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Novel therapeutic strategies in the treatment of triple-negative breast cancer

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Abstract: Triple-negative breast cancer (TNBC) is a heterogeneous subtype of breast cancer that is defined by negative estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status. Treating patients with TNBC remains clinically challenging, as patients are not candidates for endocrine or HER2-directed therapy. As a result, chemotherapy with traditional agents such as anthracyclines and taxanes remains the only available option with moderate success. Recent discoveries have revealed that TNBC is a heterogeneous disease at the clinical, histological and molecular levels. The use of biomarkers to identify distinct subsets of TNBC that derive the greatest benefit from presently approved as well as novel therapeutics has become the main focus of current research. The aim of this review is to explore the clinical and biological complexity of TNBC as well as identify novel therapeutic options that target the various molecular subsets of TNBC.

Keywords: chemotherapy, clinical trials, immunotherapy, molecular subtypes, targeted therapies, triple-negative breast cancer

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Overview

Triple-negative breast cancer (TNBC) is a unique subset of breast cancer that is characterized by negative estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status. It accounts for approximately 15–20% of all breast cancer diagnoses,[1] and has been characterized by an aggressive natural history and poor survival compared with other breast cancers. Distant recurrences peak early at 3 years following diagnosis and a majority of deaths occur in the first 5 years after initial diagnosis.[2]

In the absence of new targeted therapies, conventional chemotherapy remains the mainstay of treatment with suboptimal outcomes. Recent discoveries have revealed that TNBC is a heterogeneous disease entity at the clinical, histological and molecular levels. Advanced technologies, such as next generation sequencing, have led to the identification of several molecular characteristics including the inactivation of the BRCA pathway, MAP/ERK kinase (MEK) and Phosphatidylinositol-3-Kinase (PI3K) pathway activation, high rates of Tumor Protein p53 (TP53) mutation, high activation of MYC, loss of retinoblastoma protein (RB1), enrichment for androgen receptor (AR) and the AR gene.[1,3,4] In addition, several potentially targetable amplifications or deletions, including immune checkpoints Programmed Death-1 (PD1) and Programmed death-ligand 1 (PDL1), have also been identified.[5] These molecular features have allowed the development of promising therapeutic agents such as DNA-damaging agents, AR inhibitors and immune checkpoint inhibitors.

The objective of this article is to review the ongoing effort to identify subsets of TNBC as well as explore the promising therapeutic strategies that target them.

The clinical heterogeneity of TNBC

At the clinical level, some patients do very well, while the majority of patients have very poor outcomes. Compared with other breast cancer subtypes, TNBC is highly aggressive with less

favorable outcomes in terms of likelihood of progression, available therapeutic options and overall survival (OS). It affects more often women under 40 years of age with higher incidence among African American and Hispanic women compared with other breast cancer subtypes.[6–9]

Neoadjuvant studies have suggested that women with TNBC are more sensitive to initial anthracyclines and taxanes compared with other breast cancer subtypes, with clinical response rates of up to 85% and pathologic complete response (pCR) rates of 30–40%.[10] Although patients who achieve a pCR have a better prognosis, the vast majority of patients with TNBC have residual disease after preoperative chemotherapy and usually present with rapid disease progression in the following 3–5 years.[11–15] To date there are no predictive factors that identify subsets of patients with TNBC who will ultimately achieve pCR.

Triple negative tumors present a distinctive pattern of relapse, with a higher risk of developing distant metastasis and death compared with other breast cancer subtypes.[16–19] These tumors have a predilection for visceral, lung and brain metastasis compared with luminal breast cancers that favor relapses in bone and skin.[20–22] Harrell and colleagues reported a higher incidence of brain and lung metastases in basal-like (BL) and claudin-low breast tumors (both commonly triple negative),[23] with approximately 50% of brain relapses occurring in patients with advanced TNBC.[24]

The histologic heterogeneity of TNBC

TNBC is diagnosed immunohistochemically as breast tumors that do not overexpress ER, PR or HER2. However, the cut off used to define estrogen and PR negativity has changed over time, resulting in a discordance in the definitions used in the literature (<10% *versus* <1%). The current definition established by the American College of Pathology, the American Society of Clinical Oncology and the St Gallen guidelines, recently used a cut off of less than 1% to define estrogen and progesterone negative tumor; whereas, HER2 negativity is defined as either immunohistochemistry (IHC) expression of 0–1+ or lack of gene amplification (Fluorescence In Situ Hybridization (FISH), 2.0).[25–27]

Consequently, endocrine therapy is currently prescribed for patients with ER expression of at least 1% in all stages of breast cancer. This has resulted in a subset of patients (ER expression 1–10%) who were previously considered ER negative but who under the current recommendations would receive endocrine therapy.

The large majority of TNBC tumors are invasive ductal carcinomas characterized by high histologic grade, poor differentiation, central necrosis, high lymphocytic infiltrate and high proliferation rates.[12,13] In addition, several other high-grade histologic subtypes of breast cancer including medullary carcinoma, metaplastic carcinoma, adenoid cystic carcinoma and apocrine/histiocytoid carcinoma present with the TNBC phenotype.[28–32]

Molecular heterogeneity of TNBC

Molecular profiling has confirmed the heterogeneous nature of TNBC that had already been observed from its clinical behavior. The Cancer Genome Atlas (TCGA) Research Network analyzed primary breast cancers using six platforms, including genomic DNA copy number arrays, messenger RNA arrays, exome sequencing, DNA methylation, microRNA sequencing, and reverse-phase protein arrays.[4] The most frequent genetic alterations were found in DNA damage-repair genes, including loss of TP53, RB1 and BRCA1 in addition to activation of the PI3K pathway.

It is important to understand the difference between TNBC and the BL phenotype because TNBC is frequently assimilated into the BL molecular phenotype, although these two breast cancer subtypes are not synonymous.

