New Strategies for Triple-Negative Breast Cancer—Deciphering the Heterogeneity

Ingrid A. Mayer1,3, Vandana G. Abramson1,3, Brian D. Lehmann2,3, and Jennifer A. Pietenpol2,3

Abstract

Triple-negative breast cancer (TNBC) is a heterogeneous disease; gene expression analyses recently identified six distinct TNBC subtypes, each displaying a unique biology. Exploring novel approaches to treatment of these subtypes is critical because less than 30% of women with metastatic breast cancer survive five years and virtually all women with metastatic TNBC will ultimately die of their disease despite systemic therapy. To date, not a single targeted therapy has been approved for the treatment of TNBC and cytotoxic chemotherapy remains the standard treatment. We discuss the current and upcoming therapeutic strategies being explored in an attempt to “target” TNBC.

Disclosure of Potential Conflicts of Interest

I.A. Mayer has received commercial research support from Novartis. No potential conflicts of interest were disclosed by the other authors.

CME Staff Planners’ Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the biology and prognosis of triple-negative breast cancers (TNBC), as well as the landscape of current and future therapeutic strategies being explored in an attempt to “target” TNBC.

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Background

“Triple-negative breast cancer” (TNBC) is a term used to identify invasive breast cancers that lack the expression of estrogen and progesterone receptor (ER/PR) and HER2 (ERBB2). TNBCs, which account for 15% of all invasive breast cancers, are generally of a higher grade, occur at a higher rate in young and African-American women, and most harbor a basal-like gene expression signature (1, 2). Patients with TNBC have an increased likelihood of distant recurrence and death compared with women with other types of breast cancer (3), as well as a tendency to develop visceral metastases early in the course of their disease. Improved approaches to treatment of these cancers are critical because the median survival of patients with metastatic TNBC is only 13 months, and virtually all women with metastatic TNBC ultimately die of their disease despite systemic therapy (4).

TNBC subtyping

Although the terms “triple negative” and “basal like” are not synonymous, approximately 80% of clinical TNBCs (ER/PR/HER2 negative) are classified as basal like, based on PAM50 intrinsic subtype classification (5). Tumors arising in BRCA1 carriers have many similarities to basal-like sporadic breast tumors, including greater likelihood of being high-grade, ER/PR negative, HER2 negative, and a high frequency of p53 mutations (6). Basal keratins are expressed by both sporadic basal-like tumors and tumors with BRCA1 mutations, and both groups cluster together by gene expression profiling (6). Other studies support these data, in which familial-BRCA1 breast cancers have shared features with a subset of sporadic tumors, indicating a common or similar etiology. Hallmarks of this “BRCaness” include basal-like phenotype (associated with the BRCA1 phenotype but not with the BRCA2 phenotype), ER negativity, EGF receptor expression, c-MYC amplification, TP53 mutations, loss of RAD51-focus formation, extreme genomic instability, and sensitivity to

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DNA cross-linking agents (7). The clinical implications of the definition of this group of tumors with a "BRCAness" hallmark lie in its potential to influence the clinical management of these tumors, allowing for rational trials exploring the role of chemotherapy and biologic agents targeted toward DNA repair defects.

Using gene expression analyses, we recently identified distinct TNBC subtypes, each displaying a unique biology (8). The six TNBC subtypes include two basal like (BL1 and BL2), an immunomodulatory, a mesenchymal (M), a mesenchymal stem like (MSL), and a luminal androgen receptor (LAR) subtype, the last being characterized by androgen receptor signaling (8). We further used gene expression analysis to identify TNBC cell lines representative of these subtypes. Predicted "driver" signaling pathways were pharmacologically targeted in these cell lines as proof-of-concept and to generate preclinical data to inform future clinical trial design.

