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REVIEW

Emerging immunotherapeutic strategies for the treatment of breast cancer

Laura A. Huppert[1] · Veronica Mariotti[2] · A. Jo Chien[1] · Hatem H. Soliman[3]

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Abstract

Immunotherapy has resulted in unprecedented gains in long-term outcomes for many cancer types and has revolutionized the treatment landscape of solid tumor oncology. Checkpoint inhibition in combination with chemotherapy has proven to be effective for the treatment of a subset of advanced triple-negative breast cancer in the first-line setting. This initial success is likely just the tip of the iceberg as there is much that remains unknown about how to best harness the immune system as a therapeutic strategy in all breast cancer subtypes. Therefore, numerous ongoing studies are currently underway to evaluate the safety and efficacy of immunotherapy in breast cancer. In this review, we will discuss emerging immunotherapeutic strategies for breast cancer treatment including the following: (1) Intratumoral therapies, (2) Anti-tumor vaccines, (3) B-specific T-cell engagers, and (4) Chimeric antigen receptor T-cell therapy, and (5) Emerging systemic immunotherapy strategies. For each topic, we will review the existing preclinical and clinical literature, discuss ongoing clinical trials, and highlight future directions in the field.

Keywords Breast cancer · Immunotherapy · Novel treatment strategies · Intratumoral therapy · Vaccines

Intratumoral therapy

Intratumoral therapies can increase local drug concentration and attract immune cells to the tumor microenvironment, possibly with fewer systemic side effects. To date, intratumoral therapies have been most extensively studied in advanced and metastatic melanoma [1]. Since breast cancers are often physically accessible and locally aggressive, intratumoral drug administration is an appealing drug administration strategy. Initially, intralesional chemotherapy was studied, such as the use of intratumoral bleomycin [2, 3], cisplatin [4], and 10% Rose Bengal [5]. These studies showed limited efficacy, so the use of intralesional chemotherapy has been largely abandoned. Instead, the use of intralesional oncolytic viruses, immunotherapy, and cellular therapies has been more promising areas of study, which we will discuss further in this section (Table 1).

Intralesional oncolytic virotherapy

Oncolytic viruses have a dual mechanism of action, including an oncolytic effect through direct infection and apoptosis of tumor cells as well as an immunotherapy effect through activation of local and systemic immune responses [6]. One of the most successful examples of using a viral vector is the use of talimogene laherparepvec (T-VEC), which uses a modified herpes simplex virus (HSV). Specifically, the gene encoding the neurovirulence factor ICP34.5 is inactivated to prevent neuronal involvement and is replaced by the coding sequence for granulocyte–macrophage colony-stimulating factor (GM-CSF) [7–9]. In melanoma, intratumoral TVEC administration improved the durable response rate compared to recombinant GM-CSF alone [10, 11], ultimately leading to the FDA approval for TVEC to treat unresectable cutaneous, subcutaneous, and nodal lesions in recurrent melanoma lesions.

Laura A. Huppert and Veronica Mariotti contributed equally to this work.

✉ Hatem H. Soliman
hatem.soliman@moffitt.org

[1] Department of Medicine, University of California San Francisco, San Francisco, CA, USA

[2] College of Medicine, University of South Florida, Tampa, FL, USA

[3] Department of Breast Oncology, H Lee Moffitt Cancer Center, 12902 Magnolia Drive, Tampa, FL 33612, USA

Table 1 Select ongoing clinical trials of intratumoral therapy and combination therapies for breast cancer

NCT number	Status	Study population	Intratumoral agent	Combination therapy	Phase	Planned accrual
Intratumoral oncolytic viruses						
NCT03802604	Recruiting	Early-stage breast cancer (TNBC or LumB like primary tumor) with residual disease after neoadjuvant chemotherapy	T-VEC	Anti-PDL1 (atezolizumab)	I	30
NCT02779855	Recruiting	TNBC	T-VEC	Paclitaxel	I/II	50
NCT03802604	Recruiting	Residual breast cancer after standard neoadjuvant multi-agent chemotherapy	T-VEC	Atezolizumab	I	30
NCT03554044	Recruiting	Metastatic, unresectable, or locoregionally recurrent HER2-negative breast cancer	T-VEC	Chemotherapy: paclitaxel, nab-paclitaxel, or gemcitabine/carboplatin	Ib	15
NCT04348916	Recruiting	Advanced and/or refractory cutaneous, subcutaneous, or metastatic nodal solid tumors including breast cancer	ONCR-177, an oncolytic Herpes Simplex Virus	±PD-1 blockade	I	71
NCT04521764	Recruiting	Metastatic breast cancer	Modified measles virus, MV-s-NAP	None	I	33
NCT01846091	Active, not yet recruiting	Metastatic breast cancer (as well as head and neck cancers)	NIS-Expressing Derivative Manufactured From a Genetically Engineered Strain of Measles Virus	None	I	12
NCT04301011	Recruiting	Advanced solid tumors, including TNBC	TBio-6517, an Oncolytic Vaccinia Virus	±pembrolizumab	I/Iia	84
NCT03740256	Active, not yet recruiting	Advanced HER2 + solid tumors including HER2 + breast cancer	Binary oncolytic adenovirus	HER2-specific autologous CAR-T cells	I	39
NCT03435952	Recruiting	Treatment-refractory solid tumors including breast cancer	Clostridium novyi-NT	Pembrolizumab	I	18
Intratumoral immunotherapy						
NCT01042379	Recruiting	Locally advanced breast cancer	SD-101	Pembrolizumab + paclitaxel	II	N/A
NCT03567720	Recruiting	Inoperable locally advanced or metastatic TNBC	Tavokinogene telseplasmid (tavo) + electroporation	Pembrolizumab + nab-paclitaxel	II	65
NCT03739931	Recruiting	Multiple tumor types, including patients with TNBC s/p disease progression on at least one prior line of therapy	mRNA-2752 (a lipid nanoparticle encapsulating mRNAs encoding human OX40L, IL-23, and IL-36γ)	±durvalumab	I	126
NCT03435640	Recruiting	Metastatic solid tumor malignancies including breast cancer	NKTR-262 (PEGylated IL-2)	Nivolumab + bempegaldesleukin	I/II	393
NCT04144140	Recruiting	Advanced solid tumors including breast cancer	Stimulator of interferon genes (STING)	None	I	120
Cellular therapies						
NCT03788083	Recruiting	Early breast cancer and accessible tumor lesions (1.00 to 10 ml volume)	TriMix – mRNA encoding dendritic cell activating proteins	None	I	36

