With regard to potential research strategies relevant to the treatment of triple-negative breast cancer/basal-like breast cancer, potential targets include PTEN, INPP4B, PIK3CA, KRAS, BRAF, EGFR, FGFR1, FGFR2, IGFR1, KIT, MET, PDGFRA, and the HIF1-α/ARNT pathway. Many of these will be discussed further in this review article.

**Introduction**

Triple-negative breast cancer (TNBC) is a unique subset of breast cancer. It is characterized by the lack of the three most commonly targeted receptors in human breast cancer: the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)/neu. Given the lack of traditional targets, the propensity of early-stage TNBC to metastasize to visceral sites, and the poor survival associated with advanced TNBC, this subset of breast cancer is appropriately the subject of tremendous preclinical and clinical study.[1,2] While TNBC has historically represented a unique group of breast cancer patients, recent studies have continued to dissect the molecular heterogeneity of TNBC into smaller, distinct subsets, including the basal-like and claudin-low subtypes, both of which have unique genetic characteristics and treatment responses.[3,4] Moreover, a second molecular classification system of TNBC, also based on gene expression profiling, has identified six TNBC subtypes, including two basal-like subtypes (BL1 and BL2), an immunomodulatory subtype, mesenchymal and mesenchymal stem cell–like subtypes, and a luminal androgen receptor subtype, again with different responses to treatment.[5,6] Finally, The Cancer Genome Atlas (TCGA) project, supervised by the National Cancer Institute and the National Human Genome Research Institute, has also provided tremendous insight into the molecular heterogeneity and driver mutations specific to breast cancer, including TNBC.[7] As we unravel the biologic complexity of TNBC and develop rationally designed clinical trials rooted in strong preclinical evidence, our ability to treat this disease should continue to improve (Table).
briefly summarize this complex body of work with respect to TNBC, TCGA revealed that molecular characterizations of TNBC, specifically the basal subtype, included loss of TP53, RB1, and BRCA1 function, MYC amplification, and phosphatidylinositol 3-kinase (PIK3) pathway activation. A striking finding of this study was that basal-like breast cancers (BBCs) were found to be molecularly distinct from the luminal A, luminal B, and HER2 subtypes of breast cancer, but to share many characteristics with high-grade serous ovarian cancers (HGSOC), including loss of TP53, RB1, and BRCA1, as well as MYC amplification. This suggests that shared treatment approaches could be considered for BBC and HGSOC. Specific to BRCA loss, approximately 20% of patients with BBC had germline variants of BRCA1 or BRCA2; a significant proportion of patients with BBC could potentially benefit from therapies that target DNA repair, such as poly (ADP-ribose) polymerase (PARP) inhibitors and platinum drugs. Finally, with regard to potential research strategies relevant to the treatment of TNBC/BBC, potential targets include PTEN, INPP4B, PIK3CA, KRAS, BRAF, EGFR, FGFR1, FGFR2, IGFR1, KIT, MET, PDGFR, and the HIF1-α/ARNT pathway. Many of these will be discussed further in this review article.

**Targeting EGFR**

Preclinical data have indicated that the epidermal growth factor receptor (EGFR) may be a potential target in the treatment of advanced TNBC. In an attempt to identify molecular targets, Nielsen and colleagues conducted DNA microarray analysis on a large series of BBC cases.[8] In this series, high expression of EGFR was noted in approximately 60% of BBCs. Similarly, in a second series of BBCs, Livasy and colleagues showed that the most common immunophenotype for this subtype included EGFR expression.[9]

Despite these encouraging preclinical data, results from clinical trials utilizing cetuximab, a monoclonal antibody that targets EGFR, have shown somewhat limited benefit. A randomized, phase II trial evaluated the combination of carboplatin and cetuximab in patients with previously treated advanced TNBC.[10] Patients (n = 120) were randomized to either cetuximab alone, with carboplatin added at disease progression, or to the combination of cetuximab and carboplatin from treatment initiation. The rate of response (RR) to cetuximab monotherapy was low at 6%. The RR to combination therapy with carboplatin at progression was 16%, while the RR to the combination at initiation of treatment was 17%. Overall, combination therapy with carboplatin/cetuximab was associated with a short median time to progression (TTP) of 2.1 months and a median overall survival (OS) of 10.4 months. Combination therapy was well tolerated, with rash, fatigue, and nausea being the most common toxicities.