We also performed a direct comparison of 374 TNBC samples extracted from 14 datasets to determine the relationship between the PAM50 intrinsic and TNBC molecular subtypes. As anticipated, the majority of the TNBC samples are indeed classified as basal like (80.6%) followed by HER2 (0.2%), normal like (14.6%), luminal B (3.5%), and luminal A (1.1%) by PAM50 (Fig. 1A; modified from ref. 9). With the exception of MSL and LAR, all other TNBC subtypes are primarily composed of the basal-like intrinsic subtype. MSL TNBCs are about 50% basal like, and the remainder are composed of normal like (27.8%) and luminal B (13.9%). Unlike other subtypes, the LAR subtype is primarily classified as HER2 (74.3%) and Luminal B (14.3%) by PAM50 intrinsic subtyping (Fig. 1B). Therefore, PAM50 intrinsic subtyping alone has the potential to classify approximately 75% of TNBCs that are AR+ as HER2+.

To determine the potential clinical utility of assessing TNBC subtype, we generated a tool (TNBCtype) that determines the TNBC molecular subtype from gene expression profiles independent of platform (10). Recently, Masuda and colleagues performed a retrospective analysis on 130 TNBC cases treated with neoadjuvant Adriamycin/Cytoxan/Taxol-containing chemotherapy (11). Although the overall pathologic complete remission (pCR) rate was 28%, subtype-specific responses differed substantially with the BL1 subtype achieving highest pCR rate (52%) and the BL2, LAR, and MSL subtypes having the lowest response (0%, 10%, and 23%, respectively). Furthermore, TNBC subtype was shown to be an independent predictor of pCR status ($P = 0.022$) by a likelihood ratio test. We also used the TNBCtype tool (10) to subtype 163 primary cases in The Cancer Genome Atlas (TCGA) considered to be TNBC (Table 1 and Supplementary Table S1). In agreement with the work of Masuda and colleagues described above, we found a very similar distribution of subtypes and subtype-specific differences in survival. These findings and additional validation should guide differential use of chemotherapy-based regimens and alignment of patients with select TNBC subtypes to clinical trials investigating targeted therapies. There is a need for prospective validation of associated pCR rates among the TNBC subtypes and to determine whether subtyping will be useful for predicting long-term patient outcome.
On the Horizon

Distinguishing one subtype of TNBC from another from a histologic point of view is challenging. On the basis of the fact that the different TNBC subtypes seem to have distinct responses to a given therapy, as exemplified by Masuda and colleagues’ retrospective analysis (11), we postulate that it is inappropriate to treat all TNBC in a similar fashion. The features of TNBC described in this review, in relation to possible therapeutic targets, are discussed herein and are summarized in Fig. 2.

Platinum agents

Platinum salts, including carboplatin and cisplatin, lead to DNA cross-link strand breaks, which may be especially important in cells that are deficient in homologous recombination repair mechanisms such as BRCA-mutated cells and TNBC. Leong and colleagues (12) reported a p63-dependent tumor survival pathway that mediates cisplatin sensitivity, specifically in TNBC cells grown in vitro. Extending this observation to the clinical setting, Rocca and colleagues (13) conducted a retrospective analysis of core biopsies of patients with breast cancer treated with neoadjuvant cisplatin-based chemotherapy in breast cancer and showed that administration of cisplatin without anthracyclines yielded a higher rate of pCR in patients with p63-positive tumors. Analysis of tumor tissue from ongoing cisplatin-based neoadjuvant trials will allow further analysis of the relationship of the molecular features of various TNBC subtypes and cisplatin sensitivity.

In a small-phase II study (29 patients), Silver and colleagues showed activity of neoadjuvant cisplatin as a single agent in the treatment of patients with locally advanced TNBC. The observed pCR was 22%, 50% of patients had a partial response, and 14% had a complete response (14). In another small study, 9 of 10 patients with stage I–III breast cancer harboring BRCA1 mutations achieved a pCR after neoadjuvant therapy with cisplatin (15).

