Immunotherapy for the Treatment of Breast Cancer: Checkpoint Blockade, Cancer Vaccines, and Future Directions in Combination Immunotherapy

Heather L. McArthur, MD, MPH, and David B. Page, MD

Dr. McArthur is the medical director of Breast Oncology at Cedars Sinai Medical Center in Los Angeles, California. Dr Page is an oncologist at the Providence Cancer Center and a researcher at the Earle A. Chiles Research Institute in Portland, Oregon. Both authors contributed equally to this article.

Corresponding author:
Heather L. McArthur, MD, MPH
Samuel Oschin Comprehensive Cancer Institute
Cedars Sinai Medical Center
8700 Beverly Blvd
Los Angeles, CA 90048
Tel: (310) 423-8051
Fax: (310) 423-8300
E-mail: Heather.McArthur@cshs.org

Abstract: Immunotherapy encompasses both vaccines that direct immune responses to tumor-associated antigens, and checkpoint blocking antibodies that inhibit immune system suppression by targeting key pathways mediated by cytotoxic T-lymphocyte–associated antigen 4, programmed death 1 (PD-1), and programmed death ligand 1 (PD-L1). Both of these approaches currently are being explored as potential strategies for the treatment of breast cancer. Recent studies suggest that immunotherapy is poised to change the therapeutic landscape for some breast cancers. Specifically, overall response rates of 19% with PD-1/PD-L1–directed antibodies have been reported in 2 small studies of women with PD-L1–positive, heavily pretreated advanced triple-negative breast cancer. In combination with nab-paclitaxel, confirmed response rates were 46% in a PD-L1–unselected population in the first-line metastatic triple-negative breast cancer setting. Checkpoint-blocking antibodies also have been evaluated in small studies of women with hormone receptor–positive metastatic breast cancer, and in women whose breast cancers lack PD-L1 expression, with more modest response rates. It has been hypothesized that some breast cancers are not inherently recognized by the immune system; however, preclinical and preliminary clinical data suggest that inherently modest immunogenicity may be overcome with novel vaccination strategies, as well as strategies that combine immune checkpoint blockade with methods of optimizing antigen presentation, such as tumor ablation, radiation, chemotherapy, or other approaches. If ongoing registrational trials support the use of immunotherapy, it could revolutionize the care of early-stage and metastatic breast cancer, and ideally improve cure rates.

Keywords
Breast neoplasms, CTLA-4 antigen, immunotherapy, lymphocytes, programmed cell death 1 receptor, tumor infiltrating, vaccines

Introduction

Immune responses against cancer are initiated when the immune system recognizes abnormally expressed proteins from cancer cells, termed tumor-associated antigens (TAAs). A critical feature

of immune activation is that the ultimate response is tightly regulated by a balance between immunostimulatory mechanisms and immunosuppressive mechanisms, which prevent uncontrolled inflammation and autoimmune disease. The majority of cancer immunotherapies now in development for breast cancer aim to modulate immune regulation (assuming the presence of an endogenous antitumor immune response), to direct immune responses toward TAAs, or to combine these 2 strategies (Table). Immune checkpoint therapeutic antibodies are designed to modulate preexisting cancer immunity by shifting the balance toward immune activation and away from immune suppression. Cancer vaccines, on the other hand, are designed to produce an immune response directed against abnormally expressed cancer antigens. Both immune checkpoint antibodies and cancer vaccines have shown promise in breast cancer, and currently are being evaluated in registrational trials. However, recent preclinical and preliminary clinical data suggest that breast cancer immunotherapy may be enhanced by combining antigen-based and immune-modulating strategies. The goals of this review are to provide a rationale for immunotherapy in breast cancer, to summarize current breast cancer literature pertaining to these 2 broad classes of immunotherapy, and to illustrate promising combination approaches that aim to maximize response through the use of both immune-modulating and antigen-based immunotherapies.

A Brief Immunotherapy Primer

For TAAs to induce immune activation, peptide complexes from the antigen must be presented to the immune system via a cell surface receptor, the major histocompatibility complex (MHC). This receptor is expressed on normal cells, many tumor cells, and antigen-presenting cells (APCs), a class of immune cells that are resident in both tissue and draining lymph nodes.[1] APCs engulf both extracellular proteins and apoptotic cells, digest these materials into small peptides, and display the peptides within a pocket of the MHC receptor, which then binds to circulating T cells via the T-cell receptor (Figure). T cells that are reactive to that TAA are then activated by downstream T-cell receptor signaling. One particular type of T cells—CD4+ helper T cells—are activated by MHC class II, which is expressed on APCs. Upon activation, they facilitate antitumor responses by releasing cytokine mediators and directly activating other immune cells. Another type of T cells—CD8+ cytotoxic T cells—are activated by MHC class I, which is expressed on tumor cells. Upon activation, they are capable of directly killing tumor cells harboring the TAA.

Cancer vaccines are immunotherapies that provide an exogenous source of TAA, thereby facilitating T-cell activation and immune responses toward tumors expressing that antigen. The most investigated breast cancer antigen is the human epidermal growth factor receptor 2 (HER2) protein. Numerous HER2-directed vaccines are in clinical development, including Galena Biopharma's nelipepimut-S vaccine (NeuVax), which currently is being evaluated for clinical efficacy in early-stage breast cancer in a phase 3 clinical trial (NCT01479244).

Immune checkpoint molecules represent critical components in the T-cell activation process and in immune regulation. For a T cell to be activated, the T-cell receptor/MHC/antigen signal must be accompanied by positive costimulatory signals. Conversely, T-cell activation may be attenuated by negative coinhibitory signals.[1] Immune checkpoint therapeutic antibodies function by serving as either antagonists of coinhibitory signals or agonists of costimulatory signals. The first immune checkpoint antibodies were designed to target checkpoints in the inhibitory cascade. Thus, by administering drugs that target and inhibit key checkpoints in the inhibitory pathways, immune system activation can continue relatively unopposed, allowing for immune-mediated cancer clearance and tumor regression.

