CLINICAL INVESTIGATION

Triple-negative breast cancer and new treatment developments

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Stephen Dyar[1]
& Alvaro Moreno-Aspitia[†1]

[1]College of Medicine, Mayo Clinic, Division of Hematology & Oncology, 4500 San Pablo Road, Jacksonville, FL 32224, USA
[†]Author for correspondence:
Tel.: +1 904 953 2000
Fax: +1 904 953 2315
E-mail: morenoaspitia.alvaro@mayo.edu

Triple-negative breast cancer (TNBC) is an aggressive immunohistochemical phenotype found in approximately 15% of women with invasive breast cancer. Although TNBC is sensitive to cytotoxic chemotherapy, it is associated with poorer outcomes. Moreover, patients with TNBC are not candidates for hormonal or human epidermal growth factor 2-targeted therapies, thus underscoring the need for new treatments for TNBC. Agents targeting aberrant DNA repair, including platinum and PARP-1 inhibitors, are under evaluation in TNBC based on its overlap with BRCA1-related breast cancer. Several other cytotoxic (e.g., ixabepilone) and targeted agents (e.g., bevacizumab, cetuximab, everolimus and dasatinib) are also being investigated clinically. Results from early clinical trials suggest the potential for improving the outcomes of patients with TNBC in the future.

Keywords: basal-like breast cancer • *BRCA1*-related breast cancer • PARP-1 • targeted therapy • triple-negative breast cancer

Breast cancer is the most common malignancy and second leading cause of cancer death among women in the USA; an estimated 207,090 women were diagnosed in 2010 [1]. Approximately 15% of the cancers diagnosed as invasive breast cancer will also be classified as triple-negative breast cancer (TNBC) – a breast cancer subtype characterized by tumors that lack expression of the estrogen receptor (ER), progesterone receptor (PR) and human EGF receptor 2 (HER2) [2]. However, TNBC itself is a heterogeneous disease; it accounts for a majority of tumors classified as 'basal-like' by gene expression profiling, and also includes many tumors harboring *BRCA1* or *BRCA2* mutations.

TNBC confers an aggressive clinical course with a poor prognosis compared with other breast cancer subtypes. Owing to the lack of ER, PR and HER2 expression, TNBC is not amenable to treatment with currently approved targeted approaches such as hormone therapy or HER2-targeted drugs such as trastuzumab, and therefore cytotoxic chemotherapy remains the mainstay of treatment. This article describes the molecular and clinical features of TNBC, and then reviews traditional and experimental approaches for treating this aggressive breast cancer subtype.

Molecular & clinical features of TNBC

Molecular & histological features

Microarray-based gene expression profiling conducted in the early 2000s demonstrated that invasive breast tumors cluster into five distinct and highly reproducible molecular patterns, termed luminal A, luminal B, HER2-positive, normal breast-like and basal-like [3–5]. The luminal A and B subtypes are ER-positive, and distinguished by the presence of PR expression and absence of HER2 expression (luminal A), or the absence of progesterone expression and/or presence of HER2 expression (luminal B). Both share histological features with luminal epithelial cells arising from

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the inner layer of the breast duct [6]. The HER2-positive phenotype is characterized by HER2 overexpression and a lack of hormone receptor expression; this subset predicts an aggressive clinical course, but is sensitive to HER2-targeted therapies. The normal breast-like subset typically lacks ER and HER2 expression and exhibits characteristics of normal mammary stromal cells. Some experts believe that this subset may be an artifact caused by contamination with a large proportion of normal breast tissue [7]. The basal-like phenotype is generally associated with lack of ER, PR and HER2 expression (i.e., the triple-negative phenotype) and with expression of basal cell-like cytokeratins (CKs) 5, 6 and 17. Furthermore, these tumors are thought to arise from the outer basal layer of the breast duct [6]. More recent gene expression studies have identified other potential but less common subtypes. The claudin-low subtype is the most notable given that it typically carries a triple-negative phenotype and is enriched for features associated with stem cell function and the epithelial-to-mesenchymal transition [8,9].

The term TNBC encompasses breast cancer characterized immunohistochemically based on the lack of ER, PR and HER2 expression, whereas the molecular phenotypes are characterized by gene expression profiling. Although basal-like tumors are frequently triple negative, it is important to recognize that TNBC is not synonymous with basal-like tumors (Figure 1). Up to 30% of basal-like tumors do not exhibit a TNBC phenotype in that they show either hormone receptor or HER2 expression when evaluated by immunohistochemistry [10,11]. Moreover, as noted earlier, other molecular subtypes, such as the claudin-low subset, may fall under the TNBC umbrella.

TNBC is associated with overexpression of the basal CKs 5, 6 and 17, reflecting its overlap with the basal-like subset. In addition, the EGF receptor (EGFR; HER1) is overexpressed in up to 60% of TNBC tumors [12–15]. Other molecular markers that are expressed at rates higher in basal-like tumors than in other molecular breast cancer subtypes, and consequently may also be associated with TNBC, include c-kit, p53 (or *TP53* gene mutations), p16, cyclin E, E2F3 and α-B-crystallin [16]. Conversely, expression levels of the retinoblastoma protein and cyclin D1 are typically reduced. TNBC has been further characterized on the basis of CK 5/6 and EGFR expression: the term 'core basal phenotype' has been used to identify a subset of TNBC tumors that express either CK 5/6 and/or EGFR, whereas the term 'quintuple-negative' has been used to refer to TNBC tumors that express neither CK 5/6 or EGFR (i.e., ER, PR, HER2, CK 5/6 and EGFR negative) [2]. It should be noted, however, that the quintuple-negative subgroup among patients with TNBC is typically very small. The clinical relevance of this additional stratification remains to be determined, but several studies suggest that it may add significance to the prognostic information conferred by TNBC status [17,18].