In reality, 75–80% of TNBCs display a BL molecular phenotype on gene expression arrays, and it is identified by a basal epithelial cell gene expression cluster, including high-molecular-weight basal cytokeratin 5/6 (CK5/6), CK14, CK17, epidermal growth factor receptor (EGFR), HER1, B crystallin, vimentin, laminin, integrin-b4, fascin, caveolin 1/2 (CAV1/2), c-Kit, and P-cadherin. Similarly, not all BL tumors are TNBC; and up to 54% of BL cancers do not present the immuno-histochemical phenotype of TNBC.[33,34]

Both BL breast cancer and TNBC show an important overlap with BRCA1-mutated tumors. The prevalence of BRCA1 or two mutations in TNBC is estimated to be between 10% and 20%,[35] and these mutations play a major role in DNA repair as tumor suppressor genes. This specific genomic instability in BRCA-1 carriers may provide specific therapeutic opportunities in

TNBC. Given the limited clinical usefulness of the BL molecular phenotype, the best strategy is to identify BL tumors using an immunohistochemistry panel of antibodies (ER, HER2, CK5/6 and EGFR HER1).[36,37]

Other molecular markers, that may be targetable, have also been identified by differential gene expression, including several amplifications and deletions.[33,38] Common amplifications include PIK3CA (49%), KRAS (32%), VEGFR (>30%), BRAF (30%), EGFR (23%) whereas less frequent ones include KIT, MET, FGFR1, FGFR2, PDGFRA and IGFR1.[4] Deletions were also observed in PTEN, INPP4B in addition to deletion of chromosome 5q13–14, which harbors the RASA1 gene and regulates the RAS oncogene.[39–43]

Distinct intrinsic subtypes of TNBC were identified using gene expression and sequencing tools. The study by Lehmann and colleagues analyzed 587 TNBCs by gene expression profiling and has identified six subtypes.[1] The authors identified two BL subtypes (BL1 and BL2), mesenchymal (M), mesenchymal stem-like (MSL), immunomodulatory (IM) and finally a luminal androgen receptor (LAR) with sensitivity to an AR antagonist. BL1 tumors are characterized with high expression of cell cycle and DNA damage response gene expression signatures and BL2 tumors are characterized by enrichment in growth factor signaling and myoepithelial markers. The two mesenchymal subtypes, M and MSL, are characterized by high expression of genes involved in differentiation and growth factor pathways with high sensitivity to dual PI3K/mTOR inhibition and Abl/Src inhibitor dasatinib.[44] This study fostered a major effort to discover and develop new drugs that target specific subtypes of TNBC.

Claudin-low is another less common subtype of TNBC that shows low expression of luminal differentiation markers, low expression of genes involved in tight cell junctions such as E-cadherin, intense immune infiltrate, high enrichment for epithelial-to-mesenchymal transition (EMT) markers and stem-cell-like features that may be enriched in BRCA pathway alterations.[45,46] Response to treatment of claudin-low subtype is intermediate between luminal and BL subtypes explained by the high rate of medullary and metaplastic differentiation.

Further classification using combination copy number transcriptome analysis highlights the heterogeneity of TNBC tumors.[47,48]

Therapeutic approaches to TNBC

In the absence of new approved targeted therapies, standard chemotherapy is still the mainstay of treatment. The heterogeneity of TNBC has made it difficult to treat unselected patients. As a result there is an ongoing effort to develop more specific targets, and several new targeted treatments and immunotherapeutic drugs are under development.

DNA-damaging chemotherapy and DNA repair targets

DNA repair mechanisms play a major role in maintaining the integrity and stability of the genome. BRCA1 and BRCA2 are tumor suppressor genes that are directly involved in homologous recombination-mediated repair of double-stranded breaks. Defects in BRCA1 or BRCA2 genes result in impaired DNA repair by homologous recombination and subsequent genomic instability.[49,50]

As a result, women with germline mutations in these genes are predisposed to hereditary cancer syndromes, including breast and ovarian cancers. Breast cancers arising in BRCA1 germline mutation carriers display a triple-negative phenotype in more than 75% of cases.[51,52] Understanding DNA repair mechanism defects has allowed the development of new therapeutic approaches in TNBC due to their higher sensitivity to DNA-damaging agents, including platinum salts and poly ADP-ribose polymerase (PARP) inhibitors.

However, sporadic breast cancers have also been associated with various genetic and epigenetic disruptions to BRCA function, leading to impaired homologous repair. These sporadic breast cancers share various phenotypic characteristics with familial BRCA cancers, a concept that has been termed 'BRCAness'.[53–56]

Optimal use of platinum salts in TNBC

Platinum salts are non-cell-cycle-specific agents that bind with DNA to form intrastrand crosslinks, thus affecting DNA replication and subsequently inducing apoptosis in cancer cells. The addition of platinum salts to the treatment of TNBC in the neoadjuvant and metastatic setting stems from its proclaimed role in TNBC associated with BRCA1 mutation.

Neoadjuvant setting

Studies involving the addition of platinum compounds in the neoadjuvant setting have reported conflicting results. In the GeparSixto trial the addition of weekly carboplatin to neoadjuvant paclitaxel, liposomal doxorubicin, and bevacizumab improved pCR rates in a subset of patients with TNBC from 36.9% to 53.2% ($p = 0.005$), but at the cost of higher toxicity-associated treatment discontinuation.[57] Recently, an improvement in survival was reported for patients receiving carboplatin in this trial, with a significant increase in 3-year disease-free survival (DFS) from 76.1% to 85.8% [hazard ratio (HR) 0.56; 95% confidence interval (CI) 0.33–0.96; $p = 0.035$).[57] It should be noted that the GeparSixto trial showed better outcomes in patients with TNBC with the addition of carboplatin, independently of germ-line BRCA status.

