Immunotherapy for Breast Cancer: What Are We Missing?

Robert H. Vonderheide, Susan M. Domchek, and Amy S. Clark

Abstract

The recent demonstration of modest single-agent activity of programmed death-ligand 1 (PD-L1) and programmed death receptor-1 (PD-1) antibodies in patients with breast cancer has generated hope that breast cancer can be made amenable to immunotherapy. Depending on the subtype of breast cancer, it is now clear in both primary and metastatic disease that the extent of tumor-infiltrating T cells is not only prognostic for survival but predictive of response to nonimmune, standard therapies. Despite these findings, immune cytolytic activity in spontaneous breast tumors, the burden of nonsynonymous tumor mutations, and the predicted load of neoepitopes—factors linked to response to checkpoint blockade in other malignancies—are all relatively modest in breast cancer compared with melanoma or lung cancer. Thus, in breast cancer, combinations of immune agents with nonredundant mechanisms of action are high-priority strategies. For most breast cancers that exhibit relatively modest T-cell infiltration, major challenges include immune suppression in the tumor microenvironment as well as failed or suboptimal T-cell priming. Agents that trigger *de novo* T-cell responses may be critical for the successful development of cancer immunotherapy and immune prevention in breast cancer. Success may also require reaching beyond nonsynonymous mutations as the T-cell epitopes to target, especially as numerous unmutated proteins were validated as breast cancer–associated antigens in the pre-checkpoint era. A deeper understanding of the immunobiology of breast cancer will be critical for immunotherapy to become broadly relevant in this disease. *Clin Cancer Res; 23(11); 2640–6. ©2017 AACR.*

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Introduction

Stunning successes of cancer immunotherapy in melanoma, lung cancer, acute lymphoblastic leukemia, and other cancers reflect the power of T-cell immunity as an extrinsic tumor suppressor. After a lagging start, efforts to develop effective immune therapy for patients with breast cancer are beginning to bear fruit. Most promising among these strategies are PD-L1 or PD-1 monoclonal antibodies (mAb) for the treatment of patients with advanced breast cancer with triple-negative disease. In these studies, the objective response rates (ORR) are in the 12% to 19% range (1–3). On the other hand, cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) blockade appears minimally active (4), and combinations of PD-1 and CTLA-4 mAb are still being studied. Chimeric antigen receptor (CAR)–engineered T cells as adoptive therapy for breast cancer are at the earliest stages of development, and randomized studies of therapeutic vaccination in patients with local or advanced breast cancer as well as vaccination in the adjuvant setting have been negative (5, 6). Although the number of clinical trials testing immune therapy in breast cancer is rapidly increasing, there have been no FDA approvals of experimental immunotherapies as there have been in other tumor types (Fig. 1).

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doi: 10.1158/1078-0432.CCR-16-2569

Advancing immune therapy to a more central and applicable role in breast cancer remains a high priority, particularly given the potential for high response durability that can be achieved, sometimes uniquely so, with immune therapy of advanced cancer. Successfully developing immunotherapy in breast cancer will be best achieved as part of a collaborative effort with investigators addressing other critical areas of this disease (7–10). A chief challenge for advancing immunotherapy for patients with advanced breast cancer is to move beyond PD-1/PD-L1 blockade alone and empiric combination of other agents with PD-1/PD-L1. A deeper understanding of the immunobiology of breast cancer is considered a critical step toward eventual clinical success of these therapies. In this article, we address what is known and our limitations of understanding of breast cancer immunology and immunotherapy.

