



Nick Patten. *Gossamer View*. Oil on panel, 24" × 36".

*Research on immunotherapy for
breast cancer appears promising.*

Immunotherapy Strategies in the Treatment of Breast Cancer

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Background: Advances have been made in the field of cancer immunotherapy in understanding the role of the host immune response in affecting tumor progression and response to various treatments. Through these advances, two novel immunotherapies were introduced in the clinic for melanoma and prostate cancer.

Methods: This review discusses the available data regarding the immune-modulating effects of various treatments and how they can be utilized in the treatment of breast cancer.

Databases including PubMed and Google Scholar were searched using terms including breast cancer, vaccines, and immunotherapy.

Results: There is increasing evidence to support that certain breast tumors are intrinsically more immunogenic than others. Tumors that elicit more potent cytotoxic T-cell responses tend to have a more favorable prognosis and respond better to chemotherapy than less immunogenic tumors. Many standard treatments used in breast cancer rely in part on their immunogenic effects for their success in eradicating disease. Understanding how to use these agents to effectively augment the antitumor immune response may lead to better outcomes. Finally, many new immunomodulatory agents and vaccines that can reverse the immunosuppression caused by established tumors are in development. Combining these novel agents with current therapies may boost their efficacy.

Conclusions: Immunotherapeutic approaches combined with novel sequences of chemotherapies, radiation, vaccines, and immunomodulating agents hold promise for enhancing the treatment of breast cancer.

Introduction

The field of cancer immunotherapy is undergoing a renaissance. Many established treatments used over the years, such as Coley's adjuvant, high-dose interleukin 2 (IL-2), interferon alpha (IFN- α), intravesicular bacille Calmette-Guérin (BCG), and allogeneic stem cell transplants, utilize the immune system to eradicate tumor cells.¹⁻⁴ However, the promise of targeted

therapies developed through advances in molecular oncology has dominated the development pipelines of most pharmaceutical companies. With the recent approvals of sipuleucel-T and ipilimumab, investigators and corporations are beginning to see the fruits of years of intense research in immune regulation and cancer vaccine development.^{5,6} As the field of cancer immunotherapy develops further, new possibilities for improving cure rates will emerge without adding to the already considerable toxicities with current therapies for high-risk patients with breast cancer. This article attempts to synthesize many of these discoveries into a paradigm for integrating immunotherapy into the treatment armamentarium of breast cancer.

Immunogenicity of Breast Tumors

Breast cancer has not been perceived as an immunogenic tumor when compared with diseases such as melanoma and renal cell carcinoma, which have

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long used immunotherapy such as interleukin 2 (IL-2) with some success. The ability to profile many breast cancer tumors on a molecular level has revealed that certain tumors demonstrate a high level of immunoregulatory gene activation.⁷⁻⁹ The pattern of cytokine signaling seems to reflect a consistent trend across different datasets and with different profiling platforms. Patients with those tumors, which elicit a stronger T helper 1 (Th1) cytotoxic T-lymphocyte (CTL) response, have a better prognosis than do those whose tumors skew toward a T helper 2 (Th2) response or trigger a larger influx of tumor-associated macrophages via colony-stimulating factor 1 (CSF-1) secretion.¹⁰ Also, cells with a greater influx of tumor-infiltrating lymphocytes tend to respond better to neoadjuvant chemotherapy compared with less immunogenic tumors.¹¹

These observations suggest that intrinsic properties exist within certain breast tumors that provoke a beneficial CTL response, which synergizes with chemotherapy. Other tumors can manipulate inflammatory pathways to promote metastatic tumor spread. The goal would be to manipulate the cytokine milieu within the tumor microenvironment to trigger a beneficial immune response during neoadjuvant therapy, thereby enhancing pathological complete response rates and reducing metastatic tumor spread.

Immunogenic Effects of Standard Treatments

The success or failure of traditional cytotoxic chemotherapy and targeted agents is traditionally viewed as resulting from the tumor's sensitivity to the perturbation of key signaling or DNA repair pathways. However, many of the current treatments routinely used in breast cancer trigger immunologic changes within the tumor microenvironment. Emerging data suggest that these changes are not merely a bystander effect but contribute directly to the efficacy of these treatments.