These results were supported by The Cancer and Leukemia Group B study (CALGB 40603), a two-by-two factorial randomized phase II trial of 454 patients with stage II and III TNBC evaluating weekly paclitaxel with or without carboplatin or bevacizumab followed by dose dense doxorubicin plus cyclophosphamide. The addition of carboplatin resulted in improved pCR rates both for the breast alone from 46% to 60% (odds ratio 1.76; $p = 0.0018$) and for the breast/axillae from 41% to 54% (odds ratio 1.71; $p = 0.0029$) but not in terms of DFS or OS.[58]

Patients with TNBC who do not achieve pCR after neoadjuvant chemotherapy have a significantly worse prognosis. Unfortunately not all trials evaluating the role of platinum compounds have demonstrated improvements in pCR.[59]

In conclusion, although the incorporation of carboplatin has typically been considered in patients with locally advanced disease, especially in the setting of BRCA-associated TNBC, its clinical use remains controversial because of significant treatment-related toxicity and unclear long-term benefits. As a result, current National Comprehensive Cancer Network (NCCN) guidelines do not recommend the use of platinum-based agents in the neoadjuvant or adjuvant setting outside of a clinical trial.[60] However, several ongoing trials may provide additional information on long-term outcomes as well as on their potential use in the neoadjuvant and adjuvant setting. A phase III trial (EA1131) randomizes patients presenting TNBC with residual disease after neoadjuvant chemotherapy to four cycles of platinum chemotherapy or observation [ClinicalTrials.gov identifier: NCT02445391]. Another phase III trial (NRG BR003) is evaluating adjuvant doxorubicin plus cyclophosphamide followed by weekly paclitaxel with or without carboplatin among patients with node-positive or high-risk TNBC [ClinicalTrials.gov identifier: NCT02488967].

Metastatic setting

Similarly platinum compounds were evaluated in the metastatic setting with conflicting results. A prospective phase II study evaluated cisplatin and carboplatin in patients with metastatic TNBC with an overall response rate (ORR) of 25.6%. However, this rate was significantly increased to 54.5% in patients with germ-line BRCA1/2 mutations. The study also showed that patients who presented elevated values of homologous recombination deficiency (HRD) assays that characterize BRCA-like genomic instability also had better response to platinum-based treatments, despite the absence of germ-line BRCA1/2 mutations.[61]

A phase II trial, comparing docetaxel and cisplatin *versus* docetaxel and capecitabine showed the superiority of docetaxel plus cisplatin in the first-line treatment of patients with metastatic TNBC in terms of both ORRs (63.0% *versus* 15.4%; $p = 0.001$) and progression-free survival (PFS) (10.9 *versus* 4.8 months; $p = 0.001$).[62] Another randomized, open-label, multicenter, phase III trial enrolling 240 patients with TNBC was designed to test the noninferiority of gemcitabine plus cisplatin to gemcitabine plus paclitaxel. Results showed that the cisplatin arm was noninferior to and superior to the comparator [PFS: HR 0.692; 95% CI 0.523–0.915; $p = 0.0001$ (noninferiority) and $p = 0.009$ (superiority)].[63]

In contrast, a large phase III trial 'the Triple Negative Breast Cancer Trial' (TNT) randomized 376 patients with metastatic TNBC to receive either carboplatin or docetaxel as first-line treatment with crossover in the case of progression. In unselected patients, the primary endpoint of objective response was not met in both situations: up front (31.4% *versus* 35.6%; $p = 0.44$) and following crossover (22.8% *versus* 25.6%; $p = 0.73$). Similar results were obtained for PFS (4.5 *versus* 3.1 months) and OS (12.3 *versus* 12.4 months) for the docetaxel and carboplatin arm, respectively.[64] However, BRCA-mutant carriers in the carboplatin arm showed a significantly higher response

compared with the docetaxel arm (68% *versus* 33.3%; 95% CI 6.3–63.1; $p = 0.03$). Moreover, the median PFS for patients with BRCA1/2 mutations in the carboplatin arm 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.[64]

The use of platinum appears to be an important therapeutic option in the metastatic setting, where treatments are mainly palliative due to the lack of specific standards for TNBC but even more so in patients who are BRCA positive.

PARP inhibitors. PARP enzymes play a major role in DNA repair mechanisms, specifically in homologous recombination-mediated repair of double-stranded breaks. Any reduction in their activity leads to persistent DNA lesions that subsequently induce apoptosis. As a result, inhibitors of PARP enzymes were developed to target vulnerable cancers with specific DNA-repair deficiency, including TNBC with BRCA1/2 mutations and TNBC with BRCAness phenotype.

Several trials tested the role of PARP inhibitors alone or in combination with chemotherapy in different settings (Table 1). Olaparib is an orally active PARP inhibitor that has an impressive response rate and favorable toxicity. A phase II study assessed the efficacy, safety, and tolerability of olaparib in women with BRCA1 or BRCA2 mutations and advanced breast cancer. Patients were assigned to two sequential cohorts. The first cohort ($n = 27$) was given continuous oral olaparib at the maximum tolerated dose (400 mg twice daily), and the second ($n = 27$) was given a lower dose (100 mg twice daily). Overall responses ranged from 22% (100 mg twice per day) to 41% (400 mg twice per day) with favorable toxicity.[65] Other ongoing phase III trials are evaluating the use of olaparib in the neoadjuvant [ClinicalTrials.gov identifier: NCT02032823] and metastatic setting [ClinicalTrials.gov identifier: NCT02000622] for patients with mutations in BRCA.

Another putative PARP inhibitor, iniparib, was evaluated in an open-label, phase II trial of 123 patients with metastatic TNBC who were randomly assigned to receive gemcitabine/carboplatin with or without iniparib.[66] Patients who received iniparib had significant improvement in the form of a higher clinical benefit rate (CBR) (55.7% *versus* 33.9%) and ORR (52.5% *versus* 32.3%) in addition to a survival benefit: PFS from 3.6 to 5.9 months (HR 0.59; $p = 0.012$) and OS from 7.7 to 12.3 months (HR 0.57; $p = 0.014$).[66] Based on these impressive results, a phase III study was conducted to evaluate iniparib using the same design.[67] Unfortunately, there was no statistical benefit in terms of PFS and OS. These poor results were explained in part due to its original misclassification as a PARP inhibitor, and the subsequent discovery that iniparib lacked PARP activity.[68,69]