Immunobiology of Human Breast Cancer

Careful and comprehensive studies of human breast cancer tissue have provided a clear picture of the extent, variability, and clinical importance of leukocytes that infiltrate breast tumors, as extensively reviewed elsewhere (11–14). Tumor-infiltrating lymphocytes (TIL) are found most extensively in triple-negative breast cancer (TNBC) compared with other tumor subtypes. The extent of $CD8^+$ T cells in primary TNBC—when rigorously demonstrated to be infiltrating into tumor nests rather than only gathering in outer stroma—independently predicts survival in multivariate analyses (11, 15–17). Infiltration of follicular helper $CD4^+$ T cells, perhaps an indication of organized tertiary lymphoid structures (TLS) in the tumor, is also predictive of better survival in TNBC (18, 19). Presence of TLS themselves predict better survival in TNBC (19). Conversely, the extent of immunosuppressive regulatory T cells (Treg) predicts worse survival in TNBC (20).

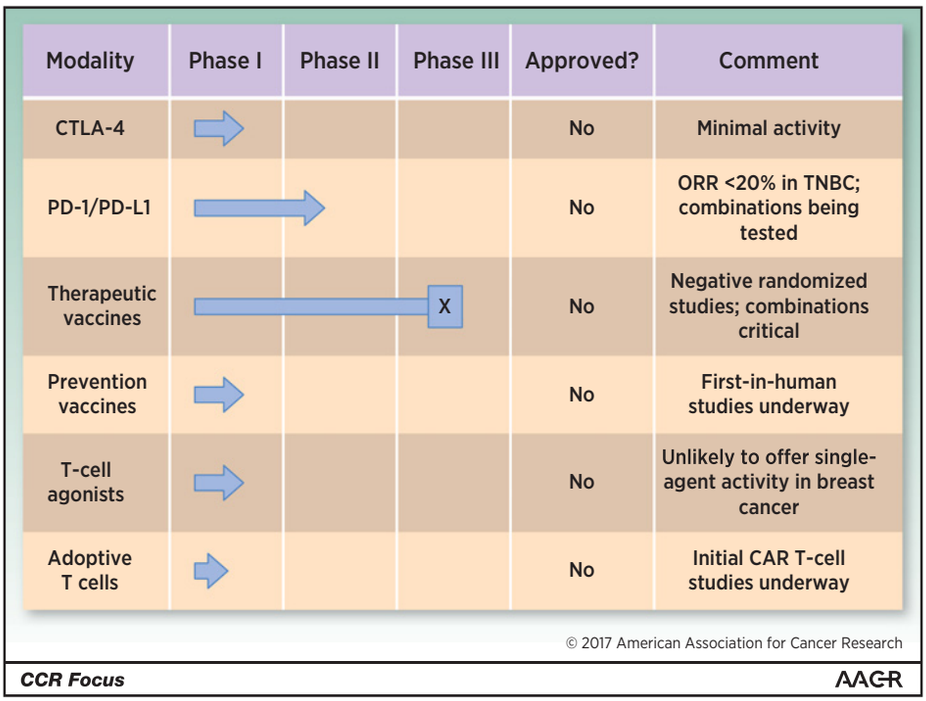

Figure 1.
Summary of status of breast cancer immunotherapy investigational trials, by phase. (Antitumor antibodies, such as trastuzumab, are not listed.) CAR, chimeric antigen receptor; TNBC, triple-negative breast cancer.

TILs in HER2$^+$ breast cancer can also be robust, and their extent similarly predicts overall survival (21). Moreover, the extent of TILs predicts improved response to certain therapies, including chemotherapy, particularly in TNBC, and trastuzumab in HER2$^+$ disease. The association also holds true across multiple clinical scenarios, including the pathologic complete response rate to chemotherapy in the neoadjuvant setting and overall response in the metastatic setting (22–25). These predictive markers hold true for both anthracycline chemotherapy and trastuzumab (11). The predictive value of TILs in estrogen receptor (ER)–positive breast tumors is less clear (26). Recently, in patients with advanced HER2$^+$ breast cancer treated with docetaxel, trastuzumab, and pertuzumab or placebo, increased TIL infiltration at baseline was associated with improved overall survival [comparing patients with >20% TILs vs. $\leq$20% TILs, median overall survival was 56.6 months vs. 44.5 months; hazard ratio (HR) 0.76, 95% confidence interval (CI), 0.60–0.96, log-rank $P = 0.021$], suggesting that antitumor immunity is also found in advanced and metastatic disease (21, 27). A consensus, standardized method for evaluating TILs in breast cancer is available (28). Pathologic analysis is able to reveal not just numbers of TILs but also the pattern of infiltration, including TLS (19).

