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Review

Clinical Cancer Research

# Breast Cancer Immunotherapy: Facts and Hopes

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## Abstract

Immunotherapy is revolutionizing the management of multiple solid tumors, and early data have revealed the clinical activity of programmed cell death-1/programmed death ligand-1 (PD-1/PD-L1) antagonists in small numbers of patients with metastatic breast cancer. Clinical activity appears more likely if the tumor is triple negative, PD-L1$^+$, and/or harbors higher levels of tumor-infiltrating leukocytes. Responses to atezolizumab and pembrolizumab appear to be durable in metastatic triple-negative breast cancer (TNBC), suggesting that these agents may transform the lives of responding patients. Current clinical efforts are focused on developing immunotherapy combinations that convert nonresponders to responders, deepen those responses that do occur, and surmount acquired resistance to immunotherapy. Identifying biomarkers that can predict the potential for response to single-agent immunotherapy, identify the best immunotherapy combinations for a particular patient, and guide salvage immunotherapy in patients with progressive disease are high priorities for clinical development. Smart clinical trials testing rational immunotherapy combinations that include robust biomarker evaluations will accelerate clinical progress, moving us closer to effective immunotherapy for almost all patients with breast cancer. *Clin Cancer Res; 24(3); 511–20. ©2017 AACR.*

## Introduction

Breast cancer remains a significant threat to the health and wellness of women in the United States, accounting for 30% of all new cancer diagnoses and almost 41,000 deaths annually (1). Although advances in early detection and therapy have resulted in a 38% decrease in the breast cancer death rate, almost all patients who develop metastatic disease will succumb to it. These sobering data illustrate a critical need for innovative approaches to breast cancer therapy that reduce relapse and death due to this disease. In recent years, accumulating data support a key role for the immune system in determining both response to standard therapy and long-term survival in patients with breast cancer (2). These data and the striking clinical success of immune checkpoint antagonists across multiple solid tumors (3, 4) have reignited interest in immune-based strategies for breast cancer treatment and prevention (5, 6).

## Setting the Stage for Modern Breast Cancer Immunotherapy

### The dualistic role of the immune system in breast cancer

The immune system plays an active role in breast cancer development, progression, and control (5). The evolving interactions between mammary tumors and host immunity are characterized by immunoediting (7). Early in mammary tumorigenesis, acute inflammation activates innate immunity, resulting in both tumor cell death and the maturation of dendritic cells (DC) that prime the tumor-specific T-cell response. At this stage, either immune-mediated rejection of incipient tumors or the selection of tumor cell variants that can escape the immune response occurs. Ultimately, there is a shift from acute to chronic inflammation, establishing a complex tumor microenvironment (TME) consisting of suppressive immune cells [regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC), and B cells] and stromal cells (fibroblasts and endothelial cells) that allow overt immune escape and tumor progression to occur (8, 9). During this shift, the CD4 T-cell response is skewed from T helper (Th) type 1 to Th type 2 (10), immune checkpoint molecules are upregulated on tumor cells and immune cells in response to early immune activation (11), and immune-suppressive metabolic pathways are activated in multiple immune cell types (12, 13). Together, these forces establish a formidable network of immune suppression within the breast TME. This microenvironment, and other factors described in this review, impinges on the immune system to sculpt antitumor immunity (Fig. 1).

### Immune biomarkers are both prognostic and predictive in breast cancer

Breast cancer is a heterogeneous disease. It can be classified into three major clinically relevant subtypes that are managed differently: luminal [expressing the estrogen receptor (ER) and/or progesterone receptor (PR)]; human epidermal growth factor receptor-2$^+$ (HER-2$^+$); and triple-negative, lacking expression of ER, PR, and HER-2 (14). Of these subtypes, HER-2$^+$ breast cancers and triple-negative breast cancers (TNBC) are more likely than luminal breast cancers to harbor stromal-infiltrating immune cells (TILs) at diagnosis, with a linear relationship between stromal TIL content and clinical outcomes (2, 15). HER-2$^+$ breast cancers and TNBCs are also more likely to express the programmed death ligand-1 (PD-L1) in the TME than luminal breast cancers (15, 16). Higher levels of TILs at diagnosis predict benefit from adjuvant and

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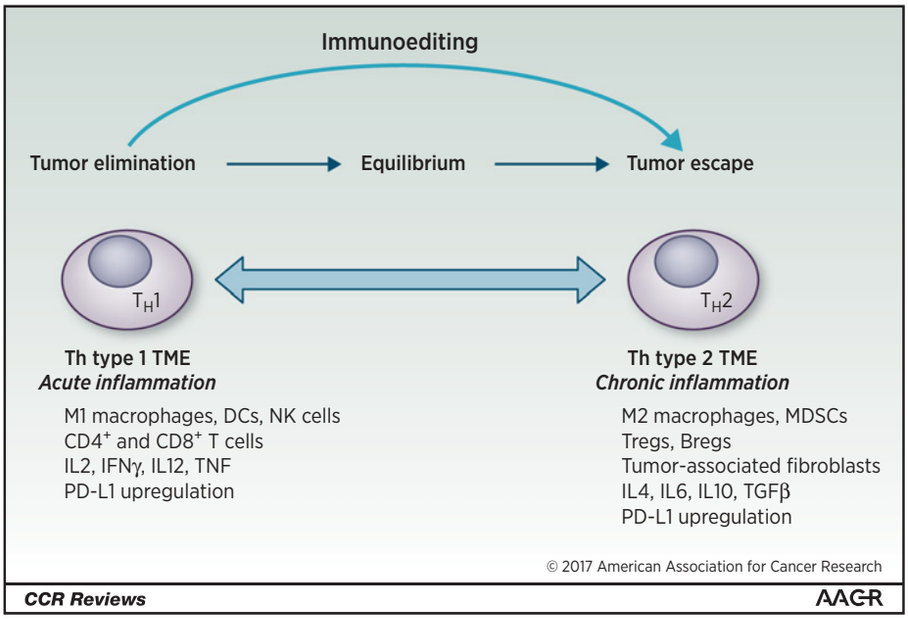


