Breast Cancer - Targets and Therapy

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REVIEW

Immunotherapy for HER2-positive breast cancer: recent advances and combination therapeutic approaches

This article was published in the following Dove Medical Press journal:
Breast Cancer - Targets and Therapy

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Abstract: Cancer immunotherapy has evolved dramatically with improved understanding of immune microenvironment and immunosurveillance. The immunogenicity of breast cancer is rather heterogeneous. Specific subtypes of breast cancer such as estrogen receptor (ER)-negative, human EGF receptor 2 (HER2)-positive, and triple-negative breast cancer (TNBC) have shown evidence of immunogenicity based on tumor–immune interactions. Several preclinical and clinical studies have explored the potential for immunotherapy to improve the clinical outcomes for different subtypes of breast cancer. This review describes the immune microenvironment of HER2-positive breast cancer and summarizes recent clinical advances of immunotherapeutic treatments in this breast cancer subtype. The review provides rationale and ongoing clinical evidence to the use of immune checkpoint inhibitors, therapeutic vaccines, and adoptive T cell immunotherapy in breast cancer. In addition, the present paper describes the most relevant clinical progress of strategies for the combination of immunotherapy with standard treatment modalities in HER2-positive breast cancer including chemotherapy, targeted therapy, and radiotherapy.

Keywords: immunotherapy, breast cancer, HER2, checkpoint inhibitors, vaccines

Background

Role of immune microenvironment in cancer

Immune cells represent a major component of the tumor microenvironment.[1] Immune elements infiltrating the tumor microenvironment include macrophages, natural killer (NK) cells, dendritic cells (DCs), and adaptive immune cells.[1,2] The role of immune system in cancer development and progression is described through immunoediting.[3] The concept of tumor immunoediting is represented by three phases designated as elimination, equilibrium, and escape.[3] The elimination phase implies a process known as tumor immune surveillance, whereby immune system identifies cancerous cells and eliminates them preventing tumor growth.[3] In the equilibrium phase, sporadic tumor cells that have escaped immune attack during elimination remain dormant and a temporary state of equilibrium develops between immune system and cancer cells. During this period, immune system will exert a selective pressure to eliminate susceptible tumor cells. However, cancer cells that acquire resistance to antitumor immune response enter the escape phase and continue to grow allowing tumors to develop aggressively.[3] Host antitumor immune responses are predominantly mediated via cellular immunity in which CD8+ cytotoxic T lymphocytes (CTLs) are considered the cornerstone cellular element in anticancer immunity.[4,5] Activated CTLs exert

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Dovepress
http://dx.doi.org/10.2147/BCTT.S175360

antitumor effects by secreting interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) along with other cytotoxins.[4,6,7] In this regard, prevention of tumor growth and development is determined, in part, by the number of CTLs invading through the tumor microenvironment and the ability of CTLs to recognize tumor-associated antigens (TAAs).[4] Other anti-oncogenic effects of immune system have been mediated through the activation of macrophages, NK cells, and CD4+ T helper (Th) 1 cells.[2]

A hallmark of cancers is the ability to evade the immune system through tumor-mediated immune escape mechanisms.[2] Tumors avoid recognition by immune system through multiple mechanisms including the downregulation of components of antigen processing and presentation machinery leading to loss of major histocompatibility complex (MHC) class I protein expression, low human leukocyte antigen (HLA) class I expression, and defects in T cell receptor (TCR) signaling.[6,8] In addition, growing tumors can avoid destruction by immune system through recruitment of immunosuppressive elements such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages.[6,8,9] These immune cells can suppress the actions of CTLs promoting tumor growth.[10] Furthermore, growing cancer cells have been shown to enhance the production of immunosuppressive cytokines within the tumor microenvironment such as TGF-β and IL-10 to escape immune attack.[8,9] Advancements in immunotherapy research have revealed a key immune evasion mechanism through the utilization of immune checkpoints by tumor cells to suppress the cellular immune response and promote immune tolerance.[2,8] Therefore, immunotherapeutic interventions are directed to enhance tumor recognition by immune system and augment CTL activity.

Breast cancer is a heterogeneous disease with a high degree of diversity between and within tumors.[11] Comprehensive gene expression profiling classifies breast tumors into three major molecular subtypes: luminal, human EGF receptor 2 (HER2)-positive, and basal-like cancers.[12,13] Recently, growing evidence is supporting the immunogenic potential of specific breast cancer subtypes.[14,15] Immunogenic nature of breast cancer was illustrated by the identification of tumor-infiltrating lymphocytes (TILs) in breast tumors.[15,16] Analysis of breast tumor samples had demonstrated higher level of TILs among patients harboring HER2-positive and triple-negative breast cancer (TNBC) than hormone-dependent subtypes.[17–19] Analysis of TILs in a large cohort of breast cancer patients indicated that the presence of CD8+ T cells is associated with a lower risk of mortality in estrogen receptor (ER)-negative and ER-positive/HER2-positive tumors.[14,20] However, the presence of CD8+ infiltrates was not associated with survival advantage for patients harboring ER-positive tumors. In the same study, Tregs that are characterized by FOXP3-positive expression were not associated with a prognostic impact among the different subsets of breast tumors evaluated.[14,20] Intra-tumoral CD4+ T cell number has been found to positively correlate with advanced tumor stages, large tumor size, positive lymph node status, and HER2 expression in breast cancer patients.[21] In addition, CD4+ TILs in breast cancer patients were positively correlated with FOXP3-positive Tregs. The CD4/CD8 ratios were negatively correlated with overall survival (OS) and relapse-free survival in breast cancer patients.[21]

HER2-positive breast cancer and the immune system

HER2 is a transmembrane tyrosine kinase receptor and a member of the ErbB protein family.[22] Amplification of the HER2/*neu* oncogene located on chromosome 17q12 is the primary pathway of HER2 receptor overexpression, which is the hallmark of HER2-positive or enriched breast tumors.[23] HER2 is amplified in 20%–30% of invasive breast cancers.[22–24] Overexpression of HER2 is an adverse prognostic factor that is associated with breast tumors of aggressive phenotype, poor survival, and increased risk of disease recurrence.[23] The introduction of HER2-directed therapy had revolutionized the treatment of HER2-positive breast cancer. Trastuzumab, a humanized monoclonal antibody, is the prototype HER2-directed therapy that was introduced in the late 1990s for the management of HER2-positive breast cancer.[25–27] The combination of trastuzumab with chemotherapy is the standard treatment for HER2-positive breast cancer in the current practice.[28,29] Trastuzumab binding to the extracellular domain of HER2 has been shown to prevent receptor dimerization, increase receptor degradation, and inhibit receptor shedding.[24] Collectively, these actions inhibit RAS-MAPK and PI3K-AKT-mTOR signaling pathways leading to the suppression of cancer cell proliferation and growth.[30] In addition, trastuzumab activity has been found to be mediated through antibody-dependent cellular cytotoxicity (ADCC) as demonstrated by the recruitment of immune cells to HER2-overexpressing breast cancers.[24,30] Other HER2-directed therapies approved for clinical use include the monoclonal antibody pertuzumab, the small molecule kinase inhibitor lapatinib, and the toxin-carrying antibody trastuzumab emtansine (T-DM1).[31–33] Despite the fact that HER2-targeted therapy had improved treatment outcomes

in breast cancer patients, several challenges of clinical relevance have been identified. Efficacy of trastuzumab therapy in combination with chemotherapy peaks at 40%–60% of breast cancer patients.[34] In addition, disease relapse has been reported in 15%–20% of patients with HER2-positive locoregional breast cancer after adequate treatment in both neoadjuvant and adjuvant settings.[35] Furthermore, pharmacological resistance to trastuzumab and other HER2-directed therapies is of particular importance as it adversely affects treatment outcomes.[34,36] Therefore, the development of newer therapies and novel approaches is of utmost importance to overcome limitations to targeted therapy and improve treatment outcomes in HER2-overexpressing breast cancer.

