Druggable Molecular Targets for the Treatment of Triple Negative Breast Cancer

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ABSTRACT

Breast cancer (BC) is still the most common cancer among women worldwide. Amongst the subtypes of BC, triple negative breast cancer (TNBC) is characterized by deficient expression of estrogen, progesterone, and human epidermal growth factor receptor 2 receptors. These patients are therefore not given the option of targeted therapy and have worse prognosis as a result. Consequently, much research has been devoted to identifying specific molecular targets that can be utilized for targeted cancer therapy, thereby limiting the progression and metastasis of this invasive tumor, and improving patient outcomes. In this review, we have focused on the molecular targets in TNBC, categorizing these into targets within the immune system such as immune checkpoint modulators, intra-nuclear targets, intracellular targets, and cell surface targets. The aim of this review is to introduce and summarize the known targets and drugs under investigation in phase II or III clinical trials, while introducing additional possible targets for future drug development. This review brings a tangible benefit to cancer researchers who seek a comprehensive comparison of TNBC treatment options.

Keywords: Clinical trial; Drug therapy; Triple negative breast neoplasms

INTRODUCTION

Triple negative breast cancer (TNBC) is one subtype of breast cancer (BC) which is defined as a group of BCs lacking the expression of 3 major receptors involved in BC; estrogen receptor (ER), progesterone receptor (PR), and overexpression of human epidermal growth factor receptor 2 (HER2). The overall incidence of TNBC is approximately 15%–20%, with higher rates seen in young women and African-Americans [1]. Compared to other types of BC, patients with TNBC will experience poorer overall survival (OS), and a higher probability of cancer recurrence.

TNBC is a heterogeneous disease which is subcategorized into various subtypes. Lehmann et al. [2] described a classification for TNBC subtypes, based on the microarray data on 587 TNBC cases. He introduced 2 basal-like (BL) subtypes. BL1 subtype showed high expression of cell cycle and DNA damage response genes and BL2 subtype showed high expression of genes involved in growth factor signaling, nerve growth factor, hepatocyte...
growth factor receptor, insulin like growth factor 1 receptor pathways, glycolysis and gluconeogenesis, and myoepithelial markers (TP63 and MME). The immunomodulatory subtype was enriched for immune cell processes and immune signaling. The mesenchymal stem-like subtype was characterized by a high expression of genes involved in motility, extracellular matrix, epithelial-to-mesenchymal transition (EMT), and cell differentiation pathways with a lower expression of genes involved in proliferation. The luminal androgen receptor (LAR) subtype is associated with a higher expression of genes responsible for steroid synthesis and androgen/estrogen metabolism. In 2015, Burstein et al. performed RNA and DNA profiling analysis on 198 TNBC tumors and defined a category consisting of 4 TNBC subtypes; BL immunosuppressed, BL immune-activated, LAR and mesenchymal. Regardless of the disease subtype and since TNBC does not express/overexpress ER, PR or HER2, systemic chemotherapy, rather than targeted therapy, has long been the major treatment option for these patients. Complications associated with single or combination chemotherapy regimens, and limitations in efficacy, has made oncologists and cancer researchers more interested in developing and administering targeted therapies to these patients. In recent years, along with the high efficacy of cancer immunotherapy in metastatic and advanced tumors such as lung and melanoma, some researchers have focused their efforts on the classification of TNBC based on immune markers. For example, Jézéquel et al. performed microarray profiling on 107 TNBC patients and described 3 TNBC subtypes: BL with low immune response and high M2-like macrophages, basal-enriched with high immune response and low M2-like macrophages, and LAR. These subtypes have incidence rates of 45%, 33%, and 22%, respectively. Highlighting the importance of immunotherapy today, drugs developed in this area have been included in clinical trials. This review paper highlights the molecular targets in TNBC, with an emphasis on well-known targets with available drugs, therapies which are mostly in phase II and III clinical trials, and discussing plausible targets for future drug development. The authors hope to give a thorough update on the basic and clinical outcomes and on the current status of clinical drug testing.

**IMMUNE CHECKPOINT MODULATORS**

**Programmed cell death protein 1 (PD-1) and its ligand**

PD-1 is a surface receptor expressed by T cells, and when engaged with its ligand has an immunosuppressive role. Therefore, PD-1 inhibitors inhibit the activation of this receptor, thereby improving immune function and allowing cancer cells to come under attack. PD-1 has 2 ligands, PD-L1 and PD-L2 (Figure 1). Reports have suggested that 40.9% of TNBC patients express PD-L1 [5]. PD-L1 is associated with increased tumor infiltrating lymphocytes (TILs) [6]. This suggests that anti-PD-1 or anti-PD-L1 agents are efficacious in these patients. Registered PD-1 and PD-L1 clinical trials are listed in Table 1. Anti-PD-1 drugs include pembrolizumab, nivolumab, toripalimab, spartalizumab, camrelizumab, FAZ053, and PF-06936308. Most studies of these drugs are in phase I clinical trials. Currently, 48 and 19 clinical trials are registered to use pembrolizumab and nivolumab, respectively, as a potential intervention in TNBC patients. Pembrolizumab is widely used in other cancers with a favorable safety profile. Pembrolizumab, as a monotherapy, was studied in a phase II clinical trial, in 170 metastatic TNBC (mTNBC) patients, 61.8% of which were PD-L1-positive. The objective response rate (ORR) in the whole population and in the PD-L1+ population in this study was 5.3% (95% confidence interval [CI], 2.7–9.9) and 5.7% (95% CI, 2.4–12.2). Likewise, the disease control rate in these 2 groups was 7.6% (95% CI, 4.4–12.7) and 9.5% (95% CI, 5.1–16.8), respectively. The median progression-free survival (PFS) and median OS.
in this study was 2 months (95% CI, 1.9–2.0) and 9 months (95% CI, 7.6–11.2), respectively. While the ORR was low, for patients who responded to treatment, disease control was durable [7].