More recently, solid evidence of the activity of platinum agents in TNBC was provided by two large phase II randomized trials in the neoadjuvant setting: the GeparSixto trial, in its TNBC subset, compared neoadjuvant paclitaxel, doxorubicin, and bevacizumab with (159 patients) or without (161 patients) carboplatin. The pCR rate improved from 37.9% to 58.7% with the addition of carboplatin. Biomarker studies in this trial are under way to determine whether certain subsets of TNBC benefit more from the addition of carboplatin (16) and should provide significant insights to the genomic alterations that mediate sensitivity to platinum agents. CALGB40603 (NCT00861705) is a randomized phase II trial with a 2 × 2 factorial design that explored the addition of carboplatin ± bevacizumab to neoadjuvant weekly paclitaxel followed by dose-dense AC in 443 patients with stage II/III TNBC (17). The pCR rate improved from 41% to 54% with the addition of carboplatin; bevacizumab had no added benefit. It is important to note that neither of these studies was powered to detect disease-free or overall survival (OS) benefit.

**PARP inhibition**

ATM, BRCA1, and TP53 are critical genes in the DNA damage response (DDR) signaling pathway, which could play a role during breast tumorigenesis. BRCA1 mutations are rare in sporadic tumors, but nevertheless, high-grade breast cancers display a high frequency of LOH and/or abnormal expression of ATM, BRCA1, and TP53. Multi-genetic analyses show that BRCA1 abnormalities are independently associated with high-grade tumors (18). Both BRCA1 and BRCA2 contribute to DNA repair and transcriptional regulation in response to DNA damage and regulate other genes involved in DNA repair, cell cycle, and apoptosis (19). Increasing evidence supports a role for BRCA1 in double-strand DNA break repair in part through its interaction with RAD51 and the Fanconi anemia proteins (20, 21). Cell lines deficient in BRCA1 or other components of the Fanconi anemia–BRCA1 pathway are more sensitive to X-ray–induced damage and DNA cross-linking agents such as cisplatin (20, 22).

**Table 1. TNBC subtyping predictions for TCGA primary breast tumors**

<table>
<thead>
<tr>
<th>TNBC subtype</th>
<th># Samples (percentage)</th>
<th>Median DFS (mo)b</th>
<th>Median OS (mo)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL1</td>
<td>27 (17%)</td>
<td>20.1</td>
<td>21.1</td>
</tr>
<tr>
<td>BL2</td>
<td>12 (7%)</td>
<td>12.5</td>
<td>8.4</td>
</tr>
<tr>
<td>IM</td>
<td>30 (18%)</td>
<td>22.7</td>
<td>24.8</td>
</tr>
<tr>
<td>M</td>
<td>39 (24%)</td>
<td>9.1</td>
<td>9.5</td>
</tr>
<tr>
<td>MSL</td>
<td>10 (6%)</td>
<td>13.9</td>
<td>20.9</td>
</tr>
<tr>
<td>EAR</td>
<td>14 (9%)</td>
<td>4.4</td>
<td>5.7</td>
</tr>
<tr>
<td>UNC</td>
<td>31 (19%)</td>
<td>22.0</td>
<td>24.9</td>
</tr>
<tr>
<td>All TNBC</td>
<td>163 (100%)</td>
<td>11.8</td>
<td>15.2</td>
</tr>
</tbody>
</table>

Abbreviations: DFS, disease-free survival; IM, immunomodulatory; M, mesenchymal.

aTNBC subtype predictions for the TCGA primary tumors were made using RSEM genes normalized (upper quartile count = 1,000) RNA-seq abundance estimates (20130606 Firehose BRCA standard run). TNBC cases were identified as previously described (8). Samples were filtered on ER, PR, and HER2 mRNA expression to identify TNBC tumors and outliers were removed after principal component analysis. The TNBC subtype tool (10) was used to make TNBC subtype predictions for the 163 TNBC samples.

bSurvival data for TCGA TNBC cases were obtained from the cBioPortal (www.cbioportal.org) on December 19, 2013.
and not on inherited depends on homologous recombination deficiency (HRD) (24). Sensitivity to PARP inhibition persistence of DNA lesions normally repaired by homologous recombination (24). Sensitivity to PARP inhibition depends on homologous recombination deficiency (HRD) and not on inherited BRCA1 or BRCA2 deficiency per se (24). Therefore, use of PARP1 inhibitors as a therapeutic strategy in the treatment of sporadic breast cancers with "BRCAness," including basal-like breast cancers, may be a promising approach.

