**Triple-Negative Breast Cancer: Molecular Subtypes and New Targets for Therapy**

Brian D. Lehmann, PhD, Jennifer A. Pietenpol, PhD, and Antoinette R. Tan, MD, MHSc

**OVERVIEW**

Triple-negative breast cancer (TNBC) is a molecularly diverse disease. This heterogeneity has limited the success of targeted therapy in unselected patients to date. Recent transcriptional analysis has divided TNBC into transcriptionally similar subtypes that may have different sensitivity to neoadjuvant chemotherapy and targeted therapy. At present, chemotherapy is the mainstay of treatment for early-stage and advanced TNBC; however, several actionable targets show promise in preclinical studies. Novel therapeutic strategies are currently being tested in phase II and phase III trials and will likely require patient stratification before therapy. Examples of these tailored approaches include poly(ADP-ribose) polymerase inhibitors for BRCA-mutated TNBC, antiandrogens for androgen receptor (AR)-positive TNBC, fibroblast growth factor receptor (FGFR) inhibitors for TNBC harboring FGFR amplifications, and gamma-secretase inhibitors for TNBC with mutations in the PEST domain of NOTCH proteins. Treatment of TNBC based on molecular subclusters represents a potential algorithm for the future. Well-designed clinical trials with incorporation of integrated biomarkers are necessary to advance the development of molecularly targeted therapy for different subgroups of TNBC.

TNBC is a heterogeneous collection of breast cancers lacking expression of estrogen receptor (ER), progesterone receptor (PR), and HER2 amplification. Together these tumors represent approximately 15% of all breast cancers, preferentially affect young women, and are more frequent in women of African and Hispanic descent.1,2 Patients with TNBC have a higher risk of both local and distant recurrence, and metastasis is more likely to occur in the brain and lungs rather than bone compared to other subtypes. The overwhelming majority of metastases of TNBC occur within the first 3 years following diagnosis, and patients who have not recurred during this time have similar survival rates as do patients with ER-positive breast cancers.3

There is a well-established association between deleterious BRCA1 mutations and the risk of developing TNBC, with lifetime risks reaching as high as 50% to 85%. BRCA1 encodes an E3 ubiquitin protein ligase essential for homologous recombination mediated-repair of DNA double-strand breaks. Retrospective analysis and previous trials have shown striking pathologic complete response (pCR) rates in BRCA1 mutation carriers (72% to 90%) with single-agent neoadjuvant DNA-crosslinking platinum salts (e.g., cisplatin).4,5 In the metastatic TNBC setting, a phase III study (Triple Negative Breast Cancer Trial, TNT) of carboplatin area under the curve (AUC) 6 every 3 weeks compared with docetaxel 100 mg/m2 every 3 weeks, between the docetaxel and carboplatin arm, respectively. However, BRCA mutant carriers who received carboplatin compared with docetaxel experienced a significantly greater response (68% vs. 33.3%; 95% CI, 6.3 to 63.1; p = 0.03). The median PFS for patients with BRCA1/2 mutations in the carboplatin group was 6.8 months compared with 3.1 months for non-BRCA mutation carriers, and 4.8 months and 4.6 months, respectively, among patients with and without BRCA1/2 mutations treated with docetaxel. These data strongly support the use of a platinum agent for metastatic TNBC with BRCA mutations.

Although germ-line BRCA1 mutations are more frequently observed in TNBC,7 only a few recurrently mutated genes across the heterogeneous group of tumors make up TNBC. Recent sequencing efforts have shown the most frequent somatic mutations occur in TP53 (62%) and PIK3CA (10%), the gene encoding the p110alpha catalytic subunit of phosphatidylinositol-3 kinase (PI3K).8,9 In addition to diverse mutations, TNBC tumors also display heterogeneity at the copy number and expression level with several clusters containing basal-like tumors.10 Unlike ER-positive and HER2-amplified breast cancers, the lack of high frequency oncogenic driver mutations in TNBC means limited molecularly targeted treatments for this disease.

