Triple-negative breast cancer: bridging the gap from cancer genomics to predictive biomarkers

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***Abstract*:** Triple-negative breast cancer (TNBC) represents a challenge clinically due to a lack of response to hormonal and HER2-targeted agents coupled with an aggressive disease course. As the biology of this breast cancer subtype is better understood, it is clear that TNBC is a heterogeneous disease and one targeted therapy is unlikely to be active in all patients. Biomarkers predictive of response to treatment are thus of great importance in TNBC. This review outlines studies evaluating biomarkers predictive of response to neoadjuvant chemotherapy and to targeted therapies in the advanced setting. The development of validated biomarkers in conjunction with novel targeted therapies represents an opportunity to improve patient outcomes in TNBC.

Keywords: biomarkers, targeted therapy, triple-negative breast cancer

Introduction

Breast cancer is well-characterized as a heterogeneous disease composed of discrete biologic subtypes which guide clinical management [Peppercorn *et al.* 2008]. Triple-negative breast cancer (TNBC) is defined by a lack of significant expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [Carey *et al.* 2010]. Patients with TNBC are generally younger than the overall population of breast cancer patients [Bauer *et al.* 2007], and they more frequently have larger and higher-grade tumors [Rakha *et al.* 2007]. TNBC is also associated with a higher risk of distant recurrence and death, especially within 3 years of diagnosis [Dent *et al.* 2007]. Owing to the lack of HER2 and hormone-receptor expression, TNBC does not respond to hormonal therapy or HER2-targeted agents, leaving cytotoxic chemotherapy as the only option for systemic therapy [Oakman *et al.* 2010].

Despite these common features recognized in TNBC, there is notable diversity within the group. Histologic variability provides one example of such, with invasive ductal, metaplastic and medullary breast cancers coexisting in this patient population [Reis-Filho and Tutt, 2008]. Furthermore, the TNBC subtype does not directly correspond to a single molecular breast cancer subgroup [Rakha and Ellis, 2009]. Though most fit into the category of basal-like cancers, these groups are overlapping rather than synonymous, with certain populations of ER-positive and HER2-positive tumors also known to express basal-like markers [Banerjee *et al.* 2006]. Further molecular evaluation has identified additional subgroups, confirming the true heterogeneity within TNBC [Lehmann *et al.* 2011].

Clearly there is a need for better therapeutic options for patients with TNBC, ideally in the form of targeted agents. However, the heterogeneity within TNBC has made achieving this goal more complex [Oakman *et al.* 2010]. Identification of biomarkers able to predict response to systemic therapies is of vital importance, as it will not only allow for better outcomes in responsive subgroups of TNBC, but also prevent unnecessary exposure of unresponsive patients to ineffective therapy. In this way, predictive biomarkers will facilitate the development of personalized medicine for TNBC. This review will detail the status of predictive biomarker development in TNBC, as well as potential directions for the future of this emerging field.

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Characterizing the heterogeneity within TNBC

Differences between TNBC and basal-like breast cancer

Though TNBC and basal-like breast cancers share many characteristics, the terms are not equivalent [Foulkes *et al.* 2010]. While TNBC is defined by lack of hormone receptor and HER2 expression, basal-like breast cancer is characterized by a gene expression profile similar to that of normal basal cells [Metzger-Filho *et al.* 2012]. There is significant overlap between these two groups, as seen in *BRCA1*-mutant breast cancers, which reliably demonstrate characteristics of both TNBC and basal-like tumors [Lakhani *et al.* 2005]. However, up to 45% of basal-like breast cancers are not TNBC, and up to 35% of TNBCs are not basal-like [Carey *et al.* 2010]. Though testing for TNBC has become quite routine in clinical practice, the identification of the basal-like cancers remains burdensome due to its requirement of gene-expression profiling. An immunohistochemistry profile consisting of CK5/6, CK14, CK17, EGFR, and C-kit has been proposed as a pattern representative of the basal-like subtype, though these do not correlate exactly. Further, the heterogeneity of immunohistochemistry (IHC) studies and lack of specific positive and negative cutoff values have limited the widespread acceptance of this method [Metzger-Filho *et al.* 2012]. The importance of routine identification of basal-like cancers remains unclear, as although this finding has potentially important prognostic implications [Cheang *et al.* 2008], it has not yet been used for therapeutic decision-making [Foulkes *et al.* 2010].

Molecular profiling in TNBC

The heterogeneity within the subtypes of breast cancer has been of great interest in recent years, with investigation of such largely focusing on genomic profiling. These evaluations have confirmed the genomic variability within the breast cancer subgroups [The Cancer Genome Atlas Network, 2012] and the profound heterogeneity specifically within TNBC [Shah *et al.* 2012]. Additional work in breast cancer genomics has led to the identification of new breast cancer driver mutations [Stephens *et al.* 2012], as well as the suggestion of a new mechanism of breast cancer subgrouping based on molecular features [Curtis *et al.* 2012].