Anti-PD-L1 drugs include atezolizumab, durvalumab, avelumab, and M7824. Atezolizumab is a promising anti-PD-L1 agent, especially in combination with taxane in the treatment of PD-L1 positive mTNBC [5]. Nabpaclitaxel in combination with atezolizumab or placebo in 902 mTNBC patients caused a statistically significant increase in the median PFS in the group receiving atezolizumab (7.2 vs. 5.5 months). In PD-L1+ tumors, the median OS of those receiving atezolizumab and nabpaclitaxel was 25 months, compared with 15.5 months for nabpaclitaxel alone. The results of these studies suggest that anti-PD-1 and anti-PD-L1 drugs may be promising therapies for these patients.

**Cytotoxic T lymphocyte-associated protein 4 (CTLA-4)**

CTLA-4 is another immune checkpoint protein which is expressed on activated T cells (Figure 1). CTLA-4 competes with its homologous molecule, CD28 for binding to CD80 and CD86 on antigen presenting cells. CLTA-4 has a higher affinity for CD80 and CD86, and unlike CD28, CTLA-4 transmits inhibitory signals to the T cell. CTLA-4 is also expressed on regulatory T cells (Tregs) mediating immunosuppressive responses. Therefore, inhibition of CTLA-4 is thought to induce proliferation of T cells leading to a boost of immune responses in the body [8]. Ipilimumab is an anti-CTLA-4 monoclonal antibody and a checkpoint blocker, currently undergoing clinical trial testing in combination with nivolumab (NCT03546686, NCT01928394) or nivolumab and INCAGN01876 (anti-human glucocorticoid-induced tumor necrosis factor [TNF] receptor) (NCT03126110, NCT03241173). Likewise, tremelimumab, a fully human anti-CTLA-4 monoclonal antibody, is under clinical investigations in combination with PF-06936308 (NCT03674827), nabpaclitaxel and carboplatin (NCT02658214), and durvalumab (NCT02527434).
### Table 1. Inhibitors of PD-1 and PD-L1 in phase II and III clinical trials

| Inhibitor      | Other treatments in the study                                      | Status | Identifier          |
|----------------|--------------------------------------------------------------------|--------|---------------------|
| Pembrolizumab* | Bemcentinib                                                       | Phase II, R | NCT03184558        |
|                | None                                                               | Phase II, R | NCT02416656        |
|                | Anastrozole, doxorubicin, exemestane, letrozole                     | Phase II, R | NCT02648477        |
|                | Valacyclovir, ADV/HSV-tk, radiation                                 | Phase II, R | NCT03004183        |
|                | Tavokinogene telseplasmid                                           | Phase II, R | NCT03567720        |
|                | Nab-paclitaxel, epirubicin, cyclophosphamide                        | Phase II, R | NCT03289819        |
|                | Carboplatin, gemcitabine                                           | Phase II, R | NCT02755272        |
|                | None                                                               | Phase II, R | NCT03145961        |
|                | Doxorubicin, cyclophosphamide, paclitaxel, carboplatin, decitabine  | Phase II, R | NCT02957968        |
|                | Carboplatin, docetaxel, pegfilgrastim                               | Phase II, R | NCT03639948        |
|                | Enobosarm                                                          | Phase II, R | NCT02977161        |
|                | Carboplatin, nab-paclitaxel                                        | Phase II, R | NCT03121352        |
|                | Radiation therapy                                                  | Phase III, R | NCT02954474        |
|                | None                                                               | Phase II, ANR | NCT02447003      |
|                | Capecitabine                                                       | Phase II, ANR | NCT03044073        |
|                | Radiotherapy                                                       | Phase II, ANR | NCT02730100        |
|                | Imprime PGG                                                        | Phase II, ANR | NCT02981303        |
|                | Cyclophosphamide                                                   | Phase II, ANR | NCT02768701        |
|                | Capecitabine, eribulin, gemcitabine, vinorelbine                    | Phase III, ANR | NCT02555657        |
|                | Nab-paclitaxel, paclitaxel, gemcitabine, carboplatin, normal saline solution | Phase III, ANR | NCT02819518        |
|                | Carboplatin, paclitaxel, doxorubicin, epirubicin, cyclophosphamide, placebo, pegfilgrastim | Phase III, ANR | NCT03036488        |
|                | Cisplatin                                                          | Phase II, NR   | NCT03644489        |
|                | Lenvatinib                                                         | Phase II, NR   | NCT03797526        |
|                | Carboplatin                                                        | Phase II, S (amendment) | NCT03213041  |
| Nivolumab*     | Cabozantinib                                                      | Phase II, R | NCT03316586        |
|                | Radiation therapy, low dose doxorubicin, cyclophosphamide, cisplatin | Phase II, R | NCT02499367        |
| Ipilimumab     | Capicitabine                                                       | Phase II, R | NCT03546686        |
|                | Carboplatin                                                        | Phase II, R | NCT03487666        |
|                | Ipilimumab, capecitabine                                           | Phase II, R | NCT03414684        |
|                | Doxorubicin                                                        | Phase II, NR   | NCT03818685        |
| Toripalimab*   | Nab-paclitaxel, placebo                                           | Phase II, NR   | NCT03775779        |
| Camrelizumab*  | Apatinib                                                           | Phase II, R | NCT03394287        |
| Atezolizumab†  | None                                                               | Phase II, R | NCT02478099        |
|                | Stereotactic radiosurgery                                          | Phase II, R | NCT03483012        |
|                | Paclitaxel, carbo/cyclo                                             | Phase II, R | NCT03189817        |
|                | Peglated liposomal doxorubicin, cyclophosphamide, placebo          | Phase II, R | NCT03164993        |
|                | Placebo, paclitaxel                                                | Phase III, R | NCT03125902        |
|                | Placebo                                                            | Phase III, R | NCT03281954        |
|                | Paclitaxel, dose-dense doxorubicin or dose-dense epirubicin, cyclophosphamide | Phase III, R | NCT03498176        |
|                | Nab-paclitaxel, placebo                                           | Phase III, ANR | NCT02425891        |
|                | Placebo, nab-paclitaxel, doxorubicin, cyclophosphamide, fegilgrastim | Phase II, NR | NCT03740893        |
|                | AZD6738, olaparib                                                  | Phase II, NR   | NCT03756298        |
|                | Capecitabine                                                       | Phase II, S   | NCT02883082        |
| Durvalumab†    | Olaparib                                                           | Phase II, R | NCT03167619        |
|                | Olaparib                                                           | Phase II, R | NCT03801369        |
|                | Placebo, nab-paclitaxel, epirubicin, cyclophosphamide              | Phase II, ANR | NCT02685059        |
|                | Tremelimumab                                                       | Phase II, S   | NCT02572434        |
|                | Carboplatin, gemcitabine, nab-paclitaxel, personalized synthetic long peptide vaccine, poly ICLC | Phase II, NR | NCT03606967        |

