Evolution of Molecular Prognostic Testing in Breast Cancer

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Disclosures

- I have no personal financial interests in any of the technologies, devices, or companies discussed herein.

Objectives

- To review the development and technologic basis for three molecular assays available for prognostic assessment of breast cancer
- To analyze the clinical data which supports the use of these assays in lymph node positive cancer, lymph node negative cancer, or DCIS
- To discuss how emerging aspects of these assays might impact the future of breast cancer

Breast Cancer in 2013

- ACS Case Estimates
  - New cases: 234,580
  - Deaths: 40,030
- “Traditional” pathologic subtypes
  - 40% Low grade ER+
  - 25% High grade ER+
  - 20% HER2+
  - 15% Triple negative

Issues in Breast Cancer Diagnosis and Treatment

- It is fairly clear that we over-treat DCIS and low grade cancer: how to determine who is at greatest risk for progression/recurrence?
- Can we predict primary versus secondary endocrine resistance?
- Can we more accurately predict anti-HER2 therapy responsiveness?
- Can we further subcategorize breast cancer in therapeutically meaningful ways?

Molecular Profiling of Breast Cancer

- Oncotype Dx (Genomic Health)
  - 21 gene expression signature
  - First major trial: 2004
  - Study population: ER+ node negative invasive cancer
  - NCCN and ASCO guidelines recommend use (CLIA compliant)
- Mammaprint (Agendia)
  - 70 gene expression signature
  - First major trial: 2002
  - Study population: women < 61 years, T1-T2, N0 disease
  - FDA 510(k) cleared (2007)
- Prosigna (Nanostring)
  - 50 gene expression signature + 5 control genes (PAM50 assay)
  - First major trial: 2013 (on the nCounter platform)
  - Study population: Stage I-III cancer
  - FDA 510(k) cleared (2013)

The "Intrinsic" Molecular Subtypes

Molecular portraits of human breast tumours

Charles M. Perou*†, Therese Sørlie†‡, Michael B. Eisen*, Matt van de Rijn§, Stefanie S. Jeffrey‖, Christian A. Rees*, Jonathan R. Pollack¶, Douglas T. Ross¶, Hilde Johnsen‡, Lars A. Akslen#, Øystein Fluge☆, Alexander Pergamenschikov*, Cheryl Williams*, Shirley X. Zhu§, Per E. Lønning**, Anne-Lise Børresen-Dale‡, Patrick O. Brown¶†† & David Botstein*

Nature 2000

PNAS 2001

Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications

Therese Sørlie[a,b,c], Charles M. Perou[a,d], Robert Tibshirani[e], Turid Aas[f], Stephanie Geisler[g], Hilde Johnsen[b], Trevor Hastie[e], Michael B. Eisen[h], Matt van de Rijn[i], Stefanie S. Jeffrey[i], Thor Thorsen[k], Hanne Quist[l], John C. Matese[c], Patrick O. Brown[m], David Botstein[c], Per Eystein Lønning[g], and Anne-Lise Børresen-Dale[b,n]

Molecular classification of breast cancer

- Array gene expression and informatics that identified 1,753 differentially expressed genes in breast cancer
  - Performed independent of receptor status
- Approximately 400-500 “intrinsic” genes defined five subtypes
  - Highest variability between different tumors
  - Highest stability between paired samples from the same tumor
- Luminal A
- Luminal B
- HER2 enriched
- Basal
- Normal breast like

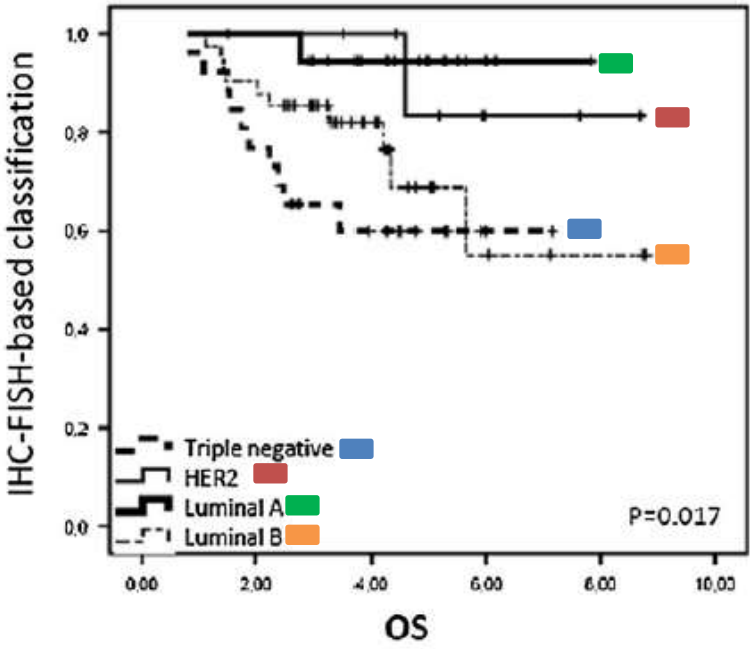
Creation of the PAM50

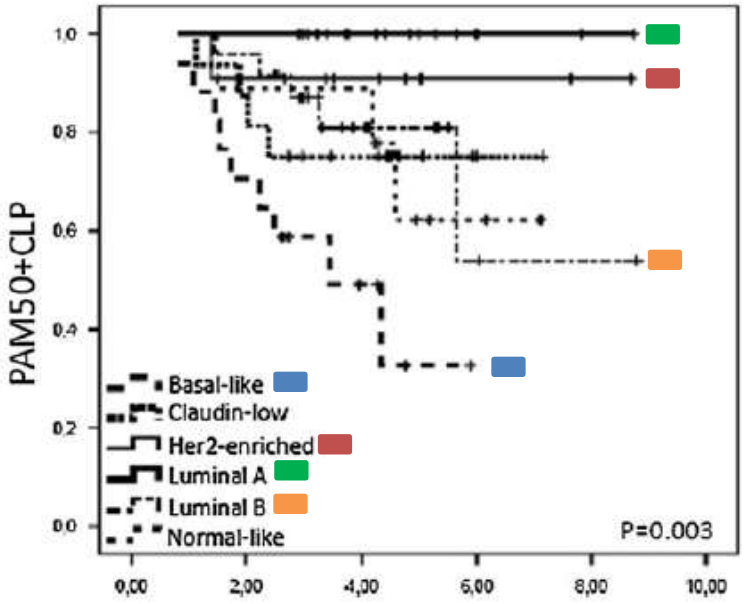
- PAM = prediction analysis of microarrays
- From these early publications, a set of 50 genes + 5 control genes was developed that best recapitulated the microarray classifier
- Reason: to convert to a FFPE compatible RT-qPCR platform
- A number of studies have examined the validity of the RT-PCR-based PAM50 assay in comparison to standard clinical molecular markers