Histologically, TNBC generally presents as a ductal carcinoma, but some cases have mixed histology with features of metaplastic or medullary carcinomas [16]. A large majority of tumors associated with TNBC are high-grade (Nottingham grade III). In the TNBC cohort from the Carolina Breast Cancer Study, 84% had tumors with Nottingham grade III whereas only 2% were low-grade tumors (grade I). Most TNBC tumors were noted to have marked nuclear pleomorphism (80%) and high mitotic index (>10 per 10 high-power fields; 87%) [19]. Consistent with this profile, TNBC is associated with high expression of the proliferation marker Ki-67 and exhibits pushing margins of invasion, with a stromal lymphocytic infiltrate at the tumor margins and multiple necrotic cores [16,20].

Clinical features

Population-based studies indicate that women with TNBC are younger at diagnosis and more likely to be African-American than those with non-TNBC [19,21,22]. In the California Cancer Registry, for example, women with TNBC were 53% more likely to be diagnosed at 40 years of age or younger and 77% more likely to be African-American compared with non-TNBC cases [21]. Similarly, in the Carolina Breast Cancer Study, women with basal-like breast cancer were diagnosed at a significantly younger age and had a higher proportion of African-Americans compared with the luminal subtypes [19]. TNBC is also associated with obesity among premenopausal women [22]. However, the higher incidence of TNBC among African-Americans appears unrelated to patient age or body mass index [23].

BRCA1 mutations – a risk factor for early-onset familial breast cancer – are also seen more frequently in TNBC than non-TNBC cases [24–27]. In a cohort of 491 breast cancer patients who underwent genetic testing for *BRCA1/2* mutations, TNBC was identified in 57% of the BRCA1-positive patients compared with 23 and 18% of BRCA2-positive and BRCA-negative patients, respectively [25]. Although a family history of breast cancer among first-degree relatives is associated with increased risk of breast cancer, it is not associated with a preferential risk increase for TNBC compared with other molecular subtypes based on recent data from the Breast Cancer Surveillance Consortium [28].

Prognosis & outcome

Early-stage TNBC is highly sensitive to neoadjuvant cytotoxic chemotherapy; however, TNBC has a paradoxical poor prognosis with increased risk of early

relapse, different patterns of metastasis (visceral > bone), and reduced survival compared with other breast cancer subtypes. In a cohort of 1601 women with early-stage breast cancer, the risk of distant recurrence in the subset with TNBC peaked at 1–3 years following diagnosis, after which it declined and matched the lower-risk levels seen in non-TNBC patients [29]. The poor survival of patients with TNBC is illustrated by data from the California Cancer Registry, which compared 6370 women with TNBC with 44,704 women with other breast cancer types [21]. Survival at 5 years following diagnosis was significantly lower among patients with TNBC compared with patients without TNBC (77 vs 93%); this survival difference was evident regardless of the disease stage at diagnosis (Figure 2). Several other smaller studies have consistently shown similar findings of shorter survival for TNBC compared with non-TNBC [24,29–31].

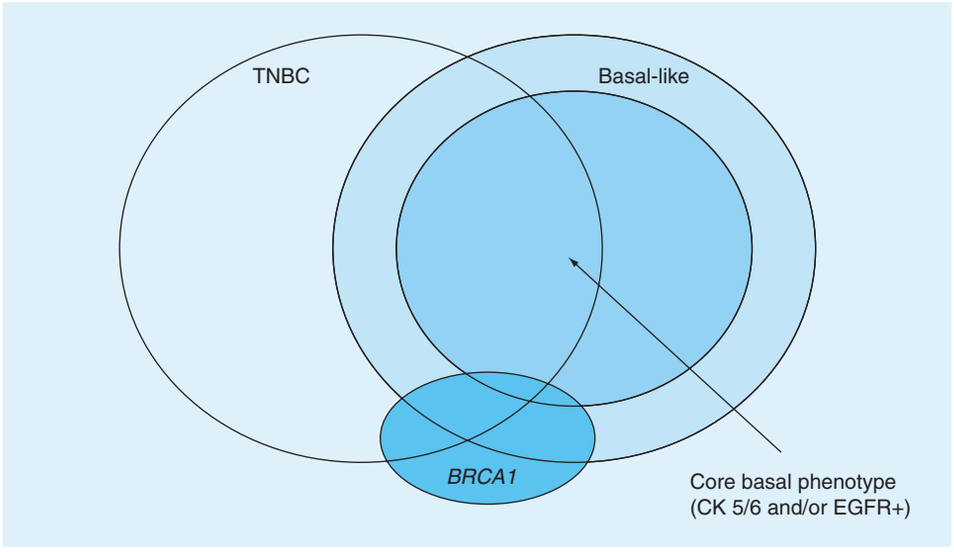

Figure 1. Overlap among triple-negative breast cancer, basal-like and *BRCA1*-related tumors.
CK: Cytokeratin; EGFR: EGF receptor; TNBC: Triple-negative breast cancer.