**Figure 1.**

The immune system plays a role in breast tumor growth and progression, and also in breast tumor elimination. Early in breast tumor development, the acute inflammatory response results in the production of IL12 and IFNγ, establishing a Th type 1 environment at the tumor site. During this phase, DCs mature, process tumor-associated antigens, and migrate to the tumor-draining lymph nodes to present antigen to naïve $CD4^+$ and $CD8^+$ T cells, resulting in an immune response that ultimately lyses tumor cells. This immune response initially results in complete tumor rejection. However, the pressure it imposes leads to the selection of tumor cell variants that escape the immune response. This process of immunoediting establishes a state of equilibrium, or dormancy. As inflammation at the tumor site shifts from acute to chronic, the TME evolves to a Th type 2 profile. A suppressive TME comprised of a complex community of tumor cells, immune cells, and host stromal cells is established, and breast tumors grow and metastasize unchecked by the immune system. Bregs, regulatory B cells; NK, natural killer; $T_H1$, Th type 1; $T_H2$, Th type 2.

neoadjuvant chemotherapy, with longer progression-free survival (PFS) and overall survival (OS; ref. 2). A higher $CD8^+$ T-cell/Treg ratio is also associated with a greater likelihood of complete pathologic response (cPR) to neoadjuvant chemotherapy (17, 18). Some solid tumors that harbor TILs and express PD-L1 are more likely to respond to programmed cell death-1 (PD-1)/PD-L1 blockade (19, 20), suggesting this may also be the case for breast cancers.

### Tumor antigens and vaccines in breast cancer

The immune system eliminates tumors by recognizing tumor antigens processed and presented by MHC class I (for $CD8^+$ T cells) and MHC class II (for $CD4^+$ T cells), resulting in the cross-priming and activation of T cells and tumor rejection. Breast cancer vaccines are designed to induce or amplify a population of tumor-specific T cells that can recognize and lyse breast tumors. Historically, breast cancer vaccines have incorporated shared tumor antigens that are overexpressed in tumors relative to normal tissues, or are restricted to mammary tissue (Fig. 2). Shared tumor antigens are typically recognized as self by the immune system. To avoid autoimmunity, thymic selection results in the deletion of high-avidity T cells specific for these tumor antigens, leaving in place a population of lower avidity (weaker) T cells for recruitment to the tumor-specific immune response.

Breast cancer vaccines that deliver shared tumor antigens have been evaluated in multiple trials that have tested peptide and/or protein vaccines specific for HER-2 or the carbohydrate antigen Mucin-1; DC-based vaccines specific for HER-2; cell-based (poly-antigen) vaccines that secrete GM-CSF; and viral vector vaccines that deliver carcinoembryonic antigen (CEA), Mucin-1, and a triad of molecules that stimulate T-cell activation (TRICOM; refs. 5, 6). These trials together have demonstrated that shared tumor antigen vaccines are safe and can induce an antigen-specific immune response. However, the magnitude of the T-cell response induced is usually low, and vaccine-induced immunity has not typically correlated with clinical benefit or response. One major limitation of the vaccines tested thus far is their potency due to both the shared tumor antigens targeted, the vaccine platforms used, and the advanced disease settings in which the vaccines were tested. As an alternative, vaccines that include mutation-specific antigens are tumor specific and perceived by the immune system as foreign. The activated T-cell repertoire would thus include high-avidity (stronger) T cells that can be effectively activated by vaccines to lyse tumor cells presenting these neoantigens (21). Consistent with this concept, tumors with a high mutational load are more likely to respond to immune checkpoint blockade (22, 23). Efforts to profile the mutational landscape of an individual patient's tumor to generate a personalized breast cancer vaccine with high potency are underway, but the extent to which mutational load influences clinically meaningful breast tumor immunity remains to be determined (24). A major limitation to the potency of both generalized and personalized tumor vaccines thus far is the activity of immune

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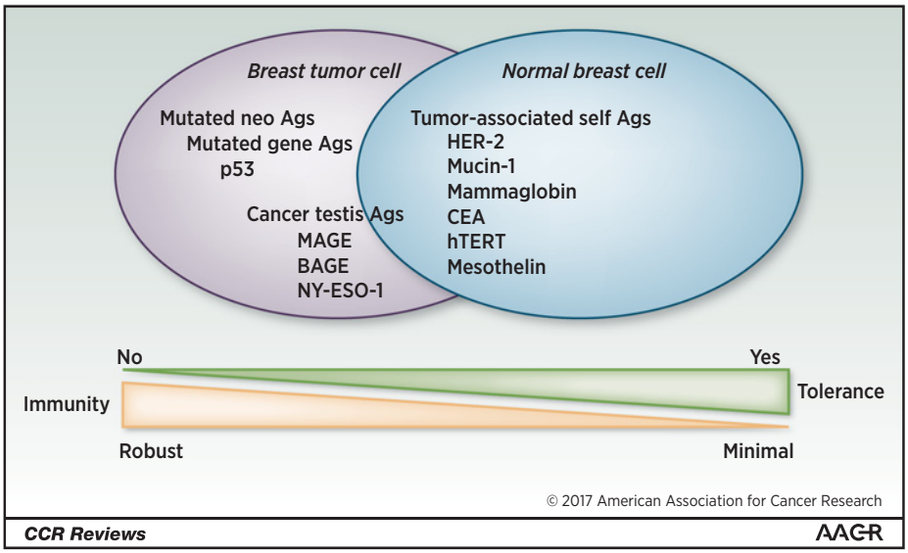


