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REVIEW

Immune Checkpoint Blockade and Emerging Combination Platforms in Breast Cancer: A Narrative Review

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Abstract: This narrative review examines recent progress in immunotherapy for breast cancer (BC), focusing on immune checkpoint inhibitors (ICIs) alone and in combination with other modalities. Landmark trials such as KEYNOTE-522 and IMpassion130 have established the efficacy of pembrolizumab and atezolizumab in triple-negative breast cancer (TNBC). However, BC remains a leading cause of cancer-related fatalities, underscoring the need for novel approaches. We synthesize combination strategies into three mechanistic categories: (I) those that remodel the immunosuppressive tumor microenvironment (chemotherapy, PARP inhibitors, oncolytic viruses); (II) those that enhance effector cell persistence (CAR-T, CAR-NK, cytokine support); and (III) those that modulate immune checkpoint axes beyond PD-1/CTLA-4 (LAG-3, TIM-3, TIGIT). Combining ICIs with CAR-T cells, CAR-NK cells, oncolytic viruses, and exosomes has been shown to improve antitumor immune responses. This review provides a translational framework for biomarker-driven patient stratification and critically evaluates the clinical maturity of emerging platforms. Further research and clinical trials are needed to expand applicability across BC subtypes and improve patient outcomes.

Keywords: breast cancer, immunotherapy, checkpoint inhibitors, CAR T cells, CAR NK cells, oncolytic viruses, exosomes

Introduction

Despite ongoing progress in medicine, breast cancer (BC) remains the second most common and deadly cancer among women. In the United States, over 270,000 women are diagnosed each year with invasive BC.[1] The rates of diagnosis and death vary by region due to differences in socioeconomic, environmental, and healthcare factors.[2]

BC is categorized by the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), which are important for prognosis and treatment. Most breast carcinomas are ER-positive; among these, more than half also express PR, while only about 2% are exclusively PR-positive. HER2-positive tumors are aggressive but respond to targeted therapies. In contrast, triple-negative breast cancers (TNBC), which lack ER, PR, and HER2, have limited treatment options and a poorer prognosis.[2–4]

Recent studies have shown that the immune system plays a significant role in BC, especially regarding the presence of tumor-infiltrating lymphocytes (TILs). These immune cells are more common in HER2-positive and TNBC subtypes than in hormone receptor-positive tumors.[5]

The tumor microenvironment (TME) in BC shows immunosuppressive features. These include the expression of immune checkpoints, immune cell exhaustion, an increase in regulatory immune cells, and the buildup of immunosuppressive metabolites. BC subtypes vary in their immune cell infiltration levels: TNBC and HER2-positive tumors generally have higher levels of infiltration, which is linked to better survival rates. In contrast, ER-positive tumors have lower immune infiltration and worse outcomes.[6] Regional lymph nodes from BC patients contain exhausted cytotoxic T cells that express

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checkpoint molecules like PD-1 and TIM-3. This finding underscores the immunosuppressive nature of the TME and its significance for immune-based treatments.[7,8]

Immune checkpoint inhibitors (ICIs) have emerged as a pivotal therapeutic option in BC by targeting inhibitory pathways that tumors exploit to evade immune surveillance, including PD-1/PD-L1 and CTLA-4-CD28 axes. Approximately 46% of BC patients in the USA may be eligible for ICI therapy.[9] These inhibitors restore the body's anti-tumor immune response, leading to tumor shrinkage and better survival rates; however, they can also trigger immune-related side effects (irAEs). These side effects differ from those caused by standard chemotherapy and can sometimes be severe, with an average onset occurring 14.5 days after treatment.[10] Since the FDA first approved ipilimumab in 2011, eleven ICIs have received approval for various cancers, including PD-1 inhibitors, PD-L1 inhibitors, CTLA-4 inhibitors, and a LAG-3-blocking antibody. In BC, only pembrolizumab has FDA approval, mainly for TNBC. Additionally, dostarlimab and pembrolizumab are approved for tumors with mismatch repair deficiency or high microsatellite instability.[11]

This narrative review examines recent advancements in immunotherapy for breast cancer (BC), focusing on immune checkpoint inhibitors (ICIs) alone and in combination with other modalities. We distinguish predictive biomarkers (PD-L1, BRCA status) from prognostic biomarkers (TIL density in early-stage TNBC) and organize combination strategies into three mechanistic categories: those that remodel the tumor microenvironment, those that enhance effector cell persistence, and those that provide multi-checkpoint blockade. The challenges of applying these treatments to a varied disease are also addressed, along with a summary of ongoing clinical trials.

Immune Checkpoint Blockade in BC

Immune checkpoint inhibitors (ICIs) are drugs that block key immunosuppressive pathways, including PD-1/PD-L1 and CTLA-4, which tumors exploit to evade immune detection. By blocking these checkpoints, ICIs remove the limits on the immune system, which boosts T-cell activity against tumors. This stronger immune response can result in tumor shrinkage, longer disease control, and better survival rates in different types of cancer, including BC.[12–14]

PD-1/PD-L1 Blockade as Monotherapy or in Combination with Other Therapeutic Agents

PD-1/PD-L1 Axis in BC

The PD-1/PD-L1 pathway plays a key role in regulating T-cell activity and tumor immune evasion in BC. PD-1 is often found at high levels on tumor-infiltrating lymphocytes and tumor cells, especially in TNBC, and its presence is linked to the aggressiveness of the disease.[15] Table 1 lists important clinical trials using immune checkpoint inhibitors

Table 1 Clinical Trials of Immune Checkpoint Inhibitors in Advanced/Metastatic TNBC

Trial Name	Primary Author	Year	Study Design	Line of Therapy	Setting	Biomarker	Patients	Drug Regimen	Results
KEYNOTE-012	Nanda	2016	Phase Ib	Heavily pretreated	mTNBC	PD-L1 ≥1%	32	Pembrolizumab	ORR 18.5%, DCR 25.9%[16]
KEYNOTE-086	Adams	2018	Phase II	≥ First line	mTNBC	Any	170	Pembrolizumab	ORR 5.3%, PD-L1+ ORR 5.7%, DCR 7.6%[17]
KEYNOTE-086	Adams	2018	Phase II	First line	mTNBC	PD-L1 ≥1%	84	Pembrolizumab	ORR 21%, PFS 2.1 mo, OS 18.0 mo[18]
ENHANCE 1/ KEYNOTE-150	Tolaney	2021	Phase Ib/II	1–3 lines	mTNBC	Any	167	Eribulin + Pembrolizumab	ORR 23.4%, PFS 4.1 mo, OS 16.1 mo
KEYNOTE-355	Cortes	2020	Phase III	First line	mTNBC	PD-L1 CPS ≥10	847	Chemo + Pembrolizumab	mOS 23.0 vs 16.1 mo, PFS 9.7 vs 5.6 mo

(*Continued*)

Table 1 (Continued).

Trial Name	Primary Author	Year	Study Design	Line of Therapy	Setting	Biomarker	Patients	Drug Regimen	Results
IMpassion130	Schmid	2018	Phase III	First line	mTNBC	PD-L1+	902	Nab-Paclitaxel + Atezolizumab	PFS 7.2 vs 5.5 mo, OS 21.3 vs 17.6 mo[19]
JAVELIN	Dirix	2018	Phase Ib	Heavily pretreated	mTNBC	Any	58	Avelumab	ORR 5.2%, PD-L1+ ORR 22.2%[20]
MEDIOLA	Domchek	2020	Phase I/II	≥ Third line	HER2-mBC	BRCA1/2 mutant	34	Durvalumab + Olaparib	ORR 63.3%, 12-wk DCR 80%[21]
TONIC	Voorwerk	2019	Phase I/II	Heavily pretreated	mTNBC	Any	67	Nivolumab ± Priming	ORR 20%
FUTURE Arm C	Liu	2023	Phase II	Heavily pretreated	mTNBC	Immunomodulatory	46	Camrelizumab + Nab-Paclitaxel	ORR 43.5%, PFS 4.6 mo
KEYLYNK-009	Rugo	2020	Phase II	First line maintenance	mTNBC	Any	271	Olaparib + Pembrolizumab	PFS 5.5 mo, OS 25.1 mo[22]
COUPLET	Kristeleit	2024	Phase Ib/II	≥ First line	mTNBC	BRCA-mut/LOH high	5	Atezolizumab + Rucaparib	ORR 40%
TORCHLIGHT	Jiang	2024	Phase III	First line	mTNBC	PD-L1 CPS ≥1	531	Nab-Paclitaxel + Toripalimab	PFS 8.4 vs 5.6 mo, OS 32.8 vs 19.5 mo
NUMBUS	Barrosa-Sousa	2020	Phase II	Any	adv/mTNBC	TMB-High	31	Ipilimumab + Nivolumab	ORR 13.3%, PFS 1.4 mo, OS 8.8 mo
IMPRIME I	O'Day	2020	Phase II	≥ First line	mHER2-	Any	44	Odetiglucan + Pembrolizumab	ORR 15.9%, DCR 54.5%
SAFIR02-BREAST IMMUNO	Bachelot	2021	Phase II	First line maintenance	mTNBC	Any	82	Durvalumab	mOS 14.0 vs 21.1 mo
DORA	Tan	2024	Phase II	First line maintenance	mBC	Any	45	Durvalumab ± Olaparib	PFS 4.0 vs 6.1 mo, CBR 44% vs 36%
FUTURE-C-PLUS	Chen	2022	Phase II	First line	adv/mBC	Immunomodulatory	48	Camrelizumab + Famitinib + Nab-Paclitaxel	ORR 81.3%, PFS 13.6 mo
FUTURE-SUPER	Fan	2024	Phase II	First line	adv/mTNBC	Immunomodulatory	139	Camrelizumab ± Famitinib + Nab-Paclitaxel	PFS 15.1 vs 6.5 mo
DART/SWOG S1609	Adams	2022	Phase II	Any	adv/mTNBC	Any	17	Ipilimumab + Nivolumab	ORR 18%

Abbreviations: adv, advanced; BC, breast cancer; CBR, clinical benefit rate; Chemo, chemotherapy; CPS, combined positive score; DCR, disease control rate; HER2, human epidermal growth factor receptor 2; LOH, loss of heterozygosity; mBC, metastatic breast cancer; mHER2-, metastatic HER2-negative; mo, months; mOS, median overall survival; mTNBC, metastatic triple-negative breast cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PD-L1, programmed death-ligand 1; TMB, tumor mutational burden; vs, versus; wk, weeks.

(ICIs) in advanced or metastatic TNBC. Conventional therapies often increase PD-L1 expression, allowing tumors to escape the immune response.[16,17] As a result, targeting the PD-1/PD-L1 axis has become an appealing treatment approach. Monoclonal antibodies (mAbs) that target PD-1 include pembrolizumab, cemiplimab, and nivolumab. PD-L1 inhibitors consist of atezolizumab, durvalumab, and avelumab.[15]

PD-1/PD-L1 Inhibitors as Monotherapy

Several clinical studies have looked at PD-1 inhibitors alone in BC. In the phase Ib KEYNOTE-012 trial (NCT01848834), pembrolizumab (10 mg/kg IV every two weeks) showed a manageable safety profile in TNBC patients.[16] The Phase II

KEYNOTE-086 trial confirmed promising anti-tumor activity with acceptable toxicity in PD-L1-positive metastatic TNBC.[17] Notably, clinical effectiveness was closely related to PD-L1 expression levels. In the Phase III KEYNOTE-119 trial, pembrolizumab alone did not improve overall survival (OS) compared to chemotherapy in later lines of metastatic TNBC.[23] Current phase III studies, including KEYNOTE-756, CHECKMATE 7FL, and KEYNOTE-B49, are further examining pembrolizumab in metastatic BC.[24]

Combination with Chemotherapy and CTLA-4 Inhibition

Combination therapies have shown better clinical outcomes. In the phase III KEYNOTE-355 trial, pembrolizumab plus chemotherapy significantly enhanced OS by seven months (23.0 vs. 16.1 months; HR 0.73; P = 0.0185) in patients with PD-L1 CPS ≥10. However, there was no OS benefit observed in the overall patient population or those with CPS ≥1.[25] Dual checkpoint inhibition, which combines PD-1/PD-L1 and CTLA-4 blockade, offers another option. A study of durvalumab plus tremelimumab in patients with refractory metastatic BC reported an overall response rate (ORR) of 17%, with the best response (43%) in TNBC. In contrast, no responses were seen in estrogen receptor-positive cases. Responders showed higher nonsynonymous mutation burdens and increased neoantigen presentation, indicating better T-cell activation.[24–26]

In luminal BC, early efforts to combine ICIs with chemotherapy were mostly ineffective. Preclinical research suggests that CDK4/6 inhibitors help improve tumor antigen presentation, limit regulatory T-cell growth, and downregulate inhibitory receptors like PD-1, thereby boosting T-cell activity.[27–29] Clinically, combining pembrolizumab with abemaciclib, with or without endocrine therapy, led to interstitial lung disease, liver toxicity, and two deaths in the triplet treatment group.[22] On the other hand, phase I/II trials found that the combination of palbociclib, letrozole, and pembrolizumab was well tolerated.[30]

In HER2-positive metastatic BC, pembrolizumab plus trastuzumab yielded a 15% response rate in PD-L1-positive, trastuzumab-resistant tumors. However, adding atezolizumab to T-DM1 did not enhance progression-free survival (PFS) and resulted in higher toxicity.[31,32]

PD-1/PD-L1 Blockade in TNBC: Metastatic and Neoadjuvant Settings

TNBC shows the most responsiveness to PD-1/PD-L1 inhibition. Atezolizumab alone and in combination with nab-paclitaxel improved recurrence-free survival in the phase III IMpassion130 trial, leading to FDA approval for TNBC patients with PD-L1-positive tumor-infiltrating immune cells.[18,33] However, the IMpassion131 trial raised concerns about OS and PFS, highlighting the need for more investigation.[34] Additional trials, including IMpassion132 and NCT04177108, are testing atezolizumab with chemotherapy and targeted therapies in locally recurrent or untreated TNBC.[19,34–36]

Beyond atezolizumab-based regimens, novel neoadjuvant combinations continue to emerge. The phase II NeoSACT trial evaluated the combination of anlotinib (a multi-target tyrosine kinase inhibitor) plus sintilimab (an anti-PD-1 antibody) in combination with chemotherapy in patients with triple-negative breast cancer, demonstrating promising antitumor activity and a manageable safety profile.[37] Furthermore, a comprehensive review of immunotherapy strategies in TNBC has highlighted the evolving landscape of clinical trials, emphasizing the need for optimizing patient selection and overcoming resistance mechanisms to improve long-term outcomes.[38]

Other PD-1 inhibitors such as avelumab and dostarlimab have shown modest success in refractory mTNBC and dMMR tumors, with overall response rates of 5.2% and 41.6%, respectively.[20,39,40]

Neoadjuvant studies have shown significant advantages. In KEYNOTE-522, pembrolizumab plus chemotherapy increased pathological complete response (pCR) from 51.2% to 64.8% and improved 3-year event-free survival from 76.8% to 84.5%, regardless of PD-L1 status.[41] IMpassion031 confirmed similar outcomes with atezolizumab,[42] while NeoTRIP and GeparNeuvo showed variable pCR improvements. GeparNeuvo demonstrated better 3-year invasive disease-free survival (iDFS) and OS with durvalumab.[43,44] However, not all trials have been positive. The IMpassion131 trial failed to show benefit with atezolizumab plus paclitaxel, underscoring that chemotherapy backbone selection matters.[34] In hormone receptor-positive disease, ICIs have shown limited efficacy due to the immunologically "cold" tumor microenvironment. Toxicity remains a concern, as seen with severe pneumonitis and hepatotoxicity when pembrolizumab was combined with CDK4/6 inhibitors.[22] These limitations do not negate the value of ICIs but emphasize the need for careful patient selection and risk-benefit assessment.