Pembrolizumab, nivolumab, toripalimab and camrelizumab are anti-PD-1 drugs. Atezolizumab and durvalumab are anti-PD-L1 drugs. PD-1 = programmed cell death protein 1; PD-L1 = programmed death-ligand 1; ADV/HSV-tk = adenoviral vector-mediated herpes simplex virus tyrosine kinase; R = recruiting; ANR = active, not recruiting; NR = not recruiting; S = suspended.  
*Anti-PD-1; †Anti-PD-L1.
Lymphocyte-activation gene 3 (LAG-3)
LAG-3 (Figure 1) is another immune checkpoint receptor which, like CTLA-4 and PD-1, is a negative regulator of the activation and proliferation of T cells and a suppressor of Tregs. About 15% of TNBC patients are reported to co-express PD-1 and LAG-3, associated with the presence of TILs [9]. Therefore, inhibition of both LAG-3 and PD-1 may have synergistic antitumor immune effects. Currently, 3 clinical trials investigating anti-LAG-3 antibodies in TNBC patients, usually in combination with an anti-PD-1 drug, are ongoing. These include LAG525 (IMP701), an anti-LAG-3 antibody, in combination with spartalizumab in mTNBC patients (NCT03742349); and TSR-033, another anti-LAG-3 monoclonal antibody, alone or in combination with anti-PD-1 antibody in various solid tumors including TNBC (NCT03250832). The third anti-LAG-3 antibody (INCAGN02385) is in phase I clinical trial in patients with advanced malignancies, including TNBC (NCT03538028).

T cell immunoglobulin and mucin-domain containing-3 (TIM-3)
TIM-3 (Figure 1) is another cell surface receptor and immune checkpoint, which together with PD-1 and LAG-3 exhausts CD8+ T cells. TIM-3 is highly expressed in TILs and tumor antigen-specific T cells, playing a role in tumor immunity [10]. Currently, INCAGN02390, an anti-TIM-3 antibody, is in phase I clinical trials in some advanced malignancies, including TNBC (NCT03652077).

Hedgehog (Hh) and neuropilin-2 (NRP-2) signaling pathway
The Hh signaling pathway is involved in the development and differentiation of embryos, angiogenesis and regulating cell fate. Importantly, this signaling pathway is a regulator of immune system, plays a role in TNBC progression [11] and contributes to cancer cell stemness in TNBC [12]. In addition, NRP-2, a VEGF receptor, is expressed in tumour-initiating cells involved in the initiation and genesis of TNBCs. In fact, activation of NRP-2 causes expression of GLI1 which induces another key stem cell factor, BMI-1. BMI-1, in an autocrine loop, enhances the expression of NRP-2. All of these steps could potentially be targeted to delay tumour initiation. In vitro studies report the successful use of Hh signaling inhibitors, such as Cyclopamine and Gant61. Vismodegib (NCT02694224) and sonidegib (NCT02027376) are 2 Hh signaling inhibitors which have been approved for use in basal-cell carcinoma, and for clinical trials in TNBC patients by the United States Food and Drug Administration (FDA).

TARGETS WITHIN THE NUCLEUS
Breast cancer susceptibility gene (BRCA) and platinum-based treatment
BRCA1 and BRCA2 are genes which are responsible for repairing double stranded DNA breaks. Mutations in these 2 genes causes DNA instability, making the cell more susceptible to DNA interacting agents, such as platinum salts. BRCA mutation is associated with inherited BC. More than 80% of the tumors with BRCA1 mutations have TNBC characteristics, and are more aggressive and have higher tumor grade [1]. Platinum-based drugs are increasingly being utilized in the adjuvant and metastatic setting as well as other standard chemotherapeutics (including microtubule inhibitors, anthracyclines and antimetabolites). The results of a phase II clinical trial suggest that platinum monotherapy is especially effective in patients with BRCA1/2 mutations [13]. Cisplatin in combination with gemcitabine has a favorable safety profile [14] and could be superior to paclitaxel plus gemcitabine, based on a phase III multicenter trial. A recent phase III clinical trial...
comparing carboplatin with docetaxel in 376 TNBC patients showed that the ORR between the 2 groups was not significantly different (31.4 vs. 34.0 months; \( p = 0.66 \)). However, in \( BRCA \) mutated patients, ORR with carboplatin (68%) was twice that of the docetaxel group (33%) \( (p = 0.01) \) [15]. A randomized phase II clinical trial studied the effects of adding carboplatin and/or bevacizumab to the chemotherapy regimen (paclitaxel, doxorubicin and cyclophosphamide) of 433 stage II and III TNBC patients. The addition of carboplatin resulted in blood toxicities including neutropenia and thrombocytopenia. However, pathologic complete responses (pCRs) in breast (60% vs. 46%; \( p = 0.0018 \)) and breast/axilla (54% vs. 41%; \( p = 0.0029 \)) were significantly increased with carboplatin, while bevacizumab only increased breast pCR (59% vs. 48%; \( p = 0.0089 \)) [16].