The first US Food and Drug Administration (FDA)-approved checkpoint blockade therapy was ipilimumab (Yervoy, Bristol-Myers Squibb), an antibody targeting cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4). Normally, after a T cell binds to tumor antigen via the T-cell receptor, B7 ligand on the tumor cell or APC binds to a T-cell costimulatory molecule (CD28) to enhance T-cell activation and proliferation. However, upon T-cell activation, the coinhibitory molecule CTLA-4 becomes upregulated on the T-cell surface, ultimately binding B7 in competition with CD28, leading to suppression of T cells.[2] This mechanism exists to ensure a balance of T-cell activation and suppression, but can be therapeutically manipulated to allow for unopposed T-cell activation. Ipilimumab, for example, which binds and blocks CTLA-4, received FDA approval for metastatic melanoma after a phase 3 trial demonstrated an improvement in median survival from 6.4 months with the gp100 vaccine alone to 10.1 months with gp100 plus ipilimumab. The addition of ipilimumab nearly doubled the survival rate at 24 months.[3] A second anti–CTLA-4 antibody, tremelimumab, currently is being evaluated across multiple phase 2 and 3 clinical trials in a variety of tumor types.[4]

The second class of FDA-approved checkpoint antibodies targets another inhibitory receptor, programmed death 1 (PD-1), or its ligand, programmed death ligand 1 (PD-L1).[5] When T cells are activated and infiltrate tumors, they release interferon gamma (IFN-γ), which

Table. Potential Immunotherapy Strategies

Therapeutic Sources of Tumor-Associated Antigens			
Class	**Mechanism**	**Examples of Clinical Trials in Breast Cancer**	**Insights and Therapeutic Challenges**
Cancer Vaccines			
Monovalent	Induction or enhancement of immunity against a known, single tumor-associated antigen	Randomized phase 3 study of nelipepimut-S in HER2-low/ intermediate early-stage breast cancer (NCT01479244)	Monovalent vaccines are ideal if an antigenic target (such as HER2) is known; heterogeneous expression or downregulation of antigen, as well as immune tolerance, may hamper efficacy
Polyvalent	Induction or enhancement of immunity against multiple tumor-associated antigens packaged together in the same vaccine product	PANVAC +/- docetaxel in metastatic breast cancer[45]	Targeting multiple antigens in one vaccine may broaden and reduce the likelihood of tumor escape due to antigen downregulation; selection of relevant antigens is still imperative
Cellular	Ex vivo modified tumor cells or antigen-presenting cells; facilitates antigen presentation	Allogeneic GM-CSF–secreting vaccine plus trastuzumab/ cyclophosphamide in metastatic HER2-negative breast cancer (NCT00971737)	Cellular vaccine preparations may more efficiently present antigens compared with peptide alone; they are costly to produce
Autophagy modulators	Manipulation of endogenous autophagy process to promote expression of cellular degradation products	Hydroxychloroquine in metastatic estrogen receptor–positive breast cancer (NCT02414776)	May facilitate antitumor responses against a broad array of endogenous tumor antigens; difficult to monitor immune responses without precise knowledge of the antigenic target
Conventional Therapies			
Chemotherapy	Chemotherapy may induce tumor cell lysis and antigen presentation, as well as Treg/ MDSC depletion and IFN-γ secretion	Phase 3 nab-paclitaxel +/- atezolizumab (anti–PD-L1) in metastatic TNBC (NCT02425891)	Combining standard-of-care chemotherapy with immunotherapy is clinically feasible; chemotherapy or supportive glucocorticoids may also be immunosuppressive
Radiotherapy	Pleiotropic immune effects including enhanced antigen release, MHC class I expression, dendritic cell function, and interferon production; may also promote MDSCs and TGF-β release	Proof-of-principle trial of GM-CSF plus radiotherapy in metastatic solid tumors[80]	Can be safely combined with immune checkpoint agents; the optimal dosing, schedule, and sequencing of radiation with immunotherapy is still being evaluated
Tumor ablation	Tumor lysis by a thermal probe; enhanced antigen presentation; release of mediators of innate and adaptive immunity	Pilot study of ipilimumab plus cryoablation in early-stage breast cancer[64]	Limited study; has not been compared with radiotherapy; being evaluated in combination with checkpoint antibody therapy
Oncolytic virus	Direct injection of virus into cancer; virally mediated expression of immunogenic proteins	Talimogene laherparepvec with neoadjuvant chemotherapy in TNBC (NCT02779855)	Safety concern of injecting live virus; potential reduced efficacy among noninjected lesions

ADCC, antibody-dependent cell-mediated cytotoxicity; CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; GM-CSF, granulocyte-macrophage colony stimulating factor; HER2, human epidermal growth factor receptor 2; IFN-γ, interferon gamma; IL-2, interleukin 2; IL-12, interleukin 12; KLH, keyhole limpet hemocyanin; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; PD-1, programmed death 1; PD-L1, programmed death ligand 1; TGF-β, transforming growth factor beta; Th1, T-helper type 1; TILs, tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer; Treg, T-regulatory cell.