The addition of cetuximab to chemotherapy was evaluated in a second, randomized phase II study in which 150 patients with advanced, HER2− breast cancer were randomly assigned to irinotecan and carboplatin either with cetuximab (ICE) or without cetuximab (IC).[11] In the overall cohort, the objective RRs (ORRs) for the ICE and IC groups were 33% and 28%, respectively; however, the group with TNBC experienced higher ORRs to both ICE and IC—49% and 33%, respectively. The most significant grade 3/4 toxicities reported in the ICE arm were diarrhea (50%) and neutropenia (91%). Baselga and colleagues conducted a phase II trial of cisplatin with or without cetuximab (randomized 2:1 to the combination arm) in 173 patients with metastatic TNBC.[12] Patients in the cisplatin arm who experienced disease progression were allowed to cross over to the combination arm. The ORR for cisplatin/cetuximab therapy was superior to that with cisplatin alone, at 20.0% vs 10.3%. Median progression-free survival (PFS) was also higher in the combination arm, at 3.7 months vs 1.5 months in the cisplatin monotherapy arm. Grade 3 and 4 adverse events were higher in patients who received combination therapy, and included rash, neutropenia, fatigue, and dyspnea.

Finally, selected studies have evaluated the efficacy of small-molecule EGFR inhibitors, including erlotinib and gefitinib, as single agents in the setting of advanced breast cancer, with disappointing results; RRs were 3.0% and 0.0%, respectively.[13,14] While there is still a strong preclinical rationale to treat advanced TNBC with EGFR inhibition with or without chemotherapy, the modest clinical activity observed has dampened the enthusiasm for this therapeutic strategy.

**Inhibition of Angiogenesis and VEGF and Its Receptors**

In breast cancer and other malignancies, the development of agents that inhibit tumor angiogenesis has been an active area of investigation. Strategies to inhibit tumor vessel growth include the use of bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor A (VEGF-A), and tyrosine kinase inhibitors (ie, sunitinib, sorafenib). These targeted agents have been studied in combination both as monotherapies and in combination with cytotoxic chemotherapy. Initial studies
New Targets for Triple-Negative Breast Cancer
Published on Cancer Network (http://www.cancernetwork.com)
in the treatment of metastatic breast cancer (MBC) formed the basis for later studies incorporating bevacizumab into earlier lines of treatment, such as the neoadjuvant setting.

Use of bevacizumab in the metastatic setting

Bevacizumab was initially studied in MBC in a series of studies that ultimately proved to have inconsistent results. The landmark E2100 study was a phase III randomized trial evaluating incorporation of bevacizumab into a weekly paclitaxel regimen in the first-line treatment of MBC.[15] In the overall analysis of 722 HER2− patients, bevacizumab demonstrated an approximate doubling of PFS compared with placebo (11.8 months vs 5.9 months, \( P < .001 \)). Despite these impressive improvements in PFS, there was no benefit in OS associated with the use of bevacizumab. Toxicities including hypertension, proteinuria, headaches, and cerebrovascular ischemia; also, infections were more common in patients treated with bevacizumab. Based on the magnitude of improvement in PFS seen in E2100, in February 2008 the US Food and Drug Administration (FDA) initially granted accelerated approval for the use of bevacizumab as first-line therapy in the treatment of HER2− MBC.

Following E2100, two other phase III studies in the first-line treatment of HER2− MBC were reported: the AVastin And DOcetaxel (AVADO) trial and the Regimens in Bevacizumab for Breast Oncology (RIBBON-1) trial.[16,17] AVADO randomized 736 patients to receive docetaxel every 3 weeks with or without bevacizumab.[16] In AVADO, bevacizumab was given at two different doses, either 7.5 mg/kg or 15 mg/kg, both every 3 weeks. In the combination therapy group, PFS was approximately 10 months, compared with 8 months with docetaxel alone; this benefit was restricted to the group that received bevacizumab at the higher dose of 15 mg/kg. Although this difference of 2 months was statistically significant, the clinical relevance of this benefit, especially in light of the cost associated with bevacizumab therapy, is controversial.