**Poly-ADP ribose-polymerases (PARP)**

PARP is responsible for repairing single stranded DNA breaks. PARP inhibitor (PARPi) agents or PARP trappers inhibit the active site of the enzyme. The PARP/PARPi complex binds to the damaged area, but without the catalytic activity necessary for PARP-dependent DNA damage repair. The stalling PARP on the DNA can induce a double strand break. In healthy cells with normal BRCA function, BRCA ultimately repairs this damage and the cell survives. In cases of \( BRCA \) mutations, the double stranded breaks persist and the cell eventually dies. Therefore, BRCA mutated patients may benefit from PARPi agents together with platinum-based drugs. There are 5 PARPi agents in phase II or III clinical trials (Table 2). The effects of olaparib (300 mg, twice a day) were compared with standard monotherapy in 302 patients with the \( BRCA \) mutation and HER2 negative metastatic BCs (OLYMPIAD study). Patients treated with olaparib had a significantly longer median PFS (7.0 months) and longer response rate (59.9%) compared to the standard therapy group (4.2 months and 28.8%) \( (p < 0.0001) \). These patients also had a lower rate of grade 3 or higher adverse events (36.6% vs. 50.5%) and drug discontinuation due to toxic events (4.9% vs. 7.7%) [17]. Olaparib was well-tolerated, however, the median OS of these patients (19.3 months) was not significantly different from the patients on standard therapy (17.1 months) \( (95\% CI, 0.66–1.23; p = 0.513) \) [18]. Olaparib is under investigation as a monotherapy and as a combination therapy with other agents such as immunotherapies, cell cycle and cell growth inhibitors. The completed trial on the combination of olaparib and carboplatin (NCT01445418) on 28 TNBC patients showed this combination is tolerable, with 1 complete response of more than 69 months, 19% partial response (median of 4 months) [19]. The safety, tolerability and efficacy of olaparib (200 mg, twice a day) plus paclitaxel was studied in another phase I clinical trial (NCT00707707) on a total of 19 mTNBC patients. Results from that trial showed that this combination caused

### Table 2

| Inhibitor | Other treatments in the study | Status | Identifier |
|-----------|------------------------------|--------|------------|
| Olaparib  | Durvalumib                   | Phase II, R | NCT03801369 |
|           | Durvalumib                   | Phase II, R | NCT03167619 |
|           | 18F-Fluoromisonidazole, cediranib maleate | Phase II, R | NCT02498613 |
|           | Paclitaxel, paclitaxol, epirubicin, cyclophosphamide | Phase II, R | NCT02789332 |
|           | Paclitaxel, carboplatin      | Phase II/III, R | NCT03150576 |
|           | BKM120, BYL719               | Phase II, ANR | NCT01623349 |
|           | None                         | Phase II, ANR | NCT00679783 |
| Veliparib | Cyclotaxol, placebo          | Phase II, C  | NCT01060392 |
|           | Cisplatin, placebo           | Phase II, R  | NCT02595905 |
| Fluzoparib| Apatinib                     | Phase I, R   | NCT03075462 |
| Rucaparib | Cisplatin                    | Phase II, ANR | NCT01074970 |
| Talazoparib| None                        | Phase II, R  | NCT02401347 |

\( R = \) recruiting; \( C = \) completed; \( ANR = \) active, not recruiting.
higher-than-expected neutropenia. However, an encouraging response rate was found in 37% (7) of the patients who had a confirmed partial response, and one patient remained on olaparib monotherapy without disease progression. The authors concluded that further dose adjustments would be required [20].

The EMBRACA clinical trial studied the effects of talazoparib in 431 patients with advanced BC and BRCA1/2 mutations. Two hundred and eighty-seven patients received 1 mg/day talazoparib and 144 patients received standard therapy. Patients in the talazoparib group had a significantly longer median PFS (8.6 months) compared to the standard therapy group (5.6 months) ($p < 0.001$). The talazoparib group had a higher ORR (62.2%) compared to standard therapy (27.2%) (95% CI, 2.9–8.8; $p < 0.001$) [21]. Talazoparib is currently in a phase II clinical trial recruiting TNBC patients (Table 2).

Veliparib is another drug in this group with some completed phase I and II clinical trials. Single agent veliparib was tested in 88 patients with and without BRCA mutations and results show that it is well-tolerated and has anti-tumor activity against both tumor types in comparison with other single PARPi agents [22]. A phase I clinical trial (NCT01104259) using a combination of veliparib, cisplatin and vinorelbine tartrate in 50 BRCA mutated TNBC patients, showed a median PFS of 5.5 months (95% CI, 4.1–6.7) [23]. In a phase III clinical trial, veliparib was added to carboplatin in 634 patients, 316 receiving paclitaxel + carboplatin + veliparib, 160 receiving paclitaxel + carboplatin and 158 receiving carboplatin alone. The first group achieved a higher pCR (53%) compared to the paclitaxel alone group (31%), but not compared to paclitaxel + carboplatin (58%). While veliparib did not add to the toxicity profile it did not provide additional benefit beyond the benefit seen with the addition of carboplatin. Importantly, the addition of veliparib + carboplatin to paclitaxel and then doxorubicin + cyclophosphamide improved the pCR in TNBC patients [24]. Other PARPi agents, including niraparib, fluzoparib, rucaparib and E7449, have fewer registered clinical trials.

**Histone deacetylase (HDAC)**

HDACs, important regulators of gene expression and transcription (Figure 1), are upregulated in BC [25]. HDAC inhibitors (HDACi) have shown a various range of effects in laboratory cancer models. Panobinostat is one of these drugs under phase I/II clinical trial in combination with tamoxifen (NCT01194908). A previous clinical trial investigating the effect of panobinostat in combination with letrozole showed that thrombocytopenia was the most common adverse reaction [26]. Romidepsin is also undergoing another clinical trial in locally recurrent or mTNBC. Entinostat was used with azacitidine in a phase II clinical trial study. The results of this study showed that this combination was well-tolerated but TNBC patients did not respond to the therapy [27]. Entinostat alone in combination with atezolizumab (NCT02708680) or azacitidine (NCT01349959) are other examples of HDACi drugs undergoing phase II clinical trials.