Emerging Combinatorial Approaches: LAG-3 and ADCs

Lymphocyte activation gene-3 (LAG-3) is an inhibitory checkpoint that suppresses effector T cells and boosts Treg function. Dual inhibition of PD-1 and LAG-3 may work together to restore antitumor immunity. IMP321 (recombinant LAG-3Ig) in combination with paclitaxel and cemiplimab or fianlimab improved pCR rates in HR+/HER2− and TNBC groups in clinical trials.[45]

Antibody-drug conjugates (ADCs), such as sacituzumab govitecan (Trop-2) and trastuzumab deruxtecan (HER2), are being tested with ICIs to enhance antigen presentation and dendritic cell activation. Early trials such as BEGONIA report ORRs of 66.7–74%, indicating potential for combined efficacy.[46–48]

CTLA-4 Blockade

Cytotoxic T lymphocyte antigen 4 (CTLA-4) is an immune checkpoint receptor found on cytotoxic T cells and regulatory T cells (Tregs). It helps limit T-cell activation by binding to CD80/CD86 on antigen-presenting cells.[49] Higher levels of CTLA-4 have been linked to poorer outcomes in BC.[50]

Clinical Investigations of CTLA-4 Blockade in BC

Research on CTLA-4 inhibitors in BC is limited. These inhibitors are often studied with other treatments like chemotherapy, aromatase inhibitors, or radiotherapy. Tremelimumab, a monoclonal antibody against CTLA-4, was tested together with exemestane in 26 patients with advanced hormone-responsive BC. The study showed stable disease in 42% of patients for at least 12 weeks, but no partial or complete responses were found. Notably, 36% of patients with stable disease had previously progressed while on exemestane alone.[51]

Ipilimumab (humanized IgG1 anti-CTLA-4) has shown strong anti-tumor effects in several cancers, including melanoma and renal cell carcinoma.[52] Preclinical data suggest that ipilimumab may encourage TNBC cells to release IL-2, which could improve local immune activity.[53] Phase I trials combining perioperative cryoablation with ipilimumab in early-stage BC showed it was safe without delaying surgery, suggesting potential for immune priming.[54]

In metastatic or resistant BC, combination therapies with ipilimumab and nivolumab have shown promising results. A Phase II trial (NCT02834013) found responses in 3 out of 17 patients with metaplastic BC, indicating a subset of about 18% who might respond. Another study that combined ipilimumab, nivolumab, and neoadjuvant paclitaxel in early-stage TNBC showed encouraging overall and complete response rates, regardless of PD-L1 status.[55]

Combination Strategies with Chemotherapy

Metronomic chemotherapy, which uses lower doses of drugs over longer periods, has been shown to improve the effectiveness of anti-CTLA-4 treatments. In preclinical models of BC, using CTLA-4 blockade followed by metronomic gemcitabine or cyclophosphamide resulted in better tumor control than CTLA-4 blockade alone, although resistance and spontaneous metastases still occurred.[56,57]

Multi-Checkpoint Blockade

Since response rates to single immune checkpoint inhibitors (ICIs) are limited (20–38%) and the treatments may cause systemic side effects, researchers are exploring combinations of CTLA-4 inhibitors with other checkpoint inhibitors. A triple blockade of LAG-3, PD-1, and CTLA-4 is currently under clinical investigation. This approach has demonstrated enhanced T-cell activation and proliferation in preclinical studies.[58,59] LAG-3, which is often overexpressed in BC tumors, may also serve as a response marker and is being studied alongside CTLA-4 targeting therapies.[60] Furthermore, donor-derived double-negative T cells (DNTs) combined with LAG-3 blockade are emerging as a new adoptive therapy in TNBC, potentially working well with CTLA-4 inhibition.[61]

Mechanisms of Resistance to Immune Checkpoint Inhibitors in BC

Resistance to immune checkpoint inhibitors in BC can be broadly classified as primary resistance, where patients fail to respond from the outset, or acquired resistance, where initial response is followed by disease progression. Several interconnected mechanisms underlie this resistance. First, adaptive immune resistance occurs when tumor cells upregulate PD-L1 in response to IFN-γ secreted by activated T cells, thereby creating a negative feedback loop that dampens

antitumor immunity.[16,25] Second, loss of antigen presentation due to downregulation of MHC class I molecules through beta-2-microglobulin (B2M) mutations or HLA loss of heterozygosity (LOH) prevents effective T cell recognition of tumor cells.[8] Third, defects in the antigen processing machinery, including mutations or downregulation of TAP1, TAP2, and immunoproteasome subunits (PSMB8, PSMB9, PSMB10), impair peptide loading onto MHC class I molecules, further compromising T cell activation.[8] Fourth, metabolic competition within the tumor microenvironment, where tumors consume glucose and produce lactate, creates a nutrient-poor, acidic milieu that suppresses T cell metabolism, proliferation, and effector function.[6] Fifth, physical T cell exclusion mediated by desmoplastic stroma, aberrant tumor vasculature, and extracellular matrix components physically prevents T cell infiltration into tumor islets, limiting immune access.[6,7] Collectively, understanding these mechanisms clarifies why specific combination strategies are rational: chemotherapy and oncolytic viruses induce immunogenic cell death, PARP inhibitors increase mutational burden and neoantigen presentation, and CAR-T cells bypass MHC dependence entirely.

PARP Inhibitors

Poly (ADP-ribose) polymerase (PARP) inhibitors focus on DNA damage repair pathways, especially in BC patients with BRCA1/2 mutations. By blocking PARP-1, these drugs prevent the repair of single-strand breaks, leading to double-strand breaks that require homologous recombination. Tumors with faulty homologous recombination, such as BRCA-mutated cells, can be killed by synthetic lethality and PARP trapping.[21,62,63]

Clinical Applications and Combinations with ICIs

PARP inhibitors like olaparib, talazoparib, rucaparib, and niraparib have shown lasting tumor-fighting effects and better progression-free survival in BC, as well as in ovarian, peritoneal, and fallopian tube cancers.[64] Combining PARP inhibitors with ICIs has become a promising way to boost immune responses against tumors in BC, especially in TNBC and BRCA-mutated tumors. Several clinical trials support the potential benefits of this strategy. In NCT04191135, researchers are examining olaparib along with pembrolizumab and chemotherapy for TNBC to improve immune activity and treatment outcomes. The MEDIOLA trial showed positive results with durvalumab and olaparib in BRCA-mutated metastatic BC, reporting a response rate of 58.5% in TNBC patients.[65] Likewise, the TOPACIO/KEYNOTE-162 study looked at pembrolizumab plus the PARP inhibitor niraparib in metastatic TNBC, yielding a 21% overall response rate, with notably higher rates in tumors with BRCA mutations. In the neoadjuvant setting, the ISPY-2 trial explored combining durvalumab and olaparib with doxorubicin and cyclophosphamide (AC) in high-risk BC, indicating a trend toward better pathological complete response rates.[66,67] Overall, these findings underline the increasing clinical rationale for combining PARP inhibition with ICIs to improve immune responses against tumors. Ongoing trials, such as DORA and KEYLYNK, aim to clarify the clinical benefits of pairing PARP inhibitors with ICIs, especially in patients selected by biomarkers.

Adoptive Cell Therapy

Adoptive T-cell therapy is a type of immunotherapy that uses and modifies a patient's T cells to improve their ability to identify and destroy cancer cells. This process includes isolating T cells from the patient's blood, modifying them in the lab to express synthetic receptors that can recognize tumor-associated antigens, expanding these modified cells in culture, and reintroducing them into the patient.[68,69] By transferring tumor-infiltrating lymphocytes (TILs), engineered T-cell receptor (TCR)-based cells, or chimeric antigen receptor (CAR)-T cells, adoptive cell therapy boosts the patient's natural immune response against tumors. This method is especially promising for those with weakened immune systems, offering a potential breakthrough in cancer treatment.

A major challenge in adoptive T-cell therapy is improving how well the infused T cells can recognize tumor antigens. Finding patient-specific neoantigens through sequencing can improve the treatment's effectiveness. Co-culturing TILs with dendritic cells (DCs) that express the corresponding neoantigens has shown to trigger strong neoantigen-specific T-cell responses. For example, in a patient with metastatic BC (HER2-/ER+), lasting regression occurred using TILs that reacted to mutant proteins, along with IL-2 and PD-1 checkpoint blockade.[70] Similarly, Assadipour et al found that mutant-reactive TILs could detect immunogenic non-synonymous somatic mutations in a TNBC patient, identifying 72 such mutations as possible therapeutic targets.[71]

Multiple clinical trials have assessed TIL therapy as a standalone treatment and in combination with chemotherapy, pembrolizumab, or trastuzumab (NCT01462903, NCT04111510, NCT01395056, NCT01232062, NCT01174121, NCT00301730). However, due to limited effectiveness, immunological tolerance, low MHC expression, and the naturally low affinity of TCRs for tumor antigens, TIL therapy is increasingly being replaced by gene transfer-based ACT methods.[72,73]

Cytokine-induced killer (CIK) cells are CD3+CD56+ lymphocytes with MHC-unrestricted cytotoxicity, allowing them to kill tumor cells directly while promoting T-cell growth. A review of 310 BC patients showed that those with PD-L1-positive tumors had better overall and recurrence-free survival when treated with CIK cells and standard treatments like chemotherapy or radiation. This suggests that PD-L1 expression may affect how well CIKs respond.[74] Additionally, combination therapy with DC/CIK and chemotherapy demonstrated higher response rates and similar safety compared to chemotherapy alone, supporting its potential as a new treatment strategy for BC.[72]

Preclinical studies consistently show that CIK cells effectively target BC stem cells and suppress tumor growth in patient-derived xenograft models.[73,75] Their ability to kill cancer cells can improve when they are engineered with chimeric antigen receptors, particularly anti-EGFR CARs, or when combined with monoclonal antibodies that target EGFR. This combination results in stronger antitumor activity.[74,76]

A retrospective analysis of 294 patients with TNBC treated with autologous CIK cells and chemotherapy showed longer survival, especially in early-stage disease. This suggests that combining CIK therapy with chemotherapy may lower the risk of recurrence and metastatic progression.[77] Additionally, a meta-analysis comparing DC/CIK plus chemotherapy with chemotherapy alone found higher objective response rates in the DC/CIK group without added toxicity. This reinforces the potential of CIK-based immunotherapy in BC.[76–78]

Together, these findings indicate that CIK-cell therapy, either on its own or combined with standard treatments, holds significant promise, especially for PD-L1–expressing tumors and TNBC. However, larger prospective clinical studies are needed to confirm long-term benefits and to determine the best way to integrate this therapy into current BC treatment plans. Table 2 summarizes ongoing and completed clinical trials investigating immune cell- and biologic-based therapies combined with immune checkpoint inhibitors in BC. This table provides an overview of the therapy type, targets, combination strategies, cancer subtype or model, and key study findings, highlighting the potential of these approaches.

Table 2 Immune Cell- and Biologic-Based Therapies Combined with Immune Checkpoint Inhibitors in BC

Therapy/ Platform	Target/ Engineering	Combination Strategy	Cancer Type/ Setting	Study Type/ Phase	Key Findings/Advantages
ADCs: Sacituzumab govitecan	Trop-2	Pembrolizumab	Metastatic TNBC, ≥2 prior lines	Clinical trials (ongoing)	FDA-approved; SN-38 payload; enhances DC activation, neoantigen presentation, PD-L1 expression; synergistic with ICIs.[46–48]
ADCs: Trastuzumab deruxtecan (T-DXd)	HER2	PD-1/PD-L1 inhibitors	HER2+ unresectable/ metastatic BC	Phase Ib/II	Improved PFS (HR 0.50) and OS (HR 0.64); ORR ~66.7%; combination enhances immune activation.[67]
ADCs: Ladiratuzumab vedotin	LIV-1	Pembrolizumab	Advanced TNBC, first-line	Phase Ib/II (NCT03310957)	Moderate tolerability; ORR 54%; supports potential synergy with ICIs.
ADCs: Datopotamab deruxtecan (Dato-DXd)	Trop-2	PD-1/PD-L1 inhibitors	Advanced TNBC	BEGONIA study	ORR 74%; enhances neoantigen generation and dendritic cell activation.[46–48]

(*Continued*)

Table 2 (Continued).

Therapy/ Platform	Target/ Engineering	Combination Strategy	Cancer Type/ Setting	Study Type/ Phase	Key Findings/Advantages
Adoptive: TILs	Patient-specific neoantigens	DC co-culture, PD-1 blockade	Metastatic $HER2^{-}/ER^{+}$ BC, TNBC	Clinical	Durable regression; personalized therapy improves tumor-specific T cell response.
Adoptive: Engineered TCR T cells	Tumor-associated antigens	ICIs, gene transfer	TNBC, $HER2^{+}$ BC	Preclinical & Clinical	Overcomes TIL limitations; enhances antigen-specific cytotoxicity.
Adoptive: CIK/ DC-CIK cells	$CD3^{+}CD56^{+}$ lymphocytes	Chemotherapy, DC co-culture, ICIs, anti-EGFR CAR	TNBC, $PD\text{-}L1^{+}$ BC	Clinical/Preclinical (310 pts)	MHC-independent cytotoxicity; targets BC stem cells; enhances OS/RFS; synergistic with CARs/antibodies.[74,78]
CAR-T: MUC1-targeted	MUC1	± anti-PD-1/PD-L1	Advanced TNBC	Clinical (NCT04020575, etc).	Early-phase trials; improved tumor-specific cytotoxicity expected.
CAR-T: MSLN-CAR	Mesothelin	PD-1 blockade	TNBC	Preclinical & Clinical	Enhanced persistence, cytokine production; 4th-gen achieved complete tumor regression in mice.
CAR-T: NKG2D-targeted (CTM-N2D)	ULBP, MICA/B	—	Refractory/ relapsed solid tumors	Phase I (NCT04107142)	Targets stress ligands upregulated in TNBC; safe dosing under investigation.[79,80]
CAR-T: ROR1-CAR	ROR1	—	$ROR1^{+}$ malignancies incl. BC	Phase I (NCT02706392, NCT04842812)	Early antitumor activity; ongoing BC clinical trials.[81]
CAR-T + PD-1/PD-L1 blockade	MUC1, MSLN, EGFR	ICIs or local anti-PD-L1	TNBC, BC	Preclinical & Clinical	Enhanced IFN-γ/TNF-α production, persistence, antitumor efficacy; reduces PD-1/PD-L1–mediated suppression.[82]
CAR-T + Oncolytic virus (CAdVEC)	HER2-specific CAR-T	OV expressing PD-L1 mini-antibody	BC	Phase I (NCT03740256)	Local PD-L1 blockade reinvigorates CAR-T; more effective than systemic anti-PD-L1.
CAR-T: CTLA-4/RASA2 modulation	CTLA-4 knockout or RASA2 modulation	—	Preclinical BC	Preclinical	Enhances proliferation, effector function, antitumor activity; potential to overcome T cell exhaustion.[83]
CAR-NK: CD44v6-CAR	CD44v6	—	TNBC	Preclinical	Strong cytotoxicity; enhanced tumor regression in vitro/in vivo.[84]
CAR-NK: PD-L1 t-haNK cells	PD-L1 + CAR + CD16	± N-803 + anti-PD-1	TNBC, bladder, oral SCC	Preclinical	Retains NK function; selectively kills MDSCs; efficacy enhanced by IFN-γ and checkpoint blockade.[85]
CAR-NK: HER2-CAR	HER2	± PD-1 blockade	$HER2^{+}$ BC	Preclinical	High cytotoxicity; safer than CAR-T; co-expression of PD-1 improves killing of $PD\text{-}L1^{+}HER2^{+}$ tumors.[86]

(*Continued*)

Table 2 (Continued).

