER2 positive metastatic or unresectable locally advanced breast cancer. The primary outcome was PFS with T-DM1, defined as the period between the first administration of T-DM1 and the first radiological or clinical assessment demonstrating progression of the disease.

**Results** We included 51 patients. One patient had to be excluded from the analyses because she no longer had HER positive disease. The median PFS was 9.01 months. The line of treatment in which T-DM1 was administered had no influence on PFS (hazard ratio 0.976, CI95 0.835-1.142).

There was no statistically significant difference in PFS between patients who had not received capecitabine / lapatinib before T-DM1 and those who had (9.11 vs 8.91 months, p-value 0.466).

Median PFS was 10.07 months when T-DM1 was administered prior to pertuzumab, and 5 months when administered after pertuzumab. Patients who received pertuzumab before T-DM1 thus tended to exhibit a shorter PFS. However, this difference is not statistically significant (p-value 0.096).

**Conclusions** Unlike with conventional chemotherapy, the line of treatment in which T-DM1 is administered does not influence PFS in metastatic breast cancer patients.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-33

A multi-scale mathematical model of combination targeted and cytotoxic therapy to evaluate treatment response in HER2+ breast cancer

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**Introduction:** We have previously established a tissue scale mathematical model with 5 coupled, ordinary differential equations (ODEs) describing the longitudinal relationship of vasculature, hypoxia, necrosis, immune infiltration, and tumor growth in a preclinical model of human epidermal growth factor receptor 2 positive (HER2+) breast cancer undergoing trastuzumab treatment. The purpose of this study is to expand this model to multiple scales (tissue and cellular) and to explicitly include the effects of combination trastuzumab-paclitaxel therapy as measured by time-resolved microscopy of *in vitro* HER2+ breast cancer cells. This requires interlacing experimental data and additional ODEs at the cellular scale to incorporate drug dynamics within the tissue scale model for overall tumor growth. An integrated multi-scale mathematical-experimental approach bridging *in vivo* and *in vitro* experimental data has potential to elucidate the optimal strategies for combination therapy for HER2+ breast cancer.

**Experimental:** *In vitro* data was collected to longitudinally evaluate BT474 HER2+ human cancer cells growth when treated with trastuzumab (25-50 ug/ml) and/or paclitaxel (10-250 nmol/L) measured by time-resolved microscopy. Changes in confluence before and after treatment are recorded every 3 hours over the course of 7 days. Ongoing studies are quantifying the change in confluence for these cells with alternating dosing and timing of the two therapies.

**Modeling:** The *in vivo*, tissue-scale model parameters were calibrated using mean and 95% confidence intervals of tumor volume from caliper measurements, vasculature and hypoxia from imaging data, and necrosis from histology. The *in vitro* scale of the model is calibrated with confluence data for controls to define intervals for growth rates and carrying capacities. Experimental results for single drug applications are used to estimate cell death, and data from combinations of both the targeted anti-HER2 therapy and cytotoxic therapies provide estimates of synergistic drug effects. After calibration of parameters, the two scales of the model are coupled *via* the tumor volume and vasculature components to simulate the effects of combination therapy *in vivo*.

**Results and Discussion:** The confluence data shows that trastuzumab dosing in combination with paclitaxel results in the greatest reduction in tumor cells than either drug alone ( $p < 0.05$ ), and there are distinct differences in alternating the order and timing of these drugs. Further, preliminary results show that the model's *in vitro* scale equations can be calibrated with the available longitudinal data. As dosing of combination therapy *in vitro* provides the opportunity to quantify the effects of combination therapy that would not be feasible to collect in an *in vivo* setting, our aim is to generate experimentally testable predictions for improved combination therapy *in vivo* with our multiscale model. This is an important step for future clinical translation to provide temporal guidance of standard-of-care, combination therapies, potentially leading to significantly improved anticancer response in HER2+ breast cancer.

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# 2018 San Antonio Breast Cancer Symposium ®

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Clinical analysis of lapatinib in combination with capecitabine versus continued use of trastuzumab in advanced HER2-positive breast cancer patients with trastuzumab-resistance

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## Purpose

Trastuzumab-refractory patients will derive clinical benefit from further anti-HER2 therapy. It is unclear whether we should adopt empiric continuation of trastuzumab beyond progression. We evaluate the efficacy and safety of lapatinibplus capecitabine (LC orLX) versus trastuzumab plus chemotherapy in patients with HER-positive metastatic breast cancer who were resistant to trastuzumab.

## Patients and methods

We retrospectively analyzed breast cancer patients linked to detailed demographic, treatment,outcome data who began the regimen of lapatinibplus capecitabine (LC or LX) or trastuzumab beyond progression (TBP) at eight hospitals between May 2010 and October 2017.Among those, 299 patients had received TBP and 255 were treated with LX. The clinicopathologic parameters explored included age, hormone receptor status, metastatic sites, primary or acquired trastuzumab resistance,previous treatment. Primary endpoint was evaluation of progression-free survival (PFS), key secondary endpoints were overall response rate (ORR), clinical benefit rate (CBR).

## Results

Among 554 patients who had developed resistance to trastuzumab, 255 in the LX group and 299 in the TBP group. The median PFS was 6.77 months in the LX group compared with 5.6 months in the TBP group (hazard ratio 0.7955; 95% CI, 0.6632 to 0.9542; log-rank  $P=0.014$ ). In the primary resistant patients, the median was significantly increased from 4.3 months for TBP to 6.8 months for LX (HR=0.4986; 95%CI, 0.3632 to 0.6844;  $P<0.001$ ). In the secondary resistant patients, no significant difference was observed (median PFS: 6.6 months for LX vs 6.3 months for TBP,  $P=0.883$ ). The central nervous system progression rate during treatment was 5.9% in LX group and 12.5% in TBP group, respectively( $P=0.018$ ). The most frequent grade III-IV AEs diarrhoea (5.1%), hand-foot syndrome (10.2%)in LX group,and ALT/AST increased (9.1%) and neutropenia (6.4%) in TBP group.

## Conclusion

The combination of lapatinib and capecitabine has shown a prolonged PFS compared with TBP in patients who had progressed on trastuzumab.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-36

Consensus and disagreement among experts for treatment of patients with HER2+ early-stage breast cancer suggests unmet need for online decision support tool

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Treatment (tx) choices for HER2+ early stage breast cancer (EBC) have become increasingly complex. Clinicians and patients must decide 1) which chemotherapy and HER2-targeted agents to use, 2) the sequence of surgery and chemotherapy: either neoadjuvant (neoadj) or adjuvant (adj) tx, and 3) whether to shorten or extend maintenance HER2-targeted tx.

As tx options expand, so does the need for online decision aids. One online decision support tool was developed in 2015 to provide specific tx recommendations for pts with EBC and showed that community healthcare providers (HCPs) did not consistently align with experts for neoadj or adj tx of many pts with EBC (SABCS 2015 Abs P5-09-04).

This study includes analysis of neoadj and adj tx practice patterns of 5 breast cancer experts based on their tx recommendations for 270 unique HER2+ EBC case scenarios made for development of a 2018 online decision tool. We aim to compare these recommendations with the intended treatment of clinicians using the tool.

## Results

Experts agree on neoadj tx approaches: initial surgery, no neoadj tx for pts with cT1a/b N0 tumors; neoadj tx before surgery for pts with  $\geq$ cT2 or N+ tumors. There was disparity among experts for pts with cT1c N0 disease: 3 experts recommend neoadj TCH $\pm$ P and 2 recommend proceeding directly to surgery.

Experts generally recommend adj TCHP for pts with stage II N+ or higher HER2+ EBC who did not receive neoadj tx. In addition, 5/5 experts would consider extended adj tx with neratinib for these pts if HR+ and 2/5 experts would also consider neratinib if HR-.

In pts who received neoadj chemo+HER2 tx, post-surgery management depends on response to neoadj tx. For pts with pCR, 5/5 experts generally agree on continuing H+P if both were given as neoadj tx or H alone if only H given as neoadj tx for a total of 1 yr of anti-HER2 Ab tx and 2/5 experts would consider extended adj tx with neratinib for HR+ disease. For pts with residual disease, experts would recommend continuing H+P if both were given as neoadj tx and most would add P for subsequent adj tx if H alone was given to complete a total of 1 yr of anti-HER2 Ab tx (Table1). All experts would consider extended adj tx with neratinib for HR+ disease and 3/5 experts would also consider neratinib for HR- disease. None of the experts recommended less than 12 mos of adj HER2-targeted tx.

We will present analyses of cases entered into our online tool and detailed comparisons of expert and the intended treatment of clinicians using the tool.

## Conclusions

Practice patterns are changing rapidly and are more complex in response to the evolving treatment landscape for HER2+ EBC. This analysis highlights several areas of expert consensus; however, disparities remain for select cases. The current tool addresses an unmet medical need for expert-led evaluation of HER2+ EBC tx choices and warrants further investigation.

Expert Recommendations: Initial Adj HER2 Ab Tx After Neoadj Tx With H Alone

	Experts				
Response	1	2	3	4	5
pCR (HR-)	H	H	H	H	H
pCR (HR+)	H	H	H	H	H
ypT1a-c N0 (HR-)	H + P	H	H + P	H + P	H + P

ypT1a-c N0 (HR+)	H + P	H	H	H + P	H
ypT2 N0 (HR-)	H + P	H + P	H + P	H + P	H + P
ypT2 N0 (HR+)	H + P	H + P	H	H + P	H + P
ypTany N+ (HR+ or HR-)	H + P	H + P	H + P	H + P	H + P

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-17-37

Therapy landscape of patients with metastatic, HER2 positive breast cancer - Data from the real world breast cancer registry PRAEGNANT (NCT02338167)

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## **Purpose:**

This analysis describes comprehensive real-world data concerning the use of anti-HER2 therapies in HER2 positive metastatic breast cancer (MBC). Specifically, it describes the therapy patterns of treatments with trastuzumab (TzM), pertuzumab+trastuzumab (PTZ/TzM), lapatinib (LAP) and trastuzumab emtansine (T-DM1).

## **Methods:**

The PRAEGNANT study is a real-time, real-world registry for patients with MBC. Patients can be registered for PRAEGNANT at any time during the course of their metastatic disease and are followed up until death. All therapy lines are documented. This analysis presents the utilization of anti-HER2 therapies as well as therapy sequences.

## **Results:**

Of 1936 patients within PRAEGNANT at the time of database closure 451 were HER2 positive (23.3%). Within the analysis set (417 patients after an unilateral breast cancer diagnosis), of which 53% were included in PRAEGNANT in the 1<sup>st</sup> line setting, 241 were treated with TzM (58%), 237 with PTZ (57%), 85 with LAP (20%) and 125 with T-DM1 (30%) during the course of their therapies. The sequence PTZ/TzMàT-DM1 was given to 51 patients (12%). Worse ECOG, negative hormone receptor status, and visceral or brain metastases were associated with a more frequent use of this therapy sequence. Most patients received T-DM1 after a therapy with pertuzumab.

## **Conclusions:**

Both novel therapies (PTZ/TzM and T-DM1) are utilized in a high proportion of HER2 positive breast cancer patients. As most patients receive T-DM1 after pertuzumab real world data might help to understand whether this sequence has similar efficacy like in the approval study.

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Clinical and pathological features of breast cancer with 'polysomy' of Chromosome 17

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**Background:** Anti-HER2 therapy is a standard of care for patients with HER2+ breast cancer. HER2 status is routinely evaluated using immunohistochemical stain and/or florescence *in situ* hybridization (FISH). Interpretation of FISH results may be challenging in tumors with 'polysomy' of chromosome 17 ('polysomy'17), which is defined by increased chromosome enumeration probe 17 (CEP17) signal number. There is evident that 'polysomy'17 might account for anti-HER2 therapy response in breast cancer (BC) with normal *HER2*/CEP17 ratio. This study aims to study the clinical and pathological features of BC with 'polysomy'17. **Method:** Primary BC were selected based on elevated CEP17 count ( $\geq 3.0$ ) in *HER2* FISH performed at MD Anderson Cancer Center between April 2014 and March 2018 (n=385). Patient charts were reviewed for detailed clinical and pathological features. These cases were further divided into four groups according to *HER2*/CEP17 and *HER2* copy number, based on ASCO-CAP guidelines: group 1 (HER2+) – *HER2*/CEP17 $\geq 2.0$ , *HER2* copies $\geq 4.0$ ; group 3 (HER2+) – ratio $<2.0$ , *HER2* copies $\geq 6.0$ ; group 4 (HER2 equivocal) – ratio  $<2.0$ , *HER2* copies  $\geq 4.0$  and  $<6.0$ ; group 5 (HER2-) – ratio $<2.0$ , *HER2* copies $<4.0$ . Chi-square tests were performed to study the difference of these characters. **Results:** In comparison with groups 1 and 3, BC in groups 4 and 5 are more commonly seen in elder patients (p=0.001). Also, these tumors show higher pathological category (p<0.001) and higher rate of lymph nodes metastasis (p<0.05). The clinical and pathological features are summarized in [Table 1]. **Conclusion:** Understanding the clinical and pathological features of BC with 'polysomy'17 may help clinically to choose patients who might be benefit from anti-HER2 therapy. Further study is to follow up the therapy response of those who received anti-HER2 treatment in this cohort of patients.

Clinical and pathological characteristics of breast cancer with 'polysomy' of chromosome 17

		Total (N=385)	Group 1 ( N=131)	Group 3 (N=25)	Group 4 (N=69)	Group 5 (N=160)	P value
Age (year)	Mean (range)	56.8 (25-94)	53.9 (25-83)	52.8 (26-75)	58 (33-92)	59.3 (29-94)	0.001
Race	Black (%)	37 (9.6)	14 (10.7)	4 (16)	5 (7.2)	14 (8.8)	0.873
	Hispanic (%)	61 (15.8)	23 (17.6)	3 (12)	12 (17.4)	23 (14.4)	
	White (%)	251 (65.2)	82 (62.6)	14 (56)	44 (63.8)	111 (69.4)	
	Other (%)	36 (9.4)	12 (9.1)	4 (16)	8 (11.6)	12 (7.4)	
Gender	Female (%)	383 (99.5)	131 (100)	25 (100)	68 (98.6)	159 (99.4)	0.478
	Male (%)	2 (0.5)	0 (0)	0 (0)	1 (1.4)	1 (0.6)	
Histology type	IDC, NOS (%)	350 (90.9)	121 (92.4)	20 (80)	66 (95.7)	143 (89.4)	0.471
	ILC (%)	9 (2.3)	3 (2.3)	1 (4)	0 (0)	5 (3.1)	
	Others (%)	26 (6.8)	7 (5.3)	4 (16)	3 (4.3)	12 (7.5)	
Nuclear grade	I (%)	4 (1.2)	2 (1.8)	0 (0)	1 (1.8)	1 (0.7)	0.044
	II (%)	122 (37)	30 (26.3)	12 (50)	19 (33.3)	61 (45.5)	
	III (%)	203 (61.8)	82 (71.9)	12 (50)	37 (64.9)	72 (53.8)	
	NA	56	17	1	12	26	
Pathological tumor category	pT0+Tis (%)	61 (21)	36 (36.3)	3 (20)	5 (10.9)	17 (13.1)	<0.001
	pT1 (%)	125 (43.2)	39 (39.4)	7 (46.7)	20 (43.5)	59 (45.4)	
	pT2+T3+T4 (%)	104 (35.8)	24 (24.3)	5 (33.3)	21 (45.6)	54 (41.5)	
	NA	95	32	10	23	30	

Pathological lymph node category	pN0 (%)	187 (65.4)	81 (82.7)	11 (73.3)	22 (47.8)	73 (57.5)	0.048
	pN1+N2+N3 (%)	99 (34.6)	17 (17.3)	4 (26.7)	24 (52.2)	54 (42.5)	
	pNx (%)	99	33	10	23	33	

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BAT8001, a potent anti-HER2 antibody-drug conjugate with a novel stable linker for the treatment of HER2-positive breast cancer

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Overexpression of HER2 occurs in approximately 20% of breast cancers and is associated with shortened survival. Trastuzumab emtansine (T-DM1), an anti-HER2 ADC, has shown efficacy in HER2-positive breast cancer patients and was approved by the FDA and EMA for advanced HER2-positive breast cancer. However T-DM1 causes grade 3 and 4 thrombocytopenia in up to 14.5% of patients as its major toxicity. The thrombocytopenia is likely caused by one of T-DM1's catabolites and payload, DM1, indicating T-DM1's linker can be cleaved. Here we adopted a novel noncleavable linker and created an anti-HER2 ADC, BAT8001, which is expected to be efficacious in HER2-positive breast cancer and have a better side effect profile relative to T-DM1 due to the stability of BAT8001's noncleavable linker. BAT8001 is internalized in HER2-positive cancer cells. It inhibits proliferation of HER2-positive tumor cells with IC<sub>50</sub>s of ~0.1 nM, similar to the potency of T-DM1. BAT8001 also induces apoptosis in HER2-positive cancer cells. In both cell-line and patient-derived mouse xenograft (PDX) models, BAT8001 demonstrates strong inhibition activity on tumor growth. For example, in a cell-line model of breast cancer (BT474), BAT8001 demonstrates potent activity with complete responses in all animals tested at the 15mg/kg dose level. Pharmacokinetics studies in monkey reveal BAT8001 has similar C<sub>max</sub>, AUC, and t<sub>1/2</sub> as T-DM1. The major catabolite of BAT8001 is the Cys-linker-payload containing product. No free payload is observed. This compares favorably with T-DM1 where free DM1, T-DM1's payload, is one of the major catabolites. In a multiple dose toxicity study, BAT8001 had a NOAEL of 15 mg/kg versus 10 mg/kg for T-DM1. BAT8001 exhibits similar potency to T-DM1 on inhibiting HER2-positive cell proliferation and tumor growth, yet demonstrates better multiple dose toxicity than T-DM1. The improved toxicity profile of BAT8001 suggests that the novel noncleavable linker utilized in BAT8001 is more stable than the linker utilized in T-DM1. BAT8001 is very efficacious in cell-line xenograft models of breast cancer. The preclinical profile of BAT8001 warrants further development for the treatment of breast cancer and other HER2-positive cancers.

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Treatment benefit of ribociclib + letrozole in patients with *de novo* disease and visceral metastases from the MONALEESA-2 study

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**Background:** Ribociclib (ribo) + letrozole (LET) demonstrated efficacy and safety in first-line treatment of patients (pts) with hormone receptor–positive (HR+), HER2– advanced breast cancer (ABC). Most endocrine therapy (ET)–naïve pts with *de novo* ABC respond to ET, but first-line monotherapy with ET or fulvestrant elicited only moderate efficacy in the FALCON study of pts with visceral metastases (mets). Visceral mets are associated with poor prognosis and may warrant novel treatment options in addition to ET. Here, we present clinical outcomes of treatment with ribo in pts with *de novo* ABC and visceral mets.

**Methods:** In the MONALEESA-2 study, postmenopausal women with HR+, HER2– ABC (N=668) were randomized (1:1) to receive either ribo 600 mg/d or placebo (3 wk on/1 wk off) + LET 2.5 mg/d (continuous). The primary endpoint was median progression-free survival (mPFS).

**Results:** All pts in this analysis (n=227) had *de novo* ABC; 108 pts had visceral mets (ribo, n=53; placebo, n=55). Baseline characteristics were similar between the ribo and placebo groups. The median follow-up time was 26.4 months. In pts with visceral mets, mPFS was significantly higher in the ribo group (30.3 months) than in the placebo group (14.1 months; hazard ratio, 0.52; 95% CI, 0.295–0.905; *P*=0.019). Rates of PFS in these pts in the ribo vs placebo group, respectively, were 76% vs 60% at 12 months and 60% vs 36% at 24 months. Among the pts with visceral mets, clinical benefit rate (CBR) was 79% vs 71% in the ribo vs placebo group, and overall response rate (ORR) in pts with measurable disease at baseline was 55% vs 46%, respectively. In pts without visceral mets (ribo, n=61; placebo, n=58), PFS rates in the ribo vs placebo group, respectively, were 87% vs 72% at 12 months and 66% vs 51% at 24 months. Safety results were consistent with the overall population, and responses were durable with ribo + LET irrespective of whether the pts had visceral or nonvisceral mets.

**Conclusions:** Clinical outcomes from this analysis of the MONALEESA-2 study are consistent with those of the overall population, and ribo + LET prolonged the mPFS and improved ORR and CBR in pts with *de novo* ABC with visceral mets vs placebo + LET.

# 2018 San Antonio Breast Cancer Symposium ®

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Primary and secondary results of the first nationwide molecular screening program in Spain for patients with advanced breast cancer (AGATA SOLTI-1301 study)

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**Background:** Metastatic breast cancer is the second leading cause of death among women globally. A better understanding of tumor biology, and the availability of high-throughput technologies, have enabled the emergence of precision medicine bringing new expectations and giving rise to molecular screening programs (MSP). SOLTI, as a collaborative Spanish network, designed AGATA, the first multi-institutional MSP ever implemented in this country. Here, we report both the primary and some of the secondary results of the pilot study.

**Methods:** A total of 10 sites within SOLTI network in Spain participated. DNA-sequencing of 56 cancer related genes was performed using FFPE tumor samples (primary or metastatic). Each clinical case was reviewed by a multidisciplinary advisory board (MAB), which recommended, in a prospective manner, potential experimental treatments, mainly in the context of clinical trials. The primary objective was to determine the success rate of matching a DNA alteration to an experimental drug or drug class. Secondary objectives included a comprehensive molecular characterization of tumor samples by PAM50 subtyping and quantification of protein expression levels by MASS-SPEC (70 proteins panel).

**Results:** 305 patients (pts) were screened from September 2014 to July 2017 and 260 (85.3%) were finally evaluated by the MAB. Pts characteristics were: mean age 54 years (29-80), ER+/HER2- (n=192; 74%), HER2+ (n=30; 11.5%) and TNBC (n=38; 14.5%). 163 primary tumors and 97 metastatic samples were profiled. Regarding the primary objective, 116 pts (45%) presented at least one mutation (range 1-6) that could be matched to a drug or drug class. Of these, 13 pts (11.2%) received therapy matched to their molecular profile according to the MAB recommendation and their follow-up is still on-going. No mutation was detected in 97 (37%) pts (WT), and 47 patients (18.1%) presented a mutation but no match was possible. The most common mutations were *PIK3CA* (34%), *TP53* (22%), *AKT1* (5%), *ESR1* (3%) and *ERBB2* (3%). Intrinsic subtype distribution in 177 samples was as follows: 34% Luminal A (n=60); 21% Luminal B (n=36); 13% HER2E (n=22); 19% Basal-like (n=34) and 13% Normal-like (n=23). Compared to primary tumors (n=110), the proportion of HER2-enriched disease in metastatic tumors (n=63) was significantly higher (6% vs 20%; p=0.005). Protein expression analysis was performed in 146 samples (94 primary and 57 metastasis). In 19 cases (13%), the outlier expression of some targetable proteins (FGFR1 [n=4, 2.7%], IGF1R [n=4, 2.7%], EGFR [n=1, 0.7%], CEACAM5 [n=6, 4.1%], IDO1 [n=2, 1.37%], TROP2 [n=2, 1.37%]) were identified. Of note, HER2 overexpression (>740 amol/μg) was observed in 4 HER2- cases. Finally, among WT tumors, 21% presented a potential drug-matched protein target.

**Conclusions:** Nationwide molecular screening in Spain is feasible. Nearly half of patients had tumors with mutation(s), mostly *PIK3CA*, that could be matched to a potential drug or drug class. PAM50 profile might be helpful to navigate towards a therapeutic decision making, although the MAB could not make any targeted-driven recommendation yet with this data. More clinical evidence is needed to use MASS-SPEC as a diagnostic tool.



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Biomarker analysis of CDK 4/6 and endocrine pathways in hormone-receptor positive (HR+) advanced breast cancer (ABC) bone only disease patients: A joint analysis of PALOMA-2 and PALOMA-3 studies

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**Background:** Palbociclib (PAL) in combination with endocrine therapy (ET) is a preferred treatment option for untreated and previously treated patients with hormone receptor–positive (HR+), human epidermal growth factor receptor 2 (HER2)–negative ABC. Patients with bone only disease could derive prolong disease control with single agent ET. Further understanding the predictive markers for sensitivity to ET alone and P+ET in both endocrine sensitive and resistant settings could help guide clinical treatment sequencing decision. We performed biomarker (BM) analysis of baseline tumor tissues in both PALOMA-2 (P2) and PALOMA-3 (P3) in bone only (BO) vs non-bone (NBO) only patients.

**Methods:** Postmenopausal women (N=666) with no prior systemic therapy for ER+/HER2-ABC were randomized 2:1 to receive PAL+LET or matching placebo (PBO)+LET in P2. In P3 women (N=521) who progressed on prior endocrine therapy were randomized 2:1 to receive PAL+ FUL vs matching PBO+FUL. All pts including those with BO disease had to consent to provide tumor tissues to participate the current study. EdgeSeq Oncology Biomarker Panel (HTG Molecular Diagnostics; Tucson, AZ) was used for mRNA profiling. Cox regression analysis was used to evaluate the association of BO disease with treatment effect as well as the association of gene expression with treatment effect in BO vs NBO pts.

**Results:** Treatment effect in the BM population from each study was consistent with that observed in the respective ITT population. In ITT population, BO disease was reported in 151 (23%) pts of P2 with mPFS 36.2mo PAL+LET arm (n=103) vs 11.2mo LET arm (n=48), HR=0.40 (0.26-0.62) and in 124 (24%) pts of P3 with mPFS 14.3mo PAL+FUL arm (n=86) vs 9.2mo FUL arm (n=38), HR=0.64(0.38-1.06). BO disease was reported in 107 (24%) pts in P2 and 81 (27%) pts in P3 BM population. In P2, longer mPFS was seen with PAL+ LET vs PBO+LET in BO vs NBO disease (30.6 mo vs 11.2 mo; HR=0.42 (0.25-0.69) in BO pts and 22.1 mo vs 13.8 mo HR=0.67 (0.52-0.88) in NBO pts) although the interaction effect was not statistically significant. Similar results were also observed in P3 with longer mPFS in PAL+FUL vs PBO+FUL in BO vs NBO disease (16.6 mo vs 11.2 mo HR=0.79 (0.40-1.56) in BO pts and 11.1mo vs 3.5 mo HR=0.47 (0.34-0.66) in NBO pts) with insignificant interaction effect. In both studies, BO pts had higher incidence of luminal A disease (BO vs NBO: P2=62% vs 47%; P3=54% vs 40%) and lower rate of luminal B disease (P2=19% vs 33%; P3: 26% vs 33%). In both studies, baseline ESR1, Cyclin E and CDK 4 gene expression level were comparable between BO vs NBO pts. Elevated CDK4 gene expression level was associated with resistance to LET in P2 and lower Cyclin E gene expression predicts the effect of PAL in P3.

**Conclusion:** mPFS was significantly prolonged in BO pts with the addition of PAL to ET. BO disease was more frequently associated with luminal A subtype in ABC setting. CDK4 expression level was similar to NBO disease pts but associated with resistance to single agent LET.

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Ribociclib with a non-steroidal aromatase inhibitor and goserelin in premenopausal women with hormone receptor-positive, HER2-negative advanced breast cancer: MONALEESA-7 age subgroup analysis

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**Background:** Younger patients (pts) with breast cancer may experience more aggressive disease and are more likely to die from their cancer vs older pts. In the Phase III MONALEESA-7 study (NCT02278120), the addition of ribociclib (RIB; cyclin-dependent kinase 4/6 inhibitor) to a non-steroidal aromatase inhibitor (NSAI) or tamoxifen (TAM) + goserelin significantly prolonged progression-free survival (PFS) in premenopausal women with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC; hazard ratio 0.553; p<0.0001). RIB treatment benefit was observed irrespective of endocrine partner (NSAI or TAM). Here we report results from a MONALEESA-7 subgroup analysis in pts aged <40 yrs and ≥40 yrs who received RIB or placebo (PBO) in combination with an NSAI + goserelin.

**Methods:** Pre- or perimenopausal women with HR+, HER2- ABC who had received no prior endocrine therapy and ≤1 line of chemotherapy for ABC were enrolled. Of the 672 pts randomized, 495 (74%) received RIB (600 mg/day, 3-weeks-on/1-week-off) or PBO + an NSAI (letrozole [2.5 mg/day] or anastrozole [1 mg/day]) and goserelin (3.6 mg every 28 days). The primary endpoint was locally assessed PFS; secondary endpoints included overall response rate (ORR), clinical benefit rate (CBR), and safety. A prespecified subgroup analysis was performed in pts aged <40 yrs and ≥40 yrs.

**Results:** A total of 144 pts were aged <40 yrs (RIB vs PBO arm: 78 vs 66) and 351 were aged ≥40 yrs (170 vs 181). As of August 20, 2017, in the RIB vs PBO arms, treatment was ongoing in 50% vs 23% of pts aged <40 yrs and 54% vs 43% of pts aged ≥40 yrs; disease progression was the most common reason for treatment discontinuation (<40 yrs: 37% vs 68%; ≥40 yrs: 35% vs 44%). Median PFS was prolonged in the RIB vs PBO arms both in pts aged <40 yrs (not reached vs 10.8 months; hazard ratio 0.435; 95% confidence interval [CI] 0.276–0.686) and in pts aged ≥40 yrs (27.5 vs 19.1 months; hazard ratio 0.625; 95% CI 0.449–0.870). In pts with measurable disease, the ORR (RIB vs PBO arm) was 49% vs 32% in pts aged <40 yrs and 51% vs 38% in pts aged ≥40 yrs; CBR was 81% vs 61% and 82% vs 65%, respectively. The most common Grade 3 adverse events (AEs; ≥5% of pts in either arm; RIB vs PBO arm) were neutropenia (<40 yrs: 47% vs 5%; ≥40 yrs: 58% vs 3%), leukopenia (<40 yrs: 18% vs 2%; ≥40 yrs: 14% vs 1%), diarrhea (<40 yrs: 5% vs 0%; ≥40 yrs: 1% vs 0%), and increased alanine aminotransferase (<40 yrs: 4% vs 2%; ≥40 yrs: 5% vs 1%); neutropenia was the only Grade 4 AE occurring in ≥5% of pts in either arm (<40 yrs: 15% vs 0%; ≥40 yrs: 8% vs 1%). New post-baseline QTcF >480 ms (RIB vs PBO arm) occurred in 3% vs 2% of pts aged <40 yrs and 7% vs 1% of pts aged ≥40 yrs.

**Conclusions:** Consistent treatment benefit was observed with RIB + NSAI vs PBO + NSAI in premenopausal women with HR+, HER2- ABC irrespective of age. RIB + NSAI had a manageable safety profile in pts aged <40 yrs and in those aged ≥40 yrs, with a safety profile similar to that observed in the full study population.

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First-line ribociclib + endocrine therapy in hormone receptor-positive, HER2-negative advanced breast cancer: A pooled efficacy analysis

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**Background:** In three separate Phase III randomized, placebo-controlled trials, ribociclib (RIB; cyclin-dependent kinase 4/6 inhibitor) + various endocrine therapy (ET) partners prolonged progression-free survival (PFS) vs placebo (PBO) + ET in patients (pts) with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC). Here we further evaluate the efficacy of RIB-based regimens of interest (i.e. with a non-steroidal aromatase inhibitor [NSAI] or fulvestrant [FUL]) in pts who were ET-naïve in the ABC setting, using pooled data from three Phase III trials: MONALEESA (ML)-2 (NCT01958021; all pts), ML-3 (NCT02422615; no prior ET for ABC subgroup only), and ML-7 (NCT02278120; RIB + NSAI subgroup only).

**Methods:** Postmenopausal pts with no prior ET for ABC received RIB (600 mg/day; 3-weeks-on/1-week-off) or PBO + either letrozole (2.5 mg/day) in ML-2 or FUL (500 mg every 28 days, with an additional dose on Day 15 of Cycle 1) in ML-3. In ML-7, premenopausal pts with no prior ET and ≤1 line of chemotherapy for ABC received RIB or PBO + goserelin (3.6 mg every 28 days) + NSAI (anastrozole [1 mg/day]/letrozole [2.5 mg/day]). The primary endpoint of all three trials was locally assessed PFS. Secondary endpoints included overall response rate (ORR), clinical benefit rate (CBR), and duration of response (DoR; ML-3 and -7). DoR was an exploratory endpoint in ML-2.

**Results:** Data were pooled for 820 pts treated with RIB + ET (ML-2: n=334; ML-3: n=238; ML-7: n=248) and 710 pts treated with PBO + ET (ML-2: n=334; ML-3: n=129; ML-7: n=247). As of the data cutoffs (ML-2: January 2, 2017; ML-3: November 3, 2017; ML-7: August 20, 2017), in the RIB + ET vs PBO + ET arms, 385 (47%) vs 234 (33%) pts remained on-treatment; the most common reason for discontinuation was disease progression (n=292 [36%] vs n=391 [55%]). In this pooled analysis, median PFS was prolonged for RIB + ET vs PBO + ET, with a hazard ratio of 0.570 (95% confidence interval [CI] 0.491–0.662); median PFS was 25.3 months (95% CI 23.9–29.6) vs 15.6 months (95% CI 14.4–16.9), respectively. Consistent PFS benefit for RIB + ET vs PBO + ET was observed across pt subgroups, including ECOG performance status, age, race, or presence/absence of liver and/or lung metastases or bone-only disease. Among all pts in the pooled analysis, the ORR was 41% for RIB + ET vs 28% for PBO + ET and the CBR was 79% vs 70%, respectively. In pts with measurable disease at baseline (RIB + ET: n=639; PBO + ET: n=542), the ORR was 51% for RIB + ET vs 37% for PBO + ET and the CBR was 79% vs 68%, respectively. In the RIB + ET vs PBO + ET arms, the median DoR was 26.7 months vs 20.0 months. A decrease in best percentage change from baseline in the sum of longest diameters per RECIST was observed in 86% of pts receiving RIB + ET vs 73% of pts receiving PBO + ET.

**Conclusions:** RIB in combination with various ET partners demonstrates improved clinical outcomes vs PBO + ET across a broad population of pts with HR+, HER2- ABC. These data provide further support for the use of RIB-based combinations in pre- and postmenopausal pts with HR+, HER2- ABC who have received no prior ET for advanced disease.

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Ribociclib treatment benefit in patients with advanced breast cancer with  $\geq 1$  dose reduction: Data from the MONALEESA-2, -3, and -7 trials

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**Background:** In the MONALEESA (ML) trials, addition of ribociclib (RIB; cyclin-dependent kinase 4/6 inhibitor) to endocrine therapy (ET) prolonged progression-free survival (PFS) in patients (pts) with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC). RIB was generally well tolerated, with adverse events (AEs) managed effectively by dose modifications. Here we present efficacy data for RIB-based regimens of interest for the proposed indication (i.e. with a non-steroidal aromatase inhibitor [NSAI] or fulvestrant [FUL]) from ML-2, -3, and -7 in pts who received no prior ET for ABC and who had  $\geq 1$  RIB dose reduction, to explore the efficacy of RIB in pts who need to dose reduce.

**Methods:** Pts included in this analysis were: postmenopausal women with HR+, HER2- ABC and no prior ET for ABC who received RIB (600 mg; 3-weeks-on/1-week-off) with letrozole (2.5 mg/day; ML-2 [NCT01958021]), or FUL (500 mg per label; ML-3 [NCT02422615]); and premenopausal women with no prior ET and  $\leq 1$  line of chemotherapy for ABC who received RIB with an NSAI (anastrozole: 1 mg/day; letrozole: 2.5 mg/day; ML-7 [NCT02278120]) plus goserelin (3.6 mg every 28 days). Dose reductions for RIB (600 to 400 to 200 mg) were permitted. Primary endpoint was PFS. Secondary endpoints included overall response rate (ORR), clinical benefit rate (CBR), and safety.

**Results:** In ML-2, -3, and -7,  $\geq 1$  RIB dose reduction occurred (n/N) in 169/334 (51%), 92/238 (39%), and 91/246 (37%) pts assigned to RIB, respectively. AEs were the main reason for dose reduction, with all-grade neutropenia the most common AE leading to dose reduction (ML-2 69%, ML-3 80%, ML-7 82%). Median PFS (months) was prolonged with RIB vs placebo in pts without a RIB dose reduction (ML-2: 27.7 vs 16.0; ML-3: not reached [NR] vs 18.3; ML-7: 23.8 vs 13.8); median PFS in pts with  $\geq 1$  RIB dose reduction was: ML-2 25.3, ML-3 NR, and ML-7 27.5 months. In pts with measurable disease and without a RIB dose reduction, ORR was 46% (ML-2), 43% (ML-3), and 48% (ML-7); CBR was 70%, 68%, and 79%, respectively. In pts with measurable disease and  $\geq 1$  RIB dose reduction, ORR was 62% (ML-2), 57% (ML-3), and 55% (ML-7); CBR was 88%, 85%, and 88%, respectively. The most common Grade 3/4 AEs in the RIB vs placebo groups ( $\geq 5\%$  of pts in either ML trial, irrespective of causality or dose reduction) were neutropenia (ML-2: 62% vs 1%; ML-3: 55% vs 0; ML-7: 65% vs 4%), leukopenia (ML-2: 21% vs 1%; ML-3: 12% vs 0; ML-7: 16% vs 1%), hypertension (ML-2: 13% vs 13%; ML-3: 5% vs 5%; ML-7: 2% vs 3%), increased alanine aminotransferase (ML-2: 10% vs 1%; ML-3: 10% vs 0; ML-7: 5% vs 1%), and increased aspartate aminotransferase (ML-2: 6% vs 1%; ML-3: 6% vs 0; ML-7: 4% vs 1%).

**Conclusions:** Results from the ML-2, -3, and -7 trials suggest that pts who start on 600 mg of RIB and require dose reduction for the management of their AEs, or for other reasons, continue to derive clinical benefit.

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Ribociclib + endocrine therapy in patients with hormone receptor-positive, HER2-negative advanced breast cancer presenting with visceral metastases: Subgroup analysis of phase III MONALEESA trials

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**Background:** Patients (pts) with advanced breast cancer (ABC) who present with visceral metastases (mets) have a poorer prognosis vs pts with non-visceral disease. In the Phase III MONALEESA (ML) trials, ribociclib (RIB) + endocrine therapy (ET) prolonged progression-free survival (PFS) vs placebo (PBO) + ET in hormone receptor-positive (HR+), HER2-negative (HER2-) ABC. Here we show data for pts with and without visceral mets from the ML-2, -3, and -7 trials.

**Methods:** Data were collated from 3 trials in HR+, HER2- ABC: in ML-2 (NCT01958021; data cutoff [DCO] Jan 2/4, 2017), postmenopausal pts (no prior ET for ABC) received RIB or PBO + letrozole; in ML-3 (NCT02422615; DCO Nov 3, 2017), postmenopausal pts (no prior ET for ABC subgroup only) received RIB or PBO + fulvestrant; in ML-7 (NCT02278120; DCO Aug 20, 2017), premenopausal pts (no prior ET and ≤1 chemotherapy for ABC) received RIB or PBO + goserelin + anastrozole/letrozole. Endpoints; primary: local PFS; secondary: overall response rate (ORR), clinical benefit rate (CBR), safety.

**Results:** Of all 820 pts treated with RIB + ET, 484 (59%) had visceral mets (ML-2 197/334; ML-3 137/238; ML-7 150/248); of all 710 pts treated with PBO + ET, 416 (59%) had visceral mets (ML-2 196/334; ML-3 77/129; ML-7 143/247). Median PFS was prolonged for RIB vs PBO in pts with and without visceral mets (Table). ORR and CBR were also higher for RIB vs PBO in pts with and without visceral mets. The most common (≥10% of pts in any arm) Grade [G] 3 and 4 adverse events (AEs) for each trial are shown in the table; no G4 AEs occurred in ≥10% of pts in ML-3.

	Visceral mets	No visceral mets
<b>ML-2</b>		
<b>Median PFS</b> (RIB/PBO), months (95% CI)	24.9 (22.2–30.9)/13.4 (12.7–16.5)	25.3 (22.2–NR)/18.2 (15.0–24.6)
Hazard ratio (95% CI)	0.538 (0.408–0.709)	0.634 (0.448–0.897)
<b>ORR</b> (RIB/PBO),* %	48/37	35/17
<b>CBR</b> (RIB/PBO),† %	79/72	82/75
<b>Most common (≥10% in any arm) G3 AEs (RIB/PBO), %</b>		
Neutropenia	56/1	47/1
Leukopenia	19/1	21/<1
Hypertension	11/11	15/15
<b>Most common (≥10% in any arm) G4 AEs (RIB/PBO), %</b>		
Neutropenia	10/0	9/0
<b>ML-3</b>		
<b>Median PFS</b> (RIB/PBO), months (95% CI)	NR (19.1–NR)/16.5 (9.0–NR)	NR (NR–NR)/21.9 (14.8–NR)
Hazard ratio (95% CI)	0.610 (0.403–0.926)	0.521 (0.295–0.921)

<b>ORR</b> (RIB/PBO),* %	46/29	31/21
<b>CBR</b> (RIB/PBO),† %	74/60	75/81
<b>Most common (≥10% in any arm) G3 AEs (RIB/PBO), %</b>		
Neutropenia	50/0	45/0
Leukopenia	12/0	10/0
Increased ALT	6/0	12/0
<b>ML-7</b>		
<b>Median PFS</b> (RIB/PBO), months (95% CI)	23.8 (14.8–NR)/10.4 (7.2–12.9)	27.5 (NR–NR)/19.3 (16.5–NR)
Hazard ratio (95% CI)	0.507 (0.367–0.700)	0.609 (0.377–0.984)
<b>ORR</b> (RIB/PBO),* %	45/36	30/19
<b>CBR</b> (RIB/PBO),† %	79/57	83/81
<b>Most common (≥10% in any arm) G3 AEs (RIB/PBO), %</b>		
Neutropenia	54/3	56/4
Leukopenia	14/1	16/1
<b>Most common (≥10% in any arm) G4 AEs (RIB/PBO), %</b>		
Neutropenia	11/<1	9/0

CI, confidence interval; NR, not reached. \*ORR = complete response + partial response; †CBR = complete response + partial response + (stable disease + non-complete response/non-progressive disease ≥24 weeks).

**Conclusions:** Although the presence of visceral mets is associated with a poorer prognosis, RIB + ET is an effective and well-tolerated treatment option for pts with HR+, HER2– ABC irrespective of the presence of visceral mets.

# 2018 San Antonio Breast Cancer Symposium ®

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Everolimus + exemestane for HR+ advanced breast cancer in routine clinical practice- Final results from the non-interventional trial, BRAWO

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**Background:** In the pivotal BOLERO-2 trial, everolimus (EVE) + exemestane (EXE) more than doubled the median progression-free survival (PFS) vs EXE alone in hormone receptor positive (HR+), human epidermal growth factor-receptor 2-negative (HER2-) advanced breast cancer (ABC) recurring/progressing on/after prior non-steroidal aromatase inhibitors (NSAIs). BRAWO is a German non-interventional study conducted in patients (pts) with HR+, HER2-ABC receiving EVE + EXE, according to Summary of Product Characteristics (SmPC), in routine clinical practice. Here we report the final PFS and safety results.

**Methods:** This multicenter study documented 2100 pts between October 2012 and December 2017 across 341 sites in Germany. Postmenopausal women with HR+, HER2- ABC with recurrence or progression after a NSAI were included. Primary observation parameters included the evaluation of the effectiveness of EVE + EXE used in routine care for the entire pt group.

**Results:** In the final analysis, out of the 2100 documented pts, 2074 were included in the full analysis set. The median time since the primary diagnosis was 7.1 years and the median time from first sign of relapse (local recurrence or distant metastases) was 2.1 years. At baseline, 54.1% of pts presented with visceral metastases and 50.1% had an ECOG performance status of 0. Approximately, 63% of pts started with EVE 10 mg (median duration of exposure: 5.1 months; 95% CI, 4.6-5.4), while 34.1% started with EVE 5 mg (median duration of exposure: 4.6 months; 95% CI, 4.1-5.2).

The distribution of treatment lines was as follows: first line, 28.7% (n=595); second line, 31.9% (n=662); third line, 18.1% (n=376); fourth line, 10.7% (n=221) and, fifth line and later, 10.6% (n=220). Treatment was discontinued by 55.7% of pts (n=1170) due to progressive disease and 26% of pts (n=546) due to adverse events. The Kaplan-Meier estimate of the median PFS was 6.6 months (95% CI, 6.2-7.0). The best overall responses, based on clinical routine, were complete response, 0.8% (n=17), partial response, 7.4% (n=150), and stable disease, 41.3% (n=842). The general safety profile was consistent with the previously reported safety findings. The most common adverse events were stomatitis (any grade: 42.6%, grade 3: 3.8%, grade 4: <0.1%) and fatigue (any grade: 19.8%, grade 3: 1.5%).

**Conclusions:** Data from BRAWO support EVE + EXE as a suitable treatment option with a reasonable safety profile for HR+, HER2- ABC recurring or progressing on/after prior NSAIs.

# 2018 San Antonio Breast Cancer Symposium ®

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Clinical management of metastatic breast cancer (MBC) after CDK 4/6 inhibitors: A retrospective single-institution study

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Cyclin dependent kinase 4 and 6 inhibitors (CDK4/6i) are standard first line (1L) and second line (2L) treatments of ER+ MBC. However, the optimal treatment strategy after progression on a CDK 4/6i is unknown. Given concern for rapid disease progression after discontinuation of CDK4/6is (Bashour, J Cancer 2017), further data on responses to subsequent lines of therapy post CDK4/6i are needed. We performed a single institution retrospective review of patients (pts) with ER+ MBC who received 1L or 2L CDK4/6i to examine the prescribing patterns and clinical responses to post-CDK4/6i treatment.

**Methods:** We identified 136 ER+ MBC pts prescribed a CDK4/6i at Mayo Clinic Rochester (from 12/2014 to 2/2018) on 1L or 2L who received at least 30 days of therapy and  $\geq$  two clinic visits during treatment. For the 1L and 2L cohorts we assessed the time to treatment failure during (TTF-1) and after CDK4/6i therapy (TTF-2). TTF was defined as time from start of therapy to discontinuation for any cause. Additionally, we assessed overall survival (OS) post CDK4/6i discontinuation (defined as the date of TTF-1 to death or last follow up).

**Results:** The study cohort included 81 and 55 pts treated with 1L and 2L CDK4/6is, respectively. In the 1L cohort, palbociclib/letrozole (82.3%) and palbociclib/fulvestrant (13.6%) were most commonly prescribed. Treatment was discontinued in 39/81 pts due to: progression (84.6%), intolerability (7.7%), and other (7.7%). The median TTF-1 was 19.7 mo (95% CI 11.2 – 25.4 mo). Subsequent treatment and response data were available in 37/39 pts that progressed on 1L CDK4/6i. The most commonly prescribed regimens included single-agent hormonal therapy (29.7%), everolimus/exemestane (27.0%), and single agent chemotherapy (21.7%). The overall median TTF-2 were as follows: everolimus/exemestane 13.2 mo [95%CI 0.33 mo – not reached (NR)]; single agent hormonal therapy 3.1 mo (95%CI 1.4 – 5.4 mo); and single agent chemotherapy 4.1 mo (95%CI 1.4 - 5.4 mo). With a median post-CDK4/6i follow up of 11.2 mo, we observed 8 deaths and the median OS-post CDK4/6i was NR. In the 2L cohort, common regimens were palbociclib/letrozole (63.6%) and palbociclib/fulvestrant (27.3%). Treatment was discontinued in 30/55 pts due to: progression (73.3%), intolerability (13.3%), and other (13.3%). The median TTF-1 in the 2L cohort was 8.3 mo (95%CI 4.6 – 12.7 mo). Subsequent treatment data were available in 24/30 pts and included single-agent hormonal therapy [n=7 (29.2%); median TTF-2 4.7 mo; 95%CI 1.9 – 14.0 mo], everolimus/exemestane [n=6 (25.0%); median TTF-2 3.2 mo, 95% CI 0.8-10.1 mo] and single agent chemotherapy [n=6 (25.0%); median TTF-2 2.6 mo; 95%CI 1.7 mo – NR]. With a median post-CDK4/6i follow up 18.7 mo in the 2L cohort, we observed 11 deaths, and the median OS-post CDK4/6i was 11.8 mo (95%CI 11.8 mo – NR).

**Conclusions:** Single and combination-based endocrine strategies are commonly administered post-CDK4/6i without clinical evidence for rapid deterioration. The observation of a median treatment duration lasting >12 months in patients receiving everolimus/exemestane post-CDK4/6i supports ongoing studies evaluating drugs that target PI3K/mTOR pathway in combination and following progression on a CDK4/6i.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-10

A phase 1b/2 study of ribociclib plus trastuzumab for the treatment of advanced, treatment-refractory HER2-positive breast cancer

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## Introduction:

Despite the success of anti-HER2 therapy, acquired resistance usually develops in the metastatic setting. CDK4/6 pathway activity has been identified as a mediator of this resistance, and in preclinical studies the combination of CDK4/6 and HER2 blockade can be more effective than either therapy alone. We conducted a single-arm phase 1b/2 study of the CDK4/6 inhibitor ribociclib given with trastuzumab or T-DM1 to subjects with advanced, treatment-refractory HER2-positive breast cancer. The results of the trastuzumab cohort are presented below. The primary objective was to determine the clinical benefit rate (CBR) at 24 weeks, and secondary endpoints included objective response rate (ORR), progression-free survival (PFS), and adverse events.

**Methods:** Individuals with locally advanced or metastatic, measurable HER2-positive breast cancer were eligible. All subjects must have previously received trastuzumab, pertuzumab, and T-DM1 as (neo)adjuvant or metastatic therapy. There was no limit on the number of prior lines of treatment. Patients with previous CDK4/6 inhibitor exposure, QTcF > 450msec on EKG, or without stable brain metastases were excluded. An initial safety run-in phase (with dose-limiting toxicity (DLT) monitoring) included six subjects who received trastuzumab (8mg/kg loading then 6mg/kg IV three-weekly) and ribociclib 400mg PO daily on a continuous schedule (cycle length 21 days). The study had a two-stage design. The first stage required 20 patients, at least 6 of whom must have demonstrated clinical benefit (CR+PR+ SD>24 weeks) in order to recruit 15 more patients to the second stage. All patients with accessible disease underwent metastatic tumor biopsies at baseline and C2D1.

**Results:** 13 patients were enrolled (6 in the safety run-in and 7 in the expansion cohort). One patient was found to have HER2-negative disease and did not receive treatment. Patient characteristics are shown in Table 1

Table 1

Age (median, range)	50.5 (42 - 71)
Number of prior lines of systemic therapy for metastatic disease (median, range)	5.5 (0-14)
Number with Hormone receptor-positive disease (%)	8 (67 %)
Number of metastatic sites (median, range)	2.5 (2 - 5)

No DLTs were observed during the safety run-in phase, and ribociclib was thus used at 400mg po daily for the expansion cohort. Grade 3/4 toxicities were observed in 5 patients (41.7%) and included neutropenia (n=2), and fatigue, pain, and muscle weakness (all n=1). No patient demonstrated QTc prolongation >480 msec, or grade 3/4 LFTs. 1/12 patients ((8.3%); 95% CI 0.2%-38.5%) achieved stable disease>24 weeks; no objective responses were observed, and median PFS was 41.5 days. The trastuzumab portion of study was closed early due to limited clinical activity observed (the T-DM1 with ribociclib cohort remains open).

**Conclusions:** The combination of trastuzumab and ribociclib (400mg daily continuous schedule) is safe, with no new safety signals observed. The limited activity seen in this heavily pretreated population suggests that future efforts to incorporate CDK4/6 inhibition should be limited to a less extensively treat population.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-11

Long-term responders to single-agent margetuximab, an Fc-modified anti-HER2 monoclonal antibody, in metastatic HER2+ breast cancer patients with prior anti-HER2 therapy

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## Background

Margetuximab is an Fc-optimized anti-HER2 antibody that recognizes the same epitope as trastuzumab. Margetuximab has increased affinity for the activating CD16A Fc-receptor on NK cells and macrophages as well as decreased affinity for the inhibitory CD32B receptor compared to trastuzumab. In a Phase 1 study (NCT01148849) of 66 patients with relapsed or metastatic HER2+ cancer across multiple indications, margetuximab was well tolerated at all doses. Among 60 response-evaluable patients, confirmed partial response (PR) and stable disease (SD) were seen in 7 (12%) and 30 (50%) patients, respectively. Tumor reductions occurred in 18/23 (78%) evaluable breast cancer patients. Ex-vivo analyses of patient peripheral blood mononuclear cell samples confirmed margetuximab's ability to enhance antibody dependent cell-mediated cytotoxicity over that from trastuzumab. We report on 3 breast cancer patients with prior anti-HER2 therapy failure with durable ( $\geq$  3.5 years) SD (1) or PR (2).

## Methods

Enrolled patients had histologically/cytologically-confirmed carcinoma with documented HER2 overexpression by immunohistochemistry (2+ or 3+) and disease progression during/following last therapy. Eligibility included life expectancy  $\geq$ 3 months; performance status  $\leq$ 1; measurable disease by Response Criteria for Solid Tumors 1.1; adequate bone marrow, renal, hepatic function; and left ventricular ejection fraction  $\geq$ 50%. Margetuximab was given by intravenous infusion at 0.1 – 6.0 mg/kg for 3 of every 4 weeks or once every 3 weeks (10 – 18 mg/kg).

## Results

Three of 17 HER2 3+ metastatic breast cancer patients received long-term margetuximab. Patient 35 had 3 prior regimens (adjuvant doxorubicin+cyclophosphamide followed by docetaxol+trastuzumab; gemcitabine+vinorelbine; lapatinib+capecitabine) and received margetuximab at 10 mg/kg q3wk, 88 cycles to date, with PR achieved Cycle 1 Day 43, maintained 4.4 years. Patient 44 had 3 prior regimens for metastatic disease (docetaxel+trastuzumab+pertuzumab; doxorubicin+cyclophosphamide; lapatinib+capecitabine) and received margetuximab at 15 mg/kg q3wk, 79 cycles to date with SD for 4.3 years. Patient 50 had 4 prior regimens for recurrent/metastatic disease (tamoxifen; anastrozole; capecitabine+trastuzumab; lapatinib+capecitabine) and received margetuximab dose of 18 mg/kg q3wk with PR achieved Cycle 1 Day 43, maintained 3.5 years. Progression was noted at Cycle 57, and margetuximab continues at 63 cycles to date. No cardiac toxicities were found during long-term follow-up for these 3 patients and there were no treatment-related adverse events  $\geq$ Grade 3.

## Conclusions

Margetuximab is well-tolerated without cardiac toxicities in long-term responders, with single-agent activity including durable responses in heavily pre-treated metastatic breast cancer. A Phase 3, randomized, multi-center clinical trial (SOPHIA; NCT02492711) is enrolling patients with metastatic breast cancer, comparing margetuximab plus chemotherapy to trastuzumab plus chemotherapy in patients who have received 1 to 3 lines of therapy for advanced disease.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-12

**EMBRACA:** Efficacy and safety of talazoparib or physician's choice of therapy in patients with advanced breast cancer and a germline *BRCA1/2* mutation: A regional analysis

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**Background:** Talazoparib (TAL) prevents DNA damage repair by inhibiting poly (ADP-ribose) polymerase (PARP) enzymes and trapping PARP on DNA, resulting in cell death in *BRCA1/2*-mutated cells.

**Methods:** EMBRACA is an open-label, randomized, 2-arm phase 3 trial in which efficacy and safety of TAL (1 mg/d) were compared with physician's choice of therapy (PCT; capecitabine, eribulin, gemcitabine, vinorelbine) in patients (pts) with locally advanced or metastatic breast cancer (ABC) and a germline *BRCA* mutation (g*BRCAm*). Outcomes were assessed by region of the world (North America [NA]; Europe [EU]; rest of world [ROW]). Progression-free survival (PFS), objective response rate (ORR), and clinical benefit rate (CBR) at 24 wks were assessed; safety was also assessed.

**Results:** 431 pts were randomized 2:1. Pt characteristics were well balanced, although a higher percentage of pts in ROW had more severe disease (eg, triple-negative breast cancer [TNBC], Disease-free interval [DFI]<12 mo, more distant metastases, more disease sites) and were on average younger than pts in NA/EU. TAL provided improvement in PFS, ORR, and CBR in all regions vs PCT. The most common toxicities with TAL included anemia, neutropenia, thrombocytopenia, fatigue, and nausea for all regions. Alopecia was less frequent with TAL in EU/ROW. Serious adverse events for pts receiving TAL were more frequent in EU than NA/ROW. Incidences of adverse events associated with permanent treatment discontinuation in pts receiving TAL were low in all regions and generally lower than for PCT.

Table 1

Category	NA* (N=156)	EU* (N=190)	ROW* (N=85)
Mean age, years	49.0	49.2	44.2
Race, %			
White	76.9	71.1	52.9
Black	5.8	-	3.5
Asian	5.8	-	42.4
Not reported	-	27.4	-
TNBC, %	42	44	47
BRCA1**, %	41	47	48
BRCA2**, %	59	53	52
DFI<12 mo, %	31	34	44
Distant metastases, %	94	93	97
≥3 disease sites, %	47	40	49
PFS, (hazard ratio [HR]; [95% CI]); P value	0.46 [0.29-0.74] P=.0009	0.52 [0.33-0.80]; P<.003	0.57 [0.31-1.07] ;P=.08
ORR (odds ratio [OR] [95% CI]); P value	5.54 [2.4-16.1];P<.0001	3.75 [1.57-9.87]; P=.001	6.7 [1.61-28.39]; P=.001

CBR (OR [95% CI]); P value	4.71 [2.20-10.57]; P<.0001	3.39 [1.56-7.36]; P=.0007	5.70 [1.70-17.13]; P=.002
Hematologic AEs, %			
Anemia	50.5	58.6	42.6
Neutropenia	31.3	32.3	46.3
Thrombocytopenia	28.3	22.6	35.2
Nonhematologic AEs, %			
Fatigue	59.6	43.6	50.0
Nausea	47.5	45.9	57.4
Headache	32.3	30.8	37.0
Alopecia	34.3	20.3	20.4
Serious adverse events, %	25.3	40.6	22.2
Treatment discontinuation, n/N, (%)			
TAL	7/99 (7.1)	12/133 (9.0)	3/54 (5.6)
PCT	7/43 (16.3)	3/54 (5.6)	2/29 (6.9)

AE, adverse event; CI, confidence interval; \*NA (United States); EU (Belgium, France, Germany, Ireland, Italy, Poland, Spain, United Kingdom, Russia, Ukraine, Israel); ROW (Brazil, Korea, Australia, Taiwan). \*\*Central laboratory.

**Conclusions:** In pts with g*BRCAm* ABC, TAL demonstrated significant improvements in clinical outcomes compared with PCT regardless of the region of the world in which they lived. However, slight differences among the regions in baseline characteristics were noted, possibly due to regional variation in diagnosis and detection of g*BRCAm* ABC as well as different treatment paradigms for metastatic breast cancer.

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## 2018 San Antonio Breast Cancer Symposium ®

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Identification of optimal combination therapy partners for PI3K/Akt/mTOR pathway inhibitor in triple negative breast cancer

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Triple-negative breast cancer (TNBC) is among the most aggressive subtypes, accounts for 10-15% of all breast cancer cases and is characterized by a lack of hormone receptors with a low overall survival rate. Due to the heterogeneity nature of this disease, the absence of validated molecular targets makes it unresponsive to conventional therapies. PI3K/Akt/mTOR pathway is aberrantly activated in TNBC, but single agent therapy is commonly subject to resistance. The goal of this study is to identify the genes that can be targeted to enhance the efficacy of mTOR inhibitor TAK228, an agent that is being investigated as a treatment for advanced solid tumors, in TNBC with PI3K pathway activation. We utilized an *in vivo* pooled barcoded shRNA library screening to determine the genes that have the potential to be used as TAK228 synthetic lethal partners. Using deep sequencing analysis of the shRNA profiles, we identified several genes whose loss of function conferred synthetic lethality in the presence of TAK228. We found that targeting the candidate genes (WEE1, BMX and MAPK15) with their inhibitors (AZD1775, Ibrutinib and Sunitinib) did not significantly affect the viability, however combination treatment of these agents with TAK228 induced a robust growth inhibition and demonstrated a significant synergy in MDA-MB-468 cell lines. Investigating the activation of relevant survival signaling pathways will further elucidate the mechanism of synthetic lethal interaction. These observations provide a promising rational strategy for the treatment of TNBC with PI3K pathway aberration.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-14

Patient-reported outcomes with ribociclib-based therapy in hormone receptor-positive, HER2-negative advanced breast cancer: results from the phase III MONALEESA-2, -3, and -7 trials

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**Background:** In the Phase III MONALEESA (ML) trials, ribociclib (RIB; cyclin-dependent kinase 4/6 inhibitor) + endocrine therapy (ET) significantly improved progression-free survival (PFS) vs placebo (PBO) + ET in patients (pts) with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC). Here we report key patient-reported outcomes (PROs) for pts treated with RIB-based regimens of interest (i.e. with a non-steroidal aromatase inhibitor [NSAI] or fulvestrant [FUL]) in the ML-2, -3, and -7 trials.

**Methods:** Postmenopausal pts with HR+, HER2- ABC and no prior ET for advanced disease received RIB (600 mg/day; 3-weeks-on/1-week-off) + letrozole (2.5 mg/day; ML-2 [NCT01958021]), or FUL (500 mg every 28 days, with an additional dose on Day 15 of Cycle [C] 1; ML-3 [no prior ET for ABC subgroup only; NCT02422615]). Premenopausal pts with no prior ET and ≤1 line of chemotherapy for advanced disease received RIB + NSAI (anastrozole [1 mg/day]/letrozole [2.5 mg/day]) + goserelin (3.6 mg every 28 days; ML-7 [NCT02278120]). The primary endpoint for all trials was PFS. PROs were a secondary endpoint of all trials and were evaluated using EORTC QLQ-C30, QLQ-BR23 (ML-2 and ML-7), EQ-5D-5L, WPAI-GH (ML-7 only), and BPI-SF (ML-3 only) questionnaires. Changes from baseline and time to 10% deterioration (TTD) in health-related quality of life (HRQoL) were analyzed using linear mixed-effect and stratified Cox regression models, respectively.

**Results:** A total of 1530 pts were included in this analysis. Questionnaire compliance was high across trials (ML-2/ML-3: >90%; ML-7: >80%). On-treatment HRQoL (EORTC QLQ-C30 global health status/quality of life [QoL] score) was maintained from baseline up to C34, C28, and C17 in both treatment arms for ML-2, ML-3, and ML-7, respectively. In ML-7, mean overall HRQoL scores continued to improve in the RIB arm from C18 to C28, but scores decreased in the PBO arm. At end of treatment, mean overall HRQoL scores decreased in both arms across trials. Median TTD (RIB vs PBO) was similar between arms, favoring the RIB arms (ML-2: 27.7 vs 27.6 months; hazard ratio 0.944; 95% confidence interval [CI] 0.720–1.237; ML-3: not reached [NR] vs 22.4 months; hazard ratio 0.721; 95% CI 0.484–1.074; ML-7: 24.0 vs 19.4 months; hazard ratio 0.759; 95% CI 0.561–1.028). Clinically meaningful reductions in EORTC QLQ-C30 pain score (>5 points from baseline) were observed in the RIB arm of ML-2 from as early as C3 and were sustained vs only at C7 and C13 in the PBO arm. Clinically meaningful reductions in pain were observed from C22 to C28 in the RIB arm of ML-7 vs only at C28 in the PBO arm. In ML-3, clinically meaningful reductions in pain were observed from C3 to C5, C11–17, and at C22 and C28 in the RIB arm vs C17–C25 in the PBO arm. Furthermore, median TTD of the BPI-SF pain severity index score was NR in either arm of ML-3 (hazard ratio 0.858; 95% CI 0.554–1.330).

**Conclusions:** In addition to significantly prolonging PFS, RIB consistently maintains QoL regardless of ET combination partner. RIB + ET is also associated with clinically meaningful reductions in pain vs PBO + ET across a broad population of pts with HR+, HER2- ABC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-15

Ribociclib + endocrine therapy in hormone receptor-positive, HER2-negative advanced breast cancer: A pooled safety analysis

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**Background:** In Phase III trials, ribociclib (RIB; cyclin-dependent kinase 4/6 inhibitor) + various endocrine therapy (ET) partners has demonstrated significantly prolonged progression-free survival vs placebo (PBO) + ET in patients (pts) with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC). Here we further evaluate the safety of RIB-based regimens of interest for the proposed indication (i.e. with a non-steroidal aromatase inhibitor [NSAI] or fulvestrant [FUL]) using pooled data from three Phase III trials (MONALEESA [ML]-2 [NCT01958021], -3 [NCT02422615], and -7 [NCT02278120]).

**Methods:** Postmenopausal pts with HR+, HER2- ABC received RIB (600 mg/day; 3-weeks-on/1-week-off) or PBO + letrozole (LET; 2.5 mg/day; ML-2 [no prior ET for ABC]) or FUL (500 mg, Days 1 and 15 of Cycle 1, then Day 1 of every cycle thereafter; ML-3; no or ≤1 prior line of ET for ABC). Premenopausal pts (ML-7; no prior ET and ≤1 chemotherapy for ABC) received RIB or PBO + anastrozole (1 mg/day)/LET (2.5 mg/day) + goserelin (3.6 mg every 28 days). Adverse events (AEs) were characterized per Common Terminology Criteria for Adverse Events v4.03; safety analyses included time to first event, duration of event, and rate of associated RIB/PBO discontinuations.

**Results:** Data for 1883 pts were pooled; 1065 pts received RIB + ET and 818 pts received PBO + ET (median exposure to study treatment: 17 and 13 months, respectively). Exposure-adjusted incidence rates for AEs of special interest were 561 and 131 per 100 pt-years in the RIB and PBO arms, respectively. The most common all-causality Grade 3/4 AEs (≥10% in any arm; RIB vs PBO) were neutropenia (59% vs 2%), leukopenia (18% vs 1%), and hypertension (13% vs 13%). A new Fridericia's corrected QT interval (QTcF) >480 ms occurred in (n/N) 52/1054 (5%) vs 11/814 (1%) pts in the RIB vs PBO arms; a new QTcF >500 ms occurred in 14/1054 (1%) vs 1/814 (<1%) pts. Median time to first event for Grade ≥2 neutropenia, elevated alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST), and QTc prolongation in the RIB arm was 2, 12, and 2 weeks, respectively; median duration of first Grade ≥2 event was 4, 4, and 2 weeks. In the RIB arm vs PBO arms, 7% vs 3% of pts discontinued study treatment due to AEs; common all-grade AEs leading to RIB/PBO discontinuation (≥2% in any arm) were elevated ALT (4% vs <1%) and elevated AST (2% vs 1%). Discontinuation due to QT prolongation occurred in 4 pts in the RIB arm and 2 in the PBO arm (both <1%). All-grade serious AEs occurred in 25% of pts in the RIB arm vs 15% of pts in the PBO arm.

**Conclusions:** RIB in combination with various ET partners continues to demonstrate a predictable and manageable tolerability profile across a broad population of pts with HR+, HER2- ABC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-16

Ribociclib (RIBO) + letrozole (LET) in older patients with hormone receptor-positive (HR+), human epidermal growth factor receptor-2–negative (HER2–) advanced breast cancer (ABC): Preliminary subgroup results from the phase 3b CompLEEmment-1 trial

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**Background:** The cyclin-dependent kinase (CDK)4/6 inhibitor RIBO is approved in combination with an aromatase inhibitor (AI) for HR+, HER2– ABC in postmenopausal women with no prior therapy for ABC, based on the MONALEESA-2 trial (Hortobagyi et al. *NEJM* 2016). Although a high proportion of patients with HR+, HER2– ABC are >65 years of age, older patients are often under-represented in clinical trials. Furthermore, treatment decisions may be complicated by comorbidities, functional status, and concurrent medications. Here, we report early safety results for patients ≥65 years of age enrolled in CompLEEmment-1, an open-label, phase 3b trial evaluating RIBO+LET as first-line endocrine-based therapy in an expanded patient population.

**Methods:** Patients with HR+, HER2– ABC, ≤1 line of prior chemotherapy (CT), and no prior endocrine therapy for ABC received RIBO (600 mg/day, 3 weeks on/1 week off) + LET (2.5 mg/day); men and premenopausal women received concomitant goserelin (3.6-mg subcutaneous implant every 28 days). The primary outcome was safety and tolerability. A pre-planned interim analysis was conducted ~15 months after first patient first visit.

**Results:** Of the first 1,008 patients enrolled who completed 56 days of follow-up or discontinued before the data cut-off date, 377 were ≥65 years of age. Of these, 157 (41.6%) were 65–<70 years, 107 (28.4%) were 70–<75 years, and 113 (30%) were ≥75 years. The majority of patients (94.4%) had an Eastern Cooperative Oncology Group performance status ≤1; 33.2% presented with stage IV disease at diagnosis; 9 patients were male. The most common sites of metastasis were bone (70.0%), lung (44.8%), and lymph nodes (29.7%). The most common all-grade adverse events (AEs) were neutropenia (58.4%), nausea (31.8%), and fatigue (24.1%). The most common grade 3/4 AEs were neutropenia (37.7%) and alanine aminotransferase increase (4.2%). QT prolongation events were mild (majority grade 1/2) and occurred in 6.1% of patients (0.5% grade 3/4). Dose reduction or interruption due to AEs occurred in 54.5% of patients; 6.9% of patients had AEs leading to treatment discontinuation. In the overall patient population, the most frequent grade 3/4 AEs were neutropenia (42.8%), leukopenia (3.4%), and increased alanine aminotransferase (2.9%); QT prolongation occurred in 5.4% of patients (0.5% grade 3/4).

**Conclusions:** Initial safety results from CompLEEmment-1, from the first 56 days of follow-up, demonstrate the tolerability of RIBO+LET in older patients, consistent with the overall patient population. NCT02941926.



# 2018 San Antonio Breast Cancer Symposium ®

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Ribociclib + letrozole in patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer (aBC) with no prior endocrine therapy (ET) for ABC: Compleement-1 trial, preliminary results from Spanish population

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**Background:** The phase III Monaleesa-2, Monaleesa-3 and Monaleesa-7 trials have shown significantly improved PFS for the combination ribociclib + ET vs ET + placebo in pre-, peri-, and postmenopausal women with HR+/HER2-, first and second line aBC. The Compleement-1 trial is a phase IIIb, single-arm, open-label, international study to assess the safety and efficacy of ribociclib + letrozole in men and women who have not received prior ET for HR+, HER2- ABC [J Clin Oncol 36, 2018 (suppl; abstr 1056)].

**Methods:** 526 patients with HR+, HER2- ABC, ≤1 line of prior CT, and no prior ET for aBC were enrolled in the Compleement-1 trial in Spain from April 2017 to January 2018. Patients received ribociclib (600 mg/day, 3 weeks on/1 week off) + letrozole (2.5 mg/day); men and premenopausal women received concomitant goserelin (3.6 mg subcutaneous implant every 28 days). The primary objective was safety and tolerability. Here we report on a sub-analysis from the Spanish population of Compleement-1 trial including baseline characteristics and early safety results for the first patients enrolled who completed at least 56 days of follow-up or discontinued before the cut-off date (3rd Oct 2017).

**Results:** One hundred fifty four patients constituted the analytical cohort for this sub-analysis. Demographics and baseline characteristics: median age was 52 years (range 24-82); 1% of patients were male, 31.8% female pre-menopausal and 67.5% female post-menopausal; 44.2% vs 38.3% of patients had visceral disease vs bone only disease; 49.9% patients had ≥2 metastatic sites; and 34.4% of patients presented as *de novo* stage IV. The median exposure for study treatment was 1.8 months (range 0.8-1.8). The grade 3/4 events reported >1% included neutropenia (50%), increased GGT levels (3.2%), leukopenia (1.3%), and increased ALT (1.3%). QTcF prolongation >480ms based on ECG data was reported in 1.2% patients. Median dose intensity for ribociclib was 600mg/day (range 476.5-600); 11% of patients required one dose reduction (8.4% due to AEs), 59.7% had at least one dose interruption (57.1% due to AEs) and 9.7% were permanently discontinued (4.5% due to AEs).

**Conclusions:** Preliminary safety results from this Compleement-1 sub-analysis including Spanish population are consistent with previous data presented from Monaleesa-2, Monaleesa-3, Monaleesa-7 and Compleement-1. These data support the predictable and manageable safety profile of ribociclib in combination with letrozole. Clinical trial information: NCT02941926

# 2018 San Antonio Breast Cancer Symposium ®

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Phase I trial of eribulin and everolimus in patients with metastatic triple negative breast cancer

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**Background:** Alteration of PI3K/Akt/mTOR pathway is the most common genomic abnormality detected in triple negative breast cancer (TNBC). Everolimus acts synergistically with eribulin in inducing apoptosis in TNBC cell lines and xenografts in our preclinical study. This phase I trial was designed to test the safety and tolerability of combining eribulin and everolimus in patients (pts) with metastatic TNBC.

**Methods:** The overall objective of this study was to describe the safety and toxicities of the combination. The secondary objective was to assess activity based on response rate (RR) and progression free survival (PFS). Eligibility criteria included pts with metastatic TNBC, ECOG 0-2, 0-3 lines of prior chemotherapy in metastatic setting, and prior treatment with anthracycline and/or taxane therapy. The study utilized the toxicity equivalence range (TEQR) design with a target equivalence range for dose-limiting toxicities (DLTs) of 0.20-0.35. The recommended phase 2 dose (RP2D) will be the dose closest to the target of 0.25 below 0.51 based on isotonic regression. Three dosing levels of the combinations were tested: level A1 (everolimus 5mg daily; eribulin 1.4 mg/m<sup>2</sup> days 1, 8 every 3 weeks), level A2 (everolimus 7.5mg daily; eribulin 1.4 mg/m<sup>2</sup>, days 1, 8 every 3 weeks), level B1 (everolimus 5mg daily; eribulin 1.1 mg/m<sup>2</sup> days 1, 8 every 3 weeks). Nanostring RNA analysis and genomic mutation analysis were conducted in 16 pts with available tumor tissue.

**Results:** A total of 27 pts were enrolled. Median age was 55 years (range 36-76). Two pts were ineligible due to HER2+ on repeat biopsy and were only included in the toxicity analysis. Dose level B1 (everolimus 5mg daily and eribulin 1.1 mg/m<sup>2</sup> days 1, 8 every 3 weeks) was determined to be the RP2D doses. The DLTs were neutropenia, stomatitis and hyperglycemia. Across all cycles, 59% (16/27) had a ≥ Gr3 toxicity attributed to treatment at the possible or above level. 44% (12/27) had Gr3 heme-toxicities. The most common toxicities were ≥ Gr3 neutropenia (10 pts), Gr3 lymphopenia (6 pts) and ≥ Gr3 leukopenia (7 pts). 33% (9/27) had Gr3 non-heme toxicities. The most common were Gr3 stomatitis (3 pts), Gr3 hyperglycemia (3 pts) and Gr3 fatigue (5 pts). The median number of cycles completed was 4 (0-8). 68% (17/25) had a dose modification or hold, 14 of 25 (56%) were for eribulin and 15 of 25 (60%) were for everolimus. Of 25 eligible pts, 8 (32%) achieved a best response as partial response, 11 (44%) had stable disease and 6 (24%) had progression. 80% (20/25) experienced progression by RECIST or showed clinical progression, and the median time to progression was 2.7 mo (95% CI (2.2, 4.6)). At the time of this analysis, 16 participants had died, median OS was 6.3 mo (95% CI (5.3, undefined)). Two pts are still being followed on treatment. PI3K-Akt-mTOR pathway genes and mutations profiles were studied.

**Conclusion:** Eribulin 1.1 mg/m<sup>2</sup> days 1, 8 and everolimus 5mg daily was defined as the RP2D. Genomic analysis is currently underway to understand the molecular mechanisms of resistance.

# 2018 San Antonio Breast Cancer Symposium ®

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Real-world survival of heavily pretreated patients with refractory HR+, HER2- metastatic breast cancer receiving single-agent chemotherapy - A comparison with MONARCH 1

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## Background

In MONARCH 1 (NCT02102490), abemaciclib demonstrated promising single-agent activity and tolerability in a population of heavily pretreated women with refractory HR+, HER2- metastatic breast cancer (MBC).<sup>1</sup> Confirmed objective response rate (ORR) was 19.7% (95% CI: 13.3, 27.5) and at 18 months minimum follow-up median overall survival (OS) was 22.3 months. Due to the single-arm trial design of MONARCH 1, there is a need to view these results in clinical context relative to available treatment options. This study compared the OS results of abemaciclib in MONARCH 1 vs that in a real-world single-agent chemotherapy cohort with similar patient and disease characteristics.

## Methods

MONARCH 1 study design and key eligibility criteria were previously described.<sup>1</sup> The real-world cohort was based on Flatiron Health electronic health records-derived, nationally representative (USA-based) database comprising patient-level structured and unstructured data, curated via technology-enabled abstraction, for patients with MBC between January 1, 2011 through February 28, 2018. A real-world single-agent chemotherapy cohort was created based on the key eligibility criteria of MONARCH 1 and included patients diagnosed with HR+, HER2- MBC who received single-agent chemotherapy (eribulin, capecitabine, gemcitabine, or vinorelbine) following 1-2 prior chemotherapy regimens in the metastatic setting, had an ECOG PS of 0-1, and no prior CDK4 & 6 therapy. The index date was the start of the eligible single-agent chemotherapy, and patients were followed from the index date until date of death, loss to follow-up, or end of the database, whichever occurred earlier. OS results were adjusted using 2 methods (Mahalanobis distance matching and entropy balancing with bootstrapping) to account for baseline demographic and clinical differences between the real-world and trial cohorts.

## Results

A real-world cohort (n=281) with eligibility criteria similar to the MONARCH 1 population (n=132) was identified. A subsequent matching based on Mahalanobis distance was performed to match MONARCH 1 population (n=108) with the real-world cohort (n=108). The matched cohorts demonstrated similar patient and disease characteristics. Median OS was 22.3 months in the abemaciclib arm vs 13.6 months in the matched cohort with an estimated hazard ratio (HR) of 0.54 (95% CI: 0.37, 0.77). Results of a sensitivity analysis performed using entropy balancing were consistent with an adjusted median OS of 12.7 months in the real-world cohort (n=281) with HR of 0.57 (95% CI from bootstrapping: 0.44, 0.78).

## Conclusion

Methodological advances to adjust for potential biases, and improvements in data quality, have evolved enabling the ability to leverage a real-world cohort as an external comparator arm. This study demonstrates the ability to create a real-world chemotherapy cohort suitable to serve as a comparator for MONARCH 1. These exploratory results suggest a survival advantage and adequately place the clinical benefit of abemaciclib monotherapy in clinical context.

## References

Dickler et al, *CCR* 2017

# 2018 San Antonio Breast Cancer Symposium ®

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Targeting mitochondrial function for the treatment of triple negative breast cancer: Development of a small molecule inhibitor against mitochondrial STAT3

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**Background:** Patients with Triple Negative Breast Cancer (TNBC) can benefit significantly from earlier diagnosis/prognosis, targeted therapy, and predictive biomarker panels for optimal therapy. However, currently there are no clinically accepted markers for the prognosis of TNBC and to predict its potential to metastasize. It is well documented that numerous cancer subtypes with increased mitochondrial oxidative phosphorylation in which enhanced mitochondrial activity is linked to aggressiveness. Also, there is greater awareness of metabolic heterogeneity within tumors, with some cells using glycolysis as their main energy source, whereas others use oxidative phosphorylation. Interestingly, TNBC has been shown to adopt increased mitochondrial biogenesis to "fuel" enhanced growth and aggressiveness. Signal Transducers and Activators of Transcription family 3 (STAT3) has been studied extensively as a transcription factor, however the finding that STAT3 also localizes to mitochondria has opened a new area to discover non-classical functions.

**Methods:** Targeting mitochondrial STAT3 functions challenge the current design of therapies that solely target STAT3 as a transcription factor and suggest the need for "design thinking," to intervene the STAT3 pathway. With this in mind, we developed an in-house mitochondrial targeting - MitoTam. Data from in vitro cell-based assays, in vivo subcutaneous xenograft and patient-derived xenograft (PDX) models of TNBC will be reported.

**Results:** Our data shows MitoTam robustly inhibited proliferation of TNBC cells at pharmacological doses and induced apoptosis. Mechanistically, we observed the MitoTam was able to target STAT3 leading to the downregulation of genes which is highly upregulated in most of the cancers. Furthermore, we show inhibition of STAT3 transcriptional activity hampers mitochondrial biogenesis, a prominent feature of cancer cell. Interestingly our *in vivo* and *in vitro* protein data showed the decreased phosphorylation of nuclear STAT3 and decreased mitochondria import of STAT3. We also found the decreased phosphorylation of STAT3 is associated with the interaction of GRIM-19 which is a cell death regulatory protein in complex1. Treatment of MitoTam was able to deplete the super complexes involved in OXPHOS and also in the regulation of mitochondrial transcription regulation. Our *in vivo* and PDX models show significant reduction of tumor size and tumor burden with treatment of MitoTam without effecting body mass. In addition we also found decrease in protein kinases associated with regulation of STAT3 for tumor survival. In addition, nuclear DNA encoded mitochondrial transcription factor A (TFAM), which enhances both transcription and replication of mitochondrial DNA is also shown to be downregulated with treatment, suggesting that MitoTam effectively inhibit TFAM binding to the mitochondrial DNA genes involved in OXPHOS regulation which was further validated by TFAM Chip-seq.

**Conclusion:** Our results places MitoTam is a promising candidate drug against TNBC and establish mitochondrial STAT3 as its molecular target.

# 2018 San Antonio Breast Cancer Symposium ®

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Real world treatment patterns and outcomes of patients receiving palbociclib plus fulvestrant in the United States: Sub-groups analysis based on age, performance status and sites of metastases

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**Background:** Ibrance Real World Insights (IRIS) is a multi-country study aimed to describe the clinical characteristics and understand treatment patterns and clinical outcomes of patients receiving palbociclib plus fulvestrant in real world clinical practice. Previously the results on the overall population within the US have been communicated. The current analyses focus on subgroups stratified by age, performance status and visceral status.

**Materials and methods:** A retrospective chart review of HR+/HER2- ABC/MBC patients was conducted between June and October 2017. Physicians extracted data from patient medical records for HR+/HER2- ABC patients who received palbociclib plus fulvestrant following disease progression with endocrine based therapy for their advanced disease. Electronic case report forms collected data covering patient demographics, clinical characteristics, treatment history/patterns and clinical outcomes. Progression free rates and survival rates at 6 and 12 months were estimated via Kaplan-Meier analysis.

**Results:** Data for the US are reported here. In total, 65 physicians extracted data for 292 patients who had a mean follow up time of 7.4 months. Majority of the patients were >65 years (54%), and had ECOG status of 0 (32%) or 1 (48%). Overall 224 (77%) patients had metastatic disease, of which 93 (42%) had visceral metastases. Across all sub-groups, majority of patients prescribed an initial palbociclib dose of 125mg did not require a change of dose while on treatment. The 6-month and 12-month progression free and survival rates across subgroups are presented in Table 1. Patients with a performance status of ECOG  $\geq$  2 had a slightly lower progression and survival rates at 6 and 12 months compared to those with a score <1. Likewise, patients with visceral disease were observed to have slightly lower progression free and survival rates than others.

Table 1: Clinical Outcomes by Patient Sub-groups.

	Patient Sub-groups						
	Age		ECOG status			Visceral Status	
	Up to 65 n=158	Over 65 n=134	0 n=93	1 n=139	2+ n=60	Visceral Metastases n=93	Non-visceral Metastases n=131
Progression free survival rate at 6 months, %	95.2	93.2	97.8	93.4	90.6	89.6	94.8
Progression free survival rate at 12 months, %	81.2	77.8	84.6	83.0	-	73.2	76.5
Survival rate at 6 months, %	98.0	96.3	100.0	98.2	90.6	92.2	98.9
Survival rate at 12 months, %	90.0	85.1	97.6	95.1	-	80.8	92.7

**Conclusions:** The analysis indicates consistent trends in different clinical outcomes were observed with palbociclib plus fulvestrant across patients sub-groups based on age, performance status and visceral metastases.

## 2018 San Antonio Breast Cancer Symposium ®

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Phase Ib study of rebastinib plus antitubulin therapy with paclitaxel (P) or eribulin (E) in patients with HER2-negative metastatic breast cancer (MBC)

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**BACKGROUND:** TMEM (Tumor Microenvironment of Metastasis) are the portal for tumor cell intravasation into the circulation and subsequent metastasis (*Harney et al Cancer Discov 2015*). The potent Tie2 kinase inhibitor rebastinib inhibits intravasation at TMEM sites, reduces circulating tumor cell (CTC) burden, increases angiopoietin (Ang) 1/2 levels, prevents distant metastases, and improves survival in breast cancer animal models when added to either P or E (*Harney et al MCT 2017*), and circumvent chemotherapy-induced pro-metastatic changes in the tumor microenvironment mediated by TMEM (*Karagiannis et al STM 2017*). We sought to determine the safety of rebastinib combined with antitubulin therapy (P or E) in patients with HER2- MBC. We also hypothesized that addition of rebastinib would reduce CTC burden and increase Ang levels by blocking Ang-mediated stimulation of VEGF release from TMEM-associated macrophages.

**METHODS:** We aimed to determine the safety and recommended phase 2 dose (RP2D) of rebastinib (2 dose levels: 50 mg or 100 mg PO BID) in combination with P (80 mg/m<sup>2</sup> x 12 weeks) or E (1.4 mg/m<sup>2</sup> on day 1 & 8 q 21 days) using a standard 3+3 design (1 cycle = 21 days). Secondary objectives included evaluating the effect of the P/E + rebastinib combination on CTCs (TelomeScan) and Ang levels. Dose limiting toxicity (DLT) was defined as grade 3-4 febrile neutropenia, thrombocytopenia, and non-hematologic toxicity during the first 6 weeks of therapy. Eligibility included HER2- MBC, ECOG PS 0-1, CDK4/6 inhibitor progression if ER+. Patients with ≤ 2 prior non-taxane chemotherapy regimens received P+ rebastinib, whereas those with ≥ 2 chemo regimens (including a taxane) received E+ rebastinib.

**RESULTS:** Of 11 treated patients, 6 received rebastinib + P and 5 received rebastinib + E (2 non-evaluable due to rapid disease progression and non-compliance). Among 11 patients who received 60 treatment cycles, only 1 patient (treated with eribulin) had grade 3 events (anemia and neuropathy after week 6) potentially related to treatment. When combined with P, the RP2D of rebastinib was 100 mg PO BID, with DLT occurring in 0/6 patients. When combined with E, 0/3 evaluable patients had a DLT at 50 mg BID of rebastinib (accrual ongoing for 100mg BID). Best response included partial response/stable disease in 4(2PR/2SD) of 6 treated with P+ rebastinib, and 1(1PR) of 5 treated with E+ rebastinib. CTCs decreased during therapy (median decrease 99.7 %) and 4/8 patients converted from CTC+ to CTC-. Ang1 levels increased during therapy in 8 patients (0.2-7.0 fold), while Ang2 levels were also increased in 8 patients (0.2-1.4 fold).

**CONCLUSIONS:** When combined with P x 12 weeks, the RP2D of rebastinib is 100 mg PO BID. When combined with E, the RP2D of rebastinib is at least 50mg PO BID; however, the 100 mg PO BID dose level is still accruing patients. The P/E + rebastinib combinations are associated with antitumor activity and exhibit pharmacodynamic evidence indicating blockade of Tie2 (increased Ang) and TMEM function (reduced CTCs) We plan to further evaluate the P+ rebastinib combination as neoadjuvant therapy in the I-SPY program, and continue further evaluation of P/E + rebastinib combinations in MBC.

# 2018 San Antonio Breast Cancer Symposium ®

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**Palbociclib and endocrine therapy in fourth line and beyond for hormone receptor-positive HER2-negative advanced breast cancer: The UK compassionate access program experience**

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## Background

Palbociclib is approved in 1st line for hormone receptor (HR)-positive HER2-negative advanced breast cancer (ABC). A Compassionate Access Program (CAP) previously allowed patients to receive it in 4th line. However, Palbociclib has not been specifically tested in this population. We aimed to determine the safety and efficacy profile of Palbociclib within the CAP across ten institutions in the United Kingdom.

## Methods

We retrospectively identified HR-positive HER2-negative ABC patients on the Palbociclib CAP between December 2015 and September 2017. Demographics, disease characteristics, prior treatments, blood tests, toxicities, treatment delays and responses were recorded. Simple statistics, Fisher's exact test, chi-squared method and Cox regression were used as appropriate.

## Results

118 patients identified had median age of 59 (32-82). 97 (82.20%) were postmenopausal and 109 (92.37%) had ECOG Performance Status 0-1. 96 (81.36%) had visceral involvement, 19 (16.10%) bony only and 15 (12.71%) visceral only disease. Patients received a median 5 (range 3-11) prior lines of treatment and 3 (range 0-8) prior chemotherapy lines.

105 patients (89.74%) developed neutropenia (grade  $\geq 3$  in 59 [56.19%]). 6 experienced febrile neutropenia (5.13%). 57 (48.72%) had a dose reduction, down to 100mg in 48 (41.03%) and 75mg in 9 (7.69%) due to hematologic toxicity in 46 (80.70%). Dose delays were in median 7 days long (range: 0-56). Palbociclib was discontinued due to disease progression in 97 (82.91%) and to toxicity in 5 (4.27%). Grade 3 neutropenia occurred in 45 patients (67.16%) who received  $\geq 3$  and in 22 (32.84%) who received  $< 3$  prior chemotherapies (p 0.351).

Palbociclib produced clinical benefit rate (CBR) of 47.52% and overall response rate of 15.84% in 101 patients assessed. CBR was 45.45% with previous endocrine treatment (ET) progression-free survival (PFS)  $< 6$  months versus 49.12% if PFS  $\geq 6$  months (p 0.714).

Overall, median PFS was 4.5 months (95% CI 3.7-5.9). The PFS seen in different subgroups showed no impact in relation to the number of lines of prior chemotherapy ( $< 3$  lines: 5.9 months [95% CI 3.7-11.0];  $\geq 3$  lines: 4.3 months [95% CI 3.3-5.5], p 0.159), but was numerically greater in those who had previous benefit from ET (PFS  $\geq 6$  months: 5.9 months [95% CI 4.4-8.0];  $< 6$  months: 3.7 months [95% CI 2.8-4.5], p. 0.055) or in those with bone only disease (bone only: 11.0 months [95% CI 2.3-not reached]; other sites involved: 4.4 months [95% CI 3.6-5.5], p 0.024).

Median OS was 15.8 months (95% CI 13.3-18.7) for the whole cohort and it was greater in patients who derived longer PFS from prior ET ( $\geq 6$  months: 18.1 months [95% CI 13.0-NR];  $< 6$  months: 14.4 months [95% CI 7.7-18.3], p. 0.052).

## Conclusions

To date, this is the most extensive analysis of Palbociclib outcomes in  $\geq 4$ th-line setting. In this heavily pretreated population clinical benefit was confirmed particularly for endocrine-sensitive disease and predominantly involving the bones and in earlier lines of treatment. Grade  $\geq 3$  neutropenia rates were similar to PALOMA trials, but the higher incidence of febrile neutropenia need to be carefully considered.

# 2018 San Antonio Breast Cancer Symposium ®

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Lapatinib-based therapies after pertuzumab and/or T-DM1 for HER2+ metastatic breast cancer patients

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## **Background:**

Trastuzumab + pertuzumab + taxane and ado-trastuzumab emtansine (T-DM1) are standard first and second-line therapies for HER2+ metastatic breast cancer. Lapatinib is approved in combination with capecitabine in trastuzumab-resistant patients and in combination with letrozole in hormone receptor positive HER2+ pts for whom endocrine treatment is indicated. In Italy, L is also approved in combination with trastuzumab for hormone receptor-negative HER2+ pts. There are only few data on the activity of lapatinib in pts who received prior pertuzumab and /or T-DM1.

**Methods:** We retrospectively analysed HER2+ metastatic breast cancer pts who received lapatinib after prior pertuzumab and/or T-DM1 from July 2013 to June 2018. Objective response rate (ORR) was assessed according to RECIST 1.1. Progression-free survival (PFS) was calculated from lapatinib-based therapy starting to disease progression (PD) or last follow-up. Overall Survival (OS) was calculated from lapatinib-based therapy starting to death or last follow up.

**Results:** Data from 32 HER2+ mBC treated with lapatinib-based therapy were recorded: 30 pts (94%) received lapatinib combined with capecitabine, 1 pt received lapatinib combined with letrozole and 1 pt received lapatinib combined with trastuzumab. All patients had been treated with prior T-DM1 and 9 (28%) with prior pertuzumab for metastatic breast cancer. Setting of treatment with lapatinib-based therapy was: 2° line (n=2, 6%), 3° line (n=17, 53%) and ≥4° line (n=13, 41%). Patients characteristics were as follows: median age 55 years (range 35-71), hormone receptor positive 66% (n=21), stage IV at diagnosis 34% (n=11), visceral involvement at lapatinib-based therapy starting 91% (n=29).

As of June 2018, lapatinib-based therapy was ongoing for 4 pts and 28 pts discontinued treatment due to disease progression (median number of courses for these pts was 8.5, range 2-27).

ORR in evaluable pts was: complete response (n=1, 3%), partial response (n=13, 41%), stable disease (n=9, 28%), disease progression (n=9, 28%). Median PFS was 6.4 months (95% CI 3.0-9.8). As of June 2018, 14 pts (44%) were alive, median OS was 11.8 months (95% CI 9.9-13.6).

**Conclusion:** These results confirm that lapatinib-based therapy retains clinical efficacy in HER2+ metastatic breast cancer pts treated with prior pertuzumab and/or T-DM1 and represents a valid therapeutic option in this setting.



# 2018 San Antonio Breast Cancer Symposium ®

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First-line treatment with ribociclib and letrozole in advanced breast cancer: First interim data from US patients enrolled in the phase 3b CompLEEmment-1 clinical trial

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**Background:** Addition of ribociclib to aromatase inhibitors significantly increased median progression-free survival in postmenopausal women in the MONALEESA-2 trial and premenopausal women in the MONALEESA-7 trial with hormone receptor–positive (HR+), human epidermal growth factor 2–negative (*HER2*–) advanced breast cancer (ABC). The global phase 3b CompLEEmment-1 trial (Clinicaltrials.gov, NCT02941926) was designed to assess this treatment approach in a cohort of patients with HR+/*HER2*– ABC that was larger and more diverse than that of the MONALEESA program, particularly in the United States where underrepresented/underserved patient groups were targeted. Here we present the first interim safety analysis of the subset of US patients in the CompLEEmment-1 trial.

**Methods:** Women (premenopausal and postmenopausal) and men with HR+/*HER2*– ABC, ≤1 line of prior chemotherapy, Eastern Cooperative Oncology Group performance status ≤2, and no prior endocrine therapy for ABC received open-label treatment with ribociclib (600 mg/d, 3 weeks on/1 week off) and letrozole (2.5 mg/d). Men and premenopausal women were required to use concomitant goserelin (3.6-mg subcutaneous implant every 28 days). Targeted enrollment in the overall study is ~3000 patients. Patients with ≥56 days of follow-up at the data cutoff (October 3, 2017) were included in the first interim analysis (n=1008). The primary endpoint is safety and tolerability.

**Results:** In the first interim analysis, the US cohort of CompLEEmment-1 included 128 patients, 94% of whom were continuing to receive treatment in the study. This cohort included 4 male patients (3%) and 18 patients not identified as white (14%); median age was 62.0 years (Table). The most common adverse events (AEs; >30%) were neutropenia (48%), nausea (45%), and fatigue (36%). The most common grade ≥3 AEs were neutropenia (30%) and leukopenia (6%); all other grade ≥3 AEs occurred in <5% of patients. Rates of AEs leading to discontinuation and dose interruption/adjustment were 5% and 52%, respectively. Four patients experienced at least 1 postbaseline QTcF >480 ms by local assessment. Grade ≥3 increases in alanine aminotransferase and aspartate aminotransferase levels occurred in 3 (2%) and 4 (3%) patients, respectively. There was 1 on-treatment death resulting from a serious AE, bradycardia, that was considered by the investigator to be treatment related.

**Conclusions:** The first interim safety data for ribociclib + letrozole from US patients in the CompLEEmment-1 study, which included a broader population reflective of real-world clinical practice, are consistent with the global study population and previously reported safety profiles from the MONALEESA trials.

Table: Baseline Characteristics

Demographics	Patients, % <sup>a</sup>
	(n=128)
Age, median, y	62.0
Female	96.9
Postmenopausal	86.7
Premenopausal	10.2
Male	3.1
Race	
White	82.8
Black	5.5

Asian	4.7
Other	3.1
Unknown	3.1
Native American	0.8
ECOG Performance Status 0/1/2	50.0/45.3/4.7
Stage at study entry II/III/IV	2.3/3.1/94.5
Current extent of disease	
Bone only	28.1
Central nervous system	0.8
Visceral	53.1
Liver	20.3
Lung	38.3
Other	8.6
Number of metastatic sites	
≤2	61.7
3 or 4	24.2
≥5	14.1

<sup>a</sup>Unless otherwise noted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-26

Ado-trastuzumab for the treatment of metastatic HER2-amplified breast cancer patients previously treated with pertuzumab

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**BACKGROUND:** Ado-trastuzumab (T-DM1) is an antibody-drug conjugate of trastuzumab and a cytotoxic microtubule-inhibitory agent, emtansine. T-DM1 is approved for the treatment of advanced HER2-amplified breast cancer that progressed following trastuzumab-based therapies based on improvement in progression-free survival (PFS) and overall survival (OS) compared to the therapy of physician choice. However, T-DM1 trials were conducted prior to the widespread adoption of docetaxel, trastuzumab, and pertuzumab as standard frontline therapy for advanced HER2-amplified breast cancer. As such, none of the patients enrolled on T-DM1 studies had been exposed to pertuzumab, and the clinical benefit of T-DM1 in patients previously treated with pertuzumab therapy is unknown.

**METHODS:** We completed a retrospective review of patients at our institution over the age of 18 with metastatic HER2-amplified breast cancer treated with pertuzumab prior to T-DM1 between February 2013 and May 2018. Data was collected on patient and tumor characteristics, number and duration of therapies in the metastatic setting, and clinical outcomes. The primary endpoint of this study was PFS in patients given T-DM1 after earlier exposure to pertuzumab. Secondary endpoints included overall response rate (ORR), prolonged duration of T-DM1 therapy (> 6 months), and OS. Adverse events following T-DM1 were collected using CTCAE 4.03, with a focus on cardiac dysfunction and peripheral neuropathy. Patient features and outcomes were summarized with descriptive statistics and time-to-event measures were analyzed using the Kaplan-Meier method and log-rank test.

**RESULTS:** Twenty patients met the inclusion criteria and are included in this study. The patient population consisted of 18 non-Hispanic white and 2 black women, with a median age of 58.5 (range 34-68) years. The number of prior systemic therapies (excluding pertuzumab) ranged from 0-8 with a median of 1 therapy. The duration of T-DM1 therapy (started, on average, 24 months after metastatic diagnosis) ranged from < 1 month to 3.5 years with a median of 6 months. T-DM1 therapy was overall very well tolerated, with all adverse events being grade ≤2. Of note, 2 patients had grade 2 neuropathy, and one patient had grade 1 cardiotoxicity, without any change in left ventricular ejection.

