Immunology and Breast Cancer: Toward a New Way of Understanding Breast Cancer and Developing Novel Therapeutic Strategies

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Abstract: Every cancer triggers an immune response that constitutes an important first-line protection against cancer progression. In breast cancer, there is an increasing awareness of the relationship between the immune system and tumor evolution. The tumor microenvironment is composed of a variety of immune cells that can control or arrest malignant progression. Chemotherapy and targeted therapy have been shown to modulate this immune microenvironment. Recently, tumor-infiltrating lymphocytes have emerged as a predictive and prognostic biomarker in early breast cancer. In addition, immune gene expression signatures have been shown to be associated with prognosis in triple-negative and human epidermal growth factor receptor 2–positive breast cancer. Such findings have increased interest in the development of immunotherapeutic agents for breast cancer, and multiple clinical trials of anticancer vaccines and immune checkpoint inhibitors are ongoing. In this review, we summarize what is known about the relationship between immunity and breast carcinoma, explore the relevance of this information to the clinical and research settings, and give a portrait of new therapeutic strategies using immunotherapy in breast cancer.

Introduction

In the last 2 decades, remarkable progress has been made in our understanding of the molecular, genetic, and biologic origins of cancer. In 2000, Hanahan and Weinberg proposed 6 hallmarks of cancer to explain the process by which a normal human cell transforms into a cell with malignant potential.[1] Eleven years later, 4 new hallmarks were added to better encompass the complexity of the cancer cell's biology and its ability to evade host defenses. One of these new traits is the ability of cancer cells to avoid immune destruction.[2]

The immune system detects and destroys abnormal neoplastic cells during a monitoring process called immunosurveillance.[3] Our understanding of this classic concept, proposed more than 50 years ago, has been refined over the last 15 years. Specifically, we

now know that the immune system shapes the character of the growing malignancy. This complex interplay between a tumor and the body's immune defenses is known as immunoediting. The interactions between the cancer cells and the immune network evolve through 3 different phases: elimination, equilibrium, and escape.[4] Most patients are diagnosed in the escape phase, during which cancer cells evade immunosurveillance. One of the hypotheses to explain this immunologic escape is loss of antigen expression by cancer cells; another is the establishment of an immunotolerant environment.[4] Although breast cancer traditionally has not been considered a highly immunogenic malignancy, researchers in multiple laboratories have demonstrated a relationship between the intratumoral immune reaction and breast cancer evolution.[5-8] Thus, it is becoming clear that the immune system plays a role in controlling breast tumor progression.

On the other hand, important progress has been made during the last decade in the treatment of breast cancer, especially with the growing use of targeted therapy in human epidermal growth factor receptor 2 (HER2)–positive breast cancer. Unfortunately, only a fraction of breast cancer patients can benefit from targeted therapies, and resistance to these therapies can emerge.[9] New therapeutic options are needed to improve the treatment of breast cancer patients, including immunotherapeutics, which recently have shown impressive results in other cancers, such as malignant melanoma.[10,11] As our understanding of the relationship between breast cancer biology and immunity expands, it should allow for new advances in immunotherapy for breast cancer patients.

In this review, we summarize what is known about the relationship between immunity and breast carcinoma, explore the relevance of this information to the clinical and research settings, and give a portrait of new therapeutic strategies using immunotherapy in breast cancer.

Tumor-Infiltrative Lymphocytes

An association between tumor-infiltrative lymphocytes (TILs) and prognosis in primary breast carcinoma has been suspected for many years.[12] Recently, a high level of TILs has raised large attention as a biomarker predicting pathologic complete response (pCR) after neoadjuvant chemotherapy (see Table 1 at www.hematologyandoncology.net). In a pivotal analysis from the German Breast Group, Denkert and colleagues demonstrated an independent association between the percentage of intratumoral TILs as a continuous variable and pCR in 1058 patients (odds ratio, 1.36; P=.01).[5] Lymphocyte-predominant breast cancer (LPBC; defined as more than 60% of stromal or intratumoral lymphocytic infiltration) was associated with an exceptionally high rate of pCR (41.7% vs 12.8%; P<.0005). In contrast, the pCR rate was only 2% in patients without any tumoral lymphocytic infiltration.

Since then, the independent predictive value of TILs has been validated in other large cohorts of patients, with more than 3000 samples analyzed.[13-17] Many of these studies made a distinction between intratumoral lymphocytes (lymphocytes in tumor nests having cell-to-cell contact with carcinoma cells) and stromal lymphocytes (lymphocytes dispersed in the tumoral stroma with no direct contact with carcinoma cells). Although intratumoral and stromal TILs generally correlate with each other, certain trials have found a stronger association with one or the other.[5,17] Stromal TILs are usually found in higher numbers and thus are easier to assess.[18]

TILs are associated with poor-prognosis clinicopathologic characteristics, including estrogen receptor (ER) negativity, higher tumor grade, high levels of Ki-67, larger tumor size, and positive lymph nodes.[7,16,17,19-21] Despite this, high TIL level as an independent indicator of good prognosis has been validated in large cohorts of patients in the adjuvant setting (see Table 2 at www.hematologyandoncology.net). A high level of TILs is associated with improved distant disease–free survival, disease-free survival (DFS), and overall survival (OS).[7,13,20-23]