There are at least three potential roles of PARP inhibitors in cancer treatment: sensitization to chemotherapy and radiotherapy, synthetic lethality in patients with hereditary mutations in BRCA1/2 genes (inherited defect in homologous recombination), and finally, leveraging of putative "BRCA-like" defective tumors and defects in DNA repair such as those seen in TNBC.

Significant single-agent activity was recently reported with the PARP inhibitor olaparib in patients with BRCA-deficient metastatic breast cancer. Overall responses ranged from 22% (100 mg twice daily) to 41% (400 mg twice daily) with minimal toxicity (25). Olaparib as a single agent was also evaluated in a phase II study in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or TNBC (26). In this trial, however, no confirmed objective responses were seen in the 26 patients with TNBC.

A phase II, open-label, two-arm randomized safety and efficacy trial (Study 20070102) investigated gemcitabine/carboplatin with or without iniparib in patients with metastatic TNBC. The final analysis of 123 randomized patients showed that addition of iniparib to gemcitabine/carboplatin improved the clinical benefit rate from 33.9% to 55.7% ($P = 0.015$) and ORR from 32.3% to 52.5% ($P = 0.023$). The addition of iniparib prolonged the median progression-free survival (PFS) from 3.6 to 5.9 months (HR $= 0.59$; $P = 0.012$) and the median OS from 7.7 to 12.3 months (HR $= 0.57$; $P = 0.014$; ref. 27). A subsequent phase III study evaluating gemcitabine/carboplatin ± iniparib with similar inclusion criteria as the phase II study did not meet the prespecified criteria for significance for the coprimary endpoints of OS and PFS. Interestingly, the results of a prespecified analysis in patients treated in the second- and third-line setting showed an improvement in OS and PFS, consistent with what was seen in the smaller phase II study (28).

More recently, a single-arm phase II study of neoadjuvant gemcitabine, carboplatin, and iniparib in patients with TNBC or BRCA 1/2 mutation-associated breast cancer showed impressive responses, especially in BRCA 1/2 carriers (29). An HRD assay was developed as part of this study to identify non-BRCA1/2 mutation carriers with “BRCA-like” cancers who might benefit from DNA repair–targeted treatment strategies. Higher HRD scores were significantly associated with higher rates of response. Of note, more recent preclinical data have shown that iniparib does not possess characteristics typical of the PARP inhibitor class. Investigations into potential targets of iniparib and its metabolites are ongoing, and additional targets are under investigation.

Modulation of p53 family signaling

The p53 family of transcription factors (p53, p63, and p73) are key regulators of signaling pathways that regulate developmental and tumor-suppressive processes (30).
p53 tumor suppressor is mutated in approximately 30% of breast cancers (31) and shows a strong association with the basal-like subgroup (32).

The transcription factor p73 plays critical roles during development and tumorigenesis. It exhibits sequence identity and structural homology with p53 and can engage p53-like tumor-suppressive programs. Rosenbluth and colleagues defined the p73 genomic-binding profile and demonstrated its modulation by rapamycin, an inhibitor of mTOR and inducer of p73. Rapamycin selectively increases p73 occupancy at a subset of its binding sites. In addition, multiple determinants of p73 binding activity and function are evident and modulated by mTOR (33). Interestingly, the combination of mTOR inhibitor, paclitaxel, and cisplatin, can synergistically regulate the p73/p63 signaling axis and promote apoptosis in breast cancer cells.