PAM50: Similar but distinct from IHC/FISH/Grade

Table 3 Histological scoring across PAM50 subtypes

	Grade	ER	PR	HER2
LumA	G1-68 (25%)	Neg-19 (7%)	Neg-16 (6%)	Neg-273 (99%)
n = 277	G2-142 (51%)	Pos-258 (93%)	Pos-261 (94%)	Pos-4 (1%)
	G3-39 (14%)			
	GX-28 (10%)			
LumB	G1-25 (10%)	Neg-22 (8%)	Neg-68 (26%)	Neg-224 (86%)
n = 261	G2-111 (43%)	Pos-239 (92%)	Pos-193 (74%)	Pos-37 (14%)
	G3-111 (43%)			
	GX-14 (5%)			
HER2-E	G1-6 (3%)	Neg-63 (36%)	Neg-93 (53%)	Neg-105 (60%)
n = 174	G2-65 (37%)	Pos-111 (64%)	Pos-81 (47%)	Pos-69 (40%)
	G3-96 (55%)			
	GX-7 (4%)			
Basal	G1-0 (0%)	Neg-63 (90%)	Neg-62 (89%)	Neg-67 (96%)
n = 70	G2-4 (6%)	Pos-7 (10%)	Pos-8 (11%)	Pos-3 (4%)
	G3-61 (87%)			
	GX-5 (7%)			





Bastien RR et al., BMC Medical Genomics 2012

Romero A et al., Clin Transl Oncol 2013

OVERVIEW OF THE ASSAYS

Mammaprint: Development

Gene expression profiling predicts clinical outcome of breast cancer

Laura J. van 't Veer*†, Hongyue Dai†‡, Marc J. van de Vijver*†, Yudong D. He‡, Augustinus A. M. Hart*, Mao Mao‡, Hans L. Peterse*, Karin van der Kooy*, Matthew J. Marton‡, Anke T. Witteveen*, George J. Schreiber‡, Ron M. Kerkhoven*, Chris Roberts‡, Peter S. Linsley‡, René Bernards* & Stephen H. Friend‡

Nature 2002

A GENE-EXPRESSION SIGNATURE AS A PREDICTOR OF SURVIVAL IN BREAST CANCER

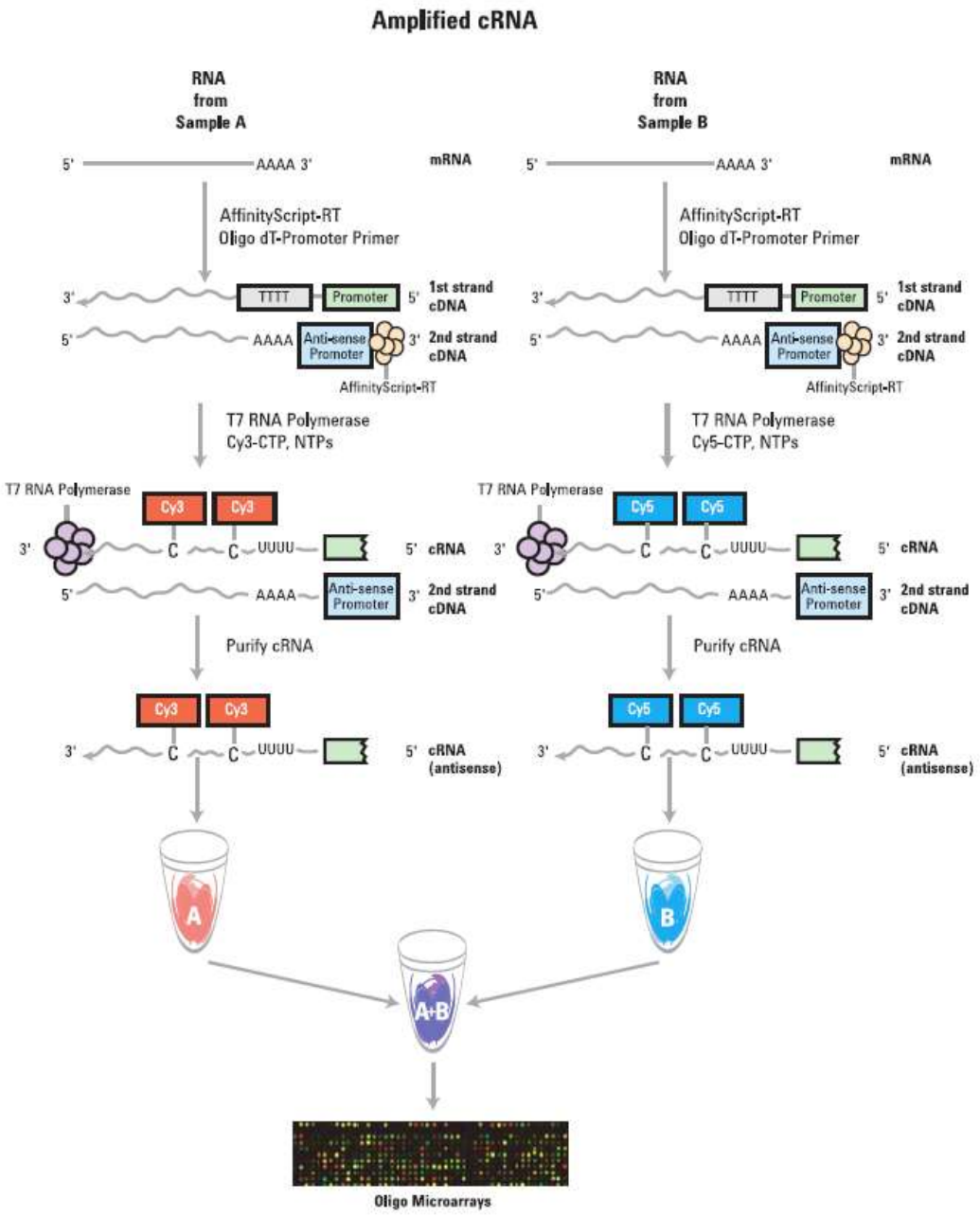
MARC J. VAN DE VIJVER, M.D., PH.D., YUDONG D. HE, PH.D., LAURA J. VAN 'T VEER, PH.D., HONGYUE DAI, PH.D., AUGUSTINUS A.M. HART, M.SC., DORIEN W. VOSKUIL, PH.D., GEORGE J. SCHREIBER, M.SC., JOHANNES L. PETERSE, M.D., CHRIS ROBERTS, PH.D., MATTHEW J. MARTON, PH.D., MARK PARRISH, DOUWE ATSMA, ANKE WITTEVEEN, ANNUSKA GLAS, PH.D., LEONIE DELAHAYE, TONY VAN DER VELDE, HARRY BARTELINK, M.D., PH.D., SJOERD RODENHUIS, M.D., PH.D., EMIEL T. RUTGERS, M.D., PH.D., STEPHEN H. FRIEND, M.D., PH.D., AND RENÉ BERNARDS, PH.D.