Therapy/ Platform	Target/ Engineering	Combination Strategy	Cancer Type/ Setting	Study Type/ Phase	Key Findings/Advantages
CAR-NK: EGFR-CAR NK-92 + HSV-1 OV	EGFR	Oncolytic HSV-1	Metastatic BC, incl. brain tumors	Preclinical/Clinical	Reduced tumor growth; effective for distant metastases.
CAR-NK: TF-CAR NK	Tissue Factor + CD16/FcγRIII	± ADCC, ICIs	TNBC	Preclinical	Potent anti-TNBC activity in CDX/PDX; combination with ICIs enhances antitumor response.
NK Cells: Herceptin-mediated	HER2	± trastuzumab	Metastatic $HER2^+$ BC	Clinical	Improved cytotoxicity; synergistic with Herceptin.
NK Cells: iPSC-derived FT-516 + Avelumab	hnCD16 NK cells	PD-L1 blockade	Advanced solid tumors incl. TNBC	Clinical (NCT04551885)	Combines high-affinity NK cells with ICIs; potential enhanced antitumor activity.
NK Cells: haNK + Avelumab	IL-15 + hnCD16 engineered NK cells	ICIs, vaccine, metronomic chemo	Metastatic/ refractory TNBC	Phase I/II (NCT03387085)	Disease control 78%; ORR 67%; CR 22%; median PFS 13.7 months.[87]
Oncolytic: CVA21 (KEYNOTE-200/STORM)	Coxsackievirus A21	+ Pembrolizumab	Metastatic TNBC	Phase 1b	Evaluating safety/dosing; preliminary efficacy ongoing.
Oncolytic: OV-IL15C + EGFR-CAR-NK	Adenovirus expressing IL-15	+ EGFR-CAR-NK	Intracranial BC (mouse)	Preclinical	Increased $CD8^+$ T/NK infiltration; enhanced tumor growth inhibition.
Oncolytic: EGFR-CAR-NK + HSV-1 OV	HSV-1–based OV	+ EGFR-CAR-NK	BC brain metastases (mouse)	Preclinical	Combination killed tumor cells, extended survival.
Oncolytic: rMV-BNiP3 + Paclitaxel	Measles virus armed with BNiP3	+ Paclitaxel	TNBC	Preclinical	Increased tumor apoptosis and antitumor efficacy.
Oncolytic: T-VEC + NAC	HSV-1 expressing GM-CSF	+ Doxorubicin/ Cyclophosphamide	TNBC	Early clinical	Increased TILs; 55% pathologic CR rate.
Oncolytic: CF33-hNIS-F14.5 + anti–PD-L1	Chimeric poxvirus + anti–PD-L1 scFv	+ ICI	TNBC (mouse)	Preclinical	Synergistic antitumor effect; enhances $CD8^+$ T-cell infiltration.[88]
Oncolytic: OV + TMZ + ICIs	Adenovirus	+ Temozolomide + ICIs	TNBC (cell)	Preclinical	Enhanced OV replication, autophagy, and tumor killing.
Oncolytic: OV-IL15/RANTES + CAR-T	Adenovirus expressing IL-15/ RANTES	+ CAR-T	Solid tumors incl. BC	Preclinical	Promotes CAR-T infiltration/persistence; improves efficacy.[89–92]

(*Continued*)

Table 2 (Continued).

Therapy/ Platform	Target/ Engineering	Combination Strategy	Cancer Type/ Setting	Study Type/ Phase	Key Findings/Advantages
Oncolytic: OV-CD19t + CD19-CAR-T	Chimeric virus encoding CD19t	+ CD19-CAR-T	Solid tumors incl. BC	Preclinical	Upregulated tumor CD19t; improved CAR-T recognition and activity.
Oncolytic: Anti-TGF-β OV + MSLN-CAR-T	Adenovirus targeting TGF-β	+ MSLN-CAR-T	BC (primary/ metastatic)	Preclinical	Reduced tumor growth/metastasis; improved CAR-T efficacy.
Exosomes: HER2-targeted vaccine	DC-derived exosomes w/ HER2 peptides	Enhance ICI response	HER2+ BC	Preclinical/ Translational	Activate CTLs/$CD4^+$ T cells; increase ICI sensitivity.[93,94]
Exosomes: OX40L-engineered	Exosomes expressing OX40L	Synergy with anti-PD-1/PD-L1	Various BC	Preclinical	Promotes T-cell proliferation; reverses exhaustion; potentiates ICIs.
Exosomes: DC-derived (DEXs)	MHC I/II peptide-loaded	+ ICIs	TNBC/HER2 + BC	Preclinical	Stimulates CTL/helper T cells; converts immunosuppressive TME into ICI-responsive.[95]
Exosomes: CAR-T derived ($RN7SL1^+$)	CAR-T exosomes carrying RN7SL1 RNA	+ ICIs	Multiple BC subtypes	Preclinical	Boosts CAR-T expansion; reduces MDSC suppression; overcomes PD-L1 evasion without CRS.[96,97]
Exosomes: Cetuximab-scFv	CAR-T–derived	+ ICIs	EGFR+ BC	Preclinical	Dose-dependent tumor inhibition; low toxicity; compatible with ICIs.[98,99]
Exosomes: Trastuzumab-scFv	CAR-T–derived	+ ICIs	HER2+ BC	Preclinical	Effective HER2+ cytotoxicity; bypasses CRS/off-tumor effects.
Exosomes: Mesothelin-CAR-T	CAR-T–derived	+ ICIs	Mesothelin+ TNBC	Preclinical	Selective killing; minimal toxicity.
Exosomes: CAR-NK (HER2-targeted)	CAR-NK exosomes w/ perforin/ granzymes, T7 peptide	+ ICIs	HER2+ BC brain mets	Preclinical	Cross BBB; strong anti-tumor activity; promising off-the-shelf adjunct.[100,101]
Exosomes: Tumor-derived exosome inhibition	Block miR-9, miR-181a, $PD\text{-}L1^+$ exosomes	+ ICIs	TNBC/ metastatic BC	Preclinical	Reduces immunosuppression; enhances $CD8^+$ T-cell responses; improves ICI efficacy.

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ADCs, antibody-drug conjugates; BC, breast cancer; BBB, blood-brain barrier; CAR, chimeric antigen receptor; CAR-NK, chimeric antigen receptor natural killer cell; CAR-T, chimeric antigen receptor T cell; CBR, clinical benefit rate; CDX, cell line-derived xenograft; CIK, cytokine-induced killer; CR, complete response; CRS, cytokine release syndrome; CTL, cytotoxic T lymphocyte; DC, dendritic cell; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FDA, Food and Drug Administration; GM-CSF, granulocyte-macrophage colony-stimulating factor; haNK, high-affinity natural killer; HER2, human epidermal growth factor receptor 2; HSV-1, herpes simplex virus type 1; ICIs, immune checkpoint inhibitors; IFN-γ, interferon-gamma; IL, interleukin; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; NAC, neoadjuvant chemotherapy; NK, natural killer; ORR, overall response rate; OS, overall survival; OV, oncolytic virus; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PDX, patient-derived xenograft; PFS, progression-free survival; RFS, recurrence-free survival; scFv, single-chain variable fragment; SCC, squamous cell carcinoma; T-VEC, talimogene laherparepvec; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; TMZ, temozolomide; TNBC, triple-negative breast cancer; TNF-α, tumor necrosis factor-alpha.

CAR-T Cell Therapy and Immune Checkpoint Blockade in BC

CAR-T cell therapy is a promising approach for metastatic, triple-negative, and HER2-positive BC. This method involves genetically modifying T cells to express chimeric antigen receptors (CARs). These receptors link tumor antigen-binding parts with co-stimulatory signals. After reinfusion, CAR-T cells can identify tumor-specific antigens, activate signaling pathways, and initiate strong antitumor responses. Some key BC antigens being studied include EGFR, FRα, AXL, NKG2D, MUC-1, c-Met, and mesothelin (Figure 1). [102] Scientists introduce CAR constructs into T cells using plasmid transfection, mRNA transduction, or viral vectors.[103]

CAR-T therapy allows for strong antigen recognition that does not depend on MHC, leading to effective T-cell activation and antitumor responses.[104,105] Preclinical and early-stage clinical trials focusing on mesothelin, which is found in high levels in tumors and low levels in normal tissues, show antitumor activity without significant on-target off-tumor toxicity. Table 3 presents major candidate antigens explored for CAR-T cell development in TNBC.

Many studies are investigating the combination of CAR-T therapy with immune checkpoint inhibition. PD-1-targeted CAR-T cells can bypass PD-L1-mediated immune suppression in BC. When combined, CAR-T therapies and checkpoint blockade show better effectiveness than using either alone, although this may increase the risk of heightened immune reactions.[115,116] Several ongoing clinical trials are assessing MUC1-targeted CAR-T cells in advanced or treatment-resistant TNBC (NCT04020575, NCT02587689, NCT04025216, NCT05812326).

PD-1/PD-L1 interactions can reduce the effectiveness of CAR-T cells in solid tumors. Combining CAR-T therapy with PD-1 blockade, including CRISPR/Cas9-mediated PD-1 knockout, enhances cytokine production and antitumor

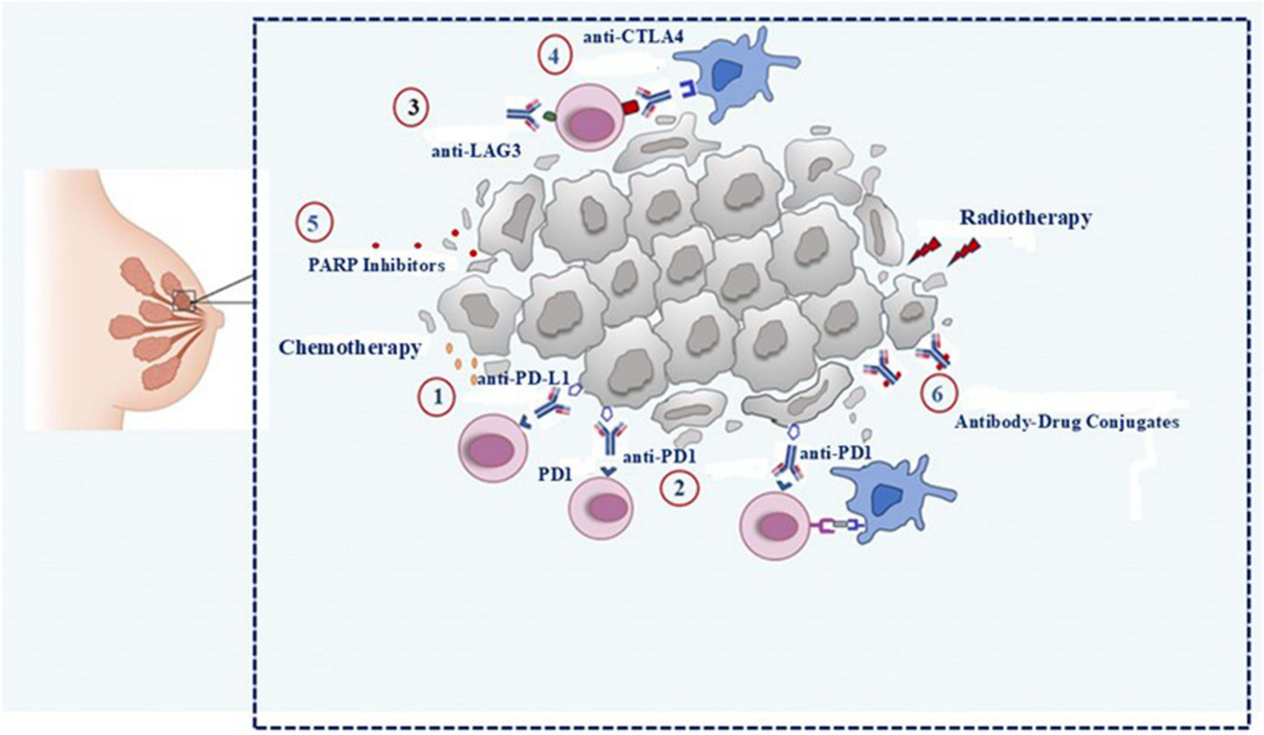

Figure 1 Therapeutic Strategies Targeting the Tumor Microenvironment in BC. Strategies targeting the tumor microenvironment in breast cancer focus on enhancing anti-tumor immunity and overcoming therapeutic resistance by utilizing immune checkpoint inhibitors, PARP inhibitors, antibody-drug conjugates, and radiotherapy to create a more hostile environment for tumor cells. These strategies include: - Immune Checkpoint Inhibitors: Antibodies like anti-PD-1/PD-L1 (e.g, pembrolizumab, atezolizumab) enhance T cell activation by blocking interactions that inhibit immune responses. Anti-LAG3 antibodies further promote T cell activation, while anti-CTLA-4 antibodies (e.g, ipilimumab) reduce regulatory T cell activity, enhancing overall immune response. - PARP Inhibitors: These disrupt DNA repair mechanisms in cancer cells, leading to increased genomic instability and apoptosis. - Antibody-Drug Conjugates (ADCs): ADCs deliver cytotoxic agents directly to tumor cells, maximizing tumor cell death while minimizing off-target effects. - Radiotherapy and Chemotherapy: Radiotherapy induces DNA damage in cancer cells, complementing other therapies, while chemotherapy targets rapidly dividing cells to further enhance tumor cell death. Together, these approaches work synergistically to overcome immune tolerance and strengthen anti-tumor responses within the TME of breast cancer.

Abbreviations: CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell death protein; ADC, antibody-drug conjugate; PD-L1, Programmed Cell Death Ligand 1; PARP Inhibitors, Poly (ADP-ribose) Polymerase inhibitors.

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Table 3 Major Candidate Antigens Driving CAR-T Cell Engineering Efforts in TNBC

No.	Target	Role in Breast Cancer	Key Finding/Preclinical Insight
1	**EGFR**	Often overexpressed in aggressive breast tumors	EGFR-redirected CAR-T cells show enhanced activity when combined with DNA-damage–inducing therapies.[104]
2	**TROP2**	Linked to proliferation and cell adhesion	CAR-T engagement leads to strong target-specific cytotoxicity in TROP2-high models.[106]
3	**AXL**	Drives epithelial-to-mesenchymal transition	AXL-directed constructs efficiently suppress invasive TNBC cell growth.
4	**ROR1**	Expressed in stem-like cancer populations	Blocking TGF-β signaling improves the therapeutic effect of ROR1-CAR-T cells.
5	**GD2**	Surface glycolipid enriched in metastatic TNBC	GD2-targeted CAR-T induces robust and selective tumor killing.
6	**MSLN (Mesothelin)**	Elevated in several breast cancer subtypes	Targeting MSLN yields strong tumor-specific elimination, especially with supportive immune modulators.[107]
7	**ICAM1**	Supports tumor migration and immune escape	Redirected CAR-T cells recognize ICAM1-positive TNBC and restrict proliferation.
8	**NKG2D-Ligands**	Upregulated under stress and transformation	NKG2D-CAR-T shows cytotoxicity across multiple ligand-positive breast models.[108]
9	**CD44v6**	Associated with metastasis and therapy resistance	CAR-immune cells blocking CD44v6 effectively impair TNBC survival.
10	**ALCAM/ CD166**	Adhesion molecule contributing to invasiveness	Early CAR designs indicate promising selective recognition.
11	**EpCAM**	Upregulated in epithelial cancers	Combining EpCAM-CAR-T with other agents boosts anti-tumor potency.[109]
12	**FRα**	Folate receptor variant in subsets of TNBC	Enables targeted elimination of FRα-positive cancer populations.
13	**c-MET**	Metastasis-associated growth receptor	CAR-T cells recognizing c-MET reduce tumor burden in MET-positive models.
14	**B7-H3**	Immune suppressive protein frequently upregulated	Shows stronger therapeutic outcomes when paired with radiotherapy.
15	**B7-H4**	Involved in immune evasion	B7-H4-CAR-T demonstrates potent cytolytic function against select cell lines.
16	**αvβ6 integrin**	Linked to aggressive TNBC behavior	CAR-T potency increases when migration-enhancing receptors are co-expressed.[110]
17	**αvβ3 integrin**	Regulates cell motility	Targeted CAR-T induces cytokine secretion and tumor cell destruction.
18	**SLC3A2**	Essential amino-acid transporter	Its targeting disrupts metabolic integrity of TNBC cells.
19	**PSMA**	Expressed in breast cancer stem-like cells	Represents a unique vulnerability in BCSC-driven tumors.
20	**TEM8/ ANTXR1**	Present in tumor vasculature and TNBC	CAR-T constructs show efficacy in both cell lines and PDX models.[111]
21	**CD70**	Immune signaling marker	Multi-target CARs engaging CD70 produce significant tumor inhibition.

(*Continued*)

Table 3 (Continued).

