Developing an Effective Breast Cancer Vaccine

Hatem Soliman, MD

**Background:** Harnessing the immune response in treating breast cancer would potentially offer a less toxic, more targeted approach to eradicating residual disease. Breast cancer vaccines are being developed to effectively train cytotoxic T cells to recognize and kill transformed cells while sparing normal ones. However, achieving this goal has been problematic due to the ability of established cancers to suppress and evade the immune response.

**Methods:** A review of the literature on vaccines and breast cancer treatment was conducted, specifically addressing strategies currently available, as well as appropriate settings, paradigms for vaccine development and response monitoring, and challenges with immunosuppression.

**Results:** Multiple issues need to be addressed in order to optimize the benefits offered by breast cancer vaccines. Primary issues include the following: (1) cancer vaccines will likely work better in a minimal residual disease state, (2) clinical trial design for immunotherapy should incorporate recommendations from expert groups such as the Cancer Vaccine Working Group and use standardized immune response measurements, (3) the presently available cancer vaccine approaches, including dendritic cell-based, tumor-associated antigen peptide-based, and whole cell-based, have various pros and cons, (4) to date, no one approach has been shown to be superior to another, and (5) vaccines will need to be combined with immunomodulatory agents to overcome tumor-related immunosuppression.

**Conclusions:** Combining a properly optimized cancer vaccine with novel immunomodulating agents that overcome tumor-related immunosuppression in a well-designed clinical trial offers the best hope for developing an effective breast cancer vaccine strategy.

**Introduction**

The notion of cancer immunotherapy is hardly new. Associations between tumor regressions and infections date back to the 1700s. In 1893, Coley reported injecting sarcomas with Gram-positive bacteria to induce a local inflammatory response and subsequent tumor regression. Subsequent breast cancer chemoimmunotherapy trials performed at the M.D. Anderson Cancer Center in the 1970s combined bacillus Calmette-Guérin (BCG) injections with 5-fluorouracil-Adriamycin-cyclophosphamide (FAC) chemotherapy. Responding patients who received BCG injections plus FAC had longer periods of progression-free and overall survival than those treated with FAC alone. The field has advanced considerably since then, with an increased understanding of candidate antigens, adjuvants, tumor-related immunosuppression pathways, and techniques. However, this improved understanding has yet to translate into a US Food and Drug Administration (FDA)-approved cancer vaccine for breast cancer.

While breast cancer cure rates with current multimodality therapy have improved, an estimated 20% to 30% of patients will have a recurrence of their disease.
A safe, effective breast cancer vaccine strategy may reduce this recurrence risk significantly and prevent more costly palliative treatments later on. Several reviews have discussed the scientific rationale and data behind specific breast cancer vaccines. There are many promising candidates, but the main challenges will be to properly select the vaccines that offer the greatest clinical benefit and to implement these treatments on a large scale. This article provides an overview of these considerations and attempts to put them into perspective.

**Appropriate Setting for Use**

The issues surrounding the ideal setting for the use of breast cancer vaccines are complicated, not because of a lack of understanding of how immunotherapy works, but because of the various practical issues in demonstrating a clinical benefit in these different settings. The possible risk vs benefit of a particular proposed treatment may mandate that the initial trials be performed in metastatic patients with poorer prognosis. This would be the case particularly if a novel immunomodulating agent with an unknown or a significant toxicity profile is combined with a breast cancer vaccine for the first time. When compared to traditional cytotoxic agents, immunotherapy usually takes longer to exert its effect, responses are variable, and the immunotherapy probably works better in patients with a lower disease burden. A lower disease burden gives the immune system time to mount a response before being overtaken, and it addresses the immunosuppressive environment seen in advanced cancer states. The well-established role of the graft-vs-leukemia effect after allogeneic stem cell transplantation provides ample evidence for this principle.

While a cancer vaccine may be less effective in the metastatic setting, it may modulate the disease in a way that renders it more susceptible to subsequent chemotherapy and/or radiation treatments. This was observed in a phase II trial for small-cell lung cancer using a p53 dendritic cell (DC) vaccine in patients who completed initial chemotherapy. While the response rate to the vaccine was typically low (5%), all patients were treated with paclitaxel in the second line. The response rate was 50%, much higher than the typical 10% rate observed in second-line treatment. In another study using CYP1B1 vaccine, Gribben et al observed similar enhanced chemosensitivity to subsequent salvage chemotherapy in metastatic cancer patients who developed an immune response to the vaccine. It remains to be seen if this can be demonstrated in breast cancer patients undergoing similar treatment. Cancer vaccines tend to exhibit lower toxicity profiles than targeted/cytotoxic agents generate, making vaccines ideal maintenance treatments. These properties seem to indicate that the greatest promise of breast cancer vaccines would be in the adjuvant or minimal residual disease state.