There is hope that such gene expression profiling may be further utilized to select treatments of greatest benefit according to the various TNBC subtypes identified. This concept was explored by Lehmann and colleagues, who identified six TNBC subtypes based on gene expression patterns, and then evaluated response to various cancer therapies in representative cell lines. Cell culture data as well as knowledge of key pathways activated in the various TNBC subtypes were used to propose specific therapies [Lehmann *et al.* 2011] (Table 1).

Though treatment according to these molecularly-defined subtypes has not yet been directly evaluated in the clinical setting, there are examples of the potential for translation to the clinic. The use of platinum chemotherapy agents for *BRCA1*-muated cancers as a representative of the basal-like subtypes is the most extensively studied, with notable effects demonstrated in the neoadjuvant setting as detailed below [Byrski *et al.* 2010, Silver *et al.* 2010]. There are early-phase trials of poly ADP ribose polymerase (PARP) inhibitors in *BRCA*-mutated cancers that correspond to these basal-like subgroups, as well [Fong *et al.* 2009; Tutt *et al.* 2010; Gelmon *et al.* 2011]. Studies of bicalutamide in androgen receptor positive TNBC as an example of the LAR subtype are also underway [Gucalp and Traina, 2010].

BRCA1 Mutations and TNBC

As noted above, the use of DNA damaging agents for the treatment of basal-like breast cancers provides some clinical relevance for treatment according to the TNBC subtypes proposed by Lehmann and colleagues. Studies evaluating such treatments have generally assessed patients with *BRCA* mutations, which are associated with defects in DNA damage repair. *BRCA1* mutations in particular are thought to be a primary mechanism of basal-like breast cancer development [De Ruijter *et al.* 2011]. A total of 75% of breast cancers harboring this mutation are triple-negative and/or basal-like [Foulkes *et al.* 2010], while *BRCA2*-mutated tumors are more commonly hormone receptor positive [Mavaddat *et al.* 2012].

Also of interest are BRCA1-like tumors, which have functional *BRCA1* losses, such as those associated with promoter methylation, rather than currently recognized *BRCA1* mutations. A classifier based on array-comparative genomic hybridization has been developed to identify such

Table 1. Molecular subtypes of triple-negative breast cancer [Lehmann *et al*. 2011].

TNBC subtypes	Molecular characteristics	Potential therapies
Basal-like 1	Cell cycle function Proliferation DNA damage response	Chemotherapy PARP inhibitor
Basal-like 2	Cell cycle function Proliferation Growth factor signaling	Chemotherapy PARP inhibitor
Mesenchymal	EMT Cell motility Differentiation Proliferation	Src inhibitor PI3K pathway inhibitor Wnt pathway inhibitor
Mesenchymal stem-like	EMT Cell motility Differentiation Growth factor signaling Angiogenesis	Src inhibitor PI3K pathway inhibitor Wnt pathway inhibitor
Luminal androgen receptor	AR signaling Luminal cytokeratine	AR antagonist Hsp90 inhibitor PI3K pathway inhibitor
Immunomodulatory	Immune cell processes	Immune targeted agents

AR, androgen receptor; EMT, epithelial mesenchymal transition; PARP, poly ADP ribose polymerase; TNBC, triple-negative breast cancer.

BRCA1-like tumors [Joosse *et al.* 2009]. This classifier was retrospectively applied to tumor samples from patients with stage III breast cancer treated in a clinical trial evaluating adjuvant 5FU, epirubicin, and cyclophosphamide (FEC) *versus* FEC followed by cyclophosphamide, thiotepa, and carboplatin. Results demonstrated a significant increase in recurrence-free survival and overall survival (OS) in patients with BRCA1-like tumors treated with a platinum-containing regimen [Vollebergh *et al.* 2011]. This classifier may thus serve as a broader biomarker of platinum-based chemotherapy response.

The use of neoadjuvant therapy trials to develop predictive biomarkers

Despite having a poorer overall prognosis, patients with nonmetastatic TNBC treated with neoadjuvant chemotherapy seem to have a better response than other breast cancer subtypes. This paradox was demonstrated in a large retrospective analysis of 1118 patients with stage I–III breast cancer treated with standard neoadjuvant chemotherapy regimens, with pathologic complete response (pCR) rates of 22% in patients with TNBC *versus* 11% in non-TNBC. When patients with TNBC who achieved pCR were compared with non-TNBC patients, OS was not significantly different ($p = 0.24$). However, when pCR was not achieved in TNBC patients after neoadjuvant chemotherapy, a significantly poorer OS was identified when compared with non-TNBC with residual disease ($p < 0.0001$) [Liedtke *et al.* 2008]. These findings highlight the importance of identifying biomarkers able to predict response to neoadjuvant chemotherapy, as well as the importance of finding alternative therapies with associated biomarkers for those patients who can be identified as likely nonresponders.

