Curr Oncol Rep (2015) 17: 5
DOI 10.1007/s11912-014-0426-9

BREAST CANCER (B OVERMOYER, SECTION EDITOR)

Immunotherapy for the Treatment of Breast Cancer

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Published online: 13 February 2015

Abstract Decades of research are now leading to therapeutics that target the molecular mechanisms of the cancer-specific immune response. These therapeutics include tumor antigen vaccines, dendritic cell activators, adjuvants that activate innate immunity, adoptive cellular therapy, and checkpoint blockade. The advances in targeted immunotherapy have led to clinical advances in the treatment of solid tumors such as melanoma, prostate cancer, lung cancer, and hematologic malignancies. Preclinical and translational studies suggest that patients with breast cancer may also benefit from augmenting effective immune responses. These results have led to early-phase clinical trials of tumor antigen vaccines, adjuvants, and combinations of checkpoint inhibitor blockade to boost breast cancer-specific immunity in patients. This review focuses on the current and emerging development of cancer immunotherapy for breast cancer.

Keywords Tumor antigen · Vaccine · Immunotherapy · Tumor immunology

Introduction

Despite significant advances in breast cancer detection, locoregional therapy, endocrine therapy, chemotherapy, and now molecular-targeted therapy, breast cancer remains the second leading cause of death from cancer in women. An estimated 232,670 new cases of invasive breast cancer will be diagnosed in the USA in 2014 and approximately 40,000 women will die from breast cancer [1]. In recent years, both immune evasion and inflammation have been recognized as hallmarks of cancer progression. In breast cancer, there is a growing body of scientific evidence that cancers induce local immune dysregulation via innate immune suppression, tumorigenic inflammation, and in situ suppression of the adaptive T and B cell immune response. While endocrine and cytotoxic therapies have long been the mainstay of systemic treatment for breast cancer, recent advances in immunotherapy in multiple cancer types highlight the potential for immunotherapy, in particular as a component of multi-modality adjuvant therapy. Even standard breast cancer therapy may function, in part, by recruitment of immune cells and local immune activation, including radiation, endocrine therapy, and chemotherapy.

The most striking evidence of effective immunotherapy in breast cancer has been the development of monoclonal antibodies directed against the HER2/neu protein. Up to one third of breast cancers contain amplifications of the *ERBB2* gene encoding the HER2 receptor tyrosine kinase, which has led to clinical use of the anti-HER2 monoclonal antibodies (MAbs) trastuzumab and pertuzumab. In addition to targeting the kinase signaling pathway, trastuzumab also functions via recruitment of NK cells and activation of antibody-dependent cytotoxicity (ADCC), which may partially account for the synergistic activity of trastuzumab and docetaxel in breast cancer [2]. Here, we will focus on the clinical translation of breast cancer vaccines, adjuvants, and checkpoint inhibitor blockade.

This article is part of the Topical Collection on *Breast Cancer*

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Immune Dysregulation in Breast Cancer: an Overview

Innate vs. Adaptive Immunity The immune system functions as a highly specific and adaptable system that recognizes

alterations in proteins, glycoproteins, and cell structure. As a result, immune surveillance can alter cancer progression, while nonspecific inflammation can be tumorigenic [3]. The innate immune response is nonspecific and is comprised of antigen-presenting cells (APCs) such as dendritic cells and macrophages, as well as cytokines and chemokines that induce local and systemic inflammatory changes (Fig. 1). In contrast, the adaptive immune system is highly specific and has long-lasting memory responses. Adaptive immunity results in the development of memory B cells which secrete antibodies, cytotoxic CD8+ T lymphocytes, and T helper CD4+ T lymphocytes. Activation of the T cell immune response occurs when tumor antigens are processed by APCs, leading to direct tumor cytolysis by CD8+ T lymphocytes, and indirect cytolysis through the production of cytokines by both CD8+ and CD4+ T lymphocytes, which recruit phagocytic cells and destroy tumor and stroma.

