Genetic Markers in Triple-Negative Breast Cancer

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Abstract

Triple-negative breast cancer (TNBC) accounts for 15% to 20% of breast cancer cases and is characterized by the absence of estrogen, progesterone, and human epidermal growth factor 2 receptors. Though TNBC is a highly heterogenic and aggressive disease, TNBC patients have better response to neoadjuvant therapy compared to other breast cancer subtypes. Nevertheless, patients with residual disease have a very poor prognosis, with higher probability of relapse and lower overall survival in the first years after diagnosis. TNBC has 6 subtypes with distinct molecular signatures with different prognoses and probably different responses to therapy. The precise stratification of TNBC is therefore crucial for the development of potent standardized and targeted therapies. In spite of intensive research into finding new molecular biomarkers and designing personalized therapeutic approaches, *BRCA* mutational status is the only clinically validated biomarker for personalized therapy in TNBC. Recent studies have reported several promising biomarkers that are currently being validated through clinical trials. The objective of this review was to summarize the clinically relevant genetic markers for TNBC that could serve as diagnostic, prognostic, or predictive or could improve personalized therapeutic strategies.

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Introduction

Breast cancer is the leading cause of cancer death in women worldwide, and triple-negative breast cancer (TNBC) accounts for approximately 15% to 20% of all new cases. All TNBC subtypes share a common gene expression pattern: the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2; also known as ERBB2) expression.[1,2] Despite these shared features, TNBC is a highly heterogeneous disease that can be divided into many distinct subgroups according to clinical, histopathologic, and molecular profiles.[3] TNBC patients are typically young (< 40 years), are African American, and have shorter progression-free survival and overall survival (OS) relative to non-TNBC breast cancer patients.[4-8] The disease also follows a more aggressive course, characterized by higher relapse rates and worse prognosis, than hormone receptor−positive tumors.[4,5] The insidiousness of TNBC lies in the high prevalence of highly proliferating grade 3 tumors at diagnosis.[9] Additional features of TNBC include a peak in recurrence between 1 and 3 years after diagnosis (hazard ratio = 2.6; $P < .0001$), as well as a majority of deaths occurring within 5 years of therapy (hazard ratio = 3.2; $P < .0001$) compared to non-TNBC phenotypes.[7] TNBC patients usually experience better pathologic complete response rates (pCR) after neoadjuvant chemotherapy (pCR rates in 30%-40%). Moreover, TNBC patients who experience pCR have excellent long-term clinical outcome. However, TNBC patients with residual disease after neoadjuvant chemotherapy have very poor prognosis.[1] The recurrence of TNBC is associated with a high risk of metastasis to the lungs or central nervous system, a lower risk of bone metastasis, and a dismal median survival of approximately 1 year.[7,10-12]

The intricacy of this disease is further illustrated by the high prevalence of rare histopathologic subtypes such as metaplastic (90%), medullary (95%), and apocrine (40%-60%) carcinomas.[13] When both the poor prognosis facing TNBC patients and the lack of a recognized predictor of therapy response are considered, the need to identify specific markers that can be targeted by tailored therapies or used to predict response to chemotherapy is indisputable.

This review focuses on genetic alterations in TNBC that could serve as predictive markers of prognosis, which will help in selecting a suitable chemotherapy approach and/or inspire further research.

Intrinsic Subgroups of Breast Cancer

Breast cancer comprises a heterogeneous group of diseases that can be, according to gene expression profiles, classified into luminal

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A, luminal B, basal-like, normal-like and HER2-enriched subgroups.[14,15] The PAM50 assay, a 50-gene subtype predictor, was developed on the basis of these expression profiles.[16] These so-called intrinsic subgroups of breast cancer show differences in incidence, age at diagnosis, prognosis, and response to treatment.[15,17]

At the morphologic level, TNBC and basal-like breast cancer (BLBC) are similar in terms of larger tumor size, higher grade, presence of geographic necrosis, enhanced invasive potential, and stromal lymphocytic infiltration.[7,12,18,19] However, the gene expression profiles of only 71% of TNBC samples are clustered as basal-like. Moreover, only 77% of the basal-like tumors bear TNBC signatures.[20] This observation was confirmed through the molecular characterization of 412 TNBC and 473 basal-like (based on PAM50 subtype prediction) breast cancer samples.[21] Using this approach, 21.4% of TNBC samples were not assigned as BLBC, and 31.5% of BLBC samples did not display a TNBC profile. Out of 412 TNBC samples, 78.6% were identified as BLBC, 7% as normal-like, 7.8% as HER2 enriched, 4.4% as luminal B, and 2.2% as luminal A.

TNBC Subtypes

Once the molecular heterogeneity of TNBC was recognized, subsequent research focused on classifying TNBC subtypes on the basis of disease prognosis or the expected response to systemic therapy. Groundbreaking work identified 6 different TNBC gene expression profile subtypes from 587 TNBC cases identified in 21 gene expression data sets using a top-down approach of hierarchical clustering.[22] The subtypes were named according to their expression patterns: basal-like 1 and 2 (BL1/2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem–like (MSL), and luminal androgen receptor (LAR) (Table 1). Following this classification, approximately 30 TNBC cell lines have been identified as models of the distinct subtypes and are used to investigate which pharmacologic strategies are most effective against each subtype.

Both BL1 and BL2 subtypes are sensitive to DNA-damaging agents (such as cisplatin) and show elevated expression of cell-cycle and DNA damage-response genes. While BL1 is characterized by heightened expression of both cell division and DNA damage-response genes, as well as elevated Ki-67 expression, BL2 displays up-regulated growth factor signaling, glycolysis, and gluconeogenesis along with increased expression of myoepithelial markers.[22]

Both M and MSL subtypes are characterized by decreased distant metastasis-free survival and positive response to phosphoinositide 3-kinase (PI3K)/mechanistic target of rapamycin (mTOR) inhibitors and dasatinib. The gene expression profiles of the M and MSL subtypes overlap with that of chemoresistant metaplastic breast cancer and display the up-regulation of genes involved in epithelial–mesenchymal transition, cell motility, extracellular matrix remodeling, and cellular differentiation. Unlike the M subtype, which displays overexpression of proliferation genes, the MSL subtype is enriched in mesenchymal stem-cell–associated genes and shows up-regulation of genes involved in angiogenesis and growth factor pathways. The MSL subtype overlaps with the previously described claudin-low subtype, as both demonstrate reduced claudin 3, 4, and 7 expression.[22,24]

The IM subtype is characterized by increased expression of immune signaling genes (immune cell and cytokine signaling, antigen processing and presentation, core immune signaling pathways). The IM expression profile overlaps with the molecular signature of medullary breast cancer, and both classifications share a good prognosis.[2,25] Expression profile of IM subtype is generated by tumor-infiltrating lymphocytes (TILs) rather than tumor cells itself.[26] The robust presence of TILs has been found in approximately 20% of TNBC and was found to be an independent prognostic marker in TNBC. The BIG 02-98, ECOG 2197, and ECOG 1199 trials demonstrated very similar results, with 15% to 20% reduction in any recurrence and mortality for every 10% increase in stromal TILs.[27-29] The presence of TILs is associated with better response to both adjuvant and neoadjuvant therapy, and could serve as marker of better outcome when detected in residual tumor after neoadjuvant therapy.[27,30-32]

The final subtype, LAR, is enriched in genes involved in hormone signaling, steroid synthesis, and androgen/estrogen metabolism, including overexpression of androgen receptor (AR) and its

Table 1 Triple-Negative Breast Cancer Subtype Characterization[22]

Subtype	Signaling Pathways	Important Markers	Chemosensitivity[23]	Potential Therapy
BL1	Cell cycle, proliferation, DNA damage pathways	*ATR, BRCA, MYC, NRAS, Ki-67*	Very good	Cisplatin, PARP inhibitors
BL2	Cell cycle, proliferation, growth factor signaling, glycolysis, gluconeogenesis	*EGFR, MET, EPHA2, TP53*	Very poor	Cisplatin; PARP and growth factor inhibitors
IM	Immune cell signaling processes	*JAK1/2, STAT1/4, IRF1/7/8, TNF*	Medium	—
M	EMT, cell motility, differentiation, proliferation	*Wnt, ALK, TGF-β*	Medium	PI3K/mTOR, Src inhibitors
MSL	EMT, cell motility, differentiation, growth factor signaling, angiogenesis	*EGFR, PDGFR, ERK1/2, VEGFR2*	Medium	PI3K/mTOR, Src inhibitors
LAR	Androgen/estrogen metabolism, steroid synthesis, porphyrin metabolism	*AR, FOXA1, KRT18, XBP1*	Poor	AR antagonist; PI3K, Hsp90 inhibitors