The neoadjuvant treatment paradigm has allowed for significant evaluation of biomarkers that may be studied in the context of pathologic response (Table 2). Though most of these studies have been performed retrospectively using tumor specimens from large neoadjuvant chemotherapy trials, a few small prospective studies have been performed [Silver *et al.* 2010; Li *et al.* 2011]. In one study of 41 Chinese patients with locally advanced TNBC, tumor tissue was evaluated for Ki-67 proliferation index, CK5/6, EGFR, cyclin D1, and nm23-H1, a subunit of a gene encoding nucleoside diphosphate kinase, by IHC prior to neoadjuvant therapy with docetaxel and epirubicin. Patients received between 4 and 6 cycles of chemotherapy, followed by resection. Multivariate

Table 2. Biomarker evaluations with classical chemotherapeutic agents in the treatment of triple-negative breast cancer in the neoadjuvant setting.

Chemotherapy regimen	Potential biomarker(s)	pCR rate	Method of testing	Type of study
Docetaxel and epirubicin [Li *et al*. 2011]	Basal-like markers negative Nm23-H1 positive	72.7% 53.8%	IHC	Prospective
Cisplatin [Byrski *et al*. 2010]	*BRCA1* mutation	83%	PCR	Retrospective
Docetaxel and doxorubicin [Keam *et al*. 2011]	High Ki-67	18.2%	IHC	Retrospective
Anthracycline-based therapy [Bidard *et al*. 2008]	p53 positive	22.5%*	IHC	Retrospective
TAC [Von Minckwitz *et al*. 2011]	High cytoplasmic PARP	41%	IHC	Retrospective
Anthracycline and taxane combinations [Darb-Esfahani *et al*. 2012]	High *TMSB15A* expression	47.2% and 36.8%**	qRT-PCR	Retrospective
Various regimens [Dennison *et al*. 2013]	High *LDHB* expression	45.5% and 36.6%**	Microarray	Retrospective
Anthracycline or taxane-based therapy [Ono *et al*. 2012]	High tumor-infiltrating lymphocytes	37%	Histopathologic evaluation	Retrospective

* Not statistically significant increase.
** Two datasets evaluated.
IHC, immunohistochemistry; PARP, poly ADP ribose polymerase; pCR, pathologic complete response; PCR, polymerase chain reaction; qRT-PCR, quantitative real-time polymerase chain reaction; TAC, docetaxel, doxorubicin, and cyclophosphamide.

analysis identified tumors with negative basal-like markers of CK5/4 and/or EGFR, as well as those with positive nm23-H1, as more likely to achieve pCR, with odds ratios of 3.15 and 1.93, respectively [Li *et al.* 2011].

Based on data suggesting improved outcomes with platinum chemotherapy in basal-like breast cancers, a second small prospective study of 28 patients with stage II and III TNBC evaluated biomarkers predictive of pCR following 4 cycles of cisplatin in the neoadjuvant setting. pCR was achieved in 21% of patients at the time of surgical resection, and good response (pCR or significant partial response) was achieved in 50%. *BRCA1* mRNA, *BRCA* methylation, *p53* mutation, and *ΔNp63/Tap73* ratio were evaluated as potential biomarkers. None correlated with complete response, though lower *BRCA1* mRNA expression and promoter methylation as well as *p53* nonsense or frameshift mutations and E2F3 activation did correlate with good response [Silver *et al.* 2010].

A larger retrospective study compared rates of pCR from 102 women with *BRCA1* mutations treated with various neoadjuvant regimens. Interestingly, 83% of the women treated with cisplatin had a pCR, as compared with 7% treated with cyclophosphamide, methotrexate, and fluorouracil (CMF), 8% treated with doxorubicin and docetaxel (AT), and 22% treated with doxorubicin and cyclophosphamide with or without fluorouracil (AC, FAC) [Byrski *et al.* 2010]. These data suggest that *BRCA1* mutational status may be a predictive marker for response to platinum-based chemotherapy. A randomized phase II study is further evaluating this possibility in patients with germline *BRCA* mutations. This ongoing study will compare pCR rates in patients treated with AC chemotherapy to those treated with cisplatin in the neoadjuvant setting [ClinicalTrials.gov identifier: NCT01670500].