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From the Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN; Levine Cancer Institute, Carolinas Healthcare System, Charlotte, NC.

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Corresponding author: Antoinette R. Tan, MD, MHSc, Levine Cancer Institute, Carolinas Healthcare System, 1021 Morehead Medical Drive, Charlotte, NC 28204; email: antoinette.tan@carolinashealthcare.org.

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Neoadjuvant chemotherapy has proven efficacy in the treatment of TNBC and the regimens include combinations of anthracyclines, alkylating agents, taxanes, and platinum salts. Patients treated with neoadjuvant chemotherapy who experience a pCR at the time of surgery have significant improvements in both disease-free survival (DFS) and OS compared to patients with residual invasive disease. The latter patients have a much poorer prognosis and are six times more likely to have recurrence and 12 times more likely to die. Currently, there are no clinically actionable biomarkers to predict which patients with TNBC will experience a pCR.

**MOLECULAR HETEROGENEITY OF TNBC**

TNBC show a remarkable diversity of histologic patterns and subtypes. Although majority are high-grade invasive ductal carcinomas, a small subset has distinct pathologic features and indolent clinical behavior. Rare cases of adenoid cystic carcinomas and secretory carcinomas share common recurrent chromosomal translocations, resulting in oncogenic chimeric fusions (MYB-NFIB and ETV6-NTRK3, respectively). In addition, several TNBC have atypical medullary and metaplastic histologies. Medullary carcinomas are characterized by infiltrating carcinomas with circumscribed pushing borders, dense peripheral lymphoid infiltrate, and have favorable outcome, whereas metaplastic carcinomas display differentiation toward squamous epithelium with mesenchymal components and cells displaying spindle, chondroid, osseous, or rhabdoid morphologies.

Given the diverse pathologic classifications, one would predict that TNBC have a diverse array of biologic subtypes that could be revealed by transcriptional profiling. Initial global transcriptional studies showed TNBC to largely display basal-like gene expression. This observation led many investigators to consider basal-like breast tumors and TNBC to be relatively synonymous. The uniform basal-like gene expression pattern in TNBC is largely a result of the significant transcriptional differences between hormonally driven cancers and TNBC. However, when analyzed independent from ER- and HER2-positive cancers, TNBC have quite heterogeneous gene expression patterns that can be used to classify the tumors into distinct subtypes.

**SUBTYPES OF TNBC**

Using gene expression analyses from 386 tumors, we recently identified six distinct TNBC subtypes, each displaying unique biologies. The TNBC molecular subtypes include two basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal AR (LAR) subtype. The BL1 subtype is characterized by elevated cell cycle and DNA damage response gene expression, while the BL2 subtype is enriched in growth factor signaling and myoepithelial markers. Both M and MSL share elevated expression of genes involved in epithelial-mesenchymal-transition (EMT) and growth factor pathways, but the MSL subtype has decreased expression of genes involved in proliferation. Consistent with the de-differentiated mesenchymal gene expression pattern is the recent analysis of metaplastic breast cancers showing the majority of chondroid and spindle cell carcinomas to be of the MSL subtype. The IM subtype is composed of immune antigens and genes involved in cytokine and core immune signaling pathways. The LAR subtype is characterized by luminal gene expression and is driven by the AR. Comparison with the intrinsic subtypes demonstrated that BL1, BL2, IM, and M are largely composed of basal-like subtype, while MSL has a large fraction of normal-like and LAR mostly composed of luminal and HER2 subtypes. In addition to the intrinsic subtypes, a claudin-low subtype has recently been described and is enriched for EMT markers, immune response, and cancer stem cell–like genes. This claudin-low subtype is mostly composed of M and MSL TNBC subtypes.