In breast cancer, several studies have reported data about the use of T-VEC alone or in combination with systemic therapies. Soliman et al. recently enrolled nine patients with stage 2–3 TNBC into a 3 + 3 phase I trial with two TVEC dose levels, given concurrently with neoadjuvant chemotherapy (NAC) [12]. The most common toxicities with TVEC were fever ($n = 8$), chills ($n = 3$), hematomas ($n = 3$), and injection site pain ($n = 3$). Thromboembolic events ($n = 2$) and bradycardia ($n = 1$) occurred during or after NAC. No dose-limiting toxicities were observed, so the addition of TVEC to NAC was deemed safe and feasible. Five patients achieved RCB0 (55%), 2 had RCB1 (22%), and 2 had RCB2 (22%). Several phase II trials are ongoing in the early stage and advanced settings, including a phase 2 study of TVEC + NAC for patients with locally advanced breast cancer (NCT02779855) and TVEC + chemotherapy or endocrine therapy for patients with unresectable, recurrent, or metastatic HER2- disease (NCT03554044). Besides TVEC, other oncolytic viruses are being studied in breast cancer (See Table 1). Future studies are needed to determine the breast cancer subtypes, stages, and combinations that are most safe and effective.

Intralesional immunotherapy

Besides oncolytic viruses, there has also been interest in directly injecting immune-modulating agents into the tumor microenvironment either alone or in combination with systemic immunotherapy. For example, synthetic oligonucleotide SD-101 is a potent and specific agonist for toll-like receptor 9 (TLR9) that induces an anti-tumor T-cell response [13]. In breast cancer, low tumor TLR9 expression at the time of diagnosis is associated with a significantly shortened disease-free-specific survival [14], suggesting that increased TLR9 expression may improve outcomes. In advanced melanoma, intratumoral SD-101 in combination with systemic pembrolizumab was safe and tolerable, with an overall response rate of 76% and a tolerable side effect profile [15, 16]. In breast cancer, intratumoral SD101 is currently being studied in combination with systemic pembrolizumab and paclitaxel followed by doxorubicin/cyclophosphamide in the neoadjuvant setting in the ongoing phase 2 I-SPY2 TRIAL (NCT01042379).

Another promising intralesional immunotherapy agent that has been studied to date is intratumoral tavokinogene telseplasmid (tavo), which is a plasmid encoding interleukin (IL)-12. IL-12 is a pivotal regulator of innate and adaptive immunity, and higher levels of IL-12 in the TME are associated with more robust anti-tumor responses [17]. Therefore, it was hypothesized that administration of IL-12 into the tumor microenvironment would augment anti-tumor activity [18]. In melanoma, the administration of intratumoral tavo in patients with unresectable or metastatic melanoma lead to an objective overall response rate of 35.7% with a complete response rate of 17.9% [19]. In breast cancer, a phase II trial is ongoing to assess the safety and efficacy of tavo in combination with systemic pembrolizumab in patients with inoperable locally advanced or metastatic TNBC (KEYNOTE-890/OMSI-141, NCT03567720). Preliminary results from 11 of the planned 25 patients were presented at the San Antonio Breast Cancer Symposium in 2018: 3/11 patients had a partial response (ORR 27.3%) [20]. Final results from this trial have not yet been reported.