In the neoadjuvant setting, a single-arm phase II trial has showed an important response rate, with iniparib especially in BRCA1/BRCA2 carriers.[70] Similarly, the addition of veliparib and carboplatin to standard neoadjuvant chemotherapy produced an improvement in pCR for patients with TNBC from 26% to 52%, but it is difficult to extract the benefit of veliparib from the benefit of carboplatin.[71]

The BROCADE trial is another randomized phase II study that evaluated the efficacy and tolerability of veliparib in combination with carboplatin and paclitaxel *versus* placebo in patients presenting with locally advanced or metastatic BRCA1/BRCA2-mutant breast cancer, with 42.4% of patients who had TNBC.[72] The ORR was 77.8% (95% CI 66.4–86.7) in the veliparib arm compared with 61.3% (95% CI 49.7–71.9) in the placebo group. The improvement in PFS in the veliparib arm (14.1 *versus* 12.3 months) was not statistically significant. The trend towards improved median survival observed with veliparib was also not statistically significant (28.3 *versus* 25.9 months; $p = 0.157$).[72] These results can be explained by the small number of patients and an ongoing phase III trial is more adequately powered to address this issue [ClinicalTrials.gov identifier: NCT02163694].

Talazoparib is a novel, dual-mechanism PARP inhibitor that potently inhibits the PARP enzyme and effectively traps PARP on DNA. The phase I trial [ClinicalTrials.gov identifier: NCT01286987] showed an important single-agent antitumor activity in BRCA-mutated breast cancer (ORR 33%).[73] A phase II trial is evaluating the activity of talazoparib in patients with BRCA1/2 wild-type breast cancer using an optimal Simon two-stage design. Patients will be assigned to one of two parallel cohorts. The first cohort ($n = 29$) includes patients with advanced TNBC with underlying homologous recombination defects as assessed by

Table 1. Selected phase II and III clinical trials of PARP inhibitors in TNBC.

Disease setting	Study, ClinicalTrials.gov identifier, NCT00494234	Phase	Treatment	Primary endpoint
Metastatic	AZD2281 in patients with known BRCA mutation status or recurrent high-grade ovarian cancer or patients with known BRCA mutation status/TNBC, NCT00679783	II, open label, single arm	Olaparib 400 mg	ORR, CR
	Efficacy and safety of KU-0059436 (olaparib) given orally twice daily in patients with advanced BRCA1- or BRCA2-associated BC	II, open label, single arm	Olaparib 100 mg twice daily, 400 mg twice daily	ORR, CR
	Efficacy and safety of olaparib given orally twice daily in patients with advanced cancers who have a confirmed genetic BRCA1 or BRCA2 mutation, NCT01078662	II, open label, single arm	Olaparib 400 mg twice daily	ORR, CR
	Efficacy and safety of talazoparib in patients with BRCA1 and BRCA2 wild type and (1) advanced TNBC and HRD and (2) advanced HER2-negative BC with either a germline or somatic mutation in HR pathway genes, NCT02401347	II, open label, single arm	Talazoparib	ORR
	Efficacy and safety of talazoparib *versus* physician's choice (capecitabine, eribulin, gemcitabine or vinorelbine) in patients with BRCA mutation and locally advanced or metastatic BC, NCT01945775	III, open label, randomized	Talazoparib	PFS
Neoadjuvant	Safety and efficacy of the addition of veliparib plus carboplatin *versus* the addition of carboplatin to standard neoadjuvant chemotherapy *versus* standard neoadjuvant chemotherapy in subjects with early stage TNBC, NCT02032277	III, randomized, double blind	Veliparib	pCR

BC, breast cancer; CR, complete response; HRD, homologous recombination deficiency; pCR, pathological complete response; PFS, progression-free survival; ORR, objective response rate; TNBC, triple-negative breast cancer.

the HRD assay and the second cohort ($n = 29$) includes patients with advanced HER2-negative breast cancer with a somatic or germline deleterious mutation in a non-BRCA1/2 HR pathway gene. Eligible patients will receive oral talazoparib (1.0 mg/day, 28-day cycles) until disease progression or unacceptable toxicity [ClinicalTrials.gov identifier: NCT02401347].[74]

An ongoing international phase III trial (EMBRACA) is comparing the safety and efficacy of talazoparib *versus* physician's choice (capecitabine, eribulin, gemcitabine or vinorelbine) in BRCA-mutation subjects with locally advanced or metastatic breast cancer. The primary objective is PFS and secondary objectives include objective response rate (ORR), OS, and safety [ClinicalTrials.gov identifier: NCT01945775] (Table 1).

Targeting growth factors. Inhibition of vascular endothelial growth factor (VEGF). VEGF is overexpressed in TNBC and has a critical role in proliferation, invasion and metastasis. This is supported by the high proliferative potential of TNBC and has led to the incorporation of antiangiogenic treatments but with limited activity in unselected patients.

In a meta-analysis of three phase III trials of bevacizumab given as first-line therapy in the metastatic setting (E2100, AVADO, RIBBON-1), a pooled analysis of 621 patients with TNBC demonstrated a significant benefit in terms of median PFS (8.1 *versus* 5.4 months), but not OS.[75] Similar findings were observed in second-line therapy in a subgroup analysis of the RIBBON-2 study, in which bevacizumab showed a benefit in PFS among the TNBC subgroup (6.0 *versus* 2.7 months for chemotherapy alone; $p = 0.0006$) but without statistically significant advantage in OS.[76] After mature survival results became available, bevacizumab lost its regulatory approval in metastatic breast cancer in the United States, but

continues to be used in other countries.[60,77] In the neoadjuvant setting, the GeparQuinto showed high rates of pCR when bevacizumab was added to anthracycline/taxane-based chemotherapy, but these findings were not confirmed in the NSABP B-40 trial.[78,79] Similar disappointments followed with adjuvant trials such as the phase III BEATRICE trial in which bevacizumab failed to show an advantage in OS.[80]

Regarding anti-VEGFR tyrosine kinase inhibitors such as sunitinib and sorafenib, they showed an activity in breast cancer in clinical studies with substantial TNBC populations. However, subsequent phase III trials were negative.