Extensive research into the ability of certain chemotherapeutic agents such as anthracyclines and platinum salts to induce "immunogenic cell death" has been published by Zitvogel et al¹² at the Institut Gustave Roussy. These agents can trigger the release of inflammatory "danger signals" within the tumor such as high-mobility group box 1 (HMGB1) and adenosine triphosphate (ATP). These signals initiate a cascade of immune activation through IL-1 β secretion and activation of Toll ligand receptor 4 (TLR4) on infiltrating dendritic cells. In addition, tumor cells undergoing disruption of their endoplasmic reticulum express calreticulin on their cell surface. Calreticulin serves as a phagocytic signal for dendritic cells to take up apoptotic bodies and process associated tumor antigens for presentation.

The neoadjuvant administration of taxanes in locally advanced breast cancer was shown to increase the levels of tumor-infiltrating lymphocytes within the tumor parenchyma.¹³ In the metastatic setting, docetaxel also has been shown to increase levels of

Th1-associated cytokines (IL-2, IFN- γ) while decreasing negative inflammatory markers such as tumor necrosis factor beta (TNF- β).¹⁴ Alkylating agents such as cyclophosphamide, especially when given at lower metronomic doses, can deplete immunosuppressive T-regulatory cells, which promote anergy toward tumor-associated antigens.¹²

The dosing and schedule of chemotherapy also help to maximize the effects of vaccines or immunotherapy when given concurrently. Emens et al¹⁵ demonstrated that optimal vaccine responses were obtained when doses of cyclophosphamide at 200 mg/m² and doxorubicin at 35 mg/m² were used during vaccination. It is likely that lower doses of chemotherapeutic agents given at more frequent intervals are an ideal schedule if they are combined with immunotherapy.

Trastuzumab given to HER2-amplified breast cancer patients not only downregulates signaling through HER2 heterodimer-activated pathways, but also activates antibody-dependent cytotoxic cellular (ADCC) killing of HER2-overexpressing cells via natural killer (NK) cell activation.^{16,17} The importance of ADCC in the outcomes of patients with metastatic breast cancer treated with taxanes plus concurrent trastuzumab was highlighted by Musolino et al,¹⁸ when they analyzed polymorphisms in the immunoglobulin G fragment C- γ (IgG Fc γ) receptor genes of patients. This receptor on NK cells docks with the IgG Fc portion of trastuzumab and activates NK cells to kill tumor cells. They found that patients with specific IgG Fc γ polymorphisms had better outcomes with trastuzumab, presumably because their IgG Fc γ receptors resulted in superior NK cell antitumor activity. These data provide an immunotherapeutic rationale for concurrent chemotherapy with monoclonal antibodies. They also provide an interesting predictive biomarker for this approach as well. Since the data from the North Central Cancer Treatment Group (NCCTG) N9831 trial already demonstrated the superiority of concurrent over sequential trastuzumab with chemotherapy,¹⁹ this approach is easy to incorporate into clinical practice.

Another approach, which was pioneered by the work of Disis et al,²⁰ demonstrated that trastuzumab concurrently administered with peptide-based HER2 vaccines may result in more potent immune activation. In a group of 22 patients with HER2-positive metastatic breast cancer, the median survival had not been reached for the group at a median follow-up of 36 months. This idea could be extended to vaccination of patients with HER2-positive breast cancer in the adjuvant setting during trastuzumab monotherapy.

These effects are not limited to systemic agents. There is mounting evidence that radiation therapy can be employed in a combination approach using certain fractionation and dosing schedules to elicit immunogenic changes within the treated tumor. They include the upregulation of major histocompatibility complex (MHC) class I expression, release of chemokine (C-X-C motif) ligand 16 (CXCL16), and presentation of radia-

tion-induced tumor epitopes.^{21,22} The combination of radiation and a cytotoxic leukocyte antigen 4 (CTLA-4) agonist can trigger a potent enough immune response that in some cases may shrink other metastatic tumors outside of the radiation field.²³ Exploiting this abscopal effect by using the radiated primary tumor as an antigen source to provoke a potent systemic antitumor response augmented by chemotherapy and immunomodulators would be an intriguing treatment approach for neoadjuvant or de novo metastatic breast cancer patients.