Antigen Presentation The identification of dendritic cells (DCs) as the most effective antigen-presenting cell led to the Nobel Prize in Physiology or Medicine for Ralph Steinman in 2011. Dendritic cells constitute a uniquely efficient subset of APC that process tumor antigen through a mechanism known as cross-presentation and then migrate to the draining lymph nodes, where they initiate T cell differentiation toward the Th1, Th2, or Th17 phenotypes. Dendritic cells in breast cancer are both reduced in number and are dysfunctional [4]. Several factors determine the direction of T cell polarization and thereby regulate the T cell response [5]. Indoleamine 2,3 deoxygenase (IDO) accumulates in tumor-infiltrating dendritic cells and correlates with T cell impairment in cancer [6], and in breast cancer specifically [7]. Many clinical trials have used ex vivo purified DCs to deliver antigen, but the cost and technical challenges have limited widespread clinical trials. Of cytokines, interleukin (IL)-12 activates DCs and promotes the development of Th1 cells, cytotoxic T lymphocytes (CTL), and natural killer (NK) cells [5]. IL-12 has been shown to induce a type I immune response likely directed toward tumor-associated antigens and neo-vascularization [8].

Immunosuppression Tumor cells actively modify the tumor microenvironment to generate both immune suppression of effector T lymphocytes and to induce tumorigenic inflammation. Immune suppression occurs through mediators such as IDO, prostaglandin E2 (PGE2), transforming growth factor β (TGFβ), IL-6, and VEGF-A. Interleukin-10 (IL-10) is an anti-inflammatory cytokine that induces immunosuppression and functions to block tumor immune surveillance. In breast cancer, IL-10 induces both tumor proliferation and inhibits immune responses [9].

As a result, the tumor environment often contains high levels of regulatory T cells and myeloid-derived suppressor cells (MDSCs). MDSCs are a population of immature myeloid cells that function to suppress both innate and adaptive immune responses [10]. Therapeutics that target MDSCs include STAT3 inhibitors, tyrosine kinase inhibitors, and aminobisphosphonates, which prevent MDSC expansion, while cytotoxic agents may directly decrease MDSC accumulation [10] [11].

T Cell Inhibition Immune regulation of the T cell response is necessary to minimize tissue destruction and autoimmunity after antigen stimulation, but these inhibitory molecules can be overexpressed in cancer tissues to cause local T cell inhibition. When T cells are activated by APCs, the co-stimulatory molecule CD28 on the naïve T cell surface binds to B7 proteins on the APC. This second signal induces T cells activation, proliferation, effector function, and migration. Additional co-stimulatory molecules include OX40, which promote clonal expansion, cytokine production, and T cell survival once bound to OX40L [12]. The inducible co-stimulatory (ICOS) and CD40 pathways promote TH2 responses (T cell-dependent Ab immunity), whereas the OX40/4-1BB pathways promote the activation of CD4+ and CD8+ T cells.

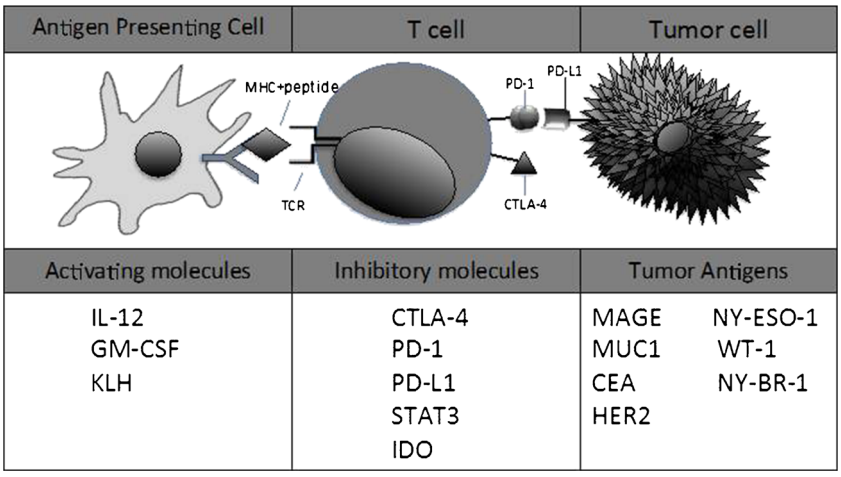

Fig. 1 Schematic of potential immunotherapy targets in breast cancer. Molecular pathways are altered in multiple cellular types at the tumor site. Antigen-presenting cells (APC, *left*), T cells (*center*), and tumor cells (*right*) all express potential targetable molecules such as tumor antigens for vaccines, adjuvants for activating APCs, and blockade of inhibitory molecules that function to impede anti-tumor immunity