Hematoxylin/eosin stains and immunohistochemistry have been the primary tools for characterizing TILs in breast cancer; RNAseq was also used to examine immune signatures in TNBC and HER2$^+$ disease in the CALGB 40603, CALGB 40601, and NeoALTTO trials; in all cases, activated signatures were significantly and independently associated with pathologic CR. Recently, RNAseq data from The Cancer Genome Atlas (TCGA) were used to quantitatively examine immune cytolytic activity in 18 histologies of human cancer, including breast cancer, based on a two-gene signature that was proposed to represent T-cell function (*perforin 1* and *granzyme A*; ref. 29). Cytolytic activity in breast cancer was reported to be modest compared with other tumors, such as kidney or lung cancer. Three driver mutations in breast cancer (*beta-2 microglobulin*, *CUL4B*, and *ARID2*) were significantly associated with cytolytic activity.

The burden of nonsynonymous DNA mutations in breast cancer is relatively low compared with other tumor types, especially carcinogen-related tumors such as sun-exposure–associated melanoma and smoking-related lung and bladder carcinoma (30). In other tumors, the extent of nonsynonymous mutations correlates with response to checkpoint therapy (31, 32). In breast cancer, there is heterogeneity in this metric among subtypes and large variation from patient to patient. In a study of more than 700 tumors, TNBC tumors were found to exhibit more nonsynonymous mutations than non-TNBC tumors (30).

The nonsynonymous mutational burden of cancer is important because it relates to a major molecular basis of antitumor T-cell reactivity (33). Somatic gene mutations resulting in single changes in amino acids can ultimately lead to the expression of mutated peptides in the groove of major histocompatibility complex (MHC) expressed on the surface of tumors cells, where these complexes can react with T-cell receptors on infiltrating T cells. Because these "neoepitopes" likely draw upon a T-cell receptor (TCR) repertoire that has avoided classically defined central tolerance, a robust T-cell response can manifest (33). Such T-cell reactivity, which has been well characterized in patients, can be specific for either driver or passenger mutations. Importantly, the mechanisms of actions of both CTLA-4 and PD-1/PD-L1 blocking mAb have been linked to activation or reinvigoration of neoepitope specific T cells in melanoma and lung cancer (31, 32). Exceptions are well described, however. For example, nivolumab has been approved for clear cell renal cell carcinoma, yet this is a cancer that exhibits modest levels of nonsynonymous mutations, at least in the primary tumor (34).

The load of classically defined neoepitopes with high predicted affinity for MHC in a given tumor is best predicted by

the burden of nonsynonymous mutations, and, as such, most breast cancers likely exhibit a relatively low number of predicted neoepitopes (30). Still, it has been predicted that just for one class I MHC allele (HLA-A2), there is likely one neoepitope for every 10 nonsynonymous mutations in breast cancer (35). Beyond the link to checkpoint therapy, neoepitopes offer a therapeutic target in cancer for a variety of antigen-specific approaches, including adoptive T-cell therapy (36) and vaccines (37, 38).