RIBBON-1 randomized 1,237 patients in a 2:1 ratio to receive chemotherapy with bevacizumab or placebo.[17] The study design allowed the investigator to choose the chemotherapy backbone from one of three approved strategies: taxane-based chemotherapy, anthracycline-based chemotherapy, or capecitabine. For each of the bevacizumab-containing combinations, PFS was prolonged compared with the PFS for placebo, and these improvements were statistically significant. For example, both the taxane-based and anthracycline-based bevacizumab combination arms resulted in PFS of 8.6 months vs 5.7 months for placebo \( (P < .001) \).

In conclusion, the magnitudes of improvement in PFS in AVADO and RIBBON-1 were not comparable to the impressive doubling of PFS observed in E2100. Furthermore, none of the studies demonstrated improvement in OS with bevacizumab. For these reasons, as well as because of the costs and toxicities associated with bevacizumab, the FDA ultimately revoked the initial approval of bevacizumab for HER2− MBC in November 2011. The use of bevacizumab to treat advanced TNBC should, therefore, be restricted to the clinical trial setting.

Use of bevacizumab in the adjuvant and neoadjuvant settings

Although the impact of bevacizumab did not appear to improve outcomes for patients with established metastatic TNBC, investigators sought to understand whether inhibition of angiogenesis would prove fruitful in the prevention of metastases. Thus, a series of adjuvant and neoadjuvant studies incorporating bevacizumab have been conducted. In the adjuvant setting, the Bevacizumab Adjuvant Therapy in Triple-Negative Breast Cancer (BEATRICE) trial randomized over 2,500 patients with TNBC to anthracycline- and/or taxane-based chemotherapy, with or without 1 year of bevacizumab.[18] At a median follow-up of 32 months, there was no statistically significant improvement in disease-free survival (DFS) associated with adjuvant bevacizumab; OS outcomes have yet to be reported. We also await the results of E5103, a randomized, phase III trial evaluating the potential benefit of adding bevacizumab to standard anthracycline/taxane-based adjuvant chemotherapy in high-risk breast cancer patients (National Cancer Institute [NCI] ClinicalTrials.gov Identifier: NCT00433511).

In the neoadjuvant setting, two randomized phase III trials investigated the addition of bevacizumab to chemotherapy with somewhat contradictory findings. The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-40 trial evaluated the addition of bevacizumab to anthracycline/taxane-based preoperative chemotherapy in 1,206 women with HER2− breast cancer.[19] The addition of bevacizumab was associated with an increased pathologic complete response (pCR) rate in the breast (34.5% vs 28.2%, \( P = .02 \)). Improvements in the pCR rate by addition of bevacizumab to chemotherapy were more pronounced in women with hormone receptor–positive (HR+) breast cancer (23.2% vs 15.1%, \( P = .007 \)) than in women with TNBC (51.5%
vs 47.1%, \( P = .34 \). The GeparQuinto trial evaluated the addition of bevacizumab to preoperative anthracycline/taxane-based chemotherapy in 1,948 women with HER2− breast cancer.[20] The rate of pCR was improved with the addition of bevacizumab to chemotherapy (18.4% vs 14.9% without bevacizumab, \( P = .04 \)). In contrast to results of the NSABP B-40 trial, the benefit of bevacizumab was more pronounced in the TNBC group (39.3% vs 27.9%, \( P = .003 \)) than in the HR+ group (7.7% vs 7.8%, \( P = 1.00 \)). Similar to the metastatic setting, incorporation of bevacizumab into neoadjuvant regimens should remain restricted to the clinical trial setting. Meanwhile, results of the Cancer and Leukemia Group B (CALGB) 40603 trial, which also seeks to determine the benefit of preoperative bevacizumab, are anticipated (NCI ClinicalTrials.gov Identifier: NCT00861705).