Abbreviations: *ALK* = anaplastic lymphoma receptor tyrosine kinase; *AR* = androgen receptor; *ATR* = ataxia telangiectasia and Rad3 related; *BL1* = basal-like 1; *BL2* = basal-like 2; *BRCA* = breast cancer gene; *EGFR* = epidermal growth factor receptor; EMT = epithelial–mesenchymal transition; *EPHA2* = ephrin type A receptor 2; *ERK1/2* = mitogen-activated protein kinase 1/2; *FOXA1* = forkhead box A1; Hsp90 = heat shock protein 90; IM = immunomodulatory; *IRF1/7/8* = interferon regulatory factor 1; *JAK1/2* = Janus kinase 1/2; Ki-67 = marker of proliferation Ki-67; *KRT18* = keratin 18; LAR = luminal androgen receptor; M = mesenchymal; *MET* = hepatocyte growth factor receptor; MSL = mesenchymal stem–like; *mTOR* = mechanistic target of rapamycin; *MYC* = MYC proto-oncogene; *NRAS* = neuroblastoma Ras; PARP = poly(ADP-ribose) polymerase; *PDGFR* = platelet-derived growth factor receptor; PI3K = phosphoinositide 3-kinase; *Src* = SRC proto-oncogene; *STAT1/4* = signal transducer and activator of transcription 1/4; *TGF-β* = transforming growth factor β; *TNF* = tumor necrosis factor; *TP53* = tumor protein P53; *VEGFR2* = vascular endothelial growth factor receptor 2; *Wnt* = Wnt family member; *XBP1* = X-box binding protein 1.

downstream targets and coactivators. Patients with the LAR subtype show shorter relapse-free survival. This subtype overlaps with the previously described molecular apocrine group.[33] One possible therapy regimen for this subtype targets the AR antagonist (ie, flutamide, enzalutamide, bicalutamide).[34] Moreover, LAR-subtype cell lines are sensitive to PI3K inhibitors as a result of a mutation in the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA) kinase domain.[2]

A study clarifying the clinical relevance of the 7-subtype classification of TNBC revealed differences in pCR after neoadjuvant chemotherapy ($P = .044$) and found the TNBC subtype to be an independent predictor of pCR status ($P = .022$) by a likelihood ratio test. BL1 showed the highest pCR rate (52%), while BL2 and LAR subtypes showed the lowest rates (0% and 10%, respectively).[23]

Genetic Markers in TNBC

The molecular and genetic profiles of TNBC, known for its enormous complexity and diversity, continue to challenge researchers all around the world. As mentioned above, TNBC tumors are characterized by the lack of ER, PR, and HER2 expression. The lack of therapeutic targets complicates efforts to characterize TNBC with certain molecular markers in a bid to improve disease outcome. To date, two large studies have focused

Table 2 Summary of Triple-Negative Breast Cancer Important Genetic Markers

Gene	Localization	Alteration Type	Main Function	Prognostic Significance	Predictive Significance	References
TP53	17p13.1	Inactivating mutation	Genome integrity, DNA repair and apoptosis	Poor prognostic factor, worse OS and increased metastatic risk	Poor response to chemotherapy	37-43
BRCA1	17q21.31	Inactivating mutation, epigenetic changes	DNA double-strand break repair	Poor prognostic factor	Higher response to neoadjuvant anthracycline and taxane therapy, response to platinum-based therapy, potential predictor for response to PARP inhibitors	23,44-50
BRCA2	13q13.1					
PIK3CA	3q26.32	Activating mutation	Survival, differentiation, proliferation	Poor prognostic factors	Potential predictors for response to PI3K/AKT/mTOR inhibitors	51-53
PTEN	10q23.31	Deletion, inactivating mutation			Higher sensitivity to combination therapy of PI3K and androgen receptor inhibitors	54,55
INPP4B	4q31.21	Deletion				
EGFR	7p11.2	Amplification, overexpression	Cell proliferation, metastasis	Poor prognostic factor	Potential predictor for response to anti-EGFR therapy	56-58
FGFR1	8p11.23	Amplification	Proliferation, survival, migration, differentiation	Unknown	In vitro sensitivity to FGFR ATP-competitive inhibitor brivanib	59-62
FGFR2	10q26.13				In vitro sensitivity to FGFR ATP-competitive inhibitor PD173074	59,63
VEGFRA	6p21.1	Overexpression, amplification, mutation	Angiogenesis, invasion, metastases	Unknown	Addition of bevacizumab to chemotherapy significantly elevates pCR rates	28,48,64-67
VEGFRB	11q13.1					
VEGFRC	4q34.3					
AR	Xq12	Overexpression	Cell signaling	Controversial; probably better DFS and OS	Lower sensitivity to chemotherapy, higher sensitivity to AR inhibitors (enzalutamide, bicalutamide), PI3K inhibitors, and their combination	23,68-72
BCL2	18q21.33	Overexpression	Antiapoptotic	Positive prognostic factor	Negative predictor of response to neoadjuvant and adjuvant anthracycline-based chemotherapy, positive predictor of response to CMF treatment	73-76

Abbreviations: *TP53* = tumor protein P53; *PTEN* = phosphatase and tensin homolog; *INPP4B* = inositol polyphosphate-4-phosphatase type II B; *EGFR* = epidermal growth factor receptor; *VEGFR A/B/C* = vascular endothelial growth factor receptor A/B/C; *AR* = androgen receptor; *BCL2* = B-cell lymphoma 2; DFS = disease-free survival; OS = overall survival; PARP = poly(ADP-ribose) polymerase; *PI3K* = phosphoinositide 3-kinase; *AKT* = AKT serine/threonine kinase; *mTOR* = mechanistic target of rapamycin; pCR = pathologic complete response; CMF = cyclophosphamide, methotrexate, 5-fluorouracil; *BRCA1/2* = breast cancer gene 1/2; *FGFR1/2* = fibroblast growth factor receptor 1/2; *PIK3CA* = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α.

on the genetic basis of TNBC.[35,36] Genetic markers that influence prognosis and/or prediction of appropriate therapy are summarized in Table 2 and visualized in Figure 1.

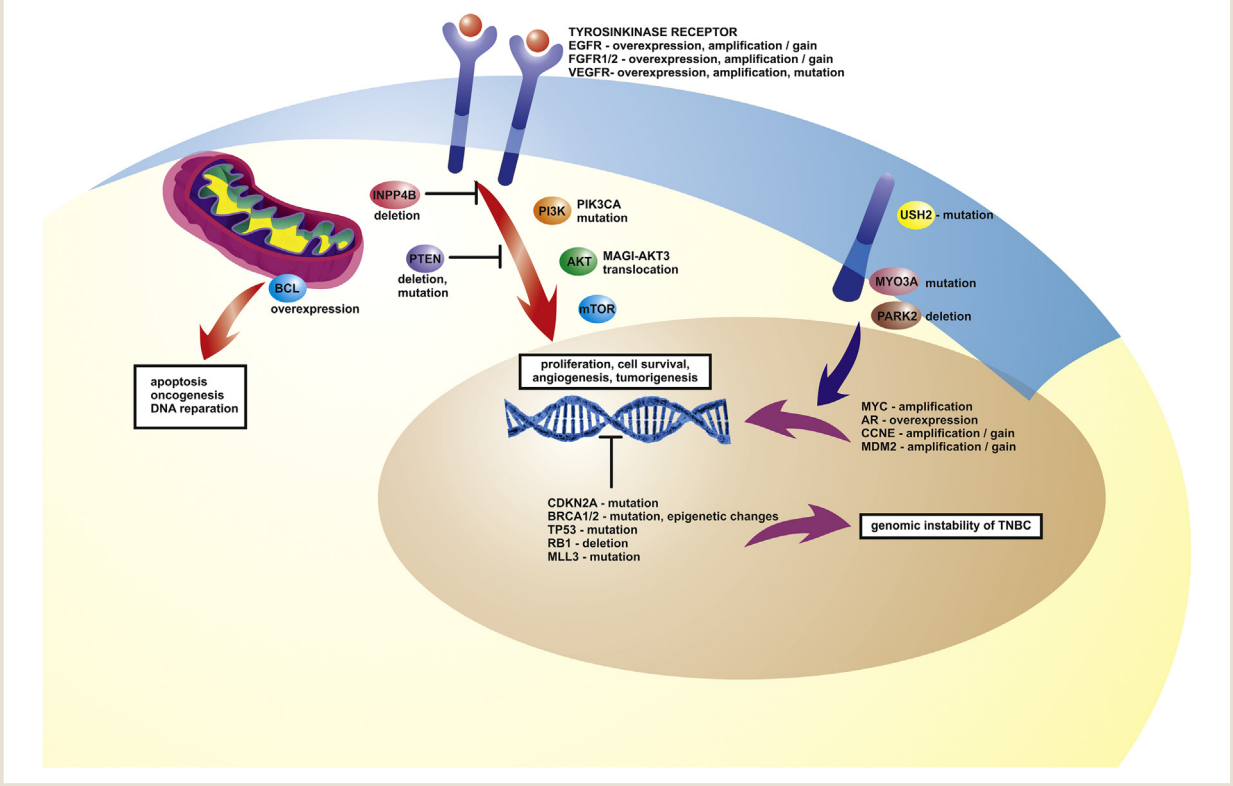
The exome-sequencing, RNA-sequencing, high-resolution single nucleotide polymorphism arrays and targeted deep resequencing were performed on 104 primary TNBC samples grouped into various subsets to reveal the patterns of somatic mutation.[35] The most frequent copy number aberrations were identified for the *PARK2* (Parkinson disease 2) (6%), *RB1* (retinoblastoma gene 1) (5%), *PTEN* (phosphatase and tensin homolog) (3%), and *EGFR* (epidermal growth factor receptor) (5%) genes. *TP53* mutations were found to be the most common somatic aberration, observed in 53.8% of cases, while the TNBC samples also showed frequent mutations in the *PIK3CA* (10.2%), *USH2A* (usher syndrome 2A) (9.2%), *MYO3A* (myosin IIIA) (9.2%), *PTEN*, and *RB1* genes (7.7%). However, only a minority of mutations (36%) were transcribed into mRNA.[35]

