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Anti-tumour Treatment

The landscape of combining immune checkpoint inhibitors with novel Therapies: Secret alliances against breast cancer

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ABSTRACT

This review focuses on the immune checkpoint inhibitors (ICIs) in the context of breast cancer (BC) management. These innovative treatments, by targeting proteins expressed on both tumor and immune cells, aim to overcome tumor-induced immune suppression and reactivate the immune system. The potential of this approach is the subject of numerous clinical studies. Here, we explore the key studies and emerging therapies related to ICIs providing a detailed analysis of their specific and combined use in BC treatment.

Introduction

Currently, BC is the most prevalent cancer worldwide, with approximately 2.3 million new cases in 2020, representing 12 % of all global cancer cases [1].

BC is a heterogeneous neoplasm with distinct subtypes. Based on the presence or absence of estrogen receptors (ER) and progesterone receptors (PR), and the expression of human epidermal growth factor receptor 2 (HER2), BC subtypes are classified as: hormone-receptor (HR)-positive (HR+; ER+, PR+), HER2-positive (HER2+) and triple-negative BC (TNBC; ER–, PR– and HER2–) [1], with different prognosis and treatment options. Certain types of immune cells, primarily Natural Killer (NK) cells and cytotoxic T cells, can suppress cancer growth [2]. However, cancer cells have developed various mechanisms to evade immune surveillance, managing to escape the control of the immune system cells [3].

A groundbreaking milestone in the field of oncology has been the advent of immunotherapy, aimed at enhancing natural defenses to counteract cancer cells [4]. Initially BC was considered not very responsive to immunotherapies, but technological advancements in immune system studies have led to the application of these treatments in this cancer type as well [5]. There are several categories of

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immunotherapeutic drugs, among which immune checkpoint inhibitors (ICIs) are one of the most studied approaches. The immune checkpoints (ICs) are regulatory inhibitory molecules crucial for ensuring immune tolerance, often exploited by tumors to evade immune surveillance by our immune system [6]. ICIs drugs block inhibitory signals allowing immune cells to attack tumor cells more effectively. The most actively targeted molecules include cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), programmed cell death receptor-1 (PD-1), and programmed cell death ligand-1 (PD-L1) [7–9]. In this review, we explore the efficacy of novel immunotherapeutic strategies for BC, with a particular emphasis on ICIs.

Breast cancer therapy

Breast cancer (BC) treatment is highly complex (Fig. 1), involving surgery, radiotherapy, chemotherapy, targeted therapy, gene therapy, and immunotherapy [1,10,11].

Conventional therapies

Conventional therapies are fundamental in the management of BC, with the aim of eliminating the tumor, preventing its return, and enhancing overall survival [12] (Fig. 1A). Surgery, including lumpectomy or partial mastectomy, and total mastectomy, is typically the primary method for removing tumors, particularly those that are localized and solid [13]. Surgery remains a critical aspect of BC treatment, offering options such as breast-conserving surgery (lumpectomy) or mastectomy, chosen based on factors such as tumor characteristics, patient preferences, and oncological considerations [14]. Lumpectomy involves removing the tumor along with a margin of healthy tissue, preserving the breast, while mastectomy involves complete removal of the breast tissue [15].

Radiotherapy is essential in BC treatment, utilizing high-energy radiation to target and eliminate cancer cells in the breast or surrounding areas. It is used at various stages of BC management, including after surgery (adjuvant radiotherapy) to reduce the risk of local recurrence and as part of definitive therapy for locally advanced or metastatic disease. Adjuvant radiotherapy is typically recommended after lumpectomy to lower the risk of cancer recurrence in the breast, while post-mastectomy radiotherapy is often advised to eradicate any residual cancer cells in the chest wall and regional lymph nodes [16,17].

Chemotherapy plays a crucial role in BC treatment, especially in cases where cancer has spread or is considered high risk. Commonly used chemotherapy agents include anthracyclines like doxorubicin and epirubicin, taxanes such as paclitaxel and docetaxel, methotrexate and fluorouracil in various regimens [18]. Chemotherapy may be given before surgery (neoadjuvant chemotherapy) to reduce tumors, after surgery (adjuvant chemotherapy) to eliminate any remaining cancer cells, or in advanced cases to control the disease and relieve symptoms [19]. Despite associated risks, chemotherapy is essential in preventing recurrence in patients with stage I-III BC. Chemotherapy has been proven effective against TNBC and complements endocrine therapy or HER2-directed treatment for hormone receptor-positive/HER2-negative or HER2-positive BC, respectively [20]. However, these approaches are not very specific and can lead to significant side effects, being frequently associated with persistent fatigue or suboptimal health-related quality of life [21].

Targeted therapy

Targeted therapy (Fig. 1A) is designed to combat specific proteins expressed by tumor cells implicated in the tumorigenic process [22–24]. Notably, among the therapeutic targets are the ER and HER2 receptors [25,26].

Drugs classified as SERMs (Selective Estrogen Receptor Modulators) are employed in hormone-dependent BC therapy due to their ability to act as either agonists or antagonists of the estrogen receptor [27]. Key examples of SERMs include tamoxifen, raloxifene, and lasofoxifene [28,29].

Aromatase Inhibitors (AIs), such as anastrozole, letrozole, and exemestane, operate by inhibiting the conversion of androgens to estrogens in postmenopausal women [30].

In BC treatment, encompassing HER2+ and TNBC subtypes, drugs targeting factors involved in the cell cycle, angiogenesis, and metastasis are used. Some examples of these therapeutic targets comprise HER2, PARP, PI3K/AKT/mammalian target of rapamycin (mTOR), vascular

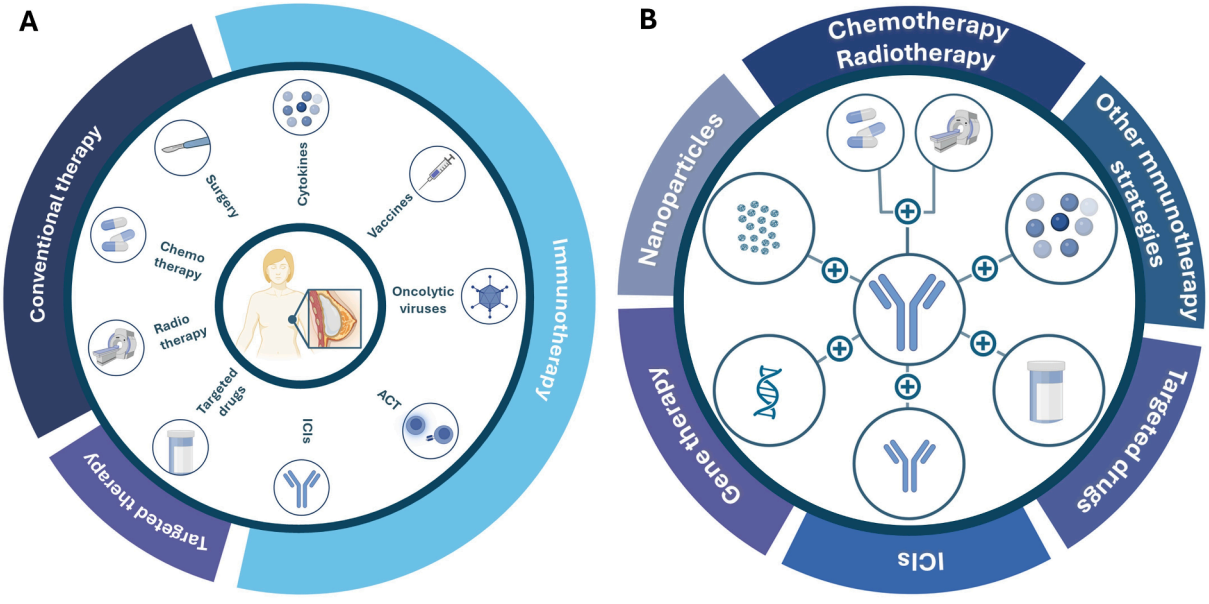

Fig. 1. Different approaches of Breast Cancer treatment. Panel A describes the currently used treatment approaches in BC involving surgical resection, chemotherapy and radiotherapy in addition to immunotherapy and targeted therapy. Panel B describes the novel treatment options and approaches currently being studied or under trial for the treatment of BC. At the center of the figure are monoclonal antibodies, which can be combined with various other therapies. These synergistic combinations aim to improve patient response and the overall efficacy of BC therapy.

endothelial growth factor (VEGF), and fibroblast growth factor receptors (FGFR) [31,32].

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor overexpressed in approximately 20 % of BCs [33]. The first approved monoclonal antibody (mAb) targeting HER2 was Trastuzumab (Herceptin®), which remains a cornerstone drug for treating early-stage and metastatic HER2 positive patients [34].

At the molecular level, it acts by inhibiting HER2 receptor dimerization, internalization, and degradation; moreover, being an IgG1, it also induces Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) [35]. However, treatment with Trastuzumab often leads to resistance, in which are involved mutations (e.g., expression of a truncated form of HER2 lacking the binding site for Trastuzumab), overexpression of mucin 4 (MUC4) that hinders the interaction between Trastuzumab and HER2, and the activation of the PI3K/mTOR pathway [36]. This type of resistance may be overcomed using combination therapies (e.g. Trastuzumab with PI3K/mTOR pathway inhibitors) [37].

Other antibodies, such as Pertuzumab and ertumaxomab, also target HER2 and have demonstrated efficacy in HER2+ BC [38,39].

The combination of Pertuzumab with Trastuzumab is approved for treatment of early-stage and metastatic HER2 positive patients [40,41].

Targeted therapy encompasses PARP inhibitors (PARPi), which specifically target Poly (ADP-ribose) polymerase (PARP), a key player in DNA repair processes activated by DNA strand breaks [42]. Olaparib, niraparib, rucaparib, and talazoparib are among the PARPi approved by the FDA. Their utilization is advised for cancers manifesting impaired homologous recombination repair mechanisms, such as those associated with BRCA1/2 mutations [43].

CDK4/6 are essential for cancer cell proliferation. Their activation by D-type cyclins phosphorylates retinoblastoma protein (Rb), which then activates E2F transcription factors, enabling cell cycle progression and division [44]. Given the frequent upregulation of cyclin D and phosphorylated Rb in HR+ BC, targeting the G1/S checkpoint is a strategic approach to stop cancer cell proliferation. CDK4/6 inhibitors effectively arrest the cell cycle at this checkpoint and are used with hormonal therapies to treat HR+ and HER2− advanced or metastatic BC [45]. FDA-approved CDK4/6 inhibitors include palbociclib, abemaciclib, and ribociclib [46,47].

