



Early-stage triple negative breast cancer: the therapeutic role of immunotherapy and the prognostic value of pathological complete response

Pierluigi De Santis¹, Martina Perrone¹, Chiara Guarini¹, Anna Natalizia Santoro¹, Carmelo Laface¹, Daniela Carrozzo¹, Gaia Rachele Oliva², Palma Fedele^{1*} 

¹Oncology Unit, Francavilla Fontana Ceglie Messapica Hospital District, 72021 Francavilla Fontana, Italy

²Department of Medicine and Translational Surgery, Università Cattolica del Sacro Cuore, 00168 Roma, Italy

***Correspondence:** Palma Fedele, Oncology Unit, Francavilla Fontana Ceglie Messapica Hospital District, 72021 Francavilla Fontana, Italy. minafedele@hotmail.com

Academic Editor: Laura Cerchia, Institute of Experimental Endocrinology and Oncology "G. Salvatore"-National Research Council (IEOS-CNR), Italy; Simona Camorani, Institute of Experimental Endocrinology and Oncology "G. Salvatore"-National Research Council (IEOS-CNR), Italy

Received: May 26, 2023 **Accepted:** December 26, 2023 **Published:** February 28, 2024

Cite this article: De Santis P, Perrone M, Guarini C, Santoro AN, Laface C, Carrozzo D, et al. Early-stage triple negative breast cancer: the therapeutic role of immunotherapy and the prognostic value of pathological complete response. Explor Target Antitumor Ther. 2024;5:232–50. <https://doi.org/10.37349/etat.2024.00215>

Abstract

Triple negative breast cancer (TNBC) represents an aggressive disease associated with a high risk of recurrence after curative treatment and a poor prognosis in the metastatic setting. Chemotherapy was for years the only treatment available in the early and metastatic setting, due to the lack of actionable targets. Clinical practice has changed following the results obtained with the addition of immunotherapy to standard chemotherapy, the development of novel drugs [i.e. antibody-drug conjugates (ADCs)], and the use of targeted treatments for patients carrying germline pathogenic breast cancer susceptibility genes (*BRCA*) 1 or *BRCA* 2 variants. The treatment of early-stage disease has had a shift in clinical practice since July 2021, after the Food and Drug Administration (FDA) approval of pembrolizumab in association with chemotherapy as neoadjuvant treatment for TNBC and as a single agent in the subsequent adjuvant setting. This intensive treatment based on the combination of a poly-chemotherapy and an immune checkpoint inhibitor (ICI) led to the improvement of short- and long-term outcomes, but it has highlighted some new unmet clinical needs in the treatment of early-stage TNBC: the selection of the most effective adjuvant therapy and the integration of pembrolizumab with other therapeutic strategies [capecitabine, poly(ADP-ribose) polymerase (PARP) inhibitors] based on the achievement of pathologic complete response (pCR); the identification of predictive biomarkers to select patients who could most benefit from the addition of ICI, to minimize toxicities and to maximize outcomes; the possibility of de-escalating chemotherapy in favor of immune-combo or novel agents, such as ADCs; the role of immunotherapy in estrogen receptor (ER)-low patients. The advent of immunotherapy not only addresses current challenges in TNBC treatment but also holds the promise of a radical transformation in its therapeutic paradigm, enhancing significantly clinical outcomes and offering new perspectives for patients grappling with this aggressive form of breast cancer.



Keywords

Triple negative breast cancer, immunotherapy, pathological complete response, neoadjuvant combination treatment, adjuvant treatment

Introduction

Triple negative Breast Cancer (TNBC) is a histological subtype of breast cancer (BC) characterized by the immunohistochemical lack of expression (< 1%) of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2). It accounts for approximately 10–20% of all BC, affecting mainly young, premenopausal women, and individuals with inherited gene alterations, such as BC susceptibility genes 1/2 (*BRCA 1/2*) mutations [1–3]. It notably presents an aggressive biological behavior with a trend to have a higher grade and an often lymph node involvement at diagnosis, an inclination to metastasize after curative treatment, and a poorer prognosis in metastatic setting when compared with other BC subtypes [4, 5].

For decades, treatment for early TNBC has been based on surgery and subsequent adjuvant chemotherapy (CHT) for the reduction of disease recurrence [6]. Therefore, conventional cytotoxic CHT has represented the backbone of systemic treatment in the early TNBC, including neoadjuvant treatment, which used to reduce tumor size in larger tumors increasing the chances of a breast-conserving surgery [7, 8]. In recent years the development of novel therapeutic approaches has been difficult, due to the heterogeneity of TNBC and lack of therapeutic targets [9, 10]. Nevertheless, immunotherapy and poly(ADP-ribose) polymerase (PARP) inhibitors have shown survival benefits in recent studies.

Specifically, combinations of immune checkpoint inhibitors (ICIs) with CHT or other alternative therapeutic compounds could emerge as a successful therapeutic approach in the management of TNBC patients. Despite the progress in ICIs representing a notable milestone in TNBC treatment, additional investigations are necessary to tackle this issue comprehensively. A profound comprehension of tumor subtypes, alongside tumor microenvironment (TME) and in terms of molecular, genetic, and immune aspects, would amplify the potential for developing targeted immunotherapy to achieve superior therapeutic effectiveness, especially in TNBC [11].

Therefore, in this review, we aimed to investigate the role of immunotherapy in early-stage TNBC, the prognostic value of pathologic complete response (pCR) with its therapeutic implications, and the future perspectives regarding the systemic treatment of early TNBC, including the discovery of new biomarkers.

The landscape of immunotherapy in TNBC

The immune system plays a crucial role in TNBC compared to the other molecular subtypes of BC. Although originally BC was considered non-immunogenic, TNBC has a high immunogenic potential, making it a promising candidate for immunotherapy, especially with ICIs [12, 13]. TNBC immunogenicity is related to intrinsic tumor cell signatures and tumoral surrounding microenvironment features.

Over the last decades thanks to emerging technologies such as next-generation sequencing (NGS), the knowledge of the molecular and genetic background of TNBC improved, bringing to light its intertumoral and intratumoral heterogeneity.

A first classification divided TNBC into six subtypes: basal-like 1 (BL1), basal-like 2 (BL2), mesenchymal (M), M stem-like (MSL), immunomodulatory (IM), and luminal androgen receptor (LAR) [14].

Subsequently, analyzing RNA and DNA-based profiles of 198 TNBC tumors, a four-type classification of TNBC was shaped: basal-like immunosuppressed (BLIS), basal-like immune-activated (BLIA), M and LAR [15]. This classification was further revised with the identification of four specific TNBC subtypes: BL1, BL2, M, and LAR, omitting IM and MSL because of the dependence of these two subtypes on the TME features [14].

In addition, TNBC could be classified into three microenvironment phenotypes or clusters:

- (1). Cluster 1: “immune-desert” with poor immune cell permeation, due to a high presence of *MYC* amplifications and, consequently, a lower recruitment of innate immune cells.
- (2). Cluster 2: “innate immune-inactivated” characterized by a hyper-activation of phosphatidylinositol 3-kinase/protein kinase B (PI3K-AKT) pathway in tumor cells, low tumor antigen burden and infiltration of deactivated innate immune cells, fibroblasts, and endothelial cells. Clusters 1 and 2 are therefore referred to as “cold tumors”.
- (3). Cluster 3: “immune-inflamed”, the so-called “hot tumor” that represents about 30% of TNBCs and is characterized by an abundant adaptive and innate immune cells infiltration and with a high expression of immune checkpoint molecules [16].

The potential “hot” conversion of “cold” tumors could improve the efficacy of cancer immunotherapy. For example, local IM therapies can express a synergistic effect with immunotherapy by acting on components of the TME and immune system function, such as elevating the expression of tumor antigens and increasing the recruitment of activated immune cells in the TME [17].

TNBC cancer cell immunological features include genomic instability and high tumor mutational burden (TMB), resulting in more somatic mutations and neoantigens [18].

Moreover, approximately 10–20% of TNBC harbor *BRCA 1* or *BRCA 2* germinal mutations, with a consequent hereditary deficit in the DNA repair mechanism and strong genomic instability. Several studies have demonstrated that TNBC-carrying BRCA mutations are more sensitive to DNA-damaging drugs such as anthracyclines, but also platinum agents and PARP inhibitors [19–21]. Sensitivity to these drugs was also observed in tumors with alterations in other genes, sharing BRCA-mutant phenotype in the absence of a *BRCA 1/2* mutation, namely “BRCAness” [22, 23].

Tumors with *BRCA 1/2* mutations or BRCAness TNBC are more immunogenic than TNBC without these genetic alterations [24–26].

Compared to the other BC subtypes the immunogenic TME features in TNBC consist of higher levels of vascular endothelial growth factor (VEGF), that promote tumor cell growth and migration such as mitogen-activated protein kinases (MAPKs), tumor-associated macrophages (TAMs), and tumor-infiltrating lymphocytes (TILs), white blood cells that migrate towards the tumor, leading to an important immunogenic effect and consequently that are involved in killing cancer cells [27–29].

TAMs regulate the interaction between the immune system and cancer cells. CD163+ M2 macrophages, which are associated with tumors characterized by higher proliferation and poorer differentiation [30], are more present in TNBC and basal-like BC [31]. A prosperous infiltration of TILs is found in TNBC tumors and the stroma surrounding them, with a recognized predictive and prognostic role, specifically for CD4+ CD8+ T cells [32]. Several studies have reported better response to neoadjuvant CHT (NACT) [33] and better clinical outcomes in BC with high TIL infiltrate [34–39]. Based on this evidence, the international TILs working group started standardizing the evaluation of BC TILs to use it in clinical practice identifying those patients that may benefit from emerging immunotherapies with ICIs or combination therapies [40].

All these TME elements contribute to TNBC immunogenicity which also appears to be closely related to the concept of TMB, depending on the ineffective DNA repair system with the consequent generation of high rates of neoantigens. The upregulated antigen presentation system leads to an increasing number of innate and adaptative immune cells and many cytokines interplaying with cancer cells. However, the exact relationship between TMB, neoantigens, and immune infiltration is not yet completely understood, and some studies have reported an inverse association between immune cells in TME and the rate of somatic copy number alterations [41, 42].

Moreover, although TMB is comparable across the three clusters of TNBC, the “immune-inflated” phenotype is characterized by a higher degree of immune cells in the TME, but also a high expression of immune checkpoints by cancer cells [16]. The rate of TILs, indeed, has been positively related to

programmed death ligand 1 (PD-L1) expression [43]. PD-L1 is an immune checkpoint that mediates local immune escape in many tumors inducing saturation of activated T cells. Even if PD-L1 prognostic role is yet controversial [44], however it results more overexpressed in TNBC compared with other BC and it can predict responsiveness to immunotherapy [16].

Therefore, TNBC represents an aggressive BC subtype, associated with high mutational load, high tumor immunogenicity and TME diversity.

New paradigms in early TNBC: from CHT to immunotherapy

CHT in adjuvant treatment for TNBC

In early TNBC patients, CHT represents the mainstay of adjuvant and neoadjuvant treatments. Adjuvant CHT is recommended for tumor sizes greater than 1.0 cm and patients with nodal involvement, regardless of tumor size. Therefore, it can be considered for tumor sizes between 0.6–1.0 cm [45]. A recent large meta-analysis demonstrated that adjuvant CHT with anthracyclines-containing regimens plus taxanes, compared with no CHT, can reduce BC mortality rates by about 40% during the first decade after diagnosis. Moreover, regimens with higher cumulative and dose-dense schedules of anthracycline (with granulocyte colony-stimulating factor support) have shown better survival benefits and more reductions in recurrence [46]. Three-weekly docetaxel and paclitaxel can be considered in adjuvant setting, but weekly paclitaxel, in a subgroup analysis, has shown improved outcomes and is preferred for TNBC [47]. In TNBC in frail patients with a known history of heart disease, to minimize the cardiotoxicity of adjuvant treatments, docetaxel combined with cyclophosphamide (TC) has proven to be a viable alternative to doxorubicin and cyclophosphamide (AC), demonstrating a favorable disease-free survival (DFS) [48]. Therefore, there is a broad spectrum of therapeutic treatments for early TNBC that should be customised according to the patient and expected toxicities.

The role of platinum in adjuvant setting for TNBC

TNBC patients commonly harbor *BRCA 1/2* or BRCAness mutations with a homologous recombination deficiency (HRD) that makes them particularly susceptible to platinum agents due to their ability to hit cancer cells that have deficient DNA repair mechanisms [49–51]. Several retrospective single-center studies have explored the role of adjuvant platinum combined with standard anthracycline and taxane-based regimens, with controversial results not showing clear clinical benefits [52, 53]. Nevertheless, a recent phase III trials have demonstrated a longer 5-year DFS (86.5% vs. 80.3%) with similar results in distant DFS and relapse-free survival (RFS) of platinum-containing adjuvant regimens (paclitaxel-carboplatin) compared to a standard anthracyclines-containing regimen followed by taxane, however with no benefit in overall survival (OS) [54].

Another important factor is platinum resistance. Platinum sensitivity may be affected by changes in the hazard ratio (HR) pathway or, in the case of patients with *BRCA 1/2* mutations, by the secondary appearance of new *BRCA 1 or 2* mutations that make cancer cells less sensitive to platinum [55, 56].

Other mechanisms of resistance to platinum compounds are:

- (1). Modification of drug transport within the tumor cell, by determining decreased influx or increased efflux.
- (2). Increase of detoxification systems.
- (3). Decrease of cell apoptosis [57].

Therefore, the benefit of adjuvant platinum-based regimens remains controversial and needs validation by prospective adjuvant ongoing trials.

Neoadjuvant treatments for TNBC

NACT

Several treatment guidelines recommend NACT as the preferred option for stage II or III TNBC and for stage I with a tumor size greater than 1 cm. It can be considered in stage I TNBC with a tumor size from 0.6 cm to 1 cm and/or in the case of tumors with nodal micrometastases. [6, 45]. There is no significant difference in survival benefits between patients receiving neoadjuvant or adjuvant CHT after surgical resection. However, neoadjuvant treatments can be useful for inoperable tumors rendering them operable and they can also downstage patients with operable BC promoting breast-conservation [58, 59]. The use of neoadjuvant treatments provides important prognostic information based on response to therapy. Achieving a pCR, defined as the lack of cancer cells in tissue samples of breast and axillary lymph nodes, after a neoadjuvant treatment, is associated with favorable disease-free and OS in early TNBC, as demonstrated in Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) pooled analysis. In this study, patients with early BC treated with NACT and followed by surgery who obtained pCR (ypT0 ypN0, ypT0/is ypN0) were associated with improved event-free survival (EFS) and OS, especially in TNBC (HR = 0.24 and HR = 0.16, respectively) [33]. Like adjuvant treatment, traditional NACT is based on anthracyclines and taxanes, and a dose-dense regimen is preferred in neoadjuvant settings based on proven improved DFS and OS in a large meta-analysis [60].

In recent years, the use of platinum-based combination regimens has been the focus of neoadjuvant treatment to increase the rate of pCR in TNBC. Three recent studies demonstrated that combining platinum with taxane and anthracycline led to an improvement in the pCR rate in TNBC, with a similar survival benefit [61–63]. In Brightness Trial patients with II–III stage TNBC were randomly assigned to receive paclitaxel alone, paclitaxel and carboplatin and this combination with a PARP inhibitor, veliparib followed by AC. Although the addition of veliparib and carboplatin was associated with an increase of patients who achieved a pCR compared to paclitaxel alone (53% vs. 31%, $P < 0.0001$), but not to paclitaxel and carboplatin, this benefit could be related to the addition of the carboplatin [63]. The initial rationale for using the combination of platinum in NACT was that sporadic TNBC can show BRCA⁺ with a major response to platinum regimens [50, 51]. However, the greatest benefit was seen in patients who were germline BRCA wild type, and only a marginal benefit was observed in the germline BRCA mutant subgroup, as was shown in the recent GeparOLA trial. In this trial, patients were randomized to neoadjuvant therapy with paclitaxel and carboplatin vs. neoadjuvant therapy with paclitaxel and olaparib (PARP inhibitor). In both arms, the combination of epirubicin and cyclophosphamide was administered next. This study, although limited by a small number of patients enrolled, showed an advantage for the carboplatin arm in patients without BRCA mutation (germ or somatic) and high HRD. The 4-year invasive DFS (iDFS) rate with olaparib-paclitaxel was 81.2% vs. 93.4% with carboplatin-paclitaxel (CP) [HR = 3.03; 95% confidence interval (CI) = 0.67–13.67; log-rank $P = 0.1290$]. The 4-year OS rate was 89.2% with the olaparib combination vs. 96.6% with carboplatin (HR = 3.27; 95% CI = 0.39–27.20; log-rank $P = 0.2444$). The trend of the iDFS curves was similar in the two treatment arms and independent of germline or somatic BRCA mutation [64].

Platinum combinations are currently recommended for selected patients with TNBC who require adequate local control before surgical resection [45]. A more recent phase III trial presented at the San Antonio Breast Cancer Symposium evaluating the efficacy and safety of adding carboplatin to standard sequential taxane-anthracycline NACT in patients with TNBC who had no evidence of metastatic disease, has observed improvements in terms of DFS (5-year DFS were 70.6% and 64.5% respectively with a HR = 0.79, 95% CI = 0.61–1.02, $P = 0.073$) and OS (5-year OS were 74.0% and 66.7% respectively with a HR = 0.75, 95% CI = 0.57–0.98, $P = 0.034$) with the addition of carboplatin, but these benefits were limited to patients who were 50 years of age or younger. Therefore, the pCR in the intention-to-treat population was 54.5% in the carboplatin arm and 40.3% in the control arm ($P < 0.001$) [65].

The inclusion of platinum agents as NACT for TNBC remains controversial. Long-term outcomes and new prospective studies are needed to clarify the role of platinum agents in this setting.

Neoadjuvant immunotherapy

The success of ICIs in metastatic TNBC led to expand their role in neoadjuvant settings. Pembrolizumab and atezolizumab have shown progression-free survival (PFS) benefits in phase III trials in advanced setting [66, 67]. In contrast to atezolizumab that showed conflicting results [68, 69], pembrolizumab consistently showed OS benefits in advanced TNBC [66, 70]. In early-stage TNBC two studies evaluated atezolizumab in neoadjuvant setting. In the NeoTRIPaPDL1 trial, no improvement in pCR was shown with the addition of atezolizumab to a non-anthracycline-containing CHT regimen [71]. More recent Impassion031 phase III study evaluating the association of atezolizumab to a standard NACT (nab-paclitaxel weekly for 12 weeks followed by 4 cycles of AC), has demonstrated a significant improvement of pCR rates in intention to treat (ITT) population (58% in atezolizumab arm vs. 41% in placebo arm, $P = 0.0044$), regardless of PD-L1 status, meeting the primary endpoint of the study [72]. Therefore, in early BC the combination of pembrolizumab with paclitaxel-carboplatin followed by anthracycline increased pCR rate and EFS rate in the KEYNOTE-522 trial, representing a turning point for the role of immunotherapy in neoadjuvant therapy of TNBC and establishing pembrolizumab as a standard treatment during neoadjuvant treatment for stage II and III TNBC. The trial evaluated the combination of pembrolizumab (18 cycles, 200 mg every 3 weeks) combined with four cycles of paclitaxel (weekly or 3-weekly) and carboplatin (3-weekly), followed by 3-weekly AC for 4 cycles, compared to placebo with CHT. Pembrolizumab arm showed a 13.6% improvement in pCR [64.8% (95% CI = 59.9–69.5%) vs. 51.2% (95% CI = 44.1–58.3%)] and in EFS rate [84.5% (95% CI = 81.7–86.9%) vs. 76.8% (95% CI = 72.2–80.7%)], meeting the primary endpoint of the study, regardless nodal involvement and PD-L1 status. The average duration of follow-up is still immature, but a trend of superiority in terms of OS in the pembrolizumab arm was nevertheless detected [73]. Limits of this study are the lack of biomarkers that predict what patient may benefit from the addition of pembrolizumab and the non-utilization of dose-dense schedule of AC which showed superior OS benefit in the neoadjuvant setting in TNBC [74].

Moreover, the recent GeparNuevo trial showed that durvalumab (1,500 mg every 4 weeks) added to NACT consisting of nab-paclitaxel 125 mg/m² weekly for 12 weeks, followed by epirubicin/cyclophosphamide every 2 weeks, in early TNBC significantly improved iDFS (85.6% with durvalumab vs. 77.2% with placebo HR = 0.48, 95% CI = 0.24–0.97, stratified log-rank $P = 0.036$) and OS (95.2% vs. 83.5% with a HR = 0.24, 95% CI = 0.08–0.72, $P = 0.006$), despite a modest pCR increase and no adjuvant component of durvalumab [75]. Future studies should aim to define the role of immunotherapy in the treatment of early TNBC, to define the ideal duration of these treatments, and should research new biomarkers to personalize treatments.

Pathological complete response: prognostic role and therapeutic implications

In clinical practice, the achievement of pCR after neoadjuvant treatment is correlated to the improvement of long-term benefits concerning EFS and OS. Its prognostic value is greatest in aggressive tumor subtypes, like in TNBC (EFS: HR = 0.24; OS: HR = 0.16) [33]. Patients who have residual invasive BC after the receipt of NACT have a high risk of relapse. Patients with TNBC who do not experience pCR have an estimated 5-year EFS of 57% and OS of 47% (compared with 90% EFS and 84% OS, respectively, for patients with early-stage TNBC who demonstrate pCR) [76, 77].

After pre-operative CHT and surgical treatment, patients can receive postoperative radiation therapy (RT). Patients with hormone receptor-positive BC [hormone receptor-positive (HR+) BC] are candidates for adjuvant endocrine treatment. However, until recently, no adjuvant CHT was expected as standard in patients with TNBC. Only follow-up was recommended in those who have pCR or in those with residual invasive BC after the receipt of neoadjuvant regimens [78]. To address the unmet clinical need for optimal adjuvant treatment in the subgroup of patients with TNBC at high risk of recurrence (those who have not achieved the pCR after NACT containing anthracycline, taxane, or both), the Capecitabine for Residual Cancer as Adjuvant Therapy (CREATE-X) was designed. The trial did not include only patients with TNBC

but also patients with HR+ HER2 negative BC [79]. The results of this phase III trial showed that the addition of adjuvant capecitabine (1,250 mg per square meter of body-surface area, twice per day, on days 1 to 14, every 3 weeks for six or eight cycles) was safe and effective in prolonging DFS and OS among the ITT population. The study showed a superior DFS in the capecitabine group than in the control group (74.1% vs. 67.6% at 5 years; HR = 0.70; P = 0.01). Therefore, OS was longer in the experimental group: 89.2% vs. 83.6% of the patients were alive at 5 years (HR = 0.59; P = 0.01). Thirty percent of the patients had triple negative (TN) disease, and they represent the subgroup with poor prognosis (approximately half the patients with TNBC who had a pCR did not have the recurrence of the disease) [33]. The benefit of capecitabine vs. control in DFS and OS was notable among this subgroup of patients (HR = 0.58 and HR = 0.52, respectively) [79].

The reflection in the treatment algorithm due to these results was significant.

Some limits of this study are the exclusion of patients who had reached the pCR, for whom only follow-up was indicated, and the lack of efficacy results selected for residual cancer burden (RCB). The RCB quantifies the extent of residual disease after neoadjuvant treatment at the time of surgery. This score uses the diameter of residual disease, percentage of vital tumor cells, and diameter of the largest involved lymph node to calculate the amount of residual disease. It has been validated with distinct prognostic RCB classes in all BC subtypes, with the most significant discriminatory power in TN and Her-2 positive BC. It is categorized as RCB-0 (equivalent to a pCR), RCB-1, RCB-2, and RCB-3, reflecting increasingly larger residual cancer and respective poor prognoses (in terms of EFS) [80]. Finally, the CREATE-X trial did not examine capecitabine efficacy in patients with germline *BRCA* 1 or *BRCA* 2 pathogenic variants (less than 15% of those enrolled) [79].

OlympiA is a phase III study designed to investigate how the PARP inhibitor olaparib might improve DFS and OS in patients with resected HR+ BC and TNBC with germline *BRCA* 1 or *BRCA* 2 mutation. It enrolled patients treated with CHT (containing anthracyclines, taxanes or the combination of both) in neoadjuvant or adjuvant setting and randomized them to receive olaparib (orally administered at the dose of 300 mg twice daily) vs. placebo for 1 year after surgical resection (and radiotherapy when indicated). Also in this trial, patients with TNBC who underwent NACT followed by surgery were required to have residual invasive BC in the breast and/or resected lymph nodes (non-pCR) [81]. Postneoadjuvant capecitabine was not foreseen in this trial. iDFS, the primary endpoint of the study, was significantly longer among patients assigned to receive olaparib than among those assigned placebo (HR = 0.58; P < 0.001). The percentage of patients alive and free of invasive disease at 3 years was 85.9% in the olaparib group and 77.1% in the placebo group. The benefit of adjuvant olaparib was observed irrespective of the germline *BRCA* mutation (*BRCA* 1 vs. *BRCA* 2), the hormone-receptor status, or the timing of previous CHT (neoadjuvant vs. adjuvant) [81] 4-year iDFS for the olaparib group was 82.7% (vs. 75.4% in placebo group) and 4-year distant DFS (DDFS) was 86.5% (vs. 79.1%). Adjuvant olaparib improves OS, with an HR of 0.68 and a P value of 0.009 at 3.5 years of median follow-up, meeting the significance threshold for OS at the second planned interim analysis. The OS benefit at 4 years in the olaparib arm compared with the placebo arm was reported (89.8% vs. 86.4%, respectively) [82].

Both studies have defined the standard of adjuvant therapy post-NACT for patients with *BRCA* wild type (CREATE-X) and *BRCA* mutated (OlympiA) TNBC, that did not reach the pCR.

The low percentage of *BRCA* mutated patients enrolled in the CREATE-X, the absence of pre-planned subgroup analyzes for this population do not allow for a description of the efficacy of capecitabine in this subgroup of patients.

Moreover, there are no prospective randomized trials between capecitabine and olaparib to guide the clinical decision in this population, nor combination or sequence data between these two drugs.

It would also be important to consider the potentially severe toxicity profile of such a combination, given their overlapping side effects (in particular, cytopenias).

The treatment paradigm of early TNBC has had a real evolution since July 2021, with the introduction of immunotherapy following the Food and Drug Administration (FDA) approval of pembrolizumab for high-

risk TNBC (tumor size > 1 cm but ≤ 2 cm in diameter with nodal involvement or tumor size > 2 cm in diameter regardless of nodal involvement), regardless of tumor PD-L1 expression, in combination with CHT as neoadjuvant treatment, and then continued as a single agent as adjuvant treatment after surgery for a total duration of approximately 1 year [83].

Results from the KEYNOTE-522 study were the basis for this approval, demonstrating a significantly higher rate of pCR at the time of definitive surgery among patients who received pembrolizumab plus NACT than among those who received placebo plus NACT and an improvement in long-term benefits [73, 84]. The aim of the trial was not to identify the contributions of the neoadjuvant and adjuvant treatment phases, so it is difficult to define if these long-term results are related to exposure to adjuvant pembrolizumab or a lesser RCB at the end of the neoadjuvant phase in the pembrolizumab-CHT group.

An exploratory analysis of the study then provided data to further describe the prognosis related to the RCB after neoadjuvant experimental treatment (Figure 1) [85].

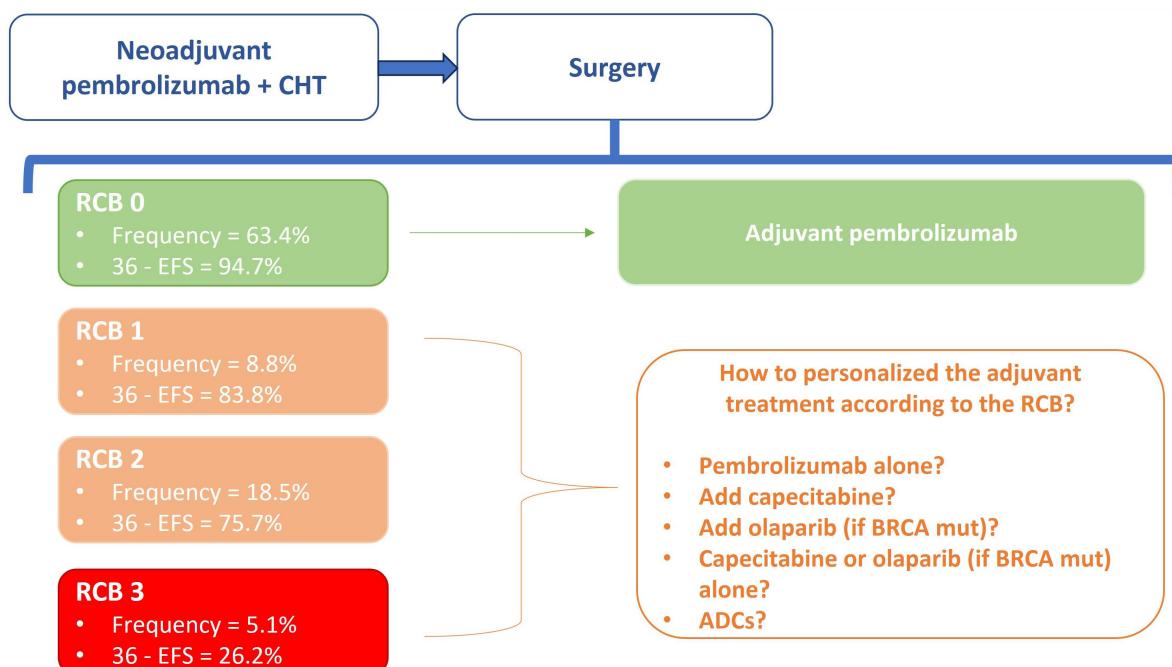


Figure 1. The unmet need for the optimal adjuvant treatment according to RCB [85]

The HR for recurrence event in subgroups RBO-0, RCB-1, RCB-2, and RCB-3 are respectively 0.70 (rates: 5.2% vs. 7.3% in the pembrolizumab + CHT vs. placebo + CHT), 0.92 (rates: 17.4% vs. 20%), 0.52 (rates: 25.5% vs. 44.3%), 1.24 (72.5% vs. 69.2%).

The rate of recurrence was numerically lower in all RCB groups with pembrolizumab + CHT, except in the small RCB-3 subset (that is represented by 5% and 7% of the population in the study, respectively in the experimental and control group). Pembrolizumab shifted RCB to lower categories in most patients (RCB-0: 63% vs. 56% of patients in the experimental vs. the control arm; RCB-1: 9% vs. 11%; RCB 18% vs. 20%).

No patients in this trial received adjuvant capecitabine, and there are no randomized efficacy and safety data showing that multiagent therapy with pembrolizumab and capecitabine is superior to single-agent therapy in high-risk patients (stage II-III) who did not reach pCR.

At the time, only results from phase II studies in metastatic TNBC demonstrated no new safety signals with this combination [86, 87].

Pembrolizumab has also not been studied in combination with olaparib in the adjuvant setting, for the treatment of patients with *BRCA* mutations. No efficacy data are reported in the literature, even if some

safety data are reported in the metastatic setting, in some early-phase studies that have evaluated the combination of PARPis and ICIs, not reporting unexpected toxicities [88, 89].

Prospective trials would be needed to define what is the optimal adjuvant strategy according to RCB (single-agent CHT or poly-CHT), how the clinician should decide between olaparib, immunotherapy or capecitabine in the treatment of the population with *BRCA* mutations and whether these therapies can be administered in combination or sequence, with data in terms of efficacy and safety.

Additional treatment strategies with new drugs are being studied as adjuvant treatment after NACT, with antibody-drug conjugates (ADCs) such as datopotamab deruxtecan (with or without durvalumab in TROPICS-Breast 03, ClinicalTrials.gov identifier: NCT05629585), and patritumab deruxtecan (HER3-DXd) which showed promising clinical response and biological changes in early TNBC [SOLTI TOT-HER3 window of opportunity trial part B, presented at European Society for Medical Oncology (ESMO) Breast 2023], or with ICIs (A-BRAVE trial, NCT02926196 and SWOG S1418/BR006 trial, NCT02954874).

New biomarkers and frontiers in TNBC

Recent progress in integrating ICIs and novel agents has revolutionized the therapeutic approach for early TNBC. Treatment strategies now emphasize escalating chemotherapeutic agents based on standard neoadjuvant regimens. An example is a phase II trial (ACTRN12617000651381) presented at the San Antonio Breast Cancer Symposium 2022 evaluating in high-risk TNBC, the addition of ipilimumab and nivolumab to neoadjuvant paclitaxel following a suboptimal response to anthracycline-based CHT (< 50% tumor reduction) and resulting in promising objective response rate (ORR) (43.7%) and pCR (18.8%) rates, regardless of PD-L1 status.

However, it is also crucial to identify subgroups of patients with favorable prognoses, where NACT could potentially be de-escalated. Therefore, discovering novel biomarkers to categorize patients with good prognoses and safely de-escalate NACT is essential.

TILs show promise as a biomarker for selecting patients who may have favorable outcomes with treatment de-escalation. In recent trials, higher TILs levels were associated with a higher pCR rate [71, 75, 90] and with a better response [75, 91]. Liquid biopsies, such as circulating tumor DNA (ctDNA), could serve as promising markers for identifying patients who might benefit from de-escalating or escalating neoadjuvant or adjuvant treatment. Rapid ctDNA clearance during NACT in early TNBC is linked to a high likelihood of achieving pCR [92]. Conversely, detecting ctDNA after completing NACT and surgery is associated with higher recurrence rates and poorer prognoses [93]. The use of dynamic biomarkers, such as ctDNA, to guide the choice of treatments in high-risk patients appears increasingly to be an important resource to be exploited in future studies.

Furthermore, ADCs are emerging. Particularly, sacituzumab govitecan (SG) an ADC targeting Trop-2 was approved in metastatic TNBC patients who received ≥ 2 prior systemic therapies in the light of the results of the phase III ASCENT study. In this trial patients were randomized (1:1) to receive sacituzumab govitecan 10 mg/kg via intravenous infusion on day 1 and day 8 of a 21-day treatment cycle or a treatment of physician's choice (TPC) achieving the primary endpoint (PFS 4.8 vs. 1.7 months) and also demonstrating an advantage in terms of OS (11.8 months vs. 6.9 months) [94]. Another single-arm phase II trial is evaluating SG and atezolizumab in combination as adjuvant treatment for patients with TNBC who have residual invasive disease after neoadjuvant therapy and detectable ctDNA (ClinicalTrials.gov identifier NCT04434040).

Finally, it is essential to redefine, with new dedicated trials, the role of ER-low (1–9%) BC which, biologically and prognostically very similar to TNBC, could potentially benefit from the addition of immunotherapy to CHT and the role of HER-2 low [score 1+ or 2+ not amplified in fluorescence *in situ* hybridization (FISH)] BC in the light of recent results of efficacy of trastuzumab deruxtecan in advanced BC HER-2 low. Therefore, future studies are likely to expand the armamentarium at our disposal in this setting.

New frontiers in early TNBC are summarized in [Figure 2](#).

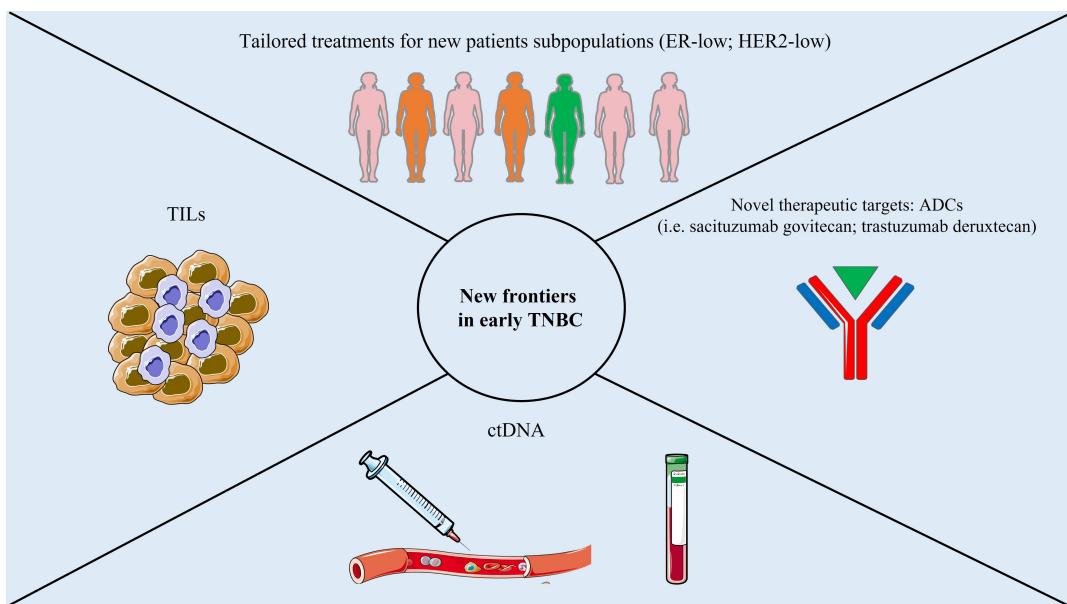


Figure 2. New frontiers in early TNBC

Interpretation and clinical implications

TNBC has long been a challenging disease to treat due to its aggressive behavior and the lack of target therapies [95].