When classified by expression profiling, the survival of patients with basal-like tumors is comparable to those with HER2-overexpressing tumors, both of which are significantly shorter than survival of patients with luminal A or B tumors [32]. However, the advent of adjuvant trastuzumab has significantly improved the prognosis of patients with early-stage HER2. No such targeted therapy exists yet for early-stage TNBC; however, early developments in adjuvant clinical trials with PARP-1 inhibitors are promising.

TNBC continues to confer a survival disadvantage even after development of distant metastases. In a cohort of 3726 patients initially diagnosed with early-stage breast cancer between 1986 and 1992 (median follow-up time of 14.8 years) and having archival tumor specimens for expression analysis, median survival following distant metastasis was 0.5 years for patients with basal-like tumors compared with 0.7 years for those with HER2-overexpressing tumors and 1.6–2.2 years for those with luminal A or B tumors [33]. Similarly, in another study, median survival from the time of distant recurrence was significantly shorter for patients with TNBC compared with patients without TNBC (9 vs 20 months; p = 0.02) [29]. This poorer survival in the metastatic disease setting associated with TNBC may reflect higher rates of visceral metastases and lower rates of bone metastases compared with non-TNBC cases [33–35]. Additionally, patients with metastatic TNBC are at increased risk of developing brain metastases.

Traditional cytotoxic therapies for TNBC

The primary treatment for TNBC is cytotoxic chemotherapy [2,16]. TNBC is highly sensitive to anthracyclines and anthracycline/taxane combinations, but patients have a relatively high risk of relapse, and as noted previously, a poorer outcome when compared with patients without TNBC. Evidence for the chemosensitivity of TNBC has been shown in several neoadjuvant and adjuvant clinical studies. In a cohort of 1118 women with early-stage breast cancer (including 255 patients with TNBC), neoadjuvant chemotherapy, consisting primarily of an anthracycline-based or anthracycline/taxane-based regimen, produced higher pathologic complete response (pCR) rates in women with TNBC compared with other breast cancer subtypes (22 vs 11%; p = 0.034) [30]. Three-year survival for women achieving pCR was comparable for TNBC and non-TNBC cases (94 vs 98%; p = 0.24). However, for those with residual disease, 3-year survival was significantly poorer in the TNBC subset (68 vs 88%; p < 0.0001; Figure 3). Comparable data were reported with neoadjuvant doxorubicin/cyclophosphamide with or without sequential taxane therapy in a cohort of 107 women [36]. Higher pCR rates were achieved among basal-like and HER2-overexpressing patients compared with those having luminal subtypes (p = 0.01). Early relapse was rare after pCR regardless of breast cancer subtype, whereas with residual disease, outcome was poorer for the basal-like and HER2-overexpressing subset compared with the luminal subsets. The addition of capecitabine to anthracycline/taxane regimens has also proven effective in studies of patients with early breast cancer. Data

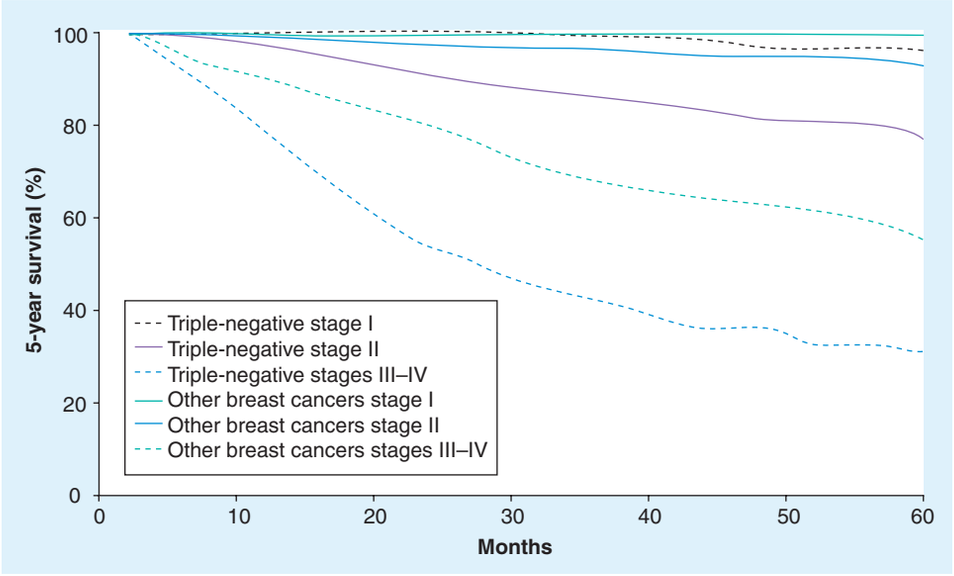

Figure 2. 5-year relative survival of patients with triple-negative breast cancer versus non-triple-negative breast cancer by stage at diagnosis in the California Cancer Registry (1999–2003) [21].

from the Phase III ABCSG-24 trial demonstrated significant improvements in pCR with the addition of capecitabine to a neoadjuvant regimen of epirubicin plus docetaxel (24.3 vs 16.0%; p = 0.02) [37]. Furthermore, a review of subgroup analyses from ABCSG-24 showed that patients with TNBC (n = 122) had a significantly greater chance of achieving a pCR than non-TNBC (n = 348; odds ratio = 5.29; 95% CI: 3.22–8.68; p < 0.0001), independent of the regimen. And in the total study population, the highest pCR rates were achieved in patients with TNBC who received epirubicin, docetaxel and capecitabine compared with those who only received epirubicin and docetaxel (47.5 vs 31.2%; p = NS) [38]. Taken together, these studies highlight the importance of achieving pCR with neoadjuvant therapy in patients with early-stage TNBC. In two recent studies, sequential anthracycline/taxane regimens produced pCR rates of 29 and 36% in patients with TNBC [39,40]. This underscores the need for more effective therapies capable of producing much higher pCR rates.