(Table continues on page 925)

Table. *(Continued from page 924)* Potential Immunotherapy Strategies

Therapeutic Sources of Immune Modulation			
Class	**Mechanism**	**Examples of Clinical Trials in Breast Cancer**	**Insights and Therapeutic Challenges**
Immune checkpoint antagonists	Blockade of inhibitory signals mediated by T-cell surface molecules; anti–CTLA-4 may also mediate clearance of suppressive Tregs by ADCC (CTLA-4, PD-1/PD-L1)	Pembrolizumab (anti–PD-1) monotherapy for metastatic TNBC[79]	Efficacy as monotherapy may be limited to tumors with preexisting immunity; combination checkpoint therapy may improve response rate
Immune checkpoint agonists	T-cell activation by binding and downstream signaling of costimulatory T-cell surface molecules (OX40, 41BB)	Stereotactic radiotherapy plus OX40 (MEDI6469) in metastatic breast cancer (NCT01862900)	May be capable of facilitating de novo immune responses against tumor antigens by lowering the threshold for T-cell activation; still early in development
Vaccine Adjuvants			
GM-CSF	Endogenous cytokine that enhances dendritic cell maturation and antigen presentation	Randomized phase 3 study of nelipepimut-S in HER2-low/intermediate early-stage breast cancer (NCT01479244)	An effective vaccine adjuvant; many randomized vaccine trials use GM-CSF as the comparator arm
Imiquimod	Agonist of Toll-like receptor 7; facilitates innate immunity, antigen presentation, and cytokine release	Phase 2 trial of topical imiquimod for breast cancer skin metastases[81]	Only available topically; has activity against basal cell carcinoma and actinic keratosis; may be combined with vaccine and administered peridermally
Saponins	Soap-like glycosides that enhance both antibody and Th1 and Th2 responses by unknown mechanism	Randomized trial of globo-H/KLH plus OPT-822/821 immune adjuvant in metastatic breast cancer[40]	Limited comparative data of saponins vs other conventional vaccine adjuvants, such as aluminum salts or emulsions
Immunostimulatory cytokines (eg, IL-2, IL-12, IFN-γ)	Enhance antitumor immunity by facilitating Th1-mediated T-cell responses; potential cytokine-mediated upregulation of PD-L1	Ad-RTS-hIL-12 with veledimex as maintenance in subjects with metastatic breast cancer (NCT02423902)	Effective as monotherapy in a variety of cancers (eg, IL-2 for melanoma); modern liposomal and intratumoral formulations may improve therapeutic index
Cytokine inhibitors	Enhance antitumor immunity by blocking adverse effects of immunosuppressive cytokines (TGF-β)	Galunisertib (TGF-β inhibitor) and paclitaxel in metastatic TNBC (NCT02672475)	Preclinical literature supports combining TGF-β with radiotherapy; TGF-β may also play a role in the pathophysiology of bone metastases

ADCC, antibody-dependent cell-mediated cytotoxicity; CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; GM-CSF, granulocyte-macrophage colony stimulating factor; HER2, human epidermal growth factor receptor 2; IFN-γ, interferon gamma; IL-2, interleukin 2; IL-12, interleukin 12; KLH, keyhole limpet hemocyanin; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; PD-1, programmed death 1; PD-L1, programmed death ligand 1; TGF-β, transforming growth factor beta; Th1, T-helper type 1; TILs, tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer; Treg, T-regulatory cell.

in turn upregulates PD-L1 expression by tumor cells. PD-L1 binds to PD-1, which is expressed by activated T cells, and generates a signal that leads to T-cell exhaustion. Thus, PD-1/PD-L1–blocking antibodies may impede the exhaustion signal, and thus reinvigorate tumor-specific T cells to destroy the cancer. Nivolumab (Opdivo, Bristol-Myers Squibb) and pembrolizumab (Keytruda, Merck) are PD-1–targeting antibodies that are FDA-approved for the treatment of metastatic melanoma[6,7] and non–small cell lung cancer.[8,9] Several PD-L1–directed antibodies in development, including the anti–PD-L1 agents atezolizumab (Tecentriq, Genentech), MedImmune's durvalumab, and EMD Serono's avelumab, are also being investigated in breast cancer.