There are several inhibitory pathways that serve to limit T cell activation and function. CTLA-4 is a CD28 homologue that binds to B7 on APC with a higher affinity and is induced by TCR signaling to out-compete the CD28 function. In effect, CTLA-4 expression inhibits T cell activation and antitumor efficacy. PD-1 is a CD28 and CTLA-4 homologue that is normally induced on activated T cells, but the chronic antigenic exposure in cancer may lead to high levels of PD-1 and T cell exhaustion [13••]. Upregulation of PD-1 has been shown to inhibit innate and adaptive immunity [14]. The ligand of PD-1, PD-L1, is upregulated in tumor cells and is correlated with progression and poor prognosis of cancer [14].

The mammalian target of rapamycin (mTOR) belongs to the phosphatidylinositol 3-kinase-related kinase protein family and is commonly dysregulated in breast cancer [15]. Hyperactivation of this pathway has been linked to resistance of endocrine and anti-HER2 therapies [16]. mTOR inhibition potentiates vaccine-induced generation of memory T cells while increasing susceptibility to cytotoxic effector cells in vitro [17, 18]. Everolimus is an inhibitor of mTOR that is currently used in combination with exemestane for advanced metastatic hormone receptor-positive (HR+) breast cancer [19], and mTOR inhibition is being evaluated as an adjuvant for cancer vaccine development.

The Tumor Microenvironment in Breast Cancer Tumor expression profiling of breast cancer has demonstrated patterns of immunoregulatory gene activation [20]. The immunomodulatory subtype of triple-negative breast cancer (TNBC) has prominent lymphocytic features, which it shares with medullary breast cancers [21••]. TNBC subsets with B cell signatures on tumor expression profiling have improved clinical outcomes [22]. Approximately 50 % of Her2/neu+ breast cancers have inflammatory signatures, which also correlate with improved clinical outcome [23]. Breast cancers with higher numbers of tumor-infiltrating lymphocytes have improved responses to neoadjuvant chemotherapy [24]. These observations suggest that subsets of breast cancers have intrinsic properties that may respond better to targeted immunotherapy [25].

Clinical Trials of Immunotherapy in Breast Cancer

Breast Cancer Vaccines Vaccines for infectious disease are designed for disease prevention and usually target B lymphocyte immunity, but cancer vaccines have primarily been developed to stimulate T lymphocyte immunity, for the treatment of preexisting disease. Cancer vaccines target tumor antigens that are altered in tumors, either by mutation, splice variation, or overexpression. The development of next-generation sequencing of cancers has led to the prediction of a large number of unique tumor antigen targets, which carries the (as yet unrealized) potential for personalized vaccine therapy targeting the proteomic alterations within an individual patient's cancer. Most early cancer vaccines were designed to activate CD8+ T cell responses using short peptides that bind MHC class I molecules [26]. These T cell responses were often short-lived and ineffective, which led to antigen delivery methods that target both CD8+ effector T lymphocytes and CD4+ helper T lymphocytes [13••]. Antigens can be delivered as peptides, proteins, naked DNA, with viral vectors, or loaded into antigen-presenting cells such as dendritic cells (Tables 1 and 2). Antigen delivery is generally combined with adjuvants that enhance antigen presentation and activation of innate immunity, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or Montanide ISA-51.

HER2 Peptide Vaccines The most well-studied antigens in breast cancer are the Her2/neu (HER2) and mucin-1 (MUC1) antigens. HER2 is overexpressed in 25–30 % of breast cancers, while MUC1 is expressed in the vast majority of breast cancers with altered glycosylation. Subsets of patients with breast cancer naturally have low levels of antibodies as well as T cell immunity specific for HER2 or for MUC1. The vaccines have been designed to amplify the preexisting immunity to a therapeutically beneficial level. The vaccines that are furthest along in clinical development are the Her2/neu vaccines that target the E75 peptide (NeuVax™, nelipepimut-S) and the GP2 peptide, both of which are limited to HLA-A2+ patients (40–50 % of the US population) [27••, 28]. An initial nonrandomized phase I/II trial of the E75 peptide with the adjuvant GM-CSF enrolled 195 early-stage HER2-positive patients and demonstrated improved 5-year disease-free survival (DFS) (89.7 %) when compared to GM-CSF-treated HLA-A2-negative controls (80.2 %) in the adjuvant setting [29••]. These results led to an ongoing phase III study evaluating E75 with GM-CSF in the prevention of recurrence for patients with node-positive HER2-positive breast cancer (NCT01479244). A phase II study evaluating the combination of E75 with trastuzumab is also ongoing (NCT01570036).