The proportion of LPBC varies widely among studies (4%-28%), but this pattern is consistently associated with a good prognosis.[5,7,17] The threshold for defining LPBC ranges from 50% to 60% in different trials. The variability in LPBC prevalence among studies can be explained by differences in patient (and hence tumor) characteristics in the trials. The proportion of LPBCs generally is higher in neoadjuvant trials than in adjuvant trials, which could result from the inclusion of patients with larger tumors in neoadjuvant trials. For instance, more than half the tumors in the neoadjuvant cohort from the GeparTrio trial from the German Breast Group (LPBC, 12%) were larger than 4 cm,[5] whereas in the adjuvant cohort from ECOG (Eastern Cooperative Oncology Group) 2197 and ECOG 1199 (LPBC, 4%), only 8% were larger than 5 cm.[22] The variability in LPBC proportion might also be explained by differences in the proportion of breast cancer subtypes, ER negativity being associated with a higher level of TILs. Some TIL trials included only patients with specific breast cancer subtypes, such as HER2-positive and triple-negative breast cancer (TNBC) in the GeparSixto trial, and TNBC in the cohort from ECOG E2197 and E1199. Other TIL trials included all breast cancer subtypes, with a majority of patients having ER-positive disease (cohorts from GeparDuo, GeparTrio, BIG [Breast International Group] 02-98). Other factors unaccounted for in these studies might also influence the prevalence of TILs; for example, patients with an immunosuppressive

state secondary to a medical condition or drug might have a lower level of TILs. In an analysis comparing the prevalence of TILs in pregnant and nonpregnant early breast cancer patients, pregnant women had a lower rate of LPBC compared with nonpregnant women, suggesting that the immunotolerant state associated with pregnancy might influence the number of TILs.[24]

Moreover, the predictive and prognostic value of TILs differs according to breast cancer subtype. In an analysis by Ono and colleagues of a cohort of 474 patients having received neoadjuvant chemotherapy, a significant correlation between pCR and a high TIL score was verified only in the subgroup of patients with TNBC.[16] Loi and colleagues investigated the relationship between TILs and clinical outcomes using 2009 samples from the BIG 02-98 adjuvant study. TILs were associated with improved DFS and OS, but only in patients with TNBC.[7] The same trend was observed in the analysis of 935 samples from the FinHER (Finland Herceptin) phase 3 adjuvant trial.[21] In this study, high TIL levels were associated with improved distant disease–free survival in patients with TNBC and HER2-positive breast cancer, but not in patients with ER-positive and HER2-negative breast cancer. These results are not surprising because a previous paper published in 1992 had suggested that lymphocytic infiltration was associated with prognosis only among rapidly proliferating tumors.[25]

Furthermore, there is increasing evidence of an interaction between TILs and sensitivity to targeted therapy and chemotherapy. Recent results of the prospective analysis of TILs on biopsy samples from GeparSixto, a phase 3 neoadjuvant trial evaluating the benefit of adding carboplatin to paclitaxel and liposomal doxorubicin in TNBC and HER2-positive breast cancer, demonstrated a significant interaction between the benefit from the addition of carboplatin and TILs.[26] In a subgroup analysis, the interaction was only significant in HER2-positive patients, which could suggest a role for anti-HER2 therapy to alleviate suppression of antitumor effector immunity.

However, the evidence regarding the association between benefit from trastuzumab (Herceptin, Genentech) and TILs is conflicting. In the FinHER trial, the association between TILs and better outcomes in 232 HER2-positive breast cancer patients was driven mostly by those enrolled in the trastuzumab arm, supporting an interaction between TILs and benefit from trastuzumab.[21] This interaction was validated in a second cohort of 156 HER2-positive breast cancer patients treated with trastuzumab in the GeparQuattro trial.[27] Despite this finding, stromal TILs were not associated with improvement in relapse-free survival (RFS) in 456 patients treated with adjuvant trastuzumab in the NCCTG (North Central Cancer Treatment Group) N9831 trial; the association between TILs and better outcome was confirmed only in the chemotherapy-alone arm.[28] In the LPBC group (10% of patients), patients treated with chemotherapy alone had an unexpectedly better RFS than patients treated with trastuzumab and chemotherapy (90.9% vs 80%; HR, 2.43; 95% CI, 0.58-10.22). The explanation for these conflicting results is not clear, but could be related to the low number of events. Larger prospective trials are needed to better define the interaction between trastuzumab and TILs.

The encouraging evidence supporting the prognostic and predictive role of TILs has triggered a serious attempt to standardize the evaluation of TILs. Recently, recommendations for the standardization of TIL evaluation in breast cancer have been published by a group of international investigators representing key breast cancer research teams.[18] Among other recommendations, the group suggests evaluating stromal TILs rather than intratumoral TILs, because stromal TILs represented a superior and more reproducible parameter in most studies. TILs should be assessed as a continuous variable and include all mononuclear cells except polymorphonuclear leukocytes. Other recommendations for the pathologic evaluation of TILs are detailed in the publication by Salgado and colleagues. This initiative should help the integration of TILs in the research field and eventually in routine clinical practice.

Gene Expression Profiling: The Immune Signatures

The growing availability of gene expression profiling has allowed the study of numerous breast cancer gene profiles. Distinct patterns of gene expression are associated with different clinical outcomes.[29,30] A classification of breast cancer into molecular subsets is now commonly used in clinical practice.[31] However, significant disparity remains in the clinical outcomes among those subtypes.