Among 18 patients evaluable for response, ORR was 16.7% (95% CI: 3.6% to 41.4%), with 3 patients achieving a partial response. No complete responses were noted. 10/18 (55.6%) patients had prolonged duration of therapy with T-DM1. Median follow-up time after initiation of T-DM1 was 15 months and 6/20 (30%) patients died while under observation. At the time of data cut-off, 10/20 patients had disease progression on T-DM1. Median PFS was 16 months, with a 1-year PFS rate of 54.5% (95% CI: 36.4% to 81.7%). The 1-year OS rate was 75.0% (95% CI: 58.2% to 96.6%). Patients with liver metastases (n=8) had a significantly worse PFS (p=0.003).

**CONCLUSION:** T-DM1 following pertuzumab is well tolerated and shows excellent efficacy in the treatment of HER2-positive metastatic breast cancer. Comparing T-DM1 following pertuzumab to T-DM1 in pertuzumab-naïve patients should be explored in this patient population.

# 2018 San Antonio Breast Cancer Symposium ®

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Patterns of treatment and outcome of palbociclib plus endocrine therapy in hormone receptor-positive (HR+)/HER2 receptor-negative (HER2-) metastatic breast cancer (MBC): A real life multicenter Italian study

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## Background

The CDK4/6 inhibitor palbociclib combined with endocrine therapy has proven to prolong progression-free survival (PFS) in previously untreated and pretreated women with HR+/HER2- MBC. Few data are available regarding the performance of such a regimen outside the clinical trials, and there is the need to understand its performances in real world.

## Methods

We report a multicenter real-life experience aimed to verify the patterns of treatment and outcome of palbociclib plus endocrine therapy in an unselected population of MBC patients (pts). The primary endpoint was clinical benefit rate (CBR: complete response [CR], partial response [PR], or stable disease for longer than 6 months [SD]); secondary aims were median progression-free survival (mPFS) and safety of the combination. Statistical analysis was performed to identify variables potentially predictive of outcomes. Patients received P at 125 mg daily, 3 wk on/1 wk off in a 28d cycle, combined with letrozole administered orally 2.5 mg on a continuous daily dosing schedule (cohort A) or Fulvestrant 500 mg (F500) i.m. q4wks with loading dose (cohort B); treatment was given until disease progression, toxicity or patient's refusal.

## Results

The study enrolled 150 postmenopausal pts (65 in cohort A, 85 in cohort B) treated from December 2016 to April 2018 at 4 Italian Institutions. Median age in the whole population was 62 years (range 47-79; mean age 59 years in cohort A, 64 in cohort B); 13 pts (20%) in cohort A and 23 pts (27%) in cohort B had *de novo* metastatic disease; 46% of pts in cohort A and 55% had visceral involvement, while 17% and 20% respectively had bone-only disease. The median number of prior lines was 2 in cohort A (range 1-3) and 3 (range 2-5) in cohort B, including ET (median 3) and chemotherapy (median 2); in cohort B 37 pts (43%) had previously received F 500, while 12% (cohort A) and 15% (cohort B) had been pretreated with everolimus. In cohort A a PR as best response was observed in 32% (20 pts), with 14 pts (23%) achieving SD lasting  $\geq 6$  months for a CBR of 52%. In cohort B CBR was 60%, with 25% PR, 48% SD lasting  $\geq 6$  months in 35 pts (41%).

The most common adverse events in both cohorts were neutropenia (grade 1-2 in 67%, grade 3-4 in 35%), grade 1 anemia (52%) and thrombocytopenia (34%), requiring dose reduction in 27% of cases. At a median follow-up of 12 months (range 1-16), mPFS was 6.3 months in cohort A and 5.5 months in cohort B. In both groups a better mPFS was observed in pts treated as  $\leq 3^{\text{rd}}$  versus  $> 3^{\text{rd}}$  line: 9.6 versus 5.2 in cohort A ( $p=0.003$ ), 8.8 versus 4.1 months in cohort B, respectively ( $p=0.002$ ). No significant outcome differences were observed according to prior endocrine therapy with F500 or everolimus, or dominant metastatic site.

## Conclusions

Our real life data, in line with the results of reported clinical trials, indicate that palbociclib plus letrozole or F500 is active and safe in HR+/HER2- MBC, also suggesting a better performance of the combinations in earlier treatment lines.

# 2018 San Antonio Breast Cancer Symposium ®

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Real-world treatment patterns and clinical outcomes with palbociclib combination therapy received in US community oncology practices

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## **Background:**

The treatment landscape for women with HR+/HER2- advanced and metastatic breast cancer (A/MBC) is changing as new agents are being combined with more established treatments to achieve greater efficacy in combating resistant and unresponsive disease. The present study is designed to describe patient characteristics, treatment patterns, and clinical outcomes in a cohort of women with HR+/HER2- A/MBC treated with palbociclib plus aromatase inhibitor (P+AI) or palbociclib plus fulvestrant (P+FV) in the US community oncology setting.

## **Methods:**

Retrospective medical record data from adult women diagnosed with HR+/HER2- A/MBC who initiated P+AI or P+FV for treatment of A/MBC on or after February 3, 2015 were collected from the Vector Oncology Data Warehouse, a network comprised of 10 community oncology practices across the US. Descriptive analyses were performed on patient characteristics, treatment patterns, and clinical outcomes. Time to event outcomes (progression-free rate (PFR) and survival rate (SR)) at 12 (PFR-12, SR-12) and 24 (PFR-24, SR-24) months for the P+AI combination as first line endocrine therapy and 12 and 18 months for the P+FV combination as treatment following prior endocrine based therapy in either the adjuvant or metastatic setting.

## **Results:**

Among 304 patients who received palbociclib combination therapy, 281 (92.4%) received it per labeled indication. Of the 281 on-label users, the focus of reporting here, 233 (82.9%) received P+AI as their initial endocrine therapy after A/MBC diagnosis; 48 (17.1%) received P+FV after prior endocrine therapy for breast cancer. Patient mean age (SD) was 63.1 (11.4) and 68.2 (10.2) years for patients receiving P+AI and P+FV, respectively. Patients were predominantly white (74.2% for P+AI and 77.1% for P+FV patients). The initial dosing for palbociclib was 125mg/day in 85.4% (n=199) of P+AI and 79.2% (n=38) of P+FV patients. Among patients who received P+AI, PFR-12 was 69.8% and PFR-24 was 46.8% with median follow up time of 10.8 months and 36.8% of progression events. The SR-12 was 89.8% and SR-24 was 71.4%. For patients who received P+FV, PFR-12 was 43.5% and PFR-18 was 39.9% with a median follow up time of 7.6 months and 50.0% of progression events. The SR-12 was 76.3% and SR-18 was 65.0%.

## **Conclusions:**

This study provides real-world assessment of treatment patterns and clinical outcomes of patients with HR+/ HER2- A/MBC who received palbociclib in combination with an AI or a FV in US community oncology settings. These findings demonstrate the benefit of palbociclib combination therapy in a diverse real world population.

**Sponsor:** Pfizer, Inc.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-30

Phase Ib/II study of capecitabine 7/7 schedule with neratinib in patients with HER2-positive metastatic breast cancer (MBC)

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**Background:** Neratinib (N) is a potent irreversible inhibitor of HER1, HER2, and HER4 and has been shown to have antitumor activity in patients (pts) with human epidermal growth factor receptor 2 (HER2) - positive breast cancer. A previous study of combination of neratinib with capecitabine (X) was associated with  $\geq$  G3 diarrhea in > 20% of patients. Currently, the NALA study is evaluating this combination of N with X at standard schedule against control. X at 7 day on and 7 day off schedule (7/7) has been shown to be well-tolerated with less  $\geq$ G3 toxicities. We are conducting a phase Ib/II study of N with X (7/7) in pts with pretreated HER2+ MBC (NCT03377387). **Methods:** Eligible pts had HER2+ MBC, normal left ventricular ejection fraction (LVEF  $\geq$  50%); pts can have any and up to 4 prior chemotherapy-based treatments in phase Ib and II portions, respectively. Primary endpoints are to define maximum tolerated dose and efficacy in phase I and phase II portions, respectively. Secondary endpoints include safety and tolerability; exploratory endpoint is to quantify cell-free DNA to correlate with response for phase II portion. There were 4 cohorts for phase Ib with dose level 1 with starting dose of X at 1500 mg BID at 7/7 schedule with N at 240 mg daily. **Results:** As of July 1, 2018 8 pts have been enrolled in 2 cohorts. The median age is 63y (range: 57-79), and median ECOG is 0 (range: 0-1). 4 patients were treated at dose level 1 and 2 of 4 patients experienced dose-limiting toxicity with G3 diarrhea during cycle 1. Other significant toxicities included G3 hand foot syndrome (n=1), G3 fatigue (n=1) and G3 nausea (n=1). Three pts have now been treated at dose level -1 (X at 1000 mg twice daily 7/7 and N at 240 mg daily) and no  $\geq$  G3 toxicities has been noted. Once MTD is reached, the phase II portion will occur to assess the efficacy and to further establish the safety and tolerability of capecitabine and neratinib at the MTD. **Conclusions:** The phase Ib/II study combining neratinib and capecitabine 7/7 is ongoing and updated result will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-31

**PROCLAIM-CX-072:** Monotherapy for advanced triple negative breast cancer with skin metastases in a phase 1-2 trial of the PD-L1 probody therapeutic CX-072

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**Background:** Probody™ therapeutics are novel, fully recombinant antibody prodrugs designed to remain relatively inactive in healthy tissue and to be specifically activated by proteases in the tumor microenvironment. In this way, Probody therapeutics may broaden the therapeutic window for effective but potentially toxic anticancer agents. CX-072 is a Probody therapeutic directed against programmed death-ligand 1 (PD-L1) for the treatment of cancer patients. In a first-in-human, open-label, multicenter, dose-escalation, 3+3 design, phase 1-2 study, PROCLAIM-CX-072 (**PRO**body **CL**inical **A**ssessment **I**n **M**an) (NCT03013491), 22 patients were enrolled in the phase 1 dose escalation portion. Twenty patients were evaluable per RECIST v1.1. Three patients had confirmed partial response (15%), including a 39-year-old woman with stage IV triple negative breast cancer (TNBC) treated with 10 mg/kg CX-072 monotherapy whose disease had progressed on one previous line of chemotherapy for metastatic disease. Metastatic sites included extensive nodal disease and skin/chest wall lesions. The tumor was negative for PD-L1 expression, was microsatellite stable, and had a low tumor mutational burden (4 mutations/megabase). Positive results from the phase 1 study suggest that additional exploration of treatment with CX-072 monotherapy in the TNBC patient population is warranted.

**Dose expansion trial design:** The phase 2 dose expansion part of the PROCLAIM-CX-072 study will include enrollment of TNBC patients with skin metastases. Key inclusion criteria for patients in the TNBC cohort are as follows: naive to immunotherapy (PD-1/PD-L1 and CTLA-4 inhibitors), approved immune checkpoint inhibitor agents not available, histologically confirmed triple negative (estrogen receptor–, progesterone receptor–, and human epidermal growth factor receptor-2–negative cancer per ASCO-CAP guidelines), previously treated with 1 to 3 systemic chemotherapy regimens, and locally advanced and recurrent skin or subcutaneous metastases not suitable for surgical resection or radiotherapy. Patients will receive doses of 10 mg/kg CX-072 intravenously every 2 weeks. Efficacy will be evaluated using RECIST v1.1 and immune-related RECIST criteria. Safety and tolerability will be assessed based on the incidence and severity of adverse events (categorized by NCI CTCAE criteria, v4.03) and relationship to study drug. Other analyses will include pharmacokinetics, incidence of anti-drug antibodies against CX-072, exploratory analysis for immune response, and CX-072 activation in the tumor.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-32

Profiling the early haematological dynamics and treatment modifications with palbociclib when used as first line treatment for ER-positive, HER2-negative metastatic breast cancer

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**Background:** Palbociclib plus endocrine therapy (ET) significantly increases progression free survival compared to ET alone. Within PALOMA2 neutropenia was the most common AE and reason for dose reductions. No real data exists regarding dose reductions (DRs), dose interruptions (DIs), toxicities and benefits of palbociclib. **Objective:** To describe the early haematological dynamics, DRs/DIs with 1st line palbociclib in the context of a routine UK clinical practice. **Methods:** A prospective record was maintained of all patients with ER-positive, HER2-negative metastatic BC registered on the Pfizer patient programme at the Clatterbridge Cancer Centre NHS Foundation Trust. The clinical records of all patients commenced on treatment between April and December 2017 were reviewed, and clinico-pathological information, haematological data & toxicity data recorded. Data lock was 31<sup>st</sup> March 2018. **Results:** 48 patients received at least one cycle of treatment. The median age was 58, 29% (14/48) premenopausal & 71% (34/48) postmenopausal. 43% (21/48) had bone only disease with 42% (20/48) having visceral disease. The median number of cycles delivered 8 (range 2-11). **DRs:** 18/48 (38%) patients had a total of 21 DRs; 14/18 (78%) had 1 DR to 100mg; 1/18 (5%) 1 DR to 75mg; & 3/18 (17%) 2 DRs to 75mg. **Reasons for DRs:** 13 neutropenia, 2 leukopenia, 1 thrombocytopenia, 2 fatigue, 1 poor appetite, 1 sore mouth & 1 non-specially unwell. **DIs:** occurred in 24/48 patients (50%). Details of DRs/DIs by cycle will be presented. 85% (41 of 48) patients remain on treatment with 59% (24/41) on 125mg; 34% (14/41) on 100mg & 7% (3/41) on 75mg. FBC were available for 41/48 (85%) cases & dynamics considered over the first 6 cycles using FBC at the time of planned treatment delivery. **Hb Baseline** all patients (AP): 129 (121 – 138), patients; patients with no dose reductions (NDR) 127 (123 – 139) & patient dose reduction (DR): 130 (118 – 136). **Hb changes to cycles 2, 4 and 6** AP: 122 (115 – 131), 121 (116 – 127) and 125 (116 – 134); NDR: 122 (110 - 135), 125 (117 - 127) and 131 (116 – 135); DR: 115 (112 - 120), 120 (115 - 124) and 122 (115 – 129). **WCC Baseline** AP: 6.8 (5.6 – 7.7); NDR: 7.2 (6.3 – 7.7); DR: 6.7 (5.2 – 7.7). **WCC changes to cycles 2, 4 and 6** AP: 3.7 (2.9 – 4.4), 3.7 (3.1 – 4.4) and 3.3 (3 – 3.9); NDR: 3.5 (2.9 – 4.1), 3.6 (3.2 – 4.3) and 3.6 (3.1 – 4.1); DR: 2.1 (1.7 – 2.5), 4.3 (3 – 4.6) and 3.3 (2.8 – 3.5). **ANC Baseline** AP: 4.0 (3.2 – 5.1); NDR: 4.4 (3.4 – 5.0); DR: 3.6 (2.9 – 5.2). **ANC changes to cycles 2, 4 and 6** AP: 1.5 (1.2 – 2.1), 1.7 (1.3 – 2.0) and 1.4 (1.2 – 1.9) NDR: 1.5 (1.1 – 2.1), 1.7 (1.4 – 2.0) and 1.3 (1.2 – 2.0); DR: 0.8 (0.6 – 0.8), 1.7 (1.2 – 2.3) and 1.4 (1.3 – 1.6). **Plts Baseline** AP: 298 (226 – 339), NDR: 252 (211 – 336); DR: 299 (253 – 339). **Plt changes to cycles 2, 4 and 6** AP: 252 (198 – 310), 221 (186 – 259) and 200 (169 – 243). NDR: 249 (185 – 334), 229 (171 – 267) and 205 (177 – 263); DR: 208 (199 – 210), 216 (199 – 243) and 194 (162 -210). **Conclusion:** These initial real world data are consistent with the PALOMA2 data. Baseline WCC & ANC show no significant difference between NDR and DR cases. Updated data will be presented as well as outcome data for first time.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-33

GI toxicities in metastatic breast cancer: A comprehensive literature review

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## Background

Treatments for advanced or metastatic breast cancer (aBC/mBC) are associated with gastrointestinal (GI) toxicities. The objective of this study was to assess the association between GI toxicities in mBC/aBC and health-related quality of life (HRQoL) and economic burden.

## Methods

We conducted a comprehensive literature search of the Cochrane Central Register of Controlled Trials (2017), NHS Economic Evaluation Database (2016), Embase (1988 – 2017 week 34), and Ovid MEDLINE (1946 to August 2017). Eligible studies evaluated an intervention/comparator treatment in adult patients (age ≥18 years) with aBC/mBC and reported a direct connection between GI toxicities (ie, diarrhea, constipation, nausea, vomiting) and HRQoL and economic evidence. All studies published from January 2000 to August 2017 were assessed for eligibility. Editorials, case reports, conference abstracts, and studies of early, locally advanced, or inflammatory BC were excluded. Abstract and title screening, and full-text screening were conducted by single reviewers. Data were extracted by a single reviewer and verified by a second. Results were synthesized narratively.

## Results

Database searches identified 3,428 articles; an additional 16 articles were identified through other sources. Ninety-four studies underwent full-text review, of which 27 reported a direct connection between GI toxicities and HRQoL (n = 11) and economic burden (n = 19). Some studies reported both HRQoL and economic data.

Patients identified treatment-related adverse events (AEs), such as GI events, as an important aspect of treatment that can affect therapy choice, discontinuation, and switching. Generally, patients with mBC had lower HRQoL than other BC groups, and increasing toxicity was associated with a greater degree of HRQoL impairment. When patients were asked to rank which AEs they most wanted to avoid, only pain ranked higher than nausea and vomiting. In a willingness to pay study, women with mBC were willing to pay \$3,894 (2014 USD) per year to avoid severe diarrhea and \$3,211 to avoid severe nausea.

Gastrointestinal events were among the costliest class of AEs, with mean costs as high as \$4,809 (2016 USD) per episode; costs increased by 24% if events were persistent or recurrent.

## Conclusions

Gastrointestinal toxicities are common in patients with aBC/mBC and have significant consequences for HRQoL and system-level economic outcomes. Frequency and implications of GI effects of treatment regimens should be considered carefully during patient counseling, prescribing and coverage decisions in metastatic breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-34

Treat ER+ight Canadian prospective observational study in HR+ advanced breast cancer: *3<sup>rd</sup> interim analysis*

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Treat ER+ight is the 1<sup>st</sup> prospective observational study in Canadian HR+ HER2– advanced breast cancer patients currently receiving endocrine therapy (ET) alone or in combination with targeted therapy (TT) in the first, second or third line setting (NCT02753686).

**Methods:** This pre-planned interim analysis describes baseline characteristics, treatment patterns and safety/toxicity of patients enrolled in ET and ET+TT cohorts. At data cut-off (November 2017), 200 patients were enrolled from 24 sites since March 2016. The median follow-up time at data cut-off was 8.1 months and 182 patients were evaluable for this analysis.

## Results:

	ET (n=73)	ET + TT (n=109)
Baseline Patient and Disease Characteristics		
Median age, years (range)	71 (37-92)	65 (39-87), p=0.017
ECOG 0-1 (%)	62	74
Median time since primary BC diagnosis, years (range)	5 (0-37)	6 (0-31)
Median time with advanced BC diagnosis, years (range)	1 (0-16)	1 (0-8)
Sites of Metastases (%)		
Bone only	40	27
Visceral	60	73
Location of Metastases (%)		
Lungs	57	47
Liver	23	48, p=0.006
Bone	48	46
Lymph Nodes	27	35
Enrollment Therapy (%)		
Everolimus+exemestane	-	29
Fulvestrant	17	-
Palbociclib+letrozole	-	16
Palbociclib+fulvestrant	-	12
Tamoxifen	11	-
Exemestane	6	-
Letrozole	3	-
Anastrozole	2	-
Palbociclib+exemestane	-	1

Everolimus+tamoxifen	-	0.5
Unknown	1	1
Start Dose of Targeted Therapy		
Everolimus (n=53); 10, 7.5, 5mg (%)	-	72, 4, 24
Palbociclib (n=54); 125, 100mg, unknown (%)	-	89, 9, 2
On-Study Adverse Events ≥15% (all-grade, %)		
Fatigue	23	32
Nausea	12	24
Diarrhea	11	20
Cough	8	15
Alopecia	3	16
Neutropenia	0	17

**Conclusions:** The majority of patients enrolled (64%) were receiving endocrine-based targeted therapy (CDK4/6 inhibitor or mTOR inhibitor) combinations. ET+TT patients were younger with more visceral disease and liver metastases compared to patients receiving endocrine therapy alone. Real-world starting dose of targeted therapies appears to be lower than the approved full dose for 11% of patients receiving palbociclib and 28% of patients receiving everolimus. Incidence of reported all-grade adverse events appears to be lower than previously reported in randomized trials and may be a consequence of real-world under-reporting of adverse events as well as the heterogeneous nature of the treatment cohorts. There does however appear to be a trend toward increased incidence of adverse events in the ET+TT cohort compared to the ET cohort.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-35

A phase 1 study of erlotinib and metformin in advanced triple negative breast cancer

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**Background:** The epidermal growth factor receptor (EGFR) is frequently overexpressed in triple negative breast cancer (TNBC). However, EGFR inhibitors have not shown efficacy as monotherapy in TNBC. One strategy for overcoming resistance to EGFR inhibition is concomitant inhibition of downstream signaling. Metformin is a LKB1-dependent AMPK activator that inhibits both MAPK and AKT signaling. The combination of the EGFR inhibitor erlotinib and metformin synergistically induces apoptosis in TNBC cell lines and decreases tumor burden in PTEN-null EGFR-amplified mouse xenograft models. We evaluated the combination of erlotinib and metformin in a phase 1 study of patients with advanced TNBC.

**Methods:** Patients with advanced TNBC who had received at least one prior line of therapy for metastatic disease were eligible. Erlotinib dose was fixed at 150mg daily. Metformin dose escalation was planned according to a 3+3 design, beginning at 850mg BID and escalating to 850mg TID. One de-escalation to 500mg BID was allowed. Dose-limiting toxicities (DLT) were assessed during the first five weeks of therapy. The primary objectives were to determine the maximum tolerated dose (MTD) of metformin with fixed dose erlotinib and to determine the potential for clinical benefit. Secondary endpoints were response rate, stable disease rate, and progression free survival. Pre- and on-treatment skin biopsies were collected to determine the effect of the study drugs on their respective cell signaling targets, particularly EGFR, AMPK, and mTOR.

**Results:** Between March 2013 and May 2015, nine patients were screened and eight were enrolled. Median age was 48 years (range 37-79). Median number of prior therapies for metastatic disease was 2.5 (range 1-6). No DLT events were reported in either of the dose escalation cohorts during the DLT assessment period. AEs occurring in three or more patients and all grade III AEs are reported in Table 1. Grade III diarrhea despite maximum supportive care required dose reduction of metformin from 850mg TID to 850mg BID in one patient. Grade III rash led to study withdrawal in one patient. No grade IV AEs were reported. Per RECIST v1.1, the best observed response was stable disease in two patients (25%). Median time on study was 2.0 months (range 1.2-3.0). Skin biopsy marker assessment is ongoing and will be reported.

**Conclusion:** The combination of erlotinib and metformin was generally well tolerated in a population of pre-treated metastatic TNBC patients. No unexpected toxicities occurred. While no responses were achieved, stable disease was observed in patients who received this non-chemotherapy combination.

## Adverse Events

Event	Metformin 850mg BID n=3		Metformin 850mg TID n=5		All patients n=8	
	Number of patients (percent)					
	All grades	Grade III	All grades	Grade III	All grades	Grade III
Rash	3 (100)	1 (33.3)	5 (100)	0	8 (100)	1 (12.5)
Diarrhea	3 (100)	0	5 (100)	2 (40.0)	8 (100)	2 (25.0)
Weight loss	1 (33.3)	0	5 (100)	0	6 (75.0)	0
Dry skin	1 (33.3)	0	5 (100)	0	6 (75.0)	0
Nausea	2 (66.7)	0	3 (60.0)	0	5 (62.5)	0
Vomiting	1 (33.3)	0	3 (60.0)	0	4 (50.0)	0
Dry mouth	1 (33.3)	0	3 (60.0)	0	4 (50.0)	0
Dysgeusia	1 (33.3)	0	2 (40.0)	0	3 (37.5)	0
Increased creatinine	2 (66.7)	0	1 (20.0)	0	3 (37.5)	0

Fatigue	1 (33.3)	0	2 (40.0)	0	3 (37.5)	0
Anorexia	1 (33.3)	0	2 (40.0)	0	3 (37.5)	0
Hyponatremia	1 (33.3)	1 (33.3)	0	0	1 (12.5)	1 (12.5)

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-36

Real world treatment patterns and outcomes of patients receiving palbociclib plus aromatase inhibitor in the United States: Sub-groups analysis based on age, performance status and sites of metastases from the IRIS study

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**Background:** Ibrance Real World Insights (IRIS) is a multi-country study aimed to describe clinical characteristics, treatment patterns and clinical outcomes of patients receiving palbociclib plus aromatase inhibitor. Previously the results on the overall population within the US have been communicated. The current analysis focuses on subgroups stratified by age, performance status and visceral status.

**Materials and methods:** A retrospective chart review of HR+/HER2- ABC/MBC patients who received palbociclib plus aromatase inhibitor as initial endocrine based therapy for their advanced disease was conducted between June and October 2017.

Physicians completed electronic case report forms, extracting data on patient demographics, clinical characteristics, treatment history/patterns and clinical outcomes. Progression free and survival rates at 12 and 24 months were estimated via Kaplan-Meier analysis.

**Results:** Data for the US are reported here. In total 63 physicians completed 360 eCRFs with a mean follow up time since palbociclib initiation of 12 months. Majority of the patients were >65 years (53%), and had ECOG status of 0 (30%) or 1 (56%). Overall 293 (81%) patients had metastatic disease, of which 50% had visceral metastases. Across all sub-groups, majority of patients prescribed an initial palbociclib dose of 125mg did not require a change of dose while on treatment. The 12-month and 24-month progression free and overall survival rates across subgroups are presented in Table 1. Patients with a performance status of ECOG=1 had a slightly lower progression and survival rates at 12 and 24 months compared to those with a score =0. Likewise, patients with visceral disease were observed to have slightly lower progression free and survival rates than others.

Table 1: Clinical Outcomes for the different sub-groups.

Patient Sub-groups						
	Age		ECOG status		Visceral Status	
	Up to 65 n=169	Over 65 n=191	0 n=107	1 n=200	Visceral metastases n=147	Non-visceral metastases n=146
Progression free survival rate at 12 months, %	86.3	82.1	94.3	86.5	80.2	85.8
Progression free survival rate at 24 months, %	59.7	69.0	71.2	67.4	50.7	80.6
Overall survival rate At 12 months, %	97.9	92.8	100.0	99.5	90.5	99.3
Overall survival rate at 24 months, %	95.1	85.6	95.7	96.4	87.2	90.7

**Conclusions:** The analysis indicates consistent trends in different clinical outcomes were observed with palbociclib plus aromatase inhibitor across patients sub-groups based on age, performance status and visceral metastases.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-37

The efficacy and safety analysis of the treatments of everolimus and exemestane combination in 101 metastatic breast cancer patients: Real-life experience from Turkey

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**Background:** Endocrine treatment and chemotherapy are a treatment options for patients with hormone receptor (HR) positive and HER2-negative metastatic breast cancer (MBC). However, response to first-line hormone treatment could not obtained in all patients, and even patients who have a response will eventually relapse. After disease progression, second-line hormonal treatment options are used sequentially. Everolimus with exemestane has demonstrated promising activity in patients with HR-positive HER2-negative endocrine-resistant MBC with respect to the results of the BOLERO-2 study. In the present study, we aimed to evaluate the efficacy and safety of this combination in the real-life clinical setting for the unselected population in Turkey.

**Material and Methods:** One hundred and one patients with HR-positive HER-2 negative MBC progressing after prior endocrine treatment who were treated with everolimus with exemestane were retrospectively analyzed. The tolerability and efficacy of this combination were evaluated in the unselected Turkish patients. **Results:** Among 101 patients, 45% of patients had visceral and 50% patients had only bone metastasis. Everolimus with exemestane treatment was administered as a second-line in 21.3% of patients, third-line in 40.4% and fourth-line and later in 38.2%. The objective response rate was 24.7% (CR+PR) and stable disease was obtained in 37.7% of patients. At the median follow-up time of 13.5 months, the median progression-free survival (PFS) time and 1-year PFS were 13.8 months and 57.2%, while the median overall survival (OS) interval and 1-year OS were 40 months and 85%. The median treatment duration was 8.3 and 6.5 months for exemestane and everolimus, respectively. The most frequent reason for discontinuation of treatment were disease progression (39%). Moreover, the most common adverse events (AE) causing permanent discontinuation were stomatitis (3%) and pneumonitis (3%). A total of 81 % of patients experienced at least one AE of any grade, 25% of patients at least one grade 3 or 4 AE. Due to AEs, everolimus dosage was reduced to 5 mg in 16 (15.8%) of patients. **Conclusions:** Our findings confirmed that the combination of everolimus with exemestane was the safe and effective treatment options for patients with HR-positive HER-2 negative MBC after second or later lines treatments.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P6-18-38

Treatment patterns and sequences among pre-menopausal women with HR+/HER2- metastatic breast cancer: A chart review study

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**Background:** Recently, a novel class of treatments, CDK4/6 inhibitors, has been approved, and is now recommended for pre-menopausal women with HR+/HER2- metastatic breast cancer (mBC). This study examined prevailing treatment patterns and sequencing among premenopausal women with mBC treated in clinical practice.

**Methods:** Patient-level data were collected from patient charts in May 2018 from 30 oncologists, mostly from community practice, in the US. Treatment sequences and patterns were assessed for pre-menopausal women diagnosed with HR+/HER2- mBC between January 2015 and January 2017 (with a minimum of 1 year of follow-up).

**Results:** Data were collected on 201 pre-menopausal women with HR+/HER2- mBC. In first-line therapy for mBC, 52.7% of the patients received a CDK4/6 inhibitor-based regimen, 23.4% received endocrine monotherapy, 20.9% received a chemotherapy-based regimen, and the remaining 3.0% received an everolimus-based regimen. The majority of patients who received a CDK4/6 inhibitor received it in combination with an AI (73.6%), fulvestrant (11.3%), or tamoxifen (6.6%). Approximately half of all patients (51.2%) received an ovarian suppression agent during first-line therapy. Overall, median time on treatment from Kaplan Meier (KM) analysis for first-line therapy was 16.1 months. Most common reason for discontinuing first line was disease progression or suboptimal response (79.0% of patients who discontinued); another common reason was the completion of the planned duration of therapy (12.6%).

Among the 106 patients who received a CDK4/6 inhibitor in the first line, median time on treatment from KM analysis was 26.8 months. Main reason for CDK4/6 inhibitor discontinuation was disease progression or suboptimal response (90.2% of patients who discontinued).

For the 109 patients for whom we observed a second-line therapy, treatment sequences are presented in **Table 1**. Median time on treatment for second and third line therapy was 9.6 and 7.8 months, respectively.

**Conclusion:** Following the introduction of novel CDK4/6 inhibitor treatments in the mBC setting, we observed that approximately half of pre-menopausal patients received a CDK4/6-based regimen in the first line of therapy.

Top 5 treatment sequences in pre-menopausal HR+/HER2- mBC patients (n=109)

Treatment Sequence	N	(%)
CDK4/6 - ET -> Everolimus - ET	21	(19.3%)
Chemo -> Chemo	16	(14.7%)
CDK4/6 - ET -> Chemo	13	(11.9%)
ET -> CDK4/6 - ET	13	(11.9%)
Chemo -> CDK4/6 - ET	10	(9.2%)

ET: endocrine therapy; Chemo: chemotherapy; -> indicates a change to the next line of therapy. Percentages calculated among patients with at least 2 lines of therapy



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-18-39

Abemaciclib after prior palbociclib exposure in patients with metastatic hormone-receptor positive (HR+)/HER2- breast cancer

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**Introduction:** The advent of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) has transformed the clinical practice of HR+/HER2- metastatic breast cancer. Palbociclib, ribociclib, and abemaciclib have been approved in conjunction with anti-estrogens, while abemaciclib has also been approved as monotherapy based on single agent activity in the MONARCH-1 trial (objective response rate/ORR: 19.7% and median progression-free-survival/PFS = 6.0 months; Dickler M et al CCR 2017). However, there is limited insight into the mechanisms governing resistance to CDK 4/6i and the potential utility of continued CDK4/6i after progression on a prior CDK 4/6i-based therapy.

**Methods:** We evaluated the clinical outcomes of patients with metastatic HR+/HER2- breast cancer who had received abemaciclib following an initial course of palbociclib-based therapy at our institution. In addition, we conducted genomic analysis utilizing next-generation sequencing of tissue samples and blood (cell-free DNA/cfDNA analysis) where available.

**Results:** From June, 2014 through July, 2018, a total of 49 patients received abemaciclib, and 14 patients had prior palbociclib exposure. One patient was deceased shortly after initiating abemaciclib and one patient was lost to follow-up. Among the 12 remaining patients, eight had sequential courses of CDK4/6-based therapy, while four patients had at least one intervening non-CDK 4/6i based regimen. At data-cutoff of 8/15/2018, five patients (41.7%) had early progression on abemaciclib (PFS equal to or less than 120 days) while three (25%) patients had ongoing benefit (PFS greater than 120 days, two of three actively on therapy). Three additional patients had recently initiated abemaciclib therapy (less than 120 days prior to current analysis). Preliminary analysis of baseline cfDNA results in patients with early progression on abemaciclib therapy after prior CDK4/6i revealed the presence of RB1 mutation, FGFR1 amplification, and TP53 mutation, among others. Additional analyses with mature clinical data (including updated PFS and ORR), toxicity assessment during secondary CDK4/6i exposure, and further analysis of genomic sequencing results will be provided at the meeting.

**Conclusions:** The majority of patients had early disease progression on abemaciclib after prior exposure to CDK4/6i suggesting potential cross-resistance to CDK4/6i mediated by common drivers. However, a subset of patients derived clinical benefit with continued exposure to CDK4/6i, highlighting the need for additional research to evaluate potential predictive biomarkers and guide rational utilization of continued CDK4/6 blockade in metastatic HR+/HER2- breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

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## Evaluation of multiple transcriptomic gene risk signatures in male breast cancer

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**Introduction:** Male breast cancer (MBC) is a rare disease accounting for less than 1% of all breast cancers (BC) and 1% of all cancers in males. The clinical management is largely extrapolated from female BC. Several multigene assays are increasingly used to guide clinical treatment decisions in female BC, however there is little data on the utility of these tests in MBC.

**Methods:** Here we present the gene expression results of 380 M0, ER+ve, HER2-ve MBCs enrolled in the Part 1 (retrospective joint analysis) International Male Breast Cancer Program of 1483 patients diagnosed between 1990-2010 (Cardoso et al. *Annals of Oncology*, 2018). Using a custom Nanostring™ panel comprised of the genes from the commercial risk tests Prosigna®, OncotypeDx® and MammaPrint®, risk scores and intrinsic subtyping data were generated to recapitulate the commercial tests as described by Bayani and Yao et al (*npjBreast Cancer*, 2017). Survival outcomes by risk classification were analyzed using Cox models with time-dependent covariates when the proportional hazard assumption was not met and adjusted for clinical and treatment variables.

**Results:** Prosigna-like risk scores identified 99 (26.1%) as low-risk, 159 (41.8%) as intermediate-risk, and 122 (32.1%) as high-risk. Using the TAILORx cut-off (25) for OncotypeDx-like risk of recurrence scoring, 158 (41.6%) were identified as low-risk, while 222 (58.4%) were identified as high-risk. MammaPrint-like results identified 175 (46.1%) as low-risk and 205 (53.9%) as high-risk. Overall, patients classified as high-risk had higher grade, more nodal involvement, larger tumors, and more frequently treated with chemotherapy than low-risk patients. Survival analyses demonstrated clear clinical utility for each test, showing patients at high-risk with poor relapse-free survival (RFS) as compared to patients classified as low-risk: Prosigna-like RFS at 3-years (HR=2.20, 95% CI, 1.28-3.80); Oncotype-like RFS at 3-years (HR=1.92, 95% CI, 1.17-3.17); MammaPrint-like RFS (HR=1.51, 95% CI, 1.00-2.27); with similar findings for distant relapse-free survival (DRFS) and overall survival (OS). Across outcomes and all gene signatures, patients with concordant Low/Low risk classification had better prognosis than those with concordant High/High risk classification. PAM50 intrinsic subtyping identified 147 (38.7%) as Luminal A, 57 (15.0%) as Luminal B, 80 (21.1%) as Her2-enriched and 96 (25.3%) as Basal-like; showing overall 34.5% concordance to clinic-pathological subtyping by central pathology (95% CI, 29.7%-39.5%). Comparison between the tests in the MBC cohort and a comparable cohort of female BC from the Tamoxifen Exemestane Adjuvant Multinational (TEAM) trial processed in the same way will be presented.

**Conclusion:** Common transcriptomic assays designed to assess residual risk, validated in female BC, provide similar information

in male BC patients. Not surprisingly, disagreement between test results at the individual patient level was observed. To our knowledge, this is the largest study of MBC assayed to generate risk scores of the current commercial BC tests to demonstrate their clinical utility and their differences and similarity to female BC.

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# 2018 San Antonio Breast Cancer Symposium ®

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Tumor subtypes and survival in male breast cancer: SEER 2010-2014

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**Background:** Male breast cancer (MaBC) is an uncommon disease, and population-based information regarding prognostic factors is limited. Most MaBC are hormone receptor (HR) positive, however, the association of tumor subtypes with overall survival (OS) and breast cancer-specific survival (BCSS) is unclear. The aim of this study was to analyze the characteristics of each tumor subtype and its impact on OS and BCSS.

**Methods:** Using Surveillance, Epidemiology, and End Results (SEER) data, we identified men with invasive breast cancer between 2010 and 2014 with known estrogen receptor and progesterone receptor (together HR) status and human epidermal growth factor receptor 2 (HER2) status. Tumor subtypes were classified as: HR+/HER2-, HR+/HER2+, HR-/HER2+ and triple negative (TN). We examined tumor subtypes by patient (pt) characteristics and performed multivariate Cox proportional hazards analyses to determine the associations of each variable with OS and BCSS.

**Results:** We included 1508 pts with a median follow-up of 24 months (range 0-60). Median age was 65 years (range 26-97). At diagnosis, 86.6% of tumors were ductal, 97.1% HR+, 42.1% T1, 55.7% N0, 7.9% M1. Tumor subtype distribution was: 85.5% HR+/HER2-, 11.6% HR+/HER2+, 0.9% HR-/HER2+ and 2% TN. Compared with other subtypes, pts with TN tumors had higher grade disease, presented with more advanced stage and died more often from breast cancer (all  $p < 0.0001$ ); whereas pts with HR+/HER2- tumors were older ( $p = 0.02$ ) and more often white ( $p = 0.02$ ). In univariate analysis, OS at 5 years for all HER2- and all HER2+ was 74.2% and 64.1%, respectively ( $p = 0.002$ ); while BCSS at 5 years for all HER2- and all HER2+ was 88.4% and 78.8%, respectively ( $p = 0.009$ ). Of all subtypes, TN had the worst OS and BCSS ( $p < 0.0001$ ). Breast cancer was the cause of death in 43.7% of HR+/HER2-, 54.2% of all HER2+ and 100% of TN ( $p < 0.0001$ ). In multivariate analyses for OS, older pts (Hazard ratio [HaR] 3 vs. <50 years;  $p = 0.001$ ), stage IV (HaR 9 vs. stage I;  $p < 0.001$ ), HR+/HER2+ tumors (HaR 1.9 vs. HR+/HER2-;  $p = 0.003$ ), TN tumors (HaR 8.5 vs. HR+/HER2-;  $p < 0.001$ ) and unmarried pts (HaR 1.9 vs. married;  $p = 0.002$ ) had significantly worse survival. In multivariate analyses for BCSS, stage IV (HaR 25.7 vs. stage I;  $p < 0.001$ ), HR+/HER2+ tumors (HaR 2.1 vs. HR+/HER2-;  $p = 0.019$ ), TN tumors (HaR 17 vs. HR+/HER2-;  $p < 0.001$ ) and unmarried pts (HaR 2.2 vs. married;  $p = 0.009$ ) had significantly worse survival.

**Conclusion:** We observed significant differences in outcomes by tumor type in men with breast cancer which mirror those previously observed for women with breast cancer. Among the limited numbers of men with HER2+ and TN disease in our sample, outcomes were poor, suggesting possible under-treatment, aggressive tumor biology, and/or more advanced of disease at presentation. Studies to better understand the inferior survival for men with these subtypes are warranted and efforts to ensure appropriate treatment are paramount.

## 2018 San Antonio Breast Cancer Symposium ®

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The role of lumpectomy and radiation therapy in men 70 years of age and older with early breast cancer on hormone therapy: A NCDB analysis

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**Background:** Current consensus guidelines for the treatment of male breast cancer are driven by female-only clinical trials despite data suggesting distinct biologic, clinicopathologic, and prognostic differences between male and female breast cancer patients. This includes a recent retrospective multicenter analysis showing greater overall survival among male breast cancer patients who underwent lumpectomy with radiation therapy (RT), compared to total mastectomy or lumpectomy alone. In light of these findings and the CALGB 9343 trial performed in women, we sought to evaluate if survival was also equivalent in men  $\geq 70$  years old with early stage breast cancer treated with hormone therapy and lumpectomy with or without radiation therapy (RT), as shown in women.

**Methods:** We performed a retrospective analysis of 752 stage I (T1N0M0), estrogen receptor (ER) positive male breast cancer patients  $\geq 70$  years of age who were treated with hormone therapy and underwent lumpectomy with or without RT or total mastectomy (without RT) from the National Cancer Database (NCDB) between the years 2004 to 2014. Chi-squared, Kruskal-Wallis, and analysis of variance tests were used to compare demographic and clinicopathologic differences between groups. Multivariable Cox proportional hazards regression analysis was used to compare overall survival between treatment groups, controlling for demographic and clinicopathologic differences.

**Results:** Most patients underwent total mastectomy (67.4%), with only 32.6% treated with lumpectomy. Of those who underwent lumpectomy, 72.6% (n=178) underwent adjuvant RT. There were significant differences in age, tumor size, histology, grade, surgical margins, nodal surgery, and chemotherapy between patients who underwent lumpectomy without RT, lumpectomy with RT, and total mastectomy ( $p < 0.05$ ). Lumpectomy without RT patients were older (78.9 vs. 76.0 & 76.9 years,  $p < 0.01$ ), more frequently presented with invasive ductal carcinoma (77.6% vs. 71.3% & 85.4%,  $p < 0.0001$ ), and less frequently underwent axillary nodal surgery including sentinel lymph node biopsy (71.7% vs. 91.6% & 94.9%,  $p < 0.0001$ ) compared to lumpectomy with RT and total mastectomy patients. In multivariate analysis, there were no significant differences in overall survival for lumpectomy without RT, lumpectomy with RT (HR 0.71, 95%CI 0.39-1.27,  $p = 0.25$ ), and total mastectomy alone (HR 0.92, 95%CI 0.55-1.56,  $p = 0.76$ ). Older age, higher Charlson-Deyo comorbidity scores, and poorly differentiated tumors were associated with poorer overall survival, while treatment at an academic/research center was associated with improved overall survival ( $p < 0.05$ ).

**Conclusion:** In this national sample of elderly ER positive male breast cancer patients with early disease on hormone therapy, lumpectomy alone was associated with equivalent survival compared to lumpectomy with RT and total mastectomy alone. These results suggest that breast conserving surgery without radiation therapy is appropriate for this subset of male breast cancer patients and greater adoption by breast surgeons should be considered.

# 2018 San Antonio Breast Cancer Symposium ®

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Male breast cancer: Tumour characteristics and treatment compared with females in Australia – 99,768 breast cancers over a 10 year period

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## Introduction

Stage and tumour characteristics in males with early breast cancer (EBC) differ from those in females. Guidelines recommend that treatment of males with EBC should be based on evidence derived from clinical trials in females but some studies suggest men may be undertreated. We assessed tumour characteristics and adjuvant treatment in Australian males as recommended by NCCN guidelines.

## Methods

Using data collected prospectively in the BreastSurgANZ Quality Audit - society of breast surgeons in Australia and New Zealand. Membership requires entry of tumour and treatment details of all breast cancer patients managed by individual surgeons onto an online secure database. No outcome data is collected. The collected information is bound by the National Privacy Principles of both countries. Patients diagnosed with EBC from 1 October 2006 to 30 September 2016 were analysed. The study period was chosen as it corresponds with the availability of trastuzumab and contemporary chemotherapy regimens. Comparisons between males and females were made using chi-square test, significance was considered with alpha of 0.05.

## Results

There were a total of 99,768 episodes, comprising 585 (0.6%) males (544 invasive; 41 DCIS) and 99,183 (99.4%) females (85,596 invasive; 13,525 DCIS; 62 unk). Mean age (range) 68y (25-94) males; 61y (15-102) females. Bilateral synchronous cancer in 6 (1%) males, 3636 (3.7%) females. Histology was ductal/lobular/other in 85%/2%/13% males; 76%/12%/12% females. DCIS in 7% males, 14% females. Triple negative (2% males, 11% females), hormone receptor positive (HR+ - 94% males, 82% females), HER2 positive (7% males, 13% females). Node positive 44% males, 35% females. No breast surgery was performed in 1.9% males, 1.3% females. Where breast surgery was done, complete local excision/mastectomy in 7%/90% males, 55%/40% females. In regard to axillary procedures; 632 were done in males with sentinel node biopsy (SLN) 53%, axillary dissection (AD) 44%, unk 3%; 100,187 done in females with SLN 65%, AD 33%, unk 2%. Table 1 shows the rate of adherence to NCCN February 2018 guidelines.

Table 1

	Treatment recommended	FEMALE % (TR/total)	MALE % (TR/total)	P value
ER/PR - & HER2 +, tumour > 10mm OR node positive	C+T	79% 2336/2943	100% 1/1	1.0
ER/PR + & HER2 +, tumour 6-10mm AND node negative	E±C/T	81% 436/539	100% 1/1	1.0
ER/PR + & HER2 +, tumour >10mm OR node positive	C+T+E	62% 3377/5481	53% 17/32	0.33
ER/PR + & HER2 -, node positive	C+E	64% 12349/19350	56% 109/194	0.028
Triple negative, 10mm OR node positive	C	85% 5756/6789	71% 5/7	0.33

C - chemotherapy, T – trastuzumab, E – Endocrine, TR – treatment received

## Discussion

In this Australian study, male breast cancer accounted for only 0.6% of all cases seen over the 10yr period. In line with other

studies, invasive lobular cancer, triple negative and HER2 positive disease was infrequently seen in males, but with a higher likelihood of being node positive. There were no cases of medullary carcinoma in males but a higher than previously reported incidence of DCIS. Males with HR+, HER2-, node positive EBC were significantly less likely to receive chemotherapy and endocrine treatment, with all other subgroups showing similar systemic treatment for both genders.

# 2018 San Antonio Breast Cancer Symposium ®

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Clinical characteristics and survival of patients with male breast cancer: The Mayo Clinic experience

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## Background:

Male breast cancer (MBC) is rare, and usually managed by extrapolation from female breast cancer. We report on the characteristics and survival outcomes of MBC patients from Mayo Clinic Rochester (MCR).

## Methods:

Medical records of MBC patients treated at MCR during a 25-year period (1990-2015) were reviewed. Demographic variables, tumor characteristics, recurrences, and overall survival (OS) were collected. Progression free survival (PFS) and OS were estimated by the Kaplan-Meier method. Multivariate Cox-proportional hazard regression was used to identify predictors of OS.

## Results:

One hundred sixty-seven patients were included in the final analysis, with a median follow-up of 58 months after diagnosis. Baseline characteristics are presented in Table 1. Eighty percent of patients with ER-positive tumors received endocrine therapy. Among men with stage I-III disease, approximately 90% underwent mastectomy, and 44% received adjuvant chemotherapy. The 5-year locoregional and distant recurrence rates for patients with stage I-III disease were 4.4% and 21.5%, respectively. The 5-year PFS and OS for patients with stage I-III disease were 65.5% and 80.1%, respectively. In a multivariate analysis assessing predictors of OS in patients with stage I-III disease, older age (HR 1.05; 95% CI: 1.02 – 1.09), stage II (HR 11.06; 95% CI: 3.84 – 31.85) or stage III disease (HR 14.74; 95% CI (3.99 – 54.45), and omission of surgery (HR 45.33; 95% CI: 3.97 – 517.32) were associated with poorer OS, while endocrine therapy (HR 0.21, 95% CI: 0.09 – 0.51) was associated with better OS. ER, PR, HER2 and grade were not independently prognostic. The median OS for stage IV patients was 10 months, though this 11-man cohort was too small to allow assessment of prognostic factors in advanced male breast cancer.

## Conclusions:

MBC remains an understudied condition. Prognostic factors in this stage I-III disease are consistent with those identified in other MBC retrospective cohorts. Prospective studies are needed to better understand the unique clinical features of MBC, and to improve outcomes, particularly for advanced disease.

Table 1: Baseline characteristics

	N=167
Median age at diagnosis (Years)	64.4
Ethnicity/Race:	
Caucasian	131 (78.4%)
African American	4 (2.4%)
Other or unknown	32 (19.2%)
Overall AJCC 7th edition stage:	
Stage I	39 (23.4%)
Stage II	80 (47.9%)
Stage III	32 (19.2%)



Stage IV	11 (6.6%)
Unknown	5 (3.0%)
Grade:	
1	8 (4.8%)
2	47 (28.1%)
3	101 (60.5%)
Unknown	12 (7.1%)
ER status:	
Negative	8 (4.8%)
Positive	153 (91.6%)
Unknown	6 (3.6%)
PR status:	
Negative	17 (10.2%)
Positive	141 (84.4%)
Unknown	9 (5.4%)
HER-2 status:	
Negative	70 (41.9%)
Positive	12 (7.2%)
Unknown	85 (50.9%)

# 2018 San Antonio Breast Cancer Symposium ®

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Breast conservation surgery in male breast cancer: A systematic literature review

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## BACKGROUND

Male breast cancer (BC) incidence is low and management is extrapolated from female BC. Breast conservation surgery (BCS) is commonly used for female BC, however, mastectomy remains the most frequently used surgical procedure for male breast cancer. We performed a literature review to assess the use of BCS in male BC as well as outcomes following BCS.

## METHODS

A systematic literature review identified peer-reviewed articles in PubMed evaluating male BC and surgery. Abstracts were screened to identify studies that measured overall survival (OS), disease-free survival (DFS), or local recurrence (LR) in patients undergoing BCS. For all studies, we extracted the total number of patients and number of BCS cases. Of patients undergoing BCS, we further extracted mean age, mean follow-up time, clinical stage, type of axillary surgery [sentinel lymph node biopsy (SLNB), axillary lymph node dissection (ALND)], radiation therapy, hormonal therapy, and chemotherapy. Weighted averages, based on number of patients in each study, were performed for LR, DFS and 5-year OS. The time period for LR and DFS was the duration of follow-up time for each study.

## RESULTS

The literature search yielded 4341 articles. Twelve studies published from 1998 to 2016 met the inclusion criteria and were selected for the systematic literature review. Among the 12,616 male breast surgery cases included, 1,633 (12.9%) underwent BCS. Only patients who underwent BCS were included in our analysis. The mean follow-up time was 54.4 months and mean age was 62.4 with stage II as the most common presentation. Two studies reported that 50-71.4% of patients underwent SLNB and 4 studies reported ALND in 14.3-100%. Seven studies reported that adjuvant radiation therapy was administered in 12.0-100% of 474 total patients undergoing BCS. Four studies reported use of hormonal therapy in 73.8-100% of patients. Four studies reported use of chemotherapy in 25-66.7% of patients. Seven studies reported LR among 122 patients, with a weighted average 11.8%. Four studies reported on 5-year OS in 1511 patients, with a weighted average of 85.8%.

## CONCLUSION

While less commonly used than mastectomy, BCS can be considered a safe alternative in the surgical treatment of male BC. We have demonstrated that the use of adjuvant radiation following BCS is variable in this patient group. Future research should focus on better standardization of local therapy for male BC and improved reporting of outcomes.

# 2018 San Antonio Breast Cancer Symposium ®

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**METRIC:** A randomized international phase 2b study of the antibody-drug conjugate (ADC) glembatumumab vedotin (GV) in gpNMB-overexpressing, metastatic, triple-negative breast cancer (mTNBC)

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## Background

gpNMB is an internalizable transmembrane protein overexpressed in ~40% of TNBC, and associated with a worse prognosis. Preclinical data implicates gpNMB in tumor invasion and metastasis. GV is a novel ADC designed to deliver monomethyl auristatin E (MMAE) to gpNMB-overexpressing cells. Prior phase 1/2 studies suggested promising activity of GV in gpNMB-overexpressing TNBC.

## Methods

In the METRIC trial (NCT#01997333), patients (pts) with mTNBC were randomized 2:1 to GV (1.88 mg/kg IV q21 days) or capecitabine (2,500 mg/m<sup>2</sup> PO daily d1-14 q21 days) until disease progression or intolerance. Key eligibility criteria included: gpNMB over-expression (≥25% tumor cells positive by central immunohistochemistry of archival tissue); estrogen and progesterone receptor expression <10% and HER2 negative; ECOG 0-1; prior taxane; prior anthracycline exposure (if indicated); ≤2 chemotherapy regimens for advanced BC. The primary endpoint was progression-free survival (PFS) per independent, blinded central review using RECIST 1.1. Secondary endpoints included overall survival (OS), objective response rate (ORR), duration of response (DOR), safety, and pharmacokinetics. The trial had 85% power to detect a PFS hazard ratio of 0.64 with two-sided  $\alpha = 0.05$ .

## Results

From Feb 2014 to Aug 2017, 327 women were randomized at 120 institutions to GV (n=218) or capecitabine (n=109). Pretreatment characteristics were well balanced between arms: median age of 55 years; 77% visceral disease; 2.4 years median duration of disease at study entry; 1 median prior anticancer regimen for metastatic disease. At the cut-off for final data analysis (Nov 30, 2017), 21 pts remained on treatment and 98 pts were alive. For GV vs. capecitabine based on independent central review, median PFS was 2.9 (95% CI: 2.8, 3.5) vs. 2.8 (95% CI: 1.6, 3.2) months (HR=0.95; p=0.76); median OS was 8.9 (95% CI: 7.9, 10.5) vs. 8.7 (95% CI: 6.9, 10.8) months (HR=1.06; p=0.73); ORR was 16% (95% CI: 11.1, 22.4) vs. 15% (95% CI: 8.6, 23.5); and median DOR was 5.6 (95% CI: 3.0, 7.8) vs. 4.2 (95% CI: 3.0, 12.2) months. A post-hoc subgroup analysis suggested the greatest benefit from GV was in potentially taxane-sensitive disease (i.e., not previously rechallenged or >6 months progression-free interval following last taxane). The incidence of grade ≥ 3 treatment-related adverse events (TRAEs) was 58% in the GV arm and 37% in the capecitabine arm. The most common grade ≥ 3 TRAEs were neutropenia (27%), rash (15%), and leukopenia (9%) in the GV arm, and diarrhea (14%) and palmar-plantar erythrodysaesthesia (8%) in the capecitabine arm. There was 1 fatal TRAE of neutropenic sepsis in the GV arm and none in the capecitabine arm.

## Conclusion

The METRIC study evaluating GV in mTNBC did not meet the primary efficacy endpoint of improved PFS over capecitabine. While anticancer activity was seen with GV, it was comparable to capecitabine with no therapeutic advantage of GV in terms of ORR, PFS, or OS. The safety profile of GV was consistent with prior experience with no new safety signals identified.

# 2018 San Antonio Breast Cancer Symposium ®

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Activity of larotrectinib, a highly selective inhibitor of tropomyosin receptor kinase, in TRK fusion breast cancers

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**Background:** Tropomyosin receptor kinase (TRK fusions) involving the genes *NTRK1*, *NTRK2*, and *NTRK3* occur in a broad range of malignancies including breast cancers (BC) with secretory features. Larotrectinib, the first selective TRK inhibitor in clinical development, has demonstrated an overall response rate of 75% by independent radiology review across various solid tumors, with a favorable safety profile. Here we report on a case series of TRK fusion BC patients treated with larotrectinib alone or in combination.

**Methods:** Patients were treated with oral larotrectinib in the NAVIGATE global phase II study or in single-patient compassionate-use protocols. All patients received the Phase 2 dose of 100mg BID on a continuous 28d schedule. Efficacy was evaluated using RECIST v1.1.

**Results:** As of June 2018, 5 TRK fusion BC patients had been treated, all with secretory characteristics, but diverse ER/PR/HER2 positivity (Table); 2 patients were triple negative. Four patients harbored *ETV6-NTRK3* fusions. Larotrectinib treatment yielded a response rate of 80% (4/5 PRs). All responses occurred within the first 2 cycles of therapy and cancer-related symptoms resolved rapidly. No treatment-related Grade  $\geq 3$  adverse events have been reported.

Patient 2 (14 yr) presented with a recurrent fungating mass (10.4x8.5 cm) having failed multiple rounds of chemotherapy. Significant reduction in tumor size was noted after one week of larotrectinib treatment with near complete resolution after 2 months.<sup>1</sup> Patient 3 (37 yr) had extensive involvement of the lung and pleura, bilateral pleural effusions, peritoneal infiltration with ascites, severe dyspnea with PS=3. There was rapid improvement with larotrectinib treatment and the patient was PS=1 after 2 weeks. At 6 weeks, there was >80% reduction in tumor size.<sup>2</sup> Patient 5 had a synchronous, locally advanced TRK fusion positive, ER+/HER2- secretory BC and TRK fusion negative, ER+/HER2- metastatic lobular BC. Prior to TRK inhibition, this patient received 2-months of palbociclib/letrozole to which the TRK fusion negative lobular cancer responded, while the TRK fusion SBC did not. The patient tolerated the larotrectinib/letrozole combination with no notable toxicities and experienced a PR in both sites, now ongoing for 11 months.

**Conclusions:** We provide the first evidence that larotrectinib is effective in the treatment of BC harboring NTRK gene fusions. Assays capable of identifying NTRK gene fusions should be considered when profiling patients with BC, especially for patients with secretory BC.

Table: Subject summary

Subject ID	Subtype	Regimen	NTRK gene fusion	ER/PR/HER2 status	Best Response	Protocol
1	Invasive ductal carcinoma (NOS)	larotrectinib	TPM3-NTRK1	ER+/PR+/HER2-	PD	NAVIGATE Ph II
2	Secretory	larotrectinib	ETV6-NTRK3	ER-/PR-/HER2-	PR	Compassionate use
3	Invasive ductal with secretory features	larotrectinib	ETV6-NTRK3	ER-/PR-/HER2-	PR	Compassionate use
4	Invasive ductal with secretory features	larotrectinib	ETV6-NTRK3	ER+/PR+/HER2+	PR	Compassionate use
5*	Secretory		ETV6-NTRK3	ER+/PR-/HER2-	PR	

		larotrectinib + letrozole				Compassionate use
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\*- synchronous TRK fusion negative invasive lobular

1. Shukla et al., *JCO Precis Oncol* 2017, epub  
2. Landman et al., *Clin Breast Cancer*, 2018, 18:e267-e270.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-20-03

Tumor epichaperome expression using  $^{124}\text{I}$  PU-H71 PET (PU-PET) as a biomarker of response for PU-H71 plus nab-paclitaxel in HER2 negative (HER2-) metastatic breast cancer (MBC)

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**Background:** The epichaperome is a new cancer target required for tumor survival (Joshi et al. Nature Reviews Cancer 2018). PU-H71 is a synthetic, purine scaffold epichaperome inhibitor that binds to the ATP-binding site of HSP90 specifically when HSP90 is integrated into the epichaperome (Rodina et al. Nature 2016). It has demonstrated antitumor activity in multiple xenograft models. Furthermore, sequential administration of nab-paclitaxel and PU-H71 in TNBC xenograft models augmented epichaperome levels, and in turn resulted in super-synergistic drug action with ablation of xenografted tumors and cures in mice.

**Methods:** This is an open label phase 1b study of PU-H71 + nab-paclitaxel in pts with HER2- MBC. Pts received nab-paclitaxel at a standard dose of 260mg/m<sup>2</sup> IV Q 3weeks. PU-H71 was administered IV 6 hrs (+/-1 hr) post nab-paclitaxel Q3weeks in 2 escalating dose levels (225mg/m<sup>2</sup> and 300 mg/m<sup>2</sup>). All pts underwent FDG PET/CT every 6 weeks. Additionally, patients had the option to enroll on a separate diagnostic PU-PET protocol to measure epichaperome expression prior to initiating treatment on the phase 1b study, wherein they received a single dose of up to 11mCi of  $^{124}\text{I}$ -PU-H71 IV and underwent imaging at 3-4hrs and 20-24 hrs. Primary objective was to establish the MTD/RP2D of this regimen. Secondary objectives were to assess PK of PU-H71 + nab-paclitaxel and clinical efficacy. Exploratory analysis included correlation of epichaperome expression at baseline using PU-PET with tumor response.

**Results:** 12 patients (5 ER+/HER2- ; 7 TNBC) were enrolled (6 at 225mg/m<sup>2</sup> of PU-H71 and 6 at 300mg/m<sup>2</sup>). Median Age: 54 yrs (range: 37-71). Median ECOG: 0. Median lines of therapy in the metastatic setting: 6 (range 1-11) including prior taxanes in 75% of pts. Most common toxicities included diarrhea G1 58%; G2 7%, G3 7%) that was easily managed with anti-diarrheal agents, G1 fatigue (25%), G1/2 peripheral neuropathy (17%), G1 hyperglycemia (67%), G1 increases in alk phos (58%), AST (50%) and ALT (42%). Hematological toxicities included G3 leukopenia (42%), G3/4 neutropenia (67%), G3 anemia (50%) and G2 thrombocytopenia (17%). There were no DLTs. 33% (4/12) had PR, 58% (7/12) achieved SD with only 1 PD at the time of first scan; 5 pts are currently ongoing including 2 TNBC pts with PR who have been on therapy > 7 months. PK data will be presented. 8/12 patients also underwent PU-PET at baseline. A higher tumor to muscle SUV ratio at 24 hrs on PU-PET predicted response and increased PU-H71 retention on PU-PET at 24 hrs correlated with a longer duration of response.

**Conclusion:** The RP2D of PU-H71 was 300mg/m<sup>2</sup> with 260mg/m<sup>2</sup> of nab-paclitaxel administered IV every 3 weeks. The regimen is well tolerated with promising clinical activity in this heavily pre-treated cohort. Tumor epichaperome expression at baseline using PU-PET has the potential to serve as a predictive biomarker of response. A Phase 2 trial of this combination along with baseline PU-PET is currently planned.

# 2018 San Antonio Breast Cancer Symposium ®

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Activation of AR inhibits growth of endocrine-resistant breast cancer

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## Introduction

Resistance to endocrine therapy is a major clinical problem in estrogen receptor positive (ER+) breast cancer. The androgen receptor (AR) is expressed in ~90% of all ER+ breast cancers and high expression of AR is associated with a better patient outcome in this subtype. In agreement, AR activation in breast cancer cell line models reduces proliferation of cells via antagonism of ER signaling. However, uncertainty surrounding the role of AR in endocrine resistance is reflected in current clinical trials in which both AR agonists and antagonists are being investigated. In this study, we sought to investigate the optimal approach in targeting AR in endocrine-resistant breast cancer.

## Methods

We evaluated the consequences of AR activation, using AR cognate ligand 5 $\alpha$ -dihydrotestosterone (DHT) and selective AR modulator enobosarm, and AR antagonism using enzalutamide on *in vitro* and *in vivo* models of endocrine-resistance. The efficacy of these AR modulators were assessed *in vitro* using tamoxifen-resistant (TamR) and long-term estrogen derived (LTED) models of MCF7 cells, and *in vivo* using *ESR1* mutant E2-dependent (HCI-005) and *ESR1* wild-type E2-independent (Gar15-13) endocrine-resistant PDX models

## Results

Treatment with DHT and enobosarm inhibited the growth of MCF7 TamR and LTED cells but enzalutamide had no effect. AR activation was associated with loss of ER in MCF7 TamR cells and loss of ER-regulated PR expression in MCF7 LTED which suggests that this growth suppression was mediated through the antagonism of ER signaling. Notably, an additive anti-proliferative effect was observed with the combination of enobosarm and CDK4/6 inhibitor palbociclib in the MCF7 TamR cells. A similar pattern was observed *in vivo* with DHT strongly inhibiting the proliferation of both PDX models. Enobosarm similarly suppressed the proliferation of HCI-005, and to a lesser extent in Gar15-13. The benefit of enobosarm in Gar15-13 was significant given that this model is fulvestrant-resistant. Antagonizing AR with enzalutamide had no effect on growth of Gar15-13 model, similar to our *in vitro* data. AR agonists reduced expression levels of ER and PR in HCI-005, and transcriptomic analysis of AR agonist-treated Gar15-13 identified significant negative enrichment of genes related to proliferation and estrogen response. These observations indicate that the growth-suppressive effects of AR activation *in vivo* were mediated through inhibiting ER signaling. We identified an AR gene signature, through RNA-seq analysis of DHT-treated Gar15-13 PDX, which is strongly associated with good outcome in the METABRIC dataset, supporting the hypothesis that an active canonical AR signaling is tumor suppressive in both endocrine-sensitive and -resistant disease contexts. Lastly, we present *in vivo* data demonstrating enhanced suppression of Ki-67 with the combination of enobosarm and palbociclib in the Gar15-13 PDX.

## Conclusion

We have demonstrated that activating AR is an effective therapeutic approach in endocrine-resistant breast cancer, and the combination of an AR agonist with a CDK4/6 inhibitor warrants further investigation in this breast cancer subtype.

## 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P6-20-05

Therapeutic targeting of *BRCA1* and *TP53* mutant breast cancer through mutant p53 reactivation

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Triple negative breast cancer (TNBC) is an aggressive subset for which novel therapeutic approaches are needed. A significant proportion of TNBC patients harbor either germline or somatic mutations in *BRCA1*, or epigenetic silencing of *BRCA1*, which renders them deficient in DNA repair. Virtually all *BRCA1* deficient breast cancers harbor mutations in *TP53* suggesting that inactivation of p53 is a requirement for tumor progression in the setting of *BRCA1* deficiency. Due to this dependency, we hypothesized that restoring wild type p53 function in *BRCA1* deficient breast cancer would be therapeutic. The majority of *TP53* mutations are missense, which generate a defective protein that potentially can be targeted with small molecules. Zinc Metallochaperones (ZMCs) are a new class of anti-cancer drugs that reactivate a class of zinc deficient mutant *TP53* alleles by restoring zinc binding. Using ZMC1 in human breast cancer cell lines expressing the zinc deficient p53<sup>R175H</sup>, we demonstrate that loss of BRCA1 sensitizes cells to mutant p53 reactivation. Using genetically engineered murine mammary tumor models with *Brca1* deficiency, we demonstrate that ZMC1 significantly improves survival in mice bearing tumors harboring the zinc deficient *Trp53*<sup>R172H</sup> allele but not the *Trp53* null allele. We synthesized a novel formulation of ZMC1 (Zn-1), in which the drug is made in complex with zinc to improve zinc delivery, and demonstrate that Zn-1 has increased efficacy over ZMC1. Furthermore, we show that ZMC1 plus olaparib is a highly effective combination for tumors expressing the p53<sup>R172H</sup> mutant. In conclusion, we have validated preclinically a novel therapeutic approach for *BRCA1* deficient breast cancer through reactivation of mutant p53.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-20-07

Efficacy and safety of CB-839, a small molecule inhibitor of glutaminase, in combination with paclitaxel in patients with advanced triple negative breast cancer (TNBC): Initial findings from a multicenter, open-label phase 2 study

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**Background:** CB-839 is an investigational first-in-class, potent, oral inhibitor of glutaminase (GLS), a mitochondrial enzyme that controls a critical step in tumor cell utilization of glutamine. TNBC is associated with high GLS expression and depends on GLS conversion of glutamine to glutamate for tumor cell survival and proliferation. Higher glutamine utilization has been observed in TNBC tumors from patients (pts) of African ancestry (AA), compared to those of European ancestry. CB-839 has demonstrated preclinical antitumor activity in both in vitro and in vivo models of TNBC and synergizes with paclitaxel (Pac) by reversing GLS-dependent mechanisms that lead to taxane resistance. In a phase 1 study, the Pac+CB-839 combination was well tolerated and demonstrated clinical activity in a cohort of heavily pretreated pts with TNBC (22% overall response rate [ORR]; 59% disease control rate [DCR] at  $\geq 600$  mg BID dose [n=37]), including those with taxane-refractory disease or of AA (Kalinsky et al. SABCS 2017). Here we present initial findings from an ongoing phase 2 study of Pac+CB-839 in pts with advanced TNBC (NCT03057600).

**Methods:** Key eligibility criteria included advanced/metastatic TNBC with ECOG PS 0-1 and either 0 (1L) or  $\geq 2$  prior lines (3L+) of therapy for metastatic disease were eligible (N=112 pts max). Prior taxane therapy for the 3L+ cohorts was required. For the 1L cohorts, prior neo/adjuvant therapy (including taxanes) was allowed if time to recurrence was  $>12$  months (mo). Pts were allocated to cohorts by line of therapy (1L or 3L+) and ancestry (AA or non-AA) in order to ensure sufficient enrollment to each of these subgroups. Pts received oral CB-839 (800 mg BID) in combination with intravenous Pac (80 mg/m<sup>2</sup> on days 1, 8, 15 of each 28-day cycle). The primary endpoint was ORR. Other endpoints included overall survival, duration of response, clinical benefit rate, biomarker analyses, and safety.

**Results:** As of the June 15, 2018 data cutoff, 44 pts have been enrolled (median age, 59 yrs; 41% AA; n=22 each in 1L or 3L+). In the 1L cohort, 64% and 36% had ECOG PS of 0 and 1, respectively. In the 3L+ cohort, 36% and 55% had ECOG PS of 0 and 1, respectively (9% unknown). Of 14 evaluable 1L pts, ORR was 43% and DCR (partial response [PR] + stable disease  $\geq 8$  weeks [SD]) was 79% (n=6 PR; n=5 SD). Of 16 evaluable 3L+ pts, ORR was 6% and DCR was 25% (n=1 PR; n=3 SD). Most common CB-839- or Pac-related treatment-emergent adverse events (TEAE) occurring in  $>15\%$  included fatigue (34%), diarrhea (23%), nausea (18%), neuropathy (18%), alopecia (16%), anemia (16%), AST increased (16%), and hypophosphatemia (16%). Treatment-related grade  $\geq 3$  TEAEs occurred in 8 (18%) pts; the most common (occurring in  $>1$  pt) were hypophosphatemia and neutrophil count decreased (n=2 each). No events of febrile neutropenia were reported.

**Conclusions:** Early analyses of this phase 2 study show that Pac+CB-839 has clinical activity and is well tolerated in pts with advanced/metastatic TNBC. Updated data including exploratory biomarker analysis will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

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Anti-tumor activity of elacestrant (RAD1901) in models harboring ESR1 mutations resistant to standard of care therapies

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**Background:** Estrogen receptor positive (ER+) breast cancers make up approximately 75% of all breast cancers diagnosed and ER, a protein encoded by the ESR1 gene, plays a major role in the initiation, growth and survival of these cancers. Current targeted therapies inhibit the ER pathway by either blocking the synthesis of the natural ligand of ER, estradiol, (aromatase inhibitors (AI)), or by antagonizing and/or degrading the receptor (selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs)). AIs are used in both the adjuvant and metastatic setting and recent clinical reports have shown that 20-50% of patients that had received AI therapy had detectable mutations in the ER ligand binding domain (ER-LBD). Two frequently found point mutations in the ER-LBD, Y537S and D538G, have been shown to result in estradiol-independence and constitutive activation of ER, consistent with their ability to cause resistance to AIs. While the selection of ESR1 mutations post-AI has been demonstrated clinically, the clinical response of ESR1 mutant tumors to fulvestrant, an approved SERD, is not fully understood. Preclinical studies have suggested that ESR1 mutations can cause decreased binding and a corresponding decrease in potency of ER antagonists, including fulvestrant (SERD) and tamoxifen (SERM). Conversely, clinical data from the SoFEA, PALOMA-3, and FERG1 trials suggested the presence of ESR1 mutations did not alter fulvestrant activity. The limited clinical data that exists, however, is based on retrospective study designs with relatively small data sets, making it difficult to accurately predict fulvestrant activity against specific mutations and the activity of fulvestrant against tumors that harbor multiple mutations. In fact, recent additional data from the PALOMA-3 trial suggests that the Y537S mutation, specifically, was selected out in clinical samples from patients treated with fulvestrant, more closely matching preclinical results. This suggests there may be certain contexts of ESR1 mutations where fulvestrant may have limited activity. It will be important to further understand the consequence of specific mutations and to utilize therapies that have activity against all ESR1 mutations. We have previously described elacestrant (RAD1901), a novel orally bioavailable SERD, that exhibited activity in multiple ER+ breast cancer models. Interestingly, elacestrant exhibited similar effects to fulvestrant in *in vitro* ESR1 mutant models, however, in some *in vivo* PDX models harboring the Y537S mutation elacestrant inhibited growth, while fulvestrant had limited activity. Here, we describe a more complete *in vivo* dataset describing elacestrant activity versus fulvestrant in multiple patient-derived xenograft (PDX) models harboring ESR1 mutations.

**Methods:** Multiple PDX models harboring natural mutations in ESR1 or genetically-engineered CRISPR models were used to assess the anti-tumor efficacy and the pharmacokinetic/pharmacodynamic properties of elacestrant and fulvestrant.

**Results:** Elacestrant significantly inhibited the growth of xenograft models harboring ESR1 mutations, including those harboring Y537S or D538G mutations and models that were insensitive to fulvestrant and tamoxifen.

# 2018 San Antonio Breast Cancer Symposium ®

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Pharmacological inhibition of TFF3 enhances chemo-sensitivity and overcomes acquired resistance in breast cancer

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## Background

Dose-dependent toxicity and acquired chemo-resistance are two major challenges in the use of doxorubicin in breast cancer treatment. Trefoil factor 3 (TFF3) is a secreted ligand that promotes breast cancer progression and predicts poor survival outcome of breast cancer patients. It has also been shown to confer resistance to anti-estrogens and trastuzumab in breast cancer. Here, the role of TFF3 in regulating the sensitivity and acquired resistance to doxorubicin in breast cancer was investigated.

## Methods

MCF7, ZR-75-1 and BT474 breast cancer cell lines with siRNA-mediated depletion of TFF3, and doxorubicin-resistant MCF7 cells generated from the pulsatile exposure to doxorubicin, were used as *in vitro* models. We have developed a novel non-toxic small molecule inhibitor of TFF3 (AMPC) that binds specifically to cysteine 57 residue of dimeric TFF3 and promotes its dissociation to monomers thereby, inhibiting its dimeric functions such as proliferation and apoptosis. Here, the effects of AMPC in enhancing doxorubicin sensitivity and overcoming acquired doxorubicin resistance in breast cancer cells were also explored.

## Results

Consistent with siRNA-mediated depletion of TFF3, pharmacological inhibition of TFF3 by AMPC enhanced doxorubicin-mediated decrease in cell viability, foci formation and 3D growth of the breast cancer cells, suggesting that TFF3 inhibition increased the sensitivity of these cells to doxorubicin treatment. Notably, AMPC combined with doxorubicin in a synergistic manner, enabling doxorubicin dose reduction for the same inhibitory effect. Doxorubicin-induced AKT activation has been reported to antagonize the effects of doxorubicin and promote its resistance in breast cancer. Here, the inhibition of TFF3 by AMPC was shown to reduce AKT activation. Mechanistically, AMPC co-treatment suppressed doxorubicin-induced AKT activation thereby enhancing doxorubicin-induced apoptosis, with an overall up-regulation of pro-apoptotic and down-regulation of anti-apoptotic proteins, as compared to doxorubicin monotherapy. TFF3 also mediated the acquired doxorubicin resistance in MCF7 cells. Elevated expression of TFF3 was observed in the doxorubicin-resistant MCF7 cells as compared to the parental MCF7 cells, while the inhibition of TFF3 by AMPC completely abrogated the resistant phenotype of these cells as shown in the cell viability, foci formation and 3D growth assays. In concordance with the elevated levels of TFF3, doxorubicin-resistant MCF7 cells also exhibited increased activation of AKT with reduced susceptibility to doxorubicin-induced apoptosis as compared to the parental MCF7 cells. Consistently, this was reversed with AMPC co-treatment, which suppressed the elevated levels of activated AKT in the doxorubicin-resistant MCF7 cells, resulting in the re-sensitization of these resistant cells to doxorubicin-induced apoptosis. Similar to that in the parental cells, AMPC also exhibited a synergistic inhibitory effect with doxorubicin in the doxorubicin-resistant MCF7 cells.

## Conclusion

The pharmacological inhibition of TFF3 with AMPC is a potential therapeutic approach to reduce the dose-dependent toxicity and to overcome the acquired resistance of doxorubicin in breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-20-10

### Role of GPR110 in breast cancer

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Our long-term goal is to discover adhesion GPCR targets in breast cancer. Our previous studies have found GPR110 to be overexpressed in tumorigenic cell population as well as in anti-HER2 drug-resistant derivatives of HER2+ breast cancer cells. In subsequent studies, we found that GPR110 knockdown inhibited anchorage-independent cell growth, mammosphere formation, and invasion/migration of HER2+ breast cancer cells. Conversely, overexpression of GPR110 by lentiviral delivery of cDNA enhanced anchorage-independent cell growth, mammosphere formation, and invasion/migration in HER2+ breast cancer cells. In addition, GPR110 overexpression led to increase in the % of Aldefluor-positive tumorigenic cell population, further emphasizing the role of GPR110 as a mediator of tumorigenesis in addition to the metastatic processes in HER2+ breast cancer. Among various subtypes of breast cancer, GPR110 expression was higher in HER2+ and basal subtypes, most of which are triple-negative (negative for ER, PR, and HER2), compared to luminal A and B subtypes. GPR110 was either gene amplified or upregulated in 4% of all breast cancers based on the publicly available TCGA dataset. GPR110 overexpression predicted poorer recurrence-free survival in triple-negative breast cancer. Furthermore, GPR110 was overexpressed in brain metastatic lesions compared to mammary tumors in patient-derived xenograft models of triple-negative breast cancer (WHIM2 and WHIM30). Knocking down GPR110 reduced anchorage-dependent and -independent cell growth, mammosphere formation, and invasion/migration of triple-negative breast cancer cells. Overall, our results suggest that GPR110 may be a potential drug target in HER2+ and triple-negative breast cancer. Drug discovery efforts to identify GPR110 antagonists will provide useful pharmacological tools for validating GPR110 as a drug target in breast cancer. Since GPR110 is also overexpressed in various other types of cancer, understanding the mechanism of GPR110 upregulation and signaling in cancer is an important future direction.

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# 2018 San Antonio Breast Cancer Symposium ®

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The stapled peptide ALRN-6924, a dual inhibitor of MDMX and MDM2, enhances antitumor efficacy of paclitaxel and Nab-paclitaxel in TP53 wild-type MCF-7 breast cancer models

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**Background:** MDMX and MDM2 are endogenous inhibitors of the p53 tumor suppressor protein. MDMX levels are frequently elevated in luminal breast cancer, which generally expresses wild-type p53. ALRN-6924, an  $\alpha$ -helical stapled peptide, is the first and only dual inhibitor of MDMX and MDM2 currently in clinical trials for solid tumors and hematological malignancies. We sought to determine the antitumor efficacy of the combination of ALRN-6924 with taxanes in models of human breast cancer.

**Methods:** Sulforhodamine B colorimetric assay was used to assess the cytotoxicity of the combination of ALRN-6924 with taxanes *in vitro*. Athymic nude mice were implanted with MCF-7 tumors and treated for four weeks with ALRN-6924 alone and in combination with paclitaxel in cremaphor (Taxol®, study #1) or a nanoparticle-albumin-bound (nab) formulation (Abraxane®, study #2). In study #1, ALRN-6924 (5, 10 mg/kg) was dosed twice weekly and paclitaxel (10, 15 mg/kg) was dosed weekly, with paclitaxel administered 6 h prior to ALRN-6924. In study #2, ALRN-6924 alone (5 mg/kg) was dosed twice weekly while nab-paclitaxel (15 mg/kg) was administered weekly in combination at -24h, -6h, 0h, +6h, or +24h relative to ALRN-6924 administration.

**Results:** ALRN-6924 was found to have synergistic activity with paclitaxel in both MCF-7 and ZR-75-1 cell lines *in vitro* (Combination index: 0.874 and 0.323 respectively). In *in vivo* study #1, the combination of ALRN-6924 and paclitaxel significantly inhibited MCF-7 tumor growth compared to either agent alone ( $p \leq 0.005$ ). Paclitaxel 15 mg/kg + ALRN-6924 5 mg/kg resulted in the greatest tumor inhibition with average tumor size decreased by 13% at four weeks versus the starting size. In study #2, the combination of nab-paclitaxel with ALRN-6924 administered -6h to +24h relative to nab-paclitaxel resulted in improved efficacy over either single agent and a significant increase in the number of tumor regressions (up to 6/10 with 3 consecutive measurements <50% of starting volume) compared to nab-paclitaxel alone (1/10,  $p < 0.005$ ). When ALRN-6924 was administered 24h prior to nab-paclitaxel, there was a marked decrease in efficacy and no tumor regressions were observed. In both studies, drug treatments were well tolerated with no significant weight loss in mice.

**Conclusion:** The significant increase in efficacy observed with ALRN-6924 in combination with paclitaxel supports further evaluation in patients with breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

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FGFR1 $\beta$  is a driver isoform of FGFR1 alternative splicing in breast cancer cells

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**Background:** Abnormal FGFR1 alternative splicing is correlated with tumorigenicity and poor prognosis in several tumor types. We sought to determine the roles of FGFR1 $\alpha$  and FGFR1 $\beta$  variants in breast cancer. **Methods:** TCGA breast cancer samples and cell lines were analyzed for FGFR1 $\alpha$  and FGFR1 $\beta$  expression. MCF-10A cells were used to overexpress these variants. Cell growth was assessed by SRB and colony formation assays. Cell transformation was assessed by 3D-Matrigel, soft agar, cell motility assays. Cell survival assay was used to determine drug IC50. **Results:** In the TCGA, compared to FGFR1 non-amplified samples, FGFR1-amplified samples had significantly higher FGFR1 $\alpha$ , but not FGFR1 $\beta$  levels. FGFR1 $\beta$  expression levels and FGFR1 $\beta$ /FGFR1 $\alpha$  ratio were higher in basal subtype samples than in luminal samples in both the TCGA and in a panel of breast cancer cell lines. Both FGFR1 $\alpha$  and FGFR1 $\beta$  induced transformation of MCF-10A cells. However, only FGFR1 $\beta$ -expressing cells, not FGFR1 $\alpha$ , enhanced cell growth, cell motility, and FGFR signaling. Cells with higher FGFR1 $\beta$  levels and FGFR1 $\beta$ /FGFR1 $\alpha$  ratio were more sensitive to FGFR inhibitor BGJ-398. Interestingly, in ER-negative cells, BGJ-398 decreased FGFR1 $\beta$  levels, likely by increasing expression of splicing repressor PTBP1. In ER-positive cells, estrogen treatment increased FGFR1 $\beta$  levels by decreasing PTBP1 expression, which was blocked by 4-OHT. Lastly, combination treatment with BGJ-398 and 4-OHT synergistically inhibited cell survival. **Conclusions:** These findings suggest that FGFR1 alternative splicing plays an important role in breast cancer, where FGFR1 $\beta$  functions as a driver isoform. Further work is needed to assess FGFR1 $\beta$  prognostic and predictive role.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-20-13

The pure progesterone receptor (PR) antagonist onapristone enhances the anti-proliferative effects of CDK4/6 inhibitors in preclinical in-vitro breast cancer models

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Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer related death in women. Around 5–10% of cases are metastatic at diagnosis, and close to 30% of patients with early stage disease will relapse with metastatic disease. Anti-estrogen therapy is an important treatment modality for hormone receptor-positive (HR+) metastatic breast cancer (mBCa) as mono- or combination (e.g. with CDK4/6 inhibitors) first-line (1L) therapy. Unfortunately, despite the high rate of clinical benefit in 1L therapy, disease progression still generally occurs and there remains a need for an efficacious 2L therapy. Although anti-estrogens continue to play a role in 2L treatment there is a critical need for targeting additional mechanisms in combination with anti-estrogens. Progesterone is a major mitogen in the adult human mammary epithelium and, in addition to ER, is a key driver of breast cancer cell proliferation. Thus, blocking both ER and PR would likely be an effective approach for inhibition of all hormone driven breast cancer cell proliferation.

Onapristone is a unique PR full antagonist that is efficacious as an antitumor agent in multiple preclinical breast cancer models and exhibits additive/synergistic effects with antiestrogens. Here, we examine the effects of onapristone in in-vitro model systems in combination with CDK4/6 inhibitors.

**Methods:** T47D cells were treated with various concentrations of onapristone or palbociclib in media containing 10%FBS. 10 days after treatment cell viability was determined using Cell Titer Glo. Cells were also treated with increasing concentrations of palbociclib in the absence or presence of onapristone and analysed for cell proliferation after 10 days and for gene expression after 3 days of treatment. The effects of onapristone were also tested in soft agar anchorage-independent growth assays as well as 3D tumorsphere assays using ER+/PR+ MCF7 and BT474 cells +/- onapristone for 21-28 days. Formed colonies or spheres were analyzed morphologically using cell stain and by quantifying the total number and size of colonies or spheres formed.

**Results:** Onapristone inhibited T47D cell proliferation in a concentration-dependent manner. Additionally, it sensitized the cells to inhibition by CDK4/6 inhibitors in the preclinical in-vitro models. Onapristone also had a marked effect on downstream target genes in the cell-based models providing a mechanistic basis for the anti-proliferative effects. Onapristone also blocked progesterone-induced breast cancer cell survival in either soft agar or 3D tumorsphere assays with effects comparable to that of anti-estrogen (fulvestrant).

**Conclusions:** Onapristone inhibits T47D proliferation and key target genes involved in cell proliferation. Additionally, onapristone enhances the anti-proliferative effects of palbociclib in-vitro. Previous studies have provided a clear rationale for combining onapristone and anti-estrogens in the clinic for the treatment of breast cancer. Our data extend these studies to provide additional rationale for the combination of onapristone with CDK4/6 inhibitors such as palbociclib as an effective new approach for the treatment of breast cancer.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-20-14

Neutralizing soluble tumor necrosis factor alpha overcomes trastuzumab-resistant breast cancer immune evasion by downregulating mucin 4, improving NK cell function and decreasing myeloid-derived suppressor cells in tumor microenvironment

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**Background:** Novel strategies aimed to overcome trastuzumab (Tz) resistance of HER2+ breast cancer (BC) are needed. Recently, we demonstrated a novel immune evasion strategy used by BC where tumor necrosis factor alpha (TNF) induces upregulation of the transmembrane glycoprotein mucin 4 (MUC4) via NF-κB activation to impair Tz binding that prevents antibody mediated killing of BC cells. Etanercept, a non-selective inhibitor of soluble and transmembrane TNF (sTNF, tmTNF), downregulated MUC4 expression and sensitized *de novo* Tz-resistant BC xenografts to Tz. Moreover, we showed that MUC4 expression is an independent predictor of poor disease-free survival in patients treated with Tz in the adjuvant setting (Clin Cancer Res 2017, 23:636). Etanercept is immunosuppressive due to off-target effects on tmTNF while selective inhibition of sTNF improves the immune response to the tumor (Cancer Immunol Res 2016, 4:441). Because of the immunosuppressive properties of etanercept, we wanted study if the dominant negative-TNF protein XPro1595 (DN-TNF; also known as INB03) that neutralizes sTNF without affecting tmTNF is able to downregulate MUC4 to inhibit Tz-resistant tumor growth and improve innate antitumor immune response.

**Methods:** To assess the effect of DN-TNF on Tz-resistant HER2+ tumor growth, JIMT-1 cells were s.c. injected in nude mice. When tumors were established, animals were treated with IgG, DN-TNF, Tz or DN-TNF+Tz, i.p. twice a week for one month. Innate immune response was determined by flow cytometry analysis of NK cells activation and degranulation and myeloid-derived suppressor cells (MDSC) subtypes in tumor microenvironment (TME) and in spleen. Tz-dependent NK cells degranulation was assessed in splenocytes using HER2+, Tz-sensitive cell line BT-474 as the target. MUC4 and phospho NF-κB expression was determined by Western blot.

**Results:** Treatment with Tz or DN-TNF had no impact on JIMT-1 tumor growth. However, co-treatment with DN-TNF and Tz resulted in significantly less growth. At day 21<sup>st</sup>, tumor volume was 75mm<sup>3</sup> in DN-TNF+Tz vs 300mm<sup>3</sup> control groups. DN-TNF+Tz treatment showed a decrease in myeloid cell infiltration and MDSC phenotype was enriched in the granulocytic-MDSC vs monocytic-MDSC suggesting a less immunosuppressive TME. DN-TNF+Tz administration significantly increased activation and degranulation of tumor infiltrating NK cells. In addition, spleen NK cells from these animals exhibited enhanced Tz-dependent degranulation vs control groups. MUC4 expression was downregulated in tumors treated with DN-TNF and NF-κB phosphorylation was inhibited (all comparisons p<0.05).

**Conclusion:** These results suggest that targeting sTNF together with Tz treatment improves antitumor immune response reducing tumor burden. Activated NK cells can more effectively attack the tumor due to a less suppressive TME and decreased MUC4 expression enhancing Tz binding in Tz-resistant HER2+ BC. Patients with increased levels of TNF expressing MUC4 in their tumors could be eligible for a combined therapy with DN-TNF and Tz to overcome/avoid resistance to therapy. These results can be translated quickly into the clinic.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-20-15

Characterization of a novel prognostic and targetable modulator of the Wnt/ $\beta$ -catenin signaling pathway in breast cancers

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Mortality in metastatic triple negative breast cancer (TNBC) remains high due to a lack of targeted therapy options. Consequently, there is a pressing need to identify prognostic markers and therapeutic targets for TNBC. Our group identified the DEAD-box RNA helicase DP103 as a novel prognostic biomarker and metastasis-driving oncogene enriched in TNBC. Aberrant Wnt/ $\beta$ -catenin signaling has been implicated for driving growth and metastasis of many cancers, like TNBC. Our studies found that the depletion of DP103 in metastatic TNBC cells resulted in decreased Wnt activity and expression of downstream Wnt target genes such as Axin2 and c-Myc. In addition, stimulation of the Wnt pathway via GSK3 $\beta$  inhibition or Wnt-3a recombinant protein significantly increased the transcriptional expression of DP103, indicating a possible positive feedback loop. To validate this new role for DP103 in regulating the Wnt/ $\beta$ -catenin signaling pathway in vivo, we developed a novel Drosophila stem cell and tumor model by generating a genetic mosaic of oncogenic Ras and  $\beta$ -catenin in Drosophila. The co-expression of these oncogenes caused benign tumor formation in Drosophila larval tissue that were visualized and used to identify key genetic modifiers necessary for growth and invasion into neighboring tissues. RX-5902, a novel Wnt/ $\beta$ -catenin modulator preventing the nuclear translocation of  $\beta$ -catenin via phosphorylated p68 decreased the transcriptional expression of DP103 and inhibited tumor formation as observed in our Drosophila model. Interestingly, overexpression of DP103 abrogated the effects of RX-5902, suggesting a requirement for RX-5902-mediated downregulation of DP103 to bring about anti-tumorigenic effects. Collectively, our data suggests a novel regulatory role for DP103 in the Wnt/ $\beta$ -catenin signaling pathway that is targetable by drugs like RX-5902.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-20-16

BAT8003, a potent anti-Trop-2 antibody-drug conjugate, for the treatment of triple negative breast cancer

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Trophoblast cell surface antigen 2 (Trop-2) is overexpressed on many epithelial carcinomas, yet is expressed at much lower level on normal tissue. Overexpression of Trop-2 has been correlated with poor prognosis in several solid tumors. These two characteristics make Trop-2 a potential drug target. An antibody-drug conjugate (ADC) targeting Trop-2, IMMU-132, has recently been demonstrated to be effective in treating triple negative breast cancer (TNBC) and gastric cancer patients. Here we present a Trop-2 ADC, BAT8003, which contains an uncleavable linker and a maytansine derivative as the payload. An A114C mutation on antibody heavy chain was introduced to BAT8003 for site-specific conjugation, in order to generate a more homogeneous product for a better pharmacokinetics profile. BAT8003 is also completely devoid of fucose modification, to allow for enhanced ADCC effect. We show that BAT8003 is effectively internalized upon binding to Trop-2, and inhibits proliferation of Trop2-overexpressed tumor cells with IC50s of ~1 nM. In a TNBC (MDA-MB-468 cell) mouse xenograft model, BAT8003 strongly inhibits tumor growth at a dose level as low as 5 mg/kg. BAT8003 also demonstrates potent activity in another TNBC (MX-1 cell) mouse tumor model, in which it shows the same inhibition activity as the naked antibody conjugated heterogeneously with almost two fold payload (DAR 3.5), suggesting the effect caused by site-specific conjugation. We are currently developing BAT8003 for clinical evaluation in TNBC and other cancer indications.

## 2018 San Antonio Breast Cancer Symposium ®

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Targeting SHP2 for the treatment of HER2-positive breast cancer

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Approximately 20% of breast cancer (BC) is caused by overexpression of the human epidermal growth factor receptor 2 (HER2). As a result, several antibody-based and small molecule-based anti-HER2 therapies have been developed. These drugs have benefited BC patients by improving overall survival and quality of life. However, development of resistance and disease recurrence have been the major clinical challenges. One way to overcome these clinical problems is to develop alternative therapeutic strategies. Multiple lines of evidence show that targeting the Src homology phosphotyrosyl phosphatase 2 (SHP2) in HER2-positive BC may prove beneficial both in treatment-naïve and anti-HER2 therapy-resistant forms of the disease. For instance, silencing SHP2 expression in HER2-positive breast cancer cells or conditional knockout in ErbB2 transgenic mice blocks HER2 overexpression and associated signaling, leading to loss of cell transformation and tumorigenesis. To test the clinical translational significance of SHP2 targeting, we have invented a specific small molecule SHP2 inhibitor named WGMDY (US 9,932,288) that has shown promising anti-cancer effects. The results obtained so far show that inhibition of SHP2 with WGMDY blocks HER2 expression, suppresses cell proliferation and anchorage independent growth, and induces regression of preformed xenograft tumors. These findings suggest that SHP2 might be an excellent drug target for HER2-positive BC and WGMDY has a promising potential to serve as a lead compound for developing anti-SHP2 drugs.

# 2018 San Antonio Breast Cancer Symposium ®

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Xentuzumab (BI 836845), an insulin-like growth factor (IGF)-neutralizing antibody (Ab), combined with exemestane and everolimus in hormone receptor-positive (HR+) locally advanced/metastatic breast cancer (LA/mBC): Randomized phase 2 results

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## Background:

Xentuzumab (Xen), an IGF-1/-2-neutralizing Ab, binds IGF-1 and IGF-2, inhibits their growth-promoting signaling, and suppresses AKT activation by everolimus (Ev). This Phase 1b/2 trial evaluates Xen in combination with Ev and exemestane (Ex) in HR+/HER2- LA/mBC.

## Methods:

The two-arm, open-label, randomized Phase 2 part enrolled female patients (pts) with HR+/HER2- LA/mBC not amenable to curative therapy and refractory to nonsteroidal aromatase inhibitors. Pts were randomized (1:1) to: oral Ev (10 mg/d) + Ex (25 mg/d); or Xen (1000 mg/wk iv) + Ev (10 mg/d) + Ex (25 mg/d). Randomization was stratified by visceral metastases (VM; Y vs N). Treatment continued in 28-day cycles until progression, intolerable adverse events (AEs) or other reasons for discontinuation. Primary endpoint was progression-free survival (PFS), with an interim futility analysis incorporated in the study design.

## Results:

Following the results of the interim analysis, the Data Monitoring Committee (DMC) advised early termination of the trial and discontinuation of Xen treatment. Thus, Xen treatment exposure time and time-to-event data for the Xen+Ev+Ex arm are limited. Of the 139 women treated (Xen+Ev+Ex 70; Ev+Ex 69), 77% had VM. Median PFS was not significantly different between arms (Xen+Ev+Ex vs Ev+Ex, 7.3 vs 5.6 months; HR [95% CI] 0.97 [0.57–1.65];  $p=0.91$ ). In a pre-specified subgroup of pts without VM, Xen+Ev+Ex showed favorable PFS vs Ev+Ex (HR 0.21 [0.05–0.98];  $P_{\text{int}}=0.0141$ ).  $P_{\text{int}}$  values  $<0.05$  were also observed for *ad hoc* subgroups: measurable disease at baseline; bone-only metastases. Rates of total AEs/grade  $\geq 3$  AEs/drug-related AEs were similar between arms (Xen+Ev+Ex, 100/60/96%; Ev+Ex, 99/58/96%). The most common AEs overall were diarrhea (44 vs 33%), mucosal inflammation (39 vs 32%), rash (34 vs 33%) and stomatitis (34 vs 38%); most were grade 1/2. 6% of pts in the Xen+Ev+Ex arm discontinued Xen due to AEs. Ev/Ex discontinuations (Xen+Ev+Ex vs Ev+Ex) occurred in 13/6% vs 23/6%; 1 pt each in the Xen+Ev+Ex arm died from pneumonitis and liver injury and 1 pt each in the Ev+Ex arm died from Burkitt's lymphoma, acute kidney injury and metastases to the peritoneum.

## Conclusion:

In the overall population, PFS did not improve with the addition of Xen to Ev+Ex and the trial was therefore discontinued early. Nevertheless, a favorable signal was observed in the pre-specified subgroup of pts without VM when treated with Xen+Ev+Ex, which warrants additional investigation. The safety profile was comparable between arms.

## 2018 San Antonio Breast Cancer Symposium ®

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Phase I trial of intratumoral (IT) administration of a NIS-expressing derivative manufactured from a genetically engineered strain of measles virus (MV)

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**Background:** The live attenuated non-pathogenic Edmonston MV vaccine strain has advantages as an oncolytic platform given its tumor specificity, potent bystander effect, and ability to be engineered and retargeted. MV-NIS expresses the human thyroidal sodium-iodide symporter (NIS) and is selectively oncolytic, entering tumor cells through CD46 (overexpressed on many cancers, including breast cancer of all subtypes) and Nectin-4. NIS expression in MV-NIS infected cells permits noninvasive monitoring of virus spread by SPECT-CT imaging of Tc-99m pertechnetate or I-123 uptake.

**Methods:** NCT01846091 is a standard 3+3 phase I trial of a single IT administration of MV-NIS in pts with recurrent/metastatic squamous cell carcinoma of the head and neck (SCCHN) or metastatic breast cancer (MBC). Primary objectives are (a) safety and tolerability and (b) maximally tolerated single dose. The secondary clinical objective is to preliminarily assess antitumor efficacy at and away from the MV injection site. Key eligibility criteria were: absence of standard therapy with life prolonging intent; at least one lesion  $\geq 1$  cm amenable to percutaneous injection; and no impending visceral crisis. MV-NIS was administered on D1 with mandatory SPECT-CT at baseline (BL) and on D3&D8; repeat SPECT-CT on D15&D21 if the prior result was positive; mandatory tumor biopsies on D3&D21; optional tumor biopsies on D8&D15; assessments for viremia and viral shedding at BL and on D3,D8,D15,D21; and standard imaging for restaging at BL,D21,W6,W12.