No.	Target	Role in Breast Cancer	Key Finding/Preclinical Insight
22	**Nectin-4**	Surface protein in advanced breast tumors	Targeting leads to functional disruption and reduced cell viability.
23	**CEA**	Tumor-associated antigen in some breast cancers	CEA-directed CAR-T induces marked cell lysis in engineered models.
24	**CD22**	Appears on selected TNBC subsets	CD22-CAR-T exhibits selective recognition and tumor reduction.
25	**FAP**	Marker of cancer-associated fibroblasts	Eliminating FAP-positive stromal cells weakens tumor support structures.
26	**CSPG4**	Glycoprotein tied to invasiveness	Targeting CSPG4 produces strong anti-tumor cytotoxicity.[112]
27	**PD-L1**	Immune checkpoint molecule	Direct CAR-T targeting of PD-L1 triggers potent killing in aggressive TNBC.
28	**RON (MST1R)**	Proto-oncogenic receptor	Its high expression in many tumors makes it a promising CAR-T candidate.[113]
29	**CLDN6**	Tight-junction protein not in normal adult tissue	Provides a tumor-restricted target for next-generation CAR-T designs.
30	**csGRP78**	Stress-induced chaperone elevated in resistant cancers	Targeting this molecule is effective especially in therapy-resistant models.[114]

Abbreviations: BC, breast cancer; BCSC, breast cancer stem cell; CAR-T, chimeric antigen receptor T cell; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; FAP, fibroblast activation protein; FRα, folate receptor alpha; MSLN, mesothelin; PD-L1, programmed death-ligand 1; PDX, patient-derived xenograft; PSMA, prostate-specific membrane antigen; ROR1, receptor tyrosine kinase-like orphan receptor 1; TGF-β, transforming growth factor beta; TNBC, triple-negative breast cancer.

activities in TNBC models.[117] Using pembrolizumab together with CAR-T cells can restore IFN-γ and TNF-α production and improve the persistence of CAR-T cells.[118,119] However, widespread PD-1 blockade can lead to high costs, uncontrolled T-cell activation, and toxic effects on various organs.[120,121] Delivering immune checkpoint inhibitors (ICIs) directly through CAR-T cells or preconditioning with specific cytokines like IL-7/IL-15 can boost effectiveness while lowering required dosages and side effects.[122] PD-1 blockade may revive exhausted CAR-T cells, but repeated treatments are necessary to avoid tumor recurrence.[123]

Administering CAR-T cells locally, such as through intrapleural infusion, has been shown to allow for lasting systemic circulation, suggesting potential for long-lasting responses in distant tumors.[124] New strategies targeting immune checkpoints, like CTLA-4 knockout or RASA2 modulation, can improve CAR-T cells growth, antitumor effects, and activity in suppressive environments.[125,126] Combining PD-L1 blockade with CAR-T cells also helps counter MDSC-mediated suppression, enhancing antitumor effectiveness.[83,127]

CAR-T cells aimed at mesothelin (MSLN) show strong ability to kill TNBC cell lines and patient-derived xenografts. PD-1 blockade further boosts their persistence and function.[82,128,129] TNBC also overexpresses NKG2D ligands (ULBP, MICA/B), which are being targeted by CTM-N2D CAR-T cells in phase I trials (NCT04107142).[79,80] ROR1-targeted CAR-T cells are currently under clinical evaluation for BC (NCT02706392, NCT04842812).[81]

EGFR-specific CAR-T cells, created using lentiviral vectors that encode dual scFv regions, effectively recognize TNBC cells in lab studies and hinder tumor growth in xenograft models.[102] Bicistronic vectors that express CARs and PD-L1-blocking scFvs in the TME reduce inhibitory receptor levels and improve CAR-T function.[130,131] EpCAM-targeted CAR-T cells are being studied clinically for recurrent metastatic BC (NCT02915445).[132] Using oncolytic adenoviruses together with CAR-T therapy (eg, CAdVEC plus HER2-CAR-T) might help overcome tumor-induced immune suppression (NCT03740256) (Figure 2).

Despite these advances, challenges remain, such as on-target off-tumor toxicity, varied tumor antigens, immunosuppressive tumor environments, cytokine release syndrome, and T-cell exhaustion. To improve specificity, effectiveness, and safety, researchers are developing strategies like trans-signaling CARs, targeting dual antigens, applying inhibitory

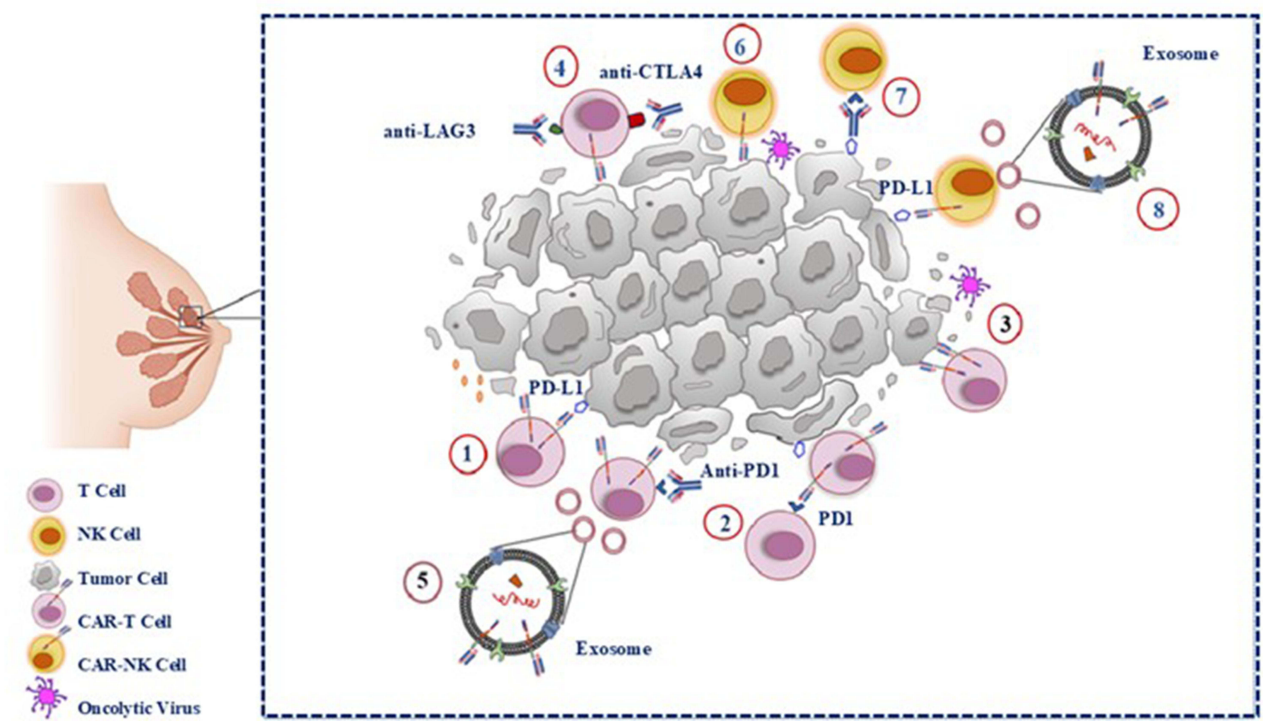

Figure 2 Synergistic Roles of CARNK Cells, CAR-T Cells, NK Cells, T Cells, Oncolytic Viruses, and Exosomes in BC Immunotherapy. This figure illustrates the multifaceted roles of various immune cells and therapeutic agents in enhancing breast cancer immunotherapy. CARNK cells are engineered to improve tumor recognition and cytotoxicity. CAR-T cells target specific tumor antigens to enhance adaptive immune responses. NK cells provide rapid immune responses to eliminate tumor cells without prior sensitization. T cells, particularly CTLs, play a crucial role in recognizing and destroying cancer cells through specific mechanisms. The figure also highlights the role of oncolytic viruses, which selectively infect and kill cancer cells while stimulating anti-tumor immune responses, and exosomes, small extracellular vesicles that facilitate communication between tumor and immune cells and may enhance the effectiveness of immunotherapies. Together, these components represent a promising combination strategy to overcome tumor evasion and improve treatment outcomes in breast cancer.
Abbreviations: CARNK cells, Chimeric Antigen Receptor Natural Killer cells; CAR-T cells, Chimeric Antigen Receptor T cells; NK cells, Natural Killer cells; CTL, cytotoxic T lymphocytes.

signals to normal tissues, and combining CAR-T therapy with ICIs.[133] With ongoing research, CAR-T therapy shows significant promise as a new treatment for BC and other solid tumors.

Immune Checkpoint Blockade in Natural Killer (NK) Cell Therapy

Natural killer (NK) cells are essential components of the innate immune system. They can eliminate tumor cells without needing prior exposure to antigens. By targeting cancer stem cells and sparing normal cells, NK cells offer unique benefits for BC therapy. However, tumor cells use strategies to evade the immune system. They shed stress-induced ligands, such as MHC class I polypeptide-related sequence A (MICA) and MICB. This leads to a downregulation of the NKG2D receptor and decreases susceptibility to NK-mediated cell death.[134] Elevated levels of soluble NKG2D ligands are linked to lymph node metastasis in BC and serve as negative predictors. Cytokines in the tumor microenvironment, such as TGF-β and IFN-γ, reduce the expression of ULBP and MICA. This reduction impairs NKG2D-mediated activation of natural killer cells.[135–137]

Research on NK cell immunotherapy mainly focuses on: (i) transferring large numbers of the patient's own NK cells that have been expanded outside the body, (ii) boosting NK cell activity or overcoming the signals from inhibitory receptors, and (iii) modifying NK cells to handle the immunosuppressive TMEs.[138,139] Autologous NK cells often show limited effectiveness because they interact with inhibitory receptors on tumor MHC class I molecules. As a result, allogeneic NK cells are receiving more attention.[140]

Clinical evidence supports NK cell-based strategies. A trial involving Herceptin-mediated NK cells in metastatic HER2+ BC showed potential benefits.[141] Additionally, NK cells can express PD-1, suggesting that ICIs can enhance NK cell-mediated killing of PD-L1+ tumors.[142,143] Preclinical studies show that blocking PD-1/PD-L1 increases NK cell growth, durability, and

Table 4 Clinical Trials of Cellular Immunotherapies in BC: CAR-T, ACT, NK, and CAR-NK Platforms with Immune Checkpoint Integration

Trial ID	Therapy Type	Target/Approach	Breast Cancer Indication	Phase	Delivery/ Combination	Immune Checkpoint Integration	Status/ Notes
NCT04020575	CAR-T	MUC1 (huMNC2-CAR44/CAR22)	Advanced $MUC1^+$ BC	I/II	Autologous CAR-T	–	Ongoing
NCT02587689	CAR-T	MUC1-CAR-T	$MUC1^+$ BC/ solid tumors	I/II	Dose-escalation	–	Ongoing
NCT04025216	CAR-T	TnMUC1-CAR-T	$TnMUC1^+$ TNBC/solid tumors	I	First-in-human	–	Terminated (risk–benefit)
NCT05812326	CAR-T	PD-1-KO Anti-MUC1 CAR-T (AJMUC1)	Advanced $MUC1^+$ BC	I	CRISPR-edited CAR-T	PD-1 knockout	Recruiting
NCT02414269	CAR-T/ACT	Mesothelin-CAR-T	$Mesothelin^+$ tumors incl. BC	I/II	± Chemotherapy/ ICIs	ICI-combination arm	Active, not recruiting
NCT06256055	CAR-T	Mesothelin (UCMYM802)	Mesothelin $^+$ BC	I	Novel CAR-T	–	Ongoing
NCT02580747	CAR-T	Mesothelin-CAR-T	Solid tumors incl. BC	I	IV infusion	–	Ongoing
NCT05623488	CAR-T	Lentiviral Mesothelin-CAR-T	Mesothelin $^+$ BC	I	Lentiviral CAR-T	–	Ongoing
NCT04107142	CAR-T/ACT	NKG2D-ligand CAR-T	Refractory solid tumors	I	Allogeneic/γδ	–	Early phase
NCT02706392	CAR-T/ACT	ROR1-CAR-T	$ROR1^+$ TNBC	I	IV infusion	–	Ongoing
NCT04842812	CAR-T	PD-1-KO TIL-CAR hybrid	Advanced solid tumors	I	PD-1 deletion + scFv	PD-1 deletion	Active
NCT02915445	CAR-T	EpCAM-CAR-T	$EpCAM^+$ BC	I	IV infusion	–	Ongoing
NCT03740256	CAR-T	HER2-CAR-T + Oncolytic virus	$HER2^+$ BC	I	OV + CAR-T	Viro-immunotherapy enhances ICI signaling	Ongoing
NCT04430595	CAR-T/ACT	Multi-target 4SCAR (CD44v6, CD70, MSLN)	BC	I/II	Multi-antigen CAR-T	–	Active
NCT02713984	CAR-T	HER2-CAR-T	$HER2^+$ BC	I/II	IV infusion	–	Ongoing
NCT04427449	CAR-T/ACT	CD44v6-CAR-T	$CD44v6^+$ BC	I/II	IV	–	Unknown
NCT03696030	ACT	HER2-CAR-T	$HER2^+$ BC	I	Intraventricular	–	Active
NCT04511871	ACT	HER2-CAR-T	$HER2^+$ BC	I	IV	–	Active
NCT01837602	ACT	TNBC/MBC CAR-T	TNBC/MBC	I	Intratumoral	–	Completed
NCT02830724	ACT	CD70-CAR-T	Advanced BC	I/II	Cy + Flu + IL-2	–	Recruiting

(*Continued*)

Table 4 (Continued).

Trial ID	Therapy Type	Target/Approach	Breast Cancer Indication	Phase	Delivery/ Combination	Immune Checkpoint Integration	Status/ Notes
NCT02792114	ACT	$HER2^{-}$/$MSLN^{+}$ CAR-T	$HER2^{-}$ BC	I	Cy preconditioning	–	Active
NCT02541370	ACT	CD133-CAR-T	Advanced BC	I/II	IV	–	Completed
NCT04348643	ACT	CEA-CAR-T	Advanced BC	I/II	IV	–	Unknown
NCT03635632	ACT	C7R-GD2 CAR-T	Advanced BC	I	Cy + Flu	–	Active
NCT00376805	NK	Mixed NK	Mixed BC	II	Cy + Flu	–	Terminated
NCT01105650	NK	Mixed NK	Mixed BC	II	Cy + Flu + Cyclosporine	–	Completed
NCT02030561	NK	$HER2^{+}$ NK	$HER2^{+}$ BC	I/II	Trastuzumab	Indirect PD-L1 modulation via ADCC	Unknown
NCT03319459	NK	$HER2^{+}$ NK	$HER2^{+}$ BC	I	Trastuzumab + Cetuximab	–	Completed
NCT04319757	NK	$HER2^{+}$ NK	$HER2^{+}$ BC	I/II	Cy + Flu	–	Completed
NCT02536625	NK	HR^{+}/$HER2^{-}$ NK	HR^{+}/$HER2^{-}$ BC	–	Everolimus	–	Completed
NCT03634501	NK	Mixed NK	Mixed BC	I/II	None	–	Unknown
NCT02839954	CAR-NK	MUC1-CAR-NK	Relapsed solid tumors	I/II	Not disclosed	–	Unknown
NCT05194709	CAR-NK	5T4-CAR-NK	Advanced tumors	I	Not disclosed	–	Recruiting
NCT05686720	CAR-NK	Unspecified CAR-NK	Advanced TNBC	I	Not disclosed	–	Not yet recruiting
NCT03415100	CAR-NK	Unspecified CAR-NK	Solid tumors incl. BC	I	Not disclosed	–	Completed

Abbreviations: ACT, adoptive cell therapy; ADCC, antibody-dependent cellular cytotoxicity; BC, breast cancer; CAR, chimeric antigen receptor; CAR-NK, chimeric antigen receptor natural killer cell; CAR-T, chimeric antigen receptor T cell; Cy, cyclophosphamide; Flu, fludarabine; HER2, human epidermal growth factor receptor 2; ICIs, immune checkpoint inhibitors; IL-2, interleukin-2; IV, intravenous; KO, knockout; MBC, metastatic breast cancer; MSLN, mesothelin; NK, natural killer; OV, oncolytic virus; PD-1, programmed cell death protein 1; scFv, single-chain variable fragment; TNBC, triple-negative breast cancer; Allogeneic γδ, allogeneic or gamma-delta T cells.

antitumor effects.[142,144] Mechanically, PD-L1 inhibition may reduce Treg induction, which normally restricts NK cell activity, and can activate AMPK to boost tumor sensitivity to NK cells.[145,146]

Beyond PD-1/PD-L1 checkpoint blockade, targeting TIGIT represents another strategy to enhance NK cell function. Chen et al developed Ociperlimab (BGB-A1217), an Fc-competent anti-TIGIT blocking antibody that effectively blocks TIGIT-CD155/CD112 interaction, promotes NK cell activation, and synergizes with anti-PD-1 antibodies in preclinical tumor models.[147] These findings suggest that TIGIT blockade, alone or in combination with ICIs, may further improve NK cell-based immunotherapies in breast cancer.