NEJM 2002

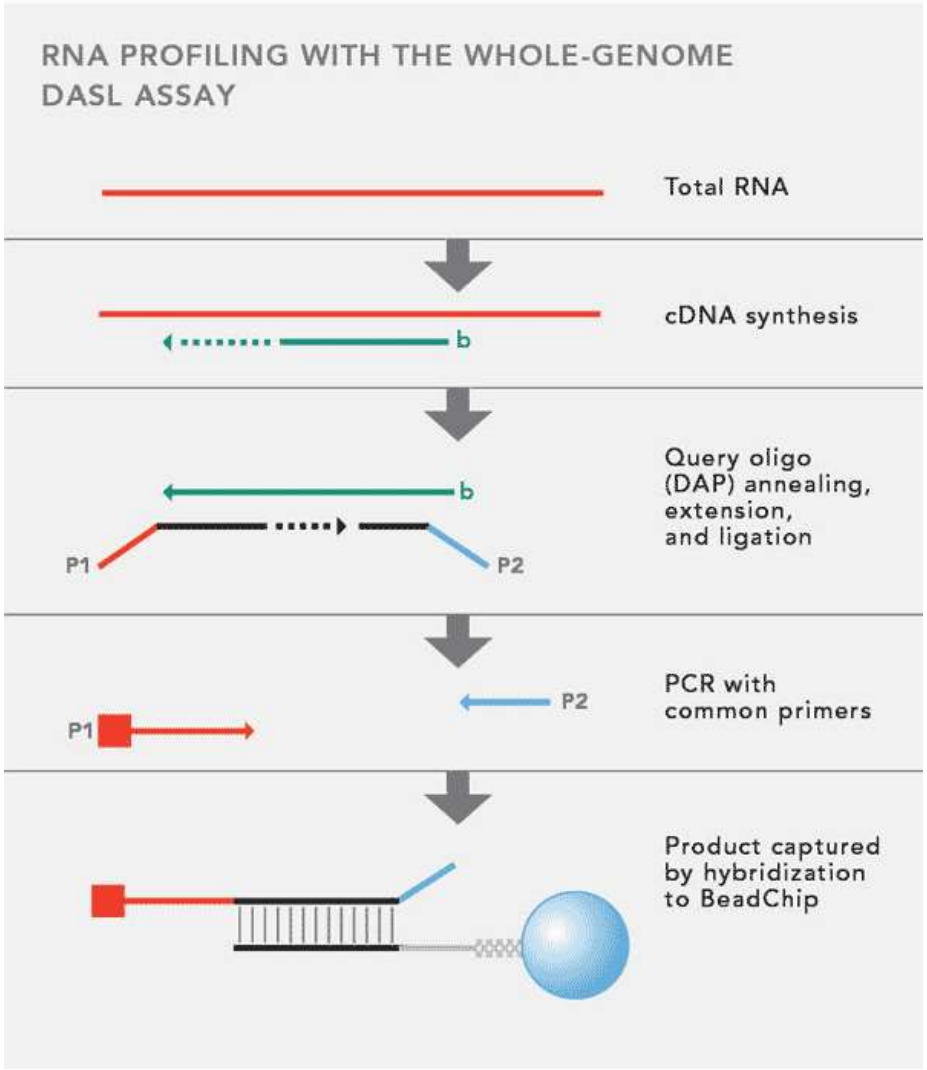
- Original development studies identified 5,000 genes (via microarray) that were deferentially expressed in good prognosis vs. poor prognosis breast cancers (based on 10 year disease free survival)
- Informatics clustering selected 70 genes that best identified these two subgroups

Mammaprint: Development

- Microarray platform (“gene chip”)
- Original chips contained ~25,000 oligo probes (RefSeq genes and ESTs)



Mammaprint: Switch to FFPE



- cDNA-mediated Annealing, Selection, extension and Ligation (DASL) assay (Illumina)
- 200 ng of RNA
- 24,500 targets queried

Mammaprint: Technology

- Input material: ideally > 50% tumor
- Reporting of the Mammaprint assay
  - Binary: either low risk or high risk for 10 year recurrence
  - NO intermediate category
- Agendia has “expanded” their panels and now offers Mammaprint as part of “Symphony”
  - Targetprint: mRNA confirmation of ER/PR/HER2 status
  - Blueprint: 80 gene signature that captures (in part) the intrinsic molecular subtypes
  - Theraprint/Discoveryprint: adaptable gene expression profiling for companion diagnostics

Oncotype Dx: Development

A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer

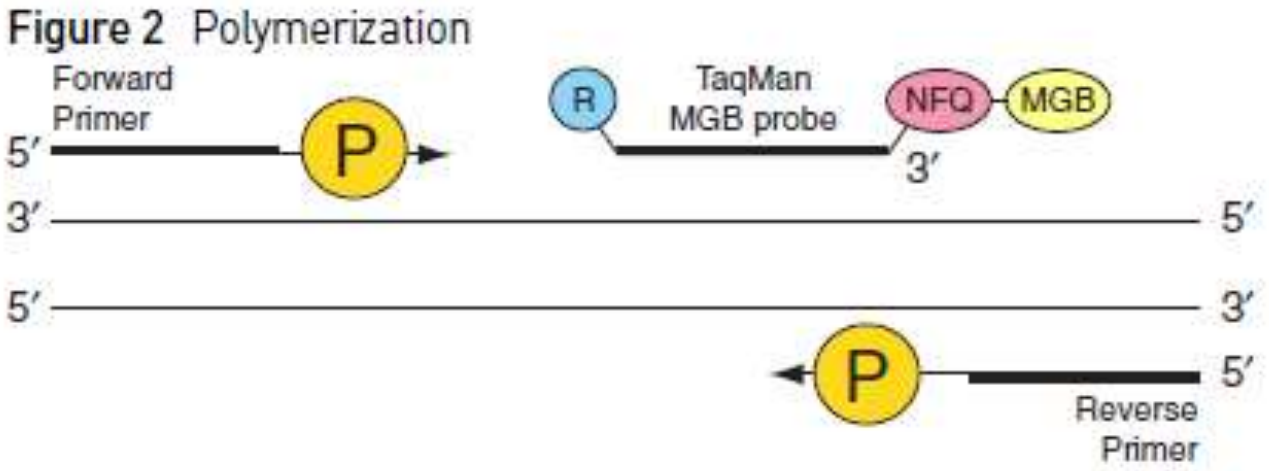
Soonmyung Paik, M.D., Steven Shak, M.D., Gong Tang, Ph.D., Chungyeul Kim, M.D., Joffre Baker, Ph.D., Maureen Cronin, Ph.D., Frederick L. Baehner, M.D., Michael G. Walker, Ph.D., Drew Watson, Ph.D., Taesung Park, Ph.D., William Hiller, H.T., Edwin R. Fisher, M.D., D. Lawrence Wickerham, M.D., John Bryant, Ph.D., and Norman Wolmark, M.D.