The Cancer Genome Atlas Group analyzed samples from 463 patients using genomic DNA copy number arrays, DNA methylation, exome sequencing, mRNA arrays, microRNA sequencing, and reverse-phase protein arrays.[36] In a group containing 93 basal-like tumors (76 TNBCs), the most commonly mutated genes were found to be *TP53* (80%), *PIK3CA* (9%), *MLL3* (lysine methyltransferase 2C) (5%), *AFF2* (AF4/FMR2 family member 2) (4%), *RB1* (4%), and *PTEN* (1%). Copy number alterations were observed in several chromosomal regions or genes, namely amplification or gain of *MYC* (MYC protooncogene) (40%), (E3 ubiquitin-protein ligase Mdm2) (14%), *CCNE* (cyclin E1) (9%), as well as the 1q and 10p regions, along with loss of *PTEN*, *RB1*, *INPP4B* (inositol polyphosphate-4-phosphatase type II B) (30%), and the 8p and 5q regions. Heightened *CDKN2A* (cyclin-dependent kinase inhibitor 2A) expression, decreased *RB1* expression, and high genomic instability were also found to be typical features of the BLBC profile.[36]

The discovery of the fusion gene *EML4-ALK* (echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase) in non–small-cell lung cancer fueled interest in finding such a structural rearrangement in breast carcinomas, particularly in TNBC.[77,78] An enrichment in most known *MAGI3-AKT3* (membrane-associated guanylate kinase–AKT serine/threonine kinase 3) translocation as well as rearrangements involving the *NOTCH1/2* (Notch 1/2) and *MAST* (microtubule-associated serine–threonine kinase) genes, were identified in TNBC by whole exome sequencing.[77,79]

As was mentioned above, TNBC disease showed higher sensitivity to neoadjuvant chemotherapy, but patients with residual

Figure 1 Genetic Markers and Their Aberrations That Influence Prognosis And/Or Prediction of TNBC



Abbreviations: *AKT* = AKT serine/threonine kinase; *BCL* = B-cell lymphoma; *BRCA1/2* = breast cancer gene 1/2; *CDKN2A* = cyclin-dependent kinase inhibitor 2A; *EGFR* = epidermal growth factor receptor; *FGFR1/2* = fibroblast growth factor receptor 1/2; *INPP4B* = inositol polyphosphate-4-phosphatase type II B; *MLL3* = lysine methyltransferase 2C; *mTOR* = mechanistic target of rapamycin; *MYO3A* = myosin IIIA; *PARK2* = Parkinson disease 2; *PI3K* = phosphoinositide 3-kinase; *PTEN* = phosphatase and tensin homolog; *RB1* = retinoblastoma gene 1; *TP53* = tumor protein P53; *USH2* = Usher syndrome 2; *VEGFR* = vascular endothelial growth factor receptor.

disease have very poor prognosis. Identification of targetable alteration in residual tumor is therefore necessary.[80] The genomic profile of tumor has been shown to be frequently altered during chemotherapy. Several studies comparing pretreatment and posttreatment biopsy samples found significant changes mainly in cell-cycle regulators and PI3K/mTOR pathway.[81,82] These genomic changes could be the reason for resistance to conventional chemotherapies and identification of new druggable targets in posttreatment biopsy samples could significantly improve TNBC outcome. Molecular analysis of posttreatment biopsy samples is therefore necessary in TNBC patients who do not experience pCR after neoadjuvant chemotherapy.

TP53 *Gene*

TP53 is one of the most important genes involved in maintaining homeostasis and genomic integrity throughout cell-cycle arrest, DNA repair, and apoptosis. Alterations of *TP53* associated with aberrant p53 expression have been described in numerous types of human cancers as well as in all breast cancer subtypes.[83] Expression of mutant *p53* was found to be associated with high proliferation rate, early disease recurrence, and early death in node-negative breast cancer.[37] In breast cancer, the DNA-binding domain is the most frequently mutated area of the *TP53* gene, and missense substitutions were identified as the culprit behind unfavorable breast cancer outcomes.[35,36,84] While missense mutations have been found to be predominantly associated with the luminal subtype, nonsense and frameshift changes are prevalent in basal-like tumors.[36] Generally, *TP53* mutations are more common in ER-negative breast cancers than in breast cancers with ER expression.[38,85] Moreover, ER-negative patients with p53 expression (TNBC and HER2-positive subtypes) were reported to have a better prognosis, while p53 expression in ER-positive patients was related to a worse prognosis.[38,39]

In TNBC, *TP53* is the most frequently mutated gene, with mutations occurring in 65% to 80% of cases.[35,36] In one of the most extensive studies to date, mutations in *TP53* were found in 62% of basal-like and 43% nonbasal TNBC.[35] In the context of TNBC, these mutations result in increased genetic instability and cytogenetic changes, as well as a higher probability of loss of heterozygosity.[86,87] Recent studies have shown worse OS and increased metastatic risk in TNBC patients with decreased p53 function.[40,56] However, another study did not confirm that mutations of *TP53* and/or p53 expression are prognostic factors; nevertheless, discrepancies between *TP53* mutation and p53 expression could be a potential predictor of poor outcome in TNBC.[88] Other studies found *TP53* mutations to be a predictor of chemoresistance in TNBC.[41-43] Taken together, *TP53* is mutated in a majority of TNBC cases and is therefore an attractive candidate for antitumor therapies.

BRCA1/2

The *BRCA1* and *BRCA2* gene products are vital to the activation and transcriptional regulation of DNA damage, control of the cell cycle, and cellular proliferation and differentiation.[89] More specifically, BRCA1/2 proteins play an essential role in DNA double-strand break repair by homologous recombination (HRR) and the maintenance of DNA stability.[90]

Over 80% of hereditary *BRCA1*-mutated breast cancers are classified as TNBC and/or BLBC, and approximately 15% TNBC patients are carriers of a *BRCA* germ-line mutation (g*BRCA*).[3,91-95] The remaining sporadic TNBC cases frequently share certain characteristics with *BRCA1/2* mutation carriers in HRR defects, sometimes collectively termed BRCAness.[59] This BRCAness status can involve the epigenetic inactivation of *BRCA1* by promoter methylation, which has been associated with poor prognosis in terms of relapse-free survival and OS after anthracycline- or taxane-based therapy.[96] Breast cancers with *BRCA1* mutations as well as BRCAness often express basal markers that correspond with the BL1 subtype and therefore respond to neoadjuvant anthracycline and taxane therapy.[2,23,97] Interesting results were recently published in the POSH study determining the effect of g*BRCA* on breast cancer outcome after systemic therapy. OS at 10 years was 78% in g*BRCA* carriers compared to 69% in *BRCA*-negative cases, suggesting that *BRCA* mutation provided some survival advantage to their carriers.[98] Better survival of g*BRCA* TNBC and probably also BRCAness might be caused by better sensitivity of g*BRCA* carriers to chemotherapy as a result of defects in HRR or higher immune activation.[98-100]