Intralesional cellular therapies

There have also been studies investigating the intratumoral administration of cellular therapies. For example, a small phase I study evaluated the safety of administering intratumoral CAR-T mRNA in patients with metastatic breast cancer [21]. In this study, tumors injected with mRNA c-Met-CAR-T cells had extensive tumor necrosis at the injection site and loss of c-Met immunoreactivity, inducing an inflammatory response. Further studies are needed to better understand the safety and efficacy of intralesional cellular therapies in the treatment of breast cancer.

Anti-tumor vaccines

Cancer vaccines are generally composed of a vector that contains a cancer antigen or epitope that can be recognized by the immune system and enhance immune activity against the malignant cells. Cancer vaccines can be classified into four categories: peptide vaccines, cell-based vaccines, virus or bacteria delivered vaccines, or dendritic cells (DCs) based vaccines. We will discuss these therapies in this section (also see Table 2).

Peptide vaccines

Peptide vaccines are composed of tumor-associated antigens (TAAs) or specific cancer peptides that are injected and captured by antigen-presenting cells (APCs) in vivo. These peptides are usually combined with adjuvant factors, such as GM-CSF, given their low intrinsic immunogenicity. Of note, GM-CSF is also produced by primary breast tumor cells and may promote tumor growth [22, 23], but endogenous administration has not been shown to have the same effect. Peptide vaccines are restricted to specific HLA subtypes, which can limit their availability to patients. The most frequently used target peptides in BCs vaccines are HER2, CEA, hTERT, and MUC-1.

HER2 has been targeted in multiple peptide vaccine trials. The NeuVax™ vaccine, composed of GM-CSF and Nelipepimut-S, a CTL-activating peptide from HER2/

Table 2 Select ongoing clinical trials of vaccines therapy for breast cancer

NCT number	Status	Study population	Therapy	Phase	Planned accrual
Peptide vaccines					
NCT04270149	Recruiting	Early-stage ER-positive BC	ESR1 peptide vaccine + GM-CSF	I	18
NCT02427581	Recruiting	Early-stage TNBC with residual disease following neoadjuvant chemotherapy	Personalized synthetic long peptide vaccine (Poly-ICLC)	I	15
NCT03606967	Recruiting	Metastatic TNBC	Personalized Synthetic Long Peptide Vaccine and poly-ICLC	II	70
NCT04144023	Recruiting	HER2-expressing DCIS	Multi-epitope HER2 Peptide Vaccine H2NVAC	I	43
NCT02826434	Active, not recruiting	Early-stage TNBC	multi-peptide vaccine PVX-410 with durvalumab	I	22
NCT02636582	Active, not recruiting	DCIS	Nelipepimut-S and GM-CSF	II	13
NCT00194714	Active, not recruiting	Metastatic HER2-positive BC or ovarian cancer	HER-2/neu Peptide Vaccine	I/II	26
NCT03689192	Recruiting	Metastatic solid tumors	Arginase-1 peptides and Montanide ISA-51	I	10
NCT03761914	Recruiting	Advanced or metastatic solid tumors	Peptide WT1 galinpepimut-S in and pembrolizumab	I/II	90
NCT01376505	Recruiting	Metastatic solid tumors	Combination of HER-2 epitopes with nor-MDP in Montanide ISA 720	I	100
NCT02276300	Recruiting	Metastatic HER2-positive cancers	HER2-derived peptide vaccine with GM-CSF, Imiquimod, and Cyclophosphamide	I	2
NCT02229084	Recruiting	High-risk ER-positive BC	P10s-PADRE formulation vaccine with neoadjuvant chemotherapy	I/II	61
NCT03362060	Recruiting	Metastatic TNBC	multi-peptide vaccine PVX-410 and pembrolizumab	I	20
NCT04197687	Recruiting	Early-stage HER2-positive BC with residual disease	HER2 peptide vaccine TPIV100	II	480
NCT03012100	Recruiting	Early-stage TNBC	Multi-epitope Folate Receptor Alpha Peptide vaccine, GM-CSF, and Cyclophosphamide	II	280
NCT02593227	Active, not recruiting	Early-stage TNBC	Folate Receptor Alpha (FRα) peptide vaccine with GM-CSF	II	80
NCT04024800	Recruiting	Advanced TNBC	AE37 Peptide Vaccine in Combination With Pembrolizumab	II	29
Cell-based vaccines					
NCT00880464	Active, not recruiting	Early-stage BC	Autologous-irradiated BC cells	I	8
NCT00317603	Active, not recruiting	MBC	GM-CSF–secreting autologous irradiate BC cells	I	20
NCT04418219	Not yet recruiting	MBC	GM-CSF-secreting breast cancer vaccine	I/II	42
NCT00722228	Recruiting	Metastatic solid tumors	Autologous or allogeneic tumor cells	I/II	50
Viral, bacterial, or DNA plasmid-delivered vaccines					
NCT03199040	Recruiting	Early-stage TNBC	Neoantigen DNA vaccine with or without Durvalumab	I	24
NCT02204098	Recruiting	Early-stage ER + HER2-negative BC	Mammaglobin-A DNA Vaccine	I	56
NCT00393783	Active, not recruiting	Metastatic or high-risk HER2-positive BC	Rat HER2 DNA	I	12
NCT02780401	Active, not recruiting	Early-stage HER2-negative BC	pUMVC3-IGFBP2-HER2-IGF1R Plasmid DNA Vaccine (WOKVAC)	I	24
NCT02157051	Recruiting	HER2-negative stage III–IV BC	CD105/Yb-1/SOX2/CDH3/MDM2-polyepitope Plasmid DNA Vaccine	I	40