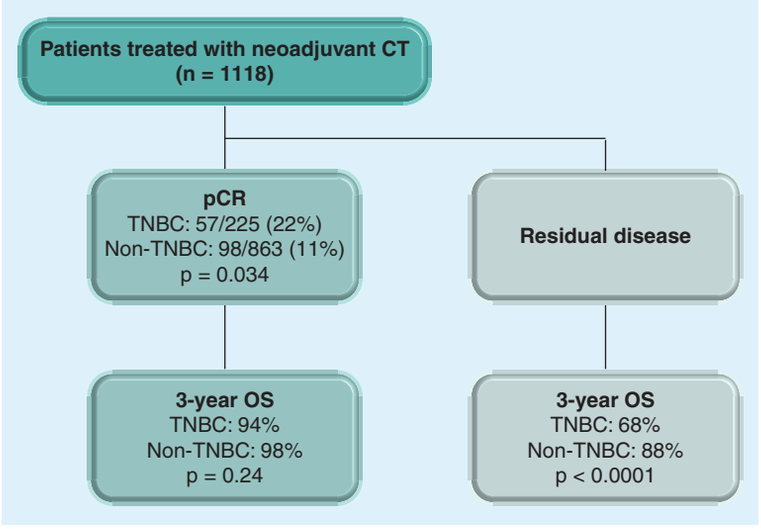

Figure 3. Impact of pathologic complete response on 3-year overall survival in triple-negative breast cancer versus non-triple-negative breast cancer patients who received neoadjuvant chemotherapy at the MD Anderson Cancer Center from 1985–2004 [30].
CT: Chemotherapy; OS: Overall survival; pCR: Pathologic complete response; TNBC: Triple-negative breast cancer.

In the adjuvant setting, a meta-analysis of four Phase III clinical trials suggested that adjuvant anthracycline-based therapy was more effective than classical cyclophosphamide, methotrexate and fluorouracil in prolonging disease-free survival (DFS) in TNBC (hazard ratio [HR] = 0.77; 95% CI: 0.54–1.09) [41]. The magnitude of benefit of anthracycline-based therapy was comparable to that of patients whose cancers overexpress HER2. In comparison, there was no difference in DFS among patients with HER2 or TNBC with regard to the two regimens. Results from the Phase III FinXX trial have also demonstrated the effectiveness of adjuvant anthracycline – as part of sequential therapy – with significant improvements in 3-year recurrence-free survival (RFS) observed with the incorporation of capecitabine to a sequential taxane-anthracycline adjuvant regimen (92.5 vs 88.9% control; HR = 0.66; 95% CI: 0.47–0.94; p = 0.020) [42]. A subsequent review of subgroup analyses from FinXX demonstrated that patients with TNBC (n = 202) had significantly shorter RFS than patients without TNBC (n = 1294; 81.7 vs 92.2%; HR = 0.43; 95% CI: 0.29–0.63; p < 0.001). Moreover, 3-year RFS was significantly longer in the capecitabine-containing treatment arm (n = 93) than in the control arm (n = 109; 87.7 vs 76.6%, respectively; HR = 0.43; 95% CI: 0.21–0.90; p = 0.024) [38]. These promising data for capecitabine in early breast cancer have provided the foundation for an ongoing randomized Phase III study conducted by the CIBOMA collaborative group. This trial is prospectively investigating capecitabine-maintenance therapy after adjuvant anthracycline/taxane treatment in patients with early TNBC (NCT00130533).

Use of anthracyclines and taxanes in early-stage disease limits their value in the metastatic disease setting, directed in part by emergence of drug resistance, as well as limitations on the maximum cumulative dose due to cardiotoxicity (anthracyclines) or neurotoxicity (taxanes). Nevertheless, patients with metastatic TNBC remain sensitive to cytotoxic chemotherapy. In a cohort of 111 patients with metastatic TNBC, many of whom were treated with neoadjuvant or adjuvant chemotherapy, the median duration of first-, second- and third-line treatment was approximately 12, 9 and 4 weeks, respectively [43]. Median survival for the entire cohort with metastatic TNBC was 13.3 months. The short durations of treatment indicate a need for more effective interventions that can be administered over longer intervals once metastatic TNBC occurs.

Newer therapies under investigation for TNBC

Numerous agents are currently being explored for use in TNBC – both in neoadjuvant/adjuvant therapy, as well as in treatment of metastatic disease (Table 1).

Agents targeting aberrant DNA repair

Patients with mutated *BRCA1* have defects in homologous recombination mechanisms that repair DNA double-strand breaks [44]. The overlap of TNBC with *BRCA1* breast cancer raises the possibility that the pathogenesis of TNBC may also involve defective DNA repair. This has led to reconsideration of alkylating agents, such as cisplatin and carboplatin, that interfere with DNA repair, as well as the development of PARP-1 inhibitors that target the key enzyme involved in base excision repair of single-strand DNA. The latter pathway is important in repairing DNA damage in cells with defective homologous recombination.