Several gene modules have been created from cancer cell lines to describe common oncogenic pathways. Desmedt and colleagues defined 7 different gene modules associated with key biological processes (proliferation, immune response, tumor invasion, evasion of apoptosis, sustained angiogenesis, self-sufficiency in growth signals, and signaling of ER and HER2) and correlated them with prognosis in more than 2100 breast cancer patients.[32] The prognostic impact of the different gene modules varied according to breast cancer subtype. Proliferation and histologic grade remained the variables most associated with survival in patients with ER-positive breast cancer. However, immune response and tumor invasion were the main variables associated with prognosis in HER2-positive breast cancer. Only immune response was associated with survival in TNBC. Another study concluded that the prognostic value of the immune-gene expression signatures was restricted

to tumors with a high proliferative index, which includes TNBC and HER2-positive breast cancer.[33]

The association between different gene modules (including immune response) and pCR was also evaluated in a pooled analysis of 996 patients having received neoadjuvant chemotherapy and for which gene expression profiling was accessible in public databases.[34] A high immune response score was associated with increased probability of pCR in all breast cancer subtypes, but the association remained significant after multivariate analysis only in ER-negative breast cancer, and was the strongest in HER2-positive breast cancer. Other trials have also confirmed that immune-gene expression signatures are better than conventional clinicopathologic criteria at identifying subgroups of patients with a better prognosis,[32,35-37] especially in ER-negative breast cancer.[38]

Recently, another group reported the results of whole transcriptome analysis of 1282 samples from the HER2-positive breast cancer adjuvant trial NCCTG-N9831. Once more, immune-gene enriched tumors were associated with improved prognosis. Moreover, an interaction between the benefit from trastuzumab and immune-gene enrichment was reported.[39] However, the same group demonstrated no significant association between TILs and RFS in patients treated with adjuvant trastuzumab in the same trial.[28] These conflicting data between TILs and the immune-gene signature are difficult to reconcile; nevertheless, because TILs are composed of a mixture of cells with stimulating and suppressive immune activity, it is possible that they do not always reflect immune-gene enrichment. Additional studies are needed to better understand these findings.

Altogether, the data suggest that including an immune-enriched component in the high-proliferative subtypes could further refine the molecular classification of breast cancer. For example, TNBC is a heterogeneous subgroup of breast cancer with wide variety in prognosis and evolution. It is typically viewed as falling into the molecular category of basal-like breast cancer. Still, a significant proportion of TNBC cannot be classified in this category. In a study analyzing gene expression profiles of 587 TNBC samples, 6 different molecular subtypes were identified, including an immunomodulatory subtype characterized by high expression of genes involved in immune processes.[40] Each molecular subtype was associated with a distinct prognosis and sensitivity to therapy, the immunomodulatory subtype being associated with an improved RFS compared with other subtypes.

The Immune Microenvironment: A Battlefield for Pro- and Anti-Tumor Activities

The immune system can play an antagonistic role in the tumoral environment. Although its primary method of preventing tumor formation is through immune surveillance, some immune cells also promote alternate inflammatory pathways that suppress adaptive immunity and create a state of immunotolerance. The global influence of the different immune cells depends on their cellular distribution and on the overall immune context.[41] Immune infiltrates in breast cancer are mainly composed of T lymphocytes (≈75%), together with B lymphocytes (<20%), monocytes (<10%), and natural killer cells (<5%).[6,42] The T CD3+ lymphocytes are divided into CD8+ and CD4+ T helper (Th) cells and CD4+ regulatory T cells (Treg). The CD4+ lymphocytes are composed of all Th subsets, with a mixture of activating and suppressive activities.[6]

The balance between the different T-cell subsets is determinant for efficient antitumor activity. The proportion of each T-cell subset varies widely from one patient to another and according to breast cancer subtype.[43] High T-helper 1 (Th1) cytotoxic response at the expense of T-helper 2 (Th2)-driven humoral immunity is associated with a good prognosis.[44,45] Tumoral infiltration by CD8+ lymphocytes, a crucial component of tumor-specific cellular adaptive immunity, is associated with an improved prognosis and a superior probability of pCR,[19,46] especially in ER-negative breast cancer.[47-49] Intriguingly, in a subgroup analysis of a study that evaluated CD8+ lymphocyte infiltration in 12,439 breast cancer tumor samples, the presence of CD8+ T-cell infiltrate was associated with a reduced risk of death from breast cancer in TNBC and HER2-positive breast cancer, but with a worse prognosis in luminal A breast cancer.[49] In another trial, the prognostic value of CD8+ T-cell infiltrate was restricted to patients with basal-like TNBC.[48]

On the other hand, Tregs play an antagonistic role by promoting an immunologic self-tolerant state. Circulating Tregs were demonstrated to be elevated in breast cancer patients.[50] Expression of CD4+ and CD25+ Tregs is elevated in breast tumor tissue, principally in high-grade and ER-negative breast cancer.[51,52] Measurement of forkhead box P3 (FOXP3) levels has been used in many studies as a surrogate marker for Treg activity. This transcription factor plays a crucial role in the generation of immunosuppressive Tregs and is one of the most specific markers for Treg activity.[53] FOXP3 expression is associated with higher tumor grade and with ER negativity.[54]

The predictive and prognostic value of FOXP3 in breast cancer is controversial. High FOXP3 expression before neoadjuvant chemotherapy was identified as an independent predictor of pCR in a trial evaluating FOXP3 and CD8+ infiltration in 180 pretherapeutic breast cancer core needle biopsies.[55] The highest rate of pCR was seen when both CD8+ lymphocytes and FOXP3 were elevated before chemotherapy. However,

elevated FOXP3 expression in the breast tumor generally is associated with an increased risk of distant relapse and decreased survival,[52,56-58] although not consistently through all studies.[54,59] These discrepancies could be partially explained by the absence of a standardized way of scoring FOXP3 expression by immunohistochemistry. There could be variability in prognostic significance depending on FOXP3 density, tissue location, or breast cancer subtypes.[54,57,59] It has been recently elucidated that FOXP3 may not be specific to Tregs, and might also be expressed by malignant cells.[19]