Table 2 (continued)

NCT number	Status	Study population	Therapy	Phase	Planned accrual
Dendritic cell vaccines					
NCT02063724	Active	Advanced or recurrent HER2-positive BC	HER-2-pulsed DCs	I	15
NCT04105582	Recruiting	non-metastatic TNBC	DCs pulsed with tumor-specific neoantigen	I	5
NCT03387553	Recruiting	early-stage HER2-positive BC	HER2-directed DCs	I	30
NCT02061423	Active	early-stage HER2-positive BC	HER2-directed DCs	I	7
NCT03384914	Recruiting	early-stage HER2-positive BC	DNA plasmid-based vaccine (WOK-VAC) and DC1 vaccine	II	110

neu, failed to demonstrate a significant clinical benefit and showed only a temporary dose-dependent immunologic response in early-stage breast cancer patient [24, 25]. Other cancer vaccines have been created utilizing peptides with MHC class II epitope capable of stimulating a helper T-lymphocyte response to increase the durability of CTL-activating peptides. Examples are the MHC class II epitopes from the HER2 protein AE36 and AE37. A phase II clinical trial tested an AE37 vaccine in early-stage BC but did not show clinical benefit in the ITT population [26].

Other peptide vaccines aimed to enhance immunogenicity and response duration to MHC I-restricted epitopes in combination with MHC II–epitopes from the same target protein. This strategy induced long-lasting HER-2-specific CTL response as well as IgG immunity by the administration of HER-2/neu helper peptide epitopes [27, 28]. The recombinant HER2 protein (dHER2), presented in HLA class I molecules combined with the AS15 immunostimulant, led to prolonged dHER2-specific antibody response and clinical response in patients with early stage and metastatic HER2-overexpressing BC, respectively [29, 30]. The peptide GP2, derived from the transmembrane domain of HER-2/neu, led to a GP2-specific CTL immune response in patients with early-stage BC expressing HER2, but failed to demonstrate a DFS benefit in the ITT analysis [31].

The oxidized mannan–MUC1 (M-FP) peptide produced a significant overall survival benefit in patients with MUC1 + breast cancer [32]. In a phase III clinical trial, the synthetic TAA sialyl-Tn, a carbohydrate epitope found on cancer-associated mucins, conjugated to the immunogenic keyhole limpet hemocyanin (KLH) carrier protein did not show significant clinical benefit in MBC [33]. The carbohydrate antigen Globo H combined with KLH and QS-21 in MBC patients generated IgM antibody titers [34].

Other potential target cancer peptides that have been studied in a clinical trial with promising results are hTERT, the catalytic subunit of human telomerase, and WT1 [35, 36].

Cell-based vaccines

In this approach whole cancer cells, either from the individual patient or from cancer cell lines, are used for vaccination. These cells need to be transfected with immune-stimulating molecules prior to infusion, given their poorly immunogenicity. The advantage of this approach is the availability of multiple TAAs without HLA restriction.

Tumor cells engineered to express GM-CSF can induce T-cell immune responses. The allogeneic HER2-positive GM-CSF-secreting breast tumor vaccine combined with trastuzumab and cyclophosphamide in patients with HER2-positive MBC led to increased HER-2-specific CD8 + T-cells response and demonstrated clinical benefit [37, 38]. In another study, MDA-MB-231 BC cells genetically modified to express the co-stimulatory molecule CD80-induced tumor-specific immune responses but did not demonstrate clinical benefit in MBC patients [39].

Virus, bacteria, or DNA-delivered vaccines

Specific TAAs can be introduced into APCs through recombinant viral or bacterial vectors, which naturally infect human cells and can be easily produced. These vectors can be directly cytotoxic (oncolytic viruses) or can be used as vaccines to elicit specific immune responses. Common viruses used for vaccine therapy are adenoviruses (Ad5), poxviruses (vaccinia, fowlpox), and herpesviruses.

ADXS31-164 is a live-attenuated Listeria monocytogenes-listeriolysin O genetically modified to express intracellular and extracellular epitopes of HER2 and currently tested in solid tumors [40].

The PANVAC poxviral vaccine, consisting of CEA/MUC-1 genes and co-stimulatory molecules TRICOM (PANVAC-V and PANVAC-F) in combination with chemotherapy, led to significant clinical responses in MBC patients [41–43].

The reconstituted influenza virosome (IRIV) coupled with three HER2 epitopes produced an increase in peptide-specific antibody titer and cellular immune responses [44].

MVA-BN®-HER2, a recombinant vaccine derived from the highly attenuated smallpox virus MVA-BN® (Modified Vaccinia Ankara virus) encoding a modified form of HER2-induced humoral and T-cell response in MBC patients [45].