**Results:** Accrual completed with 12 evaluable pts (6 SCCHN and 6 MBC) at 3 dose levels ( $10^8$ ,  $3 \times 10^8$ ,  $10^9$  TCID<sub>50</sub>). The MBC group included 5 HR+/HER2- pts and 1 pt with mixed HR+/HER2- and HR+/HER2+ disease. 5 pts had evidence of disease progression prior to study participation. No dose limiting toxicities were observed among the MBC pts; AEs possibly related to MV-NIS in this group were gr2 fatigue, gr1 flu-like illness, gr2 lymphopenia (all n=1). No SCCHN responses were observed. Best response for the MBC pts was: stable disease (SD) >6 wks, n=4; clinical response, n=1; progression, n=1. One MBC pt with SD for 12 wks had positive SPECT/CT imaging at and away from the injection site on D3&D8 and was the only pt seronegative for measles IgG antibodies prior to MV-NIS exposure. The MBC pt who responded after initial MV-NIS exposure was the only pt with low viral RNA in blood (D3); she received additional doses at W9&W13 without toxicity through an expanded access protocol exemption and had disease progression by W19. No viral shedding was detected from mouth rinse or urine in any pt. MV was detected in tumor samples from all pts treated at the highest dose level. Additional blood and tissue analyses are in progress.

**Conclusion:** These results demonstrate the safety of IT MV-NIS administration, provide early evidence of biologic activity in MBC, and support the possibility of viral replication in tumors remote from the IT injection site. A MV strain encoding the immunomodulatory neutrophil activating protein transgene has been constructed (MV-s-NAP) with preclinical evidence of improved antitumor activity and immunogenicity. The phase I MV-s-NAP trial will start recruitment in Fall 2018.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-21-04

Targeting PI3K $\beta$  alone and in combination with chemotherapy or immunotherapy in tumors with PTEN loss

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**Background:** PTEN functions as a negative regulator of the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway to promote balanced cell proliferation, survival and differentiation. PTEN loss occurs across a variety of cancer subtypes; PTEN-deficient tumors are dependent on PI3K $\beta$  activity, making PI3K $\beta$  a compelling target. We evaluated the efficacy of PI3K $\beta$  inhibitor AZD8186 as a single agent and in combination with standard chemotherapy and immune checkpoint inhibitors focused on tumors with loss of PTEN function.

**Methods:** In vitro, cell proliferation assays were performed to determine the half maximal inhibitory concentration (IC<sub>50</sub>) after 3 days of treatment and to test the effects in combination with standard chemotherapy. Colony formation assays were performed to confirm efficacy of AZD8186 in PTEN-deficient cell lines. Western blot analysis was performed to assess PTEN protein expression and to evaluate effects of AZD8186 on PI3K signaling. In vivo, antitumor efficacy of AZD8186 as a single agent as well as in combination with paclitaxel and anti-PD1 was evaluated.

**Results:** AZD8186 inhibited the cell proliferation of three of ten TNBC cell lines in vitro; PTEN loss was significantly correlated with AZD8186 sensitivity ( $p=0.008$ ). Colony formation assay confirmed sensitivity of PTEN-deficient cell lines to AZD8186. AZD8186 inhibited PI3K signaling with decreased expression of pAKT, pGSK3 $\beta$ , pPRAS40 and pS6. AZD8186 treatment of PTEN-deficient cell lines, MDA-MB-436 and MDA-MB-468, resulted in increased apoptosis. Cell proliferation assays demonstrated additive effect of the combination of paclitaxel with AZD8186. AZD8186 significantly enhanced antitumor activity of paclitaxel in MDA-MB-436 and MDA-MB-468 cell-line-derived xenografts, with disease stabilization in the latter. In syngeneic models, AZD8186 enhanced antitumor efficacy of anti-PD1 antibodies in PTEN-deficient BP murine melanoma xenograft ( $p=0.0073$ ), but not in PTEN-wildtype colon carcinoma, CT26.

**Conclusion:** AZD8186 has single agent efficacy in PTEN-deficient triple negative breast cancer cell lines in vitro, with modest single agent efficacy in vivo. AZD8186 enhanced the antitumor efficacy of paclitaxel and of Anti-PD1 antibodies in vivo. Further study is needed to determine optimal combination therapies for PTEN-deficient solid tumors.

# 2018 San Antonio Breast Cancer Symposium ®

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Concurrent exercise and chemotherapy in preclinical breast cancer models

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The benefits of exercise following a cancer diagnosis is increasingly recognized. Increased physical activity is associated with reduced breast cancer recurrence and breast cancer specific mortality. However, the mechanisms underpinning this effect are still under investigation. The role of exercise as an adjunct to systemic therapy for breast cancer remains unclear. We hypothesize that exercise may exert an anti-tumor effect and a change in tumor immune cell infiltration.

## Methods

We evaluated the effect of exercise alone and in combination with chemotherapy in preclinical patient-derived TNBC xenografts (PDX) established in Nude mice and PyMT mouse tumor models. Mice were individually housed in boxes equipped with running wheels and randomized to 1) clamped wheels (sedentary controls), 2) doxorubicin (Dox, 2mg/kg/week), 3) exercise (Ex) and 4) Ex + Dox. Daily distance run was measured. One week after randomization (acclimatization period), the intervention was commenced. Body composition was measured at randomization and at end point. Tumors were harvested after 5 weeks of intervention or at ethical endpoint. Tumor immune infiltrates were analyzed, and transcriptomic analysis performed.

## Results

In the TNBC PDX model, there was no difference in tumor volume at randomization ( $p=0.96$ ), or cumulative distance run after 1 week of acclimatization to the running wheel ( $p=0.47$ ). At 5 weeks, Ex alone significantly reduced tumor growth rate compared with controls (relative reduction 10%,  $p=0.025$ ). There was no difference between the other interventions. Mice randomized to Ex + Dox ran a shorter cumulative distance over 5 weeks compared with Ex alone ( $103.6 \pm 16.2\text{km}$  vs  $168.8 \pm 23\text{km}$ ,  $p=0.028$ ). There was no correlation between distance run and tumor volume in either of the treatment cohorts involving exercise ( $p=0.39$ ). PyMT, transcriptomic and immune cell infiltration analysis will be reported.

At baseline, there was no significant difference in mean total body mass (TBM), lean mass (LM) or fat mass (FM) between the intervention groups ( $p>0.05$ ). At 5 weeks, the mean TBM and LM in both groups treated with Dox was significantly lower than Ex only and controls. The Ex and the control mice gained weight (11%), compared with Dox only and Ex + Dox mice which did not gain weight (0% and -6% respectively). Therefore exercise had no significant impact on TBM or LM at 5 weeks, but Dox resulted in loss of TBM and LM compared with mice not treated with Dox.

## Conclusion

Exercise significantly reduced tumor growth compared with sedentary controls in our preclinical TNBC PDX model, however there was not synergistic effect seen with Dox. Cumulative doses of Dox resulted in weight loss and loss of lean mass, and reduced the cumulative running distance, compared with mice not treated with Dox.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-21-06

Target N-Ras for degradation by flunarizine to treat basal-like breast cancer

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**Background:** Basal-like breast cancer (BLBC) is an aggressive form of breast cancer that are usually triple negative for ER, PR, and HER2. There is no effective targeted therapy for BLBC due to the lack of a druggable driver. Ras GTPases are powerful drivers for tumorigenesis. We have shown that wild type N-Ras, but not K- or H-Ras, is overexpressed in BLBC and driving its growth and transforming activities. However, there is currently no treatment that directly target Ras. This study thus screened existing pharmacologically active and approved compounds for the new ability to induce N-Ras degradation in BLBC.

**Methods:** Compounds in the LOPAC library were screened by an automated microscopy system for the ability to reduce GFP-N-Ras signals in the cells. Isolated compounds were then examined to identify those that can degrade endogenous N-Ras in BLBC cells without impacting levels of other Ras proteins. Final candidate compounds were further examined to determine by which proteolytic pathway N-Ras is induced to be degraded. The potentials of the identified compound to treat BLBC were assessed by examining cell growth and soft agar colony formation *in vitro* and tumor growth *in vivo*.

**Results:** We identified flunarizine (FLN), previously approved for treating migraine and epilepsy. The FLN-induced N-Ras degradation was not affected by a 26S-proteasome inhibitor. Rather, it was blocked by autophagy inhibitors. Furthermore, N-Ras can be seen co-localized with active autophagosomes upon FLN treatment, suggesting that FLN alters the autophagy pathway to degrade N-Ras. Importantly, FLN treatment recapitulated the effect of *N-RAS* silencing *in vitro* by selectively inhibiting the growth of BLBC cells, but not that of breast cancer cells of other subtypes. In addition, *in vivo* FLN inhibited tumor growth of a BLBC xenograft model.

**Conclusion:** This proof-of-principle study presents evidence that the autophagy pathway can be coerced by small molecule inhibitors, such as FLN, to degrade Ras as a strategy to treat cancer. FLN has low toxicity and should be further investigated to enrich the toolbox of cancer therapeutics against BLBC.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-21-07

The stapled peptide ALRN-6924, a dual inhibitor of MDMX and MDM2, and the CDK4/6 inhibitors palbociclib or abemaciclib synergistically enhance each other's *in vitro* and *in vivo* anticancer activity

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**Background** ALRN-6924 is a cell-penetrating  $\alpha$ -helical stapled peptide that disrupts the interaction of the p53 tumor suppressor protein and its inhibitors, MDMX and MDM2. Reactivation of p53 with ALRN-6924 in TP53-wild-type tumors triggers cell cycle arrest and apoptosis resulting in antitumor efficacy. CDK4/6 inhibitors induce apoptosis, senescence, and cell growth arrest via the interrelated Rb pathway, and co-amplification of MDM2 and CDK4 (both on chromosome 12q13) is a known oncogenic driver, suggesting that combinations of ALRN-6924 and CDK4/6i's may be synergistic. This study evaluates the antitumor efficacy and pharmacodynamics (PD) of ALRN-6924 combined with palbociclib or abemaciclib.

**Methods** ALRN-6924 was tested in combination with palbociclib or abemaciclib in MCF-7 breast cancer cell lines and MDM2- and CDK4-co-amplified SJSA-1 sarcoma cell lines using WST-1 cell viability assays. Synergy was quantified by the Chou-Talalay combination index method. Single agents and combinations were evaluated in cell culture using assays for apoptosis (Caspase 3/7 cleavage), proliferation (BrdU), senescence ( $\beta$ -Galactosidase), colony growth (Giemsa), and Western blot analysis of p53, p21, Rb, phospho-Rb, FOXM1, and phospho-FOXM1; and E2F1 mRNA. *In vivo* combinations were tested in athymic nude mouse MCF-7 and SJSA-1 xenograft models, with cell cycle assays (EdU) measured in tumor samples by flow cytometry.

**Results** ALRN-6924 combinations with palbociclib or abemaciclib display synergistic *in vitro* anti-proliferative activity in MCF-7 and SJSA-1 cells. ALRN-6924 induces senescence *in vitro* as a monotherapy and in combination with CDK4/6i's. Western blot assays show that ALRN-6924/palbociclib combinations trigger sustained on-mechanism biomarker activation, vs. transient activation with single agents. Phospho-Rb and phospho-FOXM1 down-regulation, p53 and p21 up-regulation, and repression of E2F1 mRNA are sustained after wash-out in combination, but not in single agent-treated cells. MCF-7 and SJSA-1 tumor growth inhibition was improved in mice treated with ALRN-6924 combinations with either palbociclib or abemaciclib vs. single agent. EdU assays show that ALRN-6924/palbociclib combinations inhibit SJSA-1 tumor cell proliferation *in vivo*. Body weights and mortality data show the combination of ALRN-6924 with palbociclib 75 mg/kg/day was well tolerated; the combination with abemaciclib 100 mg/kg/day was tolerated with interruption and dose-reduction. No pharmacokinetic (PK) drug-drug interactions were noted in nude mice due to different modes of metabolism for ALRN-6924 (proteolysis) and palbociclib (CYP3A).

**Conclusions** This study demonstrates that ALRN-6924 and CDK4/6i combinations show synergistic activity. PD biomarkers indicate on-mechanism *in vitro* activity that is sustained after wash-out. *In vivo* efficacy, biomarker, PK, and tolerability results, plus clinical evidence that the most frequent and concerning safety issues for CDK4/6i's (neutropenia, leukopenia, infections) do not overlap with ALRN-6924's reported safety profile (Meric-Bernstam et al., ASCO 2017) support the development of combination regimens for breast cancer and other malignancies.

# 2018 San Antonio Breast Cancer Symposium ®

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Synergistic anti-cancer activity of cyclin-dependent kinase 4/6 inhibitor palbociclib and dual mTOR kinase inhibitor MLN0128 in pRb-expressing triple negative breast cancer

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## Background

No targeted therapies have been approved for triple negative breast cancer (TNBC) thus far. Retinoblastoma protein (pRb), a major substrate of cyclin-dependent kinase (CDK) 4/6, might be a potential target especially in chemoresistant TNBC. Palbociclib is a first approved oral CDK4/6 inhibitor for treatment of patients with estrogen receptor (ER) -positive and human epidermal growth factor receptor 2 (HER2) -negative breast cancers. Nevertheless, the usefulness of CDK4/6 inhibitors has not been established in patients with TNBC although, pRb is expressed in approximately 60% of this subtype of breast cancer. In addition, pRb expression has been shown to be associated with poor prognosis after chemotherapy. This pre-clinical study investigated the combination effects of palbociclib with oral dual mTOR kinase inhibitor MLN0128 in TNBC *in vitro* and *in vivo*.

## Methods

Four TNBC cell lines (MB231, MB453, MB468, and CAL148) were tested with the combination of two drugs *in vitro*. The combination effects on cell proliferation were investigated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and colony formation assay. Cell cycle analysis and level changes of molecules related to G1/S transition and mTOR pathway were examined. Importantly, a pRb-expressing TNBC patient-derived xenograft (PDX) model was used for confirming the combination effect *in vivo*.

## Results

Palbociclib suppressed cell proliferation in pRb-expressing cell lines (MB231 and MB453), not pRb-deficient lines (MB468 and CAL148). The combination of palbociclib with MLN0128 showed synergistic inhibition of proliferation of MB231 and MB453 cells. Western blot analysis revealed that CDK4/6-pRb and mTOR-p70S6K pathways were inhibited by palbociclib or MLN0128 alone, but considerably more effective by the combination treatment. Cell cycle analysis showed that this combination induced G1 cell cycle arrest. The combined effect of palbociclib and MLN0128 were investigated further *in vivo*. In pRb-expressing TNBC PDX, the combination treatment drastically inhibited pRb phosphorylation and tumor growth compared to control or single treatment. In addition, effective reduction of PDX tumors was also demonstrated by major suppression of Ki67-positive cells by the combination treatment compared to control or single treatment.

## Conclusions

In this pre-clinical study, we discovered that the combination treatment of CDK4/6 inhibitor palbociclib and dual mTOR kinase inhibitor MLN0128 had synergistic anti-cancer activity in pRb+ TNBC cell lines and a PDX model. Our results prove that such combination therapy is earnest to be further investigated in a clinical setting.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-21-09

muTarget.com: Linking gene expression and mutation status to identify patient cohorts eligible for targeted- and immunotherapy in breast cancer

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Selection of cancer patients for new targeted therapies reached its dead end as next generation sequencing based precision oncology approaches failed to deliver breakthrough improvements to oncology practice. The problem with current approaches is that the effect of a mutation can be indirect by influencing the expression of various other genes, which in turn can act as new therapy targets. A large-scale analysis of such cascades was not yet executed in breast cancer.

Here, we developed an analysis tool to identify targetable genes showing an altered expression in relation to a mutation in other genes.

The background database includes two independent large patient's cohorts, the TCGA and the Metabric datasets. Mutation status for each gene was determined using the VCF files from the TCGA repository. RNA-seq gene expression data for the same patients was re-normalized using a scaling normalization. Gene expression for the Metabric samples was determined using Illumina gene arrays and mutation status for the same patients is available for 174 selected genes. The Metabric database includes 1,981 patients and the TCGA breast cancer database contains 1,091 patients. Expression is linked with mutation status for each gene across all patients using Mann-Whitney test. A  $p < 0.05$  and a false discovery rate of  $< 10\%$  was accepted as significant.

We demonstrate the utility of the analysis platform by using it to uncover patient cohorts with higher expression of PD1 (PDCD1) and PD-L1 (CD274). Immune checkpoint inhibitors pembrolizumab and nivolumab target PD1. PD-L1 inhibitors include atezolizumab, avelumab, and durvalumab. None of these immunotherapy agents is approved to be used in breast cancer. In both settings, only one gene reached statistical significance. For PD1, the best performing gene was NOP14. Patients with mutation in NOP14 (1.3% of patients) had a 2.08x increased expression (4.58 in mutated vs. 2.20 in wild type) of PD1 ( $p = 8.4e-05$ , FDR=0.0239). For PD-L1, the strongest gene was CCDC88A (mutated in 2.6% of patients), which had a 2.03x increased expression (10.42 in mutated vs. 5.13 in wild type) of PD-L1 ( $p = 6.2e-05$ , FDR=0.0147). Both NOP14 and CCDC88A have been linked to cancer development and progression, but have not been investigated in relation to immune therapies. One can anticipate that patients with mutation in these genes will be prone to respond to immune checkpoint inhibitors.

In summary, an online portal was set up capable to identify genes with altered expression in relation to a given mutation. The presented approach can help to increase speed and reduce cost of development for future anticancer treatments. The analysis tool also enables identification of patient cohorts for new agents and is accessible at [www.mutarget.com](http://www.mutarget.com).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-21-10

Talazoparib and decitabine combination therapy: When DNA damage repair process impairing can be a benefit

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**Background:** *BRCA* genes (e.g. *BRCA1* and *BRCA2*) are known to play a major role in tumorigenesis. These genes are key mediators of DNA damage repair response including the homologous recombination repair (HRR) pathway. To leverage impaired DNA repair in tumors, a new class of drugs, the poly (ADP-ribose) polymerase inhibitors (PARPi), were developed and olaparib, niraparib, rucaparib have already been approved as single agents. Efforts to combine these agents with cytotoxic agents showed increased efficacy but overlapping toxicities renders combination therapy difficult to tolerate. Furthermore, the short duration of responses in diseases like breast cancer has further inspired the search for combinations with other agents including epigenetic modifiers.

To overcome those hurdles, we explored synergistic interactions between PARPi and DNA Methyltransferases inhibitors (DNMTi) such as decitabine (a global DNA methyltransferase inhibitor). This cytidine analog, when incorporated in newly synthesized DNA strands (during DNA replication phase (S phase)), will trigger a covalent protein-DNA complex formation thereby causing cell cycle arrest and ultimately causing cell death.

**Material & Methods:** Breast and Ovarian cell lines with/without *BRCA* mutations are used for *in vitro* analysis: SUM149PT (Breast, *BRCA1* mutated), JHOS-2 & COV362 (Ovary, *BRCA1* mutated), KURAMOCHI (Ovary, *BRCA2* mutated), MDA-MB-231 (Breast, *BRCA* Wild-Type), MCF10A (Breast non-tumorigenic cells, *BRCA* Wild-Type). Combinational effect of talazoparib (PARPi) and decitabine (DNMTi) analyzed by Combenefit® software. Colony assays, cell death analysis, as well as pH2AX level are evaluated. *In vivo* toxicity analysis has also been done on CD-1 IGS mice.

**Results:** Our preclinical data in *BRCA* deficient breast and ovarian cancer cell lines, demonstrated a synergistic inhibition of cell growth and cell death improvement at concentrations of talazoparib and decitabine where each agent individually had minimal efficacy. In cells with intact HRR pathways, the drug combination impact is not significant on the cell death ratio (compared to *BRCA* deficient cell lines) and only causes a cell growth arrest. The drug combination efficacy is related to a better accumulation of DNA breaks (pH2AX level is higher in the combination group than drug alone groups).

**Conclusion:** The low drug doses used in these preliminary experiments present a promising therapeutic window for patients with tumors carrying HRR pathway mutations. Ongoing experimentations are also being done in pancreatic cell lines carrying a *BRCA* mutation as well as CRISPR/Cas9 DNA editing for *BRCA1* & 2 in MDA-MB-231 cells. Translational *in-vivo* studies are currently underway to determine whether synergistic *in vitro* cytotoxicity translates to anti-tumor efficacy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-21-11

Final results from IMPROVE: A randomized, controlled, open-label, cross-over phase IV study to determine the patients' preference for either combined endocrine therapy (exemestane plus everolimus) or immunochemotherapy (capecitabine plus bevacizumab) as first line treatment for advanced HR+/HER2- breast cancer

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For patients (pts) with advanced HR+/HER2- breast cancer various treatment options exist. Endocrine therapy or chemotherapy are the recommended 1st-line treatment options according to international guidelines. With comparable efficacy, it is of utmost importance to identify the treatment that has the least negative impact on the pts' quality of life (QoL). Randomized studies to determine the pts' preference (pref) for equi-effective treatment concepts, are lacking. IMPROVE compared both therapeutic concepts by assessing the pts' pref for either a combined antihormonal approach (everolimus /exemestane (E/E)) or chemotherapy (capecitabine /bevacizumab (C/B)).

In total, 77 pts were recruited from 10/14 until 04/17 at 26 sites in GER. Upon diagnosis of locally advanced, inoperable or metastatic disease, pts were randomized to receive 1st-line C/B until disease progression followed by 2nd-line E/E (Arm A) or vice versa (Arm B). Primary objective was the patient-reported pref for either treatment protocol 12 weeks after switching therapy. Key secondary endpoints include PFS, OS, safety and QoL. Descriptive statistics were used to analyse data, PFS and OS were calculated by using the Kaplan-Meier method.

Baseline characteristics were well balanced with a slightly shorter disease-free interval after primary diagnosis and more prior treatments in Arm B. In Arm A [95% CI], 39% [13.9-68.4] vs 23% [5.0-53.8] of pts preferred E/E compared to C/B, 23% [5.0-53.8] were undecided. In Arm B, 56% [30.8-78.5] vs 11% [1.4-34.7] preferred C/B, 22% [6.4-47.6] undecided. Overall, 42% [24.5-60.9] vs 23% [9.6-41.1] of pts preferred C/B, 23% [9.6-41.1] were undecided.

Physicians' pref had a tendency for C/B treatment (Arm A, 62% [31.6-86.1] vs 39% [13.9-68.4] for C/B. Arm B, 56% [30.8-78.5] vs 28% [9.7-53.5] for C/B, no pref 17% [3.6-41.4]).

Median 1st-line PFS [months, 95% CI] was 11.1 [7.8-18.0] for C/B (Arm A) vs 3.5 [2.7-5.5] for E/E (Arm B). Median 2nd-line PFS was 3.7 [2.4-7.8] for E/E (Arm A) vs 3.6 [2.3-5.5] for C/B (Arm B). Median OS [months, 95% CI] was 28.8 [19.7-NA] (Arm A) and 24.7 [13.9-28.8] (Arm B). 73.0% and 52.6% (1<sup>st</sup> and 2<sup>nd</sup> line, C/B) vs 54.1% and 52.9% (1<sup>st</sup> and 2<sup>nd</sup> line, E/E) of pts developed grade 3/4 AEs. Most common grade 3/4 AEs (%) were hand-foot syndrome (18.9), fatigue (17.6), hypertension (13.5) for C/B and anaemia (23.5), fatigue, dyspnoea, cough (each 17.6) for E/E (either line). No treatment-related deaths occurred. Patient-reported QoL (EORTC-QLQC30) and treatment satisfaction were not significantly different between arms in either treatment phase.

In the IMPROVE study, pts had no pref for either endocrine therapy or immunochemotherapy. Overall, there was a tendency in favour of the chemotherapeutic approach (C/B), which was in line with the therapy pref reported by the physicians. C/B was found to have slightly better efficacy results but at the cost of a higher frequency of grade 3/4 AEs, bearing in mind the difference in duration of therapy between the two regimens. Patient-reported QoL, however, was similar in both arms.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-21-12

Local transdermal therapy (LTT): Drug permeation and distribution of telapristone acetate (TPA) in a pre-surgical window study of women undergoing mastectomy

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**Background:** Low uptake and poor adherence to oral drugs for breast cancer prevention and ductal carcinoma in situ has led to an interest in local routes of delivery with the intent of decreasing systemic exposure and reducing toxicity. LTT has emerged as a possible alternative; previous studies have shown selectively higher concentrations in the breast than in the serum with this delivery route. A question related to LTT is whether or not the drug will permeate and distribute throughout the breast, as is expected with oral delivery.

**Methods:** We conducted a double-blind study of oral versus LTT delivery of the selective progesterone receptor modulator, telapristone acetate (TPA), in a presurgical window setting, enrolling 82 women planning therapeutic or prophylactic mastectomy. We randomized 67 women 1:1, to oral TPA 12 mg daily, or gel TPA applied to both breasts daily (12 mg/breast), for 4 weeks  $\pm$  1 week. Mastectomy specimens were sampled at 5 non-tumor locations as well as the tumor and lymph node when available. Samples were split in two: drug concentration (conc.) assay using LC-MS/MS and histological evaluation of tissue composition (fat, fibrous stroma, epithelium). The primary endpoint was mean drug conc. across all breast locations (anticipating that the gel would deliver a mean concentration that was >50% of the mean in the oral group). A secondary endpoint was the drug distribution pattern across the breast, expecting that the distribution would be similar. The tumor sample was saved for biomarker assays related to TPA action; these are ongoing, for a pre-planned pooled analysis of data with NCT01800422 (reported in SABCS abstract 851863).

**Results:** Of 63 evaluable women (33 oral and 30 gel group), 27 had unilateral and 36 had bilateral mastectomy. The mean drug conc. in the oral group was 166.3 ng/G (SE 11.7), and in the gel group was 10.6 ng/G (SE 10.8), ( $p < .0001$ ). The conc. was variable across the 7 locations tested in both groups. High concentrations were found in the superficial and deep central locations, retroareolar and lateral locations ranked in the middle, and the medial location was discrepant, being high in the oral and low in the gel group. The variation in drug concentration across all locations was not significantly different between groups (Kolmogorov-Smirnov  $p = 0.99$ ). Among women with bilateral mastectomy, drug concentrations were similar between breasts in both oral and gel groups. In the gel group, despite low TPA concentrations, there was evidence of drug metabolism. The major metabolite, CDB 4453 was detectable in 192/193 samples with detectable parent drug. Analysis of drug concentration adjusted for tissue composition is ongoing.

**Conclusions:** The gel formulation of TPA did not permeate the skin well. However, the drug delivered to the breast was distributed throughout the breast, similar to the oral delivery route, with the highest concentration in the deep central location. These drug distribution data are novel; drug distribution at multiple locations throughout the breast has not previously been shown. Further work is needed to understand breast distribution with formulations known to have good dermal permeation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-21-13

A phase II single arm trial evaluating the efficacy and safety of eribulin in combination with bevacizumab for second-line treatment of human epidermal growth factor receptor 2 (HER2)–negative metastatic breast cancer (MBC) progressing after first-line therapy with bevacizumab and paclitaxel

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**Background:** At present, there is no standard second-line chemotherapy-based treatment in patients with HER2-negative MBC. Continued VEGF inhibition with bevacizumab is a new potential option in patients progressing to first line bevacizumab and chemotherapy. Eribulin is a non-taxane microtubule dynamics inhibitor, with a unique mechanism of action and suggested beneficial effects on tumor microenvironment and neoangiogenesis. This study evaluated efficacy and safety of eribulin plus bevacizumab as a novel second-line chemotherapy scheme, in patients progressing after first line paclitaxel and bevacizumab.

**Methods:** This is a multicenter, single-arm, Simon's two-stage, Phase II study. Patients with HER2-negative MBC progressing to paclitaxel and bevacizumab received eribulin (1.23 mg/m<sup>2</sup> intravenously on Days 1 and 8 of every 21-day cycle) plus bevacizumab (10 mg/kg every 2 weeks or 15 mg/kg every 3 weeks intravenously), as second-line chemotherapy. The primary endpoint was the overall response rate (ORR), considered as sum of partial (PR) and complete response (CR), basing on the best overall response. The present safety and efficacy analyses, as planned per study design, refer to six cycles of treatment (18 weeks).

**Results:** Among the 61 patients enrolled in the study, 55 (90,2% ) were evaluable for efficacy. ORR (PR/CR) was 9.1% [95% confidence intervals (C.I.) 3.0 to 19.9]; stable disease (SD) rate was 63.6% [95% C.I. 49.6 to 76.2]; clinical benefit rate (CR/PR/SD) at 24 weeks was 35% [95% C.I. 22.0 to 49.1]. The median progression free survival was 6.3 months [95% C.I. 4.1 to 7.8 months]. Drugs-related adverse events (AEs) were: 49.5% related to eribulin, 7.7% related to bevacizumab, and 11.8% related to both the study drugs. The most common AEs were fatigue (9.9% of all AEs), paresthesia (6.5% of all AEs) and neutropenia (6.2% of all AEs). Quality of life was well preserved among the majority of patients.

**Conclusions:** The results of our trial suggest that continuing bevacizumab in combination with eribulin, beyond first line treatment with bevacizumab and paclitaxel, offers a reasonable therapeutic option for patients with HER2-negative MBC, without detrimentally affecting quality of life.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-21-14

Targeting Rac/Cdc42 in human epidermal growth factor receptor 2 (HER2)-positive breast cancer

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Breast cancer metastasis, when cancer cells move and establish tumors in distant organs, confounds treatment options. Therefore, there is an unmet need for targeted therapeutics to address metastasis, especially in the intractable HER2 positive (+) breast cancer type. HER2 (+) breast cancer is treated with the monoclonal HER2 antibody trastuzumab and the HER2/EGFR targeting small molecule lapatinib. However, ~20% of early stage breast cancer patients and ~70% of patients with metastatic disease are resistant to trastuzumab. Trastuzumab-resistant breast cancers circumvent HER2 inhibition via bypass signaling, which includes the PI3-K/Akt/mTOR pathway. HER2/EGFR and PI-3K/Akt signaling activate the Rho GTPases Rac and Cdc42, and overexpression of Rac or its downstream effector p21-activated kinase (PAK) significantly diminishes response to anti HER2 therapy and patient survival. Therefore, targeting Rac and Cdc42, pivotal regulators of cancer cell migration/invasion, and thus, metastasis, is a viable option to overcome HER2/EGFR therapy resistance. First, we designed and developed the Rac inhibitor EHOP-016 (US patents 8,884,006 B2, 9,278,956B1), which inhibits Rac activation with an IC<sub>50</sub> of 1  $\mu$ M and reduces breast cancer growth and metastasis in mouse models of trastuzumab resistant HER2 (+) breast cancer. A more potent dual Rac and Cdc42 inhibitor, MBQ-167 (US Patent 9,981,980 B2), inhibits Rac and Cdc42 activation with IC<sub>50</sub>s of 103 nM and 78 nM, respectively. *In vivo*, MBQ-167 inhibits trastuzumab resistant HER2 (+) mammary tumor growth and metastasis by ~90-100% (Humphries-Bickley et al. 2017, *Mol Cancer Therap*). Therefore, Rac/Cdc42 inhibition blocks cell proliferation, cell cycle progression, migration, and induces apoptosis to ultimately impede tumor growth and metastasis in trastuzumab resistant HER2 (+) breast cancer models. To further investigate the role of Rac/Cdc42 inhibitors in overcoming HER2 therapy resistance, we developed a lapatinib resistant variant of the SKBR3 HER2 (+) breast cancer cell line and found that Rac was overexpressed and over activated in the therapy resistant variant compared to the parental cells. Accordingly, the Rac/Cdc42 inhibitors overcame lapatinib resistance by decreasing cell viability and inducing apoptosis in parental and SKBR3 therapy resistant variants. Pharmacokinetic analysis in mouse plasma demonstrated that the bioavailability of EHOP-016 and MBQ-167 is ~30% with a half-life of 3-4 h. Therefore, to increase the bioavailability of Rac/Cdc42 inhibitors and to facilitate sensitization of trastuzumab therapy, we designed and developed novel nanoliposomal formulations containing Rac/Cdc42 inhibitors with trastuzumab on the outer surface to target HER2 (+) breast cancer cells. This data demonstrates the utility of developing Rac/Cdc42 inhibitors as mono or combined therapy with current targeted therapeutics for HER2 (+) breast cancer.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-22-01

Repurposing sulindac sulfide as a notch inhibitor to target cancer stem-like cells in triple negative breast cancer

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Triple negative breast cancer (TNBC) is a heterogeneous group of clinically aggressive breast cancers. TNBC patients have a high risk of recurrence and metastasis, and current treatment options remain limited. There is strong evidence supporting the involvement of Notch signaling in TNBC progression. Expression of Notch1 and its ligand Jagged1 correlate with poor prognosis. Emerging evidence suggests that cancer stem-like cells (CSCs) that escape chemo or radiation therapy in TNBC are often Notch-dependent. At the same time, there is evidence that active tumor immunity predicts good response to neo-adjuvant chemotherapy in TNBC. Notch inhibitors, including Gamma Secretase Inhibitors (GSIs) are quite effective in preclinical models of TNBC, where they eliminate CSCs resistant to chemotherapy. However, the success of GSIs in clinical trials is limited by their intestinal toxicity and adverse immunological effects. CD4 and CD8 T-cells, necessary to adaptive tumor immunity, require Notch1 for activation. Our overarching goal is to replace GSIs with agents that lack their systemic toxicity and adverse immunological effects. We identified Sulindac Sulfide (SS), the active metabolite of FDA-approved NSAID Sulindac, as a potential candidate to replace GSI. SS has Gamma Secretase Modifier (GSM) activity. We confirmed that SS inhibits Notch1 cleavage in TNBC cells. SS significantly inhibited mammosphere growth in all human and murine TNBC models we tested: 1) human MDA-MB-231 cells; 2) murine TNBC model C0321, from targeted conditional knockout of Lunatic Fringe (*LFng*<sup>-/-</sup>); and 3) Two TNBC patient-derived xenograft models, 2K1 and 4IC. In contrast, SS did not inhibit Notch expression or cleavage in murine T cells. In C0321 tumors, which recapitulate human mesenchymal TNBC, we found that SS had remarkable single-agent anti-tumor activity and virtually eliminated Notch1 expression in tumors. SS caused an increase in intra-tumoral CD11c+ dendritic cells, but decreased CD4 cells, which in this model are largely PD-1 positive (exhausted). CD8 cells were modestly increased. SS did not affect the number of tumor infiltrating macrophages or myeloid-derived suppressor cells (MDSC). However, SS blocked the immunosuppressive function of bone marrow-derived MDSC. We are currently investigating the mechanisms of this anti-tumor activity. Our data support further investigation of SS for the treatment of TNBC, with standard of care or with immunotherapy agents. Repurposing an FDA-approved, safe agent for the treatment of TNBC would be significantly easier and more cost-effective than developing unproven investigational agents.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** P6-22-02

Rates of high risk and malignant lesions incidentally detected in the contralateral breast after large volume displacement oncoplastic breast surgery

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**Background:** Given the prevalence of female breast cancer in the United States, large volume displacement (Level II) oncoplastic breast surgery is becoming an increasingly common technique to remove cancerous breast tissue. It allows for the removal of the cancerous tissue with a large partial mastectomy followed by a mastopexy or breast reduction oncoplastic reconstruction design. A contralateral symmetry operation using similar mastopexy or breast reduction designs are many times also performed, often by a plastic surgeon. Our goal was to determine the rates of high risk and malignant lesions present in the contralateral breast specimen.

**Methods:** We conducted a retrospective chart review of the first consecutive 100 large volume displacement oncoplastic breast surgeries performed at our institution between August 2015 and June 2018. Of the 100 cases, 84 patients had an immediate symmetry operation performed on the contralateral breast. Our inclusion criteria for malignant lesions included invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS). Inclusion criteria for high risk lesions included atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS). We obtained this information from the patient's pathology report.

**Results:** Of the 84 patients that underwent a symmetry operation on the contralateral breast, 14 patients (16.7%) had malignant and/or high risk lesions incidentally detected. Two patients (2.4%) had IDC, 1 (1.2%) had DCIS alone, 1 (1.2%) had DCIS with ADH, 6 (7.1%) had ADH alone, 2 (2.4%) had ALH, and 2 (2.4%) had LCIS. For those who had IDC in the contralateral breast, average reported incidental tumor size was 6.5 mm at its largest point. The majority of these lesions (57%) were found when performing in superomedial pedicle inverted T (Wise) skin incision pattern specimens. 3 (21%) were found using the inferior pedicle inverted T (Wise) skin pattern, 2 (14%) were found using the superomedial pedicle circumvertical skin incision pattern, and 1 (7%) was found using the superior pedicle circumvertical skin incision pattern.

**Conclusion:** The high incidence of cancer and high risk lesions incidentally detected in the contralateral breast highlights the need for consistent histological examination of the contralateral specimen. These results suggest the need for proper orientation of the specimen and good communication between the surgical (often the plastic surgeons performing the symmetry operation) and pathology teams. Such communication is needed to help target any future surgeries that may be required due to positive margins in the contralateral symmetry specimen.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P6-22-03

Tumor phenotype and concordance in synchronous bilateral breast cancer in young women

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**Background:** Synchronous bilateral breast cancer is rare, with reported incidence from 0.3-12%; the incidence and pattern of bilateral breast cancer among younger women is unknown. Here we report the incidence and phenotypes of bilateral breast cancer in women ≤40 years of age enrolled in the Young Women's Study (YWS) cohort.

**Methods:** The YWS is a multi-center, prospective cohort study that enrolled women with newly diagnosed breast cancer at age ≤40 years from 2006-2016. Those with synchronous bilateral breast cancer (in-situ and/or invasive) formed our study cohort. Disease characteristics and treatment were obtained by medical record review. Central pathology review was performed to capture histologic features and categorize the tumor phenotype as either luminal A (hormone receptor (HR)+, HER2-, grade 1 or 2), luminal B (HR+, HER2+, or HER2- and grade 3), HER2-type (HR-, HER2+), or triple negative (TNC; HR/HER2-). Tumor phenotypes of bilateral breast cancers were compared and evaluated for concordance.

**Results:** Among 1302 patients enrolled in the YWS, 20 (1.5%) patients presented with bilateral disease, with median age of diagnosis of 38 years (range 18-40). The majority of patients (13 (65%)) presented with unilateral symptoms and contralateral disease was identified on subsequent imaging. 12 (60%) reported a positive family history of breast cancer and 17 (85%) underwent genetic testing; resulting in the identification of 6 mutation carriers (2 *BRCA1*, 3 *BRCA2*, 1 *TP53*). The majority of patients (15 (75%)) underwent bilateral mastectomy, 1 underwent unilateral mastectomy with contralateral lumpectomy, and 4 underwent bilateral lumpectomy. On pathology, 2 patients had bilateral in-situ disease, 5 had unilateral invasive and contralateral in-situ disease, and 13 had bilateral invasive disease. Of those with bilateral invasive disease, all had concordant tumor histology (92% ductal, 8% ductal and lobular), 10 (77%) patients had bilateral luminal tumors and when fully characterized 6 were of the same luminal type. Only one patient had bilateral basal-like breast cancer.

Patient ID		ER	PR	Her2 amplified	Grade	Phenotype
1	Left	+	+	-	2	Luminal A
	Right	+	+	-	3	Luminal B
3	Left	+	+	-	3	Luminal B
	Right	+	+	-	3	Luminal B
6	Left	+	+	-	3	Luminal B
	Right	+	+	-	3	Luminal B
9	Left	+	+	-	2	Luminal A
	Right	+	+	-	2	Luminal A
10	Left	+	+	+	3	Luminal B
	Right	+	+	-	2	Luminal A
12	Left	+	-	-	3	Luminal B
	Right	+	-	-	2	Luminal A
13	Left	-	-	-	NA	Basal-like
	Right	+	+	-	NA	Luminal A or B
14	Left	+	+	+	2	Luminal B
	Right	+	+	-	3	Luminal B

15	Left	+	+	-	3	Luminal B
	Right	+	+	+	3	Luminal B
16	Left	+	+	+	3	Luminal B
	Right	-	-	+	NA	HEr2-type
17	Left	-	-	-	3	Basal-like
	Right	-	-	-	3	Basal-like
19	Left	+	+	-	2	Luminal A
	Right	+	+	-	3	Luminal B
20	Left	+	+	-	1	Luminal A
	Right	+	+	-	2	Luminal A

Conclusions: Among a large cohort of young women, only 20 (1.5%) had bilateral disease, and the majority of the invasive tumors were of the luminal phenotype, yet frequently differed by grade or HER2 status; supporting the need for thorough pathologic evaluation of bilateral disease to determine risk and tailor treatment. Overall the low incidence of bilateral disease and preponderance of the luminal phenotype in this population is reassuring.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: P6-22-04

A pooled analysis of waist and hip circumference and postmenopausal breast cancer

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**Background** Many, but not all, epidemiologic studies suggest that waist and hip circumference, and waist-to-hip ratio [WHR] are positively associated with postmenopausal breast cancer. However, results are inconsistent for breast cancer subtypes, by strata of body mass index, or after adjustment for body mass index. We evaluated these associations in 10 prospective cohorts in the Pooling Project of Prospective Studies of Diet and Cancer.

**Methods** These analyses included 256,214 postmenopausal women among whom 10,738 (N=1,346 estrogen receptor [ER]- and 6,587 ER+ tumors) were diagnosed with breast cancer during follow-up ranging up to 8-20 years. Study-specific, multivariable-adjusted hazard ratios [HR] were calculated for risk of breast cancer overall and by ER status using Cox proportional hazards regression analyses and controlled for age, race, education, height, reproductive factors, physical activity, smoking status, energy and alcohol intake, and family history of breast cancer. Pooled estimates were computed using random-effects models.

**Results** The medians across studies ranged from 76.0-88.3 cm for waist circumference, 98.0-104.8 cm for hip circumference, and 0.77-0.84 for WHR. As expected, preliminary analyses showed stronger, positive associations for all three exposures for women who had never used hormone replacement therapy (HRT) compared to women who had used HRT (P for interaction <0.05). Subsequent results are presented only for never users of HRT. For waist circumference, the pooled HR of overall breast cancer was 1.58 (95% confidence interval [CI] 1.44-1.74) for women with waist circumference  $\geq 100$  cm compared to 70-80 cm. The corresponding pooled HRs were 1.65 (95% CI 1.45-1.86) for ER+ and 1.38 (95% CI 1.03-1.84) for ER- breast cancer. The associations for hip circumference and WHR were weaker than those observed for waist circumference. The pooled HR for women with hip circumference  $\geq 110$  cm compared to 95-100 cm was 1.22 (95% CI 1.11-1.35) for breast cancer risk overall. The HRs for ER+ and ER- tumors were 1.27 (95% CI 1.12-1.43) and 1.04 (95% CI 0.78-1.38), respectively. For WHR, the pooled HRs in women with WHR >0.90 compared to 0.75-0.80 were 1.37 (95% CI 1.23-1.52) for overall breast cancer, 1.33 (95% CI 1.15-1.53) for ER+, and 1.29 (95% CI 0.94-1.77) for ER- breast cancer. The findings for ER+ and ER- tumors were significantly different for waist and hip circumference (P-value, test for common effects < 0.05). After additionally adjusting for body mass index, the associations for all three exposures were attenuated and only the associations for waist circumference and WHR for overall and ER+ breast cancer remained statistically significant. Similar positive associations were observed for waist circumference across strata of body mass index: the HRs (95% CI) for a 5 cm increment were 1.06 (1.03-1.09) for women with body mass index <25 kg/m<sup>2</sup> and 1.04 (1.02-1.06) for women with body mass index  $\geq 25$  kg/m<sup>2</sup>.

**Conclusions** In postmenopausal women who had not used HRT, higher waist circumference, hip circumference and WHR were associated with a modestly higher risk of breast cancer. Results were stronger for ER+ than ER- tumors for waist and hip circumference and did not vary by body mass index for all three exposures.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD1-01

Trilaciclib (T), a CDK4/6 inhibitor, dosed with gemcitabine (G), carboplatin (C) in metastatic triple negative breast cancer (mTNBC) patients: Preliminary phase 2 results

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**Background:** Cytotoxic chemotherapy-induced damage of hematopoietic stem and progenitor cells (HSPCs) results in acute toxicities consisting of multi-lineage myelosuppression, and late onset toxicities consisting of progressive bone marrow suppression with increased incidence of therapy-related myeloid neoplasms. T is an IV CDK4/6 inhibitor in development to preserve HSPC and immune system function during cytotoxic chemotherapy (myelopreservation). Proof of concept for myelopreservation with T was observed in a randomized, placebo-controlled Phase 2 trial in small-cell lung cancer patients receiving 1<sup>st</sup>-line chemotherapy. This trial in mTNBC patients (NCT02978716) was designed to explore the utility of T in combination with GC.

**Methods:** This Phase 2, randomized, open-label study enrolled patients in the US and EU with mTNBC who had received 0-2 prior systemic cytotoxic therapies in the locally recurrent or metastatic setting and had no symptomatic brain metastases. Patients were randomized (1:1:1) to GC alone (Group 1) or T plus GC (Group 2) using a standard schedule (D1, 8 every 21 days) or to an alternative schedule (T on D1, 2, 8 and 9 with GC on D2 and 9 every 21 days; Group 3). On those days when both T and GC were scheduled, T was administered iv prior to GC infusion. Prophylactic growth factors were not administered in cycle 1; otherwise supportive care was allowed as needed. Primary objectives were safety and tolerability; tumor response was evaluated using RECIST v1.1 and PFS and OS were assessed. Myelopreservation endpoints reflecting the potential effects of T on multiple cellular lineages include occurrence of Grade 4 neutropenia (primary), RBC and platelet transfusions (primary), and lymphocyte counts with immune profiling (secondary and exploratory). A signature of CDK4/6 independence developed from preclinical data will be used to evaluate archival tumor tissue samples and data analysis is ongoing.

**Results:** 95 patients were dosed; median age 57 years (range 32,86), ECOG PS 0 (53%) or 1 (47%), 25% had liver metastases at baseline, and approximately 50% had received no systemic therapy in the recurrent/metastatic setting. Fifty-five patients remain on treatment. Disease progression was the most common reason for drug discontinuation (22/40; 55%). Overall the most common (≥ 25%) TEAEs were anemia (47%), nausea (35%), fatigue (34%), neutropenia (32%), platelet count decreased (25%), and vomiting (25%). The most frequent (≥ 15%) Grade 3 or 4 TEAEs were hematologic toxicities, i.e. neutropenia (28%), anemia (21%), neutrophil count decreased (21%) and thrombocytopenia (16%). These were also the most frequent drug-related TEAEs observed. Tumor efficacy data are being evaluated.

**Conclusions:** This trial, assessing the myelopreservation effects of T when combined with GC in patients with mTNBC, has completed enrollment. Myelopreservation data, immune profiling, as well as ORR and preliminary PFS results will be presented by study arm at the meeting.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD1-03

Single agent activity of U3-1402, a HER3-targeting antibody-drug conjugate, in HER3-overexpressing metastatic breast cancer: Updated results of a phase 1/2 trial

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**Background:** Human epidermal growth factor receptor 3 (HER3) is overexpressed in a variety of solid tumors, including breast cancer. However, there are no approved HER3-targeted anti-cancer therapies. U3-1402 is a HER3-targeted antibody drug conjugate with a novel peptide-based cleavable linker attached to a potent topoisomerase I inhibitor payload. It has a high drug-to-antibody ratio (7:1 to 8:1), a novel linker which is stable in plasma and selectively cleaved by lysosomal cathepsins up-regulated in cancer cells, and a payload with a short systemic half-life. The ongoing, phase 1/2 study (NCT02980341) was initiated to evaluate the safety, tolerability, and efficacy of U3-1402 in HER3-overexpressing metastatic breast cancer (MBC). The study has 3 parts: Dose Escalation, Dose Finding, and Dose Expansion. Here we report updated results from Dose Escalation and Dose Finding.

**Methods:** In Dose Escalation, the dose of U3-1402 was escalated based on dose-limiting toxicity data (between 1.6 and 8.0 mg/kg) and guided by the modified Continuous Reassessment Method. In Dose Finding, patients were treated with 1 of 2 doses (4.8 mg/kg or 6.4 mg/kg) identified during Dose Escalation. In both parts, U3-1402 was administered via IV infusion in 21-day cycles. The primary objectives were to determine the safety and tolerability of U3-1402, the maximum tolerated dose (MTD), and the recommended dose for expansion. Efficacy assessments included investigator-assessed objective response rate (ORR; proportion of complete response [CR] + partial response [PR]) per RECIST v1.1 and disease control rate (DCR; proportion of CR + PR + stable disease). Efficacy-evaluable patients received  $\geq 1$  dose of U3-1402 and had pre- and post-treatment tumor assessments. Pharmacokinetics and the anti-drug antibodies were also assessed.

**Results:** As of 1 June 2018, a total of 42 patients received U3-1402 across Dose Escalation and Dose Finding (34 and 8 patients, respectively). Overall, 12 patients have discontinued treatment (9 due to progressive disease, 1 due to clinical progression, 1 due to Grade 2 pneumonitis, and 1 due to withdrawal of consent). A total of 30 patients remain on treatment (33.3% [14/42] for  $\geq 6$  months). The median (range) age was 54.5 (30–81), and majorities of patients had an ECOG performance status of 0 (76.2%; 32/42) and had received  $\geq 5$  prior anti-cancer regimens (78.6%; 33/42). Among efficacy-evaluable patients, the ORR was 46.3% (19/41) and the DCR was 90.2% (37/41). Grade  $\geq 3$  treatment-related AEs (TEAEs) were reported in 61.9% (26/42) patients. TEAEs ( $>50\%$  in treated patients) regardless of causality (any grade, Grade  $\geq 3$ ) included nausea (83.3%, 4.8%), thrombocytopenia (71.4%, 33.3%), decreased appetite (64.3%, 7.1%), neutropenia (59.5%, 26.2%), and leukopenia (57.1%, 19.0%). The MTD was not reached; dose limiting toxicities included events of decreased platelet count and increases in AST or ALT.

**Conclusions:** In a preliminary analysis of this ongoing phase 1/2 clinical trial, U3-1402 demonstrated antitumor activity in a substantial number of heavily pretreated HER3-expressing MBC patients, and U3-1402 treatment was associated with a manageable safety profile.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD1-04

Nuclear localization of intracellular domain of LDL receptor-related protein 1B predicts poor outcome in breast cancer; putative relation to *NEAT1* mediated mammary gland carcinogenesis

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**Background:** Low-density lipoprotein receptor-related protein 1B (LRP1B) is thought to have a pleiotropic biological function. Notably, intracellular domain of LRP1B is released and transported to nucleus in a  $\gamma$ -secretase dependent fashion; however, pathological property, which is driven by nuclear transport of intracellular domain of LRP1B is largely unclear. In this study, we aimed to unravel the pathological significance of nuclear localization of intracellular domain of LRP1B in mammary gland carcinogenesis. **Aims:** We examine the clinical significance of LRP1B for breast cancer and we clarify mechanism of nuclear localization of LRP1B in invasive ductal carcinoma. **Methods:** Immunohistochemical staining using newly generated antibodies to intracellular domain of LRP1B was used to determine LRP1B expression in 92 invasive ductal carcinomas of the breast. The clinicopathological significance including prognosis value was statistically analyzed. Doxycycline dependent nuclear expression of intracellular domain of LRP1B was established in cultured breast cancer cells. Subsequently, a series of *in vitro* experiments were performed to explore the role of nuclear localized intracellular domain of LRP1B in cultured breast cancer cells. Comprehensive microarray-based analysis followed by quantitative RT-PCR and chromatin immunoprecipitation assay was performed to know the altered molecular signature induced by nuclear localized intracellular domain of LRP1B. **Results:** Non-tumorous mammary duct epithelial cells did not exhibit LRP1B staining, whereas different degrees of LRP1B immunoreactivity were observed in 75 of 92 invasive ductal carcinoma of the breast. LRP1B immunoreactivity was found in surface membrane and cytoplasm of 60 of 92 (65.2%) invasive ductal carcinoma cells, whereas it was detected in nucleus of 15 of 92 (16.3%) cancer cells. Interestingly, nuclear LRP1B immunoreactivity was significantly associated with poor prognosis of the patients, especially with luminal A type breast cancers. Notably, nuclear localized intracellular domain of LRP1B significantly related to status of nodal metastasis in luminal A type breast cancers. Enforced nuclear expression significantly increased Matrigel invasion activity in MCF-7 luminal A breast cancer cells without affecting cell growth. Notably, nuclear expression of intracellular domain of LRP1B decreased transcription of *LRP1B*. Comprehensive microarray-based analysis demonstrated that nuclear expression of intracellular domain of LRP1B significantly increased the expression of long non-coding RNA *nuclear paraspeckle assembly transcript1 (NEAT1)*, which facilitates breast cancer invasion with poor survival. **Conclusions:** Present findings indicated that nuclear localized intracellular domain of LRP1B promoted breast cancer progression with a poor prognostic value, possible through NEAT1 pathway. Nuclear transport of intracellular domain of LRP1B could be a therapeutic target for breast cancer patients.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD1-05

Results from the phase Ib/II clinical trial of bazedoxifene and palbociclib in hormone receptor positive metastatic breast cancer

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**Background:** Despite the efficacy of endocrine treatments (ETs), most patients with ER+ metastatic breast cancer (MBC) will ultimately develop resistance to these treatments. In most cases of endocrine resistance, ER continues to be expressed. About 30% of patients with ER+ MBC acquire an activating ER mutation that confers resistance to aromatase inhibitors. Thus, ER remains an important driver in a substantial number of patients with MBC despite resistance to currently available ETs. Bazedoxifene is a third generation selective ER modulator (SERM) with selective ER degradation (SERD) activity. The structural characteristics of bazedoxifene differ from those of tamoxifen and raloxifene. Pre-clinical studies showed that bazedoxifene has increased ER antagonistic activity compared to tamoxifen and fulvestrant and has synergistic activity in combination with the CDK4/6 inhibitor, palbociclib. We evaluated the safety and efficacy of bazedoxifene in combination with palbociclib in ER+ MBC.

**Methods:** This was a phase Ib/II study of patients with ER+/HER2- MBC who had progressed on at least one line of ET for metastatic disease or within 12 months of adjuvant ET. Treatment included daily continuous bazedoxifene 40mg and palbociclib 125 mg for 21 days out of a 28-day cycle. Primary endpoint was clinical benefit rate (CBR; CR + PR + SD  $\geq$  24 weeks). Secondary end points included objective response, progression free survival (PFS) and safety. The study used a Simon optimal two-stage design and included a safety run-in phase with the first six patients. The sample size was chosen to have 90% power and a one-sided Type I error of  $\leq$ 10% to reject the null (CBR of  $\leq$  20%). A biopsy from a metastatic site was obtained from 21 patients and serial plasma samples were collected from all patients for correlative studies.

**Results:** From July, 2015 to July, 2017 36 patients enrolled in the study (34 females, 2 males). More than 80% of the patients received at least 1 prior line of endocrine treatment for metastatic disease. 45% of the patients received  $\geq$  2 lines of endocrine treatment and 52% received 1-2 lines of chemotherapy prior to the study. More than 80% of the patients had visceral disease. The combination of bazedoxifene and palbociclib was well tolerated. The most commonly observed adverse events (any grade) were fatigue and neutropenia. Grade 4 adverse events were uncommon (2 patients). The CBR was 36% (95% CI 23-49%). The objective response rate was 8% (95% CI 2-22%). Median PFS was 3.6 months (95% CI 2-8.5 months). There were 5 patients with a durable clinical benefit of  $\geq$ 12 months. Correlative analysis of the pre-treatment tissue samples and serial plasma samples are ongoing.

**Conclusions:** The combination of bazedoxifene and palbociclib was well tolerated and demonstrated significant clinical activity in heavily pre-treated patients with ER+ MBC. Further studies investigating bazedoxifene in ER+ breast cancer are warranted.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD1-06

A phase 1b dose-escalation and expansion study of the BCL-2 inhibitor venetoclax combined with tamoxifen in ER and BCL-2-positive metastatic breast cancer (MBC)

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**Background:** Venetoclax, a potent and selective inhibitor of the survival protein BCL-2 (recently approved in CLL and in development in other hematopoietic malignancies), has yet to be evaluated in pts with solid tumors. BCL-2 is overexpressed in ~85% of ER+ breast cancer. Pre-clinical findings using patient-derived xenograft breast tumor models suggest that venetoclax synergizes with endocrine therapy by increasing apoptosis. Here we report mBEP, an investigator-initiated phase 1b study of venetoclax with tamoxifen in 33 pts with ER+ (>1%), BCL-2+ (>10%, 2-3+ intensity) and HER2- MBC.

**Methods:** We conducted a 3+3 dose escalation study comprising cohorts receiving venetoclax 200, 400, 600 or 800 mg/d with tamoxifen 20 mg/d (continued until progression). The primary endpoint was to determine the maximum tolerated dose (MTD), define dose-limiting toxicities (DLTs) and identify the recommended phase 2 dose (RP2D). In a dose expansion phase (at the RP2D), secondary endpoints including safety and tolerability, response at 24 wks (RECIST v1.1), clinical benefit rate (CBR) and progression-free survival (PFS) were studied.

**Results:** In the escalation phase (n=15 pts), treatment was well tolerated with no DLTs or high-grade (Gd 3/4) adverse events observed, apart from asymptomatic on-target lymphopenia (Gd 3, 2/15 pts). MTD was not reached. The 800 mg/d dose was selected as the RP2D and the cohort expanded to include 24 pts with ≥24 wks follow up (range 24-105 wks). Fifteen pts had received prior regimens for MBC (median 3, range 1-9) that included tamoxifen in 5/15.

For the RP2D cohort (n=24), overall responses (OR) included 1 CR (4%) and 12 PR (50%), with 5 SD (21%), corresponding to a CBR of 75%. The 9 pts treated in the first line setting experienced a 78% OR (7/9 pts) and 11% SD (1/9 pts), equating to an 89% CBR. The data are immature for determining median PFS for the RP2D cohort (currently 40+ wks).

Treatment responses were pre-empted by metabolic responses (FDG-PET) at 4 wks (seen in 13/16 (81%) pts studied), and correlated with serial changes in circulating tumor DNA (ctDNA). Intriguingly, responses and clinical benefit were observed in pts with plasma-detected *ESR1* mutations (4/10 and 7/10, respectively).

The most common treatment-related AEs (CTCAE v4.0) for all pts were lymphopenia in 29/33 (88%; 57% Gd 1-2, 30% Gd 3-4), neutropenia in 24/33 (73%; 67% Gd 1-2, 6% Gd 3), nausea in 22/33 (67%; all ≤Gd 2), anemia in 13/33 (39%; 33% Gd 1-2, 6% G3), thrombocytopenia in 11/33 (33%; all ≤Gd 2), vomiting in 11/33 (33%, all ≤Gd 2), diarrhea in 10/33 (30%; 24% Gd 1-2, 6% Gd 3), infection in 9/33 (27%; 18% Gd 2, 9% Gd 3) and fatigue in 7/33 (21%; all ≤Gd 2). There was one possible treatment-related SAE (infection).

**Conclusions:** In the first clinical study to evaluate venetoclax in a solid tumor, we demonstrate that combining venetoclax with endocrine therapy has a tolerable safety profile and elicits remarkable activity in ER+ and BCL-2+ MBC. These findings support further investigation of combination therapy for patients with BCL-2-positive breast cancer.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD1-08

Phase 1/1b study of novel oral selective estrogen receptor degrader (SERD) LSZ102 for estrogen receptor-positive (ER+) advanced breast cancer (ABC) with progression on endocrine therapy (ET)

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**Background:** LSZ102 is an orally bioavailable SERD that inhibits ER gene transcription, induces receptor degradation, and blocks ER-dependent cell growth in preclinical models. This study is evaluating LSZ102 as a single agent and in combination with the CDK 4/6 inhibitor ribociclib (LEE011) or the PI3K inhibitor alpelisib (BYL719) in patients (pts) with ER+ ABC. The LSZ102 single agent data are presented below; combination data are not discussed.

**Methods:** In the dose-escalation phase evaluating single-agent LSZ102 (Arm A), pts (age ≥18 years; ECOG PS 0-1) with histologically confirmed ER+ ABC and progression on endocrine therapy (ET) received LSZ102. The starting dose was 200 mg once daily. The primary objective of Arm A was to characterize the safety and tolerability of LSZ102 and identify a recommended dose for expansion (RDE). Secondary objectives included preliminary antitumor activity and pharmacokinetics (PK).

**Results:** As of January 22, 2018, 57 pts were enrolled to Arm A (LSZ102 200 mg, n=4; 400 mg, n=6; 450 mg fasted, n=15; 450 mg with food, n=6; 600 mg, n=20; 900 mg, n=6). Median age was 60 years, 75% (n=43) of pts had an ECOG PS of 0, 56% (n=32) had received prior fulvestrant, and 58% (n=33) had received prior CDK4/6 inhibitors; median number of prior lines of therapy (all settings) was 6. At data cut-off, 48 pts had discontinued treatment, most (n=45, 94%) due to disease progression. Dose-limiting toxicities across treatment groups included diarrhea (2 pts in the 900-mg group), vomiting (1 pt in the 600-mg group), and AST and ALT elevation (1 pt in the 450-mg with food group). The most common treatment-related adverse events (AEs) in the treatment period were diarrhea (60%), nausea (56%), and vomiting (30%). In the treatment period, treatment-related grade 3 AEs (12%) were infrequent, and there were no such grade 4 events. Six pts (11%) required dose reduction due to AEs (nausea, vomiting or diarrhea); 4/6 of the dose reductions occurred at 900 mg. Preliminary PK assessment showed rapid absorption and dose-proportional increases in LSZ102 exposure; trough concentrations were above the predicted tumorostatic concentrations at doses of ≥400 mg. Based on PK results for the 450-mg fasted and fed cohorts, LSZ102 exposure does not appear to be affected by dosing with a regular meal. Evidence of ER modulation by immunohistochemistry was observed in paired baseline and on-treatment biopsies. <sup>18</sup>F-fluoroestradiol positron emission tomography (FES-PET) analysis (n=6) demonstrated abrogation of FES-PET signal for pts in the 450-mg and 600-mg dose groups. Seventeen pts (29.8%) had a best response of stable disease, and 1 pt, who happened to be in the 600-mg group, achieved a partial response.

**Conclusion:** In heavily pretreated pts, LSZ102 was well tolerated, demonstrated antitumor activity, and achieved effective exposure levels based on PK and pharmacodynamics. Food intake did not appear to significantly alter the PK profile of LSZ102. Dose escalation for LSZ102 in combination with ribociclib or alpelisib is ongoing and will be reported in a future analysis. An update on the recommended single agent dose and schedule will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

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Phase 2 safety and efficacy results of TAK-228 in combination with exemestane or fulvestrant in postmenopausal women with ER-positive/HER2-negative metastatic breast cancer previously treated with everolimus

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**Background:** TAK-228 is an investigational, oral and highly selective ATP-competitive inhibitor of TORC1/2. Targeting the PI3K/AKT/mTOR pathway with the dual TORC1/2 inhibitor TAK-228 may restore sensitivity to endocrine therapies in patients (pts) with breast cancer who have progressed on the combination of an endocrine agent plus a TORC1 inhibitor. Here we report data from the phase 2 portion of a phase 1b/2 study of TAK-228 plus exemestane (E) or fulvestrant (F).

**Methods:** Postmenopausal women with ER+ and HER2-, inoperable or metastatic breast cancer (MBC) following everolimus (EVE) plus E or F after progression, received oral TAK-228 (4 mg QD) plus E (25 mg QD) or F (500 mg monthly) for 28-day cycles until progressive disease (PD) or unacceptable toxicity (NCT02049957). Pts were enrolled into parallel cohorts based on prior response to EVE plus E or F and were given the same prior therapy (E or F) at their established dose: EVE-sensitive, defined as disease progression after complete response (CR), partial response (PR), or  $\geq 6$  mos stable disease (SD); or EVE-resistant, defined as disease progression without a CR or PR, or after  $< 6$  mos SD. Primary endpoint was clinical benefit rate at 16 wks (CR, PR, or SD at 16 wks; CBR-16). Secondary endpoints included CBR at 24 wks (CBR-24), overall response rate (ORR), progression-free survival (PFS), overall survival (OS) and safety.

**Results:** From Oct 2015 to Dec 2017, 94 pts were enrolled. Median age was 58 y (range 32–83). At baseline, most pts (67%) had stage IV disease and others were stage IA–IIIC (24%), other (3%) or unknown (5%); 94% of EVE-sensitive (93% E vs 100% F) and 88% of EVE-resistant pts (91% E vs 75% F) had received  $\geq 4$  prior lines of therapy. Pts received a median of 3 cycles (1–15) of TAK-228. At data cutoff (24 Apr 2018), 98% of pts had discontinued treatment, mainly due to PD (76%) or adverse events (AEs; 14%). CBR-16 was 41% (n=21) in EVE-sensitive and 26% (n=11) in EVE-resistant pts (table). CBR-24 was 24% in EVE-sensitive (19% E vs 50% F) and 23% in EVE-resistant (23% E vs 25% F) pts. Eleven of 21 pts who achieved CBR-16 also achieved CBR-24 (6 SD, 5 PR) in the EVE-sensitive cohort and 8 of 11 pts in the EVE-resistant cohort (6 SD, 2 PR). The ORR was 12% in EVE-sensitive pts and 9% in EVE-resistant pts (table). Median PFS (95% CI) was 4.1 mos (2.2–5.5) and 3.4 mos (1.9–5.4), and median OS (95% CI) was 15.9 mos (14.1–19.5) and 14.0 mos (13.0–16.0) in the EVE-sensitive and -resistant cohorts, respectively. Drug-related any grade and grade  $\geq 3$  AEs were seen in 90% and 29% of pts, respectively. Most common drug-related any grade AEs were nausea (50%), fatigue (38%), hyperglycemia and diarrhea (each 29%); 22% of pts reported a serious AE. No deaths were reported. Treatment is ongoing in two pts.

**Conclusion:** TAK-228 plus E or F showed modest clinical benefit in pts with previously treated, EVE-sensitive or -resistant MBC, with an acceptable safety profile.

	EVE-sensitive (N=51)		EVE-resistant (N=43)	
	TAK-228+		TAK-228+	
Best response, n (%)	E (n=43)	F (n=8)	E (n=35)	F (n=8)
ORR=CR+PR	4 (9)	2 (25)	3 (9)	1 (13)
CR	0	0	1 (3)	0
PR	4 (9)	2 (25)	2 (6)	1 (13)

CBR-16	17 (40)	4 (50)	9 (26)	2 (25)
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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD1-10

Randomized phase II study comparing two different schedules of palbociclib plus second line endocrine therapy in women with estrogen receptor positive, HER2 negative advanced/metastatic breast cancer: CCTG MA38 (NCT02630693)

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**Background:** Palbociclib is approved for treatment of ER+, HER2- ABC at a standard dose of 125 mg po daily 3 weeks on/1 week off (STD). We evaluated the efficacy, safety, quality of life (QOL) and compliance of a 100 mg continuous daily dose (CDD) of palbociclib based on modeling data that suggested efficacy and tolerability.

**Methods:** Canadian Cancer Trials Group (CCTG) led a randomized phase II trial (CCTG MA38) to estimate the efficacy of palbociclib 100 mg po on CDD schedule (Arm 1) relative to 125 mg po STD schedule (Arm 2) with physician choice endocrine therapy. Eligible patients had ER+, HER2- ABC, post progression on first line metastatic endocrine therapy or progressed while on/within 12 months of completion of adjuvant endocrine therapy. One line of palliative chemotherapy in ABC was allowed. Stratification factors were: visceral metastases, duration of prior endocrine therapy and planned endocrine therapy. Primary Outcome measure was investigator reported PFS; secondary outcome measures included: RR, OS, safety (CTCAE V4.0), and QOL (EORTC QLQ-C30). The sample size was 180 to enable estimation of the HR between arms with the upper bound of the 90% CI 1.36 times the estimated HR and the lower bound is 0.74 times the estimated HR.

**Results:** 180 patients were enrolled across Canada from Dec 2015 to Feb 2017. The database was locked April 16 2018 after prespecified PFS events reached at a median follow-up of 19 months for all patients. For the whole population: median age was 60.5 years (21% ≥ 70 years); ECOG 0/1 95%; postmenopausal 91%; visceral metastases 67%. Planned endocrine therapy: aromatase inhibitor 33%, fulvestrant 57%, tamoxifen 10%. Efficacy analyses CDD vs STD: PFS stratified univariate HR 0.93 (90% CI 0.66-1.30); multivariate Cox model analysis (covariates ECOG PS, age, histology, grade): HR 0.92 (90% CI 0.67-1.25). Median PFS CDD: 9.33 months (90% CI 6.93-13.90) and STD: 11.30 months (90% CI 8.08- 13.83) Secondary analyses CDD vs STD: OS stratified univariate HR: 1.07 (90% CI 0.67-1.69); multivariate HR 1.14 (90%CI 0.75-1.75); median OS for CDD: 20.73 months (90% CI 19.29-23.30) and 21.39 months (90% CI 19.65 to 26.68) for STD. RR and median duration of response: 11.1% vs 12.4% (p=0.84) and 126 vs 169 days (p=0.86), respectively. Palbociclib drug exposure CDD vs STD: median daily dose (mg) 87 vs 125; median dose intensity (mg/week) 571.2 vs 613.5; ≥ 90% planned dose intensity, 41% vs 54%. Dose modification (withhold and/or reduction) rate CDD vs STD for neutropenia was 70% vs 40%. Grade 3/4 neutropenia 69% vs 53%; febrile neutropenia 3% each arm. Non-hematological toxicity profiles were comparable for both arms. No significant differences were seen in QOL domains.

**Conclusion:** Palbociclib is active and tolerable when administered on either a 100 mg CDD or a 125 mg STD schedule. CDD schedule had higher rates of grade 3/4 neutropenia and dose modifications. Post approval evaluation of alternate dosing schedules for targeted therapies provides useful information regarding drug activity, toxicity and adherence.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD1-11

nextMONARCH 1: Phase 2 study of abemaciclib plus tamoxifen or abemaciclib alone in HR+, HER2- advanced breast cancer

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## Background

Abemaciclib is a selective CDK4 & 6 inhibitor approved on a continuous dosing schedule for HR+, HER2- advanced breast cancer (ABC) as monotherapy (MONARCH 1) and in combination with endocrine therapy (ET). A previous Phase 1b (NCT01394016) cohort of HR+ ABC patients (pts) demonstrated efficacy of abemaciclib monotherapy (150mg and 200mg Q12H starting dose); given the small sample size and nonrandomized design the impact of the starting dose was unclear. nextMONARCH 1 (NCT02747004) evaluated abemaciclib in 2 monotherapy arms, in a randomized setting. Abemaciclib has been associated with dose-dependent early onset diarrhea that is well managed with antidiarrheal therapy. nextMONARCH 1 also explored the 200mg Q12H abemaciclib dose in combination with prophylactic loperamide to reduce incidence/severity of diarrhea and dose adjustments. A third arm evaluated abemaciclib + tamoxifen as a strategy to overcome endocrine resistance.

## Methods

nextMONARCH 1 is a multicenter, randomized, open-label, Phase 2 study of abemaciclib or abemaciclib + tamoxifen in women with HR+, HER2- ABC who have progressed on or after prior ET and previously received chemotherapy. Pts were stratified by presence of liver metastases and prior use of tamoxifen in the advanced setting. Randomization was 1:1:1 to abemaciclib 150mg Q12H + daily tamoxifen 20mg (Arm A) or abemaciclib 150mg Q12H (Arm B); or abemaciclib 200mg Q12H + prophylactic loperamide (Arm C). Key eligibilities were  $\geq 2$  chemotherapy regimens (1-2 administered in metastatic setting), measurable disease and no prior treatment with CDK4 & 6 inhibitors. Primary objective was progression free survival (PFS). Key secondary objectives included objective response rate (ORR), clinical benefit rate (CBR), and safety. PFS analysis tested superiority of Arm A to C at ~110 events across the 2 arms assuming a hazard ratio (HR) of 0.667 to achieve ~80% power. Arm B would be considered non-inferior to Arm C if the observed PFS HR is  $< 1.2$ .

## Results

234 pts were randomized to Arms A (n=78), B (n=79) and C (n=77). 166 PFS events have been observed (A: 57; B: 54; C: 55). Median PFS was 9.1 months in Arm A, 6.5 in Arm B and 7.4 in Arm C (A vs C: HR=.815, 95% CI, .556-1.193, p=.293; B vs C: HR=1.045, 95% CI, .711-1.535 p=.811). Investigator-assessed ORR was 34.6%, 24.1% and 32.5% (confirmed ORR: 25.6%, 19.0%, 28.6%) and CBR was 61.5%, 49.4% and 51.9% in Arms A, B and C, respectively. Prophylactic loperamide reduced the incidence and severity of diarrhea (C: 62.3%, Gr 3: 7.8%) compared to MONARCH 1 (90.2%, Gr 3: 19.7%, Dickler et al. 2017) resulting in similar rates of diarrhea with 150mg abemaciclib without prophylaxis (A: 53.8%, Gr 3: 1.3%; B: 67.1%, Gr 3: 3.8%). The adverse event profile across arms was generally consistent with other breast studies of abemaciclib.

## Conclusions

nextMONARCH 1 confirmed single-agent activity of abemaciclib in heavily pretreated pts with HR+, HER2- ABC. Efficacy of abemaciclib monotherapy at 150mg was similar to 200mg. Combining tamoxifen with abemaciclib did not demonstrate a statistically significant improvement in PFS compared to abemaciclib monotherapy. Addition of prophylactic loperamide to abemaciclib 200mg resulted in diarrhea similar to 150mg without prophylaxis.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD1-12

Omics profiling of CDK4/6 inhibitors reveals functionally important secondary targets of abemaciclib

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The recent introduction of small molecule inhibitors of cyclin-dependent kinases (CDK) 4/6 to the clinic has improved the treatment of hormone receptor positive breast cancer, and shown promise in other malignancies. The three clinically used CDK4/6 inhibitors, palbociclib, ribociclib, and abemaciclib, are reported to be broadly similar although recent data suggest that abemaciclib has distinct single-agent activity in patients and a unique adverse effects profile. Key questions are: How do these drugs differ at the molecular level? Should such differences inform their use in the clinic? Can these three agents be used interchangeably or should patient stratification differ between them? We use molecular and functional profiling by mRNA sequencing, mass spectrometry-based proteomics, and GR-based dose-response assays to obtain complementary views of the mechanisms of action of CDK4/6 inhibitors. We show that abemaciclib, but not ribociclib or palbociclib, is a potent inhibitor of kinases other than CDK4/6, including CDK1/Cyclin B, which appears to cause arrest in the G2 phase of the cell cycle, and CDK2/Cyclin E/A, which is implicated in resistance to palbociclib. We show that inhibition of these additional targets is accessible in a xenograft model. Whereas ribociclib and palbociclib induce cytostasis, and cells adapt to these drugs within 2-3 days of exposure, abemaciclib induces cell death and durably blocks cell proliferation. Abemaciclib is active even in retinoblastoma protein (pRb)-deficient cells in which CDK4/6 inhibition by palbociclib or ribociclib is completely ineffective. The degree of polypharmacology of small molecule drugs is increasingly viewed as an important consideration in their design, with implications for efficacy, toxicity, and acquired resistance. In the case of CDK4/6 inhibitors, we propose that abemaciclib polypharmacology elicits unique molecular responses. More generally, we propose that multi-omic approaches are required to fully elucidate the spectrum of targets relevant to drug action in tumor cells. We expect such understanding to assist in stratifying patient populations and ordering sequential therapies when resistance arises.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-01

Personalized serial circulating tumor DNA (ctDNA) analysis in high-risk early stage breast cancer patients to monitor and predict response to neoadjuvant therapy and outcome in the I-SPY 2 TRIAL

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ctDNA analysis offers a non-invasive approach for monitoring response and resistance to treatment. Serial ctDNA testing during neoadjuvant therapy (NAT) may provide early indicators of emerging resistance and disease progression. In this study, we analyzed ctDNA from high-risk early breast cancer patients who received NAT and definitive surgery in the I-SPY 2 TRIAL (NCT01042379). We hypothesize that (1) assessment of ctDNA levels early in treatment will improve the performance of molecular and imaging-based predictors of pathologic complete response (pCR) to NAT; and (2) levels of ctDNA after NAT are associated with residual cancer burden and recurrence [distant recurrence free survival (DRFS)].

**Methods:** ctDNA analysis was performed in 84 high-risk stage II and III breast cancer patients randomized to neoadjuvant investigational agent (n=52), AKT inhibitor MK-2206 (M) in combination with paclitaxel (T) followed by doxorubicin and cyclophosphamide (AC) (M+T->AC), or standard-of-care (T->AC) (n=32). HER2+ patients also received trastuzumab (H). Serial plasma was collected before NAT (T0), early treatment (3 weeks, T1), between regimens (12 weeks, T2), and after NAT prior to surgery (T3). Mutational profiles derived from pretreatment tumor biopsy and normal matched DNA whole exome sequencing were used to design personalized assays targeting 16 patient-specific somatic variants to detect ctDNA in serial plasma.

**Results:** Of the 84 patients in this study, 43% were HR-/HER2- (TNBC), 35% HR+/HER2-, and 23% HER2+. In total, 74% (61 of 83), 35% (28 of 79), 14% (9 of 65), and 8% (5 of 61) were positive for ctDNA at timepoints T0, T1, T2, and T3, respectively. At T0, ctDNA positivity and levels (average number of mutant molecules detected per mL) were significantly associated with increased tumor burden (by clinical and MRI examination), more aggressive tumor biology (as reflected in higher Mammprint scores and grade) and subtype (HER2+ and TNBC). Twenty-seven percent (27%) of the 84 patients achieved a pCR and all patients who were ctDNA-positive at T3 (n=5) did not achieve a pCR. Currently, data are being collected to: (1) assess the relationship of ctDNA and MRI imaging in predicting tumor response to therapy; (2) examine the relationship of ctDNA levels before and after NAT with 3-year DRFS and event-free survival (EFS). The results of these analyses will be presented at the SABCS 2018 meeting.

**Conclusions:** Our study provides a platform to evaluate the clinical significance of ctDNA for serial monitoring of response to NAT. Accurate and early response prediction by highly sensitive ctDNA analysis can facilitate a timely and judicious change in treatment to improve patients' chances of achieving a pCR. Finally, personalized ctDNA testing may complement imaging and pathologic evaluation of tumor response to fine-tune pCR as a surrogate endpoint for improved DRFS and EFS.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: PD2-02

Longitudinal ctDNA sequencing using an expanded genomic panel in the PALOMA3 trial of palbociclib plus fulvestrant

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**Background:** CDK4/6 inhibition combined with endocrine therapy (ET) is now the standard of care for advanced hormone receptor (HR) positive breast cancer patients (pts). Previous ctDNA analysis of paired pre-treatment (pre-T) and end of treatment (EoT) plasma samples from the PALOMA-3 trial of the CDK4/6 inhibitor P with/without F demonstrated that acquired *PIK3CA*, *ESR1*, *RB1* and rare growth factor receptor mutations (mut) likely contribute to resistance (Turner et al, ASCO 2018, Abstract 1001). We now report results from an extended ctDNA analysis of paired PALOMA-3 plasma samples using hybridization capture-based, next-generation sequencing (NGS) assay covering 87 breast cancer, HR signaling, and cell cycle-related genes.

**Methods:** Pre-/post-menopausal pts whose disease progressed after prior ET (N=521) were randomized 2:1 to receive F 500 mg + P (125 mg/d, 3 wk on/1 wk off) or placebo. Cell-free DNA extracted from paired plasma samples (n=194) was analyzed with a custom 87-gene NGS assay, to identify single nucleotide variants and copy number amplification (CNA), with molecular barcoding, high NGS coverage (10,000-20,000 reads per nucleotide position), and background error correction. Differences in ctDNA mut frequencies detected in P+F compared to F alone were assessed and their association to clinical outcome estimated.

**Results:** Blinded assay qualification and droplet digital PCR validation suggested a mut detection frequency cutoff at 0.3%. A high coefficient of correlation with previously presented data was observed for *ESR1* and *PIK3CA* variants (r=0.94 and 0.97, respectively), with acquisition of *PIK3CA* and *ESR1* mutations at EoT in P+F and F groups. Gene level mut analysis of EoT plasma revealed no significant difference between P+F vs F alone (Table), although there was an increase in *RB1* mutations in P+F consistent with previous data. Other acquired muts at EoT (*SMAD4*, *NOTCH2*, and *CDKN1B*) were observed at low frequencies. Muts in *CDK4* and *CDK6* were rarely observed (< 1% of pts), regardless of treatment arm.

Detected Variants, Frequency, n (%)						
Gene	P+F		F		Pre-T pval	EoT pval
	Pre-T	EoT	Pre-T	EoT		
PIK3CA	47(37)	51(40)	19(28)	22(33)	0.266	0.352
ESR1	36(28)	45(35)	19(28)	24(36)	1	1
TP53	30(24)	33(26)	23(34)	25(37)	0.128	0.137
RB1	2(2)	9(7)	2(3)	2(3)	NA	0.336
PTEN	5(4)	7(6)	3(4)	3(4)	1	1
AKT1	7(6)	7(6)	2(3)	2(3)	0.721	0.721

**Conclusions:** Our results corroborate the previously reported results demonstrating that genomic profiles of treatment resistant cancer are similar between P + F and F therapy. Expanded analysis of cell-cycle genes identified no new recurrently mutated genes, and confirmed that *RB1* mutations are selected at low frequency after P+F treatment. Alterations in cell cycle genes may not be a common mechanism of resistance to CDK4/6 inhibitors in HR+ HER2- advanced breast cancer.

Sponsor: Pfizer

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-03

Circulating tumor DNA (ctDNA) and circulating tumor cells (CTC) predictive value in HER2 negative metastatic breast cancer patients treated with first line weekly paclitaxel and bevacizumab: Results of a prospective cohort from the French Breast Cancer InterGroup Unicancer (UCBG): COMET study

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**Background:** Increased levels of CTC and a persistent elevated level after just one cycle of chemotherapy are very strong and independent markers of worse progression-free survival (PFS) and overall survival (OS) in patients (pts) with metastatic breast cancer (MBC) (Bidard et al, Lancet Oncol 2014). ctDNA can be used to detect mutation associated with resistance to treatment. It has also been shown that dynamic changes in ctDNA levels closely reflect changes in tumor burden. We prospectively monitored CTC and ctDNA early variations during first line chemotherapy for MBC.

**Patients & methods:** The French cohort COMET is a prospective study including first line HER2 negative patients (pts) receiving weekly paclitaxel and bevacizumab according to EMA approved combination. The aim of this cohort is to evaluate clinical, biological and radiological parameters associated with pts outcome (CTC, serum markers, ctDNA, pharmacogenomic polymorphisms, metabolomic parameters, visceral fat, serum estradiol level and quality of life). We present here the first planned analysis on pts evaluated for CTC (CellSearch) and ctDNA using targeted sequencing (Roche SeqCap technology) of a panel of 46 genes and 8 promoters, using unique molecular identifiers to increase ctDNA detection sensitivity. Blood samples were obtained at baseline (BL) and before the second cycle of chemotherapy (C2).

**Results:** From 09/2012 to 5/2014, 218 pts were included in this substudy. Median age was 55 years and 22% of pts had triple negative BC. At BL, 70% of pts had  $\geq 1$  detectable CTC per 7.5 ml of blood (median 4 CTC, range 1- 30,000) and 37% at C2. With a threshold of  $\geq 5$  CTC, 47% of pts were positive at BL and 22% at C2. For ctDNA, out of the first 141 pts analyzed, 105 had at least one somatic mutation detected in plasma (74%). The average number of mutations per pt was 2.7 and most commonly mutated genes were *TP53* and *PIK3CA*. *ESR1* was found mutated in 9% of all cases and restricted to the ER+ subgroup. Median Allelic Frequency was 10% (range 0.6-83%). Only 33% of pts had detectable ctDNA at C2. At BL, CTC and ctDNA levels were correlated ( $r=0.46$ ,  $p<0.0001$ ). Despite no complete overlap, 11% of pts had no CTC nor ctDNA detected. Median follow-up was 53 months and median OS was 32 months. Increased level of CTC and ctDNA were significantly associated with decreased PFS and OS. At C2,  $\geq 5$  CTC or still detectable ctDNA were strong markers of reduced OS: HR 4.6 (CI95 3.1-7) and HR 3.2 (CI95 1.8 – 5.5), respectively (both  $p<0.0001$ ). At multivariate analysis for PFS, detectable ctDNA at C2 and triple negative status were the only significant prognostic factors. None of serum marker level at BL or their early variations had prognostic value.

**Conclusion:** This is the largest prospective cohort assessing the respective prognostic values of early CTC and ctDNA changes in homogeneously treated first line MBC patients. Analysis of mutations profile variations and comparison with primary tumor and metastasis biopsies are ongoing and may reveal early mechanisms of resistance.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-04

Baseline circulating *ESR1* mutation analysis in the randomised phase III EFECT study of fulvestrant versus exemestane in advanced hormone receptor positive breast cancer

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**Background.** *ESR1* mutations are acquired frequently in hormone receptor positive breast cancer after treatment with aromatase inhibitors (AI) in the metastatic setting. In prior analysis of the SoFEA phase III randomised trial, we demonstrated the detection of *ESR1* mutations in circulating tumor DNA (ctDNA) predicted for greater benefit of fulvestrant compared to exemestane (Fribbens et al JCO 2016).

**Methods.** The phase III EFECT study randomised 693 patients with ER+ metastatic breast cancer who had progressed on a prior non-steroidal AI, between fulvestrant loading dose and then 250mg q28 days and exemestane (Chia S, et al. J Clin Oncol 2008). Baseline serum samples were available from 227 patients in EFECT, and were analysed for the 8 most common *ESR1* mutations by multiplex digital PCR. The association between baseline *ESR1* status and time to progression (TTP) was analysed using Kaplan-Meier methods.

**Results.** *ESR1* mutations were successfully analysed in 98% (222/227) of patients with baseline serum samples, with *ESR1* mutations detected in 23.4% (52/222) samples. Overall, detection of *ESR1* mutations at baseline was associated with shorter TTP (hazard ratio [HR] 2.03, 95% CI 1.26-3.29, p=0.004). In patients with *ESR1* mutation detected, TTP was 2.0 months (95%CI, 1.7-2.4) on exemestane and 3.5 months (95%CI, 1.9-5.0) on fulvestrant (HR 0.67, 95%CI 0.37-1.19, p=0.17). In patients without *ESR1* mutations detected, TTP was 4.5 months (95%CI, 3.7-5.6) on exemestane and 3.7 months (95%CI, 3.3-5.2) on fulvestrant (HR 1.05, 95%CI 0.75-1.45, p=0.78). A meta-analysis of SoFEA and EFECT will be presented at the conference.

**Conclusions.** Historical serum samples may be used for ctDNA analysis, illustrating the potential for future research of sample archives. Patients with *ESR1* mutation detected in ctDNA have poor outcome when treated with exemestane, replicating prior results from SoFEA and demonstrating clinical utility for *ESR1* mutation ctDNA analysis.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: PD2-05

Biomarker analysis by baseline circulating tumor DNA alterations in the MONALEESA-3 study

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**Background:** In the Phase III MONALEESA-3 (NCT02422615) study, ribociclib (RIB; cyclin-dependent kinase 4/6 inhibitor) + fulvestrant (FUL) significantly prolonged progression-free survival (PFS) vs placebo (PBO) + FUL in postmenopausal women with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC). Here we present MONALEESA-3 efficacy data by molecular alterations detected in circulating tumor DNA (ctDNA) at baseline.

**Methods:** Postmenopausal women (N=726) with HR+, HER2- ABC (treatment naïve or received ≤1 line of prior endocrine therapy for ABC) were randomized 2:1 to RIB or PBO (600 mg/day; 3-weeks-on/1-week-off) + FUL (500 mg per label). Biomarker analysis of baseline ctDNA was an exploratory endpoint. Baseline plasma samples were collected from 692 patients (pts), and plasma ctDNA was analyzed using next-generation sequencing with a targeted panel of ~550 genes. The subgroup analysis was performed in 600 pts (RIB + FUL n=400; PBO + FUL n=200) with evaluable ctDNA data. Results were not adjusted for multiple testing.

**Results:** Alterations (frequency) were observed in the following genes: *PIK3CA* (35%), *ESR1* (14%), *TP53* (18%), *CDH1* (12%), *FGFR1* (5%), *FGFR1/ZNF703/WHSC1L1* (11%), cell cycle-related (CCC) genes (16%), and genes involved in receptor tyrosine kinase (RTK) signaling (20%), and the mitogen-activated protein kinase (MAPK) pathway (10%). PFS hazard ratios favored RIB vs PBO regardless of baseline ctDNA gene alteration status (Table).

	Events, n/N		Median PFS, months		
Gene(s)	RIB + FUL	PBO + FUL	RIB + FUL	PBO + FUL	Hazard ratio; 95% confidence interval
PIK3CA					
WT	108/265	70/124	22.34	16.49	0.67; 0.49–0.91
Altered	75/135	49/76	16.36	11.10	0.75; 0.52–1.08
ESR1					
WT	141/338	99/176	22.34	14.88	0.66; 0.51–0.85
Altered	42/62	20/24	9.23	6.11	0.71; 0.41–1.23
TP53					
WT	132/325	98/170	22.34	14.88	0.62; 0.47–0.80
Altered	51/75	21/30	9.17	7.39	0.75; 0.44–1.27
CDH1					
WT	156/356	97/173	20.63	16.49	0.70; 0.54–0.90
Altered	27/44	22/27	12.09	5.13	0.46; 0.24–0.86
FGFR1					
WT	165/378	113/192	21.32	14.55	0.65; 0.51–0.83

Altered	18/22	6/8	8.34	6.11	0.64; 0.23–1.75	
<b><i>FGFR1/ZNF703/WHSC1L1</i></b>						
WT	155/356	105/181	21.32	14.65	0.67; 0.52–0.85	
Altered	28/44	14/19	10.97	6.67	0.73; 0.37–1.43	
<b>CCC</b>						
WT	138/331	103/174	22.08	14.65	0.61; 0.47–0.79	
Altered	45/69	16/26	12.65	9.13	0.92; 0.51–1.65	
<b>RTK</b>						
WT	126/315	95/166	22.34	14.82	0.61; 0.47–0.80	
Altered	57/85	24/34	9.17	5.78	0.82; 0.50–1.35	
<b>MAPK</b>						
WT	158/357	105/183	20.04	14.82	0.69; 0.54–0.89	
Altered	25/43	14/17	14.72	3.61	0.47; 0.22–1.00	

Numerically shorter PFS was observed in pts with altered vs wildtype (WT) genes, irrespective of treatment. A slight trend towards limited RIB benefit vs PBO was observed in pts with altered CCC and RTK genes. RIB benefit was more pronounced vs PBO in pts with altered *CDH1* and MAPK genes. *PIK3CA* and *ESR1* analyses for pts receiving treatment in the first- or second-line setting will be presented at the meeting.

**Conclusions:** Generally consistent PFS benefit for RIB + FUL vs PBO + FUL was observed irrespective of baseline ctDNA alterations; altered status trended to correlate with a shorter PFS. Results are hypothesis generating and should be interpreted with caution for some subgroups due to small sample sizes.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-06

Circulating *ESR1* mutation detection rate and early decrease under first line aromatase inhibitor and palbociclib in the PADA-1 trial (UCBG-GINECO)

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**Background:** Palbociclib (Pal) combined with an aromatase inhibitor (AI) is a standard of care as first line therapy in ER+ HER2- metastatic breast cancer (MBC). While *ESR1* mutations (*ESR1*<sub>mut</sub>) are a characterized mechanism of resistance to AI as single agent, it remains unknown how these mutations, when detected prior to treatment initiation, may affect the efficacy of Pal+AI. In a subsidiary analysis of the first line PADA-1 trial, we report the *ESR1*<sub>mut</sub> detection rate at baseline and, in patients with *ESR1*<sub>mut</sub> detected at baseline, how *ESR1* circulating tumor DNA levels changed after the first cycle of AI-Pal therapy.

**Methods:** PADA-1 (NCT03079011) is a randomized phase III trial testing the clinical utility of real time *ESR1*<sub>mut</sub> detection (baseline, at 1 month and then every 2 months) in the blood of patients treated with AI-Pal (first step). Patients with rising *ESR1*<sub>mut</sub> in circulating tumor DNA (*i.e.* increasing or appearing mutations during first line AI-Pal therapy) are randomized in a second step between keeping AI-Pal or switching to Fulvestrant-Pal. Main inclusion criteria are patients with ER+ HER2- MBC, who never received adjuvant AI or completed adjuvant AI for >12 months, with neither prior therapy for MBC nor visceral crisis. *ESR1*<sub>mut</sub> are tracked in circulating DNA from up to 4ml of plasma by a ddPCR-based assay targeting E380, L536, Y537 and D538 hotspots (*i.e.* #90% of known *ESR1* activating mutations) with #0.1% sensitivity (Bidard *et al*, AACR 2018 #3867).

**Results:** From 04/2017 to 05/2018, 803 MBC patients have been included in 80 centers. Among these patients, *ESR1*<sub>mut</sub> were detected at baseline, prior to any systemic therapy, in 17 patients (2.1%, 95%CI=[1.3;3.3%]). *ESR1*<sub>mut</sub> levels ranged from 6 to 3959 copies/ml of plasma (median: 30 copies/ml); allelic frequency ranged from 0.3% to 47% (median=3.5%). *ESR1*<sub>mut</sub> were not associated with any of the tumor pathological characteristics (including PR status, primary TNM stage, number and type of metastatic sites); a non-significant association was observed with primary tumor grade (1.7% vs 4.1% in grade I-II vs III, Fisher: p=0.15). Among patients who received a prior adjuvant endocrine therapy, *ESR1*<sub>mut</sub> detection rate was lower in patients treated with tamoxifen (0.9% with any tamoxifen exposure vs 5.7% with no tamoxifen exposure; Yates Chi2: p=0.01) and observed only in patients treated with AI (4.9% with any AI exposure vs 0% with no AI exposure, Yates Chi2: p=0.009). Among the 17 patients with *ESR1*<sub>mut</sub> detected at baseline, only 4 patients had residual detectable *ESR1*<sub>mut</sub> detected after 1 month of AI-Pal therapy (3 of these 4 had decreased levels of *ESR1* mutation).

**Conclusion:** *ESR1*<sub>mut</sub> is a rare event in untreated AI-sensitive, ER+ HER2- MBC patients. Such detection is primarily associated with prior use of AI in the adjuvant setting. Interestingly, in most MBC patients with *ESR1*<sub>mut</sub> detected at baseline, *ESR1*<sub>mut</sub> became undetectable after 1 month of AI-Pal therapy, suggesting that this combination may retain early antitumor efficacy.

**Funding:** Pfizer

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-07

mRNA signatures predict response to durvalumab therapy in triple negative breast cancer (TNBC)– Results of the translational biomarker programme of the neoadjuvant double-blind placebo controlled GeparNuevo trial

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**Background:** The GeparNuevo trial showed a numerical increase in the pCR rate to 53% vs 44%;  $p=0.281$  compared to placebo in TNBC with the addition of the anti-PD-L1 antibody durvalumab to a neoadjuvant anthracycline-taxane containing chemotherapy (Loibl S et al. ASCO 2018). In a predefined subgroup analysis, a significant increase of the pCR rate was observed for patients that received durvalumab for 2 weeks alone prior to the start of chemotherapy in a window phase (61% vs 41%,  $p$  interaction=0.048), while the pCR rate was not increased for the subset of patients that did start durvalumab together with chemotherapy.

Here we report the main results of the translational programme for GeparNuevo with a focus on mRNA signatures predictive for pCR in pretherapeutic core biopsies.

**Methods:** A total of 162 baseline FFPE core biopsies were evaluable for expression of 2560 genes using the HTG EdgeSeq® system that combines a modified nuclease protection assay with next generation sequencing. Data was processed as recommended by the HTG and median transformed for further analyses. For differential gene expression analyses, the data was scale-normalized (TMM normalization; EdgeR package) and linear models were fit (limma package). Prior to these analyses, genes were filtered based on minimal expression ( $> 4$ ) and variability ( $IQR > 1$ ). As a first step, predefined immune-genes signature (TILs signature) (Denkert et al. JCO 2016) as well as IFN-gamma signatures were evaluated for correlation with pCR in logistic regression models. Subsequently, we performed a differential gene expression analysis according to therapy response for the durvalumab-arm and the placebo arm using the pre-filtered candidate genes. Gene names are not included in this abstract to allow filing of IP, but full gene names will be presented at the SABCS meeting.

**Results:** The predefined TIL- and IFN-gamma signatures were associated with increased pCR rates in the complete cohort (TIL-signature: OR 1.44, 95% CI 1.15-1.82,  $p=0.002$ ; IFN-Gamma-signature: OR 1.63, 95% CI 1.22-2.24,  $p=0.002$ ) as well as in the durvalumab arm ( $p=0.012$  and  $0.042$ ) and the placebo arm ( $p=0.050$  and  $0.011$ ). These signatures were general pCR predictors without specificity for durvalumab response.

Additional 44 genes were significantly ( $p<0.05$ ) correlated with pCR in the durvalumab arm. Of those, 21 genes were upregulated and 23 genes were downregulated in pCR patients. 14 of the 21 upregulated genes are related to tumobiologically relevant immune cell functions. A total of 6 of the 44 genes had a positive test for interaction (interaction  $p<0.05$ ) with the therapy arm (durvalumab + NACT vs. placebo + NACT), suggesting that these genes might specifically predict response to durvalumab. Additional analyses investigating the role of molecular tumor subtypes, additional immune gene signatures and other subgroup analyses will be presented at the meeting.

**Conclusion:** Our results show that specific immune-related gene expression signatures predict response to durvalumab in primary triple negative breast cancer.

The trial was financially supported by Astra Zeneca and Celgene



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-08

Ribociclib with endocrine therapy for premenopausal patients with hormone receptor-positive, HER2-negative advanced breast cancer: Biomarker analyses from the phase III randomized MONALEESA-7 trial

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**Background:** In the MONALEESA-7 study (NCT02278120), ribociclib (RIB) + a non-steroidal aromatase inhibitor (NSAI)/tamoxifen (TAM) + goserelin (GOS) significantly improved progression-free survival (PFS) vs placebo (PBO) + NSAI/TAM + GOS in premenopausal patients (pts) with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC; hazard ratio 0.553; 95% confidence interval [CI] 0.441–0.694; p=0.0000000983). Here we present PFS data by baseline (prior to study treatment) tumor (primary and metastatic) Ki67, total Rb, and p16 protein expression, and *CCND1* (cyclin D1), *CDKN2A* (p16), and *ESR1* (estrogen receptor) messenger RNA (mRNA) levels.

**Methods:** Pre-/perimenopausal pts (N=672; ≤1 line of prior chemotherapy and no prior endocrine therapy for ABC) were randomized 1:1 to either RIB (600 mg/day; 3-weeks-on/1-week-off) or PBO + an NSAI (letrozole [2.5 mg/day]/anastrozole [1 mg/day]) or TAM (20 mg/day) + GOS (3.6 mg every 28 days). The primary endpoint was local PFS, while correlation with biomarker expression was an exploratory endpoint. Baseline tumor tissue was evaluated for Ki67, Rb, and p16 protein expression by immunohistochemistry and *CCND1*, *CDKN2A*, and *ESR1* gene expression by NanoString® nCounter. To assess correlations between protein/gene expression and PFS, pts were classified into prespecified low vs high expression subgroups; 14% of positively stained cells was used as a cutoff for Ki67, 10<sup>th</sup> percentile was used as a cutoff for total Rb, and median expression was used as a cutoff for all other proteins/genes.