The role of the B lymphocytes and adaptive humoral immunity has been less extensively studied. In an analysis of B lymphocytes in 1470 breast tumor samples, a higher CD20+ B-cell count was associated with better outcomes, independent of CD8+ T-cell count.[60] A B-cell gene expression signature has also been correlated with good prognosis in highly proliferative tumors, mainly TNBC.[61-64] However, some data suggest that a subset of B lymphocytes, named tumor-evoked regulatory B cells, play a role in promoting breast cancer metastasis by stimulating T-cell conversion to Tregs.[65]

Macrophages are directly involved in tumor progression and metastasis by promoting tumor invasion, migration, and angiogenesis.[66] Type 2 macrophages promote Th2 differentiation, favoring Treg development.[67] In breast cancer, tumors with dense macrophage infiltration usually are of higher grade, have an elevated proliferation index,[68,69] and are associated with a poor prognosis.[69-71] Myeloid-derived suppressor cells, a heterogeneous population of immature myeloid cells, are also believed to exert a variety of immune suppressive functions and to be of poor prognosis in metastatic breast cancer.[72] Myeloid-derived suppressor cells are associated with aberrant expression of the immunomodulatory enzyme indoleamine 2,3-dioxygenase (IDO), which could partly explain their immunosuppressive mechanism.[73,74] Angiogenesis is a necessary component of tumor growth. High endothelial venules are specialized capillary venules that support migration and extravasation of lymphocytes into tissue. A high endothelial venule concentration has been shown to confer a lower risk of relapse and improved survival in patients with breast cancer.[75] Although these observations highlight the diversity encountered in the tumoral immune microenvironment, there is no evidence that analysis of the different immune cell subsets is a more robust prognostic indicator than TIL level.

Relationship Between Breast Cancer Therapies and the Immune System

The immune microenvironment is not static. It evolves within the tumor, and changes with therapeutic intervention. It has been demonstrated that chemotherapy can induce drastic diminution in FOXP3-positive cells, and this reaction is associated with a higher probability of pCR.[19,57] Although chemotherapy's main mechanism of action is believed to be induction of apoptosis, because of interference with DNA replication and evidence of DNA damage, studies suggest that part of the antitumor effect occurs through modulation of the immune system.[76,77] Accumulation of CD11c+ dendritic cells and enhanced CD8+ activation in breast tumors were demonstrated in mice treated with cisplatin.[78] Elevation of TIL percentage has been demonstrated after taxane neoadjuvant therapy, and this reaction correlated with clinical response.[79] Anthracyclines can stimulate CD8+ lymphocyte proliferation in tumors.[80] Conversely, studies on CD8+ depleted mice demonstrated an increased resistance to anthracyclines, suggesting that immunity is indispensable for an optimal antitumor effect.[80] In a study that included 111 ER-negative breast cancer patients, adjuvant anthracycline treatment was associated with increased DFS only in patients with high TIL levels.[13] As mentioned earlier in this review, there is also a probable interaction between TIL level and benefit from carboplatin.[26]

On the other hand, the monoclonal antibody trastuzumab kills HER2-expressing tumor cells not only directly, by interfering with HER2 signaling, but also indirectly, via immune mechanisms such as antibody-dependent cellular cytotoxicity.[81] Peripheral blood sample analysis from breast cancer patients before and during treatment with trastuzumab revealed that the agent could modulate immune activity by reducing the level of circulating Tregs.[82] An increase in CD8+, CD4+, and natural killer cell activity after neoadjuvant trastuzumab was demonstrated on surgical samples from breast cancer patients.[83,84] A small trial analyzing an anti-HER2 antibody and specific CD4 response in metastatic breast cancer before and during treatment with trastuzumab demonstrated induction of HER2-specific immunity during trastuzumab therapy. Patients with an objective clinical response were those with a larger humoral immune sensitization.[85] The analysis of TIL level in tumor samples from HER2-positive breast cancer patients treated with trastuzumab in the FinHER and GeparQuattro trials also supports an interaction between immune response and benefit from trastuzumab,[21,27] as does the recent assessment of immune-gene enriched tumors in the NCCTG-N9831 trial.[39] Together, these trials suggest that patients with higher immune activation benefit more from the addition of trastuzumab, although the conflicting results of TIL analysis in the NCCTG-N9831 trial remind us that caution is warranted before reaching any definite conclusions.[28]

In FinHER, elevated expression of cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and programmed death 1 (PD-1)—both negative regulators of the immune system—also were associated with

increased benefit from trastuzumab in HER2-positive breast cancer patients.[27] This interesting finding suggests a role for trastuzumab as an immunomodulator. Accordingly, trastuzumab conceivably could be employed as an immunomodulating agent in HER2-negative breast cancer. A trial is currently ongoing evaluating the role of trastuzumab as adjuvant therapy in patients with HER2-negative breast cancer and persistent circulating tumor cells after adjuvant or neoadjuvant chemotherapy and surgery (NCT01548677).

Taken together, these data highlight the interplay between therapy and the immune system. As such, therapeutic agents act as modulators of the immune system and depend on it to exert maximal efficacy. More research is needed to better characterize the biological mechanisms underlying the interaction between chemotherapy, targeted therapy, and the immune system.