Current clinical efforts include iPSC-derived NK cells (FT-516) combined with avelumab, a high-affinity, non-cleavable CD16-expressing NK cell therapy. This combination is being tested in advanced solid tumors, including TNBC (NCT04551885) (Table 4). Combination strategies involving NK cells and immune-stimulating agents like antibodies,

cytokines, and chemotherapy (eg, lenalidomide) have shown promise in improving NK antitumor effects by addressing TME-induced immunosuppression.[148,149]

Notably, the QUILT-3.067 trial evaluated high-affinity NK (haNK) cells along with avelumab, IL-15 cytokine delivery, cancer vaccines, and metronomic chemotherapy in metastatic or unresectable TNBC. Among nine participants, the disease control rate was 78%, the overall response rate was 67%, and the complete response rate was 22%. The median progression-free survival (PFS) was 13.7 months, significantly better than the historical PFS of 3 months.[150]

Immune Checkpoint Blockade by CAR-NK Cells

The development of chimeric antigen receptor-engineered NK (CAR-NK) cells has broadened the treatment possibilities of NK cell therapy in BC. Integrating CAR improves cytotoxic activity, persistence, and tumor targeting while reducing side effects like cytokine release syndrome (CRS).[151] CAR-NK cells use natural killing mechanisms and are generally safer than CAR-T cells. They target BC-specific antigens like HER2, CD44v6, B7-H6, tissue factor (TF), EGFR, and PD-L1.[85] For instance, CD44v6-targeted CAR-NK cells demonstrated strong cytotoxicity in models of TNBC.[84]

Next-generation CAR-NK strategies aim to overcome inhibitory signaling and exhaustion. Checkpoint receptors like PD-1, LAG-3, TIM-3, TIGIT, KLRG1, and NKG2A negatively affect NK function. Blocking or deleting these receptors boosts cytotoxicity, metabolic health, and effector functions. Additionally, cytokine-induced CIS protein enhances survival and function.[152–154] Combining CAR-NK therapy with immune checkpoint inhibitors (ICIs), especially those that target the PD-1/PD-L1 and CTLA-4 pathways, shows better effectiveness than using single treatments.[152,153,155] Engineered PD-L1-targeting t-haNK cells express both CARs and CD16 while maintaining their native NK receptors and perforin granules. This setup allows them to effectively target TNBC and other solid tumors.

Their activity relates to PD-L1 presence and is further enhanced by pre-treating with IFN-γ. In vivo, irradiated PD-L1 t-haNK cells reduced TNBC and bladder tumor growth, with additional support from N-803 and anti-PD-1 antibodies. These cells also effectively reduced myeloid-derived suppressor cells (MDSCs) when cultured with human peripheral blood mononuclear cells (PBMCs), showing their potential to alter the TME.[156,157]

CAR-NK cells that target HER2 have shown greater effectiveness against HER2-positive BC. They maintain their ability to kill cancer cells within the TME without harming normal tissues.[87,158] They can also impact the TME by reducing myeloid-derived suppressor cells (MDSCs) and counteracting TGF-β, galectin, MMP, and ADAM-mediated suppression.[86,156,159] Beyond direct immune modulation, proteolytic enzymes such as ADAM12 contribute to tumor progression by facilitating invadopodia formation and extracellular matrix remodeling, particularly under hypoxic conditions.[160] Therefore, combining CAR-NK therapy with inhibitors of ADAM-mediated shedding may further impair BC metastasis. CAR-NK methods have demonstrated activity against metastatic lesions, such as when EGFR-CAR NK-92 cells are combined with oncolytic HSV-1 in brain metastasis models of metastatic breast cancer (MBC).[161,162]

Tissue factor (TF)-CAR-NK cells, which often express CD16 to promote antibody-dependent killing, show strong effects against TNBC cell lines and patient-derived xenograft (PDX) models, both in vitro and in vivo. Their effectiveness increases when paired with L-ICON or checkpoint inhibitors (112, 140). This combination highlights the potential of merging CAR-NK therapies with checkpoint blockade to improve tumor responses while minimizing immune evasion.[116,163,164]

Table 4 summarizes selected clinical studies of CAR-T, adoptive cell transfer (ACT), NK, and CAR-NK cell therapies in BC, focusing on trials that test combinations with ICIs.

Despite the promising early research and clinical results, CAR-NK therapy faces challenges. These include limited ability to penetrate tumors, evasion by tumor cells, difficulties in expanding cells outside the body, and brief effectiveness in living organisms. Strategies that involve cytokine preconditioning and modifications to CAR can enhance NK cell movement and killing ability, establishing a strong foundation for future clinical advancements.[165,166] Overall, CAR-NK therapy represents an innovative approach in BC immunotherapy, with potential applications for treating resistant and metastatic cancers.

Oncolytic Virotherapy in BC with Immune Checkpoint Inhibitors

Oncolytic virotherapy (OV) is a promising treatment strategy in BC. It uses natural or genetically modified viruses to infect and destroy tumor cells while leaving healthy tissues unharmed.[167] OVs not only kill tumor cells directly but also improve the adaptive immune response against tumors. This makes them a good addition to ICIs.[168,169] The FDA-

Table 5 Oncolytic Viruses Evaluated in BC Clinical Trials, Including Combinations with Immune Checkpoint Inhibitors

Oncolytic Virus Candidate	Observed Clinical Effects in Breast Cancer	Reported Toxicities	ICI-Related Notes/Combinations
Adenoviridae – dsDNA			
Ad5/3-D24-GM-CSF	Tumor regression or stable disease in a subset of patients.	Mild flu-like symptoms; no severe adverse events.	GM-CSF enhances antigen presentation; may potentiate PD-1/PD-L1 blockade (no BC-specific ICI trial).[177]
Ad5-Δ24-GM-CSF	Disease stabilization; one complete radiologic response reported.	Fever, fatigue, mild injection-site discomfort.	Mechanistically synergistic with ICIs by increasing T-cell priming.[178]
RGD-4C (ICOVIR-7)	Early biochemical responses; mild to partial radiologic responses.	No clinically meaningful toxicities.	RGD-modified adenoviruses upregulate PD-L1, supporting ICI co-therapy rationale.[179]
ONYX-015 + Etanercept	Predominantly progressive disease in BC patients.	Low-grade fever; otherwise well tolerated.	No ICI combination tested.[180]
Herpesviridae – dsDNA			
T-VEC (Talimogene laherparepvec)	Mostly stable disease; limited objective responses.	Mild fever, fatigue, GI upset.	Known synergy with pembrolizumab in melanoma; BC trials not yet combining with ICIs.[181]
T-VEC + Neoadjuvant Chemotherapy	Enhanced pathologic response rates and improved 2-year DFS in TNBC.	Typical chemo-related AEs and mild viral-associated fever/chills.	Chemotherapy enhances tumor immunogenicity, potentially augmenting future OV–ICI responses.[182]
HF10 (Oncolytic HSV)	30–100% tumor necrosis in cutaneous/subcutaneous BC lesions.	No significant toxicity.	Increases intratumoral $CD8^+$ T cells; potential synergy with PD-1/PD-L1 inhibitors.[183]
Poxviridae – dsDNA			
VVDD (Double-deleted Vaccinia Virus)	Partial responses in advanced cancers; tumor-selective replication observed.	Mostly mild symptoms; one serious pain event.	Vaccinia viruses frequently combined with ICIs in other cancers; provides rationale for BC expansion.[184]
Pelareorep (Reovirus Type 3)	Partial responses in several cases; improved OS in metastatic BC cohort.	Grade 1–2 toxicities; well tolerated.	Strongest evidence for OV–ICI synergy: pelareorep boosts PD-L1 expression, TIL infiltration, and type-I IFN signaling.[185]
Pelareorep + Paclitaxel	OS improvement despite no PFS benefit.	Mild and manageable adverse events.	Under evaluation with pembrolizumab; enhances antigen spreading and T-cell priming.[186]
Paramyxoviridae – (–)ssRNA			
PV701 (Newcastle Disease Virus)	One BC patient maintained stable disease >6 months.	Mild flu-like symptoms and injection-site inflammation.	NDV activates both NK and T cells; preclinical synergy with ICIs documented.[187]

Abbreviations: AEs, adverse events; BC, breast cancer; DFS, disease-free survival; dsDNA, double-stranded DNA; GI, gastrointestinal; GM-CSF, granulocyte-macrophage colony-stimulating factor; HSV, herpes simplex virus; ICI, immune checkpoint inhibitor; IFN, interferon; NDV, Newcastle disease virus; NK, natural killer; OS, overall survival; OV, oncolytic virus; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; RGD, arginine-glycine-aspartate; ssRNA, single-stranded RNA; T-VEC, talimogene laherparepvec; TIL, tumor-infiltrating lymphocyte; TNBC, triple-negative breast cancer.

approved Talimogene laherparepvec (T-VEC), a modified HSV-1 virus that expresses GM-CSF, has shown clinical effectiveness in advanced melanoma. When combined with checkpoint blockers, it significantly improves response rates.[170–172]

OVs induce immunogenic cell death (ICD), release pro-inflammatory cytokines and chemokines, and promote immune cell entry into the TME. These actions help fight immune suppression.[173,174] OVs can also be used to deliver genes, further boosting immune activation. Preclinical and clinical studies show that combining OVs with traditional treatments increases OV-induced apoptosis (Figure 2).

Coxsackieviruses (CV) are a group of positive-sense ssRNA enteroviruses that show potential as OVs in TNBC. Engineered strains like CV-B3 and CVA21 target tumor cells specifically, sparing normal tissues, and have shown anti-tumor effectiveness in lab settings and mouse models of TNBC.[175] Several clinical trials are testing the combinations of OVs and ICIs in BC. For instance, the KEYNOTE-200 (STORM) trial is examining CVA21 along with pembrolizumab

in metastatic TNBC. The results aim to clarify dosing, safety, and effectiveness.[176] Table 5 summarizes the oncolytic viruses investigated in clinical trials for BC, including studies evaluating their therapeutic potential alone or in combination with ICIs.

OVs combined with CAR-NK cells further boost anti-tumor responses. OV-IL15C paired with EGFR-CAR NK cells improved tumor control and survival while enhancing the presence of CD8+ T and NK cells within tumors in preclinical studies.[188] Similarly, the combination of EGFR-CAR NK cells and oncolytic HSV-1 effectively targeted brain metastases from BC, leading to longer survival in mouse models.[161]

Researchers have also explored combination therapies with standard treatments. Measles virus modified to carry BNiP39 was used with paclitaxel to induce death in TNBC cells.[189] Moreover, T-VEC combined with neoadjuvant chemotherapy (doxorubicin and cyclophosphamide) increased tumor-infiltrating lymphocytes (TILs) and achieved a 55% complete response rate in TNBC patients.[190] Chimeric poxviruses carrying anti-PD-L1 antibodies, such as CF33-hNIS-F14.5, altered the TME by increasing CD8+ T-cell infiltration and enhancing ICD in TNBC models.[191] Further clinical evidence supports the combination of oncolytic virotherapy with standard treatments. Soliman et al conducted a phase I trial evaluating Talimogene Laherparepvec (T-VEC) in combination with neoadjuvant chemotherapy (doxorubicin and cyclophosphamide) in patients with nonmetastatic TNBC.[192] This combination was found to be safe and feasible, with evidence of immune activation and tumor regression, supporting further clinical development of T-VEC plus chemotherapy in TNBC.[192] In parallel, Chaurasiya et al developed CF33-hNIS-ΔF14.5, an oncolytic poxvirus engineered to express an anti-PD-L1 antibody.[88] This novel construct not only directly infected and killed tumor cells but also favorably modulated the tumor immune microenvironment by increasing CD8+ T-cell infiltration and enhancing immunogenic cell death, leading to synergistic antitumor effects when combined with immune checkpoint inhibitors in preclinical TNBC models.[88]

The combination of OVs with CAR-T therapy also shows promise; oncolytic adenoviruses delivering IL-15 and RANTES improved CAR-T cell movement, persistence, and effectiveness within the TME.[89–92] These findings highlight OVs as powerful enhancers that work well with ICIs, CAR-NK, and CAR-T therapies in BC.

Exosomes in BC Therapy with ICIs

Exosomes can be viewed through three conceptual lenses in the context of checkpoint blockade: (I) as delivery vehicles for ICIs or antigens, (II) as circulating biomarkers for treatment response or resistance, and (III) as direct immunomodulators that reprogram the TME. In this section, we focus on their role as immunomodulators that can be combined with ICIs.

As immunomodulators, tumor-derived exosomes inhibit CD8+ and CD4+ T cells, reduce NK cell activity, and promote the activation of myeloid-derived suppressor cells (MDSCs) through miRNAs like miR-9 and miR-181a, thereby helping the tumor evade immune detection.[193–196] Beyond their direct immunomodulatory effects, exosomes also reflect the molecular characteristics of their cells of origin and have been implicated in cancer progression and therapy resistance. Mimeault and Batra highlighted that exosomes derived from cancer stem/progenitor cells carry specific molecular biomarkers associated with tumor progression, metastasis, and treatment resistance in aggressive cancers, including breast cancer.[93] Bae et al further demonstrated that both cancerous and non-cancerous cell-derived exosomes can regulate the anti-tumor response within the tumor microenvironment by modulating immune cell functions and cytokine profiles.[94] In the context of BC immunotherapy, exosome-based platforms have also been explored as cancer vaccines. Conversely, exosomes from dendritic cells (DCs) presenting MHC I/II-loaded peptides activate cytotoxic T lymphocytes (CTLs) and helper T cells, improving antitumor responses.[95,197]

CAR-T cell-derived exosomes carry RN7SL1 RNA, which activates RIG-I/MDA5 pathways in immune cells, promoting CAR-T cell growth and antitumor activity while limiting MDSC expansion.[96,97]

Exosomes from CAR-T or CAR-NK cells offer unique advantages as immunomodulators, causing little off-target damage and avoiding cytokine release syndrome (CRS). Trastuzumab- or cetuximab-scFv-expressing CAR-T exosomes slow tumor growth in HER2- or EGFR-positive BC models, and mesothelin-targeted exosomes effectively target TNBC.[98,99] CAR-NK-derived exosomes containing perforin and granzyme can cross the blood-brain barrier and target HER2-positive BC brain metastases when modified with transferrin receptor-binding peptides (T7).[100,101] Overall,

exosome-based immunomodulation, especially when combined with immune checkpoint inhibitors, shows promise for overcoming immune evasion and improving BC immunotherapy.[198–200]

Clinical Positioning of CAR-T, CAR-NK, and Exosome Platforms

The clinical translation of cellular and cell-free platforms for BC immunotherapy requires careful consideration of their respective safety profiles, persistence, manufacturing complexity, and clinical maturity. Autologous CAR-T cells, while capable of inducing potent and durable antitumor responses, carry well-documented risks of CRS, immune effector cell-associated neurotoxicity syndrome (ICANS), and on-target off-tumor toxicity.[115] Although approved for hematologic malignancies, CAR-T therapy remains in early-phase development for breast cancer, as solid tumors present unique barriers including limited infiltration and an immunosuppressive microenvironment.[135] In contrast, CAR-NK cells offer a safer alternative with lower CRS risk and intrinsic MHC-independent cytotoxicity,[159] yet their limited in vivo persistence often necessitates repeated dosing, and manufacturing complexity remains moderate.[153] Exosome-based platforms derived from CAR-T or CAR-NK cells represent a cell-free approach with minimal toxicity and the unique ability to cross the blood-brain barrier, but scalability, standardization, and batch-to-batch consistency pose unresolved challenges.[200] From a clinical maturity perspective, CAR-T is established in hematologic cancers but experimental in breast cancer, whereas CAR-NK and exosome platforms remain predominantly preclinical or in phase I trials.[84] Regarding persistence, CAR-T cells endure for months to years, CAR-NK for weeks, and exosomes for days. Collectively, safety is highest for exosomes and CAR-NK, followed by CAR-T, while manufacturing complexity follows the reverse order. These distinctions are essential for positioning each platform within the evolving landscape of BC immunotherapy.

Conclusion

Immune checkpoint inhibitors have transformed BC treatment, particularly for aggressive subtypes such as TNBC. Combining ICIs with chemotherapy, targeted therapies, CAR-T cells, NK cells, oncolytic viruses, and exosome-based platforms has improved response rates, progression-free survival, and overall survival in clinical trials. Biomarker-driven patient selection most notably PD-L1 expression and TIL density is essential for optimizing outcomes and represents a step toward precision oncology in BC.

Despite these advances, significant barriers remain. Primary and acquired resistance to ICIs, driven by MHC loss, antigen presentation defects, and metabolic competition within the tumor microenvironment, limits durable responses. Toxicity concerns, including immune-related adverse events (irAEs) and organ-specific complications from combination regimens, require careful risk-benefit assessment. Furthermore, the limited efficacy of ICIs in hormone receptor-positive BC and the negative results of trials such as IMpassion131 underscore that not all patients or combinations benefit equally.