**Figure 2.**

Breast tumor antigens and immune recognition. Tumor antigens expressed by normal cells and tumors partially overlap. Historically, tumor antigens targeted by immunotherapy have been proteins shared by normal host tissue and tumor cells for which there is established immune tolerance. Specific mutations in these proteins and/or their overexpression by tumors relative to normal tissue facilitate the preferential recognition of tumor cells relative to normal cells by the immune system. More recently, the importance of tumor neoantigens unique to a given tumor relative to other tumors or normal tissue has emerged. These neoantigens result from the genomic instability of tumors. Tumors with a high mutational load (more neoantigens) tend to have a higher response rate to immune checkpoint blockade and are thought to be more readily recognized by the immune system due to lack of antigen-specific immune tolerance to the expressed neoantigens. Ags, antigens; BAGE, B melanoma antigen; CEA, carcinoembryonic antigen; hTERT, human telomerase reverse transcriptase; MAGE, melanoma-associated antigen.

checkpoint pathways and other mechanisms of immune suppression that keep vaccine-induced T cells in check (5). Several clinical trials have tested breast cancer vaccines in combination with low-dose chemotherapy (to mitigate Treg-mediated suppression; refs. 25, 26) or full-dose chemotherapy (to reduce tumor burden, induce antigen release to support T-cell priming, and reduce immune-suppressive mechanisms in the TME; ref. 27). Other combination vaccine trials have tested peptide- and cell-based vaccines that deliver HER-2 with trastuzumab alone (which can augment immune priming, enhance effector $CD8^+$ T-cell activity, and promote immune memory; refs. 28, 29) or trastuzumab and low-dose cyclophosphamide (to abrogate Treg activity; ref. 30). Data to date are consistent with augmented vaccine-induced immunity induced by combination therapy. An area of high interest is combining breast cancer vaccines with antagonists of CTLA-4 or PD-1/PD-L1 to abrogate the signaling that shuts down vaccine-induced T cells at the tumor site.

## Recent Clinical Advances in Breast Cancer Immunotherapy

Immune checkpoint antagonists specific for CTLA-4, PD-1, and PD-L1 have revolutionized cancer therapy, inducing durable objective responses that sometimes translate into an OS benefit in multiple cancer types (3, 4). Although several drugs are now FDA approved for multiple cancers, none has yet been approved for breast cancer. Even so, the CTLA-4 antagonists tremelimumab and ipilimumab have been tested in small breast cancer trials, with evidence of immune modulation. Furthermore, accumulating data suggest that antagonists of PD-1/PD-L1 signaling can induce durable clinical responses in some patients with metastatic TNBC and likely have meaningful clinical activity in rare patients with $ER^+$ $HER\text{-}2^-$ breast cancer as well. These data are summarized in Table 1.

### CTLA-4 blockade in breast cancer

CTLA-4 is upregulated shortly after T-cell activation, binding CD80/CD86 to provide negative feedback to CD28 costimulation and limiting T-cell activation during the priming phase of the immune response (31). This helps to prevent uncontrolled immunity. Two humanized mAbs specific for CTLA-4 are in the clinic.

***Tremelimumab.*** The anti–CTLA-4 agent tremelimumab remains investigational in every tumor type. Escalating doses of tremelimumab have been tested with concurrent exemestane in 26 patients with $ER^+$ $HER\text{-}2^-$ breast cancer (32). Five patients had dose-limiting toxicity (DLT), which included diarrhea (four counts) and elevated serum transaminase levels (one count). The MTD was 6 mg/kg every 90 days. The best response was stable disease (SD) for $\geq$12 weeks in 42% of patients, and a significant increase in the ratio of $ICOS^+/FoxP3^+$ $CD4^+$ T cells was observed in most patients.

***Ipilimumab.*** Ipilimumab is currently approved as a single agent for early- and late-stage melanoma, and is under investigation in multiple other tumor types both as a single agent and in combination with PD-1/PD-L1 blockade (4). A study in breast cancer evaluated a single dose of neoadjuvant ipilimumab alone or given

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**Table 1.** PD-1/PD-L1 blockade in metastatic breast cancer

| Antibody | Target | Combination | Breast cancer subtype | Patients (*n*) | ORR | DCR |
|---|---|---|---|---|---|---|
| Avelumab | PD-L1 | Single agent | All | 168 | 4.8% | 28% |
| | | | PD-L1$^+$ all | 12 | 33.3% | NR |
| | | | TNBC | 58 | 8.6% | 31% |
| | | | PD-L1$^+$ TNBC | 9 | 44.4% | NR |
| | | | PD-L1$^-$ TNBC | 39 | 2.6% | NR |
| Pembrolizumab | PD-1 | Single agent | PD-L1$^+$ TNBC | 27 | 18.5% | 26% |
| | | Single agent | TNBC | 170 | 4.7% | 7.6% |
| | | | PD-L1$^+$ TNBC | 105 | 4.8% | 9.5% |
| | | | PD-L1$^-$ TNBC | 64 | 4.7% | 4.7% |
| | | Single agent | PD-L1$^+$ TNBC, 1st line | 52 | 23.1% | NR |
| | | | PD-L1$^+$ ER$^+$ HER-2$^-$ BC | 25 | 12% | 20% |
| Atezolizumab | PD-L1 | Single agent | TNBC | 112 | 10% | 23% |
| | | | PD-L1$^+$ TNBC | 71 | 13% | 27% |
| | | | PD-L1$^-$ TNBC | 37 | 5% | 16% |
| Atezolizumab | PD-L1 | Nab-paclitaxel | TNBC | 32 | 38% | NR |
| Pembrolizumab | PD-1 | Eribulin | TNBC | 39 | 33.3% | 41% |