AKT (Ak strain transforming) plays a central role in the PI3K/AKT/mTOR signaling pathway, driving cell growth and cancer progression [48,49]. This pathway can be activated by different stimuli, while PTEN (phosphatase and tensin homolog) acts as a negative regulator. Tumor cell can be characterized by activation of the PI3K/AKT signaling pathway, either resulting from activating mutations or loss of PTEN inhibition, depending on the subtype of BC [50]. Various AKT inhibitors have been tested in clinical trials, with ATP-competitive inhibitors like capivasertib and ipatasertib showing promise, especially in combination therapies [51].

Angiogenesis, crucial for oxygen supply to tumors, is regulated by various mechanisms in cancer cells, fostering new blood vessel growth and metastasis [52]. In BC, VEGF expression is prominent due to their association with HER2 activation [53]. Angiogenesis inhibitors, like mAbs and tyrosine kinase inhibitors (TKIs) targeting VEGF and VEGFRs, are used in advanced solid tumor therapy [54]. Anti-angiogenesis has good efficacy when combined with standard chemotherapy. Bevacizumab, a mAb against VEGF, is explored for metastatic BC treatment. TKIs (axitinib, cediranib, sunitinib, sorafenib, pazopanib and vandetanib) targeting catalytic function of VEGFR, are also used in BC therapy [55].

Another mAb used in hormone therapy for patients with BC targets the insulin-like growth factor 1 receptor (e.g., figitumumab and cixutumumab) [56].

Moreover, the FGF/FGFR axis represents a potential therapeutic target in BC. FGF/FGFR signaling is vital for regulating key cellular processes in cancer including growth, survival, and migration [57].

Activation occurs when FGFs bind to FGFRs, triggering a cascade of events leading to cell proliferation and survival via the PI3K/AKT and/or RAS/MAPK pathways. Abnormalities in FGF/FGFR are prevalent in BC, often co-occurring with other genetic abnormalities [58,59].

TKIs such as dovitinib, lucitanib, and lenvatinib show promise in clinical trials. However, challenges such as poor patient enrollment have led to the discontinuation of some trials, highlighting the need for further research in this area [23].

In conclusion, conventional BC treatments often cause side effects affecting patients' quality of life. Drug usage limitations include non-specific targeting of tumor antigens and drug resistance [60]. To mitigate these effects, emerging therapies, like immunotherapy, treatments targeting specific tumor molecules and combination of different therapies, showing promise for optimizing BC management [61].

Immunotherapy

In BC, as in many other types of cancer, a primary role is played by the tumor microenvironment (TME), a very complex and constantly evolving environment which includes blood vessels, stromal cells, mesenchymal stem cells (MSCs), tumor-associated macrophages (TAMs), tumor-infiltrating T and B lymphocytes, as well as dendritic cells (DCs) and NKs, and other components that form the extracellular matrix (ECM) [62].

The cells within the TME continuously interact with each other and with tumor cells, producing extracellular matrix complexes and soluble factors, such as cytokines and chemokines, that shape the TME [62,63]. The TME plays an essential role in tumor progression and metastasis, treatment resistance, and recurrence. The interaction between these cells is currently being extensively studied to identify tumor progression pathways and therefore provide better therapeutic targets for BC [62].

Traditionally, patients who respond to ICIs typically exhibit a "hot" (immune-inflamed) phenotype, characterized by immune cell infiltration, while non-responders may display a "cold" (immune-desert/immune-excluded) phenotype, marked by an absence or lower infiltration of immune cells in the tumor [62].

Notably, recent studies indicate that tumors with low immune infiltrate can still significantly respond to ICIs, while tumors with high tumor infiltrating lymphocytes (TILs) may exhibit a poor response to these therapies [64]. Therefore, immune cell infiltration may be necessary but insufficient for ICIs responsiveness, raising questions about how to accurately identify responders to ICIs.

NK cells are innate lymphoid cells that are considered highly effective in antitumor responses, since many tumor cells lack HLA-I molecules rendering T cell response ineffective, whereas NK cells can identify and destroy these cells [65]. Generally, their abundance is associated with increased overall survival (OS) in various cancers [66]. Tumors with high NK infiltration, termed "NK-dominant", could represent an important clinical subgroup. Furthermore, besides direct cytotoxicity, NK cells play a crucial role in shaping both innate immune responses in inflamed peripheral tissues and adaptive immune reactions in secondary lymphoid compartments [67]. In addition, NK cells can share inhibitory receptors with T cells, including PD-1, suggesting possible common targets in ICIs therapies [8,68,69].

BC was previously deemed non-immunogenic, leading to limited exploration of immunotherapeutic strategies. However, recent immunophenotypic analyses have revealed various immune cells with either pro- or anti-tumoral activities within the TME of BC [70]. The presence of TILs in BC samples has underscored the tumor's immunogenic potential. HER2+ and TNBC subtypes exhibit higher TILs counts compared to HR+ subtypes [70], highlighting the variability in immune cell composition. Immunotherapeutic strategies primarily involve ICIs that block suppressive pathways such as PD-1 and CTLA-4. Programmed death-ligand 1 (PD-L1) immunohistochemistry (IHC) expression is the most widely used biomarker, but not sufficient, as it only appears to have predictive value in metastatic TNBC. Tumor mutational burden (TMB) is a marker of tumor foreignness and immunogenicity, as mutated

antigens are recognized by T cells to initiate a cytotoxic response [71,72]. Mutational load is highly variable in BC, and tumors that present high TMB may respond more favorably to ICI [73].

Other approaches under investigation include cytokine treatments, cancer vaccines, and oncolytic viruses, in addition to adoptive cellular therapies.

The expression profile of pro-inflammatory cytokines changes during BC progression. Research on cytokines like IL-2, IFN-α, IFN-β, IFN-γ, IL-6, and IL-12 has shown modest initial effects, but newer strategies involving engineered cytokines and receptors are showing promise [74].

Cancer vaccines include peptide-based, DC-based and nucleic acid-based vaccines. The first category targeting tumor-specific antigens (TSAs) or tumor-associated antigens (TAAs) such as HER2 or Mucin 1 (MUC1), aim to elicit immune responses against tumors [75].

Oncolytic virotherapy uses replication-competent viruses to selectively infect and destroy cancer cells through mechanisms like direct lysis and immune activation. Talimogene laherparepvec (T-VEC), approved by the FDA in 2015 for melanoma [76], is an example, with ongoing trials exploring its use in BC.

Adoptive cell therapy (ACT) involves isolating and modifying immune cells *ex vivo* before reintroducing them into the patient [77]. This includes genetic engineering techniques such as chimeric antigen receptor T cell (CAR-T) therapy and T cell receptor chimeric T cell (TCR-T) therapy. CAR-NK therapy and CAR-M cells are newer approaches, focusing on improving specificity and targeting antigens on BC cells [78].

Additional ACT methods involve expanding and activating immune cells from peripheral blood or tumors *in vitro* before reintroduction. Examples include TILs therapy, cytokine-induced killer (CIK) cell therapy, lymphokine-activated killer (LAK) cell therapy, and NK cell therapy [78–81].

ICIs-based therapy

The introduction of ICIs has revolutionized cancer treatment by targeting key ICs like the PD-1/PD-L1 axis and the CTLA-4–CD28 pathway. PD-1, expressed on T cells, binds to its ligands PD-L1/PD-L2 to maintain immune tolerance and prevent autoimmunity by inducing apoptosis in cells attacking PD-Ls + cells. CTLA-4, competing with CD28 for binding to CD80/CD86 on antigen-presenting cells, acts as an "off" switch, downregulating the immune response. Tumors exploit these mechanisms by upregulating ICs/IC ligands expression to evade immune detection, a phenomenon also observed in BC [11]. ICIs work by releasing this inhibitory control, allowing the immune system to target cancer cells (Fig. 2). Anti-PD-1 and anti-PD-L1 antibodies, as well as mAbs against CTLA-4, are in clinical use for treating various tumors, with ongoing trials evaluating their efficacy [82]. Additionally, recent findings suggest ICIs also affect NK cells, which express inhibitory ICs like NKG2A, KIRs, PD-1, TIM-3, LAG-3, and TIGIT/CD96 [9,83]. Blocking antibodies targeting NK inhibitory receptors are currently under clinical assessment for solid tumors [84].

Anti-PD-1/PD-L1 therapy

Pembrolizumab is an IgG4 mAb that binds to PD-1. Phase I clinical trial KEYNOTE-012 (NCT01848834) showed that Pembrolizumab

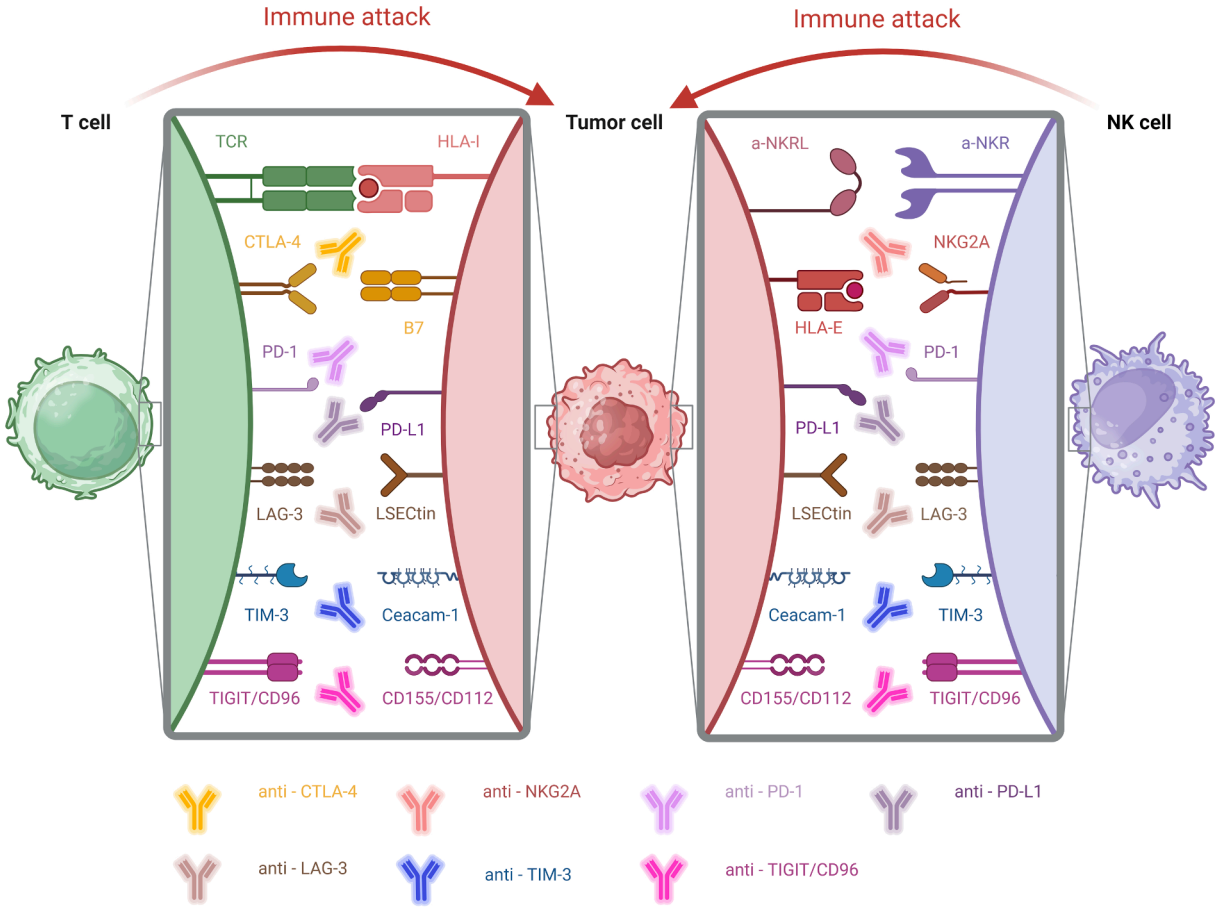

Fig. 2. ICIs permit T and NK activation against tumor cells. How different Immune checkpoint inhibitors (ICIs) can activate T and NK cells by blocking inhibitory receptors and/or their ligands, enabling effective targeting and killing of tumor cells (TCR: T cell receptor; a-NKR: activating NK receptors; a-NKR-L: ligands for a-NKR; LSECtin: LAG-3 ligand; Ceacam-1: TIM-3 ligand; CD155/CD112: TIGIT/CD96 ligands).