In a retrospective analysis of patients treated at the Royal Marsden in London, neoadjuvant platinum-based chemotherapy produced higher clinical complete response rates in TNBC than in non-TNBC (88 vs 51%; p = 0.005), although DFS tended to favor the non-TNBC subset [45]. In a Phase II neoadjuvant study, 74 patients received eight cycles of cisplatin, epirubicin and paclitaxel with granulocyte-colony stimulating factor support. This regimen produced a pCR in 46 patients (62%) with large, operable TNBC [46]. 5-year DFS was 90 and 56% among patients who did and did not achieve pCR, respectively. In a similar population, neoadjuvant cisplatin, epirubicin and infusional fluorouracil followed by weekly paclitaxel produced pCR in 12 of 30 patients with TNBC (40%) and 2-year DFS of 87.5% [47]. In comparison, four cycles of single-agent cisplatin (75 mg/m^2 every 21 days) resulted in pCR in only six of 28 patients with TNBC (22%). Interestingly, decreased BRCA1 mRNA expression was associated with good response to cisplatin therapy [48].

Platinum-based chemotherapy has also been suggested to produce favorable results in patients with advanced TNBC compared with patients without TNBC. In a retrospective cohort from the Royal Marsden, the TNBC subset had a numerically higher response rate (41 vs 31%), significantly longer progression-free survival (PFS; 6 vs 4 months; p = 0.05), and a trend for longer overall survival (OS; 11 vs 7 months; p = 0.10) [45]. In a retrospective study of 36 patients, cisplatin/gemcitabine showed a trend for longer PFS in metastatic TNBC compared with non-TNBC (5.3 vs 1.7 months; p = 0.058) [49]. However, in another retrospective study of 143 patients with metastatic breast cancer, platinum-based therapy was not associated with improvement in PFS or OS in TNBC compared with non-TNBC, despite a higher response rate (33 vs 22%) [50].

Brostallicin (PNU-166196A) is a new synthetic α-bromoacrylic derivative that belongs to the pharmacological class of DNA minor groove binding anticancer agents. In preclinical human and murine tumor models, brostallicin is a potent inducer of apoptosis, which retains activity in cancer cells resistant to alkylating agents, topoisomerase I inhibitors, and is fully active against DNA mismatch repair-deficient tumor cells [51–53]. The North Central Cancer Treatment Group is conducting a Phase II trial (N0937) of cisplatin followed by brostallicin in patients with advanced TNBC who have received up to four prior lines of therapy in the metastatic setting (NCT01091454). The rationale for this study is based on preclinical observations that cisplatin increases

Table 1. Treatment approaches under clinical investigation in triple-negative breast cancer.

Approach	Agents
Enhance aberrant DNA repair	Platinum drugs PARP-1 inhibitors Brostallicin
Block angiogenesis	Bevacizumab Sunitinib
Block EGFR	Cetuximab Erlotinib
Stabilize microtubules	Ixabepilone
Block signaling cascades	Src inhibitors (e.g., dasatinib) mTOR inhibitors (e.g., everolimus)

expression of glutathione *S*-transferase in tumor cells, and that brostallicin has greater cytotoxicity in tumor cells with elevated levels of glutathione and glutathione *S*-transferase. The PARP-1 inhibitors are novel targeted agents designed to inhibit single-strand DNA repair mechanisms that may be important to the survival of TNBC cells, especially those harboring a *BRCA* mutation. Multiple studies of PARP-1 inhibitors in TNBC are currently in progress. The furthest advanced of the PARP-1 inhibitors is iniparib (also known as BSI-201). It was evaluated in a randomized Phase II trial involving 116 patients with TNBC who had received up to two prior chemotherapy regimens for metastatic disease [54]. Patients were allocated to carboplatin/gemcitabine with or without iniparib, with the PARP-1 inhibitor administered at a dose of 5.6 mg/kg intravenous twice-weekly for the first 2 weeks of a 3-week cycle. When added to carboplatin/gemcitabine, iniparib significantly improved the response rate (52.5 vs 32.3%; p = 0.023), PFS (5.9 vs 3.6 months; p = 0.0012), and OS (12.3 vs 7.7 months; p = 0.014) compared with carboplatin/gemcitabine alone. Chemotherapy-related adverse events were not increased by the addition of iniparib to carboplatin/gemcitabine. The encouraging data from this trial have led to a randomized Phase III study using the same treatment arms. This trial is now complete and awaits final assessment (NCT00938652). Notably, on 28 January 2011, a press release from Sanofi-Aventis and its subsidiary, BiPar Sciences, stated that this Phase III trial did not meet the specified criteria for significance for co-primary end points of OS and PFS. Conversely, data from the prespecified analysis in patients treated in the second- and third-line setting do support the findings reported in the Phase II trial – improvement in OS and PFS.

The PARP-1 inhibitor olaparib (AZD2281) was evaluated in advanced breast cancer patients with *BRCA1* or *BRCA2* mutations in a multicenter, proof-of-concept, Phase II trial [55]. The study cohort had received a median of three previous chemotherapy regimens. Olaparib produced objective responses in a dose-related manner (41% at 400 mg twice daily [b.i.d.] and 22% at 100 mg b.i.d.) and was well tolerated with mainly grade 1/2 toxicities. Importantly, at an end-of-year discussion, AstraZeneca decided to discontinue the Phase III trial of olaparib in TNBC with BRCA mutations and instead, focus on development in serous ovarian cancer – a decision apparently based on iniparib not meeting its end points in the Phase III trial.