Since adjuvant trials in breast cancer generally require large sample sizes and long follow-up for recurrence and survival, the initial funding of these studies may be cost-prohibitive. However, from a public health perspective, an effective adjuvant vaccine could help stem the larger societal costs of the expensive and toxic palliative treatments administered to patients with distant recurrences. Compared with hormone receptor-positive disease, certain subsets of breast cancer such as triple-negative disease may provide a population with shorter recurrence times and higher risk of recurrence. Targeting a study population with sufficient risk of recurrence may maximize the odds that the vaccine will demonstrate a clinical benefit while potentially limiting the sample size required to do so. A possible consideration could be including enough eligible patients with a certain recurrence risk cutoff after standard adjuvant therapy (eg, > 20%) based on testing with Adjuvant! Online (http://www.adjuvantonline.com) or Oncotype DX (Genomic Health Inc, Redwood City, CA). If benefit is seen in patients with more aggressive disease expressing the immune target, it is likely that patients with more indolent disease expressing the same target would also benefit.

**Paradigms for Clinical Vaccine Development**

Since the clinical effect of cancer vaccines differs from that shown with cytotoxic agents, the clinical development paradigm for vaccines should also be different. Concepts such as a maximally tolerated dose or response rates measured by traditional response evaluation criteria in solid tumors (RECIST) criteria may not apply to early-phase cancer vaccine trials. Also, heterogeneity in immunologic monitoring tests makes it difficult to standardize definitions of immunologic efficacy. There has been a significant effort to define pathways and harmonized methods for translational cancer immunotherapy. The Translational Research Working Group published a recommended pathway incorporating various milestones in a flowchart algorithm for translating potential immunologic therapeutics from the bench to its use in clinical trials. For vaccines that are ready for clinical testing, a useful guide for appropriate clinical trial design was published in 2007 by the Cancer Vaccine Clinical Trial Working Group. Rather than performing the usual phase I-III trials, recommendations include carrying out proof-of-principle trials followed by efficacy trials for promising candidates. A proof-of-principle trial would enroll approximately 20 to 30 patients to allow for safety, limited dose ranging, and immunologic efficacy testing. Efficacy trials should be randomized trials that can be a hybrid of a phase II/III trial with well-defined time-to-event endpoints such as overall, disease-free, and progression-free survival. When using progression-free survival as an endpoint, patients with minor progression (ie, < 50% increase in overall tumor burden or < 3 new lesions) at their first evaluation time point should be allowed to continue to the next evaluation time point. This is because patients treated with cancer vaccines may show early progression followed by a delayed response to treatment. Response patterns to ipilimumab, the anti-cytotoxic T lymphocyte antigen 4 (CTLA-4) monoclonal antibody, have been described as immedi-
Vaccination Strategies

Although additional vaccine strategies are in various stages of development,17,18 this section discusses four main approaches. Each strategy carries with it a set of pros and cons that bear consideration (Table). Although there are proponents for one strategy vs another, no one approach has been shown to be superior to others in all situations. In general, a vaccine strategy is composed of a method to deliver target antigens or epitopes to the immune system and an immune-stimulating adjuvant to trigger an immune response. These various aspects of a cancer vaccine strategy and some of the related immune pathways involved are illustrated in the Figure.

**Dendritic Cell Vaccines**

DCs are the body’s most effective antigen-presenting cells (APCs). The purpose of DCs is to process cancer antigens and present pieces of these antigens bound to major histocompatibility complexes to receptive effector T cells. The DCs are collected from a patient by leukapheresis and then manipulated ex vivo to activate them with the desired tumor antigens. The activated DCs are then reintroduced into the patient, and they can go on to activate effector T cells. The DC vaccine closest to FDA approval is sipuleucel-T (Provenge, Dendreon Corp, Seattle, WA) prostate vaccine.19 The cellular immunotherapy process employed by Dendreon Corp collects the patient’s DCs using leukapheresis. The patient’s APCs then undergo ex vivo stimulation with PA2024, a fusion protein consisting of full-length human prostate acid phosphatase and full-length human granulocyte-macrophage colony-stimulating factor (GM-CSF). The stimulated APCs are then returned to the patient and infused to stimulate the effector T cells to mount an antitumor response. This is also the method for the HER2-based breast cancer vaccine lapuleucel-T (Neuvenge, Dendreon Corp), which is currently in early clinical trials, although a HER2 fusion protein is used instead.20

Another DC vaccine approach being explored in breast cancer uses leukapheresed APCs transfected with a replication defective adenoviral vector loaded with the wild-type p53 transcript under the control of a cytomegalovirus promoter (contusugene ladenovec; INGN-201).21 The viral infection triggers the activation of the DCs and, because p53 is made in the DC cells, the p53 is processed and presented on the surface. These DCs can trigger p53-specific effector T-cell responses against cancer cells with abnormally accumulated mutant p53. The advantage of this process is that, since DC vaccines use autologous cells that are primed ex vivo, the vaccine would be immunologically compatible with any patient who undergoes the process. Also, the ex vivo priming allows potentially better activation of DCs when removed from a cancer patient’s immunosuppressive milieu.