From a translational perspective, integrating ICIs with cellular platforms (CAR-T, CAR-NK) and biologic agents (oncolytic viruses, exosomes) holds promise for reshaping treatment algorithms, particularly for resistant and metastatic disease. However, clinical maturity varies widely: CAR-T therapy remains early-phase in BC, while CAR-NK and exosome approaches are largely preclinical. Cost-effectiveness and real-world applicability also remain unresolved challenges that will influence adoption into routine practice.

In conclusion, the evolving landscape of BC immunotherapy offers hope for more effective and personalized treatments. Yet, realizing this potential will require continued research into resistance mechanisms, optimized combination strategies, rigorous biomarker validation, and prospective evaluation of long-term benefits and toxicities. Only then can these advances translate into meaningful improvements in patient outcomes and durable remission.

Data Sharing Statement

All data supporting the findings of this study are included within this published article. No additional datasets were generated or analyzed during the current study (Not applicable).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare they have no conflicts of interest.

References

1. Ohl K, Tenbrock K, Kipp M. Oxidative stress in multiple sclerosis: central and peripheral mode of action. *Exp Neurol*. 2016;277:58–67. doi:10.1016/j.expneurol.2015.11.010
2. Bahrin NWS, Matusin SNI, Mustapa A, Huat LZ, Perera S, Hamid M. Exploring the effectiveness of molecular subtypes, biomarkers, and genetic variations as first-line treatment predictors in Asian breast cancer patients: a systematic review and meta-analysis. *Syst Rev*. 2024;13(1):100. doi:10.1186/s13643-024-02520-5
3. Di Palma S, Koliou P, Simonovic A, et al. Breast cancer molecular subtyping in practice: a real-world study of the APIS breast cancer subtyping assay in a consecutive series of breast core biopsies. *Int J Mol Sci*. 2024;25(5):2616. doi:10.3390/ijms25052616
4. Berg T, Jensen MB, Celik A, et al. Molecular subtyping improves breast cancer diagnosis in the Copenhagen Breast Cancer Genomics Study. *JCI Insight*. 2024;9(7). doi:10.1172/jci.insight.178114.
5. Han E, Choi HY, Kwon HJ, et al. Characterization of tumor-infiltrating lymphocytes and their spatial distribution in triple-negative breast cancer. *Breast Cancer Res*. 2024;26(1):180. doi:10.1186/s13058-024-01932-4
6. Czajka-Francuz P, Prendes MJ, Mankan A, et al. Mechanisms of immune modulation in the tumor microenvironment and implications for targeted therapy. *Front Oncol*. 2023;13:1200646. doi:10.3389/fonc.2023.1200646
7. Chow A, Perica K, Klebanoff CA, Wolchok JD. Clinical implications of T cell exhaustion for cancer immunotherapy. *Nat Rev Clin Oncol*. 2022;19(12):775–790. doi:10.1038/s41571-022-00689-z
8. Teschendorff AE, Miremadi A, Pinder SE, Ellis IO, Caldas C. An immune response gene expression module identifies a good prognosis subtype in estrogen receptor negative breast cancer. *Genome Biol*. 2007;8(8):R157. doi:10.1186/gb-2007-8-8-r157
9. Schneider BJ, Naidoo J, Santomasso BD, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: ASCO guideline update. *J Clin Oncol*. 2021;39(36):4073–4126. doi:10.1200/JCO.21.01440
10. Haslam A, Gill J, Prasad V. Estimation of the percentage of US patients with cancer who are eligible for immune checkpoint inhibitor drugs. *JAMA Network Open*. 2020;3(3):e200423. doi:10.1001/jamanetworkopen.2020.0423
11. Wong RS, Ong RJ, Lim JS. Immune checkpoint inhibitors in breast cancer: development, mechanisms of resistance and potential management strategies. *Cancer Drug Resist*. 2023;6(4):768–787. doi:10.20517/cdr.2023.58
12. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. *Nat Rev Immunol*. 2018;18(3):153–167. doi:10.1038/nri.2017.108
13. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359(6382):1350–1355. doi:10.1126/science.aar4060
14. Marei HE, Hasan A, Pozzoli G, Cenciarelli C. Cancer immunotherapy with immune checkpoint inhibitors (ICIs): potential, mechanisms of resistance, and strategies for reinvigorating T cell responsiveness when resistance is acquired. *Cancer Cell Int*. 2023;23(1):64. doi:10.1186/s12935-023-02902-0
15. Mittendorf EA, Philips AV, Meric-Bernstam F, et al. PD-L1 expression in triple-negative breast cancer. *Cancer Immunol Res*. 2014;2(4):361–370. doi:10.1158/2326-6066.CIR-13-0127
16. Nanda R, Chow LQ, Dees EC, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: phase Ib KEYNOTE-012 study. *J Clin Oncol*. 2016;34(21):2460–2467. doi:10.1200/JCO.2015.64.8931
17. Adams S, Schmid P, Rugo HS, 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(3):397–404. doi:10.1093/annonc/mdy517
18. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and Nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med*. 2018;379(22):2108–2121. doi:10.1056/NEJMoa1809615
19. Schmid P, Turner NC, Barrios CH, et al. First-line ipatasertib, atezolizumab, and taxane triplet for metastatic triple-negative breast cancer: clinical and biomarker results. *Clin Cancer Res*. 2024;30(4):767–778. doi:10.1158/1078-0432.CCR-23-2084
20. Dirix LY, Takacs I, Jerusalem G, 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 Treat*. 2018;167(3):671–686. doi:10.1007/s10549-017-4537-5
21. Domchek SM, Postel-Vinay S, Im SA, 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(9):1155–1164. doi:10.1016/S1470-2045(20)30324-7
22. Rugo HS, Kabos P, Beck JT, et al. Abemaciclib in combination with pembrolizumab for HR+, HER2- metastatic breast cancer: phase 1b study. *NPJ Breast Cancer*. 2022;8(1):118. doi:10.1038/s41523-022-00482-2

23. Winer EP, Lipatov O, Im SA, 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(4):499–511. doi:10.1016/S1470-2045(20)30754-3
24. Santa-Maria CA, Kato T, Park JH, et al. A pilot study of durvalumab and tremelimumab and immunogenomic dynamics in metastatic breast cancer. *Oncotarget*. 2018;9(27):18985–18996. doi:10.18632/oncotarget.24867
25. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*. 2014;515 (7528):568–571. doi:10.1038/nature13954
26. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357 (6349):409–413. doi:10.1126/science.aan6733
27. Tolaney SM, Barroso-Sousa R, Keenan T, 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(10):1598–1605. doi:10.1001/jamaoncol.2020.3524
28. Goel S, Wang Q, watt AC, et al. Overcoming therapeutic resistance in HER2-positive breast cancers with CDK4/6 inhibitors. *Cancer Cell*. 2016;29(3):255–269. doi:10.1016/j.ccell.2016.02.006
29. Deng J, Wang ES, Jenkins RW, et al. CDK4/6 inhibition augments antitumor immunity by enhancing T-cell activation. *Cancer Discov*. 2018;8 (2):216–233. doi:10.1158/2159-8290.CD-17-0915
30. Yuan Y, Lee JS, Yost SE, et al. Phase I/II trial of palbociclib, pembrolizumab and letrozole in patients with hormone receptor-positive metastatic breast cancer. *Eur J Cancer*. 2021;154:11–20. doi:10.1016/j.ejca.2021.05.035
31. Loi S, Giobbie-Hurder A, Gombos A, 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(3):371–382. doi:10.1016/S1470-2045(18)30812-X
32. Emens LA, Esteva FJ, Beresford M, et al. Trastuzumab emtansine plus atezolizumab versus trastuzumab emtansine plus placebo in previously treated, HER2-positive advanced breast cancer (KATE2): a Phase 2, multicentre, randomised, double-blind trial. *Lancet Oncol*. 2020;21 (10):1283–1295. doi:10.1016/S1470-2045(20)30465-4
33. Emens LA, Adams S, Barrios CH, 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(8):983–993. doi:10.1016/j.annonc.2021.05.355
34. Miles D, Gligorov J, André F, 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 (8):994–1004. doi:10.1016/j.annonc.2021.05.801
35. Cortés J, André F, Gonçalves A, et al. IMpassion132 Phase III trial: atezolizumab and chemotherapy in early relapsing metastatic triple-negative breast cancer. *Future Oncol*. 2019;15(17):1951–1961. doi:10.2217/fon-2019-0059
36. Dent R, André F, Gonçalves A, et al. IMpassion132 double-blind randomised phase III trial of chemotherapy with or without atezolizumab for early relapsing unresectable locally advanced or metastatic triple-negative breast cancer. *Ann Oncol*. 2024;35(7):630–642. doi:10.1016/j.annonc.2024.04.001
37. Zhang L, Yang L, Ge Y, et al. Neoadjuvant anlotinib/sintilimab plus chemotherapy in triple-negative breast cancer (NeoSACT): phase 2 trial. *Cell Rep Med*. 2025;6(7):102193. doi:10.1016/j.xcrm.2025.102193
38. Tiberi E, Parisi A, Pistelli M, et al. Immunotherapy in triple-negative breast cancer. *Oncol Ther*. 2025;13(3):547–575. doi:10.1007/s40487-025-00346-2
39. André T, Berton D, Curigliano G, et al. Antitumor activity and safety of dostarlimab monotherapy in patients with mismatch repair deficient solid tumors: a nonrandomized controlled trial. *JAMA Network Open*. 2023;6(11):e2341165. doi:10.1001/jamanetworkopen.2023.41165
40. Conte PF, Dieci MV, Bisagni G, et al. A-BRAVE trial: a phase III randomized trial with anti-PD-L1 avelumab in high-risk triple-negative early breast cancer patients. *Ann Oncol*. 2025;36(12):1492–1502. doi:10.1016/j.annonc.2025.08.005
41. Schmid P, Cortes J, Dent R, et al. Event-free survival with pembrolizumab in early triple-negative breast cancer. *N Engl J Med*. 2022;386 (6):556–567. doi:10.1056/NEJMoa2112651
42. Mittendorf EA, Zhang H, Barrios CH, 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(10257):1090–1100. doi:10.1016/S0140-6736(20)31953-X
43. Gianni L, Huang CS, Egle D, 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 (5):534–543. doi:10.1016/j.annonc.2022.02.004
44. Loibl S, Untch M, Burchardi N, 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 (8):1279–1288. doi:10.1093/annonc/mdz158
45. Isaacs C, Nanda R, Chien J, et al. Abstract GS5-03: evaluation of anti-PD-1 Cemiplimab plus anti-LAG-3 REGN3767 in early-stage, high-risk HER2-negative breast cancer: results from the neoadjuvant I-SPY 2 TRIAL. *Cancer Res*. 2023;83(5_Supplement):GS5–03–GS5. doi:10.1158/1538-7445.SABCS22-GS5-03
46. Du X, Tang F, Liu M, et al. A reappraisal of CTLA-4 checkpoint blockade in cancer immunotherapy. *Cell Res*. 2018;28(4):416–432. doi:10.1038/s41422-018-0011-0
47. Yu H, Yang J, Jiao S, Li Y, Zhang W, Wang J. Cytotoxic T lymphocyte antigen 4 expression in human breast cancer: implications for prognosis. *Cancer Immunol Immunother*. 2015;64(7):853–860. doi:10.1007/s00262-015-1696-2
48. Vonderheide RH, LoRusso PM, Khalil M, 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(13):3485–3494. doi:10.1158/1078-0432.CCR-10-0505
49. Navarrete-Bernal MGC, Cervantes-Badillo MG, Martínez-Herrera JF, et al. Biological landscape of triple negative breast cancers expressing CTLA-4. *Front Oncol*. 2020;10:1206. doi:10.3389/fonc.2020.01206
50. Wesolowski J, Tankiewicz-Kwedlo A, Pawlak D. Modern Immunotherapy in the Treatment of Triple-Negative Breast Cancer. *Cancers*. 2022;14 (16):3860. doi:10.3390/cancers14163860

51. McArthur HL, Diab A, Page DB, et al. A pilot study of preoperative single-dose ipilimumab and/or cryoablation in women with early-stage breast cancer with comprehensive immune profiling. *Clin Cancer Res*. 2016;22(23):5729–5737. doi:10.1158/1078-0432.CCR-16-0190
52. Banys-Paluchowski M, Schütz F, Ruckhäberle E, Krawczyk N, Fehm T. Metronomic chemotherapy for metastatic breast cancer - a systematic review of the literature. *Geburtshilfe Frauenheilkd*. 2016;76(5):525–534. doi:10.1055/s-0042-105871
53. Loi S, Francis P, Zdenkowski N, et al. Neoadjuvant ipilimumab and nivolumab in combination with paclitaxel following anthracycline-based chemotherapy in patients with treatment resistant early-stage triple-negative breast cancer (TNBC): a single-arm phase 2 trial. *J Clin Oncol*. 2022;40:602. doi:10.1200/JCO.2022.40.16_suppl.602
54. Parra K, Valenzuela P, Lerma N, et al. Impact of CTLA-4 blockade in conjunction with metronomic chemotherapy on preclinical breast cancer growth. *Br J Cancer*. 2017;116(3):324–334. doi:10.1038/bjc.2016.429
55. Long L, Zhang X, Chen F, et al. The promising immune checkpoint LAG-3: from tumor microenvironment to cancer immunotherapy. *Genes Cancer*. 2018;9(5–6):176–189. doi:10.18632/genesandcancer.180
56. Kreidieh FY, Tawbi HA. The introduction of LAG-3 checkpoint blockade in melanoma: immunotherapy landscape beyond PD-1 and CTLA-4 inhibition. *Ther Adv Med Oncol*. 2023;15:17588359231186027. doi:10.1177/17588359231186027
57. Xia ZA, Lu C, Pan C, et al. The expression profiles of signature genes from CD103(+)LAG3(+) tumour-infiltrating lymphocyte subsets predict breast cancer survival. *BMC Med*. 2023;21(1):268. doi:10.1186/s12916-023-02960-1
58. Wang M, Wei Y, Li Y, et al. Targeting breast cancer with a combination of DNT and LAG3 checkpoint blockage and its mechanism. *Immun Inflamm Dis*. 2022;10(8):e626. doi:10.1002/iid3.626
59. Zimmer AS, Gillard M, Lipkowitz S, Lee JM. Update on PARP inhibitors in breast cancer. *Curr Treat Options Oncol*. 2018;19(5):21. doi:10.1007/s11864-018-0540-2
60. Burugu S, Gao D, Leung S, Chia SK, Nielsen TO. LAG-3+ tumor infiltrating lymphocytes in breast cancer: clinical correlates and association with PD-1/PD-L1+ tumors. *Ann Oncol*. 2017;28(12):2977–2984. doi:10.1093/annonc/mdx557
61. Lord CJ, Ashworth A. PARP inhibitors: synthetic lethality in the clinic. *Science*. 2017;355(6330):1152–1158. doi:10.1126/science.aam7344
62. Fong PC, Yap TA, Boss DS, et al. Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. *J Clin Oncol*. 2010;28(15):2512–2519. doi:10.1200/JCO.2009.26.9589
63. Pusztai L, Yau C, Wolf DM, 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(7):989–98.e5. doi:10.1016/j.ccell.2021.05.009
64. Vinayak S, Tolaney SM, Schwartzberg L, 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(8):1132–1140. doi:10.1001/jamaoncol.2019.1029
65. Garrido MA, Rodriguez T, Zinchenko S, et al. HLA class I alterations in breast carcinoma are associated with a high frequency of the loss of heterozygosity at chromosomes 6 and 15. *Immunogenetics*. 2018;70(10):647–659. doi:10.1007/s00251-018-1074-2
66. Nomura T, Huang WC, Zhau HE, Josson S, Mimata H, Chung LW. β2-Microglobulin-mediated signaling as a target for cancer therapy. *Anticancer Agents Med Chem*. 2014;14(3):343–352. doi:10.2174/18715206113139990092
67. Doi T, Shitara K, Naito Y, et al. Safety, pharmacokinetics, and antitumour activity of trastuzumab deruxtecan (DS-8201), a HER2-targeting antibody-drug conjugate, in patients with advanced breast and gastric or gastro-oesophageal tumours: a phase 1 dose-escalation study. *Lancet Oncol*. 2017;18(11):1512–1522. doi:10.1016/S1470-2045(17)30604-6
68. Grinda T, Rassy E, Pistilli B. Antibody-drug conjugate revolution in breast cancer: the road ahead. *Curr Treat Options Oncol*. 2023;24(5):442–465. doi:10.1007/s11864-023-01072-5
69. Ferraro E, Drago JZ, Modi S. Implementing antibody-drug conjugates (ADCs) in HER2-positive breast cancer: state of the art and future directions. *Breast Cancer Res*. 2021;23(1):84. doi:10.1186/s13058-021-01459-y
70. Ott PA, Dotti G, Yee C, Goff SL. An update on adoptive T-cell therapy and neoantigen vaccines. *Am Soc Clin Oncol Educ Book*. 2019;39:e70–e8. doi:10.1200/EDBK_238001
71. Wang K, Xu J, Zhang T, Xue D. Tumor-infiltrating lymphocytes in breast cancer predict the response to chemotherapy and survival outcome: a meta-analysis. *Oncotarget*. 2016;7(28):44288–44298. doi:10.18632/oncotarget.9988
72. Zacharakis N, Chinnasamy H, Black M, et al. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. *Nat Med*. 2018;24(6):724–730. doi:10.1038/s41591-018-0040-8
73. Assadipour Y, Zacharakis N, Crystal JS, et al. Characterization of an immunogenic mutation in a patient with metastatic triple-negative breast cancer. *Clin Cancer Res*. 2017;23(15):4347–4353. doi:10.1158/1078-0432.CCR-16-1423
74. Pilipow K, Darwich A, Losurdo A. T-cell-based breast cancer immunotherapy. *Semin Cancer Biol*. 2021;72:90–101. doi:10.1016/j.semcancer.2020.05.019
75. Zhang Y, Wang S, Yang B, Lu S, Du Y, Liu H. Adjuvant treatment for triple-negative breast cancer: a retrospective study of immunotherapy with autologous cytokine-induced killer cells in 294 patients. *Cancer Biol Med*. 2019;16(2):350–360. doi:10.20892/j.issn.2095-3941.2018.0378
76. Hu J, Hu J, Liu X, Hu C, Li M, Han W. Effect and safety of cytokine-induced killer (CIK) cell immunotherapy in patients with breast cancer: a meta-analysis. *Medicine*. 2017;96(42):e8310. doi:10.1097/MD.0000000000008310
77. He J, Xiong X, Yang H, et al. Defined tumor antigen-specific T cells potentiate personalized TCR-T cell therapy and prediction of immunotherapy response. *Cell Res*. 2022;32(6):530–542. doi:10.1038/s41422-022-00627-9
78. Zhou ZQ, Zhao JJ, Pan QZ, et al. PD-L1 expression is a predictive biomarker for CIK cell-based immunotherapy in postoperative patients with breast cancer. *J Immunother Cancer*. 2019;7(1):228. doi:10.1186/s40425-019-0696-8
79. Yang YH, Liu JW, Lu C, Wei JF. CAR-T cell therapy for breast cancer: from basic research to clinical application. *Int J Biol Sci*. 2022;18(6):2609–2626. doi:10.7150/ijbs.70120
80. Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat Biotechnol*. 2018;36(4):346–351. doi:10.1038/nbt.4086
81. Tan G, Spillane KM, Maher J. The role and regulation of the NKG2D/NKG2D ligand system in cancer. *Biology*. 2023;12(8). doi:10.3390/biology12081079
82. Tanoue K, Rosewell Shaw A, Watanabe N, et al. Armed oncolytic adenovirus-expressing PD-L1 mini-body enhances antitumor effects of chimeric antigen receptor T cells in solid tumors. *Cancer Res*. 2017;77(8):2040–2051. doi:10.1158/0008-5472.CAN-16-1577