Therapeutic Strategies: Reversing Immunotolerance

The immune system can influence the progression of breast cancer from early evolution to metastatic spread, and also affect tumoral response to chemotherapy. It is thus an interesting target for new therapies (see the figure). Passive immunotherapy with antigen-specific monoclonal antibodies is already an integral part of the management of HER2-positive breast cancer. Active immunotherapy with vaccine administration is another approach to eliciting an immune response against tumoral antigens.

Many types of vaccines, employing diverse formulations, have been studied in breast cancer. The most common are the peptide- or protein-based vaccines, which often are derived from the intracellular or extracellular domain of HER2. Peptide- and protein-based vaccines aim at stimulating an immune response using antigenic epitopes derived from tumoral antigens. Other formulations are DNA vaccines, in which DNA is processed to an immunogenic protein by antigen-presenting cells; and whole tumor cell vaccines, which are derived from autologous cells or from malignant cell-line cultures. Infusion of dendritic cells engineered in vitro to present tumoral antigens also has been studied in breast cancer vaccine trials.

Vaccine trials have been conducted in breast cancer patients for almost 2 decades, mainly in the metastatic setting.[86-97] Overall, most of the trials evaluating vaccine therapies were phase 1 studies that demonstrated both an acceptable tolerance and an ability to generate an antigen-specific immune response. Moreover, cases of tumor regression with vaccine therapy have been reported.[91,96,98,99] Although the aforementioned trials have been successful in demonstrating antigen-specific immune responses following an anticancer vaccine, few vaccines have been further developed in clinical trials designed to demonstrate clinical efficacy against standard of care.

The only published phase 3 randomized vaccine trial in breast cancer evaluated a vaccine targeting sialyl-Tn (sTn), a known tumor-associated antigen, in 1028 patients with metastatic breast cancer.[100] Patients in the control arm received a placebo vaccine that contained only the carrier protein KLH. This large trial was not able to demonstrate a statistically significant benefit in time to progression or OS, though a vigorous immune reaction against the tumoral antigen was confirmed in the vaccine arm. Some aspects of the trial's design might explain the results. First, many patients in the control group developed an immune response to KLH that was not tumor specific, but may have boosted the immune system and blurred the results of the study. Second, patients enrolled in the study were not selected based on breast cancer subtypes. Because TNBC and HER2-positive breast cancer are associated with a stronger immune response, anticancer vaccines may be more effective in those subtypes. Furthermore, only 30% to 40% of breast cancers express the sTn antigen;[101] the vaccine may have been more successful in a population selected for sTn expression. Finally, the disease setting might not have been optimal. Anticancer vaccines might be more efficient as an adjuvant therapy—to prevent disease recurrence—than in the metastatic setting, which is associated with a more immunosuppressive environment that allows for the selection of resistant clones.[102]

In the adjuvant setting, a combined analysis of two phase 2 trials studying an anti-HER2 peptide-based vaccine compared the clinical outcome of 101 vaccinated patients with a control cohort that was not eligible for the vaccine arm because of human leukocyte antigen (HLA) type.[103] At 18 months, there were fewer recurrences in the vaccinated group (5.6% vs 14.2%; P=.04). The difference was no longer significant at 24 months, however. Interestingly, the pattern of recurrence differed between the vaccinated group and the control group, with more bone-only recurrence in the vaccinated group (P=.05) and a trend towards less mortality from recurrence in the vaccinated group.

A phase 3 adjuvant vaccine trial with already 700 patients enrolled is ongoing and will hopefully demonstrate clinical efficacy in the adjuvant setting (NCT01479244).

Many obstacles are contributing to the slow rate of anticancer vaccine development. Vaccines are technically complex to engineer, and can be difficult to produce in large quantities. Peptide vaccines often are restricted to small subgroup of patients, such as those with precise HLA types or those whose tumors express a specific antigen. Another major challenge is to achieve long-term immunity against tumoral antigens. In most