Different types of infiltrating immune cells have been clinically identified in HER2-positive breast tumors. Infiltrating immune cells have distinct prognostic and predictive significance. TILs have consistently shown a positive prognostic association in HER2-positive breast cancer patients.[17,37,38] Higher levels of TILs were associated with good prognosis in terms of improved survival and response to therapy as well as higher rates of pathological complete response (pCR).[17,38,39] The FinHER trial was the first to demonstrate an association between higher levels of TILs and improved response to trastuzumab among HER2-positive breast cancer patients.[40] In this regard, Alexe et al[41] demonstrated that strong expression of lymphocyte-associated genes was associated with reduced recurrence rates among HER2-positive breast cancer patients. In a retrospective analysis of data generated from CLEOPATRA study, Luen et al[42] found that greater TIL infiltration was significantly associated with improved OS among breast cancer patients with advanced HER2-positive disease treated with docetaxel, trastuzumab, and pertuzumab or placebo. These findings were further confirmed by the NeoALTTO trial in which the presence of TILs at diagnosis was associated with higher rates of pCR and event-free survival (EFS) in early-stage HER2-positive patients treated with lapatinib and trastuzumab.[37] Alternatively, increased fraction of Tregs in HER2-positive tumors was associated with advanced clinical stages, lower pCR rate, shorter disease-free survival (DFS), and reduced OS.[43,44] Furthermore, increased levels of circulating Tregs have been found to contribute to increased metastatic potential of HER2-positive breast cancer through suppressing CTL response.[45,46]

Collectively, these findings suggest that HER2-positive breast cancer can be targeted by immunotherapeutic interventions. This review discusses the different immunotherapeutic strategies that have been developed or being assessed in clinical trials in breast cancer, particularly the HER2-positive subtype. The review also describes the potentials for combined treatment of immunomodulating agents with other available treatments for HER2-postive breast cancer.

Immunotherapy of HER2-positive breast cancer

The goal of cancer immunotherapy is to restore the ability of the immune system to detect and eliminate cancer cells by overcoming the mechanisms by which tumors escape immune response.[47,48] Although the majority of immune-based therapeutic strategies have relied on passive immunity through the administration of antibodies with direct antitumor activity, there is a growing interest in evolving active immunotherapeutic modalities aiming at boosting host immune system ability to detect and destroy cancerous and precancerous cells.[47,49] This part discusses recent clinical progress of immune checkpoint blockade therapies, therapeutic cancer vaccines, and adoptive T cell transfer immunotherapy as immunotherapeutic approaches in breast cancer.

Immune checkpoint inhibitors

Immune checkpoints represent a plethora of immune system inhibitory pathways.[50] Under normal physiological conditions, immune checkpoints regulate immune response to promote self-tolerance and prevent autoimmunity.[51] Immunosuppressive pathways are often activated by cancer cells to escape immune detection and subsequent antitumor response and elimination.[50] Blockade of these immune checkpoints with monoclonal antibodies has been shown to restore the activity of cell-mediated immunity and promote antitumor response (Figure 1). Immune checkpoint inhibitors have been approved for the treatment of melanoma and renal cell carcinoma.[5,7,52,53] Given the immunogenic potential of HER2-positive breast cancer, the role of immune checkpoint inhibitors has been investigated.[4,5,7,52,53]

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) blockade

CTLA-4 is a key inhibitory receptor expressed on effector T cells.[54] Surface expression of CTLA-4 is upregulated after T cell activation to provide feedback inhibition of the immune response.[54] Regularly, the activation and proliferation of T cells are driven by the interaction between the costimulatory receptor CD28 expressed on T cells with its ligands expressed on antigen-presenting cells.[55] CTLA-4 and CD28 are highly homologous and compete for the binding to B7 ligands (B7-1 and B7-2) on antigen-presenting cells.[56] Binding of B7 ligands to CD28 receptor on effector T cells promotes T cell

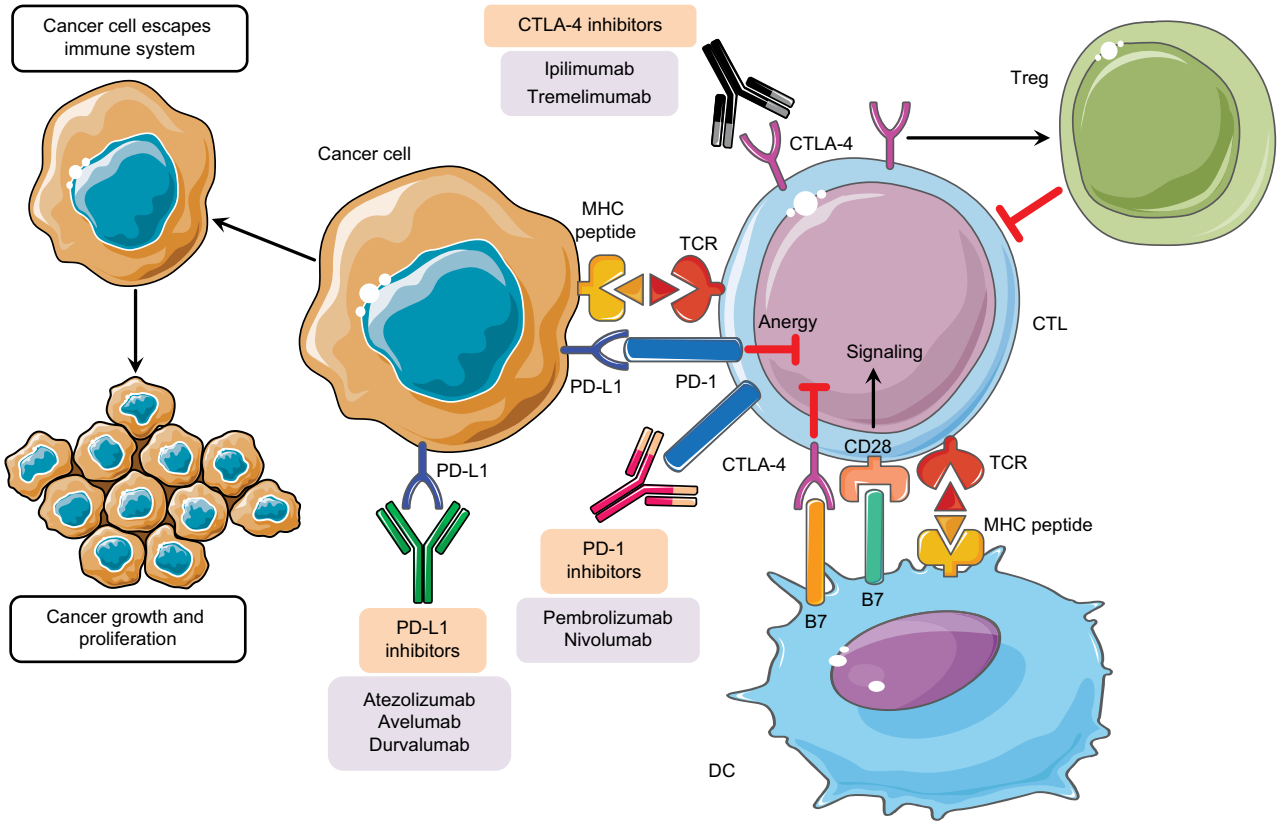

Figure 1 Immune checkpoint inhibitors in cancer treatment.
Notes: Inability to activate CTLs in tumor microenvironment through the suppressive effect of Tregs or through immune checkpoints allows cancer cells to escape immune attack, survive, and grow. B7 ligands expressed on antigen-presenting cells bind to CD28 receptor on CTL leading to T cell amplification and immune response. Alternatively, binding of B7 ligands to CTLA-4 expressed on T cells suppresses their activity. CTLA-4 also enhances the activity of Tregs leading to immunosuppressive activity. PD-1 is expressed on activated T cells. PD-1 binds to its PD-L1 leading to the anergy of CTLs further promoting inhibitory signals. Pharmacological inhibition of immune checkpoints with monoclonal antibodies restores CTL antitumor activity and relieves immunosuppression.
Abbreviations: CTLA-4, cytotoxic T-lymphocyte antigen 4; CTLs, cytotoxic T lymphocytes; DC, dendritic cell; MHC, major histocompatibility complex; PD-1, programmed cell death-1; PD-L1, programmed cell death-1 ligand; TCR, T cell receptor; Tregs, regulatory T cells.