Abbreviations: BC, breast cancer; DCR, disease control rate; NR, not reported; ORR, overall response rate.

with cryoablation in 12 patients with early breast cancer prior to mastectomy; six additional patients received preoperative cryoablation alone (33). Combination immunotherapy induced circulating Th type 1 cytokines, ICOS$^+$Ki67$^+$CD4$^+$ and ICOS$^+$Ki67$^+$CD8$^+$ T cells, and an increased CD8$^+$ T-cell /FoxP3$^+$ Treg ratio within the tumor. Clonally expanded TILs [detected by deep sequencing of T-cell receptor (TCR) DNA] correlated with the TIL score by hematoxylin and eosin (H&E; ref. 34). On the basis of these promising results, a follow-up study is evaluating cryoablation combined with CTLA-4 (ipilimumab 1 mg/kg) and PD-1 blockade (nivolumab 3 mg/kg; NCT02833233).

## PD-1/PD-L1 blockade in breast cancer

The PD-1 receptor is upregulated on activated T cells and binds two known ligands: PD-L1 and PD-L2. Through interactions with PD-L1 on the surface of tumor cells and immune cells, PD-1 signaling counters T-cell activation during the effector phase of the immune response (31). Metastatic breast cancer responds to treatment with humanized mAbs that target PD-L1 (avelumab and atezolizumab) and PD-1 (pembrolizumab; ref. 35). Side effects associated with the use of these agents in breast cancer to date have been consistent with those expected for the drug class.

***Avelumab.*** Avelumab was evaluated in multiple tumor types in the phase Ia/Ib JAVELIN study (36). The phase Ib portion of this trial enrolled 168 patients in a breast cancer–specific expansion cohort regardless of either disease subtype or PD-L1 expression (37). The subtype distribution was 42.9% ER$^+$/PR$^+$/HER-2$^-$ disease, 34.5% TNBC, and 15.5% HER-2$^+$ breast cancer; the disease subtype was unknown in 7.1% of patients. Over half of the patients had $\geq$3 prior lines of therapy for metastatic disease. The overall response rate (ORR) for the entire cohort was 4.8%, and included one complete response (CR), seven partial responses (PR), and 39 patients with stable disease (SD) for a disease control rate (DCR) of 28%. Responses were observed in all breast cancer subtypes but appeared to be higher in TNBC. Fifty-eight patients showed an ORR of 8.6%, with zero CRs, five PRs, and 13 patients with SD, and a DCR of 31%. PD-L1 expression was evaluable in 136 patients; 12 patients had $\geq$10% PD-L1$^+$ immune cells in the TME, and 124 had <10%; the PR rates in these two groups were 33.3% and 2.4%, respectively. In 48 patients with TNBC evaluable for PD-L1 expression, nine were PD-L1$^+$ and 39 were PD-L1$^-$, with ORRs of 44.4% and 2.6%, respectively. A phase III trial is testing the addition of 1 year of avelumab to curative therapy for high-risk, early-stage TNBC, with primary and secondary outcomes of disease-free survival (DFS) and OS, respectively (NCT02926196). Furthermore, a phase Ib/2 study is also evaluating avelumab in combination with the immune modulator anti-41BB in multiple advanced cancers, including TNBC (NCT02554812). 41BB (CD137) is an inducible costimulatory receptor, and agonist antibodies specific for 41BB may potentiate antitumor immunity (38).

***Atezolizumab.*** A phase Ia study evaluated single-agent atezolizumab in multiple tumor types (39), enrolling 115 patients with TNBC (40, 41). PD-L1 expression was assessed using the SP142 antibody, where tumors were positive if they had $\geq$5% PD-L1$^+$ tumor-infiltrating immune cells. Enrollment was initially restricted to PD-L1$^+$ patients and subsequently opened to patients with any level of PD-L1 expression. Ultimately, 63% of patients were PD-L1$^+$, 33% of patients were PD-L1$^-$, and 4% of patients had unknown PD-L1 status. Patients were generally heavily pretreated, with a median of seven prior lines of therapy; 17% of enrolled patients received atezolizumab as their first-line therapy for metastatic disease. The ORR in 112 evaluable patients was 10%, with an ORR of 13% in PD-L1$^+$ patients and 5% in PD-L1$^-$ patients. Although numbers are small, the ORR in patients treated first line was 26%, whereas the ORR in patients treated second and third line was 4% to 8%. The DCR was 23% in all patients, 27% in PD-L1$^+$ patients, and 16% in PD-L1$^-$ patients. At a median follow-up of 15.2 months, the median OS was 9.3 months. The OS rate at 1 year was 41%; notably, the survival rate of patients who had a CR or PR was 100% at up to 2 to 3 years. About 11% of patients who had progressive disease (PD) by standard RECIST had atypical responses/pseudoprogression and also enjoyed long-term survival.

***Pembrolizumab.*** Pembrolizumab has been evaluated in several clinical trials that demonstrated its safety and clinical activity in multiple tumor types (3, 4). KEYNOTE-012 was a phase Ib study that evaluated pembrolizumab monotherapy in advanced PD-L1$^+$ TNBC (42, 43). Tumor PD-L1 expression was evaluated by the 22C3 antibody, with staining in $\geq$1% of tumor cells or any PD-L1 expression by immune cells defined as positive. Of 111 patients prescreened for PD-L1 expression, 58.6%