**Tyrosine kinase inhibitors**

Beyond bevacizumab, small-molecule tyrosine kinase inhibitors that target pathways integral to angiogenesis, including vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR), have been evaluated as monotherapy in MBC. A phase II single-arm study evaluated sunitinib in 64 patients previously treated with an anthracycline and a taxane, and demonstrated a clinical benefit rate (CBR) of 16%.[21] Notable toxicities included fatigue, nausea, diarrhea, mucositis, and anorexia. A phase II single-arm study evaluated sorafenib as first- or second-line treatment for MBC in patients who had previously received an anthracycline and/or a taxane.[22] In the first cohort of 20 evaluable patients, sorafenib was well-tolerated, with no grade 4 and minimal grade 3 toxicities; however, the trial was stopped early due to limited efficacy. There were no partial responses (PRs) or complete responses (CRs), and only two patients (10%) had documented stable disease (SD) lasting greater than 6 months.

**Conclusions**

While preclinical data supported development of angiogenesis-inhibiting agents to treat TNBC across all stages, the most noteworthy clinical results have been in the neoadjuvant setting. Continued follow-up will be required to help determine whether higher pCR rates seen in response to inhibition of angiogenesis will translate into longer-term, durable responses.

**The Relationship Between TNBC and DNA Repair**

Over the past decade, there has been a tremendous amount of research, both in the laboratory and in the clinic, focusing on the relationship between TNBC and DNA repair capacity. Among women who develop TNBC, as compared with other subtypes of breast cancer, studies have identified not only a higher incidence of \( BRCA1 \) and \( BRCA2 \) germline mutations, with an associated impairment in homologous recombination, but also shared clinical-pathologic features between sporadic TNBC and \( BRCA \)-associated breast cancer. Specifically, among a cohort of 77 patients with TNBC, the incidence of \( BRCA1 \) and \( BRCA2 \) mutations was 19.5%; 15.6% (12 patients) harbored a mutation in \( BRCA1 \), and 3.9% (3 patients) had a mutation in \( BRCA2 \). Interestingly, clinically outcomes, including 5-year recurrence-free survival (RFS) and OS estimates, were superior for \( BRCA \)-mutation carriers compared with those with wild-type \( BRCA \) status (86.2% vs 51.7%, \( P = .031 \); and 73.3% vs 52.8%, \( P = .225 \); respectively) suggesting possible inherent sensitivity to chemotherapy among \( BRCA \)-mutated breast cancers.[23] Moreover, studies have identified shared clinicopathologic features between TNBC and \( BRCA \)-associated breast cancers, including high-grade and ER−/PR−/HER2− status, high rates of TP53 gene mutations, genome-wide aneuploidy, and BBC subtype classification, as well as sensitivity to DNA-damaging agents (ie, platinum salts).[9,24-28] Such observations have led to the expression “the ‘BRCAness’ of TNBC” and to subsequent analysis of DNA-damaging agents and inhibition of DNA repair, namely through PARP, in clinical trials focusing on this unique subset of breast cancer.

**PARP inhibition**

PARPs are a family of enzymes involved in cellular processes, such as genomic stability, DNA repair, cell cycle progression, and apoptosis.[29,30] PARP-1, the most abundant of the PARP family of enzymes, is critical for the DNA repair process of base excision repair (BER). Investigators hypothesized that PARP inhibition, in conjunction with the loss of DNA repair via \( BRCA \)-dependent mechanisms, would result in “synthetic lethality” and augmented cell death—a hypothesis that has been borne out in both the preclinical and clinical arenas.[31-34] Olaparib, an oral PARP inhibitor, has demonstrated efficacy and safety among patients with \( BRCA \)-associated solid tumors, including breast cancer, in early-phase clinical trials.[33,34] Specific to breast cancer, a multicenter phase II sequential cohort study evaluated the safety and
efficacy of olaparib in 54 patients with advanced BRCA-mutated breast cancer, of whom approximately 50% had TNBC. The RR at the optimal dose of 400 mg orally twice daily was 44%, while the median PFS was 5.7 months (range, 4.6 to 7.4 months). Treatment was generally well tolerated, with common grade 3 adverse events of fatigue, nausea, and vomiting.[33] A second investigation, Canadian Study 20, evaluated olaparib in patients within four distinct cohorts: (1) ovarian cancer, BRCA wild-type and/or unknown; (2) TNBC, BRCA wild-type and/or unknown; (3) ovarian cancer, BRCA-mutated; and (4) breast cancer, BRCA-mutated, ER− or ER+.[35] Interestingly, responses were seen in all cohorts except patients with sporadic TNBC, illustrating that additional DNA damage via chemotherapy concurrent with PARP inhibition is likely necessary to yield responses in sporadic TNBC.