Many other potential biomarkers have been retrospectively evaluated through the use of tissue and outcome data from historical data sets to correlate potential biomarkers with the rate of pCR. One such study evaluated basal-like markers similar to the prospective study described above, with microarray used to identify the expression of CK5, CK14, CK17, EGFR, E-cadherin, and Ki-67 in 56 samples from TNBC patients previously treated with standard neoadjuvant chemotherapy regimens, most commonly doxorubicin

and cyclophosphamide followed by a taxane. Interestingly, in this study, none of the markers studied correlated with pCR. Also of note, nearly all of the TNBC tumors demonstrated elevated Ki-67 index, suggesting that the predictive capabilities of this marker are limited to proliferation, with higher levels to be expected in TNBC [Kraus *et al.* 2012].

However, in an additional retrospective study evaluating only Ki-67 as a marker of response to chemotherapy, its use as biomarker was a bit more promising. Of 105 TNBC patients treated with 3 cycles of neoadjuvant docetaxel and doxorubicin, 18.2% who had high Ki-67 achieved pCR, while no patients with low Ki-67 had a pCR. High Ki-67 expression was also associated with early recurrence and poorer OS after completion of adjuvant therapy, mirroring the classic natural history of TNBC. The authors suggest Ki-67 may thus be used to separate those TNBC tumors with a more aggressive phenotype in addition to predicting chemotherapy response [Keam *et al.* 2011].

The use of p53 status as a biomarker predictive of response to neoadjuvant chemotherapy has also been evaluated in the retrospective setting. In one study, tissue from 293 breast cancer patients treated with 4–6 cycles of standard anthracycline-based neoadjuvant chemotherapy was evaluated for p53 by IHC at the time of resection. In the 120 patients with TNBC, there was a trend to association of p53 status and pCR rate, with 22% of patients with positive p53 achieving pCR and 10% of patients with negative p53 with pCR. This finding was not statistically significant in this small population of TNBC patients, however, with p value of 0.08 [Bidard *et al.* 2008].

PARP, a family of nuclear enzymes important for DNA repair, has also been evaluated as a potential predictive marker for response to standard neoadjuvant chemotherapy. A retrospective study evaluated tissue samples from participants of the GeparTrio trial for PARP expression by IHC. The breast cancer patients in this study were primarily treated with docetaxel, doxorubicin, and cyclophosphamide (TAC) chemotherapy in the neoadjuvant setting, with pCR rates evaluated at the time of surgery. Tissue samples from this patient population demonstrated increased cytoplasmic PARP expression in TNBC and HR–/HER2+ tumors, which correlated with higher pCR rates in these groups (41% and 42.9%, respectively). Interestingly, PARP expression did not correlate with survival outcomes in TNBC patients, suggesting this finding is not a single driving force of this aggressive phenotype [Von Minckwitz *et al.* 2011].

Broader gene expression profiles have also been used in hopes of identifying lesser-known markers that may predict response to neoadjuvant chemotherapy. *TMSB15A* is one example of a gene identified by expression profiling, which was found to encode regulatory protein thymosin beta 15 (TMSB15). This protein has been associated with tumor progression in cancer cell lines, though its exact function in TNBC is unknown. Expression of this gene was studied retrospectively in tumor samples from neoadjuvant phase III trials GeparTrio and GeparQuattro, which evaluated various anthracycline and taxane combination regimens in the neoadjuvant setting, and documented pCR rates at the time of surgery. Increased *TMSB15A* expression by quantitative reverse transcription polymerase chain reaction (qRT-PCR) was associated with an increased likelihood of pCR in patients with TNBC in the GeparTrio study (47.2% *versus* 16%), and was validated by data from TNBC patients in the GeparQuattro study (36.8% *versus* 17%). *TMSB15A* expression was not found to be a predictor of pCR in patients with luminal cancers, thus distinguishing this as a potential predictive marker specific to TNBC [Darb-Esfahani *et al.* 2012].

Gene expression profiling has also identified a marker of metabolic pathway activity as a potential biomarker for response to neoadjuvant chemotherapy. Lactate dehydrogenase B (*LDHB*) was identified as a strong bimodal gene by mRNA microarray, with highest expression in basal-type and TNBC cell lines. LDHB is known to be associated with lactate uptake as well as conversion to pyruvate, though can also convert pyruvate to lactate, which was demonstrated in breast cancer cell lines in this study. Analysis of two combined patient datasets, Microarray Quality Control (MAQC) II and MD Anderson Cancer Center Super Series (MDACCSS), demonstrated an association between high levels of *LDHB* expression and pCR following neoadjuvant therapy for TNBC with various standard regimens (odds ratio 3.18). Higher recurrence rates were also identified in TNBC patients with high levels of *LDHB* in the setting of residual disease following neoadjuvant therapy [Dennison *et al.* 2013].