Considering the above preclinical data, our group hypothesized that drugs that either negatively modulate p63 and/or activate p73 (such as an mTOR inhibitor, paclitaxel, and cisplatin combination) would promote increased apoptosis in TNBC. We recently completed a large randomized phase II study of neoadjuvant cisplatin and paclitaxel with or without everolimus for 3 months in 145 patients with stage II and III TNBC (NCT00930930). Despite the lack of a difference in pCR rate in both arms (38%), the pCR rate seen with this short course of taxane/platinum chemotherapy backbone, which did not contain anthracyclines, was similar to that of a more extensive, anthracycline/taxane-containing chemotherapy regimen (34). TNBC subtyping, DNA mutations and alterations, as well as markers of proliferation, apoptosis, PI3K/mTOR, and DDR signaling are part of the correlative studies being done to investigate a molecular signature or biomarker predictive of benefit from the paclitaxel/cisplatin ± everolimus combination in TNBC.

PI3K inhibition

Exome sequencing of TNBC identifies TP53 (62%) as the most frequently mutated gene in TNBC, followed by alterations in the phosphoinositide-3 kinase (PI3K) pathway that include PIK3CA (10.2%) and PTEN (9.6%) mutations (35).

Our preclinical data show that a significant fraction of TNBC cells lines are sensitive to PI3K inhibitors. The LAR subtype cell lines in particular have a high rate of PIK3CA-activating mutations and exhibit strong sensitivity to PI3K inhibitors, as well as to androgen blockers such as bicalutamide (8). The coevolution of PIK3CA mutations with androgen receptor dependency is similar to ER-positive breast cancers frequently containing PIK3CA mutations (36, 37). Our studies of combinations of PI3K inhibitors and cisplatin show either additive or synergistic decreases in tumor viability, with significant decreases in pAKT and pS6 levels and a concomitant elevation in cleaved PARP. Of note, PI3K inhibition of TNBC cells has been shown to sensitize cells to DNA-damaging agents by, in effect, creating a BRCA-deficient state (38).

Other studies provide additional preclinical rationale for the combined use of a DNA-damaging agent with PI3K inhibitors (38, 39). These studies confirm that in addition to regulating cellular processes, including growth, metabolism, and survival (40), PI3K also stabilizes double-strand breaks by interacting with the homologous recombination complex (41). PI3K blockade promotes homologous recombination deficiency by downregulating BRCA1/2 and thus sensitizing BRCA-proficient tumors to PARP inhibition. The elevated levels of pAKT and pS6 are consistent with activated PI3K in the TNBC tumors. To capitalize on these findings, a phase I study of the pan-PI3K inhibitor BKM120 (Novartis) in combination with the PARP inhibitor olaparib in patients with metastatic TNBC is ongoing. BKM120 would be expected to create a BRCA-mutant–like tumor state, thus making it susceptible to PARP inhibition.

Together, these data suggest that targeting the PI3K pathway could be clinically relevant in TNBC. Our group is now initiating a clinical trial in which therapy for patients with metastatic TNBC will be determined by androgen receptor status. Patients with tumors that express androgen receptor by immunohistochemistry will receive the antiandrogen bicalutamide with a PI3K inhibitor, and patients who have androgen receptor–negative TNBC will be randomized to chemotherapy with cisplatin or cisplatin with a PI3K inhibitor.

MEK inhibition

Hoeflich and colleagues have shown that a large number of basal-like breast cancer cell lines are sensitive to MEK inhibition (42). Several of these have a BRAF, HRAS, or KRAS mutation; however, the majority of these cell lines show upregulation of the RAS/MEK pathway without an identified oncogenic mutation. Interestingly, loss of the tumor suppressor phosphatase Pten (which occurs in 29% of TNBC; ref. 43) results in upregulation of the PI3K/Akt pathway, and is associated with lack of response to MEK inhibitors (42).

Balko and colleagues found that DUSP4 loss, a negative regulator of extracellular signal–regulated kinase (ERK)-1 and ERK2, is associated with basal-like breast cancers and Ras–ERK activation (44). In existing microarray datasets, DUSP4 mRNA levels were lowest in basal-like breast cancers. Moreover, in a dataset of 286 breast cancers from patients who did not receive adjuvant therapy, low DUSP4 expression predicted for shorter recurrence-free survival time. DUSP4 deficiency was associated with Ras–ERK pathway activation in chemotherapy-refractory basal-like breast cancers. In addition, DUSP4 mRNA expression was inversely correlated with the Ras–ERK pathway score in a series of 230 primary breast cancers. Thus, loss of DUSP4 may be a crucial biomarker in identifying activation of the Ras–ERK pathway in basal-like breast cancers (44).