In addition, we identified representative cell lines and demonstrated differential sensitivity to chemotherapy and targeted agents. BL1 cell lines are sensitive to genotoxic agents, LAR cell lines have differential sensitivity to the LAR antagonist bicalutamide and PI3K inhibitors, mesenchymal cell lines are more sensitive to the multifamily tyrosine kinase inhibitor dasatinib, and M cell lines display some sensitivity to PI3K/mTOR inhibitors. Subtyping of breast tumors from The Cancer Genome Atlas (TCGA) resulted in identification of 163 tumors and analysis of the clinical data associated with TNBC tumors demonstrated that the median OS and DFS of patients with BL1, IM, and MSL subtype tumors were nearly double that of patients with BL2, LAR, and M tumors. Further, patients with tumors of the IM subtype had the best outcome. Analysis of the gene expression data from the IM subtype and identification of transcripts associated with lymphocytes suggests that the IM tumor samples may contain...
tumor-infiltrating lymphocytes (TILs). The favorable outcome of patients with TNBC with higher levels of TILs associated with their tumors has recently been demonstrated in two adjuvant phase III trials.24

A similar transcriptional analysis was recently performed on a smaller cohort of 84 patients, and investigators identified four stable TNBC subgroups associated with distinct clinical outcomes.21 They defined these subtypes as "luminal/androgen receptor (LAR)," "mesenchymal (MES)," "basal-like/immune-suppressed (BLIS)," and "basal-like/immune activated (BLIA)" groups. Similar to the previous study, TNBC patients with tumors expressing immune component features had the best outcome. Between the two studies there is clearly evident overlap between MSL and MES, IM and BL1 with BLIA, M with BLIS, and the two LAR subtypes. In summary, these data show that reproducible and distinct transcriptional subtypes can be unmasked when TNBC samples are analyzed in the absence of ER- and HER2-expressing tumors and as sample size is increased there will likely be additional unique subtypes revealed.

Despite the rather aggressive clinical behavior of TNBC, approximately 30% of patients with TNBC benefit from chemotherapy. In a retrospective reanalysis of pretreatment biopsies, TNBC molecular subtypes were predictive of response to neoadjuvant anthracycline and cyclophosphamide followed by taxane.25 This study showed BL1 had the highest pCR rate (50%) at time of surgery and BL2 and LAR had the lowest (0% and 10%, respectively). Similar to the initial classification, patients with LAR subtype were significantly older at diagnosis, and recent preclinical data suggest that these patients may benefit from antiandrogen or PI3K inhibitors.26 We recently demonstrated that PIK3CA kinase domain mutations are a frequent event in AR-positive TNBC tumors relative to the other subtypes (40% vs. 4%), and targeting of AR in LAR cells increases sensitivity to PI3K inhibitors. These data suggest that although there are few genomic alterations shared by TNBC as a whole, individual subtypes may be enriched in select somatic alterations, several of which may afford opportunities for preclinical discovery and translation to clinical investigation.

**NOVEL APPROACHES FOR TREATMENT OF TNBC**

Chemotherapy at present is the main treatment for patients with TNBC. No specific targeted agent has U.S. Food and Drug Administration (FDA) or European Medicines Agency (EMA) approval to treat TNBC in the adjuvant, neoadjuvant, or metastatic settings. This article discusses the most recent data on promising and novel targeted, nonimmunotherapeutic approaches currently under evaluation for TNBC.

**Poly(ADP-Ribose) Polymerase Inhibition**

The PARP enzyme plays an important role in the repair of DNA single-strand breaks via the base-excision repair (BER) pathway. PARP protein binds directly to sites of DNA damage and recruits other DNA repair enzymes. In normal cells, BER and homologous recombination (HR, repair of DNA double-strand breaks) are both available to repair damaged DNA. In cancer cells of BRCA1 or BRCA2 mutation carriers, where HR is nonfunctional, PARP inhibition leads to an accumulation of DNA single-strand breaks that degenerate into double-strand breaks and result in cell death, as the cells are unable to repair DNA damage by either BER or HR.27 This "synthetic lethality" has been demonstrated in several preclinical studies where BRCA-deficient cells are markedly sensitive to PARP inhibitors.28,29 The prevalence of BRCA1 or 2 mutations in TNBC is estimated to be between 10.6% and 19.8%.7,30 Since genomic instability is common to both BRCA-mutated cancers and TNBC, investigators have tried to expand BRCA-deficient tumors to include those TNBC with transcriptional profiles similar to BRCA-mutants, referred to as BRCAness.31 These observations have led to multiple efforts in evaluating PARP inhibitors as monotherapy or in combination with cytotoxic agents in the treatment of both mutant and sporadic TNBC.