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(65 patients) were PD-L1$^+$; 32 patients were enrolled and treated. Patients had a median of two prior lines of chemotherapy for metastatic disease, with 25% having ≥5 prior lines of therapy. In 27 patients evaluable for efficacy, the ORR was 18.5%, with one CR, four PRs, and seven patients with SD; the DCR was 25.9%. The median OS was 10.2 months, and the OS rate at 1 year was 41.1%. The median duration of response (DOR) had not yet been reached. The KEYNOTE-086 phase II trial evaluated pembrolizumab monotherapy both as salvage treatment for previously treated patients with metastatic TNBC expressing any level of PD-L1 (cohort A) and as first-line therapy for patients with metastatic PD-L1$^+$ TNBC (cohort B; refs. 44, 45). In cohort A, 170 patients were enrolled and treated; about 62% (105 patients) had PD-L1$^+$ TNBC, and over 40% of patients had been treated with ≥3 prior lines of therapy. Clinical activity was modest, with no apparent impact of PD-L1 expression level on clinical benefit (ORR of 4.7%–4.8%, PFS of 1.9–2.0 months, and OS of 8.3–10.0 months). Early analyses suggest longer OS at 9 months for patients with a CR or PR (100%) relative to patients with SD (89.6%) or PD (39%), similar to atezolizumab. Notably, the ORR for the 52 patients in cohort B was higher than for patients overall, at 23.1%. This is also consistent with the atezolizumab data, where responses in metastatic TNBC appear to be highest when given first line and/or in PD-L1–selected patients (41). The KEYNOTE-119 phase III trial, comparing pembroblizumab with chemotherapy of physician's choice (NCT02555657), continues to accrue patients.

The utility of PD-1/PD-L1 blockade for TNBC in the neoadjuvant and adjuvant studies is also under investigation. Early data from the I-SPY trial revealed that adding pembrolizumab to neoadjuvant paclitaxel results in an estimated pCR rate of 46% versus 16% in HER-2$^-$ patients, 60% versus 20% in TNBC patients, and 34% versus 13% in ER$^+$/PR$^+$/HER-2$^-$ patients (46). The probability of a successful phase III trial demonstrating that the addition of pembrolizumab to paclitaxel results in a superior cPR rate was estimated at >99% for all HER-2$^-$ patient subgroups. Treatment-emergent adverse events included higher rates of thyroid dysfunction and adrenal insufficiency. KEYNOTE-173, a trial investigating pembrolizumab combined with chemotherapy for the neoadjuvant therapy of TNBC, also suggests that PD-L1$^+$ TNBC might be more likely to respond (47).

The evaluation of PD-1/PD-L1 agents in other breast cancer subtypes has been limited, but single-agent pembrolizumab was evaluated in a small cohort of advanced ER$^+$ HER-2$^-$ breast cancer as part of the KEYNOTE-028 study (48). Of 248 patients prescreened for PD-L1 expression, 19.4% (48 patients) had PD-L1$^+$ tumors. Of these, 25 patients were enrolled and treated. All had received at least one prior line of therapy for metastatic disease, and 44% had received ≥5 lines of prior therapy. The ORR was 12%, with zero CRs, three PRs, and four patients with SD; the clinical benefit rate (CBR) was 20%. All responding patients had been on study for at least 26 weeks at the data cutoff.

## Expanding the Clinical Success of Breast Cancer Immunotherapy

Early data with antagonists of PD-1/PD-L1 illustrate that some breast cancers are inherently immunogenic, with durable clinical responses in some responding patients. However, single-agent activity occurs in less than 10% of patients with metastatic disease. Current efforts focus on developing immunotherapy combinations that can convert nonresponders to responders, deepen responses that do occur, and surmount acquired resistance to immunotherapy. Promising combinations are illustrated in Fig. 3 and discussed below.

### Combining chemotherapy with immune-based therapy

Chemotherapy has a variable impact on the immune response depending on the drug, the dose, and the timing of chemotherapy in relation to immune-based therapy (49). A number of trials are looking at the addition of chemotherapy to PD-1/PD-L1 blockade, with the goal of enhancing immune priming through antigen release and DC modulation or augmenting immunity through relieving immune-suppressive signals in the TME. Nab-paclitaxel and eribulin are two drugs that can modulate immunity in these ways, and early data from two small trials have already been reported. Atezolizumab (840 mg every 2 weeks) has been tested with nab-paclitaxel (125 mg/m$^2$ on days 1, 8, and 15 every 28 days) in a phase Ib study that enrolled patients with metastatic TNBC regardless of PD-L1 status (50). Thirty-two patients were evaluable for safety and response. Grade 3 to 4 hematologic toxicity occurred in over half the patients but was manageable. At a median follow up of >5 months, the ORR was 38%, with one CR, 11 PRs, and two additional patients demonstrating a nonclassical response. Responses occurred in patients with both PD-L1$^+$ and PD-L1$^-$ disease, and there was a trend for higher response rate in patients treated first line relative to later line. A phase III, global, randomized, double-blind, placebo-controlled study is currently underway evaluating the addition of atezolizumab to nab-paclitaxel for the first-line therapy of metastatic TNBC (NCT02425891). Pembrolizumab (200 mg i.v. every 3 weeks) has been tested with eribulin (1.4 mg/m$^2$ on days 1 and 8 every 21 days) in an ongoing trial designed to enroll 95 patients with metastatic TNBC of any PD-L1 status treated with ≤2 prior lines of chemotherapy (51). An interim analysis of 39 patients demonstrated the safety of the combination, with the most common side effects of fatigue, alopecia, nausea, neutropenia, and peripheral neuropathy. The ORR for 17 patients treated with first line was 41.2%, and for those treated second or third line was 27.3%; this included one CR and 12 PRs. The CBR was 41%. PD-L1 status did not appear to impact the likelihood of response; the ORR and CBR for PD-L1$^+$ patients were 29.4% and 35.3%, and for PD-L1$^-$ patients were 33.3% and 44.4%. This trial is ongoing; a second trial designed to enroll patients with metastatic ER$^+$HER-2$^-$ breast cancer is planned. The KEYNOTE-355 phase III trial is evaluating pembrolizumab with chemotherapy relative to various chemotherapy regimens alone as first-line therapy for incurable TNBC (NCT02819518).