Though not specific to TNBC, the large I-SPY 1 trial also evaluated gene expression arrays in 237 breast cancer patients treated with neoadjuvant anthracycline with or without a taxane in hopes of identifying predictors of response. This study identified poor prognosis gene signatures as correlates to higher pCR, with rates ranging from 24% to 36%. These poor prognosis signatures included activated wound healing signature (pCR 26%), p53 mutation (pCR 34%), a high-risk 70-gene prognostic profile (pCR 24%), and moderate or high risk of recurrence score (ROR-S) (pCR 27%) [Esserman *et al.* 2012]. As these gene signatures were not evaluated according to hormone receptor status, it is difficult to say if such findings are transferrable to the TNBC subtype, though it is possible that they may predict chemotherapy response outside of the traditional breast cancer subtypes.

Pathologic features such as tumor-infiltrating lymphocytes (TIL) and apoptosis scores have also been evaluated as predictive of response to neoadjuvant chemotherapy. In a retrospective study of 92 TNBC tumors from patients with stage II–III disease treated with neoadjuvant anthracycline- or taxane-based chemotherapy alone or in combination, higher TIL scores of 3–5 were associated with higher rate of pCR (37%) than lower scores (16%) ($p = 0.05$). Increased apoptosis scores were also associated with higher rates of pCR in both the primary tumor and axillary nodes in the TNBC patients ($p = 0.04$) [Ono *et al.* 2012].

Identification of predictors of response to neoadjuvant chemotherapy is of great importance in TNBC due to the knowledge that patients without pCR will have poorer overall outcomes. Though many studies have been performed to identify such biomarkers, most were retrospective and were inconsistent with respect to neoadjuvant chemotherapy regimen. Data for some potential biomarkers, such as Ki-67, has also been inconsistent in these trials. There is still hope that further evaluation in larger clinical trials may show that Ki-67 and PARP, among others, may predict response to neoadjuvant chemotherapy.

As these studies demonstrate, neoadjuvant therapy is an attractive setting for evaluation of predictive biomarkers due to the ability to evaluate tissue pre- and post-therapy, and accessibility of an early readout of treatment response. Biomarkers predictive of response to adjuvant therapy have been more difficult to assess, largely due to limitations in outcome measures. The clinical trials evaluating such have largely used OS or disease free survival to evaluate response to chemotherapy, thus making associated biomarkers more prognostic than predictive [Jacquemier *et al.* 2011; Kashiwagi *et al.* 2011]. In one such study, 138 of 190 TNBC patients were treated with adjuvant chemotherapy with either an anthracycline-based or 5FU-based regimen, while the remaining 52 patients did not receive adjuvant therapy. Tumor tissue expression of E-cadherin, Ki67 and p53 by IHC was correlated with OS. Interestingly, tumors positive for E-cadherin and negative for Ki67 were associated with improved OS in the group treated with adjuvant chemotherapy, but not in the group treated with surgery alone. The authors suggest this pattern may be useful to predict which patients have a greater chance of benefiting from adjuvant chemotherapy [Kashiwagi *et al.* 2011].

Evaluation of biomarkers predictive of response to therapy in metastatic TNBC is quite limited, and has been performed only as a subset analysis in studies of all-comers with metastatic breast cancer. One example is a study evaluating thymidylate synthase (TS) and thymidine phosphorylase (TP) retrospectively in patients treated with capecitabine monotherapy for metastatic breast cancer. Results demonstrated significantly higher overall response rates and disease control rates in women with TS levels <100 as compared with those with levels >100. In addition, women with TNBC were more likely to have TS levels >100 than women with hormone receptor or HER2 positive disease [Lee *et al.* 2011].

Clearly additional studies of predictive biomarkers for adjuvant and metastatic therapies for TNBC are required to further direct chemotherapy administration in these settings. Such investigation is limited, however, by the reliance on response or survival data to evaluate outcomes. This explains the preponderance of predictive biomarker studies performed in patients receiving neoadjuvant therapy for TNBC, where pathologic response can be used to document degree of response more accurately. However, given the poor prognosis of TNBC patients who do not respond to neoadjuvant chemotherapy, there is just as great a need for studies to identify predictive biomarkers in these other phases of treatment.

Development of biomarkers for novel targeted agents

BRCA mutation as a predictive biomarker for targeted therapies.