amplification and immune response mediated by specific cytokines and chemokines essential for T cell activation.[54–57] On the contrary, binding of B7 ligands to CTLA-4 suppresses T cell activity and aborts further immune response.[54,57] In addition to down-modulating the activity of effector T cells, CTLA-4 has been found to promote the function of Tregs, thus enhancing their immunosuppressive activity.[58] Blockade of CTLA-4 with specific antibodies relieves immunosuppression and enhances the antitumor activity of effector T cells (Figure 1).[4,59,60] Ipilimumab was the first therapeutic immune checkpoint inhibitor targeting CTLA-4.[61] This drug is a human monoclonal antibody approved by the US Food and Drug Administration (US FDA) for the treatment of metastatic melanoma patients.[61] Tremelimumab is another CTLA-4 human monoclonal antibody currently under clinical investigations in the management of mesothelioma.[62]

There are few published reports on the use of CTLA-4 inhibitors in breast cancer. Ipilimumab was evaluated with or without cryoablation in early-stage breast cancer patients.[63] Nineteen patients were included in this study in which two patients had HER2-positive disease.[63] Findings showed a favorable increase in the proportion of activated effector T cells among patients receiving ipilimumab treatment alone or in combination with cryoablation. The combination treatment also showed a modest increase in the ratio of intratumoral T effector cells relative to Tregs.[63] In addition, ipilimumab was well tolerated when administered alone or in combination with cryoablation in which all patients reached the primary end point of the study with no reported grade III or IV adverse events (AEs) associated with treatment.[63] Rash, diarrhea, and fatigue were the most frequently reported treatment-related AEs. Tremelimumab was evaluated in combination with exemestane in hormone-dependent breast cancer patients with advanced disease.[64] The study included 26 patients who were characterized for the status of hormone receptor expression but not for HER2. Combination treatment

stabilized the disease in 42% of patients for a minimum of 12 weeks. Immunologically, tremelimumab and exemestane improved the ratio of effector T cells to Tregs and enhanced T cell activation among patients with advanced breast cancer.[64] This combination of tremelimumab and exemestane was tolerable. Most AEs were mild to moderate grade I/II, with no grade IV AEs being reported. Diarrhea was the most frequently reported treatment-related AE. Other AEs were pruritus, constipation, and fatigue.[64] Multiple ongoing clinical trials are assessing the therapeutic effect of CTLA-4 inhibitors in different breast cancer subtypes and settings. These trials are summarized in Table 1.

Programmed cell death-1 (PD-1)/ programmed cell death-1 ligand (PD-L1) blockade

PD-1 is a member of the CD28 superfamily that is expressed on activated T cells.[65] PD-1 binds to its ligands, PD-L1 and PD-L2, delivering inhibitory signals leading to decreased cytokine production and cytolytic activity of T cells.[65] PD-L1 has wide tissue expression and is the main contributor to the inhibitory signals that attenuate T cell activation leading to immune tolerance.[66] As such, multiple immunotherapeutic agents blocking the PD-1/PD-L1 axis have been approved to enhance antitumor immune response (Figure 1). Pembrolizumab and nivolumab are monoclonal antibodies against PD-1 which have been approved by the US FDA in 2014 for the treatment of patients with metastatic melanoma.[67,68] Both antibodies inhibit the interaction between PD-1 receptor and its ligand, thus restoring antitumor immunity.[67,68] Nivolumab approval has been recently expanded to include metastatic non-small-cell lung cancer (NSCLC), advanced renal cell carcinoma, and classical Hodgkin lymphoma.[69–71] PD-L1-targeting antibodies include atezolizumab, avelumab, and durvalumab. Atezolizumab has been approved for the treatment of metastatic NSCLC and advanced urothelial carcinoma, while avelumab has gained recent approval for the treatment of metastatic Merkel cell carcinoma.[72] Durvalumab was recently approved for the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following chemotherapy as well as patients with locally advanced, unresectable stage

Table 1 Ongoing clinical trials of CTLA-4 inhibitor immunotherapeutics in breast cancer

NCI identifier	Phase	Recruitment	Setting	Subtype	Immunotherapies	Combined treatments
NCT03546686	II	Not yet recruiting	Early	TNBC	Ipilimumab and nivolumab	Cryoablation
NCT03409198	II	Recruiting	Metastatic	Luminal B	Ipilimumab and nivolumab	Pegylated liposomal doxorubicin and cyclophosphamide
NCT01986426	I	Active, not recruiting	Metastatic	TNBC	Ipilimumab or pembrolizumab	LTX-315
NCT03241173	I/II	Recruiting	Metastatic or locally advanced	All	Ipilimumab and/or nivolumab	INCAGN01949
NCT03126110	I/II	Recruiting	Metastatic or locally advanced	All	Ipilimumab and/or nivolumab	INCAGN01876
NCT03328026	I/II	Recruiting	Metastatic or locally recurrent	All	Ipilimumab or pembrolizumab	SV-BR-1-GM, cyclophosphamide, and interferon inoculation
NCT02453620	I	Recruiting	Metastatic or locally advanced	$HER2^-$	Ipilimumab and nivolumab	Entinostat
NCT02983045	I/II	Recruiting	Metastatic or locally advanced	TNBC	Ipilimumab and/or nivolumab	NKTR-214
NCT03430466	II	Recruiting	Metastatic or recurrent	$HR^+/HER2^-$	Tremelimumab and durvalumab	Fulvestrant
NCT02892734	II	Recruiting	Metastatic or recurrent	$HER2^-$	Ipilimumab and nivolumab	
NCT03342417	II	Recruiting	Stage II–III Neoadjuvant	$HER2^-$	Ipilimumab and nivolumab	
NCT03132467	I	Recruiting	Stage II–III Neoadjuvant	$HR^+/HER2^-$	Tremelimumab and durvalumab	

Abbreviations: CTLA-4, cytotoxic T-lymphocyte antigen 4; HER2, human EGF receptor 2; HR, hormone receptor; TNBC, triple-negative breast cancer.