Moreover, patients with deficient BRCA1/2 function should be more susceptible to DNA-damaging agents like platinum derivatives and poly(ADP ribose) polymerase (PARP) inhibitors.[44] The Treating to New Targets (TNT) trial shown the double objective response rate to carboplatin compared to docetaxel in metastatic TNBC tumors carrying g*BRCA* mutations.[101,102] High effectivity of platinum-based therapy in metastatic g*BRCA* TNBC was also demonstrated by other studies.[45] Moreover, not only *BRCA1/2* mutation carriers but also patients with advanced TNBC with defects in the BRCA1/2 pathway (determined by higher values of loss of heterozygosity score and large-scale state transitions score) showed a positive response to platinum therapy in the TBCR009 study.[45] Indeed, the TNT trial did not demonstrate better response to carboplatin in patients with a high homologous recombination deficiency score.[101] Biomarkers of genomic instability that predict a positive response to platinum-based therapy should therefore be validated for a subset of TNBC tumors.[103] In the neoadjuvant setting, the role of g*BRCA* mutation in response to platinum-based therapy is unclear. Several studies demonstrated higher responses in g*BRCA* carriers; nevertheless, the GeparSixto study showed higher responses in patients with wild-type *BRCA*.[46,104-106]

PARP inhibitors present another promising therapeutic tool. PARP inhibition increases the occurrence of irreparable toxic DNA double-strand breaks resulting in cell death in *BRCA*-mutated patients. PARP inhibitors therefore effectively kill the tumor cells through the principle of synthetic lethality. The PARP inhibitors olaparib and rucaparib have been approved for the treatment of advanced previously treated ovarian cancer with g*BRCA*. More recently, olaparib was also approved by the US Food and Drug Administration for treatment of metastatic HER2-negative breast cancer with *BRCA* mutation previously treated with chemotherapy.[107-111] Promising results with PARP inhibitor talazoparib were shown in the EMBRACA study. Talazoparib therapy resulted in significantly prolonged progression-free survival in advanced HER2-negative breast cancer patients with g*BRCA* compared to treatment of physician's choice.[112]

PI3K Pathway

Dysregulation of the PI3K/AKT/mTOR pathway causes changes in cell survival, differentiation, and/or proliferation that are

frequently observed during carcinogenesis.[54] Increased signaling through the PI3K/Akt/mTOR pathway is very common in all breast cancer types, including TNBC.[113] In basal-like tumors, alterations in *PTEN* and *INPP4B* phosphatases are more common than mutations in *PIK3CA*.[35,54] *PIK3CA* mutations are associated with ER positivity and therefore are more frequent in ER-positive breast cancers (luminal and HER2-enriched subtypes).[77,114]

PTEN is an important negative regulator of the PI3K pathway. Loss of PTEN expression has been shown to be significantly associated with ER negativity as well as basal-like phenotype.[115] Loss of PTEN contributes to both rapid tumor cell proliferation and poor prognosis in TNBC.[55] The phosphatase INPP4B, another negative regulator of the PI3K pathway, has been shown to be frequently lost in ER-negative primary breast carcinomas. INPP4B loss is associated with high clinical grade, increased tumor size, loss of hormone receptors, and aggressive basal-like breast cancers.[36,116] In addition, oncogenic mutations of *PIK3CA*, which encodes for a catalytic subunit of PI3K (p110α), occur in about 10% of TNBC cases and can further activate the PI3K pathway. Among the TNBC subtypes, LAR shows the highest prevalence of *PIK3CA* mutations, and in this way the simultaneous therapeutic targeting of AR and PIK3CA could prove beneficial to patients.[51] In addition to the known TNBC cancer-related genes involved in PI3K pathway regulation, the novel MAGI3-AKT3 translocation has been described. This rearrangement occurs in about 7% of TNBC cases and leads to constitutive AKT3 activation and hyperactivation of the PI3K pathway.[77]

In TNBC, PI3K/AKT/mTOR pathway alterations occur frequently and are promising therapeutic targets. Preclinical data have demonstrated that TNBC tumors are more sensitive to combination therapy.[51-53] Clinical trials are currently evaluating the potency of mTOR, PI3K, AKT, and mTOR/PI3K inhibitors for treating TNBC alone or in combination with other therapies (eg, cisplatin, PARP, and AR inhibitors).[114]

Tyrosine Kinase Receptors

Tyrosine kinase receptors from the *EGFR, FGFR* (fibroblast growth factor receptor), and *VEGFR* (vascular endothelial growth factor receptor) families have been reported to be potential clinical targets for treating TNBC.[117,118] The tyrosine kinase receptor EGFR (HER1) mediates cell proliferation, angiogenesis, and metastasis as well as the inhibition of apoptosis by transducing an extracellular signal through a kinase cascade to ultimately initiate the transcription of specific genes. While EGFR overexpression has been described in approximately 60% of TNBC cases, *EGFR* amplification or high copy number has been reported in only 5% to 30% of cases. Moreover, *EGFR* mutations were found to be rare, occurring in about 11% of samples.[56,57] Studies of Asian populations did not find a correlation between EGFR expression and either increased *EGFR* copy number or *EGFR* mutations.[57,119] Nevertheless, a recent study reported *EGFR* copy number to correlate with *EGFR* overexpression and to be associated with poor clinical outcome in TNBC. EGFR overexpression is also influenced by factors other than genomic changes, and *EGFR* copy number status seems to predict the response of TNBC patients to anti-EGFR therapy.[58] A number of clinical trials have evaluated the efficacy of tyrosine kinase inhibitors as well as monoclonal antibodies.[117] However, the results from these clinical trials, which tested EGFR tyrosine kinase inhibitors alone or in combination with chemotherapy, have so far been disappointing.[117] Similarly, clinical trials investigating anti-EGFR monoclonal antibodies in monotherapy or combination therapy have not yet provided any promising results.[120-123] Despite predominantly unsuccessful studies, a small proportion of TNBC patients have disease that responds positively to anti-EGFR therapy. Therefore, identification of patients with EGFR activations who may profit from anti-EGFR therapy is crucial.

FGF receptors mediate proliferation, survival, migration, and differentiation. As such, they could be a promising target for treating a subset of TNBC patients. Amplifications of *FGFR1* or *FGFR2*, with respective frequencies of 9% and 4% in TNBC, may act as driver mutations, whereas mutations in *FGFR* genes are less common in TNBC.[59-61] Two studies have shown that *FGFR2* amplification leads to constitutive activation of the receptor in TNBC cell lines, and that this subset of cells is sensitive to the FGFR ATP-competitive inhibitor PD173074.[59,63] Cell lines with *FGFR1* amplifications were shown to be sensitive to the FGFR ATP-competitive inhibitor brivanib.[62] These results concerning FGFRs, along with the fact that alterations in *FGFR* genes occur in more than 10% of TNBC cases, make this family of tyrosine kinase receptors an attractive therapeutic target. Ongoing clinical trials will, we hope, clarify the effectiveness of FGFR inhibitors in breast cancer patients.

The VEGFR family has also been explored as a potential therapeutic target because it plays an essential role in angiogenesis, which affects cancer development, invasion, and metastasis.[64] Even though *VEGFR* amplifications or mutations are rare in TNBC, a number of clinical trials have confirmed that the addition of bevacizumab to chemotherapy significantly elevates pCR rates in TNBC patients.[28,65,66] Higher pCR rates occur in TNBC patients treated with bevacizumab; interestingly, the best responses to bevacizumab were associated with high VEGFR1 levels.[67] The effect of the multitarget tyrosine kinase receptor inhibitor sunitinib on TNBC has been evaluated in several studies, but this inhibitor was not found to be any more effective than other previously reported therapeutic approaches.[124,125]

Androgen Receptor

AR, as well as ER and PR, belongs to the nuclear steroid hormone receptor family.[126] AR plays an important role in cell signaling through the Wnt pathway and regulates genes involved in metastasis,[68] FOXA1, PTEN, and p53 along with other cell-cycle regulators, and the PI3K/AKT/mitogen-activated protein kinase signaling pathway.[127] AR expression has been found in approximately 70% of breast cancers, and it is associated with ER positivity.[47,128] In breast cancer, AR positivity is more common in older women and is associated with lower stage, nuclear grade, and risk of lymph node involvement as well as smaller tumor size at diagnosis, decreased risk of recurrence, and better OS and disease-free survival.[69,129,130] In TNBC, AR positivity is present in 13% to 37% of cases and is associated with LAR subtype and older age at presentation.[130,131] The prognostic significance of AR positivity is controversial; AR positivity has been associated with both favorable and poor prognoses in previous studies.[69,70,131-133] AR-positive

TNBC has a lower Ki-67 index than AR-negative TNBC and could therefore be less sensitive to chemotherapy,[134] which is in accordance with findings that the LAR subtype has lower pCR rates relative to other TNBC subtypes.[23]