**p53, checkpoint kinase 1 (ChK1) and ataxia telangiectasia and Rad3 related (ATR)**

The tumor suppressor gene $p53$ is a well-known oncogene. Studies show that $p53$ is a nuclear protein which is responsible for DNA repair, triggering apoptosis in cases of irreparable DNA damage or inducing senescence, cell cycle arrest, necrosis or autophagy. Mutations in $p53$ are very common in TNBC (60%–70%), and result in the loss of p53 mediated tumor-suppression [28]. Mutated $p53$ loses its ability to monitor the G1 checkpoint of cell cycle and as a result, the cell relies more on ChK1 to arrest the cell. ChK1 is downstream of activated ATR, another
coordinator of DNA repair management. The activated ATR-Chk1 pathway affects multiple DNA damage and replication checkpoint responses. Various ATR and/or Chk1/2 inhibitors have been designed and synthesized so far. LY2606368 is a ChK1/2 inhibitor which is being tested in a phase II clinical trial in patients with tumors, including TNBC, aiming to see if this therapy can help shrink tumors (NCT02203513). AZD6738 is an inhibitor of ATR currently in phase II clinical trials to assess the safety and efficacy of olaparib (PARPi) in combination with AZD6738 in TNBC patients (NCT03330847). This drug is also being tested in another phase II clinical trial to compare its effects as a monotherapy to olaparib or durvalumab (anti PD-L1) monotherapy in TNBC patients (NCT03740893).

**DNA binding agents**
Mithramycin or plicamycin is an antibiotic produced by *Streptomyces* strains. Mithramycin adheres to DNA and inhibits RNA and protein production. Mithramycin has shown efficacy in various cancers, and in *in vitro* and *in vivo* TNBC models. Trabectedin is another antineoplastic alkylating agent. Early studies showed the efficacy of trabectedin in TNBC, however, a phase II clinical trial on TNBC and HER2-overexpressing metastatic BC patients showed no confirmed responses in mTNBC patients [29].

**INTRACELLULAR TARGETS AND SIGNALING MEDIATORS**

**Androgen receptor (AR)**
AR is a member of the steroid and nuclear receptor superfamily and a transcription factor (Figure 1). The LAR class of TNBC expresses AR. Studies investigating the link between AR and decreased relapse-free survival [2,28], higher mortality rate [30] or a favorable disease-free survival [31,32] are controversial. However, this class of TNBC has become a favorable target for anti-androgen therapy. Bicalutamide, an AR inhibitor, primarily used for metastatic prostate cancer, was well tolerated with a 19% 6-month clinical benefit rate (CBR) (95% CI, 7%–39%) in a phase II trial study in metastatic BC patients [33]. Enzalutamide, an inhibitor of AR nuclear localization, was well tolerated in a phase II clinical trial, resulting in a 35% CBR at 16 weeks and a median PFS of 14% [34]. The use of seviteronel, a dual lyase-selective CYP17 inhibitor and AR antagonist in a phase II trial in AR+TNBC patients was also well tolerated [35], and more studies on the drug are underway. Table 3 shows a summary of the clinical trials on AR drugs in TNBC patients.

| Table 3. Phase II and III clinical trials ongoing on anti-androgen drugs in triple negative breast cancer patients |
|---|---|---|---|
| Treatment | Other treatments | Status/key achievement | Identifier |
| Bicalutamide | Physician’s choice, bicalutamide | Phase III, R | NCT03055312 |
| | None | Phase II, U | NCT02353988 |
| | None | Phase II, T (slow enrolment of patients) | NCT02348281 |
| Enzalutamide | Paclitaxel | Phase IIb, R | NCT02689427 |
| | None | Phase II, ANR | NCT01889238 |
| | None | Phase II, ANR | NCT02750358 |
| Enobosarm | Pembrolizumab | Phase II, R | NCT02977761 |
| | None | Phase II, T (lack of efficacy) | NCT02368691 |
| Seviteronel | None | Phase II, C | NCT02130700 |
| CR1447 | None | Phase II, ANR | NCT02067741 |
| Darolutamide | Capecitabine | Phase II, R | NCT03383679 |
| Orteronel | None | Phase II, R | NCT01990209 |

R = recruiting; U = unknown; T =terminated; ANR = active, not recruiting; C = completed.

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Heat shock protein 90 (HSP90)
HSP90 is one of the most common members of HSP class of chaperones which is involved in many important signaling pathways including those that are implicated in BC progression. Owing to its wide range of action on important proteins, inhibition of HSP90 could be very beneficial. The effects of known inhibitors of HSP90 is reviewed in [36]. In vitro studies have shown that compared to BL or mesenchymal cell lines, the LAR class of TNBC cell lines are more sensitive to the HSP90 inhibitor, 17-DMAG [2]. In the latest phase II clinical trial on the single agent ganetespib, good tolerability and regression of lung tumor metastases in TNBC patients was observed, yet it failed to meet the expected end point of ORR [37]. Ganetespib is no longer available and hence the other clinical trial on ganetespib was terminated (NCT02637375). A clinical trial of onalespib in combination with talazoparib (PARPi) was withdrawn (NCT02627430), but the organizers of this trial are recruiting for a different clinical trial to test the combination of paclitaxel (NCT02474173) and onalespib (NCT02898207). The future application of this group of drugs remains unclear.