monotherapy has a good safety and objective response rate in patients with advanced TNBC [82]. In the Phase II KEYNOTE-086 (NCT02447003) study Pembrolizumab showed a safety profile, durable antitumor activity in a small subset of patients and survival was promising [82]. The assessment of TILs levels could be useful to identify which patients with TNBC might have a higher likelihood of response to Pembrolizumab [82]. An exploratory biomarker analysis revealed that baseline levels of PD-L1, CD8, TILs, and TMB were associated with improved clinical outcomes [85]. The cTRACK-TN trial (NCT03145961) aims to shed light on the benefits of gradually escalating Pembrolizumab treatment for TNBC patients with detectable circulating tumor DNA [82]. Despite these encouraging results, the phase III KEYNOTE-119 (NCT02555657) did not show a significant survival benefit for Pembrolizumab compared to chemotherapy. Only patients with higher PD-L1 expression levels showed a favorable response to Pembrolizumab treatment [82].

Avelumab is a human anti-PD-L1 IgG1 mAb that inhibits the interaction between PD-1 and PD-L1. In the phase I JAVELIN Solid Tumor study (NCT01772004), Avelumab was administered in heavily pretreated patients with metastatic BC, showing a manageable safety profile and modest therapeutic activity. Currently, the Phase III randomized trial A-Brave (NCT02926196) is ongoing to evaluate the efficacy of Avelumab in high-risk TNBC patients. Additionally, Avelumab exhibited cytotoxic effects through NK cells in TNBC patients. This enhancement improved NK cell recognition and cytotoxicity against tumors, boosting immune responses [82].

Atezolizumab is another anti-PD-L1 IgG1 mAb. In a multicenter phase I trial (NCT01375842), Atezolizumab showed clinical benefit and good tolerance in patients with metastatic TNBC [82].

Anti-CTLA-4 therapy

CTLA-4 is an essential regulator of T-cells immune response, maintaining peripheral tolerance binding to CD80 (B7-1) and CD86 (B7-2) expressed on DCs or tumor cells. Binding B7 co-stimulatory molecules, it reduces their interaction with CD28 [86]. Upon T-cell activation, CTLA-4 translocates to the cell surface and inhibits signaling through the CD28 receptor, crucial for T-cell activation and growth. This inhibitory effect is amplified by CTLA-4′s higher binding affinity with B7s compared to CD28. Additionally, CTLA-4 binding blocks IL-2 transcription, essential for T-cell growth [11]. Blocking CTLA-4 with mAb may enhance T-cell response against cancer cells.

In a pilot study (NCT01502592), the effectiveness of Ipilimumab, an IgG1 anti-CTLA-4 mAb, was tested in women with early-stage BC. Ipilimumab monotherapy or Ipilimumab in combination with cryoablation in BC patients showed an acceptable safety profile [82].

Other ICIs

LAG-3 is an emerging inhibitory receptor highly expressed in regulatory and activated T-cells, and NK cells. LAG-3 inhibitors can also suppress Tregs while enhancing the activity of effector T-cells [87]. LAG-3 shows a simultaneous or parallel upregulation with PD-1 on TILs, and it could drive primary resistance in certain T cell-infiltrated tumors [88]. The FDA has recently approved the LAG-3 inhibitor Relatlimab for use in combination with Nivolumab for treating unresectable or metastatic melanoma [89].

TIM-3 induces immunosuppression of T and NK cells function, thereby enhancing the immunosuppressive environment by modulating T-cell proliferation and cytokine production [90]. Different clinical trials are ongoing to target TIM-3 in patients with advanced solid tumors [91].

ICIs targeting NK cell inhibitory receptors, which could enhance the activation and cytotoxic functions of NK cells, have emerged as a promising strategy in cancer immunotherapy.

NK and T cells share several ICs, including the classical NK-ICs NKG2A and KIRs [69]. NKG2A, present on NK cells and a subset of CD8 T cells [68], binds to the non-classical HLA-E molecule, thereby inhibiting immune-cell function [68,92].

Given that HLA-E molecules are frequently overexpressed on human tumor cells, using an anti-NKG2A antibody could enhance NK cell cytotoxicity against these tumors [68]. Monalizumab, for instance, binds to NKG2A, blocking the NKG2A-HLA-E axis and restoring NK cell activity, thereby mimicking the 'missing-self' response.

TIGIT (T cell immunoreceptor with immunoglobulin and ITIM domain) is a recently identified checkpoint expressed on NK cells, T cells, Tregs, and TILs, and it contributes to increased levels of immunosuppressive cytokines. Blocking TIGIT has been shown to trigger a robust NK cell-dependent, tumor-specific T cell response [69]. Currently, two distinct mAbs targeting TIGIT are in phase I clinical trials in breast cancer patients (NCT03628677 and NCT03119428) (Table 1).

ICIs resistance

Despite the significant clinical benefits observed from anti-CTLA-4 and anti-PD-1/PD-L1 antibody therapies in certain patients, a considerable number of patients still fail to respond to these treatments. The efficacy of ICIs immunotherapy relies on the infiltration of TILs into TME, and the initiation of an inflammatory response characterized by the production of IFN-γ. Tumors with this phenotype, known as T cell-inflamed, typically exhibit high PD-L1 levels [93]. TNBC with elevated PD-L1 expression, increased TMB, and higher levels of TILs has been shown to benefit from ICIs therapy [94]. ICIs treatment alongside first-line chemotherapy has enhanced survival in advanced TNBC patients expressing PD-L1. However, most patients either fail to respond to or ultimately develop resistance to ICIs that inhibit the PD-1/PD-L1 pathway leading to poor outcomes. ICIs have shown limited effectiveness in PD-L1-negative TNBC and other BC subtypes, highlighting the need for a deeper understanding of ICIs resistance in BC. ICIs activity and resistance in BC are closely linked to the tumor's intrinsic immunophenotype. Biomarkers more robust than PD-L1 expression are utterly needed. Although there are currently insufficient studies explaining the effect of MSCs on BC treatment with ICIs, evidence suggests that MSCs may nevertheless influence the expression of PD-1/PD-L1 in BC cells [95]. Conversely, tumors evade T cell-mediated immunity by reducing the expression of HLA-I molecules; these HLA-I-deficient tumor cells are not targeted by T cells, but they remain susceptible to NK cell attacks [96]. In this context, NK cell-targeted immunotherapy emerges as a promising alternative or complementary strategy to overcome the limitations of T cell immunotherapy. Future approaches could benefit from both treatments to enhance therapeutic efficacy. Furthermore, drug

Table 1
Clinical Trials in Breast Cancer: ICIs monotherapy and combination of ICIs.

ICIs monotherapy			
Treatment	**Target**	**Trial**	**BC Subtype**
Pembrolizumab	PD-1	NCT01848834	Mestastatic TNBC
	PD-1	NCT02447003	Advanced TNBC
	PD-1	NCT02555657	Metastatic TNBC
Avelumab	PD-L1	NCT02926196	High-risk TNBC
	PD-L1	NCT02926196	High-risk TNBC
Atezolizumab	PD-L1	NCT01375842	Advanced TNBC
Ipilimumab	CTLA-4	NCT01502592	Early-stage/ resectable BC
Combination of ICIs			
Treatment	**Target**	**Trial**	**BC Subtype**
Nivolumab and Ipilumab	PD-1 and CTLA-4	NCT02834013	Metaplastic breast cancer
Durvalumab and Tremelimumab	PD-L1 and CTLA-4	NCT02536794	Metastatic ER+, TNBC
Cemiplimab and Fanlimab	PD-1 and LAG-3	NCT01042379	BC
Spartalizumab and Ieramilimab	PD-1 and LAG-3	NCT02460224	TNBC

combinations are under evaluation to overcome immuno-resistance, showing encouraging results in early-phase studies. Tailored combination strategies based on patient and tumor immunophenotype hold promise for overcoming resistance and maximizing the potential of ICIs [97] (Fig. 1B).

Combination of ICIs with other treatments

ICIs with chemo- or radio- therapy

Chemotherapy and radiotherapy contribute to modify TILs and Tregs infiltrate, providing rationale for combining them with ICIs [98] (Table 2).

In the phase III KEYNOTE-355 trial (NCT02819518), the combination of Pembrolizumab and standard chemotherapy led to a greater progression-free survival compared to chemotherapy alone in advanced TNBC patients with PD-L1 combined positive score (CPS) $\geq$ 10 [82]. Similar results were obtained in the neoadjuvant setting in the KEYNOTE-173 trial (NCT02622074), phase III trial KEYNOTE-522 (NCT03036488), and phase II trial I-SPY2 (NCT01042379) in HR+HER2+ [82]. The efficacy of treatment in early-stage disease was independent of PD-L1 expression [99].

Table 2
Clinical Trials in Breast Cancer: Combination of ICIs with chemo- and radiotherapy.