In a prospective and randomized phase III study, patients with advanced breast cancer were randomly assigned to receive docetaxel with or without sunitinib as a first-line treatment. Although ORR was significantly higher with the combination compared with monotherapy (55% *versus* 42%, $p = 0.001$), the PFS was not different ($p = 0.265$) and adverse events were also more common with the combination.[81]

A randomized phase III trial (SUN 1107) evaluated single-agent sunitinib *versus* single-agent capecitabine for the treatment of patients with advanced breast cancer after failure of standard treatment, with the primary endpoint of prolonging PFS. The data showed an inferior outcome for the sunitinib *versus* the capecitabine group. The median PFS was 2.8 *versus* 4.2 months and median OS was 15.3 *versus* 24.6 months. Based on these results, the study was thought to be futile and discontinued early.[82]

Sorafenib was also evaluated in several trials. A phase II trial, demonstrated that the combination of sorafenib and capecitabine improved PFS in patients with advanced HER2-negative breast cancer (median 6.4 *versus* 4.1 months; HR 0.58; 95% CI 0.41–0.81; $p = 0.001$).[83] These results led to a phase III confirmatory study (RESILIENCE trial) in which 537 women with locally advanced or metastatic HER2-negative breast cancer who had received no more than one prior regimen were enrolled.[84] The trial excluded women previously treated with a VEGF receptor inhibitor. Patients received capecitabine at 1000 mg/m^2 twice daily plus sorafenib or placebo 600 mg/day. The study did not meet its primary endpoint, with a median PFS of 5.5 months with the combination of capecitabine and sorafenib *versus* 5.4 months for capecitabine plus placebo (HR 0.973; $p = 0.406$). Median OS was not improved (18.9 *versus* 20.3 months; HR 1.195; $p = 0.930$).[84]

Inhibition of EGFR. Overexpression of EGFR is well established in TNBC and was reported in over 50% of cases. Although EGFR plays an important role in proliferation and migration, only limited activity was seen with monoclonal antibodies against EGFR.[85] These disappointing results suggested a lack of correlation between EGFR overexpression and the activity of EGFR inhibitors in TNBC.

Patients with TNBC treated with the EGFR inhibitor cetuximab in addition to cisplatin in the phase III trial (BALI-1) reported improved ORR of 20% compared with 10% for those who received cisplatin alone, but the difference was not statistically significant. PFS was also improved from 1.5 to 3.7 months but with non-negligible toxicity, mainly in the form of rash and neutropenia.[86]

Another EGFR inhibitor, panitumumab, was investigated in a single-arm phase II clinical trial including 14 patients with locally advanced and metastatic TNBC, evaluating the combination of weekly paclitaxel, carboplatin and panitumumab. The ORR of the 13 evaluable patients was 46%, while the median time to best response was 2.4 months and the median time to disease progression was 3.6 months.[87]

A more recent phase II trial evaluated panitumumab among 71 women with metastatic TNBC in addition to carboplatin and gemcitabine. The median PFS was 4.4 months (95% CI 3.2–5.5 months) with a median follow up of 11 months and the ORR was 42%. Reported toxicity was mainly in the form of rash (70%), fatigue (52%), neutropenia (45%) and thrombocytopenia (45%).[88]

Inhibition of FGFR. FGFR1 is amplified in TNBC in approximately 9% of tumors and FGRF2 in 4%.[89] Both of them have a critical role in differentiation, proliferation, resistance to apoptosis and metastasis. Their implication in the cancer process makes them an interesting target for development of new personalized treatments.[89] To date, there is no study evaluating FGFR inhibitors in TNBC but there is an ongoing phase II, randomized study of lucitanib in patients with FGF

aberrant metastatic breast cancer when patients with TNBC were eligible [ClinicalTrials.gov identifier: NCT02202746].

Inhibition of the PI3K/AKT/mTOR pathway. The PI3K/AKT signaling pathway is hyperactivated in approximately 10% of patients with TNBC, and various oncogenic alterations may occur in this pathway, including PIK3CA mutations, loss of the tumor suppressor phosphatases inositol polyphosphate 4-phosphatase type II (INPP4B) and loss of PTEN in addition to amplification of AKT and translocation of AKT3.[1,90]

A small phase II neoadjuvant study including 50 patients with TNBC evaluated the addition of everolimus to weekly paclitaxel followed by anthracycline-based chemotherapy. The everolimus arm was associated with an improved clinical response rate (48% *versus* 30%), but this was not statistically significant and no benefit was noted in terms of pCR rate.[91]

An ongoing randomized phase II trial is testing the efficacy of adding everolimus to weekly paclitaxel plus bevacizumab in the first-line treatment of patients with HER2-negative metastatic breast cancer [ClinicalTrials.gov identifier: NCT00915603], while other ongoing studies are investigating the use of everolimus in the treatment of advanced TNBC [ClinicalTrials.gov identifier: NCT01272141, NCT01111825 and NCT00827567]. The NCT01272141 trial is testing the combination of lapatinib and everolimus in locally advanced or metastatic TNBC.

Results are also awaited from trials evaluating the impact of another mTOR inhibitor temsirolimus in the neoadjuvant and metastatic settings.

The serine/threonine kinase AKT inhibitor ipatasertib is being investigated in a neoadjuvant phase II trial (FAIRLANE) evaluating the impact of adding ipatasertib to paclitaxel in patients with stage IA–IIIA TNBC [ClinicalTrials.gov identifier: NCT02301988]. Ipatasertinib is also under investigation in metastatic setting in a randomized phase II trial (LOTUS trial) in combination with paclitaxel as a first line of treatment in patients with locally advanced or metastatic breast cancer. PFS is the primary endpoint in all patients with TNBC and patients with TNBC with PTEN-low tumors [ClinicalTrials.gov identifier: NCT02162719].

PIK3CA mutation may also be a major driver for LAR TNBC cell lines. This TNBC subtype presents with a high frequency of PIK3CA-activating mutations and shows a high sensitivity to PI3K inhibitors with a synergistic effect of the AR inhibitor with a PI3K inhibitor.[92,93] Based on these results, targeting both the AR and PI3K pathways are promising strategies that are currently under investigation.