The modified vaccinia virus Ankara (MVA), an attenuated poxvirus engineered to express p53 (p53MVA), combined with pembrolizumab led to clinical response in patients with advanced TNBC in a phase I clinical trial [46].

Plasmid DNA can be used to deliver specific TTA to the APCs. One example is the use of a Mammaglobin-A DNA vaccine in MBC patients. In a phase 1 trial, this treatment generated specific cytotoxic T-lymphocyte immune responses and showed clinical benefit [47].

Dendritic vaccines

Dendritic Cell (DC) vaccines use dendritic cells to present selected antigens to naive T cells via the major histocompatibility complexes (MHC). DCs are first collected from the patient via leukapheresis, then engineered to present the target cancer antigen, activated, and finally reinfused in the patient's bloodstream. In 2010, the FDA approved the first and only dendritic cell vaccine, sipuleucel-T, for use in metastatic castrate-resistant prostate cancer. This vaccine is obtained by pulsing a prostate acid phosphatase and granulocyte–macrophage GM-CSF into DCs [48].

In a phase I trial, the preoperative intranodal injection of HER2-pulsed DCs in patients with HER-2/neu-overexpressing DCIS led to complete response in 18.5% of the patients and eradication of HER-2/neu expression in 50% of those with residual disease [49]. In another phase I trial, DCs transduced with a HER2-expressing adenovirus produced clinical benefit in MBC patients [50].

In a phase I/II study by Soliman et al., DCs infected with the adenoviral vector contusugene ladenovec, containing the wild-type p53 sequence under the control of a cytomegalovirus promoter, were administered in combination with the IDO inhibitor indoximod in patients with p53-overexpressing MBC. Although 10% of the patients had stable disease, there was no significant difference between the immunologic responders and the non-responders [51]. In another phase I study, DCs loaded with p53 peptides led to disease stabilization or transient regression in 3 out of 10 MBC patients [52].

DCs vaccines that target CEA and Wilms' tumor protein 1 (WT1) have also been studied. In a phase I study, autologous DCs loaded with mRNA encoding CEA infused in patients with CEA-expressing metastatic adenocarcinoma, including breast cancer, lead to clinical response in 20% of the patients. DCs pulsed with WT1 peptides demonstrated clinical response in all patients with advanced breast cancer in a small phase I/II study [53].

DCs have also been loaded with individual tumor lysates. In a phase 2 study, DCs pulsed with each subject's tumor in combination with neoadjuvant chemotherapy in HER2-negative BC patients resulted in a higher percentage of pCR when compared to controls [54] In a phase I study, autologous DCs cultured in granulocyte GM-CSF, interleukin 4, and autologous plasma from metastatic solid cancers showed a clinical response in 3 out of 10 patients [55].

Bispecific T-cell engagers (BITEs)

The concept of antibodies with dual affinities was initially described in the 1960s by Nissonof when two different rabbit antibody fragments were joined to agglutinate different cells together [56]. A series of advances over the next four decades has resulted in many different formats of engineered antibodies for cancer therapy. Each of these variations, broadly classified as fragment or Fc containing, are associated with different concerns surrounding manufacturing, pharmacokinetics, and pharmacodynamics. In general, the smaller fragment-based antibodies like diabodies or dual antigen retargeting (DARTs) are simpler, penetrate tumor tissue more easily, and have much shorter half-lives compared to Fc containing antibody constructs. The initial challenge of preventing incorrect pairing of the two similar heavy chains/light chains during Fc-bispecific production has been largely solved through chemical modifications of the two different chains to ensure proper pairing. From a therapeutic perspective, the larger Fc containing constructs with their longer half-lives and greater stability are more prevalent in drug development.

The selection of cell surface targets for bispecific antibodies for breast cancer like in other diseases depends on differential expression of the target on cancer versus normal tissues, biologic effects of the target on cancer cells, along with the accessibility of the protein's extracellular domain for antibody binding [57]. If the bispecific is a T-cell engager meant to redirect killer T cells to activate against tumor cells with bound antibodies on its surface, then the other end will usually bind the CD3 receptor on the T cell leading to cell-mediated killing. One of the initial candidates in breast cancer clinical trials, 2B1, was a murine derived HER2 and Fc gamma III bispecific meant to activate natural killer cells against HER2 expressing cancer cells. The agent caused immune activation with resultant cytokine release symptoms but failed to demonstrate objective activity in patients with pretreated breast cancer. The 2B1 antibody was reformulated (HDX-210) with deleted Fc domains which resulted in better tolerability but also did not show sufficient activity [58]. A subsequent trifunctional bispecific targeting HER2 and CD3, ertumaxomab, completed two phase 1 trials showing a tolerable side effect profile at lower doses, with significant side effects, including systemic inflammatory

response syndrome and heart failure at the highest dose levels [59]. While the antibody demonstrated objective activity in metastatic breast cancer patients, the sponsor elected to terminate further development of the agent. Additional targets including EGFR, CEACAM, EphA10, prolactin, and p-cadherin have been investigated in TNBC models [59–65]. Most recently, a CD3-p-cadherin-bispecific (PF-06671008) phase 1 trial was terminated after treating 27 patients in a phase 1 trial. Limited information is available regarding efficacy, but it appears that significant cytokine release syndrome was encountered at higher dose levels. An alternative approach using autologous peripheral mononuclear cells activated ex vivo with IL-2 and a HER2-CD3 bispecific (so-called HER2 BiATCs) administered to 23 metastatic breast cancer patients demonstrated a stable disease rate of 47% [66].