Other studies of PARP-1 inhibitors in TNBC are currently in progress, including a Phase II trial of iniparib plus carboplatin/gemcitabine as neoadjuvant therapy (NCT00813956), and Phase I trials of olaparib with either carboplatin, paclitaxel, or both in metastatic disease (NCT00516724; NCT00647062; NCT00707707). Another PARP-1 inhibitor, veliparib, is being investigated with and without carboplatin in a Phase II trial in patients with *BRCA1/2* mutations (NCT01149083) and in combination with cisplatin/vinorelbine in a Phase I study in patients with metastatic TNBC (NCT01104259). Additional studies are also in development and will likely be activated by the time of this publication.

■ Angiogenesis inhibitors

VEGF is a major angiogenic factor in human malignancies. Patients with TNBC have been shown to have higher intratumoral levels of VEGF compared with patients without TNBC [31]. When added to first-line paclitaxel, the anti-VEGF monoclonal antibody bevacizumab significantly improved PFS compared with paclitaxel alone in the Phase III E2100 trial, with subset analyses showing comparable benefits among hormone receptor-negative patients (which is a TNBC cohort given the HER2-negative status of the study population) as in hormone receptor-positive patients [56]. A meta-analysis of patients with TNBC treated in the first-line setting in the Phase III E2100, AVADO, and RIBBON-1 trials assessed the difference in PFS and OS between treatment groups (chemotherapy [taxane-, anthracycline-, or capecitabine-based] plus bevacizumab compared with chemotherapy alone). Although median PFS was significantly longer in the chemotherapy plus bevacizumab cohort compared with chemotherapy alone (8.1 vs 5.4 months; unstratified HR = 0.65; 95% CI: 0.54–0.78, log-rank p < 0.0001; stratified HR = 0.68; 95% CI: 0.56–0.83, log-rank p = 0.0002), there was no significant difference in OS (18.9 vs 17.5 months, respectively; unstratified HR = 0.96; 95% CI: 0.79–1.16, log-rank p = 0.673; stratified HR = 0.99; 95% CI: 0.81–1.21, log-rank p = 0.930) [57]. In a separate study, bevacizumab added to first-line nab-paclitaxel/gemcitabine produced a clinical benefit rate of 85% in a small subgroup of patients with metastatic TNBC, which was comparable to the benefit seen in hormone receptor-positive, HER2-negative patients in a single-arm Phase II study [58]. Bevacizumab is currently being evaluated in numerous trials in patients with TNBC, including the Phase III BEATRICE trial comparing bevacizumab plus chemotherapy versus chemotherapy alone in the adjuvant setting (NCT00528567). Notable Phase II studies in first-line treatment of metastatic TNBC include bevacizumab plus carboplatin/gemcitabine (NCT01201265) and bevacizumab plus carboplatin/doxorubicin (NCT00608972), and those in the neoadjuvant setting include bevacizumab plus carboplatin/docetaxel (NCT01208480) and bevacizumab in combination with sequential nab-paclitaxel/

carboplatin followed by doxorubicin/cyclophosphamide (NCT00777673). However, the recent decision of the US FDA to remove the indication for bevacizumab in patients with metastatic breast cancer makes it very difficult to predict its future role in the management of patients with TNBC.

Sunitinib is a tyrosine kinase inhibitor that targets the VEGF receptor, PDGF receptor, and c-kit. Sunitinib administered at a dose of 50 mg/day for 4 weeks of a 6-week cycle produced objective responses in three of 20 (15%) patients with metastatic TNBC previously treated with an anthracycline and a taxane [59]. In another Phase II trial, first-line treatment with sunitinib 25 mg/day in combination with weekly paclitaxel produced responses in three of nine (33%) patients with TNBC [60]. Other outcome data for the TNBC subsets in these trials were not reported. Sunitinib is currently being evaluated in a randomized Phase II trial against standard-of-care chemotherapy in patients with advanced TNBC who had been treated previously, with up to two prior chemotherapy regimens (NCT00246571). Results based on an independent central review showed no significant improvement in median PFS in patients treated with sunitinib compared with those treated with standard-of-care chemotherapy (2.0 vs 2.7 months, respectively; HR = 1.20; 95% CI: 0.89–1.63; 1-sided p = 0.889). Similarly, sunitinib did not prolong median OS (9.4 vs 10.5 months, respectively; HR = 1.22; 95% CI: 0.89–1.68; 1-sided p = 0.892) [61]. Sunitinib is also being investigated in a Phase I/II trial as neoadjuvant therapy in combination with paclitaxel/carboplatin for TNBC (NCT00887575). However, it is unlikely that sunitinib will play any significant role in breast cancer management. SUN 1107, a Phase III trial of sunitinib versus capecitabine in a broad range of advanced breast cancer patients who had failed prior first-line standard therapy, was closed earlier than expected after an Independent Data Monitoring Committee found that sunitinib would be unable to demonstrate a statistically significant improvement in the primary PFS end point. Three other Phase III studies (SUN 1064, SUN 1099 and SUN 1094) evaluating sunitinib in combination with docetaxel, capecitabine, or paclitaxel in advanced breast cancer did not meet their primary end points.