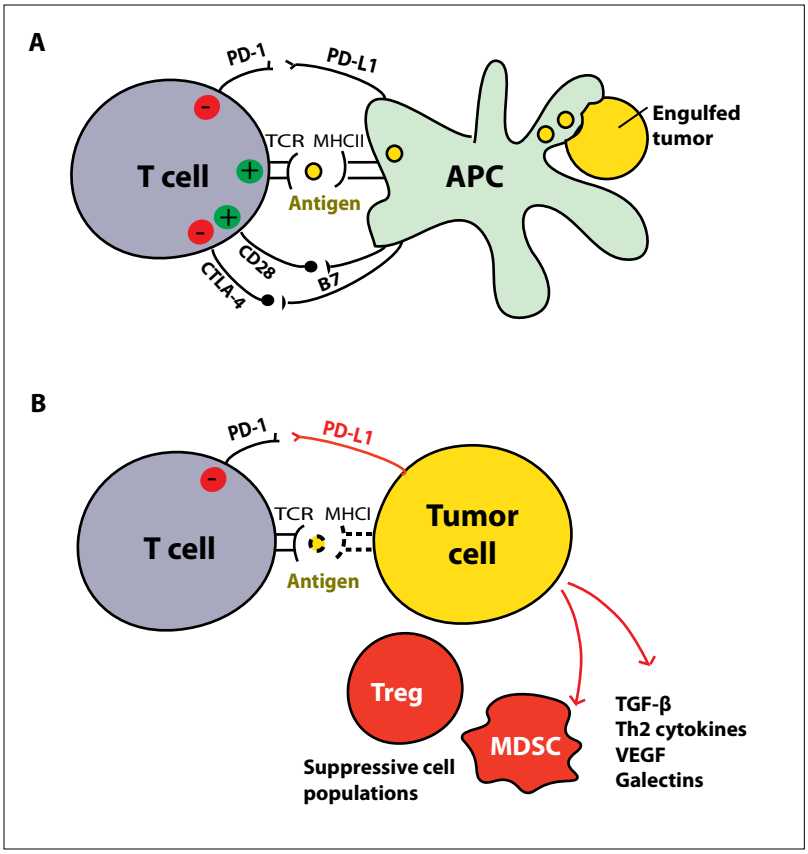

Figure. **A,** T cells recognize tumor-associated antigens in the context of the T-cell receptor/major histocompatibility complex, and T-cell activation is dictated by an interplay of costimulatory and coregulatory signals. **B,** Tumors may escape immune detection by a variety of mechanisms, including MHC class I downregulation, antigen downregulation, expression of immunosuppressive cytokines or other factors, PD-L1 expression, and recruitment of suppressive immune cells.

APC, antigen-presenting cell; CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; MHCI, MHC class I, MHCII, MHC class II; PD-1, programmed death 1; PD-L1, programmed death ligand 1; TCR, T-cell receptor; TGF-β, transforming growth factor beta; Th2, T-helper type 2; Treg, T-regulatory cell; VEGF, vascular endothelial growth factor.

Immune-Based Biomarkers in Breast Cancer: Rationale for Immunotherapy

PD-L1 Expression in Breast Cancer

Because anti–PD-1/PD-L1 antibodies function by impeding PD-1/PD-L1 interactions, one proposed method of predicting response is to assess for PD-L1 expression within tumor specimens. In the first phase 1 trial of the anti–PD-1 agent nivolumab, objective responses appeared to be restricted to tumors that were PD-L1–positive, as measured by the proportion of tumor cells with membranous staining for PD-L1 by immunohistochemistry (IHC).[10] In subsequent clinical trials in melanoma and lung cancer, PD-L1 positivity by this method has been found to enrich for tumor response, with PD-L1–negative tumors potentially responding, albeit less frequently. On the basis of these preliminary observations, attempts were made to characterize PD-L1 expression in breast cancer. One of the few published studies in this area utilized PD-L1 RNA expression data from The Cancer Genome Atlas (TCGA), in conjunction with PD-L1 IHC expression in breast cancer tissue microarrays, to demonstrate that approximately 19% of triple-negative breast cancer (TNBC) specimens expressed PD-L1, and that average PD-L1 expression was much higher in TNBC than in hormone receptor–positive breast cancers.[11] In early clinical trials of anti–PD-1 agents in metastatic breast cancer, 58% of screened TNBCs and 19% of hormone receptor–positive/HER2-negative tumors were found to be PD-L1–positive by a proprietary IHC PD-L1 assay.[12-14]

On the basis of these data and similar unpublished data, most breast cancer trials have required PD-L1 positivity (by various methodologies) for eligibility, as a method to enrich for subjects likely to respond. One potentially problematic aspect of this strategy is that the PD-L1 biomarker has not yet been standardized: there is no consensus on assay/reagent, scoring methodology, or numerical cutoff for positivity. For example, some assays measure PD-L1 expression on tumor cells, others measure PD-L1 on tumor-infiltrating immune cells, and some measure a combination of the two. Only one reported breast cancer anti–PD-1/PD-L1 monotherapy trial, the JAVELIN study (Avelumab in Metastatic or Locally Advanced Solid Tumors), has not required PD-L1 positivity for eligibility.[15] In this trial, PD-L1 expression on tumor cells was not predictive of clinical response to avelumab, regardless of the numerical threshold of PD-L1 tumor cell positivity. However, the presence of PD-L1 positive "immune cell hot spots" was associated with response. Only 10% of tumors contained these hot spots,

and the methodology for identifying these hot spots was not clearly described.

In light of the potential association between PD-L1 expression and clinical response, one possible therapeutic strategy is to combine anti–PD-1/PD-L1 agents with modulators of PD-L1 expression. The most frequently cited modulator of PD-L1 is IFN-γ. Expression of PD-L1 has been associated with IFN-γ and downstream Janus kinase 2 (JAK2) signaling in a variety of preclinical models.[16,17] In a recent atezolizumab clinical trial, on-treatment changes in IFN-γ levels over time in serial biopsies was positively associated with changes in PD-L1 protein expression.[18] Several clinical trials are evaluating anti–PD-1/PD-L1 agents with various formulations of exogenous IFN-γ (NCT02614456, NCT02339324, and others). Furthermore, PD-L1 may be indirectly modulated by other therapeutic agents, such as chemotherapy or immune checkpoint agents. Chemotherapy is a known inducer of IFN-γ secretion,[19] and has also been associated with increases in PD-L1 protein expression.[20] In a phase 1 trial evaluating the anti–PD-L1 agent atezolizumab in combination with anti-OX40 immune checkpoint agonist therapy, treatment with the anti-OX40 alone resulted in marked upregulation of intratumoral PD-L1 IHC expression in a patient with renal cell carcinoma who was experiencing progressive disease. The patient was subsequently treated with anti-OX40 plus atezolizumab, and experienced partial response that was associated with further increases in intratumoral PD-L1 expression.[21]