Thanks to recent developments on TNBC, a series of therapeutic targets have been identified for the treatment of metastatic and early setting diseases. Especially for the radically operable disease, the chances of cure are increased with treatments aimed at reducing the odds of recurrence after tumor removal.

Anthracycline and taxane-based poly-CHT remains the standard of treatment, most often administered preoperatively to assess tumor sensitivity. It aims to increase the rate of local control, making it useful to guide breast-conserving surgery and to ensure survival benefits by reaching the pCR.

The introduction of immunotherapy in association with poly-CHT in the neoadjuvant setting has increased the rate of pCR, guaranteeing better results in terms of long-term benefits in the KEYNOTE-522, the pivotal trial that led to the approval in clinical practice of the use of the anti-PD1, pembrolizumab, in the early setting disease (neoadjuvant and adjuvant setting). These clinical findings were based on preclinical investigations that overturned the previous belief that BC was not an immunogenic disease [12].

The actual need is to define the optimal adjuvant strategy after neoadjuvant chemo-immunotherapy, which must be affected by the patient's risk of recurrence based on the histological prognostic and evidence after radical surgery, the individual's tolerance of therapy-induced side effects ([Figure 3](#)).

In patients with low RCB and a low overall risk of recurrence, pembrolizumab alone should be continued. In patients with poor prognostic features of high RCB, this strategy may not be the best choice. Patients with high RCB, BRCA wild type, could benefit from capecitabine alone, although it would be reasonable to use a combination of capecitabine and pembrolizumab. Patients with high RCB, germline *BRCA* mutations, could benefit from olaparib (according to the inclusion criteria of the OlympiA trial), although it would be reasonable to use olaparib and pembrolizumab in combination or sequentially.

However, none of these strategies, in monotherapy and/or in combination, have evidence from specific randomized trials after the neoadjuvant immunotherapy. There are no data on efficacy and safety in this setting. Currently, the best schedule is not known, and new data are awaited on new adjuvant strategies.

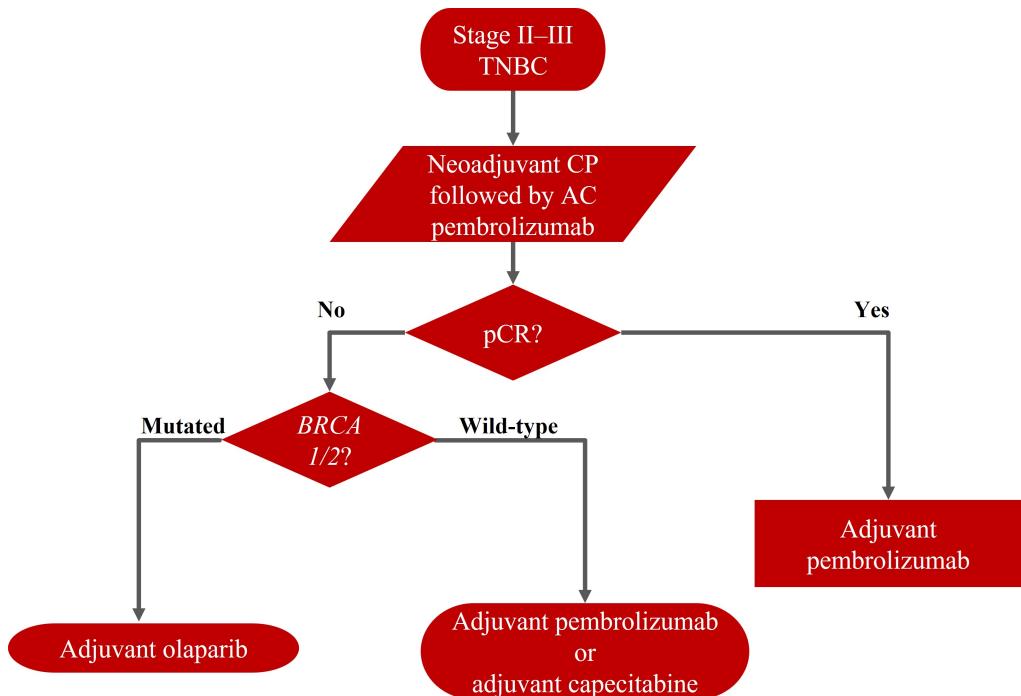


Figure 3. Current treatment algorithm for stage II–III TNBC

Extensive efforts will also be required to investigate and expand access to immunotherapy to ER-low populations (ER 1–9%), not included in KEYNOTE-522. It represents a subgroup that does not formally meet the definition of TNBC, but shares biology, with nearly 90% of these tumors harboring a basal-like intrinsic subtype, and prognosis with TNBC and could share the same benefit from the addition of immunotherapy [96, 97].

Furthermore, novel active agents are emerging for the treatment of TNBC and could provide an opportunity for a de-escalation of traditional CHT, the anti-trophoblast cell-surface antigen 2 (Trop2) sacituzumab govitecan that is currently being investigated in the early setting, including in combination with immunotherapy in the ASPRIA trial (ClinicalTrials.gov identifier NCT04434040).

Conclusions

This review highlights the multitude of advances in the treatment of early-stage TNBC and the important issues raised.

The management of triple-negative breast cancer (TNBC) has seen notable advancements with the identification of therapeutic targets and successful integration of immunotherapy in neoadjuvant treatment. However, the current challenge lies in determining the optimal adjuvant strategy post-chemoimmunotherapy, tailoring decisions to individual patient characteristics and prognostic factors. The uncertainty surrounding the efficacy and safety of these strategies necessitates further randomized studies, while ongoing research explores novel approaches, such as the potential use of innovative agents like sacituzumab govitecan in the context of de-escalating traditional CHT. The imperative to extend access to immunotherapy to subgroups, such as those with low ER expression, holds crucial promise, paving the way for a more personalized and targeted future direction in TNBC treatment.

In the next few years, it will be necessary to design new prospective clinical trials and wait for the results of those in progress, for a better knowledge of the efficacy of combination therapies, therapeutic sequences and new target drugs for the treatment of a disease which up to a few years ago was considered “untargetable”. This should be accompanied by a commitment to biomarker discovery, which could help the oncologist make the best decision for patient care.

Abbreviations

AC: doxorubicin and cyclophosphamide
ADCs: antibody-drug conjugates
BC: breast cancer
BRCA: breast cancer susceptibility genes
CHT: chemotherapy
CI: confidence interval
CREATE-X: Capecitabine for Residual Cancer as Adjuvant Therapy
ctDNA: circulating tumor DNA
DFS: disease-free survival
EFS: event-free survival
ER: estrogen receptor
FDA: Food and Drug Administration
HER2: human epidermal growth factor receptor 2
HR: hazard ratio
HR+: hormone receptor-positive
ICI: immune checkpoint inhibitor
iDFS: invasive disease-free survival
IM: immunomodulatory
LAR: luminal androgen receptor
M: mesenchymal
NACT: neoadjuvant chemotherapy
OS: overall survival
PARP: Poly(ADP-ribose) polymerase
pCR: pathologic complete response
PD-L1: programmed death ligand 1
RCB: residual cancer burden
TILs: tumor-infiltrating lymphocytes
TMB: tumor mutational burden
TME: tumor microenvironment
TNBC: triple negative breast cancer

Declarations

Author contributions

PDS: Conceptualization, Investigation, Writing—original draft, Writing—review & editing, Validation, Supervision. MP, CG, and GRO: Conceptualization, Investigation, Writing—original draft, Writing—review & editing. ANS, PF, and CL: Validation, Writing—review & editing, Supervision. DC: Investigation. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

Not applicable.

Funding

Not applicable.

Copyright

© The Author(s) 2024.

References

1. Sporikova Z, Koudelakova V, Trojanec R, Hajduch M. Genetic markers in triple-negative breast cancer. *Clin Breast Cancer*. 2018;18:e841–50.
2. Howard FM, Olopade OI. Epidemiology of triple-negative breast cancer: a review. *Cancer J*. 2021;27:8–16.
3. Almansour NM. Triple-negative breast cancer: a brief review about epidemiology, risk factors, signaling pathways, treatment and role of artificial intelligence. *Front Mol Biosci*. 2022;9:836417.
4. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*. 2012;486:346–52.
5. Azim HA, Ghosn M, Oualla K, Kassem L. Personalized treatment in metastatic triple-negative breast cancer: the outlook in 2020. *Breast J*. 2020;26:69–80.
6. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, et al.; ESMO Guidelines Committee. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up[†]. *Ann Oncol*. 2019;30:1194–220. Erratum in: *Ann Oncol*. 2019;30:1674. Erratum in: *Ann Oncol*. 2021;32:284.
7. Bear HD, Anderson S, Brown A, Smith R, Mamounas EP, Fisher B, et al.; National Surgical Adjuvant Breast and Bowel Project Protocol B-27. The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27. *J Clin Oncol*. 2003;21:4165–74.
8. Golshan M, Loibl S, Wong SM, Houben JB, O'Shaughnessy J, Rugo HS, et al. Breast conservation after neoadjuvant chemotherapy for triple-negative breast cancer: surgical results from the BrightNess randomized clinical trial. *JAMA Surg*. 2020;155:e195410. Erratum in: *JAMA Surg*. 2021;156:503.
9. Turner N, Lambros MB, Horlings HM, Pearson A, Sharpe R, Natrajan R, et al. Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. *Oncogene*. 2010;29:2013–23.
10. Marra A, Trapani D, Viale G, Criscitiello C, Curigliano G. Practical classification of triple-negative breast cancer: intratumoral heterogeneity, mechanisms of drug resistance, and novel therapies. *NPJ Breast Cancer*. 2020;6:54.

11. Ye F, Dewanjee S, Li Y, Jha NK, Chen ZS, Kumar A, et al. Advancements in clinical aspects of targeted therapy and immunotherapy in breast cancer. *Mol Cancer*. 2023;22:105.
12. Liu Z, Li M, Jiang Z, Wang X. A comprehensive immunologic portrait of triple-negative breast cancer. *Transl Oncol*. 2018;11:311–29.
13. Farshbafnadi M, Pastaki Khoshbin A, Rezaei N. Immune checkpoint inhibitors for triple-negative breast cancer: from immunological mechanisms to clinical evidence. *Int Immunopharmacol*. 2021;98:107876.
14. Lehmann BD, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y, et al. Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. *PLoS One*. 2016;11:e0157368.
15. Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, Fuqua SAW, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. *Clin Cancer Res*. 2015;21:1688–98.
16. Xiao Y, Ma D, Zhao S, Suo C, Shi J, Xue MZ, et al.; AME Breast Cancer Collaborative Group. Multi-omics profiling reveals distinct microenvironment characterization and suggests immune escape mechanisms of triple-negative breast cancer. *Clin Cancer Res*. 2019;25:5002–14.
17. Pelly VS, Moeini A, Roelofsen LM, Bonavita E, Bell CR, Hutton C, et al. Anti-inflammatory drugs remodel the tumor immune environment to enhance immune checkpoint blockade efficacy. *Cancer Discov*. 2021;11:2602–19.
18. Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, et al. PD-L1 expression in triple-negative breast cancer. *Cancer Immunol Res*. 2014;2:361–70.
19. Wang C, Zhang J, Wang Y, Ouyang T, Li J, Wang T, et al. Prevalence of *BRCA1* mutations and responses to neoadjuvant chemotherapy among *BRCA1* carriers and non-carriers with triple-negative breast cancer. *Ann Oncol*. 2015;26:523–8.
20. Hahnen E, Lederer B, Hauke J, Loibl S, Kröber S, Schneeweiss A, et al. Germline mutation status, pathological complete response, and disease-free survival in triple-negative breast cancer: secondary analysis of the GeparSixto randomized clinical trial. *JAMA Oncol*. 2017;3:1378–85.
21. Pohl-Rescigno E, Hauke J, Loibl S, Möbus V, Denkert C, Fasching PA, et al. Association of germline variant status with therapy response in high-risk early-stage breast cancer: a secondary analysis of the GeparOcto randomized clinical trial. *JAMA Oncol*. 2020;6:744–8.
22. Vollebergh MA, Lips EH, Nederlof PM, Wessels LF, Wesseling J, Vd Vijver MJ, et al. Genomic patterns resembling *BRCA1*- and *BRCA2*-mutated breast cancers predict benefit of intensified carboplatin-based chemotherapy. *Breast Cancer Res*. 2014;16:R47.
23. Belli C, Duso BA, Ferraro E, Curigliano G. Homologous recombination deficiency in triple negative breast cancer. *Breast*. 2019;45:15–21.
24. van Verschuer VMT, Hooning MJ, van Baare-Georgieva RD, Hollestelle A, Timmermans AM, Koppert LB, et al. Tumor-associated inflammation as a potential prognostic tool in *BRCA1/2*-associated breast cancer. *Hum Pathol*. 2015;46:182–90.
25. Nolan E, Savas P, Policheni AN, Darcy PK, Vaillant F, Mintoff CP, et al.; Kathleen Cunningham Foundation Consortium for Research into Familial Breast Cancer (kConFab); Perou CM, Visvader JE, Gray DHD, Loi S, Lindeman GJ. Combined immune checkpoint blockade as a therapeutic strategy for *BRCA1*-mutated breast cancer. *Sci Transl Med*. 2017;9:eaal4922.
26. Parkes EE, Walker SM, Taggart LE, McCabe N, Knight LA, Wilkinson R, et al. Activation of STING-dependent innate immune signaling by S-phase-specific DNA damage in breast cancer. *J Natl Cancer Inst*. 2016;109:djw199.
27. Castaneda CA, Mittendorf E, Casavilca S, Wu Y, Castillo M, Arboleda P, et al. Tumor infiltrating lymphocytes in triple negative breast cancer receiving neoadjuvant chemotherapy. *World J Clin Oncol*. 2016;7:387–94.

28. García-Teijido P, Cabal ML, Fernández IP, Pérez YF. Tumor-infiltrating lymphocytes in triple negative breast cancer: the future of immune targeting. *Clin Med Insights Oncol.* 2016;10:31–9.
29. Gomez-Macias GS, Molinar-Flores G, Lopez-Garcia CA, Santuario-Facio S, Decanini-Arcuate H, Valero-Elizondo J, et al. Immunotyping of tumor-infiltrating lymphocytes in triple-negative breast cancer and genetic characterization. *Oncol Lett.* 2020;20:140.
30. Sousa S, Brion R, Lintunen M, Kronqvist P, Sandholm J, Mönkkönen J, et al. Human breast cancer cells educate macrophages toward the M2 activation status. *Breast Cancer Res.* 2015;17:101.
31. Medrek C, Pontén F, Jirström K, Leandersson K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. *BMC Cancer.* 2012;12:306.
32. Savas P, Salgado R, Denkert C, Sotiriou C, Darcy PK, Smyth MJ, et al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. *Nat Rev Clin Oncol.* 2016;13:228–41.
33. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet.* 2014;384:164–72.
34. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol.* 2018;19:40–50.
35. Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. *J Clin Oncol.* 2014;32:2959–66.
36. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. *J Clin Oncol.* 2013;31:860–7.
37. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. *Ann Oncol.* 2014;25:1544–50.
38. Hida AI, Watanabe T, Sagara Y, Kashiwaba M, Sagara Y, Aogi K, et al. Diffuse distribution of tumor-infiltrating lymphocytes is a marker for better prognosis and chemotherapeutic effect in triple-negative breast cancer. *Breast Cancer Res Treat.* 2019;178:283–94.
39. Ibrahim EM, Al-Foheidi ME, Al-Mansour MM, Kazkaz GA. The prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancer: a meta-analysis. *Breast Cancer Res Treat.* 2014;148:467–76.
40. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruner G, et al.; International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol.* 2015;26:259–71.
41. Karn T, Jiang T, Hatzis C, Sänger N, El-Balat A, Rody A, et al. Association between genomic metrics and immune infiltration in triple-negative breast cancer. *JAMA Oncol.* 2017;3:1707–11.
42. Safonov A, Jiang T, Bianchini G, Győrffy B, Karn T, Hatzis C, et al. Immune gene expression is associated with genomic aberrations in breast cancer. *Cancer Res.* 2017;77:3317–24.
43. Lotfinejad P, Asghari Jafarabadi M, Abdoli Shabdar M, Kazemi T, Pashazadeh F, Sandoghchian Shotorbani S, et al. Prognostic role and clinical significance of tumor-infiltrating lymphocyte (TIL) and programmed death ligand 1 (PD-L1) expression in triple-negative breast cancer (TNBC): a systematic review and meta-analysis study. *Diagnostics (Basel).* 2020;10:704.
44. Zhu X, Zhang Q, Wang D, Liu C, Han B, Yang JM. Expression of PD-L1 attenuates the positive impacts of high-level tumor-infiltrating lymphocytes on prognosis of triple-negative breast cancer. *Cancer Biol Ther.* 2019;20:1105–12.

45. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Breast Cancer. National Comprehensive Cancer Network® (NCCN®); c2020 [cited 2023 May 21]. Available from: <https://www2.tri-kobe.org/nccn/guideline/breast/english/breast.pdf>
46. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Anthracycline-containing and taxane-containing chemotherapy for early-stage operable breast cancer: a patient-level meta-analysis of 100 000 women from 86 randomised trials. *Lancet.* 2023;401:1277-1292.
47. Sparano JA, Zhao F, Martino S, Ligibel JA, Perez EA, Saphner T, et al. Long-term follow-up of the E1199 phase III trial evaluating the role of taxane and schedule in operable breast cancer. *J Clin Oncol.* 2015; 33:2353-60.
48. Jones SE, Savin MA, Holmes FA, O'Shaughnessy JA, Blum JL, Vukelja S, et al. Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide as adjuvant therapy for operable breast cancer. *J Clin Oncol.* 2006;24:5381-7. Erratum in: *J Clin Oncol.* 2007;25:1819.
49. Wheate NJ, Collins JG. Multi-nuclear platinum drugs: a new paradigm in chemotherapy. *Curr Med Chem Anticancer Agents.* 2005;5:267-79.
50. Garutti M, Pelizzari G, Bartoletti M, Malfatti MC, Gerratana L, Tell G, et al. Platinum salts in patients with breast cancer: a focus on predictive factors. *Int J Mol Sci.* 2019;20:3390.
51. Chalasani P, Livingston R. Differential chemotherapeutic sensitivity for breast tumors with "BRCAness": a review. *Oncologist.* 2013;18:909-16.
52. Vetter M, Fokas S, Biskup E, Schmid T, Schwab F, Schoetzau A, et al. Efficacy of adjuvant chemotherapy with carboplatin for early triple negative breast cancer: a single center experience. *Oncotarget.* 2017; 8:75617-26.
53. Su YW, Hung CY, Lam HB, Chang YC, Yang PS. A single institution experience of incorporation of cisplatin into adjuvant chemotherapy for patients with triple-negative breast cancer of unknown *BRCA* mutation status. *Clin Med Insights Oncol.* 2018;12:1179554918794672.
54. Yu KD, Ye FG, He M, Fan L, Ma D, Mo M, et al. Effect of adjuvant paclitaxel and carboplatin on survival in women with triple-negative breast cancer: a phase 3 randomized clinical trial. *JAMA Oncol.* 2020;6: 1390-6.
55. Norquist B, Wurz KA, Pennil CC, Garcia R, Gross J, Sakai W, et al. Secondary Somatic Mutations Restoring *BRCA1/2* Predict Chemotherapy Resistance in Hereditary Ovarian Carcinomas. *J Clin Oncol.* 2011;29:3008-15.
56. Guillemette S, Serra RW, Peng M, Hayes JA, Konstantinopoulos PA, Green MR, et al. Resistance to therapy in *BRCA2* mutant cells due to loss of the nucleosome remodeling factor CHD4. *Genes Dev.* 2015;29:489-94.
57. Zhou J, Kang Y, Chen L, Wang H, Liu J, Zeng S, et al. The drug-resistance mechanisms of five platinum-based antitumor agents. *Front Pharmacol.* 2020;11:343.
58. Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A, et al. Preoperative chemotherapy: updates of national surgical adjuvant breast and bowel project protocols B-18 and B-27. *J Clin Oncol.* 2008;26:778-85. Erratum in: *J Clin Oncol.* 2008;26:2793.
59. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials. *Lancet Oncol.* 2018;19:27-39.
60. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Increasing the dose intensity of chemotherapy by more frequent administration or sequential scheduling: a patient-level meta-analysis of 37 298 women with early breast cancer in 26 randomised trials. *Lancet.* 2019;393: 1440-52.
61. von Minckwitz G, Schneeweiss A, Loibl S, Salat C, Denkert C, Rezai M, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. *Lancet Oncol.* 2014;15:747-56.

62. Sikov WM, Berry DA, Perou CM, Singh B, Cirrincione CT, Tolaney SM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). *J Clin Oncol.* 2015;33:13–21.
63. Loibl S, O'Shaughnessy J, Untch M, Sikov WM, Rugo HS, McKee MD, et al. Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrightNess): a randomised, phase 3 trial. *Lancet Oncol.* 2018;19: 497–509.
64. Fasching PA, Link T, Hauke J, Seither F, Jackisch C, Klare P, et al.; German Breast Group and Arbeitsgemeinschaft Gynäkologische Onkologie Breast. Neoadjuvant paclitaxel/olaparib in comparison to paclitaxel/carboplatinum in patients with HER2-negative breast cancer and homologous recombination deficiency (GeparOLA study). *Ann Oncol.* 2021;32:49–57.
65. Gupta S, Nair NS, Hawaldar R, Vanmali V, Parmar V, Gulia S, et al. Abstract GS5-01: Addition of platinum to sequential taxane-anthracycline neoadjuvant chemotherapy in patients with triple-negative breast cancer: a phase III randomized controlled trial. *Cancer Res.* 2023;83:GS5-01.
66. Cortes J, Cescon DW, Rugo HS, Nowecki Z, Im SA, Yusof MM, et al.; KEYNOTE-355 Investigators. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. *Lancet.* 2020;396:1817–28.
67. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al.; IMpassion130 Trial Investigators. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med.* 2018;379:2108–21.
68. Miles D, Gligorov J, André F, Cameron D, Schneeweiss A, Barrios C, et al.; IMpassion131 investigators. Primary results from IMpassion131, a double-blind, placebo-controlled, randomised phase III trial of first-line paclitaxel with or without atezolizumab for unresectable locally advanced/metastatic triple-negative breast cancer. *Ann Oncol.* 2021;32:994–1004.
69. Emens LA, Adams S, Barrios CH, Diéras V, Iwata H, Loi S, et al. First-line atezolizumab plus nab-paclitaxel for unresectable, locally advanced, or metastatic triple-negative breast cancer: IMpassion130 final overall survival analysis. *Ann Oncol.* 2021;32:983–93. Erratum in: *Ann Oncol.* 2021;32:1650.
70. Cortes J, Rugo HS, Cescon DW, Im SA, Yusof MM, Gallardo C, et al.; KEYNOTE-355 Investigators. Pembrolizumab plus chemotherapy in advanced triple-negative breast cancer. *N Engl J Med.* 2022; 387:217–26.
71. Gianni L, Huang CS, Egle D, Bermejo B, Zamagni C, Thill M, et al. Pathologic complete response (pCR) to neoadjuvant treatment with or without atezolizumab in triple-negative, early high-risk and locally advanced breast cancer: NeoTRIP Michelangelo randomized study. *Ann Oncol.* 2022;33:534–43.
72. Mittendorf EA, Zhang H, Barrios CH, Saji S, Jung KH, Hegg R, et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. *Lancet.* 2020;396:1090–100.
73. Schmid P, Cortes J, Dent R, Pusztai L, McArthur H, Kümmel S, et al.; KEYNOTE-522 Investigators. Event-free survival with pembrolizumab in early triple-negative breast cancer. *N Engl J Med.* 2022; 386:556–67.
74. Del Mastro L, Poggio F, Blondeaux E, De Placido S, Giuliano M, Forestieri V, et al.; Gruppo Italiano Mammella Investigators. Fluorouracil and dose-dense adjuvant chemotherapy in patients with early-stage breast cancer (GIM2): end-of-study results from a randomised, phase 3 trial. *Lancet Oncol.* 2022;23:1571–82.

75. Loibl S, Schneeweiss A, Huober J, Braun M, Rey J, Blohmer JU, et al.; GBG and AGO-B. Neoadjuvant durvalumab improves survival in early triple-negative breast cancer independent of pathological complete response. *Ann Oncol*. 2022;33:1149–58.
76. Kuroi K, Toi M, Ohno S, Nakamura S, Iwata H, Masuda N, et al. Prognostic significance of subtype and pathologic response in operable breast cancer; a pooled analysis of prospective neoadjuvant studies of JBCRG. *Breast Cancer*. 2015;22:486–95.
77. Spring LM, Fell G, Arfe A, Sharma C, Greenup R, Reynolds KL, et al. Pathologic complete response after neoadjuvant chemotherapy and impact on breast cancer recurrence and survival: a comprehensive meta-analysis. *Clin Cancer Res*. 2020;26:2838–48.
78. Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart MJ, et al.; Panel Members. Tailoring therapies—improving the management of early breast cancer: St Gallen international expert consensus on the primary therapy of early breast cancer 2015. *Ann Oncol*. 2015;26:1533–46.
79. Masuda N, Lee SJ, Ohtani S, Im YH, Lee ES, Yokota I, et al. Adjuvant Capecitabine for Breast Cancer after Preoperative Chemotherapy. *N Engl J Med*. 2017;376:2147–59.
80. Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. *J Clin Oncol*. 2007;25:4414–22.
81. Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, et al.; OlympiA Clinical Trial Steering Committee and Investigators. Adjuvant olaparib for patients with *BRCA1*- or *BRCA2*-mutated breast cancer. *N Engl J Med*. 2021;384:2394–405.
82. Geyer CE Jr, Garber JE, Gelber RD, Yothers G, Taboada M, Ross L, et al.; OlympiA Clinical Trial Steering Committee and Investigators. Overall survival in the OlympiA phase III trial of adjuvant olaparib in patients with germline pathogenic variants in *BRCA1/2* and high-risk, early breast cancer. *Ann Oncol*. 2022;33:1250–68.
83. FDA D.I.S.C.D. burst edition: FDA approval of Keytruda (pembrolizumab) for high-risk early-stage triple-negative breast cancer [Internet]. [cited 2023 May 21]. Available from: <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-disco-burst-edition-fda-approval-keytruda-pembrolizumab-high-risk-early-stage-triple-negative>
84. Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, et al.; KEYNOTE-522 Investigators. Pembrolizumab for early triple-negative breast cancer. *N Engl J Med*. 2020;382:810–21.
85. Pusztai L, Denkert C, O'Shaughnessy J, Cortes J, Dent RA, McArthur HL, et al. Event-free survival by residual cancer burden after neoadjuvant pembrolizumab + chemotherapy versus placebo + chemotherapy for early TNBC: exploratory analysis from KEYNOTE-522. *J Clin Oncol*. 2022;40:503.
86. Shah AN, Flaum L, Helenowski I, Santa-Maria CA, Jain S, Rademaker A, et al. Phase II study of pembrolizumab and capecitabine for triple negative and hormone receptor-positive, HER2-negative endocrine-refractory metastatic breast cancer. *J Immunother Cancer*. 2020;8:e000173.
87. Page D, Pucilowska J, Bennetts L, Kim I, Sanchez K, Martel M, et al. Updated efficacy of first or second-line pembrolizumab plus in metastatic triple negative breast cancer and correlations with baseline lymphocyte and naïve CD4+ T-cell count. 2018 San Antonio Breast Cancer Symposium. San Antonio: Books, Presentations, Posters, Etc. 2018.
88. Vinayak S, Tolaney SM, Schwartzberg L, Mita M, McCann G, Tan AR, et al. Open-label clinical trial of niraparib combined with pembrolizumab for treatment of advanced or metastatic triple-negative breast cancer. *JAMA Oncol*. 2019;5:1132–40.
89. Domchek SM, Postel-Vinay S, Im SA, Park YH, Delord JP, Italiano A, et al. Olaparib and durvalumab in patients with germline *BRCA*-mutated metastatic breast cancer (MEDIOLA): an open-label, multicentre, phase 1/2, basket study. *Lancet Oncol*. 2020;21:1155–64.

90. Nederlof I, De Bortoli D, Bareche Y, Hooijer GKJ, Sotiriou C, Van De Vijver MJ, et al. Relationship between tumor infiltrating lymphocytes (TILs) and response to pembrolizumab (pembro)+ chemotherapy (CT) as neoadjuvant treatment (NAT) for triple-negative breast cancer (TNBC): phase Ib KEYNOTE-173 trial. *Ann Oncol*. 2019;30:III2.
91. Doroshow DB, Bhalla S, Beasley MB, Sholl LM, Kerr KM, Gnjatic S, et al. PD-L1 as a biomarker of response to immune-checkpoint inhibitors. *Nat Rev Clin Oncol*. 2021;18:345–62.
92. Magbanua MJM, Swigart LB, Wu HT, Hirst GL, Yau C, Wolf DM, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. *Ann Oncol*. 2021;32:229–39.
93. Radovich M, Jiang G, Hancock BA, Chitambar C, Nanda R, Falkson C, et al. Association of circulating tumor DNA and circulating tumor cells after neoadjuvant chemotherapy with disease recurrence in patients with triple-negative breast cancer: preplanned secondary analysis of the BRE12-158 randomized clinical trial. *JAMA Oncol*. 2020;6:1410–5.
94. Bardia A, Hurvitz SA, Tolaney SM, Loirat D, Punie K, Oliveira M, et al.; ASCENT Clinical Trial Investigators. Sacituzumab govitecan in metastatic triple-negative breast cancer. *N Engl J Med*. 2021;384:1529–41.
95. Loibl S, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast cancer. *Lancet*. 2021;397:1750–69.
96. Schrodi S, Braun M, Andrulat A, Harbeck N, Mahner S, Kiechle M, et al. Outcome of breast cancer patients with low hormone receptor positivity: analysis of a 15-year population-based cohort. *Ann Oncol*. 2021;32:1410–24.
97. Villegas SL, Nekljudova V, Pfarr N, Engel J, Untch M, Schrodi S, et al. Therapy response and prognosis of patients with early breast cancer with low positivity for hormone receptors – An analysis of 2765 patients from neoadjuvant clinical trials. *Eur J Cancer*. 2021;148:159–70.

Review

Immunotherapy for Triple-Negative Breast Cancer: Combination Strategies to Improve Outcome

Liying Li, Fan Zhang, Zhenyu Liu and Zhimin Fan *

Department of Breast Surgery, General Surgery Centre, The First Hospital of Jilin University, Changchun 130012, China

* Correspondence: fanzm@jlu.edu.cn

Simple Summary: For decades, countless efforts have been devoted to developing targeted drugs to improve the prognosis of triple-negative breast cancer (TNBC). Among the novel therapies that have been approved for the clinical management of TNBC, immunotherapy shows great potential. Although exciting progress has been made in immunotherapy for TNBC, there are still gaps to fill. This review will analyze current immunotherapy strategies in TNBC, summarize the current landscape of clinical trials, review the results achieved, and shed light on future developments.

Abstract: Due to the absence of hormone receptor (*both estrogen receptors and progesterone receptors*) along with human epidermal growth factor receptor 2 (HER-2) amplification, the treatment of triple-negative breast cancer (TNBC) cannot benefit from endocrine or anti-HER-2 therapy. For a long time, chemotherapy was the only systemic treatment for TNBC. Due to the lack of effective treatment options, the prognosis for TNBC is extremely poor. The successful application of immune checkpoint inhibitors (ICIs) launched the era of immunotherapy in TNBC. However, the current findings show modest efficacy of programmed cell death- (ligand) 1 (PD-(L)1) inhibitors monotherapy and only a small proportion of patients can benefit from this approach. Based on the basic principles of immunotherapy and the characteristics of the tumor immune microenvironment (TIME) in TNBC, immune combination therapy is expected to further enhance the efficacy and expand the beneficiary population of patients. Given the diversity of drugs that can be combined, it is important to select effective biomarkers to identify the target population. Moreover, the side effects associated with the combination of multiple drugs should also be considered.

Keywords: triple-negative breast cancer; immunotherapy; immune checkpoint; tumor immune microenvironment; clinical trials



Citation: Li, L.; Zhang, F.; Liu, Z.; Fan, Z. Immunotherapy for Triple-Negative Breast Cancer: Combination Strategies to Improve Outcome. *Cancers* **2023**, *15*, 321. <https://doi.org/10.3390/cancers15010321>

Academic Editors: Claudia De Lorenzo and Laura Cernchia

Received: 14 November 2022

Revised: 27 December 2022

Accepted: 29 December 2022

Published: 3 January 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Triple-negative breast cancer (TNBC) accounts for approximately 15–20% of breast malignancies and is the only subtype of breast cancer that lacks targeted treatment [1]. Compared with other subtypes, TNBC is more aggressive, and most patients develop recurrence and metastasis within 3 years, with poor prognosis [2]. Anthracycline- and taxane-based chemotherapy remains the mainstay of treatment for early-stage patients, but resistance has emerged [3]; for patients with recurrence or metastasis, there are even fewer treatment options. There is an urgent need for novel and more effective treatments.

Genomic advances reveal a high degree of heterogeneity in TNBC and set the stage for the development of targeted therapies [4–6]. In the past few years, poly ADP-ribose polymerase inhibitors (PARPi), programmed cell death- (ligand) 1 (PD-(L)1) inhibitors, and antibody–drug conjugates (ADCs) have been approved successively for the treatment of TNBC. Among these, PD-1 inhibitor pembrolizumab is approved for patients with advanced PD-L1-positive and early high-risk disease, displaying great therapeutic potential.

Some studies have found that TNBC has higher PD-L1 expression and tumor-infiltrating lymphocytes (TILs) in comparison with other subtypes [7,8], making it the most likely subtype to benefit from immunotherapy. However, PD-(L)1 inhibitors benefit only a small proportion of individuals, with a single-agent effectiveness of approximately 20% in patients with PD-L1-positive metastatic TNBC (mTNBC) [9]. Numerous preclinical and clinical studies have been conducted to investigate the reasons for this disappointing result, expand the beneficiary population, and improve the efficacy of immune checkpoint inhibitors (ICIs).

In this review, we will analyze the reasons for the poor efficacy of PD-(L)1 inhibitors monotherapy in terms of tumor immune escape mechanisms and tumor immune microenvironment (TIME) characteristics of TNBC. We summarize the current landscape of clinical trials in TNBC, highlight the major immune combination therapy strategies in clinical practice and the progress achieved, and briefly discuss the biomarkers for predicting immunological response, as well as possible adverse events associated with immunotherapy.

2. Rationale of Immunotherapy and the TIME of TNBC

The cross-talk between tumor cells and the TIME can be described as “cancer immunoediting”, encompassing three stages: (1) elimination; (2) equilibrium, in which the host immune system and tumor cells that survive enter a dynamic balance; and (3) escape [10] (Figure 1).

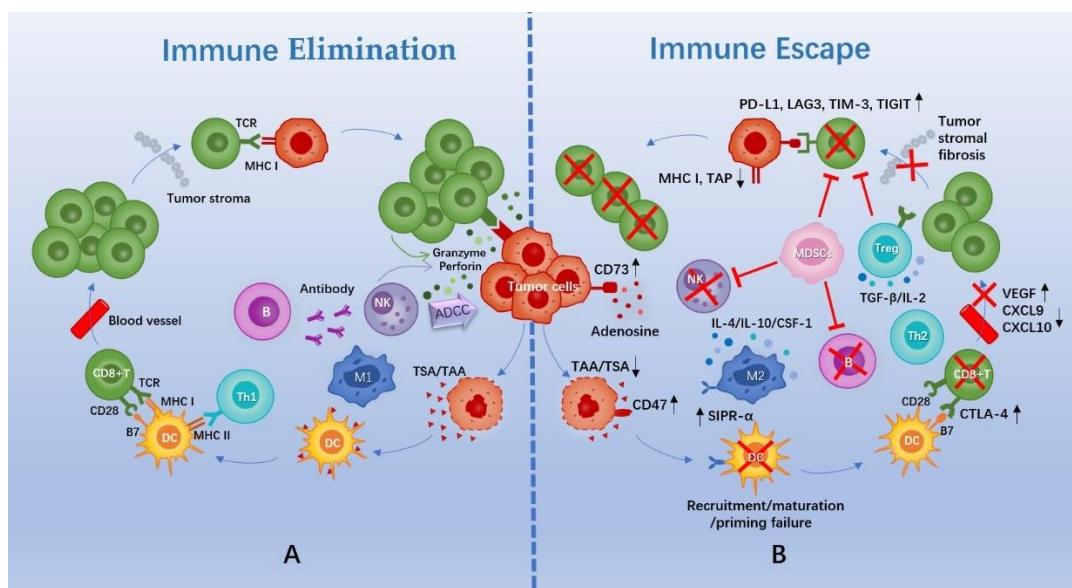


Figure 1. Schematic diagram of immune elimination and escape in TNBC. (A) Cascade events of tumor cell elimination by the immune system. (B) Mechanisms of tumor cell immune escape. Abbreviations: TAA, tumor-associated antigen; TSA, tumor-specific antigen; DC, dendritic cell; M1/2, M1/2 macrophage; Th1/2, CD4+ helper T cell 1/2; TCR, T cell receptor; MHC, major histocompatibility complex; NK, natural killer cell; ADCC, antibody-dependent cell-mediated cytotoxicity; SIPR- α , signal regulatory protein- α ; CTLA-4, cytotoxic T-lymphocyte antigen-4; VEGF, vascular endothelial growth factor; CXCL, chemokine (C-X-C motif) ligand; IL, interleukin; CSF, colony-stimulating factor; TGF, transforming growth factor; Treg, regulatory T cell; MDSCs, myeloid-derived suppressor cells; TAP, transporter associated with antigen processing; PD-L1, programmed death-ligand 1; LAG3, lymphocyte-activation gene 3; TIM-3, T cell immunoglobulin-3; TIGIT, T cell immunoreceptor with Ig and ITIM domains.