In addition to biomarkers predictive of response to traditional cytotoxic chemotherapeutic agents, multiple studies have evaluated predictive biomarkers for novel targeted agents. The most developed biomarker in this group is that of *BRCA* mutation as a predictor for response to therapy with PARP inhibitors. The PARP family of enzymes facilitate DNA repair upon activation by DNA strand breaks, and when PARP is inhibited, increase the role of DNA repair by homologous recombination [Farmer *et al.* 2005]. Since *BRCA*-mutated tumors lack capacity for homologous recombination, PARP inhibitors have been evaluated as targeted therapy in these cancers, with striking results in both preclinical and early-phase clinical trials. A phase I study of PARP inhibitor olaparib evaluated 60 patients with solid tumors, including 22 patients with *BRCA1* or *BRCA2* mutation. Notable antitumor activity was identified in patients with *BRCA* mutations, with 9 of 19 demonstrating complete or partial radiologic response (1 patient had breast cancer), and 12 of 19 with radiologic or tumor marker response or stable disease (2 patients had breast cancer) [Fong *et al.* 2009].

A phase II study of the same agent evaluated 54 patients with *BRCA1*- or *BRCA2*-mutated advanced breast cancer treated with two different doses of olaparib. Overall response rate (ORR) was 41% in the higher-dose cohort, and 22% in the lower-dose cohort [Tutt *et al.* 2010]. However, results were not as convincing in an additional phase II study of patients with ovarian cancer or TNBC treated with olaparib. In this study, 11 patients with and 15 TNBC patients without *BRCA* mutations were treated with the same dose of olaparib, with no objective responses identified [Gelmon *et al.* 2011]. Ongoing studies are evaluating olaparib in combination with chemotherapy and new targeted agents in *BRCA*-mutated breast cancer and TNBC [ClinicalTrials.gov identifiers: NCT00707707 and NCT01116648], and other PARP inhibitors are being evaluated in various settings [ClinicalTrials.gov identifiers: NCT01074970 and NCT01905592].

As it is the impairment of homologous recombination and not *BRCA* mutations in particular that are thought to predict response to PARP inhibition, it is possible that biomarkers of homologous-recombination deficiency could be used to identify additional patients that might benefit from such therapy [Fong *et al.* 2009]. This concept is further supported by preclinical investigation that has identified seven DNA repair genes as potential predictors of response to olaparib therapy based on transcription levels [Daemen *et al.* 2012]. A DNA-based homologous recombination deficiency scoring system has also been developed through analysis of loss of heterozygosity in the genome of ovarian tumors, though must still be evaluated as a predictor of disease response in this and other tumor types [Abkevich *et al.* 2012]. Furthermore, evaluation of the BRCA-like classifier described above as a predictor of response to platinum-based chemotherapy would also be of interest in the setting of PARP inhibition.

Iniparib has also been evaluated in *BRCA*-mutated TNBC. This compound was initially developed as a PARP inhibitor, but later found to lack the properties that define other agents of that class [Mateo *et al.* 2013]. Though the true mechanism of the drug remains unknown, it is still being developed in *BRCA*-mutated cancers. Most recently the agent was evaluated in the neoadjuvant setting in combination with gemcitabine and carboplatin. This study demonstrated pCR rates of 33% in *BRCA* wild-type breast cancers, 47% in *BRCA* 1 or 2 mutant breast cancers, and 56% in breast cancers that were both triple-negative and *BRCA* 1 or 2 mutant [Telli *et al.* 2013].

Other biomarkers predictive of response to targeted agents

As *BRCA*-mutated cancers represent only 20% of unselected TNBC [Gonzalez-Angulo *et al.* 2011], attempts have been made to identify genomic aberrations other than *BRCA* mutations that may serve as both biomarkers and targets for new therapies for additional TNBC subtypes. Mutations of p53 have been documented at a high frequency in TNBC (up to 80% in one study) [The Cancer Genome Atlas Network, 2012], making this an area of interest for both therapeutic and biomarker development. However, p53 mutations do not reliably affect p53 pathway function, making targeting p53 or its downstream effectors difficult. Gene signatures have been used to specifically identify markers of p53 pathway dysfunction, and though these remain to be validated, may provide p53 targets and biomarkers in the future [Turner *et al.* 2013].

Current strategies in development of p53-targeted agents are based on restoring functional p53 through two mechanisms: (1) preventing binding of p53 to MDM2 and thus associated p53 degradation; and (2) inhibiting p53 mutant proteins to restore normal transcription [Lehmann and Pietenpol, 2012]. APR-246, a novel molecule based on the second mechanism of p53 targeting, has been evaluated in a phase I clinical trial, though no breast cancer patients were enrolled. The small size of the study also prevented the evaluation of p53 mutational status as a predictive biomarker [Lehmann *et al.* 2012]. Future trials may evaluate the role for such an agent in TNBC, as well as the value of p53 mutation as a biomarker for response.