83. Carnevale J, Shifrut E, Kale N, et al. RASA2 ablation in T cells boosts antigen sensitivity and long-term function. *Nature*. 2022;609 (7925):174–182. doi:10.1038/s41586-022-05126-w
84. Zhuang X, Long EO. NK cells equipped with a chimeric antigen receptor that overcomes inhibition by HLA Class I for adoptive transfer of CAR-NK cells. *Front Immunol*. 2022;13:840844. doi:10.3389/fimmu.2022.840844
85. Kistler M, Nangia C, To C, et al. Abstract P5-04-02: 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(4_Supplement):P5–04–2–P5—2. doi:10.1158/1538-7445.SABCS19-P5-04-02
86. Xia W, Chen J, Hou W, et al. Engineering a HER2-CAR-NK cell secreting soluble programmed cell death protein with superior antitumor efficacy. *Int J Mol Sci*. 2023;24(7):6843. doi:10.3390/ijms24076843
87. Fabian KP, Padget MR, Donahue RN, et al. PD-L1 targeting high-affinity NK (t-haNK) cells induce direct antitumor effects and target suppressive MDSC populations. *J Immunother Cancer*. 2020;8(1):e000450. doi:10.1136/jitc-2019-000450
88. Chaurasiya S, Yang A, Kang S, et al. Oncolytic poxvirus CF33-hNIS-ΔF14.5 favorably modulates tumor immune microenvironment and works synergistically with anti-PD-L1 antibody in a triple-negative breast cancer model. *Oncoimmunology*. 2020;9(1):1729300. doi:10.1080/2162402X.2020.1729300
89. Garza-Morales R, Gonzalez-Ramos R, Chiba A, et al. Temozolomide enhances triple-negative breast cancer virotherapy in vitro. *Cancers*. 2018;10(5):144. doi:10.3390/cancers10050144
90. Li H, Wu Y, Chen Y, et al. Overcoming temozolomide resistance in glioma: recent advances and mechanistic insights. *Acta Neuropathol Commun*. 2025;13(1):126. doi:10.1186/s40478-025-02046-4
91. Nishio N, Diaconu I, Liu H, et al. Armed oncolytic virus enhances immune functions of chimeric antigen receptor-modified T cells in solid tumors. *Cancer Res*. 2014;74(18):5195–5205. doi:10.1158/0008-5472.CAN-14-0697
92. Park AK, Fong Y, Kim SI, et al. Effective combination immunotherapy using oncolytic viruses to deliver CAR targets to solid tumors. *Sci Transl Med*. 2020;12(559). doi:10.1126/scitranslmed.aaz1863.
93. Mimeault M, Batra SK. Molecular biomarkers of cancer stem/progenitor cells associated with progression, metastases, and treatment resistance of aggressive cancers. *Cancer Epidemiol Biomarkers Prev*. 2014;23(2):234–254. doi:10.1158/1055-9965.EPI-13-0785
94. Bae S, Brumbaugh J, Bonavida B. Exosomes derived from cancerous and non-cancerous cells regulate the anti-tumor response in the tumor microenvironment. *Genes Cancer*. 2018;9(3–4):87–100. doi:10.18632/genesandcancer.172
95. Anticoli S, Manfredi F, Chiozzini C, et al. An exosome-based vaccine platform imparts cytotoxic T lymphocyte immunity against viral antigens. *Biotechnol J*. 2018;13(4):e1700443. doi:10.1002/biot.201700443
96. Hsu DH, Paz P, Villaflor G, et al. Exosomes as a tumor vaccine: enhancing potency through direct loading of antigenic peptides. *J Immunother*. 2003;26(5):440–450. doi:10.1097/00002371-200309000-00007
97. Li R, Chibbar R, Xiang J. Novel EXO-T vaccine using polyclonal CD4(+) T cells armed with HER2-specific exosomes for HER2-positive breast cancer. *Onco Targets Ther*. 2018;11:7089–7093. doi:10.2147/OTT.S184898
98. Nutt WS, Srivastava S. Special delivery! CAR-T cells transport RN7SL1 to the tumor microenvironment. *Trends Mol Med*. 2021;27 (11):1019–1021. doi:10.1016/j.molmed.2021.09.002
99. Johnson LR, Lee DY, Eacret JS, Ye D, June CH, Minn AJ. The immunostimulatory RNA RN7SL1 enables CAR-T cells to enhance autonomous and endogenous immune function. *Cell*. 2021;184(19):4981–95.e14. doi:10.1016/j.cell.2021.08.004
100. Wang Z, Zhou G, Risu N, et al. Lenalidomide enhances CAR-T cell activity against solid tumor cells. *Cell Transplant*. 2020;29:963689720920825. doi:10.1177/0963689720920825
101. Yang P, Cao X, Cai H, et al. The exosomes derived from CAR-T cell efficiently target mesothelin and reduce triple-negative breast cancer growth. *Cell Immunol*. 2021;360:104262. doi:10.1016/j.cellimm.2020.104262
102. Sommaggio R, Cappuzzello E, Dalla Pietà A, et al. Adoptive cell therapy of triple negative breast cancer with redirected cytokine-induced killer cells. *Oncoimmunology*. 2020;9(1):1777046. doi:10.1080/2162402X.2020.1777046
103. Ren X, Ma W, Lu H, et al. Modification of cytokine-induced killer cells with chimeric antigen receptors (CARs) enhances antitumor immunity to epidermal growth factor receptor (EGFR)-positive malignancies. *Cancer Immunol Immunother*. 2015;64(12):1517–1529. doi:10.1007/s00262-015-1757-6
104. Liu Y, Zhou Y, Huang KH, et al. EGFR-specific CAR-T cells trigger cell lysis in EGFR-positive TNBC. *Aging*. 2019;11(23):11054–11072. doi:10.18632/aging.102510
105. Roselli E, Frieling JS, Thorner K, Ramello MC, Lynch CC, Abate-Daga D. CAR-T engineering: optimizing signal transduction and effector mechanisms. *BioDrugs*. 2019;33(6):647–659. doi:10.1007/s40259-019-00384-z
106. Somboonpatarakun C, Phanthaphol N, Suwanchiwasiri K, et al. Cytotoxicity of fourth-generation anti-Trop2 CAR-T cells against breast cancer. *Int Immunopharmacol*. 2024;129:111631. doi:10.1016/j.intimp.2024.111631
107. Malla M, Kumar Deshkmukh S, Wu S, et al. Mesothelin expression correlates with elevated inhibitory immune activity in patients with colorectal cancer. *Res Sq*. 2023. doi:10.21203/rs.3.rs-3787873/v1
108. Peña-López J, Gámez-Pozo A, Trilla-Fuertes L, et al. NKGD2 ligands (NKG2DLs) in breast cancer: in silico analysis and narrative review. *Int J Mol Sci*. 2026;27(4):1848. doi:10.3390/ijms27041848
109. Li D, Guo X, Yang K, et al. EpCAM-targeting CAR-T cell immunotherapy is safe and efficacious for epithelial tumors. *Sci Adv*. 2023;9(48): eadg9721. doi:10.1126/sciadv.adg9721
110. Whilding LM, Halim L, Draper B, et al. CAR T-cells targeting the integrin αvβ6 and co-expressing the chemokine receptor CXCR2 demonstrate enhanced homing and efficacy against several solid malignancies. *Cancers*. 2019;11(5):674. doi:10.3390/cancers11050674
111. Byrd TT, Fousek K, Pignata A, et al. TEM8/ANTXR1-specific CAR T cells as a targeted therapy for triple-negative breast cancer. *Cancer Res*. 2018;78(2):489–500. doi:10.1158/0008-5472.CAN-16-1911
112. Chen X, Habib S, Alexandru M, et al. Chondroitin Sulfate Proteoglycan 4 (CSPG4) as an emerging target for immunotherapy to treat melanoma. *Cancers*. 2024;16(19):3260. doi:10.3390/cancers16193260
113. An W, Kang JS, Oh S, Tu A. MST1R as a potential new target antigen of chimeric antigen receptor T cells to treat solid tumors. *Korean J Physiol Pharmacol*. 2023;27(3):241–256. doi:10.4196/kjpp.2023.27.3.241

114. Amaresan R, Gopal U. Cell surface GRP78: a potential mechanism of therapeutic resistant tumors. *Cancer Cell Int*. 2023;23(1):100. doi:10.1186/s12935-023-02931-9
115. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science*. 2018;359 (6382):1361–1365. doi:10.1126/science.aar6711
116. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. *Cancer Discov*. 2013;3(4):388–398. doi:10.1158/2159-8290.CD-12-0548
117. Chen N, Fang W, Zhan J, et al. Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-Driven NSCLC: implication for optional immune targeted therapy for NSCLC patients with EGFR mutation. *J Thorac Oncol*. 2015;10(6):910–923. doi:10.1097/JTO.0000000000000500
118. Grosser R, Cherkassky L, Chintala N, Adusumilli PS. Combination immunotherapy with CAR T cells and checkpoint blockade for the treatment of solid tumors. *Cancer Cell*. 2019;36(5):471–482. doi:10.1016/j.ccell.2019.09.006
119. Hu W, Zi Z, Jin Y, et al. CRISPR/Cas9-mediated PD-1 disruption enhances human mesothelin-targeted CAR T cell effector functions. *Cancer Immunol Immunother*. 2019;68(3):365–377. doi:10.1007/s00262-018-2281-2
120. Gargett T, Yu W, Dotti G, et al. GD2-specific CAR T cells undergo potent activation and deletion following antigen encounter but can be protected from activation-induced cell death by PD-1 blockade. *Mol Ther*. 2016;24(6):1135–1149. doi:10.1038/mt.2016.63
121. John LB, Devaud C, Duong CP, et al. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. *Clin Cancer Res*. 2013;19(20):5636–5646. doi:10.1158/1078-0432.CCR-13-0458
122. Hofmann L, Forschner A, Loquai C, et al. Cutaneous, gastrointestinal, hepatic, endocrine, and renal side-effects of anti-PD-1 therapy. *Eur J Cancer*. 2016;60:190–209. doi:10.1016/j.ejca.2016.02.025
123. Shivaji UN, Jeffery L, Gui X, et al. Immune checkpoint inhibitor-associated gastrointestinal and hepatic adverse events and their management. *Therap Adv Gastroenterol*. 2019;12:1756284819884196. doi:10.1177/1756284819884196
124. Giuffrida L, Sek K, Henderson MA, et al. IL-15 preconditioning augments CAR T cell responses to checkpoint blockade for improved treatment of solid tumors. *Mol Ther*. 2020;28(11):2379–2393. doi:10.1016/j.ymthe.2020.07.018
125. Cherkassky L, Morello A, Villena-Vargas J, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. *J Clin Invest*. 2016;126(8):3130–3144. doi:10.1172/JCI83092
126. Adusumilli PS, Zauderer MG, Rivière I, et al. A Phase I Trial of regional mesothelin-targeted CAR T-cell therapy in patients with malignant pleural disease, in combination with the Anti-PD-1 agent pembrolizumab. *Cancer Discov*. 2021;11(11):2748–2763. doi:10.1158/2159-8290.CD-21-0407
127. Agarwal S, Aznar MA, Rech AJ, et al. Deletion of the inhibitory co-receptor CTLA-4 enhances and invigorates chimeric antigen receptor T cells. *Immunity*. 2023;56(10):2388–407.e9. doi:10.1016/j.immuni.2023.09.001
128. Burga RA, Thorn M, Point GR, et al. Liver myeloid-derived suppressor cells expand in response to liver metastases in mice and inhibit the anti-tumor efficacy of anti-CEA CAR-T. *Cancer Immunol Immunother*. 2015;64(7):817–829. doi:10.1007/s00262-015-1692-6
129. Dees S, Ganesan R, Singh S, Grewal IS. Emerging CAR-T cell therapy for the treatment of triple-negative breast cancer. *Mol Cancer Ther*. 2020;19(12):2409–2421. doi:10.1158/1535-7163.MCT-20-0385
130. Boneva E, Shivarov V, Ivanova M. A concise review of the role of the NKG2D receptor and its ligands in cancer. *Immuno*. 2025;5(1):9. doi:10.3390/immuno5010009
131. Srivastava S, Furlan SN, Jaeger-Ruckstuhl CA, et al. Immunogenic chemotherapy enhances recruitment of CAR-T cells to lung tumors and improves antitumor efficacy when combined with checkpoint blockade. *Cancer Cell*. 2021;39(2):193–208.e10. doi:10.1016/j.ccell.2020.11.005
132. Li S, Siriwon N, Zhang X, et al. Enhanced cancer immunotherapy by chimeric antigen receptor-modified t cells engineered to secrete checkpoint inhibitors. *Clin Cancer Res*. 2017;23(22):6982–6992. doi:10.1158/1078-0432.CCR-17-0867
133. Rafiq S, Yeku OO, Jackson HJ, et al. Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. *Nat Biotechnol*. 2018;36(9):847–856. doi:10.1038/nbt.4195
134. Sadeghi S, Hojati Z, Tabatabaeian H. Cooverexpression of EpCAM and c-myc genes in malignant breast tumours. *J Genet*. 2017;96 (1):109–118. doi:10.1007/s12041-017-0748-0
135. Safarzadeh Kozani P, Safarzadeh Kozani P, Rahbarizadeh F, Khoshtinat Nikkhoi S. Strategies for dodging the obstacles in CAR T cell therapy. *Front Oncol*. 2021;11:627549. doi:10.3389/fonc.2021.627549
136. Chan IS, Knútsdóttir H, Ramakrishnan G, et al. Cancer cells educate natural killer cells to a metastasis-promoting cell state. *J Cell Biol*. 2020;219(9). doi:10.1083/jcb.202001134.
137. Eisele G, Wischhusen J, Mittelbronn M, et al. TGF-beta and metalloproteinases differentially suppress NKG2D ligand surface expression on malignant glioma cells. *Brain*. 2006;129(Pt 9):2416–2425. doi:10.1093/brain/awl205
138. Trinh TL, Kandell WM, Donatelli SS, et al. Immune evasion by TGFβ-induced miR-183 repression of MICA/B expression in human lung tumor cells. *Oncoimmunology*. 2019;8(4):e1557372. doi:10.1080/2162402X.2018.1557372
139. Jinushi M, Takehara T, Tatsumi T, et al. Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. *Int J Cancer*. 2003;104(3):354–361. doi:10.1002/ijc.10966
140. Lorenzo-Herrero S, López-Soto A, Sordo-Bahamonde C, Gonzalez-Rodriguez AP, Vitale M, Gonzalez S. NK cell-based immunotherapy in cancer metastasis. *Cancers*. 2018;11(1):29. doi:10.3390/cancers11010029
141. Hu Z. Tissue factor as a new target for CAR-NK cell immunotherapy of triple-negative breast cancer. *Sci Rep*. 2020;10(1):2815. doi:10.1038/s41598-020-59736-3
142. Hu W, Wang G, Huang D, Sui M, Xu Y. Cancer immunotherapy based on natural killer cells: current progress and new opportunities. *Front Immunol*. 2019;10:1205. doi:10.3389/fimmu.2019.01205
143. Tian X, Wei F, Wang L, et al. Herceptin enhances the antitumor effect of natural killer cells on breast cancer cells expressing human epidermal growth factor receptor-2. *Front Immunol*. 2017;8:1426. doi:10.3389/fimmu.2017.01426
144. American Association for Cancer Research. NK cells respond to checkpoint blockade. *Cancer Discov*. 2018;8(12):1498. doi:10.1158/2159-8290.CD-NB2018-131
145. Hsu J, Hodgins JJ, Marathe M, et al. Contribution of NK cells to immunotherapy mediated by PD-1/PD-L1 blockade. *J Clin Invest*. 2018;128 (10):4654–4668. doi:10.1172/JCI99317