Olaparib was the first oral PARP inhibitor to be approved under accelerated approval by the FDA in December 2014 as single-agent treatment of patients with a deleterious or suspected deleterious germ-line BRCA mutated advanced ovarian cancer, treated with three or more prior lines of chemotherapy. Additionally, the EMA recommended approval of olaparib for use in the maintenance treatment of platinum-sensitive relapsed BRCA-mutated (germ line and/or somatic) high-grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancer in complete response or partial response to platinum-based chemotherapy, and has been granted marketing authorization by the European Commission. The side effects of PARP inhibitors are mild and include fatigue, nausea, and vomiting.

In breast cancer, the antitumor activity of PARP inhibitors is evidenced in several metastatic trials, and the most compelling subset to benefit are the patients that harbor BRCA-mutations. Several phase II trials of PARP inhibitors as monotherapy have been conducted in patients with metastatic breast cancer (MBC). Tutt et al evaluated olaparib in 54 patients with MBC and germ-line BRCA mutations who were treated previously with a median of three prior chemotherapy regimens.32 Two cohorts were enrolled. One group (50% triple negative) was treated with olaparib 400 mg twice daily and the second group (64% triple negative) with 100 mg twice daily. In patients with TNBC, the response rate was 54% (7/13) in the higher-dose group, and 25% (4/16) in the lower-dose group; all were partial responses. Disease stabilization was 31% and 44%, respectively. This proof of concept study confirmed activity in BRCA-mutated TNBC, as well as in patients who were ER positive and HER2 positive. In an international, multicenter phase II study by Kaufman et al, 298 patients with BRCA1 and BRCA2-associated cancers, including breast, ovarian, pancreatic, and prostate, were treated with olaparib 400 mg twice daily.33 In the MBC cohort of 62 patients, the overall response rate was 12.9% (95% CI, 5.7% to 23.9%; all partial responses). In the 30 patients with ER-negative tumors, the response rate was 13.3% (95% CI,
3.8% to 30.7%). The lower efficacy in this trial may be a result of a more pretreated population in which the median number of prior chemotherapies was 4.6 and a higher percentage of patients with prior platinum exposure. In another multicenter trial, no responses were observed in 15 patients with non-BRCA TNBC who received olaparib 400 mg twice a day, indicating that single-agent PARP inhibitor is not likely to be a treatment approach for sporadic TNBC.34

Velparib, another oral PARP 1 and 2 inhibitor, has been extensively evaluated in combination with several chemotherapeutic agents as a chemopotentiator. Iaszkoff et al conducted a study in 41 patients with MBC who had at least one prior cytotoxic regimen and were given veliparib 30 mg orally twice daily day 1 through 7 (originally started at 40 mg but reduced secondary to grade 4 thrombocytopenia) and temozolomide 100 mg/m² orally daily day 1 through 5 every 28 days.35 The response rate in BRCA-mutation carriers was 37.5% (3/8), including one complete response and two partial responses, and there were no responses in non-BRCA carriers (0/33). To date, the data suggest that a monotherapy treatment strategy for PARP inhibitors is not active in sporadic TNBC, but preferentially active in BRCA-mutated breast cancer. The combination of a PARP inhibitor and DNA-damaging agents for potentiation may still have a role in a specific subset of sporadic TNBC. Given the observed activity of PARP inhibitors in these studies, major phase III clinical trial efforts are underway to evaluate the benefit of PARP inhibitors in the adjuvant and neoadjuvant settings.