Normally, a cascade of events needs to be initiated for the elimination of tumor cells. First, tumor cells release specific antigens that are captured and processed by antigen-presenting cells (APCs), which mainly include dendritic cells (DCs). Next, DCs migrate to

lymphoid tissue to present antigenic signals to T cells. Later, T cells initiate, activate, and then transport and infiltrate tumor tissues. Finally, T cells specifically recognize and kill tumor cells [11]. B cells and innate immune cells, such as natural killer (NK) cells, are also essential for the elimination of tumor cells (Figure 1).

However, tumors can evade surveillance and attack from the immune system via complex intrinsic signaling or external microenvironment mechanisms. In general, this can be summed up in these aspects. First, immunogenicity of tumors is decreased via downregulation of antigen expression, sequestering of antigens, or downregulation of major histocompatibility complex (MHC) molecules [12,13]. Second, there are functional and/or quantitative defects in intrinsic and/or adaptive immune cells, including recruitment failure, insufficient maturation, failure to activate, and impaired chemotaxis and transport [14]. Further, an immunosuppressive microenvironment is generated through an increase in the proportion of immunosuppressive cells (regulatory T cells (Tregs), M2 macrophage cells, and myeloid-derived suppressor cells (MDSCs)) and cytokines, as well as the accumulation of immunosuppressive substances [15,16]. Moreover, there is an upregulation of immune checkpoints, a negative regulatory mechanism used by the body to prevent over-activation of the immune system and to protect normal tissues from the autoimmune system [17,18] (Figure 1).

Based on the above information, immunotherapy has emerged that involves the use of various agents or means to enhance immune system function (in a tumor-localized rather than whole-body manner) or to block immune escape pathways of tumor cells (by normalizing anti-tumor immunity), thereby recognizing and eliminating tumor cells. These immunotherapy approaches include: therapeutic cancer vaccines (TCVs), which are active immunotherapies; targeted monoclonal antibodies (mAb) and their derivatives, such as ADCs, adoptive cell therapies (ACTs), and cytokines, which are passive therapies; as well as the best-known ICIs [19].

Tumors that lack immune cell infiltration and do not respond to ICIs are referred to as “cold tumors”, while tumors with high levels of immune cell infiltration and upregulated immune checkpoints that may respond to ICIs are referred as “hot tumors” [20]. Based on omics analysis, heterogeneity of the TIME in TNBC has been revealed. According to the data from these studies, the percentage of TNBC cases that present with “hot tumors” is approximately 25%, which may explain the poor clinical efficacy of ICIs as single agents [21–23]. Moreover, the suppressive TIME found in “hot tumors” and the decreased MHC of tumor cells also make ICIs less effective [14].

When all of these findings are considered, it is clear that combining ICIs with other therapies that block immune escape pathways of tumor cells or convert “cold tumors” to “hot tumors” is a potential strategy to improve the clinical response of ICIs.

3. The Landscape of Clinical Trials on TNBC Immunotherapy

As of 1 September 2022, we had screened a total of 234 clinical trials on *clinicaltrials.gov* (accessed on 1 September 2022), that primarily explore immunotherapy for TNBC, either as a monotherapy or in combination with other therapies (Figure 2). Apart from that, more than 100 immunotherapy clinical trials are being conducted on advanced solid tumors, including TNBC. Of note, these are just the tip of the iceberg as there are trials registered on other websites. In addition to observing the surge in clinical trials of immunotherapy for TNBC in the past decade, we further analyzed the data and found that: (1) there are 197 trials involving ICIs that target PD-(L)1, accounting for 84% of all trials, of which only 13 trials involved monotherapy, while in the other trials, PD-(L)1 inhibitors were combined with almost all therapies that can be applied to TNBC; (2) there are considerable numbers of clinical trials in both early and metastatic settings, which demonstrates the generalizability of immunotherapy for TNBC; and (3) there is a gradual increase in the number of window-of-opportunity (WOP) trials to test for optimal timing of interventions and changes in biomarkers.

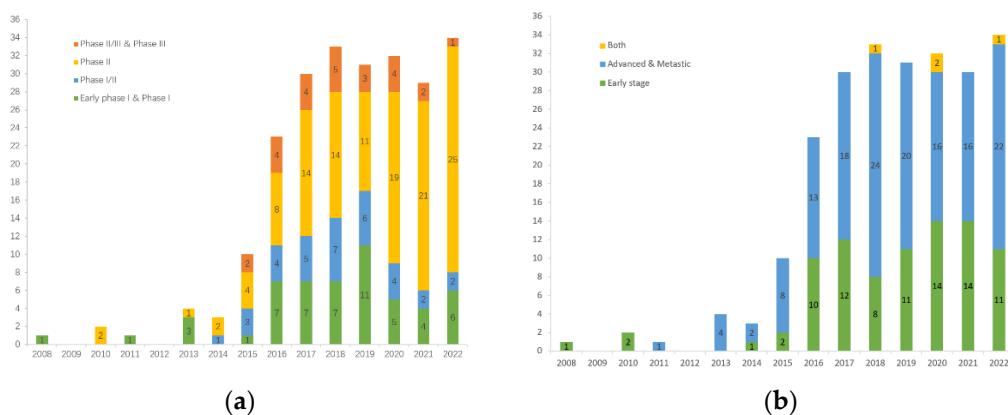


Figure 2. Clinical trials of immunotherapy for TNBC registered at [clinicaltrials.gov](#): (a) Number of clinical trials in each phase per year; (b) Number of clinical trials by disease stage per year.

4. Performance of PD-(L)1 Inhibitors Monotherapy

PD-(L)1 is the most powerful immune checkpoint found in TNBC. PD-1 can be expressed on a variety of immune cells, the most important of which are activated T cells. PD-1 has two ligands: PD-L1 and PD-L2 [24]. PD-L1 is abnormally overexpressed on the surface of tumor cells and some APCs [25]. Through binding to PD-1, it leads to lymphocyte apoptosis, unresponsiveness, and abnormal secretion of cytokines, thus mediating immune escape of tumor cells [25]. In contrast, PD-L2 has a dual role of suppressing and activating T cells, and its role in tumors is gaining attention [26]. PD-(L)1 inhibitors are monoclonal antibodies that block the PD-1/PD-L1 signaling pathway and restore the immune function of T cells to kill tumor cells.

4.1. In Advanced TNBC

The phase Ib study, KEYNOTE-012, first demonstrated acceptable safety and durable anti-tumor activity of single-agent pembrolizumab in previously treated patients with advanced PD-L1-positive TNBC. The objective response rate (ORR) in 32 TNBC patients was 18.5% [27]. The phase II KEYNOTE-086 study then further confirmed the safety and anti-tumor activity of pembrolizumab as first-line or second-line and beyond therapy in patients with mTNBC, using more appropriate dosages and dosing intervals. This study found that pembrolizumab monotherapy performed better in previously untreated PD-L1-positive patients with an ORR of 21.4%, whereas the response was flat in heavily pre-treated mTNBC patients with an ORR of 5.3%. Although there was no significant difference in ORR, progression-free survival (PFS), and overall survival (OS) between the PD-L1-positive and PD-L1-negative subgroups in pre-treated mTNBC, a more durable clinical response was observed in PD-L1-positive patients [28,29]. In the next large phase III randomized KEYNOTE-119 trial, as second- or third-line treatment for mTNBC, pembrolizumab failed to significantly improve OS compared to chemotherapy in the total population (9.9 vs. 10.8 months, HR = 0.97, 95% CI: 0.82 to 1.15) or in the PD-L1-positive population (10.7 vs. 10.2 months, HR = 0.86, 95% CI: 0.69 to 1.06, $p = 0.073$). However, the improved effect of pembrolizumab was consistent with increased tumor PD-L1 expression in the efficacy endpoints of ORR, PFS, and OS [30] (Table 1).

Table 1. Clinical trials of PD-(L)1 inhibitors monotherapy.

Clinical Trial	Phase	Status	Arms (n)	Population (n)	PD-L1 Status	Major Outcomes
Trials in advanced TNBC						
KEYNOTE-012 (NCT01848834)	Ib	Completed	Pemb	Pre-treated: PD-L1 (+) (32)	+ (stroma / ≥ 1% TC ^a)	ORR: 18.5% mPFS: 1.9 months mOS: 11.2 months
KEYNOTE-086 (NCT02447003)	II	Completed	Pemb	Cohort A (170): pre-treated	Overall	ORR: 5.3% mPFS: 2.0 months mOS: 9.0 months
					+ (CPS ^b ≥ 1)	ORR: 5.7% mPFS: 2.0 months mOS: 8.8 months
					—	ORR: 4.7% mPFS: 1.9 months mOS: 9.7 months
KEYNOTE-119 (NCT02555657)	III	Completed	Pemb (312) vs. CT ^c (310)	Pre-treated: 1–2 prior therapy (622)	Overall	ORR: 9.6 vs. 10.6% mPFS: 2.1 vs. 3.3 months mOS: 9.9 vs. 10.8 months
					+ (CPS ≥ 1)	ORR: 12.3 vs. 9.4% mPFS: 2.1 vs. 3.1 months mOS: 10.7 vs. 10.2 months
					+ (CPS ≥ 10)	ORR: 17.7 vs. 9.2% mPFS: 2.1 vs. 3.4 months mOS: 12.7 vs. 11.6 months
JAVELIN (NCT01772004)	Ib	Completed	Avel	Received a median of 2 prior therapies (58)	+ (CPS ≥ 20)	ORR: 26.3 vs. 11.5% mPFS: 3.4 vs. 2.4 months mOS: 14.9 vs. 12.5 months
					Overall	ORR: 5.2% mPFS: 5.9 months mOS: 9.2 months
					+ (≥ 10% IC ^d)	ORR: 22.2% mPFS: 2.6%
NCT01375842	Ia	Completed	Atez	mTNBC: 58% ≥ 2 prior therapies (116)	Overall	ORR: 10% mPFS: 1.4 months mOS: 8.9 months
					+ (≥ 1% IC)	ORR: 12% mOS: 10.1 months ORR: 0% mOS: 6.0 months
					—	
SAFIR02-BREAST IMMUNO (NCT02299999)	II	Completed	Durv (47) vs. CT (35)	Maintenance setting (82)	Overall	mOS: 21.2 vs. 14 months mOS: 27.3 vs. 12.1 months mOS: 19.5 vs. 14 months
Trials in early-stage TNBC as adjuvant therapy						
SWOG 1418 (NCT02954874)	III	Ongoing	Pemb vs. observation	TNBC with ≥ 1 cm RIC or LN (+) after NACT		NA
A-Brave (NCT02926196)	III	Ongoing	Avel vs. observation	High-risk TNBC		NA

Abbreviations: mTNBC, metastatic triple-negative breast cancer; Pemb, pembrolizumab; Avel, avelumab; Atez, atezolizumab; Durv, durvalumab; NACT, neoadjuvant chemotherapy; CT, chemotherapy; RIC, residual invasive cancer; LN, lymph node; ORR, objective response rate; mOS, median overall survival; mPFS, median progression-free survival; +, PD-L1 positive; −, PD-L1 negative; TC, tumor cells; CPS, combined positive score; IC, immune cells; NA, not available. ^a PD-L1 positivity was defined as membranous staining in at least 1% of cells (neoplastic and intercalated mononuclear inflammatory cells) within tumor nests. ^b Immunohistochemistry 22C3 assay, CPS = PD-L1 stained cells (including tumor cells, lymphocytes, and macrophages) / the number of all viable tumor cells × 100. ^c In KEYNOTE-119, the chemotherapy regimens included capecitabine, eribulin, gemcitabine, or vinorelbine. ^d The percentage of PD-L1 stained tumor-associated immune cells in the tumor area; immune cells including lymphocytes, macrophages, dendritic cells, plasma cells, and granulocytes.

In addition to the above, some trials also tested single-drug ICIs targeting PD-L1 in a metastatic setting. In the phase Ib JAVELIN trial, avelumab showed acceptable safety and clinical activity in the mTNBC subgroup. The ORRs were 5.2% and 22.2% for the total TNBC population and PD-L1-positive TNBC patients, respectively [31]. Moreover, a phase Ia study (NCT01375842) verified that atezolizumab monotherapy was well tolerated in patients with mTNBC and the ORR for the unselected TNBC population was 10%. The PD-L1 expression status and prior treatment history continue to strongly influence the efficacy of atezolizumab [32]. In addition, durvalumab was tested in the SAFIR02-BREAST IMMUNO trial as a maintenance therapy. Subgroup analysis showed that, compared with maintenance chemotherapy, durvalumab improved OS in patients with mTNBC (21.2 vs. 14 months, HR = 0.54, 95% CI: 0.30 to 0.97, $p = 0.0377$), especially in PD-L1-positive patients (27.3 vs. 12.1 months, HR = 0.37, 95% CI: 0.12 to 1.13, $p = 0.0678$) [33] (Table 1).

The preliminary results of these trials suggest that PD-(L)1 blockade alone has a modest clinical response across the entire mTNBC population. However, more durable responses have been observed in specific patients, such as PD-L1-positive patients receiving first-line treatment. These findings encourage further research on PD-(L)1 inhibitors.

4.2. In Early-Stage TNBC

There are studies on PD-(L)1 inhibitors monotherapy currently underway in early-stage TNBC patients. SWOG 1418 is an ongoing phase III trial investigating the efficacy of pembrolizumab on TNBC patients with ≥ 1 cm residual invasive cancer or positive lymph nodes after neoadjuvant chemotherapy (NACT) [34]. One year of postoperative intravenous avelumab is currently being evaluated for its impact on survival in high-risk TNBC patients in the A-Brave trial [35]. The results of these trials are eagerly anticipated and could provide additional options for the intensive treatment of patients with early-stage, high-risk TNBC (Table 1).

5. Research Progress of PD-(L)1 Inhibitors in Combination with Chemotherapy

Preclinical and clinical studies have shown that in addition to direct toxicity to tumor cells, some chemotherapeutic agents kill tumor cells through a pathway of immunogenic cell death (ICD), which stimulates the recruitment and maturation of APCs, enhances the antigen presentation process, and promotes the activation of T cells [36]. Chemotherapeutic agents also increase the immunogenicity of tumors by exposing MHC molecules and antigens on the surface of tumor cells [37]. The transient immunosuppression induced by chemotherapy causes a massive release of cytokines and chemokines, which increases the infiltration and activation of immune cells [38]. Furthermore, chemotherapeutic agents reduce immunosuppressive cells such as Tregs and MDSCs [39]. These chemotherapy drugs include anthracyclines, cyclophosphamide, and others commonly used for TNBC. Thus, combining PD-(L)1 inhibitors with chemotherapy is a promising approach to enhance the efficacy of immunotherapy and facilitate synergistic anti-tumor activity. Based on this concept, a number of trials combining chemotherapy and immunotherapy are being conducted in the clinic and some breakthroughs have been made (Table 2).

Table 2. Clinical trials of PD-(L)1 inhibitors in combination with chemotherapy.

Clinical Trial	Phase	Status	Arms (<i>n</i>)	Population (<i>n</i>)	PD-L1 Status	Major Outcomes
Trials in advanced TNBC						
KEYNOTE-355 (NCT02819518)	III	Ongoing	Pemb + CT ^a (566) vs. placebo + CT (281)	First-line treatment in mTNBC (847)	ITT population +(CPS ^b ≥ 1) +(CPS ≥ 10)	mPFS: 7.5 vs. 5.6 months mOS: 17.2 vs. 15.5 months mPFS: 7.6 vs. 5.6 months mOS: 17.6 vs. 16.0 months mPFS: 9.7 vs. 5.6 months mOS: 23.0 vs. 16.1 months

Table 2. Cont.

Clinical Trial	Phase	Status	Arms (n)	Population (n)	PD-L1 Status	Major Outcomes
KEYNOTE-150/ ENHANCE 1 (NCT02513472)	Ib/II	Completed	Pemb + eribulin mesylate	≤2 prior lines therapies in the metastatic setting (167)	Overall +(CPS ≥ 1)	ORR in total: 23.4% stratum 1: 25.8% stratum 2: 21.8% mPFS in total: 4.1 months stratum 1: 4.2 months stratum 2: 4.1 months mOS in total: 16.1 months stratum 1: 17.4 months stratum 2: 15.5 months ORR in stratum 1: 34.5% ORR in stratum 2: 24.4% mPFS in stratum 1: 6.1 months mPFS in stratum 2: 4.1 months mOS in stratum 1: 21.0 months mOS in stratum 2: 14.0 months ORR in stratum 1: 16.1% ORR in stratum 2: 18.2% mPFS in stratum 1: 3.5 months mPFS in stratum 2: 3.9 months mOS in stratum 1: 15.2 months mOS in stratum 2: 15.5 months
TORCHLIGHT (NCT04085276)	III	Recruiting	Tori + nab-P vs. placebo + nab-P	≤1 line of CT in the metastatic setting	—	NA
NCT04537286	II	Recruiting	Cari + nab-P + Cp	First-line treatment in mTNBC	—	NA
NCT02755272	II	Recruiting	Pemb + Cb + gemcitabine vs. Cb + gemcitabine	>2 prior lines therapies in the metastatic setting	—	NA
TONIC (NCT02499367)	II	Ongoing	A/C/Cp/ RT/no induction + Nivo (70)	mTNBC (70)	—	ORR in total: 20% Cp induction ORR: 23% A induction ORR: 35% mPFS in total: 1.9 months
TONIC-2 (NCT04159818)	II	Recruiting	Cp/ low dose A/no induction + Nivo	Metastatic or incurable locally advanced TNBC	—	NA
NCT01633970	Ib	Completed	Atez + nab-P (33)	≤2 lines prior CT in the metastatic setting (33)	—	ORR: 39.4% mPFS: 5.5 months mOS: 14.7 months
IMpassion130 (NCT02425891)	III	Completed	Atez + nab-P (451) vs. placebo + nab-P (451)	First-line treatment in mTNBC (902)	ITT population +(≥1% IC ^c)	mPFS: 7.2 vs. 5.5 months mOS: 21.0 vs. 18.7 months mPFS: 7.5 vs. 5.0 months mOS: 25.4 vs. 17.9 months
IMpassion131 (NCT03125902)	III	Ongoing	Atez + P (431) vs. placebo + P (220)	First-line treatment in mTNBC (651)	ITT population +(≥1% IC)	mPFS: 5.7 vs. 5.6 months mOS: 19.2 vs. 22.8 months mPFS: 6.0 vs. 5.7 months mOS: 22.1 vs. 28.3 months
IMpassion132 (NCT03371017)	III	Recruiting	Atez + CT ^d vs. placebo + CT	First-line treatment for locally advanced inoperable or mTNBC	—	NA
ALICE (NCT03164993)	II	Ongoing	Atez + PLD + C vs. placebo + PLD + C	≤ 1 line previous CT in the metastatic setting	—	NA
GIM25-CAPT (NCT05266937)	II	Recruiting	Atez + nab-P + Cb	First-line therapy in PD-L1-positive mTNBC	—	NA
EL1SSAR (NCT04148911)	III	Ongoing	Atez + nab-P	First-line therapy in PD-L1-positive mTNBC	—	NA

Table 2. Cont.

Clinical Trial	Phase	Status	Arms (n)	Population (n)	PD-L1 Status	Major Outcomes
Trials in early-stage TNBC as neoadjuvant therapy						
I-SPY2 (NCT01042379)						
I-SPY2 (NCT01042379)	II	Recruiting	Pemb + P → AC (29) vs. P → AC (85)	HER-2 negative, stage II or III at high risk (250, including 114 TNBC)		pCR rates in TNBC: 60% vs. 22%
KEYNOTE-173 (NCT02622074)	Ib	Completed	Pemb + (nab-P ± Cb → AC) (60)	High-risk, early-stage TNBC (60)		Overall pCR rate: 60%
KEYNOTE-522 (NCT03036488)	III	Ongoing	Pemb + (PCb → AC/EC) (784) vs. placebo + (PCb → AC/EC) (390) (→surgery → Pemb/placebo for up to 9 cycles)	Stage II-III TNBC (1174)	Overall +(CPS ≥ 1) —	pCR rates e: 64.8% vs. 51.2% 3-year EFS: 84.5% vs. 76.8% pCR rates: 68.9% vs. 54.9% pCR rates: 45.3% vs. 30.3%
NeoPACT (NCT03639948)	II	Ongoing	Pemb + Cb + docetaxel	Early-stage TNBC		NA
NCT04613674	III	Recruiting	Camr + CT vs. placebo + CT	Early or Locally Advanced TNBC		NA
GeparNuevo (NCT02685059)	II	Completed	Durv × 2w f → durv + (nab-P → EC) (88) vs. placebo + (nab-P → EC) (86) (→surgery → physician's choice)	Primary, cT1b-cT4a-d disease, centrally confirmed TNBC (174)		Overall pCR rates: 53.4% vs. 44.2% pCR rates in the window cohort: 61.0% vs. 41.4% 3-year iDFS: 85.6% vs. 77.2% 3-year DDFS: 91.7% vs. 78.4% 3-year OS: 95.2% vs. 83.5%
NeoTRIPaPDL1 (NCT02620280)	III	Ongoing	Atez + nab-P + Cb (138) vs. nab-P + Cb (142) (→surgery → adjuvant anthracycline regimen as per investigator's choice)	Early high-risk and locally advanced TNBC (280)	ITT population +(≥1% IC)	pCR rates: 48.6% vs. 44.4% pCR rates: 59.5% vs. 51.9%
IMpassion031 (NCT03197935)	III	Ongoing	Atez + (nab-P → AC) (165) vs. placebo + (nab-P → AC) (168) (→surgery → adjuvant Atez/placebo for up to 11 cycles)	Stage II-III TNBC (333)	Overall +(≥1% IC) —	pCR rates: 58% vs. 41% pCR rates: 69% vs. 49% pCR rates: 48% vs. 34%
NSABP B-59 (NCT03281954)	III	Ongoing	Atez + (PCb → AC) vs. placebo + (PCb → AC) (→surgery → adjuvant Atez/placebo until 1 year after the first dose)	Stage II-III TNBC		NA
NCT02530489	II	Ongoing	Atez + nab-P (→surgery → adjuvant Atez for 4 cycles)	TNBC that were non-responders to initial AC chemotherapy		NA
Trials in early-stage TNBC as adjuvant therapy						
NCT03487666	II	Ongoing	Nivo vs. capecitabine vs. Nivo + capecitabine	TNBC with ≥ 1 cm RIC or LN (+) after NACT		NA
IMpassion030 (NCT03498716)	III	Recruiting	Atez + A/P-based CT vs. CT	Operable-stage II-III TNBC		NA

Table 2. Cont.

Clinical Trial	Phase	Status	Arms (n)	Population (n)	PD-L1 Status	Major Outcomes
NCT03756298	II	Recruiting	Atez + capecitabine vs. capecitabine	TNBC with RIC after NACT		NA

Abbreviations: mTNBC, metastatic triple-negative breast cancer; Pemb, pembrolizumab; Atez, atezolizumab; Durv, durvalumab; Cari, carilizumab; Tori, toripalimab; Nivo, nivolumab; Camr, camrelizumab; CT, chemotherapy; RT, radiotherapy; Nab-P, nab-paclitaxel; Pa, paclitaxel; E, epirubicin; A, doxorubicin; C, cyclophosphamide; Cb, carboplatin; Cp, cisplatin; PLD, pegylated liposomal doxorubicin; mOS, median overall survival; mPFS, median progression-free survival; EFS, event-free survival; iDFS, invasive disease-free survival; DDFS, distant disease-free survival; ITT population, intention-to-treat population; ORR, objective response rate; pCR, pathological complete remission; CPS, combined positive score; IC, immune cells; +, PD-L1 positive; −, PD-L1 negative; RIC, residual invasive cancer; NA, not available. ^a In KEYNOTE-355, the chemotherapy regimens included nab-paclitaxel; paclitaxel; or gemcitabine plus carboplatin. ^b Immunohistochemistry 22C3 assay, CPS = PD-L1 stained cells (including tumor cells, lymphocytes, and macrophages) / the number of all viable tumor cells × 100. ^c The percentage of PD-L1 stained tumor-associated immune cells in the tumor area; immune cells including lymphocytes, macrophages, dendritic cells, plasma cells, and granulocytes. ^d In the IMpassion132, the chemotherapy regimens include gemcitabine, capecitabine, and carboplatin. ^e In KEYNOTE-522, the first interim pCR analysis was conducted on the first 602 patients who underwent randomization (401 patients in pembrolizumab–chemotherapy group and 201 in placebo–chemotherapy group). ^f In the GeparNuevo study, 117 patients participated in the window phase.

5.1. In Advanced TNBC

At present, a majority of clinical trials apply immunotherapy with chemotherapy concomitantly. The reason for this is that ICIs take time to work, while chemotherapy agents kill tumor cells and modify the TIME during this waiting period.

KEYNOTE-355 is a phase III randomized controlled trial (RCT) assessing the efficacy and safety of pembrolizumab plus chemotherapy versus placebo plus chemotherapy as first-line treatment for patients with advanced TNBC; chemotherapy regimens were based on the physician's choice including nab-paclitaxel, paclitaxel, and gemcitabine/carboplatin. Initial results showed that the combination of pembrolizumab with chemotherapy improved PFS in the intention-to-treat (ITT) population and in the combined positive score (CPS) ≥ 1 subgroup (7.5 vs. 5.6 months, HR = 0.82, 95% CI: 0.69 to 0.97 and 7.6 vs. 5.6 months, HR = 0.74, 95% CI: 0.61 to 0.90, $p = 0.0014$, respectively); the improvement was particularly significant in the CPS ≥ 10 subgroup (9.7 vs. 5.6 months, HR = 0.65, 95% CI: 0.49 to 0.86, $p = 0.0012$) [40]. According to the latest release of follow-up data, OS was improved by almost 7 months in the CPS ≥ 10 subgroup after the addition of pembrolizumab to chemotherapy (23.0 vs. 16.1 months, HR = 0.73, 95% CI: 0.55 to 0.95, $p = 0.0185$) and the adverse effects were manageable [41].

Another single-arm phase Ib/II trial, KEYNOTE-150, used pembrolizumab in combination with eribulin mesylate in patients with mTNBC who had received ≤ 2 lines of prior therapy in the metastatic setting. Of the 167 patients enrolled, 40% had not received previous systemic therapies and were classified in stratum 1. The results showed that the survival benefit was most significant in PD-L1-positive patients who had not received prior systemic therapy, which was consistent with previous studies. This study offers a new immuno–chemotherapy combination for the treatment of patients with mTNBC, although further confirmation is needed [42].

After a phase Ib trial (NCT01633970) demonstrated the safety and feasibility of atezolizumab plus nab-paclitaxel in patients with locally recurrent or metastatic TNBC [43], the efficacy of this immuno–chemotherapy combination for TNBC patients who did not receive systemic therapy in the metastatic setting was further validated by IMpassion130, the first phase III RCT of immunotherapy for TNBC [44]. Preliminary results showed that a PFS benefit was observed with the addition of atezolizumab in both the ITT population (7.2 vs. 5.5 months, HR = 0.80, 95% CI: 0.69 to 0.92, $p = 0.002$) and the PD-L1-positive population (7.5 vs. 5.0 months, HR = 0.62, 95% CI: 0.49 to 0.78, $p < 0.001$). The second set of interim results indicated that atezolizumab significantly improved OS from 18.0 months to 25.0 months in the PD-L1-positive subgroup (HR = 0.71, 95% CI: 0.54 to 0.94), but the difference was not significant in the ITT population (21.0 vs. 18.7 months, HR = 0.86, 95%

CI: 0.72 to 1.02, $p = 0.078$) [45]. As a result, in March 2019, atezolizumab was granted accelerated approval by the Food and Drug Administration (FDA) to be used in combination with nab-paclitaxel as a first-line treatment for late-stage TNBC patients. Additionally, the 7.5-month survival benefit shown in the final OS data further demonstrated the durable efficacy of this treatment combination for PD-L1-positive patients (25.4 vs. 17.9 months, HR = 0.67, 95% CI: 0.53 to 0.86) [46].

However, these findings were contradicted when atezolizumab was combined with paclitaxel and compared to placebo plus paclitaxel in the phase III clinical study IMpassion131, which also investigated this as first-line treatment for patients with advanced or metastatic TNBC. The study found no obvious differences in PFS between the two arms, regardless of PD-L1 expression status (5.7 vs. 5.6 months, HR = 0.86, 95% CI: 0.70 to 1.05 for the ITT population and 6.0 vs. 5.7 months, HR = 0.82, 95% CI: 0.60 to 1.12, $p = 0.20$ for the PD-L1-positive patients). With respect to OS, the atezolizumab arm appeared to be worse but not detrimental in the PD-L1-positive population (22.1 vs. 28.3 months, HR = 1.11, 95% CI: 0.76 to 1.64) and in the ITT population (19.2 vs. 22.8 months, HR = 1.12, 95% CI: 0.88 to 1.43). Different chemotherapeutic agents, steroid pre-treatment with paclitaxel, and subtle differences between study populations may explain the difference in results between IMpassion130 and IMpassion131 [47]. In addition, levels of TILs, breast cancer susceptibility gene (BRCA) mutational load, and the proportion of patients with residual disease after NACT (which were unreported in the trial) may also have contributed to the unclear results from IMpassion131 [48]. As the reason for this discrepancy remains undefined, Roche has voluntarily withdrawn the indication for atezolizumab for the treatment of PD-L1 positive advanced TNBC. Recently, a small sample-based single-cell sequencing study suggested that paclitaxel may affect the efficacy of atezolizumab by reducing key anti-tumor immune cells in the TIME but enhancing immunosuppressive macrophages, yet this finding needs to be further explored [49].

Despite some setbacks in the exploration of combination treatments with taxanes, atezolizumab is still being tested in different trials to investigate the safety and efficacy of combination treatment with other chemotherapy agents in TNBC (Table 2).

Beyond concurrent chemotherapy, the induction use of small doses of chemotherapeutic agents prior to immunotherapy is another strategy of the immuno-chemotherapy combination that is in the experimental phase. In the five cohorts of the phase II TONIC trial, patients with mTNBC received no induction or 2 weeks induction with low-dose cyclophosphamide, cisplatin, doxorubicin, and hypofractionated irradiation, respectively, all followed by the PD-1 blocking drug nivolumab. The total ORR was 20%, with a median PFS (mPFS) of 1.9 months; a higher ORR occurred in the doxorubicin and carboplatin cohorts at 35% and 23%, respectively. Analysis of patient samples suggested that short-term doxorubicin or cisplatin induction can convert the tumor microenvironment towards inflammation and improve the response of nivolumab in TNBC. However, due to the limitations of the trial itself, this conclusion needs further confirmation [50]. The subsequent trial, TONIC-2, is currently recruiting (Table 2).

5.2. In Early-Stage TNBC

Studies based on transcriptomics and immunohistochemical techniques have revealed that mTNBC has significantly reduced expression of immune activation genes as well as immunotherapeutic targets, such as PD-L1, and a lower number of TILs compared to primary TNBC [51,52]. Thus, the TIME in the early-stage disease setting is more suitable for ICIs to function and to potentially achieve a true cure.

Several trials have indicated that the combination of pembrolizumab with chemotherapy can improve pathological complete remission (pCR) rates in early-stage TNBC. One of the cohorts in the I-SPY2 trial first determined the feasibility of 4 cycles of pembrolizumab in combination with paclitaxel- and anthracycline-based chemotherapy regimen in women with early-stage, high-risk HER-2-negative breast cancer. Compared to standard NACT regimens, the addition of pembrolizumab increased the pCR rate for patients with TNBC by 38%

(60% vs. 22% for pembrolizumab vs. control) [53]. Another phase Ib KEYNOTE-173 trial with a relatively small sample volume evaluated the safety and efficacy of pembrolizumab in combination with chemotherapy regimens, including different doses of nab-paclitaxel with or without carboplatin followed by doxorubicin and cyclophosphamide; the overall pCR rate was consistent with I-PSY2 at 60% [54].

In the phase III trial KEYNOTE-522, 1174 patients with previously untreated early-stage TNBC were randomized in a 2:1 ratio to either the pembrolizumab–chemotherapy arm or the placebo–chemotherapy arm (chemotherapy backbone of 4 cycles of paclitaxel plus carboplatin, followed by 4 cycles of anthracycline plus cyclophosphamide every 3 weeks), with up to 9 cycles of adjuvant pembrolizumab or placebo after surgery. Preliminary results based on the first 602 patients showed that the addition of pembrolizumab increased the pCR rate by 13.6% compared to the placebo–chemotherapy arm (64.8% vs. 51.2%, 95% CI: 5.4% to 21.8%, $p < 0.001$). This benefit was observed in most subgroups, including PD-L1-negative patients [55]. Based on this undifferentiated benefit, in July 2021, the FDA approved pembrolizumab in combination with chemotherapy as a neoadjuvant treatment for early-stage, high-risk TNBC and for continued use as a single agent in the adjuvant phase. Furthermore, recently updated follow-up data after 39.1 months showed that pembrolizumab treatment for almost 1 year reduced the risk of disease progression by 37% (3-year event-free survival (EFS) of 84.5% vs. 76.8%, HR = 0.63, 95% CI: 0.48 to 0.82, $p < 0.001$). This EFS benefit was independent of PD-L1 expression status, which is consistent with previous results and further demonstrates the long-term effectiveness of the perioperative addition of pembrolizumab. At the time of this analysis, data on OS were immature and further follow-up data are expected [56].

In addition, the phase II NeoPACT trial is also ongoing, combining pembrolizumab with carboplatin and docetaxel as neoadjuvant therapy. The results of this study will demonstrate whether similar pCR rates and survival benefits can be achieved by removing anthracyclines from neoadjuvant chemotherapy regimens in early TNBC.

However, the situation becomes more complicated upon review of the results of clinical trials with PD-L1 inhibitors.

The phase II GeparNuevo study compared the efficacy of receiving durvalumab or placebo every 4 weeks in addition to chemotherapy of nab-paclitaxel sequentially with epirubicin and cyclophosphamide. A total of 174 patients with early TNBC were enrolled. It was noteworthy that 117 patients in this study received an additional, 2-week earlier window treatment of durvalumab or placebo before the start of nab-paclitaxel, and 87% of 158 detected patients were PD-L1 positive. The intensive postoperative treatment regimen for patients enrolled in this trial was based on the physician’s choice. In the window cohort, the pCR rates were statistically increased by the addition of durvalumab (61.0% vs. 41.4%, OR = 2.22, 95% CI: 1.06 to 4.64, $p = 0.035$), but not in the whole study population (53.4% vs. 44.2%, OR = 1.45, 95% CI: 0.80 to 2.63, $p = 0.224$). However, it remains uncertain whether this difference was due to one dose of durvalumab window treatment [57]. Surprisingly, after a median follow-up of 43.7 months, significant improvements in 3-year invasive disease-free survival (iDFS), distant disease-free survival (DDFS), and OS were observed in the durvalumab group, even without the adjuvant durvalumab treatment, which contradicts the pCR results obtained initially (iDFS was 85.6% vs. 77.2%, HR = 0.48, 95% CI: 0.24 to 0.97, $p = 0.036$; DDFS was 91.7% vs. 78.4%, HR = 0.31, 95% CI: 0.13 to 0.74, $p = 0.005$; OS was 95.2% vs. 83.5%, HR = 0.24, 95% CI: 0.08 to 0.72, $p = 0.006$) [58]. More studies are needed to elucidate this result and to explore the timing and sequence of ICIs when combined with chemotherapy to treat early-stage TNBC.

The efficacy of 8 cycles of nab-paclitaxel and carboplatin with or without atezolizumab in early-stage, high-risk TNBC was investigated in the NeoTRIPaPDL1 trial, with 4 cycles of anthracycline regimen chemotherapy administered as adjuvant treatment. The published results thus far have shown that the addition of atezolizumab to the neoadjuvant setting did not significantly increase the pCR rate in the ITT population (48.6% vs. 44.4%, OR = 1.18, 95% CI: 0.74 to 1.89, $p = 0.48$) or in the PD-L1-positive subgroup (59.5% vs.

51.9%). Nevertheless, the primary endpoint of the study, the EFS data, still requires further follow-up [59].

On the contrary, in the Impassion031 trial, a significant increase in pCR rates was observed when atezolizumab was combined with a standard nab-paclitaxel- and doxorubicin-based chemotherapy regimen and applied in the adjuvant phase as a single agent (58% vs. 41%, rate difference 17%, 95% CI: 6% to 27%, $p = 0.0044$). The mature long-term survival follow-up data are not available at present. Similar to the KEYNOTE-522 results, the benefit of pCR was not significantly related to PD-L1 expression status. Of note, platinum agents were removed from the NACT regimen in this trial [55,60].

Furthermore, it is noteworthy that anthracyclines were given preoperatively in both the KEYNOTE-522 and Impassion031 trials, whereas anthracyclines were applied postoperatively in the NeoTRIPaPDL1 trial. This may be one reason why the difference in pCR rates between the two arms in the NeoTRIPaPDL1 trial was not significant.