III NSCLC.[73,74] Ghebeh et al[75] demonstrated the increased expression of PD-L1 in breast cancer specimens as well as TILs. The expression of PD-L1 has been associated with unfavorable clinicopathologic features such as large tumor size, high tumor grade, lack of hormone receptor expression, and HER2-positive status.[75] In a Phase Ib study, pembrolizumab was well tolerated and resulted in a modest but durable overall response in a subset of patients with advanced, PD-L1-positive, ER-positive/HER2-negative breast cancer.[76] Nausea and fatigue were the most common treatment-related AEs and were mainly grade I/II. In addition, pembrolizumab and atezolizumab had shown acceptable toxicity profile and modest activity among TNBC patients in Phase I clinical trials.[77–79] In the JAVELIN Solid Tumor study, avelumab has been tested in 168 patients with metastatic breast cancer; in which 26 patients (15.5%) had HER2-positive breast cancer disease, 72 patients (42.9%) had hormone receptor-positive disease, and 58 patients (34.5%) had TNBC.[80] Overall, avelumab was well tolerated in which fatigue, nausea, and infusion-related reactions were most commonly encountered AEs. The overall objective response rate (ORR) was 3%; however, avelumab produces better clinical activity among patients with positive expression of PD-L1 and among patients with TNBC.[80] Currently ongoing clinical trials of PD-1/PD-L1 checkpoint inhibitors in breast cancer are summarized in Table 2.

Therapeutic cancer vaccines

Therapeutic cancer vaccines are intended to treat existing tumors by enhancing antitumor immune response.[81] Cancer vaccine strategies utilize specific tumor antigen-derived peptides, proteins, DNA, RNA, and whole tumor and DC lysates.[82,83] Cancer vaccines are expected to induce strong immunity by stimulating immune regulators such as antibodies, Th cells, and CTLs, thus establishing immunological memory and preventing tumor recurrence.[10,84] Multiple breast cancer-specific TAAs have been identified including HER2, mucin 1 (MUC1), carcinoembryonic antigen (CEA), human telomerase reverse transcriptase (hTERT), Wilms tumor gene (WT1), and mammaglobin-A (MAM-A).[2,85]

Nelipepimut-S (E75 peptide)

HER2/*nue* is a remarkable therapeutic target for peptide-based cancer vaccines. HER2/*neu* drives oncogenic potential, and its expression on the surface of HER2-overexpressing breast carcinomas can be up to 50-fold higher than its expression on normal cells.[85–87] Nelipepimut-S (E75) is a nine amino acid peptide from the extracellular domain of HER2/*neu*.[88] Nelipepimut-S binds HLA-A2 and HLA-A3 molecules on antigen-presenting cells which then stimulate CTLs to recognize and lyse HER2-expressing tumor cells.[89] NeuVax™ (Galena Biopharma, Inc., Portland, OR, USA) is a vaccine composed of nelipepimut-S and the hematopoietic growth factor, granulocyte macrophage colony-stimulating factor (GM-CSF).[89] GM-CSF is administered as an adjuvant known to induce proliferation, maturation, and migration of DCs to enhance nelipepimut-S-specific immunity through augmentation of antigen presentation to CTLs.[89] In addition, E75 vaccination has been shown to decrease circulating levels of Tregs in the majority of vaccinated breast cancer patients.[90] Earlier clinical trials showed that nelipepimut-S was immunogenic and of reasonable safety profile and reduced recurrence rate among high-risk breast cancer patients.[91–93] Mild pain and erythema at the site of injection were most frequent treatment-related AEs to E75 and GM-CSF vaccine. Other AEs included fever, nausea, and fatigue all of which were grade I/II.[91] Interestingly, immunogenic potential of nelipepimut-S was shown in breast cancer patients irrespective of the level of HER2 tumor expression.[94] Recently, a multicenter, multinational, prospective, randomized, double-blind, controlled Phase III study (PRESENT trial, NCT01479244) evaluating the efficacy and safety of NeuVax administered with adjuvant Leukine® (sargramostim, GM-CSF) has been completed. The study enrolled breast cancer patients who have node-positive disease and low-to-intermediate expression of HER2. A total of 758 vaccine-treated patients and control patients will be compared for DFS (NCT01479244).

Preclinical findings suggest that trastuzumab enhanced breast cancer cell killing by HER2/*neu* peptide-stimulated CTLs.[95] In this context, two clinical trials are assessing the combination of nelipepimut-S with trastuzumab. Both studies will be multicenter, prospective, randomized, single-blind, placebo-controlled Phase II trials of trastuzumab and nelipepimut-S/GM-CSF vs trastuzumab and GM-CSF treatment alone (NCT02297698 and NCT01570036). The first study (NCT02297698) is currently recruiting participants and will include high-risk HER2-positive breast cancer patients with high HER2 expression level. In the second study (NCT01570036), the target population will be node-positive breast cancer patients with low-to-intermediate HER2-expressing tumors. In both studies, the primary end point is to compare DFS between both treatment groups.

GP2

GP2 (HER2/*neu* 654–662) is a nine amino acid, MHC class I peptide from the transmembrane domain of HER2.[81,86] GP2

Table 2 Ongoing clinical trials of PD-1/PD-L1 immunotherapeutics in breast cancer

NCI identifier	Phase	Recruitment	Setting	Subtype	Immunotherapies	Combined treatments
NCT02129556	I/II	Active, not recruiting	Metastatic	$HER2^+$ trastuzumab resistant	Pembrolizumab	Trastuzumab
NCT03139851	II	Recruiting	Metastatic	$HER2^-$	Pembrolizumab	Metronomic cyclophosphamide
NCT03012230	I	Recruiting	Metastatic	TNBC	Pembrolizumab	Ruxolitinib phosphate
NCT03362060	I	Recruiting	Metastatic	TNBC	Pembrolizumab	PVX-410 vaccine
NCT02752685	II	Recruiting	Metastatic	$HER2^-$	Pembrolizumab	Nab-paclitaxel
NCT02971748	II	Recruiting	Inflammatory	$HR^+/HER2^-$ and $HR^+/HER2^+$	Pembrolizumab	Hormonal therapy
NCT03184558	II	Recruiting	Metastatic/ inflammatory	TNBC	Pembrolizumab	Bemcentinib
NCT03051659	II	Recruiting	Metastatic	$HR^+/HER2^-$	Pembrolizumab	Eribulin mesylate
NCT02513472	I/II	Recruiting	Metastatic	TNBC	Pembrolizumab	Eribulin mesylate
NCT03222856	II	Recruiting	Metastatic	$HR^+/HER2^-$	Pembrolizumab	Eribulin
NCT02999477	I	Recruiting	Nonmetastatic	$HR^+/HER2^-$	Pembrolizumab	Nab-paclitaxel
NCT03591276	I/II	Recruiting	Metastatic	$HR^+/HER2^-$ endocrine resistant	Pembrolizumab	Pegylated liposomal doxorubicin
NCT02657889	I/II	Active, not recruiting	Metastatic	TNBC	Pembrolizumab	Niraparib
NCT03454451	I	Recruiting	Advanced	TNBC	Pembrolizumab	CPI-006
NCT02957968	II	Recruiting	Neoadjuvant	$HER2^-$	Pembrolizumab	Decitabine
NCT02990845	I/II	Not yet recruiting	Metastatic	$HR^+/HER2^-$	Pembrolizumab	Exemestane and leuprolide
NCT03051672	II	Recruiting	Metastatic	$HR^+/HER2^-$	Pembrolizumab	Palliative radiotherapy
NCT03095352	II	Recruiting	Advanced	All	Pembrolizumab	Carboplatin and trastuzumab
NCT02646748	I	Recruiting	Advanced	TNBC	Pembrolizumab	Itacitinib or INCB050465
NCT03289819	II	Recruiting	Neoadjuvant	TNBC	Pembrolizumab	Nab-paclitaxel, epirubicin, and cyclophosphamide
NCT02648477	II	Recruiting	Metastatic	$HR^+/HER2^-$ TNBC	Pembrolizumab	Doxorubicin hydrochloride, anastrozole, exemestane, or letrozole
NCT03036488	III	Recruiting	Nonmetastatic	TNBC	Pembrolizumab	Carboplatin, paclitaxel, doxorubicin (or epirubicin), and cyclophosphamide
NCT02755272	II	Recruiting	Metastatic	TNBC	Pembrolizumab	Carboplatin and gemcitabine
NCT03515798	II	Not yet recruiting	Inflammatory/ neoadjuvant	$HER2^-$	Pembrolizumab	Chemotherapy
NCT02819518	III	Active, not recruiting	Metastatic	TNBC	Pembrolizumab	Nab-paclitaxel, paclitaxel, gemcitabine, or carboplatin
NCT02622074	I	Active, not recruiting	Locally advanced	TNBC	Pembrolizumab	Nab-paclitaxel, doxorubicin, cyclophosphamide, carboplatin, and paclitaxel
NCT03523572	I	Recruiting	Advanced	$HER2^+$	Nivolumab	Trastuzumab deruxtecan
NCT03414684	II	Recruiting	Metastatic	TNBC	Nivolumab	Carboplatin
NCT03430479	I/II	Recruiting	Metastatic	$HER2^-$	Nivolumab	Radiation and hormone therapy
NCT03487666	II	Recruiting	Residual disease	TNBC	Nivolumab	Capecitabine
NCT02309177	I	Active, not recruiting	Metastatic	$HER2^-$	Nivolumab	Nab-paclitaxel
NCT02997995	II	Recruiting	Neoadjuvant	$ER^+/HER2^-$	Durvalumab	Exemestane
NCT02802098	I	Recruiting	Metastatic	$HER2^-$	Durvalumab	Bevacizumab
NCT02628132	I/II	Recruiting	Metastatic	TNBC	Durvalumab	Paclitaxel
NCT01975831	I	Active, not recruiting	Advanced	Non-TNBC	Durvalumab	Tremelimumab
NCT02649686	I	Active, not recruiting	Metastatic	$HER2^+$	Durvalumab	Trastuzumab
NCT03616886	I/II	Not yet recruiting	Metastatic	TNBC	Durvalumab	Paclitaxel and carboplatin