The challenges observed with bispecific antibodies so far include limited activity in solid malignancies and toxicities related to cytokine release syndrome (CRS). Management of CRS has improved with Fc-deleted antibodies, proper monitoring, and better treatment algorithms. The limited activity noted with solid tumors relative to hematologic malignancies is likely due to more immunosuppressive solid tumor microenvironments and limited penetration of larger engineered antibody constructs. Combining engineered antibodies with other treatments designed to disrupt tumor-mediated immune suppression mechanisms and improve the delivery of antibodies within the tumor stroma will likely be required to improve the efficacy of this approach in breast cancer.

Chimeric antigen receptor (CAR) T cells

Chimeric antigen receptor (CAR)-T cells are effector T lymphocytes modified to achieve antibody-type specificity that can trigger IL-2 signaling and consequent cell lysis. The specificity of the CAR is driven by a specific tumor antigen recognition motif like a single-chain variable fragment (scFv). Over the years, CARs have been engineered to improve efficacy via the incorporation of co-stimulatory signals. Among the advantages of CAR-T cells is the recognition of the target antigens in an MHC-independent manner, with consequent avoidance of tumor immunologic escape. Furthermore, CAR-T cells are specifically home to tumor sites, where they can expand, persist, and penetrate the blood–brain barrier. CAR-T cells can be engineered to target specific TAAs, and this strategy can produce long-lasting responses.

Although CAR-T cells have been very successful in the treatment of hematological malignancies, the use of CAR therapy in solid tumors, including breast cancer, is still limited. This is partially due to the scarcity of specific TAAs, inefficient homing, and limited persistence due to tumor immunosuppressive microenvironment. CAR-T cells could be engineered to improve their expansion and persistence in the tumor microenvironment. Strategies include targeting the adverse effects of tumor-derived TGFβ, expressing cytokines that can reverse the immunosuppressive signals, and silencing genes inhibiting the T-cells' function.

Multiple clinical trials of CAR-T cells targeting TAAs in breast cancer have been conducted or are ongoing (Table 3).

Table 3 Select ongoing clinical trials of CAR-T-cell therapies for breast cancer

NCT number	Status	Study population	Target	Therapy	Phase	Planned accrual
NCT02915445	Recruiting	Nasopharyngeal carcinoma and breast cancer patients	EpCAM	CAR-T cells recognizing EpCAM	I	30
NCT04430595	Recruiting	Breast cancer patients	GD2, Her2 and CD44v6	4SCAR-T cells	I/II	100
NCT03696030	Recruiting	Breast cancer patients with brain and/or leptomeningeal metastases	HER2	HER2 CAR-T cells	I	39
NCT04511871	Recruiting	Solid tumors expressing HER2	HER2	CCT303-406 CAR-T cells	I	15
NCT04650451	Recruiting	Solid tumors expressing HER2	HER2	BPX-603 CAR-T cells	I	220
NCT04020575	Recruiting	Breast Cancers expressing MUC1	MUC1	huMNC2-CAR44 T Cells	I	69
NCT04025216	Recruiting	Cancers expressing TnMUC1	TnMUC1	CART-TnMUC1	I	112
NCT04348643	Recruiting	CEA-positive cancers	CEA	CEA CAR-T cells	I/II	40
NCT04107142	Not yet recruiting	Solid tumors including TNBC	NKG2DL	NKG2DL-targeting CAR-T	I	10
NCT02706392	Recruiting	Advanced ROR1 + Malignancies	ROR1	ROR1 CAR-T cell	I	60
NCT02830724	Recruiting	CD70 Expressing Cancers	CD70	anti-hCD70 CAR	I/II	124
NCT02792114	Recruiting	HER2-negative breast cancers	Mesothelin	Mesothelin-targeted T cells	I	36

In a study by Tchou et al., CAR-T cells were engineered to target the cell surface molecule c-Met via mRNA transfection [21]. The cells were then injected intratumorally in patients with metastatic breast cancer with accessible cutaneous or lymph node metastases and elicited a significant intratumoral inflammatory response on immunohistochemistry. The epithelial cell adhesion molecule (EpCAM) is overexpressed in breast cancer, and reduced EpCAM gene expression has been shown to decrease cancer cell proliferation [67]. Preclinical studies showed the efficacy of CAR-T cells targeting the HER2 domain. In a recent study by Toth G., T cells genetically modified to express a chimeric antigen receptor consisting of a HER2-specific scFv derived from trastuzumab in mice produced a strong response against an antibody-resistant xenografts [68]. After positive preclinical studies demonstrating the efficacy of CAR-T cells targeting [69], MUC1a growth factor receptor largely expressed in solid tumors, including breast cancer, a few ongoing clinical trials have been developed. Other TAAs currently targeted in active clinical trials are mesothelin, CEA, the natural killer group 2D, ROR1, and CD70.