The Tumor Infiltrating Lymphocyte Prognostic Biomarker

The presence of immune cells within tumors may also suggest an endogenous immune response to TAAs, and increased likelihood of response to immune checkpoint therapy. In metastatic melanoma treated with the anti–PD-1 agent pembrolizumab, patients with an objective response had higher baseline infiltration of lymphocytes measured by IHC, whereas nonresponders had low or no detectable immune cells.[22] Similarly, a growing body of data in early-stage breast cancer indicates that the presence of tumor-infiltrating lymphocytes (TILs) may be a reliable prognostic marker, with TIL-rich tumors exhibiting lower recurrence rates and improved response to neoadjuvant chemotherapy.[23-28] At the 2015 San Antonio Breast Cancer Symposium, a pooled analysis of TILs as a biomarker across 5 adjuvant anthracycline-based chemotherapy TNBC trials was presented.[23] The analysis comprised 991 chemotherapy-treated subjects, and utilized the Salgado criteria—a consensus developed by an international working group—for quantifying TILs as a continuous variable, defined as the percentage of tumor stroma infiltrated by TILs.[29] In this study, an increased quantity of stromal TILs was associated with improved recurrence-free and overall survival. This association was independent of conventional prognostic variables, such as lymph node status and tumor size. TILs have been shown to be prognostic in HER2-positive breast cancers across several datasets.[30] However, data are conflicting with regard to predictive utility of TILs in patients treated with trastuzumab (Herceptin, Genentech), given that TIL count predicted response to trastuzumab in the FinHER study (Finland Herceptin)[24] but not in the Alliance N9831 study.[28] In a separate analysis of invasive lobular carcinomas (of which 94% were hormone receptor–positive) using the Salgado criteria, the TIL count was associated with adverse prognosis, contrary to what was found in TNBC.[31] This finding did not reach significance in multivariate analysis, suggesting that confounding variables, such as tumor grade, may have accounted for the observation. Thus, there are data to suggest that some breast cancers may inherently interact with the immune system, and that this relationship may mediate antitumor immunity following systemic therapies. Baseline immune cell infiltration has not yet been evaluated in the context of anti–PD-1/PD-L1 therapy in any of the preliminary trials in breast cancer.

Immune Checkpoint Therapy Trials in Breast Cancer

Anti–PD-1/PD-L1 Monotherapy Trials in PD-L1–Positive TNBC

The first 2 clinical trials of anti–PD-1/PD-L1 agents enrolled patients with metastatic, PD-L1–positive TNBC. In the KEYNOTE-012 study, the anti–PD-1 antibody pembrolizumab achieved an overall response rate of 18.5% among 27 evaluable patients, with 1 complete response and 23% of patients being free from progression at 6 months.[12] The second study was a phase 1 expansion cohort of the anti–PD-L1 agent atezolizumab, whereby 19% of 21 treated subjects achieved an objective response and 27% of subjects achieved freedom from progression at 6 months.[32,33] The treatments were well tolerated, albeit with frequent immune adverse events, including fatigue, pyrexia, and neutropenia. In the pembrolizumab trial, 1 subject died of disseminated intravascular coagulation; however, this toxicity generally was not observed with anti–PD-1/PD-L1 therapy in other trials. These studies were promising and consistent, increasing enthusiasm for anti–PD-1/PD-L1 monotherapy in triple-negative disease. However, only data from subjects with PD-L1 positivity by IHC were presented. The KEYNOTE-012 study defined PD-L1 positivity as PD-L1 expression in greater than 1% of tumor cells, whereas the atezolizumab

study defined PD-L1 positivity as PD-L1 expression in greater than 5% of infiltrating immune cells.

Anti–PD-1/PD-L1 Trials in Hormone Receptor–Positive and PD-L1–Negative Breast Cancers

At the 2015 San Antonio Breast Cancer Symposium, 2 additional clinical trials of monotherapy were featured in plenary sessions. The first was the KEYNOTE-028 trial, which enrolled PD-L1–positive patients with hormone receptor–positive, HER2-negative breast cancer to receive pembrolizumab. The overall response rate (ORR) was 12%, with a clinical benefit rate of 20%.[13] Notably, all 25 women had received prior palliative chemotherapy, and 11 of the 25 had received at least 5 prior lines of therapy. The second trial was the JAVELIN study of avelumab.[15] This study included both PD-L1–positive and PD-L1–negative tumors, and included patients of various tumor subtypes (58 with TNBC, 26 with HER2-positive breast cancer, 72 with hormone receptor–positive/HER2-negative breast cancer, and 12 with unknown histology). The ORR for all patients was 4.8%, with an ORR of 8.6% in the TNBC cohort and 2.8% in the hormone receptor–positive/HER2-negative cohort. When responses by tumoral PD-L1 expression by IHC were interrogated, no impact on response rates was observed by various PD-L1 cutoffs; however, tumors that contained hot spots of PD-L1 immune cells exhibited response rates of 18%.

Antigen-Directed Immunotherapies in Breast Cancer: Vaccines

From the preliminary clinical trials of anti–PD-1/PD-L1 checkpoint blockade in breast cancer, it is clear that monotherapy approaches with immune-adjuvant therapies may be effective only in a minority of breast cancers. These therapies appear to work best when patients have produced an endogenous immune response. Antigen-based strategies such as cancer vaccines may serve as alternative approaches, especially for breast cancers that are inherently less immunogenic; for example, PD-L1–negative tumors or tumors with poor baseline immune infiltration. Cancer vaccines have been extensively evaluated in breast cancer, and have been the topic of several recent comprehensive reviews.[34,35] Multiple vaccine strategies have been evaluated, including monovalent vaccines, polyvalent vaccines, and cellular vaccines. Monovalent vaccines aim to facilitate immune responses against a single antigen of interest (such as HER2), whereas polyvalent vaccines aim to deliver multiple TAAs simultaneously. A third class of vaccines uses whole cell preparations or cellular products to enhance delivery of TAAs. Here, we summarize vaccine studies in breast cancer, as well as emerging developments in the field.