146. Zhang J, Larrocha PS, Zhang B, Wainwright D, Dhar P, Wu JD. Antibody targeting tumor-derived soluble NKG2D ligand sMIC provides dual co-stimulation of CD8 T cells and enables sMIC(+) tumors respond to PD1/PD-L1 blockade therapy. *J Immunother Cancer*. 2019;7(1):223. doi:10.1186/s40425-019-0693-y
147. Chen X, Xue L, Ding X, et al. An Fc-competent anti-human TIGIT blocking antibody ociperlimab (BGB-A1217) elicits strong immune responses and potent anti-tumor efficacy in pre-clinical models. *Front Immunol*. 2022;13:828319. doi:10.3389/fimmu.2022.828319
148. Li F, Chen Y, Pang M, Yang P, Jing H. Immune checkpoint inhibitors and cellular treatment for lymphoma immunotherapy. *Clin Exp Immunol*. 2021;205(1):1–11. doi:10.1111/cei.13592
149. Zhang Q, Bi J, Zheng X, et al. Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. *Nat Immunol*. 2018;19(7):723–732. doi:10.1038/s41590-018-0132-0
150. Cifaldi L, Locatelli F, Marasco E, Moretta L, Pistoia V. Boosting natural killer cell-based immunotherapy with anticancer drugs: a perspective. *Trends Mol Med*. 2017;23(12):1156–1175. doi:10.1016/j.molmed.2017.10.002
151. Xu Y, Sun J, Sheard MA, et al. Lenalidomide overcomes suppression of human natural killer cell anti-tumor functions by neuroblastoma microenvironment-associated IL-6 and TGFβ1. *Cancer Immunol Immunother*. 2013;62(10):1637–1648. doi:10.1007/s00262-013-1466-y
152. Niu Z, Wu J, Zhao Q, Zhang J, Zhang P, Yang Y. CAR-based immunotherapy for breast cancer: peculiarities, ongoing investigations, and future strategies. *Front Immunol*. 2024;15:1385571. doi:10.3389/fimmu.2024.1385571
153. Raftery MJ, Franzén AS, Radecke C, et al. Next generation CD44v6-Specific CAR-NK cells effective against triple negative breast cancer. *Int J Mol Sci*. 2023;24(10):9038. doi:10.3390/ijms24109038
154. Włodarczyk M, Pyrzynska B. CAR-NK as a rapidly developed and efficient immunotherapeutic strategy against cancer. *Cancers*. 2022;15 (1):117. doi:10.3390/cancers15010117
155. Wang Y, Jin S, Zhuang Q, et al. Chimeric antigen receptor natural killer cells: a promising antitumor immunotherapy. *MedComm*. 2023;4(6): e422. doi:10.1002/mco2.422
156. Menon T, Gopal S, Rastogi Verma S. Targeted therapies in non-small cell lung cancer and the potential role of AI interventions in cancer treatment. *Biotechnol Appl Biochem*. 2023;70(1):344–356. doi:10.1002/bab.2356
157. Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. *EMBO Mol Med*. 2017;9(9):1183–1197. doi:10.15252/emmm.201607485
158. Lee MY, Robbins Y, Sievers C, et al. Chimeric antigen receptor engineered NK cellular immunotherapy overcomes the selection of T-cell escape variant cancer cells. *J Immunother Cancer*. 2021;9(3):e002128. doi:10.1136/jitc-2020-002128
159. Ghaedrahmati F, Esmaeil N, Abbaspour M. Targeting immune checkpoints: how to use natural killer cells for fighting against solid tumors. *Cancer Commun*. 2023;43(2):177–213. doi:10.1002/cac2.12394
160. Díaz B, Yuen A, Iizuka S, Higashiyama S, Courtneidge SA. Notch increases the shedding of HB-EGF by ADAM12 to potentiate invadopodia formation in hypoxia. *J Cell Biol*. 2013;201(2):279–292. doi:10.1083/jcb.201209151
161. Knops AM, South A, Rodeck U, et al. Cancer-associated fibroblast density, prognostic characteristics, and recurrence in head and neck squamous cell carcinoma: a meta-analysis. *Front Oncol*. 2020;10:565306. doi:10.3389/fonc.2020.565306
162. Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: a systematic review. *Mol Cancer*. 2019;18(1):63. doi:10.1186/s12943-019-0983-5
163. Chen X, Han J, Chu J, et al. A combinational therapy of EGFR-CAR NK cells and oncolytic herpes simplex virus 1 for breast cancer brain metastases. *Oncotarget*. 2016;7(19):27764–27777. doi:10.18632/oncotarget.8526
164. Qi X. Advances in antitumour therapy with oncolytic herpes simplex virus combinations. *Discov Oncol*. 2024;15(1):302. doi:10.1007/s12672-024-01165-z
165. Beldi-Ferchiou A, Caillat-Zucman S. Control of NK cell activation by immune checkpoint molecules. *Int J Mol Sci*. 2017;18(10):2129. doi:10.3390/ijms18102129
166. Moscarelli J, Zahavi D, Maynard R, Weiner LM. The next generation of cellular immunotherapy: chimeric antigen receptor-natural killer cells. *Transplant Cell Ther*. 2022;28(10):650–656. doi:10.1016/j.jtct.2022.06.025
167. Cao Y, Wang X, Jin T, et al. Immune checkpoint molecules in natural killer cells as potential targets for cancer immunotherapy. *Signal Transduct Target Ther*. 2020;5(1):250. doi:10.1038/s41392-020-00348-8
168. Chesney J, Puzanov I, Collichio F, et al. Randomized, open-label phase II study evaluating the efficacy and safety of talimogene laherparepvec in combination with ipilimumab versus ipilimumab alone in patients with advanced, unresectable melanoma. *J Clin Oncol*. 2018;36 (17):1658–1667. doi:10.1200/JCO.2017.73.7379
169. Schirrmacher V. Cancer vaccines and oncolytic viruses exert profoundly lower side effects in cancer patients than other systemic therapies: a comparative analysis. *Biomedicines*. 2020;8(3):61. doi:10.3390/biomedicines8030061
170. Liu S, Galat V, Galat Y, Lee YKA, Wainwright D, Wu J. NK cell-based cancer immunotherapy: from basic biology to clinical development. *J Hematol Oncol*. 2021;14(1):7. doi:10.1186/s13045-020-01014-w
171. Russell SJ, Barber GN. Oncolytic viruses as antigen-agnostic cancer vaccines. *Cancer Cell*. 2018;33(4):599–605. doi:10.1016/j.ccell.2018.03.011
172. Andtbacka RH, Kaufman HL, Collichio F, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J Clin Oncol*. 2015;33(25):2780–2788. doi:10.1200/JCO.2014.58.3377
173. Ribas A, Dummer R, Puzanov I, et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves Anti-PD-1 immunotherapy. *Cell*. 2017;170(6):1109–19.e10. doi:10.1016/j.cell.2017.08.027
174. Marelli G, Howells A, Lemoine NR, Wang Y. Oncolytic viral therapy and the immune system: a double-edged sword against cancer. *Front Immunol*. 2018;9:866. doi:10.3389/fimmu.2018.00866
175. Tesniere A, Panaretakis T, Kepp O, et al. Molecular characteristics of immunogenic cancer cell death. *Cell Death Differ*. 2008;15(1):3–12. doi:10.1038/sj.cdd.4402269
176. Galluzzi L, Vitale I, Warren S, et al. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. *J Immunother Cancer*. 2020;8(1):e000337. doi:10.1136/jitc-2019-000337
177. Koski A, Kangasniemi L, Escutenaire S, et al. Treatment of cancer patients with a serotype 5/3 chimeric oncolytic adenovirus expressing GMCSF. *Mol Ther*. 2010;18(10):1874–1884. doi:10.1038/mt.2010.161

178. Feng Y, Yang H, Liang G, et al. Immune checkpoint inhibitors combined with oncolytic virotherapy: synergy, heterogeneity, and safety in cancer treatment. *Oncol Res*. 2025;33(12):3801–3836. doi:10.32604/or.2025.067824
179. Wang M, Hemminki A, Siegal GP, et al. Adenoviruses with an RGD-4C modification of the fiber knob elicit a neutralizing antibody response but continue to allow enhanced gene delivery. *Gynecol Oncol*. 2005;96(2):341–348. doi:10.1016/j.ygyno.2004.09.063
180. Ries S, Korn WM. ONYX-015: mechanisms of action and clinical potential of a replication-selective adenovirus. *Br J Cancer*. 2002;86(1):5–11. doi:10.1038/sj.bjc.6600006
181. Chesney JA, Ribas A, Long GV, et al. Randomized, double-blind, placebo-controlled, global Phase III trial of talimogene laherparepvec combined with pembrolizumab for advanced melanoma. *J Clin Oncol*. 2023;41(3):528–540. doi:10.1200/JCO.22.00343
182. Pascual T, Vidal M, Cejalvo JM, et al. Talimogene laherparepvec and atezolizumab in HER2-negative breast cancer following neoadjuvant chemotherapy: a window-of-opportunity phase II trial (SOLTI-1503 PROMETEO). *Nat Commun*. 2026;17(1). doi:10.1038/s41467-026-69222-5.
183. Tanaka R, Goshima F, Esaki S, et al. The efficacy of combination therapy with oncolytic herpes simplex virus HF10 and dacarbazine in a mouse melanoma model. *Am J Cancer Res*. 2017;7(8):1693–1703.
184. Lun X, Ruan Y, Jayanthan A, et al. Double-deleted vaccinia virus in virotherapy for refractory and metastatic pediatric solid tumors. *Mol Oncol*. 2013;7(5):944–954. doi:10.1016/j.molonc.2013.05.004
185. Noonan AM, Farren MR, Geyer SM, et al. Randomized Phase 2 trial of the oncolytic virus pelareorep (Reolysin) in upfront treatment of metastatic pancreatic adenocarcinoma. *Mol Ther*. 2016;24(6):1150–1158. doi:10.1038/mt.2016.66
186. Clark AS, Zhao F, Klein P, et al. A Phase II randomized study of paclitaxel alone or combined with pelareorep with or without avelumab in metastatic hormone receptor-positive breast cancer: the BRACELET-01/PrE0113 study. *Clin Cancer Res*. 2025;31(13):2655–2662. doi:10.1158/1078-0432.CCR-24-2701
187. Sun J, Wang J, Xiao M, Chen L, Guan Y. Research progress on recombinant NDV in cancer therapy. *Front Immunol*. 2025;16:1735440. doi:10.3389/fimmu.2025.1735440
188. LaRocca CJ, Warner SG. Oncolytic viruses and checkpoint inhibitors: combination therapy in clinical trials. *Clin Transl Med*. 2018;7(1):35. doi:10.1186/s40169-018-0214-5
189. Pandha H, Harrington K, Ralph C, et al. Abstract CT115: phase 1b KEYNOTE 200 (STORM study): a study of an intravenously delivered oncolytic virus, Coxsackievirus A21 in combination with pembrolizumab in advanced cancer patients. *Cancer Res*. 2017;77(13_Supplement): CT115–CT. doi:10.1158/1538-7445.AM2017-CT115
190. Ma R, Lu T, Li Z, et al. An oncolytic virus expressing IL15/IL15Rα combined with off-the-shelf EGFR-CAR NK cells targets glioblastoma. *Cancer Res*. 2021;81(13):3635–3648. doi:10.1158/0008-5472.CAN-21-0035
191. Lal G, Rajala MS. Combination of oncolytic measles virus armed with BNiP3, a pro-apoptotic gene and paclitaxel induces breast cancer cell death. *Front Oncol*. 2018;8:676. doi:10.3389/fonc.2018.00676
192. Soliman H, Hogue D, Han H, et al. A Phase I trial of talimogene laherparepvec in combination with neoadjuvant chemotherapy for the treatment of nonmetastatic triple-negative breast cancer. *Clin Cancer Res*. 2021;27(4):1012–1018. doi:10.1158/1078-0432.CCR-20-3105
193. Li Y, Xiao F, Zhang A, et al. Oncolytic adenovirus targeting TGF-β enhances anti-tumor responses of mesothelin-targeted chimeric antigen receptor T cell therapy against breast cancer. *Cell Immunol*. 2020;348:104041. doi:10.1016/j.cellimm.2020.104041
194. Zhang Y, Shi X, Shen Y, et al. Nanoengineering-armed oncolytic viruses drive antitumor response: progress and challenges. *MedComm*. 2024;5 (10):e755. doi:10.1002/mco2.755
195. Tickner JA, Urquhart AJ, Stephenson SA, Richard DJ, O'Byrne KJ. Functions and therapeutic roles of exosomes in cancer. *Front Oncol*. 2014;4:127. doi:10.3389/fonc.2014.00127
196. Wen SW, Lima LG, Lobb RJ, et al. Breast cancer-derived exosomes reflect the cell-of-origin phenotype. *Proteomics*. 2019;19(8):e1800180. doi:10.1002/pmic.201800180
197. Abbaspour M, Akbari V. Cancer vaccines as a targeted immunotherapy approach for breast cancer: an update of clinical evidence. *Expert Rev Vaccines*. 2022;21(3):337–353. doi:10.1080/14760584.2022.2021884
198. Tao B, Du R, Zhang X, et al. Engineering CAR-NK cell derived exosome disguised nano-bombs for enhanced HER2 positive breast cancer brain metastasis therapy. *J Control Release*. 2023;363:692–706. doi:10.1016/j.jconrel.2023.10.007
199. Si C, Gao J, Ma X. Natural killer cell-derived exosome-based cancer therapy: from biological roles to clinical significance and implications. *Mol Cancer*. 2024;23(1):134. doi:10.1186/s12943-024-02045-4
200. Fu W, Lei C, Liu S, et al. CAR exosomes derived from effector CAR-T cells have potent antitumour effects and low toxicity. *Nat Commun*. 2019;10(1):4355. doi:10.1038/s41467-019-12321-3