NEJM 2004

- Design Intention: develop a FFPE compliant assay to predict distant recurrence of ER+ breast cancer
- Selected 250 candidate genes (from others' array data) to test on NSABP B-14 and B-20 trials
- Refined a 16+5 gene panel that could predict recurrence
- Later studies on these trials (and SWOG-8814) demonstrated ability to predict significant benefit from adjuvant chemotherapy (2006, 2010)

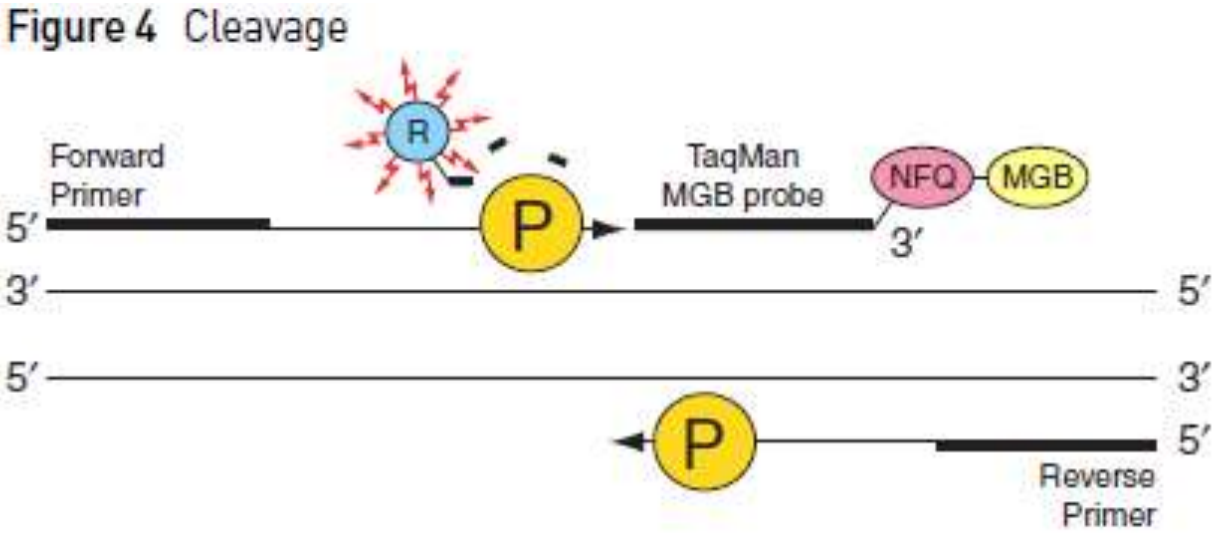
Oncotype Dx: Technology

- Reverse transcription, quantitative PCR assay
- Input material: Ideally > 50% tumor
  - RNA extracted from FFPE (15 unstained slides)
- RNA → cDNA → TaqMan assay



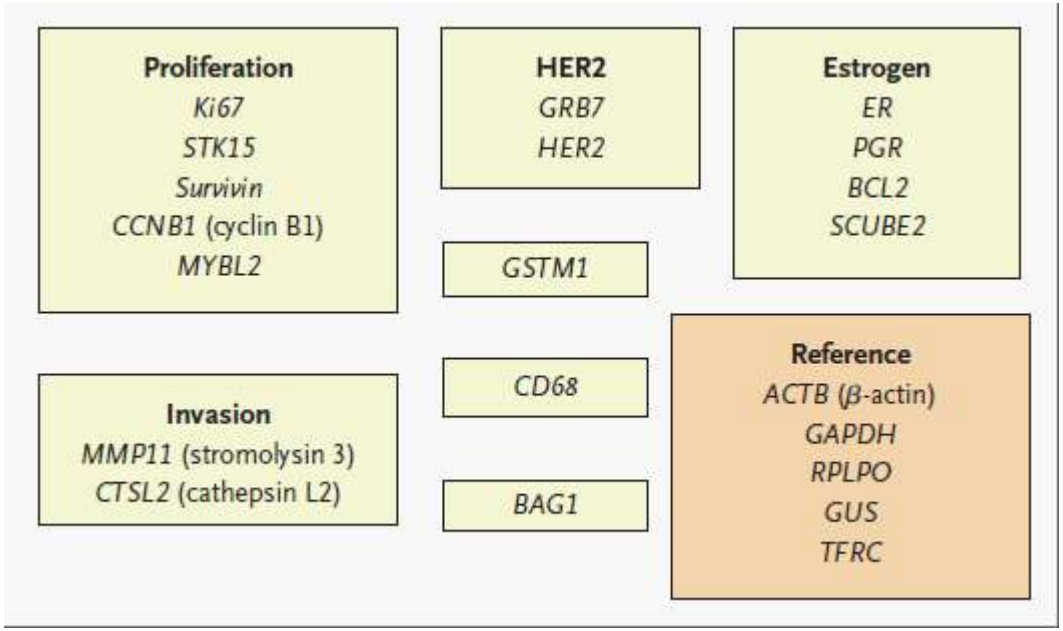
Applied Biosystems TaqMan Protocol

Oncotype Dx: Technology



- 16 target genes (selected from 250 candidate genes in preliminary studies on 447 patients)
- 5 “housekeeping” genes used for sample normalization (thus a “21 gene signature”)
- Samples are run in triplicate

Oncotype Dx: Technology



- Quantifies the standard pathologic characterization
- Complex algorithm that adds the HER2, proliferation, and invasion scores, and subtracts the estrogen score in a weighted fashion

- Reported as a Recurrence Score (RS)
- RS < 18 = low risk
- 18 ≤ RS < 31 = intermediate risk
- RS ≥ 31 = high risk

Paik et al. *NEJM*, 2004

Prosigna: PAM50 Meets the Clinic

- Thirteen years after Perou's paper, the PAM50 is entering the clinical arena
- The PAM50 gene signature has been transferred to a novel and robust method for mRNA quantification
  - Works well in FFPE
  - Does not rely on amplification of nucleic acids
  - Is intended for kit use in local labs with the proper instruments