CAR-T cells can be modified to produce cytokines that would improve their expansion and persistence in the tumor microenvironment. Strategies to improve CAR-T persistence include genetically engineering CAR-T cells to overcome the adverse effects of tumor-derived TGFβ, avoid Fas/Fas ligand-mediated apoptosis, express cytokines that can reverse the immunosuppressive signals, and silence genes that inhibit the T-cells' function. Universal CAR-T cells derived from healthy donors can be used to treat multiple patients avoiding single patients' inherent variability. Human leukocyte antigens class I (HLA-Is) and T-cell receptor (TCR) on CAR-T cells need to be removed to minimize immunogenicity and graft versus host disease (GVHD). Combining CAR-T cells with other therapies, such as blocking antibodies (CTLA4, PD-1, and PD-L1) or epigenetic modifiers, could improve anti-tumor effects.

Novel Systemic Immunotherapy Strategies: Moving Beyond PD-1

Checkpoint blockade against the programmed cell death receptor-1 (PD-1) axis has been the most widely studied and applied systemic immunotherapy strategy in breast cancer to date [70]. These gains have provided proof of concept and opened the door to the identification of additional targets and the development of novel immunologic strategies for the treatment of breast cancer. Here, we will discuss several of these novel immunotherapy strategies, including lymphocyte-associated gene 3 inhibitors, CD40 agonists, and anti-CD47 antibodies (also see Table 4).

Lymphocyte-Associated Gene 3 (Lag3) Inhibitors

Lymphocyte-associated gene 3 (LAG3) is an inhibitory receptor that is mainly found on activated immune cells and co-expressed with other inhibitory receptors like PD-1 [71–73]. There are currently several drugs targeting LAG3 being tested in clinical trials. First, there was a phase I study evaluating the safety of combining IMP321, a fusion recombinant inhibitor of LAG3, with paclitaxel in the first-line metastatic setting [74]. The authors found that toxicity was acceptable and there was a significant durable response compared to historical controls. Subsequently, a phase II placebo-controlled trial tested IMP321 and paclitaxel in patients with metastatic hormone receptor-positive breast cancer (NCT02614833). Data from the trial run-in phase presented at ASCO 2018 suggested that IMP321 enhanced antigen-presenting cell (APC) and T-cell activation [75].

Of note, studies in other tumor types suggest that IMP321 monotherapy has minimal activity [76] and only modest activity in combination approaches [77]. Indeed, several in vivo studies have demonstrated a highly significant clinical benefit when PD-1 and LAG3 are inhibited together [78]. Based on these findings, there are several trials underway studying the combination of dual PD-1 and LAG3 inhibition. For example, there is a phase Ib clinical trial studying the safety and efficacy of a LAG3 inhibitor (LAG525), a PD-1 inhibitor (spartalizumab), and an additional targeted agent (NIR178, capmatinib, MCS110, or canakinumab) in patients with triple-negative breast cancer (NCT03742349). In addition, a LAG3 monoclonal antibody (REGN3767) is being tested in combination with a PD-1 inhibitor (cemiplimab) and paclitaxel in the neoadjuvant setting in the I-SPY2 TRIAL (NCT01042379).

CD40 Agonists

CD40 is an immune co-stimulatory receptor expressed by APCs [79]. It is expressed on all malignant B-cell tumors and can be identified on the cell surface of approximately 50–70% of malignant epithelial tumors, such as breast cancer, rectal cancer, and nasopharyngeal cancer [80]. In a study of 181 breast carcinoma samples, CD40 was expressed in the breast tumor ducts in 53% of cases [81]. CD40 agonists have been shown to suppress tumor growth in both mouse models and human tissue culture [82, 83]. In early-phase human clinical trials, CD40 monoclonal antibodies demonstrated favorable anti-tumor responses in the treatment of melanoma, mesothelioma, pancreatic adenocarcinoma, and lymphoma [84–89].

There are several ongoing clinical trials of CD40 monoclonal antibodies alone or in combination with other immune-modulating drugs that include patients across tumor types. In breast cancer, NCT04616248 will study the

Table 4 Select ongoing clinical trials of novel systemic immunotherapy agents for breast cancer