Preclinical in vitro and xenograft studies have demonstrated that cell line models of LAR subtype are partially dependent on AR signaling.[22,135] Small interfering RNA knockdown and pharmacologic inhibition of AR both substantially decreased cell viability and tumor growth. Moreover, all analyzed LAR cell lines showed an activating mutation in the kinase domain of *PIK3CA* (H1047R) and were therefore sensitive to PI3K inhibitors.[2] In AR-positive TNBC, *PIK3CA* mutations were reported in approximately 40% of cases.[51] Studies using in vitro experiments and xenograft models have shown that the treatment of both LAR and non-LAR TNBC subtypes with the AR inhibitors enzalutamide and bicalutamide reduces proliferation, anchorage-independent growth, migration, and invasion, and increases apoptosis.[71,72] Therefore, a positive response to AR antagonists is probably not limited to the LAR TNBC subtype. However, the TBCRC011 study showed a relatively weak response, with a 6-month clinical benefit rate of 19% for bicalutamide in AR-positive patients compared to 18% in the intention-to-treat population.[136] In a MDV3100-11 study, enzalutamide showed higher clinical activity, with a 6-month clinical benefit rate of 28%, for AR-positive patients compared to 20% in the intention-to-treat population.[137]

Future studies should focus on elucidating the mechanisms of AR therapy resistance and how to select patients who will show the optimal response. Other therapeutic approaches, such as CYP17 (cytochrome P450 family 17 subfamily a member 1) inhibitors or the combination of AR inhibitors with CDK4/CDK6 (cyclin-dependent kinase) inhibitors, PI3K inhibitors or neoadjuvant chemotherapy, are still being investigated.[130]

The therapeutic value of screening for AR positivity is that this is an easily detectable marker than can identify subgroups of TNBC patients who will derive minimal clinical benefit from standard chemotherapy. AR-dependent TNBC patients could benefit from targeted therapy based on AR antagonists alone or in combination with other chemical agents.

BCL2 *Gene*

B-cell lymphoma 2 (BCL2) is a mitochondrial protein known for its antiapoptotic and oncogenic effects. BCL2 exerts inhibitory effects on cell growth and proliferation and DNA damage, resulting in increased genetic instability.[138,139] Many studies have proven BCL2 expression to be a promising prognostic and predictive marker, especially in hormone receptor−positive, node-negative breast cancer.[140,141] BCL2 expression is directly up-regulated by estrogens and therefore commonly shows elevated levels in ER-positive breast cancers.

The role of BCL2 in the context of TNBC has not yet been well established. BCL2 positivity was found to be a positive prognostic factor in TNBC, as the $ER^{-}BCL2^{+}$ group demonstrated a better prognosis than the $ER^{+}BCL2^{-}$ group.[73] Moreover, BCL2 positivity was shown to be a predictor of response to neoadjuvant and adjuvant anthracycline-based chemotherapy. The absence of BCL2 expression in prechemotherapy TNBC samples was associated with a higher probability of pCR to neoadjuvant doxorubicin-based chemotherapy, and the lack of BCL2 expression was also found to be an independent predictor of pCR.[74] Similarly, in an adjuvant setting, low BCL2 expression was associated with better outcome when TNBC was treated with anthracycline-based chemotherapy.[75] In addition, heightened BCL2 expression seems to predict response to cyclophosphamide, methotrexate, and 5-fluorouracil treatment.[76] The mechanism of this response is not entirely clear, but it may be influenced by expression changes in genes associated with BCL2 levels—for example, altered expression of HER3 (human epidermal growth factor receptor 3), MDM4 (Mdm2-like P53-binding protein), and p27 proteins.[142] The addition of BCL2 to the screening panel in clinical practice would be simple and could provide important prognostic and predictive information about the TNBC patient.

Cyclin-Dependent Kinases

CDKs and cyclins play key roles in cell-cycle regulation and are altered in almost all cancer types. Altered expression of cyclin D, cyclin E, CDK4/6, CDK2, and others was observed in TNBC, and CDK inhibition therapy therefore seems to be promising strategy in TNBC.[81,143,144] More than 10 CDK inhibitors are evaluating in ongoing clinical trials; the most promising are ribociclib, palbociclib, abemaciclib, and dinaciclib. The CDK4/6 inhibitors ribociclib and palbociclib have been already approved for treatment of advanced breast cancer patients with hormone receptor positivity and HER2 negativity.[145,146] In TNBC, the LAR subgroup was found to be sensitive to CDK4/6 inhibition (palbociclib/ribociclib). Moreover, CDK4/6 inhibitors were synergistic with PI3K inhibitors in TNBC cell lines with *PIK3CA* mutation.[147] Recently, inhibition of CDK4/6 was found to block breast tumor metastasis in TNBC xerograph model. Palbociclib inhibition did not affect growth of primary tumor but nevertheless significantly inhibited the spread of TNBC to distant organs through destabilization of the SNAIL1 protein.[148] Currently, palbociclib and ribociclib in combination with bicalutamide (AR antagonist) are being tested for the treatment of advanced AR-positive TNBC.[149] Abemaciclib has a different toxicity profile and is being tested in advanced TNBC with high RB1 expression as a single agent.[149] Dinaciclib (a pan-CDK inhibitor) was recently shown to have activity against TNBC both in vitro and in vivo.[150] Dinaciclib failed in combination with epirubicin because of substantial toxicity and is currently being tested in combination with pembrolizumab.[149,151]

Conclusion

TNBC encompasses a complex group of heterogeneous diseases characterized by various genetic alterations and a lack of validated biomarkers. Current research is focused on identifying genes that may serve as therapeutic targets, prognostic markers, or predictors of therapeutic response and are common in all or particular TNBC subtypes. High-throughput analysis tools such as sequencing and microarray technology have the potential to elucidate the nature of TNBC; however, results of these technologies rarely have therapeutic impact. Well-defined and extensive data sets are required for clinical validation of founded biomarkers. To date, several promising markers have been described, but they still lack validation with the stringent criteria of clinical studies. The heterogeneity of TNBC is also evident in its treatment. The different subtypes differ in both proliferative activity and response to conventional chemotherapy; as such, classic therapeutic approaches should consider which subtype is being targeted until personalized options become available.

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The authors have stated that they have no conflict of interest.