### HER-2–directed therapy and immunotherapy

Trastuzumab, a humanized mAb that specifically binds to HER-2 homodimers, is the cornerstone of therapy for both early- and late-stage HER-2–overexpressing breast cancer (52, 53). Added to standard chemotherapy, it prolongs survival in metastatic disease and decreases the risk of relapse in early-stage disease by about 50%. Pertuzumab, a second humanized mAb specific for HER-2, prevents the formation of HER-2/HER-3 heterodimers. The addition of pertuzumab to trastuzumab and taxotere for the first-line therapy of metastatic disease further prolongs survival (54), and neoadjuvant therapy with pertuzumab and trastuzumab added to taxotere and carboplatin gives the highest reported cPR rate reported to date

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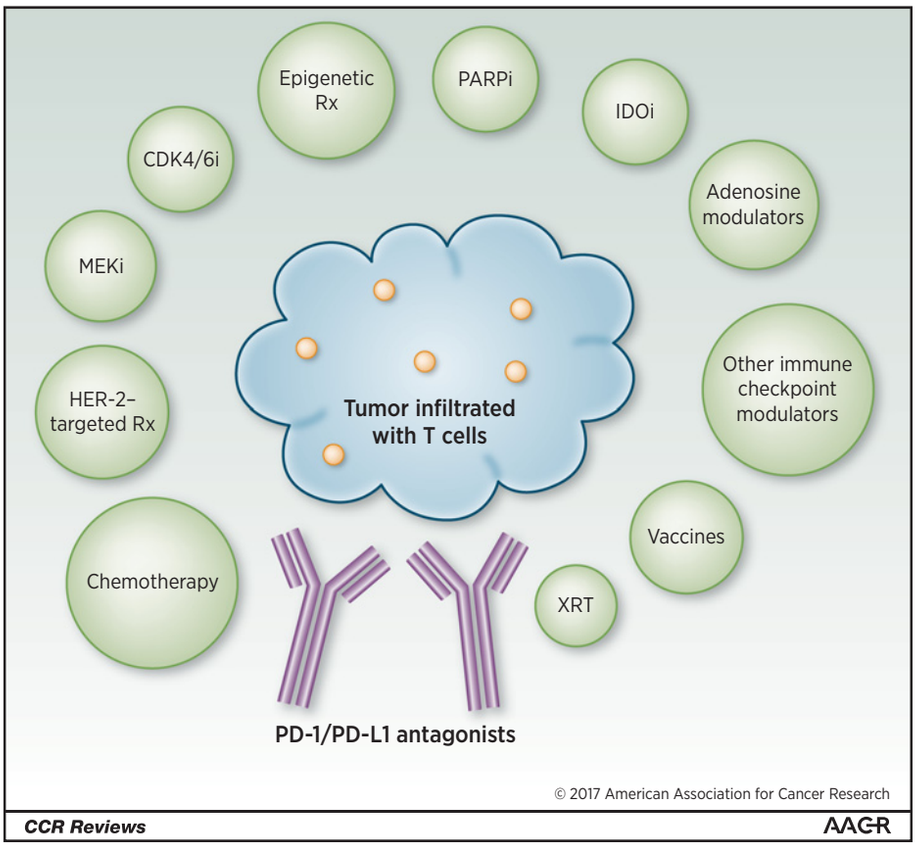


**Figure 3.**
Summary of selected immunotherapy combinations for breast cancer treatment with strong mechanistic rationale. Because blockade of the PD-1/PD-L1 pathway has clear activity in multiple cancers, many regard it as a fundamental component of future cancer immunotherapies. Current clinical efforts are focused on developing immunotherapy combinations, many based on PD-1/PD-L1 blockade, which convert nonresponders to responders, deepen responses that do occur, and surmount acquired immunotherapy resistance. Other combinations, some of which include PD-1/PD-L1 blockade, are also in development. CDK4/6i, cyclin-dependent kinase 4/6 inhibitor; IDOi, indoleamine 2,3 dioxygenase inhibitor; MEKi, mitogen-activated protein kinase (MAPK) kinase (MEK) inhibitor; PARPi, PARP inhibitor; Rx, therapy; XRT, radiotherapy.

(55). Ado-trastuzumab emtansine (TDM1) is composed of trastuzumab conjugated to a chemotherapeutic emtansine moiety, and improves both PFS and OS relative to lapatinib and capecitabine in patients with HER-2$^+$ breast cancer who have progressed on a taxane and trastuzumab (56). Trastuzumab itself has intrinsic immune-modulating activity, with the capacity to mediate antibody-dependent cellular cytotoxicity (ADCC; ref. 57) and promote an HER-2–specific T-cell response (58). The emtansine moiety of TDM1 may further augment immune priming by modulating DC activity (59). Our group has found that the preclinical equivalent of trastuzumab or trastuzumab itself can augment the activity of a cell-based vaccine in preclinical models (60, 61) and in patients (30). Exploration of the mechanism in HER-2 transgenic mice demonstrated enhanced Fc-mediated immune priming by DCs, augmented effector T-cell activity, and a durable memory T-cell response (61). Others have shown that combined therapy with a trastuzumab-like antibody plus a PD-1 antibody markedly augments the clearance of HER-2$^+$ tumors in mice (62), and that combining TDM1 with both anti–CTLA-4 and anti–PD-1 antibodies prolongs survival in over 90% of mice bearing HER-2$^+$ tumors relative to TDM1 or immunotherapy alone (63). Multiple clinical trials are underway testing the addition of PD-1/PD-L1 blockade to HER-2–based therapies for both locally advanced and metastatic HER-2$^+$ breast cancer. A phase II, global, randomized, double-blind, placebo-controlled study is currently underway evaluating whether the addition of atezolizumab to TDM1 can further improve clinical outcomes in patients with metastatic HER-2$^+$ breast cancer previously treated with trastuzumab and a taxane (NCT02924883).