(*Continued*)

Table 2 (*Continued*)

NCI identifier	Phase	Recruitment	Setting	Subtype	Immunotherapies	Combined treatments
NCT02826434	I	Recruiting	Stage II–III	TNBC	Durvalumab	PVX-410 and hiltonol
NCT03356860	I/II	Recruiting	Neoadjuvant	Luminal B and TNBC	Durvalumab	Paclitaxel, epirubicin, and cyclophosphamide
NCT02685059	II	Active, not recruiting	cT1b–cT4a-d	TNBC	Durvalumab	Nab-paclitaxel, epirubicin, and cyclophosphamide
NCT02489448	I/II	Recruiting	Neoadjuvant	TNBC	Durvalumab	Nab-paclitaxel, doxorubicin, and cyclophosphamide
NCT02484404	I/II	Recruiting	Advanced	TNBC	Durvalumab	Olaparib and/or cediranib
NCT03518606	I/II	Not yet recruiting	Advanced	All	Durvalumab	Tremelimumab and vinorelbine
NCT02924883	II	Active, not recruiting	Metastatic	$HER2^+$	Atezolizumab	T-DM1
NCT02849496	II	Recruiting	Stage III–VI	TNBC	Atezolizumab	Olaparib
NCT02708680	I/II	Recruiting	Advanced	TNBC	Atezolizumab	Entinostat
NCT03483012	II	Recruiting	Metastatic	TNBC	Atezolizumab	Stereotactic radiosurgery
NCT03292172	I	Recruiting	Advanced	TNBC	Atezolizumab	RO6870810
NCT02425891	III	Active, not recruiting	Metastatic	TNBC	Atezolizumab	Nab-paclitaxel
NCT03125902	III	Recruiting	Metastatic	TNBC	Atezolizumab	Paclitaxel
NCT02655822	I	Recruiting	Advanced	TNBC	Atezolizumab	CPI-444
NCT03566485	I/II	Recruiting	Metastatic	$ER^+/HER2^-$	Atezolizumab	Cobimetinib or idasanutlin
NCT03281954	III	Recruiting	Neoadjuvant	TNBC	Atezolizumab	Chemotherapy
NCT02914470	I	Active, not recruiting	Metastatic	All	Atezolizumab	Carboplatin and cyclophosphamide
NCT03395899	II	Recruiting	Neoadjuvant	$ER^+/HER2^-$	Atezolizumab	Cobimetinib, ipatasertib, and bevacizumab
NCT03498716	III	Recruiting	Nonmetastatic Stage II–III	TNBC	Atezolizumab	Paclitaxel, doxorubicin (or epirubicin), and cyclophosphamide
NCT03164993	II	Recruiting	Metastatic	TNBC	Atezolizumab	Pegylated liposomal doxorubicin, and cyclophosphamide
NCT03197935	III	Recruiting	Early	TNBC	Atezolizumab	Nab-paclitaxel, doxorubicin, and cyclophosphamide
NCT02605915	I	Active, not recruiting	Metastatic	$HER2^+$ and $HER2^-$	Atezolizumab	Pertuzumab, trastuzumab, T-DM1, carboplatin, doxorubicin, and cyclophosphamide
NCT03125928	II	Recruiting	Metastatic	$HER2^+$	Atezolizumab	Paclitaxel, trastuzumab, and pertuzumab
NCT02883062	II	Recruiting	Stage II–III Neoadjuvant	TNBC	Atezolizumab	Carboplatin Paclitaxel
NCT01898117	II	Recruiting	Advanced	TNBC	Atezolizumab	Carboplatin and cyclophosphamide, or paclitaxel
NCT02947165	I	Recruiting	Advanced	TNBC	Spartalizumab (PDR001, anti-PD-1 antibody)	NIS793
NCT03294694	I	Recruiting	Metastatic	$HR^+/HER2^-$	Spartalizumab	Ribociclib and fulvestrant
NCT02936102	I	Recruiting	Advanced	TNBC	Spartalizumab	FAZ053 (anti-PD-L1 antibody)
NCT02890069	I	Recruiting	Metastatic	TNBC	Spartalizumab	LCL161, everolimus, panobinostat, or QBM076
NCT03549000	I	Not yet recruiting	Advanced	TNBC	Spartalizumab	NZV930 and NIR178
NCT03147287	II	Recruiting	Metastatic	$HR^+/HER2^-$	Avelumab	Palbociclib and fulvestrant
NCT03414658	II	Recruiting	Advanced	$HER2^+$	Avelumab	Vinorelbine, trastuzumab, and utomilumab
NCT02536794	II	Recruiting	Metastatic	$HER2^-$	Tremelimumab	MEDI4736

(*Continued*)

Table 2 (*Continued*)

NCI identifier	Phase	Recruitment	Setting	Subtype	Immunotherapies	Combined treatments
NCT03394287	II	Recruiting	Advanced	TNBC	SHR-1210 (anti-PD-1 antibody)	Apatinib
NCT03151447	I	Recruiting	Metastatic	TNBC	JS001 (anti-PD-1 antibody)	Stereotactic body radiation
NCT03251313	I	Not yet recruiting	Metastatic	TNBC	JS001	Gemcitabine and cisplatin
NCT02447003	II	Active, not recruiting	Metastatic	TNBC	Pembrolizumab	
NCT02555657	III	Active, not recruiting	Metastatic	TNBC	Pembrolizumab	
NCT02411656	II	Recruiting	Metastatic/ inflammatory	TNBC	Pembrolizumab	
NCT02644369	II	Active, not recruiting	Advanced	TNBC	Pembrolizumab	
NCT02404441	I/II	Active, not recruiting	Advanced	TNBC	Spartalizumab	
NCT02926196	III	Recruiting	Nonmetastatic	TNBC	Avelumab	
NCT02838823	I	Active, not recruiting	Metastatic	TNBC	JS001	
NCT03620201	I	Recruiting	Stage II–III	$HER2^+$	M7824 (anti-PD-L1 fusion protein)	