OlympiaA (National Surgical Adjuvant Breast and Bowel Project [NSABP] B-55/BIG 6-13, NCT02032823) is a randomized, double-blind, placebo-controlled phase III study to evaluate the effect of adjuvant treatment with olaparib in 1,320 patients with germ-line BRCA1/2 mutations and TNBC who have completed definitive local treatment.36 This is a global, collaborative effort led by NRG Oncology and the Breast International Group and sponsored by the National Cancer Institute and AstraZeneca. The primary endpoint is invasive DFS. Randomization is 1:1 to either 12 months treatment with olaparib 300 mg orally twice daily or matching placebo. Eligible patients are those who did not achieve a pCR following at least six cycles of neoadjuvant chemotherapy followed by surgery or patients with either axillary node-positive disease or axillary node-negative disease with a primary tumor larger than 2 cm, who have undergone surgery and have completed at least six cycles of adjuvant chemotherapy. This is a unique trial targeting a rare population, with the potential to change the current adjuvant standard of care of observation for high-risk primary TNBC with BRCA mutations.

Another phase III clinical trial (M14-011, AFT-04, ABCSG 44, GBG 81, GEICAM/2014-02, NSABP B56-I, USO 12152, NCT02032777) is the first to evaluate the efficacy of veliparib in combination with chemotherapy for neoadjuvant treatment of TNBC.37 This is a randomized, placebo-controlled, double-blind trial enrolling women presenting with clinical stage T2-4N0-2 or T1N1-2 triple-negative disease, who are candidates for potentially curative surgery. They will be randomly assigned in a 2:1:1 ratio to one of three neoadjuvant treatment arms: (arm A) weekly paclitaxel 80 mg/m² for 12 weeks, carboplatin (AUC6), veliparib 50 mg orally twice daily followed by doxorubicin and cyclophosphamide (AC); (arm B) weekly paclitaxel 80 mg/m² for 12 weeks, carboplatin (AUC6), placebo followed by AC; or (arm C) weekly paclitaxel 80 mg/m² for 12 weeks, placebo, placebo followed by AC. All patients must have documented BRCA germ-line mutation testing. The primary endpoint is pCR in the breast and lymph node. The trial seeks to accrue 624 subjects in about 200 sites. This is another global collaboration with several groups including the Alliance for Clinical Trials in Oncology, Austrian Breast and Colorectal Cancer Study Group, German Breast Group, Grupo Espanol de Investigacion en Cancer de Mama, NSABP Foundation, and US Oncology, and it is sponsored by Abbvie. The results of this study will help answer the question of the utility of carboplatin with and without a PARP inhibitor in TNBC with and without BRCA mutations. Results from these studies will hopefully lead to the availability of PARP inhibitors in routine clinical practice for patients with BRCA-mutated breast cancers and TNBC with DNA repair pathway defects.

More effective treatment strategies are needed for sporadic TNBC. Other approaches are being developed to sensitize sporadic TNBC to PARP inhibition. One example is an ongoing clinical trial (NCT01623349) that is evaluating olaparib and BKM120 (buparlisib) or BYL719, PI3-kinase inhibitors, in advanced sporadic TNBC and high-grade serous ovarian cancer.38 Another trial (NCT01434316) is combining veliparib and dinaciclib, a cyclin-dependent kinase inhibitor (CDK) inhibitor, where the hypothesis is that CDK inhibition sensitizes BRCA-proficient breast cancers to PARP inhibition.39 After the phase I dose-finding portion, the study will enroll patients with BRCA-mutated and nonmutated advanced breast cancer in a dose-expansion cohort. These combinatorial approaches of PARP inhibitor and other targeted agents are promising strategies that may expand the clinical utility of PARP inhibitors to sporadic TNBC.