**Results:** Based on a data cutoff of August 20, 2017, PFS hazard ratios for most biomarker subgroups favored RIB + NSAI/TAM + GOS vs PBO + NSAI/TAM + GOS (Table).

	RIB + NSAI/TAM		PBO + NSAI/TAM			
	Events, n/N	Median PFS, months	Events, n/N	Median PFS, months	Hazard ratio; 95% CI	
Ki67						
Low	16/56	27.5	22/49	Not reached	0.523; 0.270–1.013	
High	52/117	19.4	71/117	12.6	0.495; 0.341–0.719	
Total Rb						
Low	8/19	27.5	4/12	Not reached	0.239; 0.034–1.667	
High	63/151	23.8	89/152	12.9	0.483; 0.342–0.681	
p16						
Low	28/85	Not reached	41/79	16.4	0.570; 0.348–0.935	
High	35/74	23.8	47/74	9.1	0.403; 0.250–0.651	
CCND1						
Low	40/88	19.1	51/92	13.6	0.672; 0.442–1.022	

High	36/97	23.8	47/83	14.6	0.381; 0.236–0.614	
<b>CDKN2A</b>						
Low	29/85	Not reached	53/95	15.6	0.476; 0.300–0.756	
High	47/100	19.4	45/80	12.6	0.485; 0.310–0.758	
<b>ESR1</b>						
Low	42/97	22.1	45/83	12.9	0.571; 0.362–0.901	
High	34/88	27.5	53/92	14.6	0.568; 0.359–0.899	

It is difficult to draw conclusions for total Rb protein expression due to the small sample size and limited number of events. RIB treatment benefit was numerically greater in pts with high vs low *CCND1* mRNA expression levels.

**Conclusions:** Generally consistent PFS benefits with RIB + NSAI/TAM + GOS vs PBO + NSAI/TAM + GOS were observed irrespective of baseline biomarker expression in cell cycle- and proliferation-related genes. Updated results will be presented at the meeting.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-09

High-throughput phosphoproteomics (HTPS) in neoadjuvant (NEO) breast cancer (BC) reveals clusters of extreme sensitivity to paclitaxel (T)

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**Background:** NGS has elucidated the mutational landscape in BC. However, the correlation between mutational landscapes and complex phenotypic traits such as drug response is yet unclear. The genomic and transcriptomic aberrations coalesce into a diverse number of phosphorylation-driven patterns of activation of the proteome. These patterns are associated to clinical outcomes of interest. The specific nature of the phosphopeptides in a profile versus another can be driven by a small number of activated kinases. In the past we relied on mass-spectrometry-based HTPS to build a kinase-based classification of triple-negative breast cancer (TNBC, Nat. Comms, *in press*), which is more parsimonious than gene-centered classifications and uncovers new actionable targets. We implemented this methodology to find predictors of response to T in early BC.

**Methods:** Training set: fresh baseline and day+15 tumor biopsies from a trial in NEO HER2-negative BC (N=139) that randomized patients to T (80 mg/m<sup>2</sup> weekly x12) plus placebo or nintedanib (150 mg b.i.d) were processed with a hybrid ion trap-orbitrap mass spectrometer after TiO<sub>2</sub> phospho-enrichment. Phosphoprofiles were compared pair-wise according to the following factors: day 0 versus day + 15, standard versus experimental arm, pathologic response Symmans/Pusztai (Symmans) 0/1 vs 2/3.

Kinases driving each phosphoprofile were solved by an *in silico* algorithm termed kinase-set enrichment analysis (KSEAS).

**Validation set:** an in-house designed and previously validated mass-spectrometry-to-immunohistochemistry translation algorithm was used to validate the kinases enriched in the profiles from the baseline samples of patients achieving Symmans 0/1 (pCR) or Symmans=3 in the training set. Probes against those kinases were validated in an independent dataset of 160 HER2-negative patients receiving NEO T followed by AC. H-score of each activated kinase was divided in quartiles (Q1 to Q4), and upper-quartiles (Q1) of each kinase were tested in a multivariate logistic regression model to predict pCR adjusted by T, N, age, G, ER/PR and Ki67.

**Results:** >2.5 millions of spectra were captured, identifying >35000 unique phosphopeptides mapping to >2500 unique proteins per sample. Training set: KSEAS revealed that a high activity of CDK4 and pP70S6K drove the baseline phosphoprofiles of the patients obtaining pCR in the T arm, whereas pSTAT3, pSrc and BARD drove that of the patients achieving Symmans=3. In the validation set, H-score cut-offs for Q1 were 1.21, 0.69, 0.79, 1.64 and 1.54 for pP70S6K, CDK4, pSTAT3, pSrc and BARD. TNBC patients with Q1 pP70S6K or Q1 CDK4 achieved pCR in 100% of the cases (versus 45% in Q2-4). In the HR+ cases, the pCR rate for Q1 patients was 50% and 61% respectively. In the multivariate model (all patients), having Q1 pP70S6K or CDK4 multiplied by 2.3- and 3.6-fold, respectively, the probability of achieving pCR (P<0.05). Regarding Symmans=3, BARD and pSTAT3 lost significance in the validation but not pSrc (2.5-fold less probability of pCR, P<0.005).

**Conclusions:** HTPS is a useful tool to find associations with complex traits. TNBC and HR+ patients with high pP70S6K or CDK4 receiving NEO T-based chemotherapy achieve very high rates of pCR.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-10

Treatment with abemaciclib modulates the immune response in gene expression analysis of the neoMONARCH neoadjuvant study of abemaciclib in postmenopausal women with HR+, HER2 negative breast cancer

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**Background:** Abemaciclib is a selective inhibitor of CDK4 & 6 approved on a continuous dosing schedule for the treatment of HR+, HER2- metastatic breast cancer (MBC) patients (pts) in combination with endocrine therapy or as monotherapy. Recent studies have demonstrated the potential for CDK4 & 6 inhibitors, including abemaciclib, to promote anti-tumor immunity. Schaer et al., (*Cell Reports* 2018) showed that abemaciclib monotherapy results in upregulation of antigen presentation on tumor cells and increases T-cell activation. These activities synergized with anti-PD-L1 therapy to further enhance immune activation leading to complete tumor rejection in murine tumor models (Schaer et al., *Cell Reports* 2018). In this exploratory analysis, we evaluated the early and late immune-modulating effects of abemaciclib in the neoadjuvant study neoMONARCH (NCT02441946).

**Methods:** NeoMONARCH is a Phase II trial in women with stage I-IIIB HR+, HER2- BC evaluating neoadjuvant treatment with 2 weeks of abemaciclib, alone or in combination with anastrozole (abemaciclib+ANZ), or ANZ alone. All patients received 14 weeks of abemaciclib +ANZ after the first 2 weeks of treatment. Serial biopsies were collected at 3 time points: Baseline (BL) - prior to treatment, Early - after 2 weeks of therapy with abemaciclib, ANZ, or abemaciclib+ANZ, and Late – after 2 weeks of initial therapy followed by 14 weeks of abemaciclib+ANZ. RNA was extracted from formalin fixed paraffin embedded (FFPE) tumor biopsies at each time point and subjected to whole transcriptome RNA sequencing. The curated data were subjected to statistical analysis using ANOVA tests followed by pathway analysis using Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA).

**Results:** Consistent with the known activity of abemaciclib to inhibit the cell cycle, we observed at the early and late time points a significant treatment induced downregulation of genes related to mitotic spindle organization, replication stress response, G2M checkpoint, and E2F targets. Abemaciclib treatment for 2 weeks, alone or in combination with ANZ, followed by 14 weeks of combination therapy was associated with upregulation of gene expression signatures related to T-cell immune response and antigen presentation. Importantly, this phenomenon was not observed with 2 weeks of ANZ treatment alone followed by 14 weeks of combination therapy.

**Conclusion:** These data lend support that continuous inhibition of CDK4 & 6 signaling by abemaciclib treatment leads to prolonged cell cycle arrest resulting in tumor cell apoptosis & senescence, which then leads to an enhanced immune activation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-11

Ki-67 (30-9) scoring and differentiation in Luminal A and Luminal B breast cancer subtypes

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## Introduction

Ki-67 labeling index is a powerful prognostic marker in breast cancer (BC). It is especially useful in assessing the risk of recurrence for estrogen receptor-positive (ER+) BC, where it may be considered a surrogate of the molecular assays for distinguishing Luminal A-like from Luminal B-like BCs. We evaluated the performance of the VENTANA anti Ki-67 (30-9) rabbit monoclonal antibody in assessing the risk of distant relapses for a large series of patients with ER+ BC treated and followed up in a single Institution.

## Patients and Methods

The initial cohort (9415 patients) comprised all women operated on for early ER+, HER2-negative (HER2-) BC at the European Institute of Oncology (IEO), who did not receive neo-adjuvant treatment<sup>1</sup>. We subsequently restricted the cohort to 3986 patients operated on between 1998-2002 and for whom long-term follow-up data was available. A case-cohort was built by randomly selecting 17% of the above cohort (679 patients, including 84 with events). Additional 303 patients who developed an event (metastasis in distant organs or death due to BC as primary events) were added to this cohort.

Ki-67 was evaluated using the anti-Ki-67 (30-9) antibody (Ventana Medical Systems, Inc., Tucson, AZ) using OptiView IHC DAB detection on the BenchMark ULTRA advanced staining platform. The stained slides were evaluated using the scoring method described by the International Ki-67 in BC Working Group.

We considered "Luminal A-like" tumors that were ER+, HER2-, with Ki-67 <14% or with Ki-67 14-19% and PgR ≥20%, and "Luminal B-like" ER+, HER2- tumors with Ki-67 14-19% and PgR <20% or with Ki-67 ≥20%<sup>1</sup>.

The main outcome was distant disease-free survival (DDFS) and was calculated from the date of surgery to the date of any first event or last contact with the patient.

Cumulative incidence curves were drawn for patients in the sub-cohort and differences between BC subtypes were assessed using the log-rank test. Multivariable Cox regression with inverse sub-cohort sampling probability weighting was used to evaluate the risk of metastasis or death from BC across groups.

## Results

In the sub-cohort, 400 (58.9%) patients had "luminal A-like" and 279 (41.1%) "luminal B-like" BC. The 10-year cumulative incidence of distant metastasis (or BC related death as first event) in the two groups were respectively 8.2% and 24.5% (log rank  $P < 0.0001$ ).

In the whole case-cohort, multivariable analysis confirmed statistically significant increased risk of events for women with "Luminal B-Like" BC compared to women with "Luminal A-Like" BC (HR=1.97; 95% CI 1.38-2.79), after adjustment for pT, pN, PVI and menopausal status.

## Conclusion

Ki-67 evaluated using the VENTANA anti-Ki67 (30-9) antibody, was able to stratify patients with endocrine responsive BC, maximizing the number of those classified as having 'Luminal A-like' intrinsic subtype for whom the use of cytotoxic drugs could be at large avoided.

**Funding source:** Ventana Medical Systems, Inc.

## References

Maisonneuve P, Disalvatore D, Rotmensz N, et al. (2014) Proposed new clinicopathological surrogate definitions of luminal A and luminal B (HER2-negative) intrinsic breast cancer subtypes. *Breast Cancer Res* **16**:R65

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD2-12

Palbociclib in combination with fulvestrant or tamoxifen as treatment for hormone receptor positive (HR+) metastatic breast cancer (MBC) with prior chemotherapy for advanced disease (TBCRC 035) A phase II study with pharmacodynamics markers

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## Background

Addition of the cyclin dependent kinase 4/6 inhibitor (CDK4/6i) palbociclib to endocrine therapy in the first and later line settings significantly improves progression free survival (PFS) in patients with HR+ MBC. The primary toxicity is neutropenia without an increase in febrile neutropenia. TBCRC035 explored rates of neutropenia in patients who had received prior chemotherapy for MBC with 2 dose levels of palbociclib, and correlated changes in retinoblastoma protein phosphorylation (pRB) and Ki67 expression in proliferating keratinocytes and tumor with response.

## Methods

TBCRC035 is a 1:1 randomized multicenter phase II study evaluating palbociclib at either 125 or 100 mg in combination with physician choice fulvestrant or tamoxifen. Eligible patients (pts) with HR+ MBC had received  $\geq 1$  but  $\leq 3$  lines of chemotherapy for MBC, any number of prior hormone therapies, and were naïve to CDK4/6i. The primary endpoint was grade 3/4 neutropenia; secondary endpoints included response, safety/tolerability, inhibition of pRB and change in Ki67 in skin and tumor at day 14-21 of treatment compared to baseline. FFPE sections of skin punch and tumor biopsies obtained before and on treatment were stained using antibodies to Ki67, total RB, and phospho-RB-S780 using BOND polymer red detection. Stained slides were scanned into the Aperio image analysis platform; the percentage of marker positive cells and H-score was determined.

## Results

70 pts were enrolled (fully accrued); 35 randomized to 100 vs 125 mg of palbociclib respectively; data for the last 3 pts on the 125 mg arm is pending. Grade 3/4 neutropenia was more common in the 125 mg vs the 100 mg arm (56 vs 34%); dose adjustments for adverse events (AEs) occurred in 47 vs 43%, 4 vs 0 pts discontinued treatment due to AEs. Grade 3 febrile neutropenia was rare (1 patient each arm). Median duration of treatment was 5.2 vs 7.2 months. Response data and correlation with changes in pRB and Ki67 expression in skin and tumor by treatment arm will be reported.

## Conclusion

In pts with prior chemotherapy for HR+ MBC, treatment with 100 mg of palbociclib in patients is associated with a lower rate of  $\geq$  grade 3 neutropenia compared to 125 mg. Correlation of response by dose with pRB and Ki67 has the potential to inform palbociclib dosing and reduce toxicity for pts with HR+ MBC.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD3-01

Results from KATE2, a randomized phase 2 study of atezolizumab (atezo)+trastuzumab emtansine (T-DM1) vs placebo (pbo)+T-DM1 in previously treated HER2+ advanced breast cancer (BC)

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## Background

T-DM1 has improved PFS and OS in HER2+ advanced BC patients (pts) who previously progressed on trastuzumab and a taxane (Verma *NEJM* 2012). Preclinical data show that atezo (anti-PD-L1) inhibits PD-L1 binding to PD-1 and B7.1 to restore antitumor immunity. PD-L1 is often expressed on tumor-infiltrating immune cells (IC) in BC. KATE2 (NCT02924883) is the first study to assess atezo combined with T-DM1 in previously treated HER2+ advanced BC.

## Method

Eligible pts had HER2+ advanced BC previously treated with trastuzumab and a taxane and progressed on treatment for metastatic disease or within 6 mo of adjuvant therapy. Pts were randomized 2:1 to atezo 1200 mg or pbo+T-DM1 3.6 mg/kg IV q3w (crossover not permitted). The primary endpoint was investigator-assessed PFS per RECIST v1.1. Additional endpoints included OS, ORR, DoR (secondary), PFS in the PD-L1+ subgroup (exploratory) and safety. Data cutoff: 11 Dec 2017.

## Result

133 pts were randomized to atezo+T-DM1 and 69 pts to pbo+T-DM1. 49% and 46% received prior pertuzumab for metastatic BC; median duration of pertuzumab treatment was 10 and 13 mo, respectively. Median follow-up was 8.5 and 8.4 mo, respectively. Efficacy in the ITT population and biomarker subgroups are shown.

## ITT and Biomarker Subgroups: Efficacy

	Atezo+T-DM1 (n=133)		Pbo+T-DM1 (n=69)	
mPFS, mo	8.2		6.8	
6-mo PFS, %	58		51	
12-mo PFS, %	38		34	
ORR, % <sup>a</sup>	45		43	
CR	6		7	
PR	39		36	
PD-L1 IHC <sup>b</sup>				
	PD-L1+	PD-L1-	PD-L1+	PD-L1-
Patients, n	57	76	27	42
mPFS, mo	8.5	6.8	4.1	8.2
ORR, % <sup>a</sup>	54	40	33	50
Stromal TILs <sup>c</sup>				
	TIL-high (≥5%)	TIL-low (<5%)	TIL-high (≥5%)	TIL-low (<5%)
Patients, n	80	45	40	25
mPFS, mo	8.5	5.5	5.3	NA

ORR, % <sup>a</sup>	48	38	38	48
HER2 <sup>c</sup>				
	IHC3+	IHC0-2+	IHC3+	IHC0-2+
Patients, n	103	30	56	13
mPFS, mo	8.5	5.9	8.0	4.0
ORR, % <sup>a</sup>	52	23	46	31

<sup>a</sup>ORR-evaluable pts (measurable disease at baseline): n=132 in atezo+T-DM1 arm. <sup>b</sup>PD-L1+, IC1/2/3; PD-L1–, IC0; measured per the VENTANA SP142 IHC assay. <sup>c</sup>Post hoc exploratory analyses.

PFS HR was 0.82 (95% CI: 0.55, 1.23);  $p=0.3332$ . At data cutoff, 13 OS events (10%) in the atezo+T-DM1 arm and 8 (12%) in the pbo+T-DM1 arm had occurred. mDoR was not reached. 44% and 41% of safety-evaluable pts had an AE of Gr  $\geq 3$  with atezo+T-DM1 and pbo+T-DM1, respectively. The most common was thrombocytopenia (13% and 4%). The incidence of serious AEs (SAEs) was 33% with atezo+T-DM1 and 19% with pbo+T-DM1, with the most common being Gr 1-2 pyrexia with atezo+T-DM1 (5%) and abdominal pain (3%) and seizure (3%) with pbo+T-DM1. AE rates leading to atezo/pbo or T-DM1 discontinuation were 25% and 15%, respectively, with atezo+T-DM1 and 15% and 13% with pbo+T-DM1. 1 patient in the atezo+T-DM1 arm had a drug-related Gr 5 AE (hemophagocytic syndrome).

#### Conclusion

Atezo+T-DM1 did not demonstrate a clinically significant PFS benefit vs pbo+T-DM1; OS and DoR data are not yet mature. Numerically higher PFS and ORR were seen with atezo+T-DM1 in PD-L1+ pts. T-DM1 safety in both arms was consistent with its known profile. Although the combination of atezo+T-DM1 showed a numerically higher incidence of SAEs and discontinuation of atezo due to an AE, rates of Gr 3-5 AEs were similar between arms. Additional biomarker data, including gene expression and mutation data, will be presented.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD3-02

Incidence of late relapse in HER2-positive (HER2+) breast cancer patients receiving adjuvant trastuzumab: Combined analysis of NCCTG (Alliance) N9831 and NSABP (NRG) B31

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**Background:** Recent trials showed potential benefit of extended adjuvant endocrine therapy and relatively high risk of relapse after 5 years (yr) in hormone receptor-positive (HR+) HER2- breast cancer. While risk of late relapse in HR+ HER2- is fairly well defined, the risk of late relapse in HER2+ remains largely unknown. **Method:** 4547 HER2+ patients treated with adjuvant chemotherapy alone or combined with trastuzumab (TH) were included (3132 from North Central Cancer Treatment Group [NCCTG, now Alliance] N9831; 1415 from National Surgical Adjuvant Breast and Bowel Project [NSABP, now NRG] B-31). Intrinsic subtypes were assessed by Prosigna test. Kaplan-Meier method and Cox proportional hazards model were used for analysis. **Results:** Median follow-up was 10.4 yr in N9831 and 7.0 yr in NSABP-B31. 54.5% of pts had HR+ disease. Pts were classified as HER2 enriched (77.4%), Luminal B (10.1%), Luminal A (7.7%), and Basal (4.8%). In multivariate Cox regression analysis in both treatment groups, HR+ was significantly associated with improved recurrence-free survival (RFS) during the first 5 yr (HR 0.65, 95% CI 0.56-0.77, p<0.001) but there was no significant difference during yr 5-10 (HR 1.32, 95% CI 0.93-1.88, p=0.12). In HR+HER2+ pts treated with TH, cumulative hazard for relapse or death was lower in the first 5 yr (10.96%, 95%CI 8.78-13.08) compared to HR-HER2+ (17.48%, 95%CI 14.59-20.27), with adjusted HR 0.60 (95%CI: 0.45-0.79, p<0.001). Unlike HR+HER2- disease, cumulative hazard of relapse or death in yr 5-10 in HR+HER2+ pts treated with TH was lower at 8.6% (95%CI 6.16-10.97). This is slightly higher than pts with HR-HER2+ at 5.75% (95%CI 3.18-8.24, adjusted HR=1.7, 95%CI 1.01-2.85, p=0.046). Compared to 22% and 31% risk of relapse after 5 yr in N0 and N1 HR+HER2- in recent publication, 3.23% (95%CI 0-9.25) of pts with HR+HER2+ with N0 developed recurrence in yr 5-10 and 6.39% (95%CI 3.09-9.59) in those with N1 disease. Albeit rare, pts with Luminal A tumors had 89.8% relapse-free survival at 10 yr compared to Luminal B 82.3%, HER2 enriched 76.8%, and Basal 74.19%. **Conclusions:** Even without extended adjuvant endocrine therapy in both trials, risk of late relapse in HR+ HER2+ appeared to be low, particularly in pts with N0 or N1 disease. The benefit of extended adjuvant endocrine therapy in this group of TH pts, particularly those with no lymph node involvement, may not outweigh side effects of continuing endocrine therapy beyond 5 yr.

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**ClinicalTrials.gov Id:** NCT00005970, NCT00004067

Cumulative probability (%) of relapse or death.

Nodal status	Year 0-5		Year 5-10*	
	HR+	HR-	HR+	HR-
	Cumulative hazard (95%CI)	Cumulative hazard (95%CI)	Cumulative hazard (95%CI)	Cumulative hazard (95%CI)
0	7.71 ( 0.00, 15.74)	16.05 ( 7.98, 23.42)	3.23 ( 0.00, 9.25)	8.86 ( 1.05, 16.04)
1	6.70 ( 3.66, 9.64)	10.63 ( 5.99, 15.04)	6.39 ( 3.09, 9.59)	5.57 ( 1.46, 9.51)
2	13.68 ( 7.42, 19.52)	20.99 (12.98, 28.26)	5.10 ( 0.64, 9.35)	1.22 ( 0.00, 3.57)
3	10.25 ( 2.76, 17.17)	39.76 (24.11, 52.18)	13.19 ( 3.54, 21.88)	8.52 ( 0.00, 19.16)

\*Based on pts who survived 5 yr without relapse

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD3-03

SOLTI-1303 PATRICIA phase II trial (STAGE 1) -- Palbociclib and trastuzumab in postmenopausal patients with HER2-positive metastatic breast cancer

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**Background:** CDK4/6 inhibition combined with anti-HER2 therapy is currently being explored in HER2-positive (HER2+) BC. Here, we report the efficacy, safety and genomic analysis of STAGE 1 of the PATRICIA phase II trial evaluating palbociclib in combination with trastuzumab (TTZ) in advanced HER2+ BC.

**Methods:** PATRICIA is an exploratory, prospective, open-label, multicenter phase II trial. Patients (pts) had received 2-4 prior lines of anti-HER2-based regimens. Treatment consisted of TTZ 6 mg/kg every 3w and palbociclib 200 mg daily for 2w and 1w off. The study was based on a Simon 2-stage design comprising 3 cohorts: estrogen receptor (ER)-negative (cohort A), ER+ (cohort B1) and ER+ with letrozole (cohort B2). Pts ER+ were randomized to cohorts B1 or B2. The trial included a safety run-in phase of the first 12 pts. For part 1 to be successful, at least 6 pts of 15 had to be progression-free at 6 months (PFS6R of 40%) in each cohort. Secondary objectives included safety and the association of the research-based PAM50 intrinsic subtyping with PFS. PAM50 was performed from FFPE samples using the nCounter platform. Multivariable Cox regression analyses evaluating PAM50 subtypes, age, performance status, treatment line, type of biopsy and endocrine treatment were performed.

**Results:** A total of 45 pts were recruited (n=15 in each cohort). Median age was 59.5y, and median number of prior lines was 3.0. The PFS6R in cohorts A, B1 and B2 were 33.3% (5/15), 40.0% (6/15) and 53.3% (8/15), respectively. Regarding safety, grade 1-2 and 3-4 toxicities occurred in 97.7% and 84.4% of pts. The most common grade 3-4 toxicities were neutropenia (80%) and thrombocytopenia (17%). Dose reductions were required in 60% of pts. Regarding PAM50, a total of 40 (83.9%) tumors samples (22 primary and 18 metastasis) were profiled. Subtype distribution was as follows: 92.9% HER2-enriched (HER2-E) and 7.1% Basal-like in cohort A, and 46.2% HER2-E, 23.1% Luminal B, 19.2% Luminal A and 11.5% Normal-like in cohorts B1+2. No significant differences in PFS were observed across the 3 cohorts. In cohorts A+B1+B2, Luminal disease defined by PAM50 showed a higher median PFS compared to non-luminal disease (12.4 vs. 4.1 months; adjusted hazard ratio=0.37; p-value=0.052). Clinical Benefit Rate (CBR6) was 73% in Luminal Vs. 31% in non-luminals (p=0.031). In cohorts B1+B2, Luminal disease defined by PAM50 showed a higher median PFS compared to non-luminal disease (12.4 vs. 4.1 months; adjusted hazard ratio=0.30; p-value=0.025). CBR6 was 73% in Luminal Vs. 25% in non-luminals (p=0.022). No clinical-pathological variable was found associated with PFS or CBR6 in all pts or in cohorts B1+B2.

**Conclusion:** Palbociclib in combination with TTZ is safe and active in TTZ pre-treated HER2+ advanced BC, specially within ER+ disease. Identification of the non-luminal subtypes by PAM50 might help identify pts who might not derive a large benefit from this treatment strategy regardless ER status. Our results might have important implications for current and future clinical trials evaluating CDK4/6 inhibitors in HER2+ disease. In Part 2, a total of 92 pts in cohorts B1 and B2 will be included to better assess the efficacy of this treatment strategy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD3-04

Analysis of ERBB2 (HER2) amplification by ctDNA in a phase Ib dose-escalation trial evaluating trastuzumab emtansine (T-DM1) with neratinib in women with metastatic disease with initially diagnosed HER2+ breast cancer: NSABP FB-10

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## Background:

In this phase Ib study, the activity of T-DM1 plus N was assessed in patients (pt) previously treated with trastuzumab, pertuzumab, and a taxane (H+P+T). Several mechanisms of resistance have been hypothesized in pts progressing following H+P+T, including acquired alterations in the ERBB (HER) family proteins, reactivation of bypass or parallel pathways, or selective elimination of HER2-overexpressing clones. Loss of HER2 amp has been shown to occur in 25-35% of pts with residual tumor after neoadjuvant therapy or in metastatic disease after initial therapy with chemotherapy and HER2-targeted agents. Data on concordance of HER2 status between tissue and blood is limited. In 7 pts with cfDNA HER2 amp, concomitant tissue was concordant in all 7 pairs and response to anti-HER2 therapy occurred in 6. In our study we have retrospectively analyzed cfDNA in blood samples obtained at study entry.

## Methods:

Eligible pts had prior H+P+T as neoadjuvant therapy, or 1st-line metastatic disease, measurable disease, ECOG PS  $\leq 2$ , and adequate hematologic, renal, and liver function. Pts with stable brain metastases were eligible. Treatment consisted of T-DM1 3.6 mg/kg iv q3wk and N 120, 160, 200, or 240 mg/d using a 3+3 dose-escalation design. HER2+ was determined at initial diagnosis; tissue confirmation at study entry (after H+P+T progression) was not required. Blood was collected in for pharmacokinetic analyses of N peak and trough, and for cfDNA using the Guardant360 assay, which is a 73-gene next-generation cfDNA-sequencing panel that detects SNVs, indels, CNAs, and fusions, utilizing Digital Sequencing and custom bioinformatics methods for error correction. The cut-off for HER2 amp was a copy number of  $\geq 2.0$  established by Guardant based on training-set data.

## Results:

There were 27 H+P+T-resistant pts enrolled and all pts had a blood sample analyzed for HER2 amp. Eighteen pts were evaluable for efficacy at 6 wks and 11 pts at 12 wks. Dose-limiting toxicity occurred in 6 pts during cycle 1, 1 pt was withdrawn for non-compliance, and 2 pts were withdrawn for disease complications. The recommended phase II dose of N was determined to be 160 mg/d. Responses were seen at all dose-levels of N. Pharmacokinetic analyses did not show a clear relationship with either peak or trough and dose-level. Ten pts showed HER2 amp in blood and 17 were non-amp. Of 18 pts evaluable after 2 cycles (6 wks), 12 pts had an objective response (7 amp; 5 non-amp) and 5 had progressive disease (1 amp; 4 non-amp). At 12 wks, there were 3 CRs and 8 PRs (7 amp; 4 non-amp). All CRs were in amp pts and lasted 364, 510, and 859+ days.

## Conclusions:

HER2 amp as determined by cfDNA was found in 10 of 27 pts. The deeper and more prolonged ( $>12$  wk) responses occurred in 7 of 10 amp HER2 pts v 4 of 17 non-amp HER2 pts ( $p=0.04$ ). In our ongoing phase II study of this regimen concomitant tissue and blood will be analyzed to better understand potential benefit or lack of benefit, with continued use of anti-HER2 therapy after progression on anti-HER2 therapies.

**Support:** Puma Biotechnology, Inc.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD3-05

Co-occurring gain-of-function mutations in HER2 and HER3 cooperate to enhance HER2/HER3 binding, HER-dependent signaling, and breast cancer growth

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*ERBB2*, the gene encoding HER2, is mutated in 2-4% of breast cancers. The HER2 tyrosine kinase inhibitor neratinib has shown clinical activity against breast cancers harboring HER2 activating mutations, suggesting these tumors depend on HER2 signaling. Co-occurring HER2 and HER3 (*ERBB3*) mutations have been reported in patients who respond to neratinib (Hanker et al., *Cancer Discov.* 2017) suggesting the possibility of cooperativity of both oncogenes. Co-expression of the mutant intracellular domains of HER2 and HER3 in HEK293 cells enhanced phosphorylation of HER3 and ERK compared to expression of either mutant alone, which was blocked by 100 nM neratinib. Interrogation of TCGA, METABRIC, Project GENIE, and Foundation Medicine datasets revealed that gain-of-function mutations in *ERBB2* and *ERBB3* co-occur with a statistically significant frequency. For example, in GENIE, *ERBB2* mutations co-occur with mutations in *ERBB3* (8.3% of *ERBB2*-mutant vs 2.3% of *ERBB2* WT;  $q=1.37 \times 10^{-10}$ ).

We hypothesized that co-occurring mutations in HER2 and HER3 cooperate to enhance HER2 signaling and dependence and breast cancer progression.

Thirty-four unique breast cancers were found to harbor co-occurring mutations in HER2 and HER3, the most common of which were *ERBB2*<sup>L755S</sup>/*ERBB3*<sup>E928G</sup> (n=10), *ERBB2*<sup>V777L</sup>/*ERBB3*<sup>E928G</sup> (n=6), and *ERBB2*<sup>L869R/Q</sup>/*ERBB3*<sup>E928G</sup> (n=4). Using co-immunoprecipitation assays with HER2 and HER3 antibodies in transfected HEK293 cells, we found that co-expression of HER3<sup>E928G</sup> with wild type (WT) HER2, or co-expression of HER2<sup>L755S</sup> or HER2<sup>L869R</sup> with HER3<sup>WT</sup>, slightly increased HER2-HER3 dimerization. However, binding was strongest between double mutants. This was accompanied by the highest levels of Y1289 p-HER3 in cells expressing both HER3<sup>E928G</sup> and each HER2<sup>L755S</sup>, HER2<sup>V777L</sup>, or HER2<sup>L869R</sup> compared to cells expressing each HER2 or HER3 mutant with a respective WT heterodimer partner. Structural modeling of the HER2<sup>L869R</sup>/HER3<sup>E928G</sup> double-mutant predicted that the HER3 mutation, located at the dimer interface, may enhance heterodimerization of the kinase domains through decreased bulk and electrostatic repulsion. We also noted that the HER2<sup>L755S</sup> mutation is predicted to be in close proximity to HER3<sup>E928G</sup> (<4 Å) and may impact binding affinity. Investigation of the structural basis for the enhanced binding of other double mutants is in progress.

MCF7 “knock-in” cells incorporating HER2<sup>L755S</sup>, HER2<sup>V777L</sup>, or HER2<sup>L869R</sup> (or HER2<sup>WT</sup>) were stably transduced with HER3<sup>E928G</sup> or HER3<sup>WT</sup>. Co-expression of double mutants strongly enhanced estrogen-independent growth in 3D Matrigel over cells expressing either mutant alone. We are currently testing inhibitors of HER2/HER3 signaling, including neratinib ± trastuzumab, trastuzumab + pertuzumab, and the ERBB1-3 antibody mixture Sym013, to determine therapeutic strategies to block the cooperative growth induced by co-occurring HER2 and HER2 mutations.

**Conclusions:** Co-expression of mutant HER2 and mutant HER3 promotes HER2/HER binding, HER3 phosphorylation, and breast tumor cell proliferation. We aim to identify therapeutic vulnerabilities for patients with co-occurring HER2 and HER3 mutations.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD3-06

Neratinib + fulvestrant for HER2-mutant, HR-positive, metastatic breast cancer: Updated results from the phase 2 SUMMIT trial

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**Background:** HER2 mutations define a rare subset of metastatic breast cancer (MBC) with a unique mechanism of oncogenic addiction to HER2 signaling. Neratinib, an irreversible pan-HER tyrosine kinase inhibitor, has demonstrated single-agent clinical activity in HER2-mutant MBC. In HER2-mutant, HR+ MBC, neratinib + fulvestrant (N+F) appears synergistic vs single-agent neratinib, possibly due to more complete inhibition of bi-directional signaling between HER2 and estrogen receptors. Here we describe interim efficacy results of the expanded HER2-mutant, HR+ MBC cohort treated with N+F from SUMMIT (NCT01953926).

**Methods:** HR+ MBC patients (pts) with HER2 mutations documented by local testing received oral neratinib 240mg qd and intramuscular fulvestrant (labeled dose). Intensive loperamide prophylaxis was mandatory during cycle 1. Efficacy endpoints include objective response rate at week 8 (ORR<sub>8</sub>); confirmed objective response rate (ORR); clinical benefit rate (CBR); duration of response (DOR); progression-free survival (PFS); response was assessed by RECIST 1.1 and/or PET Response Criteria. Genomic profiling from fresh/archival tumor tissues and/or plasma cfDNA was performed retrospectively by next-generation sequencing (MSK-IMPACT).

**Results:** As of 18 May 2018, 46 HER2-mutant HR+ MBC pts have been treated with N+F. Most pts were pretreated, with 91% having received prior anti-cancer medication for MBC (range 0–10). ORR was 33% and median DOR in the 15 pts with a confirmed response was 9.2 months (95% CI 3.9–18.5). Twenty-four pts had prior fulvestrant exposure, and 19 had received prior CDK4/6i-based therapy. Clinical activity was observed with ORRs of 17% and 26% in prior fulvestrant-treated and prior CDK4/6i-treated pts, respectively. ORRs by HER2 mutation were: V777L 63% (5/8 pts); S310F/Y 67% (4/6 pts); G778\_P780dup 50% (3/6 pts). Diarrhea was the most common adverse event (grade 3, 24%; grade 4, 0%). Median cumulative duration of grade 3 diarrhea was 3 days. There were no treatment discontinuations due to diarrhea.

	Neratinib + fulvestrant		
Outcome <sup>a</sup>	All patients (N=46)	Prior fulvestrant (N=24)	Prior CDK4/6i-based therapy (N=19)
ORR <sub>8</sub> – n (%)	19 (41.3)	8 (33.3)	7 (36.8)
95% CI	27.0–56.8	15.6–55.3	16.3–61.6
ORR – n (%)	15 (32.6)	4 (16.7)	5 (26.3)
95% CI	19.5–48.0	4.7–37.4	9.1–51.2
DOR for each responder, months		5.6 <sup>b</sup> ; 9.2; 9.6 <sup>b</sup> ; 18.5	5.6 <sup>b</sup> ; 5.7 <sup>b</sup> ; 9.3; 9.6 <sup>b</sup> ; 12.9 <sup>b</sup>

CBR – n (%)	27 (58.7)	11 (45.8)	9 (47.4)
95% CI	43.2–73.0	25.6–67.2	24.4–71.1
Median (95% CI) time to event, <sup>c</sup> months			
PFS	3.9 (3.6–5.7)	3.7 (3.5–12.8)	3.9 (1.9–NA)
DOR	9.2 (3.9–18.5)	NA	NA

<sup>a</sup>For pts with both RECIST- and PET-evaluable lesions, the best of either RECIST or PET response was used to determine response; the earliest progression by RECIST or PET was used for progression; <sup>b</sup>Pt has not progressed; <sup>c</sup>Kaplan-Meier analysis; NA, not applicable

**Conclusions:** N+F demonstrates encouraging clinical activity with durable responses in heavily pretreated pts with HER2-mutant, HR+ MBC. Of note, responses were observed in pts who had received prior fulvestrant or CDK4/6 inhibitors. No new safety signals were identified; the rate of diarrhea was similar to single-agent neratinib and not dose limiting. Updated data after additional follow-up and genomic data will be presented.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD3-07

A phase II pre-surgical trial of lapatinib for the treatment of women with HER2 positive or EGFR positive ductal carcinoma in situ

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**Background:** Estrogen receptor (ER)-negative tumors and human epidermal growth factor 2-Neu (HER2) positive breast cancers are known to be more clinically aggressive subtypes of breast cancer and account for 30% of all breast cancers. Women with HER2 + breast cancers, whether ER+ or ER -, require cytotoxic chemotherapy with a HER2-targeting agent, and often have adverse outcomes. Thus, preventive agents are needed to reduce the incidence of these subtypes of aggressive breast cancer. Lapatinib, a dual tyrosine kinase inhibitor, inhibits epidermal growth factor receptors (EGFR) and HER2 kinases and has shown to decrease breast cell proliferation in invasive breast cancer and adjacent premalignant lesions. Therefore, we conducted a multi-institutional randomized Phase II clinical trial to study the effects of the signal transduction inhibitor lapatinib in women with HER2-positive or EGFR-positive ductal carcinoma in situ (DCIS).

**Methods:** Randomized participants received either lapatinib (750mg, 1000mg, or 1500mg) or placebo daily for 2-6 weeks prior to their surgery. After minimal accrual, the trial was later amended to lapatinib 1000mg or placebo. Pre-treatment breast tissue was obtained from initial diagnostic core biopsy and post-treatment breast tissue was obtained from surgical excision specimen. Blood was obtained prior to surgery to assess serum lapatinib level. Participants kept a daily symptom assessment log and had a cardiac assessment at baseline and prior to surgery. Patients were instructed to take drug up to and including the day before surgery. The dual primary endpoint for this study was change in proliferation in pre- versus post-treatment biopsies between the two treatment arms, as measured by Ki67 as well as toxicity assessment. Secondary endpoints included incidence of DCIS at surgery and modulation of tissue biomarker expression in growth factor receptors (EGFR, ErbB2); phosphorylated growth factor receptor (phospho-ErbB2); signal transduction markers (MAPK, phospho-MAPK); hormone receptors (ER, PR); and p27.

**Results:** Twenty-two women (mean age: 51; range: 32-66) with HER2+ or EGFR+ DCIS were treated with lapatinib (1,000 or 1,500 mg) or placebo for 2–6 weeks prior to surgical excision. Ki67 expression was significantly decreased in the lapatinib treatment arms compared to placebo ( $p=0.0122$ ). Diarrhea, fatigue, and skin reactions were notable adverse events that occurred predominately in the lapatinib arm compared to placebo. No grade 3 or 4 events related to the study drug were noted during the study. No changes were noted in cardiac function. DCIS was present in all surgical specimens in both arms. Invasive breast cancer was noted in 1 patient on lapatinib 1000mg and 3 patients on placebo. No statistically significant changes were noted in signal transduction biomarkers

**Conclusion:** These results demonstrate the effectiveness of lapatinib in reducing proliferation in women with EGFR+ or HER2+ DCIS. Even low-grade toxicities can deter use of an agent in the prevention setting. This and the lack of a risk model for HER2+ and triple negative breast cancer make the development of larger scale clinical prevention trials of lapatinib for the prevention a challenge.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD3-08

Assessment of the tumor immune environment in inflammatory breast cancer treated with neoadjuvant dual-HER2 blockade

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**Background:** Inflammatory breast cancer (IBC) is an aggressive form of breast cancer that remains relatively understudied. We examined the efficacy of neoadjuvant dual-HER2 blockade (trastuzumab (H) and pertuzumab (P)) combined with paclitaxel (T) in HER2+ IBC, including a planned analysis to elucidate associations between the tumor immune microenvironment profile and response to therapy.

**Methods:** An IRB-approved, single-arm phase II trial for patients (pts) with newly diagnosed HER2+ IBC was conducted. Pts had a pre-treatment biopsy of the affected breast (D1) followed by a loading dose of HP. A second biopsy was performed 1 week (wk) later (D8), when T (80mg/m<sup>2</sup>/wk x 16 wks) was added to HP. Responding pts underwent modified radical mastectomy (MRM) where residual disease was collected. The primary objective was to determine the rate of pathologic complete response (pCR) defined as ypT0/isN0. Residual Cancer Burden (RCB) was also determined. Tumor specimens from D1, D8 and MRM were assessed for disease cellularity and scored for percentage of tumor infiltrating lymphocytes (TILs): low=0-10%, intermediate=11-59%, high≥60%. RNA-sequencing was performed on tumor tissue from D1 and D8 to explore the impact of short-term HP treatment on the tumor transcriptomic profile and to identify potential predictors of pCR.

**Results:** 23 pts with HER2+ IBC were enrolled between 8/2013-6/2017. Mean age was 48 years (range 32-74); 11 pts (48%) had estrogen and progesterone receptor (ER/PR) negative disease. Matched tumor biopsies (D1, D8) were obtained in all 23 pts; 21 underwent MRM; 1 was lost to follow-up and 1 had disease progression. In the intent to treat analysis, 10/23 (43%) pts achieved a pCR and 7 (30%) had RCB-1. Ten of the 22 evaluable pts achieved a pCR (45.5%). TILs were evaluable in 20/23 (87%) matched tumor biopsies (D1, D8). Among the D1 biopsy specimens: 19 (95%) had low levels, 2 (10%) had intermediate levels, and none had high levels. When D1 TIL levels were compared with D8 levels, 3(15%) had an increase in TILs, 16(80%) had no change in TIL levels, and 1(5%) had a decrease in the level of TILs. Both samples with intermediate levels and 2 of 3 samples with high levels of TILs on D1 and D8 were seen in ER/PR negative disease. An evaluation of biopsy specimens associated with subsequent pCR using GO enrichment analysis from the RNA-Seq data showed significant upregulation of several immune-process related gene expression signatures both at D1 and D8 (e.g. antigen processing and presentation, TCR signaling, NK cell cytotoxicity, p-value: 2.99E-48 to 1.39E-16) when compared with those associated with residual disease at the time of MRM. Across the entire cohort, D8 biopsies showed evidence of upregulated anti-tumor immunity compared to D1 biopsies (p-value: 9.57E-06 to 0.012). Notably, this change from D1 to D8 was largely restricted to tumors that achieved a pCR.

**Conclusion:** THP for 16 weeks was a highly effective treatment for HER2+ IBC. Immune activation as determined by gene expression signatures predicted pCR, and moreover upregulation of anti-tumor immunity after 1 wk of HP might further predict a complete pathologic response to therapy. ClinicalTrials.gov identifier: NCT01796197



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD3-09

Efficacy of HER2 inhibitors in metastatic breast cancer by discordance in HER2 amplification status between primary and metastatic breast cancer

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## **Purpose:**

In stage IV breast cancer (BC), discordance in the human epidermal growth factor receptor 2 (HER2) amplification status between primary and metastatic BC might affect efficacy of HER2-targeted agents. We studied progression free (PFS) and overall survival (OS) dependent on HER2 concordance in patients treated with a first line taxane-trastuzumab combination and later line trastuzumab-emtansine (T-DM1).

## **Patients and Methods:**

This retrospective monocentric study included 76 patients with metastatic BC under treatment with trastuzumab in which a biopsy from both the primary and metastatic site was available. HER2 amplification status, sex-steroid receptor status, Nottingham prognostic index, distant metastasis-free interval and consecutive lines of therapy were retrieved from patients' reports. The Kaplan-Meier method was used for estimating PFS/OS and log-rank test for analyzing between group differences. A Cox model is used for testing difference between groups while correcting for Pertuzumab. Multivariable Cox regression is used to model OS as a function group, correcting for possible confounders.

## **Results:**

Discordance in HER2 amplification status was seen in 30 out of 76 patients (39%), 11 patients lost HER2 amplification in the metastatic lesion (HER2<sup>lost</sup>) while 19 acquired HER2 amplification (HER2<sup>acquired</sup>). The other 46 patients had a HER2 amplification on both primary and metastatic site (HER2<sup>stable</sup>). The HER2<sup>lost</sup> group had a significant lower median PFS (PFS= 5.5 months) for taxane-trastuzumab, after correcting for pertuzumab, compared to the HER2<sup>stable</sup> group (PFS= 9 months, corrected p= 0.0146) and HER2<sup>acquired</sup> group (PFS=14 months, corrected p=0.0121). For T-DM1 treatment, both discordant groups, HER2<sup>acquired</sup> (PFS=1.1 months, p=0.0373) and HER2<sup>lost</sup> (PFS=1.5 months, p=0.0116), had a significant lower PFS compared to the HER2<sup>stable</sup> group (PFS=6.0 months). After correcting for possible confounders, HER2<sup>lost</sup> had a significant worse OS compared to HER2<sup>stable</sup> (HR 0.187, 95% CI 0.079 – 0.439) and HER2<sup>acquired</sup> (HR 0.147, 95% 0.058-0.378).

## **Conclusion:**

Loss of HER2 amplification in metastatic lesions seems to have a negative predictive value for PFS on HER2-targeted agents and negative prognostic impact on OS. Acquiring of HER2 amplification was predictive for lower PFS on T-DM1 but wasn't predictive for lower PFS on taxane-trastuzumab.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: PD3-10

Higher serum PD-L1 predicts for increased overall survival to lapatinib vs trastuzumab in the phase 3 CCTG MA.31 trial

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**Background:** In the CCTG (Canadian Clinical Trials Group) MA.31 randomized phase 3 trial, the trastuzumab-taxane combination led to longer PFS than lapatinib-taxane in HER2-positive metastatic breast cancer (MBC). We previously reported the prognostic utility of pretreatment serum PD-L1 in the trastuzumab arm of MA.31 (ASCO 2018, #1031), and here we evaluate serum PD-L1 in the lapatinib arm, and in the whole trial. Higher serum PD-L1 has been reported to be associated with reduced response to treatment with the immune checkpoint inhibitors in melanoma and lung cancer.

**Methods:** MA.31 accrued 652 centrally and/or locally-identified HER2-positive patients; 186 in the trastuzumab arm, and 202 in the lapatinib arm had pretreatment serum available. The ELLA immunoassay platform (ProteinSimple, San Jose, CA) was used to quantitate serum PD-L1. Step-wise forward Cox multivariate analysis was used for PFS and OS, and testing for treatment-biomarker interaction was based on the local partial-likelihood method (Liu Y, Jiang W, and Chen BE, *Statistics in Medicine* 34, 3516-3530, 2015).

**Results:** In the total study population, pretreatment serum PD-L1 concentration had a median of 86.2 pg/ml, and 25% and 75% interquartiles of 64.1 and 134.3 pg/ml, respectively. In univariate analysis in the whole trial, and within both treatment arms, serum PD-L1 was not a significant biomarker for PFS. For OS, higher serum PD-L1 (as a continuous variable) was significant for shorter OS within the trastuzumab arm (HR=3.84, p=0.04), but was not associated with OS in the lapatinib arm (p=0.37). In the whole trial, in multivariate analysis for OS [15 covariates included: age, race, ECOG status, anthracyclines, other chemo, endocrine, radio, other prior adjuvant therapy, disease status, ER status, PR status, Ki67 (log transformed), CK5, EGFR, treatment arm, and serum PD-L1 (with median cut point)], serum PD-L1 remained a significant independent covariate (HR= 2.27, p= 0.001 (Table)). There was significant interaction between treatment arm and continuous serum PD-L1 (Bootstrap method, p=0.0025); above 214.2 pg/ml serum PD-L1 (89% percentile), higher pretreatment serum PD-L1 was associated with a shorter OS to trastuzumab treatment, but longer OS to lapatinib treatment.

**Conclusions:** In the CCTG MA.31 trial, serum PD-L1 was a significant predictive factor: higher pretreatment serum PD-L1 was associated with a shorter OS to trastuzumab treatment, but longer OS to lapatinib treatment. Immune evasion may decrease the effectiveness of trastuzumab therapy. Further evaluation of elevated serum PD-L1 in the advanced breast cancer setting is warranted to identify HER2-positive MBC patients who may benefit from novel immune-targeted therapies in addition to trastuzumab.

Multivariate Analysis (whole trial): Significant Independent Covariates

Covariate	P-Value	HR	Lower 95% CI	Higher 95% CI
Serum PD-L1 (pretreatment) (>median vs <median)	0.001	2.27	1.40	3.68
EGFR Status (continuous IHC score)	0.003	1.012	1.004	1.019
Other Chemotherapy (yes vs no)	0.008	1.91	1.19	3.07
Treatment Arm (trastuzumab vs. lapatinib)	0.010	0.53	0.33	0.86
ECOG Performance Status (0 vs 1 or 2)	0.025	0.59	0.37	0.94
Ki67 (log)	0.046	1.45	1.006	2.081



# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: PD3-11

*HER2/ERBB2* status in “*HER2* equivocal” breast cancers by FISH and ASCO-CAP guidelines: False-positives due to heterozygous deletions of alternative control loci

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**Background.** The ASCO-CAP guidelines for *HER2* testing by fluorescence in situ hybridization (FISH) have a category, referred to as “equivocal” (average *HER2* copies per tumor cell  $\geq 4$ -6 with *HER2*/CEP17 ratio  $< 2.0$ ), which is neither “*HER2*-positive” nor “*HER2*-negative”. Approximately 4% - 12% of invasive breast cancers are “*HER2*-equivocal” based on FISH. Cancers in this category may be resolved as “negative” or “positive” by FISH alternative control probes (2013/2014 guidelines) or *HER2* immunohistochemistry (IHC) (2018 update). Our objectives were to evaluate the following hypotheses: 1.) Genetic loci used as alternative controls show heterozygous deletion in a substantial proportion of breast cancers; 2.) Use of these loci for assessment of *HER2* by FISH leads to false-positives; 3.) *HER2* FISH false-positive breast cancer patients have outcomes that do not differ from clinical outcomes for *HER2*-negative breast cancer patients; and 4.) *HER2*-equivocal breast cancers seldom show *HER2* protein overexpression (IHC 3+).

**Methods.** We retrospectively assessed the use of chromosome 17 p-arm and q-arm alternative control genomic sites (*TP53*, *D17S122*, *SMS*, *RARA*, *TOP2A*), as recommended by the 2013/2014 ASCO-CAP guidelines, in patients whose data were available through the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC)(N=1980) or whose tissues were available from the BCIRG-005 clinical trial (N=3298). We used either FDA-approved *HER2* IHC (HercepTest) or laboratory-developed *HER2* (10H8) IHC assays to assess *HER2* protein expression.

**Results.** Using METABRIC we found heterozygous deletions, particularly in specific p-arm sites, were common in both *HER2*-amplified and *HER2*-not-amplified breast cancers. Use of alternative control probes from these regions to assess *HER2* by FISH in “*HER2* equivocal” as well as *HER2*-not-amplified breast cancers resulted in high rates of false-positive ratios (*HER2*-to-alternative control ratio  $\geq 2.0$ ) due to heterozygous deletions of control p-arm genomic sites used as ratio denominators. Misclassifications of *HER2* status was observed not only in breast cancers with ASCO-CAP “equivocal” status but also in breast cancers with an average of  $< 4.0$  *HER2* copies per tumor cell. These deletions were also identified by FISH. IHC demonstrated  $< 1\%$  of FISH “*HER2*-equivocal” breast cancers in BCIRG-005 had IHC3+ immunostaining, consistent with *HER2*-not-amplified status. Clinical outcomes of “*HER2*-equivocal” breast cancer patients with *HER2*-to-alternative control ratio  $\geq 2.0$  did not differ significantly from clinical outcomes of those with *HER2*-to-alternative control ratio  $< 2.0$ .

**Conclusion.** Using chromosome 17 p-arm alternative controls, as recommended by 2013/2014 ASCO-CAP guidelines, instead of CEP17 for resolution of “*HER2* equivocal” cases, is problematic due to frequent heterozygous deletions of these loci in breast cancers. The indiscriminate use of alternative control probes to calculate a *HER2* FISH ratio in “*HER2*-equivocal” breast cancers leads to false-positive interpretations of *HER2* status resulting from unrecognized heterozygous deletions in one or more of these alternative control genomic sites and incorrect *HER2* ratio determinations.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: PD3-12

Central nervous system as first site of relapse in patients with HER2 positive early breast cancer treated in the BCIRG-006 trial

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**Introduction:** Central Nervous System (CNS) metastases as first site of relapse is seen in 2-3% of patients with HER2+ early breast cancer (EBC) during or after treatment with adjuvant trastuzumab. Data about long-term follow-up outcomes in this population is scarce. **Methodology:** BCIRG-006 was designed to assess the efficacy and safety of two trastuzumab-based regimens compared to a standard (non-trastuzumab) regimen in the adjuvant treatment of HER2+ EBC. 3,222 patients were randomized to standard AC-T or two trastuzumab-based regimens (AC-TH or TCH). Ten year follow-up outcomes were previously presented; we have used this data to assess the frequency and course of CNS relapses as first site of distant recurrence. DFS and OS in these patients were estimated and compared using the Kaplan-Meier method and Log-Rank test respectively. Univariate and multivariate analyses for DFS were conducted considering patient's age, nodal status, tumor size and estrogen receptor (ER) status in the primary tumor. **Results:** Of the 3,222 patients randomized, 575 (17.8%) experienced a distant relapse and in 17.5% of these (n=101) CNS was the first site of recurrence. With a median follow-up of 10.3 years, the frequency of CNS relapses did not differ when comparing the trastuzumab containing arms with the control arm (OR 0.86, 95% CI: 0.56-1.33;  $p=0.519$ ). No difference was observed either between AC-TH and TCH (OR 1.14, 95% CI: 0.67-1.94;  $p=0.704$ ). There were no differences in DFS (HR 1.21, 95% CI: 0.74-1.99;  $p=0.621$ ) nor in OS (HR 0.74, 95% CI: 0.43-1.27;  $p=0.377$ ) between AC-T, AC-TH and TCH arms. Positive axillary nodes ( $\geq 4$  nodes) and ER negative status at baseline remained independent risk factors for CNS relapse after univariate and multivariate analysis (HR 0.60, 95% CI: 0.8-0.95;  $p=0.007$  for nodal status and HR 0.56, 95% CI: 0.37-0.85;  $p=0.029$  for ER status). **Conclusion:** Among the pivotal adjuvant trastuzumab trials, BCIRG-006 is the one with the longest median FUP in which data about CNS relapses has been presented. CNS relapse in patients in this trial was an infrequent event. Its frequency and outcomes were similar across the three treatment arms. Patients with ER negative and/or  $\geq 4$  positive nodes are at higher risk of CNS relapse irrespective of trastuzumab therapy and may be the patient population where research efforts should be focused.

BC outcomes in patients with CNS metastases per treatment arm in the BCIRG-006 trial

	AC-T N=1073 n(%)	AC-TH N=1074 n(%)	TCH N=1075 n(%)	p value
Frequency of CNS relapse	37 (3.44%)	30 (2.79%)	34 (3.16%)	0.519 ^
Median DFS (months; 95% CI)	23.8 (13.3-30.4)	19.9 (16.6-25.1)	19.9 (15.0-27.2)	0.621
Median OS (months; 95% CI)	42.5 (28.3-62.7)	53.2 (31.2-103.6)	30.3 (23.4-39.0)	0.377

^ comparing control vs. trastuzumab-containing arm using Fisher's Exact test.  
comparing control vs. trastuzumab-containing arms using Log Rank test.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD4-01

A new additive diagnostic assay for breast cancer screening - Biochemical infrared analysis of immune cells and plasma

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## Background:

Screening for early detection of breast cancer is currently based on breast imaging. Screening mammography, the most commonly used imaging modality, has limited sensitivity, especially in dense breast tissue, and a significant rate of false positive results. An adjuvant blood-test based assay may improve breast cancer detection irrespective of breast density. In previously reported studies, Total Biochemical Infrared Analysis (TBIA) of peripheral blood mononuclear cells (PBMCs) and plasma was able to differentiate between patients with breast cancer and healthy controls or patients with benign breast disease. The TM-B1<sup>TM</sup> assay is based on TBIA technology and is intended for breast cancer screening. In the present study, the performance of the TM-B1<sup>TM</sup> assay was evaluated for cancer detection across breast densities.

## Patients and Methods:

A total of 220 women were recruited to this IRB approved study: 50 women with newly diagnosed breast cancer, and two control groups – 79 patients with benign findings and 61 patients with no detectable abnormality. Twenty-nine women were withdrawn from the study (for eligibility or technical reasons), and the data presented refer to 190 cases. Breast cancer patients included 6 cases of DCIS, 1 of LCIS, 42 of IDC and 1 of ILC. Ten milliliters of blood were drawn and separated by Ficoll gradient method into PBMCs and plasma. The samples were dried on a zinc selenide optical window and analyzed by a FTIR spectrometer. The spectra were analyzed by the proprietary software TodoSpectra to distinguish between infrared spectra of cancer patients vs. patients with benign findings and healthy controls. The influence of age and breast density on TM-B1<sup>TM</sup> results were evaluated.

## Results:

The TM-B1 assay obtained a sensitivity of 86 % and specificity of 98 % for breast cancer detection. The positive predictive value (PPV) was 95.1% and the negative predictive value (NPV) was 93.5%. Dense breast tissue (BIRADS categories C&D) was present in 55 % of the subjects. The specificity and sensitivity for patients with dense breasts was 99 % and 83 % respectively. No major differences in accuracy of TM-B1 were found due to patients' age.

## Conclusions:

TM-B1 in conjunction with current imaging techniques may contribute to early detection of breast cancer by increasing sensitivity and reducing false positive results and unnecessary biopsies. Further studies with larger patient numbers are required and under way to establish the utility of adding the TM-B1 assay to current standard screening for breast cancer.

# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: PD4-03

MRI detection of residual disease following neoadjuvant chemotherapy (NAC) in the I-SPY 2 TRIAL

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**Background:** Detecting residual disease accurately using MRI after NAC to identify both responders and non-responders is essential for de-escalating therapy or redirecting patients to more effective treatment. The purpose of this study is to determine if the combination of longest diameter (LD) and functional tumor volume (FTV) from dynamic contrast enhanced (DCE-) MRI is superior to FTV alone or LD alone for assessing treatment response after neoadjuvant therapy in breast cancer patients.

**Methods:** Data from patients in the graduated drug arms of the I-SPY 2 trial were included in the analysis. Both LD and FTV were assessed using DCE-MRI after neoadjuvant therapy. LD was measured by the site radiologist as the longest dimension of the enhanced area on early post-contrast images. Functional tumor volume (FTV) was assessed as the sum of voxels with enhancement above specific thresholds within the pre-defined region-of-interest (ROI). A linearized variable was derived to represent the combination of FTV and LD. The area under the receiver operating characteristic curve (AUC) was used to evaluate the assessment of treatment response, pathologic complete response (pCR), defined as no invasive disease in the breast and lymph nodes, and in-breast pCR, defined as no invasive disease in the breast only. The analysis was performed in the full cohort and in breast cancer subtype defined by hormone receptor status and HER2 status.

**Results:** Among the patient cohort of N=675 with FTV and LD, 247 (37%) did and 428 (41%) did not achieve pCR after neoadjuvant therapy. pCR rates varied among HR/HER2 subtypes (HR+/HER2-: 19%; HR+/HER2+: 38%; HR-/HER2+: 71%; HR-/HER2- (triple negative, TN): 43%). In-breast pathologic complete response rates were slightly higher in each group (full: 41%; HR+/HER2-: 23%; HR+/HER2+: 43%; HR-/HER2+: 72%; HR-/HER2-: 49%). Table 1 shows AUCs for assessing pCR using FTV alone, LD alone, and the variable combining FTV and LD. Higher AUCs were observed in all patient groups using the combined variable. AUC of 0.79 (95% CI: 0.77, 0.81) was observed for the combined variable to assess pCR in the full cohort. AUCs varied from 0.69 to 0.86 among HR/HER2 subgroups (HR+/HER2-: 0.69; HR+/HER2+: 0.74; HR-/HER2+: 0.86; HR-/HER2-: 0.80), with no difference in assessing pCR or in-breast pCR. The performance is best for the HR- subtypes.

**Conclusions:** Both FTV and LD can be used in the assessment of invasive disease residual after neoadjuvant therapy. The combined variable of FTV and LD achieved highest AUCs, compared to using individual variable alone. Tools to improve performance in the HR+ subsets are underway.

AUCs of MR measurements for identifying pCR

		FTV alone (95% CI)	LD alone (95% CI)	Combined (95% CI)
Full	With subtype adj.	0.73 (0.71, 0.75)	0.77 (0.74, 0.79)	0.79 (0.77, 0.81)
Full	Without subtype adj	0.69 (0.65, 0.73)	0.72 (0.68, 0.76)	0.75 (0.71, 0.79)
HR+/HER2-		0.68 (0.60, 0.77)	0.68 (0.59, 0.77)	0.69 (0.61, 0.77)
HR+/HER2+		0.65 (0.56, 0.75)	0.72 (0.64, 0.80)	0.74 (0.66, 0.82)
HR-/HER2+		0.69 (0.55, 0.83)	0.82 (0.71, 0.92)	0.86 (0.77, 0.95)

HR-/HER2- (TN)		0.72 (0.66, 0.79)	0.73 (0.67, 0.80)	0.80 (0.74, 0.85)
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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD4-04

Role of breast MRI in predicting pathologically negative nodes after neoadjuvant chemotherapy in cN0 patients in the I-SPY2 trial

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## Background

In clinically node-negative (cN0) breast cancer patients with triple negative (TN) and HER2+ disease and breast pathological complete response (breast pCR), low rates of nodal positivity after neoadjuvant chemotherapy (NAC) have been demonstrated. In these patients, the omission of surgical axillary staging has been proposed. However, this information is not routinely known preoperatively. We aimed to validate the correlation between pathologic breast response and pathologic nodal status, and evaluate the relationship between response of the breast tumor on MRI and pathologic nodal status after NAC in cN0 patients in the I-SPY2 trial.

## Methods

We identified all patients with cT1-4 cN0 breast cancer prior to NAC from graduated arms of the I-SPY2 trial, a prospective neoadjuvant chemotherapy trial. Absence of residual disease post-NAC was defined as longest diameter (LD) of 0 mm on MRI. Breast pCR was defined as the absence of invasive tumor in the breast at surgery. Associations between ypN0 and patient, MRI, and tumor characteristics were assessed using chi-square tests and univariate regression.

## Results

Of 365 cT1-4 cN0 patients included, 128 had HR+/HER2- tumors (35%), 60 HR+/HER2+ tumors (16%), 34 HR-/HER2+ tumors (9%) and 143 TN tumors (39%). Overall, 283 patients (78%) were ypN0 after NAC and 152 patients (42%) had a breast pCR. ypN0 rate was higher in patients with a breast pCR than those with residual disease (93% vs 66%,  $p < 0.001$ ). Patients with HR-/HER2+ and TN tumors were more likely to be ypN0 (97% and 87% respectively) than patients with HR+/HER2- and HR+/HER2+ disease (66% and 71% respectively,  $p < 0.001$ ). Other characteristics associated with ypN0 were tumor grade (grade I 57%, grade II 66%, grade III 84%;  $p = 0.002$ ), MammaPrint Classification (High Risk 1 68% and High Risk 2 87%;  $p < 0.001$ ) and absence of residual tumor in the breast on MRI (87% vs 72% in patients with evidence of tumor on MRI post-NAC/pre-surgery;  $p = 0.003$ ).

In patients with HR-/HER2+, HR+/HER2+, HR-/HER2+ or TN disease and a breast pCR, ypN0 rate was respectively 82%, 96%, 96% and 97% (table 1). In patients with HR+/HER2-, HR+/HER2+, HR-/HER2+ or TN disease and with no evidence of residual disease in the breast on MRI, rate of ypN0 was 71%, 80%, 94% and 96% respectively.

## Conclusion

In cT1-4 cN0 breast cancer patients with HR+/HER2+, HR-/HER2+ and TN tumors and a breast pCR, ypN0 rates after NAC are extremely high. In patients with HR-/HER2+ and TN tumors with no residual breast disease on MRI after NAC and pre-surgery, ypN0 rates are high enough to consider omission of axillary surgery. In patients with HR+ tumors, MRI is unsufficiently predictive for pathological response and can therefore not be used to select ypN0 patients. Research on the prediction of ypN0 in cN+ I-SPY2 patients is ongoing.

Nodal status in patients with pCR and absence of residual disease on MRI

	Number of positive nodes					
Breast Cancer Subtype	0	1	2	3	All	
Breast pCR						
HR+/HER2-	27(82)	2(6)	4(12)	0	33(100)	
HR+/HER2+	24(96)	0	1(4)	0	25(100)	
HR-/HER2+	24(96)	1(4)	0	0	25(100)	
TN	67(97)	2(3)	0	0	69(100)	
Absence of residual disease on MRI						
HR+/HER2-	24(71)	7(21)	3(9)	0	34(100)	
HR+/HER2+	16(80)	3(15)	0	1(5)	20(100)	
HR-/HER2+	15(94)	1(6)	0	0	16(100)	
TN	54(96)	2(4)	0	0	56(100)	

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD4-05

Molecular breast imaging and tomosynthesis to eliminate the reservoir of undetected cancer in dense breasts: The Density MATTERS trial

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## Background

High mammographic breast density is the primary reason for missed cancers or delayed detection on mammography, and is associated with a higher rate of advanced and interval cancers which increase breast cancer mortality. Digital breast tomosynthesis (DBT) has been shown to reduce false positive findings relative to 2D mammography but does not eliminate the potential for tumor masking in dense breasts due to the similar x-ray attenuation characteristics of tumors and normal fibroglandular tissue. Molecular Breast Imaging (MBI) performed with a dedicated gamma camera to detect functional uptake of a radiotracer, Tc-99m sestamibi, has been shown to reveal breast cancers obscured by density on mammography. In single-institution studies, adding MBI to 2D mammography in women with dense breasts detected an additional 5 to 10 invasive cancers per 1000 screened, with modest increases in recall rate (6 to 8%) at a lower cost-per-cancer detected than mammography alone. Despite this promising evidence, the lack of multicenter trial data has limited wider acceptance. Also, MBI has yet to be compared to DBT, which in some centers has replaced 2D mammography screening. We present interim results from a multicenter trial comparing cancer detection rate of DBT and MBI in screening of women with mammographically dense breasts.

## Methods

In this ongoing, prospective, multicenter clinical trial, asymptomatic women aged 40-75 years with dense breasts on prior mammogram and no prior history of supplemental screening are invited to undergo two annual rounds of concurrent DBT and MBI. MBI is performed with injection of 300 MBq Tc-99m sestamibi with a dual-head semiconductor-based gamma camera. Screening tests are interpreted independently. Here, preliminary cancer detection rates (cancers per 1000 women screened), recall rates, and biopsy rates of DBT and MBI for initial screening are reported.

## Results

In 537 women out of a planned 3000 who have completed the first round of screening, 7 cancers were detected: one by DBT only and 6 by MBI only, giving cancer detection rates of 1.9 for DBT vs. 11.2 for MBI and incremental cancer detection rate of 9.3 for MBI. The one DBT-only cancer was a node-negative 0.8 cm invasive lobular carcinoma. All 6 cancers detected by MBI were invasive; 5 of 6 were node negative (median size 1.0 cm; range 0.6 to 2.6 cm). Recall rate was 11% (60/537) for DBT alone; 16% (84/537) for MBI alone, and 21% (115/537) for the combination. Biopsy was prompted by DBT in 13 patients (PPV 8% [1/13]); by MBI in 23 patients (PPV 26% [6/23]); and by the combination of modalities in 33 (PPV 21% [7/33]).

## Conclusion

These preliminary results demonstrate that MBI detects invasive breast cancers occult on DBT in dense breasts. Data from a second screening round will allow calculation of sensitivity and specificity, and determination of the impact of screening MBI in reducing advanced (> 2 cm) and interval cancers. Additional planned analyses will evaluate a denoising algorithm for further reduction in MBI radiation dose to match that of DBT.

# 2018 San Antonio Breast Cancer Symposium ®

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PET imaging of PARP-1 expression in breast cancer

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<sup>18</sup>F-FluorThanatrace ([<sup>18</sup>F]-FTT) is a novel radiotracer shown to quantify Poly [ADP-ribose] polymerase 1 (PARP-1) expression *in vitro* and *in vivo* through a receptor-ligand interaction. A recent study at the University of Pennsylvania in women with ovarian cancer demonstrated *in vivo* visualization of PARP-1 expression in tumors using this radiotracer that closely correlated with an *in vitro* assay of PARP-1 in tumor tissue (Makvandi, M. J. Clin. Invest. 128:2116, 2018). A radioligand with PARP-1 specificity, [<sup>125</sup>I]-KX1, was also developed as a companion tool for *ex vivo* evaluation of PARP-1 expression and PARP inhibitor (PARPi) drug occupancy by radioligand binding assay (Makvandi, M. Cancer Res. 76:4516, 2016). As the first step in validating this biomarker in breast cancer, we performed a prospective clinical trial comparing *in vivo* [<sup>18</sup>F]-FTT uptake and *ex vivo* PARP-1 expression in women with primary breast cancer.

**Methods:** 24 patients with Stage I-IV primary breast cancer were imaged with [<sup>18</sup>F]-FTT prior to any therapy including surgery. We correlated *in vivo* uptake with *ex vivo* immunohistochemistry (IHC) for PARP-1 and [<sup>125</sup>I]-KX1 autoradiography in untreated surgical specimens. Tumors were analyzed for alterations in DNA repair genes, copy number-based as well as mutational signatures indicative of homologous recombination deficiency (HRD) and mutational burden, using our established protocol (Maxwell, KN, Nature Commun. 8:319, 2017).

**Results:** [<sup>18</sup>F]-FTT uptake was visualized above background in all primary breast tumors and known metastases. Two areas of unexpected uptake revealed an unknown contralateral breast cancer and an ovarian carcinoid, respectively. We expected that uptake might be highest in triple negative breast cancer (TNBC), where PARPi have been most heavily studied. However, a range of tracer uptake was observed in tumors independent of breast cancer subtype (hormone receptor positive/HER2 negative, TNBC, HER2+) and BRCA status. Uptake ratios (SUVmax tumor/SUV max opposite breast) ranged from 1.2-10.5 with a median 4.0. *Ex vivo* [<sup>125</sup>I]-KX1 autoradiography was performed on a subset of untreated primary tumors (n=5) and compared with IHC staining for PARP-1 on sequential sections. This revealed a close spatial correspondence between elevated PARP-1 expression by IHC and regions of elevated [<sup>125</sup>I]-KX1 binding radiographically. There was also a strong positive correlation between *in vivo* [<sup>18</sup>F]-FTT uptake and *ex vivo* quantitative [<sup>125</sup>I]-KX1 autoradiography (r=0.78). Genomic analysis of HRD in all tumors is pending and will be reported.

**Conclusion:** Initial analyses support the ability of [<sup>18</sup>F]-FTT to visualize and measure PARP-1 expression in breast cancer. This is the first step toward developing an imaging companion diagnostic to help guide PARP inhibitor treatment in breast cancer. Ongoing studies are expanding upon these results, testing the extent to which expression of PARP-1 by [<sup>18</sup>F]-FTT can predict response to PARP inhibitors and measure target engagement during therapy.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD4-08

Relationship between FDG-uptake and expression level of PD-L1 in primary ER positive/HER2 negative breast cancer

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**Background:** <sup>18</sup>F-Fluorodeoxyglucose-positron emission tomography (FDG-PET) is used to evaluate the glucose metabolic rates of cancers. Several studies have reported that high FDG uptake is predictive of poor prognosis and aggressive features in patients with breast cancer (BC). FDG-uptake is influenced by many factors including inflammation. In this study, we investigated the relationship between FDG uptake and immunological factors, including degrees of stromal tumor-infiltrating lymphocytes (TILs), CD8 and programmed cell death ligand 1 (PD-L1) in ER positive/HER2 negative (ER+/HER2-) BC. No published study, to our knowledge, has assessed the association between FDG uptake and PD-L1 in BC cases, even though both represent prognosis. **Methods:** Invasive carcinoma tissues of 79 ER+/HER2- BC patients who underwent surgery without preoperative therapy were examined. PD-L1, CD8 and TILs expression were evaluated by immunohistochemically (IHC) method. The evaluation of PET was determined by standardized uptake value max (SUVmax). Multivariate linear regression analysis, including expression of PD-L1, CD8 and TILs, was performed to identify independent variable correlation with SUVmax. **Results:** Among the 79 ER+/HER2- BC (T1-4, N0-2, M0) patients, the analysis revealed that PD-L1 (P=0.005), lymphovascular invasion (P=0.011), large tumor size (p=0.001), lymph node metastasis (P=0.010), and high nuclear grade (P=0.011) and premenopausal status (P=0.014) were significantly associated with high SUVmax in the primary tumor. To define predictive value for the expression of PD-L1, SUVmax cut off was determined using receiver operating characteristic (ROC) analysis; low (<3.0) and high (≥3.0). Among the 30 cases with high SUVmax, 10 cases (33.3%) had PD-L1 positive expression in the primary tumor, while only 2 (4.1%) of the 49 cases with low SUVmax had PD-L1 positive expression in the primary tumor. **Conclusions:** The present study demonstrated that the finding of preoperative FDG uptake is associated with the expression of PD-L1 in ER+/HER2- BC. In light of our results, FDG uptake may be predictive of the expression of PD-L1 and may be reflective of immunological features as well as prognostic features among patients with ER+/HER2- BC.

# 2018 San Antonio Breast Cancer Symposium ®

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Non-invasive estrogen receptor assessment by [<sup>18</sup>F]-fluorestradiol(FES)-PET or circulating tumor cells predicts receptor status in patients with metastatic breast cancer

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**Introduction:** Estrogen receptor (ER) expression largely determines the therapy choice for patients diagnosed with metastatic breast cancer (MBC). Given potential conversion of ER-status, the current golden standard for ER assessment is by immunohistochemistry (IHC) on metastatic tissue. Since a biopsy is cumbersome and sometimes not feasible, ER-status assessments by means of [<sup>18</sup>F]-fluorestradiol (FES)-PET or circulating tumor cells (CTCs) might be potential alternatives. We hypothesize that FES-PET or CTCs can determine ER status in patients with MBC, in a more efficient and patient friendly way than IHC.

**Methods:** In the Dutch multicenter IMPACT-MBC trial (NCT01832051) patients with non-rapidly progressive MBC at first presentation, regardless of subtype, underwent extensive molecular imaging (including FES-PET, <sup>89</sup>Zr-trastuzumab-PET and serial [<sup>18</sup>F]-fluorodeoxyglucose (FDG)-PET), a metastasis biopsy and blood sampling to obtain a whole body molecular profile of their disease. ER-status on the metastasis biopsy was evaluated by IHC and considered positive if ≥10% of the tumor cells showed ER expression. The FES-PET was considered positive when the maximum standardized uptake value (SUV<sub>max</sub>) of at least one lesion was ≥ 1.5. Reverse transcription polymerase chain reaction was used to quantify the *ESR1* expression in CTCs. ER-positivity was defined as an *ESR1* mRNA ΔCq level ≥ -7.86, corrected for background healthy donor blood signal, only in samples with ≥5 CTCs/7.5ml and a sufficient mRNA signal of reference and epithelial genes. Sensitivity, specificity, positive and negative predictive values were calculated (PPV and NPV).

**Results:** From 178 patients of 201 evaluable patients both metastasis IHC and FES-PET could be assessed. 129 of these 178 patients had an IHC ER positive metastasis (72%) and 133 patients had a positive FES-PET (75%). The PPV and NPV for IHC by FES-PET were 91% and 82%, respectively. From 54 of the 178 patients, blood samples contained sufficient CTCs for *ESR1* analysis, allowing combined assessment of IHC, FES-PET and CTCs. ER positive CTCs were present in 45 patients (83%).

Table 1

	Metastasis ER positive (n=42)	Metastasis ER negative (n=12)	Sensitivity(%)	PPV(%)	Specificity(%)	NPV(%)
FES-PET positive	41	5	98	89		
CTC ER positive	38	7	90	84		
FES-PET and CTC positive	38	4	90	90		
FES-PET negative	1	7			58	88
CTC ER negative	4	5			42	56
FES-PET and CTC negative	1	4			33	80

**Conclusion:** In newly diagnosed patients with MBC, ER-status in metastatic lesions could be predicted by means of non-invasive FES-PET scan or CTCs. Prediction was most optimal with FES-PET compared to CTCs, and combining FES-PET with CTCs did not essentially improve this. Furthermore, CTCs were not detectable in most of these patients with non-rapidly progressive MBC, limiting their applicability for the present purpose. However, as CTC assessment is most patient friendly, CTCs might be considered as first step in determining MBC ER-status. If ER positive CTCs are detected, ER positive disease is very likely; if no- or ER negative CTCs are detected, MBC ER-status needs to be further assessed by means of tissue confirmation or FES-PET.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD4-10

<sup>18</sup>F-fluoroestradiol (FES) and <sup>18</sup>F-fluorodeoxyglucose (FDG) PET imaging in staging extent of disease in metastatic lobular breast cancer

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**Background:** The histology and pattern of spread in lobular breast cancer has presented challenges in estimating extent of disease and identifying treatment options. <sup>18</sup>F-FES is an estrogen analogue PET imaging tracer which measures tumor ER expression at multiple tumor sites simultaneously and predicts response to endocrine therapy. We analyzed FES-PET and FDG-PET SUV uptake in patients with metastatic lobular and ductal carcinoma to identify sites of tumor and responsiveness to therapy.

**Methods:** We retrospectively reviewed FES and FDG SUV uptake between ER+ lobular (n = 36) and ductal (n= 173, including 6 men) metastatic breast cancer patients enrolled in various institutional studies. Up to 3 lesions in each patient were evaluated by FES SUVmax and/or FDG SUVmax for a total of 475 lesions in FES images and 462 lesions in FDG images. Classification into three categories (low FDG, high FDG/high FES, and high FDG/low FES) was generated using recursive partitioning with 5-fold internal cross validation. Using a Pearson Chi-squared test, we compared degree of uptake in FES and FDG between lobular and ductal carcinomas. We used linear mixed effects model to assess association of FES SUL<sub>mean3</sub> (Lean body mass adjusted SUV) and FDG SUL<sub>mean3</sub> with histology. Overall survival (OS), from time of FES-PET scan to death, and progression free survival (PFS) was evaluated between classification groups in both histologies using Kaplan-Meier curves and Cox model.

**Results:** In patients with metastatic breast cancer, 72 patients had low FDG, 96 had high FES/high FDG, and 41 with high FES/low FDG. Lobular lesions tended to have a higher proportion of patients in the risk group with lower FDG (42% vs 33%) and a lower proportion in the risk group with high FDG/low FES (11% vs 21%) but the difference was not statistically significant (p = 0.32). Mean (range) FES SUL<sub>mean3</sub> and FDG SUL<sub>max3</sub> respectively for ductal was 1.38 (0.10, 6.7) and 3.17 (0.88, 12.26) and for lobular was 1.42 (0.34, 3.43) and 3.13 (1.04, 13.87). There was no significant difference between in FES SUL<sub>mean3</sub> and FDG SUL<sub>max3</sub> between histologies. Following FES-PET imaging, patients with lobular carcinomas and low FDG demonstrated a higher median survival time (7.7 years) compared to high FDG/low FES (4.3 years) and high FDG/high FES (2.6 years). Similarly, patients with ductal carcinomas and low FDG had an improved median survival time (5.6 years) compared to both high FDG/high FES (2.9 years) and high FDG/low FES (2.5 years). However, the interaction between histology and the FDG/FES classifications was not significant (p = 0.86). Across a variety of tumor sites, lobular histology can be detected by both FES and FDG with no difference between the imaging modalities.

**Conclusions:** In the metastatic setting, quantitative FES and FDG can be used to discriminate indolent and aggressive phenotypes in both lobular and ductal breast cancer. A greater proportion of lobular carcinoma lesions had higher FES/lower FDG and would be anticipated to be more sensitive to endocrine therapy. Further prospective trials are needed to confirm the utility of FES to stage extent of disease in metastatic breast cancer.



# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: PD4-11

[<sup>18</sup>F]Fluciclovine PET tracks cellular glutamine pool size in breast cancer and changes in response to metabolic inhibition

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**Background:** Some forms of triple negative breast cancer (TNBC) rely on glutamine (Gln) metabolism for survival and growth (1), therefore, targeting this metabolic pathway provides a viable strategy for managing TNBC. Drugs that inhibit glutaminase (GLS), a key enzyme of glutaminolysis, are being developed (1,2). [<sup>18</sup>F]Fluciclovine is a PET imaging agent that enters/exits cells via glutamine transporters and undergoes minimal metabolism. Therefore, we hypothesize, that akin to our prior work with [<sup>18</sup>F]fluoroglutamine (3), the distribution volume ( $V_T$ ) of fluciclovine obtained from dynamic PET can be used to estimate the cellular glutamine level (pool size) and to mark the effect of pharmacological inhibitors of tumor glutaminase (GLS). We tested this hypothesis in human TNBC and ER+ breast cancer xenograft exhibiting a high and low GLS activity, respectively.

**Methods:** To make [<sup>18</sup>F]fluciclovine preparation suitable for mouse imaging, citrate in the formulation was removed and replaced with PBS by eluting through a column (Bio-Rad). Cellular uptake was performed in the presence and absence of Gln transporter inhibitors and GLS inhibitor. *In vivo* dynamic PET imaging were performed on mice bearing HCC1806 (TNBC) and MCF-7 (ER+ BC) xenografts. Dynamic PET images were analyzed by Logan Plot (PMOD) to estimate  $V_T$ .

**Results:** Cellular uptake of [<sup>18</sup>F]fluciclovine in HCC1806 and MCF-7 cells were sensitively inhibited by cold glutamine (Gln) and GPNA (a pharmacologic inhibitor of ASCT-2), confirming that the uptake is mediated by Gln transporters. The peak uptake in MCF-7 cells was 5-fold higher than HCC1806. In mouse models,  $V_T$  from *in vivo* [<sup>18</sup>F]Fluciclovine PET in MCF-7 tumor is 1.4-fold of HCC1806. These data are consistent with a higher cellular Gln pool size in MCF-7 as the result of its lower GLS activity. After inhibition of tumor GLS activity,  $V_T$  of [<sup>18</sup>F]fluciclovine in HCC-1806 tumors was increased by 56% from baseline values (n=2), whereas  $V_T$  in MCF-7 tumors decreased 1% after treatment (n=2). Only a small change of FDG PET signal (5% decrease, n=5) was detected in TNBC tumors after GLS inhibitor treatment.

**Discussions:** These data suggest that  $V_T$  obtained from [<sup>18</sup>F]fluciclovine PET is sensitive to changes of the Gln pool size induced by GLS inhibition whereas FDG PET is not. Since the Gln pool size is inversely related to the GLS activity, increased  $V_T$  is consistent with the increased intracellular Gln level when metabolic conversion of Gln to glutamate by GLS is inhibited. Our results suggest that [<sup>18</sup>F]fluciclovine, an imaging agent approved for prostate cancer imaging, may be useful for assessing glutamine pool size in breast cancer and changes in response to GLS inhibition

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD4-12

Use of  $^{64}\text{Cu}$ -DOTA-trastuzumab positron emission tomography (PET) to predict response to ado-trastuzumab emtansine (TDM1)

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**Background:** We have demonstrated that  $^{64}\text{Cu}$ -DOTA trastuzumab is an effective PET imaging agent for HER2 positive breast cancer and are now seeking to evaluate the methodology for prediction of response and benefit to ado-trastuzumab emtansine (TDM1) in women with metastatic disease.

**Methods:** Patients with metastatic breast cancer were eligible if they had biopsy confirmed HER2 + disease, at least 1 site of metastasis 2.0 cm or larger outside the biopsy site, and were to receive TDM1 as therapy. Pretreatment staging included  $^{18}\text{F}$ -FDG/PET. Prior to injection of  $^{64}\text{Cu}$ -DOTA-trastuzumab, patients received 45 mg of cold trastuzumab to reduce liver uptake. PET-CT scans were obtained at 21-25 h and 47-48 h over fields of view chosen in reference to  $^{18}\text{F}$ -FDG scans. TDM1 was administered at a dose of 3.6 mg/kg every 3 weeks. Restaging  $^{18}\text{F}$ -FDG/PET was performed every 2 cycles, and response to therapy was determined by PERCIST criteria. The radiolabel uptake was measured in terms of maximum voxel standardized uptake value ( $\text{SUV}_{\text{max}}$ ).

**Results:** Ten women enrolled on study and are evaluable for response; three continue on TDM1 for 22-40 months and four patients remained on treatment for at least 1 year. The median age was 54.5 years (48-83 years); seven received prior trastuzumab-containing chemotherapy 3 wks to 55 months prior to study entry. HER2 was positive by IHC in 5 and by FISH in 5 (3 were 2+ by IHC; 1 was 1+ and 1 indeterminate). Complete or partial metabolic response was observed in 5 patients. Patients had their average  $\text{SUV}_{\text{max}}$  on  $^{64}\text{Cu}$ -DOTA trastuzumab PET ( $\text{aSUV}_{\text{max}}$ ) assessed in addition to individual assessments on up to 8 lesions on both Day1 and Day2. The mean  $\text{aSUV}_{\text{max}}$  was (6.3, 8.8) for responding patients and (4.4, 5.2) for non-responder (day1, day2). The difference between responders and non-responders on Day1  $\text{aSUV}_{\text{max}}$  was marginally significant ( $p=0.06$ ), but significant on Day2 ( $p=0.04$ ). The three highest  $\text{aSUV}_{\text{max}}$  on both day1 and day2 were three of the four patients with PFS>1 year. Data on the relationship of  $^{64}\text{Cu}$ -DOTA trastuzumab PET to IHC and FISH, and individual lesion  $\text{SUV}_{\text{max}}$  including evidence suggesting a potential threshold effect will be presented.

**Conclusions:** In women with biopsy confirmed HER2 positive metastatic disease,  $^{64}\text{Cu}$ -DOTA-trastuzumab PET imaging is predictive for response to TDM1.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD5-01

**KEYNOTE-173:** Phase 1b multicohort study of pembrolizumab (Pembro) in combination with chemotherapy as neoadjuvant treatment for triple-negative breast cancer (TNBC)

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**Background:** TNBC has poor outcome and limited therapeutic options. Pembro monotherapy demonstrated antitumor activity and acceptable safety in metastatic TNBC. KEYNOTE-173 (NCT02622074) is a 6-cohort, phase 1b study of different doses and schedules of platinum and taxanes in combination with pembro as neoadjuvant treatment in patients (pts) with locally advanced TNBC.

**Methods:** Eligible pts were women aged  $\geq 18$  y with newly diagnosed, locally advanced, previously untreated TNBC. In all cohorts, patients received single pembro 200 mg administration on day 1 of cycle 1. Cycles 2-5 included pembro 200 mg with either nab-paclitaxel 125 mg/m<sup>2</sup> QW (cohort A), nab-paclitaxel 100 mg/m<sup>2</sup> QW plus carboplatin AUC6 Q3W (cohort B), nab-paclitaxel 125 mg/m<sup>2</sup> QW plus carboplatin AUC5 Q3W (cohort C), nab-paclitaxel 125 mg/m<sup>2</sup> QW plus carboplatin AUC2 QW (cohort D), paclitaxel 80 mg/m<sup>2</sup> QW plus carboplatin AUC5 Q3W (cohort E) and paclitaxel 80 mg/m<sup>2</sup> QW plus carboplatin AUC2 QW (cohort F) followed by doxorubicin 60 mg/m<sup>2</sup> Q3W and cyclophosphamide 600mg/m<sup>2</sup> Q3W with pembro 200 mg Q3W in all cohorts (Cycles 6-9) prior to surgery. Breast MRI was performed at screening and after cycles 5 and 9. Dose-limiting toxicities (DLTs) were assessed during cycles 1-3 and 6-7. Dose levels were deemed toxic if  $\geq 3$  of the first 6 pts or  $\geq 4$  of 10 pts had DLT. Primary end points were safety and recommended phase 2 dose (RP2D). Key efficacy end points were pCR (ypT0/Tis ypN0, or ypT0 ypN0), objective response rate (ORR) based on RECIST v1.1 as assessed by investigator, event-free survival (EFS), and overall survival (OS).

**Results:** 60 pts were enrolled by 5-31-18 (10/cohort): median age, 48.5 y (range 26-71); most had ductal histology (83.3%), T2/T3 (88.3%) and nodal involvement (66.7%). 22 pts had DLTs (2, A; 4, B/F; 6, C/D); most common was febrile neutropenia (9 pts: 1, A; 2, B/C; 4, D). The most common  $\geq 3$  TRAEs were neutropenia (73%), febrile neutropenia (22%), anemia (20%) and thrombocytopenia (8%). 18 pts had immune-related AEs; most common were G2 hypothyroidism (4 pts), G1 hyperthyroidism (3 pts), G3 colitis (2 pts), and G3 rash (2 pts). 11 pts discontinued pembro due to TRAEs (1, A/B/E; 4, D/F). The overall pCR rate (ypT0/Tis ypN0) was 60% (90% CI 30-85). ORR was 100% (74-100) in B/C, 90% (61-100) in D/F, 80% (49-96) in A and 70% (39-91) in E. At a median follow-up of 19.6 mo, EFS rate at 12 mo was 100% in B/C/E/F, 90% (58-98) in D, and 80% (49-93) in A. EFS rate at 12 mo was 100% and 88% for pts who did and did not achieve pCR (71-95).

**Conclusion:** Pembro+chemo as TNBC neoadjuvant therapy results in promising antitumor activity with manageable toxicity. Results support the ongoing KEYNOTE-522 trial.

# 2018 San Antonio Breast Cancer Symposium ®

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Durability of clinical benefit with niraparib + pembrolizumab in patients with advanced triple-negative breast cancer beyond *BRCA*: (TOPACIO/Keynote-162)

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**Background:** PARP inhibitor (PARPi) monotherapy has previously demonstrated clinical activity only in patients with a germline *BRCA* mutation (*BRCAmut*), while single-agent anti-programmed cell death protein 1 (PD-1) therapy has achieved response rates of only 5–20% in advanced triple negative breast cancer (TNBC). In preclinical studies, PARP inhibition enhanced anti-tumor immunity, increased infiltration of proliferating CD8+ T cells, and synergized with anti-PD-1 agents in *BRCA* wildtype (wt) tumors. TOPACIO is a fully enrolled, phase I/II trial of niraparib + pembrolizumab (pembro) in advanced TNBC. This combination achieved 28% objective response rate (ORR) and 50% disease control rate (DCR) in evaluable patients. Although activity was highest in patients with *BRCAmut* (ORR=60%; DCR=80%), durable clinical benefit was also observed in patients without *BRCA* mutations. In this study, we conducted exploratory biomarker analyses to evaluate their potential correlation with durable clinical benefit (any complete response [CR] or partial response [PR] regardless of duration or stable disease [SD] for ≥16 weeks) beyond *BRCA* mutations.

**Method:** ORR was assessed by RECIST v1.1. Duration of disease control (DDC) was defined as time from first dose of study treatment to radiologic disease progression or death. Tumor mutational status of homologous recombination repair (HRR) and other DNA damage repair (DDR) pathway genes was determined using an NGS panel. Immunoprofiling was conducted using NanoString IO360 panel complimented with 30 DNA repair spike-ins. Tumor immune micro-environment was characterized using multiplex immuno-fluorescence (CyclIF). PD-L1 status was determined using the Agilent/DAKO 22C3 IHC clinical trial assay.

**Results:** Of 46 evaluable patients, 20 achieved durable clinical benefit (any CR/PR or SD≥16 weeks) with niraparib + pembro combination, of which 8 were tumor *BRCA* wild type (wt), and 1 was *BRCA* unknown. Of the 9 *BRCAwt/unknown* patients, 5 had deleterious mutations in HRR/DDR pathway genes, whereas the remaining 4 had no mutations (HRR/DDR wt). Mutations that were associated with response include *CHEK1* (CR; DDC=10.3 mos), *ATR* (CR; DDC=6.4 mos), *PALB2* (PR; DDC=3.5 mos), *BLM* (SD; DDC=8.1 mos), and *NBN/RAD51C* (SD; DDC=3.7 mos). Of 4 patients that had no identified mutations (HRR/DDR wt), 1 patient had CR; DDC=10.3 mos, and the remaining 3 patients had SD with DDC ranging from ~4-8 mos. Of note, all 4 HRR/DDR wt patients were also PD-L1-negative.

Table: Durable clinical benefit in *BRCAwt/unknown* patients

HRR/DDR Mutations	PD-L1 Status	Best Response	DDC (Months)
<i>CHEK1</i>	+	CR	10.3†
<i>ATR</i>	+	CR	6.4
<i>PALB2</i> *	Unknown	PR	3.5
<i>BLM</i>	-	SD	8.1
<i>NBN/RAD51C</i>	+	SD	3.7

None	-	CR	10.3 <sup>†</sup>
None	-	SD	8.2
None	-	SD	4.2
None	-	SD	3.9

\**BRCA* status unknown; <sup>†</sup>Treatment is ongoing.

**Conclusion:** Patients with mutations beyond *BRCA* achieved durable clinical benefit with niraparib + pembro treatment; five of these patients had DDC >6 mos. Mutations in genes that are associated with the HRR/DDR pathway appear to confer sensitivity to niraparib + anti-PD1. Additional translational analyses, including immunoprofiling and CyclIF, will be presented.

**Funding:** TESARO, Inc., Waltham, MA, USA sponsored the study.

# 2018 San Antonio Breast Cancer Symposium ®

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Characterization of high TIL breast cancers reveals a prognostic and functionally distinct tissue-resident memory subpopulation

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**Background:** Tumor infiltrating lymphocytes (TILs) assessed via light microscopy are prognostic and predictive in the early stage and advanced triple negative and HER2-amplified breast cancer (BC). Higher TILs can also identify patients more likely to benefit from anti-PD-1 therapy. In this study we interrogated T cell subsets that comprise high TILs to determine if distinct subpopulations are key mediators of anti-tumor immunity.

**Methods:** We characterised TILs with a focus on CD3+ T cells in 129 primary and metastatic BC samples using flow cytometry, bulk RNASeq on flow sorted T cell populations, multiplex immunohistochemistry and microdroplet based single cell 3' mRNA sequencing on the 10X Genomics Chromium platform. Cell type specific gene expression signatures were determined from differential expression between putative T cell subpopulations. These signatures were investigated in clinical cohorts, including trial cohorts treated with pembrolizumab.

**Results:** High TIL Infiltrates consisted primarily of CD3+ T cells, with both CD8 and CD4 populations. Unsupervised clustering of single cell sequencing identified 9 CD8 and CD4 subpopulations with distinct gene expression profiles. In addition to Tregs and CD8 effector memory (TEM) T cells, we found a CD8+ tissue resident memory (TRM) population expressing greater levels of T-cell checkpoints and cytotoxic markers compared to effector memory cells. In 2 primary tumours and 1 liver metastasis, bulk RNASeq of flow sorted TEM and TRM corroborated the single cell mRNASeq results. T cell receptor profiling (TCR) in the 3 samples found non-overlapping repertoires in the 2 primary tumours, but overlap in one metastatic lesion, suggesting divergent developmental origins in the breast, but the potential for nascent TRM differentiation in a metastatic niche. Clustering of these TCRs suggested differing antigen specificities between TRM and non-TRM CD8 T cells. Using Metabric data, the CD8 TRM gene expression signature was prognostic for disease free survival (DFS) in primary TNBCs (n=329, log-rank p=0.003), and was able to further stratify cases with high and low *CD8A* expression for DFS (log-rank p = 0.03). The CD8 TRM signature was enriched in baseline tumour samples of responders (n = 9) compared with non-responders (n=36) in 45 patients with metastatic melanoma treated with T cell checkpoint blockade (p < 0.0001). Additional single cell sequencing data with TCR sequencing will be combined with these initial results, and an independent data set of single cell mRNASeq and TCR Seq on CD3+ BC TILs will be used to confirm our findings. Cell type specific signatures will be explored in additional clinical cohorts including KEYNOTE-086, and presented at the meeting.

**Conclusion:** Using single cell profiling of the immune microenvironment in BC we demonstrate that high TIL BCs contain multiple T cell subpopulations with different functional and prognostic significance. Our approach identified a CD8 TRM population with a distinct gene expression profile and strong expression of key immune checkpoints likely representing the presence of true tumor specific immunity. This population may be a key target of immune checkpoint blockade.

# 2018 San Antonio Breast Cancer Symposium ®

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An open-label, phase II basket study of olaparib and durvalumab (MEDIOLA): Updated results in patients with germline *BRCA*-mutated (g*BRCA*m) metastatic breast cancer (MBC)

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**Background:** Olaparib (Lynparza®) is approved for the treatment of g*BRCA*m HER2-negative MBC based on the OlympiAD study results. Olaparib-induced DNA repair defects may attract tumor-infiltrating lymphocytes, upregulate programmed cell death ligand-1 (PD-L1) and release tumor neo-antigens upon cell death. Here, the objective was to assess the efficacy and safety of olaparib in combination with durvalumab (Imfinzi), a PD-L1 agent, in g*BRCA*m HER2-negative MBC (NCT02734004).

**Methods:** Patients (pts) with HER2-negative, g*BRCA*m MBC were eligible; prior platinum therapy without disease progression was allowed. Pts received olaparib 300 mg tablet BID for a 4-wk run-in, followed by a combination of olaparib 300 mg BID and durvalumab 1.5 g IV q 4 wks. The combination was continued until progressive disease by RECIST 1.1. Tumor assessments were done at baseline, 4 wks, and every 8 wks thereafter. The primary endpoints were disease control rate (DCR) at 12 wks, safety and tolerability. The secondary endpoints included DCR at 28 wks, objective response rate (ORR), duration of response (DoR), progression-free survival (PFS), overall survival (OS) and a biomarker analysis. A Bayesian predictive probability design was used for the statistical analysis of the primary endpoint.