Abbreviations: ER, estrogen receptor; HER2, human EGF receptor 2; HR, hormone receptor; T-DM1, trastuzumab emtansine; TNBC, triple-negative breast cancer.

is immunogenic. The peptide has been shown to stimulate CTLs and has more potential in HER2-overexpressing breast cancer, particularly when combined with trastuzumab.[86,96] Results of early Phase I clinical trial revealed safety and tolerability of GP2 peptide vaccine among high-risk, lymph node-negative breast cancer patients.[97] No dose-limiting toxicities were reported with the GP2 and GM-CSF vaccine, and local reactions were managed by dose reductions in GM-CSF.[97] This was further followed by Phase II trial to assess the efficacy of GP2 vaccine in preventing tumor recurrence among breast cancer patients who were HLA-A2+ with any level of expression of HER2.[98] Overall, the vaccinated group did not show a statistically significant difference in the rate of recurrence compared to the control group. However, this trial further confirmed the safety of GP2 vaccine and suggested that vaccination may have clinical benefit, particularly in patients with HER2 overexpression.[98]

AE37

AE37 is a 15 amino acid MHC class II epitope capable of stimulating CD4+ Th lymphocytes.[86] It is the Ii-Key hybrid of the MHC class II peptide, AE36 (HER2/*neu* 776–790).[86] AE37 vaccine has been found to reduce the number of Tregs in peripheral blood from breast cancer patients.[99] A Phase I clinical trial showed that AE37 vaccine is safe and well tolerated with minimal toxicity in breast cancer patients. AE37 was shown to induce HER2-specific immune response without the use of an immunoadjuvant.[100] A prospective, randomized, multicenter, Phase II adjuvant trial was conducted to evaluate the efficacy of AE37 vaccine among node-positive and high-risk node-negative breast cancer patients with tumors expressing any degree of HER2.[101] Results demonstrated similar rate of recurrence among vaccinated and control groups suggesting no benefit of vaccination.[101] However, findings confirm the safety of AE37 vaccine suggesting that vaccination may have clinical benefit in patients with low HER2-expressing tumors.[101] Patients receiving AE37 and GM-CSF had mild local toxicities which were grade I/II. Maximum systemic toxicities were grade I–III.

Mucin

Mucin glycoproteins are expressed by several epithelial cell types and malignancies.[102] MUC-1 is expressed in 90% of human breast cancers.[83] Mucin glycoproteins drive tumorigenesis by promoting cell adhesion, enhancing antiapoptotic signaling, and modulating intracellular growth pathways.[103] Preliminary MUC-1-directed vaccines have been shown to be safe and occasionally effective at generating antigen-specific T cell and antibody responses.[104,105] In a pilot Phase III randomized, double-blind study, patients with stage II breast cancer received subcutaneous injections of either placebo or oxidized mannan-MUC1. The recurrence rate in patients receiving the placebo was 27%, while patients who received immunotherapy had no recurrences, which was

statistically significant. Most patients receiving oxidized mannan-MUC1 had measurable antibodies to MUC1 and some reported T cell responses.[106] A Phase III trial of oxidized mannan-conjugated MUC-1 peptide vaccine demonstrated a significant reduction in recurrence rate in breast cancer patients receiving conjugated MUC-1 compared to patients receiving placebo.[107] After 12–15 years of follow-up period, no signs of toxicity or autoimmunity were developed in patients who administered the vaccine.

MAM-A

MAM-A is a secretory protein that has been found to be overexpressed in 90% of invasive ductal carcinomas of the breast.[108] Being a membrane-associated protein that is absent or expressed at very low levels in normal tissues, MAM-A had served as an attractive molecular marker for breast cancer immunotherapy.[108,109] Preclinical evidence indicated that MAM-A is highly immunogenic in which MAM-A-expressing cells can be used to generate MAM-A-specific CTLs and CD4+ T cells that are capable of recognizing and destroying MAM-A-expressing breast cancers.[109] Interestingly, MAM-A-specific CTLs were detected in breast cancer patients but not in patients without the disease.[109] A Phase I clinical trial of an MAM-A DNA vaccine showed that MAM-A was immunogenic through eliciting MAM-A-specific CD8 T cell responses, increased INF-γ levels, and reduced Tregs among patients receiving the vaccine.[110,111] Preliminary evidence also suggested the safety of the vaccine and improved progression-free survival (PFS) among treatment groups compared to control patients.[111] Malaise/flu-like symptoms were the most common grade I AEs attributed to MAM-A DNA vaccine. No grade III/IV AEs were reported among patients receiving the vaccine.[111] In the meantime, a Phase Ib (NCT02204098) randomized clinical trial is undergoing to evaluate the safety and immunogenicity of the MAM-A DNA vaccine in breast cancer patients receiving neoadjuvant endocrine therapy. Another Phase I study (NCT00807781) evaluating safety and immunogenicity of MAM-A DNA vaccine in terms of generating measurable CD8 T cell responses in metastatic breast cancer patients has been completed. These studies would provide further insights into the safety and efficacy profile of MAM-A vaccine in breast cancer.

DCs

The utilization of DCs as a vaccine strategy provides the advantage of presenting vaccine antigens to other cell types of the immune system. Preclinical studies demonstrated the potential for generating HER2-loaded DCs as well as DCs engineered to express HER2 antigen epitopes.[87,112,113] The first clinical evidence for the activity of DC vaccine in breast cancer patients was reported by Brossart et al.[114] The pilot study revealed the safety and efficacy of DC vaccinations among patients with advanced disease and tumors expressing HLA-A2 and HER2 or MUC1 who administered peptide-pulsed DCs subcutaneously. Immune response was detected by the presence of peptide-specific CTLs suggesting effective anti-tumor immune response for DC vaccination.[114] Czerniecki et al[115] conducted a clinical trial using vaccinations of DCs pulsed with HER2/*neu* HLA class I and II peptides administered to patients with HER2-positive ductal carcinoma in situ (DCIS) before surgical resection. Vaccine DCs were activated in vitro into fully functional DCs with IFN-γ and bacterial lipopolysaccharide to become highly polarized DC1 type secreting high levels of IL-12p70. Thirteen patients received four weekly intranodal doses of DC1 vaccine. The vaccinated subjects showed remarkable accumulation of T and B lymphocytes and induction of complement-dependent, tumor-lytic antibodies in breast tissue. Most patients showed reduced expression of HER2 in surgical tumor specimens. DC vaccine was well tolerated by patients with grade I/II AEs including fever, nausea, fatigue, and injection tenderness. The same strategy was applied by Koski[116] et al in another pilot trial of HER2-based DC1 vaccine in a neoadjuvant immunization trial among HER2-postive breast cancer patients with DCIS. Overall, DC vaccine sensitized CD8+ as well as CD4+ T cells against HER2 peptides and enhanced lymphocyte infiltration to breast tumor tissue in a large proportion of immunized patients.[116] A clinical trial assessing toxicity of HER2 DC vaccine in patients with HER2-overexpressing DCIS demonstrated tolerability of vaccine treatments with only grade I and II toxicities observed.[117] Furthermore, the expression of HER2 has been significantly reduced for most immunized patients suggesting HER2 DC vaccine treatment either promoting destruction of cancer cells or suppressing expression of HER2.[117] Recently, HER2 peptide-pulsed DC1 vaccine has been further tested in HER2-positive DCIS breast cancer patients (NCT02061332). In this trial, 54 patients were randomized to receive DC1 vaccine via intralesional, intranodal, or a combination of both routes of administration. In agreement with previous studies, findings confirmed the safety and immunogenicity of neoadjuvant DC1-based vaccination in DCIS HER2-positive patients which was similar among the different vaccination routes indicated.[118] Recently, a Phase II clinical trial with autologous DC vaccination in HER2-negative stage II–III breast cancer has been completed and the primary outcome was to assess

pCR (NCT01431196). Ongoing clinical trials evaluating peptide-based and DC-based vaccinations in breast cancer are summarized in Table 3.