The HER2-derived GP2 peptide is a subdominant T cell epitope that, like E75, is naturally immunogenic in a subset of patients with HER2-positive breast cancers. A phase II trial of vaccination of GP2 with GM-CSF for breast cancer patients in the adjuvant setting is ongoing. Early results with a median follow-up of 17.9 months suggest improvement in recurrence rates [27••].

Because of the short duration of immunity to MHC class I-targeted peptides, multi-epitope vaccines have been designed to elicit both CTL and CD4+ T_H cell responses. AE37 is a HER2-derived class II epitope that targets CD4+ T lymphocytes. A phase II trial that combines the AE37 peptide with the GP2 peptide and GM-CSF for the adjuvant treatment of early-stage breast cancer is currently underway, with interim

Table 1 Ongoing clinical trials of immunotherapy in breast cancer

Phase	Study	Stage	NCI identifier
DNA and viral vaccines			
I	AVX901 HER-2-expressing viral vaccine	Metastatic	NCT01526473
I	Plasma mammaglobin-A DNA	Metastatic	NCT00807781
I	AdHER-2/neu dendritic cell	Metastatic	NCT01730118
I	HER2/neu peptide+measles virus+nor-MDP in ISA 720	Metastatic	NCTO1376505
I	Human MUC1 in adenovirus	Metastatic	NCT02140996
I/II	CEA/TRICOM vaccine with chemotherapy[a]	Metastatic	NCT00048893
Peptide and protein vaccine			
I	Folate receptor binding peptide vaccine	Early	NCT02019524
I	Sialyl Lewis-KLH vaccine and QS21	Metastatic	NCT00470574
I	Multiple-peptide vaccine	Metastatic	NCT01259505
I	Synthetic peptides+tetanus toxoid+Montanide ISA-51[a]	Metastatic	NCT00304096
I	GP2+GM-CSF vs AE37+GMCSF vs GMCSF	Early	NCT00524277
I	NY-ESO-1 vaccine±sirolimus	Early	NCT01522820
I	MUC-1 peptide for TNBC	Early	NCT00986609
I	Multi-peptide vaccine with basilixumab (anti-CD25)	Metastatic	NCT01660529
II	Trastuzumab+GM-CSF±HER-2 E75 peptide	Early	NCT01570036
II	Globo H-KLH immunostimulant	Metastatic	NCT01516307
III	NeuVax™ (nelipepimut-S or E75)	Early	NCT01479244
I/II	HER-2 intracellular protein+trastuzumab±polysaccharide-K	Metastatic	NCT01922921
Cellular vaccines			
I	Pilot study of a breast cancer vaccine plus poly-ICLC	Early	NCT01532960
I/II	HER-2 peptide vaccine+cyclophosphamide+adoptive HER2-specific T cells	Metastatic	NCT00791037
I/II	Allogeneic whole-cell vaccine	Metastatic	NCT00722228
II	GSK2302024A (WT-1-specific therapy)+standard neoadjuvant	Early	NCT01220128
II	Cyclophosphamide+GM-CSF+allogeneic vaccine±trastuzumab	Metastatic	NCT00971737
II	PANVAC+docetaxel	Metastatic	NCT00179309
II/III	Allo-stim breast cancer vaccine	Metastatic	NCT01741038
Checkpoint inhibitors			
I	Lirilumab (anti-KIR)+nivolumab (anti-PD1)	Metastatic	NCT01714739
I	BMS 936558 (anti-PDL1)	Metastatic	NCT00729664
I	MPDL3280A (anti-PDL1)	Metastatic	NCT01375842
I	MEDI4736 (anti-PD1) in TNBC	Metastatic	NCT01693562
I/II	Nivolumab (anti-PD1)±ipilimumab (anti-CTLA4) in TNBC	Metastatic	NCT01928394
II	Preoperative cryotherapy±ipilimumab (anti-CTLA4)	Early	NCT01502592

TNBC triple-negative breast cancer

[a] Completed, data not yet published

evidence of induction of T cell immunity [30, 31]. Clinical trials using long overlapping peptides derived from the extracellular and intracellular domains of HER2 are also ongoing.