Monovalent Vaccine Strategies

Monovalent vaccines rely on the presence of a known antigenic target. For such a strategy to be successful, the antigen must be both enriched in the tumor relative to normal cells, and expressed in a sufficient proportion of tumor cells. In a phenomenon called epitope spreading, monovalent cancer vaccines may effectively initiate immune responses against a broad array of TAAs, potentially mediated by initial immune responses that lead to subsequent cancer cell lysis and presentation of other TAAs.

One of the most studied targets is the HER2 protein, which may be an ideal antigen because it is overexpressed frequently in breast cancer, is enriched on tumors relative to normal tissues, and functionally drives tumor growth and metastatic potential. The most studied HER2 vaccine is the E75 peptide vaccine, named nelipepimut-S. The E75 peptide is derived from the extracellular domain of the HER2 protein and has been found to stimulate HER2-specific cytotoxic T-cell responses.[36] The vaccine has been evaluated in combination with the immune adjuvant, granulocyte-macrophage colony-stimulating factor (GM-CSF), and has been found to be safe in several phase 1 and 2 clinical trials. Furthermore, subset analyses of two phase 2 studies identified that both immune responses (as measured by positive delayed-type hypersensitivity to intradermal E75) and 5-year disease-free survival (89.7% vs 80.2%; P=.08) were improved in tumors with low HER2 expression (1-2+ by IHC), whereas subjects with HER2-positive tumors (3+ by IHC) did not benefit from therapy.[36] The hypothesis is that overexpression on tumors may engender immune tolerance against the protein. As a result of these promising data, a phase 3 randomized trial of nelipepimut-S/GM-CSF compared with GM-CSF treatment alone is currently ongoing (NCT01479244), with a primary endpoint of disease-free survival.

Additional monovalent vaccine targets under development include mucin 1 (MUC1; a GP2 HER2 peptide) and globo-H. GP2 is an MHC class I peptide vaccine derived from the transmembrane domain of the HER2 protein that was recently shown in a phase 2 study to improve disease-free survival compared with control (88% vs 81%; n=180).[37] Another antigen target of interest is MUC1, a glycoprotein expressed in breast cancer that is highly expressed in breast cancer and implicated in tumor cell growth and metastasis.[38] The sialyl-Tn (STn) epitope of MUC1 was effective in mediating antitumor immunity in preclinical models.[38] An STn-based vaccine called Theratope was compared with immune adjuvant therapy using keyhole limpet hemocyanin (KLH) in a phase 3, randomized double-blind study that enrolled patients with metastatic breast cancer who experienced either objective response or stable disease following

chemotherapy. Despite effective induction of antibody responses against STn, the study did not meet its clinical endpoint of improved time to progression (3.4 vs 3.0 months; overall survival, 23.1 vs 22.3 months).[39] A similar phase 3 trial compared maintenance dosing of the globo-H–KLH vaccine vs placebo in patients with metastatic breast cancer who achieved an objective response or stable disease following hormonal therapy or chemotherapy. Globo-H is a cancer-associated carbohydrate antigen that is expressed frequently in breast cancer. The OPT-822/821 vaccine was developed by conjugating the carbohydrate to the KLH carrier protein and combining this vaccine product with a saponin-based immune adjuvant in an effort to induce antibody responses. The trial failed to meet its primary endpoint of progression-free survival; however, progression-free survival was improved among patients who received the vaccine and developed elevated antibody titers against globo-H compared with patients who received placebo or those who received the vaccine but had low antibody titers. T-cell responses against globo-H were not reported. Although the improvement in PFS could be related to vaccine-induced antitumor immunity, it could also be explained by underlying differences in immunocompetency among subjects able to mount an immune response against the vaccine.[40]

An abundance of additional breast cancer antigens have been identified and evaluated in preliminary clinical trials.[41] Recent preclinical data support the premise that vaccine efficacy can be maximized by selecting antigens that are more likely to promote a Th1-mediated immune response. Th1 responses are characterized by secretion of Th1-type cytokines such as IFN-γ, interleukin 2 (IL-2), and IL-12. These cytokines are strongly associated with effective antigen presentation and antitumor T-cell response.[42] As proof of concept in a TgMMTV-neu breast cancer mouse model, vaccination against insulin growth factor binding protein 2 (IGFBP2) was only effective when IGFBP2 peptides were selected on the basis of their ability to promote secretion of Th1 cytokines.[43]

Polyvalent Vaccine Strategies

Monovalent vaccines, even if effective in directing immune responses toward the antigen of interest, may facilitate outgrowth of resistant tumor cells that downregulate the target of interest. For example, a HER2-based dendritic cell vaccine was evaluated in humans with ductal carcinoma in situ (DCIS) and was found to induce DCIS regression in the majority of tumors; however, the residual tumor exhibited loss of HER2 expression.[44] To mitigate the effects of antigen loss, polyvalent vaccines have been developed that employ multiple TAAs to facilitate more robust and diverse antitumor responses. PANVAC (Pancreatic Vaccine) is a recombinant poxvirus-vector therapeutic vaccine that encodes for breast cancer TAAs, carcinoembryonic antigens (CEAs), and MUC1, as well as costimulatory molecules including B7, intercellular adhesion molecule 1 (ICAM1), and lymphocyte function-associated antigen 3 (LFA-3). In a phase 2 study, patients were randomized to receive docetaxel with or without PANVAC. This study met its primary endpoint of demonstrating a trend toward improved PFS (7.9 months for the combination vs 3.9 months for docetaxel alone; 1-sided P=.09). The trend was retained in multivariate analysis after adjusting for potential confounders, including hormone receptor status. Using intracellular cytokine staining, immune responses against the tumor antigens (CEA, MUC1) were detected more frequently in the vaccination arm compared with the chemotherapy-alone arm (69% vs 53%).[45]