Cyclin-dependent kinases (CDKs)
CDKs are tightly controlled regulators of cell cycle and transcriptional machinery. Aberrant expression of CDKs, such as CDK4 and CDK6, is one of the characteristics of many tumors and in TNBC. Various inhibitors of CDKs have been successfully tested in in vivo and in vitro TNBC models with promising results. A phase I clinical trial on dinaciclib, a pan-CDK inhibitor, in combination with epirubicine (dinaciclib 20 mg/m² in day 1 and epirubicine 75 mg/m² on day 2 of a 3-week cycle) in 9 mTNBC patients, was closed due to toxicity issues [38]. Dinaciclib is currently in another phase I clinical trial in combination with pembrolizumab (NCT01676753). However, other agents in this group are in phase II clinical trials: trilaciclib, an inhibitor of CDK4/6 (NCT02978716), ribociclib, an inhibitor of CDK6 and cyclin D1/CDK4 (NCT03090165), PF-06873600 (NCT03519178) and abemaciclib, inhibitors of CDK2/4 (NCT03130439).

Proteins involved in apoptosis
Programmed cell death or apoptosis is a tightly controlled process. Dysregulation in apoptosis results in uncontrolled cell proliferation in cancer. Inhibitors of apoptosis proteins (IAPs) are endogenously produced to inhibit apoptosis. Second mitochondrial-derived activator of caspase (SMAC) is a mitochondrial protein which binds to IAPs, facilitating apoptosis in the cells. The SMAC mimetic LCL161 is an orally bioavailable small molecule which is under investigation for use in TNBC patients. In a study, a TNFα-based gene expression signature (GS) which is predictive of sensitivity to LCL161 was used and evaluated as a clinical assay. The results of this phase II clinical trial on adding LCL161 to paclitaxel in TNBC patients showed that patients who received the combination therapy had a higher (38.2%) pCR compared to the control (17.2%) GS-positive group. This study also revealed an array of adverse effects in the combination group, suggesting the importance of biomarker-driven targeted therapy approach for these patients [39]. LCL161 in combination with PDR001 (anti PD-1) is currently in phase Ib clinical trials in TNBC patients (NCT02890069).

Other intracellular targets are summarized in Table 4. These include inducible nitric oxide synthase, bromodomain and extra-terminal, cyclooxygenase-2 and mitotic protein kinases. TNBC has a high proliferation rate. Certain protein kinases were found to be important in the oncogenic transformation of TNBC. Important mitotic protein kinases in TNBC include polo-like kinase, aurora, dual specificity protein kinase TTK, never in mitosis A-related kinases and Src tyrosine kinases. These kinases generally phosphorylate either tyrosine, serine/threonine or all these 3 amino acids, known as dual-specificity protein kinases.

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https://doi.org/10.4048/jbc.2019.22.e39
Phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway

PI3K is a signal transducer downstream of activated receptor tyrosine kinases (RTKs). The signaling pathway of PI3K is in association with AKT and mTOR, known as the PI3K/AKT/mTOR pathway (Figure 2). This pathway is activated in 10%–21% of TNBCs [40] and plays a role in cell cycle regulation, cell proliferation and quiescence. Activated mTOR is also implicated in the regulation of cell metabolism and migration. Inhibition of this pathway is achieved by inhibitors of PI3K, AKT or mTOR. Inhibitors of PI3K including CUDC-907, AZD8186, taselisib, BKM120, BYL719, BEZ235, GDC-0941, PQR309 and gedatolisib are in phase I clinical trials for TNBC. Likewise, most of the AKT inhibitors including AZD5363, ONC201, ARQ 092, ritonavir and GSK2141795 are also in phase I or II clinical trials.

The phase II clinical trial on MK2206 was terminated due to toxicity and no significant decline in pAKT [41]. Ipatasertib in combination with paclitaxel increased the PFS (by 1.3 months, \( p = 0.037 \)) in a phase II clinical trial [42] and is currently in 2 other phase II (NCT03337724) and II/III (NCT02162719) clinical trials in combination with paclitaxel. ONC201, an AKT/ERK inhibitor, is in a phase II clinical trial in a methionine-restricted diet (NCT03733119).

Inhibitors of mTOR are an important class of inhibitors in this pathway. Everolimus is an mTOR inhibitor which, in combination with carboplatin in mTNBC patients in a phase II clinical trial, showed efficacy with 36% CBR (95% CI, 21.1%–54.4%), 3 months median PFS (95% CI, 1.6–4.6) and 16.6 months OS (95% CI, 7.3–not reached). Hemotoxicity, as the dose limiting factor and was observed with carboplatin AUC5/6 but not with AUC4 [43]. In a more recent phase II study of cisplatin, paclitaxel with or without everolimus in stage II and
III TNBC patients, the combination of paclitaxel and cisplatin was well tolerated with 50% complete responses. Everolimus increased the adverse event profile of this combination and did not improve the desirable clinical responses [44]. Everolimus is currently in another phase II study with paclitaxel, 5-fluorouracil, epirubicin, and cyclophosphamide (NCT00499603). Temsirolimus, another mTOR inhibitor, was well tolerated in combination with cisplatin and erlotinib [45] and was investigated in another phase I study in combination with neratinib (NCT01111825). AZD2014 is also in phase I/II clinical trial (NCT02208375).