**Results:** Thirty-four pts were included in the safety, and 32 pts in the efficacy analysis. At data cut-off (DCO) on 30 Mar 18, the 12-wk DCR was 81% (90% CI: 66%, 92%) and 28-wk DCR 47% (90% CI: 32%, 63%). The ORR for the overall cohort was 56% (95% CI: 38%, 74%), with 1 (3%) complete response (CR), 17 (53%) partial response (PR), 8 (25%) stable disease (SD), and 6 (19%) progressive disease (PD). Median DoR was 9.2 months (interquartile range, 5.5 to 12.9 months). First- and second-line pts showed higher response rates (70% and 73%, respectively) compared to pts with 3 or more lines of prior chemotherapy (27%) (Table 1). The median PFS for the overall cohort was 6.7 months (95% CI of 4.6, 11.7 months). With median follow-up of 14.5 months, the median OS has not been reached. At the time of DCO, 8 (25%) pts remained on combination therapy. The most common Grade 3 or 4 adverse events reported were anemia (11.8%), neutropenia (8.8%), and pancreatitis (5.9%). Efficacy and safety results with longer follow-up will be presented. Biomarker analysis on CD3, CD8 and PD-L1 expression in the archival tissue and in paired biopsies obtained at baseline and after olaparib monotherapy run-in, will also be presented.

**Conclusions:** The addition of durvalumab to olaparib showed promising activity with the ORR in the early line g*BRCA*m MBC pts, comparing favorably with olaparib monotherapy (OlympiAD) (>70% in 1–2L MEDIOLA vs 60% overall in OlympiAD). In addition, the median DoR appeared longer with the combination (9.2 months in MEDIOLA vs 6.4 months in OlympiAD). Approximately one-third of the pts in MEDIOLA were heavily pretreated (3L+) and as expected, the ORR was lower in these pts. Due to the small number of pts, these results warrant confirmation of activity in the early-line setting. Further MBC cohorts are in development.

Best responses by line of therapy

	1L	2L	3L+
n	10	11	11

CR	0	1	0
PR	7	7	3
SD	1	2	5
PD	2	1	3
ORR, %	70	73	27



# 2018 San Antonio Breast Cancer Symposium ®

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Pre-therapeutic PD-L1 expression and dynamics of Ki-67 and gene expression during neoadjuvant immune-checkpoint blockade and chemotherapy to predict response within the GeparNuevo trial

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## Background

In the GeparNuevo trial, the PD-L1 inhibitor durvalumab increased the rate of pathologic complete response (pCR; ypT0 ypN0) in triple-negative breast cancer if treatment started in a two-week window before neoadjuvant taxane/anthracycline chemotherapy (61 % pCR vs. 41%; p = 0.048; Loibl et al. ASCO 2018). Overall, pCR rates increased only numerically from 53 % to 44 % (p = 0.281). Herein, we aimed to evaluate the predictive value of PD-L1 immunohistochemistry in pre-therapeutic core biopsies. In addition, we identified dynamics in gene expression using repeated biopsies.

## Patients and Methods

174 patients were randomized to receive durvalumab or placebo with neoadjuvant chemotherapy. In the window part, 117 patients received a single dose of durvalumab (or placebo) before chemotherapy. Core biopsies were taken at three times: pre-treatment ("A"; N=174), after the window part ("B"; N=88) and after 12 weeks of nab-Paclitaxel ("C"; N=33). PD-L1 immunohistochemistry in A-biopsies (Ventana SP263 Assay) was recorded as percentage of cells with membranous staining in tumor cells and lymphocytes (TILs). We defined a tumor as PD-L1 high if  $\geq 25$  % of either compartment was stained. Ki-67 was stained on all available A, B and C biopsies (MIB-1, Dako, 1:100) and recorded as the percentage of tumor cells with nuclear staining. We profiled all available biopsies with targeted RNASeq using the HTG EdgeSeq platform (Oncology Biomarker panel, 2560 genes). Sequencing (IonTorrent S5) was successful in 162 A-, 79 B- and 31 C-biopsies.

## Results

PD-L1 expression was high in 24 % of A-biopsies and was predictive for pCR in the complete cohort (OR 2.561; 1.183-5.554; p = 0.017). PD-L1 status of the TILs, but not of the tumor cells, was predictive (OR 1.313; 1.040-1.656; P= 0.022). The effect was not specific for durvalumab treatment. Higher levels of Ki-67 were predictive for pCR in B- biopsies in all patients (OR 1.399; 1.053-1.858; P =0.021) and in the placebo arm, but not in the durvalumab arm. Ki-67 levels in C-biopsies were not predictive; neither was the change in Ki-67 between pre-treatment and later time points (B vs. A or C vs. A).

In a differential mRNA expression analysis (A vs. B), we found seven differentially expressed genes after one dose of durvalumab. We observed strong effects on gene expression after taxane treatment (A vs. C), but no significant difference according to treatment. These genes were associated with biological processes involved in therapy response. The pre-treatment levels of 12 of 69 markedly differentially expressed genes were associated with worse response to chemotherapy.

## Conclusion

In A-biopsies, PD-L1 in TILs was predictive for response, and in B-biopsies, Ki-67 was predictive, but neither marker could specifically predict response to durvalumab. We observed limited effects of a single half-dose of durvalumab on global gene expression, but could identify substantial differential expression after taxane treatment. The evaluation of gene expression dynamic offers a promising approach for the identification of resistance-associated markers.

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# 2018 San Antonio Breast Cancer Symposium ®

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Comprehensive profiling of poor-risk paired primary and recurrent triple-negative breast cancers reveals immune phenotype shifts

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**Background:** Prognosis for triple-negative breast cancer (TNBC) patients remains poor, due in part to the lack of effective targeted therapies in the advanced setting. Emerging clinical data indicates reduced efficacy of immune checkpoint inhibitors in heavily pre-treated TNBC, but the underlying mechanisms are poorly understood. To better understand the immune phenotypic evolution of paired TNBCs, we studied the genomic and transcriptomic profiles of tumors from patients undergoing treatment for TNBC.

**Methods:** We analyzed primary and recurrent TNBCs from 55 poor-risk patients, including 44 paired primary-metastatic samples and 11 paired metastatic tumors. FoundationOne® and RNAseq was successful on 89 specimens and 97 specimens, respectively. In addition to somatic alterations, FoundationOne® provided tumor mutational burden (TMB). From RNAseq, we ascertained the TNBC molecular subtypes, and the mRNA expression of immune-related genes. Stromal tumor-infiltrating lymphocytes (stromal TILs), recurrence-free survival, and overall survival were also studied.

**Results:** From FoundationOne® sequencing, a mutational landscape typical of TNBCs was observed across both primary and recurrent disease specimens, with *TP53* mutated in 82.0% of specimens, and *BRCA1* and *BRCA2* mutated in 4.5% and 16.9% of specimens, respectively. Sample profiles revealed minimal shifts in copy number alterations and TMB over time, however, notable TNBC subtype shifts were observed between primary and recurrent tumors. These included an increase in the Lehmann/Pietenpol-defined basal-like 1 phenotype (BL1, 12.8% to 20.9%), an increase in the mesenchymal phenotype (M, 12.8% to 20.9%), and a significant decrease in the immunomodulatory phenotype (IM, 27.1% to 2.3%). Similarly, tumors exhibited a downward shift in gene expression delineating the Burstein-defined basal-like immune-activated phenotype (BLIA, 37.0% to 14.3%). Composite expression of immunomodulatory gene signatures representative of Th1/Th2 responses, IFN $\gamma$ -related inflammation, M1/M2 macrophage activation and suppression, etc., was decreased in the recurrent tumors compared to the primaries ( $p = 0.01$ ), and histopathology-derived percent stromal TILs were significantly decreased in the recurrent TNBCs ( $p = 0.02$ ). However, higher stromal TILs ( $\geq 30\%$ ) were not associated with improved overall survival when measured in primary specimens ( $p = 0.15$ ), or with the time from relapse to death when measured in recurrent specimens ( $p = 0.65$ ) in this cohort of immunotherapy-naïve patients.

**Conclusion:** In this retrospective study of paired TNBCs, significant transcriptomic phenotype shifts were observed as patients progressed, while only minor genomic shifts were seen. Selective immune profiling showed significantly reduced TILs and immune-activating gene expression signatures in recurrent TNBCs, which may explain the lack of efficacy of immunotherapeutic agents in heavily pretreated TNBCs. Further studies are ongoing to understand the proteomic landscape shifts in TNBCs over time and to identify novel targeted agents appropriate for recurrent disease.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD5-08

Neoadjuvant chemotherapy alters the genomic landscape and immune microenvironment of breast cancers

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Understanding how standard-of-care drug treatments affect tumor intrinsic biology and microenvironment is critical for elucidating drug resistance mechanisms and developing better combination therapies as well as new therapies. To characterize the effects of neoadjuvant chemotherapy (NAC) on the genome, transcriptome and tumor infiltrating leukocytes (TILs), we have conducted whole exome and whole transcriptome sequencing of a large longitudinal breast cancer cohort consisting of 146 cases and 281 paired tumor samples. In total, 52 (38%) patients achieved pathologic complete response (pCR) while 85 patients (62%) had residual disease with standard chemotherapy regimen. Tumor biopsies were collected for each patient at three time points – pre-treatment, three weeks after the first cycle of anthracycline and cyclophosphamide (AC) and at the time of surgery after 3 more cycles of AC followed by 4 cycles of taxane or taxane plus Herceptin in case of HER2+ subtype. We detected 5,955 protein-altering somatic mutations affecting 4,414 genes in pretreatment samples and 502 acquired mutations in surgery samples affecting 477 genes including 19 recurrently mutated genes such as *TP53* and *NOTCH1*. Across all subtypes, 4,346 genes were differentially expressed (DE) following NAC treatment and significantly enriched in pathways such as cell cycle, ER signaling, PI3K/mTOR, immune and metabolism. Expression-based virtual microdissection analysis indicated that NAC treatment induced an increase in the fractions of stromal and adjacent normal tissue compartment, consistent with observed reduction in tumor cellularity. To assess the NAC induced changes in the molecular landscape of these tumors, we compared molecular features including gene expression signatures, mutation prevalence and copy number alteration between three time points while adjusting for confounding effects of molecular subtype and tumor cellularity. We found that NAC induced dynamic changes in gene expression signatures associated with proliferation and immunomodulatory treatment response. We further validated the observed pattern of change in TILs through histopathology and digital imaging analyses. In pretreatment tumors, 116 genes were DE between patients with pCR vs. those with residual disease with significant enrichment in immune/inflammatory pathways. Further, pre-treatment TIL levels were found to be significantly associated with pCR, echoing previous reports in breast cancers that implicated anti-tumor immunity in mediating the efficacy of chemotherapies. Our analyses also revealed associations between NAC response and baseline genomic attributes such as genomic alterations that affect DNA damage repair pathways. Taken together, these results suggest that NAC induced a multitude of changes on the genomic landscape and immune microenvironment of breast cancers, some of which point to combination strategies with immunomodulatory therapies and therapies that target DNA damage repair.

# 2018 San Antonio Breast Cancer Symposium ®

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The immunomodulatory potential of denosumab in breast cancer: results from D-BEYOND, a window of opportunity trial evaluating a RANKL-ligand (RANKL) inhibitor and its biological effects in young pre-menopausal women diagnosed with early breast cancer

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## Background

Breast cancer (BC) in young women has unique biology and poor prognosis. Previous reports suggest that they often express *RANKL*, which was also shown to play a role in mammary tumorigenesis and various immune processes. Here, we present the primary results of D-BEYOND, a window study investigating the biological activity of the *RANKL* inhibitor; denosumab in pre-menopausal BC patients.

## Methods

D-BEYOND is a prospective, phase IIA, single-arm, multicenter study assessing the effect of denosumab on BC biology in premenopausal women with early BC (NCT01864798). Patients received two subcutaneous injections of denosumab (120mg), one week apart, followed by breast surgery. The primary endpoint was geometric mean change in tumor Ki67 assessed by immunohistochemistry (IHC). Blood, tumor and normal adjacent breast tissue were collected pre- and post-treatment. Serum levels of RANKL, OPG and CTX were assessed by ELISA. RNA was extracted from fresh-frozen tissue and RNAseq was performed. DESeq2 was used for differential expression analysis, GAGE was used for pathway analysis and CIBERSORT was used to infer immune cell subsets between pre- and post-treatment. Ki67, CD4/Foxp3 and CD4/CD8 IHC were performed on FFPE tissue to further assess the immune microenvironment. The percentage of TILs was independently evaluated by two pathologists on H&E slides. Pre- and post-treatment values were compared using a paired t-test.

## Results

A total of 27 patients were enrolled in the study between October 2013 and July 2016. The median age was 45 years (range 35-51 years). Tumors of 21 patients were hormone receptor positive (77.8%), 4 were HER2 positive (14.8%) and 2 were triple negative (7.4%). No serious adverse events were reported, the most frequent non-serious adverse event being arthralgia (14.8%). After treatment, serum levels of CTX and RANKL decreased in all patients ( $P < 0.001$ ) whereas OPG increased in 76.9% of patients ( $P = 0.009$ , 95% CI 0.56-0.91). There was no significant reduction of Ki67 values from baseline (geometric mean [GM] change after treatment; 0.98, 95% CI 0.76-1.26;  $P = 0.90$ ). Twenty-four pre- and post-treatment tumor pairs were available for RNAseq, IHC and TILs evaluation. There was a significant increase in the percentage of stromal TILs after treatment (GM change of 1.75, 95% CI 1.28–2.39;  $P = 0.001$ ). 1084 differentially expressed genes were identified and pathway analysis revealed enrichment of several immune processes. CIBERSORT revealed an enrichment of CD8+ T cells (GM change 1.72, 95% CI 1.19–2.48;  $P = 0.006$ ) and a decrease of Treg cells (0.71, 95% CI 0.52–0.98,  $P = 0.040$ ). These results were confirmed by IHC of CD8+ and CD4+/Foxp3+ cells (GM change 1.59, 95% CI 1.14–2.21;  $P = 0.008$  and 0.63, 95% CI 0.49–0.83,  $P = 0.001$ , respectively).

## Conclusion

Short course of denosumab did not reduce tumor proliferation rate. However, it induced a significant increase in TILs and CD8 cytotoxic T cells, while Treg infiltration decreased. These findings suggest an immunomodulatory role for denosumab in young breast cancer and that its use in combination could boost immunotherapy efficacy.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD5-09

Immune parameters associated with survival in triple negative and HER2-positive breast cancer patients with 10 years of follow-up

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The clinical utility of tumor-infiltrating lymphocytes (TIL) is actively being investigated in breast cancer (BC). It is unclear whether TIL spatial location and organization in tertiary lymphoid structures (TLS) have an impact on prognosis. Additionally, the significance of PD-1 and PD-L1 expression is being debated due to conflicting data from several studies. We hypothesize that the presence, extent and spatial location of multiple immune biomarkers, reflecting ongoing immune responses, will be consistently associated with a good prognosis in highly infiltrated BC [triple-negative (TNBC) and HER2+].

The relationship between these immune biomarkers and clinical outcome was examined in the TNBC and HER2+ cohorts of node-positive BC patients enrolled in the BIG 02-98 adjuvant phase III trial with available material for immunohistochemical (IHC) labeling (N=113 and N=136, respectively). HER2+ patients did not receive trastuzumab. Dual IHC staining was performed on full-face consecutive tissue sections. Scoring was independently performed by two pathologists, blinded to the clinical data, and included: global, intratumoral and stromal TIL and TLS, assessed on CD3/CD20 slides; the percentage and location of PD-1 and PD-L1 expression, assessed on PD-1/PD-L1 slides. TIL were considered as a categorical variable with different cut-offs used for each parameter and for each cohort (TNBC and HER2+). Invasive disease-free survival (I-DFS) and overall survival (OS) were analyzed (median follow-up: 10 years). Cox proportional hazard models were used for survival analyses.

The TNBC cohort revealed an association between global TIL and outcome [adjusted hazard ratio (HR) for I-DFS: 0.27 (0.15-0.51); OS: 0.26 (0.13-0.53)]. Similar results were observed for stromal and intratumoral TIL. PD-L1 expression within TLS was an independent predictor of OS, after adjustment for tumor size and age [HR: 0.30 (0.09-0.99)]. Multivariate analysis reveals this effect was principally driven by high stromal TIL ( $\geq 17.5\%$  based on CD3/CD20 assessment) ( $\chi^2$  OS: p=0.009). In contrast, no significant prognostic associations were found in the overall HER2+ cohort. However high T cell TIL were associated with improved I-DFS and OS in the ER-/HER2+ group [I-DFS: 0.34 (0.14-0.80); OS: 0.32 (0.12-0.86)] and stromal TIL were associated with improved I-DFS in the ER+/HER2+ group [HR: 0.29 (0.09-0.94)] (univariate analyses). No significant associations between the number of TLS nor the expression of PD-1 with outcomes were observed in either cohorts.

The presence of PD-L1+ TLS, driven by high baseline TIL, was associated with an excellent prognosis in node-positive TNBC. This observation might reflect specific immune activities taking place in these mini lymph node-like structures adjacent to the tumor bed where specific antitumor memory immune responses could be generated. No different prognostic impact was observed when analyzing TIL spatial location. Although the statistical power of the study might be limited, in line with previous findings our data reveal that, among the immune parameters evaluated, TIL are the strongest predictor of outcome in TNBC, while PD-L1+ TLS could be a new and important parameter that requires further investigation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD5-10

Immune characterization of matched primary and multiple metastatic samples issued from an institutional autopsy cohort

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## Introduction

While immune infiltrates have already been extensively characterized in primary tumors (P), data on breast cancer metastases (M) remains limited. To this end we quantified and qualified the immune cells in a unique cohort of multiple matched P and M samples selected from an institutional breast cancer autopsy cohort.

## Patients and methods

Twenty-three patients were selected from an institutional autopsy program (Semmelweis University, Budapest, Hungary) based on matched P and M sample availability (124 samples). All samples were centrally characterized for estrogen (ER), progesterone (PR) and HER2 receptors. The primary molecular subtypes were as follows: 9 ER+/PR+/HER2-, 8 triple negative and 6 HER2+. Ten patients relapsed  $\leq 1$  year after diagnosis and were further referred to as “early relapsers”, as opposed to the remaining qualified as “late relapsers”. Immunohistochemistry (IHC) was carried out against CD3/CD20 and CD4/CD8 in 21 patients (119 samples). Tumor infiltrating lymphocytes (TILs) were assessed on hematoxylin and eosin (H&E) and CD3-stained slides. Gene expression data were generated using the NanoString nCounter assay (PanCancer Immune Profiling Panel) for 11 patients (35 samples) and analyzed using the R package *NanoStringQCPro*. The scores from published immune gene signatures were calculated as a weighted sum of the expressions of their genes. All samples were analyzed for 22 immune cell subtypes relative abundance using CIBERSORT.

## Results

TILs assessed on H&E and CD3-stained slides were weakly correlated ( $Rho = 0.38$ ,  $p < .001$ ). TIL levels as well as the number of tertiary lymphoid structures (TLS) were significantly lower in Ms as compared to Ps ( $p < .001$ ). Among the different metastatic sites, the lung was more infiltrated when considering CD3+ and CD4+ cells ( $p = .01$  and  $.02$ , respectively). We further observed significantly higher levels of TILs, CD3+, CD4+ and CD8+ cells in the Ms but not in the Ps from late relapsers as opposed to those from early relapsers. Gene expression analyses further confirmed these observations as several immune gene signatures displayed significantly higher scores in the Ms from late compared to early relapsers. An unsupervised analysis identified 13 genes significantly differentially expressed between Ps and Ms: CSF1R, CXCL14, CYBB, IL21R, IL2RB, TNF and TNFSF15 were upregulated in Ps while BCL2L1, C7, HSD11B1, and PSMB7 were upregulated in Ms. The matched P/M CIBERSORT analyses revealed a distinct composition of immune cell types between P and M of a same patient. Apart from a potential increase in M0 macrophages, no common trait was observed in immune cell composition between the Ms from the different patients.

## Conclusion

This is to the best of our knowledge the first study characterizing the immune infiltration in patients with multiple matched P and M samples. The results suggest that Ms have not only a globally lower immune infiltration as compared to Ps, but also a different immune composition. Additionally, Ms from late relapsers are more infiltrated as compared to early relapsers. The present data also uncovers not only important inter-patient but also intra-patient immune heterogeneity, which should be taken into consideration for optimal treatment decision.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD5-11

Differential prognostic value of TILs in BC subtypes is attributed to antigen load and T cell suppression

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In breast cancer (BC), tumor infiltrating lymphocytes (TILs), in particular CD8 T cells, have prognostic value, yet response rates to immune therapies are generally low and differ significantly across subtypes.

To define immune markers that would facilitate the identification of patient subgroups which respond to immune therapies, we assessed the quality and quantity of T cell evasive mechanisms in BC subtypes, using large gene expression datasets (microarray GSE2034/5327 and DNA/RNAseq: EGAS00001001178). To this end, we generated a 152 immune-gene signature, based on quantitative measurements of TILs. This TIL signature highly correlated with numbers of TCR reads (MIXCR,  $r=0.91$ ,  $p<0.0001$ ) and frequencies of activated lymphocytes (Cibersort, such as CD8 T cells, follicular helper CD4 T cells and plasma cells), whereas this signature inversely correlated with frequencies of immune suppressor cells (such as M2 macrophages, MDSCs and neutrophils). We tested this signature for its prognostic value in our cohort of 344 LNN patients, not adjuvantly treated with hormone- or chemotherapy (GSE2034/5327). Luminal A and B subtypes had low average TIL scores. Her2 and basal-like tumors had equally high TIL scores, yet an association between TIL score and survival was only observed in the Her2 molecular subtype ( $HR=0.24$ ,  $p<0.001$ ). Multi-immune parameter analysis demonstrated that Her2 BC is characterized by a high antigen load (extent of neo and cancer germline antigens) and relatively low frequencies of suppressor cells. In contrast, basal-like BC is characterized by the highest antigen load ( $p=0.003$ ), the highest number of clonally expanded T cells ( $p<0.001$ ), the highest frequency of immune suppressor cells ( $p<0.001$ ), and significantly enhanced expression of co-inhibitory molecules (such as LAG3 and PD-L1). Notably, a subset of basal-like tumors had mutations in antigen presentation/processing pathways and/or lacked gene-expression of molecules involved in this pathway, suggesting selection of lesser immunogenic tumor cells. Interestingly, regardless of low antigen load, also normal-like BC had a high TIL score, yet no clonally expanded T cell clones, which suggests lack of a tumor-specific T cell response.

Collectively, these data suggest that not TIL score per se but rather the extent of immunogenicity and T cell suppressive mechanisms discriminate BC subtypes. These results point toward the requirement of different combinatorial approaches to boost the efficacy of immune therapies across BC subtypes.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD5-12

Spatial mapping of the immune microenvironment in primary triple-negative breast cancer (TNBC) and association with neoadjuvant therapy response

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**Background:** Emerging data suggest that some patients with TNBC could benefit from the addition of immune-based therapy. This observation is in part due to the significant association between distinct tumor-infiltrating lymphocytes (TILs) and prognosis in TNBC. Efforts to comprehensively characterize the immune microenvironment of TNBCs are critically important to gain a better understanding of the immune landscape and how it influences response to both standard chemotherapy and immunotherapy. We previously assessed both stromal TILs (sTILs) and intraepithelial TILs (iTILs) from pre-treatment tumor samples from patients enrolled on a phase II study of neoadjuvant gemcitabine, carboplatin and iniparib (PrECOG 0105; NCT00813956). We found that both iTILs and sTILs significantly associated with pathologic response. Furthermore, we assessed a novel 'in silico flow cytometry' gene expression-based method, CIBERSORT, designed to assess overall immune content and deconvolute the relative levels of distinct leukocyte subsets in tumors. Specific leukocyte subsets significantly associated with pCR included activated memory CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and M1 macrophages (all  $p < 0.05$ ). To validate these findings and further explore spatial mapping of these immune cell subsets we undertook this analysis.

**Methods:** We performed SPARTA - Spatial Perception And Regional Tumor Analysis - a multiplexed immunohistochemistry-based technology with data visualization and analysis modules that is designed to capture clinically relevant information about the tumor microenvironment in 72 pre-treatment FFPE tumor sections from patients enrolled on PrECOG 0105. SPARTA was used to test and analyze the coordinated expression of PD-L1, PD-1, CD45RO, CD4, CD8 and HLA-DRA in the study cohort from whole slide scanned images. Pathologic response at the time of surgery was evaluated using the residual cancer burden index. Germline BRCA1 and BRCA2 status was known for all patients.

**Results:** Of 72 samples, 67 were evaluable for SPARTA analysis. Within a subset of tumors separately profiled by both SPARTA (FFPE/IHC microscopy) and CIBERSORT (Frozen/RNA GEP), we found significant correlation for clinically relevant TIL subpopulations, including for CD8<sup>+</sup> T cells ( $r = 0.83$ ,  $p < 0.0001$ ), and activated memory CD4<sup>+</sup> T cells ( $r = 0.78$ ,  $p < 0.0001$ ). On average, the SPARTA frequency of CD8, PD-L1 and CD4 expressing cell types were higher in samples from the pCR group when compared to the no pCR group. Subpopulation analysis revealed that, on average, the frequency of CD4<sup>+</sup>HLA-DRA<sup>+</sup> cells were higher in samples from the pCR group when compared to the no pCR group. Samples from the pCR group were associated with a higher local spatial density of CD45RO<sup>+</sup>HLA-DRA<sup>+</sup>CD4<sup>+</sup> cells when compared to the no pCR group. Location- and proximity-based analyses are ongoing and will be presented. Analyses based on germline BRCA1/2 mutant versus wild-type status, as well as associations between tumor mutation burden and neoantigen burden will also be presented.

**Conclusions:** Spatial mapping of the immune microenvironment in primary TNBC reveals distinct immune cell populations associated with response to neoadjuvant platinum-based therapy.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-07

Trends in the cost of care for breast cancer among women with commercial insurance

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**Background:** Breast cancer care imposes a significant financial burden to U.S. healthcare systems and has become a key focus in the health care debate. Therapies for breast cancer are expensive, and the economic burden of these therapies may be rising due to the rapid introduction of pricey new drugs and techniques. There are limited data on the health care costs of individuals with breast cancer after initial diagnosis and how these costs have changed over time.

**Methods:** We conducted a retrospective analysis of commercially insured adult women with newly diagnosed non-metastatic breast cancer (identified via previously published claims-based algorithms) using 2007-2016 data from a large US health plan available in OptumLabs® Data Warehouse. We included patients with continuous health plan coverage for at least 2 years after initial diagnosis 2007-2014 and assessed how total health care spending and out-of-pocket costs (paid amounts) changed over this time. Costs were adjusted to 2016 US dollars using the general Consumer Price Index. Inpatient, outpatient, and outpatient pharmacy costs were evaluated. A multivariable logistic regression model was used to examine predictors of above average cost (cost > mean for that year of diagnosis).

**Results:** A total of 12,446 newly diagnosed breast cancer patients were identified (mean age, 51.6 years). Forty percent had undergone mastectomy, 38% chemotherapy, and 63% radiation. After adjustment for inflation, total healthcare costs increased 29.7% from 2007 to 2014 (Table 1), with increases primarily observed during the first year after diagnosis. Out-of-pocket costs remained relatively stable, and accounted for 5.3% of the total spending. Approximately 80% of the total costs were related to care received in the outpatient setting. Factors independently associated with above average spending included treatment with mastectomy [OR 1.78 (95% CI 1.5-2.1)], reconstruction [OR 3.0 (95% CI 2.6-3.5)], radiation [OR 4.0 (95% CI 3.4-4.7)] and chemotherapy [OR 18.4 (95% CI 16.6-20.3)].

Table 1. Average healthcare spending over time

Year of diagnosis	Mean cost during first year after diagnosis		Mean cost during second year after diagnosis	
	total	out-of-pocket	total	out-of-pocket
2007	\$80,296.17	\$4,271.25	\$16,559.21	\$1,907.01
2008	\$84,126.70	\$4,445.78	\$16,785.43	\$2,205.98
2009	\$88,331.45	\$4,728.42	\$17,005.68	\$2,214.93
2010	\$91,502.58	\$5,067.78	\$17,243.91	\$2,126.19
2011	\$93,826.40	\$5,089.45	\$16,862.45	\$2,027.96
2012	\$96,690.06	\$5,449.91	\$17,814.09	\$2,179.26
2013	\$104,064.93	\$5,678.19	\$17,087.47	\$2,115.97
2014	\$104,169.74	\$5,620.51	\$16,714.12	\$1,590.67

**Conclusions:** Breast cancer care is increasingly expensive during the first year after diagnosis, and costs are greatest for the recipients of more aggressive treatments. Costs during the second year after diagnosis have remained relatively stable.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-08

Medicare costs for women after breast cancer: Preparing for survivorship

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## **BACKGROUND**

Improvements in breast cancer survival and increasing population life expectancy have resulted in a growing number of women receiving subsequent health care after breast cancer diagnosis and treatment. We sought to determine the magnitude of increases in healthcare costs related to breast cancer survivorship, in anticipation of predicted increases in enrollment, higher intensity utilization, and greater healthcare spending among Medicare beneficiaries.

## **METHODS**

Women age 65+ diagnosed with stage 0-III breast cancer in 1998, 2003 or 2008, were identified from the SEER database linked to Medicare records. After restrictions they were propensity score matched to a comparable group of non-breast-cancer women on demographic characteristics and co-morbidities at time of diagnosis based on the Elixhauser co-morbidity index. Line payments to care providers were then calculated for the first year of care after diagnosis (cases only) as well as years 2-6, 8-11 and 12-16 post-diagnosis (cases and controls). Direct Medicare costs were adjusted for inflation using the experimental Medicare costs Price Index and compared under real world and alternative survival scenarios.

## **RESULTS**

Overall, the costs of care were progressively higher in later cohorts across all time periods. Differences in survivorship were the primary driver of differences in costs between breast cancer cases and controls. All-stage costs in years 2-6 were higher in the cancer group (\$2,499, \$10,261 and \$12,029 higher per-person in 1998, 2003 and 2008 respectively), however, higher mortality in the cancer group reduced the costs and quantity of care received in later years [years 7-11 (\$2,183 lower per-person in 1998) and 12-16 (\$2,431 lower per-person in 1998)]. In pairs with identical survival, costs in the cancer group were significantly higher than in matched non-breast-cancer controls across all time periods (years 2-6: \$4,799, \$9,545 and \$12,245 higher in 1998/2003/2008; years 7-11: \$2,922 and \$5,597 higher in 1998/2003). Stratification by stage changed the magnitude but not the general pattern of our results. The first year of care in 2003 was on average \$4,933 dollars higher than in 1998; in 2008 costs again increased by \$4,223 per-person. In years 2-6 the cost of cancer care increased by \$12,440 (2003 vs 1998) and \$3,456 (2008 vs 2003) per-person; Finally, cancer care for years 7-11 in 2003 \$3,964 higher than in 1998 per-person.

## **CONCLUSION**

Improved breast cancer survival and increased overall life expectancy among women in the United States will contribute to higher Medicare expenditures. Future risk-based capitation schemes should account for these advancements when preparing for healthcare delivery after cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-09

Cost effectiveness of bilateral prophylactic mastectomy with and without different breast reconstruction techniques versus screening in women with high risk of breast cancer in the Canadian Province of Ontario

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**Purpose:** We aimed to investigate the cost-effectiveness of mastectomy with and without different reconstruction for the purpose of determining which strategies represent value for money and identify the most cost-effective technique from the perspective of Ontario's health care system.

**Methods:** We developed a decision analytic model to project the lifetime clinical and economic consequences of different strategies. The decision model was parameterized using 10-year follow up and cost data from Ontario administrative health databases and Ontario Cancer registry and utility data from secondary Canadian sources. Costs are presented in 2018 Canadian dollars. Future costs and benefits were discounted at 5%.

**Results:** Compared to organized screening-based strategy, surgical strategies ranged from being more effective and cost-saving and up to being associated with an incremental cost effectiveness ratio (ICER) of \$63,010 per quality-adjusted life year (QALY) gained

Table 1 Baseline life-time outcomes of the decision model.

			Extensive breast cancer screening alone vs. surgical interventions		
Strategy	Overall QALYs	Overall cost	Inc. QALY	Inc. cost	ICER per QALY gained
Extensive breast cancer screening	18.549	\$90,231	Ref.	Ref.	Ref.
Prophylactic bilateral mastectomy without breast reconstruction	19.057	\$82,011	+0.508	-\$8,220	Cost-saving
Prophylactic bilateral mastectomy with two-stage traditional TE-implant immediate breast reconstruction	19.364	\$111,319	+0.815	+\$21,088	\$25,868 (dominated)
Prophylactic bilateral mastectomy with one-stage ADM-assisted implant immediate breast reconstruction	19.706	\$101,359	+1.157	+\$11,128	\$9,615
Prophylactic bilateral mastectomy with two-stage ADM-assisted TE-implant immediate breast reconstruction	19.065	\$122,757	+0.516	+\$32,526	\$63,010 (dominated)
Prophylactic bilateral mastectomy with any type of autologous immediate breast reconstruction (with or without TE or breast implant)	19.501	\$114,014	+0.951	+\$23,784	\$24,988 (dominated)
Prophylactic bilateral mastectomy with one-stage non-ADM immediate breast reconstruction	19.408	\$103,512	+0.859	+\$13,282	\$15,457 (dominated)
Prophylactic bilateral mastectomy with delayed breast reconstruction	19.241	\$107,582	+0.691	+\$17,351	\$25,087 (dominated)

ADM;acellular dermal matrix ; TE=Tissue Expander; ICER=Incremental cost-effectiveness ratio; QALY=Quality adjusted life year

, with BPM with immediate one-stage acellular dermal matrix (ADM)-assisted implant breast reconstruction having the greatest incremental QALY of 1.157 and lowest ICER of \$9,615. Incorporating the PBM with one-stage ADM-assisted implant immediate breast reconstruction as the standard surgical strategy in Ontario would result in the largest total annual net gains of 20 QALYs and \$ 1.7 million.

**Conclusion:** The choice of breast reconstruction needs to be decided based on the patient body habitus, general condition and

goals . BPM with and without reconstruction is likely both clinically and economically attractive. However ,all other things being equal , BPM with immediate one-stage ADM-assisted implant breast reconstruction is the most cost effective strategy and appears to offer the highest value for money.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-10

Association between adherence to cardiovascular medications and cardiovascular events following a diagnosis of early stage breast cancer

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**Background:** Studies show that patients diagnosed with early-stage breast cancer (BC) are more likely to die from cardiovascular disease (CVD) than BC. Adherence to CVD medications, such as statins and antihypertensives, is poor in BC survivors, particularly in the year following diagnosis. The impact of non-adherence to CVD medications on cardiovascular events in BC survivors is unknown.

**Methods:** Using the Surveillance, Epidemiology, and End Results (SEER)-Medicare linked dataset, we evaluated patients with non-metastatic BC who were diagnosed between 2006-2014. Prescriptions were identified for the treatment of hypertension, hyperlipidemia and diabetes. The pre-cancer diagnosis study period for adherence was defined as 1 year prior to the diagnosis of cancer. The follow up adherence period was between years 1 and 2 following the diagnosis of cancer, so the BC treatment period was not included. Adherence was defined as a medication possession ratio of  $\geq 80\%$ . A CVD event was defined as an ischemic event or acute heart failure. Patients with a CVD event prior to diagnosis were excluded. Logistic regression was performed for each non-cancer condition to define factors associated with medication non-adherence. Cox regression was used to calculate the association between CVD medication adherence and time-to-subsequent cardiac events, adjusted for baseline factors. Cox regression was performed separately for each non-cancer condition.

**Results:** Among 23,080 women with BC in the cohort, 15,576 were adherent to at least one CVD medication prior to diagnosis, and of these, 2732 (17.5%) were non-adherent to at least one medication following treatment. Among the women adherent to medications prior to diagnosis, 19.2% were non-adherent to hypertension medications, 26.2% were non-adherent to cholesterol medications, and 30.6% were non-adherent to diabetes medications following the first year of BC treatment. Factors that were associated with non-adherence to anti-hypertensives included receipt of chemotherapy (OR 1.24,  $p < 0.001$ ), other comorbidities (OR 1.34,  $p < 0.001$ ), higher stage (OR 1.18,  $p < 0.001$ ) and hormone receptor negative tumors (OR 1.15,  $p < 0.001$ ). Similar factors were associated with non-adherence to cholesterol medications, whereas only stage and tumor type were associated with non-adherence to diabetes medications. Non-adherence to hypertension medications compared to adherence following diagnosis was associated with an increased risk of having a CVD event (HR 1.33, 95% CI 1.18-1.51,  $p < 0.001$ ; 5-year cumulative incidence of 32% vs 26%, respectively,  $p < 0.001$ ). Similar results were seen for adherence to cholesterol medications (HR 1.21, 95% CI 1.05-1.40,  $p = 0.009$ ) and diabetes medications (HR 1.31, 95% CI 1.09-1.56,  $p = 0.003$ ).

**Conclusions:** In summary, we found that a large proportion of women who were previously adherent to their medications to prevent CVD events prior to their breast cancer diagnosis were non-adherent following treatment. Of concern, non-adherence to any of these classes of medications resulted in an increased risk of having a cardiovascular event. Improving outcomes and reducing morbidity following a breast cancer diagnosis also requires focused attention on non-breast cancer conditions.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-12

Evaluating racial disparities in breast cancer referrals for hereditary risk assessment

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**Background:** There is a pronounced onco-racial disparity in Washington, D.C., which had the highest national incidence of breast cancer in African Americans (AA) patients in between 2010-2015 and the worst outcomes (American Cancer Society). Recent data indicates a higher incidence of deleterious BRCA1 [and BRCA2] mutations and variants of uncertain significance (VUS) in AA patients compared to Caucasian (C) patients when controlled for patients of Ashkenazi Jewish (AJ) populations (Hall 2009). Despite this, AA women meeting National Comprehensive Cancer Network (NCCN) criteria for genetic testing are less likely to complete such testing compared to C women nationally. Studies have investigated psycho-social drivers of minority patient aversion to genetic testing. We hypothesize that lack of physician referral for cancer genetic counseling and testing for AA women contributes to this disparity.

**Methods:** The George Washington Cancer Center (GWCC) Registry was used to identify non-Hispanic African Americans (BNH) and non-Hispanic whites (WNH) treated for breast cancer between 2014-2018. Of those individuals selected for inclusion were those who met select NCCN criteria for referral for genetic evaluation including breast cancer diagnosis under age 50, triple negative breast cancer (TNBC) under age 60, and two primary breast cancers. Only BNH and WNH individuals were included. Excluded patients were those who were not BNH or WNH or who did not meet the NCCN criteria listed above. Patients were then stratified by race according to who underwent genetic evaluation, whether at our Ruth Paul Cancer Genetics and Prevention Service (RPCGPS) or elsewhere, by reviewing GWCC, RPCGPS, and patient clinic records for genetic testing results from outside institutions. Patient charts were used to identify individuals who received a physician referral over the course of their cancer care.

**Results:** 1180 patients were treated at the GWCC for breast cancer (both in situ and invasive carcinoma) between 2014–2018. Of those, BNH n=502; WNH n=435. Twenty-seven percent of BNH and WNH patients met the study criteria for referral for genetic evaluation (n=252; BNH n=115, WNH n=137), including breast cancer diagnosis under age 50 (BNH n=76; WNH n=108), TNBC under age 60 (BNH n=14; WNH n=5), and two primary breast cancers (BNH n=18, WNH n=16). Several patients identified met two or more criteria for referral (BNH n=7, WNH n=8). Physician referral rates differed significantly by race (BNH 76%, n=87 and WNH 91%; n=125;  $\chi^2=11.4$ , p-value<0.001). Of referred patients, there was no significant difference in those who followed-up at RPCGPS by race (BNH 93%, n=81; WNH= 93%, n=116,  $\chi^2=0.0072$ , p-value=0.93).

**Conclusions:** Low genetic testing rates for AA breast cancer patients are an impediment to resolving the prominent onco-racial breast cancer disparities. This study identified physician referral as a potential contributor to racial disparity in the utilization of cancer genetics services. Potential reasons for the discrepancy in referral may include lag in physician education on the topic of hereditary risk and barriers in physician-patient communication. These findings need to be confirmed and explored at other sites to help improve the identification of at-risk women in the AA community.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-01

Prevalence and predictors of self-reported memory ability in a large sample of breast cancer survivors

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**Background:** A substantial subset of women previously treated for breast cancer report deficits in cognitive abilities such as memory. Cancer-related cognitive dysfunction (CRCDD) has been linked to a variety of factors including chemotherapy. However, the reported prevalence of symptoms is variable and investigations of CRCDD correlates in large samples are limited. This study aimed to 1) investigate whether the prevalence of patient-reported memory problems differs as a function of having received chemotherapy and time-since-treatment; and 2) identify additional factors associated with patient-reported memory in a large sample of breast cancer survivors.

**Method:** In this cross-sectional cohort study, self-administered questionnaires including those assessing memory (Multifactorial Memory Questionnaire) and lifestyle behaviors were mailed to 1500 disease-free breast cancer survivors from three time-since-treatment cohorts (early: 6-18 months, middle: 2-4 years, or late: 5-12 years post-treatment). Demographic and clinical information was collected and confirmed from chart review. The prevalence of clinically significant memory dysfunction was estimated using published normative cut-off scores. We tested whether chemotherapy and time-since-treatment affected memory (analysis of variance), or increased the risk of significant memory dysfunction (odds ratio chi-squared test). Using a forward stepwise regression model, we explored whether patient characteristics (age, education, comorbidities, concussion history, adverse life events), type of treatment (chemotherapy, radiotherapy, hormonal therapy), or lifestyle behaviors (adherence to a Mediterranean diet, physical activity, sleep efficiency, stress management practices) were associated with patient-reported memory.

**Results:** 773 questionnaire packages were returned (mean age=60.4±11.7). 436 (56%) survivors had received chemotherapy (Ch+), and 337 (44%) had not (Ch-). 314 (41%) were early survivors, 244 (32%) were middle, and 215 (28%) were late. Ch+ reported poorer memory than Ch- ( $F(1, 764)=12.752$ ,  $p<0.001$ ), with no effect of time-since-treatment or interaction. Prevalence of significant memory dysfunction was higher in Ch+ (28%) than in Ch- (15%) (OR=2.130, 95% CI 1.479-3.066). Younger age and history of concussion were significantly associated with worse patient-reported memory ( $p=0.002$ ,  $p<0.001$ ). Unlike chemotherapy ( $p=0.018$ ), neither radiation nor hormonal treatment was a significant predictor of memory symptoms. Increased physical activity ( $p=0.002$ ) and higher sleep efficiency ( $p<0.001$ ) were associated with better memory. Survivors reporting greater memory symptoms also reported greater use of stress management techniques ( $p=0.026$ ).

**Conclusion:** This large study indicates that chemotherapy doubles the risk of memory symptoms up to at least 10 years post-treatment. Results also point to sleep hygiene and physical activity as potentially meaningful targets for self-management training to reduce CRCDD in breast cancer survivors.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-02

A randomized, controlled trial of high dose vs. standard dose vitamin D for aromatase inhibitor-induced arthralgia in breast cancer survivors

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**Background:** Approximately half of women on aromatase inhibitor (AI) therapy develop AI-induced arthralgia (AIA), and many discontinue the medication because of this common side effect. While Vitamin D has been studied as a treatment for AIA, trial results have been conflicting thus far.

**Patients and Methods:** All subjects were post menopausal women who were beginning adjuvant AI therapy for stage I-III hormone receptor positive breast cancer. Patients were randomized 1:1 to receive standard dose vitamin D3 (800 IU daily for 52 weeks) or high dose vitamin D3 (50,000 IU weekly for 12 weeks, followed by 2000 IU daily for 40 weeks). All patients also took oral calcium 600 mg daily. The primary endpoint was development of AIA, as defined by pre-specified changes in the Health Assessment Questionnaire II (HAQ-II). Secondary endpoints include compliance with AI therapy, and correlation between grip strength and development of AIA. Exploratory endpoint was measurement of inflammatory cytokine reduction in each arm. The trial was designed to enroll 184 patients, but this futility analysis was performed after 93 patients were enrolled. The futility boundary for stopping the trial early was calculated as  $p = 0.47$ .

**Results:** All 93 patients (46 in the high dose arm, and 47 in the standard dose arm) enrolled in the study at the time of the interim analysis were evaluable. The HAQ-II was completed at 12 weeks in 76% on the high dose arm, and 68% in the standard dose arm. Subjects who did not complete the questionnaire were deemed as study failures (i.e. development of AIA was assumed). In the high dose arm, 25 patients (54%) developed AIA, compared to 27 patients (57%) in the standard dose arm. The one-tailed p value is 0.3818, and the Z-score is 0.3, yielding only a 38% conditional power that that study would find a significant difference between the two arms. Thus, the study was terminated early for futility. There was no significant difference between the two arms in adherence to AI therapy. The grip strength and inflammatory cytokine data are pending at this time. They will be ready by the time of the conference.

**Conclusions:** There was no significant signal for benefit of high dose vitamin D supplementation, as compared to standard dose vitamin D, for AIA prevention in post menopausal women taking adjuvant AI therapy. These results further characterize the role of Vitamin D in AIA, and they inform future clinical trials in this arena. Further research is necessary, as this remains an important cause of non-adherence to this highly effective therapy.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-04

Lifestyle intervention study (LISA) in early breast cancer (BC): An RCT of the effects of a telephone-based weight loss intervention (with educational materials) vs educational materials alone on disease-free survival (DFS)

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**Background:** Obesity has been associated with poor BC outcomes. We investigated whether a standardized, telephone-based weight loss lifestyle intervention in recently diagnosed BC patients would lower recurrence and death rates.

**Methods:** We conducted a multicenter RCT comparing mail-based educational material alone (control arm) or combined with a standardized, telephone-based lifestyle intervention (19 calls over 2 years, (intervention arm) that focused on diet (500-100 kcal/day deficit), physical activity (150-200 minutes of moderate-intensity activity per week) and behavior (compliance, relapse prevention) to achieve up to 10% weight loss. 338 (of 2150 planned) T1-3, N0-3, M0 ER/PgR+ BC patients with body mass index (BMI)  $\geq 24$  kg/m<sup>2</sup> receiving adjuvant letrozole were randomized Aug 2007 to Jan 2010 (enrolment ended due to funding loss). Primary outcome was DFS; secondary outcome OS. Weight loss (5.3 vs 0.7% at 6 months and 3.6 vs 0.4% at 24 months in the intervention vs control arms, respectively) has been reported (JCO 2014;32:2331). At 8 years median follow-up (May 2018), DFS and OS were compared using Cox proportional hazards regression.

**Results:** Mean age was 61.6 vs 60.4 years, mean BMI 31.4 vs 31.0 kg/m<sup>2</sup> and adjuvant chemotherapy was received by 56.1 vs 57.5% in intervention vs controls arms respectively. T1/T2/T3 66.7/27.5/5.9% vs 61.7/33.5/3.6% and N0/1/2+ 62.6/28.7/8.8 vs 63.5/32.3/4.2% in intervention vs controls arms respectively. HER2+ in 8.8 vs 15.0% (intervention vs control). 20 of 171 (11.7%) in the lifestyle intervention arm vs 30 of 167 (18.0%) in the mail-based arm had DFS events, HR 0.71, 95%CI 0.41-1.24, p=0.23). DFS curves separated at 2 yrs; beyond 3-3.5 yrs separation approximated 5%. In a landmark DFS analysis of women alive at 24 months, DFS HR=0.68 (0.34-1.37, p=0.28).

**Conclusions:** We identified fewer DFS events in the lifestyle intervention arm. Although loss of funding reduced sample size and lowered power, these results are consistent with a potential beneficial effect of a lifestyle intervention on DFS in postmenopausal ER/PgR+ BC patients. They provide strong support for completion of ongoing RCTs (e.g. BWEL) that will provide definitive evidence regarding the effect of lifestyle based weight loss on BC outcomes.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-05

Recreational physical activity and breast cancer risk for women across the familial risk continuum

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**Significance:** Physical activity is associated with a 20-30% reduced breast cancer risk for women unselected for risk, yet it is not known if this benefit extends to women spanning the range of familial risk. We addressed this gap by examining the association of recreational physical activity and breast cancer risk using a large cohort of women over-sampled for having breast cancer family history.

**Methods:** We studied 15,622 women, including 1,171 *BRCA1* and *BRCA2* gene mutation carriers, who were unaffected with breast cancer at baseline and followed prospectively for cancer (median follow-up = 10.2 years). At baseline, women self-reported average hours per week of recreational moderate and strenuous physical activity during the past 3 years. We categorized women into quintiles based on total metabolic equivalents (METs) per week (1 hour moderate = 4 METs; 1 hour strenuous = 7 METs) and examined associations with risk of invasive breast cancer, overall and stratified by baseline age, using multivariable Cox proportional hazards regression. We tested for multiplicative and additive interaction by familial risk, defined as the estimated absolute lifetime breast cancer risk predicted using the Breast Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) model.

**Results:** Combining women of all ages, doing any recreational physical activity (versus none) was associated with a decreased breast cancer risk (hazard ratio (HR) = 0.75, 95% confidence interval (CI) = 0.63-0.90), but there was no decrease in risk with increasing METs per week (p-trend = 0.27). Only for women 50-59 years at baseline was there evidence for a dose-response trend with increasing physical activity (p-trend = 0.04). The most physically active women, quintile 5 (Q5), had an estimated 39% reduced breast cancer risk compared to the least physically active women, quintile 1 (Q1), in this age group (HR = 0.61, 95% CI = 0.40-0.93). We found no evidence of interaction with familial risk, overall or stratified by baseline age (all p > 0.05).

**Conclusion:** Performing recreational physical activity appears to be associated with a reduced breast cancer risk for women spanning the familial risk spectrum.

Table 1. Recreational physical activity and breast cancer risk

	Any Physical Activity		Average Metabolic Equivalents per Week				
			Q2	Q3	Q4	Q5	
	N	HR (95% CI) <sup>a</sup> <sup>b</sup>	HR (95% CI) <sup>a</sup> <sup>c</sup>	HR (95% CI) <sup>a</sup> <sup>c</sup>	HR (95% CI) <sup>a</sup> <sup>c</sup>	HR (95% CI) <sup>a</sup> <sup>c</sup>	P-Trend
Total Sample (N=15,622)	13,416	0.75 (0.63, 0.90)	0.81 (0.67, 1.00)	0.81 (0.67, 0.98)	0.85 (0.70, 1.05)	0.88 (0.71, 1.08)	0.27
Baseline Age							0.50 <sup>d</sup>
18-39 years (N=5,586)	5,096	0.93 (0.61, 1.42)	0.77 (0.50, 1.18)	0.96 (0.66, 1.40)	0.99 (0.66, 1.48)	1.00 (0.67, 1.50)	0.73
40-49 years (N=3,646)	3,151	0.58 (0.42, 0.79)	0.88 (0.61, 1.27)	0.70 (0.47, 1.05)	0.67 (0.46, 0.98)	0.85 (0.57, 1.27)	0.18
50-59 years (N=3,185)	2,656	0.66 (0.48, 0.90)	0.69 (0.46, 1.03)	0.73 (0.52, 1.03)	0.76 (0.52, 1.12)	0.61 (0.40, 0.93)	0.04
60-79 years (N=3,205)	2,513	1.03 (0.72, 1.50)	0.94 (0.60, 1.48)	0.95 (0.62, 1.45)	1.21 (0.75, 1.95)	1.21 (0.75, 1.95)	0.29

<sup>a</sup>Adjusted for race/ethnicity, study center, baseline age, education, family history and body mass index; <sup>b</sup>Reference group: no recreational physical activity in past 3 years; <sup>c</sup>Reference group: least physically active quintile (Q1); <sup>d</sup>Wald chi-square test

comparing trends across baseline age groups.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-06

Impact of *BRCA* mutations on chemotherapy-induced loss of ovarian reserve: A prospective longitudinal study

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**Background:** The *BRCA1/2* genes are key members of the ataxia-telangiectasia mutated (ATM)-mediated DNA double strand break (DSB) repair pathway. Recent research showed that germline mutations in these genes result in DNA repair deficiency in oocytes, leading to accelerated ovarian aging as manifested by lower ovarian reserve and earlier age at natural menopause. Because we discovered that oocyte DNA repair is similarly critical in chemotherapy-induced ovarian follicle loss, we hypothesized that women with pathogenic mutations in *BRCA1/2* genes may experience larger declines in ovarian reserve after chemotherapy. To gauge the degree of the chemotherapy-induced ovarian damage, we utilized serum anti-mullerian hormone (AMH), which is the most reliable current marker for assessing oocyte reserve.

**Methods:** Women with early stage breast cancer were enrolled before chemotherapy (Trial registration number: NCT00823654) between January 2009 and November 2017. Sera were obtained at baseline, before the initiation of treatment, and 18 to 24 months after the completion of chemotherapy. Stored sera were assayed at once for anti-mullerian hormone (AMH) and the results were adjusted for the women's age at sample collection. Of the 235 enrolled, 117 evaluable women were stratified into three groups, those never tested (based on NCCN Guidelines V 1.2018 ; n=38) and those negative (n=65) or positive (n=14) for a pathogenic *BRCA* mutation. Ovarian recovery was defined as the geometric mean of the post chemotherapy age-adjusted AMH levels compared to baseline.

**Results:** Compared to the lower risk (*BRCA*-untested) control group, AMH levels averaged 76% and 66% in those negative or positive for *BRCA* mutations (p=0.078). The geometric mean recoveries for the three groups (not tested, *BRCA* negative and *BRCA* positive) were 3.7%, 5.2% and 1.6%, respectively. The mean recovery in the *BRCA* mutation positive group was about one-third the 4.6% recovery in the other two groups combined (two group ANOVA, p=0.034, F=4.89). Given the potential of the ovarian recovery to be dependent on type of chemotherapy, the data were reanalyzed for all three *BRCA* groups after restriction to those treated with the AC-T (doxorubicin and cyclophosphamide followed by paclitaxel) regimen. Of the 108 women in the previous analysis, 83 (77%) were treated with AC-T; 25, 46 and 12 women in the three groups, respectively. The geometric mean AMH recoveries for these new groups were 3.2%, 4.7% and 1.3%. When the *BRCA* mutation positive group was compared with other two groups, the former had significantly worse recovery of serum AMH levels (ANOVA, p=0.044, F=4.2).

**Conclusions:** These data show that women with breast cancer and pathogenic *BRCA* mutations have striking liability to chemotherapy-induced ovarian reserve loss and may have to be preferentially counselled on fertility preservation methods. In addition, taken together with the previous data showing that women with *BRCA* mutations may have accelerated ovarian aging, even unaffected reproductive age individuals may have to be proactive about family building or early preservation of their fertility (Supported by NIH R01HD053112).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD6-03

Pilot project assessing the impact of self-care techniques on post-surgical pain, fatigue, and inflammation

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More than one third of women diagnosed with breast cancer experience significant emotional distress, which may then effect pain perception, wound healing, quality of life (QOL), and return to physical function. Several studies have shown that physical and cognitive relaxation strategies may relieve perioperative anxiety, leading to improved postoperative pain and QOL.

One hundred women from Brooke Army Medical Center with non-metastatic breast cancer, planning to undergo surgery as initial treatment, were randomly assigned using an intention-to-treat model to either the treatment as usual group (TAU; n = 49) or TAU plus a self-care toolkit (SCT; n=51). The SCT included audio-recordings of guided mind-body techniques (breathing, progressive muscle relaxation, meditation, guided imagery, and self-hypnosis), an acupuncture anti-nausea wristband, and a workbook with instructions for use of the tools plus a section to journal the cancer experience.

Pain, anxiety, nausea, sleep, fatigue, global health, and QOL were assessed using the Defense and Veterans Pain Rating Scale (DVPRS), the 10-cm Visual Analog Scale (VAS), and the NIH PROMIS-57 subscales. Data was collected at baseline (T1), immediately prior to surgery (T2), within 10 hours post-operatively (T3), and approximately two weeks post-surgery (T4). Two inflammatory blood markers (ESR and CRP) were measured at T1, T2, and T4. Due to diurnal variability of ESR and CRP, laboratory draws were generally collected prior to 10:00 AM. Categorical variables and frequency counts were analyzed using Chi-Squared or Fisher's Exact tests, whichever was most appropriate. Means and standard deviations were used as summary statistics for continuous variables and analyzed using Student's t-test, ANOVA, and/or Wilcoxon's Test. For data measured at two time points, the delta change in values was calculated to detect within-group differences in SCT and TAU using Wilcoxon's rank sum test or paired t-test. For factors measured at more than two time points, a two-way repeated measures ANOVA was implemented with a Bonferroni corrected post-hoc analysis to determine between-group differences at each time point.

Between T1 and T4, there were significant between group differences in PROMIS-57 scores of Pain Interference, Fatigue, and Satisfaction With Social Roles, favoring the SCT group compared to TAU (p=0.005, p=0.023, and p=0.021, respectively). There was a significant mean change in DVPRS scores from T2 to T3, with the SCT group having significantly smaller increases in post-operative pain (p=0.008) and in post-operative ESR (p=0.0197) compared with the TAU group. Clinically significant reductions in anxiety occurred in the SCT group during the main intervention period. These results suggest that using the SCT in the perioperative period decreased pain perceptions, fatigue, and inflammatory cytokine secretion.

The view(s) expressed herein are those of the author(s) and do not reflect the official policy or position of Brooke Army Medical Center, the U.S. Army Medical Department, the U.S. Army Office of the Surgeon General, the Department of the Army, the Department of the Air Force and Department of Defense or the U.S. Government.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD7-01

Bromodomain protein 3 is a novel therapeutic target in invasive lobular carcinoma

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Invasive lobular carcinoma (ILC) is a subtype of breast cancer comprising 10% of breast tumours. ILC is characterised by a loss of E-cadherin, and is generally estrogen receptor (ER) positive. The majority of ILC breast cancers are treated with endocrine therapy, although approximately one in three women are de novo resistant to therapy. To identify novel therapeutic targets for the treatment of ILC, we carried out RNA sequencing on 61 primary ILC samples. We found that high expression of the epigenetic reader, bromodomain protein 3 (BRD3) was associated with poor recurrence free survival. We also validated this finding in a separate cohort of 99 ILC patient samples using the METABRIC cohort. Next, we assessed ILC cell lines for sensitivity to JQ1, an inhibitor of BET family proteins. We found that JQ1 inhibited cell growth in all the cell lines tested. Interestingly, two of the ILC cell lines were sensitive to JQ1-induced apoptosis, whereas two of the cell lines were inherently resistant to JQ1-induced apoptosis. Using dynamic BH3 profiling we showed that the JQ1 resistant cell lines were dependent on anti-apoptotic protein BCL-XL following JQ1 treatment. Interestingly, we show both in 2D and 3D cultures that JQ1 is synergistic when combined with the BH3 mimetic, ABT-263. Highlighting that combination treatment with JQ1 and ABT-263 may be a novel potential therapeutic option for ILC.

To unveil the mechanism underlying resistance to JQ1-induced apoptosis, we performed paired-end RNA sequencing and compared differentially expressed genes in JQ1 sensitive and JQ1 resistant ILC cell lines. DAVID gene ontology analysis identified 6 pathways differentially upregulated in the JQ1 resistant ILC cell line including MAPK, Wnt, and insulin resistance signaling. Interestingly, we found that ILC cell lines, which were resistant to BET inhibition with JQ1, demonstrated high levels of FGFR1-4 both at the mRNA level and the protein level. Combination treatment with JQ1 and the FGFR1 inhibitor PD173074 or following knockdown of FGFR with siRNA, resulted in increased cell death in JQ1 resistant cells. Currently, we are assessing how FGFR signaling enables survival of ILC cells following JQ1 treatment and determining the exact function of BRD3 in ILC. In conclusion, we have identified a novel therapeutic target, BRD3, which may be inhibited using JQ1 in combination with BH3 mimetic ABT-263 or FGFR1 inhibitor for a more effective treatment strategy for ILC.

## 2018 San Antonio Breast Cancer Symposium ®

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Novel human cell line xenograft models of ER $\alpha$ -positive metastatic invasive lobular breast carcinoma as pre-clinical platforms for validating candidate genetic drivers

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Invasive lobular breast carcinoma (ILC) is the second most common subtype of breast cancer following invasive ductal breast carcinoma, accounting for 10-15% of all cases. Despite the intriguing histological and clinical differences between these two subtypes, ILC has been chronically understudied. Human ILC cell lines and xenografts are regarded to be of limited utility for ILC research due to slow proliferation rates in culture and low reported intake rates. Data on human ILC cell line xenografts remains limited and knowledge of their metastatic capacity is currently lacking. Using Estrogen Receptor alpha (ER)-positive human ILC cell lines labeled with dual bioluminescent and fluorescent reporters, herein we performed a comprehensive *in vivo* characterization of their growth as primary tumors orthotopically in the mammary fat pad, as well as at secondary sites following spontaneous or experimental metastasis. Primary tumors exhibited the characteristic dyscohesive, single-file growth of ILC cells, faithfully recapitulating the histology of human ILC tumors. *In vivo* bioluminescence imaging coupled with *ex vivo* fluorescence analysis revealed spontaneous metastases to the bone, brain, lungs and ovaries, closely mirroring the clinical patterns of ILC tumor dissemination. This was in stark contrast to experimental metastasis, where none of the cell lines colonized the lungs when injected into the tail vein and bone colonization was observed with only one cell line following intracardiac injections. The metastatic lesions harbored the classical histological features of ILC, including single strand growth, loss of E-cadherin-mediated adherens junctions and mislocalization of p120-catenin to the cytoplasm. Importantly, both the primary tumors and the metastases exhibited high ER expression and significant response to the anti-endocrine agent fulvestrant - a selective ER downregulator. Using these models, we will also present data from functional studies validating candidate genetic drivers of ILC disease progression, which are associated with poor recurrence-free survival in patients with ILC. Ongoing work focused on genomic and transcriptional analyses of metastatic lesions and isolated cell lines from these models will pinpoint additional candidate drivers of ILC dissemination. This is the first report of a hormone responsive ER+ metastatic xenograft model faithfully representing unique ILC features such as ovarian metastases, which will serve as a valuable pre-clinical platform for testing novel therapeutics towards translation into the clinic.

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Investigating cortactin as a genetic driver of disease progression in invasive lobular carcinoma

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Invasive lobular carcinoma (ILC) is the second most common type of breast cancer following invasive ductal carcinoma (IDC) and accounts for 10-15% of all cases. Unlike the masses or lumps formed by IDCs, ILCs grow as small, dyscohesive cells in a single-file pattern within dense layers of extracellular matrix. Paradoxically, while patients with ILC display favorable prognostic and predictive factors (Estrogen Receptor [ER]+, Progesterone Receptor+, HER2-, low Ki67), they present with more frequent long-term recurrences compared to those with IDC, indicative of endocrine resistance. Thus, there is urgent need to investigate genetic drivers of ILC disease progression in order to develop more effective therapeutic strategies and improve patient outcome. To this end, we used the Nanostring platform to measure the expression of 577 copy number variation-associated genes in 131 primary ILC tumors with long-term clinical data. This analysis identified CTTN (Cortactin; cortical actin binding protein) as a candidate ILC driver that exhibited higher expression in tumors from patients with subsequent recurrent (n=33) versus non-current disease (n=98). We further validated high CTTN mRNA/protein expression in human ILC cell lines, tumors and patient-derived xenografts, and CTTN locus (11q13.3) amplification in clinical ILC metastases to the brain, bone and ovaries. In follow-up *in vitro* studies, RNAi-mediated inhibition of CTTN diminished the adhesion and haptotaxis of human ILC cell lines to Collagen I, as well as impairing their growth in 3D Collagen I culture. To assess the functional role of CTTN in ILC tumor growth and metastasis, we transplanted control and CTTN knockdown human ILC cell lines into the mammary fat pads and ducts of immuno-compromised mice and monitored disease progression via bioluminescence imaging. While CTTN inhibition did not lead to a substantial impact on measurable tumor burden, both CTTN shRNAs resulted in a significant survival benefit in the fat pad model. Ongoing work is aimed at pinpointing the underlying mechanisms of CTTN's role in mediating growth in 3D Collagen I culture *in vitro* and disease progression *in vivo*. Collectively, the results of these studies will advance our understanding of ILC disease mechanisms and serve as a pre-clinical basis for improving the clinical outcome of patients with this understudied subtype of breast cancer.



# 2018 San Antonio Breast Cancer Symposium ®

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Metabolic syndrome increases risk of recurrence and impacts immune pathways in invasive lobular carcinoma

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**Background:** Invasive lobular carcinoma (ILC) of the breast has been shown to be more strongly associated with risk factors that modulate hormone levels, including obesity, as compared with invasive ductal carcinoma (IDC). Our previous study indicated the biology of disease engages disparately for ILC and IDC. This study further evaluated the effect of metabolic syndrome (MS) in patients with ILC histopathological tumor types using whole transcriptome gene expression (GEA) and pathway analyses.

**Methods:** This analysis included 76 patients with ILC from the PROMIS and IMPACt studies for whom metabolic characteristics were captured with informed consent. To be classified as having MS, the patient had to exhibit any 3 of the 5 metabolic factors (obesity, hypertension, hypercholesterolemia, hypertriglyceridemia, diabetes mellitus). Risk of recurrence was established using the 70-gene signature (70-GS). Pathway enrichment analysis was performed in DAVID v 6.8 (<https://david.ncifcrf.gov/>). Benjamini-Hochberg corrected  $P < 0.05$  was considered significant.

**Results:** Thirty-four percent (26/76) of patients with ILC had MS. Significantly more ILC patients with MS than without MS were 70-GS high risk (42% vs. 12%, respectively, [ $P=0.007$ ]).

## ILC MS Status by 70-GS Risk Classification

ILC MS Status	70-GS High Risk	70-GS Low Risk	P-value	Total
No	6 (12%)	44 (88%)	0.007	50
Yes	11 (42%)	15 (58%)		26

GEA identified 486 significant differentially expressed genes between MS and non-MS ILC patients. These differentially expressed genes indicated that the immune pathways were affected rather than growth factor pathways. The significantly enriched pathways were driven by genes upregulated on average across all MS ILC patients, and represented in the immune response ( $P < 0.001$ ), adaptive immune response ( $P=0.001$ ), B cell signaling ( $P < 0.001$ ), inflammatory response ( $P < 0.05$ ), chemokine-mediated signaling ( $P < 0.05$ ), T cell co-stimulation ( $P < 0.05$ ), and intracellular signal transduction processes ( $P < 0.05$ ); as well as enriched in systemic lupus erythematosus (largely overlapping with nucleosome assembly,  $P=0.001$ ) and osteoclast differentiation ( $P < 0.05$ ) pathways. While early infiltration of cytolytic T-lymphocytes may protect against tumor development, humoral-mediated immunity and a sustained state of chronic inflammation may result in polarization toward a pro-tumor microenvironment.

**Conclusions:** ILC is generally considered to have a low risk of recurrence, but with poorer long- term prognosis. We show that ILC patients with MS have a significantly higher 70-GS risk of recurrence than their non-metabolic counterparts. Our gene expression analysis suggests that ILC in the setting of metabolic syndrome, likely results in chronic immune activation and has distinct drivers of disease progression and metastasis, specifically to bone. The current study underscores the complex interplay between inflammation and immune suppression. Studies are needed to further characterize differences in enriched pathways as it relates to optimizing targeted therapeutics.

# 2018 San Antonio Breast Cancer Symposium ®

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Correlation of *CDH1* alterations and aberrant E-cadherin expression in lobular carcinomas

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**Background:** E-cadherin (ECAD), encoded by the *CDH1* gene, is expressed in the majority of invasive ductal carcinomas (IDC). In contrast, ECAD expression is lost in ~90% of invasive lobular carcinomas (ILC) due to genomic alterations of *CDH1*. Immunohistochemical (IHC) staining for ECAD has thus increasingly been utilized to differentiate between ductal and lobular lesions. This study examines the correlation between *CDH1* mutation profile, morphology and ECAD IHC status in invasive breast carcinomas (BC).

**Methods:** The Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) platform is a hybrid capture next-generation sequencing assay to detect somatic alterations in exons and selected introns of 468 cancer-related genes. Invasive BC tested from 1/2014 – 5/2018 which yielded a *CDH1* alteration were identified and only cases with ECAD IHC results were included. Histologic features were extracted from pathology reports. All available H&E/ECAD slides were reviewed by two pathologists to confirm the histology and immunophenotype.

**Results:** *CDH1* alterations were identified in 455 out of 4103 BC analyzed (11%) and ECAD IHC were available for 209 (46%) tumors. Most of the tumors were reported as ILC (70%, 147/209). The majority (77%, 161/209) were ECAD-negative but 23% (48/209) showed some ECAD staining. Table 1 summarizes the histologic subtypes and IHC results. Upon review of H&E/ECAD slides in cases with ECAD staining, the histologic subtype was re-classified in 52% (25/48) of cases (Table 2). For ECAD-negative BC, at least 61% (98/161) of *CDH1* alterations were truncating mutations (frameshift deletion 47/161, nonsense 51/161) and 39% (63/161) were variants of unknown significance (VUS). In cases with ECAD staining, 48% (23/48) of *CDH1* alterations were truncating mutations (frameshift deletion 12/48, nonsense 11/48) however 52% (25/48) were VUS.

**Conclusion:** In this study, 88% (141/161) of cases with negative ECAD staining and *CDH1* alteration were classified as ILC, confirming the known observation that *CDH1* alteration and loss of ECAD expression is relatively specific for a lobular phenotype. The correlation is, however, imperfect. A subset of BC (23%; 48/209) with *CDH1* alteration still exhibits ECAD expression. Morphologically this subset consists of ILC in 60% (29/48), IMC in 25% (12/48) and IDC in 15% (7/48). Our preliminary findings suggest that aberrant ECAD expression can occur in BC with morphologic features of ILC due to certain *CDH1* alterations that most likely result in non-functional ECAD complex. BC with histologic features of ILC should therefore not be re-classified as IDC/IMC based solely on the status of ECAD expression. Given that ILC is clinically distinct from IDC, correct classification based on morphological and molecular features is prudent.

Table 1: Histologic subtypes and ECAD IHC

	ILC (n = 147)	IDC (n = 13)	IMC (n = 49)
ECAD staining			
Negative	141	0	20
Strong, diffuse	4	13	5
Heterogenous	0	0	20
Reduced/weak	2	0	4

IMC: invasive mammary with mixed ductal/lobular features

Table 2: Morphologic review of cases with ECAD staining

ECAD staining	Original DX (n)	Morphology Review DX		
		ILC	IDC	IMC
Strong, diffuse	ILC (4)	3	0	1
	IDC (13)	6	7	0
	IMC (5)	5	0	0
Heterogenous	IMC (20)	10	0	10
Reduced/weak	ILC (2)	2	0	0
	IMC (4)	3	0	1

# 2018 San Antonio Breast Cancer Symposium ®

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Molecular subtypes of invasive lobular breast cancer in the I-SPY2 TRIAL

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**Background:** Invasive lobular carcinoma (ILC) of the breast has distinct histological and molecular variations compared to invasive ductal carcinoma (IDC), including absence of the adhesion protein E-cadherin. Recently, molecular subtypes within ILC have been described, with an analysis from The Cancer Genome Atlas (*Ciriello et al*) identifying three distinct groups within ILC based on gene expression—reactive-like, immune-related, and proliferative. In this study, we applied this 60-gene classifier to a locally advanced cohort of ILC and mixed ILC/IDC cases from patients screening for the I-SPY 2 neoadjuvant chemotherapy trial.

**Methods:** The I-SPY 2 TRIAL is open to women with more locally advanced, clinically/molecularly (as assessed by MammaPrint) high risk breast cancer. HR+HER2- MammaPrint Low risk patients ineligible for I-SPY 2 randomization are invited to join a MP Low risk registry. 131 ILC and mixed ILC/IDC tumors from these cohorts (I-SPY 2: n=80; low risk registry: n=51) with pre-treatment Agilent microarrays were available for analysis. We used the Classification to Nearest Centroid technique to assign TCGA subtype to our cohort. We assessed association between TCGA subtype, clinical covariates and response to therapy using a chi-square test. We also evaluated the Euclidean distance between each sample and the three subtype centroids. In an exploratory analysis, we used consensus clustering based on the 1000 most varying genes within the HR+HER2- I-SPY ILC cases to generate new unsupervised groupings, and assessed the concordance with the TCGA reactive-like, immune-related and proliferative subtype assignments.

**Results:** Of the 131 patients included, most (79%) were HR+HER2-, 11% were HR+HER2+, 2% were HR-HER2+ and 8% were HR-HER2- for a total of 10% HR-. 66 were pure ILC, while 65 were mixed ILC/IDC. Upon applying the TCGA 60-gene classifier, the distribution of ILC subtypes was as follows: 33 (25%) were classified as reactive-like, 50 (38%) were immune-related, and 48 (37%) were proliferative. 64% of triple negative cases were reactive-like; while the HR+HER2- and HER2+ cases were more likely to be in the proliferative or immune-related subtype (p=0.037). Among the 80 I-SPY 2 cases, the overall pathologic complete response rate was low (16%) but equivalent to the overall HR+HER2- I-SPY2 population (16%). This did not differ across the groups defined by the TCGA ILC subtypes (p=0.79).

Interestingly, a subset of cases assigned as reactive-like and immune-related were of similar distance to the proliferative subtype centroid as patients assigned to the proliferative subtype. When we used consensus clustering to identify new subsets within our locally advanced ILC cohort, our unsupervised groupings had only 32% concordance with the TCGA ILC subtype assignments.

**Conclusion:** The low concordance between our consensus cluster groupings and the TCGA subtype groupings may reflect underlying differences within a locally advanced cohort of ILC cases, like I-SPY, that may not be captured in the 60-gene classifier developed from the overall lower stage TCGA cohort. These findings suggest that considerable molecular heterogeneity exists in lobular cancers, which merits further investigation.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD7-07

Synergistic targeting of CDK4/6 and BCL-2 pathways in estrogen receptor positive breast cancer

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**Background:** Despite incremental advances in chemotherapy and endocrine therapy, survival outcomes for patients with ER-positive (ER+) metastatic breast cancer (MBC) remain poor. The majority of relapsing tumors exhibit deregulation of the cyclin-dependent kinase 4 and 6 (CDK4/6)/cyclin D1 (CCND1)/Rb signaling pathway. CDK4/6 inhibitors (such as palbociclib) in combination with endocrine therapy have been shown to significantly improve progression free survival in patients who are in 1<sup>st</sup> or 2<sup>nd</sup> line relapse, although overall survival benefit has yet to be demonstrated. This may reflect their largely cytostatic mechanism of action, with minimal induction of tumor cell death. Thus, combinatorial strategies that also induce apoptosis could be beneficial. Notably, the pro-survival protein BCL-2 is overexpressed in the majority of ER+ tumors and the potent and specific BCL-2 inhibitor venetoclax (ABT-199) has been found to synergize with endocrine therapy in patient derived xenograft (PDX) models. Promising activity has also been observed in an early phase clinical trial. We therefore investigated dual targeting of the CDK4/6 and BCL-2 pathways in pre-clinical models of ER+ and BCL-2+ breast cancer.

**Results:** We first examined endocrine sensitive or resistant cell-lines and found that pro-survival BCL-2 proteins were upregulated in resistant cells. BCL-2 family protein levels were also found to be elevated in palbociclib resistant cells, suggesting that BCL-2 could represent a therapeutic target. We next determined whether venetoclax improved response to dual therapy comprising the selective estrogen receptor degrader fulvestrant and palbociclib. In clonogenic assays of endocrine sensitive breast cancer cell lines, triple therapy containing venetoclax significantly reduced the number and size of colonies, when compared to double therapy. The addition of venetoclax to fulvestrant/palbociclib also augmented cell death in tumor organoid models derived from either ER+ BCL-2+ primary tumors or PDX models. Moreover, triple therapy improved tumor response and overall survival in mice bearing ER+ BCL-2+ PDX tumors. Mechanistically, this was accompanied by increased apoptosis and reduced cellular proliferation (as determined by cleaved caspase-3 and Ki67 levels, respectively). As CDK4/6 inhibitors have recently been shown to promote anti-tumor immunity, we evaluated immune modulation using the ER+ 67NR cell line in a syngeneic (BALB/c) mouse mammary tumor model. Similar to the PDX models, triple therapy comprising fulvestrant, palbociclib and venetoclax was more effective than double therapy comprising either fulvestrant/palbociclib or fulvestrant/venetoclax. Flow cytometric analysis of tumors revealed that this was accompanied by a reduced intratumoral FOXP3<sup>+</sup>:cytotoxic CD8 T-cell ratio.

**Conclusions:** The addition of the BCL-2 inhibitor venetoclax to conventional therapy comprising endocrine therapy and a CDK4/6 inhibitor augments tumor response and elicits a favorable intratumoral immune profile. Collectively, these findings support investigation of combination therapy in the clinic for patients with ER+ BCL-2+ MBC.

# 2018 San Antonio Breast Cancer Symposium ®

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Immune checkpoint upregulation and T-cell exhaustion in aggressive hormone-receptor positive breast cancer patients

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**Background:** Early reports from studies focused on assessing the clinical efficacy for immune-checkpoint (IC) inhibitors in breast cancer suggest lower activity than in cancers caused by exogenous mutagens such as melanoma and smoking-related lung cancer. Nonetheless, triple negative breast cancer is under active investigation. In contrast, estrogen receptor positive disease has been considered “immunologically silent” with very few IC studies focused on this disease subset. We therefore undertook an analysis of ER+ breast cancer to determine if there are subsets of patients with poor prognosis ER+ disease that exhibit IC pathway upregulation hence potential candidates for IC-based clinical trials.

**Methods:** In an unbiased integrative analysis of patient samples, mRNA expression data for ~15500 genes across 66 Luminal B patient set was used to identify genes for which the expression correlated positively with proliferation marker Ki67 (in aromatase-inhibitor treated tumors) and were upregulated (>2 fold) in endocrine-therapy (ET) resistant samples as compared against the sensitive counterparts. Resulting genes were queried to identify underlying activated pathways and prioritize genes for further analysis. Validation of shortlisted genes was performed on independent patient cohorts (TCGA and METABRIC) using Kaplan-Meier disease-specific survival analysis. Amplification and methylation data for TCGA samples were obtained from CBioportal and Wanderer respectively. Proteomics data for TCGA samples was extracted using LikedOmics server. T-cell activation (TCA) and exhaustion (TCE) scores were devised using mean of standardized expression of genes reported to contribute in these two T cell states.

**Result:** Approximately 30 genes were found to be differentially upregulated in ET-resistant aggressive ER+ breast cancer. This gene set was enriched in immune-tolerance biological processes ( $p < 0.005$ ). The three immune-checkpoints constituting these processes are IDO1, LAG3 and PDCD1 (PD1). Upregulation of IDO1 was a confirmed poor prognosis factor across independent patient cohorts. Furthermore, comparative analysis of TCA and TCE scores between ET-resistant and sensitive tumors revealed that resistant tumors have high TCE, despite having high TCA, hence circumventing immunogenic apoptosis.

**Conclusion:** To date, no substantial study showing prognostic relevance of mRNA levels of ICs in context of ER+ breast cancer patients has been reported. Higher IDO1 and T-cell exhaustion levels in ET-resistant ER+ disease provides clinical evidence for a role for immune-checkpoints in evading immune-driven cell death. These results suggest a potential for IC targeted immunotherapy for ~ 80% of endocrine therapy resistant clinically aggressive ER+ breast cancers.

## 2018 San Antonio Breast Cancer Symposium ®

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Lasofloxifene decreases breast cancer lung and liver metastasis in a mammary intraductal (MIND) xenograft model of mutant ER $\alpha$ + breast cancer

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The standard of care for early postmenopausal ER $\alpha$ + breast cancer patients is adjuvant endocrine therapy, typically an aromatase inhibitor or tamoxifen, and endocrine therapy with or without a CDK 4/6 inhibitor in the metastatic setting. However, a number of these patients are not sensitive to endocrine therapy or experience breast cancer recurrence 10 to 15 years after endocrine treatment, and all eventually progress in the metastatic setting. Metastasis of ER+ therapy-resistant breast cancer correlates with the acquisition of ESR1 activating mutations in up to 40% or more of affected patients. The two most common ER $\alpha$  mutations are Y537S and D538G, both of which confer ER $\alpha$  constitutive activity. Lasofloxifene is a SERM originally developed to treat vulvovaginal atrophy and osteoporosis. In this study, we analyzed the efficacy of lasofloxifene in the treatment of MCF7 tumor explant models that were engineered with CRISPR/Cas9 to express Y537S or D538G ER $\alpha$ . To better mimic the natural micro-environment of infiltrating ductal breast cancer, we used the mammary intraductal mouse model (MIND), in which tumor cells are introduced into the ducts via the nipple. Three MCF7 cell variants, MCF7 WT, MCF7 Y537S and MCF7 D538G were injected into the mammary ducts of NSG mice. Prior to injection, cells were labeled with a luciferase reporter to monitor tumor growth in vivo using a Xenogen IVIS imager. Mice were treated with three different doses of lasofloxifene (1, 5 and 10mg/kg; 5days/wk SQ), vehicle, or the SERD, fulvestrant, (5 mg SQ once per week = 250 mg/kg). After 70 days of treatment, primary tumor growth, as measured by endpoint tumor weight, of MCF7 WT, D538G and Y537S explants was significantly inhibited versus vehicle by 10 mg/kg lasofloxifene and fulvestrant. Compared to fulvestrant, lasofloxifene was significantly more effective at 5 and 10 mg/kg for the MCF7 Y537S and D538G tumors. Notably, the two MCF7 mutants metastasized to the lung and liver, whereas WT MCF-7 cells were only very weakly metastatic by the end of the study. Lasofloxifene significantly inhibited the metastasis of both MCF7 Y537S and D538G to the lungs and liver at 5 and 10 mg/ml. In contrast, fulvestrant only inhibited metastasis of the MCF7 D538G mutant to both organs. In a follow-up combination therapy study of MCF-7 WT and Y537S tumor explants, in which mice were co-treated with either fulvestrant and palbociclib or lasofloxifene and palbociclib, lasofloxifene plus palbociclib demonstrated a better outcome than fulvestrant and palbociclib in terms of primary tumor weight inhibition. Results for lung and liver metastasis inhibition are forthcoming. In conclusion, these data suggest that lasofloxifene may have the potential to be utilized as a treatment for metastatic breast cancers, including those that express constitutively active ER $\alpha$  mutations, including D538G and Y537S, and warrants further study.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD7-10

Neoadjuvant trial with letrozole identifies *PRR11* in 17q21-23 amplicon as a resistance mechanism to endocrine therapy in ER-positive breast cancer

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Approximately 20% of patients with early ER+ breast cancer (BC) treated with adjuvant antiestrogen therapy relapse with metastatic disease. Previously, we identified 3 amplicons (11q11.3, 8p11.23, and 17q21-23) associated with endocrine-resistance (Giltane *et al. Sci Transl Med* 2017). The 17q21-23 amplicon has been associated with highly proliferative luminal B tumors and cancers with high genomic instability. A causal role of this region in endocrine resistance is unclear. We performed whole transcriptome analysis on RNA extracted from 58 ER+ breast cancers of patients treated with letrozole for 5.4-9.2 months (median 7.2 months). *PRR11* (Proline rich 11), located in 17q21-23, was significantly upregulated in non-responding tumors as defined by cancer relapse after a median follow up of 5 years and/or a preoperative endocrine prognostic index (PEPI)  $\geq 4$ . Differential gene expression analysis between tumors expressing low vs high *PRR11* mRNA showed that BC signatures associated with proliferation, cell cycle, IGF-1 and PI3K signaling were enriched in tumors with high *PRR11* expression. In the Metastatic Breast Cancer Project and TCGA, *PRR11* amplification was higher in metastatic vs. primary BCs (16.5% and 8.5%, respectively; Fisher's  $p=0.0088$ ). Gene Set Enrichment Analysis of mRNA expression in METABRIC and TCGA revealed significant enrichment of hallmark gene sets associated with proliferation in *PRR11* amplified ER+ BCs. Genome-scale RNAi screening in Project Achilles showed that among all genes in the 17q21-23 amplicon, *PRR11* knockdown results in the 4<sup>th</sup> strongest anti-proliferative effect in MCF7 cells. *PRR11* knockdown with siRNA inhibited proliferation, cell cycle progression, and RB phosphorylation in HCC1428 LTED (long-term estrogen deprived), MCF7 LTED, and fulvestrant-resistant MCF7 cells. Using a PCR array with 84-cell cycle genes, we identified *SKP2*, *CDKN1A*, *CCNB2*, *CCNA2*, *CKS2* and *CCNB1* as genes downregulated by *PRR11* knockdown. Except for *CDKN1A*, expression of all those genes was elevated ER+ BCs with *PRR11* gain or amplification in TCGA. *PRR11* associates with the p85 regulatory subunit of PI3K via its SH3 domain. We speculated this association would suppress p85 homodimers, thus permitting binding of PI3K $\alpha$  (p110 $\alpha$ )-p85 dimers to IRS1 and, hence, activating PI3K/AKT. To test this, we co-transfected HEK293T cells with HA-p85 and FLAG-p85. Transfection of *PRR11* into these HEK293T cells reduced HA-p85 and FLAG-p85 homodimers as shown by HA and FLAG pulldowns followed by FLAG and HA immunoblots, respectively. Finally, *PRR11* knockdown resulted in a reduction of p110a and S473 P-AKT levels and inhibition of IGF-1/2 stimulated P-AKT. Not inconsistent with these data, *PRR11* amplification and *PIK3CA* mutations in METABRIC and TCGA are exclusive of each other, suggesting these alterations are functionally linked with the same signaling pathway. These data support a role of *PRR11* in PI3K/AKT activation that may be causal to resistance to estrogen deprivation. We propose *PRR11*, located in the 17q21-23 amplicon, is a potential mediator of resistance to antiestrogen therapy by amplifying PI3K/AKT signaling, suggesting PI3K may be a therapeutic target in ER+ BCs harboring *PRR11* amplification.



# 2018 San Antonio Breast Cancer Symposium ®

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Therapeutic strategy for *ESR1* mutation driven-endocrine resistance in ER-positive breast cancers

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**Background:** Endocrine therapy is used in estrogen receptor (ER)-positive breast cancers, however, 25% of these patients are at risk of distant relapse and the development of acquired endocrine resistance. Recently mutations in the ER gene (*ESR1*) have been validated to be acquired during the development of endocrine resistance. The most frequent *ESR1* mutation, Y537S, promotes ligand-independent ER activity and emerges subclonally during aromatase inhibitor treatment. In this study, we examined the effects of the Y537S *ESR1* mutation on cell cycle signaling and therapeutic response to a novel checkpoint inhibitor.

**Material and Methods:** MCF-7 cells expressing the Y537S *ESR1* mutation were generated by CRISPR-Cas9 knock-in techniques. Cells were incubated in steroid deprived conditions. Cell cycle analysis and apoptosis were examined by flow cytometry annexin-V assays. Proliferation was analyzed by BrdU incorporation. Cell cycle checkpoint kinases were examined by western blot analysis. Cell growth was analyzed using soft agar and MTT assays. Replication stress was identified by RPA32 and gamma-H2AX foci formation assay. For *in vivo* studies, MCF-7 *ESR1* Y537S mutant cells were injected into female athymic nude mice with 17 $\beta$ -estradiol (E2) supplemented water. When tumors reached 350 mm<sup>3</sup>, tamoxifen (20 mg/kg; s.c.; three times a week), fulvestrant (200 mg/kg; s.c; once a week) and/or PF477736 Chk1 inhibitor (7.5 mg/kg; i.p.; twice a day and twice a week) was treated without E2.

**Results:** *ESR1* Y537S mutant cells accumulated approximately 5 fold in S phase and 1.7 fold in G2/M phase compared to control cells in estrogen-deprived (ED) conditions. BrdU incorporation also increased about 2.5-fold, however, apoptosis was decreased about 60 % compared with wild-type ER parental cells. *ESR1* Y537S mutant cells induced significant replication stress, showing increased RPA32 foci together with increased gamma H2AX foci, a marker of DNA double-stranded breaks. ChIP-seq analysis revealed binding sites on *ATR* and *CHEK1* genomic locations. ATR/Chk1-mediated checkpoint signaling was activated in *ESR1* Y537S mutant cells, and was repressed with fulvestrant, tamoxifen, or *ESR1* siRNA treatment. The Chk1 inhibitor, PF477736, sensitized MCF-7 expressing the *ESR1* Y537S mutation to endocrine treatments such as fulvestrant, tamoxifen, and the ER degrader AZD9496 in cell proliferation assays. In MCF-7 *ESR1* Y537S mutant xenograft and patient derived mouse models, tamoxifen treatment combined with the Chk1 inhibitor PF477736 repressed primary xenograft tumor doubling times ( $P=0.038$ , Wilcoxon test). Treatment of mutant tumors with PF477736 together with fulvestrant significantly inhibited the frequency of distant lung metastases by 80% ( $P=0.0031$ , *t*-test), suggesting that these combinations may be useful in second line treatment of metastatic breast cancer patients resistant to endocrine therapies.

**Conclusion:** These preclinical results suggest that *ESR1* mutant tumors have a therapeutic vulnerability to combination endocrine therapy with cell cycle checkpoint kinase inhibitors. These data demonstrate that this new therapeutic approach may be useful to restore endocrine sensitivity in metastatic breast cancer patients with *ESR1* mutation driven-endocrine resistance.

# 2018 San Antonio Breast Cancer Symposium ®

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Inhibition of CDK7 overcomes resistance to CDK4/6 inhibitors in hormone receptor positive breast cancer cells

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**Background:** Despite the efficacy of the combinations of endocrine therapy (ET) and CDK4/6 inhibitors in the hormone receptor positive (HR+) metastatic breast cancer (BC) setting, the majority of patients eventually acquire resistance to these treatments. The loss of Rb activity is an important mechanism of acquired resistance to CDK4/6 inhibitors. Preclinical and clinical data support the *RB1* genetic aberrations, including mutations and deletions with loss of expression, as mechanism of palbociclib (palbo) resistance. To study vulnerabilities and potential treatment targets to overcome resistance to CDK4/6 inhibitors associated with Rb loss we studied a T47D palbo-resistant (T47D PDR) HR+ BC cell model obtained by exposing palbo-sensitive cells to increasing concentrations of palbo. These cells harbour deletion of *RB1*, and show increased cyclin E1 and decreased ER expression.

**Methods:** To identify genes that are essential for cell growth and genes whose loss enhances cell growth in palbo-resistant cells with loss of Rb, we performed genome-wide CRISPR-Cas9 knockout (KO) screens in T47D palbo-sensitive (T47D PDS) and T47D PDR cells. We searched for significantly essential genes that are targets with available drugs and tested the efficacy of these compounds in T47D PDS and T47D PDR cells.

**Results:** By employing a genome wide CRISPR KO screen, we identified 29 genes that were significantly positively selected in the T47D PDR cells. Among these genes were known tumor suppressor genes, such as TSC2 ( $\beta$  score: 0.89) and PTEN ( $\beta$  score: 0.68). We identified close to 600 genes that were significantly essential (negatively selected) for T47D PDR growth. *CDK4*, *CDK6* and *CCND1* were essential in the T47D PDS parental cells but lost essentiality with the acquisition of palbo resistance (FDR > 0.05). *CDK7* ( $\beta$  score: -1.21) and *CDK2* ( $\beta$  score: -1.67) were among the top ranked essential genes (FDR: 0). *CDK7* is a transcriptional CDK and has CDK activating kinase (CAK) activity. The CAK activity includes phosphorylation of *CDK2* and *CDK9*. To follow up on our CRISPR screen results we tested the activity of the selective *CDK7* inhibitors SY-1365 (currently in phase 1 clinical development) and THZ1 in the T47D PDS and T47D PDR cells. We saw dose dependent activity in both cell line models. Moreover, the IC50 values were comparable in T47D PDS and T47D PDR cells (THZ1; PDS: 9.93 nM, PDR with Palbo: 32.8 nM, PDR without Palbo: 1.08 nM) (SY1365; PDS: 1.60 nM, PDR with Palbo: 6.70 nM, PDR without Palbo: 1.87 nM). Combination studies of SY-1365 and fulvestrant in T47D PDR showed synergistic activity at lower concentrations.

**Conclusions:** In a model of palbo resistance with loss of Rb we found that cyclin D1, *CDK4* and *CDK6* are not essential, suggesting cross-resistance to other CDK4/6 inhibitors in this setting. We identified *CDK7* as a significant essential gene and potential therapeutic target. We validated these findings with the selective *CDK7* inhibitors, THZ1 and SY-1365. Our results offer a new therapeutic strategy for the treatment of palbo-resistant HR+ BC.

# 2018 San Antonio Breast Cancer Symposium ®

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Microscopic extracapsular extension in sentinel lymph nodes does not mandate axillary dissection in Z0011-eligible patients

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## Background

In ACOSOG Z0011 and AMAROS, matted nodes with gross extracapsular extension (ECE)—a risk factor for locoregional recurrence—were an indication for axillary dissection (ALND), but the effect of *microscopic ECE* (mECE) in the sentinel nodes (SLNs) on recurrence was not examined.

## Methods

Between 2010-2017, 815 patients with cT1-2N0 breast cancer and SLN metastasis were prospectively managed according to Z0011 criteria, with ALND for those with >2 positive SLNs. Management of mECE was not specified. Here we report outcomes of patients with 1-2 positive SLNs treated with SLN biopsy alone (n=685) and evaluate the impact of mECE on nodal recurrence. Outcomes of the 118 patients treated with ALND, of which 70% had >2 positive SLNs, are provided for comparison.

## Results

Median patient age was 58 years and median tumor size was 1.7 cm. In the SLN group, 210 (31%) had mECE. Patients with mECE were older, had larger tumors, were more likely to be hormone receptor positive (HR+) and HER2-, have 2 positive SLNs, and to receive nodal radiation (Table).

## Patient Characteristics

	Overall n=685	No mECE n=475	mECE n=210	P value
Age, years (median, range)	58 (30, 92)	57 (30, 85)	61 (34, 92)	0.0002
Pathologic tumor size, cm (median, range)	1.7 (0.1, 5.2)	1.6 (0.1, 5.2)	1.8 (0.4, 5.2)	0.008
Histology				0.24
Ductal	598 (87%)	421 (89%)	177 (84%)	
Lobular	59 (9%)	34 (7%)	25 (12%)	
Mixed	24 (4%)	17 (4%)	7 (3%)	
Other	4 (<1%)	3 (<1%)	1 (<1%)	
Subtype				0.006
HR+/HER2-	574 (84%)	386 (81%)	188 (90%)	
HR+/HER2+	54 (8%)	38 (8%)	16 (8%)	
HR-/HER2+	21 (3%)	18 (4%)	3 (1%)	
HR-/HER2-	36 (5%)	33 (7%)	3 (1%)	
Total positive SLNs				0.03
1	561 (82%)	399 (84%)	162 (77%)	
2	124 (18%)	76 (16%)	48 (23%)	
mECE size				NA
≤2mm	NA	NA	117 (56%)	
>2mm	NA	NA	93 (44%)	
Systemic therapy*				0.03
Chemotherapy + endocrine	424 (62%)	284 (60%)	140 (67%)	
Chemotherapy only	67 (10%)	55 (12%)	12 (5%)	

Endocrine only	172 (25%)	118 (25%)	54 (26%)	
Unknown§	22 (3%)	18 (4%)	4 (2%)	
Radiation field*				<0.0001
Supine	345 (50%)	260 (55%)	85 (40%)	
Prone	111 (16%)	93 (20%)	18 (9%)	
Breast + nodes	161 (24%)	80 (17%)	81 (39%)	
Unknown§	68 (10%)	42 (9%)	26 (12%)	

**\*Analysis excludes unknowns; §Unknown includes no treatment, pending, and unknown; Abbreviations: mECE, microscopic ECE; HR, hormone receptor; SLNs, sentinel nodes**

At a median follow-up of 41 months, no isolated axillary failures were observed. There were 11 nodal recurrences; 2 isolated, 4 synchronous with breast, and 5 with distant failure. The 5-year rate of any nodal recurrence was 1.6% and did not differ by mECE (2.3% vs 1.3%,  $p=0.84$ ). No differences were observed in local (0% mECE vs. 1.9% no mECE,  $p=0.08$ ) or distant (1.2% mECE vs. 4.6% no mECE,  $p=0.31$ ) recurrence rates by mECE status. In comparison, in the 118 patients having ALND, 101 (86%) had mECE, and 1 combined nodal and distant recurrence was seen.

### Conclusions

In Z0011-eligible patients, rates of nodal recurrence in patients with mECE are low after treatment with SLN biopsy alone, even in the absence of routine nodal radiation. The presence of mECE should not be considered a routine indication for ALND.

# 2018 San Antonio Breast Cancer Symposium ®

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Residual axillary involvement in early breast cancer in patients with positive sentinel nodes after neoadjuvant chemotherapy (NACT)

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**Background:** The association between pathological complete remission (pCR) in the breast and clinical/pathological parameters is well established, whereas the role of these parameters in the prediction of residual axillary involvement after NACT is unclear. The goal is to identify a subset of patients who do not need axillary treatment. We used data from Arm B of the SENTINA trial to analyze this association.

**Methods:** Patients from arm B of the SENTINA trial with clinically and sonographically unsuspicious axillary nodes but with histologically proven involvement of SLNs prior to NACT were analyzed. All patients had SLNB and axillary dissection after NACT. Univariate analyses were performed to evaluate the association between clinical/pathological parameters and axillary involvement after NACT.

**Results:** Arm B of the SENTINA study contained 360 patients, 318 of which were evaluable with respect to the above parameters. After NACT 71/318 (22.3%) patients had involved SLNs or non-SLNs; 71/318 (22.3%) had a pCR in the breast. We observed a significant association between pCR in the breast and negative ER status, negative PR status, positive HER2 status, triple negative (TN) status, tumor size before and after NACT, multifocality, lobular morphology and axillary involvement after NACT. Regarding residual axillary burden only the associations with lobular morphology, extracapsular invasion, multifocality, positive HER2 status and pCR in the breast were statistically significant.

**Conclusion:** Our analysis demonstrates that patients enrolled in the SENTINA trial with clinically and sonographically unsuspicious axillary nodes but proven histological involvement of SLNs prior to NACT have positive axillary nodes in 22.3 % after NACT. This rate is confirming similar results from other groups. Although we found statistically significant associations between pCR in the breast and clinical/pathological parameters, only the association between lobular type, extracapsular invasion, positive HER2 status and pCR in the breast and residual axillary involvement after NACT were statistically significant. We cannot clearly identify a subset of patients for whom axillary treatment after NACT could be safely omitted if SLNs were positive. Our data are well in line with recently presented data demonstrating that the association between pCR in the breast and free axillary nodes after NACT is particularly strong in patients with TN and HER2 positive tumors. This question will be addressed in future trials currently under development.

# 2018 San Antonio Breast Cancer Symposium ®

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Conversion rates from positive to negative axillary involvement in breast cancer patients presenting with biopsy-proven axillary metastases prior to primary systemic therapy (PST) – A transSENTINA subproject

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## **Background:**

Modern chemotherapy carries a high potential of converting patients with clinically suspicious axillary nodes (cN+) prior to PST to clinically (ycN0) or pathologically (ypN0) negative nodes after PST. Clinical and sonographical axillary assessment, however, may be inadequate and therefore pathological assessment of suspicious axillary nodes is recommended. We analyzed the association between clinical / pathological parameters and positive to negative conversion in patients with biopsy-proven axillary metastases in Arm C of the SENTINA trial (i.e. patients with “true conversion”).

## **Methods:**

Arm C of the SENTINA trial included patients converting from cN+ to ycN0 through PST from a prospective study. We limited our analysis to patients who had biopsy-proven axillary involvement. Univariate regression analysis was carried out to assess the association between patients with vs. without axillary disease after PST in i) patients with biopsy-proven involvement and ii) patients without biopsy proof of metastases.