Inhibition of the PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR pathway mediates multiple cellular processes including cell survival, metabolism, proliferation, motility, migration, invasion, and angiogenesis.40 Hyperactivation of the PI3K/AKT signaling pathway is frequent oncogenic alteration in TNBC, occurring in approximately 10% of patients.9 Activating PIK3CA mutations are the most frequent in TNBC.41 Other alterations that result in PI3K pathway activation include loss of the tumor suppressor phosphatases inositol polyphosphate 4-phosphatase type II (INPP4B) and loss of phosphatase and tensin homolog (PTEN).9,42 Additionally, amplification of AKT and translocation of AKT3 occurs in a small subset of TNBC.43 The PIK3CA activating mutations appear to be enriched in mesenchymal and LAR molecular subtypes.20 Targeting the PI3K/AKT pathway represents a compelling and rational potential treatment strategy for a subset of TNBC.
Ipatasertib (GDC-0068) is a novel, selective, ATP-competitive small molecule inhibitor of all three isoforms of the serine/threonine kinase AKT.\textsuperscript{44} It shows single-agent activity in several xenograft models with AKT pathway activation via PTEN loss and/or PIK3CA mutation, including breast. In a phase Ib study of ipatasertib and paclitaxel in patients with MBC, the most commonly reported side effects were diarrhea, nausea, fatigue, vomiting, anorexia, and rash.\textsuperscript{46} LOTUS (NCT02162719) is a randomized, double-blind, placebo-controlled international phase II study to evaluate the efficacy of ipatasertib combined with paclitaxel compared with placebo with paclitaxel in approximately 120 patients with previously untreated locally advanced or MBC.\textsuperscript{46} The primary endpoint is PFS in all patients with TNBC, the most commonly reported side effects were diarrhea, nausea, fatigue, vomiting, anorexia, and rash.\textsuperscript{48} LOTUS (NCT02162719) is a randomized, double-blind, placebo-controlled international phase II study to evaluate the efficacy of ipatasertib combined with paclitaxel compared with placebo with paclitaxel in approximately 120 patients with previously untreated locally advanced or MBC.\textsuperscript{46} The primary endpoint is PFS in all patients with TNBC and patients with TNBC with PTEN-low tumors. Randomization is 1:1 to either paclitaxel 80 mg/m\textsuperscript{2} intravenously on days 1, 8, and 15 of each 28-day cycle and ipatasertib 400 mg orally daily on days 1 through 21 of each cycle or paclitaxel 80 mg/m\textsuperscript{2} intravenously on days 1, 8, and 15 of each 28-day cycle and placebo. The trial is currently accruing.

The combination of weekly paclitaxel and ipatasertib is also being evaluated in the neoadjuvant setting. FAIRLANE (NCT02301988) is a randomized, double-blind, placebo-controlled, multicenter, preoperative phase II study designed to estimate the efficacy of ipatasertib combined with paclitaxel compared with placebo combined with paclitaxel in women with stage IA-IIIA (primary tumors \(\geq 1.5\) cm) TNBC.\textsuperscript{47} The primary endpoint is pCR in the breast and lymph nodes. Approximately 150 patients will be enrolled at approximately 30 centers. Patients will be randomly assigned in a 1:1 ratio, stratified by the following three factors: PTEN status (null, low, medium), prior adjuvant/neoadjuvant treatment including chemotherapy with or without radiation, and disease-free interval from last dose of chemotherapy. Following surgical resection of primary tumor, patients are expected to continue postoperative treatment with a standard adjuvant anthracycline-based chemotherapy regimen. The trial is being conducted in collaboration with the SOLTI Breast Cancer Research Group and is ongoing.

OVEREXPRESSED GROWTH FACTORS IN TNBC
Several growth factor receptors are overexpressed in TNBC, including epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR). Phase II and III clinical trials with drugs that interrupt the EGFR and VEGFR signaling pathways have been conducted in breast cancer, but these approaches are no longer being pursued because of limited activity in an unselected TNBC population.\textsuperscript{48-52} However, there still is promise in targeting other growth factor receptors, such as the FGFR.