EGFR inhibitors

EGFR overexpression is seen in up to 60% of TNBC tumors [12–15]. The combination of the EGFR inhibitor cetuximab with paclitaxel produced a major response against skin metastases in a case report of a heavily pretreated woman with metastatic, EGFR-expressing TNBC [62]. In a randomized Phase II trial, 102 patients with metastatic TNBC who had not previously received platinum chemotherapy received single-agent cetuximab with carboplatin added at disease progression, or combination therapy with cetuximab plus carboplatin [63]. Overall, 54% had been treated previously for metastatic disease, including 24% who received more than one regimen. Single-agent cetuximab exhibited limited activity (6% partial responses, 4% stable disease). In comparison, the cetuximab/carboplatin doublet was more active, producing partial responses in 17% and stable disease in 9%. The response rate did not differ by the line of therapy. Unfortunately, the median PFS was disappointing for both arms; 1.4 months for single-agent cetuximab and only 2 months in the combination arm. In another Phase II trial in metastatic disease, cetuximab plus weekly carboplatin/irinotecan produced a higher response rate in the TNBC subgroup compared with chemotherapy alone (49 vs 30%) [64]. However, the addition of cetuximab to chemotherapy did not improve the PFS or OS of the entire cohort of patients or in the TNBC population (PFS of 6.4 vs 5.2 months for the TNBC patients treated with and without cetuximab). Additionally, grade 3/4 toxicity was higher in the cetuximab arm, particularly diarrhea, neutropenia and thrombocytopenia. Other ongoing studies are evaluating cetuximab in the TNBC population. These include a series of Phase II trials comparing cetuximab plus ixabepilone versus ixabepilone in neoadjuvant therapy (NCT01097642) and in first-line treatment of advanced breast cancer in women previously treated with anthracycline-based therapy in the adjuvant or neoadjuvant setting (NCT00633464). Results from a trial comparing cetuximab plus cisplatin with cisplatin alone in patients with metastatic TNBC treated with up to one prior regimen (NCT00463788) demonstrated that the addition of cetuximab to cisplatin nearly doubled the overall response rate (ORR): 20.0 (95% CI: 13.1–28.5) versus 10.3% (95% CI: 3.9–21.2; p = 0.5 for testing ORR against 20.0%). Moreover, cetuximab in combination with cisplatin was associated with a significant 32.5% reduction in the risk of disease progression compared with cisplatin alone (HR = 0.675; 95% CI: 0.470–0.969; p = 0.032), with a manageable safety and toxicity profile [65].

The EGFR tyrosine kinase inhibitor erlotinib is also being evaluated in Phase II clinical trials in TNBC. In one study, patients with advanced breast cancer are given first-line nab-paclitaxel plus bevacizumab over a 24-week period, and those without disease progression receive maintenance therapy with bevacizumab plus erlotinib until disease progression (NCT00733408). Other trials are evaluating erlotinib in combination with neoadjuvant chemotherapy (NCT00491816) and in combination with bendamustine in women with advanced breast cancer (NCT00834678). However,

a Phase II trial of erlotinib in patients with EGFR-overexpressing TNBC was recently terminated due to poor accrual (NCT00739063). Although, as previously noted, up to 60% of TNBC may express EGFR, the results of some of the above trials raise serious questions as to whether EGFR is an appropriate target in this malignancy.

Src inhibitors

Src tyrosine kinase is an important messenger in numerous steps of oncogenesis, including tumor cell proliferation, invasion and metastasis [66]. Preclinical studies suggest that basal-like/TNBC cell lines are more likely to respond to src inhibitors, such as dasatinib, than other breast cancer subtypes [67]. Moreover, breast cancer cells that have undergone an epithelial-to-mesenchymal transition – which correlates with the basal-like phenotype – are also highly sensitive to src kinase inhibition [68]. Dasatinib exhibited modest single-agent activity in a Phase II trial involving women with advanced TNBC who had received prior anthracycline and/or taxane therapy and up to two previous regimens in the advanced disease setting [69]. Dasatinib was initially given at a dose of 100 mg b.i.d. but it caused toxicity necessitating dose interruption or delay. The starting dose was subsequently lowered to 70 mg b.i.d., and the drug was generally well tolerated with fatigue (9%) as the most common grade 3 event. Because both of the starting doses resulted in similar exposure, the efficacy analysis was combined. Overall, patients responded to dasatinib unfavorably, with partial responses observed in only two of 43 evaluable patients (5%) lasting 54 and 8 weeks, respectively. The disease control rate was 9.3% and median PFS was 8.3 weeks. Dasatinib is still being evaluated in multiple clinical trials in breast cancer, but only one trial is specifically evaluating the drug in TNBC: a Phase II trial of single-agent dasatinib in locally advanced disease (NCT00817531). Patients with TNBC are also eligible for some of the other trials. Subset analyses of these trials will be needed to determine if the drug has preferential clinical activity in TNBC compared with other breast cancer subtypes.