## **Results:**

Among 596 patients in arm C of the SENTINA trial with clinically and or sonographically suspicious ipsilateral axillary nodes, 439 (73,7%) 157 (26,3%) patients had a biopsy. In 152 patients (96,8%), lymph node metastases were confirmed and in 5 patients (3,2%), no malignant cells were identified. In both groups, we found a significant association ( $p < 0,05$ ) between increased rate of axillary conversion and small tumor diameter after PST, absence of multifocality, absence of lymphovascular invasion (LVI), ER and/or PR negativity, HER2 negativity, triple negative disease, and complete pathological response (pCR). No multiple testing corrections were performed due to an exploratory setting. However, only among patients with biopsy-proven involvement prior to PST, we found grade-3-tumors to be significantly associated with reduced probability of residual axillary involvement (76.1 vs. 33.8%, compared to G1 and G2,  $p = 0.0323$ ).

## **Conclusion:**

Our analysis demonstrates that in patients with biopsy-proven axillary involvement before NST, parameters associated with axillary conversion are similar to those among patients classified as having nodal disease based on clinical and or sonographical assessment (cN+). Our analyses demonstrate that in biopsy-proven axillary metastases before NST, modern chemotherapy regimens result in significant rates of axillary conversion. This underscores the need to deescalate axillary staging / treatment with the goal to further avoid unnecessary axillary surgery.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD8-04

Neoadjuvant chemotherapy for breast cancer: Nodal downstaging is highly correlated with pathological complete response

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**Background:** Neoadjuvant chemotherapy (NAC) is employed in patients with larger tumors to attempt to downstage locally advanced cancers to allow breast conservation and to assess in vivo tumor response. The Multi-Institutional Neoadjuvant Therapy MammaPrint Project I (MINT) study asked a secondary question of whether complete nodal downstaging could also be achieved with NAC.

**Methods:** This analysis included 147 eligible invasive breast cancer patients with high tumor burdens, classified as cT2-4N0-3M0 (T2 greater than 3.5cm if N0). Patients who had a positive core biopsy and/or fine needle aspiration (FNA) on an axillary node prior to starting NAC were included in this analysis. Those who had a surgical sentinel lymph node biopsy were not included. Nodal involvement was established following neoadjuvant treatment by axillary lymph node dissection (ALND).

**Results:** This population was 54% postmenopausal, average age 53 yrs (range 25 to 80 yrs). Tumor characteristics were 91% invasive ductal carcinoma; 65% T2, 29% T3, 6% T4; 87% LN1, 13% LN2-3; 3% low grade, 38% intermediate grade, 59% high grade; 65% ER-positive, 49% PR-positive, and 28% HER2-positive by immunohistochemistry; 84% High Risk (HR) and 16% Low Risk (LR) by MammaPrint (MP). After NAC, 45% (66/147) of these LN-positive patients were down-staged to ypN0 and also achieved a complete pathological response in the primary tumor.

Pre vs Post NAC Nodal Stage

Pre NAC Nodal Stage	ypN0	ypN1	ypN2	ypN3	Total
cN1	60	44	22	2	128
cN2	6	2	6	3	17
cN3			1	1	2
Total	66	46	29	6	147

The potential for down-staging was inversely-related to tumor burden, where 47% (60/128) of N1, 35% (6/17) of N2, and 0% (0/2) of N3 patients were down-staged to ypN0. There were 3 patients who were down-staged (2 N2 to N1, and 1 N3 to N2), but not to ypN0. At surgery, 34% (44/128) of patients had no change, and 19% (24/129) progressed in LN staging.

**Conclusions:** We confirmed that upon achieving a complete response of the primary tumor that there was also a pathologic complete response in the LN. About 53% of patients had no change or progression of LN involvement following NAC.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD8-05

POSNOC - Positive sentinel node: Adjuvant therapy alone versus adjuvant therapy plus clearance or axillary radiotherapy

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**Background:** Role of additional axillary treatment (AxT) (axillary lymph node dissection (ALND) or axillary radiotherapy (ART)) in women with  $\leq 2$  macrometastases and undergoing systemic therapy remains unclear. Z11 included both micro and macrometastases (around 40% micrometastases) and showed that ALND may be omitted in women with  $\leq 2$  positive nodes undergoing breast conserving surgery (BCS) and receiving whole breast RT. Paradoxically, NCIC MA20, demonstrated improved DFS following the addition of regional RT. 51.8% (949/1832) had 1 or 2 positive nodes. 98.9% (1812/1832) had T1/T2 tumours. A post-Z11 survey shows that most US radiation oncologists treat the undissected axilla in women with macrometastases with ART rather than omitting AxT. Therefore, a confirmatory study is needed to clarify the role of additional AxT in women with  $\leq 2$  macrometastases undergoing BCS and other subgroups that were not included in Z11 e.g. mastectomy, microscopic extranodal invasion and sentinel node biopsy (SNB) before neoadjuvant chemotherapy.

**Methods:** Primary objective is to assess whether for women with  $\leq 2$  macrometastases at SNB, systemic therapy alone is non-inferior to systemic therapy plus AxT in terms of axillary recurrence at 5 years. Secondary objectives are arm morbidity assessed by LBCQ and QuickDASH questionnaires; QoL assessed by FACT-B+4 questionnaire; anxiety assessed by STAI; loco-regional recurrence; distant metastasis; time to axillary recurrence; axillary recurrence-free survival; DFS; OS; contralateral breast cancer; non-breast malignancy; and economic evaluation. Eligibility criteria include:  $\geq 18$  y, uni or multifocal invasive cancer, T1/T2, 1 or 2 macrometastases, with or without extranodal invasion. Target sample size is 1900 with a projected drop-out and non-compliance with treatment allocation rate of 10%. Primary analysis will be per protocol. The following pre-specified subgroup analyses shall be performed: number of macrometastases (1, 2), age ( $<50$ ,  $\geq 50$ ), breast surgery (mastectomy, BCS), ER (positive, negative), tumour grade (1 or 2, 3), SN assessment technique (OSNA, non-OSNA), extranodal invasion (present, absent). POSNOC opened to recruitment in July 2014. To date 1100 women have been recruited at 82 sites in the UK and 18 sites in Australia and New Zealand. Clinicaltrials.gov NCT02401685.



# 2018 San Antonio Breast Cancer Symposium ®

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Fluorescence guided breast conserving surgery

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**Background.** Palpation guided breast conserving surgery for breast cancer is associated with tumor involved margins in up to 41% of cases. Ultrasound guided breast conserving surgery and frozen section biopsy result in a significant reduction in margin positivity. However, intra-operative USG and Frozen section biopsy are not widely available. We aimed to find a simple and effective technique for intra-operative margin assessment during breast conserving surgery.

**Hypothesis.** Intravenously injected Fluorescein reaches tumor tissue in high concentration due to increased tumor blood flow and increased capillary permeability which can be detected by blue light (494 nm).

**Objective.** To find diagnostic accuracy of fluorescence guided identification of tumor margins during breast conserving surgery.

**Methods.** A total of 60 patients with T1–T2 invasive breast cancer who underwent breast conserving surgery at AIIMS, New Delhi between March, 2016 to Feb, 2018 were included. Each patient received 2 ml of intra-venous 20% Fluorescein sodium just before skin incision. Breast conserving surgery was performed under USG guidance. Specimen was bisected and examined under blue light. Fluorescent tumor margins were identified and six biopsies taken from non-fluorescent area 5mm outside from fluorescent tumor margins at 2,4,6,8,10 and 12 o'clock position. The involvement of USG guided margin was compared with Fluorescence guided margins on histopathology.

**Results.** Mean age of the patient was 51.2 (SD=6.4) years. Fourteen patients (20.3 %) had T1 tumors while 46 patients(69.7%) had T2 tumors. Axillary nodes were involved in 19 patients (20.1%) and all underwent axillary node dissection. Eight patients (13.3%) received NACT. Fluorescent margins in two (3.3%) out of 60 patients were involved by tumor while USG guided margins in all the 60 patients were free. The specificity of fluorescence in identifying uninvolved tumor margins during breast conserving surgery is 96.7%.

No patient developed adverse drug reactions.

**Conclusion.** The fluorescence identifies tumor free margins with 96.7% accuracy. It is simple, effective and affordable method of identifying margin positivity in breast conservation surgery.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD8-07

Does resection of cavity shave margins result in lower positive margin and re-excision rates in patients with stage 0-III breast cancer? Results from a prospective multicenter randomized controlled trial

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**INTRODUCTION:** Routine resection of cavity shave margins has been shown in single center studies to result in a significant reduction in positive margin and re-excision rates. In this prospective multicenter randomized controlled trial, we sought to validate these findings across practice settings.

**METHODS:** Nine centers across the United States, varying in practice setting and patient population, participated in this clinical trial of 398 stage 0-III breast cancer patients undergoing partial mastectomy (with or without resection of selective cavity margins). Participants were stratified by clinical stage and randomized 1:1 to either have routine cavity shave margins resected ("shave") or not ("no shave"). Randomization group was revealed to the surgeon intraoperatively, after they had completed their standard partial mastectomy and were ready to close. Positive margins were defined as "tumor at ink" for invasive cancer or within 2 mm for ductal carcinoma in situ (DCIS). Adverse events were defined as seromas requiring percutaneous drainage, and/or hematomas or abscesses requiring operative intervention.

**RESULTS:** Median patient age was 65 (range; 29-94). 116 patients had invasive disease, 74 had DCIS, 179 had both, and 29 had no residual cancer at the time of partial mastectomy. The median invasive cancer size was 1.2 cm (range; 0.05-8.00 cm); the median extent of DCIS was 0.9 cm (range; 0.05-6.40 cm). The "shave" and "no shave" groups were well matched at baseline for clinicopathologic and demographic factors.

Factor	Shave (n=197)	No Shave (n=201)	p-value
Age (years); median (range)	67 (36-94)	64 (29-89)	0.585
Race			0.062
-- White	173 (87.8%)	164 (81.6%)	
-- Black	20 (10.2%)	33 (16.4%)	
-- Asian	2 (1.0%)	2 (1.0%)	
-- Native American	0 (0%)	2 (1.0%)	
-- Unknown/Declined	2 (1.0%)	0 (0%)	
Hispanic ethnicity	28 (14.2%)	32 (15.9%)	0.806
Invasive tumor size (cm); median (range)	1.30 (0.09-8.00)	1.20 (0.05-7.50)	0.282
DCIS extent (cm); median (range)	0.80 (0.10-6.40)	1.00 (0.05-5.50)	0.906
Invasive histology			0.556
-- Ductal	177 (89.8%)	186 (92.5%)	
-- Lobular	16 (8.1%)	13 (6.5%)	
-- Mucinous	3 (1.5%)	2 (1.0%)	

-- Other	1 (0.5%)	0 (0%)	
Neoadjuvant therapy	15 (7.6%)	19 (9.5%)	0.592
Palpable tumor	57 (28.9%)	56 (27.9%)	0.825
Node positive*	24 (16.3%)	16 (10.6%)	0.175

\*Of the 298 patients who had lymph nodes evaluated

Prior to randomization, positive margin rates were similar in the “shave” and “no shave” groups (38.1% vs. 37.3%, respectively,  $p=0.918$ ). After randomization, however, those in the “shave” group were significantly less likely than those in the “no shave” group to have positive margins (8.6% vs. 37.3%, respectively,  $p<0.001$ ). They were also less likely to require re-excision or mastectomy for margin clearance (8.6% vs. 23.9%,  $p<0.001$ ). There were no significant differences between the two groups in terms of adverse events ( $p=0.280$ ). Rates of seroma (1.5% vs. 0.5%,  $p=0.368$ ), hematoma (0.5% vs. 0.5%,  $p=1.000$ ) and abscess (0.3% vs. 0%,  $p=0.495$ ) were similar between the “shave” and “no shave” groups, respectively.

**CONCLUSION:** Resection of cavity shave margins significantly reduces positive margin and re-excision rates in patients with stage 0-III breast cancer undergoing partial mastectomy.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD8-08

Development of humanized immune DCIS models using patient peripheral blood derived hematopoietic stem cells (CD34+)

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## **Background:**

Ductal carcinoma in situ (DCIS) is the most common form of non-invasive breast cancer. To accurately study the natural progression of DCIS lesions in mice, we devised the mouse-intraductal (MIND) animal model, which involves intraductal injection of human DCIS epithelial cells into the mammary ducts of immunocompromised mice. To improve the translational application of the MIND model, we aimed to mimic the natural microenvironment of human DCIS with patient-derived immune cells and assess the role of engrafted immune cells on human DCIS progression. In order to achieve successful engraftment of the entire immune system in mice, we utilized MISTRG mice. These mice were developed by Rongvaux *et al.*, on an immunodeficient (Rag2<sup>-/-</sup>IL2r<sup>-/-</sup>) background. The genes encoding human M-CSF (M), human IL-3 (I), SIRP1 $\alpha$  (S), human thrombopoietin (TPO)(TR), and GM-CSF (G) were knocked into their respective mouse loci. As such, MISTRG mice are highly permissive for human hematopoiesis, supporting the development and function of lymphocytes, monocytes, and natural killer (NK) cells. In contrast, previous studies have used the humanized CD34+ NOD-SCID IL2r<sup>-/-</sup> mice (CD34+NSG), which are unable to support myeloid cell differentiation due to lack of expression of human-specific cytokines. Moreover, prior xenograft studies in the CD34+NSG mice have not used immune cells derived from the same patient as the tumor.

## **Results:**

Human CD34+ cells derived from patients' peripheral blood were expanded *ex vivo* ~100-fold using a novel formulation of culture medium. Transplantation of *ex vivo* expanded CD34+ cells via tail vein injection of MISTRG mice resulted in the successful engraftment of human immune cells as early as 4 weeks following injection. Successful engraftment was confirmed by flow cytometry using human specific antibodies that recognize human leukocytes (anti-CD45), T cells (anti-CD3), B cells (anti-CD20), and myeloid cells (anti-CD33) in spleen, bone marrow, and peripheral blood of MISTRG mice. Once engraftment was confirmed, DCIS epithelial cells from the same DCIS patients or DCIS cell lines were injected intraductally. Recruitment of patient-derived immune cells to the DCIS lesions was confirmed by immunofluorescence using human-specific antibodies that recognize neutrophils (anti-myeloperoxidase), macrophages (anti-CD68), M2-polarized macrophages (anti-c-MAF), natural killer cells (anti-CD56), dendritic cells (anti-CD21), T cells (anti-CD3) and B cells (anti-CD20).

## **Conclusion:**

This model represents the first to enable the study of mechanisms of DCIS progression in a manner that fully represents the heterogeneity of human disease, including the influence of the patients' own immune cells on DCIS progression.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD8-09

Approximately 40% of invasive recurrences after treatment of ductal carcinoma in situ is likely to be a second primary tumor

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**Background.** Ductal carcinoma in situ (DCIS) is a potential precursor of invasive breast cancer, because: DCIS often accompanies invasive breast cancer; its risk factors are similar to those of invasive breast cancer; and genetic markers found in DCIS are similar to the ones found in invasive breast cancer. However, clinical behavior of DCIS is still poorly understood, as there is only limited information on its long-term natural history. Altogether, this makes it difficult to understand the relatedness of DCIS and its subsequent ipsilateral invasive breast cancer (iIBC). Here, we set-up a comparison between primary DCIS and matched subsequent iIBC, by making use of pathological and molecular data.

**Patients and methods.** For this study, we used a unique series of 155 DCIS cases which developed a subsequent iIBC during a median follow up period of 12.6 years. We assessed histological characteristics, tumor location, estrogen and progesterone receptor status, p16 expression, and HER2 and p53 overexpression. RNA sequencing and copy number sequencing was done on 78 DCIS lesions and 78 matched invasive breast cancer relapses. We determined if the iIBC lesion and DCIS lesion were related, with respect to tumor location, immunohistochemical (IHC) markers, and genomic features.

**Results.** Based on tumor location and histological grade, >95% of the subsequent invasive breast cancers reflected outgrowth of residual disease. HER2 was the only IHC marker that showed a significant difference in expression between DCIS and matched iIBC: 40% of the HER2 positive DCIS was followed by a HER2 negative invasive recurrence. In addition, RNAseq data was used to classify DCIS and IBC lesions into PAM50 subtypes. 77% of the DCIS IBC pair belonged to the same subtype. The DCIS lesions showed copy number aberrations on typical breast cancer-associated loci. However, when we compared the DCIS with its matched iIBC, we saw in 41% of the cases very distinct copy number profiles, indicating either outgrowth of a different tumor subclone or a second primary.

**Conclusion.** This is the first time that a sound comparison could be made between primary DCIS and its subsequent invasive breast cancer with such a large patient group, integrating pathological and molecular data. Our results strongly suggest that many subsequent iIBCs after treatment of pure DCIS could be considered as second primary breast cancer lesions. To provide definite proof for this, in depth DNA sequencing and heterogeneity studies will be presented at SABCS 2018.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD8-10

No increased cardiovascular mortality after twenty years in a randomized trial of radiotherapy after breast conserving surgery, SweBCG 91RT, from the Swedish breast cancer group

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## Introduction

Breast conserving surgery (BCS) with postoperative radiotherapy (RT) is recommended for the majority of patients with early breast cancer. Meta-analyses of randomized trials have shown reduction of breast cancer and overall mortality after RT but increased cardiovascular mortality

## Aim

To evaluate side effects of RT after BCS

## Patients and Methods

Trial SweBCG 91RT randomized 1187 women with stage 1-IIA breast cancer after BCS to RT or to no further treatment. RT was administered as tangential opposing beams to the breast to a target dose of 48-54 Gy. Three-dimensional dose planning on multiple CT slices 10 mm apart was used for 69% of the patients and for the remainder two-dimensional planning. All hospital records and radiotherapy charts were monitored. Update of mortality, cause of death and morbidity was made using the Swedish personal identification numbers and the following Swedish national registers: the Cancer Register, the Population Register, the Cause of Death Register, the Inpatient and the SWEDEHEART registers. For analyses the diagnoses were grouped as follows: breast cancer, cardiovascular disease, cerebrovascular disease, lung cancer and benign pulmonary disease

## Statistics

Overall mortality was calculated using the Kaplan-Meier method, whereas cumulative incidence functions with other causes of death as competing events were used for cause-specific mortality and morbidities. For all outcomes, log-rank tests were used to compare between treatment groups up to 20 years. We studied two populations: 1) patients according to intention to treat for overall and breast cancer mortality and 2) patients treated per protocol for side effects. Cause-specific death was allocated to a certain group when either the underlying or a contributing cause belonged to the disease group

## Results

After 20 years, overall mortality was 42.9% after BCS and 42.5% after BCS+RT ( $p=0.8$ ), and breast cancer mortality was 18.0% vs 15.8% ( $p=0.3$ ). The cumulative incidence of mortality from heart disease was 12.4% after BCS and 13.0% after BCS+RT ( $p=0.8$ ) with no difference for left or right side. Ischemic heart disease and congestive heart failure were the most common cardiac diagnoses. The cumulative incidence of cerebrovascular mortality was 3.4% among controls and 6.7% after RT ( $p=0.016$ ). Of the patients with cerebrovascular death, 50% also had a cardiac cause of death. Other cause-specific mortalities investigated were similar regardless of RT: lung cancer 1.7% vs 1.9% ( $p=1.0$ ), benign pulmonary disease 7.1% vs 6.4% ( $p=0.5$ )

## Morbidity

Morbidity outcomes were also similar in control and irradiated patients: the cumulative incidence of hospital admissions with cardiac diagnoses 29.7% vs 31.0% ( $p=0.7$ ), and for cerebrovascular morbidity 11.6% vs 13.7% ( $p=0.33$ ) respectively

## Radiation exposure

The original dose plans were retrieved from 125 patients. Doses to organs at risk were recalculated. Median of mean heart dose was 3.0 Gy (1.1-8.2) for left and 1.0 Gy (0.5-2.5) for right-sided RT

## Conclusions

After 20 years tangential RT in a randomized trial was not associated with increased cardiac mortality. A minor increase in cerebrovascular mortality was seen, but the causality is unclear.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD8-11

Distribution of locoregional breast cancer recurrences in relation to previous radiotherapy and biological subtype

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**Background:** Adjuvant locoregional radiotherapy (LRRT) reduces risk of locoregional failures (LRF) and improves survival for node positive breast cancer (BC) patients. However, LRRT increases the risk for toxicity as edema in the arm, lung cancer and cardiac mortality. Modern radiotherapy allows a more conform therapy which makes knowledge of LRF-patterns very important, in order not to underdose volumes with high risk for microscopic disease and simultaneously restrict dose to risk organs. In addition, BC-subtype may affect radiosensitivity and could possibly be used to individualize LRRT in the future.

**Methods:** We investigated outcome for BC-patients receiving LRRT in the Southwest Sweden (2004-2008) in order to identify LRFs. During this period patients with >3 positive lymph nodes were given LRRT (50Gy in 2Gy fractions) to the breast/thoracic wall, axilla level II+III, supra- and infraclavicular lymph nodes according to a target definition atlas introduced in 2002. Patients with LRF as first event were identified, with distant failures and death considered as competing risks. The anatomical distribution of LRF was compared with the contouring atlas, radiotherapy given, and biological subtype based on immunohistochemistry.

**Results:** 904 patients received LRRT. Median follow-up time was 9.8 years (0.2-14.6) for patients without an event. 59 patients (6,5%) developed a LRF, 30 of which were local failures (LF) and 31 regional failures (RF) (2 simultaneous LF/RF). Median time to LRF was 2.8 years. 37 of the 59 (63%) LRF-patients developed generalized disease within 3 months from the LRF. Of the 845 patients without LRF 316 developed distant metastases as first recurrence, 1 an isolated RF in the contralateral axilla, 64 died from other causes, and for 5 patients recurrence-status was unclear. 459 were alive at end of follow-up.

Of the LF 19 developed after mastectomy (MRM) and 11 after breast conserving surgery (BCS). LF-location after MRM: 9 in-field, 6 at field margin, 2 both in/out of field, 1 out of field, and for 1 not yet determined. After BCS: 8 in-field, 1 at field margin, and for 2 not yet determined. Of the RF 28 developed after MRM and 3 after BCS. Location of RF after MRM: 11 in-field, 6 at field margin, 1 both in/out of field, 2 out of field, and for 8 not yet determined. After BCS: 1 in-field, 1 at field margin, and 1 not yet determined. The most common location for RF close to /out of field was superior to the treated area in fossa supraclavicularis.

Biological approximate subtype was available for 885 of the primary tumours; luminal (ER+ and/or PR+ HER2-/? ) 67% (589/885), HER2+ 19% (169/885), triple negative (ER- and PR- HER2-/? ) (TN) 14% (127/885). Subtype distribution of BC later causing LRF despite LRRT was: luminal 44% (26/59), HER2+ 27% (16/59), TN 29% (17/59). Among primary tumours causing a LRF within irradiated volume 77% (24/31) were HER2+ or TN.

**Conclusion:** In this high risk cohort of BC-patients, we found a low incidence of LRFs. The majority of LRFs developed within irradiated volume. BCs of the HER2+ and TN-subtype may be more radioresistant and have a higher risk of LRF. Updated information as well as figures mapping all recurrences in relation to previous LRRT will be presented at the symposium.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD8-12

Safety and efficacy of palbociclib and radiotherapy in metastatic breast cancer patients: Initial results of a novel combination

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**Purpose:** Palbociclib is a selective CDK4/6 inhibitor approved for the treatment of metastatic ER+/HER2- breast cancer. Inhibition of CDK4/6 prevents cell cycle progression from G1 to the more radioresistant S phase, raising the possibility of an enhanced therapeutic effect if combined with radiotherapy (RT). Despite this potential benefit, clinicians seldom use this combination due to fear that RT may exacerbate palbociclib toxicity, particularly leukopenia. Our aim is to report the preliminary results of patients with metastatic breast cancer who received RT while receiving palbociclib.

**Methods:** We retrospectively reviewed records of all patients who were treated with palbociclib at our institution from 2015-2018. Patients who received RT for symptomatic metastases concurrently or within 14 days of last drug administration were included in our analysis. Local treatment effect was assessed by clinical exam and subsequent CT or MRI imaging, if applicable. Toxicity was graded based on CTCAE v5.0.

**Results:** A total of 16 females received palliative RT in association with palbociclib. The median age of the treated patients was 59.6 (range 33.3-91.0) years. The median time of closest palbociclib use to RT administration was 5 (range 0-14) days. The following sites were treated in order of frequency: bone (10-axial skeleton [8-vertebra]; 1-ilium), brain (4: 3-WBRT & 1-SRS), and mediastinum (1). RT dose/fractionation for bone was 30 Gy/10 fxn (7), 35 Gy/14 fxn (2), 37.5 Gy/15 fxn (1), and 18 Gy/1 fxn (1). WBRT dose/fractionation was 30 Gy/10 fxn for all patients. SRS brain dose was 25 Gy/5 fxn. The patient treated to the mediastinum received 36 Gy/18 fxn.

At most recent follow-up, 12 patients are still living. The median time from RT to last known follow-up or death is 10.3 (range 1.7-29.6) months. Pain relief was achieved in 15 of 16 (93.8%) patients. No radiographic local failure was noted in the 13 patients with evaluable follow-up imaging.

The combination of RT and palbociclib was well-tolerated. Grade 1 fatigue, dermatitis, and nausea was noted in 5, 3, and 1 patient, respectively. One patient with WBRT developed Grade 1 headache. Six of 16 patients were leukopenic prior to RT initiation. Following RT, 7 patients were observed to have a drop in WBC count, of which 2 dropped into the leukopenic range. Only a total of 5 patients were leukopenic following RT, of which 3 were leukopenic before receiving RT. No acute or late Grade 2 or higher cutaneous, neurological, gastrointestinal, or hematologic toxicities were noted.

**Conclusions:** The use of RT in patients receiving palbociclib resulted in minimal Grade 1 and no Grade 2+ toxicities, including leukopenia. This treatment can be used safely in symptomatic patients without discontinuation of systemic therapy. Further larger prospective studies with longer follow-up are needed to confirm these results.



# 2018 San Antonio Breast Cancer Symposium ®

Publication Number: PD9-01

Genomic alterations associated with loss of HR expression in metastatic breast cancer

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**Background:** Discordance in hormone receptor (HR) status between primary (p) tumors and metastatic (m) recurrences has been widely described. Loss of estrogen and progesterone receptor expression occurs in ~12% of asynchronous recurrences, leading to triple-negative (TN) status in the metastasis. Genomic mechanisms driving HR loss and its prognostic and therapeutic implications have not been fully elucidated.

**Methods:** Targeted NGS (Oncopanel, OP) at Dana-Farber Cancer Institute using multiplexed copy number variation and mutation (mut) detection across the full coding regions of 300 genes and selected intronic regions of 35 genes was prospectively performed on either archival primary or metastatic samples collected in patients (pts) with metastatic breast cancer (MBC). Receptor status at initial diagnosis and recurrence were reviewed using a 1% cutoff to define HR-positivity and excluding HER2+ cases. Fisher's exact test was used to compare frequency of alterations. Tumor mut burden (TMB) was computed normalizing the sum of reported exon mut in each pt by the exonic-bait-set size of the panel.

**Results:** Between 8/2013-9/2016, 929 pts with MBC underwent OP testing. Of 517 pts diagnosed with primary HR+/HER2- breast cancer, at time of recurrence 388 remained HR+/HER2- (pHR+/mHR+), 39 switched to HR-/HER2- (pHR+/mTN, of which 23 (59%) had initial HR expression >10%), 10 switched to HER2+ and 80 had unknown metastatic receptor status. Comparison between primary samples in pHR+/mHR+ (n=245) and pHR+/mTN (n=24) showed that pHR+/mTN was significantly more likely to harbor mut in *TP53*, *STK11* and *MSH6*, amplifications (amp) in *CCNE1* and *FGFR2*, and less likely to have *PIK3CA* mut or *CCND1* amp.

	pHR+/mHR+ (n=245)		pHR+/mTN (n=24)		p value
	N	Freq (%)	N	Freq (%)	
<b>Mut</b>					
TP53	63	25.7	20	83.3	<0.00001
PIK3CA	94	38.4	0	0	0
GATA3	35	14.3	0	0	0.053
STK11	5	2.0	3	12.5	0.026
MSH6	4	1.6	3	12.5	0.017
<b>Amp</b>					
FGFR2	0	0	2	8.3	0.008
CCNE1	0	0	2	8.3	0.008
CCND1	44	18.0	0	0	0.018

Median TMB in primary pHR+/mHR+ was 6.05 mut/Mb (0-37.5) and 5.68 mut/Mb (1.2-10.9) in pHR+/mTN (p=0.45). Metastatic samples in pHR+/mTN (n=15) were enriched in *ARID1A*, *CRTC2* and *CDH1* mut compared to metastases (n=40) in pts who remained TN (pTN/mTN). Deletions in *CDKN2A/2B* and *RB1*, and mut in *TP53*, *NOTCH2* and *ERCC2* were more prevalent in recurrent tumors of pHR+/mTN than pHR+/mHR+. In metastases, TMB was higher in pHR+/mTN than pTN/mTN or pHR+/mHR+.

(10.9 vs. 7.0 vs. 7.3 mut/Mb, respectively;  $p=0.002$ ). Median OS from initial diagnosis was 9.4 yrs in pHR+/mTN, less than pHR+/mHR+ (15.9 yrs;  $p=0.009$ ) and greater than pTN/mTN (4.3 yrs;  $p=0.008$ ). Median OS from MBC diagnosis was 1.8 yrs in pHR+/mTN, less than pHR+/mHR+ (6.4 yrs;  $p=0.001$ ) but not significantly different than pTN/mTN (1.5 yrs,  $p=0.3$ ).

**Conclusion:** Targeted NGS shows that alterations in DNA damage and cell-cycle regulation pathways in primary HR+ tumors are associated with HR loss in the metastatic setting. Primary tumors that lose HR appear more similar to basal-like than luminal tumors, despite >10% baseline HR expression in most pts, and once metastatic, survival is comparable to pTN/mTN. Metastases with HR loss have higher TMB than those that remain HR+ or TN throughout the course of the disease. These findings, if confirmed, may influence treatment and pt selection for clinical trials.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-02

Evolutionary analysis of 462 serial metastatic biopsies from 208 patients with estrogen receptor-positive (ER+) metastatic breast cancer (MBC) using whole exome sequencing (WES)

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**Background:** While great strides have been made in the treatment of ER+ MBC, therapeutic resistance is nearly universal. The genomic evolution of ER+ breast cancer in the metastatic setting under the selective pressure of multiple lines of therapies is not well understood. To address this, we analyzed the clonal dynamics of serial metastatic samples (mets) to evaluate how tumors evolve and to identify acquired resistance mechanisms.

**Methods:** We performed WES on 462 clinically annotated samples from 208 patients (pts) with ER+ MBC, including 67 primary tumor biopsies, 229 metastatic biopsies and 160 blood samples (cfDNA). Pts with multiple mets included cases with temporally concordant metastatic tumor and blood samples (48 pts) and cases with serial mets obtained over the course of treatment in the metastatic setting (69 pts). Treatments given between the serial mets included CDK4/6 inhibitors (23 pts), and selective estrogen receptor degraders (19 pts), among others.

**Results:** In the temporally-concordant mets, we found that cfDNA mutations (mut) largely overlap with muts found in tumor biopsies, capturing >85% of clonal tumor muts. However, we observed a higher level of heterogeneity in cfDNA compared to biopsies (p.value< 1.05e-19, Welch test) and a subset of high-confidence muts that were only detected in cfDNA, including in clinically important genes such as *ESR1*, *PIK3CA*, *KRAS*, and *ERBB2*. Analysis of serial mets was used to elucidate the evolutionary dynamics within the metastatic setting under the selective pressure of treatment. The median duration between mets was 112 days and the median number of inter-biopsy unique treatments was two. Most tumors continued to evolve within the metastatic setting, with 50 out of 69 pts (72%) acquiring a meaningful sub-clone (50% increase in relative cancer cell fraction) and 31 out of 69 (45%) acquiring muts in known cancer genes, including a subset acquiring a plausible resistance alteration such as alterations that dysregulate ER (5 out of 69 pts, 7%; *ESR1* mut, *FOXA1* amplification (amp), *NCOR1* bi-allelic deletion (del)), *ERBB* (4%; *ERBB2* amp, *ERBB3* mut), *RAS* (4%; *KRAS* mut, *NRAS* amp, NF1 del), *FGF/FGFR* (12%; *FGFR2* mut, *FGFR1/2* amp, *FGF3* amp), and cell cycle (13%; *RB1* del, *CDK4* amp, *AURKA* amp, *CDKN2A* del). Finally, in pts who had multiple mets, we observed several cases of evolutionary convergence toward equivalent resistance mechanisms including convergent *RB1* loss as a mechanism of resistance to a CDK4/6 inhibitor and convergent *BRCA2* reversion following resistance to a PARP inhibitor.

**Conclusions:** This study demonstrates that ER+ MBC continues to evolve under the selective pressure of treatments in the metastatic setting. These findings elucidate the challenge of studying high complexity and heavily treated tumors, while also highlighting some commonalities in the evolutionary trajectories selected by these treatments. The multiplicity of clinically relevant genomic alterations acquired in these advanced stages highlights the need for serial biopsies and the potential to inform post-progression therapeutic choices through targeting the acquired dependencies in post-progression tumors.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-03

The genomic landscape of *de novo* metastatic breast cancer (MBC)

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**Background:** Approximately 5-10% of newly diagnosed breast cancers (BC) are *de novo* MBC, which means that metastatic disease was identified at the time of initial diagnosis. Patients with *de novo* MBC are underrepresented in currently available genomic studies. In The Cancer Genome Atlas (TCGA) dataset, only 15 out of ~980 BC patients can be classified as having *de novo* MBC. The objective of this study is to analyze the genomic landscape of *de novo* MBC and to study the genomic differences of this cohort with early stage BC. To enhance our ability to study *de novo* MBC, we utilized data from the Metastatic Breast Cancer Project (MBCproject), a patient-partnered research project that aims to generate a large public database of clinical, genomic, and patient reported data (PRD) from patients with MBC.

**Methods:** We defined *de novo* MBC as patients diagnosed with metastatic disease less than 4 months after their initial diagnosis with BC. Out of 127 patients in the MBCproject with publicly released whole exome sequencing (WES) data, we identified 33 patients with *de novo* MBC. We combined this data with 15 *de novo* MBC patients in TCGA. For patients with *de novo* MBC with multiple tumor biopsies available, we used WES from breast biopsies to enable appropriate comparison to the early stage biopsies. Somatic mutations were evaluated and significantly recurring genes were identified using MutSig2CV. We compared the mutations found in the *de novo* cohort with early stage tumors. 10 patients in the *de novo* MBC cohort had paired simultaneous primary and metastatic biopsies; genomic alterations in these samples were compared. Finally, we used RNA sequencing data to compare cytolytic signatures among the *de novo* and early stage biopsies.

**Results:** Among the 48 patients analyzed the receptor subtype distribution was: HR+/HER2-(23), HR+/HER2+(13), HR-/HER2+(2), HR-/HER2-(3), HR+/HER2 unknown(5), and HR-/HER2 unknown(2). Histology subtype distribution was as follows: IDC(39), MDLC(6), ILC(2) and Other (1). Significantly recurrent genes in the *de novo* MBC cohort (q<0.1) included *TP53*(27%), *PIK3CA*(30%), *CDH1*(8%) and *MAP3K1*(11%). Mutations in *PTEN*, *EGFR*, and *MDM4* were significantly enriched (p <0.05) in the *de novo* cohort when compared to early stage BC

Gene	% Mutation Rate in De Novo MBC (N=48)	% Mutation rate in Early Stage BC (N= 997)	p-value
<i>PTEN</i>	10.40	3.51	0.0324
<i>EGFR</i>	6.25	0.50	0.00435
<i>MDM4</i>	4.17	0.30	0.0189

Evolutionary analysis of paired primary and metastatic biopsies for *de novo* MBC patients demonstrated the presence of shared clonal mutations, indicating that these were highly evolutionarily related. RNA-seq immune cytolytic signature was downregulated in *de novo* MBC as compared to early stage BC (p <0.2).

**Conclusions:** Our initial results highlight genomic differences between *de novo* MBC and early stage BC, including increased frequency of *PTEN*, *EGFR*, and *MDM4* mutations. Enrichment of *PTEN* mutations (implicated in tumor immune surveillance), and downregulation of cytolytic signature potentially suggests that *de novo* MBC may have immunosuppressive tumor microenvironment. To date, ~1200 patients with self-reported *de novo* MBC have registered for the MBCproject. We anticipate that additional study of genomic and clinical data from these patients will greatly improve our understanding of *de novo* MBC.

# 2018 San Antonio Breast Cancer Symposium ®

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FGFR4 as a key regulator of HER2E subtype in the primary and metastatic setting

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**Background:** Therapeutic targets in TNBC remain a challenge. We have observed that some Luminal A primary breast tumors give rise to HER2-enriched (HER2E) subtype metastases but remain clinically HER2 negative (HER2E/cHER2-). Molecular features that drive these HER2E/cHER2- tumors may represent key targets of metastatic progression.

**Methods:** A comparative genetic and transcriptomic analysis in TCGA (1100 patients) related to the FGFR family was performed. We focused on FGFR4, in part, due to its unique association with the HER2E expression subtype and we developed a robust FGFR4-signature based upon a supervised analysis of a HER2E/cHER2- PDX (WHIM11) treated with a FGFR4 inhibitor (BLU9931). We also constructed a new Luminal Tumor Score (LTS) to identify the optimal axis of separation between Luminal A versus HER2E tumors (higher scores represent greater Luminal A phenotype). Univariate and multivariate analyses were performed using TCGA and METABRIC (1971 samples). Finally, we performed RNA-seq on a cohort of 77 matched primary breast cancer and metastatic tissues pairs from the GEICAM/2009-03 and Hospital Clinic of Barcelona study, and did multiple analyses on these cohorts using our FGFR4-signatures.

**Results:** FGFR4 was significantly higher in HER2E subtype ( $P<0.0001$ ), independent of HER2 clinical status. FGFR4 amplification/deletions and mutations were rare and did not correlate with FGFR4 high expression. In vivo, BLU9931 treatment of WHIM11 showed a significant tumor growth inhibition ( $P=0.01$ ), prolonged survival, and a significant higher LTS ( $P=0.016$ ). We also identified 745 up-regulated genes called FGFR4-repressed (FGFR4-rep) and 427 down regulated genes called FGFR4-induced (FGFR4-ind), after BLU9931 treatment. Gene set enrichment analysis revealed that FGFR4-ind genes were enriched for STAT3, PI3K/AKT/mTOR pathway and KRAS activation, proliferation, hypoxia, glycolysis and metastasis. FGFR4-rep genes were involved with KRAS inhibition, cell polarity, p53 pathway and upregulation of IFN $\gamma$  response. In the METABRIC cohort, FGFR4-ind and FGFR4-rep each predicted OS (HR=6.30,  $P<0.0001$ ; HR=0.33;  $P<0.0001$ , respectively). Multivariate analysis showed FGFR4-ind (HR=2.34,  $P=0.014$ ) as a significant independent prognostic factor beyond subtype for OS. Supervised analysis of the 77 primary-met cohort revealed that the FGFR4-ind was significantly higher in luminal metastases compared with their primaries counterparts ( $P<0.001$ ), along with proliferation, angiogenesis, and a M2 macrophage signature (with most other immune features being unchanged). Finally, univariate and multivariate analysis demonstrated that the FGFR4-related signatures predicted site-specific metastasis for lung, liver and brain, but not for bone and lymph nodes.

**Conclusion:** FGFR4 is one of the drivers of HER2-enriched subtype tumors, including those that are clinically HER2-. The FGFR4-ind signature was predictive of worse survival, progression in the metastatic setting, and site-specific metastasis. Treatment options in HER2-enriched TNBC, and for HER2E/cHER2+ patients, may benefit from targeting FGFR4, whose high expression is not based upon genomic or genetic features.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-05

The importance of the metastatic biopsy: Clinical and translational relevance in a real world series of patients with metastatic breast cancer

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## Background

Metastatic breast cancer (MBC) is a heterogeneous disease, whose clinical course and prognosis may be unpredictable, creating significant uncertainty for patients and their families. Heterogeneity in breast cancer subtypes is now well recognized as a potential reason for treatment resistance. Sampling metastatic sites at the point of diagnosis or upon progression, when safe, is recommended to better guide therapy.

## Purpose

This study evaluated patients currently undergoing treatment for MBC in the clinic to determine the clinical and translational significance of a metastatic sample.

## Methods

Patients currently undergoing treatment for MBC at the Olivia Newton John Centre were identified. Data was collected on patient demographics, clinicopathological information, treatment and duration of response. Translational research tissue was collected, with consent, for DNA and RNA analysis.

## Results

Between January 2017 and May 2018 **111 patients were identified**. The mean age of MBC diagnosis was 60 years (range 30-87), with a mean follow up time of 2.4 years (range 0.8-16). Fifteen patients died during the study period. Sixty-seven (60%) patients were initially treated for early breast cancer (EBC), with a median disease free interval (DFI) 4.7 years. Half (51%) these patients relapsed after five years.

At MBC diagnosis, multiple sites of disease were identified including bone, visceral, brain, nodal and skin/chest wall disease. Bone only disease was common (25%), whereas brain disease was rare overall (9%). **Metastatic tissue was collected in 67 (60%) patients**, where up to four different sites were biopsied. The most commonly biopsied site was bone (n=21), followed by soft tissue (n=20), chest wall/skin disease (n=12), liver (n=9), lung (n=8) and brain (n=8). Serous disease was collected in 16 patients, including pleural, pericardial, ascitic and cerebrospinal fluid.

Based on the EBC subtype (n=67), 70% had luminal disease, 19% had Her2 positive disease and 7% had TNBC. However, based on a metastatic biopsy (n=67), only 61% of patients had luminal disease, 21% had HER2 positive, and 18% had TNBC. Paired EBC and MBC samples were available in 48 patients, with **significant change in breast cancer subtype demonstrated in 12 of these patients (25%)**. The most common change was a loss in ER staining, which included 6 patients from ER positive, HER2 negative to TNBC and three patients who became ER negative but remained HER2 positive. Molecular profiling was performed thus far on 8 samples at the single cell and bulk level. These results highlight a large level of inter- and intra-tumoral heterogeneity, and may result in a better understanding of the molecular pathways specifically deregulated in patients at the point of progression.

## Conclusion

In this single institution series of patients with MBC, **over half of the cohort underwent at least one metastatic biopsy**. Strikingly, **a quarter of patients demonstrated a change in their breast cancer subtype**, which directly guided subsequent therapy. Metastatic tissue can provide vital information to inform treatment decisions, which may be guided by translational laboratories having access to fresh tissue at the point of metastatic diagnosis or disease progression.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-06

Paired pre- and post-treatment DNA sequencing identifies genomic alterations mediating clinical resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer

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**Background:** The combination of cyclin-dependent kinase 4 and 6 inhibitors (CDK 4/6 inh) and endocrine therapy have changed the treatment paradigm for hormone receptor-positive metastatic breast cancer (HR+ MBC). Three CDK 4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) are now approved for HR+ MBC in combination with either an aromatase inhibitor (AI) or fulvestrant (a selective estrogen receptor degrader, SERD) after demonstrating significant improvement in progression-free survival compared to endocrine monotherapy. However, both primary and secondary resistance has been observed clinically, and the mechanisms of such resistance are not well understood. Here we describe emergence of molecular alterations following treatment with the combination of CDK 4/6 inhibitors and endocrine therapy.

**Methods:** We evaluated molecular alterations by next-generation sequencing (NGS) prior to and following treatment with CDK 4/6 + endocrine therapy pre and post CDK inh among patients with HR+ MBC. We performed circulating tumor (ct) DNA analysis via the Guardant360 assay, an NGS-based assay that identifies potential tumor-related (somatic) genomic alterations through sequencing of the critical exons within 73 cancer-related genes, and/or tissue-based genomic analysis utilizing our institutional NGS platform (SNaPshot), which targets hotspots and exons in 89 genes.

**Results:** A total of 33 HR+ MBC patients had paired pre/post CDK 4/6 tissue/liquid biopsies. 19 (57.6%) received palbociclib and 14 (42.4%) ribociclib, with 25 (75%) receiving an AI and 8 (24%) a SERD. Eight patients (24.2%) were first line. The median duration of response was 8 months (range 1-35 months). The most common acquired alterations at the time of progression that were not present in the pre-treatment specimens included mutations in *ESR1* (30.3%), *TP53* (30.3%), *RB1* (12.1%) and *PIK3CA* (9.1%), and *FGFR1* amplification (27.3%). We then evaluated these results in a subset of patients (N=17) who had paired pre/post liquid biopsies only and found similar results. We observed alterations in oncogenic pathways both upstream and downstream of CDK 4/6-cyclin-D complex (N=17), including upstream receptor tyrosine kinase alterations (29.4%), PI3K/AKT/mTOR alterations (5.9%), and MAPK alterations (17.6%), as well as downstream cell-cycle (35.3%) and DNA repair gene alterations (17.6%). Furthermore, 58.8% of patients had more than one acquired potential driver mutation, and 41.2% had both upstream and downstream alterations possibly reflecting emergence of different subclones and tumor heterogeneity under selective pressure from CDK 4/6 inhibitors.

**Conclusion:** Both upstream and downstream genomic alterations may mediate acquired clinical resistance to CDK 4/6 inhibitors, reflecting genomic evolution and diversification after exposure to CDK 4/6 inhibitors. Additional studies are needed to confirm these findings and develop potential therapeutic strategies to overcome clinical resistance to CDK 4/6 inhibitors for patients with MBC.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-07

A phase II study of copper-depletion using tetrathiomolybdate (TM) in patients (pts) with high risk breast cancer (BC): Role of collagen processing and tumor microenvironment

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**Background:** Copper is an important catalytic cofactor in several biological functions and is essential for lysyl oxidase (LOX), a key enzyme in cross-linking collagen, which may play a role in tumor metastasis. We hypothesized that tetrathiomolybdate (TM)-associated copper depletion (CD) would inhibit tumor metastases by altering copper dependent collagen remodeling in the pre-metastatic niche. These results are an update of our previously reported clinical outcomes with longer follow-up and translational outcomes implicating the tumor microenvironment in metastatic transformation of BC. **Methods:** Pts at high risk for recurrence, node+ triple negative (TNBC) or stage 3/4 BC with no evidence of disease (NED), were enrolled on a phase II study of CD with TM. TM was given to maintain ceruloplasmin (Cp) levels between 8-16 mg/dl for two years (yrs) with an extension phase or until relapse. Median Cp levels were monitored with each cycle. Clinical endpoints included safety/tolerability and progression of disease (POD)/death. Event-free (EFS) and overall survival (OS) were calculated using Kaplan Meier survival analyses. Translational endpoints included markers of collagen cross-linking (LOXL-2), formation (PRO-C3), and degradation (C1M and C6M). **Results:** Seventy-five pts received 2993 cycles of TM on the primary (24 cycles, 28 days per cycle) and extension study. Median age was 51 yrs (range 29-66). Forty-five pts had stage 2/3 BC, and 30 pts were stage 4 NED. At a median follow-up of 8.4 yrs, the overall EFS was 71.4% and OS was 78.8%. The EFS and OS for the 36 pts with TNBC were 71.7% and 81%, and the EFS and OS for the 39 pts with Luminal/HER2+ BC were 71.2% and 78.6% respectively. TM was well tolerated with grade 3/4 toxicities including: neutropenia (1.9%), febrile neutropenia (0.03%), and fatigue (0.2%). LOXL2 levels were significantly decreased at 12 and 24 cycles compared with baseline ( $p<0.01$ ) in those who were NED but not in those who had progressive disease (POD). LOXL2 levels were significantly correlated with C1M levels (spearman coefficient -0.34,  $p=0.02$ ). C1M levels were significantly increased at 5, 11 and 24 cycles as compared with baseline ( $p<0.01$ ) in those who were NED and were significantly higher as compared to levels in those experiencing POD/death,  $p<0.05$ . This difference may be more pronounced in those not achieving adequate CD ( $<50\%$ ) and in luminal/HER2+ BC. Interestingly, the ratio of C1M/PRO-C3 was significantly more elevated over time in those NED as compared to those experiencing POD/death. No associations were found with other collagen markers (PRO-C3 and C6M). **Conclusions:** TM is safe, well-tolerated and associated with decreased LOXL-2 and increased C1M levels over time in NED pts. This suggests that copper depletion may result in decreased collagen crosslinking and increased collagen degradation over formation, potentially “normalizing” the collagen microenvironment to create an inhospitable environment for tumor metastases. Larger randomized trials in high risk populations with translational outcomes are needed to further investigate the role of collagen processing in the tumor microenvironment and its potential as a biomarker of response.



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-08

Breast cancer liver metastases vascularize by vessel co-option, not angiogenesis, and have a desert immune phenotype: A histopathological and gene expression study

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Phase 3 trials of bevacizumab combined with chemotherapy in metastatic breast cancer have consistently failed to demonstrate a survival benefit for the addition of bevacizumab. When cancers metastasize to highly vascular organs (including the liver), they can utilize vessel co-option, instead of angiogenesis, as a mechanism to obtain a vascular supply (1). We have repeatedly shown by histopathological analyses that almost all (95%, 2 cohorts) breast cancer liver metastases utilize vessel co-option instead of angiogenesis to vascularize (2,3). The prevalence of vessel co-option in breast cancer could explain, at least in part, why anti-angiogenic therapy has been a disappointing therapeutic approach in metastatic breast cancer. Animal models of non-angiogenic liver and lung metastases also displayed resistance to anti-VEGF treatment (3,4).

We have now undertaken a gene expression study (mRNA sequencing) of targeted samples at the tumor-liver interface to discover gene expression patterns and signaling pathways that are associated with non-angiogenic growth of metastatic cancer in the liver (n = 70).

A network to detect biological themes of non-angiogenic growth was built by gene set enrichment analysis. Key components of this network are: cancer cell motility and invasion, epithelial-to-mesenchymal transition, stemness and proliferation. This contrasts with the network of angiogenic liver metastases of which the most important components are inflammation and ECM remodeling. Semi-automated image analyses of CD8-immunostained section of liver metastases confirms that non-angiogenic liver metastases have a significantly lower density of CD8-positive cytotoxic T-lymphocytes at the tumor-liver interface when compared with angiogenic liver metastases (300 cells/mm<sup>2</sup> and 1000 cells/mm<sup>2</sup>, respectively (p<0.0001)). In addition, a clear CXCL13-driven B-cell gene expression signature is associated with angiogenic growth of liver mets but is absent in non-angiogenic growth of breast cancer liver metastases. Gene expression patterns that may play a role in vessel co-option are the up-regulation of *LAMA3*, *LAMB3*, *LAMC2*, coding for the 3 subunits of laminin-5, and of *ITGA3*, *ITGB1*, *ITGA6* and *ITGB4*, coding for both integrin-receptors of laminin-5. This supports the concept of 'adhesive' vessel co-option during which cancer cells use the basement membrane of the co-opted blood vessels as a soil (5). In addition, the claudin-2 gene (*CLDN2*) is significantly overexpressed in non-angiogenic liver metastases which is consistent with earlier reports on the role of claudin-2 during breast cancer metastasis to the liver (6).

In conclusion, we provide evidence, based on morphology and gene expression, for the almost exclusive non-angiogenic growth of breast cancer liver metastases. In addition, non-angiogenic breast cancer liver metastases are characterized by a desert immune phenotype. Both observations can have an impact on the treatment strategy of patients with metastatic breast cancer.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-09

Proteomic analysis of extracellular matrix helps define drivers of metastatic progression

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Tumor microenvironment (TME) plays a central role in the development of distant metastasis. However, given its complexity, successful targeting of TME will require a detailed understanding of its composition. Cancer-associated fibroblasts represent a large component of TME and are a major contributor to the extracellular matrix (ECM). Previously, we demonstrated the presence of two fibroblast sub-populations (CDCP1<sup>pos</sup> vs CD146<sup>pos</sup> fibroblasts) in breast cancer TME, which determine therapeutic response in estrogen receptor (ER) positive breast cancer. We hypothesized that the same fibroblast subtypes would also influence ECM composition and alter the metastatic potential of breast cancer cells. Here, we present the development of a novel fibroblast driven orthotopic model of ER+ breast cancer metastasis, which we combined with an innovative proteomics approach to precisely quantify extracellular matrix proteins.

**Results:** 1. CDCP1<sup>pos</sup> fibroblasts promote increased breast cancer cell mobility, invasion and metastasis compared to CD146<sup>pos</sup> fibroblasts. We demonstrate that CDCP1<sup>pos</sup> fibroblasts significantly increase the invasion potential of breast cancer cells, when compared to CD146<sup>pos</sup> fibroblasts. Orthotopic co-implantation of ER+ tumor cells with CDCP1<sup>pos</sup> fibroblasts into the mammary fat pad of mice more frequently drives distant organ metastases to lung and brain when compared with tumors implanted with CD146<sup>pos</sup> fibroblasts. 2. Proteomic analysis of ER+ tumors influenced by CDCP1<sup>pos</sup> fibroblasts revealed known and novel drivers of breast cancer metastasis. Breast cancer cells mixed with CDCP1<sup>pos</sup> fibroblasts produce a non-uniform collagen orientation to the tumor boarder. Our novel proteomic analysis of TME specific proteins revealed that tumors influenced by CDCP1<sup>pos</sup> fibroblasts have high expression of many ECM proteins linked to increased risk of breast cancer metastasis, including TNC, FN1, COL5A3, and FBN1 among others. Derived proteomic TME signature accurately predicted lymph node involvement in patients who presented with early stage (T0 and T1) tumors in a cohort of 1,009 breast cancer patients from Cancer Genome Atlas Database. 3. Inhibiting fibroblast production of Tenascin C (TNC) results in decreased breast cancer cell invasion. Our *in vitro* mixed co-culture models, which contain ER+ breast cancer cells with both fibroblast subtypes, demonstrate that only CDCP1<sup>pos</sup> fibroblasts produce TNC. Furthermore, in spheroid assays with CDCP1<sup>pos</sup> fibroblasts and breast cancer tumor cells, invasion is inhibited by TNC knockdown. The invasion phenotype can be rescued by addition of EGF, which suggests TNC promotes invasion via EGFR signaling.

**Conclusion:** Metastatic spread of cancer cells relies heavily on TME alterations and makeup of the extracellular matrix. Our data suggest that fibroblast composition directly influences ECM properties and metastatic potential in breast cancer. Taken together, we believe that a better understanding of ECM composition will lead to a more personalized approach to breast cancer treatment.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-10

Circulating CAF/cancer stem cell co-clusters bolster breast cancer metastasis

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**Background:** Metastatic disease is the primary cause of breast cancer (BC) mortality. Cancer associated fibroblasts (CAFs) are the majority of stroma in BC and critical players in BC malignancy. For example, CAFs are the main source of SDF-1, a prominent chemokine in the tumor microenvironment (TME) that also imparts stem cell-like characteristics to BC cells. Metastasis occurs due to the transport of circulating tumor cells (CTC) and clusters of CTCs through the vasculature. Stem-like CTCs and clusters have a greater propensity to establish metastasis. We recently identified circulating CAFs (cCAFs) in blood from patients with BC and in spontaneous, syngeneic, and xenograft mouse models of BC. cCAFs not only circulate individually, but are also found in clusters with CTCs. In this study, we examine the role of CAFs in promoting egress of stem-like CTCs (cCSCs), determine the capacity of stem-like CTCs to cluster with CAFs, and evaluate the involvement of CTC/cCAF clustering in augmenting BC metastasis.

**Methods:** Our model employs NSG mice with orthotopic xenograft implantation of BC cells, primary CAF cell lines, or co-implantation of BC and CAF cell lines. We used two different BC cell lines: the non-metastatic BC cell line, MCF-7, and the highly metastatic primary BC cell line, DT28. We also employed the MMTV-PyMT spontaneous model of BC metastasis, and we used BALB/c mice injected with syngeneic 4T1 or 67nR cells to evaluate cCAFs, CTCs, and cluster egress in preclinical models. Mice were sacrificed at specific time points, and cardiac blood was collected. Blood was filtered using the faCTChecker microfluidic filtration instrument (Circulogix). Filters were stained for IF and cCAFs, CTCs, cCSCs, and clusters were enumerated. Tumors from CAF co-injected mice were evaluated for their stem cell-like phenotype and re-implanted in mice to evaluate tumorigenicity and metastasis.

**Results:** In spontaneous, syngeneic, and orthotopic xenograft models of BC, cCAFs, CTCs, and cCAF/CTCs co-clusters appear early in tumor development. cCAF/CTC clusters increase in correlation with tumor burden and metastasis. Co-inoculation of CAFs with BC cells resulted in a significant increase in tumor progression, metastasis, and in a substantially higher number of both individual cells and clusters in circulation. Dissociated tumor cells from CAF co-injected tumors had a higher proportion of CD44+stem cell-like cells (CSCs), enhanced ALDH-1 expression, and enhanced mammosphere formation. CD44+ CSCs, individually and in clusters, are found early on in the circulation of mice injected with dissociated tumor cells from CAF co-injected tumors. Upon re-implantation of CAF co-injected dissociated tumor cells without CAFs, dissociated tumor cells showed enhanced tumorigenicity and malignancy.

**Conclusion:** CAFs are highly motile and cCAFs precede CTCs into circulation and can do so independently of tumor cells. CAFs sustain egress of tumor cells by augmenting malignancy and stemness of BC cells. cCAF clusters with the highly metastatic stem cell-like subset of CTCs bolster metastatic colonization. Targeting primary CAF function and/or cCAF/cCSC co-clusters may provide novel avenues to abrogate BC metastasis.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-11

Platelet derived growth factor receptor- $\beta$  signaling: A novel therapeutic target for breast cancer associated brain metastasis

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PDGFR $\beta$  is a receptor tyrosine kinase found in cells of mesenchymal origin such as fibroblasts and pericytes. Activation of this receptor is dependent on paracrine ligand induction, and its preferred ligand, PDGFB, is released by neighboring epithelial and endothelial cells. While expression of both PDGFR $\beta$  and PDGFB has been noted in patient breast tumors for decades, how PDGFB-to-PDGFR $\beta$  tumor-stromal signaling mediates breast cancer initiation, progression, and metastasis remains unclear. To test this important research question, we developed a mouse model of mesenchymal-specific PDGFR $\beta$  hyper-activation. PDGFR $\beta$  mutant mammary glands exhibit increased tertiary side-branching and epithelial proliferation confirming a stromal-specific PDGFR $\beta$  effect on neighboring epithelium during normal development. To test the effect of hyper-active mesenchymal PDGFR $\beta$  on disease progression, experimental tail vein metastasis assays were performed where we observed prominent brain metastases in 50% of the PDGFR $\beta$  mutant mice (n=5/10) with no brain lesions seen in controls (n=0/19). There was no difference in the incidence of lung or liver metastases in the mutant mice suggesting a pro-metastatic function for PDGFR $\beta$  in the brain metastatic niche. To rule out dysfunction of the blood brain barrier contributing to the observed metastatic spread, we then intracranially injected mammary tumor cells, and as expected based on our metastasis assay, found that larger tumors formed in the brains of PDGFR $\beta$  mutant mice versus controls. To our knowledge, these combined findings are the first example where genetic manipulation of the stroma increases breast cancer associated brain metastases (BCBM). Given that these pre-clinical data suggest that primary breast tumors expressing high PDGFB could preferentially metastasize to the brain, we analyzed PDGFB protein expression in a tissue microarray comprised of HER2-positive and triple negative breast cancer (TNBC) primary tumors (total n=425). While high PDGFB did not correlate with site-independent metastatic recurrence, it was prognostic of brain metastasis, mirroring our mouse data. Evaluation of PDGFB in a small cohort of matched primary breast tumors with associated brain (n=5) and lung metastases (n=2) revealed intense PDGFB staining in 100% of the brain metastases, but only 50% of the lung metastases. These findings further suggest that high primary tumor PDGFB expression defines a subset of breast cancer patients predisposed to brain metastases and that these patients may benefit from therapeutic inhibition of PDGFR $\beta$  signaling. To test this pre-clinically, we treated mice harboring intracranial tumors with the PDGFR specific inhibitor crenolanib. Excitingly, crenolanib treatment significantly inhibited the brain tumor burden in these mice. Combined, our findings to date (1) advocate that primary tumor expression of PDGFB is a novel prognostic biomarker for the development of BCBM and (2) support clinical trial evaluation of PDGFR inhibitors for the prevention and treatment of BCBM. Ongoing studies are evaluating how the PDGFR $\beta$ -expressing mesenchymal cells within the brain promote a pro-metastatic niche.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** PD9-12

Integrative molecular profiling of breast cancer brain metastasis and patient-derived xenograft organoids from resected breast cancer brain metastases to interrogate and prioritize therapeutic personalized strategies

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**Background:** Breast cancer brain metastasis (BM) is an area of unmet need in metastatic breast cancer patients. Novel therapeutic interventions to help prevent and treat BM are warranted. We conducted integrative molecular profiling of BM and matched primary tumors (PT) using next-generation DNA and RNA sequencing to examine the molecular landscape. In addition, we established patient-derived xenograft/organoid (PDX/PDO) to examine drug sensitivity according to the molecular and clinical features of the BM.

**Methods:** Archived, formalin fixed paraffin-embedded BM was collected retrospectively. BM were also collected prospectively at the time of clinically indicated surgical resection through the central nervous system tissue banking and the Michigan Oncology Sequencing Center (MI-ONCOSEQ) protocols. Matched archived PT tissues were collected when available. Integrative next-generation sequencing was conducted using the MI-ONCOSEQ platform. The prospectively collected BM were further used to establish PDXs/ PDOs. Successfully established PDXs/PDOs were used for ex vivo drug testing via MiDrugScreen, a novel drug sensitivity testing platform, where testing was performed in a dose-response format with drug selection prioritized by clinical scenario and molecular alterations if known a priori.

**Results:** 12 matched BM-PT pairs were analyzed: 6 triple negative, 5 HER2 positive, and 1 ER positive HER2 negative. All except one (11/12) had TP53 mutations. When present, TP53 mutations in BM were also found in PT (except for 1 unknown case in PT due to low coverage). ER+HER2- was the only one without TP53 mutation but had hyper-mutation (APOBEC signature). Driver mutations and unique copy number alterations (CDKN2A loss in 1/12, mutations in PIK3CA in 1/12 and ESR1 in 1/12, CCNE1 amplification in 1/12) were noted in BMs. In 75% of cases, mutational burden was higher in BM vs. PT. 2 PDX/PDO were available for drug testing. PDO-BC9 was noted to have RB1 (splice acceptor) and LOH. As predicted by this alteration, PDO-BC9 was insensitive to CDK4/6 inhibitors (palbociclib, abemaciclib) tested on MiDrugScreen panel. PDX-BC4 was established from PIK3CA and ESR1 mutated BM from an ER+HER2- patient who had previously progressed on endocrine therapy with a CDK4/6 inhibitor. As predicted, the PDX-BC4 was resistant to CDK4/6 inhibitor but interestingly sensitive to PIK3CA, ERK, and MEK inhibitors.

**Conclusions:** TP53 mutation was highly prevalent and may be a biomarker for increased risk of BM. Further study is warranted to see if specific TP53 mutations are associated with a risk of BM development and can be used in risk stratification for BM specific intervention. Unique molecular alterations in BM compared to matched PT may have a therapeutic implication as a target or resistance biomarker. Conducting drug testing in addition to molecular profiling has the strong potential of being informative in tailoring or prioritizing therapeutic agents in the era of precision medicine. Additional BM PDXs/PDOs from breast and other solid tumors are being examined using this novel therapeutic tailoring approach with the combination of MIONCOSEQ and MiDrugScreen.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-01-01

The NEO-LET-EXE-trial: An intra-patient cross-over trial to explore the "lack of cross-resistance" between steroidal and non-steroidal aromatase inhibitors

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**Background.** The aromatase inhibitor letrozole and the aromatase inactivator exemestane currently belong to the most widely used antihormonal drugs for breast cancer worldwide. Both compounds are strongly suppressing estradiol levels in postmenopausal patients with breast cancer. However, in the metastatic setting, these drugs may be used after another, causing new responses in selected patients following switching to the alternative drug after progressing on the first choice. This well-known "lack of cross resistance" has been recognized for some time and is documented by several trials. However, the precise explanation for this clinical observation is still unknown. The solution may potentially lead us to a novel strategy to treat hormone-sensitive breast cancer.

**Trial design.** NEO-LET-EXE is a neoadjuvant, randomized, open-label, intra-patient cross-over trial .

**Eligibility criteria.** Postmenopausal patients suffering from estrogen receptor (ER) positive ( $\geq 50\%$ ), HER-2 negative, locally advanced breast cancer, suitable for neoadjuvant/presurgical antihormonal therapy, may be enrolled. Age: 18+ (no upper limit).

**Specific aims.** To explain the phenomenon of a lack of cross-resistance between steroidal and non-steroidal aromatase inhibitors *in vivo*. Sequential tumor biopsies and blood samples, obtained at baseline and following 2 months of therapy with each drug given in sequence, will be used to perform a comprehensive exploration of the consequences of each drug therapy. The influence on plasma and tissue steroids (estrogens, androgens, etc.) will be compared. In addition, whole genome sequencing, whole exome sequencing, epigenetics, proteomics and plasma analysis (cytokines, tumor DNA fragments, etc.) will be performed.

**Statistical methods.** Data will be analyzed using mixed effects models.

**Present accrual and target accrual.** 49 out of planned 100 patients have been enrolled so far. The last patient is expected to enter the trial in Q4 2019.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-01-02

An open-label, randomized, multi-center phase 2 study evaluating the activity of lasofoxifene relative to fulvestrant for the treatment of postmenopausal women with locally advanced or metastatic ER+/HER2 - breast cancer (MBC) with an ESR1 mutation

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Endocrine based therapy is the standard treatment for estrogen receptor positive (ER+) MBC. Agents targeting the ER pathway including aromatase inhibitors (AIs), fulvestrant and tamoxifen along with CDK 4/6 inhibitors are considered standard for first and 2nd line treatment. However, endocrine resistance develops in nearly all patients and the optimal systemic therapy after progression on a CDK 4/6 inhibitor is unknown.

Lasofoxifene is a third generation SERM previously investigated for the treatment of osteoporosis and vulvo-vaginal atrophy (VVA). In a large phase 3 trial evaluating the efficacy of lasofoxifene for the postmenopausal treatment of osteoporosis, lasofoxifene significantly reduced the incidence of ER+ breast cancer. Further unpublished preclinical data have demonstrated significant *in vitro* and *in vivo* efficacy in non-clinical breast cancer models including models with and without ESR1 mutants. Moreover, lasofoxifene significantly reduced metastases in ESR1 mutated models. These non-clinical and clinical data provide a strong rationale to pursue a phase 2 clinical trial in women with ER+, ESR1 mutated MBC.

This open-label, multi-center study will compare the efficacy and tolerability of lasofoxifene (5 mg orally daily) to fulvestrant (IM 500 mg D1,15,29 and then q30 D) in a 1:1 randomization. Inclusion criteria include postmenopausal women with ER+ advanced breast cancer; progression on a non-steroidal AI in combination with a CDK 4/6 inhibitor; and a known ESR1 mutation.

Approximately 90 patients with measurable or evaluable disease (i.e. bone only) will be recruited to have at least 40 patients per treatment arm. The primary endpoint will be progression free survival (PFS) with secondary endpoints of objective response rate (ORR), clinical benefit rate (CBR), duration of response (DoR) and time to response (TTR). It is assumed that lasofoxifene will double the median PFS compared to fulvestrant in this ESR1 mutation patient population for a hazard ratio 0.5 and a power of 89% to reach a 1-sided p of <0.05.

The study will commence in 4Q2018 and will complete recruitment in 1 year. It is anticipated that 25-30 centers in the US will be participating.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-01-03

The WinPro study: A window of opportunity study of endocrine therapy with and without prometrium in postmenopausal women with early stage hormone receptor-positive breast cancer

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## Background

There is bidirectional interplay between PR and ER in human breast cancers (Lim et al, Endo Rel Can 2016). There is evidence for a reprogramming of ER chromatin binding sites with 470 genes differentially regulated by dual treatment with estrogen plus progestogen compared to estrogen alone in breast cancer cell lines (Mohammed et al, Nature 2015). Functionally, there was an additive anti-cancer effect with the addition of natural progesterone to endocrine therapy in preclinical breast cancer models.

## Trial Design

This is a phase II multi-site, randomised, open-label, three-arm, study in 200 postmenopausal women with early-stage ER+, PR+, HER2-negative breast cancer. Eligible patients will be randomised 1:1:1 to receive 14 days of intervention with either letrozole 2.5mg PO daily (**arm 1**), letrozole 2.5mg + prometrium 300mg PO daily (**arm 2**) or tamoxifen 20mg + prometrium 300mg PO daily (**arm 3**), between diagnosis of breast cancer and definite surgery.

Australian Clinical Trials Registry: ACTRN1261800092813

## Eligibility Criteria

### Inclusion Criteria

- a) Histologically confirmed ER+ **and** PR+ breast cancers ( $\geq 10\%$  positive staining cells)
- b) HER2/CEP17 ratio of  $< 2$  and mean HER2 copy number  $< 6$  (ASCO CAP 2013 guidelines)
- c) Tumour size  $\geq 1$ cm on ultrasound and/or mammogram
- d) Aged  $\geq 18$  years

### Exclusion Criteria

- a) Currently on hormone therapies (HRT and OCP)
- b) Locally advanced/inoperable and inflammatory breast cancer
- c) Clinical evidence of metastatic disease
- d) Received other preoperative systemic therapies
- e) Nut allergy (prometrium contains peanut oil)
- f) Prior history of uterine cancer, deep vein thrombosis, pulmonary embolism or clotting disorder
- g) Women who are pregnant/breast feeding

## Specific Aims

- a) Primary Endpoint

The geometric mean suppression of the centrally assessed proliferation marker Ki67, after two weeks of intervention, compared with baseline. This will be obtained by comparing the mean difference in Ki67 staining between pre and post-treated samples in each intervention arm.

- b) Secondary Endpoint

Safety and tolerability of combination therapy (NCI-CTCAE v4.0)

- c) Translational Endpoints

1. Define a gene set as a predictive biomarker for a reduction in Ki67
2. Evaluate changes in the apoptotic markers Bcl-2 and Caspase 3 in the tumors following intervention
3. Evaluate changes in ER, PR, AR, FoxA1, Cyclin D1 protein and mRNA expression in the tumors following intervention

## Statistical Methods

The IMPACT study reported a geometric mean reduction in Ki67 after 2 weeks of preoperative tamoxifen of 59.5% and anastrozole of 76% (Dowsett et al, JNCI 2007). This allows estimation of power to detect differences between Arm 1 and either



Arm 2 or Arm 3 with a p-value of 0.025. For the third possible comparison of Arm 2 vs Arm 3, there is no prior evidence, therefore this as a purely exploratory comparison. With a total trial recruitment of 200 and allowing 4% dropouts, this would give 80% power to detect an improvement in Ki67 suppression from 76% in the letrozole alone control arm to 92% in either experimental arm.

**Accrual**

Present: 5 (1 site open)

Target: 200 (8 sites total)

**Contact Information**

Elgene Lim, MBBS, FRACP, PhD. [e.lim@garvan.com.au](mailto:e.lim@garvan.com.au)

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-01-04

Phase 1 study of D-0502, an orally bioavailable SERD with optimized pharmacological and PK/PD property for ER-positive breast cancer

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**Background:** Endocrine therapy such as selective estrogen receptor degrader (SERD) fulvestrant has been used effectively to extend the life of HR<sub>+</sub> (ER<sub>+</sub> and PR<sub>+</sub>) and HER2<sub>-</sub> breast cancer patient, either alone or in combination with CDK4/6 inhibitors such as palbociclib or abemaciclib. D-0502 is an orally bioavailable SERD with potent activity in various HR<sub>+</sub> and HER2<sub>-</sub> breast cancer cell lines and xenograft models. Its combination with palbociclib in both MCF-7 xenograft model and ESR-1 mutated (Y537S) patient derived breast cancer xenograft models resulted in further tumor growth inhibition or regression. Drug metabolism and pharmacokinetic studies both in vitro and in vivo demonstrated that D-0502 exhibits favorable PK profiles suitable for clinical development.

**Trial Design:** D-0502 is currently being evaluated in a phase 1 trial of women with advanced or metastatic HR<sub>+</sub>, HER2<sub>-</sub> breast cancer (MBC) (NCT03471663). This is a multicenter, open-label phase I study of D-0502 single agent and D-0502 in combination with standard dose of palbociclib. The primary objective is to characterize the safety and tolerability of D-0502 and D-0502 in combination with palbociclib, to identify an MTD and/or RP2D. The secondary objective is to evaluate the PK properties and the preliminary anti-tumor activities. Patients will receive D-0502 orally every day and treatment will be administered as 28-day cycles. The study has two parts: Dose Escalation (phase 1a) and Dose Expansion and Combination (phase 1b). In phase 1a, patients will be enrolled using a conventional dose-escalation algorithm (3+3 subjects per dose level) with 4 sequential dose cohorts to identify the MTD and RDE (recommended dose for expansion) in phase 1b) which will be at or below MTD. In phase 1b, there will be 2 cohorts, one is D-0502 single agent administered at RDE and the other is D-0502 in combination with standard dose of palbociclib, each with approximately 12 patients.

**Key Eligibility Criteria:** Eligible patients included women with confirmed HR<sub>+</sub>, HER2<sub>-</sub> MBC who have previously received no more than 2 prior chemotherapies for MBC; ECOG 0-1; evaluable (phase 1a) or measurable (phase 1b) disease (RECIST v1.1); premenopausal or postmenopausal status; adequate hematologic, hepatic and renal functions.

**Current Status and Contact Information:** At the time of abstract submission, the first cohort of 50 mg patients have started the study treatment. For inquiry of the study, please contact ling.zhang@inventisbio.com.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-01-05

Endocrine treatment alone as primary treatment for elderly patients with estrogen receptor positive good prognosis operable breast cancer: A single arm phase II, single institution study

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**Background:** It is estimated that approximately 46,000 women age >75 are diagnosed annually with breast cancer. Due to competing co-morbidities, there is wide variation in treatment recommendations which can lead to over- or under-treatment. Though surgery for breast cancer is considered low-morbidity, many elderly women given a choice, choose not to have surgery. Previous randomized trials comparing surgery with tamoxifen versus endocrine therapy alone in women age >70 unselected for ER status demonstrated similar overall survival with poorer local control in the latter group. A new standard of care needs to be defined for elderly women with good prognosis ER+ tumors, since these women may benefit from endocrine therapy alone to treat their cancer without compromising local and distant control.

**Hypothesis:** We hypothesize that endocrine therapy alone provides adequate local and systemic control of breast cancer in a subpopulation of women age 70 or older with ER+ breast cancer and good prognostic characteristics.

## **Primary Objective**

To correlate response to neoadjuvant endocrine treatment at 6 months with Oncotype DX Recurrence Score (**RS**) in women with early-stage ER+ breast cancer who are age >70.

## **Secondary Objective**

1. To determine the breast cancer-specific survival of women with early-stage ER+ breast cancer, age >70, treated with endocrine therapy alone.
2. To determine the rate of overall survival of women with early-stage ER+ breast cancer, age >70 treated with endocrine therapy alone.

**Study Design:** This is a prospective single arm phase II study. Patients with clinical stage I/II ER+ breast cancer, grade 1-2, Ki67<30 or RS <18 (performed on the diagnostic core biopsy) continue to be enrolled and followed for time to progression. A Kaplan-Meier model will be used to estimate the 5-year local progression rate. If the true 5-year progression rate is 10%, then 50 patients will provide power = .90 at a one-sided .05 significance level to demonstrate that the rate is less than 25.5%. Exploratory objectives include: evaluation of the molecular characteristics of breast cancers of responders versus non-responders, determine compliance with medications, evaluate cost-effectiveness, and quality of life.

**Results:** Between February 2017 and April 2018, 11 patients were enrolled into the study. Two patients could not tolerate endocrine therapy and received standard of care treatment. For the 9 patients on study, average tumor size was 1.7cm, average Ki67 was 15%, average RS was 14. All of the patients received an aromatase inhibitor. At 6 months, 71% of the patients had a partial response, 28% had stable disease. None of the patients developed progressive disease.

**Conclusion:** A new standard of care needs to be defined for women age >70 with good prognosis ER+ tumors, since these women may benefit from endocrine therapy alone to treat their cancer without compromising local and distant control. We continue to enroll patients to determine the optimal tumor markers for identifying women who can be treated with PET only to control their cancer.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-01-06

**POSITIVE:** A study evaluating Pregnancy, disease outcome and safety of interrupting endocrine therapy for premenopausal women with endocrine responsive breast cancer who desire pregnancy (IBCSG 48-14/BIG 8-13)

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## Background

Young patients with breast cancer (BC) are often diagnosed with the disease before completing their families. The best available retrospective evidence suggests that pregnancy after BC does not negatively impact disease outcomes in patients with endocrine responsive BC and is safe for the offspring. However, given the possibility of extended adjuvant endocrine therapy (ET) (5-10 years), it is not feasible for many of these women to delay pregnancy until completion of therapy and thus there is a need to study the safety of interrupting ET to allow pregnancy. To date, no prospective study has been conducted in BC survivors attempting future pregnancy.

## Trial Design

Young patients with endocrine responsive early BC who desire pregnancy will interrupt ET for up to 2 yrs to attempt pregnancy. As resumption of menses and conception depends on many factors (e.g. patient's age and adjuvant treatment received), the 2-yr interruption period is approximate, intended to include treatment wash-out (3 mos), conception (~3-6 mos), delivery (~9 mos), and breast feeding (~6 mos). Patients will be strongly advised to resume ET as soon as pregnancy attempts/deliveries are concluded, and to complete the planned 5-10 yrs of ET.

## Major Eligibility Criteria

- Histologically-proven stage I-III endocrine-responsive BC.
- Patient's wish to become pregnant.
- Age  $\geq 18$  and  $\leq 42$  years at enrollment.
- Adjuvant ET (selective estrogen receptor modulator [SERM] alone, GnRH analogue plus SERM or aromatase inhibitor) for  $\geq 18$  months but  $\leq 30$  months, stopped within 1 month prior to enrollment.
- Premenopausal status at BC diagnosis.

## Specific Aim

To assess the risk of BC relapse associated with the interruption of ET to permit pregnancy, and to evaluate pregnancy success rate and offspring outcome.

## Statistical Methods

With 500 pts enrolled and followed for a median of 3 years, the statistical design is based on the 95% CI for the 3-year BC recurrence rate. Interim monitoring assumes a 2% BC recurrence risk/yr with continuous ET and a recommendation to stop the study early if the BC risk exceeds 4%/yr with ET interruption.

**Translational Research** will investigate various ovarian function and uterine parameters and circulating tumour DNA. Fresh frozen paraffin embedded tissue of the primary tumour will be collected to evaluate parameters related to the biology of BC in young women. All material will be banked centrally.

**Psycho-oncological Companion Study (POCS)** will evaluate fertility concerns, psychological well-being and decisional conflict. It is mandatory in North America and open to interested centers elsewhere.

**Accrual:** Target: 500; Actual: 262 (30 June 2018)

**Psycho-oncological Companion Study Accrual:** Target: 200; Actual: 138 (30 June 2018)

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-01-07

**PIONEER-** Pre-operative window study of letrozole plus PR agonist megestrol acetate versus letrozole alone in post-menopausal patients with ER-positive breast cancer

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## **Background:**

Published preclinical findings provided new insights into the functional 'cross-talk' between the oestrogen receptor (ER) and the progesterone receptor (PR) in breast cancer (BC) (Mohammed et al., Nature, 2015). Addition of a PR agonist to anti-oestrogens directly modifies ERα chromatin binding and the transcriptional response in breast cancer cells, and is anti-proliferative *in vitro* and *in vivo*.

Megestrol Acetate (MA), an off-patent semi-synthetic derivative of progesterone, has been licensed for many years as treatment for ER+ metastatic BC. There is also good evidence for the effectiveness of MA as a supportive therapy to ameliorate endocrine therapy-related hot flushes.

## **Trial design:**

PIONEER is a three-arm, open label, multi-centre randomized phase II pre-surgical window trial evaluating effects of 15 days of preoperative therapy with Letrozole (LET), or LET plus MA 40mg, or LET plus MA 160mg in postmenopausal women with ER+ HER2- invasive primary BC.

## **Eligibility criteria:**

### Inclusion Criteria

Postmenopausal women with histologically confirmed invasive BC,  $\geq T1c$ , clinical NX or N0-N3, ER+ (Allred $\geq 3$ ), and HER2-  
2 groups of patients are potentially eligible:

Cohort A: Patients whose cancers have been deemed to be operable by the Multi-Disciplinary Team (MDT) with surgery planned for the next 2-6 weeks

Cohort B: Patients with early or locoregionally advanced BC planned for primary endocrine therapy either in lieu of surgery or as neoadjuvant therapy before surgery

ECOG performance status of 0-2

Adequate Liver, Renal, Bone marrow function

### Exclusion Criteria

Hormone replacement therapy in the last 6 months

Treatment with tamoxifen or an aromatase inhibitor (AI) in the last 6 months

A progestogen-containing intrauterine system in situ, unless removed prior to randomisation

Metastatic disease on presentation

Recurrent BC (patients with a new primary invasive BC are eligible)

## **Specific aims:**

The primary endpoint is % change in proliferation between baseline and day 15 tumor biopsies, measured by Ki67 IHC assessment. Secondary endpoints include: expression of Aurora Kinase A, Caspase 3 and AR/PR/EMT markers by IHC; and safety endpoints. Exploratory endpoints: transcription factor mapping (ChIP-seq) and identification of differential ERα-associated proteins (RIME) on paired fresh-frozen tumor samples.

PIONEER will help determine whether there is value in conducting a Phase III adjuvant study to investigate the longer term benefit of combining an AI with MA, and if so, at what dose (40mg vs. 160mg).

## **Statistical methods:**

Patients are randomized in a 1:1.5:1.5 ratio into arms A:B:C. Based on results from previous clinical trials, a mean 66% reduction in Ki67 is anticipated for LET alone (arm A), and a 77.5% reduction for the combination arms B and C, based on preclinical data.

**Present and target accrual:**

Patients are being recruited in Cambridge, Cornwall, Belfast & London with 6 other UK sites due to open q3/4 2018. At the time of submission, 29 patients had been recruited. A recruitment total of 189 patients is required.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-02-01

Phase I trial of bicalutamide and ribociclib in androgen receptor-positive triple negative breast cancer

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**Background:** To date, the use of anti-androgens in the subset of triple negative breast cancers (TNBC) that express androgen receptor (AR) has shown modest response rates, indicating anti-androgen-resistance in the majority of these tumors. Based on data that Cyclin D kinase (CDK) inhibitors reverse resistance to anti-androgens in prostate cancer cell lines, we hypothesize that the use of CDK inhibition may enhance the activity of anti-androgens in AR-positive TNBC.

**Methods:** Key eligibility include: patients with centrally confirmed AR-positive TNBC, defined as AR expression >0%; 0 to 1 line of prior therapy for metastatic disease; and measurable disease. Patients are treated with bicalutamide 150mg orally once daily plus ribociclib at one of 3 dose levels (see table).

**Table 1**

Dose level	Bicalutamide	Ribociclib
1	150mg orally once daily (D1 to 28)	400mg daily (D1 to 21)
2	150mg orally once daily (D1 to 28)	400mg daily (D1 to 28)
3	150mg orally once daily (D1 to 28)	600mg daily (D1 to 21)

**Results:** AR expression was positive by trial criteria in 74% of screened patients. Three patients have been accrued at each dose level. Median age is 56 and 6 and 3 patients were treated in first and second-line settings, respectively. Median AR expression was 50% (range 5 to 75%). Toxicity data is available for 6-patients treated on dose levels 1 and 2. No dose-limiting toxicities were noted. As anticipated with ribociclib, the most common toxicity is neutropenia (1 patient grade 4 and 2 patients grade 3). Two patients experienced grade 3 hypertension and 1 experienced grade 3 lymphopenia. Grade 2 or lower toxicities included fatigue, nausea, hyperglycemia and mucositis. One patient experienced grade 1 QT interval prolongation.

**Conclusion:** The combination of bicalutamide and ribociclib is tolerable without unexpected toxicities. Data on the 3-patients treated at dose level 3 and dose expansion will be included. Phase 2 dosing schedule will be decided based on phase 1 results.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-02-02

A phase 1, first-in-human, multi-part study of RAD140, an oral nonsteroidal selective androgen receptor modulator, in postmenopausal women with hormone receptor positive breast cancer

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**Background:** Hormone receptor-positive (HR+) breast cancer accounts for about 75% of breast cancer cases. Despite initial response to ER-targeted therapies, subsequent tumor progression remains an important clinical problem, highlighting the need for new therapies with activity in endocrine-resistant tumors.

The androgen receptor (AR) is expressed in up to 90% of ER+ breast cancers, and until the 1970s, breast cancer was commonly treated with androgens with single-agent response rates ranging from 20% to 25%. However, androgen-based therapy for breast cancer declined due to lack of tissue selectivity, potential for aromatization to estrogen, and the emergence of ER-targeted agents such as tamoxifen.

RAD140 is an oral nonsteroidal selective androgen receptor modulator (SARM) that cannot be converted to estrogen by aromatase. RAD140 has high AR affinity and target selectivity, demonstrating marked tissue-selective AR agonist activity comparable to natural androgens in breast cancer cells, but lacking agonist activity in prostate cancer cells. Preclinical efficacy studies in multiple *in vivo* and *in vitro* models of AR+/ER+ breast cancer demonstrate potent anti-tumor activity of RAD140 as a single agent and in combination with a CDK4/6 inhibitor. This first-in-human study will evaluate the safety, tolerability, pharmacokinetics (PK) and clinical activity of RAD140 in patients with HR+ breast cancer.

**Trial Design and Specific Aims:** This is a Phase I, open-label dose escalation and safety expansion study of RAD140 in postmenopausal patients (pts) with advanced HR+ breast cancer for whom no standard therapy is available. During dose escalation (Part A), pts are assigned sequentially to escalating doses of RAD140 using a standard 3+3 design to establish a maximum tolerated dose (MTD) and/or recommended dose (RD) based on safety, PK and preliminary clinical activity. The Safety Expansion (Part B) will further evaluate the safety, tolerability and clinical activity of the RD.

**Eligibility Criteria:** Pts must be post-menopausal females ≥18 years old with locally advanced or metastatic ER+/HER2- (Part A) or ER+/AR+/HER2- (Part B) breast cancer and ECOG 0 or 1. Part A pts must not be eligible for standard therapy. Pts in Part B must have had at least 1 line of prior systemic therapy in the metastatic setting and at least 6 months of prior endocrine therapy in the metastatic setting and progressed on the most recent endocrine therapy. Measurable disease by RECIST 1.1 is also required for enrollment in Part B.

**Statistical Methods:** Descriptive statistics (including mean, standard deviation, median for continuous variables; frequency counts and percentages for categorical variables) will be presented by dose. Plasma or serum PK parameters for RAD140 will be estimated using non-compartmental methods.

**Present Accrual and Target Accrual:** The study will enroll up to 40 pts, including up to approximately 24 pts enrolled in cohorts of 3 to 6 in Part A, and another 15 pts enrolled in Part B. As of June 2018, 11 pts have enrolled at 5 sites. (NCT03088527)



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-03-01

Phase 1/1b study of novel oral selective estrogen receptor degrader (SERD) LSZ102 in combination with alpelisib (BYL719) in estrogen receptor-positive (ER+), human epidermal growth factor receptor-2–negative (HER2–) advanced breast cancer (ABC) with progression on endocrine therapy (ET)

Giuseppe Curigliano<sup>1</sup>, Sara Cresta<sup>2</sup>, Yoon-Sim Yap<sup>3</sup>, Dejan Juric<sup>4</sup>, Francois P Duhoux<sup>5</sup>, Catherine Terret<sup>6</sup>, Shunji Takahashi<sup>7</sup>, Rachel M Layman<sup>8</sup>, Nicole Kundamal<sup>9</sup>, Daniela Baldoni<sup>10</sup>, Serena Liao<sup>11</sup>, Adam Crystal<sup>11</sup> and Komal Jhaveri<sup>12</sup>. <sup>1</sup>University of Milan, Istituto Europeo di Oncologia – IRCCS, Milan, Italy; <sup>2</sup>Fondazione IRCCS – Istituto Nazionale dei Tumori, Milan, Italy; <sup>3</sup>National Cancer Centre Singapore, Singapore, Singapore; <sup>4</sup>Massachusetts General Hospital, Boston, MA; <sup>5</sup>Cliniques Universitaires Saint-Luc, Brussels, Belgium; <sup>6</sup>Centre Léon Bérard, Lyon, France; <sup>7</sup>The Cancer Institute Hospital of JFCR, Tokyo, Japan; <sup>8</sup>The University of Texas MD Anderson Cancer Center, Houston, TX; <sup>9</sup>Novartis Institutes for Biomedical Research, East Hanover, NJ; <sup>10</sup>Novartis Pharma AG, Basel, Switzerland; <sup>11</sup>Novartis Institutes for Biomedical Research, Cambridge, MA and <sup>12</sup>Memorial Sloan Kettering Cancer Center, New York, NY.

**Background:** Although ET remains the basis of therapy for ER+, HER2– ABC, treatment resistance frequently occurs. Novel strategies to target the receptor and/or alternative pathways to overcome therapeutic resistance are under investigation. LSZ102 is a novel, orally bioavailable, nonsteroidal SERD. Preclinically, LSZ102 inhibits ER gene transcription, induces receptor degradation, blocks ER-dependent cell growth, and has synergistic activity with the phosphoinositide 3-kinase (PI3K)-alpha inhibitor alpelisib (BYL719). The present study is evaluating the safety and tolerability of LSZ102 plus alpelisib in patients with ER+, HER2– ABC with progression on ET.

**Trial Design:** This phase 1/1b, open-label study is enrolling ~18-30 patients (men and women of any menopausal status) in Arm C of the dose-escalation part of the study, which investigates the combination of LSZ102 and alpelisib; additional study arms will investigate LSZ102 as a single agent or in combination with ribociclib. Enrollment in Arm C started after identification of a safe and tolerable single-agent dose for LSZ102. Alpelisib dosing began at 200 mg/day and will not be escalated beyond the maximum tolerated dose (MTD) determined in the alpelisib single-agent arm of study CBYL719X2101 (400 mg/day). Dose escalation of alpelisib in combination with LSZ102 is guided by BLRM and integrates Cycle 1 DLT rates, lower grade and later cycle AE, PK, PD and preliminary activity to identify a recommended dose for expansion (RDE). Patients will receive treatment until disease progression, unacceptable toxicity, or withdrawal of consent. For inclusion in the study, patients must have histologically confirmed ER+, HER2– ABC and disease progression after ET for ABC or recurrence on/within 12 months of completion of adjuvant ET. In the escalation part of the study, patients are eligible regardless of PIK3CA status. Premenopausal women must receive concomitant treatment with a gonadotropin-releasing hormone agonist. Eligible patients must have adequate bone marrow and organ function, Eastern Cooperative Oncology Group performance status of 0 or 1, and have completed and recovered from acute toxicities of radiotherapy and/or prior anticancer therapy. Exclusion criteria include symptomatic central nervous system metastases, clinically significant cardiac disease or impaired cardiac function (including a QT interval corrected for heart rate using Fridericia's formula [QTcF] >460 ms in women or >450 ms in men), uncontrolled diabetes mellitus type II (or type I), and prior treatment with a PI3K inhibitor. The primary objectives are characterization of safety and tolerability for the combination and identification of a recommended dose. Secondary objectives include characterization of pharmacokinetic properties and pharmacodynamic effects. Recruitment for Arm C is ongoing. NCT02734615

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-03-02

CICLADES: Monitoring of *ESR1*, *PIK3CA* and *AKT1* ctDNA mutations during real-life follow-up of patients with advanced breast cancer treated with endocrine therapy

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Activating *ESR1* mutations have recently been reported as a key mechanism leading to Aromatase Inhibitor (AI) resistance. *ESR1* mutations occur rarely in primary breast cancers. However, in large retrospective studies, *ESR1* mutations occurred in up to 39% of Estrogen-Receptor(ER)-positive metastatic breast cancer resistant to AI. Numerous hotspot mutations have been identified, most of them affecting the ligand-binding domain (LBD) and leading to ligand-independent activation of the ER and to resistance to AI.

Phosphatidylinositol 3-kinase (PI3K)/AKT pathway is involved in key cellular mechanisms and mutations in *PIK3CA* and *AKT1* are frequently reported in breast cancer.

In this study, we propose to use a capture-based Next Generation Sequencing (NGS) assay and to use the barcoding and polishing features in our analysis pipeline. This assay will be able to detect all mutations on *AKT1*, *PIK3CA*, *ESR1* and other genes on circulating tumor DNA (ctDNA) extracted from blood samples of patients with breast cancer. We consider that this exon-screening strategy is relevant according to the recent knowledge.

We plan to prospectively include women with advanced breast cancer about to begin standard-of-care first line endocrine therapy (ET). Patients will be required to have histologically confirmed ER-positive, HER2-negative breast cancer and documented loco-regionally advanced or metastatic disease, not amenable to surgery or radiation with curative intent. Patients with endocrine sensitive disease (no prior ET or relapse more than 12 months after completing adjuvant ET) as well as patients with endocrine resistant disease (relapse while on adjuvant ET or within 12 months of completing adjuvant ET) will be enrolled.

ET can be prescribed alone or in combination with a targeted therapy. Nevertheless, we will recruit at least 25% of patients with exclusive ET in the endocrine sensitive group.

Peripheral-blood samples, for analysis of ctDNA, will be obtained from participating patients at pre-specified time points: at start of ET to determine the baseline mutational status of *ESR1*, *PIK3CA*, *AKT1* and other genes included in a panel of genes of interest in solid tumors, and then, at evaluation of response to therapy until disease progression or end of study.

Patients will be followed for 36 months or until disease progression. Determination of progression will be done per local investigator.

The primary objective is to describe the prevalence of activating *ESR1* mutations affecting the LBD, using NGS, from the start of ET to progression or end of study. Secondary objectives include to describe the prevalence of *ESR1* mutations affecting other domains, the prevalence of *ESR1* mutations in patients with and without endocrine resistance at enrolment and the prevalence of *PIK3CA* and *AKT1* mutations, to demonstrate that *ESR1*, *PIK3CA* and *AKT1* mutations whatever their times of onset are predictors of progression free survival.

As of June 2018, 8 sites were opened to recruitment and 18 pts were included; the target enrollment is 146. The trial is supported by AstraZeneca.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-03-03

**STEM:** SFX-01 in the treatment and evaluation of metastatic breast cancer

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**Background:** Sulforaphane, a plant secondary metabolite, was first identified as an anti-cancer agent in 1992, although its inherent instability has thus far held back its clinical development. SFX-01 is a proprietary synthetic pharmaceutical development product based upon a stabilised sulforaphane. Preclinical data show sulforaphane and SFX-01 inhibit breast cancer stem cells<sup>1,2</sup> and SFX-01 potently suppresses STAT3 in ER+ metastatic breast cancer (mBC) PDX tumours<sup>3</sup>. It is therefore hypothesised that SFX-01 could become a potential therapy for reducing resistance to hormone therapy in patients with mBC.

**Trial Design:** An open label trial with a maximum target of 60 patients with ER+, HER2-negative, mBC who are on a third generation AI, tamoxifen (Tam) or fulvestrant (Fulv) and have evidence of emerging secondary endocrine resistance. Participants remain on AI, Tam or Fulv and take this in combination with 300mg SFX-01 orally, twice-daily, and are scanned every 6 weeks (wks) until disease progression. Patients come off study at disease progression or at the full term of 24wks. Patients who are progression free as they approach 24wks can be enrolled in a compassionate use phase.

## **Key Eligibility Criteria**

- Male or female patients 18yrs or older
- Histological confirmation of ER+ HER2-negative breast cancer
- Clinical or histological confirmation of metastatic or locally advanced disease not amenable to curative surgical resection
- At least 1 site of measurable disease
- Must be on a third generation AI, Tam or Fulv and have evidence of emerging secondary endocrine resistance as evidenced by either: a) Progressive disease while on adjuvant ET but after the first 2 years, or b) Progressive disease within 12 months of completing adjuvant ET, or c) Progressive disease while on ET, ≥6 months after initiating ET for metastatic breast cancer
- No more than 3 lines of endocrine therapy including the treatment at the time of study entry
- No more than 1 prior line of chemotherapy for metastatic/locally advanced breast cancer

**Specific Aims:** The primary objectives are to determine the safety & tolerability of SFX-01 in combination with AI, Tam or Fulv and to determine clinical benefit rate (CBR) at 24wks using RECIST v1.1. Time to response, objective response rate, progression free survival interval and the PK of SFX-01, AI, Tam & Fulv will also be assessed.

**Statistical Methods:** Demographic, baseline characteristics, safety and efficacy data will primarily be summarised descriptively. The sample size of 20 patients in each treatment cohort is such that if the observed CBR on any arm is 15%, it will provide a 90% exact binomial confidence interval (CI) for the true CBR of 4.2% to 34.4%. Duration of response will be compared to duration of response on prior ET.

**Accrual:** To date, 46 of the maximum target of 60 patients have been enrolled across 10 centres in UK, Belgium, France and Spain. Final study results are expected end of 2018.

## **References**

1. Li *et al*, Clin Cancer Res 2010 May1;16(9):2580-2590
2. Simões *et al*, AACR 106th Annual Meeting 2015, Philadelphia
3. Simões *et al*, 1st UK Interdisciplinary Breast Cancer Symposium 2018; Manchester, UK

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-03-04

**INTERACT-** INTegrated Evaluation of Resistance and Actionability using Circulating Tumor DNA in hormone receptor (HR) positive metastatic breast cancers (MBC)

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<sup>1</sup>The University of Texas MD Anderson Cancer Center, Houston, TX and <sup>2</sup>Guardant Health, Redwood City.

## Background

Mutations in the ligand-binding domain of *ESR1* have been demonstrated to mediate resistance to aromatase inhibitors (AI) and are associated with poor survival. Analyses of circulating tumor DNA (ctDNA) offer a minimally invasive and real-time approach to characterize genomic landscape, clonal evolution, and treatment response. Early detection and intervention with alternate therapy to overcome resistance at minimal disease burden progression could have a larger impact than treating higher burden disease at clinical progression. However, whether treatment decisions made based on the emergence of secondary resistance mutations or mutant allele fraction (MAF) changes in ctDNA can improve clinical outcomes is unknown. Currently, the most effective therapy for patients harboring *ESR1* mutations is unclear; although, pre-clinical and retrospective clinical trial analyses have suggested that some of these mutations may exhibit greater sensitivity to fulvestrant, a selective estrogen receptor down-regulator, compared to AI. This study hypothesizes that real-time monitoring of ctDNA for secondary *ESR1* alterations can identify subclinical progression and early intervention with a targeted-agent that has greater efficacy against *ESR1* mutations can improve disease-free survival.

## Trial Design

This is a randomized, open-label, Phase-2 study for HR-positive MBC patients who are on AI and CDK 4/6 inhibitor as first line therapy. Patients on treatment for at least 12 months without evidence of clinical progression would be screened for *ESR1* mutations using Guardant360 ctDNA assay. Patients with positive *ESR1* mutations would be randomized to change of endocrine therapy to fulvestrant vs. continuing AI.