References

1. Abramson VG, Lehmann BD, Ballinger TJ, Pietenpol JA. Subtyping of triple-negative breast cancer: implications for therapy. *Cancer* 2015; 121:8-16.
2. Lehmann BD, Pietenpol JA. Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. *J Pathol* 2014; 232: 142-50.
3. Oakman C, Viale G, Di Leo A. Management of triple negative breast cancer. *Breast* 2010; 19:312-21.
4. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California Cancer Registry. *Cancer* 2007; 109: 1721-8.
5. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* 2006; 295:2492.
6. Liedtke C, Hess KR, Karn T, et al. The prognostic impact of age in patients with triple-negative breast cancer. *Breast Cancer Res Treat* 2013; 138:591-9.
7. Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007; 13:4429-34.
8. Morris GJ, Mitchell EP. Higher incidence of aggressive breast cancers in African-American women: a review. *J Natl Med Assoc* 2008; 100:698-702.
9. Heitz F, Harter P, Lueck HJ, et al. Triple-negative and HER2-overexpressing breast cancers exhibit an elevated risk and an earlier occurrence of cerebral metastases. *Eur J Cancer* 2009; 45:2792-8.
10. Lin NU, Vanderplas A, Hughes ME, et al. Clinicopathologic features, patterns of recurrence, and survival among women with triple-negative breast cancer in the National Comprehensive Cancer Network. *Cancer* 2012; 118:5463-72.
11. Kennecke H, Yerushalmi R, Woods R, et al. Metastatic behavior of breast cancer subtypes. *J Clin Oncol* 2010; 28:3271-7.
12. Livasy CA, Karaca G, Nanda R, et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol* 2006; 19:264-71.
13. Criscitiello C, Azim HA, Schouten PC, Linn SC, Sotiriou C. Understanding the biology of triple-negative breast cancer. *Ann Oncol* 2012; 23(suppl 6): vi13-8.
14. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature* 2000; 406:747-52.
15. Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001; 98:10869-74.
16. Parker JS, Mullins M, Cheang MCU, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol* 2009; 27:1160-7.
17. Sotiriou C, Neo SY, McShane LM, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci U S A* 2003; 100:10393-8.
18. Fulford LG, Easton DF, Reis-Filho JS, et al. Specific morphological features predictive for the basal phenotype in grade 3 invasive ductal carcinoma of breast. *Histopathology* 2006; 49:22-34.
19. Rakha EA, Elsheikh SE, Aleskandarany MA, et al. Triple-negative breast cancer: distinguishing between basal and nonbasal subtypes. *Clin Cancer Res* 2009; 15: 2302-10.
20. Bertucci F, Finetti P, Cervera N, et al. How basal are triple-negative breast cancers? *Int J Cancer* 2008; 123:236-40.
21. Prat A, Adamo B, Cheang MCU, Anders CK, Carey LA, Perou CM. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. *Oncologist* 2013; 18:123-33.
22. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* 2011; 121:2750-67.
23. Masuda H, Baggerly KA, Wang Y, et al. Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. *Clin Cancer Res* 2013; 19:5533-40.
24. Prat A, Parker JS, Karginova O, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Res* 2010; 12: R68.
25. Bertucci F, Finetti P, Cervera N, et al. Gene expression profiling shows medullary breast cancer is a subgroup of basal breast cancers. *Cancer Res* 2006; 66:4636-44.
26. Lehmann BD, Jovanović B, Chen X, et al. Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. *PLoS One* 2016; 11, e0157368.
27. Adams S, Gray RJ, Demaria S, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. *J Clin Oncol* 2014; 32:2959-66.
28. Gerber B, Loibl S, Eidtmann H, et al. Neoadjuvant bevacizumab and anthracycline–taxane–based chemotherapy in 678 triple-negative primary breast cancers; results from the geparquinto study (GBG 44). *Ann Oncol* 2013; 24: 2978-84.
29. Loi S, Michiels S, Salgado R, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. *Ann Oncol* 2014; 25:1544-50.
30. Dieci MV, Criscitiello C, Goubar A, et al. Prognostic value of tumor-infiltrating lymphocytes on residual disease after primary chemotherapy for triple-negative breast cancer: a retrospective multicenter study. *Ann Oncol* 2014; 25:611-8.
31. García-Martínez E, Gil GL, Benito AC, et al. Tumor-infiltrating immune cell profiles and their change after neoadjuvant chemotherapy predict response and prognosis of breast cancer. *Breast Cancer Res* 2014; 16:488.
32. Denkert C, von Minckwitz G, Brase JC, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2–positive and triple-negative primary breast cancers. *J Clin Oncol* 2015; 33:983-91.
33. Farmer P, Bonnefoi H, Becette V, et al. Identification of molecular apocrine breast tumours by microarray analysis. *Oncogene* 2005; 24:4660-71.
34. Rampurwala M, Wisinski KB, O'Regan R. Role of the androgen receptor in triple-negative breast cancer. *Clin Adv Hematol Oncol* 2016; 14:186-93.
35. Shah SP, Roth A, Goya R, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature* 2012; 486:395-9.
36. Koboldt DC, Fulton RS, McLellan MD, et al. Comprehensive molecular portraits of human breast tumours. *Nature* 2012; 490:61-70.
37. Allred DC, Clark GM, Elledge R, et al. Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. *J Natl Cancer Inst* 1993; 85:200-6.
38. Coates AS, Millar EKA, O'Toole SA, et al. Prognostic interaction between expression of p53 and estrogen receptor in patients with node-negative breast cancer: results from IBCSG trials VIII and IX. *Breast Cancer Res* 2012; 14:R143.
39. Kurosumi K, Kurosumi M, Oba H, et al. Pathological tumor response to neoadjuvant chemotherapy using anthracycline and taxanes in patients with triple-negative breast cancer. *Exp Ther Med* 2011; 2:257-64.
40. Powell E, Shao J, Yuan Y, et al. p53 deficiency linked to B cell translocation gene 2 (*BTG2*) loss enhances metastatic potential by promoting tumor growth in primary and metastatic sites in patient-derived xenograft (PDX) models of triple-negative breast cancer. *Breast Cancer Res* 2016; 18:13.
41. Aas T, Børresen AL, Geisler S, et al. Specific *P53* mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nat Med* 1996; 2:811-4.
42. Geisler S, Lønning PE, Aas T, et al. Influence of *TP53* gene alterations and c-erbB-2 expression on the response to treatment with doxorubicin in locally advanced breast cancer. *Cancer Res* 2001; 61:2505-12.
43. Chae BJ, Bae JS, Lee A, et al. p53 as a specific prognostic factor in triple-negative breast cancer. *Jpn J Clin Oncol* 2009; 39:217-24.
44. Plummer R. Poly(ADP-ribose) polymerase inhibition: a new direction for *BRCA* and triple-negative breast cancer? *Breast Cancer Res* 2011; 13:218.
45. Isakoff SJ, Mayer EL, He L, et al. TBCRC009: a multicenter phase II clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. *J Clin Oncol* 2015; 33:1902-9.
46. Silver DP, Richardson AL, Eklund AC, et al. Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. *J Clin Oncol* 2010; 28:1145-53.
47. Loibl S, Müller BM, von Minckwitz G, et al. Androgen receptor expression in primary breast cancer and its predictive and prognostic value in patients treated with neoadjuvant chemotherapy. *Breast Cancer Res Treat* 2011; 130:477-87.
48. Chen X, Yuan Y, Garfield DH, Wu J, Huang O, Shen K. Both carboplatin and bevacizumab improve pathological complete remission rate in neoadjuvant treatment of triple negative breast cancer: a meta-analysis. *PLoS One* 2014; 9, e108405.
49. von Minckwitz G, Schneeweiss A, Loibl S, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. *Lancet Oncol* 2014; 15:747-56.
50. Rugo HS, Barry WT, Moreno-Aspitia A, et al. Randomized phase III trial of paclitaxel once per week compared with nanoparticle albumin-bound nab-paclitaxel once per week or ixabepilone with bevacizumab as first-line chemotherapy for locally recurrent or metastatic breast cancer: CALGB 40502/NCCTG N063H (Alliance). *J Clin Oncol* 2015; 33:2361-9.
51. Lehmann BD, Bauer JA, Schafer JM, et al. *PIK3CA* mutations in androgen receptor–positive triple negative breast cancer confer sensitivity to the combination of PI3K and androgen receptor inhibitors. *Breast Cancer Res* 2014; 16:406.
52. De P, Sun Y, Carlson JH, Friedman LS, Leyland-Jones BR, Dey N. Doubling down on the PI3K-AKT-mTOR pathway enhances the antitumor efficacy of PARP inhibitor in triple negative breast cancer model beyond BRCA-ness. *Neoplasia* 2014; 16:43-72.
53. Gordon V, Banerji S. Molecular pathways: PI3K pathway targets in triple-negative breast cancers. *Clin Cancer Res* 2013; 19:3738-44.
54. Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002; 296:1655-7.
55. Beg S, Siraj AK, Prabhakaran S, et al. Loss of PTEN expression is associated with aggressive behavior and poor prognosis in Middle Eastern triple-negative breast cancer. *Breast Cancer Res Treat* 2015; 151:541-53.
56. Kim Y, Kim J, Lee HD, Jeong J, Lee W, Lee KA. Spectrum of *EGFR* gene copy number changes and *KRAS* gene mutation status in Korean triple negative breast cancer patients. *PLoS One* 2013; 8, e79014.
57. Teng YHF, Tan WJ, Thike AA, et al. Mutations in the epidermal growth factor receptor (*EGFR*) gene in triple negative breast cancer: possible implications for targeted therapy. *Breast Cancer Res* 2011; 13:R35.