Advantages of Immunotherapy

The use of immunotherapy has some key advantages in the treatment of breast cancer over traditional chemotherapies and molecularly targeted agents. Targeted therapies depend on the cell's oncogenic dependency on specific pathways. However, it is known that many cells can activate compensatory signaling to make them resistant to these therapies.²⁴ Efforts are underway to combine multiple receptor tyrosine kinase inhibitors to prevent this emergence of resistance, but with these combinations come greater toxicities, which may limit their feasibility. In contrast, the ability of immunotherapy to target and eradicate micrometastatic disease is dependent on tumor cells' protein expression. This makes it difficult for individual cells to overcome a potent immune response. Immune evasion can occur at a population level, with some cells that downregulate the expression of targets or MHCs escaping elimination. Unlike the additional toxicity that is seen when multiple tyrosine kinase inhibitors are combined, additional epitopes can be easily included in cancer vaccines to reduce the chance of immune evasion.

Another advantage with some immunotherapies such as vaccines is that they can be given over much longer periods in the adjuvant setting. Delayed tumor recurrence after standard adjuvant therapy is a significant problem, especially in patients with hormone receptor-positive disease. An activated immune response maintained through booster vaccines can patrol the body for residual disease over an extended period without significant toxicity to the patient or a lengthy time commitment. The initial results of the HER2 E75 peptide vaccine studies demonstrated initial protection over the first few years, but this protection waned as the immune response declined. This finding led the investigators to incorporate a booster schedule in the ongoing phase III E75 adjuvant trial.²⁵

Breast Cancer Vaccines

Many of the various types of vaccines employed in cancer immunotherapy clinical trials have been previously reviewed in detail.²⁶ The main purpose of this discussion is to outline how breast cancer vaccines can be optimally used in a multimodality regimen. In general, the greatest benefit of these vaccines appears to be in the minimally residual disease state settings

in patients who have not been heavily pretreated. The reason for this is the typical numbers of antigen-specific cytotoxic T cells that are activated as a result of a vaccination schedule represent a small subset of the total cell population. Vaccines targeting micrometastatic disease in the adjuvant setting even the odds by targeting a fewer number of isolated tumor cells before they have a chance to establish larger tumors with supporting stromal elements.

In addition, vaccine trial designs should target higher risk patients such as those with luminal B, triple, N2-3 disease or those with triple-negative/HER2-positive residual disease after neoadjuvant therapy. Since adjuvant studies are event-driven, looking at tumor recurrence and death rates over time, patients with a good prognosis are unlikely to demonstrate a meaningful improvement with a vaccine unless the sample size is large.

Data from Sharma et al²⁷ suggest that HER2-directed dendritic cells administered to patients with ductal carcinoma in situ (DCIS) preoperatively exhibited excellent activity, with a pathological complete response rate of 18.5%. This finding suggests that eradication of breast cancer by the immune system may be more successful early on in the natural history of the disease. Although this approach is not likely to impact the already excellent survival of patients with DCIS, an optimized breast cancer vaccine could be used in place of adjuvant radiation to prevent local tumor recurrences after lumpectomy.

A Pilot Study of MUC-1/CEA/TRICOM Poxviral-Based Vaccine

In the metastatic setting, the objective activity of vaccine treatments is low due to the sheer tumor burden and underlying immunosuppression caused by the tumors. In a recently reported pilot study of MUC-1/CEA/TRICOM poxviral-based vaccine, only 1 of 12 patients with breast cancer had an objective complete response, whereas all of the others progressed quickly.²⁸ The sole responder also had minimal disease in 1 lymph node and was not as heavily pretreated as the other patients. Cancer vaccine monotherapy in patients with heavy tumor burdens or many prior chemotherapies is not likely to produce significant clinical benefit. However, vaccines may result in slower progression of disease or enhanced sensitivity to subsequent lines of salvage chemotherapy in this setting. This effect was seen particularly in a phase II trial utilizing the Ad-p53 dendritic cell vaccine in patients with small cell lung cancer treated with first-line platinum salt/etoposide.²⁹ The typical response rate to salvage therapy is approximately 10%; however, Ad-p53 responders had a 61.9% response rate to salvage paclitaxel.²⁹