PD-1/PD-L1 in Human Breast Cancer

Expression of PD-L1 on breast tumors cells as well as on associated stromal cells has also been extensively studied by analyzing pathologic specimens (2). This is important because the binding of PD-L1 to PD-1 on the surface of infiltrating T cells can lead to T-cell inactivation or exhaustion. Overall, tumor cell expression of PD-L1 in breast cancer is modest and variable. For TNBC and ER^+ HER2-negative tumors (2, 3, 27), only 20% of samples have been shown to exhibit more than 1% of tumor cells expressing PD-L1. In contrast, leukocytes in the stroma of TNBC, especially myeloid cells, are typically PD-$L1^+$ (27). Ductal carcinoma *in situ* (DCIS) has very low levels of PD-L1 (39). These observations are important because PD-L1 expression in some tumors predicts, to some extent, improved clinical response to PD-1 or PD-L1 checkpoint mAb such as pembrolizumab and nivolumab (each anti–PD-1) or atezolizumab (anti–PD-L1). Pembrolizumab has an indication in the first-line therapy of non–small cell lung cancer expressing PD-L1 in more than 50% of tumor cells based on a survival advantage of pembrolizumab compared to chemotherapy (40). On the other hand, a fraction of patients with PD-L1–negative tumors across a variety of histologies have also been shown to respond to these agents.

Clinical results of PD-1/PD-L1 checkpoint blockade in metastatic breast cancer are now emerging. In a small study of 27 evaluable patients with advanced TNBC, for which tumors had at least 1% PD-L1 expression, the ORR following pembrolizumab was 18.5% (1). Toxicities observed in this study were similar to those well described for PD-1 blockade in other settings (41). Similarly, in 21 patients with metastatic TNBC, treatment with atezolizumab yielded a preliminary assessment of the ORR as 19% (2). Further investigation is required to understand if these patients with a clinical response were enriched for TNBC patients with detectable levels of PD-L1 in the tumor (2). In an unselected cohort of 168 breast cancer patients, the anti–PD-L1 antibody avelumab showed an ORR of only 4.8%; most of the responders were those with TNBC in this cohort (2, 42). In this study, known as JAVELIN, patients with PD-L1 expression on immune cells in the tumor showed greater overall response than patients with PD-L1–negative immune cells (4 of 12, or 33.3%, vs. 3 of 124, or 2.4%); in TNBC patients, PD-L1 expression also appeared to be associated with response (4 of 9, or 44%, for PD-L1–positive patients vs. 1 of 39, or 2.0%, for PD-L1–negative patients; ref. 42). Clinical trials combining PD-1/PD-L1 antibodies with other therapies such as trastuzumab or antiestrogens are underway, including in the neoadjuvant and adjuvant settings.

CTLA-4 in Breast Cancer

We tested the CTLA-4–blocking antibody tremelimumab in combination with exemestane in 26 patients with metastatic ER^+ breast cancer but found only limited signs of clinical activity (4). Best overall response was stable disease for at least 12 weeks in 42% of patients. Interestingly, treatment was associated in most patients with increased peripheral $CD4^+$ and $CD8^+$ T cells expressing ICOS and a marked increase in the ratio of $ICOS^+$ T cells to $FoxP3^+$ Tregs—findings that likely signal immune activation secondary to CTLA-4. In a recent pilot study of 19 presurgical patients with breast cancer, the combination of cryoablation with single-dose ipilimumab (anti–CTLA-4) demonstrated favorable intratumoral and systemic immune effects (43).

Therapeutic Vaccines in Breast Cancer

Extensive efforts have explored a role for therapeutic vaccination in breast cancer. Tumor-associated antigens targeted in these clinical trials have included HER2, sialyl-Tn-KLH, MUC1, telomerase, survivin, mammoglobin, mutant p53, and others (12, 44). Multiple formulations have been used, including peptides, proteins, and recombinant or genetically engineered constructs. In some strategies, such as GVAX cell–based vaccines (irradiated autologous or allogeneic tumor cells engineered to express GM-CSF; ref. 45), the antigen is not defined per se, but tumor antigen–specific T-cell responses can be triggered. In some cases, an immunodominant antigen, such as mesothelin, has been discerned (46). For nearly every vaccine formulation, the generation of T-cell immunity can be readily demonstrated, but positive clinical effects have been less clear. For example, in our studies in metastatic breast cancer using MHC-binding peptides derived from telomerase, we observed robust generation of functional effector CD8 T cells following vaccination, but the best response in any patient was stable disease (47). Presentation of telomerase-derived peptides on the surface of tumor cells has been demonstrated (48). However, in this small cohort of patients, those who demonstrated high-level immune response to telomerase peptides exhibited statistically significant improvement in overall survival compared to patients who failed to respond immunologically to vaccination (47). Nevertheless, two vaccination strategies (HER2 peptides or sialyl-Tn-KLH) that showed promise in early-phase testing disappointingly failed to meet the primary clinical endpoints in randomized studies (5, 6), which dampened enthusiasm for vaccine-only strategies in breast cancer. Notably, checkpoint blocking antibodies were not used in these randomized vaccine trials.