In summary, the preclinical data above suggest that cases of TNBC with Pten expression and low expression of DUSP4 have a dependency on Ras–ERK activation, and therefore could be sensitive to agents being investigated in clinical trials combining MEK inhibitors with chemotherapy.
Table 2. Targeted therapies clinical trials in TNBC

<table>
<thead>
<tr>
<th>Study design</th>
<th>Clinical trial</th>
<th>Type of inhibitor</th>
<th>Patient population</th>
<th>Clinicaltrials.gov</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>Phase I of BKM120/olaparib for TNBC or high-grade serous ovarian cancer</td>
<td>pan-PI3K PARP</td>
<td>Patients with TNBC or high-grade serous ovarian cancer</td>
<td>NCT01623349</td>
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<tr>
<td>Phase II</td>
<td>A trial of BKM120 (a PI3K inhibitor) in patients with TNBC</td>
<td>pan-PI3K</td>
<td>TNBC</td>
<td>NCT01629615</td>
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<td></td>
<td>A trial of cisplatin ± GDC0941 (a PI3K inhibitor) in patients with androgen-receptor–negative TNBC</td>
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<td>NCT01918306</td>
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<tr>
<td>Phase Ib/II</td>
<td>Everolimus (RAD001) and carboplatin in pretreated metastatic breast cancer</td>
<td>TORC1 inhibitor</td>
<td>HER2-negative MBC</td>
<td>NCT00930475</td>
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<td>Phase II</td>
<td>Cisplatin and paclitaxel with or without everolimus in treating patients with stage II or stage III breast cancer</td>
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<td>Stage II/III TNBC</td>
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<td>Phase I</td>
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<td>Metastatic TNBC</td>
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<td>Phase II</td>
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<td>Androgen receptor-positive metastatic TNBC</td>
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<td>Phase II</td>
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<td>HDAC inhibitor</td>
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<td>Carboplatin and paclitaxel albumin-stabilized nanoparticle formulation with or without vorinostat in treating women with breast cancer that can be removed by surgery</td>
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<td>Phase I</td>
<td>Olaparib in combination with carboplatin for refractory or recurrent women’s cancers</td>
<td>PARP inhibitor</td>
<td>Women’s cancers; men with BRCA mutations</td>
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<td>Axd2281 plus carboplatin to treat breast and ovarian cancer</td>
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<td>Breast and ovarian metastatic cancers</td>
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Conclusions

TNBC is a very heterogeneous disease, for which a number of therapeutic strategies are being explored. Studies described above clearly indicate that TNBC cannot be treated in a uniform fashion; for instance, TNBCs that have a basal-like genotype are more likely sensitive to DNA cross-linking agents, such as platinum-based chemotherapy and PARP inhibitors. On the other hand, the androgen receptor–expressing tumors may derive greater benefit from a combination of an androgen blocker and a PI3K inhibitor. Numerous experimental approaches are under way with the goal of identifying “targets” in TNBC, with PI3K inhibitors, MEK inhibitors, HSP-90 inhibitors, histone deacetylase inhibitors, PD-1 (programmed death 1) inhibitors, etc. under consideration or currently being investigated in the clinical setting (Table 2).