Combination of ICIs with chemo- and radio- therapy			
Treatment	**Target**	**Trial**	**BC Subtype**
Pembrolizumab with nab-paclitaxel or paclitaxel or Gemcitabine/carboplatin	PD-1	NCT02819518	Metastatic TNBC
Neoadjuvant pembrolizumab with chemotherapy combination	PD-1	NCT02622074	Advanced and metastatic TNBC
Neoadjuvant pembrolizumab with paclitaxel-carboplatin and adjuvant pembrolizumab	PD-1	NCT03036488	Advanced and metastatic TNBC
Neoadjuvant pembrolizumab with standard chemotherapy	PD-1	NCT01042379	High-risk clinical stage II or III BC
Pembrolizumab with eribulin	PD-1	NCT02513472	Metastatic TNBC
Pembrolizumab with eribulin	PD-1	NCT03222856	Metastatic or recurrent HR+HER2-
Pembrolizumab with neoadjuvant nab-paclitaxel	PD-1	NCT03289819	TNBC
Pembrolizumab with radiotherapy	PD-1	NCT02730130	TNBC
Cemiplimab with neoadjuvant chemotherapy	PD-1	NCT04243616	High-risk or progressive HR+HER2-, TNBC
Nivolumab following chemo- or radio- therapy	PD-1	NCT02499367	Metastatic TNBC
Toripalimab with nab-paclitaxel	PD-1	NCT04085276	Metastatic or recurrent TNBC
Atezolizumab with nab-paclitaxel and anthracycline	PD-L1	NCT03197935	Early-stage TNBC
Atezolizumab with nab-paclitaxel	PD-L1	NCT 02425891	Unresectable locally advanced or metastatic TNBC
Atezolizumab with carboplatin and nab-paclitaxel	PD-L1	NCT002620280	Advanced or metastatic TNBC
Avelumab with liposomal doxorubicin	PD-L1	NCT03971409	Unresectable and recurrent TNBC
Durvalumab with neoadjuvant anthracycline or taxane	PD-L1	NCT02685059	Early-stage TNBC
Durvalumab and olaparib with neoadjuvant paclitaxel	PD-L1	NCT01042379	BC
IMP321 with paclitaxel	LAG-3	NCT00349934	Metastatic BC

Combination of Pembrolizumab with eribulin has been investigated in different trials, with promising therapeutic efficacy in metastatic TNBC patients (NCT02513472) [82] and in metastatic or recurrent HR+ HER2− BC patients (NCT03222856) [82]; on the contrary, it did not show promising clinical outcomes in metastatic HR+HER2− BC patients [100].

The NeoImmunoboost trial (NCT03289819) investigated the combination of neoadjuvant nab-paclitaxel chemotherapy with Pembrolizumab, showing favorable outcomes [101].

Pembrolizumab combined with radiotherapy was both safe and effective for metastatic TNBC patients in a phase II trial (NCT02730130) [102].

The combination of neoadjuvant chemotherapy and Cemiplimab (anti-PD1) is under investigation in a phase II trial (NCT04243616) [82] in TNBC and high-risk or progressive HR+ HER2− disease.

In the TONIC phase II study (NCT02499367) [82] chemotherapy or radiotherapy followed by maintenance Nivolumab (anti PD-1) exhibited therapeutic efficacy in metastatic TNBC patients, higher than expected in metastatic TNBC. These data indicate immunoregulatory properties by chemotherapeutics in developing a TME favorable for PD-1 blockers.

The humanized PD-1 antibody Toripalimab, demonstrated significant antitumor activity as a monotherapy in patients undergoing salvage treatment for advanced TNBC [103]. The TORCHLIGHT evaluated the combination with nab-paclitaxel versus placebo, showing a reduction in disease progression and mortality in patients with metastatic or recurrent TNBC [104].

According to the IMpassion130 trial (NCT 02425891) [82], the combination of Atezolizumab with paclitaxel is approved in first line PD-L1-positive TNBC patients. In early stage TNBC, the phase III trial IMpassion031 (NCT03197935) [82], the combination of Atezolizumab with chemotherapy, significantly improved complete response rates with a safety profile, regardless of PD-L1 expression [105]. However, the results of the phase III NeoTRIPaPDL1 clinical trial (NCT002620280) did not confirm these data.

Similar clinical studies have shown variable results, which may be related with different methods of PD-L1 detection, different clinical protocols, and different clinical characteristics of the patients enrolled in the study [11].

Given the divergent results of the PD-1/PD-L1 blockade plus chemotherapy-based trials for the first-line treatment of TNBC [105–108], such as those conducted in IMpassion031, KEYNOTE-355, and IMpassion130, additional clinical research is essential to pinpoint the optimal chemo-immunotherapy combinations and to elucidate the associated tumor biology for the treatment of metastatic TNBC. While these trials have demonstrated the potential of combining ICIs with chemotherapy, they have also highlighted variability in efficacy, patient response, and survival outcomes. For instance, the IMpassion130 trial showed significant improvement in progression-free survival with atezolizumab plus nab-paclitaxel, particularly in PD-L1-positive patients, whereas the KEYNOTE-355 trial found pembrolizumab plus chemotherapy to be effective but with variable outcomes depending on PD-L1 expression levels. The IMpassion031 trial, focusing on neoadjuvant therapy, demonstrated the potential for increased pathologic complete response rates, yet questions remain about long-term benefits and optimal patient selection. Thus, a comprehensive understanding of these strengths and limitations is crucial for advancing therapeutic strategies in TNBC [105–108].

The phase II trial NCT03971409 is investigating the clinical effectiveness of Avelumab combined with liposomal doxorubicin with or without the MEK inhibitor binimetinib, or Avelumab combined with the anti-TROP-2 Sacituzumab govitecan, in advanced TNBC [82].

Durvalumab, when combined with various chemotherapy regimens, showed clinical benefits in BC patients. In the GeparNuevo phase II trial (NCT02685059), Durvalumab combined with anthracycline- or taxane-based neoadjuvant chemotherapy slightly improved pathological

complete response in TNBC patients. Notably, the treatment was effective despite the absence of Durvalumab maintenance [82].

Combining LAG-3Ig fusion protein, IMP321, with paclitaxel (NCT00349934) showed an improved response via activation of APCs, CD8 + T and NK cells in metastatic BC patients [82].

ICIs with targeted therapy

Several studies have evaluated ICIs therapy combined with targeted therapy (Table 3).

Combination of PARP-I (niraparib or talazoparib) with ICIs (respectively Pembrolizumab or Avelumab) in two distinct studies (NCT02657889; NCT03330405) showed an improved response in advanced or metastatic TNBC [82]. Antitumor activity was observed in some patients with BRCA1/2-associated BC when treated with Avelumab plus talazoparib [109].

In a phase I/II trial (NCT02734004) Durvalumab combined with PARPi, Olaparib, showed a safety antitumor effect in metastatic BC patients [82].

The recent NEWFLAME trial evaluated the efficacy and safety of Nivolumab in combination with the CDK4/6 inhibitor abemaciclib, but although this combination demonstrated activity, it also resulted in severe and prolonged immune-related adverse effects [110,111].

Atezolizumab used in combination with the AKT inhibitor, Ipatasertib, and Paclitaxel/Nab-paclitaxel demonstrated efficacy in advanced and metastatic TNBC (NCT03800836) [82].

Anti-VEGF drugs exhibit dosage-dependent immunostimulatory effects. Low doses of antiangiogenetics can enhance anti-tumor immunity. Targeting VEGFR can increase immune cell infiltration, leading to osteopontin (OPN) secretion by CD8+ T cells and macrophages. OPN, in turn, promotes TGF-β production, upregulating PD-1 expression on immune cells [112].

Combination of Camrelizumab (anti PD-1) with low-dose VEGFR inhibitor apatinib showed benefit in advanced TNBC patients, especially PD-L1 negative tumors or those treated with chemotherapy for a prolonged period (NCT03394287) [82]. The combination of Camrelizumab, apatinib and eribulin exhibits promising efficacy and a good safety profile in patients with heavily pretreated advanced TNBC (NCT04303741) [82]. The phase II FUTURE-C-Plus trial (NCT04129996) evaluated the combination of Camrelizumab, famitinib (antiangiogenetic), and chemotherapy in advanced TNBC. The trial confirmed the efficacy and safety of this triplet regimen and highlighted the potential of using CD8, PD-L1, and somatic mutations to guide clinical decision-making and treatment strategies [82].

Conversely, in another phase II trial (NCT03316586), cabozantinib plus Nivolumab did not show any encouraging clinical outcomes in patients with metastatic TNBC [82].

Encouraging data for hormone therapy combinations come from a phase I, where Tremelimumab in combination with exemestane enhanced co-stimulators induces activation on CD8+ and CD4+ T-cells [113].

Monalizumab (anti-NKG2A), when combined with anti-EGFR, was found to enhance Cetuximab's ADCC capability [68].

Combining anti-PD1/PD-L1 with an anti-HER2 agent revealed promising responses in HER2+ patients with positivity for PD-L1 (PANACEA trial NCT02129556 and NCT02924883) [82]. In another study (NCT03726879) [82], the lack of the same responses could depend on low TILs levels, suggesting that the immune escape mechanism in HER2+ BC involves intricate immune interactions, necessitating the exploration of alternative immunomodulatory strategies.

The novel combination of Monalizumab and Trastuzumab (anti-HER2) in the MIMOSA-trial (NCT04307329) did not induce objective responses in heavily pre-treated HER2+ metastatic BC patients. The lack of responders could be due to the enrollment of patients with unfavorable immunological characteristics [82].

ICIs with other ICIs or alternative immunotherapy strategies

Literature suggests that combining different ICIs may improve response rates compared to monotherapy [114] (Table 1).

For example, the combination of Nivolumab (anti-PD-1 mAb) with Ipilimumab in a phase II trial (NCT02834013) showed promising results in patients with refractory metastatic BC, a rare and aggressive subtype that responds poorly to chemotherapy. This suggests that targeting both adaptive (T-cell mediated) and innate (NK cell-mediated) immune pathways may yield better outcomes in difficult-to-treat cancers [82].

Dual blockade with Durvalumab (anti-PD-L1 mAb) and Tremelimumab (a humanized IgG2 mAb targeting CTLA-4) further supports this idea, as it was effective in activating and expanding T cells (NCT02536794). These inhibitors may influence B/T cell crosstalk, leading plasma cells to produce specific antibodies within the TME. While negative outcomes were observed in the general population, patients with TNBC showed better responses, particularly with Durvalumab monotherapy. This highlights the potential for combining Durvalumab with Tremelimumab to be especially beneficial for TNBC patients, as it leverages both adaptive and innate immune responses to enhance efficacy [82].

Additionally, the phase II I-SPY2 trial (NCT01042379) demonstrated that the concurrent administration of Cemiplimab (an anti-PD-1 IgG4) and Fanlimab (a LAG-3 inhibitor) with paclitaxel significantly increased the rate of pathologic complete response compared to paclitaxel monotherapy in patients with HR+ and HER2− BC or TNBC. This further underscores the advantage of combining different ICIs to target multiple immune pathways simultaneously [82].

Moreover, a recent study described the immune profile of a complete tumor response in a patient with metastatic TNBC undergoing dual blockade of PD-1 and LAG-3 receptors within the CLAG525X2101C

Table 3
Clinical Trials in Breast Cancer: Combination of ICIs with targeted therapy and other immunotherapy strategies.