Adoptive T cell transfer immunotherapy

Adoptive T cell transfer immunotherapy involves the isolation and generation of antitumor T lymphocytes from the primary tumor tissues, further expansion and activation of T cells ex vivo, and subsequently reinfusion of antitumor T cells into cancer patients to cure the disease.[83,119] Thus, the ultimate objective of this process is the stimulation and expansion of potent and antigen-specific T cell immunity.[119] Chimeric antigen receptor (CAR) technology represents one of the most advanced gene recombination technologies incorporated in

Table 3 Ongoing clinical trials of peptide- and DC-based vaccine immunotherapeutics in breast cancer

NCI identifier	Phase	Recruitment	Setting	Subtype	Vaccines	Combined treatments
NCT02479230	I	Recruiting	Metastatic	All	TBVA peptide-pulsed alpha-type-1 polarized DC vaccine	Gemcitabine hydrochloride
NCT00622401	I/II	Terminated	Metastatic	All	DC/tumor fusion vaccine	IL-12
NCT02826434	I	Recruiting	Stage II/III	TNBC	PVX-410 (a peptide vaccine)	Durvalumab and hiltonol
NCT02593227	II	Active, not recruiting	Stage IIb–III	TNBC	FRα peptide vaccine	Cyclophosphamide
NCT03362060	I	Recruiting	Metastatic	TNBC	PVX-410	Pembrolizumab
NCT02938442	II	Not yet recruiting	Stage II/III	TNBC	P10s-PADRE with MONTANIDE™ ISA 51 VG	Standard Chemotherapy
NCT01355393	I/II	Active, not recruiting	Stage II–IV	HER2$^+$	HER-2/*neu* peptide vaccine	Rintatolimod and/or GM-CSF
NCT01922921	I/II	Active, not recruiting	Metastatic	HER2$^+$	HER2 peptide-based intracellular domain protein	Trastuzumab and pertuzumab
NCT00343109	II	Active, not recruiting	Stage III/IV	HER2$^+$	HER2 intracellular domain protein peptide-based vaccine	Sargramostim
NCT03606967	II	Not yet recruiting	Metastatic	TNBC	Personalized synthetic long peptide vaccine	Durvalumab, nab-paclitaxel, and hiltonol
NCT03012100	II	Recruiting	Stage I–IV	TNBC	Multi-epitope FRa peptide vaccine	Cyclophosphamide and sargramostim
NCT02336984	I/II	Active, not recruiting	DCIS	HER2$^+$	HER2-pulsed DC1	Trastuzumab and pertuzumab
NCT02636582	II	Recruiting	DCIS	All/HLA-A2 positive	Nelipepimut-S	Sargramostim
NCT02063724	I	Active, not recruiting	At least stage IIIA with N2 (four positive nodes)	HER2$^+$ or HER2 IHC 2+ and FISH negative	HER2-pulsed DC1 vaccine	
NCT02061423	I	Active, not recruiting	Stage I–III	HER2$^+$	HER2-pulsed DC vaccine	
NCT01390064	I	Active, not recruiting	Metastatic	All	Mimotope P10s-PADRE/ MONTANIDE ISA 51 VG	
NCT03384914	II	Recruiting	Stage I–III	HER2$^+$	DC1 vaccine	
NCT03387553	I	Recruiting	Stage II/III	HER2$^+$	DC1 vaccine	
NCT02364492	I	Recruiting	Nonmetastatic	HER2$^-$	MAG-TN3 + AS15	
NCT01730118	I	Recruiting	Metastatic	HER2 1+, 2+, or 3+ by IHC	AdHER2/neu DC vaccine	
NCT03113019	I	Active, not recruiting	Stage II–IV	HER2$^+$ and TNBC	DC-based vaccine	
NCT03630809	II	Not yet recruiting	DCIS or inflammatory	HER2$^+$	HER2-pulsed DC1	
NCT03450044	I/II	Recruiting	Stage II–IV	All	DC	
NCT01376505	I	Recruiting	Metastatic	HER2 1+, 2+, or 3+ by IHC	MVF-HER-2 (597–626)-MVF-HER-2 (266–296) peptide vaccine	

Abbreviations: DCIS, ductal carcinoma in situ; DCs, dendritic cells; FISH, fluorescence in situ hybridization; FRa, folate receptor alpha; GM-CSF, granulocyte macrophage colony-stimulating factor; HER2, human EGF receptor 2; HLA, human leukocyte antigen; IHC, immunohistochemistry; TBVA, tumor blood vessel antigen; TNBC, triple-negative breast cancer.

adoptive T cell transfer. CAR is a modular fusion protein comprising extracellular target-binding domain usually derived from the single-chain variable fragment (scFv) of antibody, spacer domain, transmembrane domain, and intracellular signaling domain.[53] CAR-modified T cells (CAR-T cells) showed remarkable efficacy in B cell malignancies, mostly in anti-CD19 CAR-T cells for B cell acute lymphoblastic leukemia with up to a 90% complete remission rate.[120] CAR-T cell technology effectively triggers specific and prolonged T cell-mediated immunity against target antigen.[53] Targeting breast cancer using CAR technology has been well documented with preclinical studies reporting the generation of CAR for breast cancer-associated tumor antigens such as mesothelin,[121] MAM-A2,[122] and MUC-1.[123]

Multiple preclinical and clinical studies had evaluated adoptive T cell transfer immunotherapy in HER2-overexpressing breast cancer utilizing HER2 as a TAA. In this context, Lanitis et al[124] showed that ErbB2-redirected T cells prepared by the isolation of a novel TCR with ErbB2 (369–377) by TCR gene transfer delayed progression of ErbB2-positive HLA-A2+ human tumor in a novel xenograft animal model. Kuznetsova et al[87] established a genetically modified T cell-expressing CAR specific for the ErbB2 antigen which was directly transduced into CD3+ cells which were able to specifically target and induce apoptosis in ERbB2-overexpressing breast cancer cells cocultured with CAR-T cells. A dual targeted T cells co-expressing an ErbB2- and MUC-1-specific CAR have been found to effectively kill HER2-positive breast cancer cells which express both targets.[123] In addition, adoptive T cell immunotherapy had also demonstrated immune antitumor efficacy in suppressing tumor growth and both micro- and macrometastasis in HER2-overexpressing breast tumors in animal models.[125–127]

Few clinical studies evaluated adoptive T cell immunotherapy in breast cancer. Bernhard et al[128] assessed the impact of the adoptive transfer of autologous HER2-specific T cell clones to patients with HER2-overexpressing metastatic breast cancer refractory to standard regimens. Results indicated the accumulation of the transferred T cells into bone marrow (BM) of breast cancer patients which was further associated with the disappearance of BM-residing disseminated tumor cells.[128] A completed Phase I/II clinical trial (NCT00791037) involved autologous ex vivo expanded HER2-specific T cell administration to patients with metastatic HER2-overexpressing breast cancer. The study enrolled 23 patients, and the major outcomes are to evaluate toxicity and immunogenicity of infusing HER2-specific T cells.