MUC1 Peptide Vaccines MUC1 is an epithelial membrane antigen whose overexpression has been linked with breast cancer and other human epithelial cancers and is the target of the CA27.29 and CA15-3 biomarkers. The presence of abnormally glycosylated MUC1 on cancer cells can trigger a cytotoxic T cell response and the presence of antibodies in serum of patients with early breast cancer is associated with better outcomes [32]. Sialyl-Tn (STn) is a naturally occurring carbohydrate epitope found on a variety of glycoproteins, including MUC1, expressed by many types of tumor cells and is believed to have functional significance in tumor growth and metastasis [33]. Theratope® is a therapeutic cancer vaccine that consists of a synthetic antigen that mimics the STn antigen. The STn antigen is conjugated to the high-molecular-weight carrier protein keyhole limpet hemocyanin (KLH) and administered with the adjuvant Detox B (later renamed Enhanzyn).

Table 2 Recently completed clinical trials of immunotherapy in breast cancer

Therapy	Adjuvant	Number of participants	Results	Stage	Ref
Nelipepimut-S E75 (HER2) NeuVax™	GM-CSF	182	2-year DFS: overall, 94.3 vs. 86.8 % (P=0.08) HER2-low tumors, 94.0 vs. 79.4 % (P=0.04) HER2-positive tumors, 90.3 vs. 83.3 % (P=0.44)	Early	[29••]
GP2 (HER2)	GM-CSF	172	Recurrence rate, 4.3 vs. 11.6 % (P=0.41)	Early	[80]
AE37 (HER2)	GM-CSF	298	Recurrence rate, overall 12 % RRR P=0.70	Early	[81]
AE37/GP2	GMCSF	28	Immune response observed	Early	[82]
Theratope™ Sialyl-Tn	KLH	1028	OS, 23.1 vs 22.3 months (P=0.916) TTP, 3.4 vs 3.0 months (P=0.353) ER+: OS, 39.6 vs 25.4 months (P=0.005)	Metastatic	[37••]

KLH keyhole limpet hemocyanin

Theratope has now been studied in phase I, II, and III clinical trials [34–36]. In a large phase III trial of 1028 women with metastatic breast cancer, treatment with Theratope failed to result in prolonged survival, but subset analysis of patients receiving concomitant endocrine therapy demonstrated improvements in time to progression and overall survival [37••].

Cancer-Testis Antigen Vaccines Cancer-testis (CT) antigens such as MAGE-A3 and NY-ESO-1 are defined by their selective expression in germ line cells and absent expression in normal tissues. Most of the clinical trials targeting CT antigens have been for melanoma and lung cancer [38]. Vaccination with NY-ESO-1 has been recently evaluated in metastatic melanoma in combination with ipilimumab [39, 40] and sirolimus [39]. Therapeutic tumor vaccines targeting MAGE-A3 are also being tested in clinical trials in NSCLC (NCT00480025). In breast cancer, the CT antigens MAGE-A3 and NY-ESO-1 are preferentially expressed in ER-negative cancers [41]. When expressed in breast cancer, the cancer-testes antigens are highly immunogenic [42••], and the titer of antibodies to NY-ESO-1 has been shown to correlate with breast cancer progression, and these antigens remain potential targets for breast cancer immunotherapy [43].

Other Breast Cancer Target Antigens A number of other tumor antigens have increased expression in breast cancers, and early-phase clinical trials have targeted breast cancer as well as other cancers. Most of these vaccine trials have demonstrated induction of T cell-specific immunity, but these studies have been too small or too recent to demonstrate evidence of clinical impact. Carcinoembryonic antigen (CEA) is a glycosylated membrane-bound protein of 180 kDa expressed in a high percentage of several human carcinomas, including colorectal, gastric, pancreatic (90 %), non-small cell lung (70 %), and breast carcinomas (50 %) [44]. A number of CEA-based cancer vaccines have been tested in early-phase clinical trials for the treatment of breast and other cancers [45]. Telomerase (hTERT) is a widely expressed tumor antigen, present in more than 85 % of human cancers and as many as 99 % of breast cancer while absent in normal cells [46]. Clinical trials of dendritic cells pulsed hTERT-derived peptide vaccines resulted in increased hTERT specific immunity, but did lead to anti-tumor immunity [47].