However, TNBC is clearly a complex disease. As such, it is likely that its biology involves multiple redundancies and pathway cross-talk. If only one pathway is selectively inhibited, the efficacy of the therapeutic strategy would likely be undermined by activation of a compensatory pathway. Therefore, it is not surprising that to date, not a single targeted therapy has been approved for treatment of TNBC, for which cytotoxic chemotherapy remains the standard treatment. Combining two or more targeted agents may be required for a more rational and optimal approach to TNBC treatment. Efforts in this direction are evidenced by novel clinical trials involving different/complementary pathway

<table>
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<tr>
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<tr>
<td>Phase II</td>
<td>A phase II study of standard chemotherapy plus BSI-201 (a PARP inhibitor) in the neoadjuvant treatment of TNBC</td>
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<td>TNBC metastatic BRCA mutant cancers</td>
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<td>PARP inhibition for TNBC (ER-/PR-/HER2-)</td>
<td>PARP inhibition for TNBC (ER-/PR-/HER2-) with BRCA1/2 mutations</td>
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<td>ABT-888 with cyclophosphamide</td>
<td>Study of SAR240550 (BSI-201) in combination with gemcitabine/cisplatin, in patients with metastatic TNBC</td>
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<td>ABT-888 and temozolomide for metastatic breast cancer and BRCA1/2 breast cancer</td>
<td>Two regimens of SAR240550/weekly paclitaxel and paclitaxel alone as neoadjuvant therapy in patients with TNBC</td>
<td>Stage II/III TNBC</td>
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<td>Rucaparib (CO-338; formally called AG-014699 or PF-0136738) in treating patients with locally advanced or metastatic breast cancer or advanced ovarian cancer</td>
<td>Rucaparib (CO-338; formally called AG-014699 or PF-0136738) in treating patients with locally advanced or metastatic breast cancer or advanced ovarian cancer</td>
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Table 2. Targeted therapies clinical trials in TNBC (Cont’d)
inhibitors, such as phase I and II studies combining PI3K inhibitors with PARP inhibitors or with androgen blockers, platinum agents with PI3K or mTOR inhibitors or HDAC inhibitors, etc. (Table 2).

It is generally established that patients with breast cancer who achieve a pCR (lack of residual disease in both breast and axilla) after neoadjuvant therapy exhibit a good long-term outcome (45). A high residual disease burden (RDB) in the posttreatment, surgically excised cancer has been shown to correlate with a high rate of recurrence and death (46, 47). More specifically, at least 40% of patients with TNBC who do not achieve a pCR after anthracycline and taxane–based neoadjuvant chemotherapy will have a recurrence within 36 months (48). However, approximately 30% of TNBC treated with anthracycline and taxane–based chemotherapy will have a pCR after treatment (48), and consistent with the above data, achieving a pCR to neoadjuvant chemotherapy in this group of patients has been shown to be a strong positive prognostic factor. Patients with TNBC who complete neoadjuvant therapy and have no clinical evidence of metastatic disease after surgical excision of the cancer, regardless of their RDB, are usually observed without further treatment. This conduct might not be appropriate for patients at a very high risk of early recurrence such as those with a high RDB in the residual drug-resistant tumor. However, the appropriate therapy for those patients is unknown, and personalized treatment strategies using adjuvant therapies that molecularly target tumor-specific dependencies are sorely needed. The intertumor heterogeneity of TNBCs before and after neoadjuvant chemotherapy underscores the need for powerful and broad molecular approaches to identify actionable molecular alterations and, in turn, better inform personalized therapy of this aggressive disease. Incorporation of these approaches into clinical studies and eventually standards of care will aid in the prioritization of patients with residual disease after neoadjuvant chemotherapy into rational adjuvant studies. The postneoadjuvant treatment setting could be a valuable source for clinical trials initiated to align patients with treatments best suited to target their cancer subtypes.

In summary, TNBC is a complex disease. Its relative uncommonness, aggressiveness, and impressive heterogeneity have been only a few of the challenges researchers and clinicians face in making strides against this disease. Therefore, future clinical trial design for TNBC should focus on (i) continued efforts to select appropriate targeted therapies based on TNBC subtyping, (ii) continued efforts for tissue collection in the postneoadjuvant setting and metastatic setting for a better understanding of the relevant pathways that are associated with TNBC pathogenesis and therapeutic resistance, and (iii) combination of "complementary" pathway inhibitors to maximize efficacy and minimize therapeutic resistance.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): I.A. Mayer, J.A. Pietenpol
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): I.A. Mayer, B.D. Lehmann
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): I.A. Mayer
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