### MEK inhibitors and immunotherapy combinations

Inhibitors of MAPK kinase (MEK) can cause the regression of tumors with activating mutations in the Ras signaling pathway. Although MEK inhibitors have been shown to block naïve T-cell priming in tumor-bearing mice, they can increase the number of effector CD8$^+$ T cells by preventing activation-induced cell death, leaving effector T-cell activity intact (64). Combining MEK inhibitors with PD-L1 antibodies resulted in synergistic, durable antitumor activity. In breast cancer, an analysis of the residual disease of TNBC treated with neoadjuvant chemotherapy showed that TILs are associated with better prognosis, and that alterations in Ras/MAPK signaling were associated with lower levels of TILs (65). MEK inhibition upregulated cell-surface expression of MHC class I and II as well as PD-L1 on TNBC *in vitro* and *in vivo*. In mouse models of breast cancer, combined treatment with MEK inhibitors and PD-1 pathway antagonists resulted in enhanced tumor-specific immune responses and augmented tumor control. Clinical trials testing the combination of MEK inhibition and blockade of the PD-1 pathway for breast cancer are in development or underway.

### CDK4/6 inhibitors and immune-based therapy

Several targeted agents that block CDK4/6 signaling have demonstrated clinical activity in combination with an aromatase inhibitor and faslodex for the first- and second-line therapy of metastatic ER$^+$ breast cancer, respectively (66). Notably, data are beginning to emerge that these agents can induce TILs (67). As discussed previously, ER$^+$ HER-2$^-$ breast cancers are unlikely to contain TILs or respond to monotherapy with agents that target

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the PD-1 pathways (48). These observations together suggest that these agents might be one strategy for transforming a cold $ER^+$ breast cancer into an inflamed tumor poised to respond to immune checkpoint blockade. It will be interesting to evaluate the addition of PD-1/PD-L1 blockade to the combination of endocrine therapy and CDK4/6 antagonists in relevant models.

**Epigenetic therapy integrated with immunotherapy**

There is great interest in the potential of epigenetic therapy to prime for response to immunotherapy in breast cancer. Studies have shown that epigenetic modulation can promote a type I IFN response and restore production of Th type 1 cytokines and chemokines (68, 69). Another preclinical study showed that treating tumor-bearing mice (including the breast tumor 4T1) with entinostat combined with CTLA-4 and PD-1 antibodies could eradicate both primary tumors and metastases by reducing granulocytic MDSCs (70). A phase II clinical trial showed that the addition of entinostat to exemestane for patients with advanced $ER^+$ breast cancer resulted in an 8.3-month improvement in median OS relative to patients treated with exemestane alone (71). Exploratory studies of blood samples from 34 patients showed both lower numbers of MDSCs and decreased MDSC CD40 expression as well as increased MHC class II expression on $CD14^+$ monocytes 2 weeks after initiating therapy; no alterations of T-cell phenotypes were observed. Multiple clinical trials evaluating the combination of epigenetic modulation with PD-1/PD-L1 blockade or the combination of CTLA-4 and PD-1 blockade are underway.

**PARP inhibition and immunotherapy**

PARP inhibitors have recently been reported to modulate the immune microenvironment by upregulating PD-L1 expression in breast cancer cell lines and animal models (72). Antibodies that block PD-L1 restored the sensitivity of PARP inhibitor–treated cells to T-cell–mediated killing. In addition, polymeric adenosine diphosphate ribose [poly(ADP-ribose), or PAR] and PD-L1 expression were shown to be inversely correlated in human breast tumors. The combination of a PARP inhibitor and PD-L1 blockade significantly delayed tumor outgrowth relative to either agent alone in mouse models of breast cancer. Studies have shown synergy between CTLA-4 blockade and PARP inhibition in BRCA-deficient ovarian cancer models (73). In breast and ovarian cancer patients, the combination of PD-L1 blockade and PARP inhibition or VEGF inhibition has shown promise (74). These data support trials exploring the combination of PARP inhibitors and/or antiangiogenic therapies on a backbone of PD-1/PD-L1 blockade in BRCA-mutated breast and ovarian cancer.

**Integrating indoleamine 2,3-dioxygenase inhibitors and immune checkpoint blockade**

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that converts tryptophan to kynurenine, thereby suppressing immunity in the TME (75). Like PD-L1, IDO is upregulated by IFNγ-secreting T cells in the TME as a means of immune escape, and these two pathways are potentially redundant pathways of immune suppression in breast cancers that have TILs. The combination of the oral IDO inhibitor indoximod and taxotere has been tested in solid tumors (including breast cancer), with evidence of safety and clinical activity (two PRs and two minor regressions in breast cancer; ref. 76). Several clinical trials are evaluating the activity of combined inhibition of IDO and the PD-1 pathway in multiple tumor types, including breast cancer. Promising activity was recently reported with the combination of indoximod and pembrolizumab in melanoma (77).