Velparib (ABT-888) is an oral inhibitor of PARP 1 & 2, and it has been studied in early-phase clinical trials that included patients with advanced breast cancer.[36] Combination therapy with velparib and the oral alkylating agent temozolomide (TMZ) was studied in 41 patients with heavily pretreated MBC, of whom 8 harbored a BRCA1/2 mutation and 23 had advanced TNBC.[37] Patients received velparib at a dosage of 40 mg twice daily for 7 days in combination with temozolomide at 150 mg/m² orally daily for 5 days of a 28-day treatment cycle. Across the entire patient population, the CBR was 17% and PFS was 1.9 months. Among BRCA mutation carriers, the CBR was 62.5% and median PFS was 5.5 months, illustrating the impact of dysfunctional homologous recombination on patients’ response to PARP inhibition. Based on these provocative data, an expansion cohort of 21 additional BRCA mutation carriers, of whom 7 had TNBC, were treated with TMZ/velparib, yielding a CBR of 42.9% and a median PFS of approximately 3.5 months, further supporting the assertion that the efficacy of this combination therapy is limited to patients with germline BRCA mutations.[38] Iniparib was initially developed as a PARP inhibitor and later found to exert its DNA-damaging effects via alterations in metabolism of reactive oxygen species in cancer cells.[39-41] In a randomized phase II trial, iniparib yielded improvements in ORR, PFS, and OS when combined with gemcitabine and carboplatin chemotherapy in 123 patients with advanced TNBC.[42] Efficacy was not duplicated in a subsequent and definitive randomized phase III trial in 519 patients with advanced TNBC; however, patients receiving second- and third-line treatment appeared to derive benefit from the addition of iniparib, with an improvement in median PFS from 2.9 to 4.2 months (hazard ratio [HR] = 0.67) and an improvement in median OS from 8.1 to 10.8 months (HR = 0.65).[43] A randomized phase III study of carboplatin/gemcitabine with or without iniparib in patients with advanced TNBC in the second- and third-line setting is in development.

Platinum salts

Given the BRCAness of TNBC and the putative sensitivity of these cancer cells to DNA damage, the platinum salts (carboplatin and cisplatin)—which bind and crosslink DNA, ultimately triggering apoptosis and programmed cell death—are a rational drug class to study in the treatment of this disease. The neoadjuvant setting has provided an interesting platform for studying the effects of platinum agents on BRCA-mutant and BRCA–wild-type TNBC. Byrski et al published comparative effects of chemotherapy among 102 patients with BRCA1-mutated breast cancer.[44] The overall pCR rate across the entire patient population was 24% (24 of 102 patients); the pCR rate in patients treated with cisplatin was 83%, compared with a pCR rate of 22% (11 of 51 patients) in those treated with anthracycline-containing regimens. Silver et al reported a pCR rate of 21% (6 of 28 patients) in women with sporadic TNBC treated with 4 cycles of preoperative cisplatin.[45] More recently, the results of GeparSixto, a randomized phase II trial evaluating the efficacy of the addition of carboplatin to neoadjuvant therapy in 595 patients with TNBC and HER2+ disease, were reported.[46] All patients received preoperative bevacizumab, and those with HER2+ breast cancer also received preoperative trastuzumab and lapatinib. The impact of adding carboplatin to standard anthracycline/taxane-based preoperative therapy was more profound in those with TNBC (the pCR rate with carboplatin was 58.7% vs 37.9% without carboplatin; P < .05) than in the HER2+ subset of patients. (The pCR rate was 33.1% with carboplatin vs 36.3% with no carboplatin; P > .05.) Results of an ongoing large phase III randomized trial, CALGB 40603 (NCI ClinicalTrials.gov Identifier: NCT00861705), are also anticipated. This study will evaluate the additive effect of preoperative carboplatin used with standard anthracycline/taxane-containing chemotherapy in patients with early-stage TNBC. In non–BRCA mutation carriers with TNBC, investigators have also identified tissue-based biomarkers, specifically intratumoral quantification of telomeric allelic imbalances, which may prove capable of predicting response to preoperative platinum-based chemotherapy.[47] While platinum salts have often been combined with targeted agents in clinical trials, single-agent platinum therapy is an accepted treatment for advanced TNBC and is associated with respectable...
RRs. (See, for example, data from the National Comprehensive Cancer Network [NCCN], at www.nccn.org.) Perhaps the most extensive experience with single-agent platinum therapy in advanced TNBC was reported from the Translational Breast Cancer Research Consortium (TBCRC) 009 study.[37] In this study, 86 platinum therapy-naive patients with advanced TNBC received either carboplatin to AUC (area under the curve) 6 or cisplatin at a dose of 75 mg/m², per physician’s choice, in the first or second line of treatment. The overall RR was 30.2% (4 CRs and 22 PRs). An exploratory subgroup analysis of response rate by platinum type showed an RR of 37% with cisplatin therapy and an RR of 23% with carboplatin. Treatment was well tolerated, with the most common grade 3/4 toxicities being neutropenia, hypertension, fatigue, anemia, and hyponatremia. Biomarker analyses, including BRCA and p63/p73 status, are ongoing to identify responders vs nonresponders.