The overall upshot of these findings is that although tolerance can be broken to breast cancer–associated antigens, effector cells generated are either poorly potent or subsequently suppressed by peripheral tolerance mechanisms in the tumor microenvironment. This hypothesis has prompted efforts to add other agents that block specific pathways of immune suppression to generate more effective T-cell responses. In breast cancer, the negative role of intratumoral $CD25^+$ $FoxP3^+$ $CD4^+$ Tregs is well appreciated, and vaccination efforts in combination with strategies to deplete Tregs have been tested. Two examples include (i) adding low-dose cyclophosphamide and other immunomodulatory agents to GVAX (49, 50), or (ii) adding the CD25-blocking mAb daclizumab to telomerase and survivin peptide vaccination (51). The latter approach was shown to reduce systemic $CD25^+$ $CD4^+$ $FoxP3^+$ and total $CD4^+$ $FoxP3^+$ T cells for up to 2 months following a single infusion of daclizumab. Only recently has the opportunity become available to combine breast cancer vaccination with PD-1 or PD-L1 antibodies—a high-priority potential strategy. Likewise, second-generation vaccine approaches are molecularly defined and likely to be more potent. Thus, many

strategies to simultaneously vaccinate and therapeutically block immune suppressive pathways are being developed. Recent preclinical data from breast cancer models point to a role for abrogating myeloid inflammation, such as by blockade of CSF1R or PI3K gamma (52, 53), in order to activate a T-cell response.

CAR T-cell and Other Therapies in Breast Cancer

In lymphoid leukemia and other hematologic malignancies, impressive clinical results have been obtained with adoptive cell therapy using autologous T cells engineered to express CAR specific for CD19 (54). Progress of CAR T cells for breast cancer has been limited. Adoptive transfer of autologous HER2-specific polyclonal T cells generated from peripheral blood mononuclear cells (PBMC) after vaccine priming has been shown to be safe and with regressions observed in a few patients with advanced $HER2^{+}$ cancers (55). However, respiratory distress and fatal cytokine storm acutely following therapy with ERBB2-specific CAR T cells in one patient with metastatic colon cancer (56) has underscored the importance of developing new CAR targets with scrutiny of "off-tumor, on-target" toxicity of organs expressing the CAR target. For example, based on wide expression of c-Met in TNBC, investigators at the Abramson Cancer Center have developed c-Met CAR T cells for breast cancer in which initial clinical trials involve the use of mRNA (for transient CAR expression), rather than integrating lentivirus, to accomplish gene transfer; route of administration is intratumoral rather than intravenous as another potential safety feature (NCT01837602).

What Are We Missing?

The way forward for cancer immunotherapy in breast cancer is tantalizing but challenging. Although many critical areas of investigation remain, the following sections focus on four areas we wish to highlight.