**Inhibiting adenosine signaling and PD-1/PD-L1 blockade**

Adenosine is another metabolite that creates a network of immune suppression in the TME (78). Nucleotides released by tumor cells are hydrolyzed by CD39 from ATP to AMP, and then by CD73 from AMP to adenosine. This creates a cloud of adenosine in the TME that binds to adenosine receptors (particularly the adenosine A2a receptor) on the surface of immune cells, skewing the TME to a state of immune suppression. Agents that target this pathway to reverse immune suppression include therapeutic antibodies specific for the cell-surface ectonucleases CD39 and CD73, and small-molecule inhibitors of adenosine receptor signaling. CPI-444 is a small-molecule antagonist of the A2aR currently in testing alone and with atezolizumab in a multicohort study of a range of advanced, treatment-refractory cancers, including TNBC (79). Preliminary data reveal a favorable safety profile to date, with evidence of clinical activity both as a single agent and in combination in multiple tumor types, including in PD-1/PD-L1–resistant or refractory patients. This and other trials evaluating agents that target the adenosine network are ongoing.

**Integrating radiotherapy with immune checkpoint blockade**

Modulation of tumor immunity underlies the abscopal effect, where irradiation of an index lesion is associated with regression of distant, unirradatiated tumor lesions. Accumulating data demonstrate that radiotherapy can enhance tumor immunity in multiple ways (4, 80). It releases tumor antigens, and facilitates their processing and cross-presentation by dendritic cells, thus promoting T-cell priming. Furthermore, radiotherapy upregulates chemokines and vascular adhesion molecules to support trafficking of T cells to tumors, and further augments tumor immunogenicity by upregulating the expression of MHC molecules, stress-induced ligands, and death receptors on cancer cells themselves. However, the impact of radiotherapy dose and schedule on these mechanisms remains unclear. Hypofractionated radiotherapy sequenced with ipilimumab did produce significant abscopal effects in melanoma and lung cancer (4). There are several ongoing clinical trials testing radiotherapy with distinct immunotherapies, including PD-1/PD-L1 blockade.

**Vaccination strategies with immune checkpoint blockade**

As reviewed here earlier, vaccines for breast cancer therapy have shown evidence of modest immunity but limited clinical activity when given to patients with advanced disease as a single agent or in combination with standard chemotherapy. This is likely due in large part to dominant repression of tumor immunity by immune checkpoint signaling, particularly through the PD-1 pathway. Giving vaccines as a single agent may be much like stepping on the immune accelerator while the parking brake is engaged. With the advent of immune checkpoint modulators that have clear clinical activity, we are poised to bring vaccination strategies back to the clinic to accelerate T-cell priming and activation while releasing the brakes from tumor immunity with immune checkpoint blockade. Combining DC-based vaccines that deliver multiple tumor

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antigens, genetically engineered poly-antigen vaccines like TRICOM, or whole-cell tumor vaccines with agents that abrogate PD-1 signaling or other dominant pathways of immune suppression have a much greater chance of success than past strategies using vaccines alone. Even these vaccine platforms may be limited, however, by the fact that they are based on known tumor antigens that tend to be recognized as self by the immune system. The correlation of clinical responses induced by immune checkpoint blockade with endogenous immune responses specific for the neoantigens present in highly mutated tumors has created intense interest in generating personalized vaccines based on the unique neoantigens present in a given patient's tumor (21). Platforms for neoantigen-based breast cancer vaccines include peptides, genetically engineered bacterial or viral vectors, or nucleic acid–based vaccines (5). The cryoablation strategy described earlier is also a unique strategy for inducing immunity tailored to an individual's tumor and has already been tested with CTLA-4 blockade in patients with breast cancer (33, 34). Another novel approach to personalized vaccination is the intratumoral delivery of agonists that activate innate immunity, for example, through the stimulator of interferon genes (STING) pathway. The intratumoral delivery of the STING agonist ADU-S100 induces IFNβ production and DC maturation, and establishes a gradient of T-cell–recruiting chemokines that promotes effective T-cell priming and trafficking to the tumor site (81). Furthermore, sequencing ADU-S100 with PD-L1 blockade and modulation of the OX40 receptor can break antigen-specific tolerance to mediate tumor regression in a tolerized mouse breast cancer model (81). Single-agent ADU-S100 is currently in clinical trials (NCT02675439).

## Conclusions and Future Directions

Immune checkpoint blockade has shown promise for breast cancer treatment, illustrating the potential of harnessing the immune system for clinical benefit in this disease. Antagonists of the PD-1/PD-L1 pathway can induce durable clinical responses in some patients with metastatic TNBC. Both validation of these early findings and efforts to extend immunotherapy to patients with HER-2$^+$ and luminal breast cancer are underway. In addition, clinical trials evaluating the integration of immunotherapy into the adjuvant and neoadjuvant settings have already begun. The near-term future will see the development of combination immunotherapies that can convert breast cancers from immunologically cold lesions to immune-activated tumors poised for response to immunotherapy. Personalized immunotherapy strategies that utilize vaccines that deliver tumor-specific neoantigens and/or immune-modulating agents chosen based on the immunologic milieu of a given tumor are under rapid development. Developing biomarkers that predict response and resistance to therapy, and identifying environmental modifiers of immunity (the microbiome, metabolic and hormonal parameters, and concurrent drug therapy) are areas of growing investigation (82). Applying vaccination approaches integrated with the lessons learned from modern breast cancer immunotherapy will undoubtedly also bring us closer to the ultimate goal of immune-based breast cancer prevention.

## Disclosure of Potential Conflicts of Interest

L.A. Emens reports receiving commercial research grants from Aduro Biotech, AstraZeneca, Corvus, EMD Serono, Genentech/Roche, and Merck, and is a consultant/advisory board member for Amgen, AstraZeneca, Bayer, Celgene, eTheRNA, Gritstone, Molecuvax, Peregrine, Syndax, and Vaccinex. Under a licensing agreement between Johns Hopkins University and Aduro Biotech, L.A. Emens and the University are entitled to milestone payments and royalty on the sales of the GM-CSF–secreting breast cancer vaccine. The terms of these arrangements are being managed by Johns Hopkins University in accordance with its conflict of interest policies.

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# Breast Cancer Immunotherapy: Facts and Hopes

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