Combination of ICIs with targeted therapy			
Treatment	**Target**	**Trial**	**BC Subtype**
PARP inhibitors			
Pembrolizumab with Niraparib	PD-1	NCT02657889	Advanced or metastatic TNBC
Nivolumab with Cabozantinib	PD-1	NCT03316586	Metastatic TNBC
Avelumab with Talazoparib	PD-L1	NCT03330405	Advanced TNBC
Durvalumab with Olaparib	PDL-1	NCT02734004	Metastatic BC
CDK4/6 inhibitors			
Nivolumab with Abemaciclib	PD-1	UMIN000036970	Metastatic HR+HER2- BC
AKT inhibitors			
Atezolizumab with Ipatasertib and Paclitaxel/Nab-paclitaxel	PD-L1	NCT03800836	Metastatic TNBC
Angiogenesis inhibitors			
Camrelizumab with low dose of Apatinib	PD-1	NCT03394287	Advanced TNBC
Camrelizumab with Apatinib and Eribulin	PD-1	NCT04303741	Advanced TNBC
Camrelizumab with Famitinib and chemotherapy	PD-1	NCT04129996	Advanced TNBC
Anti-HER2 agents			
Pembrolizumab with Trastuzumab	PD-1	NCT02129556	Advanced HER2 + BC
Atezolizumab with Trastuzumab	PD-L1	NCT02924883	Metastatic HER2 + BC
Atezolizumab with Trastuzumab and chemotherapy	PD-L1	NCT03726879	Early-stage HER2 + BC
Monalizumab and Trastuzumab	NKG2A	NCT04307329	Metastatic HER2 + BC
Combination of ICIs with other immunotherapy strategies			
Treatment	**Target**	**Trial**	**BC Subtype**
Nivolumab with IL-2	PD-1	NCT03435640	TNBC
Avelumab with NK cells	PD-L1	NCT03387085	TNBC

phase I/II trial (NCT02460224). The combination of these inhibitors may enhance the immune system's ability to eliminate cancer cells by simultaneously targeting different immune evasion mechanisms employed by the tumor [82].

The combination of anti-NKG2A Monalizumab with anti-PD1 [115] or anti-PDL1 [116] mAbs also demonstrates the potential of this approach. Monalizumab enhanced NK cell effector functions, and its combination with PD-L1 blockade showed synergistic effects in patients with advanced non-small-cell lung cancer (NSCLC) [117] In TNBC models, dual blockade of NKG2A and PD-L1 effectively inhibited in vivo tumor growth, suggesting that this strategy could also be effective in treating TNBC [118] by enhancing both adaptive and innate immune responses.

Finally, anti-TIGIT/CD69 antibodies (Vibostolimab or Etigilimab), either alone or combined with anti-PD-1, have shown promising efficacy in refractory solid tumors [119,120], including TNBC. The combination of Tiragolumab, an anti-TIGIT mAb, with the anti-PD-L1 antibody Atezolizumab demonstrated a significant advantage over Atezolizumab monotherapy [121].

These findings suggest that targeting both adaptive and innate immunity signals through combination therapy could represent a powerful strategy for improving outcomes in TNBC and other challenging cancers.

Some studies are trying to combine ICIs with other immunotherapy strategies (Table 3).

The administration of ICIs and IL-2 in BC patients (NCT03435640) showed a limited efficacy, probably due to compensatory immunosuppressive pathways, including elevated expression of ICs and release of inhibitory cytokines, like IL-10 and TGF-β, that halt the anti-tumor response [82].

The QUILT-3.067 (NCT03387085) trial has investigated the combined use of NK cells and ICIs in patients with metastatic, refractory, or unresectable TNBC. This innovative study integrates Avelumab with IL-15 cytokine administration, NK cell therapy, cancer vaccines, and chemoradiation to activate both innate and adaptive immune responses [82].

Atezolizumab significantly enhances the inhibitory effect of FAK (focal adhesion kinase) inhibitors on cancer cells [122]. The combined administration of FAK inhibitors with CIK cells in TNBC patients, increased cellular mortality rates, implying that FAK amplifies the vulnerability of tumor cells to CIK cells [123]. Hence, the incorporation of CIK cell therapy with PD-L1 and FAK inhibitors presents a promising and potentially effective innovative treatment for TNBC patients.

ICIs with gene therapy

A fusion protein Ad.sT, generated by combining an adenovirus with a TGF-β receptor II IgG Fc fragment (sTGFβ RIIFc), which hampers TGF β-1 binding, has the potential to enhance the levels of CD4+ and CD8+ lymphocytes in peripheral blood, thereby augmenting the immunotherapeutic efficacy of ICIs. The co-administration of anti-PD-1 and anti-CTLA-4 enhances the effectiveness of Ad.sT in suppressing tumor metastasis and proliferation [124,125].

A study evaluated the administration of Adenovirus-delivered Herpes Simplex Virus (HSV) thymidine kinase into metastatic TNBC tumors. The injection site underwent stereotactic body radiotherapy (SBRT), after which patients were treated with Pembrolizumab. Combining SBRT with HSV thymidine kinase gene therapy before Pembrolizumab administration has the potential to enhance the therapeutic effectiveness of ICIs therapy [126].

ICIs with recombinant nanoparticles

Some studies demonstrated that combining nanotechnology with immunotherapy ensures targeted drug delivery and stability via enhancing drug uptake and biocompatibility.

The integration of nanotechnology with anti-PD-1 demonstrated potent inhibition of tumor growth, leading to increased infiltration of CD4+ and CD8+ T cells in primary tumors. This approach also enhances immunogenic cell death and the release of new antigens, effectively boosting the anti-tumor efficacy of anti-PD-1 [127,128].

ICIs in the contest of bi- or tri- specific antibodies

A bispecific antibody (BsAb) is an artificially engineered antibody capable of simultaneously binding two antigens or two different epitopes, acting as a bridge between them. A BsAb can block different signaling pathways to enhance the synergistic effect against cell tumors replication, improve T cells or NK cytotoxicity by their recruitment and, binding different antigens or epitopes, potentiating its specificity to cancer cells.

F7AK3 is a BsAb engineered to bind to the trophoblast cell surface antigen 2 (TROP2) and CD3 on TNBC cell lines and primary tumor cells, thereby facilitating T cell recruitment and inhibiting tumor growth in xenograft models [129]. F7AK3, used as monotherapy or in combination with ICIs, holds potential for advanced TNBC immunotherapy.

MesobsFab, a Fab-like format, targets mesothelin and CD16, promoting the recruitment and infiltration of NK cells into tumor spheres, resulting in potent, dose-dependent cell-mediated cytotoxicity in mesothelin-positive TNBC [130].

BiTP, another type of BsAb, was designed to target TGF-β and human PD-L1 using the Check-BODY platform. The introduction of BiTP in the humanized TNBC model led to increased infiltration of TILs, CD8+ T cells, and NK cells, thereby reshaping the TME [131]. The interaction between the Fc domain of certain BsAbs and its receptors or complements may initiate ADCC, resulting in nonspecific immune responses.

T-cells can be activated by engaging anti-CD3 and anti-4-1BB nanobodies in the bispecific (Bi-specific T-cell Engagers – BiTEs) and trispecific (Tri-specific T-cell Engagers – TriTEs) modalities [132]. BiTEs, a subtype of BsAbs, lacks the Fc domain, thereby avoiding cytotoxicity. BiTEs demonstrated enhanced tissue permeability, improved efficiency in eliminating tumor cells, required lower doses, and generated more potent therapeutic effects [133].

An anti-PD-L1/TGFβRII bifunctional antibody (M7824) is undergoing clinical assessment either alone in stage II–IIIm HER2+ BC (NCT03620201) or in combination with radiation (NCT03524170), eribulin (NCT03579472), or a brachyury-targeting virus-based vaccine plus Trastuzumab emtansine or the class I HDAC inhibitor entinostat in TNBC patients (NCT04296942). A phase II trial (NCT03872791) seeks to assess the effectiveness and safety of KN046, a bispecific antibody targeting PD-L1 and CTLA-4, in combination with nab-paclitaxel as the initial treatment for individuals diagnosed with metastatic TNBC [82].

In a phase I study on the bispecific PD-1 × LAG-3 molecule Tebotelimab, both as a monotherapy and combined with the anti-HER2 Margetuximab, it was found that Margetuximab-induced IFN-γ targets PD-L1 and LAG-3 in various immune cell subsets, including NK cells. Combining Tebotelimab with Margetuximab enhanced ADCC *in vitro*. Preliminary results showed that this combination produced strong responses in patients with HER2+ refractory and PD-L1-low/LAG-3-low tumors, who would not typically respond to either therapy alone. In HER2+ BC, approximately 20% of PD-L1 – tumors responded, especially patients previously treated with anti-HER2 therapy [134]. This compares favorably to a similar cohort treated with Trastuzumab plus Pembrolizumab, where no responses were seen in PD-L1– tumors [135].

The antitumor activity of endogenous NK cells can be stimulated by the administration of Bispecific and Trispecific Killer Cell Engagers (BiKEs and TriKEs, respectively), composed of antibodies targeting CD16 or NKG2D and one or two tumor antigens [136]. TriKEs can also be engineered to support NK cell proliferation in vivo by incorporating a modified IL-15 cross-linker [137]. BiKEs and TriKEs specific for HER2 or epithelial cell adhesion molecule (EpCAM) were developed for BC [136].

ICIs and aptamers

The use of aptamers as innovative therapeutic agents in various human cancers, including TNBC, is rapidly growing. Aptamers are highly selective, synthetic, short, single-stranded DNA or RNA oligonucleotides that specifically bind to a variety of targets. They offer several advantages compared to antibodies, such as reduced immunogenicity, enhanced tissue penetration due to their smaller molecular size, simpler production procedures, increased stability, decreased toxicity, and reduced costs [138].

As antagonistic therapy, aptamers block pathways that promote tumor growth binding to surface targets on cancer cells. In the contest of targeted drug delivery, they facilitate selective intracellular drug delivery, while, in gene therapy, are utilized to decorate nanoparticles carrying small interfering RNAs (siRNAs) or are directly conjugated to siRNAs. This enables them to bind to cell surface targets and internalize into cancer cells, leading to selective gene silencing.

In immunotherapy, aptamers serve to stimulate immune cells against cancer cells [139].

The Platelet-derived growth factor receptor β (PDGFRβ) is a tyrosine kinase receptor present on the surface of tumor cells within a subset of TNBC with a mesenchymal phenotype, thus promoting metastatic potential [138]. In a study by Camorani *et al.*, the Gint4.T aptamer, an antagonist of PDGFRβ, enhanced the efficacy of anti-PD-L1 in both human and murine TNBC cell cultures and in a well-established murine model of TNBC. PDGFRβ blockade directly inhibits tumor growth and metastasis formation by acting on tumor cells and enhances tumor immunity, potentially synergizing with anti-PD-L1 to exert anti-tumor effects [140].