Other potential target antigens include the Wilms' tumor antigen (WT1), a transcription factor involved in cell proliferation, differentiation, and apoptosis in breast cancer, leukemia, and other cancers [48, 49••]. A phase I/II trial of a WT1 peptide with adjuvant Montanide ISA resulted in a partial response in one of ten patients, with stable disease in five patients [50]. NY-BR-1 is a tumor-associated antigen detected in up to 60 % of primary breast carcinomas and represents a potential target [43, 51]. Mammaglobin-A (Mam-A) is a secretory protein that is overexpressed in 80 % of primary and metastatic breast cancers. A phase I clinical trial of a mammaglobin-A DNA vaccine resulted in stimulation of IFN-γ producing CD4+ICOS^{hi} T cells with tumoricidal function [52••].

Viral Vectors for Antigen Delivery Due to the HLA limitations of short peptides and the production and cost of recombinant proteins and pools of long peptides, a variety of vectors have been developed to deliver antigens with co-stimulatory molecules to enhance immunity. These vectors include the poxvirus family (fowlpox, canarypox, and vaccinia), measles, and adenoviral vectors. Viral vectors, in general, generate longer-lasting and broader immunity than either naked DNA or peptide delivery, but repeat vaccination can induce antibodies to viral antigens that limit immunogenicity. Therefore, many viral vector vaccine designs use different vectors for immune priming and boosting. PROSTVAC is a recombinant vaccinia viral vaccine that contains genes encoding PSA and three T cell co-stimulatory molecules (TRICOM: ICAM-1, B7.1, and LFA-3) [53]. PROSTVAC is currently in phase III clinical trials for prostate cancer. With a similar design, PANVAC is a recombinant poxviral vaccine that contains genes encoding MUC1, CEA, and TRICOM. Of the 12 metastatic breast cancer patients enrolled, the median time to progression was 2.5 months, with one patient TTP greater than

37 months, and median overall survival was 13.7 months [54]. Vaccinia virus engineered to produce MUC1 and IL-2 has been evaluated in metastatic breast cancer, with two of 31 patients having a clinical response [55].

Autologous Dendritic Cell Vaccines Dendritic cells can be generated from the peripheral blood of patients, loaded with tumor antigen proteins or peptides, and used as vaccines. Dendritic cell vaccines are potent, but technically and logistically challenging, as they require specialized cell processing laboratories. There are many methods for generating DCs, but monocyte-derived dendritic cells from breast cancer patients are thought to preferentially induce CD4+CD25+Foxp3+ regulatory T cells [56, 57].

Sipuleucel-T (Provenge) was the first cellular vaccine that was FDA-approved in 2010. Sipuleucel-T consists of autologous APCs that have been pulsed with a fusion protein of prostatic acid phosphatase and GM-CSF and then re-infused into the patient. In the phase III IMPACT trial [58], sipuleucel-T demonstrated improved median overall survival by 4.1 months in metastatic castrate-resistant prostate cancer. Sipuleucel-T is used for the treatment of advanced prostate cancer [3].

The HER2-targeted version of sipuleucel-T, termed lapuleucel-T (APC8024, Neuvenge) remains an investigational agent. Lapuleucel-T consists of autologous peripheral blood mononuclear cells (containing APCs), which are cultured ex vivo with a recombinant fusion protein of portions of HER2 linked to GM-CSF. An initial phase I study of lapuleucel-T showed modest activity in advanced breast cancer and is currently being evaluated in bladder cancer.

Dendritic cell vaccines have been evaluated in combination with IL-2 for breast cancer and other cancers [59]. HER2 peptides have been delivered by pulsing peptides onto ex vivo-generated autologous dendritic cells. Since HER2 overexpression plays a critical role in breast cancer development and is expressed in a subset of patients with high-risk ductal carcinoma in situ (DCIS), these HER2-targeted vaccines have also been tested in the neoadjuvant setting for DCIS [60] [61]. In another trial for patients with DCIS, peripheral blood monocytes were activated with IFN and combined with a bacterial toxin, LPS and HER2/neu peptides, which resulted in durable immunity up to 52 months against HER2 [62].