58. Park HS, Jang MH, Kim EJ, et al. High *EGFR* gene copy number predicts poor outcome in triple-negative breast cancer. *Mod Pathol* 2014; 27:1212-22.
59. Turner N, Lambros MB, Horlings HM, et al. Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. *Oncogene* 2010; 29:2013-23.
60. Lehmann BD, Pietenpol JA, Tan AR. Triple-negative breast cancer: molecular subtypes and new targets for therapy. *Am Soc Clin Oncol Educ B* 2015; 35:e31-9.
61. Cerami E, Gao J, Dogrusoz U, et al. The cBio Cancer Genomics Portal: an open platform for exploring multidimensional cancer genomics data: figure 1. *Cancer Discov* 2012; 2:401-4.
62. Shiang CY, Qi Y, Wang B, et al. Amplification of fibroblast growth factor receptor-1 in breast cancer and the effects of brivanib alaninate. *Breast Cancer Res Treat* 2010; 123:747-55.
63. Sharpe R, Pearson A, Herrera-Abreu MT, et al. FGFR signaling promotes the growth of triple-negative and basal-like breast cancer cell lines both in vitro and in vivo. *Clin Cancer Res* 2011; 17:5275-86.
64. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003; 9:669-76.
65. Sikov WM, Berry DA, Perou CM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603. *J Clin Oncol* 2015; 33:13-21.
66. von Minckwitz G, Eidtmann H, Rezai M, et al. Neoadjuvant chemotherapy and bevacizumab for HER2-negative breast cancer. *N Engl J Med* 2012; 366:299-309.
67. Tolaney SM, Boucher Y, Duda DG, et al. Role of vascular density and normalization in response to neoadjuvant bevacizumab and chemotherapy in breast cancer patients. *Proc Natl Acad Sci U S A* 2015; 112:14325-30.
68. Naderi A, Hughes-Davies L. A functionally significant cross-talk between androgen receptor and ErbB2 pathways in estrogen receptor negative breast cancer. *Neoplasia* 2008; 10:542-8.
69. Qu Q, Mao Y, Fei X, Shen K. The impact of androgen receptor expression on breast cancer survival: a retrospective study and meta-analysis. *PLoS One* 2013; 8, e82650.
70. Tang D, Xu S, Zhang Q, Zhao W. The expression and clinical significance of the androgen receptor and E-cadherin in triple-negative breast cancer. *Med Oncol* 2012; 29:526-33.
71. Zhu A, Li Y, Song W, et al. Antiproliferative effect of androgen receptor inhibition in mesenchymal stem-like triple-negative breast cancer. *Cell Physiol Biochem* 2016; 38:1003-14.
72. Barton VN, D'Amato NC, Gordon MA, et al. Multiple molecular subtypes of triple-negative breast cancer critically rely on androgen receptor and respond to enzalutamide in vivo. *Mol Cancer Ther* 2015; 14:769-78.
73. Dawson SJ, Makretsov N, Blows FM, et al. *BCL2* in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received. *Br J Cancer* 2010; 103:668-75.
74. Pusztai L, Krishnamurti S, Perez Cardona J, et al. Expression of BAG-1 and BcL-2 proteins before and after neoadjuvant chemotherapy of locally advanced breast cancer. *Cancer Invest* 2004; 22:248-56.
75. Bouchalova K, Svoboda M, Kharaishvili G, et al. *BCL2* is an independent predictor of outcome in basal-like triple-negative breast cancers treated with adjuvant anthracycline-based chemotherapy. *Tumour Biol* 2015; 36:4243-52.
76. Bouchalova K, Kharaishvili G, Bouchal J, Vrbkova J, Megova M, Hlobilkova A. Triple negative breast cancer—*BCL2* in prognosis and prediction. *Curr Drug Targets* 2014; 15:1166-75.
77. Banerji S, Cibulskis K, Rangel-Escareno C, et al. Sequence analysis of mutations and translocations across breast cancer subtypes. *Nature* 2012; 486:405-9.
78. Shaver TM, Lehmann BD, Beeler JS, et al. Diverse, biologically relevant, and targetable gene rearrangements in triple-negative breast cancer and other malignancies. *Cancer Res* 2016; 76:4850-60.
79. Robinson DR, Kalyana-Sundaram S, Wu YM, et al. Functionally recurrent rearrangements of the MAST kinase and Notch gene families in breast cancer. *Nat Med* 2011; 17:1646-51.
80. Pareja F, Geyer FC, Marchiò C, Burke KA, Weigelt B, Reis-Filho JS. Triple-negative breast cancer: the importance of molecular and histologic subtyping, and recognition of low-grade variants. *NPJ Breast Cancer* 2016; 2:16036.
81. Balko JM, Giltnane JM, Wang K, et al. Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. *Cancer Discov* 2014; 4:232-45.
82. Tan SH, Sapari NS, Miao H, et al. High-Throughput mutation profiling changes before and 3 weeks after chemotherapy in newly diagnosed breast cancer patients. *PLoS One* 2015; 10, e0142466.
83. Hussain SP, Harris CC. p53 biological network: at the crossroads of the cellular-stress response pathway and molecular carcinogenesis. *J Nippon Med Sch* 2006; 73:54-64.
84. Végran F, Rebucci M, Chevrier S, Cadouot M, Boidot R, Lizard-Nacol S. Only missense mutations affecting the dna binding domain of p53 influence outcomes in patients with breast carcinoma. *PLoS One* 2013; 8, e55103.
85. Langerød A, Zhao H, Borgan Ø, et al. *TP53* mutation status and gene expression profiles are powerful prognostic markers of breast cancer. *Breast Cancer Res* 2007; 9:R30.
86. Olivier M, Taniere P. Somatic mutations in cancer prognosis and prediction: lessons from *TP53* and *EGFR* genes. *Curr Opin Oncol* 2011; 23:88-92.
87. Mizuno H, Spike BT, Wahl GM, Levine AJ. Inactivation of p53 in breast cancers correlates with stem cell transcriptional signatures. *Proc Natl Acad Sci U S A* 2010; 107:22745-50.
88. Kim JY, Park K, Jung HH, et al. Association between mutation and expression of *TP53* as a potential prognostic marker of triple-negative breast cancer. *Cancer Res Treat* 2016; 48:1338-50.
89. Venkitaraman AR. Cancer susceptibility and the functions of *BRCA1* and *BRCA2*. *Cell* 2002; 108:171-82.
90. D'Andrea AD, Grompe M. The Fanconi anaemia/*BRCA* pathway. *Nat Rev Cancer* 2003; 3:23-34.
91. Chacón RD, Costanzo MV. Triple-negative breast cancer. *Breast Cancer Res* 2010; 12:S3.
92. Foulkes WD, Stefansson IM, Chappuis PO, et al. Germline *BRCA1* mutations and a basal epithelial phenotype in breast cancer. *J Natl Cancer Inst* 2003; 95: 1482-5.
93. Atchley DP, Albarracin CT, Lopez A, et al. Clinical and pathologic characteristics of patients with *BRCA*-positive and *BRCA*-negative breast cancer. *J Clin Oncol* 2008; 26:4282-8.
94. Couch FJ, Hart SN, Sharma P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. *J Clin Oncol* 2015; 33:304-11.
95. Engel C, Rhiem K, Hahnen E, et al. Prevalence of pathogenic *BRCA1/2* germline mutations among 802 women with unilateral triple-negative breast cancer without family cancer history. *BMC Cancer* 2018; 18:265.
96. Sharma P, Stecklein SR, Kimler BF, et al. The prognostic value of *BRCA1* promoter methylation in early stage triple negative breast cancer. *J Cancer Ther Res* 2014; 3:1-11.
97. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A* 2003; 100:8418-23.
98. Copson ER, Maishman TC, Tapper WJ, et al. Germline *BRCA* mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. *Lancet Oncol* 2018; 19:169-80.
99. Han HS, Diéras V, Robson M, et al. Veliparib with temozolomide or carboplatin/paclitaxel versus placebo with carboplatin/paclitaxel in patients with *BRCA1/2* locally recurrent/metastatic breast cancer: randomized phase II study. *Ann Oncol* 2018; 29:154-61.
100. Jiang T, Shi W, Wali VB, et al. Predictors of chemosensitivity in triple negative breast cancer: an integrated genomic analysis. *PLoS Med* 2016; 13, e1002193.
101. Tutt A, Tovey H, Cheang MCU, et al. Carboplatin in *BRCA1/2*-mutated and triple-negative breast cancer BRCAness subgroups: the TNT trial. *Nat Med* 2018; 24:628-37.
102. Tutt A, Paul E, Kilburn L, et al. The TNT trial: a randomized phase III trial carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or *BRCA1/2* breast cancer (CRUK/07/012), Proceedings of the Thirty-Seventh Annual CTRC-AACR San Antonio Breast Cancer Symposium; December 9-13, 2014; San Antonio, TX. Philadelphia, PA: AACR; 2015. *Cancer Res* 2015; 75(9 suppl) (abstract S3-01).
103. Anders CK, Abramson V, Tan T, Dent R. The evolution of triple-negative breast cancer: from biology to novel therapeutics. *Am Soc Clin Oncol Educ B* 2016; 36:34-42.
104. Hahnen E, Lederer B, Hauke J, et al. Germline mutation status, pathological complete response, and disease-free survival in triple-negative breast cancer. *JAMA Oncol* 2017; 3:1378.
105. Byrski T, Huzarski T, Dent R, et al. Response to neoadjuvant therapy with cisplatin in *BRCA1*-positive breast cancer patients. *Breast Cancer Res Treat* 2009; 115:359-63.
106. Gronwald J, Byrski T, Huzarski T, et al. Neoadjuvant therapy with cisplatin in *BRCA1*-positive breast cancer patients. *J Clin Oncol* 2009; 27:502.
107. Tutt A, Robson M, Garber JE, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and advanced breast cancer: a proof-of-concept trial. *Lancet* 2010; 376:235-44.
108. Shen Y, Rehman FL, Feng Y, et al. BMN 673, a novel and highly potent PARP1/2 inhibitor for the treatment of human cancers with DNA repair deficiency. *Clin Cancer Res* 2013; 19:5003-15.
109. Sandhu SK, Schelman WR, Wilding G, et al. The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in *BRCA* mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. *Lancet Oncol* 2013; 14:882-92.
110. Kummar S, Ji J, Morgan R, et al. A phase I study of veliparib in combination with metronomic cyclophosphamide in adults with refractory solid tumors and lymphomas. *Clin Cancer Res* 2012; 18:1726-34.
111. Robson M, Im SA, Senkus E, et al. Olaparib for metastatic breast cancer in patients with a germline *BRCA* mutation. *N Engl J Med* 2017; 377:523-33.
112. Litton J, Rugo HS, Ettl J, et al. EMBRACA: a phase 3 trial comparing talazoparib, an oral PARP inhibitor, to physician's choice of therapy in patients with advanced breast cancer and a germline *BRCA* mutation, Proceedings of the 2017 San Antonio Breast Cancer Symposium; December 5-9, 2017; San Antonio, TX. Philadelphia, PA: AACR; 2018. *Cancer Res* 2018; 78(4 suppl) (abstract GS6-07).
113. Gonzalez-Angulo AM, Stemke-Hale K, Palla SL, et al. Androgen receptor levels and association with *PIK3CA* mutations and prognosis in breast cancer. *Clin Cancer Res* 2009; 15:2472-8.
114. Dey N, De P, Leyland-Jones B. PI3K-AKT-mTOR inhibitors in breast cancers: from tumor cell signaling to clinical trials. *Pharmacol Ther* 2017; 175:91-106.
115. Jones N, Bonnet F, Sfar S, et al. Comprehensive analysis of PTEN status in breast carcinomas. *Int J Cancer* 2013; 133:323-34.