The safety and efficacy of other combinations of PD-(L)1 inhibitors with chemotherapy drugs are also being tested in clinical trials. Last but not least, trials using ICIs plus chemotherapy in the adjuvant phase of early-stage TNBC are underway and the results are awaited with great interest (Table 2).

6. Research Progress of PD-(L)1 Inhibitors in Combination with Radiotherapy

Similar to chemotherapy, radiotherapy (RT) has a dual role of mediating DNA damage-induced tumor cell death and immunomodulation, which can make the TIME more inflammatory and facilitate the role of ICIs [61]. Whereas RT acts locally, the systemic side effects are less severe and well tolerated.

A small single-arm phase II trial (NCT02730130) enrolled 17 unselected patients with mTNBC with a median of 3 lines on prior systemic therapy. They received RT with 3000 centigrays (cGy) in five fractions over 5–7 days and pembrolizumab within 1 to 3 days after the first fraction. The median follow-up was 34.5 weeks, with an ORR of 17.6%, mPFS of 2.6 months, and median OS (mOS) of 8.25 months. Although the 3 patients who experienced complete remission were all PD-L1 positive, the analysis showed that PD-L1 status was not associated with therapeutic effects [62].

Another phase II AZTEC trial enrolled 50 patients who had received less than 2 lines of prior systemic therapy to receive RT combined with atezolizumab. Participants were randomly assigned to 20 Gy stereotactic ablative body RT (SABR) in one fraction or 24 Gy SABR in three fractions to irradiate 1–4 lesions with at least one metastasis left unirradiated. Atezolizumab was initiated within 5 days after the last part of RT. The median follow-up was 17 months, with mPFS of 3.1 months. No difference was observed in mPFS between the two groups. PD-L1 expression status and TIL levels (5%) had little effect on the efficacy [63].

In these studies, the combination of pembrolizumab and RT showed modest but encouraging clinical activity in unselected patients and was well tolerated, offering a new treatment idea for pre-treated patients with advanced TNBC. Additional trials are still being explored (Table 3).

7. Research Progress of PD-(L)1 Inhibitors in Combination with Targeted Therapy

7.1. Combination with PARPi

PARPi are drugs that block the repair of single-strand DNA damage. These drugs kill tumor cells through synthetic lethal effects that are formed by the accumulation of homologous recombination (HR) repair defects for DNA double-strand breaks due to mutations in BRCA1/2. In addition to direct killing of tumor cells, previous *in vitro* studies have shown that PARPi can stimulate intrinsic immunity and upregulate interferon (IFN) release by activating the cyclic GMP–AMP synthase-stimulator of interferon genes (cGAS-STING) signaling pathway, further upregulating tumor PD-L1 expression and infiltration of CD8+ T cells [64–66]. In short, PARPi have the potential to turn cold tumors into hot tumors and set the stage for the application of PD-(L)1 blockers.

KEYNOTE-162 is a single-arm phase I/II trial evaluating the efficacy and safety of pembrolizumab in combination with niraparib in 55 patients with advanced TNBC. The total ORR was 21%, with ORR of 47% vs. 11% and 32% vs. 8% for the two subgroups, respectively, when considering tumor BRCA mutations as well as PD-L1 status [67]. Remarkably, the mPFS in patients with BRCA mutations was 8.3 months, which was nearly 3 months longer than the mPFS of 5.6 months for olaparib reported in the OlympiAD trial or 5.8 months for talazoparib reported in the TALA trial [68,69].

A cohort in the I-SPY2 trial studied the efficacy of adding durvalumab and olaparib to standard NACT regimens of paclitaxel compared to paclitaxel alone. In the TNBC subgroup analysis, although the addition of durvalumab and olaparib increased the pCR rate in the experimental arm by 20% (47% vs. 27%), by comparison with related trials, the investigators concluded that the contribution from olaparib to the increased pCR rate in the I-SPY2 experimental arm was relatively modest [70]. However, survival data from this experiment have not been published and a more reasonable random grouping should also be considered.

PD-(L)1 blockers combined with PARPi have shown initial efficacy in both advanced and early-stage TNBC patients, with more trials ongoing (Table 3).

Table 3. Clinical trials of PD-(L)1 inhibitors in combination with radiotherapy, targeted therapy, and other immunotherapies.

Clinical Trial	Phase	Status	Arms	Population
NCT02730130	II	Ongoing	Pemb + RT	mTNBC: a median of 3 lines prior systemic therapy
AZTEC (NCT03464942)	II	Ongoing	Atez + RT	Advanced TNBC: <2 lines of prior systemic therapy
NCT03483012	II	Ongoing	Atez + RT	mTNBC with brain metastases
KEYNOTE-162 (NCT02657889)	I/II	Completed	Pemb + niraparib	Advanced TNBC: a median of 1 prior line of therapy (range, 0–3) in the metastatic setting
I-SPY2 (NCT01042379)	II	Recruiting	Durv + olaparib + paclitaxel vs. paclitaxel	Stage II–III TNBC: preoperative treatment
DORA (NCT03167619)	II	Ongoing	Durv + olaparib	Platinum-treated mTNBC
KEYLYNK-009 (NCT04191135)	II/III	Ongoing	Pemb + olaparib vs. Pemb + Cb + gemcitabine	Locally recurrent inoperable or metastatic TNBC: after induction with first-line CT + Pemb
NCT03594396	I/II	Ongoing	Olaparib + Durv	Stage II/III TNBC or low ER breast cancer: preoperative treatment
NCT03310957	Ib/II	Recruiting	Pemb + ladiratuzumab vedotin	Unresectable locally advanced or metastatic TNBC: first-line treatment
ASCENT-04 (NCT05382286)	III	Recruiting	Pemb + SG vs. pemb + TPC	Previously untreated, locally advanced inoperable, or metastatic PD-L1-positive TNBC
NCT04468061	II	Recruiting	Pemb + SG vs. SG	PD-L1-negative mTNBC
ASPRIA (NCT04434040)	II	Recruiting	Atez + SG	Early-stage TNBC with RIC after NACT
NCT03394287	II	Completed	Camr + apatinib	Advanced TNBC: <3 lines of systemic therapy

Table 3. Cont.

Clinical Trial	Phase	Status	Arms	Population
NCT05447702	II	Not yet recruiting	Camr + apatinib + CT	Neoadjuvant therapy for stage II-III TNBC
NCT04303741	II	Ongoing	Camr + apatinib + eribulin	Unresectable recurrent or mTNBC; pre-treated with anthracycline and taxane
NCT04427293	I	Recruiting	Pemb + Lenvatinib	Early-stage TNBC: preoperative treatment
NCT04335006	III	Recruiting	Care + nab-P + apatinib vs. Care + nab-P vs. nab-P	Locally advanced or metastatic TNBC: first-line treatment
NCT03800836	Ib	Completed	Atez + ipatasertib + P/nab-P	mTNBC: first-line treatment
BARBICAN (NCT05498896)	II	Ongoing	Atez + PAC + ipatasertib vs. Atez + PAC	Early-stage TNBC: preoperative treatment
NCT04177108	III	Ongoing	Atez/placebo + ipatasertib/placebo + P	Locally advanced unresectable or metastatic TNBC
COLET (NCT02322814)	II	Completed	Atez + cobimetinib + P (cohorts II)/Atez + cobimetinib + nab-P (cohort III)	First-line treatment for mTNBC
NCT02536794	II	Completed	Durv + tremelimumab	Pre-treated mTNBC
NCT03872791	Ib/II	Ongoing	KN046 vs. KN046 + nab-P	mTNBC
SYNERGY (NCT03616886)	Ib/II	Ongoing	Durv + oleclumab + PCb vs. Durv + PCb	First-line treatment for mTNBC
NCT04584112	Ib	Ongoing	Atez + tiragolumab + CT	First-line treatment for PD-L1 (+) mTNBC
NCT05227664	II	Recruiting	AK117 + P/nab-P vs. AK112 + P/nab-P vs. AK117+AK112 + P/nab-P	First-line treatment for mTNBC
NCT03362060	I	Ongoing	Pemb + PVX-410 vaccine	Pre-treated HLA-A2 (+) mTNBC
NCT02826434	I	Ongoing	Durv + PVX-410	HLA-A2 (+) stage II or III TNBC
NCT03606967	II	Recruiting	CT → Durv + tremelimumab + Vaccine vs. CT → Durv + tremelimumab	First-line treatment for PD-L1-negative mTNBC
NCT03199040	I	Ongoing	Durv + DNA vaccine vs. DNA vaccine	Early-stage TNBC
NSABP FB-14 (NCT04024800)	II	Ongoing	AE37 peptide vaccine + Pemb	Advanced TNBC: ≤ 1 line of systemic therapy
NCT03387085	Ib/II	Ongoing	Combination of multiple treatments	mTNBC: ≥ 2 lines of prior therapy
NCT04445844	II	Recruiting	Retifanlimab + pelareorep	mTNBC: received 1–2 prior lines of systemic therapy
NCT03004183	II	Ongoing	ADV/HSV-tk + RT + Pemb +	Pre-treated mTNBC
NCT03256344	I	Completed	Atez + talimogene laherparepvec	mTNBC with liver metastases
NCT05081492	I	Recruiting	CF33-hNIS-antiPDL1	mTNBC: ≥ 2 prior lines of therapy for metastatic disease
NCT04185311	I	Ongoing	Talimogene laherparepvec + nivolumab + ipilimumab	Localized, palpable HER-2 negative breast cancer

Abbreviations: mTNBC, metastatic triple-negative breast cancer; Pemb, pembrolizumab; Camr, camrelizumab; Atez, atezolizumab; Durv, durvalumab; Nivo, nivolumab; Care, carelizumab; CT, chemotherapy; RT, radiotherapy; Nab-P, nab-paclitaxel; P, paclitaxel; E, epirubicin; A, doxorubicin; C, cyclophosphamide; Cb, carboplatin; mOS, median overall survival; mPFS, median progression-free survival; ORR, objective response rate; pCR, pathological complete remission; DOR, median duration of response; SG, sacituzumab govitecan; TPC, treatment of physician's choice; HLA, human leukocyte antigen; RIC, residual invasive cancer; NACT, neoadjuvant chemotherapy.

7.2. Combination with ADCs

ADCs consist of three components: mAb, linker, and cytotoxic payload. In addition to targeting antigen-expressing tumor cells for payload delivery, the mAb mediates antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), and/or complement-dependent cytotoxicity (CDC), as well as the unique bystander killing effect of ADCs to clear tumor cells [71,72]. The cytotoxic payload, apart from directly killing tumor cells, also has immunomodulatory effects, as with the chemotherapeutic agents discussed above [73,74]. Furthermore, payload microtubule inhibitors and topoisomerase inhibitors can directly activate DCs and promote their maturation [75,76]. Therefore, ADCs may create a more conducive TIME for the enhancement of PD-(L)1 inhibitors and work synergistically with PD-(L)1 inhibitors to fight against tumors.

Sacituzumab govitecan, an ADC that targets the tumor cell surface antigen trop2 and has the irinotecan metabolite SN-38 as its payload, has been approved by the FDA for patients with advanced TNBC who have received 2 or more lines treatments. Clinical trials are currently underway to evaluate the potential of sacituzumab govitecan in combination with pembrolizumab as first-line treatment for mTNBC.

Ladiratuzumab vedotin is an ADC that targets the zinc transporter protein LIV-1 with the microtubule inhibitor monomethyl auristatin E (MMAE) as a payload. A phase Ib/II trial (NCT03310957) evaluated the safety and efficacy of its combination with pembrolizumab as first-line treatment for advanced TNBC. The initial 51 patients included showed moderate tolerability and a manageable safety profile. Among the 26 patients evaluable for efficacy, the ORR was 54% [77]. This trial is currently underway and initial results are encouraging.

Several additional trials are testing the safety and efficacy of PD-(L)1 inhibitors in combination with ADCs in both early-stage and advanced TNBC (Table 3).

7.3. Combination with Small Molecule Inhibitors

The serine/threonine kinase AKT is a key component of the phosphatidylinositol-3-kinase (PI3K)/AKT and mammalian target of rapamycin (mTOR) signaling pathways. Activation of this pathway and its downstream pathways is associated with the growth, invasion, and drug resistance of a variety of tumors and is cross-linked with multiple signaling pathways, such as the mitogen-activated protein kinase (MAPK) pathway [78]. It has been shown that activation of these two pathways is associated with an increase in immunosuppressive cells and cytokines as well as a decrease in IFN γ , interleukin-2 (IL-2), and tumor necrosis factor α (TNF α) [79–81]. Therefore, a simultaneous blockade of these pathways as well as PD-(L)1 would confer a better therapeutic effect. Results of a phase Ib trial (NCT03800836) combining the AKT inhibitors ipatasertib, atezolizumab, and paclitaxel or nab-paclitaxel as a first-line treatment for mTNBC showed an ORR of 54% and mPFS of 7.2 months in 114 patients. Subgroup analysis according to PD-L1 status, PIK3CA/AKT1/phosphatase and tensin homolog (PTEN) alteration status, or taxane backbone showed no consistent trend across endpoints. Treatment was generally tolerable [82]. Cobimetinib is a MAPK/extracellular signal-regulated kinase (MEK) inhibitor. In the phase II COLET trial, a combination regimen of cobimetinib with atezolizumab and paclitaxel or nab-paclitaxel as first-line treatment failed to significantly improve ORRs in mTNBC (34.4% for the paclitaxel cohort and 29.0% for the nab-paclitaxel cohort) [83]. These findings suggest that more effort is still needed in the understanding of classical pathways and in clinical translation.

Abnormal morphological and functional vascularity within solid tumors results in hypoxia of tumor tissue and increased immunosuppressive TIME, as well as reduced and suppressed immune cell infiltration and activity [84,85]. Preclinical studies have shown that anti-vascular therapy increases immune cell infiltration and PD-L1 expression in tumor tissues [86]. Thus, anti-tumor vascular therapy is a potential method to convert cold tumors into hot tumors. A phase II clinical trial (NCT03394287) combined camrelizumab with the vascular endothelial growth factor receptor 2 (VEGFR2) tyrosine kinase inhibitor apatinib

in patients with advanced TNBC with fewer than 3 lines of systemic therapy. Of the 40 patients included, 10 were treated intermittently with apatinib and 30 were treated continuously. The ORR in the continuous dosing cohort was 43.3%, while no objective response was observed in the intermittent dosing cohort. This trial demonstrated that the combination of the two drugs is safe and it shows a superior clinical response to single drug application [87].

More trials on the combination of ICIs with small molecule inhibitors are underway (Table 3).

8. Exploration of PD-(L)1 Inhibitors in Combination with Other Immunotherapies

8.1. Combination with Other ICIs

In addition to PD-(L)1, immune checkpoints such as cytotoxic T-lymphocyte antigen-4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), and T cell immunoreceptor with Ig and ITIM domains (TIGIT) are also significantly upregulated in TNBC; their expression levels are further boosted by PD-(L)1 blockade, which may mediate acquired resistance to PD-(L)1 blockade [88,89]. Therefore, to further reverse the tumor immunosuppressive microenvironment and overcome PD-(L)1 inhibitor resistance, dual ICIs therapies have been developed.

CTLA-4 is a co-suppressor molecule expressed by T cells. As a homologous receptor of CD28, CTLA-4 can replace CD28 and bind to the B7 ligand on the surface of APCs, preventing the activation and proliferation of T cells [90]. Anti-CTLA-4 antibodies enhance tumor cell killing by blocking the CTLA-4-B7 checkpoint pathway or by selectively depleting Treg cells, although this requires further validation [91]. In a small single-arm study (NCT02536794), durvalumab in combination with tremelimumab demonstrated preliminary efficacy and a tolerable safety profile in 7 patients with mTNBC, with an ORR of 43% [92]. In particular, a combination regimen of KN046, a bispecific antibody that targets both PD-L1 and CTLA-4, with nab-paclitaxel for advanced TNBC showed initially promising results in a phase Ib/II trial (NCT03872791), which may herald the coming of the era of bispecific antibodies [93].

Nevertheless, the idea of combining ICIs for the treatment of TNBC met a Waterloo in the SYNERGY trial. CD73, a metabolic immune checkpoint, is an ecto-5'-nucleotidase that is expressed on a wide range of cells and works synergistically with CD39 to convert ATP into adenosine. Adenosine is a potent immunosuppressive molecule that suppresses the function of a wide range of immune cells, especially T cells [94]. CD73 is highly expressed in TNBC and is associated with poor prognosis [95]. The preliminary results at week 24 were presented at the European Society for Medical Oncology (ESMO) 2022 and showed that the addition of the CD73 inhibitor oleclumab to chemotherapy and durvalumab did not improve clinical benefits as a first-line treatment for advanced TNBC (the clinical benefit rates were 42.9% vs. 43.3%, respectively) [96].

The mixed results suggest that the functions and interactions of various immune checkpoints still need to be more thoroughly explored. Clinical trials on the effects of PD-(L)1 inhibitors in combination with other ICIs, such as novel phagocytosis checkpoints, are in full swing (Table 3).

8.2. TCVs and PD-(L)1 Inhibitors

The practice of utilizing vaccines against breast tumors predates even ICI uses. However, due to limited efficacy, this approach is not widely applied clinically. Personalized TCVs based on neoantigens may benefit specific patients via injection of tumor neoantigens that were extracted from tumor tissues or human body fluids along with adjuvants. Such therapy amplifies the process of antigen capture and presentation, increases the number of tumor-specific effector T cells, and establishes long-term memory to inhibit tumor recurrence [97,98]. The major types currently in trials include: autologous cells, whole/genetically modified tumor cells, or DCs; cancer antigens, DNA/RNA/peptide vaccines; and tumor cell products, such as exosomes [99].

TCVs and PD-(L)1 inhibitors act in different steps of tumor elimination, and thus combination treatment will synergistically activate the entire immune system. Several clinical trials are currently testing the safety and efficacy of TCVs in combination with PD-(L)1 inhibitors for the treatment of TNBC, in both early and advanced stages (Table 3). Notably, a preclinical study showed that the sequence of PD-(L)1 inhibitors and vaccine combinations is critical to treatment efficacy [100], which deserves special attention when conducting clinical trials.

8.3. Oncolytic Virus (OVs) and PD-(L)1 Inhibitors

OVs immunotherapy utilizes natural or modified viruses to selectively infect tumor cells and replicate in large numbers, thereby lysing tumor cells without harming normal cells [101]. In addition to direct killing of tumor cells, OVs also enhance host anti-tumor immunity by mediating ICD, promoting the release of TAAs, increasing the recruitment and maturation of immune cells, and regulating the suppressive TIME, all together rapidly and effectively transforming cold tumors into inflammatory tumors [102]. The efficacy of OVs alone or in combination with therapies such as ICIs has been demonstrated in preclinical tumor models of TNBC [103]. While oncorine (H101) and talimogene laherparepvec (T-VEC) have been approved by the Chinese Food and Drug Administration and the FDA for the treatment of head and neck cancer and melanoma, respectively, clinical studies of OVs in TNBC are still in their infancy with few results published. A phase I trial (NCT03256344) evaluated the safety of intrahepatic injection of T-VEC in combination with intravenous atezolizumab in patients with mTNBC or colorectal cancer, and no dose-limiting toxicity (DLT) was seen in the four TNBC patients who could be evaluated [104]. In addition, scientists have developed chimeric oncolytic poxvirus that can express anti-PD-L1 antibodies and are currently in a phase I trial. Several trials combining PD-(L)1 blockade and OVs are underway (Table 3).

8.4. ACT and PD-(L)1 Inhibitors

ACT refers to a therapy in which immune-active cells are isolated from tumor patients, expanded, modified, and characterized in vitro, and then infused back for the purpose of directly killing tumor cells or stimulating an immune response to kill tumor cells. Adoptive TILs and genetically modified T cells expressing modified T cell receptors (TCR-T) or chimeric antigen receptors (CAR-T) are currently the most studied, while therapies such as adoptive NK cells and cytokine-induced killer cells (CIK) are also gaining attention. However, in the field of TNBC, this treatment is still in early phase trials. ACT can directly increase populations of immune killer cells in cold tumors, but its efficacy may be greatly reduced due to the presence of immune checkpoints. Therefore, combining ACT with PD-(L)1 inhibitors is a promising approach to enhance anti-tumor efficacy. A phase Ib/II trial (NCT03387085) first demonstrated a safe and tolerable combination treatment of low-dose chemoradiation, TCV, NK cells therapy, and a PD-L1 inhibitor as third- or greater-line therapy for mTNBC. The ORR was 56% and the disease control rate was 78% in the initial enrollment of 9 patients [105] (Table 3). These preliminary encouraging results provide ideas for additional combination therapies. More outcomes are to be expected.

9. Potential Therapeutic Targets for Reversing Cold Tumors

Although considerable clinical trials have been conducted on TNBC patients with some achievements, the mechanisms of tumor immunity are still being explored. Meanwhile, some potential therapeutic targets that can convert cold tumors have been identified.

According to a fundamental study, the mRNA N⁶-methyladenosine (m⁶A)-binding protein YTHDF1 can recognize and bind transcripts encoding lysosomal proteases, which in turn increases the translation of lysosomal histone proteases in DCs, resulting in the impaired presentation of tumor neoantigens and T cell initiation. In addition, the anti-tumor effect of PD-L1 blockade was enhanced in the YTHDF1^{-/-} tumor-bearing mouse models [106]. These suggest that the combination of ICIs and YTHDF1 depletion may be

a potential new therapeutic strategy. Research on innate immunity activation by STING agonists are also proceeding in full swing.

Tumor stromal fibrosis is one mechanism by which T cell infiltration is restricted in cold tumors. A recent study showed that discoidin domain receptor 1 (DDR1), a collagen receptor with tyrosine kinase activity, can enhance the collagen fibril alignment and impede immune infiltration through the binding of its extracellular domain (ECD) to collagen. Conversely, ECD-neutralizing antibodies could disrupt this alignment, attenuate immune rejection, and inhibit tumor growth [107]. This study suggests that disruption of tumor stromal fibrosis is one way to convert cold tumors and holds promise to improve anti-tumor efficacy in combination with ICIs in the future.

There are also a growing number of studies focusing on the impact of the host nervous system and commensal microbes on anti-tumor immunity. The effects of sympathetic- β -adrenergic signaling on MDSCs' survival, expression of immunosuppressive molecules such as arginase-I and PD-L1, and proliferation and function of effector T cells in tumor tissues have been revealed in mouse tumor models. A reduction of this signaling contributed to the conversion of tumors to an immunoreactive tumor microenvironment, and this conversion significantly improved the efficacy of PD-1 ICI [108,109]. A multi-omics analysis of a TNBC cohort showed that genera under Clostridiales and the related metabolite trimethylamine N-oxide (TMAO) were positively associated with the TIME activation and immunotherapy efficacy [110]. Although showing promising prospects for converting cold tumors and improving the efficacy of immunotherapy, these aspects of TNBC have not been studied sufficiently as of now, and more research is required.

10. Biomarkers for Predicting Immunological Response

10.1. PD-L1 Expression and TILs

The predictive value of PD-L1 expression for the efficacy of PD-(L)1 inhibitors in TNBC has been demonstrated in several trials [27,40,44]. However, there are still limitations related to choosing PD-L1 as a predictive marker. First, PD-L1 expression is spatiotemporally variable. It not only evolves over time with disease progression but also varies by metastatic location, with the highest prevalence of positivity in lymph nodes and the lowest in the liver [111]. Moreover, PD-L1 status was found to be less predictive of efficacy in early-stage TNBC compared to late-stage disease, as discussed previously [55,60]. Second, there are a variety of immunohistochemistry assays for PD-L1 expression status detection, but a lack of standard test methods. The five mainstream assays commonly used from two companies are the 22C3, 28-8, and 73-10 assays on the DAKO AutoStainer Link 48 platform and the SP142 and SP263 assays on the Ventana Benchmark Ultra platform. These assays use different primary antibodies to assess PD-L1 expression in tumor cells and/or tumor-infiltrating immune cells with different scoring criteria and definitions of PD-L1 positivity [112,113]. The 22C3 assay uses a combined positive score (CPS) based on both tumor cells and immune cells (lymphocytes and macrophages) staining to determine PD-L1-positive tumors in mTNBC patients for pembrolizumab, with a cutoff value of 10 in KEYNOTE-355. In contrast, the SP142 assay uses the percentage of stained tumor-infiltrating immune cells (IC) to the tumor area to determine PD-L1-positive tumors for atezolizumab, with a cutoff value of 1% in IMpassion130. Occasionally, in some trials, the percentage of tumor cells (TC) stained is also used to assess PD-L1 expression [27,57]. It is worth noting that the three scoring systems differ significantly in terms of algorithms and the types of cells evaluated. A comparative study analyzed the concordance between different PD-L1 assays and the relationship with patient clinical outcomes. The results showed poor equivalence between the different assays and they were not analytically interchangeable. SP142 assay ($\geq 1\%$ IC) detected the least prevalence of PD-L1 positivity at 46.4% (74.9% for SP263 ($\geq 1\%$ IC) and 80.9% for 22C3 (CPS ≥ 1)), with almost all of these patients captured by the other two assays, and these patients had better clinical outcome improvement with the application of atezolizumab [114]. In addition, tissue fixation methods and subjective factors of pathologists may also affect PD-L1 results [115,116]. Finally, with the advent of some new

treatment combinations, some patients who are negative for PD-L1 can also profit from ICIs, since some drugs upregulate PD-L1 expression during treatment, which is unpredictable before therapy. Conclusively, the predictive value of PD-L1 expression for the efficacy of PD-(L)1 blockade is undeniable, but is not a determinant. Therefore, caution should be exercised when making treatment decisions based on PD-L1 status.

TILs are a cell population consisting of T cells, B cells, and NK cells, including both tumor-killing and immunosuppressive cells [117]. As with PD-L1 expression, TILs are also spatiotemporally variable [51,111]. In KEYNOTE-086, higher levels of TILs were associated with better ORR [118]. In a biomarker analysis of KEYNOTE-119, high TIL levels were related to better clinical outcomes with pembrolizumab, but not with chemotherapy. Patients with TNBC and TILs $\geq 5\%$ survived longer with pembrolizumab than with chemotherapy, but this difference was not significant [119]. In contrast, IMpassion130 showed that stromal TIL (sTIL) levels were synergistic with PD-L1 expression; when assessed independent of PD-L1, TILs failed to provide prognostic value [111]. A simple method of section staining is recommended to quantify the extent of TIL infiltration [120]. TILs appear to be a promising biomarker for predicting the efficacy of ICIs at a lower cost, but more evidence is needed. Furthermore, in addition to the numerical level, the composition ratio of cellular components, activation status, and spatial location distribution of TILs are additional important factors that deserve further investigation when exploring the predictive effect of TILs on the efficacy of immunotherapy.

10.2. TMB and Microsatellite Instability (MSI)/Mismatch Repair Deficiency (dMMR)

Tumor mutational burden (TMB) is a measurement of the number of nonsynonymous somatic mutations in the genome of tumor cells [121]. When TMB > 10 mutations/Mb, neoantigen production becomes common to tumor cells and can be recognized by TILs [121]. High TMB has been associated with efficacy benefits for ICIs in various tumors [122,123]. Despite being the highest TMB subtype of breast cancer, TNBC still has a low mutational load compared to other tumors such as melanoma. One study showed that the median TMB in breast cancer was 2.63 mut/Mb and only 5% of patients had high TMB (>10 mut/Mb), with metastatic tumors having higher TMB. Of these, the median mutational burden in TNBC was 1.8 mut/Mb [124]. Data from 149 TNBC patients in the GeparNuevo trial showed a median TMB of 1.52 mut/Mb, and continuous TMB independently predicted pCR [125]. Data from 253 patients in the KEYNOTE-119 trial showed a positive correlation between TMB and clinical response to pembrolizumab, but not to chemotherapy [126]. However, in another study, high TMB failed to predict response to ICIs. In the TNBC subgroup, 10 patients with high TMB (>10 mut/Mb) had an ORR of 0, compared to 20.5% in patients with low TMB. The reason for the immaturity of TMB as a predictor for the efficacy of ICIs is mainly due to the fact that antigens generated by tumor mutations may not be immunogenic [127].

In fact, MSI/dMMR is one possible cause of high TMB [128]. Although MSI-high/dMMR has been shown to be associated with immunotherapy efficacy in a variety of tumors and has been approved by the FDA as a biomarker for the application of PD-1 blockers in solid tumors [129–131], its frequency is extremely low in TNBC, even in the high-level TIL subtype [132,133]. Based on the available evidence, MSI-high/dMMR is not a practical biomarker for screening TNBC patients who are or are not suitable for immunotherapy.

The aforementioned biomarkers predict PD-(L)1 blockade responses either from the perspective of the TIME or the tumor itself, but none of them are perfect. For now, the combination of several biomarkers to screen suitable patients may be more reliable. The most critical point in selecting immunotherapy-sensitive individuals and giving the most appropriate therapy is to identify the immune deficiency at the tumor site; this is quite difficult, especially in patients with metastases, as many mechanisms of immune escape may exist. However, with further understanding of tumor immune mechanisms, individualized and precise immunotherapy becomes increasingly possible, especially with the influx of novel genomics, single-cell sequencing, and artificial intelligence technologies.

11. Pseudoprogression and Immune-Related Adverse Events

The unique biological mechanisms of immunotherapy-fighting tumors enable a long-term or even complete response. However, they also require a long response time, which may lead to the emergence of immune-related patterns of pseudoprogression, hyperprogression, or a mixed response [134–137]. Pseudoprogression refers to an initial increase in tumor size followed by a decrease of tumor burden, and is associated with immune cell infiltration, edema, or necrosis due to immunotherapy [138]. Pseudoprogression after immunotherapy for TNBC has been reported [139], but incidence rates based on large samples are lacking. Previous data suggest that the incidence of pseudoprogression in solid tumors is less than 10%, which implies that some patients who present with progression after treatment are likely to have true progression [140]. Although the immune-related response criteria (irRC), immune-related RECIST (irRECIST), and immune RECIST (iRECIST) have been published to assist clinicians in evaluating response to immunotherapy, these are not yet widely used in clinical practice [141–143]. Therefore, in patients presenting with tumor progression after initial immune-based therapy, clinicians must assess patients' clinical conditions and toxicity responses thoroughly before carefully deciding on subsequent treatments. This is especially important when immunotherapy is used in combination with other therapies that have tumor-killing effects.

Along with durable anti-tumor activity comes immune-related adverse events (irAEs) that are distinct from the toxicity of conventional chemotherapy. These irAEs vary according to the type of immunotherapy, but there are some common features among ICIs [144]. First, irAEs are mostly organ-specific, occurring mainly in immune-related organs, with rare cases reported involving multiple organ events at the same time [144,145]. Second, while some irAEs occur rapidly, others are regularly delayed, even after treatment [144]. Finally, there is no clear relationship between irAEs and ICI dose [144,146]. These features remind us that irAEs require long-term management that cannot be limited to the period of dosing. Furthermore, the appearance of some toxicities often requires interruptions or even permanent discontinuation of dosing. The most common irAEs in breast cancer patients are rashes, followed by thyroid dysfunction (hypothyroidism > hyperthyroidism), and infusion reactions [147]. Although these irAEs are not usually fatal, they often require high-dose corticosteroid treatment, which may lead to a reduced efficacy of immunotherapy along with additional side effects. On the other hand, patients with permanent endocrine organ damage (such as the thyroid) are required to take therapeutic drugs for the rest of their life and their quality of life is therefore compromised. Notably, the current addition of PD-(L)1 inhibitors to conventional therapies in TNBC patients has already increased the incidence of associated irAEs, although severe incident rates are less than 10% [147–149]. Lessons from other tumor types show us that some novel immunotherapies, as well as combination treatments with immunotherapies, can lead to a higher incidence of irAEs and even severe cytokine release syndromes [150–152]. Therefore, the introduction of novel immunotherapies and new combination regimens is something that should be given extra attention. Early identification and management of irAEs is extremely important. Of particular consideration is the impact of immunotherapy on fertility, as a significant proportion of patients with TNBC are younger than 40 years of age.

12. Conclusions

TNBC is the subtype of breast cancer with the worst prognosis. To date, although several targeted drugs have been approved for the treatment of TNBC, the urgent need for improved survival has not been met. The practice of immunotherapy in TNBC is just beginning to take off. An advantage of the later start in this field is that experience can be learned from other tumor types, both successful and failed. Although some progress has been made with respect to ICIs for TNBC, many challenges remain. Clinical results show that only a small proportion of patients with TNBC actually benefit from immunotherapy. Thus, identifying the target population and expanding the efficacy is a top priority. Overall, combination treatment is the way forward, but the combination treatment mode, sequence,

dosage, and duration require further exploration and careful attention should be focused on balancing economics and toxicity. As a growing number of preclinical and clinical studies are conducted in this field, we expect to reach the ultimate goal: to select the most suitable patients for immunotherapy, to give the most appropriate immunotherapy or immune-combination therapy, to accurately assess the efficacy of the treatment, and to achieve optimal therapeutic results with minimal toxic damage.