Inhibition of the FGFR
FGFR signaling stimulates cell growth, survival, migration, and differentiation.\textsuperscript{53} Approximately 9% of TNBC has \(FGFR1\) amplification, and approximately 4% of TNBC has amplification of the \(FGFR2\) gene.\textsuperscript{9,54} Mutations in FGFR are less common in TNBC (<1%).\textsuperscript{55} Cell lines with \(FGFR1\) amplification or \(FGFR2\) or \(FGFR4\) mutations were sensitive to a FGFR inhibitor in cell line models.\textsuperscript{54} Additionally, inhibition of FGFR in basal-like TNBC cell lines with \(FGFR2\) amplification led to decreased growth.\textsuperscript{56} These data support the clinical investigation of FGFR inhibitors in TNBC, which may only benefit a very small subgroup. To date, there are several multitargeted kinase inhibitors in clinical development with relatively high potencies against FGFRs, and include dovitinib (TKI258), nintedanib (BIBF1120), ponatinib (AP24534), and lucitanib (CO-3810). Selective FGFR TKIs are AZD4547 and [N]-42756493. Although no current study specifically targets the TNBC population, a phase II trial (NCT02202746) is ongoing in MBC evaluating oral lucitanib in tumors that have an FGFR1-amplification or \(11q\)- amplification, and patients with TNBC are eligible.\textsuperscript{57} A phase I trial is evaluating [N]-42756493 in patients with solid tumors, and one cohort includes patients with breast cancer of any subtype as long as the tumors harbor an FGFR translocation or FGFR activating mutation (NCT01703481).\textsuperscript{58} These trials with inclusion criteria more selective to specific FGFR alterations may prove more effective than previous trials treating unselected patients with breast cancer.

OTHER STRATEGIES
Other notable targets have led to additional clinical trial efforts to evaluate more tailored therapies for specific subpopulations within TNBC.

Blockade of the AR
Approximately 10% to 15% of TNBC express the AR.\textsuperscript{59} The LAR subclass of TNBC is characterized by luminal gene expression and enriched for AR and AR gene targets.\textsuperscript{20} This is the basis for targeting this subset of TNBC with antiandrogen therapy. In a multicenter phase II study by Gucalp et al, 150 mg of bicalutamide, an oral nonsteroidal antiandrogen, was administered daily to 26 patients with AR-positive, ER-negative, and PR-negative MBC, who were determined to be evaluable for the primary endpoint of clinical benefit rate (CBR; complete response + partial response + stable disease > 6 months).\textsuperscript{60} A CBR of 18% (95% CI, 6% to 37%), consisting of all stable disease, and a median PFS of 12 weeks (95% CI, 11 to 22 weeks) was reported. The most common treatment-related toxicities were fatigue (21%), hot flashes (21%), limb edema (21%), aspartate aminotransferase elevation (25%), and alanine aminotransferase elevation (21%). Of note, 452 patients with ER-negative and PR-negative breast cancer were screened for AR expression, and 12% tested AR-positive, consistent with an earlier report.\textsuperscript{59} The activity of a next-generation antiandrogen, enzalutamide, was evaluated in advanced AR-positive TNBC. In this multicenter trial, 26 patients evaluable for the primary endpoint of CBR (complete response + partial response + stable disease at 16 weeks) received enzalutamide 160 mg orally daily.\textsuperscript{61} The
stage I result of this Simon 2-stage, phase II trial was a CBR of 42% (95% CI, 24% to 62%), including one complete response and one partial response. This met the prespecified efficacy endpoint. Study enrollment has been met and final results are expected to be reported later in 2015. In this prescreened sample of 404 patients, 55% of samples expressed AR—a much higher percentage than anticipated. Further analysis of the appropriate diagnostic to assess AR expression in tumor specimens is ongoing. Another androgen-directed therapy under evaluation in AR-positive TNBC is orteronel (TAK-700; NCT01990209), which is a nonsteroidal, androgen synthesis inhibitor that has been shown in preclinical studies to selectively inhibit the 17,20-lyase enzymes, critical to the production of androgens. These studies show that AR is a promising and suitable therapeutic target in a small subset of TNBC, particularly the LAR subtype.