Prosigna: Technology

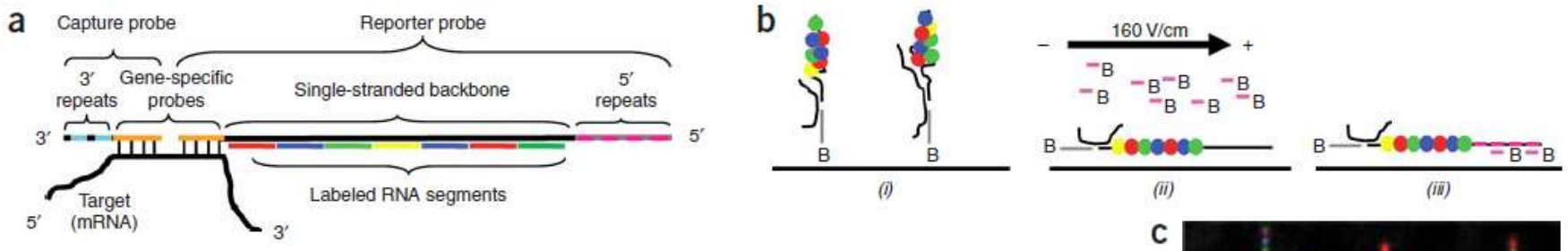
Direct multiplexed measurement of gene expression with color-coded probe pairs

Gary K Geiss[1], Roger E Bumgarner[2], Brian Birditt[1], Timothy Dahl[1], Naeem Dowidar[1], Dwayne L Dunaway[1], H Perry Fell[1], Sean Ferree[1], Renee D George[1,5], Tammy Grogan[1], Jeffrey J James[1], Malini Maysuria[1], Jeffrey D Mitton[1], Paola Oliveri[3,5], Jennifer L Osborn[1,5], Tao Peng[2], Amber L Ratcliffe[1], Philippa J Webster[1], Eric H Davidson[3], Leroy Hood[4] & Krassen Dimitrov[4,5]

nature biotechnology

- nCounter System (Nanostring)
- The brainchild of the Institute for Systems Biology, University of Washington, and Cal Tech
- Linear dynamic range of 500-fold
- Similar sensitivity to real-time PCR without amplification
  - Both have increased sensitivity over microarray
- Good correlation of results between platforms

Prosigna: Technology



- RNA (total) is isolated and loaded into the reaction
- Two probes: 1) Capture and 2) Reporter
  - Reporter has a color-coded molecular "barcode"
- Gene-specific portions of each probe hybridize to the target mRNA
- Repeat segments are used for biotin-conjugated capture and alignment → direct molecule counting by imaging analysis
- Advantages: *highly sensitive*, comparable results with qPCR, lack of amplification-induced bias, high multiplex capability with minimal input (500 targets with 100 ng of RNA), *minimal hands-on tech time*

Prosigna: Technology

- The PAM50 expression results are used to calculate a risk of recurrence score (ROR)
  - Low, intermediate, and high risk groups
  - The score is based on the intrinsic subtype and pathologic characteristics (T,N), with special weighting given to a set of proliferation associated genes

Completed Trials

- Oncotype Dx
  - ATAC Trial: confirmation of performance in node negative and node positive HR+ disease
  - ECOG E5194: adaptation to DCIS prognosis
- Mammaprint
  - TRANSBIG: performance in node positive disease
- Prosigna
  - ATAC Trial: comparison of PAM50 vs. Oncotype vs. IHC4

GENOMIC HEALTH: ONCOTYPE EXTENSION AND DCIS DEVELOPMENT

Prediction of Risk of Distant Recurrence Using the 21-Gene Recurrence Score in Node-Negative and Node-Positive Postmenopausal Patients With Breast Cancer Treated With Anastrozole or Tamoxifen: A TransATAC Study

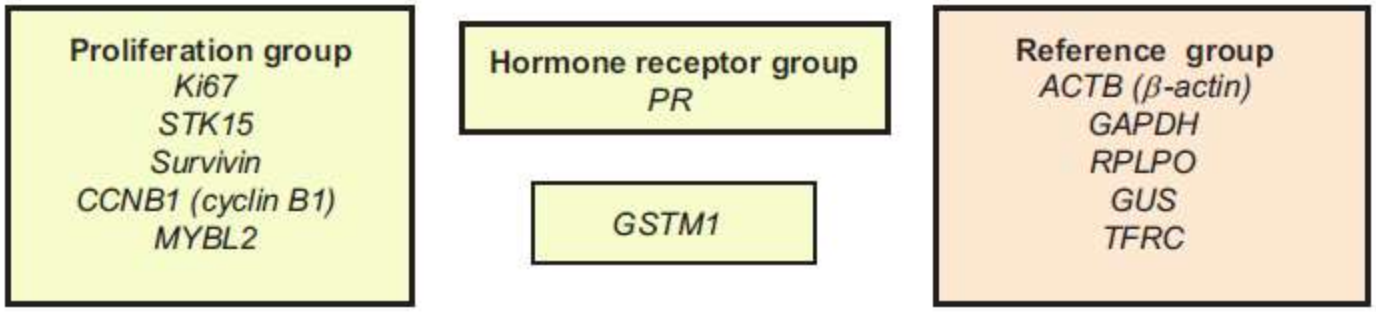
Mitch Dowsett, Jack Cuzick, Christopher Wale, John Forbes, Elizabeth A. Mallon, Janine Salter, Emma Quinn, Anita Dunbier, Michael Baum, Aman Buzdar, Anthony Howell, Roberto Bugarini, Frederick L. Baehner, and Steven Shak

JCO 2010

- Postmenopausal women, localized breast cancer, treated with 5 years of tamoxifen or anastrazole
- 1,231 patients evaluated
  - N0 = 872 N1+ = 306

DR Rates	N0	N1+
RS low (<18)	4%	17%
RS int (18-30)	12%	28%
RS high (>30)	25%	49%

Genomic Health: Expansion to DCIS



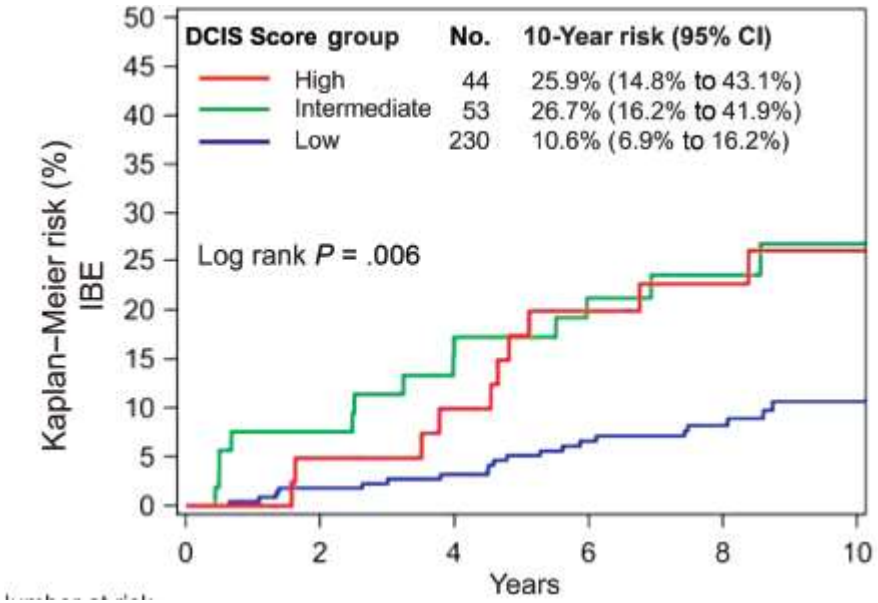
- A subset of the 21 gene panel
- Question: define the 10 year risk for an ipsilateral breast event (IBE) in women who are treated with surgery alone (no XRT)
- 327 patients from ECOG E5195