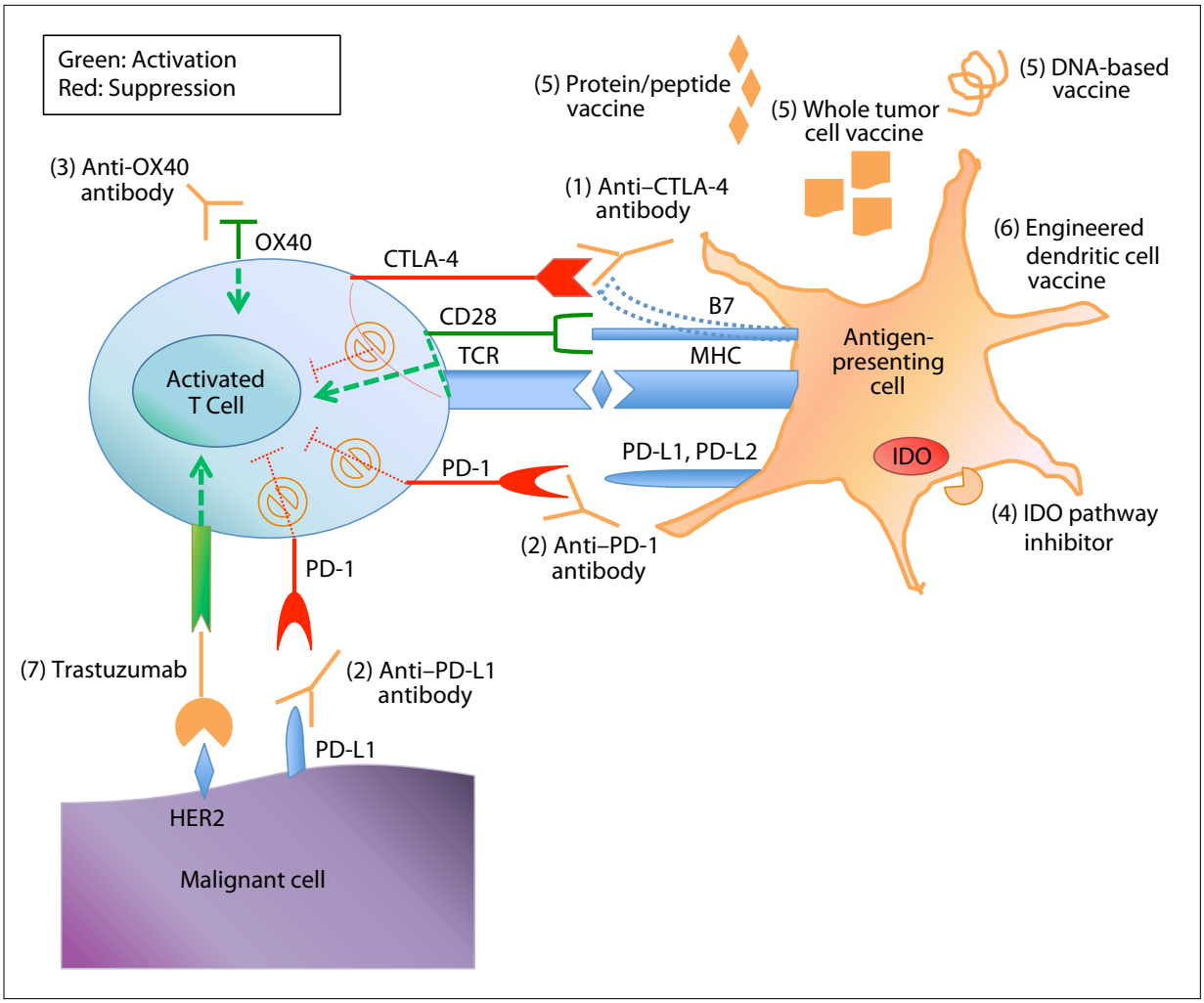

Figure. Overview of immunotherapies in breast cancer.

(1) By blocking binding between CTLA-4 and B7, monoclonal antibodies against CTLA-4 allow for unopposed T-cell activation.

(2) Anti–PD-1/PD-L1 monoclonal antibodies can relieve T-cell inhibition pathways, thereby permitting unopposed T-cell activation.

(3) Selective monoclonal antibodies can bind receptor OX40 and stimulate T-cell activity.

(4) IDO pathway inhibitors alleviate immune suppression mediated by the enzyme IDO.

(5) Antigen-specific immunization can be elicited using proteins or peptides derived from tumoral antigens, whole tumor cells, or DNA that will be processed and presented by antigen-presenting cells.

(6) Dendritic cells can be engineered in vitro to present tumoral antigens.

(7) Trastuzumab can elicit an antibody-dependent cell-mediated cytotoxicity.

CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; HER2, human epidermal growth factor receptor 2; IDO, indoleamine 2,3-dioxygenase; MHC, major histocompatibility complex; PD-1, programmed death 1; PD-L1, programmed death ligand 1; TCR, T-cell receptor.

trials, only a fraction of patients continue to show a specific immune response when tested after the first 6 to 12 months.[87,89,103,104] However, some data support the possibility of inducing durable immune responses with antitumoral vaccines in breast cancer.[88,105,106] In a retrospective analysis of 52 HER2-positive breast cancer patients immunized in vaccine trials between 1996 and 1999, 75% of the patients who were still alive had persistent immunity after a median follow-up of 112 months. Interestingly, the development of anti-HER2 antibodies against epitopes not contained in the vaccine, a phenomenon called epitope spreading, was an independent predictor of survival.[107]

An alternative immunotherapeutic approach is to reverse immunotolerance by blocking T-lymphocyte antigens that are suppressing the immune system.

CTLA-4 is a protein receptor found on the surface of activated T cells that downregulates their activity. Tremelimumab, an experimental monoclonal antibody specific for CTLA-4, was coupled with exemestane for treatment of advanced breast cancer in a phase 1 trial.[108] Treatment-related adverse events were mild to moderate, with no grade 3 or 4 treatment-related diarrhea. Stable disease for 12 weeks or longer was obtained in 42% of patients. A phase 2 trial has also been completed in stage IV breast cancer with ipilimumab (Yervoy, Bristol-Myers Squibb), another anti–CTLA-4 monoclonal antibody, with no results available yet (NCT00083278).

PD-1 is an antigen expressed on activated T cells, pro-B cells, natural killer cells, dendritic cells, and monocytes. PD-1 and its ligands, programmed death ligands 1 and 2 (PD-L1 and PD-L2), also play major role in maintenance of T-cell tolerance. PD-1 and PD-L1 are aberrantly expressed in breast cancer.[109,110] Their expression parallels that of the TILs, suggesting negative feedback activation as part of the immune reaction. There is emerging evidence of the clinical efficacy of agents targeting PD-1/PD-L1 in breast cancer. The results were recently presented of a phase 1 trial evaluating MK-3575, a monoclonal antibody specific for PD-1, in 32 heavily pretreated TNBC patients. The overall response rate was 18.5%, including 1 complete response and 4 partial responses.[111] The drug will be further developed in a phase 1b/2 trial enrolling HER2-positive breast cancer patients resistant to trastuzumab (NCT02129556). Another phase 1 clinical trial involving 9 metastatic TNBC patients treated with MPDL3280A, an anti–PD-L1 monoclonal antibody, demonstrated similar outcomes, with an overall response rate of 33%, including 1 complete response and 2 partial responses.[112] Other possible targets for immunotherapeutic agents are OX40, a receptor stimulating T-cell activity, and IDO, an enzyme with immunomodulatory activities. Many trials are currently ongoing with these promising new therapies (see Table 3 at www.hematologyandoncology.net).