Targeting Epigenetics, Including Use of HDAC Inhibitors, in TNBC

While driver mutation events are commonly targeted in breast cancer, epigenetic alterations, which may include histone hypoacetylation and abnormal DNA methylation in promoter regions of important oncogenes, are also known to promote cancer initiation and progression. Targeting such epigenetic events via histone deacetylase (HDAC) inhibitors has been explored in the preclinical and clinical treatment of TNBC. In the in vitro setting, treatment of three different TNBC cell lines (MDA-MB-231, MDA-MB-435, and Hs578t) with the HDAC inhibitor Scriptaid not only resulted in growth inhibition and increased acetylation of histone tails, but also in a significant increase in levels of ER mRNA transcript.[48] Moreover, the ability of the HDAC inhibitor vorinostat to overcome endocrine-therapy resistance in 43 women with hormone therapy-refractory, ER+ breast cancer was investigated in a phase II clinical trial.[49,50] The ORR in patients treated with vorinostat/tamoxifen was 19% and the CBR was 40%, illustrating the ability of HDAC inhibition to possibly abrogate resistance to endocrine therapy. This strategy of pharmacologically manipulating ER expression via HDAC inhibition, specifically with panobinostat plus letrozole, is currently being studied in the setting of advanced TNBC (NCI ClinicalTrials.gov Identifier: NCT01105312). Moreover, the addition of vorinostat to carboplatin and nab-paclitaxel chemotherapy has also been evaluated in the neoadjuvant treatment of TNBC; in a phase II study of 62 women with early-stage TNBC, the pCR rates were similar in patients treated with carboplatin/nab-paclitaxel/vorinostat (27.6%) and in those treated with carboplatin/nab-paclitaxel/placebo (26.7%). Biomarker analyses from this study are ongoing to help identify which patients with TNBC may derive benefit from addition of HDAC inhibition to chemotherapy.[51]

The Role of the Androgen Receptor in TNBC

Given the paucity of effective and nontoxic treatment options for TNBC, the observation that the androgen receptor (AR) may be expressed in 60% to 80% of human breast cancers has generated enthusiasm.[52] Furthermore, recent work in gene expression profiling has identified a subset of ER−/PR− tumors with an active hormonally regulated transcriptional program.[53] These observations have led to a phase II single-arm study of the nonsteroidal anti-androgen bicalutamide in patients with ER−/PR− and AR+ MBC as defined by immunohistochemistry (IHC). (See Traina et al, NCI ClinicalTrials.gov Identifier: NCT00468715.) Patient accrual is completed and efficacy results are anticipated.