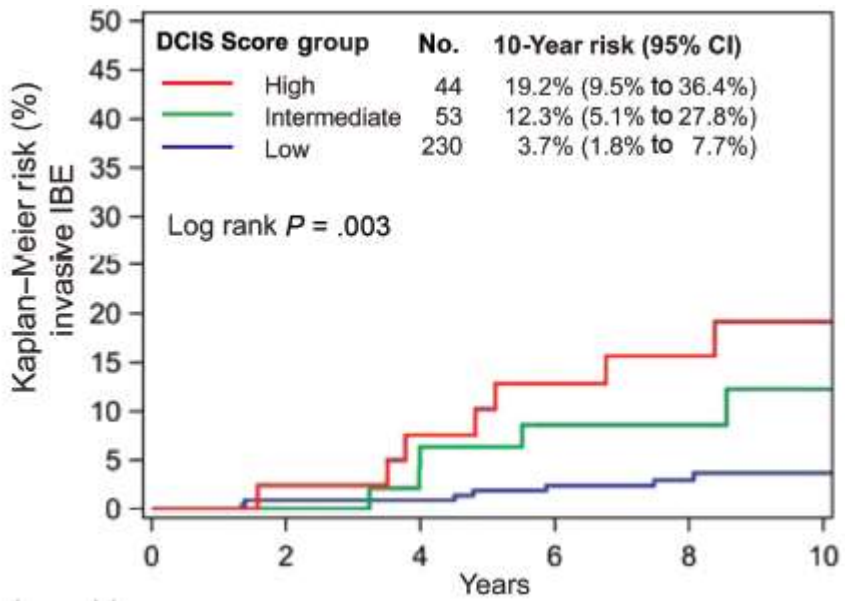
Solin LJ et al., A Multigene Expression Assay to Predict Local Recurrence Risk for Ductal Carcinoma In Situ of the Breast. J Natl Cancer Inst;2013;105:701–710.

Genomic Health: Expansion to DCIS

- Two cohorts
  - Low to intermediate grade DCIS < 2.6 cm (N = 273)
  - High grade DCIS < 1.1 cm (N = 54)
- 46 Patients with an “IBE” in 10 year follow up
  - 26 DCIS only; 20 invasive carcinoma
  - Low/int: 14.6%
  - High: 19.0%
  - Not statistically signficant based on these parameters alone

Genomic Health: Expansion to DCIS

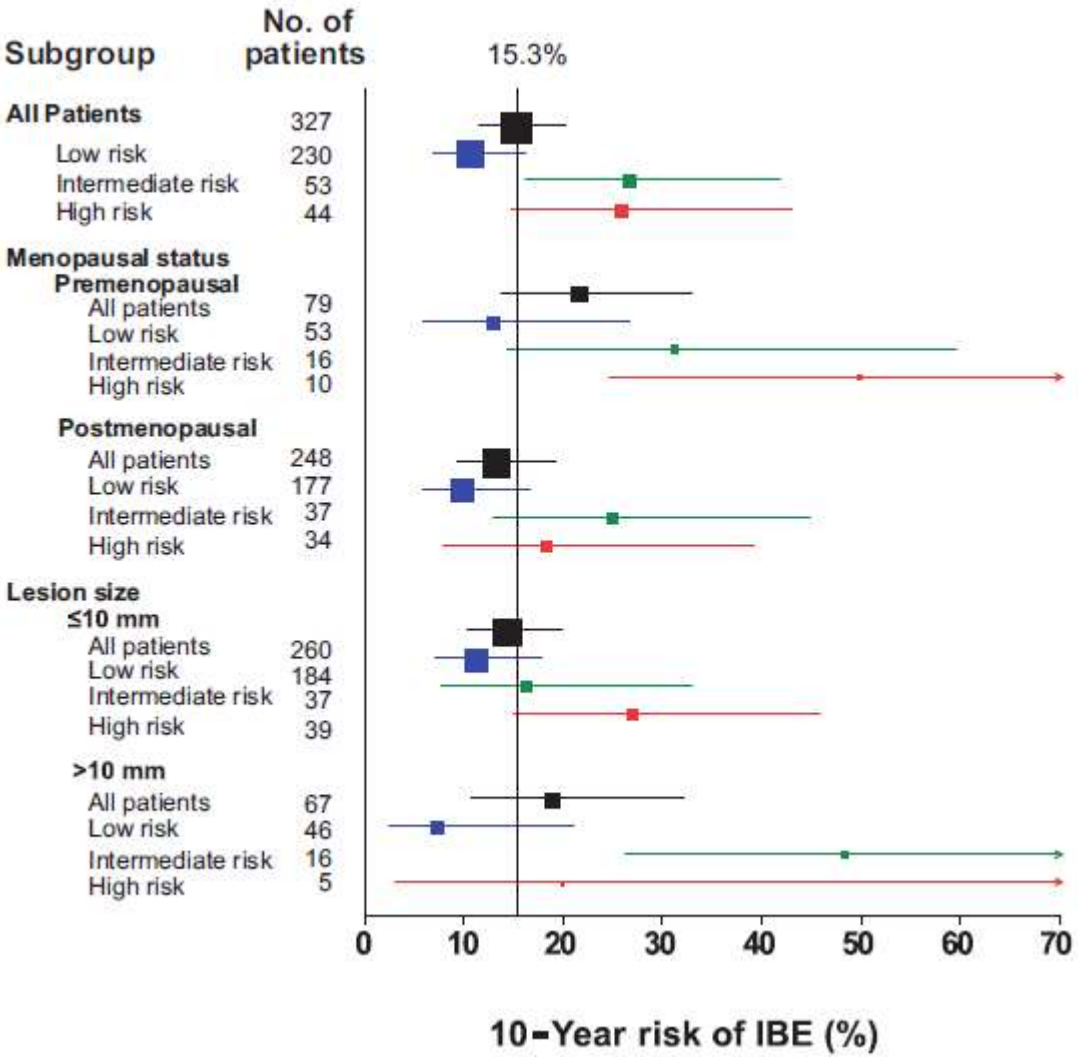




- In multi-variate analysis, the DCIS score, tumor size, and menopausal status were significantly associated with IBE ($P \leq 0.02$)
- The Oncotype RS score was NOT associated with development of an IBE
- Neither the DCIS score nor RS were associated with contralateral cancer