Innovations in ICIs-based therapy

Many open questions remain on combination therapies with ICIs including immune-related adverse events (irAEs), low response rates and time to first response, and the absence of optimal strategies for combinatorial cancer immunotherapy.

Targeted delivery of ICIs using nanoparticles (NPs) has shown feasibility and efficacy [141,142]. Additionally, nanotechnology can remodel the TME [143,144] and has the potential to overcome delivery challenges and facilitate the advancement of NK cell-based cancer immunotherapies [145].

NPs can conjugate or encapsulate ICIs, including antibodies, RNAs, and small molecule inhibitors [141,142].

Various studies investigated the impact of siRNA-mediated knockdown of PD-1, PD-L1, and CTLA-4 on enhancing antitumor immune responses in BC models. These studies demonstrate that silencing these ICs can significantly improve the efficacy of immunotherapy by boosting the ability of immune cells to target and destroy cancer cells [140,146,147].

Co-administration of PD-L1 siRNA could potentially counteract the upregulation of PD-L1 preventing the associated adaptive resistance and yielding more effective cancer treatment outcomes [148].

Several nanocarriers represent an improvement in siRNA delivery systems into tumor region and TILs as they appear to overcome issues related to this treatment, such as vulnerability to enzymatic degradation, inadequate pharmacokinetics, and possible unintended target interactions [143,148,149].

Therefore, the combination of novel delivery systems and ICIs-based therapies, including siRNA to downregulate the inhibitory ICs, has the potential to enhance drug delivery precision and efficiency, improve tumor cell targeting, and overcome existing limitations of ICIs therapy.

Conclusions

The development of ICIs by Nobel laureates Allison and Honjo has revolutionized cancer treatment by harnessing the immune system to target tumors. While ICIs have shown remarkable success in treating melanoma, lung cancer, and hematologic malignancies, their impact on BC remains limited, benefiting only a subset of patients, with secondary resistance posing a significant challenge.

ICIs work by enhancing the immune system's ability to recognize and attack cancer cells, specifically by targeting molecules that inhibit T lymphocytes and NK cells. Their effectiveness is closely tied to the presence of TILs and the overall inflammatory response. TNBC, characterized by high PD-L1 expression and increased TILs, appears to be particularly promising for ICI therapy.

A deeper understanding of the molecular and immune landscape of BC is essential for the development of more effective therapies. Combining ICIs with chemotherapy has shown potential for improving outcomes, emphasizing the importance of combination strategies. This review highlights the promise of various combinatorial approaches, including chemotherapy, radiotherapy, and cellular therapies, some of which have already gained FDA approval following successful clinical trials.

Looking forward, the future of immunotherapy in BC should broaden its scope to include not only ICIs but also NK cells and emerging technologies such as aptamers, siRNA, nanotechnology, and exosomes. These innovations hold the potential to further enhance treatment efficacy and improve patient outcomes, representing a promising direction for future research and clinical application.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Gennari A, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer ☆. Ann Oncol 2021;32.
[2] Di Vito, C. *et al.* NK cells to cure cancer. *Seminars in Immunology* vol. 41 Preprint at Doi: 10.1016/j.smim.2019.03.004 (2019).
[3] Thomas DA, Massagué J. TGF-β directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. Cancer Cell 2005;8.
[4] Hoos A, Britten CM. The immuno-oncology framework enabling a new era of cancer therapy a new era of cancer therapy. Oncoimmunology 2012;1.
[5] Thomas A, et al. Tumor mutational burden is a determinant of immune-mediated survival in breast cancer. Oncoimmunology 2018;7.
[6] Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews Cancer* vol. 12 Preprint at Doi: 10.1038/nrc3239 (2012).
[7] Sharma, P. & Allison, J. P. Immune checkpoint targeting in cancer therapy: Toward combination strategies with curative potential. *Cell* vol. 161 Preprint at Doi: 10.1016/j.cell.2015.03.030 (2015).
[8] Pesce S, et al. Identification of a subset of human natural killer cells expressing high levels of programmed death 1: A phenotypic and functional characterization. J Allergy Clin Immunol 2017;139.
[9] Pesce, S. *et al.* Cancer immunotherapy by blocking immune checkpoints on innate lymphocytes. *Cancers* vol. 12 Preprint at Doi: 10.3390/cancers12123504 (2020).
[10] Loibl S, et al. Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up ☆. Ann Oncol 2024;35.
[11] Liu, Y. *et al.* Advances in immunotherapy for triple-negative breast cancer. *Molecular Cancer* vol. 22 Preprint at Doi: 10.1186/s12943-023-01850-7 (2023).
[12] Costa, B., Amorim, I., Gärtner, F. & Vale, N. Understanding Breast cancer: from conventional therapies to repurposed drugs. *European Journal of Pharmaceutical Sciences* vol. 151 Preprint at Doi: 10.1016/j.ejps.2020.105401 (2020).
[13] Moo, T. A., Sanford, R., Dang, C. & Morrow, M. Overview of Breast Cancer Therapy. *PET Clinics* vol. 13 Preprint at Doi: 10.1016/j.cpet.2018.02.006 (2018).