Mutated p53 has been identified as a potential biomarker for non-p53 targeted agents, including novel agent ENMD-2076, a small-molecule Aurora A/B and VEGFR2/KDR inhibitor. Preclinical evaluation of this agent demonstrated anticancer activity in both *in vitro* and *in vivo* models of breast cancer, with gene array data from tumor cell lines used to help identify markers associated with greater sensitivity and resistance. Such analysis identified HER2 pathway upregulation as a marker of resistance to ENMD-2076, and TNBC cell lines as overall more sensitive. Amongst these TNBC cell lines, increased p53 mRNA and protein expression was associated with greater sensitivity to the agent [Diamond *et al.* 2013]. This biomarker will be further evaluated in an ongoing phase II trial of the drug in TNBC [ClinicalTrials.gov identifier: NCT01639248].

In addition to mutations in p53, upregulation of the phosphoinositide 3-kinase (PI3K) signaling pathway has also been identified as a common aberration in TNBC tumors. This pathway has been explored in drug and biomarker development for this disease [Gordon and Banerji, 2013], with evaluation of the mTOR inhibitor everolimus an example of such. In one study, five of nine TNBC cell lines exposed to the agent showed decreased proliferation. Further evaluation of potential predictive biomarkers revealed all five sensitive lines expressed basal markers EGFR or CK5/6, while resistant lines did not [Yunokawa *et al.* 2012]. Early-phase clinical trial data has not shown a significant treatment response in the subpopulation of TNBC patients included in such trials [Ellard *et al.* 2009], though larger studies of this subpopulation and further biomarker development to identify patients more likely to benefit may prove otherwise. However, the challenges of the development of a predictive biomarker for everolimus in the treatment of ER+ breast cancer highlight the difficulties of this endeavor, with such a biomarker yet to be identified in the years following completion of the BOLERO-2 trial [Chavez-Macgregor and Gonzalez-Angulo, 2012].

There are many additional clinical trials evaluating PI3K pathway inhibitors in TNBC currently underway, including other mTOR inhibitors, PI3K inhibitors, and combination PI3K/mTOR inhibitors, as well as AKT inhibitors. Preclinical data has demonstrated greater efficacy of these agents in combination with other therapies including PARP inhibitors[Gordon and Banerji, 2013], and some of these studies include predictive biomarker analysis [ClinicalTrials.gov identifier: NCT01629615].

Less common genomic alterations evaluated for therapeutic and biomarker activity in TNBC include overexpression and overactivation of Src, which has been implicated in breast cancer pathogenesis. Dasatinib is a multikinase inhibitor that targets BCR-ABL and the Src family of kinases, among others. A preclinical study evaluated gene signatures that correlate with response to dasatinib in various breast cancer cell lines, revealing a greater response in tumors with lower expression of ER, PR, and HER2, consistent with the triple-negative subgroup of breast cancer [Huang *et al.* 2007]. A second similar study in breast cancer cell lines confirmed this finding, and also revealed a correlation between expression of moesin, caveolin, and *yes*-associated protein-1 (YAP-1) expression and response to dasatinib. All three are known to interact with the Src family of kinases, giving a rationale to these potential biomarkers [Finn *et al.* 2007]. A phase II study of 44 patients with TNBC treated with dasatinib demonstrated a response rate of only 4.7%, with no biomarker correlates reported [Finn *et al.* 2011]. There is hope that such correlative studies may provide insight into the subpopulation that might benefit from the agent.

The MAP kinase pathway has also been evaluated as a potential target in TNBC, with corresponding biomarker studies performed. Preclinical evaluation of 21 breast cancer cell lines treated with MEK1/2 inhibitor trametinib demonstrated

Table 3. Biomarker evaluations in novel targeted agents in triple-negative breast cancer.

Drug	Target	Potential biomarker	Phase of study	Ongoing evaluation
Olaparib	Poly ADP ribose polymerase	*BRCA* mutation	Phase II	Phase I and II in combination
Iniparib	Unknown	*BRCA* mutation	Phase II	None documented
APR-246 [Lehmann *et al*. 2012]	Mutant p53	P53 mutation	Phase I	None documented
ENMD-2076 [Diamond *et al*. 2013]	Aurora kinase VEGFR2/KDR	P53 mRNA and protein expression	Preclinical	Phase II [ClinicalTrials.gov identifier: NCT01639248]
Everolimus [Yunokawa *et al*. 2012]	mTOR	EGFR CK5/6	Preclinical	No studies specific to TNBC
Dasatinib [Finn *et al*. 2007]	Src and other kinases BCR-ABL	Moesin Caveloin YAP-1	Preclinical	Phase II did not report biomarkers [Finn *et al*. 2011]
Trametinib [Jing *et al*. 2012]	MEK 1/2	DUSP6	Preclinical	Phase I kinome study [ClinicalTrials.gov identifier: NCT01467310]

greatest sensitivity in the 11 TNBC cell lines evaluated. The authors ultimately identified DUSP6, a phosphatase that decreases pERK2 activity upon MAPK pathway activation, as a potential marker of sensitivity to the drug. Expression of this gene was associated with greater sensitivity to trametinib in multiple solid tumor cell lines, though was not evaluated in the breast cancer models [Jing *et al.* 2012]. Current clinical trials are accruing to further evaluate the kinome of patients treated with trametinib in hopes of identifying additional biomarkers [ClinicalTrials.gov identifier: NCT01467310].