Novel Vaccine Strategies

Either tumor cells or immune cells may be modified ex vivo to produce potential vaccination products. For example, one such vaccine in development for breast cancer is GVAX, a cellular vaccine of allogeneic (derived from unrelated donors) irradiated human breast cancer cells transduced to express the immune adjuvant GM-CSF. The GVAX breast vaccine is currently being evaluated in a phase 2 trial in combination with low-dose cyclophosphamide and trastuzumab in patients with non–HER2-overexpressing metastatic breast cancer (NCT00971737). Similarly, immune cells—such as dendritic cells capable of presenting tumor antigens to T cells—can be harvested and manipulated ex vivo to produce potent vaccines. The only FDA-approved cancer vaccine, sipuleucel-T (Provenge, Dendreon), is an autologous peripheral blood cell–based vaccine against prostatic acid phosphatase that improves survival in metastatic prostate cancer.[46] In a preoperative DCIS trial, autologous dendritic cells were harvested; treated ex vivo with cytokines, lipopolysaccharide, and synthetic HER2 peptides; and injected into patients intranodally. Tumor shrinkage was observed, with possible complete response in 18.5% of treated patients (ie, no DCIS in the resection specimen). The vaccine also was associated with a median reduction in HER2 expression of 88% among patients with residual disease, suggesting that it either mediated destruction of HER2-positive cells or mediated downregulation of the HER2 protein.[44] Furthermore, T-cell immune responses against HER2 were observed in the majority of treated patients.[47]

With the advent of genomic sequencing, novel constructs are being developed to personalize vaccines for individual patients. Cancers are caused by somatic mutations, which are ultimately translated to abnormal protein products that drive tumor growth and invasion. Across several studies of immune checkpoint blockade, tumors with larger

numbers of somatic mutations were more likely to respond to immune checkpoint therapy, suggesting that these abnormal protein products, or "neoantigens," can potentially induce antitumor immune responses.[48,49] Several groups are now harnessing this principle to generate personalized vaccines against neoantigens, or to genetically engineer T cells against neoantigens.[50] Other groups are utilizing the autophagy process to generate personalized vaccine products. One such autophagy-based vaccine, called DRibble, is comprised of tumor-derived autophagosomes that contain a diverse array of intracellular proteins, as well as mediators of innate immunity and antigen presentation. The DRibble vaccine is manufactured by treating patient-derived (autologous) or unrelated donor–derived (allogeneic) tumor cells with compounds that interfere with intracellular protein processing and degradation. The DRibble vaccine product has been shown to contain known TAAs such as p53 and cyclin B1, as well as potential neoantigens such as mutated epidermal growth factor receptor (EGFR) or KRAS.[51]

Immune adjuvants—ie, agents delivered in tandem with vaccines to enhance or shape immune responses—may be critical to the efficacy of breast cancer vaccines. Immune adjuvants may enhance immune responses against weak antigens, enable the use of lower or fewer vaccine doses, and facilitate broader activation of B cells (antibody responses), T-helper cells, and T-effector cells.[52] A variety of adjuvants with unique mechanisms of action have been developed in the context of breast cancer vaccines (Table); however, one challenge going forward will be to determine the optimal adjuvant for a given vaccine. To date, few or no trials have specifically addressed the relative efficacy of immune adjuvants in the context of a breast cancer vaccine. Furthermore, there is growing interest in evaluating immune checkpoint antibodies as a modern immune adjuvant, delivered in combination with a vaccine.

Future Directions in Breast Cancer Immunotherapy: Combination Immunotherapy

Immunotherapy approaches that combine antigen-directed immunotherapies with immune-adjuvant therapies may hold the most promise in facilitating antitumor immunity, particularly in poorly immunogenic subsets of breast cancer. In breast cancer, no combination trials of immune checkpoint plus vaccine have been reported to date. However, a recent phase 2 trial in melanoma that combined the polyvalent dendritic cell–based vaccine TriMixDC-MEL with ipilimumab demonstrated encouraging, durable responses (ORR, 38%).[53] In breast cancer, vaccine/checkpoint antibody trials are planned, as well as numerous combination approaches with conventional therapies such as radiotherapy or chemotherapy, which may function as in situ vaccines by inducing cancer cell death and release of TAAs.

Immune Checkpoint Plus Cytotoxic Chemotherapy

Cytotoxic chemotherapy has been safely administered in conjunction with immune checkpoint antibody therapy across multiple tumor types, including melanoma and lung cancer, with potential synergistic effects.[54-56] The rationale is that chemotherapy may induce favorable immunologic effects, such as release of TAAs, depletion of suppressive immune cell populations, and release of cytokine mediators such as IFN-γ.[19,57] The first such study reported in breast cancer was a phase 1b single-arm study of nab-paclitaxel (Abraxane, Celgene) plus atezolizumab.[58,59] Among the 32 patients with metastatic TNBC who were evaluable for efficacy, the confirmed ORR by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 was 38% (95% CI, 21%-56%), with patients treated first-line demonstrating a confirmed ORR of 46% (95% CI, 19%-75%; n=13). Compared with historical controls, response rates were favorable. Furthermore, responses were similar in both PD-L1–positive and PD-L1–negative tumors, as measured on immune cells by IHC. Of the 12 responders, 6 (50%) remained on therapy at the time of analysis, and median duration of response was not reached after 6.1 months of median follow-up. Additional clinical trials are evaluating immune checkpoint therapy with other chemotherapy regimens, including the anti–PD-1 agent nivolumab plus nab-paclitaxel, and pembrolizumab plus a variety of chemotherapies including paclitaxel, capecitabine, doxorubicin, gemcitabine, and eribulin (Halaven, Eisai) (NCT02309177, NCT02648477, NCT02622074, NCT02331251, and others).