[14] Rapisarda F, et al. Sentinel lymph node biopsy in older patients with breast cancer: Which subset can be omitted? Eur J Surg Oncol 2023;49.
[15] Christiansen P, Mele M, Bodilsen A, Rocco N, Zachariae R. Breast-Conserving Surgery or Mastectomy?: Impact on Survival. Ann Surg Open 2022;3:e205.
[16] Corradini S, et al. Adjuvant radiotherapy after breast conserving surgery - A comparative effectiveness research study. Radiother Oncol 2015;114.
[17] Hennequin C, et al. Radiotherapy of breast cancer. Cancer Radiother 2022;26: 221–30.
[18] Shen SJ, Liu CM. Chemotherapy for early-stage breast cancer: the more the better? Lancet 2023;401:1243–5.
[19] Verrill M. Chemotherapy for early-stage breast cancer: A brief history. Br J Cancer 2009;101.
[20] Waks AG, Winer EP. Breast Cancer Treatment: A Review. JAMA 2019;321: 288–300.
[21] Cooper L, et al. Identifying pre-habilitation targets for the mitigation of long-term side effects of chemotherapy in patients with early breast cancer. Support Care Cancer 2024;32:1–11.
[22] Baudino T. Targeted Cancer Therapy: The Next Generation of Cancer Treatment. Curr Drug Discov Technol 2015;12.
[23] Ye, F. *et al.* Advancements in clinical aspects of targeted therapy and immunotherapy in breast cancer. *Molecular Cancer* vol. 22 Preprint at Doi: 10.1186/s12943-023-01805-y (2023).
[24] Jordan, V. C. & Brodie, A. M. H. Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer. *Steroids* vol. 72 Preprint at Doi: 10.1016/j.steroids.2006.10.009 (2007).
[25] Shastry, M. & Hamilton, E. Novel Estrogen Receptor-Targeted Agents for Breast Cancer. *Current Treatment Options in Oncology* vol. 24 Preprint at Doi: 10.1007/s11864-023-01079-y (2023).
[26] Patel, R., Klein, P., Tiersten, A. & Sparano, J. A. An emerging generation of endocrine therapies in breast cancer: a clinical perspective. *npj Breast Cancer* vol. 9 Preprint at Doi: 10.1038/s41523-023-00523-4 (2023).
[27] Patel, H. K. & Bihani, T. Selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs) in cancer treatment. *Pharmacology and Therapeutics* vol. 186 Preprint at Doi: 10.1016/j.pharmthera.2017.12.012 (2018).
[28] Fisher B, et al. Tamoxifen for the prevention of breast cancer: Current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. J Natl Cancer Inst 2005;97.
[29] Howell, A. & Evans, D. G. Breast cancer prevention: SERMs come of age. *The Lancet* vol. 381 Preprint at Doi: 10.1016/S0140-6736(13)60443-2 (2013).
[30] den Hollander P, Savage MI, Brown PH. Targeted therapy for breast cancer prevention. *Front.* Oncol 2013;3 SEP.
[31] Tung, N. & Garber, J. E. PARP inhibition in breast cancer: progress made and future hopes. *npj Breast Cancer* vol. 8 Preprint at Doi: 10.1038/s41523-022-00411-3 (2022).
[32] Zhu, K. *et al.* PI3K/AKT/mTOR-Targeted Therapy for Breast Cancer. *Cells* vol. 11 Preprint at Doi: 10.3390/cells11162508 (2022).
[33] Baselga J, Swain SM. CLEOPATRA: A phase III evaluation of pertuzumab and trastuzumab for HER2-positive metastatic breast cancer. Clin Breast Cancer 2010; 10.
[34] Wilson FR, et al. Herceptin® (trastuzumab) in HER2-positive early breast cancer: Protocol for a systematic review and cumulative network meta-analysis. Syst Rev 2017;6.
[35] Klapper LN, Waterman H, Sela M, Yarden Y. Tumor-inhibitory antibodies to HER-2/ErbB-2 may act by recruiting c-Cbl and enhancing ubiquitination of HER-2. Cancer Res 2000;60.
[36] Cruz, E. & Kayser, V. Monoclonal antibody therapy of solid tumors: Clinical limitations and novel strategies to enhance treatment efficacy. *Biologics: Targets and Therapy* vol. 13 Preprint at Doi: 10.2147/BTT.S166310 (2019).
[37] Luque-Cabal M, García-Teijido P, Fernández-Pérez Y, Sánchez-Lorenzo L, Palacio-Vázquez I. Mechanisms behind the resistance to trastuzumab in HER2-amplified breast cancer and strategies to overcome It. Clin Med Insights Oncol 2016;10.
[38] von Minckwitz G, et al. Adjuvant Pertuzumab and Trastuzumab in Early HER2-Positive Breast Cancer. N Engl J Med 2017;377.
[39] Yu, S. *et al.* Development and clinical application of anti-HER2 monoclonal and bispecific antibodies for cancer treatment. *Experimental Hematology and Oncology* vol. 6 Preprint at Doi: 10.1186/s40164-017-0091-4 (2017).
[40] Blumenthal GM, et al. First FDA Approval of dual anti-HER2 regimen: Pertuzumab in combination with trastuzumab and docetaxel for HER2-positive metastatic breast cancer. Clin Cancer Res 2013;19.
[41] Amiri-Kordestani L, et al. First FDA approval of neoadjuvant therapy for breast cancer: Pertuzumab for the treatment of patients with HER2-positive breast cancer. Clin Cancer Res 2014;20.
[42] Groelly, F. J., Fawkes, M., Dagg, R. A., Blackford, A. N. & Tarsounas, M. Targeting DNA damage response pathways in cancer. *Nature Reviews Cancer* vol. 23 Preprint at Doi: 10.1038/s41568-022-00535-5 (2023).
[43] Tutt ANJ, et al. Adjuvant Olaparib for Patients with BRCA1 - or BRCA2 -Mutated Breast Cancer. N Engl J Med 2021;384.
[44] Morrison, L., Loibl, S. & Turner, N. C. The CDK4/6 inhibitor revolution — a game-changing era for breast cancer treatment. *Nature Reviews Clinical Oncology* vol. 21 Preprint at Doi: 10.1038/s41571-023-00840-4 (2024).
[45] Saatci, O., Huynh-Dam, K. T. & Sahin, O. Endocrine resistance in breast cancer: from molecular mechanisms to therapeutic strategies. *Journal of Molecular Medicine* vol. 99 Preprint at Doi: 10.1007/s00109-021-02136-5 (2021).
[46] Cristofanilli M, et al. Overall Survival with Palbociclib and Fulvestrant in Women with HRþ/HER2− ABC: Updated Exploratory Analyses of PALOMA-3, a Double-blind, Phase III Randomized Study. Clin Cancer Res 2022;28.
[47] Cejuela M, et al. Abemaciclib, Palbociclib, and Ribociclib in Real-World Data: A Direct Comparison of First-Line Treatment for Endocrine-Receptor-Positive Metastatic Breast Cancer. Int J Mol Sci 2023;24.
[48] Ortega MA, et al. Signal Transduction Pathways in Breast Cancer: The Important Role of PI3K/Akt/mTOR. J Oncol 2020;2020.
[49] Porta, C., Paglino, C. & Mosca, A. Targeting PI3K/Akt/mTOR signaling in cancer. *Frontiers in Oncology* vol. 4 APR Preprint at Doi: 10.3389/fonc.2014.00064 (2014).
[50] Nitulescu, G. M. *et al.* Akt inhibitors in cancer treatment: The long journey from drug discovery to clinical use (Review). *International Journal of Oncology* vol. 48 Preprint at Doi: 10.3892/ijo.2015.3306 (2016).
[51] Martorana, F. *et al.* AKT Inhibitors: New Weapons in the Fight Against Breast Cancer? *Frontiers in Pharmacology* vol. 12 Preprint at Doi: 10.3389/fphar.2021.662232 (2021).
[52] Liu, Z. L., Chen, H. H., Zheng, L. L., Sun, L. P. & Shi, L. Angiogenic signaling pathways and anti-angiogenic therapy for cancer. *Signal Transduction and Targeted Therapy* vol. 8 Preprint at Doi: 10.1038/s41392-023-01460-1 (2023).
[53] Kumar, R. & Yarmand-Bagheri, R. The role of HER2 in angiogenesis. in *Seminars in Oncology* vol. 28 (2001).
[54] Madu, C. O., Wang, S., Madu, C. O. & Lu, Y. Angiogenesis in breast cancer progression, diagnosis, and treatment. *Journal of Cancer* vol. 11 Preprint at Doi: 10.7150/jca.44313 (2020).
[55] Ayoub, N. M., Jaradat, S. K., Al-Shami, K. M. & Alkhalifa, A. E. Targeting Angiogenesis in Breast Cancer: Current Evidence and Future Perspectives of Novel Anti-Angiogenic Approaches. *Frontiers in Pharmacology* vol. 13 Preprint at Doi: 10.3389/fphar.2022.838133 (2022).
[56] Luey BC, May FEB. Insulin-like growth factors are essential to prevent anoikis in oestrogen-responsive breast cancer cells: Importance of the type I IGF receptor and PI3-kinase/Akt pathway. Mol Cancer 2016;15.
[57] Farooq, M., Khan, A. W., Kim, M. S. & Choi, S. The role of fibroblast growth factor (FGF) signaling in tissue repair and regeneration. *Cells* vol. 10 Preprint at Doi: 10.3390/cells10113242 (2021).
[58] Santolla, M. F. & Maggiolini, M. The FGF/FGFR system in breast cancer: Oncogenic features and therapeutic perspectives. *Cancers* vol. 12 Preprint at Doi: 10.3390/cancers12103029 (2020).
[59] Francavilla, C. & Obrien, C. S. Fibroblast growth factor receptor signalling dysregulation and targeting in breast cancer. *Open Biology* vol. 12 Preprint at Doi: 10.1098/rsob.210373 (2022).
[60] Shen, S. J. & Liu, C. M. Chemotherapy for early-stage breast cancer: the more the better? *The Lancet* vol. 401 Preprint at Doi: 10.1016/S0140-6736(23)00094-6 (2023).
[61] Esteva, F. J., Hubbard-Lucey, V. M., Tang, J. & Pusztai, L. Immunotherapy and targeted therapy combinations in metastatic breast cancer. *The Lancet Oncology* vol. 20 Preprint at Doi: 10.1016/S1470-2045(19)30026-9 (2019).
[62] Chen, D. S. & Mellman, I. Elements of cancer immunity and the cancer-immune set point. *Nature* vol. 541 Preprint at Doi: 10.1038/nature21349 (2017).
[63] Li, J. J., Tsang, J. Y. & Tse, G. M. Tumor microenvironment in breast cancer—Updates on therapeutic implications and pathologic assessment. *Cancers* vol. 13 Preprint at Doi: 10.3390/cancers13164233 (2021).
[64] Dong W, et al. The mechanism of anti–pd-l1 antibody efficacy against pd-l1–negative tumors identifies nk cells expressing pd-l1 as a cytolytic effector. Cancer Discov 2019;9.
[65] Montesion M, et al. Somatic hla class i loss is a widespread mechanism of immune evasion which refines the use of tumor mutational burden as a biomarker of checkpoint inhibitor response. Cancer Discov 2021;11.
[66] Bald, T., Krummel, M. F., Smyth, M. J. & Barry, K. C. The NK cell–cancer cycle: advances and new challenges in NK cell–based immunotherapies. *Nature Immunology* vol. 21 Preprint at Doi: 10.1038/s41590-020-0728-z (2020).
[67] Marcenaro E, et al. KIR2DS1-dependent acquisition of CCR7 and migratory properties by human NK cells interacting with allogeneic HLA-C2+ DCs or T-cell blasts. Blood 2013;121.
[68] André P, et al. Anti-NKG2A mAb Is a Checkpoint Inhibitor that Promotes Anti-tumor Immunity by Unleashing Both T and NK Cells. Cell 2018;175.
[69] Zhang Q, et al. Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. Nat Immunol 2018;19.
[70] Čelešnik, H. & Potočnik, U. Peripheral Blood Transcriptome in Breast Cancer Patients as a Source of Less Invasive Immune Biomarkers for Personalized Medicine, and Implications for Triple Negative Breast Cancer. *Cancers* vol. 14 Preprint at Doi: 10.3390/cancers14030591 (2022).
[71] Bareche Y, et al. Unraveling Triple-Negative Breast Cancer Tumor Microenvironment Heterogeneity: Towards an Optimized Treatment Approach. J Natl Cancer Inst 2020;112:708–19.
[72] Fridman, W. H., Pagès, F., Sautès-Fridman, C. & Galon, J. The immune contexture in human tumours: Impact on clinical outcome. *Nature Reviews Cancer* vol. 12 Preprint at Doi: 10.1038/nrc3245 (2012).
[73] Karn T, 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;31:1216–22.
[74] Reschke R, Enk AH, Hassel JC. Chemokines and Cytokines in Immunotherapy of Melanoma and Other Tumors: From Biomarkers to Therapeutic Targets. Int J Mol Sci 2024.
[75] Behravan J, Razazan A, Behravan G. Towards Breast Cancer Vaccines, Progress and Challenges. Curr Drug Discov Technol 2019;16.