Similarly, PI3K has an important role in the stabilization of double-strand breaks by interacting with the homologous recombination complex and creating a BRCA1/2-deficient-like state. PI3K inhibition promotes HRD by downregulating BRCA1/2 and creating a BRCA-mutant-like tumor state. This down modulation of BRCA1/2 was accompanied by concomitant activation of the extracellular signal-regulated kinase (ERK) pathway following PI3K inhibition.[94] This rationale has led to the evaluation of combined use of DNA-damaging agents with PI3K inhibitors.[95] Some studies have shown that the combinations of PI3K inhibitors and cisplatin produce additive or synergistic effect. There is also an ongoing phase I trial testing the pan ongoing PI3K inhibitor BKM120 combined with olaparib in patients with metastatic TNBC [ClinicalTrials.gov identifier: NCT01623349].

AR blockade. Approximately 10% of TNBCs are classified as the LAR subtype. This subclass is characterized by the expression of luminal gene and the enrichment for AR and the AR gene.[1] As a result, the use of antiandrogen therapy to target this subtype is currently under investigation.[96] Several trials have investigated the role of antiandrogen agents in TNBC (Table 2). A phase II multicenter study has evaluated the efficacy of an oral nonsteroidal antiandrogen, bicalutamide, in patients with metastatic AR-positive TNBC.[97] A 6-month CBR of 19% (95% CI 7–39%) was reported for bicalutamide with a median PFS of 12 weeks (95% CI 11–22 weeks). The treatment was tolerable and side effects included hot flashes, fatigue, limb edema and transaminase elevations. A newer AR inhibitor, enzalutamide, has also shown activity in AR-positive TNBC with promising results from a multicenter phase II trial conducted in two stages. In stage 1, a CBR of at least 16 weeks, which was the primary endpoint of the study, was achieved in 42% of the 26 evaluable patients who received oral enzalutamide. For stage 2 of the study, 75 patients had AR expression of at

Table 2. Selected phase II clinical trials of antiandrogen agents in TNBC.

Disease setting	Study, ClinicalTrials.gov identifier	Phase	Drug	Primary endpoint
Metastatic	Bicalutamide for the treatment of patients with AR+, ER–, PR– metastatic BC, NCT00468715	II, open label, single arm	Bicalutamide 150 mg once daily	CBR at 6 months
	Bicalutamide as a treatment in patients with AR+ metastatic TNBC, NCT02348281	II, open label, single arm	Bicalutamide 150 mg once daily	CBR
	Bicalutamide in treating patients with TNBC, NCT02353988	II, open label, single arm	Bicalutamide 150 mg once daily	CBR
	Clinical activity and safety of enzalutamide in patients with advanced AR+ TNBC, stage 2, NCT01889238	II, open label, single arm	Enzalutamide 160 mg once daily	CBR at 16 weeks
	Clinical activity and safety of enzalutamide in patients with advanced AR+ TNBC, stage 1, NCT01889238	II, open label, single arm	Enzalutamide 160 mg once daily	CBR at 16 weeks
	Activity of abiraterone acetate plus prednisone in patients with molecular apocrine HER2– locally advanced or metastatic BC, NCT01842321	II, open label, single arm	Abiraterone acetate 160 mg once daily	CBR at 6 months
	Efficacy and safety of GTx-024 in patients with AR+ TNBC, NCT02368691	II, open label, single arm	GTx-024 18 mg once daily	CBR

AR, androgen receptor; BC, breast cancer; CBR, clinical benefit rate; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; TNBC, triple-negative breast cancer.

least 10% among the 165 patients screened, and the CBR of at least 16 weeks was estimated at 35% with a median PFS of 14.7 weeks.[98] These promising results have led to the development of the ongoing ENDEAR phase III trial. This study is evaluating the efficacy and safety of enzalutamide, as monotherapy or in combination with paclitaxel chemotherapy, in patients with locally advanced or metastatic TNBC [ClinicalTrials.gov identifier: NCT02677896].

Inhibition of C-kit. Several studies have investigated the expression of C-kit in breast cancers and have observed different percentages of expression of 11–31% of BL breast cancers.[1,99,100] This overexpression of C-kit implied that these patients might potentially benefit from tyrosine kinase inhibitors (TKIs). However, there is no correlation between the overexpression of C-kit in TNBC and the activating mutations in C-kit and PDGFRA gene. It explains why only a few patients achieve a response to imatinib.[101,102]

A Chinese study that included 171 patients with TNBC has shown 42.1% of C-kit overexpression but only one activating mutation was detected.[103] Further investigations among larger and more heterogeneous populations are required to select patients who can benefit from targeting c-kit in TNBC. Dasatinib is another small molecule that inhibits not only the src and abl kinases but also the C-kit, and could be a potential molecular targeted drug for C-kit-positive TNBC.[104]

Inhibition of the JAK2/STAT3 pathway. Janus kinases (JAKs) are tyrosine kinases that activate STAT3 proteins, which are involved in regulation of cell growth, survival, angiogenesis and immunosuppression. The JAK-STAT signaling pathway is frequently deregulated in several cancers, including BL breast cancer, and subsequently may be an effective clinical strategy to treat TNBC.[105]

TCGA reports have shown a high rate of JAK2 amplifications among women with TNBC who received preoperative chemotherapy compared with women with primary untreated BL breast cancers.[4] Consequently, inhibition of the JAK pathway could be promising in the subgroup of patients with JAK2-amplified residual disease. An ongoing phase II study is evaluating the combination of ruxolitinib with preoperative chemotherapy for triple-negative inflammatory breast cancer

[Clinical Trials.gov identifier: NCT02041429]. The trial is evaluating the expression of pSTAT3 and expects a decrease in its expression after treatment. Ruxolitinib is also being evaluated in a phase II trial comparing the OS of women with advanced or metastatic HER2-negative breast cancer who receive treatment with capecitabine in combination with ruxolitinib *versus* those who receive treatment with capecitabine alone [Clinical Trials.gov identifier: NCT02120417].