## Eligibility criteria

- Histologically confirmed HR-positive (ER and/or PR >10%) and HER2-negative MBC
- On AI with CDK4/6 inhibitor as first line therapy for 12 months without evidence of clinical progression
- Activating *ESR1* mutation identified on ctDNA
- ECOG performance status ≤1
- Normal organ and marrow function

## Specific aims

- To assess progression-free survival (PFS) with transition to fulvestrant compared with continuing AI therapy in patients with emergence of *ESR1* mutations in plasma
- To assess ctDNA *ESR1* mutant allele fraction and kinetics with transition to fulvestrant compared with AI
- To assess the prevalence of *ESR1* mutations in patients with exposure to endocrine therapy
- To assess overall survival with fulvestrant transition compared with continuing AI therapy in patients with emergence of *ESR1* mutations

## Statistical methods

To detect a change in median PFS from 5 months (for AI arm) to 9 months (with fulvestrant arm) would require about 124 patients (5% two-sided alpha, 80% power, log rank testing). Interim analysis will be performed when 42 PFS events are observed. Using O'Brien-Fleming stopping boundaries, we will stop for futility if the log rank test p-value > 0.72 and stop for success if it is < 0.004.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-04-01

A phase IIB pre-surgical trial of oral tamoxifen (TAM) versus transdermal 4-hydroxytamoxifen (4-OHT) in women with DCIS of the breast

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## Background

Ductal carcinoma in situ (DCIS) is diagnosed in 60,000 women annually in the US. TAM is proven to reduce risk of local recurrence and new primary breast cancer in women with estrogen receptor (ER) positive DCIS. However, acceptance of TAM has been low, primarily because of toxicity related to systemic exposure. Local delivery to the breast is an attractive alternative since low systemic levels could minimize toxicity. 4-OHT is an active metabolite of TAM. When formulated as a gel and applied to the breast skin, it is well tolerated, and results in 4-OHT breast tissue drug levels comparable oral TAM. In small pilot studies, its anti-proliferative effects on invasive breast tumors and DCIS are also similar to oral TAM [Lee O, et al. PMID 25028506]. The goal of our study is to validate these results in preparation for a Phase III trial of 4-OHT gel in comparison to oral TAM.

## Methods

We are conducting a randomized, double-blinded, placebo-controlled, Phase IIB pre-surgical trial to demonstrate that daily application of 4-OHT gel will result in a reduction in the Ki-67 labeling index of DCIS lesions that is not inferior to that seen in women receiving daily oral TAM 20 mg daily. Ki-67 of the base-line diagnostic core needle biopsy will be compared to that of the therapeutic surgical excision sample after oral TAM or 4-OHT gel for  $8 \pm 2$  weeks. Secondary endpoints include changes in Oncotype DCIS-Score, IHC markers (CD68, COX2, p16), hormone levels, coagulation markers, drug concentration in the plasma and breast tissue, the fraction of women with no residual DCIS in the surgical sample, and experienced symptoms. 100 women (assuming 20% non-evaluable samples or compliance issues) with DCIS (10% ER-positive) will be enrolled across six institutions into two intervention arms: oral TAM 20 mg daily, placebo gel and 4-OHT gel 4mg daily (2mg/breast), placebo capsule. All participants will be evaluable for toxicity from their first dose. All samples from all participants who receive drug will be evaluated and included in the primary analysis, which will be based on intent to treat principle. To date 15 of 100 participants have been enrolled across six institutions including: Northwestern University in Chicago, IL, St. Elizabeth Healthcare in Edgewood, KY, Duke University Medical Center in Durham, NC, Cleveland Clinic in Cleveland, OH, Memorial Sloan Kettering Cancer Center in New York, NY, and Mayo Clinic in Rochester, MN. Since study open, 69 potential participants have been contacted, 52 did not consent for screening, 17 consented for screening, 2 are pending consent, and 15 have started study intervention. The most common reasons potential participants chose not to consent are wanting to schedule surgery as soon as possible, attitudes toward medical research, and current use of a prohibited concomitant medication such as a potent inhibitor of tamoxifen metabolism or exogenous sex steroid.

**Funding Source:** NCI Contract # HHSN2612201200035I.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-05-01

A pilot study of the combination of entinostat with capecitabine in metastatic and high risk breast cancer after neoadjuvant therapy

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## Background:

HDAC inhibitors (HDACi) upregulate thymidine phosphorylase resulting in enhanced conversion of capecitabine to active 5-fluorouracil and in synergistic anti-proliferative effects. HDACi's down regulate thymidine synthase and may prevent resistance to fluoropyrimidines. Entinostat is a well-tolerated class I HDACi in phase III trials for metastatic breast cancer.

## Specific Aims:

The primary objective is to determine the maximum tolerated dose combination (MTDC) of entinostat and capecitabine in participants with metastatic breast cancer. It is hypothesized that entinostat and capecitabine is a synergistic, safe, and tolerable combination.

An expansion phase will assess the safety of the MTDC from Part A in participants with high risk breast cancer after neoadjuvant therapy. The expansion phase will generate a preliminary estimate of disease-free survival. Exploratory objectives include estimates of the association of volume of circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) with the presence of residual disease and if it decreases following treatment with the combination of entinostat and capecitabine.

## Trial Design:

The dose escalation phase (Part A) will accrue participants with metastatic breast cancer using an adaptive design to determine the MTDC. The starting doses will be entinostat 3mg po weekly and capecitabine 800mg/m<sup>2</sup> po bid, 14 days on and 7 days off with 21 day cycles. Patients will be monitored for toxicity and adverse events. The MTDC is defined as the dose combination with DLT rate closest to the target DLT rate of 25%.

The expansion phase (Part B) will accrue breast cancer participants with residual invasive disease after neoadjuvant therapy starting at the MTDC estimated in Part A. The adaptive modeling design will be used in Part B to establish the MTDC in this patient population. Participants will be treated with the MTDC for a total of 8 cycles.

Blood samples will be obtained from all enrolled patients in the expansion phase prior to the start of adjuvant treatment with entinostat and capecitabine so that ctDNA and CTCs can be measured as correlative studies. Measurements will be repeated at the end of the eighth cycle.

## Eligibility Criteria:

**Part A: Dose Escalation Phase:** Stage IV breast cancer patients; Receptor Status: hormone receptor positive or negative, triple negative patients; Age: 18 year and older

**Part B: Expansion Phase:** Stage I-III high risk breast cancer patients; Completed at least four cycles of neoadjuvant taxane or anthracycline based chemotherapy; Residual invasive disease (ypT1a or greater) or known positive lymph nodes (ypN0(itc) or greater); Receptor Status: hormone receptor positive or negative, triple negative patients; Age: 18 years and older

## Statistical Methods:

The trial is designed to determine the MTDC, defined by acceptable toxicity of the combination, for two study populations. A Bayesian adaptive design is being used to guide accrual decisions based on the occurrence of DLTs, and the minimum follow-up period for determination of escalation is 3 weeks.

## Accrual:

Maximum target accrual is 55 participants. Accrual is estimated at 1-2 participants per month.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-05-02

**OPTIMA:** A prospective randomized trial to validate the clinical utility and cost-effectiveness of gene expression test-directed chemotherapy decisions in high clinical risk early breast cancer

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**Background:** Multi-parameter tumour gene expression assays (MPAs) are widely used to estimate individual patient residual risk and to guide chemotherapy use in hormone-sensitive, HER2-negative early breast cancer. The TAILORx trial supports MPA use in a node-negative population. Evidence for MPA use in node-positive breast cancer is limited. OPTIMA (Optimal Personalised Treatment of early breast cancer using Multi-parameter Analysis) (ISRCTN42400492) aims to validate MPAs as predictors of chemotherapy sensitivity in a largely node-positive breast cancer population where prospective RCT (Randomised Controlled Trial) evidence is lacking.

**Methods:** OPTIMA is a partially blinded multi-center RCT with an adaptive two-stage design. The main eligibility criteria are women and men age 40 or older with resected ER-positive, HER2-negative invasive breast cancer and up to 9 involved axillary lymph nodes. Randomisation is to standard management (chemotherapy and endocrine therapy) or to MPA-directed treatment using the Prosigna (PAM50) test. Those with a Prosigna tumour score (ROR\_PT) >60 receive standard management whilst those with a low score (≤60) are treated with endocrine therapy alone. Endocrine therapy for pre-menopausal women includes ovarian suppression. The co-primary outcomes are (1) Invasive Disease Free Survival (IDFS) and (2) cost-effectiveness of test-directed treatment. Secondary outcomes include IDFS in patients with low-score tumours and quality of life. An integrated qualitative recruitment study addresses challenges to consent and recruitment and will build on experience from the feasibility study that a multidisciplinary approach at sites is important for recruitment success. Tumour blocks will be banked to allow evaluation of additional MPA technologies. Recruitment of 4500 patients over 5 years will permit demonstration of 3% non-inferiority of test-directed treatment, assuming 5-year IDFS of 85% with standard management, equivalent to a HR of 1.22. Inclusion of patients from the feasibility study will increase the power to test for non-inferiority.

**Results:** The OPTIMA main trial opened in January 2017. Overall recruitment (including the feasibility study) will reach 1000 in August 2018. Recruitment in Norway will commence in July 2018. Characteristics of the OPTIMA main participants recruited to 31<sup>st</sup> May 2018 are shown in the table.

Main study patient characteristics

Characteristic		%
Median age in years (range)	57 (40-80)	
Menopause status	Pre	34

	Post	66
	Male	1
Tumour size	<30mm	58
	>=30mm	42
Node status	pN0	4
	pN1mi(sn)	7
	pN1(sn)	20
	pN1	55
	pN2	14
Historic grade	1	6
	2	58
	3	36

**Conclusion:** OPTIMA is one of two large scale prospective trials validating the use of test-guided chemotherapy decisions in node-positive early breast cancer. It is expected to have a global impact on breast cancer treatment. Experience from the preliminary study and close engagement with centres will aid trial success.

**Funding:** OPTIMA is funded by the UK NIHR HTA Programme (10/34/501). Views expressed are those of the authors and not those of the HTA Programme, NIHR, NHS or the DoH.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-05-03

Efficacy and safety of scalp cooling device for prevention of alopecia in patients with breast cancer

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## **Background**

Chemotherapy for breast cancer causes alopecia as a side effect. Some patients refuse chemotherapy because of alopecia, resulting in the omission of a standard therapy. It is believed that a scalp cooling device can prevent alopecia by promoting vasoconstriction of the scalp and reducing exposure of the hair root cells to anticancer agents. There are phenotypic differences of the efficacy of a scalp cooling device for alopecia. In fact, a Dutch scalp cooling registry reported that the success rate of scalp cooling was 51% in European women and 33% in Asian women. Therefore, we aimed to investigate the efficacy of scalp cooling device for chemotherapy-induced alopecia among Asian women with breast cancer.

## **Trial design**

This is a phase II trial to evaluate the efficacy and safety of scalp cooling device for risk reduction of alopecia in women with stage I/II/III breast cancer treated with adjuvant/neoadjuvant chemotherapy in a single institute.

## **Eligibility criteria**

Women diagnosed with Stage I to III breast cancer who are scheduled to receive preoperative or postoperative adjuvant chemotherapy containing anthracycline and/or taxanes are enrolled. Patients who have blood malignancies (leukemia, non-Hodgkin lymphoma, other systemic lymphoma), and cold allergy, are excluded.

## **Specific aims**

The primary endpoint is the proportion of patients with Common Terminology Criteria for Adverse Events (CTCAE) grade 0-1 alopecia after the completion of all cycles of chemotherapy (success rate). Secondary endpoints are safety, quality of life, use of wig or cap, and success rates after the completion of all cycles of chemotherapy distinguished by anthracycline (AC) and taxane. The cooling device is the Paxman scalp cooling system. Scalp cooling was performed from 30 mins before initiation until 90 mins (25 min for taxane) after chemotherapy. Pictures of the scalp were taken at the time of the initiation of each course.

## **Statistical methods**

Successful treatment was defined as the presence of less than 50% of hair-loss area. The sample size was calculated using the Simon method, with a type I error of 10% (two-sided) and a study power of 80%. The expected success rate is 30%, with a threshold success rate of 10%, and the required number of patients was estimated to be 19.

## **Present and target accrual**

Patient accrual was started in April 2018 and present accrual is 3. We plan to enroll a total of 20 patients in the trial.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-05-04

Phase 3 trial of carboplatin in triple negative breast cancer (TNBC) patients with residual invasive carcinoma after neoadjuvant chemotherapy (JONIE4:J-CAT trial)

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**Background:** It is well known that the prognosis of non pCR TNBC patients was poor after anthracycline and taxan treatment. For such patients, capecitabine seems to be effective to reduce recurrence based on the HR 0.58 of the CREATE X trial (Masuda, N. et al. Adjuvant Capecitabine for Breast Cancer after Preoperative Chemotherapy. N Engl J Med. 376, 2147. 2017) . However, the target of capecitabine is still unclear for TNBC. We classified non pCR tumors as BRCAness and Sporadic using BRCAness test(MRC-Holland, Amsterdam, the Netherlands). The recurrence rate of the BRCAness group was about 70%. Carboplatine is expected to be effective against BRCAness tumors, as it is a DNA damaging agent. In this study BRCAness can be checked just before carboplatin treatment using surgical specimens. Then the efficacy of carboplatin will be directly known to make comparison between DFS in the carboplatin group and that of the observation group.

**Trial design:** This is an open label, randomized phase III study that will enroll TNBC with residual invasive cancer after surgery with preoperative chemotherapy including both anthracycline and taxan. Patients are randomly assigned to either the carboplatin group or observation group. The patients in the carboplatin group are treated with carboplatin at AUC 6 and those in the observation group are observed at only 3 years.

**Eligibility criteria:**

- 1) ER and PgR<1%, HER2 0, 1+ or 2+ with FISH negative on core needle biopsy before the chemotherapy and surgical specimens.
- 2) Preoperative chemotherapy including both anthracycline and taxan.
- 3) Residual invasive cancer on breast tumors or lymph node metastasis in surgical specimens.
- 4) 20-79 year old women.
- 5) No chemotherapy within 5 years.
- 6) Not bilateral breast cancer, without metastasis, no prior breast cancer.
- 7) No severe bone marrow suppression.

**Specific aims:** Primary objective is DFS (Disease Free Survival). Secondary objectives are overall survival and safety.

**STATISTICAL METHODS:**

The 3 years recurrence rate of the observation group was estimated as 40% and hazard ratio at 0.58 based on the CREATE X trial. For both groups, 135 patients are necessary. This study is powered to approximately 80% to test the superiority of carboplatin group at a 2-sided  $\alpha=0.05$  using a stratified log-rank test.

**Activation Date:** 22<sup>nd</sup> March 2018. No patients had been enrolled till 3rd July.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-05-05

**REASSURE-** Effects of Reiki as supportive treatment during chemotherapy of breast cancer: A prospective, randomized, controlled clinical trial

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**Background:** Every seventh to eighth woman is diagnosed with breast cancer in her life. Next to surgery and radiotherapy most of them receive (neo)adjuvant chemotherapy, which comes along with adverse effects. Complementary and alternative medicine (CAM) like Reiki can reduce these effects. Reiki is a Far Eastern method that promotes healing on a physical, mental and emotional level and activates self-healing powers. REASSURE examines the effects of Reiki on quality of life and taxane-induced polyneuropathy during chemotherapy.

**Methods:** REASSURE is a prospective, randomized, controlled, two-armed clinical trial, in which patients with breast cancer receive chemotherapy and Reiki (18 times) or chemotherapy and sport (18 times). During chemotherapy and before and after every Reiki- or sport-session the patients fill out standardized questionnaires (e.g. FACT/GOG-NTX Version 4). Primary endpoint is the comparison of quality of life at the end of chemotherapy between Reiki and sport based on the FACT/GOG-NTX score by T-Test. Secondary endpoint is the comparison of the taxane-induced polyneuropathy at the end of chemotherapy between the two groups. Also short-term effects before and after the Reiki- and sport-session will be examined. A power of  $1-\beta = 0.8$ , a bilateral probability of error of  $\alpha = 0.05$ , a minimum relevant difference  $\Delta\theta = 4$  and a pooled standard deviation of 11 for the two-sided T-Test result in case numbers of  $2 \times 120 = 240$  patients.

**Conclusion:** REASSURE is the most comprehensive prospective study to the effects and the feasibility of Reiki on breast cancer patients during chemotherapy so far.

Since July 2015, 138 patients have been enrolled at three different centers. Currently 63 patients in total (39 patients of the Reiki-group and 24 patients of the sport-group) have completed the study. 24 Reiki-patients and 36 sport-patients are counted as dropouts because of reasons like incomplete data records, discontinuation of chemotherapy, not enough time or energy for Reiki- or sport-sessions or other reasons.

**Sponsor:** This is a collaborative study of the Department of Obstetrics and Gynecology, Klinikum rechts der Isar, Technische Universität München (TUM), Munich, Germany, Rotkreuzklinikum München, Frauenklinik, Munich, Germany, Department of Gynaecology, Städtisches Klinikum München Harlaching, Munich, Germany and the ProReiki – der Berufsverband e.V., Berlin, Germany.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-06-01

ANG1005 in leptomeningeal disease (ANGLEd) trial: A randomized, open-label, phase 3 study of ANG1005 compared with Physician's Best Choice in HER2-negative breast cancer patients with newly diagnosed leptomeningeal carcinomatosis and previously treated brain metastases

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**Background:** Annually, more than 4,000 patients are diagnosed with leptomeningeal carcinomatosis (LC) from breast cancer in the U.S. Since most therapies cannot cross the Blood-CSF-Barrier (BCB) and the Blood-Brain-Barrier (BBB), treatment options for LC are limited to local radiation and few chemotherapy agents, none of which have provided durable clinical benefit, leading to short overall survival (OS) of 3-4 months. ANG1005 is a novel peptide-drug conjugate, consisting of 3 paclitaxel molecules covalently linked to a peptide that targets the LRP-1 receptor to cross both BCB and BBB, and enter the tumor cells, where the paclitaxel is cleaved off to exert its anti-tumor activity. ANG1005 has previously shown in a phase 2 study to prolong OS in LC patients from breast cancer with brain metastases (BM) to 8 months.

**Trial Design:** This is an open-label, multi-center phase 3 randomized study to evaluate the efficacy of ANG1005 in HER2-negative breast cancer patients with newly diagnosed LC and documented history of previously treated BM when compared to Physician Best Choice (PBC). Hundred and fifty (150) patients will be randomized 1:1 to receive either ANG1005 experimental treatment or an Investigator assigned PBC control treatment. ANG1005 will be administered by intravenous (IV) infusion at a starting dose of 600 mg/m<sup>2</sup> every 3 weeks. Allowed PBC therapies include capecitabine, eribulin, or high-dose IV methotrexate. CNS disease (LC and BM) will be evaluated at screening and every 6 weeks by MRI, CSF cytology and neurological assessments according to LANO and RANO-BM criteria. Extracranial disease will be evaluated by CT scans according to RECIST at screening and every 6-12 weeks. All patients will be followed for survival every 6-8 weeks from the date of the last dose until death, lost to follow-up or consent withdrawal.

**Eligibility Criteria:** Eligible patients are adults (≥ 18 years old) with HER2-negative breast cancer, newly diagnosed LC and documented history of previously treated BM. Patients must be neurologically stable and have adequate blood counts, organ and bone marrow function with an ECOG status grade ≤2. Patients previously treated with ANG1005 or with known allergy to paclitaxel or its components are excluded.

**Specific aims:** The primary endpoint is OS. Secondary endpoints include CNS (LC and BM) progression-free survival and clinical benefit rate, 6- and 12-month OS rates, LC response rate and duration of response, OS for triple negative patients and safety.

**Statistical Methods:** This study is sized for testing the hypothesis of improved OS for ANG1005 versus PBC in all patients (HR=0.58, two-sided test, overall type 1 error of 5%). Interim analysis for OS (using O'Brien Fleming boundaries for efficacy and a fixed HR=1 for futility) will be performed when a total of 55 death events are reached across both arms.

**Study Accrual:** Target accrual is 150 patients. The study is currently planned to start in the fall of 2018.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-07-01

Phase 1/2 study of topical SOR007 (nanoparticle paclitaxel ointment) for cutaneous metastasis

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**Background:** Cutaneous metastases are common in non-skin cancers, resulting in considerable morbidity, infection, pain, and negatively impacting quality of life. No standard of care exists for effective control of cutaneous metastases, which is a significant unmet need in oncology. Paclitaxel has shown effectiveness in the treatment of ovarian, breast, non-small cell lung, and pancreatic cancers and is approved by the United States Food and Drug Administration for the systemic treatment of many types of malignancies. We hypothesize that SOR007, or nanoparticulate paclitaxel ointment may be well tolerated and effective in the treatment of cutaneous metastases.

**Trial Design:** This trial is a Phase 1/2, open-label study evaluating the safety, tolerability, and preliminary efficacy of three concentrations of SOR007 (0.15%, 1.0%, and 2.0%) ointment applied topically, twice daily, to non-melanoma cutaneous metastases during a 3+3 dose-ascending or dose expansion phase. Subjects will self-apply SOR007 ointment twice daily on Days 1 to 28, to a 50 cm<sup>2</sup> area containing an eligible lesion. If a single dose limiting toxicity (DLT) is identified in the three subjects, an additional three subjects will be enrolled at the same dose level. If one or more DLT occur in the three additional subjects, dose escalation will stop and the prior dose level will be determined as the Maximum Tolerated Dose (MTD) and taken forward into the dose expansion phase. If no further DLT are identified, dose escalation will continue, until either a DLT is identified at a higher dose or the top dose of 2% is reached. Once DLT is determined, additional subjects will be enrolled in the expansion phase, to total up to 12 subjects at the top dose.

**Specific Aims:** 1) The primary objective of this study is to determine preliminary safety and tolerability of topical SOR007.

Secondary endpoints include: 2) evaluating preliminary efficacy based on RECIST criteria, photograph documentation of lesion size in longest diameter, and objective clinical response. 3) We will evaluate potential reduction in pain at the treatment area. 4) We will determine the peripheral blood pharmacokinetics of topical SOR007 applied to cutaneous metastases.

**Eligibility criteria:** Male and female patients over the age of 18 with malignancies resulting in cutaneous metastasis originating from: breast, lung, head and neck, pancreatic, urinary bladder, prostate, testicular, ovarian, uterine, cervical, gastric, adrenal, thyroid, parathyroid cancers or other solid tumors which previously responded to taxane treatment are eligible to participate.

Patients' last dose of any systemic non-taxane cytotoxic chemotherapy should be completed at least one day prior to the trial.

Patients' last dose of any systemic taxane cytotoxic chemotherapy should be completed at least 4 weeks prior to the trial.

Patients should be ECOG 0-2 with a minimum life expectancy of at least 3 months. Open or ulcerated wound(s) extending through the dermis are not eligible.

**Statistical methods:** Data will be analyzed by descriptive statistics.

**Present accrual and target accrual:** To date, we have enrolled 3 of a maximum of 24 subjects.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-08-01

A pilot randomized usual care controlled study of yoga for persistent chemotherapy-induced peripheral neuropathy (CIPN) in breast and gynecological cancer survivors

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**Background:** CIPN is a common, painful, and debilitating side effect of many standard chemotherapy regimens. Patients with CIPN typically experience paresthesia (tingling, numbness), pain, and muscle weakness, and may exhibit significant functional decline and diminished quality of life. Our prior study showed that more than half of breast cancer survivors experience persistent CIPN up to a mean duration of 5.6 years and that this symptom is associated with a doubled fall risk. There is an urgent need to identify nonpharmacological approaches to reduce CIPN symptoms and improve cancer survivors' functional outcomes. Yoga is a mind-body modality that includes stretching, flexibility, and balance training; however, little is known about its effects on symptoms and functional outcomes among cancer survivors with CIPN.

**Trial Design:** We are conducting a two-arm pilot randomized usual care controlled trial in breast and gynecological cancer survivors at Memorial Sloan Kettering Cancer Center (MSK), New York, NY. Eligible subjects in the intervention arm receive one-hour Hatha Yoga classes taught twice weekly for eight weeks, and practice home-based yoga for a total of 12 weeks. Subjects in the wait list control (WLC) arm continue usual care for 12 weeks, followed by eight weeks of yoga classes and home-based yoga.

**Eligibility Criteria:** 1) Patients with a primary diagnosis of stage I-III breast, ovarian, uterine, or endometrial cancer; 2) moderate to severe CIPN, defined by four or greater on a 0–10 Numeric Rating Scale (NRS); 3) completion of neurotoxic chemotherapy at least three months prior; 4) no changes in anti-neuropathy medications within three months of enrollment; and 5) an ECOG performance status of 0–2.

**Specific Aims:** The primary endpoint is safety, feasibility, and NRS changes at eight weeks (end of treatment). The secondary endpoints include the Neuropathic Pain Scale (NPS) and Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity (FACT/GOG-Ntx) at eight, 12, and 20 weeks.

**Statistical Methods:** We will accrue 40 patients to get 36 patients evaluable for the primary endpoint at eight weeks. Using an ANCOVA analysis with a sample size of 36, we will be able to detect an effect size of 0.58 standard deviations (SD) of NRS (moderate effect size) between yoga and WLC assuming a NRS correlation between pre- and post-yoga of 0.5 SD. If we assume a 10% dropout rate based on our recently completed trial, we will need to recruit 20 subjects per arm (total of 40) to fall within the precision noted in the sample size calculation. We recognize that the sample size calculation was based on detecting a moderate effect between yoga and WLC and may miss small but clinically meaningful effects that can be used to design a future trial that is sufficiently powered.

**Present accrual and target accrual:** 40 participants. We have accrued 25 participants as of June 2018 and anticipate accrual completion by October 2018.

# 2018 San Antonio Breast Cancer Symposium ®

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Phase II trial assessing accuracy of tumor bed biopsies in predicting pathologic response in patients with clinical/radiological complete response after neoadjuvant chemotherapy in order to explore the feasibility of breast-conserving surgery without surgery: NRG Oncology BR005

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The increased use of neoadjuvant chemotherapy (NCT) has enabled higher rates of breast-conserving surgery (BCS) as well as provided prognostic information for women with breast cancer. High pathological complete response (pCR) rates question the requirement for surgery, with its attendant morbidity. In order to avoid surgery, the ability to predict pCR prior to it must be very high. Trimodality imaging alone is inadequate to predict pCR prior to surgery. We hypothesize that performing core-needle biopsy (bx) of the tumor bed in addition to trimodality imaging in patients (pts) having had a clinical complete response (cCR) will increase the ability to predict pCR. Utilizing predetermined imaging response criteria of complete or near-complete response coupled with a stereotactic core-needle bx of the tumor bed, BR005 aims to determine the predictive value of imaging followed by tumor bed bx for pCR and demonstrate its reproducibility across a multi-institutional setting.

**Methods:** 175 pts with operable focal or multifocal (T1-T3), stage II/IIIA invasive ductal carcinoma (all receptor subtypes) will be entered. Pts must have completed a minimum of 8 wks of standard NCT and achieved a complete or near-complete radiologic tumor response on breast imaging with mammogram, ultrasound, and MRI, and undergo BCS. Following cCR and prior to surgery, pts will undergo a stereotactic-vacuum-assisted breast bx with clip placement. The primary endpoint is the proportion of pts with post-NCT neg image-directed bx who have a pCR. Residual cancer burden scores and core bx pathology will be collected along with trimodality imaging data. Evaluation after 135 pts will allow for the possibility of early termination of the study. Results will provide the first step toward a paradigm change in the treatment of breast cancer, enabling a study to assess the criteria for successful avoidance of surgery in pts with high response rates to NCT.

Accrual as of 6-15-18: 39 (22.3%).

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# 2018 San Antonio Breast Cancer Symposium ®

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**DETECT III/IV study trial** – The multicenter study program in patients with HER2-negative metastatic breast cancer and circulating tumor cells

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## Background:

The collaborative DETECT study program represents one of the largest study program on metastatic breast cancer worldwide. The main objective of the DETECT trial is to evaluate the efficacy of individualized breast cancer treatment based on the presence and phenotype setting of circulating tumor cells (CTCs). Thus, the DETECT study program is one of the first clinical trial translating the role of CTC enumeration/phenotyping directly into treatment intervention within different breast cancer subtypes.

## Trial design:

The DETECT III trial is a multicenter, randomized, phase III study to compare standard therapy alone versus standard therapy plus lapatinib in patients with initially HER2-negative metastatic breast cancer and HER2-positive circulating tumor cells. Patients with persisting HER2-negative circulating tumor cells (CTCs) can be included within the DETECT IV trial, a prospective, multicenter, open-label, phase II study including patients with HER2-negative metastatic breast cancer. Within the DETECT IV study setting postmenopausal patients with hormone-receptor positive metastatic breast cancer are treated with everolimus or ribociclib and endocrine therapy, while women with triple negative metastatic breast cancer or a hormone-receptor positive tumor and indication for chemotherapy receive eribulin.

## Specific aims:

The DETECT study program comprises all breast cancer subtypes and therefore offers various up-to-date treatment options, generating a wealth of clinical data including long-term follow-up data, evaluated in a controlled setting of a single large clinical trial. The primary endpoint of the DETECT III trial is the comparison of patients receiving standard anticancer therapy with lapatinib and patients receiving standard anticancer therapy alone, with regard to the CTC clearance rate. The secondary objective of this trial is to assess the level of compliance to study procedures comparing the efficacy of lapatinib between given treatment groups (Progression free survival, overall response rate and dynamic of CTCs). Primary objective of the DETECT IV trial is to evaluate CTC clearance rate within the everolimus/ribociclib cohort and additionally assess progression-free survival defined as time interval from date of recruitment until progressive disease within the eribulin cohort. The main focal point of the extensive collaborative translational oncology research projects is to apply innovative biomarkers and assays focusing on molecular characteristics of CTCs. This “biological status” may give new information about CTCs potential function as liquid biopsy in order to determine their relevance for therapy prediction.



# 2018 San Antonio Breast Cancer Symposium ®

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The BRandO BiO registry – A multicenter regional registry for patients with primary breast and ovarian cancer with longitudinal biobanking and evaluation of epidemiological, life style and quality of life factors

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## Background:

Further progress in the treatment of breast cancer will likely come from contributions of molecular biology and immunologic approaches. The search for druggable molecular aberrations may enable treatment based on the molecular profile. A better identification of patients with a high risk of relapse facilitates the selection of these pts for clinical trials investigating early therapeutic molecular-based interventions.

## Trial Design:

The BRandO BiO Registry is a multi-center regional registry to record clinical, epidemiological, and biological data from patients with newly diagnosed breast and ovarian cancer at the University of Ulm, Dept. of Gynecology and 19 affiliated network hospitals and practices in the Alb-Allgäu Bodensee region (outreach area of the Comprehensive Cancer Center Ulm). Longitudinal biobanking is included with collection of paraffin-embedded samples of the primary tumor as well as blood samples at first diagnosis, after 6 and 12 months and at first relapse to isolate and investigate cell-free and germline DNA. Epidemiological, life style and quality of life (QOL) questionnaires are collected at first diagnosis, after 12, 36 and 60 months. The follow up is planned for 10 years.

## Eligibility criteria:

Patients with primary newly diagnosed untreated breast or ovarian cancer of  $\geq 18$  years are eligible; primary metastatic untreated disease is allowed. Exclusion criteria comprise severe neurological or psychiatric disorders interfering with the ability to give an informed consent, no consent for registration, storage and processing of the individual disease characteristics and bio samples, and any malignant tumor in the last 3 years (except in situ disease).

## Specific aims:

To register the majority of patients with newly diagnosed breast or ovarian cancer in all BRandO-BiO participating centers of a well-defined geographical area. To assess clinical characteristics and outcome data (event-free survival, overall survival) of these patients. To evaluate the primary tumor of all patients for mutational (druggable) aberrations. Further to assess cell-free DNA in the serial blood samples at baseline, 6 and 12 months and correlate these results with clinical outcome data as well as tumor and patient characteristics to look for early markers predicting relapse. To perform a longitudinal assessment of the patients' sociodemographic factors, comorbidities, lifestyle and QOL factors by analyzing serial questionnaires collected at recruitment and at 12, 36 and 60 months.

## Present accrual and target accrual:

The BRandO BiO Registry started January 2016 in the Dept. of Gynecology, University of Ulm and February 2017 at the network hospitals and practices. Until June 2018, 1180 patients with primary breast or ovarian cancer have been enrolled. The current adherence to serial blood testing and serial questionnaires is good with a return rate of 90%. A sample size of 3000 patients is planned.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-11-02

**PRAEGNANT** - Real world evidence, translational research, big and smart data: A prospective academic translational research network for the optimization of the oncological health care quality in the adjuvant and advanced/ metastatic setting (NCT02338167)

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## Background

During the last decades the treatment of advanced breast cancer (ABC) patients has improved due to a variety of new treatment strategies. Nevertheless, many ABC patients are faced with limited prognosis, and their treatment remains a challenge. New targeted therapies complement the well-established treatment options for ABC (CDK4/6 inhibition, anti-endocrine, chemotherapy, antibody based and bone related therapies), leading to improved treatment regimens tailored to the needs of special patient sub-populations.

## SPECIFIC AIMS/TRIAL DESIGN

The PRAEGNANT network study is conducted as an academic, prospective registry and diagnostic translational study, accompanied by biomaterial collection. The pilot phase in more than 60 centers aims at including 3500 ABC patients. The primary objective is to discover biomarkers, which predict progression free survival (PFS). Secondary objectives include overall survival (OS), breast cancer specific survival, objective response rate, patient reported outcomes (PRO), description of therapies used in the metastatic setting, therapy adherence, health economics for patients with ABC, incidence of (serious) adverse events and big data/ machine learning algorithms. The exploratory objectives comprise correlations of gene alterations and their influence on OS, PFS, side effects and PRO. Exploratory biomarkers are assessed at baseline and at every change of therapy. These biomarkers include gene expression profiling of the primary tumor and corresponding metastasis, somatic mutations (measured in the tumor and in circulating tumor DNA), germline genetic variations, epigenetic changes and miRNA variations. Furthermore, plasma and serum markers are assessed. If actionable molecular alterations are detected patients are informed and recruited into suitable studies if available.

## ELIGIBILITY CRITERIA:

Any adult patient (>18 years) with the diagnosis of ABC and who is willing and able to sign the informed consent can be enrolled.

## STATISTICAL METHODS/TARGET ACCRUAL:

The PRAEGNANT study as a prospective real world registry and diagnostic translational study aims to identify biomarkers in ABC patients, which may predict PFS. Target accrual for the pilot phase is 3500 patients. Each patient will be documented for up to 36 months with an estimated median PFS for all patients of 11 months across all treatment lines.

# 2018 San Antonio Breast Cancer Symposium ®

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Exploration of factors associated with imminent risk of late recurrence in hormone receptor positive breast cancer

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**Research objectives:** To conduct a prospective observational study of patient and tumor-related factors in women with high risk hormone receptor (HR)+/HER2- breast cancer (BC) following at least 5 years of adjuvant hormonal therapy, in order to identify risk factors for imminent recurrence.

**Rationale:** Many of the life-threatening BC recurrences in women with HR+HER2- BC take place more than 5 years post-diagnosis, often after completion of adjuvant hormonal therapy. The identification of a biomarker(s) for late BC recurrence could lead to interventional trials to evaluate preventive therapies. We will evaluate whether the presence of blood-based biomarkers [(i) Circulating Tumor Cells (CTCs), (ii) circulating tumor DNA (ctDNA), (iii) tumor markers (CA 15-3, CEA)] and patient factors may predict BC recurrence.

**Trial design:** A prospective cohort of eligible women with previously treated HR+HER2- BC who have not experienced a distant recurrence will be enrolled; patient and circulating factors will be measured annually until distant recurrence or study completion. Host factors (including BMI, lifestyle, medical illness, surgery, trauma and stress, as well as circulating PIGF, VEGF-1 and inflammatory markers) that may contribute to exit of BC cells from dormancy will also be assessed.

The primary outcome is distant BC recurrence. Any BC event, including loco-regional recurrence, new breast or other primary cancer will be evaluated as a secondary endpoint. Outcomes will be ascertained by regular self-report (via annual telephone calls) and/or physician report and confirmed by medical record review.

**Key eligibility criteria:** i) Diagnosis of ER and/or PR positive (either or both 10% positive), HER2 negative invasive BC, ii) predicted >1.5-2% annual risk of recurrence (T2, T3 or T4 with any N+; T1 N2+; T2N0 or T1 N1 cancers with high risk genomic scores), iii) receipt of adjuvant endocrine therapy for at least 4 years, with discontinuation planned in the next 12 months or completion of endocrine therapy within the last 5 years, iv) prior adjuvant chemotherapy, targeted therapy and bone targeted therapies are allowed provided they have been completed.

**Specific aims:** 1) Determine if the presence of (i) CTCs, (ii) ctDNA, (iii) CA15-3 and CEA are associated with imminent risk (within 1-2 years) of distant recurrence in the study population. 2) Identify host factors associated with these blood-based biomarkers, as well as clinical outcomes.

**Statistical methods:** A matched case control design (matching for time since completion of adjuvant hormone therapy, baseline T, N and grade) will be used to investigate associations of key study variables with imminent risk of distant recurrence within the next 1-2 years. Measurements of patients who do versus do not recur will be compared over the 1-2 years prior to relapse. Each variable will be allocated one third of a study-wide type one error of 0.05 (2-sided). ROC analyses and multivariable modelling will be used to optimize sensitivity, specificity, PPV and NPV. Available questionnaire data will be summarized at all time-points to generate descriptive survivorship data.

**Accrual:** Starting in August 2018, we plan to recruit 1,000 patients over 2 years at selected Canadian cancer centres.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-12-02

Biomarker study of patients with HER2-negative metastatic breast cancer receiving combination therapy with nivolumab, bevacizumab and paclitaxel as first-line treatment (WJOG9917BTR)

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**Background:** In recent years, anti-PD-1 antibody, an immune checkpoint inhibitor, has been developed for the treatment of various types of cancer, including breast cancer. Synergistic effects of nivolumab, paclitaxel and bevacizumab are expected, based on various preclinical data, when these drugs are administered in combination. A biomarker study is ongoing to evaluate the immune status of patients participating in the NEWBEAT trial, which is a phase II trial of nivolumab + paclitaxel + bevacizumab therapy as first-line treatment for patients with metastatic or recurrent HER2-negative breast cancer. **Methods:** HER2-negative breast cancer patients from the WJOG9917B (NEWBEAT) trial are enrolled in this biomarker study. To explore new biomarkers for combined treatment of breast cancer with immune-checkpoint inhibitors and anti-vascular endothelial growth factor antibodies, we propose to conduct multicolor immunohistochemistry (IHC) assays for immunomonitoring of the intra-tumor environment, such as the expressions of PD-L1, CD4 and CD8. Blood samples are collected before the start of treatment and at four time-points during the treatment, to determine, using a multicolor flow cytometry panel, the numbers of circulating immunosuppressive cells, such as regulatory T cells, myeloid-derived suppressor cells and tumor-associated macrophages (M2). In the NEWBEAT trial, patients receive nivolumab 240 mg/body on days 1 and 15, paclitaxel 90 mg/m<sup>2</sup> on days 1, 8 and 15, and bevacizumab 10 mg/kg on days 1 and 15 every 4 weeks until disease progression. The primary endpoint is the objective response rate, and the key secondary endpoints include progression-free survival, overall survival, and toxicity of the protocol treatment. A total of 51 patients will be enrolled and the enrollment period will be one year. This trial opened to accrual in February 2018. Clinical trial registry number: UMIN000029590

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-12-03

**RESCUE:** Reaching for Evidence-based Chemotherapy Use in Endocrine sensitive breast cancer - A prospective health care study on risk assessment by the clinicomolecular test EndoPredict® and long-term patient outcome in early luminal breast cancer

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**Background:** In node negative and 1-3 positive nodes breast cancer patients with hormone receptor positive (HR+), HER2-negative (HER2-) early-stage breast cancer the indication for chemotherapy is based on clinical and pathologic risk stratification (tumor size, nodal status, grading, quantitative ER, progesterone receptor and Ki67). For further decision-making, the EndoPredict test, which combines a molecular signature with the clinical risk factors tumor size and nodal status, stratifies patients into "low risk" or "high risk" groups. Level I-B- evidence demonstrates, that EndoPredict predicts the 10 year cumulative risk of relapse and metastases in patients with HR+/HER2- primary breast cancer with endocrine treatment.

**Aim:** In the RESCUE-Trial we document distant metastasis-free survival (DMFS), disease free survival (DFS) and overall survival (OS) events in patients who had an EndoPredict test. The primary objective is to show that 10-year DMFS of patients tested as "low risk" by EndoPredict and treated with adjuvant endocrine therapy alone is >90 %. Secondary endpoints among others include DMFS, DFS, OS in patients with EPclin "low risk" versus "high risk". Also the proportion of patients whose treatment was concordant and non-concordant with EndoPredict test results, will be analyzed for survival. The prognostic performance of classical prognostic factors (like tumor size, nodal status, grading, quantitative ER, progesterone receptor and Ki67 level) with respect to survival will also be assessed.

**Eligibility:** Patient with HR+/HER2- primary invasive breast cancer stage I/II and T1 to T3 with 0 to 3 positive lymph nodes will be eligible, if they had an EndoPredict test within three months before inclusion.

**Methods:** The EndoPredict test results, tumor board decision and anti-tumor therapy will be assessed. After one year, annually (for 10 years), patients will be evaluated for treatment compliance, recurrence, metastases, and survival. The primary endpoint will be analyzed by a Kaplan-Meier estimate for which a one-sided lower 95 % confidence interval will be given. Several secondary endpoints will be assessed in three interim analyses after completion of the 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> year and then finally after 10 years.

**Accrual:** Start of accrual is planned for July 2018. At least 26 sites in Germany and one site in Switzerland will be active.

**Sponsor:** The study is sponsored by the North-Eastern-German Society of Gynecological Oncology (NOGGO) e.V.

**Contact Information:** For further information, contact NOGGO via [studies@noggo.de](mailto:studies@noggo.de) or the leading physician Dr. Johannes Ettl via [johannes.ettl@tum.de](mailto:johannes.ettl@tum.de).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-12-04

A phase IIIb, open-label, local, multicenter study of the molecular features of postmenopausal women with hormone receptor-positive (HR+) HER2-negative advanced breast cancer on first-line treatment with ribociclib and letrozole (BioltaLEE)

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**Background:** characterizing the molecular features associated with prolonged benefit from CDK 4/6 inhibitors in HR+ HER2- BC and the acquired genomic alterations following treatment progression remains an unmet need and is crucial for leveraging the efficacy of CDK4/6 inhibitors and for elucidating resistance mechanisms. Identifying pre-treatment or pharmacodynamic predictive markers of treatment benefit, as well as predictive markers of toxicity by correlating pharmacogenomics with adverse events, could help physicians to select patients who may benefit the most from these therapies and improve the clinical management.

**Trial design:** this is an Italian, multicenter, open-label, single-arm trial (NCT03439046) enrolling approximately 350 HR+ HER2-aBC first line patients in 48 sites. Patients are treated with ribociclib and letrozole and eligibility criteria are similar to the MONALEESA-2 trial. Patients will be followed for safety and efficacy outcomes. An extensive prospective collection of biological samples at different time points will be performed as follow: whole blood and plasma at baseline, cycle 1-D15, cycle 2-D1, at first imaging evaluation, at cycle 24-D1, as well as upon progression of disease; newly obtained tissue biopsies at baseline and at progression; a buccal swab for pharmacogenetics at baseline. Efficacy and safety data will be collected for all patients.

**Aims:** the primary objective of this study is to identify circulating tumor DNA (ctDNA) alterations at baseline, to describe their evolution during treatment and to evaluate the association with clinical outcome. An optimized Next Generation Sequencing approach for the detection of low abundance events in ctDNA will be adopted. Single nucleotide variants and copy number alterations in a customized panel of genes relevant for BC will be analyzed. Secondary objectives include the evaluation of: serum thymidine kinase 1 activity over time as blood marker of early response; ctDNA alterations across different patient profiles and clonal evolution of ctDNA alterations under treatment; ctDNA alterations at time of tumor progression; correlation between mutational status detected in ctDNA and matched tissue samples; features of tumor microenvironment before and after treatment; association of pharmacogenomics patterns with adverse events and clinical outcomes. Clinical efficacy and safety of ribociclib + letrozole will be correlated with all biological endpoints.

**Statistical methods:** the study is descriptive in nature and no formal statistical testing is necessary or applicable. Sample size is aligned with other biomarker studies and is based on a feasibility analysis of the trial and relative timelines.

**Present accrual:** The first study patient was screened in Feb 2018, as in June 2018, 126 patients have been screened and 78 patients have been enrolled.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-13-01

MammaPrint, Blueprint, and full-genome data linked with clinical data to evaluate new gene expression profiles (FLEX)

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**BACKGROUND:** Genomic signatures are revolutionizing the definition, identification, and treatment of breast cancer subtypes. The ability of genomic signatures to enable fine grained stratification of breast cancers to the granular disease level is still generally untested because of the difficulties in aggregating large clinical data sets. In order to stratify breast cancers into actionable subtypes both the full genome data and clinical data must be collected for patients at scale.

**DESIGN & METHODS:** FLEX is designed as a novel, large-scale, population based, prospective registry. All patients with stage I-III breast cancer who receive MammaPrint (MP) or Blueprint (BP) testing on a primary breast tumor are eligible. FLEX utilizes an adaptive design which enables additional study arms at low incremental effort and cost by allowing targeted substudies to be added. Patients who are enrolled in the initial study will also be eligible for inclusion in any additional study arm where they meet all criteria. Additional study arms and substudies may be investigator-initiated.

## **SPECIFIC AIMS:**

Primary: Create a big-data registry of full genome expression data and clinical data to investigate new gene associations with prognostic and/or predictive value.

Secondary: Generate hypotheses for targeted subset analyses and trials based on full genome data. To date the following substudies have been proposed:

DR. JENNIFER A. CROZIER, BAPTIST MD ANDERSON CANCER CENTER

(1) MP and BP in male breast cancer TYPE: SUBSTUDY; NO ADDITIONAL CONSENT (ICF) REQUIRED. ARMS: ALL (2) MP BP evaluation in breast cancer patients ≥70. TYPE: SUBSTUDY; NO ADDITIONAL ICF REQUIRED. ARMS: ALL (3) FG evaluation in ILC. TYPE: SUBSTUDY; NO ADDITIONAL ICF REQUIRED. ARMS: ALL (4,5) MP BP relation to PR positivity, Ki67. TYPE: SUBSTUDY; NO ADDITIONAL ICF REQUIRED. ARMS: ALL (6) MP BP in metaplastic breast cancer. TYPE: SUBSTUDY; NO ADDITIONAL ICF REQUIRED. ARMS: ALL

DR. ADAM M. BRUFISKY, UNIVERSITY OF PITTSBURGH MEDICAL CENTER MAGEE WOMENS HOSPITAL

(1) Response to standard chemotherapy regimens in clinically ER+/PR+/HER2+ (triple positive) patients according to BP molecular subtypes. (2) Expression signatures by response to bisphosphonates in ER+ patients receiving adjuvant therapy, or for osteoporosis after primary treatment. (3) Gene expression in breast cancer patients with obesity. TYPE: SUBSTUDY; DUAL ICF UTILIZED. ARMS: NEOADJUVANT AND ADJUVANT

DR. IAN GRADY, NORTH VALLEY BREAST CLINIC

Impact of genomic risk classification on travel time to receive breast cancer care. TYPE: SUBSTUDY; NO ADDITIONAL ICF REQUIRED. ARMS: ALL

DR. THOMAS LOMIS, VALLEY BREAST CARE

Complementary data collection for patients participating in the ODM-201 trial. FLEX provides gene expression for exploratory and signature discovery. TYPE: COMPLEMENTARY; DUAL ICF UTILIZED. ARM: NEOADJUVANT

DR. PAT WHITWORTH, NASHVILLE BREAST CENTER

Genomic reclassification of large tumors eligible to receive NCT therapy. TYPE: SUBSTUDY; NO ADDITIONAL ICF REQUIRED. ARM: NEOADJUVANT

## **ELIGIBILITY, ACCRUAL**

FLEX will enroll a minimum of 10000 patients aged ≥18 with stage I-III breast cancer who sign ICF. Enrollment began April 2017 and 623 patients have been enrolled as of June 2018.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT1-13-02

Molecular testing for minority patients with or at high risk for cancer

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**PURPOSE:** Meharry Medical College is a participant in eMERGE (Electronic Medical Records and Genomics); a multicenter network sponsored by NHGRI/NIMHD with the primary goal to develop, disseminate, and apply approaches to research that combine biorepositories with electronic medical records (EMR) for genomic discovery and medicine implementation research. The consortium also focuses on ethical issues involving privacy, confidentiality, and interaction with the broader community. Individual institutions created protocols around research questions individualized to their populations.

**METHODS:** We enrolled 500 African Americans with or at high-risk for the four most common cancers (prostate, colorectal, breast, lung) to examine possible genetic and proteomic differences to account for health disparities in this population. We will perform DNA, RNA, and proteomics analyses pertinent to these cancers and obtain corresponding clinical history from the EMR with planned long-term follow up.

**RESULTS:** 500 subjects (211 female) were enrolled over 11 months from Nashville General Hospital including the following cancer/at-risk participants (Breast 59/37; Colorectal 17/128; prostate 31/136; lung 16/76). Most individuals stated that they participated for potential benefit to themselves, family members, or humankind and only 11 percent of potential participants declined. Little concern has been voiced for providing samples for genetic analysis. A genetic counselor will meet with the participants that are found to have pathogenic or likely pathogenic mutations while study investigators will share results with those that are not found to have mutations. Participants will be queried regarding understanding of the genetic testing results and followed for one year to evaluate if they underwent recommended testing and to follow for cancer outcomes.

**CONCLUSION:** The inclusion of diverse groups in genomic research is critical to identify possible reasons for health disparities and to study the understanding of genetic testing and ethical issues surrounding this topic. In this study, African-Americans are participating willingly in clinical research to examine possible genetic and/or social bases for cancer disparities.

**ACKNOWLEDGEMENT:** NIMHD (U54MD007593) to the Meharry Translational Research Center (MeTRC); National Human Genome Research Institute (NHGRI); National Institute of Allergy and Infectious Disease (NIAID).



## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-01-01

**CBCSG-040:** A randomized multicenter, open, prospective, controlled study-comparison of the safety of immediate one-stage implant based breast reconstruction (IBBR) versus two-stage expander-implant IBBR augmented with TiLoop® bra

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A research from Netherlands comparing two-stage implant-based breast reconstruction with immediate one-stage implant-based breast reconstruction augmented with an acellular dermal matrix was published on Lancet Oncology in 2017. It showed that immediate one-stage IBBR with ADM was associated with more severe adverse events. However, the ADM used in this research was heterogeneous. And there were limitations in the research. An Austrian perspective study compared the safety, cosmetic outcomes and satisfaction in patients received IBBR with ADM or TiLoop® Bra. Results showed that there was no statistical difference between these two groups. A German multicenter, retrospective study focusing on IBBR with TiLoop® Bra showed that overall complication rates of one-stage and two-stage method were 29.2% and 27.6% respectively. So far, the results of these prospective and retrospective studies have inconsistent results and we find that there is a lack of high quality evidence focusing on comparing one-stage and two-stage implant-based breast reconstruction with TiLoop® Bra.

Our study is the first prospective randomized study assessing the safety and patient-reported outcomes of immediate one-stage IBBR compared with those of two-stage IBBR with TiLoop® Bra. Our hypothesis is that one step method is not inferior to two step method in implant based breast reconstruction following mastectomy using TiLoop® Bra. In the present randomized multicenter, open, prospective, controlled study, patients were enrolled at six hospitals in different places of China. All patients will undergo Skin Sparing Mastectomy (SSM)/Nipple Sparing Mastectomy (NSM) with one-stage or two stage IBBR with TiLoop® Bra.

Randomization was done electronically, stratified per center in each hospital to achieve roughly balanced groups. The study was open label, and surgeons and patients were informed about the allocated treatment at least 3 days before surgery.

Based on earlier experience, we calculated that the surgical complication rate was approximately 30 percent in the one-stage group and 25 percent in the two-stage group. Consider a dropout rate of 5%, a total of 450 patients, with 225 in each group, are needed.

The present study aims to compare the safety of immediate implant reconstruction following mastectomy using TiLoop® Bra (one step method) versus immediate-delayed implant reconstruction (two step method). The primary endpoint is the rate of complication between the two groups in one year after surgery. The secondary endpoint is assessing the quality of life, patient satisfaction, aesthetic score of reconstructed breast, type of additional surgery following breast reconstruction, angiopathology-related assessment of TiLoop® Bra. The recording surgery complications including hematoma, seroma, burn wound, redness without signs of infection, wound infection, skin necrosis, implant exposure, the implants removal and contracture of cyst. All the complications are staged according to Clavien-Dindo score system.

The study is expected to end the enrollment in 2019. We are looking forward to deliver the latest result in the future.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-01-02

Thoracic interfascial nerve blocks versus paravertebral block for improving quality of recovery after breast cancer surgery: A randomized, double-blind, non-inferiority trial

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**Background:** Chronic pain after breast cancer surgery has harmful effects on patients' daily life. Paravertebral block (PVB) can prevent not only acute but chronic pain after breast cancer surgery, although the block is not easily put into practice because of technical difficulty or necessity to change patients' position. Thoracic interfascial nerve blocks (TINB) have been reported that it gives similar analgesic efficacy as PVB as with fewer risks, however there are no reports comparing analgesic efficacy of PVB with TINB. Although there are several scales evaluating postoperative pain including visual analog scale (VAS) or numerical rating scale, Quality of Recovery (QoR) have been set up to assess the impact of postoperative morbidity on patients' ability to return to normal function and quality of life. Among those assessment scales, QoR-40 has been mainly applied. It consists of 40 questions including five dimensions: psychologic support, physical comfort, emotional state, physical independence, and pain. There is a report suggesting that PVB improve QoR-40 after ambulatory breast tumor resection.

**Methods:** This is a single center single arm phase 2 study for early breast cancer patients. Exclusion criteria are pregnant and parturient women, allergy to local anesthetics, significant psychiatric or mental disorders, and patients with chronic pain. All blocks are subjected to ultrasound guidance. Patients were randomized to receive PVB with 40 ml ropivacaine or TINB with 60 ml ropivacaine. TINB consisted of modified PECS II block and transversus thoracic muscle plane block. For assessment of QoR, QoR-40 score which was consisted of 5 elements including postoperative pain was used. The trial was activated in July 2016.

**Statistical Method:** We conducted a pilot study on 16 patients who received a PVB during breast cancer surgery. Based on the standard deviation (SD) of QoR-40 on postoperative day (POD) 1, the SD was set to 7.2. Since the non-inferiority limit needs to be reduced to about half as much as the effect quantity used in the average value superiority test, it is set to 8, which is half of the effect quantity 16 in the pilot study. When one-sided test with  $\alpha$  error = 0.025 and  $\beta$  error = 0.2, about 13 cases in each group are required. Given that dropout rate is about 30-40%, 18 cases in each group were taken as the number of subject cases.

**Results:** Thirty-six patients were accrued, 18 of PVB group and 18 of TINB group. Two patients declined the trial, 36/38 (95%) acceptors completed the trial. Total of QoR-40 scores on POD 1 was designated as the primary outcome. For secondary outcomes, both QoR-40 score and pain score were assessed on POD 3 and in postoperative month(s) 1, 3, 6, 12. Other secondary outcomes included pain score by POD 1, incidence of rescue analgesia, time to rescue analgesia, incidence of nausea and vomiting. All patients provided written informed consent before undergoing any study-related procedures.

**Conclusions:** This trial will provide non-inferiority that TINB preserve the effect of QoR as good as PVB after breast cancer surgery.

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**Clinical trial information:** UMIN000023340

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-01-03

A prospective pilot study of simultaneous robotic assisted nipple sparing mastectomy and immediate reconstruction

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Endoscopic breast surgery was expected to be an adequate technique to complete cancer clearance and preservation of the patient's body image. However, this technique has limitations including incomplete internal movements and unstable vision of a two dimensional camera due to inflexible endoscopic instruments especially during the skin flap formation. High resolution, ten-fold image magnification, and three-dimensional optics of robotic surgery help overcome the limitations of endoscopic surgery, and thus robotic surgery has been adopted in a wide range of intracorporeal procedures including breast surgery. However, few studies have evaluated feasibility and safety of robotic assisted nipple sparing mastectomy (RANSM) and immediate breast reconstruction (IBR) for the treatment of breast cancer. There were not any investigation to assess patients' satisfaction of cosmetic effect after performing RANSM and IBR. This study is aim to verify the feasibility and the safety of RANSM and IBR and to analyze cosmetic effect of the procedure and satisfaction of patients. The target number of enrollments is 15 patients. Patients who are diagnosed with early breast cancer or BRCA 1/2 mutation carriers are enrolled. Female patients over 20 years old who are candidates to preserve nipple areolar complex and considered to perform reconstruction with implants are prospectively collected. Written informed consents are mandatory. Patients who are considered the high possibility of postoperative radiation therapy according to preoperative stage are not included in this study. We exclude patients who want to undergo other methods of breast reconstruction than breast reconstruction with implants. Patients will undergo RANSM and IBR through a single axillary skin incision simultaneously. Regular follow-up at 1 month and 6 months after RANSM and IBR is scheduled to record recovery of a patient, amount of a drain, date of drain removal, and postoperative complications. Patient satisfaction questionnaire will be completed on the last follow-up day. To evaluate the safety of robotic assisted surgery, the oncologic safety (margin status of nipple areolar complex), postoperative recovery of a patient, and postoperative complications are investigated. We compare preoperative and postoperative 6 month photographs of patients and estimate the surgical outcome by objective indicators to evaluate the cosmetic grading by plastic surgeons. Patients' satisfaction are assessed by questionnaire (BREAST-Q) at the 6-month visit.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-01-04

Towards omitting breast cancer surgery in patients with pathologic complete response after neoadjuvant systemic therapy: The MICRA trial (minimally invasive complete response assessment)

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## Background

Improvements in neoadjuvant systemic therapy (NST) for breast cancer patients have led to increasing rates of pathologic complete response (pCR). Breast-conserving surgery (BCS) after NST is considered safe, despite the fact that the original tumor bed is not entirely excised. It can therefore be hypothesized that breast surgery could be omitted in patients achieving pCR. However, since imaging modalities are insufficiently accurate to predict pCR after NST, the need for surgery is unchanged. The MICRA trial is designed to determine the value of ultrasound guided biopsy of the breast in identifying pCR after NST. The ultimate aim of our study is to eliminate surgery of the breast in patients achieving pCR, consequently improving quality of life of these patients.

## Trial design

The MICRA trial is a multi-center observational prospective cohort study. Inclusion and exclusion criteria are presented in table 1. In all patients receiving NST, a marker is placed in the center of the tumor area pre-NST. Magnetic resonance imaging (MRI) is performed pre-NST and just before or after the last course of NST. Patients with radiologic complete response (rCR; complete absence of pathologic contrast enhancement) or partial response (rPR, 0.1-2.0 cm residual contrast enhancement,  $\geq 30\%$  decrease in tumour size) are eligible for participation. In these patients, 8 ultrasound guided biopsies are obtained in the region surrounding the marker: 4 central ( $<0.5$  cm) and 4 peripheral biopsies (0.5-1.5cm). Hereafter, conventional surgery is performed (BCS or mastectomy) and pathology results of the biopsies and resected specimen are compared. Pathology findings are scored using Miller-Payne criteria. To evaluate the quality and representativeness of the biopsies, biopsies are categorized according to length and pathology results.

## Statistical analysis and accrual

The primary endpoint of the trial is the false-negative rate (FNR) of the biopsy procedure. If the true FNR is 3%, 130 patients without pCR in specimen are sufficient to show that the FNR does not exceed 8% using a one-sided binomial test with a significance  $\alpha$ -level of 0.05. With an expected average pCR rate of 65%, 375 patients with rCR will be included. In the rPR-group the expected pCR rate is 12% and therefore 150 patients will be included. In total 525 patients will be included. Until now, 144 patients have been included.

## Conclusion

The ultimate aim of the MICRA trial is to eliminate surgery of the breast in patients achieving pCR, by identifying pCR with use of ultrasound guided biopsy. In this scenario, local therapy in patients with pCR would be restricted to radiotherapy.

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Women with invasive breast cancer >18 years (all histological subtypes and tumor subtypes)	DCIS as shown by core biopsy prior to NST
Tumor histology and receptor status established by pre-NST biopsy	Women with distant metastatic disease
Complete or partial response on post-NST MRI	History of ipsilateral breast cancer
Marker placed in tumor prior to NST	
Correct position of marker confirmed by mammography or ultrasound	



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-01-05

A randomized controlled trial comparing post-operative intensive follow-up with standard follow-up in high-risk breast cancer patients (JCOG1204: INSPIRE)

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**Background:** The standard follow-up after surgery for breast cancer includes periodic interviews, clinical examinations, and mammography, but many institutions are conducting intensive follow-up including periodic computed tomography(CT), magnetic resonance imaging(MRI), and bone scintigraphy in the world, despite the lack of evidence to support this approach. While intensive follow-up may contribute to prolonged survival through earlier diagnosis and treatment of relapse, it has the disadvantages of high effort and costs placed on patients(pts) and healthcare workers, radiation exposure for imaging examinations, and overtreatment owing to false-positive results. Although past two randomized trials could not show significant difference in overall survival (OS), as imaging methods have remarkably improved, leading to the earlier detection of relapse, and medical therapies have remarkably improved in recent years, randomized controlled trials are needed to confirm whether intensive follow-up can really prolong survival sufficiently to offset these disadvantages in high-risk breast cancer pts.

**Trial design:** This study is a multi-institutional two-arm open label randomized controlled phase III trial being conducted with the participation of 42 hospitals belonging to the Breast Cancer Study Group of Japan Clinical Oncology Group. Eligible pts are randomized either to the intensive follow-up group or to the standard follow-up group; the former will undergo physical examination, bone scintigraphy, chest and abdominal CT, brain MRI/CT and frequent tumor markers, whereas the latter will undergo physical examination at the same frequency and tumor markers will be evaluated once a year. Mammography once a year is planned for both groups. This trial has been registered at the UMIN Clinical Trials Registry as UMIN000012429.

**Eligibility criteria:** High-risk breast cancer pts, who are expected to have recurrence rates of over 30% within 5 years after surgery. The main inclusion criteria are as follows: four or more axillary nodal metastases in the estrogen receptor (ER) positive pts without neoadjuvant chemotherapy(NC)., axillary node metastases in ER-negative pts without NC, axillary nodal metastases in ER-positive pts with NC, histologically proven residual invasive cancer in the breast or axilla in ER-negative with NC.

**Specific Aims:** The primary endpoint is OS, and secondary endpoints are disease-free survival, relapse-free survival, distant metastasis-free survival, OS in intrinsic subtypes, actual number of implemented examinations, compliance with pre-specified examinations, and adverse events.

**Statistical methods:** The primary endpoint will require a total of 538 events to be assessed in order to obtain a statistical power of 80% with a one-sided significance level of 0.05. Thus, the planned sample size to compare the two survival curves is set at 1500 pts, assuming an accrual time of 6 years and a follow-up time of 7 years according to the Schoenfeld and Richter's method.

**Present accrual and target accrual:** The trial was activated in November 2013. 773 pts have been enrolled by the end of June 2018.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-02-01

A confirmation study of omitting axillary dissection in patients with breast cancer and positive sentinel nodes

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## Background

The omission of axillary dissection for positive sentinel-node breast cancer is considered the standard treatment for patients who undergo breast conserving surgery and radiation therapy, according to the results of ACOSOG-Z0011 and AMAROS trials. On the other hand, some surgeons still think that the surgical stress of axillary dissection is minimal, and dissection is permitted. Furthermore, Z0011 contains several problems, such as insufficient number of entry cases and lack of radiation field unity. Thus, we planned a prospective trial to confirm the safety of omitting axillary dissection in patients with breast cancer and positive sentinel nodes.

## Trial design

This is a single arm, confirmation study of three medical centers. Prior to surgery, informed consent is obtained, and patients are registered primarily. After surgery, patients with 1 to 2 positive sentinel nodes, for whom axillary dissection was omitted, are finally included in this trial at final registration.

## Eligibility criteria

Patients with histologically-diagnosed breast cancer, Tis–2, N0 based on a core needle biopsy, will be included in this trial. Eligible patients must be between 20 and 80 years of age, with a performance status of 0–2 and adequate organ function. They must not have undergone any prior operation, radiation therapy, chemotherapy, endocrine therapy, or immunotherapy.

## Specific aims

The primary endpoint is 5-year (y) axillary recurrence rate. Secondary endpoints are 5-y overall survival, 5-y recurrence-free survival, 5-y local recurrence-free survival, the rate of upper-limb lymphedema, quality of life, and comparison of axillary recurrence rates between patients with two or more dissected nodes and those with only one positive node.

## Statistical methods

The expected rate of axillary recurrence is 2.0%, and non-inferiority is defined as an axillary recurrence lesser than or equal to 5% in the axillary radiotherapy group. The sample size was calculated with a study power of 80% and type I error of 10% (two-sided). The required number of patients is estimated to be 189.

## Present and target accrual

Patient accrual from the three medical centers was initiated in July 2016. We plan to enroll a total of 189 patients at final registration in this trial.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-03-01

Multicenter study to standardize and evaluate the efficacy of radiofrequency ablation therapy for early breast cancer (RAFAELO study)

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**Background:** Early-stage breast cancer is increasingly detected by screening mammography, and we aim to establish radiofrequency ablation therapy (RFA) as a minimally invasive, cost-efficient, and cosmetically acceptable local treatment. In our Phase I study, localized tumors with a maximum diameter of 2 cm, preoperatively diagnosed by imaging and histopathology, were treated with RFA. A 90% complete ablation rate was confirmed histopathologically. Our phase II multicenter study of RFA without resection for early breast cancer will evaluate the long-term safety and efficacy of RFA as well as its cosmetic results, which are a perceived advantage of this technique.

**Trial design:** This study is a Phase III, single-arm, multicenter study being conducted with the participation of 11 hospitals. In our experimental therapy, a radiofrequency electrode needle is inserted through the skin into the breast lesion under imaging guidance, followed by thermal ablation with radiofrequency waves. RFA will be conducted under general anesthesia. The Cool-tip™ RF Ablation Single Electrode Kit (Medtronic, CO, USA) will be used to standardize the evaluation of the ablation effect. After RFA, all patients will receive radiotherapy and systemic therapy according to the ER, HER2, tumor grade, and lymph node status of the primary tumor. Residual lesions after RFA will be assessed approximately 3 months after radiotherapy using imaging and pathological studies. All patients will undergo vacuum-assisted biopsy regardless of imaging results. If specimens show viable tumor tissue, additional excision will be performed. Follow-up evaluation for residual tumor, including clinical breast examination and diagnostic imaging (ultrasound, MRI, and mammography), will be performed yearly after RFA. This trial has been registered at the UMIN Clinical Trials Registry as UMIN000012429.

**Eligibility criteria:** Early breast cancer patients, who are lack of prior treatment for breast cancer and histologically confirmed ductal carcinoma with a single localized tumor of 1.5 cm or less in the greatest dimension and clinically node negative on preoperative imaging.

**Specific Aims:** The primary endpoint is 5-year ipsilateral breast tumor recurrence (IBTR) rate, and the secondary endpoints are residual lesion rate, overall survival, DFS, and adverse events of RFA.

**Statistical methods:** The IBTR rate should be similar to that with standard treatment (BCS). However, the IBTR after RFA alone may be higher by up to 10% than in the NSABP-B06 study; therefore the 5-year IBTR rate is estimated to be 6.3%. We calculated the required sample size based on the following assumptions: a clinically acceptable 5-year local recurrence-free survival rate of 90%, a one-sided significance level of 5% ( $\alpha = 0.1$ , 2-sided), and power of 80% ( $\beta = 0.2$ ). Taking into account a drop-out rate of 10%, approximately 372 patients will be enrolled in the study.

**Present accrual and target accrual:** The trial was activated in August 2013, with a total of 372 patients enrolled by the end of June 2018.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-04-01

Phase III trial to determine if chest wall and regional nodal radiotherapy (CWRNRT) post mastectomy (Mx) or the addition of RNRT to whole breast RT post breast-conserving surgery (BCS) reduces invasive breast cancer recurrence-free interval (IBCR-FI) in patients (pts) with pathologically positive axillary (PPAx) nodes who are ypN0 after neoadjuvant chemotherapy (NC): NRG Oncology/NSABP B-51/RTOG 1304

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This phase III post-NC trial evaluates if CWRNRT post-Mx or whole breast irradiation (WBI) with RNRT after BCS significantly reduces the IBCR-FI rate in pts with PPAx nodes that are pathologically negative after NC. Secondary aims are OS, LRR-FI, DR-FI, DFS-DCIS, second primary cancer, and comparison of RT effect on cosmesis in reconstructed Mx pts. Correlative science examines RT effect by tumor subtype, molecular outcome predictors for residual disease, and predictors for the degree of reduction in loco-regional recurrence.

**Methods:** Clinical T1-3, N1 IBC PPAx nodes (FNA or core needle biopsy) pts complete ≥8 weeks of NC (anthracycline and/or taxane). HER2+ pts receive anti-HER2 therapy. Following NC, BCS or Mx, sentinel node biopsy (≥2 nodes) and/or Ax dissection with histologically negative nodes is performed. ER/PR and HER-2neu status before NC is required. Pts may receive appropriate adjuvant systemic therapy. Radiation credentialing with a facility questionnaire/case benchmark is required. Random assignment for Mx pts is to no CWRNRT or CWRNRT and for BCS pts to WBI or WBI+RNRT.

**Statistics:** 1,636 pts are to be enrolled over 5 yrs (definitive analysis at 7.5 yrs). Study is powered at 80% to test that RT reduces the annual hazard rate of events for IBCR-FI by 35% for an absolute risk reduction of 4.6% (5-yr cumulative rate). Intent-to-treat analysis with 3 interim analyses (43, 86, and 129 events) and a 4th/final analysis at 172 events. Pt-reported outcomes focusing on RT effect will be provided by 736 pts before random assignment and at 3, 6, 12, and 24 mos. Accrual as of 6-21-18 is 967 (59.11%).

**Contacts:** Protocol: CTSU member website <https://www.ctsuo.org>. Questions: NRG Oncology Pgh Clin Coord Dpt: 1-800-477-7227 or [ccd@nsabp.org](mailto:ccd@nsabp.org). Pt entry: OPEN at <https://open.ctsu.org> or the OPEN tab on CTSU member website.

**NCT01872975**

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-04-02

A phase 3 study of post-lumpectomy radiotherapy to whole breast + regional lymph nodes vs whole breast alone for patients with pN1 breast cancer treated with taxane-based chemotherapy (KROG 1701): Trial in progress

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## Background

In patients with early stage breast cancer, regional nodal irradiation (RNI) is added to whole breast irradiation (WBI) in order to control microscopic regional disease and to prevent systemic spread of cancer. According to recent randomized trials (MA.20 and EORTC 22922-10925), prophylactic RNI was associated with improvement in disease-free survival (DFS) in the patients with high-risk node negative or pN1 breast cancer. However, systemic agents now known to improve loco-regional control, such as taxane or endocrine therapy, were prescribed to a small percentage of patients in the studies. The benefit of RNI found in the previous studies might be attributed to incorporation of less effective systemic treatments. The impact of prophylactic RNI in pN1 breast cancer should be evaluated in the patients receiving modern systemic treatment. The current study was conducted to compare the effect of post-lumpectomy WBI vs WBI plus RNI on DFS in pN1 breast cancer patients who received adjuvant taxane-based chemotherapy.

## Methods

This study is a multicenter, phase 3, randomized controlled non-inferiority trial (NCT03269981). Eligibility criteria are  $\geq 20$  years female; pathologically proven invasive carcinoma of the breast; one to three positive axillary lymph nodes (pN1) in pathologic specimen; receiving breast-conserving surgery followed by taxane-based chemotherapy; having adjuvant endocrine therapy or anti-HER2 treatment according to molecular subtype of tumor. Patients are randomly assigned in a 1:1 ratio to receive WBI or WBI plus RNI. Patient randomization was stratified by molecular subtype of tumor (i.e. luminal A/luminal B/luminal HER2/HER2-enriched/triple-negative) and methods of axillary management (i.e. sentinel lymph node biopsy/axillary lymph node dissection). The primary outcome is DFS. The secondary outcomes include DFS according to molecular subtype, treatment-related toxicity, and patient's quality of life per EORTC QLQ-C30 and QLQ-BR23. Patients will be followed for survival and disease recurrence for seven years. A total of 1,926 patients are planned to be enrolled, with recruitment initiated in April 2017. As of June 2018, a total of 236 patients were enrolled.

## Acknowledgement

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-04-03

Examining personalized radiation therapy (EXPERT): A randomised phase III trial of adjuvant radiotherapy vs observation in patients with molecularly characterized luminal A breast cancer

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## *Background*

Radiation therapy (RT) after breast conserving surgery (BCS) is the current standard of care for patients with early stage breast cancer. However, individual absolute recurrence risks and hence benefits of RT vary substantially. A study showed significant association between local recurrence (LR) risk and PAM50-defined intrinsic subtypes and Risk of Recurrence scores (ROR).<sup>1</sup> The objective of EXPERT, a co-lead study of Breast Cancer Trials-Australia & New Zealand (BCT-ANZ), and Breast International Group (BIG), is to optimize local therapy for early breast cancer through precise individualized quantification of LR risk to identify patients for whom RT after BCS may be safely omitted.

## *Trial design*

This is a randomized, non-inferiority, phase III study of women who plan to receive adjuvant endocrine therapy for Prosigna (PAM50)-defined luminal A breast cancer with ROR  $\leq 60$  resected by BCS.

Women are randomized to receive adjuvant whole breast RT and endocrine therapy or endocrine therapy alone and followed-up for 10 years after randomization.

## *Major eligibility criteria*

Females aged  $\geq 50$  years; histologically confirmed invasive breast carcinoma  $\leq 2$  cm, grade 1 or 2, ER and PgR  $\geq 10\%$ , HER2-negative and node-negative; treated by BCS with negative margins for invasive carcinoma and associated DCIS; Prosigna (PAM50)-defined Luminal A subtype and ROR  $\leq 60$ ; and plan to receive adjuvant endocrine therapy.

## *Specific aims*

Primary: To determine if omission of RT is not inferior to RT in terms of LR-free interval after BCS.

Secondary: To evaluate the impact of omission of RT on regional, local-regional and distant recurrence-free interval; disease-free survival (DFS); invasive DFS; overall survival; salvage RT or mastectomy rate; toxicity; endocrine therapy adherence; patient reported outcomes; and health economic outcomes.

## *Statistical methods*

An estimated 5-year LR rate in the target population is expected to be 1% with RT. A rate of 4% is considered non-inferior as a worthwhile trade-off against RT toxicity. Using O'Brien-Fleming boundary for rejecting non-inferiority, 29 LR events are required for final analysis expected 8 years after the first patient is randomized. Two interim analyses will be conducted after 10 and 21 events. If the stratified log-rank test statistic exceeds the upper boundary at interim or final analysis, the hypothesis of non-inferiority will be rejected and it will be concluded that no RT is inferior to RT.

*Accrual: Target (1170), actual: 82 (June 2018)*

The study was activated in Australia in August 2017, with global activation planned for Q4 2018. Recruitment is expected to be completed in 4.5 years.

## *Contact information*

Professor Boon Chua, UNSW Sydney and Prince of Wales Hospital, NSW, Australia; email boon.chua@health.nsw.gov.au; T +61 2 49255239. Registration: NCT02889874

## *References*

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-04-04

Multi-institution phase II trial of intraoperative electron beam radiotherapy boost at the time of breast conserving surgery with oncoplastic reconstruction in women with early-stage breast cancer

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**Background:** In women amenable to breast conserving therapy, lumpectomy followed by adjuvant whole breast irradiation (WBI) remains the standard of care. Randomized trials demonstrate that addition of a lumpectomy cavity boost significantly reduces the risk of ipsilateral breast tumor recurrences but also increases the risk of breast fibrosis. Contemporary randomized trials define the lumpectomy cavity boost volume as a 1.7 cm isometric expansion on the lumpectomy cavity as delineated on CT. However, identifying the lumpectomy cavity can be challenging, especially in women that receive adjuvant chemotherapy and in cases in which surgical clips are not present. Recently, the use of oncoplastic techniques in breast conserving surgery has increased. These techniques are used to prevent the poor cosmetic results that can occur when a large volume of breast tissue is resected. Women that undergo oncoplastic reconstruction represent especially difficult cases for lumpectomy cavity delineation. Retrospective series have evaluated the use of intraoperative electron radiotherapy (IOERT) as a boost prior to WBI in women receiving lumpectomy without oncoplastic reconstruction. In the largest series of IOERT boost prior to WBI the local control rate of this approach was >99%. Prospective data regarding IOERT boost in women undergoing oncoplastic reconstruction are limited. The advantages of this approach include direct visualization/irradiation of the tumor bed, sparing the skin of irradiation, and reducing the treatment time by ~1 week. We hypothesize that IOERT boost followed by WBI will result in acceptably low rates of grade 3 fibrosis in women undergoing lumpectomy with oncoplastic reconstruction.

**Trial Design:** This is a single-arm, prospective study to evaluate the safety, toxicity and efficacy of IOERT boost at the time of breast conserving surgery in women with early-stage breast cancer undergoing oncoplastic reconstruction. Eligible women will receive 1 dose of 8 Gy to the surgical bed after lumpectomy but prior to oncoplastic reconstruction. Women will then receive adjuvant WBI of 40 Gy in 15 fractions or 50 Gy in 25 fractions.

**Eligibility:** Key criteria include age ≥18 yo, clinically node-negative stage I/II, any breast cancer subtype.

**Specific Aims:** To determine the rate of grade 3 breast fibrosis at 1 year. Additional aims include surgical complication rates, cosmesis, and local regional cancer control.

**Statistical Methods:** Safety will be evaluated by the rate of surgical complications necessitating hospital readmission or return to the operating room within 30 days of surgery+IOERT. If ≥4 events in the first 10 patients, ≥7 events in the first 20 patients, or ≥9 events in the first 30 patients are seen, the study will be halted. We hypothesize that the grade 3 fibrosis rate in our study will be ≤5%. Assuming an actual rate of 4%, an unacceptable rate of 9%, and a drop-out rate of 10%, the expected sample size is 176.

**Sites:** Ohio State University, Avera Medical Group, University of North Carolina-Chapel Hill

**Patient Accrual:** Current accrual is 5/176.

**Contact Information:** Jose Bazan (jose.bazan2@osumc.edu)

**Funding Source:** Intraop Medical

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-04-05

Phase II trial of pre-operative stereotactic ablative radiotherapy (SABR) in early-stage breast cancer

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## **Background:**

Post-operative accelerated partial breast irradiation (APBI) has demonstrated efficacy in preventing in-breast tumor recurrence. Pre-operative administration of APBI may be advantageous as an intact breast tumor is smaller than its corresponding lumpectomy cavity, is easier to distinguish on treatment-planning images, and results in smaller and more accurately delineated target volumes. Pre-operative APBI may reduce the incidence of positive margins following breast-conserving surgery (BCS). Investigation is needed in the correlation of MR imaging with pathologic response 6 weeks after SABR. Also, evidence suggests that SABR induces immune activation in the tumor microenvironment; evaluation of excised tumor tissue will give insight into these processes.

## **Trial Design:**

Treatment Planning and Delivery: CT simulation and treatment are performed in the prone position. Diagnostic MRI is fused to planning CT. GTV is delineated on registered breast MRI and includes the intact breast tumor. CTV is 15mm expansion of GTV. PTV is 3 mm expansion of CTV. VMAT or IMRT are permitted. Daily image-guidance aligning to tumor and biopsy-fiducial is mandatory. All subjects undergo pre-operative SABR to 28.5 Gy in 3 fractions of 9.5 Gy on different days separated by  $\leq 48$  hours. CTCAE v4 is used to assess toxicity 4-5 weeks after SABR. Pre-operative diagnostic MRI is performed 5-6 weeks following SABR. Imaging parameters to be evaluated include changes in tumor size, enhancement, and tumor margin description. BCS will be 6-8 weeks following SABR.

Tissue pathology: Margin status and degree of pathologic response are recorded from breast-conserving excisions, specimens are archived for future analysis.

## **Eligibility Criteria:**

Inclusion criteria are women age  $\geq 50$  with biopsy proven invasive breast adenocarcinoma with tumor size  $\leq 2$ cm on MRI, cN0 M0, ER+/HER2-, without history of invasive malignancy or prior breast/thoracic radiotherapy.

Exclusion criteria are active scleroderma or lupus erythematosus with skin involvement, MRI defined tumor within 10 mm of skin, implanted hardware prohibiting appropriate treatment planning or delivery, neoadjuvant chemotherapy, carrier of BRCA1 or 2 gene mutation, pregnancy.

## **Specific Aims:**

The primary endpoint is pathologic complete response (pCR) in the breast tumor, secondary endpoints are incidence of adequate surgical margins (defined as "no tumor on ink") and MRI response following SABR. Analyses of tumor immune response and microenvironment on pathologic specimens following SABR will also be performed.

## **Statistical Methods:**

Fisher's exact test will be performed to examine associations between patient/tumor characteristics and pCR and surgical margins; these associations will be explored with multivariable logistic and linear regressions.

## **Accrual:**

Present accrual is 9 subjects.

Expected accrual is 22 subjects; if  $\geq 3$  pCR are noted in the initial cohort, accrual will be expanded to 40 subjects.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-05-01

**FAMILY:** A randomized, multicenter, open-label, phase III trial of fulvestrant versus capecitabine as maintenance therapy after first-line combination chemotherapy in patients with hormone receptor-positive, human epidermal growth factor receptor-2 negative metastatic breast cancer

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**Background:** Metastatic breast cancer (MBC) is incurable. Although first-line endocrine therapy is preferred to hormone receptor positive (HR+), human epidermal growth factor receptor-2 negative (HER2-) MBC, combination chemotherapy should be reserved as the initial treatment for patients with rapid clinical progression, life-threatening visceral metastases, and need for rapidly symptom control. Either prolonged chemotherapy or endocrine therapy may be used as maintenance after disease control. However, which maintenance strategy is superior in terms of delaying disease progression as well as maintaining quality of life (QOL) remains uncertain. This phase III trial aims to compare the efficacy and safety of fulvestrant or capecitabine as maintenance therapy after first-line combined chemotherapy in HR+/HER2- MBC.

**Trial Design:** FAMILY is a multicenter, randomized, open-label phase III trial for HR+ and HER2- MBC. Eligible participants are randomized (1:1) to receive capecitabine (2000mg/m<sup>2</sup> twice daily x 14 days followed by 7 days off) or fulvestrant (500mg Days 0, 14, 28, then every 28 days) until disease progression, unacceptable toxicity, or patient refusal. Stratification factor for randomization is sensitivity to adjuvant hormonal therapy (disease-free interval ≤24 months vs. >24 months).

**Eligibility Criteria:** Eligible patients must have HR+ (ER and/or PR>1%, by IHC) and HER2- MBC; achieved a complete or partial response or stable disease (investigator assessed) after 4-8 cycles of first-line combination chemotherapy. Patients with central nervous system metastasis and/or prior use of endocrine therapy for advanced breast cancer are excluded.

**Specific Aims:** The primary endpoint is progression free survival (PFS). Secondary endpoints include overall survival, overall response rate, disease control rate, safety and QOL. A prospective translational research is also planned to assess the correlations between biomarkers and response.

**Statistical Design:** The planned sample size of 256 patients provides approximately 80% of power to detect a 6 months difference of PFS using a log-rank test with two-sided alpha of 0.05.

**Target Accrual:** Recruitment is ongoing. Up to 256 evaluable subjects will be enrolled within 24 months. (ChiCTR-IIR-17014036).

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-05-02

International retrospective cohort study of locoregional and systemic therapy in oligometastatic breast cancer (OLIGO-BC1)

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Breast cancer (BC) is so-called “systemic disease”, because disseminated cancer cells in bone marrow and blood are detected even in early BC patients. Despite adjuvant therapy and postoperative radiation therapy, patients with triple negative BC and Luminal B-like BC often relapse early and systemic therapy is the only way to control disease progression. On the other hand, some BC patients relapse several years later. In such patients, oligometastases are occasionally diagnosed, because metastatic cancer cells are slowly growing and indolent. Oligometastatic BC is defined as low volume metastatic disease with limited number and size of metastatic lesions (up to five and not necessarily in the same organ). This definition is proposed in the Advanced Breast Cancer guidelines that are developed as a joint effort from European School of Oncology and European Society of Medical Oncology. Several retrospective studies demonstrated survival benefit of locoregional therapy in addition to systemic therapy. Locoregional therapy consisted of surgical resection, radiation therapy, ablation therapy, etc. However, it remains unclear about survival benefit of combined therapy in oligometastatic BC. To improve the standard of cancer treatment through the cooperate studies on more effective therapeutic strategies based on drugs, surgery and/or radiotherapy, Federation of Asian Clinical Oncology (FACO) was established in 2012 by Chinese Society of Clinical Oncology (CSCO), Korean Society of Medical Oncology (KSMO) and Japan Society of Clinical Oncology (JSCO). Thus, FACO conducted a retrospective cohort study on oligometastatic BC. The primary endpoint is to compare the estimated 5-year overall survival (OS) of oligometastatic BC patients treated with combined therapy and systemic therapy alone. To hypothesize that combined therapy has more advantage of OS in oligometastatic BC, the 5-year OS rates are expected to be 50% and 40%, respectively. The estimated sample size is calculated to be the number of 698 cases (349 cases in each group) needed to prove the superiority of survival with a two-sided type I error rate of 5% and a statistical power of 80%. Case registry opened in February 2018 and will close in January 2019. We planned to register 700 cases, i.e., 234 cases each from investigators of CSCO, KSMO and JSCO. Update information will be discussed.

# 2018 San Antonio Breast Cancer Symposium ®

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Highly innovative personalized RNA-immunotherapy for patients with triple negative breast cancer

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**Background:** The treatment of triple negative breast cancer (TNBC) is hampered by the lack of established therapeutic targets such as hormone receptors or HER-2. Chemotherapy and radiotherapy is the standard of care, yet survival rates in TNBC remain poor. Approaches tailored to the patient's individual tumor signature may lead to improved therapeutic outcome. We have set up a clinical workflow covering drug development (from target discovery to manufacturing) and drug release providing a custom-made investigational medicinal product (IMP) for each individual patient.

**Trial Design:** A phase I/II trial assesses the feasibility, safety and biological efficacy of this personalized immunotherapy in three clinical sites in Germany and Sweden. TNBC patients (pT1cN0M0 – T<sub>x</sub>N<sub>x</sub>M0) after completion of initial standard of care therapy will be allocated to one of two study arms. Patients in ARM1 receive 8 vaccination cycles with a personalized combination of shared tumor-associated antigens, selected based on each patient tumor's antigen-expression profile out of a WAREHOUSE of pre-manufactured mRNA vaccine. Patients in ARM2 receive the personalized mRNA WAREHOUSE vaccine followed by 8 vaccination cycles of an on-demand manufactured mRNA MUTANOME vaccine encoding up to twenty unique neo-epitopes of the individual patient identified by next generation sequencing. The mRNAs are administered intravenously as a nanoparticulate lipoplex formulation, which protects RNA from degradation, activates innate immunity, transfects APCs and consequently induces highly potent antigen-specific T-cell responses. The treatment of 12 patients in ARM1 is completed and enrolment of patients for ARM2 has started. Preliminary data show that the RNA-WAREHOUSE approach is feasible and can be applied safely. Biomarker analysis is ongoing. This approach is promising as it addresses the heterogeneity of TNBC.

The TNBC-MERIT trial was initially funded by the EU Commission's FP7 and led by BioNTech AG.



# 2018 San Antonio Breast Cancer Symposium ®

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Chemokine modulation to enhance the effectiveness of pembrolizumab in patients with metastatic triple negative breast cancer

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**Background:** Triple negative breast cancer (TNBC) is found in approximately 15-20% of all breast cancer, and is associated with poor prognosis, early relapse and a significantly shorter survival following recurrence. Early phase trials with immune checkpoint inhibitors have shown modest yet durable clinical responses with a tolerable safety profile. Strategies to sensitize these tumors to checkpoint inhibition will result in decreased morbidity and mortality.

Tumor infiltrating immune cells and intratumoral expression of PD-1/PD-L1/PD-L2 can predict patients' benefit from Pembrolizumab. Pre-clinical *ex-vivo* data show that chemokine modulating regimen consisting of Rintatolimod, IFN $\alpha$  and COX2 blocker (CKM), selectively attracts cytotoxic T cells into tumors, and increases intratumoral expression of PD-1/PD-L1/PD-L2, without enhancing soluble suppressive mechanisms. Furthermore, mouse data has shown safety of CKM and PD-1 blockade combination and efficacy in inducing long-term survival of mice with resistant tumors.

Clinical trials of CKM in Colon and ovarian cancer (NCT01545141, NCT02432378) demonstrated safety of Rintatolimod given with IFN $\alpha$ /COX2, and preliminary data show local efficacy in tumor microenvironment modulation.

**Design:** This is an open-label, single center, phase IIa study to test Chemokine modulating regimen (CKM) pre-treatment followed by Pembrolizumab in patients with metastatic TNBC, regardless of PD-1 expression, who progressed on  $\geq 1$  lines of therapy.

Patients are given pre-treatment CKM, which consists of Rintatolimod (200 mg IV), IFN $\alpha$ -2 (20 million units/m<sup>2</sup> IV) and Celecoxib (200 mg po BID), on 3 consecutive days for a total of 2 cycles, one week apart. The patient is then treated with Pembrolizumab 200 mg IV every 3 weeks until disease progression, intolerable side effects or withdrawal from study for up to 24 months. Study includes pre- and post CKM treatment biopsies.

**Eligibility:** Major criteria include age  $\geq 18$  years, ECOG  $\leq 1$ , histologically proven metastatic TNBC, normal organ and marrow function, no active autoimmune disease or history of transplant, no prior anti-PD1/PDL1 therapy.

**Aims:** Primary objective is to evaluate the overall response rate to the combination therapy per irRECIST criteria. Secondary objectives include safety profile of the combination therapy, determining progression free survival, overall survival and disease control rate. Other immune exploratory objectives will include baseline and CKM-induced predictive biomarkers of clinical activity of the combination treatment.

**Statistical Methods:** The study includes a safety lead-in of 6 patients and utilizes a Simon two-stage minimax design. 18 patients are enrolled into stage 1 of the study. If  $< 4$  responses are observed, the treatment combination will not be considered promising and enrollment will be terminated. However, if  $\geq 4$  responses are observed, then an additional 19 patients will be enrolled into stage 2, for a total number of 37 patients. In stage 2, if  $\geq 9$  responses are observed, then the treatment combination will be considered promising for future study.

## 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-06-03

A phase 2 study of intratumoral tavokinogene telseplasmid (tavo) plus electroporation with pembrolizumab in patients with inoperable locally advanced or metastatic triple negative breast cancer

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Triple negative breast cancer (TNBC) accounts for 10-20% of breast cancer diagnoses and is associated with a high risk of recurrence and a more aggressive course in the metastatic setting. Emerging data suggest that some patients with TNBC could benefit from the addition of immune-based therapy due to the important role of tumor infiltrating lymphocytes (TILs) on outcome. Reports show that early-stage TNBC patients with at least 50% TILs demonstrate longer disease-free survival. Additionally, immune-modulating therapies, like the anti-PD-1/PD-L1 monoclonal antibodies, have demonstrated modest activity in the pre-treated metastatic TNBC population with objective response rates (ORR) <10%, but appear to require an immunogenic tumor, characterized by CD8+ TILs, in order to be effective. Plasmid IL-12 (tavo) with electroporation (tavo-EP) is a gene therapy approach yielding sustained intratumoral expression of the proinflammatory cytokine IL-12. Combining tavo-EP with an anti-PD-1 therapy, such as pembrolizumab, may improve responses for TNBC subjects by potentially converting poorly-immunogenic/low TIL tumors into high TIL/immune-responsive tumors while providing a favorable side-effect profile. OMS-I141 is a phase 2, non-randomized, study of intratumoral tavo-EP with pembrolizumab in patients with locally-advanced, inoperable, metastatic and/or treatment-refractory TNBC. Key inclusion criteria include documented inoperable locally advanced or metastatic TNBC, at least 1 prior line of approved systemic chemotherapy or immunotherapy and an accessible lesion for intratumoral injection and electroporation. Eligible subjects will receive intratumoral tavo-EP to the accessible lesions on Days 1, 5 and 8 every 6 weeks and intravenous (IV) pembrolizumab (200 mg) on Day 1 of each 3-week cycle. The primary objective of the study is to assess the ORR by blinded independent central review based on RECIST v 1.1. Secondary objectives include assessment of safety and tolerability of the combined treatment, duration of response (DOR), ORR (investigator-assessed), immune ORR (iORR), progression-free survival (PFS), immune PFS (iPFS) and overall survival (OS). A Simon 2-Stage design will be employed and a total of 25 patients will be accrued. In Stage 1 up to 15 subjects will be enrolled. If the number of responders meet the pre-specified number ( $N \geq 1/15$ ), then the enrollment for Stage 2 will include an additional 10 subjects (for a total of  $\geq 6$  responders out of 25 subjects). For more information regarding the study, contact OncoSec at [info@oncosec.com](mailto:info@oncosec.com).

# 2018 San Antonio Breast Cancer Symposium ®

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**MORPHEUS:** A phase Ib/II trial platform evaluating the safety and efficacy of multiple cancer immunotherapy combinations in patients with hormone receptor–positive and triple-negative breast cancer

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Kingdom; <sup>12</sup>Genentech, Inc., South San Francisco, CA and <sup>13</sup>University of Tennessee West Cancer Center, Germantown, TN.

## **Background:**

Cancer immunotherapy (CIT) has significantly improved overall survival across multiple tumor types, but only subsets of patients experience durable response with single-agent CIT. Combinations of CIT with targeted therapy or chemotherapy may be needed in order to target multiple cancer immune escape mechanisms simultaneously, thus providing personalized treatment options that extend clinical benefit to more patients. The MORPHEUS platform includes multiple phase Ib/II trials designed to identify early signals of safety and activity of CIT combinations. Using a randomized trial design, multiple CIT combination arms are compared with a single standard-of-care control arm. These trials have the flexibility to open new treatment arms with novel CIT combinations as they become available and to close arms that show minimal activity or unacceptable toxicity. Here we describe MORPHEUS trials in patients with metastatic or unresectable locally advanced hormone receptor–positive (HR+BC) or triple-negative breast cancer (TNBC), 2 patient populations in need of more treatment options.

## **Trial design:**

MORPHEUS-HR+BC (NCT03280563) will enroll patients with metastatic or unresectable locally advanced HR+BC who have progressed during or after first-line treatment with a cyclin-dependent kinase (CDK) 4/6 inhibitor and whose tumors do not express human epidermal growth factor 2 (HER2). MORPHEUS-TNBC (NCT03424005) will enroll patients with metastatic or unresectable locally advanced TNBC who have progressed during or after first-line treatment with chemotherapy. For both studies, key inclusion criteria include Eastern Cooperative Oncology Group performance status of 0-1 (stage 1) or 0-2 (stage 2) and measurable disease per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Key exclusion criteria include prior treatment with T-cell co-stimulating or immune checkpoint blockade therapies, and symptomatic, untreated, or actively progressing central nervous system metastases. Patients in both trials will be randomized to one of the CIT atezolizumab combination arms or a control arm (up to 5 arms in HR+BC and up to 6 arms in TNBC). Patients experiencing loss of clinical benefit or unacceptable toxicity in stage 1 may be eligible to switch to a different CIT atezolizumab combination arm in stage 2. Primary endpoints are safety measures and investigator-assessed objective response rate per RECIST v1.1. Progression-free survival, overall survival, duration of response, clinical benefit rate (HR+BC) or disease control rate (TNBC) are among the secondary endpoints. Exploratory biomarkers will also be examined.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-06-05

A randomized phase II study of peri-operative ipilimumab, nivolumab and cryoablation versus standard peri-operative care in women with residual triple negative early stage/resectable breast cancer after standard-of-care neoadjuvant chemotherapy

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**Background:** Triple negative breast cancer (TNBC) is a biologically distinct subtype with high risk of early relapse, particularly for patients who do not achieve a pathological complete response (pCR) after neoadjuvant chemotherapy (NAC), with an event free survival of < 60% at 3 years. Physical disruption of tumors with cryoablation (cryo) induces inflammation and releases antigens that can activate tumor-specific immune responses. In pre-clinical studies, the combination of cryo with checkpoint inhibition augmented tumor-specific immune responses and prevented cancer recurrence. In clinical studies, the combination of pre-operative ipilimumab (ipi)- +/- nivolumab (nivo)-mediated checkpoint blockade with cryo was safely administered in women with operable, early stage breast cancer and generated intra-tumoral and systemic immune responses (NCT01502592, NCT02833233). In this multi-center, randomized study, we evaluate the disease specific impact of peri-operative ipi, nivo and cryo versus standard care in women with residual TNBC after neoadjuvant taxane-based chemotherapy (NCT03546686).

**Methods:** Eligible pts are aged ≥18 years, with ER, PR and HER2 negative operable tumors ≥ 1.0 cm after neoadjuvant taxane-based chemotherapy. Approximately 160 patients will be randomized to one of two arms: standard-of-care breast surgery (control arm) or ipi/nivo/cryo followed by standard-of-care breast surgery (intervention arm). Subjects randomized to the intervention arm will undergo percutaneous, ultrasound- (or MRI-) guided cryoablation with concurrent research core biopsy 7-10 days prior to surgery, and will receive a pre-operative infusion with ipilimumab at the dose of 1mg/kg IV, and nivolumab 240mg flat dose IV (1 to 5 days prior to cryoablation). After surgery, patients will receive three additional doses of nivolumab 240mg flat dose IV Q2 weeks. Adjuvant capecitabine is recommended for all participants and will be administered per standard-of-care at the treating physician's discretion. Patients will be stratified by prior platinum administration, prior anthracycline administration, and clinical nodal status (positive versus negative) at enrollment. The primary endpoint is 3-year Event Free Survival (EFS). Secondary end points include Invasive Disease-Free Survival (IDFS), Distant Disease-Free Survival (DDFS), overall survival (OS) and safety. Exploratory correlative studies will be performed on tumor and serum to characterize the immunologic impact of the intervention and to explore predictors of efficacy and toxicity.

# 2018 San Antonio Breast Cancer Symposium ®

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**DETECT V/CHEVENDO – Comparison of dual HER2-targeted therapy with trastuzumab plus pertuzumab in combination with chemo- or endocrine therapy in addition with CDK4/6 inhibition in patients with HER2-positive and hormone-receptor positive metastatic breast cancer**

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## Background:

Metastatic breast cancer (MBC) is usually an incurable disease and maintenance of quality of life (QoL) is one of the main aims of therapy. In patients with HER2-positive MBC taxane-based chemotherapy in combination with dual HER2 targeted therapy with trastuzumab and pertuzumab is the standard of care. Adverse events are well-known side effects of any cytostatic treatment and can seriously impact the patients' QoL. The synergistic combination of dual HER2-targeted therapy with trastuzumab and pertuzumab plus endocrine therapy might offer a better treatment option for these patients. First clinical trials suggest an additional benefit when a CDK4/6 inhibitor is added to the combination of endocrine therapy and anti HER2 treatment. DETECT V is a randomized phase III study comparing the safety and efficacy of trastuzumab plus pertuzumab and the CDK 4/6 inhibitor ribociclib in combination with either endocrine therapy or chemotherapy.