Inhibition of the JAK2/STAT3 Pathway

Janus kinases (JAKs) are tyrosine kinases, and signal transducer and activation of transcription 3 (STAT3) proteins are major components of several cytokine receptor systems that regulate cell growth and survival. Binding of the cytokine to the receptor induces dimerization, which activates the associated JAKs. The JAKs also phosphorylate STATs, which lead to their dimerization, nuclear translocation, and transcriptional regulation of genes that regulate cell differentiation, proliferation, and apoptosis. There is emerging preclinical evidence that disruption of the JAK2/STAT3 signaling could be an effective clinical strategy to treat TNBC. The IM subtype is also enriched with genes involved in immune cell signaling and cytokine signaling. A preclinical study showed the JAK/STAT3 pathway was preferentially active in basal-like breast cancer cells, and inhibition of JAK2 resulted in reduced growth of xenografts. Unlike myeloproliferative neoplasms, mutations in JAKs and STATs have not been well characterized. However, JAK2 amplifications were found more frequent in TNBC treated with neoadjuvant chemotherapy than in primary untreated basal-like breast tumors in the TCGA. This observation may provide rationale to investigate JAK inhibitors in patients who have JAK2-amplified residual disease.

Ruxolitinib, a potent oral inhibitor of JAK1 and JAK2, is approved for the treatment of intermediate or high-risk myelofibrosis and is now being evaluated in breast cancer. A phase I trial (NCT02041429) is evaluating the combination of ruxolitinib given twice daily with weekly paclitaxel 80 mg/m², 3 weeks out of 4 weeks, in patients with MBC. Once a recommended phase II dose is determined, the study will treat patients with triple-negative inflammatory breast cancer with ruxolitinib orally twice daily for 21 days in a 28-day cycle and weekly paclitaxel for 12 weeks followed by dose-dense AC for four cycles. The primary endpoint is biologic, and the trial will evaluate expression of pSTAT3 in triple-negative inflammatory breast cancer tumors before and after treatment, with an expected decrease in pSTAT3 expression post-therapy.

Targeting Trop-2

Trop-2, also referred to as M1S1, TACSTD2, EGP-1, is a cell surface protein overexpressed in several epithelial cancers, but not in corresponding normal tissues. Trop-2 is a transmembrane calcium signal transducer and is involved in the regulation of cell-cell adhesion. Membrane-associated Trop-2 was found to be associated with poor prognosis in breast cancer. There is a growing interest in targeting Trop-2 in TNBC. IMMU-132 (isactuzumab govitecan) is an antibody-drug conjugate containing the humanized mono-
clonal antibody, hRS7, against Trop-2, which is linked to the active metabolite of irinotecan, 7-ethyl-10-hydroxycamptothecin (SN-38). The antibody moiety of IMMU-132 selectively binds to Trop-2. After internalization and proteolytic cleavage, SN-38 is delivered preferentially to the tumor cells. Preclinical data shows that IMMU-132 resulted in increased tumor regression in MDA-MD-468 TNBC xenograft models, compared to irinotecan or to the antibody-drug conjugate control. IMMU-132 received Fast Track designation in January 2015 from the FDA for treatment of patients with TNBC who have progressed on prior therapies for metastatic disease. A phase I/II trial of IMMU-132 was conducted in advanced epithelial cancers, including TNBC. There was no prescreening for Trop-2 expression. The recommended phase II dose of IMMU-132 was 10 mg/kg intravenously on days 1 and 8 of a 21-day cycle. The toxicity was mostly neutropenia, and diarrhea was low grade. An expansion cohort enrolled 23 patients with pretreated metastatic TNBC (prior number of median regimens was 4) with a response rate of 30% consisting of 7 partial responses, and a CBR (partial response + stable disease > 6 months) of 40%. Immunohistochemical data on Trop-2 scoring is being collected. A phase II trial will treat 80 patients with metastatic TNBC who have received two or more prior regimens with IMMU-132 alone or in combination with carboplatin (NCT02161679). Further research is necessary to evaluate the strategy of using antitrop-2 therapeutics for breast cancer and the relationship of Trop-2 expression to response.

CONCLUSION
TNBC is a heterogeneous disease. The identification of several specific subtypes characterized by different biologic pathways and various sensitivities to chemotherapy is instrumental in delivering more personalized therapy for TNBC. Ongoing and future clinical research in selected subsets of TNBC will validate the efficacy of such novel treatment strategies.

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