NCT number	Status	Study population	Novel immunotherapy agent	Combination therapy	Phase	Planned accrual
LAG3 inhibitors						
NCT00349934	Active, not recruiting	Metastatic breast carcinoma	IMP321	Paclitaxel	I	33
NCT03742349	Recruiting	Triple-negative breast cancer	LAG525	Spartalizumab (anti-PD-1) + NIR178, capmatinib, MCS110, or canakinumab	IB	220
NCT01042379	Recruiting	Locally advanced breast cancer	REGN3767	Cemiplimab (anti-PD1) + paclitaxel	II	N/A
CD40 antagonists						
NCT04616248	Not yet recruiting	Unresectable or metastatic breast cancer	CDX-1140	CXD-301 (recombinant Flt3 ligand), Poly-ICLC, radiotherapy	I	36
NCT03329950	Recruiting	Advanced malignancy, including breast cancer	CDX-1140	Alone or in combination with CDX-301 (recombinant Flt3 ligand), pembrolizumab, or chemotherapy	I	260
NCT02829099	Active, not yet recruiting	Advanced malignancy	JNJ-64457107	N/A (anti-CD40 monotherapy)	I	95
CD47 antagonists						
NCT03013218	Recruiting	Advanced solid tumors or lymphomas	ALX148	± pembrolizumab, trastuzumab, rituziman, ramucirumab/paclitaxel, 5-FU/cisplatin	I	184
NCT04306224	Recruiting	Advanced solid tumors or lymphomas	IMC-001	N/A (anti-CD47 monotherapy)	I	24
NCT04257617	Recruiting	Advanced malignancy	ZL1201	N/A (anti-CD47 monotherapy)	I	65
NCT04588324	Recruiting	Unresectable/metastatic solid tumors	Anti-CD47 antibody	SHR2150 (toll-like receptor agonist), anti-PD1 antibody	I/II	50
NCT04728334	Recruiting	Advanced solid tumors or lymphomas	AK117	N/A (anti-CD47 monotherapy)	I	162
NCT04349969	Not yet recruiting	Advanced or metastatic solid tumors or lymphomas	AK117	N/A (anti-CD47 monotherapy)	I	159

anti-CD40 agent CDX-1140 in combination with radiation therapy and other immunomodulatory agents in patients with unresectable or metastatic breast cancer. Additional breast cancer-specific trials are expected in the future.

Of note, one major challenge with the use of anti-CD40 therapy is the treatment-related adverse events such as IL6 and TNF-alpha CRS and hepatoxicity [84]. Fortunately, these side effects can be adequately managed in most cases. To limit systemic toxicities, intratumoral injection of anti-CD40 agents has been tested in mouse tumor models with promising results, and intratumoral injection of anti-CD40 (ADC-1013) is now being tested in early-phase human clinical trials to try to limit these systemic toxicities (NCT02379741).

Anti-CD47 Antibodies

CD47 is an immunoglobulin that is overexpressed on the surface of many types of malignant cells, forming a signaling complex with signal-regulatory protein alpha (SIRP-alpha) and enabling these cells to escape from macrophage-mediated phagocytosis. Therefore, anti-CD47 therapies have been developed and shown promising anti-neoplastic activity [90].

In breast cancer, CD47 mediates the killing of breast cancer cells via Gi-dependent inhibition of protein kinase [91], leading to investigation of CD47 agents alone or in combination with other immunotherapy agents. In a preclinical model, Feliz-Mosquea et al. demonstrated that targeting

CD47 enhanced the effect of doxorubicin chemotherapy in vivo in breast cancer cell lines by reducing tumor growth and metastatic spread by activation of an anti-tumor innate immune response [92]. In another orthotopic mouse breast cancer model, Willingham et al. demonstrated the safety and efficacy of targeting CD47 [93]. There are also preclinical data that suggest that CD47 blockade augments the effect of trastuzumab in HER2 + cell lines [94]. Given this promising preclinical data, early-phase clinical trials are underway (e.g., NCT03013218, NCT04306224).

One potential challenge of anti-CD47 agents is off-target effects. CD47 is expressed by non-malignant cells of the hematopoietic system, including red blood cells and platelets [95]. Anemia has been a challenge in both preclinical and clinical studies [96, 97]. The toxicity of anti-CD47 antibodies appears to be Fc-dependent, so it is possible that optimization of the anti-CD47 antibody structure may improve off-target effects [93].

Other Novel Systemic Immunotherapy Agents

A number of other immunotherapy agents are also currently being studied. For example, Ox40 agonists bind to the Ox40 protein receptor and trigger a co-stimulatory signal associated with increased production of T cells and inflammatory cytokines [98]. 4-1BB agonists target the co-stimulatory receptor 4-1BB and can activate anti-tumor lymphocytes [99]. To date, these agents have been mostly studied in diseases highly responsive to immunotherapy, so it has been more challenging to show an effect, and the future of these agents is uncertain for the treatment of breast cancer.

Conclusion

Multiple emerging immunotherapy strategies have been implemented over the past few years in the treatment of breast cancer. While preliminary data for these immunotherapy strategies in breast cancer are promising, further work is needed to better understand these therapies' safety and efficacy. Additional studies should specifically focus on the optimization of specificity and duration of the immune response. Finally, it will be critical to determine if the anti-tumor effect can be augmented to further improve long-term outcomes in the future.

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Declarations

Conflicts of interest LAH has no disclosures. VM is an employee of BMS and owns BMS stocks. AJC receives research funding from Merck, Amgen, Puma, and Seagen. HHS consults for Eisai, Seattle Genetics, Immunomedics, PUMA, and Novartis.

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