Author Contributions: Conceptualization, L.L. and Z.F.; methodology, L.L.; software, L.L.; validation, L.L., F.Z., and Z.L.; resources, L.L.; data curation, L.L. and F.Z.; writing—original draft preparation, L.L.; writing—review and editing, L.L., F.Z., and Z.L.; visualization, L.L.; supervision, Z.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Lin, N.U.; Vanderplas, A.; Hughes, M.E.; Theriault, R.L.; Edge, S.B.; Wong, Y.N.; Blayney, D.W.; Niland, J.C.; Winer, E.P.; Weeks, J.C. Clinicopathologic features, patterns of recurrence, and survival among women with triple-negative breast cancer in the National Comprehensive Cancer Network. *Cancer* **2012**, *118*, 5463–5472. [CrossRef] [PubMed]
- Dent, R.; Trudeau, M.; Pritchard, K.I.; Hanna, W.M.; Kahn, H.K.; Sawka, C.A.; Lickley, L.A.; Rawlinson, E.; Sun, P.; Narod, S.A. Triple-negative breast cancer: Clinical features and patterns of recurrence. *Clin. Cancer Res.* **2007**, *13*, 4429–4434. [CrossRef]
- Nedeljkovic, M.; Damjanovic, A. Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer—How We Can Rise to the Challenge. *Cells* **2019**, *8*, 957. [CrossRef]
- Lehmann, B.D.; Jovanovic, B.; Chen, X.; Estrada, M.V.; Johnson, K.N.; Shyr, Y.; Moses, H.L.; Sanders, M.E.; Pienpol, J.A. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. *PLoS One* **2016**, *11*, e0157368. [CrossRef]
- Burstein, M.D.; Tsimelzon, A.; Poage, G.M.; Covington, K.R.; Contreras, A.; Fuqua, S.A.; Savage, M.I.; Osborne, C.K.; Hilsenbeck, S.G.; Chang, J.C.; et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. *Clin. Cancer Res.* **2015**, *21*, 1688–1698. [CrossRef]
- Jiang, Y.Z.; Ma, D.; Suo, C.; Shi, J.; Xue, M.; Hu, X.; Xiao, Y.; Yu, K.D.; Liu, Y.R.; Yu, Y.; et al. Genomic and Transcriptomic Landscape of Triple-Negative Breast Cancers: Subtypes and Treatment Strategies. *Cancer Cell* **2019**, *35*, 428–440.e5. [CrossRef] [PubMed]
- De Melo Gagliato, D.; Cortes, J.; Curigliano, G.; Loi, S.; Denkert, C.; Perez-Garcia, J.; Holgado, E. Tumor-infiltrating lymphocytes in Breast Cancer and implications for clinical practice. *Biochim. Biophys. Acta. Rev. Cancer* **2017**, *1868*, 527–537. [CrossRef] [PubMed]
- Mittendorf, E.A.; Philips, A.V.; Meric-Bernstam, F.; Qiao, N.; Wu, Y.; Harrington, S.; Su, X.; Wang, Y.; Gonzalez-Angulo, A.M.; Akcakanat, A.; et al. PD-L1 expression in triple-negative breast cancer. *Cancer Immunol. Res.* **2014**, *2*, 361–370. [CrossRef] [PubMed]
- Kwa, M.J.; Adams, S. Checkpoint inhibitors in triple-negative breast cancer (TNBC): Where to go from here. *Cancer* **2018**, *124*, 2086–2103. [CrossRef]
- Dunn, G.P.; Bruce, A.T.; Ikeda, H.; Old, L.J.; Schreiber, R.D. Cancer immunoediting: From immunosurveillance to tumor escape. *Nat. Immunol.* **2002**, *3*, 991–998. [CrossRef] [PubMed]
- Chen, D.S.; Mellman, I. Oncology meets immunology: The cancer-immunity cycle. *Immunity* **2013**, *39*, 1–10. [CrossRef]
- Campoli, M.; Ferrone, S. HLA antigen changes in malignant cells: Epigenetic mechanisms and biologic significance. *Oncogene* **2008**, *27*, 5869–5885. [CrossRef]
- Schreiber, R.D.; Old, L.J.; Smyth, M.J. Cancer immunoediting: Integrating immunity’s roles in cancer suppression and promotion. *Science* **2011**, *331*, 1565–1570. [CrossRef] [PubMed]
- Kim, J.M.; Chen, D.S. Immune escape to PD-L1/PD-1 blockade: Seven steps to success (or failure). *Ann. Oncol.* **2016**, *27*, 1492–1504. [CrossRef] [PubMed]
- Dou, A.; Fang, J. Heterogeneous Myeloid Cells in Tumors. *Cancers* **2021**, *13*, 3772. [CrossRef] [PubMed]
- Spranger, S. Mechanisms of tumor escape in the context of the T-cell-inflamed and the non-T-cell-inflamed tumor microenvironment. *Int. Immunopharmacol.* **2016**, *28*, 383–391. [CrossRef]
- Dyck, L.; Mills, K.H.G. Immune checkpoints and their inhibition in cancer and infectious diseases. *Eur. J. Immunol.* **2017**, *47*, 765–779. [CrossRef] [PubMed]
- Feng, M.; Jiang, W.; Kim, B.Y.S.; Zhang, C.C.; Fu, Y.X.; Weissman, I.L. Phagocytosis checkpoints as new targets for cancer immunotherapy. *Nat. Rev. Cancer* **2019**, *19*, 568–586. [CrossRef]
- Sanmamed, M.F.; Chen, L. A Paradigm Shift in Cancer Immunotherapy: From Enhancement to Normalization. *Cell* **2018**, *175*, 313–326. [CrossRef]

20. Chen, D.S.; Mellman, I. Elements of cancer immunity and the cancer-immune set point. *Nature* **2017**, *541*, 321–330. [CrossRef]
21. Xiao, Y.; Ma, D.; Zhao, S.; Suo, C.; Shi, J.; Xue, M.Z.; Ruan, M.; Wang, H.; Zhao, J.; Li, Q.; et al. Multi-Omics Profiling Reveals Distinct Microenvironment Characterization and Suggests Immune Escape Mechanisms of Triple-Negative Breast Cancer. *Clin. Cancer Res.* **2019**, *25*, 5002–5014. [CrossRef] [PubMed]
22. Gruoso, T.; Gigoux, M.; Manem, V.S.K.; Bertos, N.; Zuo, D.; Perlitch, I.; Saleh, S.M.I.; Zhao, H.; Souleimanova, M.; Johnson, R.M.; et al. Spatially distinct tumor immune microenvironments stratify triple-negative breast cancers. *J. Clin. Investig.* **2019**, *129*, 1785–1800. [CrossRef] [PubMed]
23. Bareche, Y.; Buisseret, L.; Gruoso, T.; Girard, E.; Venet, D.; Dupont, F.; Desmedt, C.; Larsimont, D.; Park, M.; Rothe, F.; et al. Unraveling Triple-Negative Breast Cancer Tumor Microenvironment Heterogeneity: Towards an Optimized Treatment Approach. *J. Natl. Cancer Inst.* **2020**, *112*, 708–719. [CrossRef] [PubMed]
24. Keir, M.E.; Butte, M.J.; Freeman, G.J.; Sharpe, A.H. PD-1 and its ligands in tolerance and immunity. *Annu. Rev. Immunol.* **2008**, *26*, 677–704. [CrossRef] [PubMed]
25. Balar, A.V.; Weber, J.S. PD-1 and PD-L1 antibodies in cancer: Current status and future directions. *Cancer Immunol. Immunother.* **2017**, *66*, 551–564. [CrossRef]
26. Solinas, C.; Aiello, M.; Rozali, E.; Lambertini, M.; Willard-Gallo, K.; Migliori, E. Programmed cell death-ligand 2: A neglected but important target in the immune response to cancer? *Transl. Oncol.* **2020**, *13*, 100811. [CrossRef]
27. Nanda, R.; Chow, L.Q.; Dees, E.C.; Berger, R.; Gupta, S.; Geva, R.; Pusztai, L.; Pathiraja, K.; Aktan, G.; Cheng, J.D.; et al. Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. *J. Clin. Oncol.* **2016**, *34*, 2460–2467. [CrossRef]
28. Adams, S.; Loi, S.; Toppmeyer, D.; Cescon, D.W.; De Laurentiis, M.; Nanda, R.; Winer, E.P.; Mukai, H.; Tamura, K.; Armstrong, A.; et al. Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer: Cohort B of the phase II KEYNOTE-086 study. *Ann. Oncol.* **2019**, *30*, 405–411. [CrossRef]
29. Adams, S.; Schmid, P.; Rugo, H.S.; Winer, E.P.; Loirat, D.; Awada, A.; Cescon, D.W.; Iwata, H.; Campone, M.; Nanda, R.; et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: Cohort A of the phase II KEYNOTE-086 study. *Ann. Oncol.* **2019**, *30*, 397–404. [CrossRef]
30. Winer, E.P.; Lipatov, O.; Im, S.A.; Goncalves, A.; Munoz-Couselo, E.; Lee, K.S.; Schmid, P.; Tamura, K.; Testa, L.; Witzel, I.; et al. Pembrolizumab versus investigator-choice chemotherapy for metastatic triple-negative breast cancer (KEYNOTE-119): A randomised, open-label, phase 3 trial. *Lancet Oncol.* **2021**, *22*, 499–511. [CrossRef]
31. Dirix, L.Y.; Takacs, I.; Jerusalem, G.; Nikolinakos, P.; Arkenau, H.T.; Forero-Torres, A.; Boccia, R.; Lippman, M.E.; Somer, R.; Smakal, M.; et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: A phase 1b JAVELIN Solid Tumor study. *Breast. Cancer Res. Tr.* **2018**, *167*, 671–686. [CrossRef] [PubMed]
32. Emens, L.A.; Cruz, C.; Eder, J.P.; Braiteh, F.; Chung, C.; Tolaney, S.M.; Kuter, I.; Nanda, R.; Cassier, P.A.; Delord, J.P.; et al. Long-term Clinical Outcomes and Biomarker Analyses of Atezolizumab Therapy for Patients With Metastatic Triple-Negative Breast Cancer A Phase 1 Study. *Jama. Oncol.* **2019**, *5*, 74–82. [CrossRef] [PubMed]
33. Bachet, T.; Filleron, T.; Bieche, I.; Arnedos, M.; Campone, M.; Dalenc, F.; Coussy, F.; Sablin, M.P.; Debled, M.; Lefevre-Plesse, C.; et al. Durvalumab compared to maintenance chemotherapy in metastatic breast cancer: The randomized phase II SAFIR02-BREAST IMMUNO trial. *Nat. Med.* **2021**, *27*, 250–255. [CrossRef] [PubMed]
34. Pusztai, L.; Barlow, W.E.; Ganz, P.A.; Henry, N.L.; White, J.; Jaggi, R.; Mammen, J.M.V.; Lew, D.; Mejia, J.; Karantza, V.; et al. SWOG S1418/NRG-BR006: A randomized, phase III trial to evaluate the efficacy and safety of MK-3475 as adjuvant therapy for triple receptor-negative breast cancer with ≥ 1 cm residual invasive cancer or positive lymph nodes ($> pN1mic$) after neoadjuvant chemotherapy. *Cancer Res.* **2018**, *78* (Suppl. 4), OT1-02-04. [CrossRef]
35. Conte, P.F.; Dieci, M.V.; Bisagni, G.; De Laurentiis, M.; Tondini, C.A.; Schmid, P.; De Salvo, G.L.; Moratello, G.; Guarneri, V. Phase III randomized study of adjuvant treatment with the ANTI-PD-L1 antibody avelumab for high-risk triple negative breast cancer patients: The A-BRAVE trial. *J. Clin. Oncol.* **2020**, *38*, TPS598. [CrossRef]
36. Galluzzi, L.; Buque, A.; Kepp, O.; Zitvogel, L.; Kroemer, G. Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. *Cancer Cell* **2015**, *28*, 690–714. [CrossRef]
37. Zitvogel, L.; Tesniere, A.; Kroemer, G. Cancer despite immunosurveillance: Immunoselection and immunosubversion. *Nat. Rev. Immunol.* **2006**, *6*, 715–727. [CrossRef]
38. Wu, J.; Waxman, D.J. Immunogenic chemotherapy: Dose and schedule dependence and combination with immunotherapy. *Cancer Lett.* **2018**, *419*, 210–221. [CrossRef]
39. Ahlmann, M.; Hempel, G. The effect of cyclophosphamide on the immune system: Implications for clinical cancer therapy. *Cancer Chemoth. Pharm.* **2016**, *78*, 661–671. [CrossRef]
40. Cortes, J.; Cescon, D.W.; Rugo, H.S.; Nowecki, Z.; Im, S.A.; Yusof, M.M.; Gallardo, C.; Lipatov, O.; Barrios, C.H.; Holgado, E.; et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): A randomised, placebo-controlled, double-blind, phase 3 clinical trial. *Lancet* **2020**, *396*, 1817–1828. [CrossRef]
41. Cortes, J.; Rugo, H.S.; Cescon, D.W.; Im, S.A.; Yusof, M.M.; Gallardo, C.; Lipatov, O.; Barrios, C.H.; Perez-Garcia, J.; Iwata, H.; et al. Pembrolizumab plus Chemotherapy in Advanced Triple-Negative Breast Cancer. *N. Engl. J. Med.* **2022**, *387*, 217–226. [CrossRef] [PubMed]

42. Tolaney, S.M.; Kalinsky, K.; Kaklamani, V.G.; D'Adamo, D.R.; Aktan, G.; Tsai, M.L.; O'Regan, R.M.; Kaufman, P.A.; Wilks, S.T.; Andreopoulou, E.; et al. Eribulin Plus Pembrolizumab in Patients with Metastatic Triple-Negative Breast Cancer (ENHANCE 1): A Phase Ib/II Study. *Clin. Cancer Res.* **2021**, *27*, 3061–3068. [CrossRef]
43. Adams, S.; Diamond, J.R.; Hamilton, E.; Pohlmann, P.R.; Tolaney, S.M.; Chang, C.W.; Zhang, W.; Iizuka, K.; Foster, P.G.; Molinero, L.; et al. Atezolizumab Plus nab-Paclitaxel in the Treatment of Metastatic Triple-Negative Breast Cancer With 2-Year Survival Follow-up: A Phase 1b Clinical Trial. *Jama. Oncol.* **2019**, *5*, 334–342. [CrossRef] [PubMed]
44. Schmid, P.; Adams, S.; Rugo, H.S.; Schneeweiss, A.; Barrios, C.H.; Iwata, H.; Dieras, V.; Hegg, R.; Im, S.A.; Shaw Wright, G.; et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. *N. Engl. J. Med.* **2018**, *379*, 2108–2121. [CrossRef] [PubMed]
45. Schmid, P.; Rugo, H.S.; Adams, S.; Schneeweiss, A.; Barrios, C.H.; Iwata, H.; Dieras, V.; Henschel, V.; Molinero, L.; Chui, S.Y.; et al. Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): Updated efficacy results from a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* **2020**, *21*, 44–59. [CrossRef]
46. Emens, L.A.; Adams, S.; Barrios, C.H.; Dieras, V.; Iwata, H.; Loi, S.; Rugo, H.S.; Schneeweiss, A.; Winer, E.P.; Patel, S.; et al. First-line atezolizumab plus nab-paclitaxel for unresectable, locally advanced, or metastatic triple-negative breast cancer: IMpassion130 final overall survival analysis. *Ann. Oncol.* **2021**, *32*, 983–993. [CrossRef]
47. Miles, D.; Gligorov, J.; Andre, F.; Cameron, D.; Schneeweiss, A.; Barrios, C.; Xu, B.; Wardley, A.; Kaen, D.; Andrade, L.; et al. Primary results from IMpassion131, a double-blind, placebo-controlled, randomised phase III trial of first-line paclitaxel with or without atezolizumab for unresectable locally advanced/metastatic triple-negative breast cancer. *Ann. Oncol.* **2021**, *32*, 994–1004. [CrossRef]
48. Franzoi, M.A.; de Azambuja, E. Atezolizumab in metastatic triple-negative breast cancer: IMpassion130 and 131 trials—how to explain different results? *ESMO Open* **2020**, *5*, e001112. [CrossRef]
49. Zhang, Y.Y.; Chen, H.Y.; Mo, H.N.; Hu, X.D.; Gao, R.R.; Zhao, Y.H.; Liu, B.L.; Niu, L.J.; Sun, X.Y.; Yu, X.; et al. Single-cell analyses reveal key immune cell subsets associated with response to PD-L1 blockade in triple-negative breast cancer. *Cancer Cell* **2021**, *39*, 1578–1593.e8. [CrossRef]
50. Voorwerk, L.; Slagter, M.; Horlings, H.M.; Sikorska, K.; van de Vijver, K.K.; de Maaker, M.; Nederlof, I.; Kluin, R.J.C.; Warren, S.; Ong, S.; et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: The TONIC trial. *Nat. Med.* **2019**, *25*, 920–928. [CrossRef]
51. Hutchinson, K.E.; Yost, S.E.; Chang, C.W.; Johnson, R.M.; Carr, A.R.; McAdam, P.R.; Halligan, D.L.; Chang, C.C.; Schmolze, D.; Liang, J.; et al. Comprehensive Profiling of Poor-Risk Paired Primary and Recurrent Triple-Negative Breast Cancers Reveals Immune Phenotype Shifts. *Clin. Cancer Res.* **2020**, *26*, 657–668. [CrossRef]
52. Szekely, B.; Bossuyt, V.; Li, X.; Wali, V.B.; Patwardhan, G.A.; Frederick, C.; Silber, A.; Park, T.; Harigopal, M.; Pelekanou, V.; et al. Immunological differences between primary and metastatic breast cancer. *Ann. Oncol.* **2018**, *29*, 2232–2239. [CrossRef] [PubMed]
53. Nanda, R.; Liu, M.C.; Yau, C.; Shatsky, R.; Pusztai, L.; Wallace, A.; Chien, A.J.; Forero-Torres, A.; Ellis, E.; Han, H.; et al. Effect of Pembrolizumab Plus Neoadjuvant Chemotherapy on Pathologic Complete Response in Women With Early-Stage Breast Cancer: An Analysis of the Ongoing Phase 2 Adaptively Randomized I-SPY2 Trial. *JAMA Oncol.* **2020**, *6*, 676–684. [CrossRef] [PubMed]
54. Schmid, P.; Salgado, R.; Park, Y.H.; Munoz-Couselo, E.; Kim, S.B.; Sohn, J.; Im, S.A.; Foukakis, T.; Kuemmel, S.; Dent, R.; et al. Pembrolizumab plus chemotherapy as neoadjuvant treatment of high-risk, early-stage triple-negative breast cancer: Results from the phase 1b open-label, multicohort KEYNOTE-173 study. *Ann. Oncol.* **2020**, *31*, 569–581. [CrossRef] [PubMed]
55. Schmid, P.; Cortes, J.; Pusztai, L.; McArthur, H.; Kummel, S.; Bergh, J.; Denkert, C.; Park, Y.H.; Hui, R.; Harbeck, N.; et al. Pembrolizumab for Early Triple-Negative Breast Cancer. *N. Engl. J. Med.* **2020**, *382*, 810–821. [CrossRef]
56. Schmid, P.; Cortes, J.; Dent, R.; Pusztai, L.; McArthur, H.; Kummel, S.; Bergh, J.; Denkert, C.; Park, Y.H.; Hui, R.; et al. Event-free Survival with Pembrolizumab in Early Triple-Negative Breast Cancer. *N. Engl. J. Med.* **2022**, *386*, 556–567. [CrossRef]
57. Loibl, S.; Untch, M.; Burchardi, N.; Huober, J.; Sinn, B.V.; Blohmer, J.U.; Grischke, E.M.; Furlanetto, J.; Tesch, H.; Hanusch, C.; et al. A randomised phase II study investigating durvalumab in addition to an anthracycline taxane-based neoadjuvant therapy in early triple-negative breast cancer: Clinical results and biomarker analysis of GeparNuevo study. *Ann. Oncol.* **2019**, *30*, 1279–1288. [CrossRef]
58. Loibl, S.; Schneeweiss, A.; Huober, J.; Braun, M.; Rey, J.; Blohmer, J.U.; Furlanetto, J.; Zahm, D.M.; Hanusch, C.; Thomalla, J.; et al. Neoadjuvant durvalumab improves survival in early triple-negative breast cancer independent of pathological complete response. *Ann. Oncol.* **2022**, *33*, 1149–1158. [CrossRef]
59. Gianni, L.; Huang, C.S.; Egle, D.; Bermejo, B.; Zamagni, C.; Thill, M.; Anton, A.; Zambelli, S.; Bianchini, G.; Russo, S.; et al. Pathologic complete response (pCR) to neoadjuvant treatment with or without atezolizumab in triple-negative, early high-risk and locally advanced breast cancer: NeoTRIP Michelangelo randomized study. *Ann. Oncol.* **2022**, *33*, 534–543. [CrossRef]
60. Mittendorf, E.A.; Zhang, H.; Barrios, C.H.; Saji, S.; Jung, K.H.; Hegg, R.; Koehler, A.; Sohn, J.; Iwata, H.; Telli, M.L.; et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): A randomised, double-blind, phase 3 trial. *Lancet* **2020**, *396*, 1090–1100. [CrossRef]
61. McLaughlin, M.; Patin, E.C.; Pedersen, M.; Wilkins, A.; Dillon, M.T.; Melcher, A.A.; Harrington, K.J. Inflammatory microenvironment remodelling by tumour cells after radiotherapy. *Nat. Rev. Cancer* **2020**, *20*, 203–217. [CrossRef]

62. Ho, A.Y.; Barker, C.A.; Arnold, B.B.; Powell, S.N.; Hu, Z.I.; Gucalp, A.; Lebron-Zapata, L.; Wen, H.Y.; Kallman, C.; D’Agnolo, A.; et al. A phase 2 clinical trial assessing the efficacy and safety of pembrolizumab and radiotherapy in patients with metastatic triple-negative breast cancer. *Cancer* **2020**, *126*, 850–860. [CrossRef] [PubMed]
63. David, S.; Savas, P.; Siva, S.; White, M.; Neeson, M.W.; White, S.; Marx, G.; Cheuk, R.; Grogan, M.; Farrell, M.; et al. A randomised phase II trial of single fraction or multi-fraction SABR (stereotactic ablative body radiotherapy) with atezolizumab in patients with advanced triple negative breast cancer (AZTEC trial). *Cancer Res.* **2022**, *82*, PD10–02. [CrossRef]
64. Pantelidou, C.; Sonzogni, O.; De Oliveria Taveira, M.; Mehta, A.K.; Kothari, A.; Wang, D.; Visal, T.; Li, M.K.; Pinto, J.; Castrillon, J.A.; et al. PARP Inhibitor Efficacy Depends on CD8(+) T-cell Recruitment via Intratumoral STING Pathway Activation in BRCA-Deficient Models of Triple-Negative Breast Cancer. *Cancer Discov.* **2019**, *9*, 722–737. [CrossRef] [PubMed]
65. Sen, T.; Rodriguez, B.L.; Chen, L.; Corte, C.M.D.; Morikawa, N.; Fujimoto, J.; Cristea, S.; Nguyen, T.; Diao, L.; Li, L.; et al. Targeting DNA Damage Response Promotes Antitumor Immunity through STING-Mediated T-cell Activation in Small Cell Lung Cancer. *Cancer Discov.* **2019**, *9*, 646–661. [CrossRef] [PubMed]
66. Reislander, T.; Lombardi, E.P.; Groelly, F.J.; Miar, A.; Porru, M.; Di Vito, S.; Wright, B.; Lockstone, H.; Biocchio, A.; Harris, A.; et al. BRCA2 abrogation triggers innate immune responses potentiated by treatment with PARP inhibitors. *Nat. Commun.* **2019**, *10*, 3143. [CrossRef]
67. Vinayak, S.; Tolaney, S.M.; Schwartzberg, L.; Mita, M.; McCann, G.; Tan, A.R.; Wahner-Hendrickson, A.E.; Forero, A.; Anders, C.; Wulf, G.M.; et al. Open-label Clinical Trial of Niraparib Combined With Pembrolizumab for Treatment of Advanced or Metastatic Triple-Negative Breast Cancer. *JAMA Oncol.* **2019**, *5*, 1132–1140. [CrossRef]
68. Senkus-Konefka, E.; Domchek, S.M.; Im, S.A.; Xu, B.; Armstrong, A.; Masuda, N.; Delaloge, S.; Li, W.; Tung, N.; Conte, P.; et al. Subgroup analysis of olaparib monotherapy versus chemotherapy by hormone receptor and BRCA mutation status in patients with HER2-negative metastatic breast cancer and a germline BRCA mutation: Olympiad. *Eur. J. Cancer* **2018**, *92*, S19–S20. [CrossRef]
69. Eiermann, W.; Rugo, H.S.; Diab, S.; Ettl, J.; Hurvitz, S.A.; Goncalves, A. Analysis of germline BRCA1/2 mutated (gBRCA(mut)) hormone receptor-positive (HR plus) and triple negative breast cancer (TNBC) treated with talazoparib (TALA). *J. Clin. Oncol.* **2018**, *36*, 1070. [CrossRef]
70. Pusztai, L.; Yau, C.; Wolf, D.M.; Han, H.S.; Du, L.; Wallace, A.M.; String-Reasor, E.; Boughey, J.C.; Chien, A.J.; Elias, A.D.; et al. Durvalumab with olaparib and paclitaxel for high-risk HER2-negative stage II/III breast cancer: Results from the adaptively randomized I-SPY2 trial. *Cancer Cell.* **2021**, *39*, 989–998 e985. [CrossRef]
71. Yu, J.F.; Song, Y.P.; Tian, W.Z. How to select IgG subclasses in developing anti-tumor therapeutic antibodies. *J. Hematol. Oncol.* **2020**, *13*, 45. [CrossRef]
72. Li, F.; Ulrich, M.; Jonas, M.; Stone, I.J.; Linares, G.; Zhang, X.Q.; Westendorf, L.; Benjamin, D.R.; Law, C.L. Tumor-Associated Macrophages Can Contribute to Antitumor Activity through Fc gamma R-Mediated Processing of Antibody-Drug Conjugates. *Mol. Cancer Ther.* **2017**, *16*, 1347–1354. [CrossRef]
73. Cao, A.T.; Higgins, S.; Stevens, N.; Gardai, S.J.; Sussman, D. Additional mechanisms of action of ladiratuzumab vedotin contribute to increased immune cell activation within the tumor. *Cancer Res.* **2018**, *78*, 2742. [CrossRef]
74. Bauzon, M.; Drake, P.M.; Barfield, R.M.; Cornali, B.M.; Rupniewski, I.; Rabuka, D. Maytansine-bearing antibody-drug conjugates induce *in vitro* hallmarks of immunogenic cell death selectively in antigen-positive target cells. *Oncoimmunology* **2019**, *8*, e1565859. [CrossRef]
75. Muller, P.; Martin, K.; Theurich, S.; Schreiner, J.; Savic, S.; Terszowski, G.; Lardinois, D.; Heinzelmann-Schwarz, V.A.; Schlaak, M.; Kvasnicka, H.M.; et al. Microtubule-Depolymerizing Agents Used in Antibody-Drug Conjugates Induce Antitumor Immunity by Stimulation of Dendritic Cells. *Cancer Immunol. Res.* **2014**, *2*, 741–755. [CrossRef] [PubMed]
76. McKenzie, J.A.; Mbofung, R.M.; Malu, S.; Zhang, M.; Ashkin, E.; Devi, S.; Williams, L.; Tieu, T.; Peng, W.Y.; Pradeep, S.; et al. The Effect of Topoisomerase I Inhibitors on the Efficacy of T-Cell-Based Cancer Immunotherapy. *Jnci.-J. Natl. Cancer Inst.* **2018**, *110*, 777–786. [CrossRef] [PubMed]
77. Han, H.; Diab, S.; Alemany, C.; Basho, R.; Brown-Glberman, U.; Meisel, J.; Pluard, T.; Cortes, J.; Dillon, P.; Ettl, J.; et al. Open label phase 1b/2 study of ladiratuzumab vedotin in combination with pembrolizumab for first-line treatment of patients with unresectable locally-advanced or metastatic triple-negative breast cancer. *Cancer Res.* **2020**, *80*, PD1–06. [CrossRef]
78. Bergholz, J.S.; Zhao, J.J. How Compensatory Mechanisms and Adaptive Rewiring Have Shaped Our Understanding of Therapeutic Resistance in Cancer. *Cancer Res.* **2021**, *81*, 6074–6077. [CrossRef] [PubMed]
79. Zhang, Z.; Richmond, A.; Yan, C. Immunomodulatory Properties of PI3K/AKT/mTOR and MAPK/MEK/ERK Inhibition Augment Response to Immune Checkpoint Blockade in Melanoma and Triple-Negative Breast Cancer. *Int. J. Mol. Sci.* **2022**, *23*, 7353. [CrossRef]
80. Zhang, Z.; Richmond, A. The Role of PI3K Inhibition in the Treatment of Breast Cancer, Alone or Combined With Immune Checkpoint Inhibitors. *Front Mol. Biosci.* **2021**, *8*, 648663. [CrossRef]
81. Ho, P.C.; Meeth, K.M.; Tsui, Y.C.; Srivastava, B.; Bosenberg, M.W.; Kaech, S.M. Immune-based antitumor effects of BRAF inhibitors rely on signaling by CD40L and IFNgamma. *Cancer Res.* **2014**, *74*, 3205–3217. [CrossRef] [PubMed]
82. Schmid, P.; Savas, P.; Espinosa, E.; Boni, V.; Italiano, A.; White, S.; Cheng, K.; Lam, L.; Robert, L.; Laliman, V.; et al. Phase 1b study evaluating a triplet combination of ipatasertib (IPAT), atezolizumab, and a taxane as first-line therapy for locally advanced/metastatic triple-negative breast cancer (TNBC). *Cancer Res.* **2021**, *81*, PS12–28. [CrossRef]

83. Brufsky, A.; Kim, S.B.; Zvirbuli, Z.; Eniu, A.; Mebis, J.; Sohn, J.H.; Wongchenko, M.; Chohan, S.; Amin, R.; Yan, Y.; et al. A phase II randomized trial of cobimetinib plus chemotherapy, with or without atezolizumab, as first-line treatment for patients with locally advanced or metastatic triple-negative breast cancer (COLET): Primary analysis. *Ann. Oncol.* **2021**, *32*, 652–660. [CrossRef]
84. Lanitis, E.; Irving, M.; Coukos, G. Targeting the tumor vasculature to enhance T cell activity. *Curr. Opin. Immunol.* **2015**, *33*, 55–63. [CrossRef]
85. Corzo, C.A.; Condamine, T.; Lu, L.; Cotter, M.J.; Youn, J.I.; Cheng, P.; Cho, H.I.; Celis, E.; Quiceno, D.G.; Padhya, T.; et al. HIF-1 α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J. Exp. Med.* **2010**, *207*, 2439–2453. [CrossRef] [PubMed]
86. Li, Q.; Wang, Y.; Jia, W.; Deng, H.; Li, G.; Deng, W.; Chen, J.; Kim, B.Y.S.; Jiang, W.; Liu, Q.; et al. Low-Dose Anti-Angiogenic Therapy Sensitizes Breast Cancer to PD-1 Blockade. *Clin. Cancer Res.* **2020**, *26*, 1712–1724. [CrossRef] [PubMed]
87. Liu, J.Q.; Liu, Q.; Li, Y.; Li, Q.; Su, F.X.; Yao, H.R.; Su, S.C.; Wang, Q.R.; Jin, L.; Wang, Y.; et al. Efficacy and safety of camrelizumab combined with apatinib in advanced triple-negative breast cancer: An open-label phase II trial. *J. Immunother. Cancer* **2020**, *8*, e000696. [CrossRef] [PubMed]
88. Liu, Z.; Li, M.; Jiang, Z.; Wang, X. A Comprehensive Immunologic Portrait of Triple-Negative Breast Cancer. *Transl. Oncol.* **2018**, *11*, 311–329. [CrossRef] [PubMed]
89. Saleh, R.; Toor, S.M.; Khalaf, S.; Elkord, E. Breast Cancer Cells and PD-1/PD-L1 Blockade Upregulate the Expression of PD-1, CTLA-4, TIM-3 and LAG-3 Immune Checkpoints in CD4(+) T Cells. *Vaccines* **2019**, *7*, 149. [CrossRef] [PubMed]
90. Rowshanravan, B.; Halliday, N.; Sansom, D.M. CTLA-4: A moving target in immunotherapy. *Blood* **2018**, *131*, 58–67. [CrossRef]
91. Tang, F.; Du, X.; Liu, M.; Zheng, P.; Liu, Y. Anti-CTLA-4 antibodies in cancer immunotherapy: Selective depletion of intratumoral regulatory T cells or checkpoint blockade? *Cell Biosci.* **2018**, *8*, 30. [CrossRef]
92. Santa-Maria, C.A.; Kato, T.; Park, J.H.; Flaum, L.E.; Jain, S.; Tellez, C.; Stein, R.M.; Shah, A.N.; Gross, L.; Uthe, R.; et al. Durvalumab and tremelimumab in metastatic breast cancer (MBC): Immunotherapy and immunopharmacogenomic dynamics. *J. Clin. Oncol.* **2017**, *35*, 3052. [CrossRef]
93. Xu, B.H.; Li, Q.; Zhang, Q.Y.; Zhang, Y.; Ouyang, Q.C.; Zhang, Y.; Liu, Q.; Sun, T.; Xu, J.; Yang, J.; et al. Preliminary safety tolerability & efficacy results of KN046 (an anti-PD-L1/CTLA-4 bispecific antibody) in combination with Nab-paclitaxel in patients with metastatic triple-negative breast cancer (mTNBC). *Cancer Res.* **2021**, *81*, 1660.
94. Ghalamfarsa, G.; Kazemi, M.H.; Mohseni, S.R.; Masjedi, A.; Hojjat-Farsangi, M.; Azizi, G.; Yousefi, M.; Jadidi-Niaragh, F. CD73 as a potential opportunity for cancer immunotherapy. *Expert. Opin. Ther. Tar.* **2019**, *23*, 127–142. [CrossRef] [PubMed]
95. Loi, S.; Pommey, S.; Haibe-Kains, B.; Beavis, P.A.; Darcy, P.K.; Smyth, M.J.; Stagg, J. CD73 promotes anthracycline resistance and poor prognosis in triple negative breast cancer. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 11091–11096. [CrossRef]
96. Buisseret, L.; Loirat, D.; Aftimos, P.G.; Punie, K.; Maurer, C.; Debien, V.; Goncalves, A.; Ghiringhelli, F.; Taylor, D.; Clatot, F.; et al. Primary endpoint results of SYNERGY, a randomized phase II trial, first-line chemo-immunotherapy trial of durvalumab, paclitaxel, and carboplatin with or without the anti-CD73 antibody oleclumab in patients with advanced or metastatic triple-negative breast cancer (TNBC). *Ann. Oncol.* **2022**, *33* (Suppl.7), S808–S869. [CrossRef]
97. Shemesh, C.S.; Hsu, J.C.; Hosseini, I.; Shen, B.Q.; Rotte, A.; Twomey, P.; Girish, S.; Wu, B. Personalized Cancer Vaccines: Clinical Landscape, Challenges, and Opportunities. *Mol. Ther.* **2021**, *29*, 555–570. [CrossRef]
98. Schumacher, T.N.; Scheper, W.; Kvistborg, P. Cancer Neoantigens. *Annu. Rev. Immunol.* **2019**, *37*, 173–200. [CrossRef]
99. Fritah, H.; Rovelli, R.; Chiang, C.L.; Kandalaft, L.E. The current clinical landscape of personalized cancer vaccines. *Cancer Treat. Rev.* **2022**, *106*, 102383. [CrossRef] [PubMed]
100. Verma, V.; Shrimali, R.K.; Ahmad, S.; Dai, W.; Wang, H.; Lu, S.; Nandre, R.; Gaur, P.; Lopez, J.; Sade-Feldman, M.; et al. PD-1 blockade in subprimed CD8 cells induces dysfunctional PD-1(+)CD38(hi) cells and anti-PD-1 resistance. *Nat. Immunol.* **2019**, *20*, 1231–1243. [CrossRef]
101. Hemminki, O.; dos Santos, J.M.; Hemminki, A. Oncolytic viruses for cancer immunotherapy. *J. Hematol. Oncol.* **2020**, *13*, 84. [CrossRef] [PubMed]
102. Ylosmaki, E.; Cerullo, V. Design and application of oncolytic viruses for cancer immunotherapy. *Curr. Opin. Biotech.* **2020**, *65*, 25–36. [CrossRef]
103. Jin, S.; Wang, Q.; Wu, H.; Pang, D.; Xu, S. Oncolytic viruses for triple negative breast cancer and beyond. *Biomark Res.* **2021**, *9*, 71. [CrossRef] [PubMed]
104. Hecht, J.R.; Chan, A.; Baurain, J.F.; Martin, M.; Longo-Munoz, F.; Kalinsky, K.; Raman, S.; Liu, C.X.; Cha, E.; Chan, E. Preliminary safety data of intrahepatic talimogene laherparepvec and intravenous atezolizumab in patients with triple negative breast cancer. *Cancer Res.* **2020**, *80*, P3-09. [CrossRef]
105. Kistler, M.; Nangia, C.; To, C.; Sender, L.; Lee, J.; Jones, F.; Jafari, O.; Seery, T.; Rabizadeh, S.; Niazi, K.; et al. Safety and efficacy from first-in-human immunotherapy combining NK and T cell activation with off-the-shelf high-affinity CD16 NK cell line (haNK) in patients with 2nd-line or greater metastatic triple-negative breast cancer (TNBC). *Cancer Res.* **2020**, *80*, P5-04-02. [CrossRef]
106. Han, D.; Liu, J.; Chen, C.; Dong, L.; Liu, Y.; Chang, R.; Huang, X.; Liu, Y.; Wang, J.; Dougherty, U.; et al. Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells. *Nature* **2019**, *566*, 270–274. [CrossRef]
107. Sun, X.; Wu, B.; Chiang, H.C.; Deng, H.; Zhang, X.; Xiong, W.; Liu, J.; Rozeboom, A.M.; Harris, B.T.; Blommaert, E.; et al. Tumour DDR1 promotes collagen fibre alignment to instigate immune exclusion. *Nature* **2021**, *599*, 673–678. [CrossRef]