Turning cold tumors hot

As noted above, most breast cancers exhibit relatively low T-cell infiltration, a feature that has been termed as being immunologically "cold" (57); accordingly, single-agent checkpoint therapy is ineffective for the majority of patients with breast cancer. In contrast, immunologically "hot" tumors have marked tumor-infiltrating T cells that may be reinvigorated with single-agent or dual checkpoint blockade (Fig. 2). One hypothesis to explain cold tumors is that a network of immunosuppression develops in the tumor microenvironment as a reaction to a nascent antitumor T-cell response and actively excludes further T-cell infiltration. A recent study of TCGA data indicates that the lack of spontaneous immune infiltration in noninflamed human melanoma tumors is unlikely due to lack of antigens per se (58). In patients with pancreas cancer, we have reported that the cytolytic index, defined by expression of granzyme A and perforin 1, is inversely proportional to the number of predicted neoepitopes (59). In tumors with a high degree of T-cell exclusion, blocking only PD-1 or CTLA-4 may be insufficient to achieve a clinical response if other suppressive pathways such as IDO, CD73, TIGIT, VISTA, and many others are also expressed and function in a nonredundant fashion regardless of PD-1 or CTLA-4 blockade. Tumor cells themselves can orchestrate T-cell exclusion, for example by oncogene-driven alterations in chemokine production that recruit suppressive myeloid cells (60, 61). Oncogene-driven upregulation of tumor PD-L1 has also been described and further contributes to immunosuppression (62).

A second hypothesis is that the primary immunologic deficit in cold tumors is poor T-cell priming and expansion. Here, the problem is not exhausted T cells or T cells that do not penetrate tumor stroma but T cells that have not been adequately generated in the first place. Thus, rather than checkpoint blockade, there are emerging efforts focused on "agonist therapy" to generate and amplify T-cell responses. These include CAR T cells, second-generation cancer vaccines (such as prime boost GVAX/*Listeria*

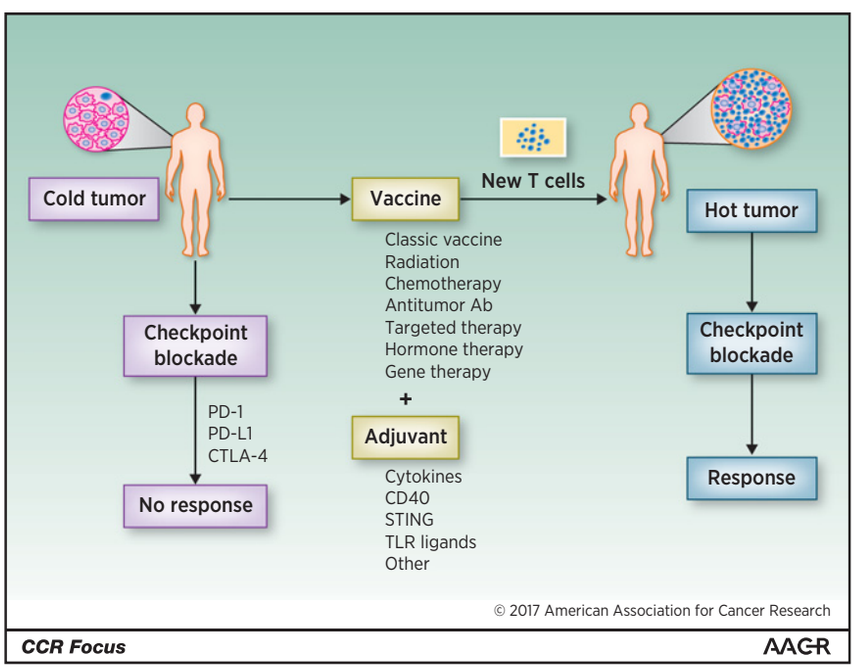

Figure 2.
Cold to hot immunologic conversion. Vaccination may be able to convert "cold" breast tumors, devoid of T cells, to "hot" tumors, with robust T-cell infiltration and responsiveness to checkpoint blockade. In addition to a classical experimental vaccine, many types of standard therapy may function to vaccinate the patient against tumor antigens released upon therapy-induced tumor cytotoxicity. STING, stimulator of interferon genes; TLR, Toll-like receptor.

combinations), dendritic cell activators (such as agonist CD40 antibodies or Toll-like receptor ligands), and T-cell agonists (such as OX40 or CD137 antibodies or cytokines including IL7 and IL15). Defining tumor-associated T-cell antigens for which potent T-cell responses can be generated will also be critical in converting cold breast cancer to hot and may require reaching beyond nonsynonymous mutations as the epitopes to rely on.