Combination of immunotherapeutic strategies in HER2-positive breast cancer

There has been a growing preclinical evidence for the potential synergy of immunotherapy with other treatment modalities in cancer.[129,130] Meaningful combinations may enhance immunogenicity by increasing antigen and MHC class I expression on tumor cells, promoting TIL infiltration and vascular permeability, positively modulating CTL antitumor activity, or by relieving immunosuppressive signals within tumors.[131] Multiple therapies are currently being investigated in combination with immunotherapy for breast cancer treatment including chemotherapy, targeted therapy, and radiation therapy.[52,131,132]

Combinations of immunotherapy and chemotherapy

Chemotherapy has been shown to augment antitumor immunogenicity through mediating the activation and functionality of CTLs and NK cells,[133–136] enhancing the activity and maturation of DCs,[136] and depleting the immunosuppressive Tregs.[134,137,138] Collectively, these findings directed the design and evaluation of several combinations of chemotherapeutic agents and immunotherapy in breast cancer. Several groups have evaluated the combinations of chemotherapy and immune checkpoint inhibitors in breast cancer. Multiple ongoing clinical trials combining ipilimumab with liposomal doxorubicin and cyclophosphamide are being conducted in breast cancer patients (Table 1; NCT03409198 and NCT03328026). Pembrolizumab is being currently investigated in advanced breast cancer in combination with carboplatin and trastuzumab in a large Phase II study (Table 2; NCT03095352). Atezolizumab is also being investigated in early Phase I studies with carboplatin, cyclophosphamide, and doxorubicin in metastatic HER2-positive breast cancer patients (Table 2; NCT02914470 and NCT02605915).

Combinations of immunotherapy and targeted therapy

Trastuzumab treatment has been shown to upregulate the levels of PD-L1 in a transgenic mouse model of breast cancer, thus mediating resistance to trastuzumab therapy.[139] In line with this, preclinical studies have shown that the combination of PD-L1 and HER2 inhibitors was synergistic against HER2-positive breast cancer in animal models.[140] In addition, the presence of TILs was associated with improved outcomes in patients receiving trastuzumab treatment.[17,37] Recent evidence also demonstrated increased Tregs in HER2-positive breast

cancer patients who failed to achieve pCR suggesting the development of an immunosuppressive phenotype.[141] Overall, these findings encouraged clinical trials utilizing checkpoint blocking immunotherapy in combination with anti-HER2 treatments in patients with HER2-positive breast cancer.

The currently ongoing Phase II PANACEA study is investigating pembrolizumab in combination with trastuzumab in HER2-positive metastatic breast cancer patients who progressed while on trastuzumab treatment (Table 2; NCT02129556). Further, pembrolizumab is being evaluated in combination with carboplatin and trastuzumab in advanced breast cancer patients in a Phase II clinical study (Table 2; NCT03095352). A Phase I study is ongoing to evaluate the safety and tolerability of atezolizumab combination with T-DM1 or with trastuzumab plus pertuzumab in HER2-positive disease (Table 2; NCT02605915). Atezolizumab is also being evaluated in combination with paclitaxel, trastuzumab, and pertuzumab in metastatic HER2-positive patients for the assessment of antitumor activity and OS (Table 2; NCT03125928). A Phase II double-blind, randomized, placebo-controlled multicenter study is investigating the efficacy and safety of T-DM1 in combination with atezolizumab in patients with HER2-positive locally advanced or metastatic breast cancer who have progressed on previously received trastuzumab and taxane-based therapy. Almost 200 participants have been enrolled, and the estimated study completion date is by the end of the year 2020 (Table 2; NCT02924883). Durvalumab and nivolumab are also being evaluated among advanced HER2-postive breast cancer patients in combination with trastuzumab (NCT02649686 and NCT03523572, respectively).

Two early Phase I/II clinical trials are currently ongoing to investigate the safety and efficacy of combined vaccine therapy with anti-HER2 treatment among HER2-postive breast cancer patients. The first study is combining HER2 peptide-based intracellular domain protein with trastuzumab and pertuzumab among metastatic HER2-overexpressing patients (Table 3; NCT01922921). The second study is investigating the combination of HER2-pulsed DC1 vaccine with trastuzumab and pertuzumab in DCIS patients with HER2-positive disease (Table 3; NCT02336984). A recently completed Phase II study was conducted among patients with locally recurrent or metastatic HER2-positive disease who received autologous DC vaccine therapy along with trastuzumab and vinorelbine (NCT00266110). Patients were monitored for disease progression and toxicity. In the same context, a completed Phase I study revealed the safety and efficacy for the combination of GP2 peptide and trastuzumab in the treatment of patients with HER2-overexpressing breast cancer in the adjuvant setting (NCT03014076).

Several clinical trials are ongoing to investigate immunotherapy in combination with other targeted treatments among other molecular subtypes of breast cancer; these include poly (ADP-ribose) polymerase (PARP) inhibitors, hormonal treatments, angiogenesis inhibitors, AKT inhibitors, mTOR inhibitors, MEK inhibitors, and other targeted therapies (Tables 1–3).

Combinations of immunotherapy and radiation therapy

Radiation therapy has been recently shown to promote several immunostimulatory effects. Radiation therapy enhances tumor neoantigen presentation, thus improving the recognition of irradiated cells by CTLs.[142–144] Although radiation therapy represents an appealing strategy to combine with immunotherapy, limited number of clinical trials is currently investigating the effect of this combination in breast cancer. A recently completed Phase I study (NCT02303366) described the safety and activity of combining ablative body radiosurgery (SABR) treatment and pembrolizumab in 15 patients with oligometastatic breast cancer. Investigators hypothesize that the combination would be tolerable and will result in systemic immune activation.

Conclusion

Immunotherapy is evolving dramatically in the treatment of several types of advanced cancers, particularly after the recent success of the use of novel immunotherapy in the treatment of melanoma. Alongside, breast cancer is being recognized as immunogenic as a result of the remarkable progress in understanding the immune landscape of tumor microenvironment for the different subtypes of breast cancer. Accordingly, immunotherapy has emerged as a promising targeted treatment in breast cancer, especially with promising results from clinical trials in patients with TNBC. In this regard, assessing the efficacy of immunotherapeutic treatments based on molecular stratification of breast cancer may allow a greater window of opportunity to individualize breast cancer treatment and drive future research to find the best immunotherapeutic agents for patients. In this review, we have highlighted recent clinical evidence for immunotherapeutic treatments in breast cancer focusing on HER2-overexpressing subtype. Overall, no immunotherapy has been approved so far for preventive or therapeutic treatments in breast cancer. Ongoing clinical trials are investigating immune checkpoint inhibitors, CTLA-4 inhibitors, and anti-PD-1 or anti-PD-L1

antibodies, in early clinical trials for advanced HER2-positive breast cancer. Taking into account the modest benefit for the former immunotherapeutic agents in breast cancer, there is a growing interest in testing activity along with tolerability for the combination treatment of immune checkpoint inhibitors with standard breast cancer treatments such as chemotherapy and targeted and radiation therapy. HER2-specific peptide-based vaccines are most advanced in clinical trials at present. Nelipepimut-S (E75) is currently in Phase III clinical trials and in Phase II trials in combination with trastuzumab. So far, limited data are available for adoptive T cell transfer immunotherapy in clinical breast cancer settings; however, preclinical evidence highlights potentials for this technology.

Future perspective

The combination of immunotherapeutics with established cancer therapies is emerging. Therefore, rational design of combination treatments of immunotherapy with other standard breast cancer treatments requires deep understanding of the effect of each treatment on antitumor immunity utilizing nonredundant mechanisms of actions. In addition, the combination treatment approach requires careful evaluation for issues of treatment cost and toxicity. A major challenge for combination treatments with immunotherapy is the great number of potential combinations which requires crucial selection of breast cancer patients who would benefit most from immunotherapy.

Disclosure

The authors report no conflicts of interest in this work.

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