108. Mohammadpour, H.; MacDonald, C.R.; Qiao, G.; Chen, M.; Dong, B.; Hylander, B.L.; McCarthy, P.L.; Abrams, S.I.; Repasky, E.A. β 2 adrenergic receptor-mediated signaling regulates the immunosuppressive potential of myeloid-derived suppressor cells. *J. Clin. Investig.* **2019**, *129*, 5537–5552. [[CrossRef](#)]
109. Bucsek, M.J.; Qiao, G.; MacDonald, C.R.; Giridharan, T.; Evans, L.; Niedzwecki, B.; Liu, H.; Kokolus, K.M.; Eng, J.W.; Messmer, M.N.; et al. β -Adrenergic Signaling in Mice Housed at Standard Temperatures Suppresses an Effector Phenotype in CD8(+) T Cells and Undermines Checkpoint Inhibitor Therapy. *Cancer Res.* **2017**, *77*, 5639–5651. [[CrossRef](#)] [[PubMed](#)]
110. Wang, H.; Rong, X.; Zhao, G.; Zhou, Y.; Xiao, Y.; Ma, D.; Jin, X.; Wu, Y.; Yan, Y.; Yang, H.; et al. The microbial metabolite trimethylamine N-oxide promotes antitumor immunity in triple-negative breast cancer. *Cell Metab.* **2022**, *34*, 581–594 e588. [[CrossRef](#)]
111. Emens, L.A.; Molinero, L.; Loi, S.; Rugo, H.S.; Schneeweiss, A.; Dieras, V.; Iwata, H.; Barrios, C.H.; Nechaeva, M.; Nguyen-Duc, A.; et al. Atezolizumab and nab-Paclitaxel in Advanced Triple-Negative Breast Cancer: Biomarker Evaluation of the IMpassion130 Study. *J. Natl. Cancer Inst.* **2021**, *113*, 1005–1016. [[CrossRef](#)]
112. Badve, S.S.; Penault-Llorca, F.; Reis-Filho, J.S.; Deurloo, R.; Siziopikou, K.P.; D’Arrigo, C.; Viale, G. Determining PD-L1 Status in Patients With Triple-Negative Breast Cancer: Lessons Learned From IMpassion130. *J. Natl. Cancer Inst.* **2022**, *114*, 664–675. [[CrossRef](#)]
113. Chebib, I.; Mino-Kenudson, M. PD-L1 immunohistochemistry: Clones, cutoffs, and controversies. *APMIS* **2022**, *130*, 295–313. [[CrossRef](#)]
114. Rugo, H.S.; Loi, S.; Adams, S.; Schmid, P.; Schneeweiss, A.; Barrios, C.H.; Iwata, H.; Dieras, V.; Winer, E.P.; Kockx, M.M.; et al. PD-L1 Immunohistochemistry Assay Comparison in Atezolizumab plus nab-Paclitaxel-Treated Advanced Triple-Negative Breast Cancer. *J. Natl. Cancer Inst.* **2021**, *113*, 1733–1743. [[CrossRef](#)] [[PubMed](#)]
115. Ghebeh, H.; Mansour, F.A.; Colak, D.; Alfuraydi, A.A.; Al-Thubiti, A.A.; Monies, D.; Al-Alwan, M.; Al-Tweigeri, T.; Tulbah, A. Higher PD-L1 Immunohistochemical Detection Signal in Frozen Compared to Matched Paraffin-Embedded Formalin-Fixed Tissues. *Antibodies* **2021**, *10*, 24. [[CrossRef](#)] [[PubMed](#)]
116. Reisenbichler, E.S.; Han, G.; Bellizzi, A.; Bossuyt, V.; Brock, J.; Cole, K.; Fadare, O.; Hameed, O.; Hanley, K.; Harrison, B.T.; et al. Prospective multi-institutional evaluation of pathologist assessment of PD-L1 assays for patient selection in triple negative breast cancer. *Mod. Pathol.* **2020**, *33*, 1746–1752. [[CrossRef](#)] [[PubMed](#)]
117. Paijens, S.T.; Vledder, A.; de Bruyn, M.; Nijman, H.W. Tumor-infiltrating lymphocytes in the immunotherapy era. *Cell Mol. Immunol.* **2021**, *18*, 842–859. [[CrossRef](#)]
118. Loi, S.; Adams, S.; Schmid, P.; Cortes, J.; Cescon, D.W.; Winer, E.P.; Toppmeyer, D.L.; Rugo, H.S.; De Laurentiis, M.; Nanda, R.; et al. Relationship between tumor infiltrating lymphocyte (TIL) levels and response to pembrolizumab (pembro) in metastatic triple-negative breast cancer (mTNBC): Results from KEYNOTE-086. *Ann. Oncol.* **2017**, *28*, v608. [[CrossRef](#)]
119. Loi, S.; Winer, E.; Lipatov, O.; Im, S.A.; Goncalves, A.; Cortes, J.; Lee, K.S.; Schmid, P.; Testa, L.; Witzel, I.; et al. Relationship between tumor-infiltrating lymphocytes (TILs) and outcomes in the KEYNOTE-119 study of pembrolizumab vs chemotherapy for previously treated metastatic triple-negative breast cancer (mTNBC). *Cancer Res.* **2020**, *80*, PD5-03. [[CrossRef](#)]
120. Loi, S.; Michiels, S.; Adams, S.; Loibl, S.; Budczies, J.; Denkert, C.; Salgado, R. The journey of tumor-infiltrating lymphocytes as a biomarker in breast cancer: Clinical utility in an era of checkpoint inhibition. *Ann. Oncol.* **2021**, *32*, 1236–1244. [[CrossRef](#)]
121. Schumacher, T.N.; Schreiber, R.D. Neoantigens in cancer immunotherapy. *Science* **2015**, *348*, 69–74. [[CrossRef](#)]
122. Ott, P.A.; Bang, Y.J.; Piha-Paul, S.A.; Razak, A.R.A.; Bennouna, J.; Soria, J.C.; Rugo, H.S.; Cohen, R.B.; O’Neil, B.H.; Mehnert, J.M.; et al. T-Cell-Inflamed Gene-Expression Profile, Programmed Death Ligand 1 Expression, and Tumor Mutational Burden Predict Efficacy in Patients Treated With Pembrolizumab Across 20 Cancers: KEYNOTE-028. *J. Clin. Oncol.* **2019**, *37*, 318–327. [[CrossRef](#)] [[PubMed](#)]
123. Samstein, R.M.; Lee, C.H.; Shoushtari, A.N.; Hellmann, M.D.; Shen, R.; Janjigian, Y.Y.; Barron, D.A.; Zehir, A.; Jordan, E.J.; Omuro, A.; et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat. Genet.* **2019**, *51*, 202–206. [[CrossRef](#)] [[PubMed](#)]
124. Barroso-Sousa, R.; Jain, E.; Cohen, O.; Kim, D.; Buendia-Buendia, J.; Winer, E.; Lin, N.; Tolane, S.M.; Wagle, N. Prevalence and mutational determinants of high tumor mutation burden in breast cancer. *Ann. Oncol.* **2020**, *31*, 387–394. [[CrossRef](#)]
125. Karn, T.; Denkert, C.; Weber, K.E.; Holtrich, U.; Hanusch, C.; Sinn, B.V.; Higgs, B.W.; Jank, P.; Sinn, H.P.; Huober, J.; et al. Tumor mutational burden and immune infiltration as independent predictors of response to neoadjuvant immune checkpoint inhibition in early TNBC in GeparNuevo. *Ann. Oncol.* **2020**, *31*, 1216–1222. [[CrossRef](#)] [[PubMed](#)]
126. Winer, E.P.; Lipatov, O.; Im, S.A.; Goncalves, A.; Munoz-Couselo, E.; Lee, K.S.; Schmid, P.; Testa, L.; Witzel, I.; Ohtani, S.; et al. Association of tumor mutational burden (TMB) and clinical outcomes with pembrolizumab (pembro) versus chemotherapy (chemo) in patients with metastatic triple-negative breast cancer (mTNBC) from KEYNOTE-119. *J. Clin. Oncol.* **2020**, *38*, 1013. [[CrossRef](#)]
127. Jardim, D.L.; Goodman, A.; de Melo Gagliato, D.; Kurzrock, R. The Challenges of Tumor Mutational Burden as an Immunotherapy Biomarker. *Cancer Cell* **2021**, *39*, 154–173. [[CrossRef](#)]
128. Chalmers, Z.R.; Connelly, C.F.; Fabrizio, D.; Gay, L.; Ali, S.M.; Ennis, R.; Schrock, A.; Campbell, B.; Shlien, A.; Chmielecki, J.; et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med.* **2017**, *9*, 34. [[CrossRef](#)]

129. Zhao, P.; Li, L.; Jiang, X.; Li, Q. Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. *J. Hematol. Oncol.* **2019**, *12*, 54. [[CrossRef](#)]
130. Prasad, V.; Kaestner, V.; Mailankody, S. Cancer Drugs Approved Based on Biomarkers and Not Tumor Type-FDA Approval of Pembrolizumab for Mismatch Repair-Deficient Solid Cancers. *JAMA Oncol.* **2018**, *4*, 157–158. [[CrossRef](#)]
131. Marcus, L.; Lemery, S.J.; Keegan, P.; Pazdur, R. FDA Approval Summary: Pembrolizumab for the Treatment of Microsatellite Instability-High Solid Tumors. *Clin. Cancer Res.* **2019**, *25*, 3753–3758. [[CrossRef](#)]
132. Ren, X.Y.; Song, Y.; Wang, J.; Chen, L.Y.; Pang, J.Y.; Zhou, L.R.; Shen, S.J.; Cao, X.; Wang, Y.X.; Shao, M.M.; et al. Mismatch Repair Deficiency and Microsatellite Instability in Triple-Negative Breast Cancer: A Retrospective Study of 440 Patients. *Front Oncol.* **2021**, *11*, 570623. [[CrossRef](#)]
133. Horimoto, Y.; Hlaing, M.T.; Saeki, H.; Kitano, S.; Nakai, K.; Sasaki, R.; Kurisaki-Arakawa, A.; Arakawa, A.; Otsuji, N.; Matsuoka, S.; et al. Microsatellite instability and mismatch repair protein expressions in lymphocyte-predominant breast cancer. *Cancer Sci.* **2020**, *111*, 2647–2654. [[CrossRef](#)] [[PubMed](#)]
134. Schadendorf, D.; Hodi, F.S.; Robert, C.; Weber, J.S.; Margolin, K.; Hamid, O.; Patt, D.; Chen, T.T.; Berman, D.M.; Wolchok, J.D. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. *J. Clin. Oncol.* **2015**, *33*, 1889–1894. [[CrossRef](#)] [[PubMed](#)]
135. Wolchok, J.D.; Hamid, O.; Ribas, A.; Robert, C.; Kefford, R.; Hwu, W.J.; Weber, J.S.; Joshua, A.M.; Gangadhar, T.C.; Dronca, R.S.; et al. Atypical patterns of response in patients (pts) with metastatic melanoma treated with pembrolizumab (MK-3475) in KEYNOTE-001. *J. Clin. Oncol.* **2015**, *33*, 3000. [[CrossRef](#)]
136. Champiat, S.; Dercle, L.; Ammari, S.; Massard, C.; Hollebecque, A.; Postel-Vinay, S.; Chaput, N.; Eggermont, A.; Marabelle, A.; Soria, J.C.; et al. Hyperprogressive Disease Is a New Pattern of Progression in Cancer Patients Treated by Anti-PD-1/PD-L1. *Clinical. Cancer Res.* **2017**, *23*, 1920–1928. [[CrossRef](#)]
137. Tazdait, M.; Mezquita, L.; Lahmar, J.; Ferrara, R.; Bidault, F.; Ammari, S.; Balleyguier, C.; Planchard, D.; Gazzah, A.; Soria, J.C.; et al. Patterns of responses in metastatic NSCLC during PD-1 or PDL-1 inhibitor therapy: Comparison of RECIST 1.1, irRECIST and iRECIST criteria. *Eur. J. Cancer* **2018**, *88*, 38–47. [[CrossRef](#)]
138. Chiou, V.L.; Burotto, M. Pseudoprogression and Immune-Related Response in Solid Tumors. *J. Clin. Oncol.* **2015**, *33*, 3541–3543. [[CrossRef](#)]
139. Schmid, P.; Cruz, C.; Braiteh, F.S.; Eder, J.P.; Tolane, S.; Kuter, I.; Nanda, R.; Chung, C.; Cassier, P.; Delord, J.P.; et al. Atezolizumab in metastatic TNBC (mTNBC): Long-term clinical outcomes and biomarker analyses. *Cancer Res.* **2017**, *77*, 2986. [[CrossRef](#)]
140. Borcoman, E.; Kanjanapan, Y.; Champiat, S.; Kato, S.; Servois, V.; Kurzrock, R.; Goel, S.; Bedard, P.; Le Tourneau, C. Novel patterns of response under immunotherapy. *Ann. Oncol.* **2019**, *30*, 385–396. [[CrossRef](#)]
141. Wolchok, J.D.; Hoos, A.; O’Day, S.; Weber, J.S.; Hamid, O.; Lebbe, C.; Maio, M.; Binder, M.; Bohnsack, O.; Nichol, G.; et al. Guidelines for the evaluation of immune therapy activity in solid tumors: Immune-related response criteria. *Clin. Cancer Res.* **2009**, *15*, 7412–7420. [[CrossRef](#)] [[PubMed](#)]
142. Seymour, L.; Bogaerts, J.; Perrone, A.; Ford, R.; Schwartz, L.H.; Mandrekar, S.; Lin, N.U.; Litiere, S.; Dancey, J.; Chen, A.; et al. iRECIST: Guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol.* **2017**, *18*, E143–E152. [[CrossRef](#)] [[PubMed](#)]
143. Nishino, M.; Giobbie-Hurder, A.; Gargano, M.; Suda, M.; Ramaiya, N.H.; Hodi, F.S. Developing a Common Language for Tumor Response to Immunotherapy: Immune-Related Response Criteria Using Unidimensional Measurements. *Clin. Cancer Res.* **2013**, *19*, 3936–3943. [[CrossRef](#)]
144. Majd, N.; de Groot, J. Challenges and strategies for successful clinical development of immune checkpoint inhibitors in glioblastoma. *Expert Opin Pharm.* **2019**, *20*, 1609–1624. [[CrossRef](#)]
145. Yang, Y.; Wu, Q.; Chen, L.; Qian, K.; Xu, X. Severe immune-related hepatitis and myocarditis caused by PD-1 inhibitors in the treatment of triple-negative breast cancer: A case report. *Ann. Transl. Med.* **2022**, *10*, 424. [[CrossRef](#)]
146. Wang, P.F.; Chen, Y.; Song, S.Y.; Wang, T.J.; Ji, W.J.; Li, S.W.; Liu, N.; Yan, C.X. Immune-Related Adverse Events Associated with Anti-PD-1/PD-L1 Treatment for Malignancies: A Meta-Analysis. *Front Pharm.* **2017**, *8*, 730. [[CrossRef](#)]
147. Balibegloo, M.; Nejadghaderi, S.A.; Sadeghalvad, M.; Soleymānītabar, A.; Nezamabadi, S.S.; Saghazadeh, A.; Rezaei, N. Adverse events associated with immune checkpoint inhibitors in patients with breast cancer: A systematic review and meta-analysis. *Int. Immunopharmacol.* **2021**, *96*, 107796. [[CrossRef](#)] [[PubMed](#)]
148. Xin, Y.; Shen, G.; Zheng, Y.; Guan, Y.; Huo, X.; Li, J.; Ren, D.; Zhao, F.; Liu, Z.; Li, Z.; et al. Immune checkpoint inhibitors plus neoadjuvant chemotherapy in early triple-negative breast cancer: A systematic review and meta-analysis. *BMC Cancer* **2021**, *21*, 1261. [[CrossRef](#)] [[PubMed](#)]
149. Villacampa, G.; Tolosa, P.; Salvador, F.; Sanchez-Bayona, R.; Villanueva, L.; Dienstmann, R.; Ciruelos, E.; Pascual, T. Addition of immune checkpoint inhibitors to chemotherapy versus chemotherapy alone in first-line metastatic triple-negative breast cancer: A systematic review and meta-analysis. *Cancer Treat. Rev.* **2022**, *104*, 102352. [[CrossRef](#)] [[PubMed](#)]
150. Wolchok, J.D.; Chiarioti-Silenti, V.; Gonzalez, R.; Rutkowski, P.; Grob, J.J.; Cowey, C.L.; Lao, C.D.; Wagstaff, J.; Schadendorf, D.; Ferrucci, P.F.; et al. Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N. Engl. J. Med.* **2017**, *377*, 1345–1356. [[CrossRef](#)]

151. Ceschi, A.; Noseda, R.; Palin, K.; Verhamme, K. Immune Checkpoint Inhibitor-Related Cytokine Release Syndrome: Analysis of WHO Global Pharmacovigilance Database. *Front Pharmacol.* **2020**, *11*, 557. [[CrossRef](#)] [[PubMed](#)]
152. Ciner, A.T.; Hochster, H.S.; August, D.A.; Carpizo, D.R.; Spencer, K.R. Delayed cytokine release syndrome after neoadjuvant nivolumab: A case report and literature review. *Immunotherapy* **2021**, *13*, 1071–1078. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Triple-negative breast cancer therapy: Current and future perspectives (Review)

KWANG-AI WON^{1,2} and CHARLES SPRUCK³

¹ConsultantCA, Moraga, CA 94556; ²Pin Pharmaceuticals, Inc., South San Francisco, CA 94080;

³Tumor Initiation and Maintenance Program, NCI-Designated Cancer Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA 92037, USA

Received July 14, 2020; Accepted September 9, 2020

DOI: 10.3892/ijo.2020.5135

Abstract. Triple-negative breast cancer (TNBC) accounts for 10–15% of all breast cancer cases. TNBCs lack estrogen and progesterone receptors and express low levels of HER2, and therefore do not respond to hormonal or anti-HER2 therapies. TNBC is a particularly aggressive form of breast cancer that generally displays poorer prognosis compared to other breast cancer subtypes. TNBC is chemotherapy sensitive, and this treatment remains the standard of care despite its limited benefit. Recent advances with novel agents have been made for specific subgroups with PD-L1⁺ tumors or germline *Brcal*-mutated tumors. However, only a fraction of these patients responds to immune checkpoint or PARP inhibitors and even those who do respond often develop resistance and relapse. Various new agents and combination strategies have been explored to further understand molecular and immunological aspects of TNBC. In this review, we discuss clinical trials in the management of TNBC as well as perspectives for potential future treatments.

Contents

1. Introduction
2. Current treatment paradigm
3. Investigational drugs
4. New potential therapeutic strategies
5. Conclusion

1. Introduction

Breast cancer is characterized by heterogeneity at the molecular and clinical levels. Several biomarkers including

estrogen receptor α (ERα), progesterone receptor (PR), and human epidermal growth factor receptor-2 (ERBB2/HER2) have been established, and the main breast cancer subtypes are classified according to their molecular profile (1,2). Traditional staging of breast cancer is based on tumor size, lymph node involvement, and presence of metastasis, and recently biologic markers have been incorporated in the 8th edition of the American Joint Committee on Cancer (AJCC), improving the prognostic discrimination over anatomic staging alone (3).

Triple-negative breast cancer (TNBC) is characterized as having ≤1% cellular expression of ER and PR as determined by immunohistochemistry (IHC), and having HER2 expression of 0 to 1+ by IHC, or 2+ by IHC and fluorescence *in situ* hybridization (FISH) negative (i.e. not an amplified gene copy number), according to American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines (4,5). TNBCs are comprised of at least four distinct transcriptional subtypes: Two basal subtypes, BL1 and BL2; a mesenchymal subtype M, which is devoid of immune cells; and a luminal androgen receptor (AR) subtype LAR (1,2). TNBC is also subdivided into 6 different subgroups based on molecular heterogeneity: Basal-like; mesenchymal-like; mesenchymal stem-like; luminal AR expression; immunomodulatory; and unstable type (6). TNBC represents approximately 15–20% of all newly diagnosed breast cancers and is generally a more aggressive disease with a poorer prognosis and higher grade than other types of breast cancer, accounting for 5% of all cancer-related deaths annually. The median overall survival (OS) for the disease is 10.2 months with current therapies, with a 5-year survival rate of ~65% for regional tumors and 11% for those that have spread to distant organs (7,8).

In this review, we discuss current TNBC treatments and key examples of improved clinical benefit, as well as new therapeutic strategies with which to treat the disease.

2. Current treatment paradigm

TNBC is chemotherapy sensitive, and this treatment remains the standard of care (SOC). Common chemotherapies include anthracycline (e.g., DNA intercalating agent and topoisomerase II blocker doxorubicin), alkylating agents (e.g., cyclophosphamide), an anti-microtubule agent taxane, and an anti-metabolite fluorouracil (5-FU). The current SOC for newly

Correspondence to: Dr Kwang-Ai Won, ConsultantCA, 1988 Ascot Drive, Unit A, Moraga, CA 94556, USA
E-mail: wonk12pharm@yahoo.com

Key words: triple-negative breast cancer, clinical studies, immunotherapy, DNA-damage response, targeted therapy, therapeutic strategy

diagnosed early TNBC consists of neoadjuvant chemotherapy, followed by surgery. For patients with relapsed/refractory TNBC, there is no standard chemotherapy regimen. Responses to treatment are usually short in duration and followed by rapid relapse, and visceral and brain metastases are common. Available therapies for patients with advanced TNBC include anti-metabolites capecitabine and gemcitabine, non-taxane microtubule inhibitor eribulin, and DNA cross-linker platinums. The median progression-free survival (PFS) with chemotherapy ranges from 1.7 to 3.7 months; the median OS from the onset of metastasis is 10 to 13 months. In clinical trials, patients with advanced TNBC treated with single-agent taxane- or platinum-based chemotherapy had a median PFS of 4 to 6 months and a median OS of 11 to 17 months (9-11).

New treatment options for patients with advanced TNBC have recently emerged, especially in cases where surgery is not an option.

TNBC is more immunogenic than other breast cancer subtypes with tumor-infiltrating lymphocytes (TILs) in its microenvironment. However, TNBC also displays a high level of programmed cell death-ligand 1 (PD-L1) expression (12,13). Thus, immunotherapies targeting the programmed cell death-1 (PD-1) receptor/PD-L1 pathway that maintains immunosuppression in the tumor environment in TNBC have been explored and atezolizumab (anti-PD-L1 antibody) in combination with nanoparticle albumin-bound (nab)-paclitaxel was approved as a first-line therapy by the US Food and Drug Administration (FDA) based on the IMpassion130 trial (NCT02425891) in 2019. This immuno-chemotherapy became SOC for patients with PD-L1⁺, unresectable, locally advanced or metastatic TNBC. Note that the survival benefit was exclusively in PD-L1⁺ TNBC patients. The threshold is 1% PD-L1 expression on infiltrating immune cells by an approved companion diagnostic SP142 IHC assay and 41% of enrolled patients showed PD-L1-positive expression in the IMpassion130 trial. This is in contrast to studies in other types of cancer which showed benefit for checkpoint inhibitor therapy even in patients with negative PD-L1 expression. In the first interim analysis of IMpassion130, the median PFS was 7.5 vs. 5.0 months with chemotherapy and the median OS was 25.0 vs. 15.5 months with chemotherapy among patients with PD-L1⁺ tumors (14). In the pre-specified second interim analysis (data cutoff January 2, 2019), the median OS was 25.0 vs. 18.0 months with chemotherapy. Overall, the combination was well-tolerated and immune-related adverse events (AEs) included rash, hypothyroidism, and pneumonitis (15). Another immunotherapy, pembrolizumab (anti-PD-1 antibody), was approved in 2017 as a histology agnostic immunotherapy in all microsatellite instability-high (MSI-H) and/or mismatch repair deficient (dMMR) tumors. This is the first FDA-approved cancer treatment based on a tumor biomarker without regard to the original location of the tumor. However, MSI-H is rare in breast cancer (<2%) (16-18).

BRCA1 and BRCA2-deficient tumors exhibit impaired homologous recombination repair (HRR) and synthetic lethality with poly(ADP-ribose) polymerase (PARP) inhibitors (19,20). The FDA approved olaparib and talazoparib in 2018 to treat advanced-stage HER2-negative breast cancer in individuals with a *Brcal* or *Brca2* mutation. The FDA also approved the companion diagnostic test to identify germline *Brca*-mutated

(gBRCAm) breast cancer patients. Approximately 5% of patients with breast cancer carry a gBRCAm. Olaparib approval was based on data from the OlympiAD Phase III (NCT02000622) trial comparing olaparib to physician's choice of chemotherapy (capecitabine, vinorelbine or eribulin). Olaparib was associated with a 42% increase in median PFS as compared to the control group (7 vs. 4 months) in gBRCAm HER2-negative metastatic breast cancer patients with previous chemotherapy (21). There was no statistically significant improvement in OS with olaparib compared to the control group (19.3 vs. 17.1 months), but there was potential OS benefit among patients with no prior chemotherapy for metastatic breast cancer (HR 0.51, 95% CI 0.29-0.90) (22). Olaparib was generally well-tolerated, with no evidence of cumulative toxicity including the risk of developing anemia during extended exposure. Talazoparib approval was based on data from the EMBRACA Phase III (NCT01945775) trial comparing talazoparib to gemcitabine or to the same physician choice of standard therapy as the OlympiAD trial. Talazoparib increased median PFS by 46% (8.6 vs. 5.6 months) in gBRCAm HER2-negative locally advanced or metastatic breast cancer patients with previous chemotherapy including an anthracycline and/or taxane. Talazoparib presented with hematologic grade 3-4 AEs (primarily anemia), which occurred in 55 vs. 38% of the patients with standard therapy, and an improved side-effect profile in patient-reported outcomes (23).

3. Investigational drugs

To improve therapeutic benefit in TNBC treatment, various agents have been explored in clinical studies. They include immuno- and targeted-therapies in the networks of tumor-stroma, DNA damage response (DDR), cell surface or intracellular receptors, and signaling pathways as well as cell surface markers for selective drug delivery, and antibody-drug conjugates (ADCs) (Fig. 1). As of March 2020, 399 ongoing studies for TNBC have been listed on ClinicalTrials.gov and select Phase III studies are listed in Table I.

Immunotherapy: Immune checkpoint. TILs are frequent in TNBC, correlate with increased pathologic complete response (pCR) to neoadjuvant chemotherapy, and are predictive of disease-free survival (DFS) and OS in early-stage TNBC (24-26). Expression of immune regulatory checkpoints is an adaptive method of tumor resistance to infiltrating lymphocytes within the tumor microenvironment. Multiple strategies have been used to enhance the response to PD-1/PD-L1 blockade in pre-clinical and early clinical studies, including several intratumoral immune modulators and targeted agents (27). The activity of immunotherapy, such as immune checkpoint inhibitors, can be enhanced by chemotherapeutic agents through the stimulation/release of antigens, thus leading to promotion of immunogenic cell death. Currently, clinical trials investigating the use of immune checkpoint inhibitors are ongoing either as a single agent or in various combinations with other agents beyond the metastatic setting and even in the first-line setting (28).

Neoadjuvant treatment. Studies determining benefit from neoadjuvant checkpoint inhibitor therapy have yielded mixed outcomes. Neoadjuvant chemotherapy with pembrolizumab

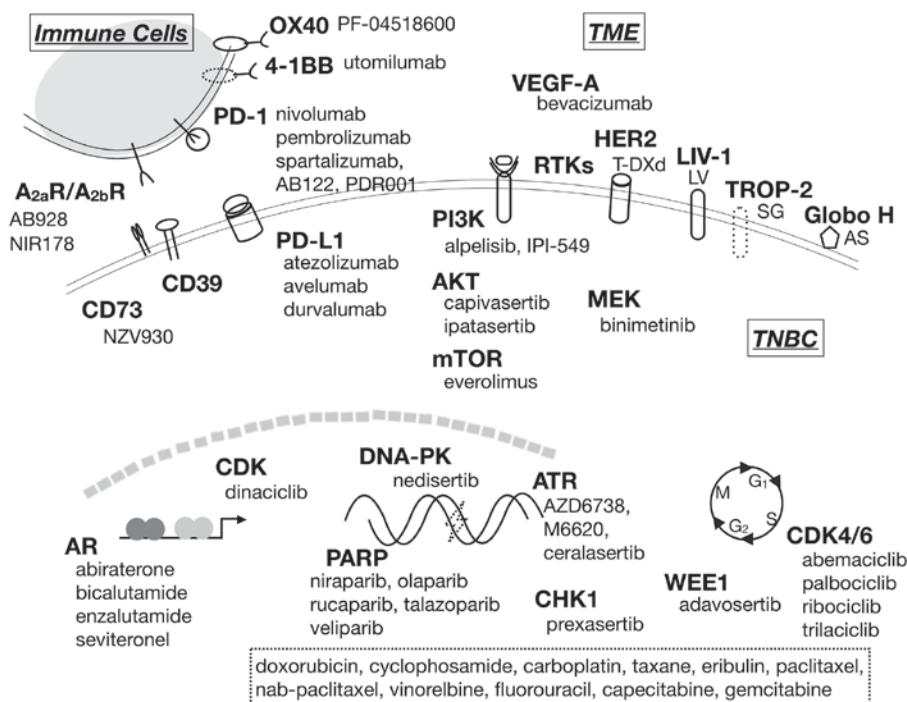


Figure 1. Immuno- and targeted-therapies in key TNBC clinical studies. Various agents in the networks of TNBCs and immune cells have been explored, as well as tumor-stroma interactions in the tumor microenvironment (TME). Targets and agents relevant to immune checkpoint, cell surface or intracellular receptors, signaling pathways, DNA damage response, and cell cycle checkpoint are shown. Various chemotherapy agents are listed in the box. AS, Adagloxad simolenin; LV, Ladiratuzumab vedotin; SG, Sacituzumab govitecan-hziy; T-DXd, Tastuzumab deruxtecan; TNBC, triple-negative breast cancer; A_{2a}R, adenosine 2A receptor; A_{2b}R, 2B receptor; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; VEGF-A, vascular endothelial growth factor A; RTKs, receptor tyrosine kinases; PARP, poly(ADP-ribose) polymerase; CDK, cyclin-dependent kinase; CD, cluster of differentiation; ATR, ataxia telangiectasia and Rad3-related kinase; CHK1, checkpoint kinase 1; DNA-PK, DNA-dependent protein kinase; AR, androgen receptor; PI3K, phosphatidylinositol 3-kinase.

have demonstrated manageable safety and promising anti-tumor activity for patients with early-stage TNBC in the KEYNOTE-173 Phase 1b (NCT02622074) (29) and I-SPY2 Phase II (NCT01042379) trials (30). The KEYNOTE-522 Phase III trial (NCT03036488) further explored neoadjuvant chemotherapy with or without pembrolizumab followed by surgery and pembrolizumab or placebo adjuvantly. The neoadjuvant combination showed a significantly higher pCR rate than the placebo-chemotherapy group (65 vs. 51%). Note that a similar pCR benefit (~15%) in both the PD-L-positive and -negative subgroups was observed, suggesting that neoadjuvant pembrolizumab may benefit patients regardless of PD-L1 levels. This is different from the advanced setting where only the PD-L1-positive patients benefit from atezolizumab. The toxicity profiles were as expected for each treatment, with similar rates (78 vs. 73%) of grade ≥ 3 treatment-related AEs (TRAEs) (31).

NeoTRIPaPDL1 Phase III (NCT02620280) trial also explored neoadjuvant chemotherapy with or without atezolizumab followed by surgery and four cycles of an anthracycline regimen. However, in this trial for patients with early-stage high-risk or locally advanced unilateral breast cancer there was no improvement in pCR with the combination therapy (44 vs. 41% with the control arm) (32). Note that the neoadjuvant chemo-regimen was different from KEYNOTE-522 which included another round of chemotherapy following carboplatin and nab-paclitaxel. The difference in the targets, PD-1 for pembrolizumab vs. PD-L1 for atezolizumab, may

also have contributed to the different outcomes. Another Phase III (NCT03197935) trial, IMpassion031 study also explored atezolizumab in combination with chemotherapy (nab-paclitaxel followed by doxorubicin and cyclophosphamide) in comparison to placebo plus chemotherapy in the neoadjuvant setting. Treatment with atezolizumab continued adjuvantly for those in the combination arm of the study (33). The primary endpoint was pCR.

In the advanced setting. As a first-line treatment option for patients with locally recurrent, inoperable or metastatic TNBC, pembrolizumab was evaluated in combination with investigator's choice of chemotherapy (*i.e.* nab-paclitaxel, paclitaxel or gemcitabine/carboplatin), compared to placebo plus chemotherapy (KEYNOTE-355 Phase III trial, NCT02819518). A significant PFS benefit with the pembrolizumab-chemo combination in patients whose tumors expressed PD-L1 (CPS ≥ 10) was reported (9.7 vs. 5.6 months for chemotherapy alone) (34). The study is currently in progress to evaluate OS, the other primary endpoint of the trial.

In contrast to other studies of immunotherapy combined with SOC chemotherapy, the Tonic trial (NCT02499367) in metastatic TNBC was based on an adaptive trial design that explores a sequential treatment with anti-PD-1 antibody nivolumab after 2 weeks of chemotherapy or radiotherapy. The hypothesis is that short-term treatment induces a more favorable tumor microenvironment that would enhance sensitivity to immune checkpoint blockade in TNBC. The highest overall

Table I. Current phase III studies concerning TNBC.

Therapeutic approach	Treatment	TNBC patient population	Recruitment status	No. of patients	Study start; Primary completion(month/day/year)	ClinicalTrials.gov Identifier
Neoadjuvant therapy: Immuno + chemotherapy (NeoTRIPaPDL1)	(Carbo/nab-pac) +/- atezolizumab. Then, four cycles of AC, EC or FEC as adjuvant chemotherapy	Early high-risk and locally advanced	Active, not recruiting	278	4/1/2016; May 2022	NCT02620280
Immuno + chemotherapy as neoadjuvant therapy and immunotherapy as adjuvant therapy (KEYNOTE-522)	(Pac/carbo, followed by AC or EC) +/- pembrolizumab as neoadjuvant therapy prior to surgery. Then, pembrolizumab vs. placebo as adjuvant therapy post-surgery	Locally advanced	Active, not recruiting	1,174	3/7/2017; 9/30/2025	NCT03036488
Neoadjuvant therapy: Immuno + chemotherapy (IMpassion031)	(Nab-pac +/- atezolizumab), followed by AC	Eligible for surgery with initial clinically assessed primary invasive (early stage)	Active, not recruiting	324	7/24/2017; 9/30/2020	NCT03197935
Neoadjuvant therapy: Immuno + chemotherapy	(Carbo/pac, then AC or EC) +/- atezolizumab, followed after surgery by atezolizumab or placebo	No metastatic disease	Recruiting	1,520	12/19/2017; 12/31/2023	NCT03281954
Immuno + chemotherapy as neoadjuvant therapy and immunotherapy as adjuvant therapy	(Nab-pac/carbo, followed by AC or EC) +/- HLX10 (anti-PD-1) as neoadjuvant therapy prior to surgery. Then, HLX10 vs. placebo as adjuvant therapy post-surgery	Previously untreated and potentially resectable patients without distant metastasis	Not yet recruiting	522	4/17/2020; 9/7/2022	NCT04301739
Immunotherapy: Immune stimulant following neoadjuvant or adjuvant chemotherapy	Adagloxad simolenin (OBI 822)/OBI-821 vs. placebo	Early stage at high risk for recurrence; defined as residual invasive disease following neoadjuvant chemotherapy or ≥4 positive axillary nodes. The Global IHC assay for identifying eligible patient	Recruiting	668	12/5/2018; 11/30/2025	NCT03562637
PARP inhibitor + chemotherapy as neoadjuvant therapy (BrightTNess)	[(Veliparib/pac/carbo) vs. (pac/carbo) vs. pac], followed by AC	Early stage	Active, not recruiting	634	4/2/2014; 3/18/2016; (10/18/2020 ^a)	NCT02032277

Table I. Continued.

Therapeutic approach	Treatment	TNBC patient population	Recruitment status	No. of patients	Study start; Primary completion(month/day/year)	ClinicalTrials.gov Identifier
PARP inhibitor + chemotherapy as neoadjuvant therapy (PARTNER, Phase II/III)	(Pac/carbo) +/- olaparib	TNBC and/or gBRCAm positive breast cancer	Recruiting	527	5/1/2016; January 2022	NCT03150576
Adjuvant therapy: Immunotherapy (A-Brave)	Avelumab (anti-PD-L1) vs. observation	High-risk; completed treatment with curative intent including surgery of the primary tumor, neo- or adjuvant chemotherapy, and (if indicated) radiotherapy TNBC or low ER-positive and/or HER2 borderline breast cancer who have ≥1 cm residual invasive breast cancer and/or positive lymph nodes after neoadjuvant chemotherapy	Recruiting	335	June 2016; June 2021	NCT02926196
Adjuvant therapy: Immunotherapy	Pembrolizumab adjuvant therapy vs. no therapy	Stage II-III operable	Recruiting	1,000	11/15/2016; 5/31/2026	NCT02954874
Adjuvant therapy: Immuno + chemotherapy (IMpassion030)	Pac +/- atezolizumab, followed by dose-dense AC or EC alone	Previously untreated locally advanced or metastatic	Active, not recruiting	2,300	8/2/2018; 1/15/2022	NCT03498716
Immuno + chemotherapy (IMpassion130)	Nab-pac +/- atezolizumab	Metastatic (second/third lines)	Active, not recruiting	900	6/23/2015; 4/14/2020	NCT02425891
Single agent immunotherapy (KEYNOTE-119)	Pembrolizumab vs. chemotherapy (capecitabine, eribulin, gem, or vinorelbine)	Previously untreated locally recurrent inoperable or metastatic	Active, not recruiting	622	10/13/2015; 4/11/2019	NCT02555657
Immuno + chemotherapy (KEYNOTE-355)	(Nab-pac or pac or gem/carbo) +/- pembrolizumab	Previously untreated, inoperable locally advanced or metastatic	Active, not recruiting	882	7/27/2016; 12/30/2019	NCT02819518
Immuno + chemotherapy (IMpassion131)	Pac +/- atezolizumab	Early relapsing recurrent (inoperable locally advanced or metastatic)	Recruiting	600	8/25/2017; 11/15/2019	NCT03125902
Immuno + chemotherapy (IMpassion132)	(Gem/capecitabine) +/- atezolizumab		Recruiting	540	1/11/2018; 1/1/2023	NCT03371017

Table I. Continued.

Therapeutic approach	Treatment	TNBC patient population	Recruitment status	No. of patients	Study start; Primary completion(month/day/year)	ClinicalTrials.gov Identifier
Immuno + chemotherapy (TORCHLIGHT)	Nab-pac +/- toripalimab (anti-PD-1)	First/second-line treatment of metastatic or recurrent	Recruiting	600	12/21/2018; 2/28/2022	NCT04085276
Immuno + chemotherapy (ELISSAR)	Single arm: (Nab-pac or pac) + atezolizumab	PD-L1-positive unresectable locally advanced or metastatic; not received prior systemic cytotoxic therapy	Recruiting	280	12/17/2019; 6/28/2024	NCT04148911
PARP inhibitor + immunotherapy as the post-induction therapy (KEYLYNK-009, Phase II/III)	Olaparib + pembrolizumab vs. (carbo/gem) + pembrolizumab after induction with first-line (carbo/gem) + pembrolizumab	Locally recurrent inoperable or metastatic	Recruiting	932	12/19/2019; 1/26/2026	NCT04191135
AKT inhibitor + immuno + chemotherapy	Cohort 1 (PD-L1 non-positive): paclitaxel (P)/ipatasertib (I)/atezolizumab (A) vs. P/I vs. P; Cohort 2 (PD-L1 positive): P/I/A vs. P/A	Locally advanced unresectable or metastatic	Recruiting	1,155	11/25/2019; 10/10/2025	NCT04177108
AKT inhibitor + chemotherapy (IPATunity130)	Pac +/- ipatasertib	PIK3CA/AKT1/PTEN-altered, locally advanced or metastatic TNBC and locally advanced or metastatic HR ⁺ /HER2/breast adenocarcinoma, not suitable for endocrine therapy	Recruiting	450	1/6/2018; 12/22/2021	NCT03337724
AKT inhibitor + chemotherapy as first line therapy (Capitello290)	Pac +/- capivasertib	Locally advanced (inoperable) or metastatic	Recruiting	800	6/25/2019; 9/1/2021	NCT03997123
PI3K inhibitor + chemotherapy (EPIK-B3)	Nab-pac +/- alpelisib	Advanced; a PIK3CA mutation (Study Part A) or PTEN loss without PIK3CA mutation (Study Parts B1 and B2)	Not yet recruiting	566	4/22/2020; 3/19/2024	NCT04251533

Table I. Continued.