These results set up a clinical trial scenario where patients with metastatic breast cancer could receive a brief priming course of a relevant cancer vaccine and then proceed to additional treatment with lower-inten-

sity weekly or metronomic chemotherapy treatments. If patients respond and do well with the treatment, they can receive booster shots during scheduled interruptions in therapy used in a “stop-and-go” fashion. The endpoint of these studies can include response rates between vaccinated and nonvaccinated patients and should be powered to detect a significant difference in progression-free or overall survival. Neoadjuvant trials could exploit the chemosensitization effect by giving a brief priming sequence of an effective breast cancer vaccine before starting chemotherapy to improve pathological response rates.

Use of Immunomodulators in Breast Cancer

The current understanding of the interaction among tumor cells, immune cells, and the tumor microenvironment has expanded greatly over the past several years. One major limiting factor in the efficacy of prior attempts at immunotherapy is the immunosuppressive environment created by the tumor cells and associated stromal elements.³⁰⁻³³ Multiple mechanisms are employed by tumor cells in preventing cytotoxic T-cell activity and facilitating metastatic tumor spread. Abnormal catabolism of amino acids such as tryptophan by indoleamine 2,3 dioxygenase (IDO) is a key pathway in causing T-cell dysfunction in cancer.³⁴ Preclinical data have shown that the use of inhibitors of IDO such as 1-methyl-tryptophan can synergize with chemotherapy in killing tumors.³⁵ The trials utilizing this agent in conjunction with vaccines and docetaxel are ongoing, and other IDO inhibitors are moving forward in clinical development.

Another pathway gaining interest is CSF-1 and its role in attracting tumor-associated macrophages (TAMs). Data published by DeNardo et al¹⁰ demonstrated that TAMs were integral in the metastatic progression of tumors in MMTV-PyMT mouse models. Furthermore, blockade of CSF-1 not only inhibited the formation of metastases in these mice but also synergized with paclitaxel-based chemotherapy in killing established tumors. These data have led to the initiation of phase I/II clinical trials with a small molecule inhibitor of CSF-1 (PLX3397) in patients with metastatic breast cancer. Additional data point to programmed death 1 (PD-1/PD-L1), CTLA-4, and Toll ligand receptor pathways as other targets of interest in breast cancer immunotherapy.³⁶⁻³⁸ The challenge will be to find appropriate settings and dose schedules for these agents to maximize their benefits and minimize the risks of autoimmune-related long-term toxicities, especially in the adjuvant setting.

Conclusions

We are just beginning to understand the complex interplay among the host immune response, tumor cells, tumor microenvironment, and effects of various treatments on these elements. Optimization of the sequence and dosing of the various possible treatment modalities is important to maximally leverage the ben-

efit from the antitumor response. Also, molecular and cytokine profiling to characterize the immune activation status of tumors may be used to select the appropriate immunotherapeutic agents required to elicit the most effective antitumor response.

In the metastatic setting, the use of a vaccine priming schedule with either an immune stimulator or metronomic cyclophosphamide can be used prior to additional chemotherapy as a sensitizer or as maintenance therapy for low-bulk/indolent disease. For bulkier or rapidly progressing disease, weekly chemotherapy combined with antibody-dependent cytotoxic cellular (ADCC)-inducing monoclonal antibodies such as trastuzumab and/or immunomodulators would be a reasonable approach. In the neoadjuvant setting, a combination of vaccinations, systemic/intratumorally administered immunomodulators, or a few fractions of targeted radiation given immediately prior to weekly paclitaxel may trigger sufficient immune activation to enhance pathological complete response rates. In the adjuvant setting, micrometastatic disease can be targeted using optimized vaccines given over a prolonged period of 3 to 5 years for cohorts at high risk of tumor recurrence.

These various scenarios need to be explored in relevant preclinical models and translated into clinical trials when possible. It is clear that cancer immunotherapy will become a more dominant force in the treatment of a wide variety of cancers. Breast cancer should be no exception to this trend, and research focused on harnessing the immune response is needed for the sake of our patients.

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