Inhibition of notch signaling. Studies have shown the aberrant activation of notch signaling in breast cancer and its involvement in proliferation, apoptosis and cancer stem cell activity. The high expression of Notch signaling pathway components including Jagged1-2, Dll1, Dll3 and Dll4, Notch receptors, and Hes and Hey target genes have been demonstrated in breast cancer.[106] The activation of notch receptor *via* the interaction between the membrane-bound ligand and notch receptor leads to a conformational change within the negative regulatory region. This results in sequential cleavage by the ADAM17/TACE metalloprotease and γ secretase, which releases the notch intracellular domain (NICD). Finally NICD translocates to the nucleus and then activates the transcriptional process.[107]

Based on these findings, the inhibition of Notch signaling may be a promising therapeutic option for patients with TNBC. Preclinical data demonstrated that TNBC xenograft models with NOTCH1 rearrangements, retaining the γ-secretase cleavage site, were associated with high levels of activated NOTCH1 and conferred sensitivity to γ-secretase inhibitors.

A phase II study is evaluating the efficacy and safety of an oral γ-secretase inhibitor PF-03084014 as a single treatment in patients with advanced TNBC, harboring genomic alterations in Notch receptors (NA+), and in a smaller subset of patients with advanced TNBC whose tumor tests negative for genomic alterations in Notch receptors (NA–). The primary endpoint is the ORR [ClinicalTrials.gov identifier: NCT02299635]. The same γ-secretase inhibitor is under investigation in the neoadjuvant setting among patients with residual disease after anthracycline and taxane based chemotherapy [ClinicalTrials.gov identifier: NCT02338531].

Targeting Trop-2. Trop-2 is a cell-surface glycoprotein present in limited amounts in normal human tissues but widely expressed in several epithelial carcinomas.[108] It has a crucial role in the regulation of cell–cell adhesion and has been associated with increased tumor aggressiveness and poor prognosis in breast cancer.[109] It is reported to be expressed in over 80% of cases of TNBC.

IMMU-132 (isactuzumab govitecan) is an antibody–drug conjugate developed by linking approximately eight molecules of an active metabolite of irinotecan SN-38 to an antibody that binds to Trop-2.[110] A multicenter phase II trial has evaluated isactuzumab govitecan in 83 patients with metastatic TNBC whose disease has failed to respond to at least two prior therapies.[111] In April 2016, the preliminary analysis showed a median PFS of 5.6 months and a median OS of 14.3 months, with 60% of patients still alive. The ORR was 31.5% including two complete responses. Isactuzumab govitecan was given at the dose of 10 mg/kg intravenously on days 1 and 8 of a 21-day cycle and was well tolerated with the most common severe adverse effects being diarrhea and low blood counts, but there was no treatment interruption because of toxicity.[111] In consideration of these results, isactuzumab govitecan was recently granted 'breakthrough' status by the US Food and Drugs Administration.

Other molecular targets. Several molecular alterations in metastatic TNBC are currently under investigation as potential therapeutic targets. Sensitivity to MEK inhibition has been demonstrated *in vitro* for cell lines derived from TNBC or BL breast cancers, and TNBC cell lines which are sensitive to MEK inhibitors harbor mutations that affect the Ras/MAPK pathway.[112,113] The activation of MEK can support the stabilization of c-Myc, and therefore MEK inhibition can induce c-Myc degradation in TNBC but it has been demonstrated that this inhibition can simultaneously induce the activation of receptor tyrosine kinases that can cause resistance to therapy.[114] These findings suggest that combining MEK inhibitors with agents targeting receptor tyrosine kinases could be a promising strategy.

HDAC inhibitors are under investigation either as single agents [ClinicalTrials.gov identifier: NCT02623751] or in combination with cisplatin in patients with metastatic TNBC [ClinicalTrials.gov identifier: NCT02393794]. Additional molecular targets of interest include c-Met [ClinicalTrials.gov identifier: NCT01738438][115]

and hypoxia-inducible factor 1 α (HIF1-α) pathway.[116]

Role of immunotherapy

Overview of immune gene signature and prognostic implications. Breast cancer was not considered to be an immunogenic cancer, but over recent years, studies have demonstrated the prognostic value and the importance of infiltrating lymphocytes (TILs) in tumors in controlling the clinical progression of numerous cancers, including breast cancer.[117,118]

Breast cancer subtypes have different degrees of immune infiltration and studies have shown that TNBC and Her2 positive breast cancers are most frequently associated with TILs compared with hormone receptor positive cancer. Subsequently the use of immunotherapy among these patients, especially those with TNBC who express high levels of TILs, could lead to better tumor responses.[119,120]

Recent studies have revealed that a higher level of TILs (>50%) was associated with worse clinicopathologic features, such as higher grade, higher expression of the proliferation marker Ki-67, and positivity of lymph nodes, but paradoxically, it was associated with better pCR in the neoadjuvant setting in addition to improved PFS and OS in the metastatic setting.[121–124]

However, research on gene expression profiling has also revealed that TNBC had higher rates of CD8+ T-cell infiltration, which was predictive of good prognosis.[125] Similarly, intratumoral B cells were found to be associated with favorable outcomes in breast cancer. In contrast, CD4+ T cells, including tumor-associated macrophages and T-regulatory cells, were associated with worse prognosis.

The gene expression analysis of Lehman and colleagues has identified six TNBC subtypes, among them the IM subtype which is composed of immune activated and associated signaling components contributed from both the tumor and the infiltrating lymphocytes. This subtype was associated with better outcome (relapse-free survival) in comparison with other TNBC subtypes.[1] The same analysis revealed that the IM subtype presented higher expression of PDL1, PD1 and CTLA4 that may be attractive targets for checkpoint inhibitors which increase the antitumor immune response by blocking immune-regulating proteins that downregulate the immune system.