Therapeutic approach	Treatment	TNBC patient population	Recruitment status	No. of patients	Study start; Primary completion(month/day/year)	ClinicalTrials.gov Identifier
AR antagonist as first line therapy (SYSUCC-007)	Bicalutamide vs. (docetaxel/capecitabine or gem/docetaxel or gem/carbo)	AR-positive metastatic	Recruiting	262	12/1/2016; December 2020	NCT03055312
Amino acid metabolism target + chemotherapy as first line therapy (TRYbeCA-2, Phase II/III)	(Gem/carbo) +/- eryaspase (L-asparaginase encapsulated inside a donor-derived red blood cell)	Locally recurrent or metastatic; not received prior systemic therapy	Recruiting	64	6/13/2019; December 2020	NCT03674242
Antibody-drug conjugate (ASCENT)	Sacituzumab Govitecan vs. (eribulin, capecitabine, gemcitabine, vinorelbine)	Refractory/relapsed metastatic	Active, not recruiting	529	11/3/2017; April 2020	NCT02574455

TNBC, triple-negative breast cancer; AR, androgen receptor; A, doxorubicin; C, cyclophosphamide; Cape, capecitabine; Carbo, carboplatin; E, epirubicin; F, fluorouracil; Gem, gemcitabine; Nab-paclitaxel; Pac, paclitaxel; gBRCAm, germline BRCA-mutated. ^aEstimated Study Completion Date.

response rate (ORR) was observed with doxorubicin induction (35%) followed by nivolumab/doxorubicin. Doxorubicin induction also upregulated immune-related genes as well as inflammation, JAK-STAT, and TNF- α signaling-related genes, suggesting a more favorable tumor microenvironment induced by these chemotherapies (35). The InCITE Phase II trial (NCT03971409) also includes a two-week induction of binimetinib (MEK inhibitor), utomilumab (4-1BB agonist), or PF-04518600 (anti-OX40 antibody) which may help activate the immune system. The trial explores how well anti-PD-L1 antibody avelumab might work with one of those agents after induction in stage IV or unresectable and recurrent TNBC.

Immunotherapy: Adenosine pathway. Adenosine is catabolized from ATP and often overproduced and released by tumor cells. It is also converted from extracellular nucleotides by the plasma membrane protein, cluster of differentiation 73 (CD73), which is upregulated in many cancer types (36,37). The excess adenosine in the tumor microenvironment activates the adenosine 2A receptor (A_{2a} R) and 2B receptor (A_{2b} R) (38,39) which are highly expressed on the cell surfaces of lymphocytes and myeloid cells, respectively, leading to immunosuppressive effects (Fig. 2). Targeting these receptors and enzymes could lead to reactivation of antitumor immunity by abrogating the inhibitory effect on the immune system and enhancing the cytotoxic T lymphocyte (CTL)-mediated immune response (40,41).

Combinations of adenosine pathway inhibitors and immune checkpoint inhibitors have been explored in clinical trials. NZV930 (SRF373) is an anti-CD73 monoclonal antibody that binds to CD73 on tumor cells, leading to internalization of CD73, thereby preventing CD73-mediated conversion of extracellular AMP to adenosine. A Phase I/Ib study (NCT03549000) is underway to evaluate NZV930 alone and in combination with PD-1 inhibitor PDR001 and/or A_{2a} R antagonist NIR178 in patients with advanced malignancies including TNBC. NIR178 is an antagonist of A_{2a} R, blocking adenosine/ A_{2a} R-mediated inhibition of T lymphocytes. A Phase II study (NCT03207867) is underway for NIR178 in combination with PD-1 inhibitor spartalizumab in multiple solid tumors and diffuse large B-cell lymphoma (DLBCL) to assess if the addition of the adenosine antagonist improves the efficacy of PD-1 inhibition. A dual adenosine A_{2a} R/ A_{2b} R receptor antagonist, AB928, is currently being evaluated in a Phase I study (NCT03629756) in combination with the PD-1 inhibitor AB122 in patients with advanced malignancies. Early results show a favorable safety profile of AB928 combination therapy and predictable PK/PD correlation (42).

DNA-damage response: PARP. Approximately 60-70% of breast cancer patients with an inherited *Brcal/2* mutation are TNBC subtype and 10-30% of TNBC patients harbor a *Brca* pathogenic variant (43,44). A condition defined as 'BRCAne' (45), which includes mutations in HRR genes through genetic or epigenetic inactivation, leads to susceptibility to both platinum and PARP inhibitors. Various PARP inhibitors (e.g. veliparib, niraparib, and rucaparib as well as olaparib and talazoparib) have been assessed in the neoadjuvant and adjuvant settings and in combination with other agents.

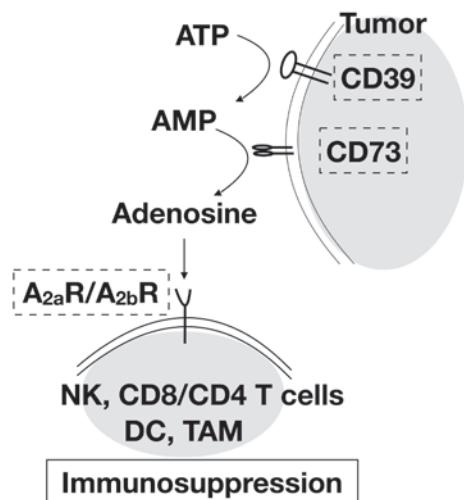


Figure 2. ATP-adenosine pathway. Adenosine is generated from ATP by CD39 and CD73. It binds to A2 receptors on immune cells and blocks T cell priming, expansion, and activation, natural killer (NK) cell degranulation, dendritic cell (DC) maturation and activation, and tumor-associated macrophage (TAM) M1 polarization, thus leading to immunosuppression. ATP, adenosine triphosphate; AMP, adenosine monophosphate; CD, cluster of differentiation.

Neoadjuvant and adjuvant settings. A PARP inhibitor appears to have efficacy for neoadjuvant treatment of patients with gBRCAm TNBC. Talazoparib achieved encouraging pCR in patients with gBRCAm breast cancer, including TNBC, and HR⁺ breast cancer, as a neoadjuvant single-agent without the addition of chemotherapy (46). Currently a larger, multi-center, neoadjuvant Phase II trial (NCT03499353) is ongoing. However, the addition of a PARP inhibitor to standard neoadjuvant chemotherapy was found to be not beneficial. In the BrightTNess Phase III trial (NCT02032277) the addition of PARP inhibitor veliparib to carboplatin and paclitaxel followed by doxorubicin and cyclophosphamide did not improve pCR whereas the addition of veliparib and carboplatin to paclitaxel did. Therefore, the addition of carboplatin but not veliparib to paclitaxel was proposed as a potential component of neoadjuvant chemotherapy for patients with high-risk TNBC (47).

PARP inhibitors have also been studied as an adjuvant single-agent therapy. The OlympiA Phase III trial (NCT02032823) was designed to assess olaparib in patients with gBRCAm and high-risk HER2-negative breast cancer who completed definitive local treatment and neoadjuvant or adjuvant chemotherapy. The primary outcome measure will be invasive DFS with a time frame of up to 10 years.

In combination with immunotherapy. A crosstalk exists between PARP inhibition and the PD-L1/PD-1 immune checkpoint axis. PARP inhibitors upregulate PD-L1 expression on tumor cells by inhibiting glycogen synthase kinase 3 beta (GSK3β) and activating the cGAS-STING pathway (48). Thus, primary/acquired resistance to PARP inhibitors seems to be associated with the development of immune evasion mechanisms. Multiple clinical studies are underway to assess synergy between therapeutic strategies of PARP inhibition and immune checkpoint blockers.

In platinum-resistant, advanced, or metastatic TNBC, niraparib combined with pembrolizumab

(TOPACIO/KEYNOTE-162 Phase II trial, NCT02657889) showed higher response rates in patients with tumor *Brca* mutations (tBRCAm): ORR of 28% in all (biomarker-unselected) patients vs. 60% for tBRCAm patients. The combination therapy was safe with a tolerable safety profile (49).

In MEDIOLA Phase I/II trial (NCT02734004) the combination of olaparib and durvalumab showed ORR of 63% in a cohort of patients with gBRCAm metastatic breast cancer (50). In the I-SPY 2 Phase II study (NCT01042379), adding the same combination to neoadjuvant paclitaxel led to improved pCR rates in patients with high-risk, HER2-negative stage II/III breast cancer compared with single-agent paclitaxel. In those with TNBC, the pCR rate was 47 vs. 27% with paclitaxel alone. AEs were consistent with the known safety profiles of each agent alone (51). In metastatic TNBC, the efficacy of induction treatment of olaparib followed by the combination treatment of olaparib and durvalumab is being assessed in a Phase II study (NCT03801369) (52). Patients with ≤2 prior chemotherapy regimens for metastatic breast cancer are eligible, but patients with gBRCAm TNBC are excluded. The primary end point is ORR.

The DORA Phase II trial (NCT03167619) is evaluating olaparib as a maintenance therapy with or without durvalumab in patients with advanced TNBC who achieve at least stable disease after 3 cycles of platinum-based chemotherapy. Another study of a PARP inhibitor as a maintenance therapy, KEFLYNK-009 Phase II/III trial (NCT04191135), is underway in metastatic TNBC to assess the efficacy of olaparib plus pembrolizumab vs. chemotherapy plus pembrolizumab after induction with first-line chemotherapy plus pembrolizumab (53).

In combination with DDR-HRR pathway inhibitors. Resistance to PARP inhibitors can occur in certain cancer contexts by various mechanisms, including increased HRR capacity and decreased cell cycle progression and DNA replication stress. RAD51 overexpression has been observed in a wide range of human cancers, particularly TNBCs and serous ovarian cancers (54,55). Upregulation of RAD51 in BRCA1-defective cells is also associated with resistance to PARP inhibitor (56,57). Inhibitors of key mediators of DNA repair and replication, such as ataxia telangiectasia mutated kinase (ATM), ataxia telangiectasia and Rad3-related kinase (ATR), checkpoint kinase 1 (CHK1) and checkpoint kinase 2 (CHK2), DNA-dependent protein kinase (DNA-PK), and WEE1 kinase (Fig. 3) have been assessed to determine if they can sensitize tumor cells to treatment with PARP inhibitors, as these inhibitors were found to prevent the accumulation of RAD51 in TNBC (58).

The VIOLETTE Phase II study (NCT03330847) was set up to assess the combinatory inhibition of PARP and a component of the ATR-CHK1-WEE1 axis. Olaparib with DDR kinase ATR inhibitor AZD6738 was compared to olaparib monotherapy in the second- or third-line setting of metastatic TNBC. Patients were stratified by *Brca* and HRR gene mutation status and the primary endpoint was PFS (59). The study also included a combination arm of olaparib with the first-in-class WEE1 inhibitor adavosertib. WEE1 inhibitor was found to potentiate the activity of DNA-damaging agents in preclinical TNBC models (60,61) and its potential clinical

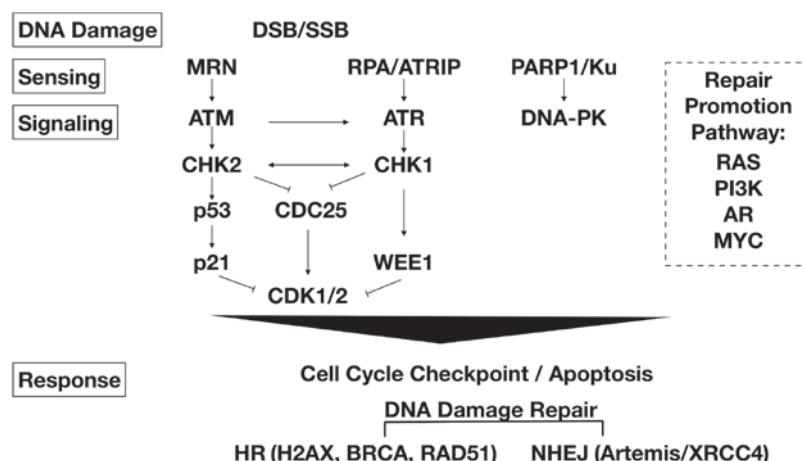


Figure 3. DNA damage response pathways. Double-strand breaks (DSB) or single-strand breaks (SSB) activate DNA damage response (DDR) pathways, leading to cell cycle arrest and DNA repair or cell death depending on cell context. PARP1 senses DNA breaks and is involved in SSB repair. Oncogenic pathways including RAS, PI3K, AR, and MYC signaling can affect HR repair activity and contribute to resistance to PARP inhibitor treatment. MRN, MRE11-RAD50-NBS1 complex; ATRIP, ATP interacting protein; HR, homologous recombination; NHEJ, nonhomologous end joining; H2AX, histone H2AX; XRCC4, X-ray repair cross-complementing protein 4; ATR, ataxia telangiectasia and Rad3-related protein; CHK1/2, checkpoint kinase 1/2; CDK1/2, cyclin-dependent kinase 1/2; DNA-PK, DNA-dependent protein kinase; AR, androgen receptor; PI3K, phosphatidylinositol 3-kinase.

value was observed in a Phase I study in patients with *Brca* mutations (62). However, the combination treatment arm of olaparib and adavosertib was discontinued in the VIOLETTE study and patients were offered the opportunity to continue treatment on olaparib monotherapy. The CHK1 inhibitor prexasertib in combination with olaparib was also explored in early clinical trials (63), but development of prexasertib was discontinued by the sponsor in 2019.

Intracellular signaling pathway targets

PI3K/AKT pathway. A wide range of malignancies including TNBC show dysregulated phosphatase and tensin homolog (PTEN)/phosphoinositide 3-kinases (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling due to mutations in multiple signaling components. Loss of PTEN, a negative regulator of AKT, was found to be correlated with decreased T-cell infiltration at tumor sites in patients, and inhibition of the PI3K-AKT pathway re-sensitized to T-cell-mediated immunotherapy (64). As the PI3K/AKT pathway has emerged as a potential mechanism of resistance to immunotherapy and chemotherapy, multiple clinical trials have assessed inhibitors of the various pathway components.

Alpelisib is an oral PI3K inhibitor that selectively inhibits p110 α . It showed efficacy in targeting *Pik3ca*-mutated breast cancer (65) and was FDA approved in 2019 in combination with fulvestrant for postmenopausal women and men, with HR $^+$, HER2-negative, *Pik3ca*-mutated, advanced or metastatic breast cancer following progression on or after an endocrine-based regimen. For patients with advanced TNBC, the EPIK-B3 Phase III trial (NCT04251533) is planned with study start date of April 2020 to assess alpelisib in combination with nab-paclitaxel. Patients have *Pik3ca* mutations or PTEN loss with ≤ 1 prior line of therapy for metastatic disease.

IPI-549 is a selective PI3K-gamma inhibitor targeting immune-suppressive tumor-associated myeloid cells. The MARIO-3 Phase II study (NCT03961698) was designed to explore the addition of IPI-549 to the FDA approved regimen

atezolizumab/nab-paclitaxel in front-line TNBC. Cohort A will be composed of patients with locally advanced, metastatic TNBC, which will include two sub-cohorts based on PD-L1 IHC status. The primary objective is CR rate.

Ipatasertib and capivasertib are pan-AKT inhibitors that bind to all three isoforms of AKT. Both are now in Phase III trials evaluating the efficacy of combination with paclitaxel as first-line therapy for locally advanced or metastatic TNBC. In the LOTUS Phase II trial, adding ipatasertib to first-line paclitaxel improved PFS, particularly in patients with PTEN/PI3K/AKT-altered tumors (HR, 0.44) (66). In this subgroup of patients, median OS was 23.1 vs. 16.2 months with placebo (HR, 0.65) (67). To confirm the findings from LOTUS, the IPATunity130 Phase III trial (NCT03337724) is evaluating ipatasertib + paclitaxel for PTEN/PI3K/AKT-altered advanced TNBC or HR $^+$, HER2-negative breast cancers. The primary endpoint is PFS (68). An independent trial also supported the potential benefit for addition of AKT inhibitor to chemotherapy. In the PAKT Phase II study (NCT02423603), addition of the oral AKT inhibitor capivasertib to first-line paclitaxel resulted in significantly longer PFS and OS in patients with advanced TNBC, especially in patients with PTEN/PI3K/AKT-altered tumors. The median PFS duration was 5.9 vs. 4.2 months with placebo, meeting the predefined significance level, and better benefit in patients with PTEN/PI3K/AKT-altered tumors with median PFS of 9.3 months (HR, 0.30). The median OS was prolonged by 6.5 months with capivasertib (69). The most common AEs of grade ≥ 3 were diarrhea, infection, rash, and fatigue, similar to those observed with ipatasertib in the LOTUS trial. The CAPItello-290 Phase III trial (NCT03997123) is underway and the primary endpoints are PFS and OS (70).

Efficacy of immunotherapy was also found to be enhanced by AKT inhibitors as a first-line therapy for locally advanced/metastatic TNBC. Phase Ib study (NCT03800836) was designed to evaluate the triplet combination of ipatasertib (I), atezolizumab (A), and paclitaxel or nab-paclitaxel (P). Preliminary efficacy and safety data up to January 5, 2019 showed that the triplet regimen had promising antitumor

activity (73% confirmed ORR), irrespective of biomarker PD-L1 status or PTEN/PI3K/AKT alteration status, and manageable toxicity (71). In Phase III trial (NCT04177108), patients were enrolled in two cohorts according to PD-L1 status: Cohort 1 for PD-L1-negative tumors and cohort 2 for PD-L1-positive tumors. Three arms, P + I + A vs. P + I vs. P, will be evaluated in cohort 1 and 2 arms, P + I + A vs. P + A, will be evaluated in cohort 2.

CDK4/6/Rb/E2F pathway. The G₁-S phase checkpoint of the cell cycle is regulated by CDK4/6 activity which is controlled by their binding partners D-type cyclins and p16 INK4 inhibitor. The active CDK4/6-cyclin D complex phosphorylates the retinoblastoma (Rb) protein, thereby activating E2F function and transition from G₁ to S phase of the cell cycle (72). The FDA approved CDK4/6 inhibitors palbociclib, ribociclib, and abemaciclib based on improvements in PFS for the treatment of ER⁺, HER2-negative advanced or metastatic breast cancer in combination with an endocrine therapy. TNBCs with a Rb⁺, p16 INK4-negative profile might represent the subpopulation of TNBC suitable for treatment with CDK4/6 inhibitors.

Preclinical combination studies of CDK4/6 inhibitors with chemotherapy suggest that the timing and sequence of drug exposure/drug delivery schedule might play a critical role in drug activity, and the evaluation of different schedules of treatment may represent a new approach (73,74). The hypothesis was that reversible G₁ arrest of palbociclib could synchronize tumor cells in the cell cycle and following their re-entry later would ensure a higher fraction in mitosis (M) phase when exposed to paclitaxel. In the first combination trial for palbociclib and paclitaxel (NCT01320592) an alternative dosing schedule was feasible and safe, without evidence of additive toxicity in Rb⁺ breast cancer regardless of subtype (75). Phase I follow-up trial (NCT02599363) of ribociclib and weekly paclitaxel is in progress in patients with Rb⁺ advanced breast cancer. In this study, pharmacodynamic, histologic, and imaging biomarkers will be utilized to confirm synchronization and schedule and identify a patient population that benefits from this treatment approach.

The standard chemotherapy regimen causes treatment-limiting cumulative myelosuppression that may compromise antitumor efficacy in TNBC. CDK4/6 inhibitors induce transient G₁ arrest in immune cells and hematopoietic stem and progenitor cells, potentially helping to preserve T-cell function and bone marrow. To test this hypothesis, an investigational CDK4/6 inhibitor trilaciclib in combination with gemcitabine and carboplatin was explored to evaluate benefit for patients with ≤ 2 prior chemotherapy regimens in metastatic TNBC. Phase II trial (NCT02978716) was negative for a safety-related primary endpoint (i.e. no difference in the frequency or duration of severe grade 4 neutropenia). However, the median OS was improved by more than 60%, which was likely due to increased chemotherapy duration and exposure. Trilaciclib-treated patients also had a higher number of activated CD8⁺ T cells over the first 5 cycles of chemotherapy, which potentially enhanced antitumor immunity (76).

MYC and CDK. Transcription factor c-MYC triggers selective gene expression to promote cell growth and proliferation. It is amplified in several different cancer types including TNBC,

functioning as a proto-oncogene (77). c-MYC compensates for BRCA loss by upregulating HRR through increased RAD51 expression (55,78). TNBC patients with high c-MYC and RAD51 expression exhibit poor prognosis and less favorable response to chemotherapy and PARP inhibitors (55,57,79). c-MYC blockade in TNBC was found to be synthetic lethal with PARP inhibitors, independent of BRCA status (80). c-MYC pathway activation in TNBC is also synthetic lethal with CDK inhibition (81). Dinaciclib is a pan-CDK (CDK1/2/5/9) inhibitor and the combination with PARP1 inhibitor veliparib is currently being pursued in patients with advanced solid tumors for which no curative therapy exists (Phase I trial, NCT01434316). Dinaciclib induced immunogenic cell death (ICD) but also increased expression of PD1 on tumor-infiltrating T cells and expression of PD-L1 on tumor cells, thus limiting its antitumor effect in preclinical studies. However, dinaciclib inhibits tumor growth in combination with anti-PD-1 (82). Phase Ib trial (NCT01676753) was designed to evaluate the efficacy of combined dinaciclib and pembrolizumab in patients with metastatic or locally advanced and unresectable TNBC. Its clinical benefit rate was 47% in preliminary efficacy analysis and high c-MYC expression correlated significantly with clinical response, warranting further validation of c-MYC as a predicative biomarker of response to CDK/checkpoint inhibitors (83).

AR antagonists. The androgen receptor (AR) is an intracellular steroid receptor that dimerizes and translocates to the nucleus after binding androgen ligands. In the nucleus, AR binds to androgen response elements to promote target gene transcription in a tissue-specific manner. AR can also be activated in a ligand-independent manner through crosstalk with key signaling pathways, including PI3K/AKT and ERK (84). AR is involved in cell cycle regulation and the epithelial-to-mesenchymal transition (EMT) (85,86). AR has emerged as a new biomarker and a potential therapeutic target in TNBC. AR is expressed in $\geq 40\%$ of TNBCs and its expression level varies considerably among TNBC molecular subtypes. It has been associated with favorable prognosis, with better DFS and higher OS in the LAR subtype (87,88). However, patients with AR⁺ TNBCs have a decreased chance of achieving pCR to neoadjuvant chemotherapy and the LAR subtype has been linked to poorer response to chemotherapy compared to other TNBC patients (89-91). Multiple selective AR inhibitors have been approved by the FDA for the treatment of prostate cancer and are currently part of standard care (92). The role of the AR in signaling pathways in TNBC is still not clear and clinical studies are underway to provide more insight into the role of the AR as well as to assess whether AR targeting is a valuable therapeutic strategy in TNBC.

The first proof-of-concept trial of AR-targeted treatment established activity of the first-generation AR antagonist bicalutamide in patients with advanced AR⁺ TNBC. The TBCRC 011 Phase II trial (NCT00468715) showed a modest clinical benefit rate (CBR) of 19% at 6 months and a median PFS duration of 12 weeks (93).

AR⁺ TNBC expresses a luminal profile with intact Rb protein, the target of CDK4/6 activity. Thus, CDK4/6 inhibitors may increase the efficacy of AR antagonists in metastatic AR⁺ TNBC. The single group Phase I/II trial (NCT02605486)

was carried out to explore this hypothesis. The combination of palbociclib and bicalutamide was well-tolerated with no unexpected toxicity (94). It also met its prespecified efficacy endpoint as measured by PFS with 11 patients (31 evaluable patients) at 6 months (95).

As one of the second-generation anti-androgen therapies, abiraterone is a steroidal CYP17 inhibitor with potent hydroxylase activity, targeting androgen biosynthesis. The French Breast Cancer Intergroup (UCBG) 12-1 Phase II trial (NCT01842321) was designed to evaluate abiraterone acetate (AA) with its requisite concomitant medication prednisone in AR⁺ advanced or metastatic TNBC. Androgen deprivation by AA resulted in 20% of the 6-month CBR. This treatment appeared to be beneficial for some patients with molecular apocrine tumors, a subtype that expresses AR but not ERα (96). Considering that prednisone stimulates the glucocorticoid receptor (GR), which is expressed in approximately 25% of TNBCs, GR activity might limit the efficacy of AA.

Seviteronel is an investigational lyase-selective non-steroidal CYP17 inhibitor that targets androgen and estrogen production. The CLARITY-01 Phase I/II trial (NCT02580448) was set up to evaluate seviteronel in locally advanced or metastatic TNBC or ER⁺ breast cancer. It revealed that seviteronel was generally well-tolerated and provided clinical benefit. A total of 26 and 11% of patients reached at least a CBR at 4 and 6 months, respectively. Levels of circulating tumor cells (CTCs) also decreased (97,98).

A second-generation AR antagonist enzalutamide not only competitively binds to the AR ligand-binding domain, but also inhibits nuclear translocation of AR, DNA binding, and coactivator recruitment. Phase II single arm study (NCT01889238) assessed the efficacy of enzalutamide in patients with locally advanced or metastatic, AR⁺ TNBC. The primary endpoint was CBR at 16 weeks, which was 25% in the intention-to-treat (ITT) population and 33% in the evaluable subgroup whose tumors expressed ≥10% nuclear AR. The only treatment-related grade 3 or greater AE occurring in ≥2% of patients was fatigue (3.4%) (99). The randomized ENDEAR Phase III study (NCT02929576) comparing enzalutamide and paclitaxel to placebo and paclitaxel in advanced TNBC was in place (100) but withdrawn in 2018, citing that further understanding about the role of androgen signaling in TNBC was required. The TBCRC 032 Phase Ib/II trial (NCT02457910) investigated the safety and efficacy of enzalutamide alone or in combination with PI3K inhibitor taselisib in patients with metastatic AR⁺ TNBC. Primary endpoint of CBR at 16 weeks was 36% and median PFS was 3.4 months. The trial was not completed due to termination of the development of taselisib. Although this study was exploratory due to sample size limitation, it revealed subtype-specific treatment response (favorable trend for luminal over non-luminal) and identified novel *Fgfr2* gene fusions that likely activate the PI3K pathway and AR splice variants that may contribute to enzalutamide resistance. Therefore, an AR IHC score of ≥10% alone may not identify patients with AR-dependent tumors, and LAR subtype and AR splice variants may help identify patients likely to benefit from AR antagonists (101).

Cell surface targets

Tumor-associated carbohydrate antigens. The Globo H antigen is a hexasaccharide sphingolipid expressed on the

surface of various cancer types and has been explored as a potential target for vaccine therapy. Adagloxad simolenin (AS) is an immune stimulant comprising the Globo H hexasaccharide epitope linked to the carrier protein keyhole limpet hemocyanin (KLH). KLH facilitates a more vigorous immune response given the weak antigen, Globo H. As a first-in-class active immunotherapy in development for metastatic breast cancer, AS with the saponin-based adjuvant OBI-821 induced antibodies reactive with Globo H⁺ tumor cells that mediate antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) (102). Phase II trial (NCT01516307) assessed low-dose cyclophosphamide with or without active immunotherapy (AS + adjuvant) in post-treated metastatic breast cancer subjects with stable disease or response to treatment. Although it did not meet its primary efficacy endpoint of PFS, patients who developed an immune response to the vaccine showed significantly improved PFS and OS (103). Based on these subgroup data, Phase III study (NCT03562637) of AS with adjuvant vs. placebo treatment is in progress for high-risk early-stage TNBC patients following neoadjuvant or adjuvant chemotherapy. Patients will be screened for Globo H expression (IHC H-score ≥15) and the primary objective is improvement of invasive disease-free survival (IDFS) in the time frame of 5 years.

Antibody-drug conjugates (ADCs). An ADC is designed to be stable in plasma, target a tumor cell surface antigen with a high affinity and specificity, and is internalized, cleaved, and releases a payload drug which drives antitumor activity through direct cytotoxic cell killing and induces ICD.

Sacituzumab govitecan-hziy (SG) targets a glycoprotein, the human trophoblast cell-surface antigen 2 (TROP-2), that is expressed in more than 90% of TNBCs. Its payload is the active metabolite of irinotecan (SN-38), which is conjugated to the anti-TROP-2 antibody by a cleavable linker. Phase I/II single group study (NCT01631552) included 108 patients with TNBC and 80% of patients had visceral metastases. The median number of prior regimens was 3 (range, 2-10), which included chemotherapies and checkpoint inhibitors. Although it did not include biomarker selection of patients, 57 patients had moderate (2+) to strong (3+) and 5 patients had weak or absent TROP-2 expression by IHC according to available data. The ORR was 33% and the median duration of response (DOR) was 7.7 months. The median PFS was 5.5 months and the median OS was 13.0 months. Myelotoxic effects were the main adverse reactions and grade 3 or 4 AEs included anemia and neutropenia (104). The confirmatory ASCENT Phase III study (NCT02574455) of SG in comparison with treatment of physician's choice for patients with metastatic TNBC was stopped due to compelling evidence of efficacy across multiple endpoints and SG was granted accelerated approval by the FDA based on the results of the IMMU-132-01 Phase II clinical trial for the treatment of adult patients with metastatic TNBC who have received ≥2 prior therapies for metastatic disease. It is the first ADC approved by the FDA specifically for relapsed or refractory metastatic TNBC as well as the first FDA-approved anti-TROP-2 ADC.

Ladiratuzumab vedotin (LV) targets LIV-1, which is expressed in >90% of breast tumors with limited expression in normal tissues. LIV-1 is a transmembrane protein with

zinc transporter and metalloproteinase activity. The payload of LV is the microtubule disrupting agent monomethyl auristatin E (MMAE). Phase I study (NCT01969643) in patients with heavily pretreated metastatic TNBC showed 25% ORR and medium PFS of 11 weeks. Treatment was generally well-tolerated and related AEs were neutropenia, anemia, and neuropathy (105). LV was further explored in combination studies and in earlier lines of treatment. The SGNLVA-002 Phase Ib/II trial (NCT03310957) was designed to assess whether combining LV and pembrolizumab results in synergistic activity through LV-induced ICD that creates a microenvironment favorable for enhanced anti-PD-L1 activity. It was for first-line treatment of patients with unresectable locally advanced or metastatic TNBC. Initial dose-finding studies revealed ORR of 35% with responses independent of PD-L1 status and manageable toxicity (106).

ADC has also been explored for HER2-low or negative breast cancer. The rationale is based on the bystander effect, that is, the cleaved drug from an ADC may leak from the targeted tumor cell and affect cells in close proximity regardless of their target antigen expression status. Thus, an ADC having a high drug-to-antibody ratio and high-potency payload would increase the killing of tumor cells even with low HER2 expression. Trastuzumab deruxtecan (T-DXd) is the first HER2-targeted agent to demonstrate promising clinical antitumor activity with a manageable safety profile in patients considered to be HER2-negative. T-DXd delivers a potent topoisomerase I inhibitor payload (an exatecan derivative) which is linked to a humanized anti-HER2 antibody. In Phase Ib (NCT02564900) trial of T-DXd for heavily pretreated patients with advanced HER2-low breast cancer, ORR was 37% with the median DOR being 10.4 months. Most toxicities were gastrointestinal or hematologic-related, and interstitial lung disease (ILD) was an important identified risk (107). The DESTINY-Breast04 Phase III (NCT03734029) was initiated to compare the efficacy and safety of T-DXd to physician's choice (capecitabine, eribulin, gemcitabine, paclitaxel, or nab-paclitaxel) in patients with HER2-low, unresectable, and/or metastatic breast cancer (108).

4. New potential therapeutic strategies

Conversion of TNBC: Access to endocrine therapy. Gene expression analysis and functional studies have revealed a high degree of plasticity and heterogeneity in luminal and basal-like tumors. Expression of ER α , FOXA1 or GATA3 can result in transition from basal-like breast cancer to luminal type whereas epigenetic reprogramming can result in a reverse transition (109-111). The CDK2-EZH2 axis in tumors with TNBC phenotype (i.e. basal-like breast cancer) has been explored for conversion to the ER α ⁺ subtype. Epigenetic enzyme EZH2, a histone-lysine N-methyltransferase that promotes histone H3 lysine 27 mono-, di- and tri-methylation (H3K27me1/2/3), drives transcriptional repression (112,113). EZH2 can be phosphorylated at T416 (pT416-EZH2) by cyclin E/CDK2 and >80% of TNBC patient specimens exhibit high pT416-EZH2 levels, which correlate with poorer survival (114). In preclinical studies, transgenic expression of a phospho-mimicking mutant

EZH2(T416D) in the mammary glands of mice reprogrammed the committed luminal breast cancer cells into the basal-like TNBC phenotype. In this setting inhibition of the CDK2-EZH2 axis by EZH2 inhibitors reactivated ER α expression and thus combination with tamoxifen suppressed tumor growth and improved the survival of mice bearing tumors with the TNBC phenotype (115). Therefore, inhibitors of CDK2 or EZH2 combined with hormonal therapy may be a novel therapeutic strategy in TNBC with especially high pT416-EZH2 levels.

Another mechanism-based therapy exploits the lack of ER expression due to hypermethylation of the ER α promoter. A combination epigenetic therapy of a DNA methyltransferase (DNMT) inhibitor and a histone deacetylase (HDAC) inhibitor led to re-expression of genes including ER α and restored tamoxifen sensitivity in ER-negative breast cancer models (116,117). However, Phase II study (NCT01349959) in patients with advanced hormone-resistant breast cancer or TNBC revealed that combination of DNMT inhibitor 5-azacitidine and HDAC inhibitor entinostat did not induce ER α expression and primary endpoint ORR was not met (118). ER α re-expression induced by DNMT/HDAC inhibition might be attenuated by an active CDK2-EZH2 axis, which affected outcomes in this study.

The conversion of basal-like breast cancer into ER α ⁺ is also under microenvironmental control. A paracrine signaling network involving platelet-derived growth factor (PDGF)-CC and PDGF receptor- α accelerated tumor growth through recruitment and activation of different subsets of cancer-associated fibroblasts (119). In mouse models, impairing PDGF signaling was found to convert basal-like breast cancers into ER α ⁺, and thus enhanced sensitivity to tamoxifen in previously resistant tumors (120). Therefore, PDGF inhibitors combined with endocrine therapy may be a novel therapeutic strategy in TNBC treatment.

Adaptive clinical studies: Molecular markers. Under the master protocol framework, basket trials, where a targeted therapy is evaluated for multiple diseases that share common molecular alterations, and umbrella trials, where multiple targeted therapies are evaluated for a single disease that is stratified into multiple subgroups based on different molecular factors, have been developed (121). Recently there have been more adaptive, signal-finding clinical trial designs coupled with correlative studies to investigate mechanisms of action. They also facilitate identifying active drug combinations as well as novel tumor indications. Patients are enrolled based on molecular markers from genetic profiling performed on their tumors. Some examples are listed below.

In the OLAPCO Phase II trial (NCT02576444), PARP inhibitor olaparib was assessed in combination with various agents according to identified tumor mutations. It included AKT inhibitor capivasertib for tumors with mutations in the PI3K-AKT pathway, WEE1 inhibitor adavosertib for tumors with *tp53* or/and *Kras* mutations, and ATR inhibitor ceralasertib for tumors with mutations in HRR genes. Primary outcome measure was ORR, and the trial also identified genetic determinants of response and resistance. Another Phase II trial (NCT03718091) evaluated ATR inhibitor M6620 in selected solid tumors. Patients were enrolled in different cohorts based on tumor mutation status, including truncating

Atm mutations, germline *Brcas* mutations, somatic *Brcas* mutations or other HRR gene mutations, c-MYC amplification, *Fbxw7* mutations, cyclin E amplification, and *Arid1a* mutations. Primary outcome measures included disease control rate (DCR) and changes in pCHK1 and γH2AX levels. The I-SPY 2 Phase II trial (NCT01042379) was a neoadjuvant breast cancer trial using response-adaptive randomization. It had multiple concurrent experimental arms with shared controls. Each biomarker signature was established at trial entry. A new regimen of combination with standard chemotherapy will be moved up to Phase III trial if it shows a high probability of improved pCR over standard chemotherapy.

5. Conclusion

Developing novel treatments in both early and advanced TNBC settings remains a significant unmet need. Recent advances with novel agents have been made for specific subgroups with PD-L1⁺ tumors or gBRCAm tumors. However, only a fraction of those patients respond to immune checkpoint or PARP inhibitors, and even those who do respond often develop resistance and relapse. In diverse tumor microenvironments, a given therapeutic agent shows variable responses, thus compromising the survival endpoints especially in an unselected TNBC population. Therefore, developing novel predictive biomarkers are crucial for selecting patients that will benefit the most from a given therapy. Single cell technologies will provide additional insight on tumor-stroma interactions and facilitate compelling rationale for new treatments based on novel biomarkers. A non-invasive testing of plasma circulating tumor DNA (ctDNA) and CTCs can potentially provide real-time disease monitoring and even early therapy modification. However, their prognostic value needs further evaluation. With recent advances in multiomic analyses of cancers, there appears to be genomic and molecular similarities between TNBC and high-grade serous ovarian carcinoma (HGSOC), suggesting that similar biological mechanisms drive some aspects of both cancer types. Therefore, treatment strategies for HGSOC can be explored in TNBC as well. The recent increase in the number of clinical trials investigating various new agents and combination strategies reflects further efforts to understand molecular and immunological aspects of TNBC. This may lead to more meaningful clinical benefits, including event-free and overall survival.