Challenges in Immunotherapy

The use of immunotherapy in breast cancer faces many challenges. First, a fundamental principle of immunoediting is that with recognition and elimination of tumoral antigens, T cells are driving the selection of clones lacking expression of strong rejection antigens.[4,113] There is evidence that immunotherapy can lead to immunoediting and loss of antigen expression.[114-116] Strategic approaches to avoid the development of resistance would be to simultaneously target multiple antigens, or to combine immunotherapy with chemotherapy or targeted therapy. Low-dose cyclophosphamide, paclitaxel, and doxorubicin have been shown to enhance the antitumor effect of vaccines in mouse models,[117] and the combination proved safe in 1 clinical trial.[95] Groups of investigators also have addressed the combination of anti-HER2 systemic agents (trastuzumab and lapatinib [Tykerb, GlaxoSmithKline]) with anti-HER2 vaccines.[94,97,118] Data about clinical efficacy are not yet available.

Second, the local immunosuppressive environment is an obstacle for the action of immunotherapy. Reversion of immune activity inhibition with an anti–PD-1 or anti–CTLA-4 monoclonal antibody can overcome the immunotolerant state. In mouse models, the efficacy of treatment with a monoclonal anti-HER2 antibody was enhanced by the addition of an anti–PD-1 monoclonal antibody.[119] It would thus be interesting to combine these 2 therapies. Third, all patients may not experience the same benefit from immunotherapy. For example, because the prognostic impact of the immune response is the strongest in TNBC and HER2-positive breast cancer, it is possible that patients with these subtypes would benefit the most from immunotherapy. There is no validated biomarker at this time to predict response to immune checkpoint blockade inhibitors, though genomic analysis of tumor DNA in 64 patients with malignant melanoma suggested a genetic basis to explain response to ipilimumab.[120] The pattern of PD-L1 expression, especially in the immune microenvironment, might predict response to anti–PD-L1 agents.[121]

Conclusion

Data demonstrating crosstalk between breast carcinoma cells and the immune system have been accumulating over the last few years. Exciting trials have raised awareness about the importance of immunity in the evolution of breast cancer. The prognostic and predictive value of elevated TILs has been validated in large cohorts of patients, and is especially convincing in TNBC and HER2-positive breast cancer. TNBC with high immune gene expression could represent a distinct molecular subtype of breast cancer with a different prognosis and sensitivity to therapy.

An assessment of immune activation could improve prognostic stratification of TNBC and HER2-positive breast cancer patients. Prospectively integrating TILs into trials of neoadjuvant/adjuvant therapy for TNBC and HER2-positive breast cancer could help to better stratify patients and identify good-prognosis subgroups that may not need therapy intensification. Patients with a poor immune response have a poor prognosis, with increased resistance to standard therapy. Those patients would be ideal candidates for investigational therapy.

The integration of immunotherapy into the management of breast cancer is challenging. Vaccines can elicit an antigen-specific immune response, but the immunity

they confer tends to fade with time. At present, it is unclear whether vaccines can elicit long-term antitumoral protection and sustained clinical benefit in breast cancer patients. Studies with new monoclonal antibodies that relieve immune inhibition are still at their beginning, and many clinical trials with immunotherapeutic agents are presently ongoing. Our knowledge of the relationship between therapies, breast carcinoma, and immunity will hopefully continue to evolve over the next decade.

Disclosures

The authors have declared no relevant conflicts of interest.

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Supporting Online Material

Table 1. Overview of Neoadjuvant Trials on TILs

Reference	Study	N	Breast Cancer Subtype	Proportion of LPBC According to Subtype	Outcome
Denkert,[5] 2010	GeparDuo	218	ER+: 67% ER–: 22%	ER+: 9.5% ER–: 14%	iTu-Ly (continuous variable) associated with pCR (OR, 1.38; *P*=.012) LPBC associated with pCR (*P*<.0005)
	GeparTrio	840	ER+: 60% ER–: 26% HER2+: 30% HER2–: 52%	ER+: 6% ER–: 27% HER2+: 11% HER2–: 12%	iTu-Ly (continuous variable) associated with pCR (OR, 1.36; *P*=.01) LPBC associated with pCR (*P*<.0005)
Yamaguchi,[14] 2011	Institutional cohort	68	ER+/HER2–: 40% HER2+: 43% TN: 16%	N/S	High TILs correlate with pCR (OR, 4.7; *P*<.0001)
Ono,[16] 2012	Institutional cohort	180	ER+/HER2–: 26% ER–/HER2+: 23% TN: 51%	N/S	High TILs associated with pCR (*P*=.0001)
Issa-Nummer,[17] 2013	PREDICT (substudy of GeparQuinto)	313	ER+/HER2–: 67% TN: 33%	ER+/HER2–: 12% TN: 36.5%	Str-Ly associated with pCR (OR, 1.2; *P*=.01) LPBC associated with pCR (OR, 2.7; *P*=.01)
Lee,[15] 2013	Institutional cohort	175	ER+: 55% HER2+: 38% TN: 19%	N/S	TIL associated with pCR (OR, 1.26; *P*=.024)
Dieci,[23] 2014	Institutional cohort	278	TN: 100%	TN: 10%	iTu-Ly and str-Ly (continuous variable) associated with MFS (HR, 0.85; *P*=.02) and OS (HR, 0.86; *P*=.03)
Denkert,[26] 2014	GeparSixto	580	HER2+: 46% TN: 54%	HER2+: 20% TN: 28%	Str-Ly (continuous variable) associated with pCR (*P*<.001) LPBC associated with pCR (*P*<.001)

ER, estrogen receptor; GeparDuo, German Preoperative Adriamycin Docetaxel Study; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; iTu-Ly, intratumoral lymphocytes; LPBC, lymphocyte-predominant breast cancer, defined as more than 60% of stromal or intratumoral lymphocyte infiltration; MFS, metastasis-free survival; N/S, not specified; OR, odds ratio; OS, overall survival; pCR, pathologic complete response; str-Ly, stromal lymphocytes; TILs, tumor-infiltrative lymphocytes; TN, triple-negative.