Cellular Vaccines Polyvalent vaccines (autologous or allogeneic) are derived from whole tumor cells or dendritic cells fused with tumor cells, loaded with tumor lysates, or transfected with tumor-derived RNA or DNA. Immune monitoring of these complex immunotherapies is difficult, since identifying which tumor antigens are immunogenic is technically difficult; as with dendritic cells, the production of cellular vaccines is labor-intensive [13••].

Adjuvants for Cancer Vaccines

Adjuvants are substances that enhance antigen immunogenicity, such as the activation of IFN-γ-producing T cells. Classical adjuvants including either alum or water-in-oil emulsions have been generally ineffective at producing strong T_H1 responses [63] [13••]. GM-CSF is a common adjuvant used in immunotherapy clinical trials, as is Montanide ISA-51. The identification of molecular pathways involved in innate immune responses has led to the development of targeted adjuvants [64]. IDO blunts T cell function by promoting enzymatic degradation of tryptophan in the tumor microenvironment. IDO is overexpressed in many tumor types, including breast cancer. IDO inhibition has been evaluated following induction chemotherapy and concurrent chemoradiation for lung cancer [65]. In breast cancer, IDO inhibition by indoximod was well tolerated as an adjuvant in combination with a P53-directed dendritic cell vaccine in a phase I clinical trial (ASCO 2013 abstract 3069).

Additional targeted adjuvants, such as antibodies that target 4-1BB or OX40 or STAT3 inhibitors, enhance T cell activity by activation of antigen-presenting cells and are being evaluated in many combination immunotherapy trials. IMP321 is a soluble form of LAG-3, which is an MHC class II agonist and activates antigen-presenting cells, resulting in secondary activation of CD8+ memory cells. In a recent phase I/II trial with paclitaxel as first-line therapy for metastatic breast cancer (n= 30), both enhanced immunity and overall response rate (ORR) of 50 % were observed [66].

Checkpoint Blockade (CTLA-4, PD-1/PD-L1)

Lessons From Melanoma Most of the seminal advances in immunotherapy have emerged from the study of melanoma, which has long been recognized as an immune-sensitive tumor [67]. Starting with cytokine therapy with IL-2 [67], immune therapy in melanoma has focused on peptide-based vaccines [68] and adoptive T cell therapy with in vitro-expanded CD4+ and CD8+ T cells [69]. A major clinical breakthrough of immunotherapy was the identification of the T cell checkpoint inhibitor pathways, CTLA-4 and PD-1/PDL1, which function to dampen both CD4+ and CD8+ T cell responses. Ipilimumab was the first therapy to target this checkpoint mechanism by activating memory T cell immunity against tumor antigens. Because checkpoint blockade is nonspecific, off-target effects include induction of autoimmunity and immune-related adverse events (irAEs). Ipilimumab increases the frequency of CD4+ and CD8+ T lymphocytes in the peripheral blood in addition to antibody responses against tumor antigens. In the landmark phase III clinical trial in metastatic melanoma, treatment with ipilimumab (3 mg/kg every

3 weeks) was associated with an improved median survival of 3.7 months [63]. The effect of ipilimumab in some patients was durable, with 24 % of patients alive after 2 years. This led to FDA approval of ipilimumab in March of 2011 for first- or second-line therapy of unresectable stage III or stage IV melanoma [3]. Because blockade of the checkpoint CTLA-4 molecule inhibits T cell regulation, careful evaluation of the dosing frequency and timing is therefore critical to minimize irAEs while preserving anti-tumor efficacy.

Checkpoint Blockade in Breast Cancer There are emerging trials of checkpoint inhibition and breast cancer, but evidence of clinical efficacy awaits the larger phase II clinical trials. The combination of tremelimumab (anti-CTLA-4) and exemestane in advanced breast cancer resulted in induction of activated T cells [70]. IMP321 is a soluble form of LAG-3, an MHC class II agonist, which activates antigen-presenting cells, resulting in secondary activation of CD8+ memory cells. In a recent phase I/II trial with paclitaxel as first-line therapy for metastatic breast cancer (n=30), both enhanced immunity and ORR of 50 % were observed [66].