Advancing combination immunotherapy

Combining immune therapies with nonredundant mechanisms of action is considered crucial but complicated. Nivolumab in combination with ipilimumab is FDA approved for melanoma, at the cost of increased toxicity and price. In some cases, inhibiting multiple immune checkpoints may prove to be effective, whereas in other situations, a T-cell response will need to be generated first and then necessarily protected from exhaustion with checkpoint blockade. Meaningful combinations are not likely to be restricted to only immune/immune combinations; many standard drugs—including chemotherapy, radiotherapy, targeted therapy, or antitumor antibodies—may be immune modulatory, kill tumor cells in an immunogenic fashion, or otherwise synergize with approved immune therapies such as checkpoint blockade antibodies. However, issues of toxicity, dosing, and sequencing remain formidable, especially for agonists. The ultimate challenge in breast cancer "combination immunotherapy" is the vast number of potential combinations that likely overwhelm the number of patients and the capacity of the current clinical trial infrastructures to test them all. Priority combinations will be those with nonredundant mechanisms and those that are supported by preclinical work that includes a reliable immune pharmacodynamic biomarker.

Establishing routine immune profiling

Another opportunity is the development of methods for deep and routine immune profiling of patients and tumors. This approach can be built upon—and merged with—existing infrastructure established over the past years for next-generation sequencing and mutation panel test of patient tumors. For example, tumor and normal whole exome sequencing and tumor RNA sequencing can establish a patient's HLA type, mutational and neoepitope burden, the tumor genome, and transcriptome from which the composition of the cellular infiltration as well as the constellation of primary suppressive pathways can be bioinformatically determined (59). If successful, based on the immune profile, patients could be matched to effective therapies (and similarly, guided away from costly or toxic ineffective therapies). We suggest immune precision medicine will become an important element of cancer therapeutics over time.

Developing immune prevention

Finally, as cancer immunotherapy continues to progress, we should look for ways to deploy this new art of immune cell activation for the prevention of cancer in the first place. This notion to develop vaccines and immunotherapies to prevent cancer was underscored by the Cancer Moonshot Blue Ribbon Panel in the 2016 report (63). For example, one of our goals is to develop a vaccine to prevent breast and other cancers in healthy individuals who carry a germline mutation in BRCA1 or BRCA2. A first-in-human study of a vaccine formulation that could be used one day for this purpose is under active clinical evaluation in the high-risk, postadjuvant setting (NCT02960594). Using DNA plasmid and *in vivo* electroporation for intramuscular delivery, this vaccine targets the human telomerase reverse transcriptase in concert with IL12 as an adjuvant. Preclinical studies have shown marked resistance to tumor development in vaccinated versus unvaccinated mice (64).

Disclosure of Potential Conflicts of Interest

R.H. Vonderheide reports receiving commercial research grants from Lilly and is listed as an inventor on a patent for telomerase-specific cancer immunotherapy held by the Dana-Farber and Whitehead Institutes. S.M. Domchek is listed as an inventor on a patent for telomerase-specific cancer immunotherapy held by the Dana-Farber and Whitehead Institutes. No potential conflicts of interest were disclosed by the other author.

Acknowledgments

The authors thank Drs. Julia Tchou, Angela DeMichele, Kevin Fox, Jennifer Matro, Kate Nathanson, David Weiner, John Wherry, and Carl June for valuable discussions. This work was supported by the Breast Cancer Research Foundation (R.H. Vonderheide and S.M. Domchek), the Basser Research Center for BRCA (R.H. Vonderheide and S.M. Domchek), Susan G. Komen (S.M. Domchek and A.S. Clark), and the Parker Institute for Cancer Immunotherapy (R.H. Vonderheide).

Received December 27, 2016; revised February 15, 2017; accepted April 5, 2017; published online June 1, 2017.

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Clin Cancer Res 2017;23:2640-2646.