Targeting the Folate Receptor in TNBC

Another promising new strategy in the treatment of TNBC may be targeting the folate receptor (FR) through the use of vintafolide (EC145), a conjugate of folate linked to the vinca alkaloid desacetylvinblastine hydrazide. In a phase I study of 32 patients with advanced solid tumors, including breast and ovarian cancer, vintafolide was tolerated, with constipation as the dose-limiting toxicity.[54] Other toxicities included fatigue, nausea, and vomiting. In this heavily pretreated population, 7 patients demonstrated SD ranging in duration from 42 to 211 days, and 1 patient with advanced ovarian cancer demonstrated a PR to therapy. The phase I experience with vintafolide led to the Platinum Resistant Ovarian Cancer Evaluation of Doxil and EC145 Combination Therapy (PRECEDENT) trial, a randomized phase II study evaluating pegylated liposomal doxorubicin (PLD) alone or in combination with vintafolide.[55] Compared with patients treated with PLD alone, patients who received the combination of PLD and vintafolide experienced an improved PFS, 24 weeks vs 11.7 weeks with just PLD ($P = .014$). Expression of the FR
as measured by the EC20 scan, a nuclear medicine scan utilizing technetium-labeled folate, may prove to be predictive of benefit from vintafolide. Specifically, in PRECEDENT, patients with tumors that were 100% EC20+ demonstrated particular benefit in PFS with combination therapy compared with PLD alone, with PFS of 24.0 weeks vs 6.6 weeks, respectively (P = .018). These data have fueled enthusiasm for the Study for Women With Platinum Resistant Ovarian Cancer Evaluating EC145 in Combination With Doxil (PROCEED; NCI ClinicalTrials.gov Identifier: NCT01170650). This ongoing phase III trial in advanced platinum-resistant ovarian cancer utilizes the same study design as the PRECEDENT trial. Based on molecular and genomic commonalities between HGSOC and BBC, vintafolide is also being developed in combination with taxane therapy in advanced TNBC.

**Promising Preclinical and Early-Phase Investigations in the Treatment of TNBC**

Meaningful improvement in the care of women with advanced TNBC will require a true partnership between physicians, translational investigators, and basic scientists. The clinical care of our patients with TNBC will continue to drive a wealth of critical questions in need of attention. In parallel, preclinical investigations of TNBC biology will continue to dissect this aggressive, heterogeneous disease and elucidate novel targets to treat it more optimally. As described above, several studies that include the comprehensive analysis of TNBC/BBC per TCGA have identified several additional targets that are being investigated in both preclinical work and early-phase clinical trials.

**p53 mutations and inhibitors of Chk1**

As described in TCGA, BBC exhibits comparatively high rates of p53 mutations (~80%) compared with luminal A (12%) and luminal B (32%) breast cancers.[7] Mutations in p53 result in loss of the G1 checkpoint in the cell cycle and shifts toward reliance on checkpoint kinase 1 (Chk1) to arrest cells in response to DNA damage. In an elegant series of preclinical experiments, Ma and colleagues showed that Chk1 inhibition potentiated cytotoxicity of the DNA-damaging agent irinotecan in two p53-mutant TNBC cell lines that were derived from human tumors and subsequently passaged in xenograft tumor models.[56] Interestingly, cytotoxicity in response to combination Chk1 inhibitor/irinotecan therapy was not duplicated in a p53-wild-type TNBC cell line. In vitro results were confirmed in vivo, as Chk1 inhibition plus irinotecan inhibited tumor growth and prolonged survival in a p53-mutant xenograft model but not a p53-wild-type model. Based on these preclinical results, development strategies for Chk1 inhibitors in TNBC will most certainly need to take p53 mutation status into account in order to optimize response and clinical outcomes.

**Inhibition of the PI3K/mTOR and MEK pathways**

The *PIK3CA* gene is commonly mutated in TNBC/BBC, with a mutational frequency rate of 9% per TCGA; furthermore, activation of the PI3K pathway at the gene and/or protein level was highest in TNBC/BBC compared with other subtypes.[7] In contrast to luminal breast cancer, in which *PIK3CA* gene mutation rates are 30% to 50%, alternate means of PI3K pathway activation in TNBC/BBC may occur through loss of either *PTEN* or *INPP4B*.[57] Inhibition of the PI3K pathway and of downstream mammalian target of rapamycin (mTOR) has been identified as a promising therapeutic strategy for treating TNBC. Moreover, compensatory activation of the PI3K pathway has also been shown in response to mitogen-activated protein/extracellular signal-regulated kinase (MEK) inhibition in results from in vitro studies of TNBC.[58] Synergy has also been observed with dual inhibition of PI3K/mTOR and MEK in genetically engineered mouse models (GEMMs) of both basal-like TNBC (C3-Tag GEMM) and claudin-low TNBC (T-11), as evidenced by improved tumor response and relative improvement in survival compared with placebo and most single-agent treatments.[59]