CTLA4 plays a crucial role in normal immunologic homeostasis by regulating immune responses early in T-cell activation. Then its inhibition by ipilimumab does not allow the T cell to interact with the receptor *via* CD28 on its cell surface. CTLA4 enhances the antitumor activity of CD8+ T cells, increases the ratio of CD8+ T cells to Foxp3+ T regulatory cells, and inhibits the suppressive function of T-regulatory cells.[126]

PD1 negatively regulates T-cell activity by blocking T cells and modulating immune responses at different phases. Research evaluating the expression of PD1 in different breast cancer subtypes has found that PD1 was more frequently expressed in TNBC compared with the other subtypes. This immune checkpoint receptor is expressed not only on activated T cells, but also on other lymphocytes, including B cells and natural killer cells, that are all active in the cancer process.[127,128] PD1 interacts with two ligands, PDL1 and PDL2, and the interaction between PD1 and PDL1 acts to suppress antitumor immunity by exerting a negative regulation on T cells, cytolytic activity and production of cytokine.[129] PDL1 is expressed in approximately 20–30% of TNBC cases and was found to be associated with TILs, in addition to being correlated with worse clinicopathologic features, such as greater tumor size, higher grade and higher rate of proliferation. These findings suggest that targeting PD1 and PDL1 is a new promising approach in the treatment of TNBC.

Clinical trials of immunotherapy in TNBC. Several trials are ongoing to evaluate the role of immune-checkpoint inhibitors alone or in combination, and of other immunotherapies in TNBC (Table 3).

PD1 monoclonal antibody pembrolizumab was recently evaluated in a single-arm phase IB study that enrolled 32 patients with recurrent or metastatic TNBC. All patients expressed PDL1 and 47% of them had received more than three lines of treatment and 21.9% had received five or more treatments.[130] Pembrolizumab was administrated intravenously at the dose of 10 mg/kg every 2 weeks and was well tolerated, with mainly low-grade joint and muscle pain, fatigue and nausea. Among the 27 patients with measurable disease, one participant (3.7%) had a complete response,

Table 3. Selected phase II or phase III clinical trials of immunotherapy in TNBC.

	Study, ClinicalTrials.gov identifier	Phase	Drug	Primary endpoint
Metastatic	Single-agent pembrolizumab (MK-3475) *versus* single-agent chemotherapy as per physician's choice for metastatic TNBC, NCT02555657	Phase III, randomized, open label	Pembrolizumab	PFS, OS
	Pembrolizumab (MK-3475) as monotherapy for metastatic TNBC, NCT02447003	Phase II, single arm, open label	Pembrolizumab	ORR, safety
	Nivolumab after induction treatment (four arms: radiotherapy, doxorubicin, cisplatin, cyclophosphamide) or noninduction treatment) in patients with TNBC: TONiC trial, NCT02499367	II, open label, single arm	Nivolumab	PFS
	Atezolizumab (MPDL3280A) (anti-PDL1 antibody) in combination with Nab-paclitaxel compared with placebo with Nab-paclitaxel for patients with previously untreated metastatic TNBC, NCT02425891	Phase III, randomized, open label	Atezolizumab	PFS
Neoadjuvant	Triple-negative first-line study: neoadjuvant trial of Nab-paclitaxel and atezolizumab in patients with TNBC, NCT02530489	II Open-label single-arm	Atezolizumab (anti PD1)	pCR
	Neoadjuvant study with atezolizumab in patients with locally advanced TNBC undergoing treatment with Nab-paclitaxel and carboplatin, NCT02620280	Phase III, randomized, open label	Atezolizumab (anti PD1)	EFS
Adjuvant	Vaccine (DC-CIK)/EC followed by docetaxel, NCT02539017	Phase II	Vaccine (DC-CIK)	DFS, OS

DFS, disease-free survival; EFS, event-free survival; OS, overall survival; ORR, objective response rate; OS, overall survival ; pCR, pathologic complete response; PFS, progression-free survival; TNBC, triple-negative breast cancer.

four participants (14.8%) had a partial response, and 25.9% had stable disease. The median time to response was 18 weeks and the median duration of response had not been reached with a median PFS just under 2 months.[130]

Others studies are currently evaluating pembrolizumab in metastatic and neoadjuvant settings. A phase III trial is testing pembrolizumab *versus* single-agent chemotherapy as per the physician's choice for metastatic TNBC and the primary outcomes are PFS and OS [ClinicalTrials.gov identifier: NCT02555657]. Another PD1 antibody nivolumab is under evaluation in a phase II trial after induction treatment in patients with TNBC (TONIC trial) [ClinicalTrials.gov identifier: NCT02499367].

Inhibition of PDL1 with atezolizumab was tested in a phase I trial in patients with metastatic TNBC. Grade 3–4 toxicities were observed in 8% of patients and immune-related adverse events occurred in a minority of patients. The study reported an ORR of 33% in the nine evaluable patients with one complete response and two partial responses. All responses were seen within the first 6 weeks of treatment.[131]

A neoadjuvant phase III study with atezolizumab is currently ongoing in patients with locally advanced TNBC undergoing treatment with nab-paclitaxel and carboplatin. The primary endpoint is event-free survival [ClinicalTrials.gov identifier: NCT02620280]. Another phase III randomized trial is evaluating atezolizumab with nab-paclitaxel for first-line treatment of patients with locally advanced or metastatic TNBC [ClinicalTrials.gov identifier: NCT02425891].

Another anti-PDL1 agent avelumab was recently evaluated and the study revealed attractive results in the TNBC subgroup. Avelumab produced an improvement in clinical response among patients with PDL1 expression on immune cells estimated at 44.4% *versus* 2.6% in the absence of expression.[132] Regarding inhibition of CTLA4, a phase I study is currently evaluating tremelimumab, which is an anti-CTLA4 agent, in patients with advanced solid tumors including breast cancer [ClinicalTrials.gov identifier: NCT02527434].

Conclusion

TNBC is a heterogeneous disease characterized by a variety of molecular subtypes, various biologic pathways, with distinct sensitivities to chemotherapy and different clinical outcomes. Standard chemotherapy remains the mainstay of treatment in TNBC, but new targeted therapies and immunotherapeutic agents have shown promising results. The future challenge is to further identify specific targets within subsets of patients diagnosed with TNBC tumors, with the aim of improving the outcome of this aggressive disease.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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