Solin LJ et al., *JNCI*, May 2013

Genomic Health: Expansion to DCIS



The DCIS score provides additional prognostic information

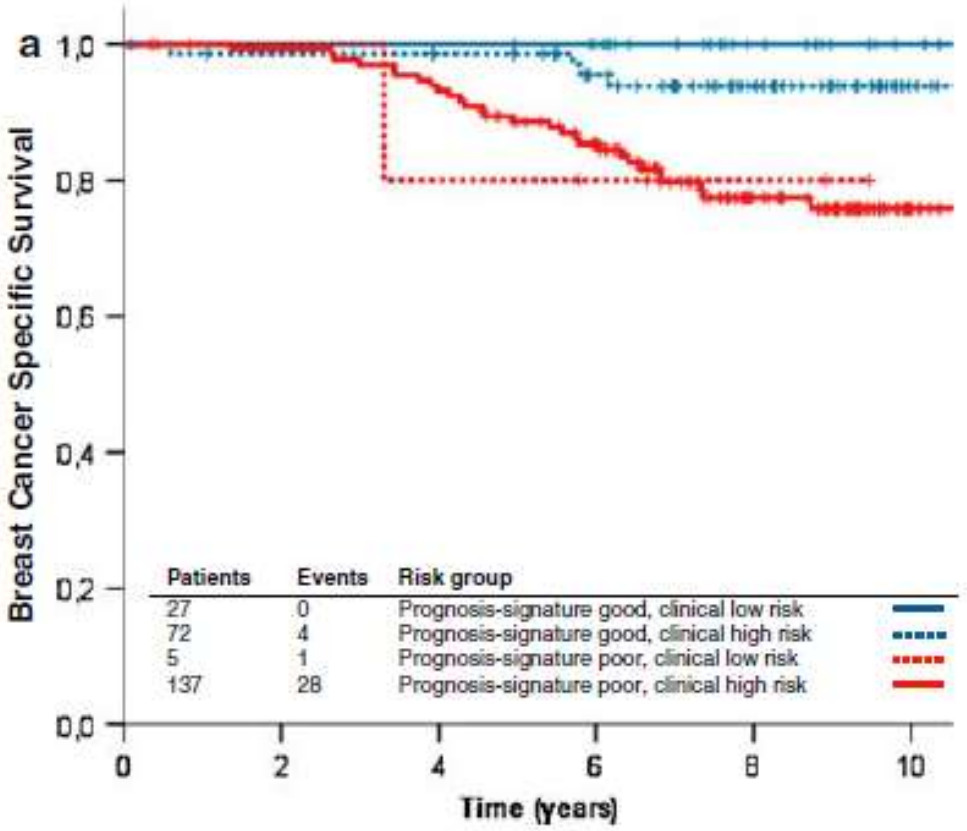
MAMMAPRINT: VALIDATION IN LN+ DZ AND MOLECULAR SUBTYPES

The 70-gene prognosis-signature predicts disease outcome in breast cancer patients with 1–3 positive lymph nodes in an independent validation study

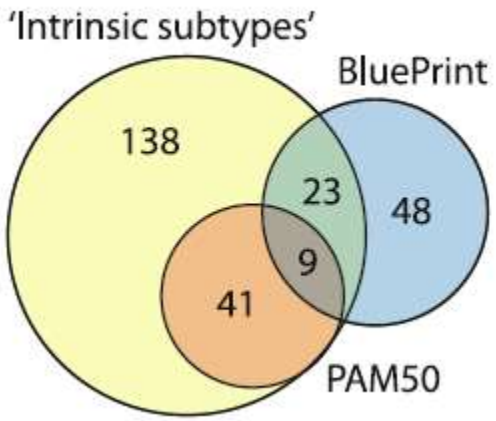
Stella Mook · Marjanka K. Schmidt · Giuseppe Viale · Giancarlo Pruneri · Inge Eekhout · Arno Floore · Annuska M. Glas · Jan Bogaerts · Fatima Cardoso · Martine J. Piccart-Gebhart · Emiel T. Rutgers · Laura J. van't Veer · On behalf of the TRANSBIG consortium

Breast Cancer Res Treat (2009) 116:295–302

- 241 patients with T1-3, N1 disease
  - 79% ER+
  - 15% HER2+
- 99 (41%) good signature
- 142 (59%) poor signature
- HR=7.17 (95% CI 1.81-28.43) in a multivariate model
- Added value to clincal-pathologic factors



Agendia BluePrint: Concordance with the Intrinsic Gene Set



- The Perou classifier was developed without consideration of ER/PR/HER2
- BluePrint explicitly started with ER/PR/HER2
- Included in the 9 overlapping genes:
  - ESR1, PGR, ERBB2
- Overall 83% concordance

Molecular subtyping by MSP	Clustering of patients by "intrinsic gene set"					
	Luminal A	Luminal B	Basal-like	Her2-like	"Normal-like"	Total
Low-risk MammaPrint Luminal-type	**83**	**9**	0	0	14	106
High-risk MammaPrint Luminal-type	**34**	**39**	2	0	9	84
Basal-type	0	0	**44**	0	2	46
HER2-type	6	7	7	**35**	4	59
Total	123	55	53	35	29	**295**

The number in bold indicates the number of samples for which the classification by both methods is in agreement

Krijgsman O et al., Breast Cancer Res Treat (2012) 133:37–47

PAM50: HEAD TO HEAD WITH ONCOTYPE AND IHC4 IN ATAC

Comparison of PAM50 Risk of Recurrence Score With Oncotype DX and IHC4 for Predicting Risk of Distant Recurrence After Endocrine Therapy