Immune Checkpoint Plus Tumor Ablation or Radiation

Therapeutic mechanisms that physically disrupt tumors may release TAAs and facilitate antitumor immune responses. Cryoablation, or tumor freezing, represents one such mechanism that has been shown to synergize with anti–CTLA-4 agents in preclinical models.[60] Freezing temperatures are administered via a thermal probe inserted into a tumor, leading to mechanical disruption that has been shown to increase antigen presentation and facilitate release of mediators of innate immunity.[61] Other ablative techniques, such as radiofrequency ablation, have been shown to synergize with checkpoint blockade in mice.[62] In a pilot preoperative study in early-stage breast cancer, cryoablation plus a single dose of ipilimumab was administered safely, with no delays in standard-of-care mastectomy.[63] Favorable immunologic effects were observed in both the tumor bed and peripheral blood, including expansion of intratumoral T-cell clones by T-cell receptor sequencing, and peripheral blood T-cell

activation and proliferation (by flow cytometric inducible T-cell costimulator [ICOS] and Ki67 expression).[64]

Radiation is another modality that can mechanically disrupt tumors, induce immune responses, and potentially synergize with checkpoint blockade, antibody therapy, or other immunotherapies. In a TNBC mouse model, for example, radiation synergized with CTLA-4 blockade to decrease tumor volume and improve survival.[65] Similarly, radiation has been demonstrated in preclinical models to synergize with anti–CTLA-4 plus anti–PD-1[66] or agents targeting tumor-associated macrophages, for example, transforming growth factor beta (TGF-β) inhibitors.[67] Radiation with checkpoint blockade has been well tolerated in patients with melanoma and prostate cancer.[68,69] When administered together, radiotherapy and checkpoint blockade may induce an abscopal effect, or reduction of tumor burden at distant sites (ie, beyond the radiation field).[70] There are numerous trials evaluating radiation plus immunotherapy in breast cancer, including a trial combining pembrolizumab with stereotactic radiosurgery (NCT02303366) and a trial combining an anti–CTLA-4 agent (tremelimumab) with brain radiotherapy with or without trastuzumab (NCT02563925).

Immune Checkpoint Therapy Plus Hormonal Therapy

One of the first trials of checkpoint blockade in breast cancer was a phase 1 study of tremelimumab, a CTLA-4–directed antibody, with exemestane in 26 women with heavily pretreated hormone receptor–positive metastatic breast cancer.[71] The combination resulted in dose-limiting diarrhea in numerous patients, and the maximum tolerated dose was lower than anticipated compared with other tremelimumab trials. Although it is plausible that diarrhea was exacerbated by the combination, another possible explanation is that effective, algorithm-based management of immune-related diarrhea/colitis[72] was not yet available and instituted for this trial. Although no responses were observed, 11 of the 26 women experienced disease stabilization, including 4 who previously progressed on exemestane, with evidence of T-cell activation in the periphery (as measured by ICOS expression).

There is growing interest in combination endocrine/immune checkpoint therapy, with multiple ongoing clinical trials, including a trial of pembrolizumab plus antiestrogen therapy (NCT02648477). Because resistance to antiestrogen therapy can be mediated by downstream cell signaling, hormonal therapies are now being combined with targeted therapy such as phosphoinositide 3-kinase (PI3K) inhibitors. In a murine model of breast cancer, constitutive PI3K activity (achieved by upstream knockdown of the PTEN tumor suppressor) was associated with increases in tumor PD-L1 expression, whereas conversely, PI3K pathway inhibition using an AKT inhibitor was associated with decreases in PD-L1 expression.[11] Increases in PD-L1 expression were associated with decreased T-cell proliferation, providing indirect evidence that PI3K inhibition may increase immune responses and potentially could enhance the therapeutic benefit of anti–PD-1/PD-L1 agents.[73]

Dual Checkpoint Blockade Therapy

In melanoma, the combination of anti–CTLA-4 (ipilimumab) and anti–PD-1 (nivolumab) agents was more toxic than monotherapy, but was associated with deep, durable clinical responses comparing favorably to monotherapy with ipilimumab or nivolumab alone.[74,75] Both PD-L1–negative and PD-L1–positive patients appeared to benefit from therapy, with ORRs of 55% and 72%, respectively, suggesting that combination therapy could "rescue" participants who were less likely to respond to anti–PD-1/PD-L1 agents alone on the basis of PD-L1 expression. These data have inspired clinical trials across a variety of malignancies, including metastatic breast cancer. A phase 1/2 clinical trial of ipilimumab plus nivolumab, unselected for PD-L1 expression, was conducted in metastatic TNBC; however, results have not yet been disclosed. Other studies evaluating dual checkpoint blockade in breast cancer are ongoing (NCT02536794), including studies with novel checkpoint antibodies targeting other inhibitory signaling proteins (such as OX40),[76] agonist checkpoint antibodies targeting stimulatory signaling proteins (such as GITR[77] or CD27), agents targeting suppressive macrophage populations (ie, by targeting CSF 1 receptor or TGF-β),[78] or histone deacetylase inhibitors (which may facilitate TAA expression).

Conclusion

Checkpoint blockade strategies have demonstrated impressive benefits in melanoma, hematologic malignancies, and numerous solid tumors in recent years. To date, only a handful of small, related studies have been reported in breast cancer, with encouraging results. It is anticipated that various iterations of related strategies incorporating cytotoxic agents, local strategies, and dual checkpoint blockade will continue to form the cornerstone of future studies. A wealth of data will likely be generated in this space over the next decade, and it is hoped that these efforts will ultimately translate into breast cancer–specific benefits, and ideally provide a cure.

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