In addition to preclinical studies, clinical evaluation of inhibitors of PI3K, mTOR, and MEK are underway, with varying results. In the preoperative setting, Gonzalez-Angulo et al treated 50 women with early-stage TNBC by coupling the mTOR inhibitor everolimus with weekly paclitaxel, followed by 4 cycles of every-3-weeks preoperative FEC chemotherapy (5-fluorouracil [5-FU], epirubicin, cyclophosphamide).[60] Combination mTOR/taxane therapy yielded numerically higher rates of pCR, although this difference was not statistically significant (30.4% vs 25.9%, P = .76). Biomarker studies using tissue specimens obtained from this study are ongoing. More recently, a second study evaluated the CBR of everolimus plus carboplatin chemotherapy in 25 women with advanced TNBC.[61] This study met its prespecified endpoint, as the CBR was 36% (95% confidence interval [CI], 23%-55%) and median PFS was 3.3 months (95% CI, 2.4 months to 7.7 months).
Thrombocytopenia was the most common dose-limiting toxicity, requiring carboplatin dose reduction from AUC 6 to AUC 4 IV every 3 weeks, with everolimus administered at 5 mg orally daily. Similar to the neoadjuvant study, real-time biopsies were collected at baseline and following 2 cycles of therapy to help identify the subset of patients most likely to benefit from mTOR inhibition in combination with standard chemotherapy.

To investigate the strategy of MEK inhibition, a preoperative “window of opportunity” study evaluated reprogramming of the kinome among nine women with operative TNBC.[62] Eligible patients were treated with the MEK 1/2 inhibitor trametinib at 1.5 mg to 2.0 mg orally daily for 7 days preceding definitive surgery. Fresh tumor was obtained at baseline and from the operative specimen. Reported treatment toxicities were minimal and included grade 1 nausea, diarrhea, and rash.

Results of correlative tissue analysis from this study showed kinome reprogramming in response to MEK 1/2 inhibition similar to that seen in preclinical models. This novel trial design showed that TNBC kinome response differs by subtype (claudin-low and basal-like) and provided evidence that MEK inhibition upregulates “druggable” targets (PDGFR-beta, VEGFR2, the receptor tyrosine kinase Axl). These observations may provide a rationale for combining MEK inhibitors with other relevant targeted therapies in future studies.

Finally, inhibition of the PI3K/mTOR pathway has shown activity in TNBC in preclinical studies, particularly the mesenchymal and mesenchymal stem-like subsets of TNBC.[5] Additional preclinical studies have illustrated synergy between PI3K inhibitors and PARP inhibitors in the treatment of TNBC cell lines and animal models.[63,64] While clinical experience with PI3K inhibition is quite limited to date, the results of several ongoing clinical studies (SOLTI, NCI ClinicalTrials.gov Identifier: NCT01629615; and LCCC1024, NCI ClinicalTrials.gov Identifier: NCT01300962) should yield valuable information regarding the treatment of patients with advanced TNBC.

**Conclusions and Future Directions**

The landscape of TNBC treatment is changing rapidly. At present and based on guidelines set forth by the National Comprehensive Cancer Network, the mainstay of treatment for TNBC, in both the curative and metastatic settings, is traditional cytotoxic chemotherapy. Concurrent with unraveling the biologic underpinnings of TNBC at the preclinical level to identify “druggable” targets, we are charged as a medical community with continuing to develop novel clinical trials rooted in sound science and to expand our armamentarium of targeted therapies to treat this aggressive disease more effectively. Perhaps our greatest hurdle is the amount of time necessary to carefully translate promising preclinical findings to the bedside, with the ultimate goal being FDA approval of new drugs that yield both superior survival and quality of life outcomes for patients with TNBC. In the meantime, we can only continue to stress the importance of clinical trial participation to move the entire field forward and provide patients with TNBC access to promising therapies.

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