**RAF-MEK-ERK pathway**

TNBC is the only subtype of BC that has a higher expression of various genes involved in the Raf/MEK/ERK pathway [46], highlighting the importance of targeting this signaling pathway in TNBC (Figure 2). An analysis of reprogramming of the kinome in 9 TNBC patients showed that targeting MEK in these patients might be a valuable option and pre- and post- administration of trametinib, a MEK1/2 inhibitor, showed that BL and claudin-low tumors responded differently to the treatment, with BL tumors showing more upregulation and activation of RTKs (NCT01467310) [47]. This drug alone or in combination with the an AKT inhibitor, GSK2141795, was tested in a clinical trial (NCT01138085) and showed limited efficacy in 50 TNBC patients. Only 2 patients in the first group had a partial response and one had stable disease after 8 cycles [48]. The same combination is being tested in a different clinical trial (NCT01964924). The other clinical trial still recruiting includes trametinib in combination with spatralizumab (anti-PD-1) (NCT02900664). Trials of selumetinib in combination with chemotherapy agents (NCT02685657), in combination with paclitaxel and durvalumab (NCT03742102), or in combination with the mTOR inhibitor, AZD2014 (NCT02583542) are also recruiting. MEK162, another MEK inhibitor, completed clinical trial testing in combination with BKM120 (NCT01363232) and BEZ235 (NCT01337765), with no posted results.
Janus kinase (JAK)

JAK proteins are a family of 4 tyrosine kinases involved in the JAK-STAT pathway (Figure 2). This pathway, along with its various roles, alters the transcription of genes involved in cell proliferation, mammary glands development during the puberty and pregnancy and cancers. Studies have shown that TNBC tumors are enriched with the amplicon of JAK2 [49] and that the subsets of TNBC with amplified JAK2 are more sensitive to the effects of specific inhibitors [50]. Ruxolitinib is an important inhibitor of JAK1 and JAK2, approved by FDA for the treatment of myelofibrosis. Given the effects of this drug and the importance of JAK2 in TNBC, currently, 3 clinical trials focusing on this signaling pathway are currently underway. These include using ruxolitinib combination with pembrolizumab in advanced TNBC patients (NCT03012230), in combination with paclitaxel, doxorubicin or cyclophosphamide (NCT02876302), and in combination with paclitaxel for the treatment of triple negative inflammatory BC (NCT02041429).

CELL SURFACE TARGETS

Vascular endothelial growth factor receptor 2 (VEGFR2)

VEGFR2, an RTK and a key mediator of angiogenesis is implicated in BC pathogenesis. VEGF, the ligand to VEGFR2, has prognostic importance in TNBC. Sixty percent of TNBCs show high VEGF-A expression and the prognosis of this group of patients is even worse, with less than 5 years of survival [51], along with metastasis and poor treatment responses [52]. Mesenchymal stem-like TNBC tumors express higher levels of VEGF-C [53]. The expression of VEGFR2 is linked to TNBC, tumor invasion and metastasis [54]. Inhibition of this pathway would include blocking the activation of the VEGF receptor by neutralizing VEGF (e.g., bevacizumab), blocking the receptor (e.g., ramucirumab), using receptor mimetics (e.g., aflibercept), or using small molecule tyrosine kinase inhibitors that have a wide spectrum of action on RTKs (e.g., sorafenib).

Bevacizumab, a monoclonal antibody, was withdrawn for use in BC by the FDA, following 2 trials showing increased toxicity and no survival benefit in HER2 negative BC [55]. However, it was tested in adjuvant/neoadjuvant settings and clinical trials in TNBC have suggested an improved pCR [16,56], although, the long-term outcomes of adding bevacizumab to chemotherapy regimens is still unclear [16]. The primary results of a phase III clinical trial on invasive BC patients receiving bevacizumab as an adjuvant drug did not improve the OS of the patients [57]. Docetaxel, carboplatin and bevacizumab were administered to stage II/III TNBC patients (phase II). Encouragingly, positive results were observed including positive pCR and clinical response of 42% and 96%, for stage II and stage III TNBC, respectively [58]. Bevacizumab plus chemotherapy (epirubicin, cyclophosphamide, docetaxel) was studied in another phase III clinical trial of 678 TNBC patients in the neoadjuvant setting. The pCR in the combination vs chemotherapy only groups were 39.3% vs. 27.9%, and the OR, 1.73 (95% CI, 2.3–2.42; \( p = 0.002 \)). This study showed a significant improvement in pCR in the patients that received bevacizumab [59].

Bevacizumab was also tested in patients with metastatic tumors. A phase III clinical trial on HER2 negative metastatic breast tumors receiving chemotherapy (capcitabine, anthracycline, taxane) with or without bevacizumab showed that the combination group had a longer median PFS, but this was not statistically significant [60]. The other phase II study on mTNBC patients receiving second-line chemotherapy (taxane, gemcitabine,
capecitabine, or vinorelbine) with bevacizumab showed a median PFS of 6 months in the combination group vs. 2.7 months in the chemotherapy alone group. Likewise, the median OS (17.9 months and 12.6 months, respectively) and ORR (41% and 18%, respectively) was also improved with bevacizumab [61]. Nab-paclitaxel, carboplatin and bevacizumab were administered to mTNBC patients in a phase II study. These combinations showed a median PFS of 9.2 months (95% CI, 7.8–25.1), an ORR of 85% (95% CI, 69–95), a CBR of 94% (95% CI, 80–99), and was well-tolerated [62].

The results of other completed phase II clinical trials including bevacizumab (NCT00608972, NCT00472693, NCT01094184, NCT02456857, and NCT00861705) have not yet been reported. Ramucirumab is a similar drug currently in a phase III clinical trial with docetaxel (NCT00703326). Small tyrosine kinase inhibitors like sorafenib (NCT02624700) and lenvatinib (NCT03797326) are in phase II clinical trials. Data on the effects of lucitanib has not yet been reported (NCT02202746). In a phase II study on previously treated advanced TNBC patients sunitinib did not improve efficacy in comparison with single-agent standard-of-care [63].

Epidermal growth factor receptor (EGFR)

EGFR has important roles in the survival of many solid tumors, including tumor metastasis, cell cycle progression, cell proliferation, differentiation, angiogenesis and apoptosis. At least 50% of TNBCs overexpress EGFR [64] which is negatively correlated with patient survival [65]. Two classes of EGFR inhibitors are currently available, including small molecule tyrosine kinase inhibitors, such as gefitinib and erlotinib, and monoclonal antibodies, such as cetuximab (SCT200). Gefitinib (NCT01732276) and afatinib (NCT02511847) are both registered for clinical trials (unknown status). Erlotinib in combination with paclitaxel albumin-stabilized nanoparticle formulation and bevacizumab in a phase II study was well-tolerated. The expected PFS was not reached but clinical benefit in most patients was achieved [66].