## Trial design:

Patients with HER2 positive and hormone-receptor positive MBC are 1:1 randomized to receive trastuzumab and pertuzumab combined with endocrine therapy and ribociclib or to chemotherapy with trastuzumab and pertuzumab followed by maintenance therapy with trastuzumab, pertuzumab, endocrine therapy and ribociclib. Chemotherapy and the endocrine agents can be chosen from a variety of available regimens according to the physicians discretion.

## Specific aims:

The primary objective of this study is to compare safety and tolerability in both arms, as assessed by the occurrence of AEs during the treatment period. Secondary endpoints are progression free survival, overall survival, quality-adjusted survival using the quality-adjusted time without symptoms and toxicity (Q-TWiST) method. A translational program is included investigating detection and phenotyping of circulating tumor cells (CTC)-and the assessment of marker expression on CTCs in order to validate an endocrine responsiveness score.

## Present accrual and target accrual:

The DETECT V trial started 2015 in the Dept. of Gynecology, University of Ulm and at the up to 120 sites in Germany. Until June 2018 97 patients with HER2-positive, hormone-receptor positive metastatic breast cancer have been enrolled. A sample size of 270 patients is planned.

## Contact information:

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-07-02

Trastuzumab deruxtecan (DS-8201a) vs investigator's choice of treatment in subjects with HER2-positive, unresectable and/or metastatic breast cancer who previously received T-DM1: A randomized, phase 3 study

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**Background:** There is no uniform standard of care for HER2-positive breast cancer (BC) after disease progression on ado-trastuzumab emtansine (T-DM1). DS-8201a is a novel HER2-targeted antibody-drug conjugate (ADC) with a humanized HER2 antibody attached to a topoisomerase I inhibitor payload by a cleavable peptide-based linker, and with a drug-to-antibody ratio of 7 to 8. It is designed with the goal of improving critical attributes of an ADC. In an ongoing phase 1 trial, DS-8201a showed promising antitumor activity in HER2-positive BC previously treated with T-DM1 (confirmed objective response rate [ORR] of 54.5%; April 2018 data cutoff; Iwata et al, ASCO 2018). Based on preliminary results from the phase 1 trial, DS-8201a received FDA breakthrough therapy and fast track designations for metastatic BC that progressed after prior treatment with T-DM1. The pivotal, phase 2 DESTINY-BREAST01 trial in this population with HER2-positive BC who received prior T-DM1 is ongoing (Baselga et al, ASCO 2018).

**Study Description:** This multicenter, open-label, phase 3 trial will assess the efficacy and safety of DS-8201a in subjects with HER2-positive (IHC 3+ or IHC 2+/ISH+; confirmed by centralized testing) unresectable and/or metastatic BC whose disease progressed on or after T-DM1 (NCT03523585, DESTINY-BREAST02). Approximately 600 subjects will be randomized (2:1) to DS-8201a or investigator's choice of treatment (trastuzumab plus capecitabine or lapatinib plus capecitabine). Randomization is stratified by hormone receptor status, prior pertuzumab treatment, and history of visceral disease. DS-8201a (5.4 mg/kg) will be administered IV once every 3 weeks. Progression free survival (PFS) based on blinded, independent central review using RECIST v1.1 criteria is primary efficacy endpoint; overall survival (OS) is the key secondary endpoint. Other secondary efficacy endpoints are ORR, duration of response, clinical benefit rate, and PFS based on investigator assessment. Safety assessments include serious and treatment-emergent adverse events, physical examinations, vital signs, and clinical laboratory parameters. Health-related quality of life will also be measured. The primary analysis for PFS will occur when approximately 372 PFS events have been observed; providing 90% power to detect a hazard ratio of 0.70 in PFS (a 43% improvement in median PFS from 3.3 months with investigator's choice to 4.7 months with DS-8201a) with a 1-sided alpha of 0.025. An interim OS analysis is planned at the time of the PFS analysis. Final OS analysis will occur when approximately 428 OS events have been observed. Long-term follow-up will continue after the primary analysis every 3 months until death, withdrawal of consent, loss to follow-up, or study closure. Efficacy analyses will include all randomized subjects, and safety analyses will include all randomized subjects who received ≥1 dose of study treatment. The study will enroll subjects from approximately 160 sites including in North and South America, Europe, and Asia. For further information on this trial, contact Fabrice André at FABRICE.ANDRE@gustaveroussy.fr or visit [clinicaltrials.gov](https://clinicaltrials.gov).

# 2018 San Antonio Breast Cancer Symposium ®

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Trastuzumab deruxtecan (DS-8201a) vs ado-trastuzumab emtansine (T-DM1) for subjects with HER2-positive, unresectable and/or metastatic breast cancer who previously received trastuzumab and a taxane: A phase 3, randomized study

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**Background:** Ado-trastuzumab emtansine (T-DM1), a HER2-targeted antibody drug conjugate (ADC), is approved for patients with HER2-positive metastatic breast cancer (BC) after disease progression on a trastuzumab-based regimen. Approval of T-DM1 was based on the EMILIA trial in which T-DM1 demonstrated an objective response rate (ORR) of 43.6%, a median progression-free survival (PFS) of 9.6 months, and an overall survival (OS) of 30.9 months (Verma S, et al. *NEJM*. 2012). DS-8201a is a novel HER2-targeted ADC with a humanized HER2 antibody attached to a topoisomerase I inhibitor payload by a cleavable peptide-based linker, and with a high drug-to-antibody ratio of 7 to 8. In an ongoing phase 1 trial, DS-8201a showed a manageable safety profile and promising antitumor activity in HER2-positive BC previously treated with T-DM1 (confirmed ORR of 54.5%; April 2018 data cutoff) (Iwata et al, ASCO 2018). The pivotal, phase 2 DESTINY-BREAST01 trial in this population with HER2-positive BC who received prior T-DM1 is ongoing (Baselga et al, ASCO 2018).

**Study Description:** This multicenter, open-label, phase 3 trial will assess the efficacy and safety of DS-8201a vs T-DM1 in subjects with HER2-positive (IHC 3+ or IHC 2+/ISH+; confirmed by centralized testing) unresectable and/or metastatic BC previously treated with trastuzumab and a taxane (NCT03529110, DESTINY-BREAST03). Subjects who previously received a HER2-targeted ADC are excluded. Approximately 500 eligible subjects will be randomized (1:1) to receive DS-8201a (5.4 mg/kg) or T-DM1 (3.6 mg/kg) IV once every 3 weeks. Randomization will be stratified by hormone receptor status, prior pertuzumab treatment, and history of visceral disease. For subjects randomized to T-DM1, the treatment will be in accordance with the approved label. The primary efficacy endpoint is PFS based on blinded, independent central review using RECIST v1.1 criteria. Secondary efficacy endpoints include OS, ORR, duration of response, clinical benefit rate, and PFS based on investigator assessment. Safety assessments include serious and treatment-emergent adverse events, physical examinations, vital signs, and clinical laboratory parameters. Health related quality of life will also be measured. The primary analysis for PFS will be performed when approximately 331 PFS events have been observed. This will provide 90% power to detect a hazard ratio of 0.70 for PFS with a 1-sided alpha of 0.025, assuming a median PFS with T-DM1 of 9.6 months and that PFS follows an exponential distribution. Long-term follow-up will continue after the primary analysis every 3 months until death, withdrawal of consent, loss to follow-up, or study closure. Efficacy analyses will include all randomized subjects, and safety analyses will include all randomized subjects who received  $\geq 1$  dose of study treatment. The study will enroll subjects from approximately 150 sites including in North America, Europe, and Asia.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-07-04

A phase 1b study of poziotinib in combination with T-DM1 in women with advanced or metastatic HER2-positive breast cancer

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**Background:** Poziotinib represents a new class of irreversible quinazoline inhibitors of ErbB receptor tyrosine kinases that inhibit the proliferation of tumor cells in culture and in vivo by inhibiting HER-1 (EGFR), HER-2, HER-4. ErbB signaling plays important roles in the progression of HER2+ breast cancer. Poziotinib has promising clinical activity in breast cancer, and other solid tumors including lung, gastric, and colorectal cancers.

Study SPI-POZ-101, is being conducted to evaluate the safety and efficacy of the combination of daily poziotinib and T-DM1, HER2 antibody-drug-conjugate every three weeks in patients with HER2+ advanced or metastatic breast cancer.

**Trial Objectives and Design:** The primary objectives of the study are to determine the maximum tolerated dose (MTD) or maximum administered dose (MAD) of daily poziotinib plus T-DM1 (every 3 weeks) in women with advanced or metastatic HER2 positive breast cancer; and to evaluate the Objective Response Rate (ORR) in these patients.

The secondary objectives include disease control rate (DCR), progression-free-survival (PFS), safety and pharmacokinetics at the MTD/MAD dose level of poziotinib plus T-DM1.

In Part 1, the dose of poziotinib plus standard dose of T-DM1 (3.6 mg/kg IV) on Day 1 of each cycle will be determined using a “3+3” design with up to 3 escalating dose levels, 8, 10 and 12 mg with no DLT or to de-escalate to 6 mg with DLT observed in Cycle 1. Patients in current dose cohort, if not discontinued, will continue treatment until discontinuation of therapy.

In Part 2 of the study, approximately 10 patients will be treated at the MTD/MAD to confirm dose for safety of the combination and to evaluate preliminary efficacy.

**Eligibility Criteria:** The study will enroll female patients between 18 and 90 years with confirmed HER2 overexpression or gene-amplified tumor via immunohistochemistry [IHC] with IHC 3+ or IHC 2+ with confirmatory fluorescence in situ hybridization [FISH]+ or [ISH]+ and must have had at least 2 lines of anti-HER2 directed therapies either in the metastatic or early-stage disease setting. Patients must have adequate hematologic, hepatic, cardiac and renal functions and have at least one measurable lesion per RECIST 1.1 criteria. Exclusion criteria includes unstable CNS metastases or seizure disorder; anticancer chemotherapy, TKIs, biologics, immunotherapy, radiotherapy, or investigational treatment within 15 days; ≥ Grade 2 adverse events; known hypersensitivity to receptor tyrosine kinase inhibitors or any of the components of poziotinib tablets or T-DM1 IV solution.

**Statistical Methods:** Part 1 of the study will enroll 3 to 6 patients at each dose using 3+3 design. Part 2 will enroll 10 patients at the MTD/MAD. The efficacy analysis will be conducted using the Evaluable Population based on RECIST 1.1. The Clopper-Pearson 95% confidence interval will be estimated using exact method based on binomial distribution.

**Target Accrual:** Part 1: 6-18 patients Part 2: 10 patients

**ClinicalTrials.gov Identifier:** NCT03429101

**Contact Information:** Spectrum Pharmaceuticals. SPI-POZ-101@sppirx.com

Poziotinib is currently under clinical investigation and has not been approved for use in breast cancer.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-07-05

A phase III trial to compare eribulin mesylate + trastuzumab (H) + pertuzumab (P) with paclitaxel or docetaxel + HP for HER2-positive advanced or metastatic breast cancer (JBCRG-M06/ EMERALD)

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**Background:** Docetaxel + Trastuzumab (H) + Pertuzumab (P) provided progression-free survival (PFS) and overall survival (OS) benefits in HER2-positive advanced or metastatic breast cancer (AMBC) in the CLEOPATRA study as a first-line therapy. However, long-term administration of docetaxel at a dose of 75 mg/m<sup>2</sup> every 3 weeks in AMBC patients (pts) is difficult due to the toxicities. Eribulin mesylate (E) is a well-tolerated microtubule inhibitor, and we have reported the efficacy and safety of EHP regimen as first- and second-line therapy for AMBC in a multicenter, phase II study (JBCRG-M03/UMIN000012232). In this M06 study, we address the clinical question as to which is the better chemotherapy partner for HP as first line regimen, in terms of efficacy, toxicity and QOL.

**Methods:** JBCRG-M06 is a multicenter open-label randomized phase III study for HER2-positive AMBC pts who have received no prior chemotherapy except for the HER2- Antibody-Drug Conjugate (ADC). Pts will be randomized 1:1 to E (1.4mg/m<sup>2</sup> on day 1 and 8) + H (8 mg/kg loading dose followed by 6 mg/kg) +P (840 mg loading dose followed by 420 mg) q3wks or standard taxanes (docetaxel 75mg/m<sup>2</sup> on day1 or paclitaxel 80mg/m<sup>2</sup> on day 1, 8 and 15) + HP q3wks. Stratification factors for randomization are; presence of visceral metastases, number of prior taxanes on perioperative adjuvant treatment, and treatment with prior anti-HER2-ADC. Primary endpoint is PFS and secondary endpoints include overall response rate, duration of response, OS, patient-reported outcomes (PRO) relating to QOL and peripheral neuropathy, new-metastases free survival, and safety. Translational research to search for biomarker for individual precision therapy will be performed. Main eligibility criteria are as follows: pts with HER2-positive AMBC, female aged 20-70 years old, ECOG PS of 0-1, LVEF ≥ 50% at baseline and adequate organ function. Pts who had progressive MBC within 6 months after the end of primary adjuvant systemic chemotherapy are excluded. The sample size was calculated by type1 error (2-sided) of 0.05 and 80% power to estimate the noninferiority margin 1.33 with an expected median PFS of 14.2 months. The target number of pts is 480 recruited over the duration of 3-years. The first patient in was achieved on August 2017. (ClinicalTrials.gov Identifier:NCT03264547).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-07-06

Antibody-coupled T cell receptor (ACTR) engineered autologous T cells in combination with trastuzumab for the treatment of HER2-positive malignancies

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Antibody-Coupled T cell Receptor (ACTR) is an autologous engineered T cell therapy developed to combine with tumor-targeting antibodies to exert potent anti-tumor immune responses and tumor cell killing. The ACTR construct is composed of the extracellular domain of CD16 fused to CD3ζ signaling and T cell co-stimulatory domains. ACTR-expressing T cells are universal in that they can be paired with a therapeutic antibody to target specific antigens on tumors. Unum has two ACTR constructs, ACTR087 and ACTR707, currently in clinical testing. ACTR087 and ACTR707 are being tested in combination with rituximab in subjects with CD20+ B cell lymphoma in two separate trials (NCT02776813 and NCT03189836, respectively). Preliminary data with ACTR087 + rituximab has demonstrated clinical proof-of-concept and a dose-response relationship in subjects with relapsed/refractory B cell lymphoma. ACTR087 is also being tested in combination with a novel BCMA-targeting antibody in subjects with multiple myeloma (NCT03266692).

While T cell therapies, such as chimeric antigen receptor (CAR) T cells, have demonstrated clinical activity in hematological cancers, the therapeutic potential of this approach has yet to be established in solid tumors. Challenges associated with targeting solid tumors with CAR-T cells include tumor antigen heterogeneity and antigen expression on normal tissues. HER2 is a well-established therapeutic target that is over-expressed in a number of cancer indications. HER2 is also expressed at low levels on normal epithelial cells, creating a risk for on-target/off-tumor toxicities of HER2-targeted CAR-T cells. Here we present nonclinical studies demonstrating that ACTR T cells in combination with trastuzumab have antigen density-dependent activity on HER2-expressing tumor cell lines, while trastuzumab-based CAR-T cells do not. We observed that ACTR + trastuzumab had robust activity against HER2-amplified tumor cells and more modest activity against non-amplified tumor cells, whereas HER2-targeting CAR-T cells had comparable activity against HER2-amplified and non-amplified tumor cells. On normal human primary cells, ACTR + trastuzumab had minimal activity in comparison to HER2 CAR-T cells, suggesting that ACTR + trastuzumab may exhibit a superior clinical therapeutic index. Furthermore, the activity of ACTR T cells against HER2-amplified tumor cells was titratable with antibody concentration, allowing for control of ACTR activity by modulation of trastuzumab concentration. Together, these data demonstrate the specificity of the ACTR T cell therapeutic approach to target HER2-amplified tumors and support clinical testing in combination with trastuzumab.

A phase 1, multicenter, single-arm, open-label dose escalation study, ATTCK-34-01, is proposed to evaluate ACTR T cells in combination with trastuzumab in subjects with advanced HER2-positive malignancies. The primary study objectives are to assess the safety and tolerability of the combination, and to define the recommended phase 2 dose combination for further study. Additional objectives include assessment of anti-tumor activity, ACTR T cell persistence and trastuzumab pharmacokinetics. Enrollment is expected to commence in early 2019.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-07-07

Image-guided de-escalation of neoadjuvant chemotherapy in HER2-positive breast cancer: The TRAIN-3 study

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## **Background**

The addition of pertuzumab to trastuzumab containing chemotherapy has boosted pathologic complete response (pCR) rates after neoadjuvant chemotherapy for HER2-positive breast cancer. PCR rates over 80% have been described and achieving a pCR is associated with a favorable long-term outcome. In addition, achieving a radiologic complete response (rCR) is predictive of the pathologic response in HER2-positive tumors. Therefore it is hypothesized that image-guided evaluation based on the early occurrence of rCR can be used to tailor the number of chemotherapy cycles.

## **Trial design**

This is a single arm, multicenter study evaluating the efficacy of image-guided de-escalation of neoadjuvant treatment with paclitaxel, Herceptin®, carboplatin, and pertuzumab (PTC-ptz). Radiologic evaluation with contrast-enhanced breast MRI and ultrasound of the axilla (in cN+ patients) is performed at baseline and after 3, 6, and 9 cycles of treatment. In case of rCR of the breast (and axilla) after 3 or 6 cycles, early surgery will be performed. If residual tumor is present after 3 and 6 cycles, patients will continue the PTC-ptz regimen to complete a total of 9 cycles. All patients will receive adjuvant Herceptin® and pertuzumab to complete 1 year of anti-HER2 blockade and endocrine treatment according to local guidelines if HR-positive. The study will be performed in the Netherlands in approximately 35 centers.

## **Eligibility criteria**

Eligible patients have histologically proven stage II/III HER2-positive primary breast cancer with known hormone-receptor status. Patients must have a measurable breast tumor on baseline MRI and can be either node negative or node positive.

## **Specific aims**

The aim is to evaluate the efficacy of image-guided de-escalation of neoadjuvant chemotherapy in HER2-positive breast cancer on event-free survival (EFS) at 3 years as primary endpoint. Secondary endpoints are overall survival, rCR, concordance between rCR and pCR (ypT0/is, ypN0), differences in EFS and OS following pCR between patients who received 3, 6, or 9 cycles, and toxicity.

## **Statistical methods**

This is a single-arm, two stage study with one interim-analysis and a final analysis. Statistics will be performed for each hormone receptor subgroup separately. Stopping rules are based on 3-year EFS-rates described in literature (88% for HR-negative tumors and 90% for HR-positive tumors) and calculated using the exact conditional Poisson distribution. The study is successful with  $\leq 34$  EFS-events in the HR-negative subgroup and  $\leq 38$  events in the HR-positive subgroup after 700 patient-years of follow-up. The three-year EFS-estimate will be calculated using Kaplan-Meier statistics.

## **Present accrual and target accrual**

Target accrual is 231 patients for the HR-negative group and 231 patients for the HR-positive group. Present accrual will follow.

## **Funding**

Investigator initiated trial sponsored by the Dutch Breast Cancer Research Group (BOOG), funded by Roche.

## **Contact information for people with a specific interest in the trial**

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-07-09

A phase Ib study of neratinib, pertuzumab, and trastuzumab with paclitaxel in patients with metastatic and locally advanced breast cancer

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**Background:** Neratinib, a potent irreversible pan-ErbB tyrosine kinase inhibitor that blocks signal transduction through HER1, HER2, and HER4, has demonstrated activity against metastatic HER2-positive breast cancer (HER2+ BC) in patients pretreated with trastuzumab. The FDA recently approved neratinib as an adjuvant treatment for HER2+ BC patients who have received trastuzumab for at least 1 year. Preclinical data demonstrate that trastuzumab-resistant BC cells remain sensitive to neratinib. Also, neratinib enhances responses to trastuzumab possibly by increasing trastuzumab's internalization, immune-mediated action, and other mechanisms. Taken together, these findings provide the rationale for adding neratinib to the standard of care combination of trastuzumab and pertuzumab with paclitaxel to enhance anti-HER2 efficacy in advanced HER2+ BC. Here, we report on the phase Ib portion of an ongoing phase Ib/II trial of this drug combination.

**Trial Design:** Patients with metastatic or locally advanced HER2+ BC will be enrolled in the phase Ib portion of the trial. Neratinib is given orally in 3-week cycles. The initial neratinib dose of 80 mg daily is increased to 120, 160, and 200 mg daily after safety assessments of each dose level. Other agents are administered as per the standard of care. Patients continue therapy with per-protocol dose escalation and de-escalation according to toxicity until the maximum tolerated dose (MTD) of neratinib is reached. The target maximum dose-limiting toxicity rate is 20%. All patients receive 4 cycles of the combination therapy. If patients do not have disease progression or excessive toxicity, they may receive 2-4 additional cycles at the treating physician's discretion. During therapy, patients undergo blood tests every week and have clinical visits and restaging scans every 3 weeks. Because gastrointestinal toxicity, mainly diarrhea, is anticipated, patients receive prophylactic antidiarrheal medication (e.g., loperamide, budesonide) beginning with the first dose of neratinib.

**Eligibility Criteria:** Eligible patients must have histologically confirmed metastatic or locally advanced HER2+ BC (BC may be inflammatory or non-inflammatory and have any hormone receptor status); an ECOG performance status score of 0 or 1; and adequate hematologic and organ function, including adequate cardiac function (as indicated by a left ventricle ejection fraction of  $\geq 50\%$ ).

**Specific Aims:**

- 1- To determine the MTD of neratinib in combination with paclitaxel, pertuzumab, and trastuzumab.
- 2- Pharmacodynamic markers will be measured on biologic specimens. Neratinib-induced changes in pEGFR and/or HER2 expression will be analyzed and compared between dose levels.

**Statistical Methods:** The Bayesian modified toxicity probability interval is used to determine dose adjustment.

**Accrual:** The target enrollment for the phase Ib cohort is 20 patients. The trial has enrolled 3 patients since its activation in January 2018. This trial is supported by Puma Biotechnology, Aggressive Breast Cancer Research Program Grant.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-08-01

Personalized breast cancer screening in a population based study: Women Informed to Screen Depending On Measures of risk (WISDOM)

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**Background:** WISDOM is a 100,000 healthy women preference-tolerant, pragmatic study comparing annual to personalized risk-based breast screening. The novelty of WISDOM personalized screening is the integration of previously validated genetic and clinical risk factors (age, family history, breast biopsy results, ethnicity, mammographic density) into a single risk assessment model that directs the starting age, timing, and frequency of screening. The goal of WISDOM is to determine if personalized screening, compared to annual screening, is as safe, less morbid, enables prevention, and is preferred by women. The study is registered on ClinicalTrials.gov, NCT02620852.

**Methods:** Women aged 40-74 years with no history of breast cancer or DCIS, and no previous double mastectomy can join the study online at [wisdomstudy.org](http://wisdomstudy.org). Participants can elect randomization or self-select a study arm, and provide electronic consent and Release for Medical Information using DocuSign. For all participants, 5-year risk of developing breast cancer is calculated according to the Breast Cancer Screening Consortium (BCSC) model. Participants in the personalized arm undergo panel-based mutation testing, and their 5-year risk is calculated using the BCSC score combined with a Polygenic Risk Score (BCSC-PRS) that includes 75 single nucleotide polymorphisms (SNPs, increase to 229) known to increase breast cancer risk. SNPs and mutations (BRCA1, BRCA2, TP53, PTEN, STK11, CDH1, ATM, PALB2, and CHEK2) are assessed by saliva-based testing through Color Genomics. 5-year risk level thresholds are used to stratify for low-, moderate- and high risk. Risk stratification determines age to start, stop, and frequency of screening.

**Enrollment:** As of July 2018, the WISDOM study is open to all eligible women in California, North Dakota, South Dakota, Minnesota and Iowa. To date, 23,329 eligible women have registered and 14,393 women have consented to participate in the trial. We analyzed 3,255 participants who have completed risk assessment in the personalized arm. The median age was 56 years. 82% were Caucasian, 1% African-American, and 6% Asian. 9% self-reported as Hispanic. We are partnering with health insurers and self-insured companies using coverage with evidence progression. To strengthen generalizability, we are expanding to other states. WISDOM enrollment will continue past 2019.

**Feasibility:** To evaluate the addition of PRS, we used paired statistical tests (McNemar) to compare the distributions of BCSC, and BCSC-PRS risk estimates around low-risk (<1.3%), and very-high risk (>6%) thresholds, the latter corresponding to 5-year risk of a BRCA mutation carrier. The median 5-year risk was 1.5% (IQR 1.0-2.1%) using the BCSC model, and 1.4% (IQR 0.8-2.5%) using the BCSC-PRS model. The BCSC-PRS model classified more women into the low (<1%) and very high (≥6%) risk categories compared to the BCSC model (p < 0.001).

**Conclusions:** Our findings demonstrate that incorporating genetic variants into a validated clinical model is feasible and impacts risk classification compared to a model without genetic risk factors. Results at 5 years will reveal if this classification improves healthcare value by reducing screen volumes and costs without jeopardizing outcomes.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-09-01

Pilot study of denosumab in *BRCA1/2* mutation carriers scheduling for risk-reducing salpingo-oophorectomy

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**Background:** Denosumab is a monoclonal antibody that inhibits RANKL and is approved for the prevention of fractures in patients with osteoporosis or bone metastases. The RANKL signaling pathway is also involved in *BRCA1*-associated mammary tumorigenesis via a progesterone-induced paracrine effect of RANKL on luminal progenitor cells. Pre-clinical studies have demonstrated that RANKL inhibition resulted in reduced proliferation of mammary tumors. Early findings from an ongoing pre-surgical study demonstrated that denosumab treatment resulted in decreased Ki67 proliferation index in benign breast tissue. Based on these data, denosumab is being pursued as a potential preventive agent for breast cancer in *BRCA1* mutation carriers. While promising, the effect of RANKL inhibition on gynecologic tissues such as the ovaries and fallopian tubes, in which progesterone has a protective effect, is unknown.

**Trial design:** We will conduct a multicenter, open-label randomized pilot study of presurgical administration of denosumab versus no treatment in premenopausal women with *BRCA1/2* mutations undergoing risk-reducing salpingo-oophorectomy (RRSO). A total of 60 women will be randomized 1:1 to Arm 1) 3-4 doses of 120 mg denosumab subcutaneously every 4 weeks or Arm 2) No treatment. Participants will be stratified by 1) *BRCA1* versus *BRCA2* mutation status and 2) Use of hormonal contraceptives within the past 3 months (yes/no). Assuming a 10% unevaluable rate, we expect to have 54 evaluable participants (27 per arm).

**Eligibility criteria:** 1) Premenopausal women (defined as < 3 months since last menstrual period OR serum follicle-stimulating hormone (FSH) < 20 mIU/mL), age ≥ 18 years; 2) Documented germline pathogenic mutation or likely pathogenic variant in the *BRCA1* or *BRCA2* gene; 3) Plan for RRSO with or without hysterectomy; 4) ECOG performance status ≤ 1 (Karnofsky ≥ 70%); 5) Normal organ and marrow function; 6) Negative pregnancy test and use of adequate contraception; 7) Willingness to take supplemental oral calcium and vitamin D3; 8) Dental examination within 6 months of enrollment and no evidence of active dental issues; 9) Ability to understand and willingness to provide informed consent.

**Specific aims:** Our primary objective is to compare the effect of denosumab to no treatment on Ki67 expression in the fimbrial end of the fallopian tube. Secondary objectives are to assess Ki67 in ovary and endometrium; cleaved caspase-3, RANK/RANKL, ER/PR, CD44, and STAT3/pSTAT3 expression in fallopian tube, ovary, and endometrium; gene expression profiling in the fallopian tube and ovary; serum markers (progesterone, estradiol, C-terminal telopeptide) and denosumab levels; and toxicity.

**Statistical methods:** The primary endpoint is post-treatment Ki67 expression in the fimbrial end of the fallopian tube in the denosumab arm compared to the no treatment arm. Assuming a standard deviation of 5.0%, we will have 82% power to detect a 4.0% absolute difference (or effect size of 0.8) in Ki67 proliferation index between the denosumab and no treatment groups by applying a 2-sample t-test at a 0.05 significance level.

**Target accrual:** 60 participants, to be activated in Summer 2018.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT2-09-02

A phase I dose escalation study of topical bexarotene in women at high risk for breast cancer

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**Background:** Breast cancer prevention with anti-estrogens, including tamoxifen, raloxifene, and exemestane, has been shown to reduce the incidence of hormone receptor-positive breast cancer. However, agents that can reduce the incidence of hormone receptor negative breast cancer are currently lacking. Rexinoids such as bexarotene are vitamin A analogues that have been shown to be involved in cell differentiation, growth, and apoptosis. In preclinical mouse models that develop ER-negative breast cancers, bexarotene showed a significant reduction in mammary tumor development. Oral bexarotene has been evaluated in BRCA mutation carriers and significant decreases in cyclin D1 were noted in breast cells suggesting biological activity of bexarotene on breast tissue. Systemic side effects of hyperlipidemia and hypothyroidism were also found. Data from chemoprevention studies with topical 4-hydroxytamoxifen support the concept of topical agents penetrating into the breast tissue and exhibiting biological activity in the tissue. We hypothesize that topical bexarotene can be applied to the breast as a chemoprevention agent with penetration to the breast tissue without subsequent systemic side effects and toxicity as seen with oral bexarotene.

**Trial Design:** Women at high risk for breast cancer will be recruited and assigned to one of three different dose levels: 10mg (1ml) every other day, 10mg (1ml) daily, 20mg (2ml) daily to one unaffected breast for 4 weeks. The primary endpoint of the study is to determine the recommended phase II dose of topical bexarotene 1% gel for evaluation in healthy at-risk women. Dose Limiting Toxicity (DLT) is defined as a grade 2 skin adverse event that persists for at least 6 days or any grade 3 or greater adverse event related to the study drug. A grade 2 skin adverse event that recurs and persists for at least 3 days is also a DLT. The Maximum Tolerated Dose (MTD) will be defined as the highest dose level at which no more than 2 participants experience a DLT among 10 participants treated. A conservative modification of the standard "3+3" design will be applied. The first three participants will be assigned to the lowest dose level. New cohorts of 3-4 participants will not be treated until toxicity has been fully evaluated for all current participants through 4 weeks. Once the MTD has been determined, an expansion cohort of an additional 10 patients will be recruited at the MTD to further evaluate safety and toxicity at this dose level as well bexarotene concentration in the breast tissue. Secondary endpoints include serum bexarotene level, tissue bexarotene levels, and changes in thyroid function tests, lipid profile, and calcium. The planned accrual for this study if maximally accrued to all dose levels and the dose expansion cohort will be 40 participants.

# 2018 San Antonio Breast Cancer Symposium ®

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Treatment burden and capacity to manage care among patients with breast cancer

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Patients with breast cancer spend significant time<sup>1</sup>, effort, and financial resources<sup>2</sup> to combat the disease for years after their diagnosis. The large volume of healthcare tasks can cause patients to become overburdened, leading to reduced adherence with care plans and worse outcomes<sup>3</sup>. On the other hand, certain patient characteristics such as physical resilience, financial well-being, and supportive family environments increase patients' capacity to manage care<sup>4</sup>. Assessing treatment burden and capacity when prescribing care has been applied to populations such as diabetes patients<sup>5</sup>. We are investigating this paradigm in treatment of patients with breast cancer. The goal of this preliminary study is to identify significant factors that contribute to treatment burden, capacity to manage care, and outcomes of overburden for patients with breast cancer.

Examples of treatment burden, capacity to manage care, and outcomes of overburden in patients with breast cancer

Treatment burden	Capacity to manage care	Outcomes of overburden
Traveling long distances for care	Access to reliable transportation	Reduced spending on food, utilities, or other necessities
Paying for child care during chemotherapy	Flexibility in informal caregivers' schedules	Missed appointment with medical oncologist
Remembering to take medications with meals	Medical understanding or knowledge	Worse than expected side effects
Reporting adverse events	Proficiency with mobile device	Trip to emergency room

Through literature review, interviews with survivors, and expert panels of navigators and providers, we will develop a survey instrument given to patients at the time of diagnosis. The survey will assess patient capacity and help providers give treatment options based on attributes of the patient. Additionally, we will attempt to correlate survey results with treatment burden measures derived from electronic health record data at a population level<sup>1</sup>. With treatment personalized for patient capacity, patients should be better able to adhere to care plans leading to improved quality of life during treatment and beyond.

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# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-01-01

**BEGONIA:** Phase Ib/II open-label, platform study of safety and efficacy of durvalumab, paclitaxel and other novel oncology therapy agents as first-line (1L) therapy in patients with metastatic triple negative breast cancer (mTNBC)

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**Background:** Immuno-oncology therapies have shown durable clinical responses in a subset of patients with mTNBC. Combination therapy with checkpoint inhibition and chemotherapy is under investigation; preliminary research showed improved objective response rate (ORR) with combination therapy versus chemotherapy alone.<sup>1</sup> Durvalumab is a selective, high-affinity, engineered, human monoclonal antibody that blocks programmed cell death-ligand 1 (PD-L1) binding to programmed cell death 1 (PD-1) and CD80 allowing T cells to recognize and kill tumor cells. This study is designed to assess the efficacy and safety of durvalumab + paclitaxel as 1L treatment in patients with mTNBC. Additionally, this study will also evaluate potential novel triplet treatment regimens of durvalumab + paclitaxel in combination with immune-modulating agents, selumetinib (ARRY-142886; AZD6244, an inhibitor of mitogen activated protein kinase/extracellular signal regulated kinase [MAPK/ERK]), danvatirsen (AZD9150, an antisense oligonucleotide designed to down-regulate expression of signal transducer and activator of transcription 3 protein), oleclumab (MEDI9447, an anti-CD73 monoclonal antibody), and capivasertib (AZD5363, a highly selective, oral, small molecule AKT inhibitor) that may provide further benefit to patients with mTNBC.

**Methods:** BEGONIA is a phase Ib/II, open-label, multicenter, platform study (EudraCT No: 2018-000764-29) consisting of 2 parts: Part 1 is a phase Ib study planned to be conducted in approximately 100 patients (20 per arm) to assess the safety and tolerability of durvalumab (1500 mg intravenous [IV], q4w) + paclitaxel (90 mg/m<sup>2</sup> IV, 4 week cycle, 3 weeks once weekly [days 1, 8, 15], 1 week off) (arm 1); and durvalumab + paclitaxel in combination with selumetinib (arm 2), danvatirsen (arm 3), oleclumab (arm 4) and capivasertib (arm 5) until disease progression. Dosing of the immune-modulating agents will be based on the previously defined recommended phase 2 doses of the component doublets (where available) in combination with durvalumab + paclitaxel using a rolling 6-patient design to evaluate for toxicity. Part 2 is a phase II study planned to be conducted in approximately 150 to 225 patients to evaluate efficacy of up to 2 best triplet combination arms based on their safety and efficacy outcomes in Part 1. The primary objective of Part 1 is safety and of Part 2 is efficacy (primary endpoint: progression free survival [PFS]); additionally, efficacy will be assessed in both parts, including overall survival, ORR, PFS, and duration of response (RECIST 1.1). Immunotherapy naïve adult patients (≥18 years) with locally assessed and confirmed TNBC, ECOG PS 0 or 1, stage IV breast adenocarcinoma and no prior systemic treatment for metastatic disease will be enrolled.

<sup>1</sup>Adams et al., J Clin Oncol 2016;34(Suppl):abstr 1009

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-01-02

**PARTNERING / PARTNER :** Phase II sub-study to establish if the addition of combinations of new agents (olaparib, cell cycle and immune checkpoint inhibitors) can improve the rate of pathological complete response (pCR) and minimal residual disease (MRD) in triple negative breast cancer (TNBC) and / or germline BRCA mutated (gBRCAm) patients with evidence of residual disease after PARTNER therapy

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## **Background:**

In patients with TNBC, following standard neoadjuvant chemotherapy, residual disease (RD) is correlated with poor prognosis and 50% relapse within 5 years [1]. PARTNER is a neoadjuvant clinical trial which randomises TNBC and gBRCAm patients to carboplatin and paclitaxel +/- olaparib followed by anthracycline-based chemotherapy. Patients with RD after neoadjuvant treatment in this trial also face poorer survival outcomes, due to the paucity of treatment options. PARTNERING, develops a new strategy using novel agent combinations as an alternative pathway for patients with RD within the PARTNER trial.

**Methods:** PARTNERING is a phase II open label, sub-study with a two-stage Simon design with biomarker guided treatment cohorts open only to patients in the PARTNER trial. A maximum of 15 patients will be included in each cohort. Patients with RD > 10% tumour cellularity (TC) on biopsy after neoadjuvant therapy will be eligible. Patients who have no tumour cells or < 10% TC, and those with progressive disease will be excluded. Allocation of patients into the cohorts will be based on tumour infiltrating lymphocytes (TILs) expression either on diagnostic or post treatment biopsy. Patients with tumours with TILs score ≤20% are considered “non-immunogenic” They will be stratified according to HRD status and allocated to receive a cell cycle checkpoint inhibitor + olaparib. Patients with a TILs score >20% are considered “immunogenic” and will be allocated to receive an immune checkpoint inhibitor with olaparib or a cell cycle checkpoint inhibitor.

Primary outcome measure is pCR / MRD rate at surgery after the administration of 2 cycles / 8 weeks of a combination of new agents. The rate of conversion to pCR/MRD will be correlated with TC, TILs, BRCA and homologous recombination deficiency (HRD) status, Ki67% and previous olaparib treatment.

**Progress:** The PARTNERING pathway in the PARTNER trial will be open late 2018.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-02-01

**RIBANNA** — Real-world evidence of ribociclib plus aromatase inhibitor, or endocrine monotherapy, or chemotherapy as first-line therapy for postmenopausal women with HR+, HER2– advanced breast cancer (aBC)

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**Background:** In the pivotal phase 3 MONALEESA-2 trial, ribociclib, a highly selective oral CDK4/6 inhibitor in combination with letrozole significantly prolonged progression-free survival (PFS) compared to letrozole alone. In the year 2017, based on the results of MONALEESA-2, ribociclib in combination with an aromatase inhibitor (AI) was approved for the treatment of HR+, HER2– aBC in postmenopausal women with no prior therapy for their advanced disease (first-line treatment). Beyond phase 3 trials, further data for ribociclib are now becoming available from the phase 3b trials, RIBECCA and COMPLEMENT-1, involving approximately 500 and 3000 patients with aBC, respectively. However, real-world evidence for the effectiveness, safety, and tolerability of ribociclib + AI in the routine clinical practice is needed to further support the use of this combination in the first-line therapy.

**Methods:** RIBANNA is a non-interventional study, which started in October 2017 and plans to enroll 3020 patients across ~400 sites in Germany. Postmenopausal women diagnosed with aBC will be enrolled into 3 cohorts (cohort 1: ribociclib + AI; cohort 2: endocrine monotherapy; and cohort 3: chemotherapy). Across all cohorts, patients will be treated in accordance with the respective German-prescribing guidelines. Data related to efficacy (with PFS as the primary efficacy criterion), safety, tolerability, duration of therapy, and impact on quality of life (QoL) will be collected. QoL will be assessed using the validated patient questionnaires. This study was especially designed to analyze the patient data from sequential lines of therapy in all three cohorts over a period of up to 7 years. For this purpose, RIBANNA will collect the data on each line of treatment and the reason for changing treatment in all 3 cohorts. The same accounts for QoL, which will also be assessed periodically, regardless of disease progression and initiation of subsequent therapies.

RIBANNA is the first study to provide the real-world evidence regarding treatment of HR+, HER2– aBC with the CDK4/6 inhibitor, ribociclib. This study includes 3 treatment cohorts (ribociclib + AI, endocrine monotherapy, and chemotherapy) with subsequent treatment algorithms and assessment of QoL over the entire study period.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-02-02

**PATHWAY:** Asian, multicenter, phase 3 trial of tamoxifen with or without palbociclib ± goserelin in women with hormone receptor-positive, HER2-negative advanced or metastatic breast cancer

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## **BACKGROUND:**

The incidence rates of breast cancer (BC) in Asian countries have been rising rapidly. The age-specific female BC incidence rates peak before menopause (around 40-50 years of age) in Asia, however treatment options for pre/perimenopausal patients are limited. Palbociclib (P) is an oral novel cyclin-dependent kinase 4/6 (CDK4/6) inhibitor. The addition of P to endocrine therapy (ET) such as aromatase inhibitor or fulvestrant has been demonstrated improved progression-free survival (PFS) in phase 3 studies PALOMA-2 and PALOMA-3. This study is designed to evaluate efficacy and safety of P plus tamoxifen (TAM) in patients with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced or metastatic BC regardless of menopausal status. This study is conducted as a Clinical Research Collaboration by National Cancer Center Hospital with research funding from Pfizer.

## **TRIAL DESIGN:**

PATHWAY/NCCH1607 is a double-blind, placebo-controlled, randomized, phase 3 study. Patients will be randomized 1:1 to receive either P (125 mg once daily, days 1-21 of a 28-day cycle) or placebo in combination with TAM (20 mg once daily, continuously). Pre/perimenopausal women should receive concurrent ovarian function suppression with goserelin. Randomization will be stratified by prior ET for advanced/metastatic BC (1st line ET vs. 2nd line ET) and menopausal status (pre/perimenopausal vs. postmenopausal).

## **KEY ELIGIBILITY CRITERIA:**

Eligible patients include women of any menopausal status with HR-positive, HER2-negative advanced or metastatic BC; candidates to receive TAM as 1st line or 2nd line ET for advanced/metastatic disease; ≥18 years of age; measurable or non-measurable disease (RECIST v.1.1); ECOG performance status 0-1; adequate organ function; have not received treatment with TAM (except for patients who have had more than 12 months from completion of adjuvant therapy with TAM); and have not received any CDK4/6 or phosphoinositide 3-kinase (PI3K) - mammalian target of rapamycin (mTOR) inhibitors.

## **SPECIFIC AIMS:**

The primary endpoint is PFS as assessed by the investigator. Secondary endpoints include overall survival (OS), 1, 2, and 3-year survival probabilities, objective response (OR), duration of response, clinical benefit rate (CBR), pharmacokinetics, safety, and patient-reported outcomes.

## **STATISTICAL METHODS:**

The sample size was determined to detect a 38% reduction in the hazard of disease progression or death in P plus TAM arm with a 1-sided significance level of 2.5% and power of 80%. A stratified log rank test will be used to compare PFS between the 2 treatment arms.

## **PRESENT ACCRUAL AND TARGET ACCRUAL:**

Target accrual of 180 patients will be enrolled within 23 sites among Japan, Korea, Taiwan, and Singapore. As of June 2018, 46 patients have been enrolled.

## **CONTACT INFORMATION:**

This trial is registered at ClinicalTrials.gov NCT03423199 and UMIN000030816. For more information, email NCCH1607\_office@ml.res.ncc.go.jp

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-02-03

IMMUNe mOdulation in early stage estrogen receptor positive breast cancer treated with neoADjuvant Avelumab, Palbociclib, and Tamoxifen: The ImmunoADAPT study (NCT03573648)

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## **Background:**

While some patients with early stage endocrine receptor positive (ER+) breast cancer experience excellent prognosis, a subset of patients with more aggressive phenotypes still have a high rate of recurrence despite optimal adjuvant endocrine therapy and chemotherapy, thus novel therapies are needed for patients with high risk disease.

Although immune checkpoint blockade has shown significant benefit in numerous types of cancer, initial reports demonstrate low response rates to single agent programmed cell death ligand 1 (PD-L1) inhibition in ER+ breast cancer. Inhibitors of cyclin dependent kinases (CDK) 4 and 6 in combination with endocrine therapy are highly active in breast cancer, and recently have been demonstrated to recruit immune cells, and increase PD-L1 on tumor cells in preclinical models. Increased tumor infiltrating lymphocytes (TILs) has been observed with neoadjuvant treatment with CDK4/6 inhibitors in patients with ER+ breast cancer. We thus hypothesize that the addition of palbociclib (CDK4/6 inhibitor) will improve responses to avelumab (PD-L1) inhibitor in patients with high risk ER+ breast cancer.

## **Trial Design:**

Eligible participants are those stage II or III ER+HER2- breast cancer (T2N0 must have  $\geq$  grade 2, T1N+ must have at least a 1.5cm breast primary). Patients will undergo a baseline MRI and biopsy, start tamoxifen +/- palbociclib for 1 cycle (1 cycle =28 days), and then undergo a repeat MRI and biopsy. Avelumab will be added to both arms in cycle 2. Patients will be treated for 3 cycles of avelumab with tamoxifen +/- palbociclib (thus 4 cycles total, including run-in without avelumab). Patients will be treated as long as there is no evidence of progression and therapy is tolerated, and then undergo MRI and surgery. The primary objective is to determine the clinical complete response (cCR) rate by MRI. Secondary objectives include evaluation of TILs (H&E), CD8 and FOXP3 by immunohistochemistry (IHC), T cell receptor (TCR) repertoire (TCR sequencing), multiplex gene expression panel (Nanostring), and multiplex IHC. Changes in these immune biomarkers will be assessed to determine differential immunophenotypic effects of palbociclib, and correlated to cCR in each arm.

The sample size of this pilot study is determined by primary analysis on the cCR rate. We hypothesize that the addition of palbociclib to tamoxifen will result in an increase rate of cCR in patients receiving avelumab. We hypothesize that the addition of avelumab will increase the response rate to palbociclib and tamoxifen by 30%. We thus estimate that a total of 40 evaluable patients (20 to each arm) will provide close to 80% power to detect a difference on cCR rates of 10% vs 40% at two-sided alpha level 10%. We will evaluate and compare cCR rates between arms by conducting Fisher's Exact test and reporting the estimated proportions together with their exact confidence intervals. Logistic regression analysis will also be conducted to explore the association between cCR and immune biomarkers.

This study has received IRB approval and is open as of Summer 2018.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-02-04

Selecting the optimal position of CDK4/6 inhibitors in hormone-receptor-positive advanced breast cancer: The BOOG 2017-03 SONIA study

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## BACKGROUND

Combining cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors with endocrine therapy (ET) is an effective strategy to improve progression-free survival (PFS) in hormone receptor-positive (HR+), human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer (ABC). There is a lack of comparative data to help clinicians decide whether CDK4/6 inhibitors can best be added to first- or second-line ET. The former strategy may provide longer PFS benefit, but is associated with longer use of the drug, which results in more toxicity and costs, whereas no clear benefit on overall survival (OS) or quality of life (QoL) has been proven thus far. No predictive biomarker exists to select patients who are most likely to benefit from the addition of CDK4/6 inhibition.

## TRIAL DESIGN AND AIMS

The SONIA study is an investigator-initiated, multicenter, randomized phase III study, funded by 'ZonMw' and 'Zorgverzekeraars Nederland'. Patients are randomly assigned to receive either strategy A (first-line treatment with a non-steroidal aromatase inhibitor (NSAI) + CDK4/6 inhibition, followed on progression by fulvestrant) or strategy B (first-line treatment with NSAI, followed on progression by fulvestrant + CDK4/6 inhibition). Each CDK4/6 inhibitor can be used according to its approved EMA label. The primary objective is to test whether strategy A is superior to strategy B. The primary endpoint is time from randomization to second objective progression (PFS2). Secondary endpoints include OS, safety, QoL, and cost-effectiveness. Additional biomarker analyses will be performed to optimize patient selection.

## ELIGIBILITY CRITERIA

Patients with a proven diagnosis of HR+/HER2-negative advanced breast cancer without prior systemic therapy for advanced disease who are candidates to receive NSAIs as first-line treatment, are eligible for the study. Exclusion criteria include advanced visceral spread with the risk of life-threatening complications in the short term. Other conditions excluding a patient from participating are other malignancies, prolonged QTc time (>480ms) or any other medical condition that interferes with study procedures or compliance.

## STATISTICAL METHODS

The difference in PFS2 will be estimated using the intention-to-treat population in a Cox proportional hazards model accounting for all stratification factors (visceral versus non-visceral disease, yes versus no prior ET in (neo)adjuvant setting, hospital, and type of CDK4/6 inhibitor). Five-hundred seventy-four primary outcome events yield 89% power to show that strategy A has statistically significant, clinically meaningful (according to European Society for Medical Oncology - Magnitude of Clinical Benefit Scale) superior PFS2 in a log-rank test at the two-sided 95% confidence level.

## ACCRUAL

**TARGET:** with an accrual period of 42 months and an additional 18 months follow-up, inclusion of 1050 evaluable patients is required. A total of 76 Dutch hospitals will participate.

**PRESENT:** the study is open in 51 hospitals and 106 patients are included.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-02-05

**CORALLEEN:** A phase 2 clinical trial of chemotherapy or letrozole plus ribociclib as neoadjuvant treatment for postmenopausal patients with luminal B/HER2-negative breast cancer

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**Background:** Dysregulation of cyclin D-CDK4/6-Rb pathway is associated with endocrine resistance in hormone receptor–positive (HR+) breast cancer. Recently, a CDK4/6 inhibitor has shown unprecedented efficacy in metastatic disease, leading to its regulatory approval. Several others are currently in clinical development for the management of HR+ breast cancer in the early and advanced settings. However, it is vital to gain insights into the molecular and biological effects of this class of agents and could identify patients who can benefit the most, delaying or avoiding the use of chemotherapy. The neoadjuvant setting provides an ideal scenario to carry out these investigations. Hence, we propose to conduct an exploratory study to evaluate the biological effects and the efficacy of ribociclib in patients with primary luminal B tumors. We hypothesize that the combination of ribociclib plus letrozole may offer clinical benefit in the preoperative setting. **Methods:** This is a parallel, multicenter, two-arm, randomized exploratory study in postmenopausal women with primary operable HR+/HER2-negative Luminal B breast cancer designed to evaluate the clinical benefit of ribociclib plus letrozole. Eligibility includes stage I-III operable breast cancer, Luminal B by PAM50, ECOG 0-1. They will be randomized 1:1 to receive either six 28-days cycles of ribociclib (600mg; 3-weeks-on/1-week-off) plus daily letrozole (2.5mg) or chemotherapy: four cycles of AC (doxorubicin 60 mg/m<sup>2</sup>, cyclophosphamide 600 mg/m<sup>2</sup> every 21 days) followed by weekly paclitaxel during 12 weeks. Baseline, Day 15 on-treatment, and surgical specimens will be collected for molecular characterization and evaluation of response (decrease in Ki67, change to ROR low disease) The primary endpoint is the rate of Residual Cancer Burden (RCB) per MD Anderson Cancer Center procedures. A rate of RCB 0 and 1 score at surgery, with a rank between 20% to 25% with 47 evaluable patients by group of treatment will offer a precision between 11.5% and 12.4%, respectively (95%CI). Ninety-four patients will be enrolled in 21 sites across Spain. The trial was activated in July 2017. As of June 2018, 78 patients have been recruited.

# 2018 San Antonio Breast Cancer Symposium ®

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Utilizing multiomic advanced diagnostics to identify CDK4/6 inhibitor response predictors and a post-treatment multiomic signature for patients with ER+/HER2- metastatic breast cancer (SIDEOUT-3)

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**Background:** Palbociclib, ribociclib and abemaciclib are 3 cyclin-dependent kinase (CDK) 4/6 inhibitors (inh) approved by the FDA for treatment of patients (pts) with hormone receptor–positive (HR+) metastatic breast cancer (MBC). We hypothesize that measuring the signaling architecture of CDK 4/6 kinase signaling network will predict response to CDK 4/6 inh and identify pts who are unlikely to respond to CDK4/6 inh and can be treated with other FDA approved drugs or a clinical trial. Patients who develop disease progression on CDK 4/6 inh within 12 months of starting therapy are eligible for a unique multi-omic based molecular analysis that can be used as a therapeutic decision support tool.

**Trial design:** This is an open label, multicenter study prospectively evaluating the phosphoprotein-based CDK 4/6 kinase network within the tumors of 100 pts with HR + MBC who are candidates for standard 1<sup>st</sup> line treatment with a CDK 4/6 inh plus endocrine therapy (ET). Eligible pts must have pretreatment tissue from a metastatic lesion sufficient to complete baseline biomarker analysis using a novel Laser Capture Microdissection (LCM) reverse phase protein array (RPPA) coupled approach to quantitatively analyze 8 specific proteins/phosphoproteins within the CDK 4/6 kinase signaling network (Total Rb; phospho Rb (S780); total Cyclin D1; phospho Cyclin D1 (S286); total p16INK; total p27KIP; phosphop27KIP (T187); phosphoFoxM1 (T600). Pts who develop disease progression within 12 months of starting CDK4/6 inh plus ET will be eligible for an optional biopsy of a soft tissue or bone metastatic lesion at time of disease progression for molecular analysis using a multi-omic CAP/CLIA laboratory assays to analyze post-therapy biopsies by RPPA, IHC analysis, RNA-Seq, and targeted exome sequencing. A molecular tumor board then provides the results to the treating physician as a therapeutic decision support tool outlining potential targets and possible therapies which may be used for further treatment. The primary objective is to demonstrate a correlation between one-year progression-free survival (PFS) and pre-treatment phospho RB levels. Secondary endpoints will examine the association between one-year PFS and 7 pre-specified CDK 4/6 signaling network markers. Thirteen of planned 100 patients have been enrolled. The study will be conducted at 12 centers.

**Clinical trial registry number 03195192**



# 2018 San Antonio Breast Cancer Symposium ®

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**PATINA:** A randomized, open label, phase III trial to evaluate the efficacy and safety of palbociclib + anti-HER2 therapy + endocrine therapy (ET) vs. anti-HER2 therapy + ET after induction treatment for hormone receptor positive (HR+)/HER2-positive metastatic breast cancer (MBC)

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## **Background:**

Pre-clinical data and results from early phase clinical trials point to synergistic antitumor activity and potential efficacy of palbociclib when given in combination with anti-HER2 therapy (tx). The aim of PATINA is to determine the effect of adding palbociclib to anti-HER2 and ET maintenance after induction tx in the 1st line setting for HR+/HER2+ MBC.

## **Trial Design:**

The PATINA trial (AFT-38/NCT02947685) is a pivotal, open-label, international, phase III study. Following 6-8 cycles of chemotherapy (taxane or vinorelbine) with anti-HER2 tx for MBC participants will be randomized 1:1 to standard anti-HER2 tx (trastuzumab +/- pertuzumab) in combination with ET with or without palbociclib until disease progression. ET options are either an aromatase inhibitor or fulvestrant. Premenopausal patients (pts) must receive ovarian suppression. The trial is open to men or women with histologically confirmed HR+/HER2+ MBC provided they are without evidence of disease progression by local assessment after induction tx. Total planned accrual is 496 pts. Primary objective is to demonstrate that the combination of palbociclib with anti-HER2 tx plus ET is superior to anti-HER2 tx plus ET alone in prolonging progression-free survival (PFS). Key secondary objectives are measures of tumor control, overall survival, safety and Quality of Life. The main translational science objective is to compare PFS estimates according to PIK3CA mutation status. The study has a 90% power to detect a hazard ratio of 0.667 in favor of the palbociclib arm. All pts approached to participate in PATINA will be asked to share remaining biospecimens and clinical data with the Mastering Breast Cancer Initiative. This initiative was created in order to understand the natural history of MBC and how it evolves over time with the aim to develop new treatments for this patient population. Recruitment has started in 07/2017 and is planned for approximately 22 months in 140 sites in Australia, Germany, Italy, France, New Zealand, Spain, and the US. 69 pts have been recruited so far.

**The study is sponsored by Alliance Foundation Trials (AFT) and financially supported by Pfizer**

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-02-08

Genetic analysis in blood, urine, stool and tumor samples from patients with advanced or metastatic estrogen receptor positive and HER2 negative breast cancer receiving palbociclib and endocrine therapy (PROMISE)

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**Background:** Combining cyclin dependent 4/6 kinase inhibitors (CDK4/6i) with endocrine therapy (ET) has resulted in clinically significant improvements in progression-free survival (PFS) in persons with hormone-receptor (HR)-positive metastatic breast cancer (MBC). However, nearly all patients will progress on CDK4/6i and ET and the mechanisms associated with primary and secondary resistance are mostly unknown. The identification of biomarkers associated with response to CDK4/6i is therefore a major research priority. PROMISE is a multicenter prospective cohort study designed to perform a comprehensive “omic” assessment of blood, tumor, urine and the fecal microbiome to identify alterations in molecular or cellular features associated with primary endocrine resistance (e.g. disease progression  $\leq$  12 months on treatment) and acquired resistance to CDK4/6i. Additionally, patient derived xenografts (PDX) and organoids are generated to test new drug strategies designed to reverse resistance to CDK4/6i and ET. **Methods:** Eligible participants include women for whom palbociclib is recommended in combination with either letrozole (first-line) or fulvestrant (second-line) therapy for HR-positive MBC. As of July 2018, 14 patients have been enrolled; the target accrual is 250 patients, who will be followed over the course of 36 months and followed for at least 12 months after the close of enrollment. Blood and tumor biopsies are obtained at baseline, at the end of cycle 2, and at progression. Whole exome sequencing (germline and tumor) and RNAseq (whole transcriptome) are performed on tumor samples in a CAP/CLIA lab (TEMPUS) and results will be provided back to all patients for later use. Additionally, PDX and organoids are generated from tumor biopsies at baseline and during progression. Blood, stool, and urine samples are collected for additional correlative studies. The primary objective of this trial is to identify novel genomic variants and pathways associated with early progression ( $\leq$  12 months) among women with advanced HR-positive breast cancer treated with palbociclib and ET. Secondary objectives include identification of the biomolecular features within the gut (stool) microbiome and exploration of the metabolomics and proteomics of ER-positive MBC. Results from the interrogation of these biospecimens will be critical to gain insights into treatment resistance. Funding is provided by Mayo Clinic Center for Individualized Medicine, TEMPUS, and an ASCO Career Development Award (COS). ClinicalTrials.gov Identifier: NCT03281902.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-03-01

Open-label, single-arm study evaluating the antitumor activity and safety of niraparib as neoadjuvant treatment in patients with localized, *HER2*-negative, *BRCA*-mutant breast cancer

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**Background:** Neoadjuvant chemotherapy is administered to patients with operable breast cancer to downstage the tumor to allow for less extensive surgery and to provide prognostic information about drug efficacy and risk of disease recurrence. Patients who achieve a pathological complete response (pCR) following neoadjuvant treatment have a more favorable outcome than patients with residual invasive disease. Single-agent poly(ADP-ribose) polymerase (PARP) inhibitors have clinical efficacy in *BRCA*-mutated breast cancer. Niraparib, a potent and selective PARP1/2 inhibitor, is approved for maintenance treatment of patients with recurrent ovarian cancer and has demonstrated strong antitumor activity in in vivo studies with *BRCA1*-mutant breast cancer. The objective of this study is to evaluate the antitumor activity of single-agent niraparib in the neoadjuvant treatment of patients with localized, human epidermal growth factor receptor 2 (*HER2*)-negative, *BRCA*mut breast cancer.

**Trial Design:** This is an open-label, single-arm pilot study with a target enrollment of 20 evaluable patients. Eligible patients are those  $\geq 18$  years old with histologically-confirmed *HER2*-negative localized breast cancer and either a *BRCA1* or *BRCA2* mutation (germline or somatic) and no prior anti-cancer therapies for the current malignancy. Patients will receive 200 mg of oral niraparib once daily for 2 months, after which they may either proceed directly to surgery or receive chemotherapy at the discretion of the physician. The primary endpoint is tumor response rate based on the change in tumor volume as measured by breast MRI after 2 months of treatment with niraparib; a response is defined as  $\geq 30\%$  reduction of tumor volume from baseline. Secondary endpoints include pCR rate, tumor response rate based on the change in tumor volume as measured by breast ultrasound, and safety and tolerability. Data will be summarized in a descriptive nature by frequency distributions (number and percentage of patients) for categorical variables and by the mean, median, and standard deviation for continuous variables. Tumor response rate will be tabulated together with its 95% binomial exact confidence interval.

**Funding:** TESARO, Inc., Waltham, MA, USA sponsored the study.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-03-02

A phase 2, open-label, single-arm, multi-center study of talazoparib for neoadjuvant treatment of germline *BRCA1/2* mutation patients with early-stage triple-negative breast cancer (TNBC)

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**Background:** Approximately 15% of all breast cancers are triple negative and deleterious *BRCA1/2* mutations are found in ~11% of unselected TNBC. In the phase 3 EMBRACA trial (NCT01945775), the poly (ADP-ribose) polymerase (PARP) inhibitor talazoparib was superior to chemotherapy in prolonging progression-free survival in *BRCA1/2* mutation patients with advanced breast cancer. A recent pilot study (NCT02282345) of 20 patients, explored the feasibility of neoadjuvant talazoparib in *BRCA1/2* mutation patients; pathologic complete response (pCR) was reported at 53% with 6 months of single agent talazoparib.

**Trial Design:** This phase 2, single-arm, open-label, multi-center study has a Simon 2-stage design. Eligible pts have stage I-III invasive TNBC (ER and PR <10%), with germline *BRCA1/2* mutations who are suitable for neoadjuvant therapy. Pts will receive talazoparib 1 mg daily for 24 weeks, followed by breast surgery, which should occur within 4 to 6 weeks of the last dose. Ultrasound will be performed serially to assess tumor response. The primary objective is to evaluate pCR after 24 weeks of neoadjuvant talazoparib. pCR (ypT0/is ypN0) will be assessed by independent central review. Safety will also be assessed. Pts will be followed for at least 5 years to assess long term outcomes (event-free and overall survival). After surgery, any further adjuvant therapy will be given at the discretion of the treating physician. Pt reported outcomes will be assessed electronically including the global health status/quality of life, functions, and symptoms using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaires C30 and BR23. Plasma pharmacokinetic (PK) samples for determination of talazoparib concentrations will be collected at defined timepoints to describe the steady-state PK of talazoparib. Exploratory biomarker research will also take place. Approximately 122 men and women will be enrolled in the study, of which 112 evaluable pts are planned. With 112 evaluable pts and one interim futility look, the null hypothesis that the true pCR rate is 35% will be tested against a 1-sided alternative. This design yields a 1-sided type 1 error rate of 2.5% and power of 90% when the true pCR rate is 50%. An interim analysis will be performed to evaluate the efficacy of talazoparib after 28 evaluable pts undergo talazoparib treatment for 24 weeks, followed by surgery, and are assessed for pCR by central review. This trial is currently recruiting and is registered at [clinicaltrials.gov](https://clinicaltrials.gov) (NCT03499353).

**Funding:** This study is sponsored by Pfizer, Inc.

# 2018 San Antonio Breast Cancer Symposium ®

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**PARTNER:** Randomised, phase II/III trial to evaluate the safety and efficacy of the addition of olaparib to platinum-based neoadjuvant chemotherapy in triple negative and/or germline BRCA mutated breast cancer patients

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**Background:** No specific targeted therapies are available for Triple Negative Breast Cancers (TNBC), an aggressive and diverse subgroup. The basal TNBC sub-group share some phenotypic and molecular similarities with germline BRCA (gBRCA) tumours. In gBRCA patients, and potentially other homologous recombination deficiencies, these already compromised pathways may allow drugs called PARP inhibitors (Olaparib) to work more effectively. **Aims:** To establish if the addition of olaparib to neoadjuvant platinum based chemotherapy for basal TNBC and/or gBRCA breast cancer is safe and improves efficacy (pathological complete response (pCR)).

**Methods: Trial design:** 3-stage open label randomised phase II/III trial of neoadjuvant paclitaxel and carboplatin +/- olaparib, followed by clinicians' choice of anthracycline regimen. Stage 1 and 2: Randomisation (1:1:1) to either control (3 weekly carboplatin AUC5/weekly paclitaxel 80mg/m<sup>2</sup> for 4 cycles) or one of two research arms with the same chemotherapy regimen but with two different schedules of olaparib 150mg BD for 12 days. Stage 3: Patients are randomised (1:1) to either control arm or to the research arm selected in stage 2. **End-points:** Stage 1: Safety; Stage 2: Schedule selection using pCR rate and completion rate of olaparib using a "pick-the-winner" design. Stage 3: pCR rate. Enrichment design is applied with an overall significance level 0.05( $\alpha$ ) and 80% power. A total of 527 patients will be included to detect an absolute improvement of 15% (all patients) and 20% (gBRCA patients) by adding olaparib to platinum based chemotherapy.

**Trial Progress:** PARTNER has been recruiting in UK since 27th May 2016. IDSMC recommended to continue the trial without change after reviewing the Stage 1 safety data. The recruitment of stage 2 was completed in April 2018 and results to be reviewed by the IDSMC in early 2019. The trial is open and enrolling patients to national and international sites.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-04-01

Nivolumab or capecitabine or combination therapy as adjuvant therapy for triple negative breast cancer (TNBC) with residual disease following neoadjuvant chemotherapy: The OXEL study

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**Background:** Long-term follow-up of neoadjuvant studies demonstrates poor clinical outcomes in patients with TNBC who do not achieve pathologic complete response, with only 35% remaining free of recurrence at 10 years. The addition of adjuvant capecitabine in the CREATE-X study prolonged disease free survival and overall survival (OS) in patients with HER2 negative breast cancer with residual invasive disease, with more striking benefit in patients with TNBC. Checkpoint inhibitors have not been approved in breast cancer yet, but recent studies suggest a benefit in combination with chemotherapy and low burden of disease. In the current study, we will evaluate the role of chemoimmunotherapy in the adjuvant setting for patients with TNBC with residual disease after neoadjuvant therapy. We will also investigate the role of the peripheral immunoscore (PIS) in predicting the benefit of immune checkpoint inhibition with or without chemotherapy.

**Trial design:** OXEL is a pilot open-label three arm randomized study of nivolumab, capecitabine or the combination as adjuvant therapy for 45 patients with residual TNBC after adequate neoadjuvant chemotherapy. Patients enrolled will be randomly assigned to 1 of 3 treatment arms: nivolumab 360 mg iv q3weeks for x 6 cycles; capecitabine 1250mg/m<sup>2</sup> po bid D1-D14 q3 weeks x 6 cycles; nivolumab 360mg iv q3weeks + capecitabine 1250mg/m<sup>2</sup> po bid D1-D14 q3 weeks x 6 cycles.

**Main eligibility criteria:** Patients ≥18 years of age with TNBC and ≥1cm of residual disease in the breast and/or node positive disease; receipt of neoadjuvant taxane +/- anthracycline, or platinum, and having completed definitive resection of primary tumor, with no prior use of capecitabine, fluorouracil or immunotherapy, and with no active autoimmune disease or chronic use of systemic steroids.

**Specific aims:** The primary endpoint is assessing the immunologic effects of capecitabine, nivolumab or the combination in the adjuvant setting by PIS. Additional endpoints include toxicity assessment, distant recurrence free survival (DRFS) and OS at 3-years, association between changes in PIS and circulating tumor DNA at different timepoints with clinical outcome variables and characterization of the immune contexture in residual tumors.

**Statistical methods:** The study is designed to assess the change in PIS at 6 weeks from baseline in each arm. The sample size of 15 per arm (45 total for 3 arms) will provide preliminary results. A sample size of 15 per arm will have 85% power to detect an effect size of 1 (the difference of the change in PIS from baseline to week 6 between two arms divided by the standard deviation) at 5% significance level.

**Present accrual and target accrual:** The Institutional Review Board at Georgetown University Medical Center has approved the study. Clinicaltrials.gov NCT03487666. Enrollment of the first patient is expected in July 2018 with a total of 45 patients planned to be recruited. Recruitment sites are MedStar Georgetown University Hospital, MedStar Washington Hospital Center, Hackensack University Medical Center. This trial is supported by Bristol-Meyers Squibb, P30CA051008-25 from NCI, Inivata and the Nina Hyde Center for Breast Cancer Research.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-04-02

Neoadjuvant Her2-targeted therapy +/- immunotherapy with pembrolizumab (neoHIP): An open label randomized phase II trial

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**Background:** In preclinical models HER2-directed therapy administered with checkpoint blockade is synergistic. Clinically, trastuzumab administered with pembrolizumab-mediated checkpoint blockade in trastuzumab-resistant HER2-positive metastatic breast cancer was safe and demonstrated modest activity. However, because checkpoint blockade can confer improved responses when administered earlier in the course of disease, trastuzumab with pembrolizumab administered in the curative-intent, treatment-naïve setting may confer life-long, tumor-specific immunity and ultimately, improve cure rates. Moreover, the potential synergy of trastuzumab and pembrolizumab with paclitaxel may overcome the need for dual HER2-blockade. The neo-HIP study is a randomized, multicenter, phase II, open-label trial to evaluate the efficacy and safety of weekly paclitaxel, trastuzumab plus pertuzumab (THP) vs weekly THP plus pembrolizumab (THP-K) vs a HER2 monotherapy regimen (TH-K) as neoadjuvant treatment in patients with HER2-positive early stage invasive breast cancer.

**Methods:** Patients ≥18 years old with previously untreated, non-metastatic, stage II-III, HER2-positive (by ASCO/CAP guidelines) breast cancer are eligible. Patients with inflammatory breast cancer or bilateral primary tumors are excluded. Adequate organ function and ECOG PS 0-1 are required. Approximately 174 patients will be randomly assigned to 1 of 3 arms with stratification by clinical nodal status (positive vs. negative) and hormone receptor status (positive vs. negative). In arm A, patients will receive T at 80mg/m<sup>2</sup> weekly for 12 weeks, H at 8mg/Kg (1 loading dose) and then 6mg/Kg IV every 3 weeks x 3 doses, P at 840mg (1 loading dose) and then 420mg/Kg IV every 3 weeks x 3 doses (THP). In arm B, patients will receive the same regimen as arm A with the addition of pembrolizumab 200mg IV every 3 weeks x 4 doses (THP-K). In arm C, patients will receive the same regimen as arm B, but without pertuzumab (TH-K). Definitive surgery will be 3-6 weeks after the last treatment dose. After surgery, patients in all arms will be treated per the treating physician's discretion. After completion of post-operative chemotherapy, patients will receive radiotherapy per local clinical standard and those patients whose tumors are hormone-receptor positive will receive hormone therapy as per local standard-of-care. The purpose of this phase II study is to identify whether Arm B (THP-K) and/or Arm C (TH-K) demonstrate a clinically significant improvement in pCR rate when compared with Arm A (THP). The primary end point is pCR rate in the breast and axilla (ypT0/Tis ypN0). Secondary end points include pCR rate by ypT0ypN0 and ypT0/Tis, residual cancer burden index, event free survival, breast conserving surgery rate, safety and overall survival. Exploratory correlative studies will characterize the immunologic responses to the interventions and explore potential predictors of efficacy and toxicity.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-04-03

**KEYNOTE-756:** A randomized, double-blind, phase III study of pembrolizumab versus placebo in combination with neoadjuvant chemotherapy and adjuvant endocrine therapy for high-risk early-stage ER<sup>+</sup>/HER2<sup>-</sup> breast cancer

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**Background:** Although ER<sup>+</sup>/HER2<sup>-</sup> breast cancer has a better overall prognosis than other breast cancer subtypes, there is a high-risk subpopulation characterized by high-grade tumors and decreased sensitivity to endocrine therapy, higher responsiveness to chemotherapy and worse prognosis. A large meta-analysis of prospective studies focusing on neoadjuvant chemotherapy (NAC) for treatment of stage I-III breast cancer demonstrated that increased pathologic complete response (pCR) rates at surgery were associated with improved survival. This correlation was observed across triple-negative breast cancer (TNBC), HER2<sup>+</sup> breast cancer, and high-grade HR<sup>+</sup>/HER2<sup>-</sup> breast cancer. Specifically, patients with a pCR after NAC had a 5-year event-free survival (EFS) rate of 90%, whereas patients who did not achieve a pCR had a 5-year EFS rate of 60%. Therefore, increasing pCR rates after NAC may have a substantial impact for patients with high-risk early-stage HR<sup>+</sup>/HER2<sup>-</sup> breast cancer. KEYNOTE-756 is a global, randomized, double-blind, phase III study of pembrolizumab (vs placebo) + chemotherapy as neoadjuvant treatment, followed by pembrolizumab (vs placebo) plus endocrine therapy as adjuvant treatment for patients with high-risk, early-stage ER<sup>+</sup>/HER2<sup>-</sup> breast cancer.

**Methods:** Patients with T1c-2 cN1-2 or T3-4 cN0-2 grade 3 or grade 2 with Ki-67 ≥30%, invasive, ductal ER<sup>+</sup>/HER2<sup>-</sup> breast cancer will be stratified by lymph node involvement (positive vs negative), tumor PD-L1 status (positive vs negative), ER positivity (≥10% vs <10%), and anthracycline dosing schedule (Q3W vs Q2W), and then randomized 1:1 to receive neoadjuvant treatment with pembrolizumab 200 mg Q3W or placebo in combination with paclitaxel (80 mg/m<sup>2</sup> QW) for 4 cycles followed by (doxorubicin [60 mg/m<sup>2</sup>] or epirubicin [100 mg/m<sup>2</sup>]) plus cyclophosphamide (600 mg/m<sup>2</sup>) Q2/3W for another 4 cycles. After definitive surgery (± radiation therapy, as indicated), patients will receive adjuvant treatment with pembrolizumab (200 mg Q3W) or placebo for 9 additional administrations, in combination with endocrine therapy, which can be given for up to 10 years. Co-primary end points are pCR rate and EFS. Secondary end points are safety and overall survival. The global study will open in North America and Latin America, Europe, and Asia Pacific in the second half of 2018.



# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-04-04

A randomized phase II study of pembrolizumab in combination with carboplatin versus carboplatin alone in breast cancer patients with chest wall disease, with immunologic and genomic correlative studies

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**Background:** Thirty percent of patients with breast cancer may experience chest wall recurrence, which is associated with a higher risk of developing distant metastases and a poor prognosis. Cancer cells may evade immune rejection through the programmed cell death 1 (PD-1) pathway. Pembrolizumab, an anti-PD-1 antibody, binds PD-1 and inhibits its interaction with the programmed death ligand 1 (PD-L1) to facilitate tumor immune rejection. We hypothesize that pembrolizumab may be an effective therapy in chest wall recurrence, given the inflammatory nature, and the high expression of PD-1 in tumors with lymphovascular invasion. Platinum agents may enhance anti-tumor immunity in a synergistic manner, and the combination of pembrolizumab and carboplatin has demonstrated efficacy in advanced lung cancer. In this study, the combination of pembrolizumab and carboplatin is being evaluated in breast cancer patients with chest wall disease.

**Methods:** This is a randomized phase II study of breast cancer patients with advanced, unresectable breast cancer involving the chest wall, being conducted through the Translational Breast Cancer Research Consortium (TBCRC). Patients may have hormone resistant disease (at least 2 prior lines of hormone therapy), triple negative breast cancer, or refractory HER2+ disease for enrollment. They may have other sites of distant metastases, have received any number of prior lines of therapy, have had prior surgery, but prior chest wall radiation is not necessary. Eighty-four patients at 7 TBCRC sites will be randomized 2:1 to treatment with pembrolizumab 200 mg IV and carboplatin AUC 5 IV every 3 weeks followed by pembrolizumab 200 mg IV alone every 3 weeks (Arm A, n=56) or carboplatin AUC 5 IV every 3 weeks (Arm B, n=28), with an option for patients in Arm B to cross-over to single agent pembrolizumab 200 mg IV every 3 weeks (arm Bx) on progression. Patients will undergo imaging with CT chest, abdomen, and pelvis at baseline and every 2 cycles of treatment for response evaluation. The primary endpoint is the disease control rate in the chest wall and distant sites at 18 weeks of treatment, and this study is powered to determine a 20% difference in disease control rates between arms A and B (hazard ratio of 0.52,  $\alpha=0.10$ ,  $\beta=0.20$ ). After 18 patients are enrolled into Arm B, an interim analysis for futility will be conducted to enable early closure of that arm for lack of efficacy. Secondary endpoints in the study are toxicity, progression free survival, and response based on PD-L1 expression and irRECIST. Exploratory endpoints, which will be studied using peripheral blood testing and chest wall tumor biopsies at baseline and after 2 cycles of treatment, include determining associations of response with changes in tumor and peripheral blood immune composition, soluble PD-L1 expression, circulating tumor cells, cell free DNA, and tumor PD-L1 and MYC genomic expression. Ultimately this study promises to improve our understanding of checkpoint inhibition and chemotherapy for chest wall disease, and the underlying mechanism of action. This study is open for enrollment and 2 patients are currently enrolled. (NCT03095352).

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-05-01

A randomized double-blind phase III clinical trial of neoadjuvant chemotherapy (NAC) with atezolizumab or placebo in patients (pts) with triple negative breast cancer (TNBC) followed by adjuvant atezolizumab or placebo: NSABP B-59/GBG 96-GeparDouze

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## **Background:**

TNBC is associated with higher percentages of pathological complete response (pCR) to neoadjuvant chemotherapy (NAC), and women with a pCR have a favorable prognosis. However, Liedtke (2008) and Loibl (2017) found that women with residual disease have a substantially higher risk of recurrence than women with other subtypes of breast cancer. Additionally, Adams (2017) and Schmid (2017) found that therapeutic blockade of PD-L1 binding by atezolizumab has resulted in relevant anti-tumor efficacy.

## **Methods:**

### **Design**

This is a phase III, double blind, placebo-control trial evaluating neoadjuvant atezolizumab with NAC followed by adjuvant atezolizumab in TNBC. Pts are stratified by region (North America; Europe), tumor size (1.1-3.0cm; >3.0cm), AC/EC schedule (q2w; q3w), and nodal status (positive; negative), then randomized 1:1 to receive atezolizumab/placebo 1200 mg IV every 3 wks concurrently with both sequential regimens of weekly paclitaxel 80 mg/m<sup>2</sup> IV for 12 doses with every 3-wk carboplatin AUC of 5 IV for 4 doses followed by AC/EC every 2-3 wks (per investigator discretion) for 4 cycles. Following surgery, pts resume atezolizumab/placebo 1200 mg IV every 3 wks as adjuvant therapy for 6 months. Radiotherapy based on local standards is co-administered with atezolizumab/placebo.

### **Eligibility criteria**

Centrally-confirmed ER-neg, PR-neg, HER2-neg invasive breast cancer by ASCO/CAP guidelines. Primary tumor must be stage T2 or T3 if cN0 or cN1 with negative biopsy or T1c, T2, or T3 if cN1 with positive biopsy or cN2 or cN3. LVEF >55% and no significant cardiac history.

### **Statistical methods**

Co-primary endpoints are event-free survival (EFS) and pCR breast/nodes. Secondary endpoints include pCR breast, overall survival, distant disease-free survival, safety and toxicity. Trial is an academic collaboration between NSABP and GBG with support from Genentech/Roche.

**NCT03281954**

**Support:** Genentech/Roche

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-05-02

**ALEXANDRA/IMpassion030:** A phase III study of standard adjuvant chemotherapy with or without atezolizumab in early triple negative breast cancer

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**Background:** Triple negative breast cancer (TNBC) is a subtype with a high risk of relapse in the early disease setting. Because TNBC does not currently have specific targeted agents approved for use in the early setting it is treated primarily with chemotherapy. A growing body of evidence indicates that TNBC is more immunogenic than other subtypes of breast cancer and promising clinical activity has been reported with atezolizumab (an anti-PD-L1 antibody) in Phase 1/1b metastatic TNBC trials. Furthermore, the anti-tumor activity of PD-1/PD-L1 targeting drugs is hypothesized to be enhanced when co-administered with chemotherapy. ALEXANDRA/IMpassion030 will evaluate the efficacy and safety of atezolizumab in combination with standard adjuvant chemotherapy in early TNBC.

**Methods:** ALEXANDRA/IMpassion030 is a global, prospective, randomised, open-label Phase 3 trial investigating the efficacy, safety and pharmacokinetic (PK) profile of adjuvant atezolizumab plus standard chemotherapy versus chemotherapy alone in early TNBC. In total, 2300 patients diagnosed with non-metastatic operable stage II or III TNBC confirmed by central pathology review will be randomised. Tumour PD-L1 evaluation will be performed centrally. Patients will be stratified by type of surgery, nodal status, and PD-L1 status. The adjuvant treatment will consist of weekly paclitaxel 80 mg/m<sup>2</sup> for 12 weeks followed by dose dense anthracycline (epirubicin 90 mg/m<sup>2</sup> or doxorubicin 60 mg/m<sup>2</sup>) and cyclophosphamide 600 mg/m<sup>2</sup> for 4 doses every 2 weeks or the same chemotherapy regimen (T-EC/AC) given concomitantly with atezolizumab 840 mg every 2 weeks followed by maintenance atezolizumab 1200 mg every 3 weeks until completion of 1 year of atezolizumab. Primary end-point is invasive disease-free survival (iDFS) and secondary end-points include iDFS by PD-L1 and lymph node status, overall survival, safety, patient functioning and health related quality of life (HRQoL). Tumour tissue and blood samples will be collected for biomarker research. The first site was activated in May 4th, and approximately 430 sites are expected to be open globally in 30 countries. This trial is sponsored by Roche and conducted in partnership with the Breast International Group, Frontier Science and Technology Research Foundation, Institute Jules Bordet and Alliance Foundation Trials. Clinicaltrials.gov NCT03498716.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-05-03

**MEDIOLA:** An open-label, phase I/II basket study of olaparib (PARP inhibitor) and durvalumab (anti-PD-L1 antibody)—Additional breast cancer cohorts

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**Background:** Olaparib (Lynparza®) is a PARP inhibitor that alters the repair of single-strand DNA breaks. Durvalumab (Imfinzi®) is a monoclonal antibody against programmed cell death ligand 1 (anti-PD-L1) that promotes antitumor immune responses. MEDIOLA (NCT02734004) is a Ph I/II open-label, multicenter study enrolling patients (pts) across several tumor types (small-cell lung cancer, gastric cancer, germline *BRCA*-mutated [*gBRCAm*] BC, or platinum sensitive relapsed *gBRCAm* ovarian cancer). 34 pts with *gBRCAm* BC received olaparib 300 mg po bid for a 4-wk run-in, followed by olaparib 300 mg po bid and durvalumab 1.5 g IV q4 wks. Encouraging preliminary results support an expansion cohort in a *BRCAm* popn. Evidence suggests that mutations in other homologous recombination repair (HRR) genes may confer a *BRCA*-like phenotype, warranting an expansion of the study population beyond *BRCAm*.

Inhibition of vascular endothelial growth factor (VEGF) has been reported to enhance the efficacy of chemotherapy in TNBC. In addition, VEGF inhibition potentiates PARP inhibitor activity, particularly in pts who do not carry *BRCAm*. Combinations of immune checkpoint inhibitors and bevacizumab, an anti-VEGF-A antibody, have shown promising results in other tumor types. Thus, MEDIOLA will additionally explore the efficacy and safety of olaparib + durvalumab in combination with bevacizumab in TNBC pts.

**Trial design:** Pts in the additional MBC cohorts will receive combination olaparib and durvalumab with no olaparib run-in. Pts in the TNBC cohort will also receive concurrent bevacizumab 10 mg/kg q 2 wks. Tumor assessments will be performed at baseline and every 8 wks thereafter.

**Eligibility criteria:** Pts with histologically confirmed, locally advanced or metastatic HER2-neg BC, who are PARP-inhibitor and immunotherapy naïve. Prior anthracycline and/or taxane therapy in early or MBC is required. Prior platinum therapy is allowed, if there was no disease progression while receiving treatment and at least 12 mths has elapsed since the last dose. Pts will undergo *BRCA* and HRR mutation testing and will be assigned to a cohort based on their mutation status, as illustrated in Table 1.

**Specific aims:** Cohort-specific primary efficacy endpoint targets were calculated aiming for superiority to standard of care treatment (Table 1). Other primary outcomes are safety and tolerability. Secondary endpoints are PK, DCR at 24 wks, objective response rate, duration of response, progression-free survival and overall survival. Exploratory endpoints include the analysis of tumor-infiltrating lymphocytes and PD-L1 expression.

**Statistical methods:** The expansion cohort will have a single-stage statistical design. Bayesian predictive probability design will be used for the analysis of the HRRm and TNBC triplet cohorts.

**Accrual:** Accrual targets are shown in Table 1. First subject will be enrolled in Nov 2018.

New MEDIOLA BC cohorts

Cohort name	Target accrual	Drugs	Popn	Primary endpoint
Breast <i>BRCAm</i> expansion	80	Olap+Durv	HER2-neg, t <i>BRCAm</i>	ORR 70%

Breast HRRm cohort	29	Olap+Durv	HER2-neg, non <i>BRC</i> Am, HRRm-pos	DCR at 16 wks 80%
TNBC triplet cohort	30	Olap+Durv+Bev	TNBC, non <i>BRC</i> Am, non HRRm	DCR at 16 wks 80%
Bev, bevacizumab; durv, durvalumab; olap, olaparib				

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-05-04

Phase II study of atezolizumab, cobimetinib, and eribulin in patients with recurrent or metastatic inflammatory breast cancer (IBC)

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**Background:** IBCs that do not completely respond to chemotherapy often have dysregulated immune pathways, and novel therapies are needed to improve outcomes in recurrent/metastatic disease. One-third of IBCs express the atezolizumab target PD-L1, and cobimetinib increases PD-L1 expression; thus, we hypothesize that atezolizumab and cobimetinib may act synergistically in IBC. The FDA-approved agent eribulin is active in IBC and has anti-stem cell activity and can reverse the IBC phenotype of epithelial-to-mesenchymal transition. Hence the use of eribulin as a chemotherapy backbone in combination with other novel agents is well justified.

**Trial Design:** This single-arm, open-label trial is enrolling patients with recurrent IBC or de novo metastatic IBC that has progressed on at least 1 line of standard chemotherapy. During a 4-week pharmacodynamic window, patients have an upfront biopsy, receive atezolizumab and cobimetinib treatment for 4 weeks, and have a second biopsy. Triple-combination treatment then commences, with standard eribulin dosing. After 4 cycles of eribulin, patients receive maintenance targeted therapy until disease progression or intolerable toxicity.

**Eligibility Criteria:** Patients with metastatic IBC of any molecular subtype must have measurable disease (per RECIST 1.1) amenable to biopsy. Patients with HER2+ disease must have received both pertuzumab and T-DM1. Patients with treated stable brain metastases are allowed. Patients must have recovered from the acute effects of any prior therapies and have adequate hematologic, organ, and cardiac function. Patients with autoimmune diseases or a history of pneumonitis are ineligible.

**Specific Aims:** The primary objective is to determine the overall response rate (ORR) of the combination therapy. Secondary objectives include determining the safety and tolerability, clinical benefit rate, response duration, progression-free survival, 2-year overall survival rate and predictive biomarker analyses.

**Statistical Methods:** The trial will enroll up to 9 patients in its phase I/safety lead-in portion and up to 33 patients total. A Bayesian optimal interval design is used to efficiently determine the maximum tolerated cobimetinib dose in phase I. Patients start cobimetinib at the FDA-approved dose of 60 mg/day with a target toxicity rate is 0.3. Phase II will enroll 24 patients to determine the efficacy of the triple-combination therapy. The historical ORR in metastatic IBC is 10%; our sample size provides 80% power to detect an ORR improvement to 25%.

**Accrual:** The trial has enrolled 7 patients since its start in August 2017.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-06-01

Phase Ib clinical trial of coPANlisib in combination with Trastuzumab emtansine (T-DM1) in pre-treated unresectable locally advanced or metastatic HER2-positive breast cancer (BC) “PANTHERA”-CTRIAL-IE 17-13

Anees Hassan<sup>1,2,7</sup>, Giuseppe Gullo<sup>3</sup>, Seamus O'Reilly<sup>4</sup>, Manuel Ruiz-Borrego<sup>5,6</sup>, Sinead Toomey<sup>1</sup>, Liam Grogan<sup>1,2,7</sup>, Oscar Breathnach<sup>1,2,7</sup>, Patrick G Morris<sup>1,2,7</sup>, Janice M Walshe<sup>3</sup>, John Crown<sup>3</sup>, Deirdre O'Mahony<sup>4</sup>, Alejandro Falcon<sup>5</sup>, Keith Egan<sup>7</sup>, Andrés Hernando<sup>8</sup>, Ausra Teiserskiene<sup>8</sup>, Catherine M Kelly<sup>9</sup>, Linda Coate<sup>8</sup> and Bryan T Hennessy<sup>1,2,7,8</sup>. <sup>1</sup>RCSI, Dublin 9, Ireland; <sup>2</sup>Beaumont Hospital, Dublin 9, Ireland; <sup>3</sup>St Vincent's University Hospital, Dublin 4, Ireland; <sup>4</sup>Cork University Hospital, Cork, Ireland; <sup>5</sup>Hospital Universitario Virgen Del Rocío, Sevilla, Spain; <sup>6</sup>GEICAM, Spanish Breast Cancer Group, Madrid, Spain; <sup>7</sup>Cancer Clinical Trials and Research Unit, Beaumont Hospital, Dublin, Ireland; <sup>8</sup>Cancer Trials Ireland, Dublin, Ireland and <sup>9</sup>Mater Misericordiae University Hospital, Dublin, Ireland.

**Background:** The phosphoinositide 3 kinase (PI3K) pathway is important in the oncogenic function of HER2. Aberrant activation of PI3K is implicated in resistance to trastuzumab and other HER2-targeted therapies and is frequent, with up to 22% of HER2 positive breast cancer having a PIK3CA mutation. Copanlisib is a pan-class 1 PI3K inhibitor administered i.v. with low nanomolar activity against both PI3K $\alpha$  and PI3K $\beta$ . Copanlisib has been shown to re-sensitise trastuzumab resistant cell lines to trastuzumab with synergism seen in some cell lines between copanlisib and HER2 targeted therapy.

**Trial design:** This is a phase Ib open label, single arm adaptive, multi-centre trial of copanlisib in combination with T-DM1. Eligible patients will receive T-DM1 at 3.6mg/kg i.v. on day 1 of a 21-day cycle plus copanlisib. Copanlisib will be administered i.v. according to the dose escalation scheme (dose level 1 is 45mg on days 1 and 8, dose level 2 is 60mg on days 1 and 8, dose level 3 is 60mg on days 1, 8, and 15). Dose level -1 will be 45 mg on day 1 in case dose de-escalation is needed. We will enrol 3 to 6 patients per dose level. All patients in each level must have completed at least the first cycle of therapy before enrolment in the next dose level. Patients not completing the first cycle for a reason other than toxicity will be replaced. Dose escalation and determination of the Maximum Tolerated Dose (MTD) will be based on the occurrence of Dose Limiting Toxicities (DLT).

**Eligibility criteria:** Eligible patients are those with unresectable locally advanced or metastatic HER2-positive BC who previously received trastuzumab and a taxane, separately or in combination. Participants must have adequate organ function and ECOG PS  $\leq 2$

**Objectives:** The primary objective is to determine the MTD for copanlisib in combination with T-DM1 in patients with pre-treated unresectable locally advanced or metastatic HER2-positive BC. Secondary objectives include evaluating the safety, efficacy and cardiotoxicity in patients treated with this regimen. Exploratory objectives include examining for predictive biomarkers in tumour tissue and blood or plasma and to examine molecular tumour adaptation to clinical trial therapy.

**Statistical methods:** Patients will be accrued in cohorts of 3 patients according to a standard 3+3 algorithm, with dose escalation and determination of MTD based on the occurrence of DLT, using the usual threshold probability of 33%. The final dose level will be expanded to include a total of 6 additional patients (expansion cohort).

**Present accrual and target accrual:** The trial will start accrual in October 2018. Maximum of 24 patients will be enrolled.

# 2018 San Antonio Breast Cancer Symposium ®

**Publication Number:** OT3-06-02

An initial safety study of gedatolisib plus PTK7-ADC for metastatic triple-negative breast cancer

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**Background:** The PI3K pathway is dysregulated in the majority of triple-negative breast cancer (TNBCs). Contrary to the theory of oncogene addiction, single agent inhibition of the PI3K pathway in TNBC has had only modest activity. Our group has demonstrated preclinically that when PI3K is inhibited, an immediate compensatory up-regulation of the Wnt pathway occurs. The Wnt pathway is known for its role in cancer metastases and can confer resistance to initial PI3K inhibition. Simultaneous dual targeting of both pathways is highly synergistic against TNBC models in vitro and in vivo.

We have initiated a Phase I clinical trial using Gedatolisib (PI3K/mTOR inhibitor) and PTK7-ADC (Wnt pathway) for patients with metastatic TNBC (NCT03243331). Gedatolisib is a pan-class I isoform PI3K/mTOR inhibitor, and PTK7-ADC is an antibody-drug conjugate against the cell-surface PTK7 protein (Wnt pathway co-receptor) with an Auristatin payload. PTK7 is an attractive second target due to its up-regulation after PI3K inhibition and its known overexpression in TNBC. Further data has shown that the PTK7-payload, Auristatin, is in itself synergistic with Gedatolisib. The combination of using both of these drugs suggests a unique concept of “double synergy”. Where Gedatolisib increases the expression of the target of PTK7-ADC leading to one mechanism of synergy, and the Auristatin payload on PTK7-ADC is synergistic with Gedatolisib providing a second mechanism.

**Study Design:** This is an open-label, Phase I, dose-escalation study with a 3 + 3 cohort design. The trial will enroll 12-18 patients. 3 cohorts of at least 3 patients will receive Gedatolisib (weekly) & PTK-ADC (q3w) at 110mg+1.4mg/kg, 180mg+1.4mg/kg, and 180mg+2.8mg/kg dose levels.

**Eligibility Criteria:** This trial enrolls patients with metastatic triple negative (ER-, PgR-, HER2-) or low estrogen expressing (ER and PgR <5%, HER2-) breast cancer. Patients must have received at least one prior chemotherapy for advanced disease and have adequate hematologic, renal, and hepatic function. Patients with previously treated CNS involvement are eligible. Patients with uncontrolled diabetes are excluded, given the potential for hyperglycemia with Gedatolisib. Patients must have disease amenable and consent to biopsy for correlative endpoints.

**Objectives:** The primary objective is to evaluate the safety of Gedatolisib plus PTK7-ADC. The secondary objective is to evaluate efficacy as determined by objective response rate, clinical benefit at 18 weeks, and progression free survival (PFS). Exploratory objectives will evaluate efficacy in patients with genomic aberrations in the PI3K pathway; and association of tumor DNA, RNA, plasma and circulating tumor cell sequencing with clinical efficacy to identify putative biomarkers.

**Correlative Sciences:** We are collecting matched pre-/post-treatment tumor biopsies and serial blood samples to determine biomarkers of clinical response to inform subsequent trials. We plan to evaluate: 1) PI3K activity; 2) genomic aberrations in the PI3K pathway; 3) baseline PTK7 expression; 4) PTK7 upregulation after Gedatolisib treatment; and 5) mutations in plasma circulating tumor DNA.

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Ixazomib in combination with carboplatin in pretreated women with advanced triple negative breast cancer, an ongoing phase I/II trial (AGMT MBC-10 trial)

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**Background:** Triple-negative breast cancer (TNBC) comprises a heterogeneous group of diseases which are generally associated with poor prognosis. Recently, the PARP inhibitor olaparib was approved as first targeted treatment beyond antiVEGF therapy for the BRCA1/2 mutated subgroup of TNBC. However, cytotoxic agents still remain the mainstay of treatment for this breast cancer subtype. Ixazomib is a selective and reversible inhibitor of the proteasome, which has been mainly investigated as treatment of multiple myeloma. In a preclinical cell line model for TNBC the first-generation proteasome inhibitor bortezomib showed synergistic efficacy with cisplatin. Clinical data are available for carboplatin plus bortezomib in metastatic ovarian and lung cancers showing remarkable antitumor activity (47% and 38% response rate, respectively) and good tolerability. In solid tumors cytotoxic effect of proteasome inhibitors is thought to be mediated through different mechanisms: (1) Inhibition of the Fanconi Anemia and BRCA1 DNA repair mechanism (2) Inhibition of p53 degradation (3) Inhibition of NF-kappa B signaling cascade. Based on this evidence, the phase I/II MBC-10 trial will evaluate the toxicity profile and efficacy of the oral second-generation proteasome inhibitor ixazomib in combination with carboplatin in patients with advanced TNBC. **Trial Design:** Patients with metastatic TNBC pretreated with at least one prior line of chemotherapy for advanced disease with a confirmed disease progression and measurable disease are eligible for this study. Patients will receive ixazomib in combination with carboplatin on days 1, 8, and 15 in a 28-day cycle. The phase I part of this study uses an alternate dose escalation accelerated titration design. After establishing the maximum tolerated dose (MTD), accrual continues to evaluate the efficacy and safety of the combination (phase II, including 41 evaluable patients). All patients will continue on study drugs until disease progression, unacceptable toxicity or discontinuation for any other reason. Primary endpoint of the phase II is overall response rate, secondary endpoints include safety profile, progression-free survival and quality of life. The MBC-10 trial is accompanied by a broad biomarker program investigating predictive biomarkers for treatment response and potential resistance mechanisms to the investigational drug combination. This trial is open for patient enrollment since November 2016 in six Austrian cancer centers. Accrual is planned to be completed within two years. ClinicalTrials.gov Identifier: NCT02993094

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A phase Ib/II clinical trial investigating the efficacy of nitric oxide deprivation and docetaxel in triple negative breast cancer

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Triple negative breast cancer (TNBC) is an aggressive disease that currently lacks an efficacious form of therapy. Although chemotherapy is the current standard of care for metastatic TNBC, the 5-year prognosis remains grim with a high rate of disease recurrence. Cancer relapse is thought to be initiated by chemotherapy-resistant breast cancer stem cells (BCSCs). These BCSCs give rise to a diverse clonal population that results in a heterogeneous cancer, which complicates targeted therapeutic strategies. Our previous studies revealed that BCSCs utilize inducible nitric oxide synthase (iNOS)-derived nitric oxide to promote their proliferation, migration, and self-renewal capacity. In an effort to target the BCSC population, we found that iNOS inhibition with NG-monomethyl-L-arginine (L-NMMA) sensitized BCSCs to docetaxel *in vivo* in TNBC xenograft models, leading to decreased BCSC viability and tumor burden. These findings suggest that BCSC resist conventional therapy in a nitric oxide-dependent manner and that combination of L-NMMA with docetaxel will effectively target BCSCs to prevent further relapse. A phase Ib/II clinical trial was conducted to determine the maximum tolerated dose, recommended phase 2 dose (R2PD), dose-limiting toxicities (DLTs), and efficacy of the L-NMMA and docetaxel combination in TNBC patients with chemotherapy-refractory locally advanced or metastatic disease. For the phase Ib portion of the study, a standard Bayesian continual reassessment method is being used to investigate 7 dose levels of L-NMMA (5, 7.5, 10, 12.5, 15, 17.5, and 20 mg/kg) and two dose levels of docetaxel (75 and 100 mg/m<sup>2</sup>). Sixteen patients have been recruited to date, and based on current pharmacokinetics, pharmacodynamics, and safety data, the RP2D is expected to be docetaxel 100 mg/m<sup>2</sup> (Day 1) and L-NMMA 20 mg/kg (Days 1-5) every 3 weeks. Two and three patients received 15 mg/kg L-NMMA + 75 mg/m<sup>2</sup> docetaxel and 17.5 mg/kg L-NMMA + 100 mg/m<sup>2</sup> docetaxel, respectively. Of these 5 patients, one partial responder completed 8 cycles before discontinuing treatment due to taxane-associated neuropathy. Among the five patients treated at the RP2D, only one taxane-associated DLT occurred. The overall response rate for patients treated at the higher doses was 22.2%. Early results of the phase Ib/II trial indicate the safety, tolerability, and promising activity of the first-in-class pan-NOS inhibitor L-NMMA in combination with chemotherapy in the treatment of chemotherapy-refractory TNBC.