In addition to CTLA-4, the PD-1/PD-L1 inhibitory pathway has developed as a promising target for activating T cell immunity in cancer. Nivolumab, the first anti-PD-1 antibody in clinical trials, has also led to durable remissions in melanoma [71] and is currently approved in Japan for clinical use. The PD-1 inhibitor pembrolizumab (Keytruda) was recently approved by the FDA for refractory metastatic melanoma and is in trials in advanced non-small cell lung cancer. Antibodies to PD-1, such as nivolumab, are also being evaluated in melanoma, renal cell carcinoma, prostate cancer, non-small cell lung cancer, colorectal cancer, and breast cancer. In general, the immune-related toxicities associated with PD-1 blockade have been lower than previously seen with ipilimumab. In melanoma, the combination of nivolumab plus ipilimumab was associated with a >80 % decline in tumor burden at 12 weeks in respondents (ORR 53 %) [72]. These exciting results have led to the evaluation of checkpoint blockade inhibitors in breast cancer. In a recent analysis of PD-L1, CTLA-4, and IDO-1 in TNBC patients, cancer cell-specific overexpression of PD-L1 protein was present in 50 % of TNBC tumors and more often seen with androgen receptor co-expression (ASCO 2014 abstract 1001). Several early phase clinical trials of PD-1/PD-L1 blockade, alone or in combination with CTLA-4 blockade, are ongoing in advanced triple-negative breast cancer. Similar early-phase trials are underway to target the costimulatory OX40 pathway, as high OX40 expression is associated with malignant transformation, progression, invasion, and metastasis in breast cancers [73]. A phase I/II trial is underway with anti-OX40 antibodies for patients with metastatic breast cancer.

Targeting Tregs: the Synergy of Chemotherapy and Immunotherapy

Certain chemotherapies, including cyclophosphamide, taxanes, and anthracyclines, alter immune suppressor mechanisms that are induced by the tumor microenvironment [74]. Cyclophosphamide in low doses decreases Treg populations and enhances anti-tumor responses [75]. The neoadjuvant administration of taxanes in locally advanced breast cancer increases CD8+ T lymphocytes within the tumor parenchyma [76].

These findings have led to the use of low-dose cyclophosphamide prior to vaccination to decrease Treg populations. For example, a single-arm phase II study evaluated the combination of cyclophosphamide, trastuzumab, and an allogeneic GM-CSF-secreting breast cancer vaccine for 20 patients with advanced breast cancer. The overall median progression-free survival (PFS) was 7 months and overall survival (OS) was 42 months, with a 5-year survival rate of 30 %. As is commonly observed in immunotherapy trials, there was a trend toward longer PFS and OS in patients who developed antigen-specific immunity relative to those who did not [77••]. CD25-targeted antibodies can also deplete regulatory T cells to allow for more effective antigen presentation and boost vaccine responses. CD25 blockade with daclizumab depleted regulatory T cells and enhanced the immunogenicity of a multi-peptide vaccine [78]. Monoclonal antibodies that target tumor antigens (such as the anti-HER2 MAb trastuzumab) may improve CD8+ T cell immunity by enhanced antigen presentation [79].

Future Directions

Immunotherapy has demonstrated clinical benefit in phase II/III clinical trials for several solid tumors, such as melanoma, lung and prostate cancer. Multiple clinical trials are now underway to evaluate breast cancer immunotherapy, including vaccines, adjuvants, and checkpoint blockade, alone or as multimodality therapy. Successful targeted immunotherapy requires not only the persistent expression of antigen by cancer cells but also the successful and sustained mobilization of sufficient numbers of effector T cells that recognize these antigens. The highest likelihood for success will be in the setting of disease prevention for high-risk individuals, treatment of DCIS, and early-stage cancers to eradicate minimal residual disease that may not be responsive to cytotoxic or endocrine therapies. Unlike cytotoxic therapies, the slower response rates and delayed effects of immune therapy require novel approaches for clinical trial design. Identification of immune biomarkers that correlate with meaningful clinical benefit is needed to identify patients that are likely to respond to these agents [3]. The rapid development of proteome-wide immune

monitoring and tumor genomic sequencing in concert with epitope prediction is leading to the identification of novel antigenic targets in breast cancer [13••]. These findings may lead to personalized vaccines and immunotherapy for breast cancer patients. It is likely that immunotherapy will soon join multimodality therapy for the treatment of breast cancer.

Compliance with Ethics Guidelines

Conflict of Interest Brenda Ernst and Karen S. Anderson declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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