Mutations that serve as targets for currently available agents, including *EGFR, HER2, KRAS*, and *BRAF* mutation as well as *EML4-ALK* fusion and *EGFR* copy gain have also been studied in TNBC. Of 65 TNBC specimens, the only abnormalities identified were a *HER2* mutation in one patient and *EGFR* gene amplification in a second patient [Grob *et al.* 2012]. This suggests little utility for use of these mutations as biomarkers or as potential targets for therapy.

These numerous studies are encouraging in their efforts to identify biomarkers for TNBC in the setting of novel targeted agent development (Table 3). However, the best data for any of these biomarkers has still been developed in only small preclinical or retrospective studies. There is hope that these promising findings will be further evaluated in large, prospective trials, and that coordinated drug and biomarker development may be central to such trials in the future.

Conclusion

TNBC is an important example of the heterogeneity within the currently described breast cancer subtypes. It is clear that a general approach to treatment of TNBC is not possible, and the development of predictive biomarkers to identify patients who will benefit from individual therapies is truly a necessity. This review demonstrates that the field of biomarker-driven therapy remains underdeveloped in TNBC, with a reliable and clinically relevant biomarker predictive of therapeutic response yet to be identified. This is true despite great efforts made in a large number of studies, and is likely related to the many limitations to the current research in this area. Importantly, the majority of the studies evaluating predictive biomarkers in TNBC are small and retrospective, limiting the potential for clinical application due to concern for bias. The retrospective nature of these trials also highlights the lack of simultaneous development of biomarkers with the therapies they are predictive for, as well as the concept that the majority of predictive biomarkers are not identified according to the underlying biology of the disease or the effect of the treatment of interest.

This concept of biomarker development without a clear biologic rationale is quite evident in studies focused on markers of response to cytotoxic neoadjuvant chemotherapy, which represent the greatest proportion of data evaluating predictive biomarkers in TNBC. The wide variability of chemotherapy regimens used as treatment, often within studies evaluating a single biomarker,

exemplify the lack of consideration for the underlying biology. As each chemotherapeutic drug has a unique mechanism, it seems unlikely that a single biomarker would be predictive for all agents. This concept has been the focus of development of biomarkers for new biologic agents, and incorporation of such into the search for predictive biomarkers for traditional chemotherapeutic agents may help further advance this important area.

Owing to the poor prognosis of TNBC despite currently available cytotoxic therapies, the area of greatest need is for development of more effective targeted agents with companion biomarkers. The evaluation of *BRCA* mutations as biomarkers for response to PARP inhibitors in addition to DNA-damaging chemotherapy agents provides an important example of such an effort in TNBC. However, there is still much work to be done to integrate BRCA-like and other tumors with impairment of homologous recombination into biomarker evaluation, as well as to determine the cause of differential response rates in *BRCA*-mutated breast and ovarian cancers. Furthermore, the utility of such biomarkers is limited to a small proportion of TNBC patients. Evaluation of genomic aberrations in other subtypes of TNBC that may serve as biomarkers for novel targeted agents is gaining interest. However, years of development of agents targeting frequent genomic abnormalities such as p53 mutation and PI3K pathway activation without overwhelming success in TNBC support the need for an even greater focus in this area.

Though improved understanding of TNBC has revealed the incredible complexity of this breast cancer subtype, it has also allowed development of new agents and exploration of associated biomarkers of treatment response. However, the field of biomarker development in TNBC remains immature, without clinically relevant predictive biomarkers to show for the efforts put forward in this area. The poor prognosis of TNBC and importance of complete response to neoadjuvant therapy in improving such highlight the need for biomarkers to guide therapy in the future. To facilitate the development of predictive biomarkers, important changes must be made in this field. A focus on simultaneous development of biomarkers with new therapeutic agents, beginning in the preclinical phase, is vital. Not only does this method of development support the link of biomarkers to the biology of the underlying disease and treatment, but also ensures prospective evaluation, allowing for more direct translation to the clinic. Similarly, evaluation of biomarkers for currently available chemotherapeutic agents should focus on the mechanism and characteristics of each agent separately, rather than on chemotherapy in general. With these changes in the development of predictive biomarkers for TNBC comes the possibility for exciting advances in this field for the future.

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