Table 2. Overview of Adjuvant Trials on TILs

Reference	Study	N	Subtype	Proportion of LPBC According to Subtype	Outcome
Mohammed,[20] 2012	Institutional cohort	468	ER+: 61% HER2+: 16% TN: N/S	N/S	TILs associated with improved cancer-specific survival (*P*=.001)
West,[13] 2011	Institutional cohort	255	ER–/HER2+: 39% TN: 61%	N/S	High CD3 associated with DFS (HR, 0.25; *P*=.0056)
Loi,[7] 2012	BIG 02-98	2009	ER+/HER2–: 54% HER2+: 15% TN: 13%	ER+/HER2–: 3% HER2+: 11% TN: 11%	In TN group only: iTu-Ly/str-Ly (continuous variable) associated with OS (HR, 0.73; *P*=.035)/(HR, 0.83; *P*=.23) LPBC associated with DFS and OS (HR, 0.3; *P*=.018)/(HR 0.29; *P*=.036)
Adams,[22] 2014	ECOG E2197 and E1199	481	TN: 100%	TN: 4%	str-Ly (continuous variable) correlated with DFS and OS (HR, 0.84; *P*=.005)/(HR, 0.79; *P*=.003)
Loi,[21] 2014	FinHER	934	ER+/HER2–: 63% HER2+: 22% TN: 14%	HER2+: 11%	In TN group: str-Ly (continuous variable) correlated with DDFS (HR, 0.77; *P*=.02) In HER2+ group: str-Ly (continuous variable) correlated with DDFS (*P*=.025)
Perez,[28] 2014	NCCTG-N9831	945	HER2+: 100%	HER2+: 10%	Chemotherapy alone group: str-Ly correlated with RFS (HR, 0.2; *P*=.007) Chemotherapy + trastuzumab group: no correlation between str-Ly and RFS (HR, 1.1; *P*=.87)

BIG, Breast International Group; DDFS, distant disease–free survival; DFS, disease-free survival; ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; FinHER, Finland Herceptin; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; iTu-Ly, intratumoral lymphocytes; LPBC, lymphocyte-predominant breast cancer, defined as more than 50% of stromal or intratumoral lymphocyte infiltration; NCCTG, North Central Cancer Treatment Group; N/S, not specified; OS, overall survival; RFS, relapse-free survival; str-Ly, stromal lymphocytes; TILs, tumor-infiltrative lymphocytes; TN, triple-negative.

Table 3. Overview of Novel Immunotherapy Trials in Breast Cancer, Including Immune Checkpoint Blockade Inhibitors

Reference	Status	Drug	Target	Patients	N	Results
Vonderheid,[108] 2010	Phase 1, completed	Tremelimumab + exemestane	CTLA-4	Metastatic ER+, HER2– BC	26	SD ≥12 weeks in 42%
Brahmer,[122] 2012	Phase 1, completed	BMS-936559	PD-1	Advanced carcinoma	207; 4 patients with BC	No efficacy data for patients with BC
Emens,[112] 2014	Phase 1, completed	MPDL3280A	PD-L1	Metastatic TNBC	9	ORR 33%; 1 CR
Nanda,[111] 2014	Phase 1, completed	MK-3475 (pembrolizumab)	PD-1	Metastatic TNBC	32	ORR 18.5%; 1 CR
NCT00083278	Phase 2, completed	MDX-10	CTLA-4	Metastatic BC	33	Not disclosed
NCT01502591	Phase 1, completed	Ipilimumab + cryoablation	CTLA-4	Early-stage BC before surgery	19	Not disclosed
NCT01792050	Phase 2, recruiting	Indoximod + taxane	IDO	Metastatic ER+, HER2– BC	≈154	
NCT01862900	Phase 1/2, recruiting	Anti-OX40 antibody + stereotactic radiation	OX40	Metastatic BC	≈40	
PANACEA NCT02129556	Phase 1b/2, not yet recruiting	MK-3475 (pembrolizumab)	PD-1	HER2+ BC resistant to trastuzumab	≈46	
BOSTON II NCT02303366	Phase 1, not yet recruiting	MK-3475 + stereotactic ablation	PD-1	Oligometastatic (1-5) BC	≈15	
NCT02309177	Phase 1, not yet recruiting	Nivolumab + nab-paclitaxel + gemcitabine + carboplatin	PD-1	Metastatic pancreatic cancer, NSCLC, and BC	≈138	

BC, breast cancer; CR, complete response; CTLA-4, cytotoxic T-lymphocyte–associated protein 4; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IDO, indoleamine 2,3-dioxygenase; NSCLC, non–small cell lung cancer; ORR, overall response rate; SD, stable disease; PD-1, programmed death 1; PD-L1, programmed death ligand 1; TNBC, triple-negative breast cancer.