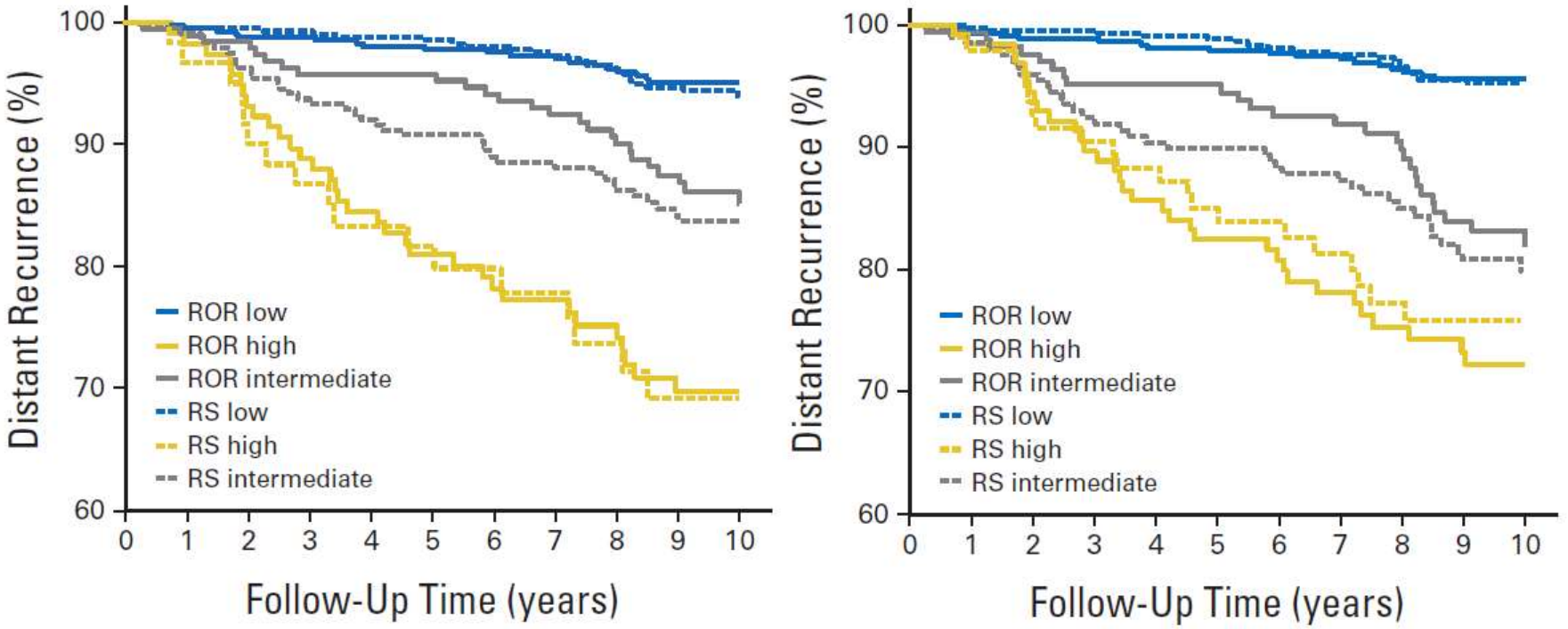
Mitch Dowsett, Ivana Sestak, Elena Lopez-Knowles, Kalvinder Sidhu, Anita K. Dunbier, J. Wayne Cowens, Sean Ferree, James Storhoff, Carl Schaper, and Jack Cuzick

VOLUME 31 · NUMBER 22 · AUGUST 1 2013

JOURNAL OF CLINICAL ONCOLOGY

- 1,017 patients from ATAC trial
  - ER+ breast cancer
  - Received either anastrazole or tamoxifen
- Assessments compared for prediction of DR
  - **ROR** (PAM50) by nCounter
  - **RS** (Oncotype)
  - **IHC4**: a recurrence risk score based on IHC for ER, PR, HER2, and Ki67
    - Largely equivalent to the RS
    - Cuzick, J et al., *J Clin Oncol 29:4273-4278* (2011)

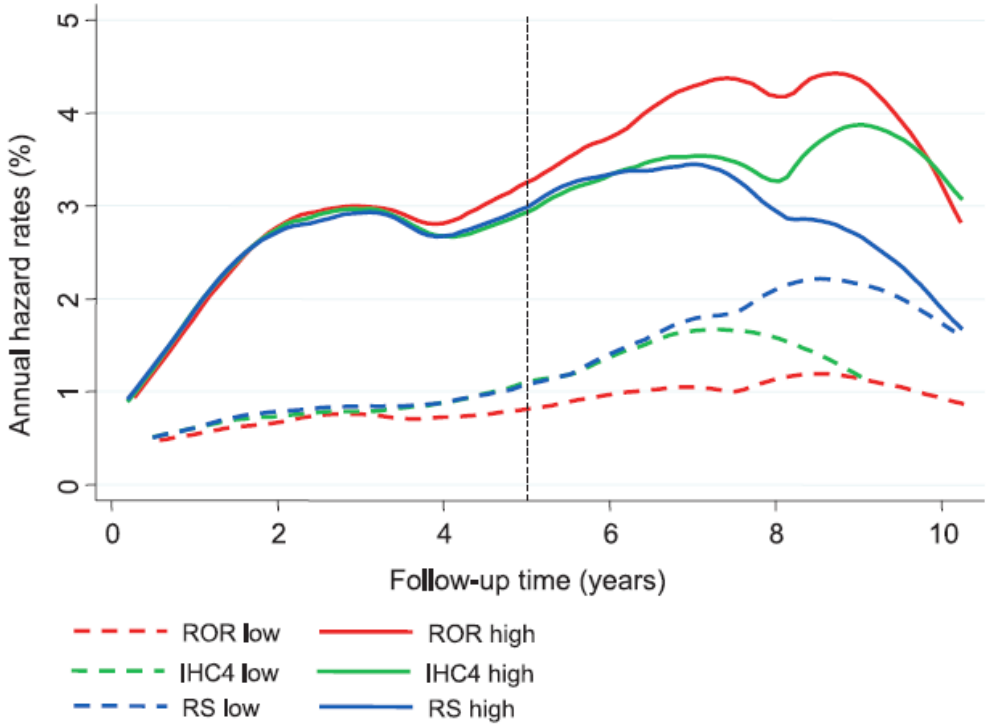
PAM50 v. Oncotype



- KM curves without (left) and with (right) clinicopathologic variables considered
- Major advantage to PAM50: fewer patients scored intermediate and thus better risk discrimination for that category

PAM50 v. Oncotype v. IHC4

- Prognostic value of each assay was compared by changes in LR values (LR – Δ χ^2) and by concordance indices
- ROR performed objectively better (by a small margin) in prognostication



Sestak, I et al., *JNCI,* 2013 Oct 2;105(19):1504-1511

Ongoing Trials

- Oncotype Dx
  - TAILORx: Prospective ER+, node negative disease to shrink the "intermediate" category to 11-25
  - RxPONDER: Prospective ER+, node positive disease with RS < 25, randomized to endocrine alone or endocrine + chemotherapy
- Mammaprint
  - MINDACT: Prospective N0-N1 disease to compare clinical vs. genomic risk prediction for adjuvant chemotherapy decision
  - I-SPY2: Neoadjuvant therapy for locally advanced breast cancer (tumor > 3cm)
- Prosigna
  - RxPONDER secondary endpoint: head to head comparison of Oncotype and PAM50
    - Should further clarify the performance in "intermediate" cases

Summary

	Mammaprint	Oncotype Dx	Prosigna
Input Material	Fresh frozen FFPE	FFPE	FFPE
Platform	Microarray	qPCR	nCounter
# Genes Analyzed	70	21	50+5
Target Patient Population	Stage I-II	ER+ Stage I-II	Stage I-II (Stage I-III)
Regulatory	FDA Cleared (Frozen)	NCCN/ASCO Guidelines	FDA Cleared
Performance Site	Central	Central	Decentralized Kit Format
Features	Binary stratification; Molecular subtypes; Lots of data	Gold standard ER+ cancer; Now in DCIS	ROR compares well; Innovative technology; *Intrinsic subtypes*