[76] Andtbacka RHI, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol 2015;33.
[77] Dudley, M. E. & Rosenberg, S. A. Adoptive-cell-transfer therapy for the treatment of patients with cancer. *Nature Reviews Cancer* vol. 3 Preprint at Doi: 10.1038/nrc1167 (2003).
[78] Li X, et al. Adoptive cell immunotherapy for breast cancer: harnessing the power of immune cells. J Leukoc Biol 2023. https://doi.org/10.1093/jleuko/qiad144.
[79] Hall ML, et al. Expansion of tumor-infiltrating lymphocytes (TIL) from human pancreatic tumors. J Immunother Cancer 2016;4.
[80] Kew, K. What is CAR T-cell therapy? *Drug and Therapeutics Bulletin* vol. 59 Preprint at Doi: 10.1136/dtb.2020.000040 (2021).
[81] Laskowski, T. J., Biederstädt, A. & Rezvani, K. Natural killer cells in antitumour adoptive cell immunotherapy. *Nature Reviews Cancer* vol. 22 Preprint at Doi: 10.1038/s41568-022-00491-0 (2022).
[82] clinicaltrial.gov.
[83] Greppi M, et al. Identification of a novel cord blood NK cell subpopulation expressing functional programmed death receptor-1. Front Immunol 2023;14.
[84] Poggi A, Zocchi MR. Natural killer cells and immune-checkpoint inhibitor therapy: Current knowledge and new challenges. Mol Ther Oncolytics 2021;24:26–42.
[85] Adams S, 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.
[86] Hosseini, A., Gharibi, T., Marofi, F., Babaloo, Z. & Baradaran, B. CTLA-4: From mechanism to autoimmune therapy. *International Immunopharmacology* vol. 80 Preprint at Doi: 10.1016/j.intimp.2020.106221 (2020).
[87] Li, Y. *et al.* Recent Progress on Immunotherapy for Breast Cancer: Tumor Microenvironment, Nanotechnology and More. *Frontiers in Bioengineering and Biotechnology* vol. 9 Preprint at Doi: 10.3389/fbioe.2021.680315 (2021).
[88] Andrews LP, et al. Resistance to PD1 blockade in the absence of metalloprotease-mediated LAG3 shedding. Sci Immunol 2020;5.
[89] Amaria RN, et al. Neoadjuvant relatlimab and nivolumab in resectable melanoma. Nature 2022;611.
[90] Yasinska IM, et al. The TiM-3-galectin-9 pathway and its regulatory mechanisms in human breast cancer. Front Immunol 2019;10.
[91] Gomes de Morais, A. L., Cerdá, S. & de Miguel, M. New Checkpoint Inhibitors on the Road: Targeting TIM-3 in Solid Tumors. *Current Oncology Reports* vol. 24 Preprint at Doi: 10.1007/s11912-022-01218-y (2022).
[92] Le Dréan E, et al. Inhibition of antigen-induced T cell response and antibody-induced NK cell cytotoxicity by NKG2A: Association of NKG2A with SHP-1 and SHP-2 protein-tyrosine phosphatases. Eur J Immunol 1998;28.
[93] Trujillo JA, Sweis RF, Bao R, Luke JJ. T cell–inflamed versus Non-T cell–inflamed tumors: a conceptual framework for cancer immunotherapy drug development and combination therapy selection. Cancer Immunol Res 2018;6.
[94] Jiang YZ, et al. Molecular subtyping and genomic profiling expand precision medicine in refractory metastatic triple-negative breast cancer: the FUTURE trial. Cell Res 2021;31.
[95] Zhang, L. The Role of Mesenchymal Stem Cells in Modulating the Breast Cancer Microenvironment. *Cell Transplantation* vol. 32 Preprint at Doi: 10.1177/09636897231220073 (2023).
[96] Stojanovic, A. & Cerwenka, A. Checkpoint inhibition: NK cells enter the scene. *Nature Immunology* vol. 19 Preprint at Doi: 10.1038/s41590-018-0142-y (2018).
[97] Tarantino P, et al. Understanding resistance to immune checkpoint inhibitors in advanced breast cancer. Expert Rev Anticancer Ther 2022;22.
[98] Voorwerk L, 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.
[99] Schmid P, et al. Event-free Survival with Pembrolizumab in Early Triple-Negative Breast Cancer. N Engl J Med 2022;386.
[100] Tolaney SM, et al. Effect of Eribulin with or without Pembrolizumab on Progression-Free Survival for Patients with Hormone Receptor-Positive, ERBB2-Negative Metastatic Breast Cancer: A Randomized Clinical Trial. JAMA Oncol 2020;6.
[101] Fasching PA, et al. Pembrolizumab in combination with nab-paclitaxel for the treatment of patients with early-stage triple-negative breast cancer – A single-arm phase II trial (NeoImmunoboost, AGO-B-041). Eur J Cancer 2023;184.
[102] Ho AY, 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;126:850–60.
[103] Bian L, et al. JS001, an anti-PD-1 mAb for advanced triple negative breast cancer patients after multi-line systemic therapy in a phase I trial. Ann Transl Med 2019;7.
[104] Jiang Z, et al. Toripalimab plus nab-paclitaxel in metastatic or recurrent triple-negative breast cancer: a randomized phase 3 trial. Nat Med 2024;30.
[105] Mittendorf EA, 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.
[106] Cortes J, 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.
[107] Schmid P, 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.
[108] Miles D, 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.
[109] Yap TA, et al. Avelumab Plus Talazoparib in Patients with Advanced Solid Tumors: The JAVELIN PARP Medley Nonrandomized Controlled Trial. JAMA Oncol 2023;9.
[110] Masuda J, et al. Efficacy, safety, and biomarker analysis of nivolumab in combination with abemaciclib plus endocrine therapy in patients with HR-positive HER2-negative metastatic breast cancer: A phase II study (WJOG11418B NEWFLAME trial). J Immunother Cancer 2023;11.
[111] Rampioni Vinciguerra, G. L. *et al.* CDK4/6 Inhibitors in Combination Therapies: Better in Company Than Alone: A Mini Review. *Frontiers in Oncology* vol. 12 Preprint at Doi: 10.3389/fonc.2022.891580 (2022).
[112] Zhao, H. *et al.* The role of osteopontin in the progression of solid organ tumour. *Cell Death and Disease* vol. 9 Preprint at Doi: 10.1038/s41419-018-0391-6 (2018).
[113] Vonderheide RH, et al. Tremelimumab in combination with exemestane in patients with advanced breast cancer and treatment-associated modulation of inducible costimulator expression on patient T cells. Clin Cancer Res 2010;16.
[114] Torres ETR, Emens LA. Emerging combination immunotherapy strategies for breast cancer: dual immune checkpoint modulation, antibody-drug conjugates and bispecific antibodies. Breast Cancer Res Treat 2022;191:291–302.
[115] Van Hall, T. *et al.* Monalizumab: Inhibiting the novel immune checkpoint NKG2A. *Journal for ImmunoTherapy of Cancer* vol. 7 Preprint at Doi: 10.1186/s40425-019-0761-3 (2019).
[116] Borst L, van der Burg SH, van Hall T. The NKG2A-HLA-E axis as a novel checkpoint in the tumor microenvironment. Clin Cancer Res 2021;26.
[117] Herbst RS, et al. COAST: An Open-Label, Phase II, Multidrug Platform Study of Durvalumab Alone or in Combination with Oleclumab or Monalizumab in Patients with Unresectable, Stage III Non-Small-Cell Lung Cancer. J Clin Oncol 2022;3.
[118] Taylor BC, et al. NKG2A Is a Therapeutic Vulnerability in Immunotherapy Resistant MHC-I Heterogeneous Triple-Negative Breast Cancer. Cancer Discov 2024;14.
[119] Niu J, et al. First-in-human phase 1 study of the anti-TIGIT antibody vibostolimab as monotherapy or with pembrolizumab for advanced solid tumors, including non-small-cell lung cancer☆. Ann Oncol 2022;33.
[120] Mettu NB, et al. A Phase 1a/b Open-Label, Dose-Escalation Study of Etigilimab Alone or in Combination with Nivolumab in Patients with Locally Advanced or Metastatic Solid Tumors. Clin Cancer Res 2022;28.
[121] Cho BC, et al. Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study. Lancet Oncol 2022;23.
[122] Mohan N, et al. Atezolizumab potentiates Tcell-mediated cytotoxicity and coordinates with FAK to suppress cell invasion and motility in PD-L1+ triple negative breast cancer cells. Oncoimmunology 2019;8.
[123] Wu CC, et al. Combination of FAK inhibitor and cytokine-induced killer cell therapy: An alternative therapeutic strategy for patients with triple-negative breast cancer. Biomed Pharmacother 2023;163.
[124] Seth P, et al. Development of oncolytic adenovirus armed with a fusion of soluble transforming growth factor-β receptor II and human immunoglobulin Fc for breast cancer therapy. Hum Gene Ther 2006;17.
[125] Yang Y, et al. An Oncolytic Adenovirus Targeting Transforming Growth Factor β Inhibits Protumorigenic Signals and Produces Immune Activation: A Novel Approach to Enhance Anti-PD-1 and Anti-CTLA-4 Therapy. Hum Gene Ther 2019;30.
[126] Sun K, et al. A Phase 2 Trial of Enhancing Immune Checkpoint Blockade by Stereotactic Radiation and In Situ Virus Gene Therapy in Metastatic Triple-Negative Breast Cancer. Clin Cancer Res 2022;28.
[127] Song L, et al. Improvement of TNBC immune checkpoint blockade with a microwave-controlled ozone release nanosystem. J Control Release 2022;351.
[128] Zhou W, et al. Landscape of the Peripheral Immune Response Induced by Local Microwave Ablation in Patients with Breast Cancer. Adv Sci 2022;9.
[129] Liu H, et al. Bispecific antibody targeting TROP2xCD3 suppresses tumor growth of triple negative breast cancer. J Immunother Cancer 2021;9.
[130] Bano JD, et al. A bispecific antibody-based approach for targeting mesothelin in triple negative breast cancer. Front Immunol 2019;10.
[131] Yi M, et al. Anti-TGF-β/PD-L1 bispecific antibody promotes T cell infiltration and exhibits enhanced antitumor activity in triple-negative breast cancer. J Immunother Cancer 2022;10.
[132] Maali, A. *et al.* Nanobodies in cell-mediated immunotherapy: On the road to fight cancer. *Frontiers in Immunology* vol. 14 Preprint at Doi: 10.3389/fimmu.2023.1012841 (2023).
[133] Huehls, A. M., Coupet, T. A. & Sentman, C. L. Bispecific T-cell engagers for cancer immunotherapy. *Immunology and Cell Biology* vol. 93 Preprint at Doi: 10.1038/icb.2014.93 (2015).
[134] Luke JJ, et al. The PD-1- and LAG-3-targeting bispecific molecule tebotelimab in solid tumors and hematologic cancers: a phase 1 trial. Nat Med 2023;29.
[135] Loi S, et al. Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer (PANACEA): a single-arm, multicentre, phase 1b–2 trial. Lancet Oncol 2019;20.
[136] Bassani, B. *et al.* Natural killer cells as key players of tumor progression and angiogenesis: Old and novel tools to divert their pro-tumor activities into potent anti-tumor effects. *Cancers* vol. 11 Preprint at Doi: 10.3390/cancers11040461 (2019).

[137] Hu, W., Wang, G., Huang, D., Sui, M. & Xu, Y. Cancer immunotherapy based on natural killer cells: Current progress and new opportunities. *Frontiers in Immunology* vol. 10 Preprint at Doi: 10.3389/fimmu.2019.01205 (2019).
[138] Camorani, S., Fedele, M., Zannetti, A. & Cerchia, L. TNBC challenge: Oligonucleotide aptamers for new imaging and therapy modalities. *Pharmaceuticals* vol. 11 Preprint at Doi: 10.3390/ph11040123 (2018).
[139] Agnello, L. *et al.* Aptamer-Based Strategies to Boost Immunotherapy in TNBC. *Cancers* vol. 15 Preprint at Doi: 10.3390/cancers15072010 (2023).
[140] Camorani S, et al. Aptamer targeted therapy potentiates immune checkpoint blockade in triple-negative breast cancer. J Exp Clin Cancer Res 2020;39.
[141] Kiaie, S. H. *et al.* Nano-immunotherapy: overcoming delivery challenge of immune checkpoint therapy. *Journal of Nanobiotechnology* vol. 21 Preprint at Doi: 10.1186/s12951-023-02083-y (2023).
[142] Wang R, et al. Nanotechnology Applications in Breast Cancer Immunotherapy. *Small* Preprint at 2023. https://doi.org/10.1002/smll.202308639.
[143] Ashrafizadeh, M. *et al.* (Nano)platforms in breast cancer therapy: Drug/gene delivery, advanced nanocarriers and immunotherapy. *Medicinal Research Reviews* vol. 43 Preprint at Doi: 10.1002/med.21971 (2023).
[144] Yadav, D. *et al.* Cancer immunotherapy by immune checkpoint blockade and its advanced application using bio-nanomaterials. *Seminars in Cancer Biology* vol. 86 Preprint at Doi: 10.1016/j.semcancer.2022.02.016 (2022).
[145] Kim, K. S., Kim, D. H. & Kim, D. H. Recent advances to augment NK cell cancer immunotherapy using nanoparticles. *Pharmaceutics* vol. 13 Preprint at Doi: 10.3390/pharmaceutics13040525 (2021).
[146] Wu Y, Gu W, Li J, Chen C, Xu ZP. Silencing PD-1 and PD-L1 with nanoparticle-delivered small interfering RNA increases cytotoxicity of tumor-infiltrating lymphocytes. Nanomedicine 2019;14.
[147] Esmaily M, et al. Blockade of CTLA-4 increases anti-tumor response inducing potential of dendritic cell vaccine. J Control Release 2020;326.
[148] Youngjin Choi Su, Seok H, Yoon HY, Ryu JH, Kwon IC. Advancing cancer immunotherapy through siRNA-based gene silencing for immune checkpoint blockade. Adv Drug Deliv Rev 2024.
[149] Zhang J, et al. Hybrid spherical nucleotide nanoparticles can enhance the synergistic anti-tumor effect of CTLA-4 and PD-1 blockades. Biomater Sci 2020;8.