116. Fedele CG, Ooms LM, Ho M, et al. Inositol polyphosphate 4-phosphatase II regulates PI3K/Akt signaling and is lost in human basal-like breast cancers. *Proc Natl Acad Sci U S A* 2010; 107:22231-6.
117. Nakai K, Hung MC, Yamaguchi H. A perspective on anti-EGFR therapies targeting triple-negative breast cancer. *Am J Cancer Res* 2016; 6:1609-23.
118. Cheng CL, Thike AA, Tan SYJ, Chua PJ, Bay BH, Tan PH. Expression of *FGFR1* is an independent prognostic factor in triple-negative breast cancer. *Breast Cancer Res Treat* 2015; 151:99-111.
119. Toyama T, Yamashita H, Kondo N, et al. Frequently increased epidermal growth factor receptor (*EGFR*) copy numbers and decreased *BRCA1* mRNA expression in Japanese triple-negative breast cancers. *BMC Cancer* 2008; 8:309.
120. Carey LA, Rugo HS, Marcom PK, et al. TBCRC 001: randomized phase II study of cetuximab in combination with carboplatin in stage IV triple-negative breast cancer. *J Clin Oncol* 2012; 30:2615-23.
121. Trédan O, Campone M, Jassem J, et al. Ixabepilone alone or with cetuximab as first-line treatment for advanced/metastatic triple-negative breast cancer. *Clin Breast Cancer* 2015; 15:8-15.
122. Baselga J, Gómez P, Greil R, et al. Randomized phase II study of the anti–epidermal growth factor receptor monoclonal antibody cetuximab with cisplatin versus cisplatin alone in patients with metastatic triple-negative breast cancer. *J Clin Oncol* 2013; 31:2586-92.
123. Nabholtz JM, Abrial C, Mouret-Reynier MA, et al. Multicentric neoadjuvant phase II study of panitumumab combined with an anthracycline/taxane-based chemotherapy in operable triple-negative breast cancer: identification of biologically defined signatures predicting treatment impact. *Ann Oncol* 2014; 25:1570-7.
124. Yardley DA, Shipley DL, Peacock NW, et al. Phase I/II trial of neoadjuvant sunitinib administered with weekly paclitaxel/carboplatin in patients with locally advanced triple-negative breast cancer. *Breast Cancer Res Treat* 2015; 152:557-67.
125. Barrios CH, Liu MC, Lee SC, et al. Phase III randomized trial of sunitinib versus capecitabine in patients with previously treated HER2-negative advanced breast cancer. *Breast Cancer Res Treat* 2010; 121:121-31.
126. McGhan LJ, McCullough AE, Protheroe CA, et al. Androgen receptor–positive triple negative breast cancer: a unique breast cancer subtype. *Ann Surg Oncol* 2014; 21:361-7.
127. Peters AA, Buchanan G, Ricciardelli C, et al. Androgen receptor inhibits estrogen receptor–activity and is prognostic in breast cancer. *Cancer Res* 2009; 69:6131-40.
128. He J, Peng R, Yuan Z, et al. Prognostic value of androgen receptor expression in operable triple-negative breast cancer: a retrospective analysis based on a tissue microarray. *Med Oncol* 2012; 29:406-10.
129. Vera-Badillo FE, Templeton AJ, de Gouveia P, et al. Androgen receptor expression and outcomes in early breast cancer: a systematic review and meta-analysis. *J Natl Cancer Inst* 2014; 106:djt319.
130. Mina A, Yoder R, Sharma P. Targeting the androgen receptor in triple-negative breast cancer: current perspectives. *Onco Targets Ther* 2017; 10:4675-85.
131. Cauley JA, Lucas FL, Kuller LH, Stone K, Browner W, Cummings SR. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of Osteoporotic Fractures Research Group. *Ann Intern Med* 1999; 130:270-7.
132. Choi JE, Kang SH, Lee SJ, Bae YK. Androgen receptor expression predicts decreased survival in early stage triple-negative breast cancer. *Ann Surg Oncol* 2015; 22:82-9.
133. Aleskandarany MA, Abduljabbar R, Ashankyty I, et al. Prognostic significance of androgen receptor expression in invasive breast cancer: transcriptomic and protein expression analysis. *Breast Cancer Res Treat* 2016; 159:215-27.
134. Barton VN, D'Amato NC, Gordon MA, Christenson JL, Elias A, Richer JK. Androgen receptor biology in triple negative breast cancer: a case for classification as AR^{+} or quadruple negative disease. *Horm Cancer* 2015; 6:206-13.
135. Cochrane DR, Bernales S, Jacobsen BM, et al. Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide. *Breast Cancer Res* 2014; 16:R7.
136. Gucalp A, Tolaney S, Isakoff SJ, et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. *Clin Cancer Res* 2013; 19:5505-12.
137. Traina TA, Miller K, Yardley DA, et al. Enzalutamide for the treatment of androgen receptor–expressing triple-negative breast cancer. *J Clin Oncol* 2018; 36:884-90.
138. Wang Q, Gao F, May WS, Zhang Y, Flagg T, Deng X. *Bcl2* negatively regulates DNA double-strand–break repair through a nonhomologous end-joining pathway. *Mol Cell* 2008; 29:488-98.
139. Basu A, Haldar S. The relationship between *Bcl2, Bax* and *p53*: consequences for cell cycle progression and cell death. *Mol Hum Reprod* 1998; 4:1099-109.
140. Ali HR, Dawson SJ, Blows FM, et al. A Ki67/BCL2 index based on immunohistochemistry is highly prognostic in ER-positive breast cancer. *J Pathol* 2012; 226:97-107.
141. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004; 351:2817-26.
142. Abdel-Fatah TMA, Perry C, Dickinson P, et al. *Bcl2* is an independent prognostic marker of triple negative breast cancer (TNBC) and predicts response to anthracycline combination (ATC) chemotherapy (CT) in adjuvant and neo-adjuvant settings. *Ann Oncol* 2013; 24:2801-7.
143. Keyomarsi K, Tucker SL, Buchholz TA, et al. Cyclin E and survival in patients with breast cancer. *N Engl J Med* 2002; 347:1566-75.
144. Velasco-Velázquez MA, Li Z, Casimiro M, Loro E, Homsi N, Pestell RG. Examining the role of cyclin D1 in breast cancer. *Future Oncol* 2011; 7:753-65.
145. Walker AJ, Wedam S, Amiri-Kordestani L, et al. FDA approval of palbociclib in combination with fulvestrant for the treatment of hormone receptor–positive, HER2-negative metastatic breast cancer. *Clin Cancer Res* 2016; 22:4968-72.
146. US Food and Drug Administration, Available at: https://www.fda.gov/. Accessed: June 21, 2018.
147. Asghar US, Barr AR, Cutts R, et al. Single-cell dynamics determines response to CDK4/6 inhibition in triple-negative breast cancer. *Clin Cancer Res* 2017; 23:5561-72.
148. Liu T, Yu J, Deng M, et al. CDK4/6-dependent activation of DUB3 regulates cancer metastasis through SNAIL1. *Nat Commun* 2017; 8:13923.
149. National Library of Medicine; National Institute of Health. Registred clinical studies information, Available at: https://clinicaltrials.gov/. Accessed: June 21, 2018.
150. Rajput S, Khera N, Guo Z, Hoog J, Li S, Ma CX. Inhibition of cyclin dependent kinase 9 by dinaciclib suppresses cyclin B1 expression and tumor growth in triple negative breast cancer. *Oncotarget* 2016; 7:56864-75.
151. Mitri Z, Karakas C, Wei C, et al. A phase 1 study with dose expansion of the CDK inhibitor dinaciclib (SCH 727965) in combination with epirubicin in patients with metastatic triple negative breast cancer. *Invest New Drugs* 2015; 33:890-4.