Cetuximab is an anti-EGFR antibody, which has been trialed in several early phase studies in TNBC with only modest benefit. Ixabepilone alone and in combination with cetuximab was tested in 77 advanced TNBC patients. The combination group had 35.9% ORR (95% CI, 21.2–52.8) vs. 30% (95% CI, 16.6–46.5) in the monotherapy group. Median PFS was equal in both groups (4.1 months) and overall, both groups showed similar clinical results [67]. In another phase II clinical study, cisplatin alone or in combination with cetuximab showed an ORR 20% (95% CI, 13–29) in the combination group and 10% in the monotherapy group (95% CI, 4–21). Cetuximab improved PFS (3.7 months vs. 1.5 months) and OS (12.9 months vs. 9.4 months). Yet the primary endpoints of the study were not met [68]. Cetuximab plus docetaxel was tested in another phase II clinical trial showing modest activity and acceptable toxicity. The study showed a 24% pCR rate (95% CI, 7.3–40.7) and 22% complete clinical response rate [69]. Cetuximab plus irinotecan was tested in another phase II clinical trial on BC patients with 58% being TNBC. The combination was tolerable but showed low overall activity. The treatment resulted in 11% ORR (95% CI, 1–33), one partial response and one complete response. TNBC patients had 18% response rate vs. no response in non-TNBC. The median time to progression and median OS was 1.4 months (95% CI, 1.0–2.2) and 9.4 months (95% CI, 2.8–16.1), respectively [70].

Fibroblast growth factor receptor (FGFR)

FGFR is another RTKs protein of which there are 5 (FGFR1–5). A total 4% of TNBCs overexpress FGFR2. FGFR1 and FGFR2 are reported to account for about 16 and 13% of TNBC patients [71]. Expression of FGFR2 could be considered as an independent prognostic
factor of OS in TNBC patients [72]. Therefore, small molecule tyrosine kinase inhibitors or monoclonal antibodies (e.g., IM-412) may be applicable in certain types of TNBC [73].

**Trophoblast antigen 2 (Trop-2)**
Trop-2 is a cell surface receptor and an epithelial glycoprotein-1, involved in various aspects of cancer including cancer cell proliferation, EMT, migration, invasion and metastasis. Overexpression of Trop-2 is common in TNBC [74]. Irinotecan is a prodrug to the topoisomerase I inhibitor, SN-38. Sacituzumab govitecan-hziy (or IMMU-132 or hRS7-SN-38) is a conjugate of SN-38 and humanized anti-trop-2 monoclonal antibody. This drug (10 mg/kg) was studied in a phase I/II clinical trial on 108 TNBC patients who had received 2–10 therapies and was associated with durable objective responses. The response rate was 33.3% (95% CI, 24.6–43.1) of which 1 had a complete response and 33 had partial responses. The median PFS and OS were 5.5 months (95% CI, 4.1–6.3) and 13.0 months (95% CI, 11.2–13.7), respectively, with the CBR of 45.4%. The main adverse reactions were myelotoxic effects [75]. The efficacy of sacituzumab govitecan in mTNBC patients refractory or relapsing after at least 2 prior chemotherapies is currently being tested in a phase III clinical trial. Approximately 150 institutions across the United States and Europe are involved in this clinical trial (NCT02574455). The drug is also being tested in another phase I/II clinical trial in various tumors including TNBC (NCT01631552). The other preclinical compound under investigation, is anti-Trop-2 conjugated with a nano-carrier-linked with doxorubicin.

**Glycoprotein non-metastatic B (GPNMB)**
GPNMB, or osteoactivin, is a type I transmembrane glycoprotein, which is overexpressed in 40%–60% of BCs [76], associated with triple-negativity [77] and poor patient outcome [77]. CDX-011 or glembatumumab vedotin, an antibody targeting GPNMB, in a phase I/II study on 42 patients with locally advanced or metastatic BC showed an acceptable safety profile [76]. Out of these patients 16 out of 19 were GPNMB+. The overall median PFS was 9.1, 17.9 and 18 weeks for all patients, TNBC patients and GPNMB+ patients, respectively [76]. Yet, despite being well tolerated, the drug alone could not meet the endpoint of ORR in a different phase II study [78]. In this study with 124 patients, more than 25% of the tumors were GPNMB+ and the ORR was 30% (7 of 23) in the glembatumumab vedotin group vs. 9% in the chemotherapy group. In TNBC patients, the ORR was 18% vs. 0%, and in GPNMP overexpressing TNBC patients, it was 40% vs. 0%, respectively. The results of another phase II clinical trial on the effect of CDX-011 in patients with metastatic GPNMB overexpressing TNBC is not yet published.

**CONCLUSION**
The development targeted therapy of TNBC is still a challenge that is being investigated by many cancer researchers around the world with the hope of finding improved treatment regimens for these patients. Due to the high rate of PD-1 expression in TNBC patients, the immune system has been uncovered as a major contributing factor to TNBC pathogenesis as revealed by newer TNBC classifications, and the success rate of drugs in this category, today, much attention is paid to immune checkpoint modulators, especially anti-PD-1 and anti-PD-L1 agents. Favorable clinical response rates of phase III studies on PARPi and platinum salts are impressive, and due to the high rate of mutations in $p53$, the ongoing clinical trials on CDK and ATR inhibitors might lead to phase III clinical trials. Inhibitors of VEGFR2 are also in phase III clinical trials, and anti-androgen drugs, due to specific application in LAR TNBC patients, are attracting some attention. There are many drugs in phase I, II and III clinical...
trials, investigating novel molecules either alone or in combination with other novel agents or standard chemotherapeutics that are promising for improved clinical outcomes. The results of these studies may pave the way for researchers and open new doors for better treatment and improvement in outcomes of TNBC patients. However, there are many completed clinical trials with no accessible published data, placing the outcomes under question.

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