Acknowledgements

The authors would like to thank Professor Ian Collins of the Institute of Cancer Research, UK for valuable discussions on the DNA damage response pathways and checkpoint kinases.

Funding

No funding was declared.

Availability of data and materials

All information provided in this review is documented with relevant and current references.

Authors' contributions

KAW was responsible for conceptualization, design, interpretation and visualization. KAW and CS were responsible for writing, reviewing and editing. Both authors approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

No competing interests are declared.

References

- Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y and Pienpol JA: Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* 121: 2750-2767, 2011.
- Lehmann BD, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y, Moses HL, Sanders ME and Pienpol JA: Refinement of triple-negative breast cancer molecular subtypes: Implications for neoadjuvant chemotherapy selection. *PLoS One* 11: e0157368, 2016.
- Giuliano AE, Connolly JL, Edge SB, Mittendorf EA, Rugo HS, Solin LJ, Weaver DL, Winchester DJ and Hortobagyi GN: Breast cancer-major changes in the American Joint Committee on cancer eighth edition cancer staging manual. *CA Cancer J Clin* 67: 290-303, 2017.
- Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, et al: Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American pathologists clinical practice guideline focused update. *J Clin Oncol* 36: 2105-2122, 2018.
- Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, et al: Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. *J Clin Oncol* 38: 1346-1366, 2020.
- Yam C, Mani SA and Moulder SL: Targeting the molecular subtypes of triple negative breast cancer: Understanding the diversity to progress the field. *Oncologist* 22: 1086-1093, 2017.
- Bonotto M, Gerratana L, Poletto E, Driol P, Giangreco M, Russo S, Minisini AM, Andreetta C, Mansutti M, Pisa FE, et al: Measures of outcome in metastatic breast cancer: Insights from a real-world scenario. *Oncologist* 19: 608-615, 2014.
- Kohler BA, Sherman RL, Howlader N, Jemal A, Ryerson AB, Henry KA, Boscoe FP, Cronin KA, Lake A, Noone AM, et al: Annual report to the nation on the status of cancer, 1975-2011, featuring incidence of breast cancer subtypes by race/ethnicity, poverty, and state. *J Natl Cancer Inst* 107: djv048, 2015.
- O'Shaughnessy J, Schwartzberg L, Danso MA, Miller KD, Rugo HS, Neubauer M, Robert N, Hellerstedt B, Saleh M, Richards P, et al: Phase III study of iniparib plus gemcitabine and carboplatin versus gemcitabine and carboplatin in patients with metastatic triple-negative breast cancer. *J Clin Oncol* 32: 3840-3847, 2014.
- Caswell-Jin JL, Plevritis SK, Tian L, Cadham CJ, Xu C, Stout NK, Sledge GW, Mandelblatt JS and Kurian AW: Change in survival in metastatic breast cancer with treatment advances: Meta-analysis and systematic review. *JNCI Cancer Spectr* 2: pky062, 2018.
- Plevritis SK, Munoz D, Kurian AW, Stout NK, Alagoz O, Near AM, Lee SJ, van den Broek JJ, Huang X, Schechter CB, et al: Association of screening and treatment with breast cancer mortality by molecular subtype in US women, 2000-2012. *JAMA* 319: 154-164, 2018.

12. Stanton SE, Adams S and Disis ML: Variation in the incidence and magnitude of tumor-infiltrating lymphocytes in breast cancer subtypes: A systematic review. *JAMA Oncol* 2: 1354-1360, 2016.
13. Safonov A, Jiang T, Bianchini G, Győrffy B, Karn T, Hatzis C and Pusztai L: Immune gene expression is associated with genomic aberrations in breast cancer. *Cancer Res* 77: 3317-3324, 2017.
14. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, Diéras V, Hegg R, Im SA, Shaw Wright G, et al: Atezolizumab and Nab-Paclitaxel in advanced triple-negative breast cancer. *N Engl J Med* 379: 2108-2121, 2018.
15. Schmid P, Rugo HS, Adams S, Schneeweiss A, Barrios CH, Iwata H, Diéras V, Henschel V, Molinero L, Chui SY, et al: Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): Updated efficacy results from a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 21: 44-59, 2020.
16. Dudley JC, Lin MT, Le DT and Eshleman JR: Microsatellite instability as a biomarker for PD-1 blockade. *Clin Cancer Res* 22: 813-820, 2016.
17. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen HZ, Reeser JW, Yu L and Roychowdhury S: Landscape of microsatellite instability across 39 cancer types. *JCO Precis Oncol* 2017: PO.17.00073, 2017.
18. Kurata K, Kubo M, Mori H, Kawaji H, Motoyama Y, Kuroki L, Yamada M, Kaneshiro K, Kai M and Nakamura M: Microsatellite instability in triple negative breast cancers. In: Proceedings of the 2018 San Antonio Breast Cancer Symposium. *Cancer Res* 79 (Suppl 4): Abstract nr P1-06-11, 2019.
19. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ and Helleday T: Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434: 913-917, 2005.
20. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, et al: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434: 917-921, 2005.
21. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, Delalodge S, Li W, Tung N, Armstrong A, et al: Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *N Engl J Med* 377: 523-533, 2017.
22. Robson ME, Tung N, Conte P, Im SA, Senkus E, Xu B, Masuda N, Delalodge S, Li W, Armstrong A, et al: OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. *Ann Oncol* 30: 558-566, 2019.
23. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee KH, Fehrenbacher L, Yerushalmi R, Mina LA, Martin M, et al: Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. *N Engl J Med* 379: 753-763, 2018.
24. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, Budczies J, Huober J, Klauschen F, Furlanetto J, et al: Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: A pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol* 19: 40-50, 2018.
25. Hida AI, Watanabe T, Sagara Y, Kashiwaba M, Sagara Y, Aogi K, Ohi Y and Tanimoto A: Diffuse distribution of tumor-infiltrating lymphocytes is a marker for better prognosis and chemotherapeutic effect in triple-negative breast cancer. *Breast Cancer Res Treat* 178: 283-294, 2019.
26. Loi S, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, Joensuu H, Dieci MV, Badve S, Demaria S, et al: Tumor-infiltrating lymphocytes and prognosis: A pooled individual patient analysis of early-stage triple-negative breast cancers. *J Clin Oncol* 37: 559-569, 2019.
27. Galon J and Bruni D: Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nat Rev Drug Discov* 18: 197-218, 2019.
28. Adams S, Gatti-Mays ME, Kalinsky K, Korde LA, Sharon E, Amiri-Kordestani L, Bear H, McArthur HL, Frank E, Perlmutter J, et al: Current landscape of immunotherapy in breast cancer: A review. *JAMA Oncol*: Apr 11, 2019 (Epub ahead of print).
29. Schmid P, Salgado R, Park YH, Muñoz-Couselo E, Kim SB, Sohn J, Im S-A, Foukakis T, Kuemmel S, Dent R, et al: Pembrolizumab plus chemotherapy as neoadjuvant treatment of high-risk, early-stage triple-negative breast cancer: Results from the phase 1b open-label, multicohort KEYNOTE-173 study. *Ann Oncol* 31: 569-581, 2020.
30. Nanda R, Liu MC, Yau C, Shatsky R, Pusztai L, Wallace A, Chien AJ, Forero-Torres A, Ellis E, Han H, et al: Effect of pembrolizumab plus neoadjuvant chemotherapy on Pathologic complete response in women with early-stage breast cancer: An analysis of the ongoing phase 2 adaptively randomized I-SPY2 trial. *JAMA Oncol* 6: 1-9, 2020.
31. Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, Denkert C, Park YH, Hui R, Harbeck N, et al: Pembrolizumab for early triple-negative breast cancer. *N Engl J Med* 382: 810-821, 2020.
32. Gianni L, Huang CS, Egle D, Bermejo B, Zamagni C, Thill M, Anton A, Zambelli S, Bianchini G, Russo S and Ciruelos E: Pathologic complete response (pCR) to neoadjuvant treatment with or without atezolizumab in triple negative, early high-risk and locally advanced breast cancer. NeoTRIPaPDL1 Michelangelo randomized study. In: Proceedings of the 2019 San Antonio Breast Cancer Symposium. *Cancer Res* 80 (Suppl 4): Abstract nr GS3-04, 2020.
33. Mittendorf E, Barrios CH, Harbeck N, Miles D, Saji S, Zhang H, Duc AN, Rafii S and Lai C: IMpassion031: A phase III study comparing neoadjuvant atezolizumab vs placebo in combination with nab-paclitaxel-based chemotherapy in early triple-negative breast cancer (TNBC). In: Proceedings of the 2017 San Antonio Breast Cancer Symposium. *Cancer Res* 78 (Suppl 4): Abstract nr OT2-07-03, 2018.
34. Cortes J, Cescon DW, Rugo HS, Nowecki Z, Im SA, Yusof MM, Gallardo C, Lipatov Ö, Barrios CH, Holgado E, et al: KEYNOTE-355: Randomized, double-blind, phase III study of pembrolizumab + chemotherapy versus placebo + chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer. *J Clin Oncol* 38: (Suppl 15): S1000-S1000, 2020.
35. Voorwerk L, Slagter M, Horlings HM, Sikorska K, van de Vijver KK, de Maaker M, Nederlof I, Kluij RJC, Warren S, Ong S, et al: Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: The TONIC trial. *Nat Med* 25: 920-928, 2019.
36. Allard B, Longhi MS, Robson SC and Stagg J: The ectonucleotidases CD39 and CD73: Novel checkpoint inhibitor targets. *Immunol Rev* 276: 121-144, 2017.
37. Ghalamfarsa G, Kazemi MH, Raoofi Mohseni S, Masjedi A, Hojjat-Farsangi M, Azizi G, Yousefi M and Jadidi-Niaragh F: CD73 as a potential opportunity for cancer immunotherapy. *Expert Opin Ther Targets* 23: 127-142, 2019.
38. Duham X, Schandené L, Bruyns C, Gonzalez NS, Goldman M, Boeynaems JM and Communi D: Extracellular adenine nucleotides inhibit the activation of human CD4+ T lymphocytes. *J Immunol* 169: 15-21, 2002.
39. Allard B, Beavis PA, Darcy PK and Stagg J: Immunosuppressive activities of adenosine in cancer. *Curr Opin Pharmacol* 29: 7-16, 2016.
40. Ohta A: A metabolic immune checkpoint: Adenosine in tumor microenvironment. *Front Immunol* 7: 109, 2016.
41. Buisseret L, Pommy S, Allard B, Garaud S, Bergeron M, Cousineau I, Ameye L, Bareche Y, Paesmans M, Crown JPA, et al: Clinical significance of CD73 in triple-negative breast cancer: Multiplex analysis of a phase III clinical trial. *Ann Oncol* 29: 1056-1062, 2018.
42. Powderly J, Spira A, Gutierrez R, DiRenzo D, Udyavar A, Karakunnel JJ, Rieger A, Colabella J, Lai DW and de Souza P: Phase 1 evaluation of AB928, a novel dual adenosine receptor antagonist, combined with chemotherapy or AB122 (anti-PD-1) in patients with advanced malignancies. *Ann Oncol* 30 (Suppl 5): v475-v532, 2019.
43. Hartman AR, Kaldate RR, Sailer LM, Painter L, Grier CE, Endsley RR, Griffin M, Hamilton SA, Frye CA, Silberman MA, et al: Prevalence of BRCA mutations in an unselected population of triple-negative breast cancer. *Cancer* 118: 2787-2795, 2012.
44. Okuma HS and Yonetomori K: BRCA gene mutations and poly(ADP-Ribose) polymerase inhibitors in triple-negative breast cancer. *Adv Exp Med Biol* 1026: 271-286, 2017.
45. Lord CJ and Ashworth A: BRCAneSS revisited. *Nat Rev Cancer* 16: 110-120, 2016.
46. Litton JK, Scoggins ME, Hess KR, Adrada BE, Murthy RK, Damodaran S, DeSnyder SM, Brewster AM, Barcenas CH, Valero V, et al: Neoadjuvant talazoparib for patients with operable breast cancer with a germline BRCA pathogenic variant. *J Clin Oncol* 38: 388-394, 2020.

47. Loibl S, O'Shaughnessy J, Untch M, Sikov WM, Rugo HS, McKee MD, Huober J, Golshan M, von Minckwitz G, Maag D, *et al*: Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrighTNess): A randomised, phase 3 trial. *Lancet Oncol* 19: 497-509, 2018.
48. Jiao S, Xia W, Yamaguchi H, Wei Y, Chen MK, Hsu JM, Hsu JL, Yu WH, Du Y, Lee HH, *et al*: PARP inhibitor upregulates PD-L1 expression and enhances cancer-associated immunosuppression. *Clin Cancer Res* 23: 3711-3720, 2017.
49. Vinayak S, Tolaney SM, Schwartzberg L, Mita M, McCann G, Tan AR, Wahner-Hendrickson AE, Forero A, Anders C, Wulf GM, *et al*: Open-label clinical trial of niraparib combined with pembrolizumab for treatment of advanced or metastatic triple-negative breast cancer. *JAMA Oncol* 5: 1132-1140, 2019.
50. Domchek S, Postel-Vinay S, Im S, Park YH, Delord J, Italiano A, Alexandre J, You B, Bastian S, Krebs MG, *et al*: Phase II study of olaparib (o) and durvalumab (d) (MEDIOLA): Updated results in patients (pts) with germline BRCA-mutated (gBRCAm) metastatic breast cancer (mbc). *Ann Oncol* 30 (Suppl 5): v475-v532, 2019.
51. Pusztai L, Han HS, Yau C, Wolf D, Wallace AM, Shatsky R, Helsten T, Boughey JC, Haddad T, Stringer-Reasor E, *et al*: Durvalumab in combination with olaparib and paclitaxel in high-risk HER2 negative stage II/III breast cancer: Results from the I-SPY 2 trial. In: Proceedings of the Annual Meeting of the American Association for Cancer Research 2020. *Cancer Res* 80 (Suppl 16): Abstract nr CT011, 2020.
52. Mitri ZI, Vuky J, Kemmer KA, Savin MA, Parmar S, Kolodzie AK, Johnson B, Williams-Belizaire R, Gray JW and Mills GB: A phase II trial of olaparib and durvalumab in metastatic BRCA wild type triple-negative breast cancer. *J Clin Oncol* 37: TPS1111, 2019.
53. Rugo HS, Lloimbart-Cussac A, Andre F, Robson ME, Saji S, Harbeck N, Schmid P, Cescon DW, Ahn JS, Nanda R, *et al*: KEYLYNK-009: A phase II/III, open-label, randomized study of pembrolizumab (pembro) plus olaparib vs pembro plus chemotherapy after induction with first-line pembro plus chemotherapy in patients with locally recurrent inoperable or metastatic triple-negative breast cancer (TNBC). *J Clin Oncol* 38: TPS596, 2020.
54. Maacke H, Opitz S, Jost K, Hamdorf W, Henning W, Krüger S, Feller AC, Lopens A, Diedrich K, Schwinger E and Stürzbecher HW: Over-expression of wild-type Rad51 correlates with histological grading of invasive ductal breast cancer. *Int J Cancer* 88: 907-913, 2000.
55. Martin RW, Orelli BJ, Yamazoe M, Minn AJ, Takeda S and Bishop DK: RAD51 up-regulation bypasses BRCA1 function and is a common feature of BRCA1-deficient breast tumors. *Cancer Res* 67: 9658-9665, 2007.
56. Wiegmans AP, Yap PY, Ward A, Lim YC and Khanna KK: Differences in expression of key DNA damage repair genes after epigenetic-induced BRCAneSS dictate synthetic lethality with PARP1 inhibition. *Mol Cancer Ther* 14: 2321-2331, 2015.
57. Liu Y, Burness ML, Martin-Trevino R, Guy J, Bai S, Harouaka R, Brooks MD, Shang L, Fox A, Luther TK, *et al*: RAD51 mediates resistance of cancer stem cells to PARP inhibition in triple-negative breast cancer. *Clin Cancer Res* 23: 514-522, 2017.
58. Marzio A, Puccini J, Kwon Y, Maverakis NK, Arbini A, Sung P, Bar-Sagi D and Pagano M: The F-Box domain-dependent activity of EM11 regulates PARPi sensitivity in triple-negative breast cancers. *Mol Cell* 73: 224-237.e6, 2019.
59. Tutt A, Stephens C, Frewer P, Pierce A, Rhee J, So K, Ottesen L, Dean E and Hollingsworth SJ: VIOLETTE: A randomized phase II study to assess DNA damage response inhibitors in combination with olaparib (Ola) vs. Ola monotherapy in patients (pts) with metastatic, triple-negative breast cancer (TNBC) stratified by alterations in homologous recombination repair (HRR)-related genes. *J Clin Oncol* 36 (Suppl 15): TPS112, 2018.
60. Hirai H, Arai T, Okada M, Nishibata T, Kobayashi M, Sakai N, Imagaki K, Ohtani J, Sakai T, Yoshizumi T, *et al*: MK-1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-fluorouracil. *Cancer Biol Ther* 9: 514-522, 2010.
61. Pitts TM, Simmons DM, Bagby SM, Hartman SJ, Yacob BW, Gittleman B, Tentler JJ, Cittelly D, Ormond DR, Messersmith WA, *et al*: Wee1 inhibition enhances the anti-tumor effects of capecitabine in preclinical models of triple-negative breast cancer. *Cancers (Basel)* 12: 719, 2020.
62. Do K, Wilsker D, Ji J, Zlott J, Freshwater T, Kinders RJ, Collins J, Chen AP, Doroshow JH and Kummar S: Phase I study of single-agent AZD1775 (MK-1775), a Wee1 kinase inhibitor, in patients with refractory solid tumors. *J Clin Oncol* 33: 3409-3415, 2015.
63. Do KT, Hill SJ, Kochupurakkal B, Supko JG, Gannon C, Anderson A, Muzikansky A, Wolanski A, Hedglin J, Parmar K, *et al*: Abstract CT232: Phase I combination study of the CHK1 inhibitor prexasertib (LY2606368) and olaparib in patients with high-grade serous ovarian cancer and other advanced solid tumors. In: Proceedings of the American Association for Cancer Research Annual Meeting 2019. *Cancer Res* 79 (Suppl 13): Abstract nr CT232, 2019.
64. Peng W, Chen JQ, Liu C, Malu S, Creasy C, Tetzlaff MT, Xu C, McKenzie JA, Zhang C, Liang X, *et al*: Loss of PTEN promotes resistance to T cell-mediated immunotherapy. *Cancer Discov* 6: 202-216, 2016.
65. André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, Iwata H, Conte P, Mayer IA, Kaufman B, *et al*: Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. *N Engl J Med* 380: 1929-1940, 2019.
66. Kim SB, Dent R, Im SA, Espie M, Blau S, Tan AR, Isakoff SJ, Oliveira M, Saura C, Wongchenko MJ, *et al*: Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (LOTUS): A multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Oncol* 18: 1360-1372, 2017.
67. Dent R, Im SA, Espie M, Blau S, Tan AR, Isakoff SJ, Oliveira M, Saura C, Wongchenko M, Kapp AV, *et al*: Overall survival (OS) update of the double-blind placebo (PBO)-controlled randomized phase 2 LOTUS trial of first-line ipatasertib (IPAT) + paclitaxel (PAC) for locally advanced/metastatic triple-negative breast cancer (mTNBC). *J Clin Oncol* 36: 1008, 2018.
68. Dent R, Kim SB, Oliveira M, Isakoff SJ, Barrios CH, O'Shaughnessy J, Lu X, Wongchenko M, Bradley D, Mani A, *et al*: IPATunity130: A pivotal randomized phase III trial evaluating ipatasertib (IPAT) + paclitaxel (PAC) for PIK3CA/AKT1/PTEN-altered advanced triple-negative (TN) or hormone receptor-positive HER2-negative (HR+/HER2-) breast cancer (BC). *J Clin Oncol* 36 (Suppl 15): TPS117, 2018.
69. Schmid P, Abraham J, Chan S, Wheatley D, Brunt AM, Nemadze G, Baird RD, Park YH, Hall PS, Perren T, *et al*: Capivasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer: The PAKT trial. *J Clin Oncol* 38: 423-433, 2020.
70. Schmid P, Cortes J, Robson M, Iwata H, Hegg R, Verma S, Nechaeva M, Xu B, Haddad V, Imedio RE, *et al*: Abstract OT2-08-02: Capivasertib and paclitaxel in first-line treatment of patients with metastatic triple-negative breast cancer: A phase III trial (CAPTello-290). In: Proceedings of the 2019 San Antonio Breast Cancer Symposium. *Cancer Res* 80 (Suppl 4): Abstract nr OT2-08-02, 2020.
71. Schmid P, Loirat D, Savas P, Espinosa E, Boni V, Italiano A, White S, Singel MS, Withana N, Mani A, *et al*: Phase Ib study evaluating a triplet combination of ipatasertib (IPAT), atezolizumab (atezo), and paclitaxel (PAC) or nab-PAC as first-line (1L) therapy for locally advanced/metastatic triple-negative breast cancer (TNBC). In: Proceedings of the American Association for Cancer Research Annual Meeting 2019. *Cancer Res* 79 (Suppl 13): Abstract nr CT049, 2019.
72. Hanahan D and Weinberg RA: Hallmarks of cancer: The next generation. *Cell* 144: 646-674, 2011.
73. Dean JL, McClendon AK and Knudsen ES: Modification of the DNA damage response by therapeutic CDK4/6 inhibition. *J Biol Chem* 287: 29075-29087, 2012.
74. Cretella D, Fumarola C, Bonelli M, Alfieri R, La Monica S, Di Giacomo G, Cavazzoni A, Galetti M, Generali D and Petronini PG: Pre-treatment with the CDK4/6 inhibitor palbociclib improves the efficacy of paclitaxel in TNBC cells. *Sci Rep* 9: 13014, 2019.
75. Clark AS, McAndrew NP, Troxel A, Feldman M, Lal P, Rosen M, Burrell J, Redlinger C, Gallagher M, Bradbury AR, *et al*: Combination paclitaxel and palbociclib: Results of a phase I trial in advanced breast cancer. *Clin Cancer Res* 25: 2072-2079, 2019.
76. Tan AR, Wright GS, Thummala AR, Danso MA, Popovic L, Pluard TJ, Han HS, Vojnović Ž, Vasev N, Ma L, *et al*: Trilaciclib plus chemotherapy versus chemotherapy alone in patients with metastatic triple-negative breast cancer: A multicentre, randomised, open-label, phase 2 trial. *Lancet Oncol* 20: 1587-1601, 2019.
77. Stine ZE, Walton ZE, Altman BJ, Hsieh AL and Dang CV: MYC, metabolism, and cancer. *Cancer Discov* 5: 1024-1039, 2015.

78. Ambrosio S, Amento S, Napolitano G, Di Palo G, Lania L and Majello B: MYC impairs resolution of site-specific DNA double-strand breaks repair. *Mutat Res* 774: 6-13, 2015.
79. Wiegmans AP, Al-Ejeh F, Chee N, Yap PY, Gorski JJ, Da Silva L, Bolderson E, Chenevix-Trench G, Anderson R, Simpson PT, *et al*: Rad51 supports triple negative breast cancer metastasis. *Oncotarget* 5: 3261-3272, 2014.
80. Carey JPW, Karakas C, Bui T, Chen X, Vijayaraghavan S, Zhao Y, Wang J, Mikule K, Litton JK, Hunt KK and Keyomarsi K: Synthetic lethality of PARP inhibitors in combination with MYC blockade is independent of BRCA status in triple negative breast cancer. *Cancer Res* 78: 742-757, 2018.
81. Horiuchi D, Kusdra L, Huskey NE, Chandriani S, Lenburg ME, Gonzalez-Angulo AM, Creasman KJ, Bazarov AV, Smyth JW, Davis SE, *et al*: MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. *J Exp Med* 209: 679-696, 2012.
82. Hossain DMS, Javaid S, Cai M, Zhang C, Sawant A, Hinton M, Sathe M, Grein J, Blumenschein W, Pinheiro EM and Chackerman A: Dinaciclib induces immunogenic cell death and enhances anti-PD1-mediated tumor suppression. *J Clin Invest* 128: 644-654, 2018.
83. Chien AJ, Gliwa AS, Rahmaputri S, Dittrich HF, Majure MC, Rugo HS, Melisko ME, Munster PN, Park JW, Moasser MM, *et al*: A phase Ib trial of the cyclin-dependent kinase inhibitor dinaciclib (dina) in combination with pembrolizumab (P) in patients with advanced triple-negative breast cancer (TNBC) and response correlation with MYC-overexpression. *J Clin Oncol* 38: 1076, 2020.
84. Kono M, Fujii T, Lim B, Karuturi MS, Tripathy D and Ueno NT: Androgen receptor function and androgen receptor-targeted therapies in breast cancer: A Review. *JAMA Oncol* 3: 1266-1273, 2017.
85. Gerratana L, Basile D, Buono G, De Placido S, Giuliano M, Minichillo S, Coinu A, Martorana F, De Santo I, Del Mastro L, *et al*: Androgen receptor in triple negative breast cancer: A potential target for the targetless subtype. *Cancer Treat Rev* 68: 102-110, 2018.
86. Anestis A, Zoi I, Papavassiliou AG and Karamouzis MV: Androgen receptor in breast cancer-clinical and preclinical research insights. *Molecules* 25: 358, 2020.
87. Masuda H, Baggerly KA, Wang Y, Zhang Y, Gonzalez-Angulo AM, Meric-Bernstam F, Valero V, Lehmann BD, Pietenpol JA, Hortobagyi GN, *et al*: Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. *Clin Cancer Res* 19: 5533-5540, 2013.
88. Thike AA, Yong-Zheng Chong L, Cheok PY, Li HH, Wai-Cheong Yip G, Huat Bay B, Tse GM, Iqbal J and Tan PH: Loss of androgen receptor expression predicts early recurrence in triple-negative and basal-like breast cancer. *Mod Pathol* 27: 352-360, 2014.
89. Echavarria I, Lopez-Tarruella S, Picornell A, García-Saenz JA, Jerez Y, Hoadley K, Gómez HL, Moreno F, Monte-Millan MD, Márquez-Rodas I, *et al*: Pathological response in a triple-negative breast cancer cohort treated with neoadjuvant carboplatin and docetaxel according to Lehmann's refined classification. *Clin Cancer Res* 24: 1845-1852, 2018.
90. Santonja A, Sánchez-Muñoz A, Lluch A, Chica-Parrado MR, Albanell J, Chacón JI, Antolín S, Jerez JM, de la Haba J, de Luque V, *et al*: Triple negative breast cancer subtypes and pathologic complete response rate to neoadjuvant chemotherapy. *Oncotarget* 9: 26406-26416, 2018.
91. Venema CM, Bense RD, Steenbruggen TG, Nienhuis HH, Qiu SQ, van Krachten M, Brown M, Tamimi RM, Hospers GAP, Schröder CP, *et al*: Consideration of breast cancer subtype in targeting the androgen receptor. *Pharmacol Ther* 200: 135-147, 2019.
92. Rice MA, Malhotra SV and Stoyanova T: Second-generation antiandrogens: From discovery to standard of care in castration resistant prostate cancer. *Front Oncol* 9: 801, 2019.
93. Gucalp A, Tolaney S, Isakoff SJ, Ingle JN, Liu MC, Carey LA, Blackwell K, Rugo H, Nabell L, Forero A, *et al*: Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. *Clin Cancer Res* 19: 5505-5512, 2013.
94. Gucalp A, Edelweiss M, Patil S, Gounder MM, Feigin KN, Corben A, Arumov A and Traina TA: Abstract P3-11-04: Phase I/II trial of palbociclib in combination with bicalutamide for the treatment of androgen receptor (AR)+ metastatic breast cancer (MBC). In: Proceedings of the 2017 San Antonio Breast Cancer Symposium. *Cancer Res* 2018;78 (Suppl 4): Abstract nr P3-11-04, 2018.
95. Gucalp A, Boyle LA, Alano T, Arumov A, Gounder MM, Patil S, Feigin K, Edelweiss M, D'Andrea G, Bromberg J, *et al*: Phase II trial of bicalutamide in combination with palbociclib for the treatment of androgen receptor (+) metastatic breast cancer. *J Clin Oncol* 38, 2020.
96. Bonnefoi H, Grellety T, Tredan O, Saghatelian M, Dalenc F, Maillez A, L'Haridon T, Cottu P, Abadie-Lacourtoisie S, You B, *et al*: A phase II trial of abiraterone acetate plus prednisone in patients with triple-negative androgen receptor positive locally advanced or metastatic breast cancer (UCBG 12-1). *Ann Oncol* 27: 812-818, 2016.
97. Gucalp A, Danso MA, Elias AD, Bardia A, Ali HY, Potter D, Gabrail NY, Haley BB, Khong HT, Riley EC, *et al*: Phase (Ph) 2 stage 1 clinical activity of seviteronel, a selective CYP17-lase and androgen receptor (AR) inhibitor, in women with advanced AR+ triple-negative breast cancer (TNBC) or estrogen receptor (ER)+ BC: CLARITY-01. *J Clin Oncol* 35: 1102, 2017.
98. Bardia A, Gucalp A, DaCosta N, Gabrail N, Danso M, Ali H, Blackwell KL, Carey LA, Eisner JR, Baskin-Bey ES and Traina TA: Phase 1 study of seviteronel, a selective CYP17 lyase and androgen receptor inhibitor, in women with estrogen receptor-positive or triple-negative breast cancer. *Breast Cancer Res Treat* 171: 111-120, 2018.
99. Traina TA, Miller K, Yardley DA, Eakle J, Schwartzberg LS, O'Shaughnessy J, Gradishar W, Schmid P, Winer E, Kelly C, *et al*: Enzalutamide for the treatment of androgen receptor-expressing triple-negative breast cancer. *J Clin Oncol* 36: 884-890, 2018.
100. Dent R, Schmid P, Cortes J, Kim SB, Andre F, Abramson V, Cardoso F, Colleoni M, Morris P, Steinberg J, *et al*: Abstract OT3-02-02: ENDEAR: A randomized international phase 3 study comparing the efficacy and safety of enzalutamide in combination with paclitaxel chemotherapy or as monotherapy vs placebo with paclitaxel in patients with advanced diagnostic-positive triple-negative breast cancer. *Cancer Res* 77: Abstract OT3-02-02, 2017.
101. Lehmann BD, Abramson VG, Sanders ME, Mayer EL, Haddad TC, Nanda R, Van Poznak C, Storniolo AM, Nangia JR, Gonzalez-Ericsson PI, *et al*: TBCRC 032 IB/II multicenter study: Molecular insights to AR antagonist and PI3K inhibitor efficacy in patients with AR+ metastatic triple-negative breast cancer. *Clin Cancer Res* 26: 2111-2123, 2020.
102. Gilewski T, Ragupathi G, Bhuta S, Williams LJ, Musselli C, Zhang XF, Bornmann WG, Spassova M, Bencsath KP, Panageas KS, *et al*: Immunization of metastatic breast cancer patients with a fully synthetic globo H conjugate: A phase I trial. *Proc Natl Acad Sci USA* 98: 3270-3275, 2001.
103. Huang CS, Yu AL, Tseng LM, Chow LWC, Hou MF, Hurvitz SA, Schwab RB, Wong CH, Murray JL, Chang SC, *et al*: Randomized phase II/III trial of active immunotherapy with OPT-822/OPT-821 in patients with metastatic breast cancer. *J Clin Oncol* 34 (Suppl 15): S1003, 2016.
104. Bardia A, Mayer IA, Vahdat LT, Tolaney SM, Isakoff SJ, Diamond JR, O'Shaughnessy J, Moroese RL, Santin AD, Abramson VG, *et al*: Sacituzumab govitecan-hziy in refractory metastatic triple-negative breast cancer. *N Eng J Med* 380: 741-751, 2019.
105. Modi S, Pusztai L, Forero A, Mita M, Miller KD, Weise A, Burris H III, Kalinsky K, Tsai M, Liu MC, *et al*: Abstract PD3-14: Phase 1 study of the antibody-drug conjugate SGN-LIV1A in patients with heavily pretreated triple-negative metastatic breast cancer. *Cancer Res* 78, 2018.
106. Han HS, Alemany CA, Brown-Glberman UA, Pluard TJ, Sinha R, Sterrenberg D, Albain KS, Basho RK, Biggs D, Boni V, *et al*: SGN-LVA-002: Single-arm, open label phase Ib/II study of ladiratumab vedotin (LV) in combination with pembrolizumab for first-line treatment of patients with unresectable locally advanced or metastatic triple-negative breast cancer. *J Clin Oncol* 37 (Suppl 15): TPS1110, 2019.
107. Modi S, Park H, Murthy RK, Iwata H, Tamura K, Tsurutani J, Moreno-Aspitia A, Doi T, Sagara Y, Redfern C, *et al*: Antitumor activity and safety of trastuzumab Deruxtecan in patients with HER2-low-expressing advanced breast cancer: Results from a phase Ib study. *J Clin Oncol* 38: 1887-1896, 2020.
108. Modi S, Ohtani S, Lee CC, Wang K, Saxena K and Cameron DA: A phase III, multicenter, randomized, open label trial of [fam]-trastuzumab deruxtecan (DS-8201a) versus investigator's choice in HER2-low breast cancer. *J Clin Oncol* 37: (Suppl 15): TPS1102, 2019.

109. Molyneux G, Geyer FC, Magnay FA, McCarthy A, Kendrick H, Natrajan R, Mackay A, Grigoriadis A, Tutt A, Ashworth A, *et al*: BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. *Cell Stem Cell* 7: 403-417, 2010.
110. Bernardo GM, Bebek G, Ginther CL, Sizemore ST, Lozada KL, Miedler JD, Anderson LA, Godwin AK, Abdul-Karim FW, Slamon DJ and Keri RA: FOXA1 represses the molecular phenotype of basal breast cancer cells. *Oncogene* 32: 554-563, 2013.
111. Su Y, Subedee A, Bloushtain-Qimron N, Savova V, Krzystanek M, Li L, Marusyk A, Tabassum DP, Zak A, Flacker MJ, *et al*: Somatic cell fusions reveal extensive heterogeneity in basal-like breast cancer. *Cell Rep* 11: 1549-1563, 2015.
112. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS and Zhang Y: Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* 298: 1039-1043, 2002.
113. Yamagishi M and Uchimaru K: Targeting EZH2 in Cancer Therapy. *Curr Opin Oncol* 29: 375-381, 2017.
114. Yang CC, LaBaff A, Wei Y, Nie L, Xia W, Huo L, Yamaguchi H, Hsu YH, Hsu JL, Liu D, *et al*: Phosphorylation of EZH2 at T416 by CDK2 contributes to the malignancy of triple negative breast cancers. *Am J Transl Res* 7: 1009-1020, 2015.
115. Nie L, Wei Y, Zhang F, Hsu YH, Chan LC, Xia W, Ke B, Zhu C, Deng R, Tang J, *et al*: CDK2-mediated site-specific phosphorylation of EZH2 drives and maintains triple-negative breast cancer. *Nat Commun* 10: 5114, 2019.
116. Yang X, Phillips DL, Ferguson AT, Nelson WG, Herman JG and Davidson NE: Synergistic activation of functional estrogen receptor (ER)-alpha by DNA methyltransferase and histone deacetylase inhibition in human ER-alpha-negative breast cancer cells. *Cancer Res* 61: 7025-7029, 2001.
117. Sharma D, Saxena NK, Davidson NE and Vertino PM: Restoration of tamoxifen sensitivity in estrogen receptor-negative breast cancer cells: Tamoxifen-bound reactivated ER recruits distinctive corepressor complexes. *Cancer Res* 66: 6370-6378, 2006.
118. Connolly RM, Li H, Jankowitz RC, Zhang Z, Rudek MA, Jeter SC, Slater SA, Powers P, Wolff AC, Fetting JH, *et al*: Combination epigenetic therapy in advanced breast cancer with 5-azacitidine and entinostat: A phase II National Cancer Institute/Stand up to cancer study. *Clin Cancer Res* 23: 2691-2701, 2017.
119. Anderberg C, Li H, Fredriksson L, Andrae J, Betsholtz C, Li X, Eriksson U and Pietras K: Paracrine signaling by platelet-derived growth factor-CC promotes tumor growth by recruitment of cancer-associated fibroblasts. *Cancer Res* 69: 369-378, 2009.
120. Roswall P, Bocci M, Bartoschek M, Li H, Kristiansen G, Jansson S, Lehn S, Sjölund J, Reid S, Larsson C, *et al*: Microenvironmental control of breast cancer subtype elicited through paracrine platelet-derived growth factor-CC signaling. *Nat Med* 24: 463-473, 2018.
121. Park JJH, Hsu G, Siden EG, Thorlund K and Mills EJ: An overview of precision oncology basket and umbrella trials for clinicians. *CA Cancer J Clin* 70: 125-137, 2020.



This work is licensed under a Creative Commons
Attribution-NonCommercial-NoDerivatives 4.0
International (CC BY-NC-ND 4.0) License.