Microtubule-stabilizing agents

Ixabepilone is the first member of the epothilone class to be approved for use in breast cancer. Like the taxanes, it stabilizes microtubules and leads to cell-cycle arrest and apoptosis. However, ixabepilone differs structurally from the taxanes and binds uniquely to β-tubulin, which may account for its low susceptibility to mechanisms that confer resistance to taxanes and anthracyclines, notably P-glycoprotein overexpression [70]. In the neoadjuvant setting, ixabepilone administered for up to four cycles produced a pCR of 26% in patients with TNBC compared with 15% in patients without TNBC [71]. Phase II trials of single-agent ixabepilone were conducted in various populations of patients with metastatic breast cancer, ranging from those previously untreated to those who had progressed on multiple lines of therapy. A retrospective analysis of data from five Phase II trials indicated that response rates for ixabepilone in patients with TNBC were comparable to those seen in the overall study cohorts [72]. In the two pivotal Phase III trials of ixabepilone plus capecitabine versus capecitabine alone, the combination significantly improved PFS in anthracycline/taxane-pretreated women with metastatic breast cancer [73,74]. Many women in these trials were resistant to anthracyclines, taxanes, or both. A prospective pooled analysis was conducted to compare treatment arms in the TNBC subset: the ixabepilone/capecitabine combination significantly improved PFS (4.2 vs 1.7 months) and also increased the response rate (31 vs 15%) compared with capecitabine alone [72]. Besides the aforementioned trials of ixabepilone with cetuximab, several other studies are evaluating ixabepilone in TNBC. Two Phase III trials are evaluating the role of ixabepilone in the adjuvant setting: the TITAN trial is comparing sequential adjuvant therapy with doxorubicin plus cyclophosphamide followed by either ixabepilone or paclitaxel in patients with TNBC (NCT00789581), and the PACS-08 trial is comparing sequential adjuvant therapy with 5-fluorouracil, epirubicin and cyclophosphamide, followed by either ixabepilone or docetaxel (NCT00630032) in patients with TNBC or ER positive – but PR/HER2-negative with poor prognosis – patients. In addition, the Phase II ECLIPSE study is evaluating ixabepilone plus carboplatin in patients with metastatic breast cancer treated with up to two lines of prior therapy and includes a predefined analysis of the TNBC subgroup (NCT01075100).

PI3K-Akt pathway inhibitors

The PI3K/Akt pathway utilizes signals from the cell surface to drive a variety of cellular functions including proliferation, survival and apoptosis. Approximately 70% of breast cancers have mutations in components of the PI3K/Akt pathway [75]. Loss of the phosphatase and tensin homolog is common in TNBC and results in activation of the mTOR, a downstream kinase in the PI3K/Akt pathway that regulates G_1 cell-cycle protein synthesis prior to cell replication [76,77]. Blocking mTOR suppresses proliferative signals causing cell-cycle arrest.

The mTOR inhibitor everolimus was evaluated in first- or second-line treatment of advanced breast cancer in a Phase II trial [77]. When administered at a dose of 10 mg/day, everolimus produced objective responses in 12% of patients, with HER2-negative status being predictive of clinical benefit. However, the activity of

everolimus in patients with TNBC in this trial was not specified. Of patients receiving daily everolimus, 16% discontinued due to pneumonitis. Everolimus is currently being evaluated in several Phase II trials specifically targeting TNBC: as single-agent therapy (NCT00827567), in combination with carboplatin for metastatic disease (NCT01127763), and in combination with cisplatin/paclitaxel in the neoadjuvant setting (NCT00930930). Another mTOR inhibitor, temsirolimus, is also being evaluated in breast cancer. However, none of the ongoing trials is specifically targeting the TNBC population.

Future perspective

Although multiple targeted therapy approaches are being explored in clinical trials, cytotoxic chemotherapy continues to be the mainstay of treatment for TNBC. Understanding the role of *BRCA1* in DNA repair and its overlap with TNBC has led to reconsideration of platinum agents and development of PARP-1 inhibitors. Similarly, identification of EGFR overexpression as a common marker in basal-like tumors and recognition of common mutations in downstream effector pathways has led to multiple targeted approaches. As a greater understanding is obtained about the mechanisms driving this aggressive phenotype, new targeted strategies for TNBC should continue to evolve over the next 5–10 years. Those therapies with proven benefit will be integrated into current treatment paradigms. Apart from targeted therapy, newer cytotoxic agents with low susceptibility to common resistance mechanisms will provide additional treatment options for patients with TNBC.

Triple-negative breast cancer is a heterogeneous subtype and consequently, a 'one-size-fits-all' treatment strategy is unlikely to be optimal. It is anticipated that predictive biomarkers identified in the coming years can be used to select patients with TNBC who are most likely to respond to a specific treatment. The development of targeted therapies and identification of predictive biomarkers are expected to have a substantial impact on TNBC. Such an impact would be analogous to the benefit of hormone therapy in hormone receptor-positive breast cancer and HER2-targeted therapies in HER2-positive disease. As a result, it is anticipated that outcomes for patients with TNBC will improve over the next 10 years.

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Executive summary

- Triple-negative breast cancer (TNBC) is an aggressive phenotype identified by immunohistochemical analysis. However, TNBC is actually a heterogeneous classification that overlaps with the basal-like subtype identified by gene expression analysis, as well as with *BRCA1*-related breast cancer.
- Cytotoxic chemotherapy is the mainstay of treatment for TNBC. Despite high chemosensitivity, TNBC is associated with poor patient outcomes underscoring the need for new treatments.
- Newer treatment approaches – both in terms of chemotherapy and targeted agents – are currently being evaluated in ongoing clinical trials. Initial results with several agents appear promising both in early-stage and metastatic disease.
- Predictive biomarkers need to be identified to select those patients with TNBC who are most likely to benefit from various treatments.

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