When designing a clinical trial for a DC vaccine, many technical considerations need to be considered, such as appropriate APC selection, optimized ex vivo APC maturation protocols, and methods to optimize APC homing to draining lymph nodes.22,23 A major dis-
The advantage of the DC approach is the need for leukapheresis facilities at the treatment center to generate the product. This may necessitate insertion of large-bore indwelling catheters in some cases, and leukapheresis carries with it the risks of hypotension, electrolyte derangements, and vascular injury. Another issue with DC vaccines is the difficulty in ensuring a consistent vaccine product due to varying leukapheresis yields, the number of activated APCs, and possible bacterial contamination issues. The logistical complexity of administering DC vaccines on a larger commercial scale may make it less viable, especially if a less complex vaccine with equal efficacy becomes available. However, advantages offered by DC vaccines may justify the inconveniences.

**Viral-Based Vaccination Vectors**

Viral-based vaccination vectors represent an active field of investigation in cancer immunotherapy. Selection of viral vectors makes use of their natural ability to trigger immune responses and carry genetic material into cells for production of the target antigen. The antigens are processed intracellularly by APCs and are presented on major histocompatibility complexes to receptive effector T cells. One ongoing trial in metastatic breast cancer utilizes the vaccinia and fowlpox viruses loaded with a carcinoembryonic antigen (CEA) peptide and three costimulatory molecules (B7.1, ICAM-1, LFA-3), collectively designated TRICOM. The TRICOM molecules serve to augment the activation signal that an effector T cell receives from the APCs that take up the virus, leading to a more robust immunologic response against CEA. The vaccinia virus is used for the initial vaccinations, and the fowlpox virus is used for booster vaccines. This is because the body mounts an immune response to the vaccinia vector after the initial vaccines so another strain must be used for the subsequent booster shots. In a completed phase I trial, 40% of patients had stable disease and 1 patient developed a pathologic complete response.

An ongoing trial at the National Cancer Institute is studying the use of the CEA-TRICOM vaccine in conjunction with an intense chemotherapy regimen in patients with metastatic CEA-expressing breast cancer. The scalability of this approach is easier than that of DC vaccines as the viral vectors and peptide can be consistently mass-produced. However, a significant infrastructure is still required. One drawback of using CEA alone is a common one to any single epitope vaccine strategy: the potential for immune escape. Clones that

---

**Figure.** — An illustration of some aspects involved in optimizing a breast cancer vaccine. The method of antigen delivery (eg, whole cell, lysate, DNA, pulsed DCs) is selected based on a variety of factors, such as the number of antigens selected for targeting, the feasibility of the approach, and immunologic compatibility. Equally important is enhancing APC activity through the use of vaccine adjuvants (GM-CSF, CpG) and combining the vaccine with costimulatory agents and/or immunomodulators that can abrogate inhibitory pathways (CTLA-4, 1-MT). ABH = 2(S)-amino-6-boronohexanoic acid, APC = antigen-presenting cell, CTL = cytotoxic T cell, DC = dendritic cell, IDO = indoleamine 2,3-dioxygenase, M = major histocompatibility complex, P = peptide, T = T-cell receptor.
express less CEA can escape the selective pressure of CEA reactive T cells. The PANVAC-VF uses the same fowlpox and vaccinia TRICOM technology but includes both CEA and MUC1 peptides, making it more difficult for immune escape to occur. Therion Biologics, which was the main commercial partner with the Cancer Therapy Evaluation Program (CTEP), recently ceased operations, so the commercial development plan for this vaccine is uncertain.

**Peptide-Based Vaccines**

Peptide-based vaccines are based on the premise that cancer cells express certain tumor-associated antigens (TAAs) that are either absent or expressed at low levels in normal tissues. The prevalent TAAs that have been targeted in breast cancer involve the HER2 extracellular domain, CEA, and MUC-1. Current manufacturing techniques allow production of large amounts of highly purified, pharmaceutical-grade peptides for use on a larger scale. The peptides consist of specific amino acid sequences from these TAAs selected on the basis of their immunogenicity and their compatibility with specific human leukocyte antigen (HLA) receptor subtypes. Since different HLA subtypes can present different pieces of TAA proteins, it is important to select these peptides to match the target population (eg, HLA-A2+ patients). This HLA restriction limits the potential number of patients who can receive these vaccines, thus making it necessary to screen a larger pool of patients to yield the desired study population size. These peptides are generally not very immunogenic on their own and require additional measures to stimulate an adequate response. Some strategies to augment the immune response include using a potent APC-stimulating adjuvant such as GM-CSF or CpG oligonucleotides, utilizing computer algorithms to select the most immunogenic epitopes (parts of the protein that are targeted by the immune response), and simultaneously activating both cytotoxic T cells (CTLs) and helper T lymphocytes (HTLs). This approach is based on the fact that different epitopes of a TAA activate either CTLs or HTLs; therefore, creating fusion peptides that combine a CTL- and HTL-activating portion may offer enhanced and more prolonged immune responses. Another factor to consider is that such specifically targeted vaccines make it easier for tumors to evade the immune response as surviving clones downregulate expression of the target antigen. To make this immune evasion more difficult, multiple peptides from different TAAs can be used in the vaccine.

The clinical trials performed by Peoples et al using an HER2-based CTL-activating peptide E75 plus GM-CSF showed promising reductions in recurrence. For 171 enrolled patients, the recurrence rate in the vaccinated group was 5.6% compared with 14.2% in the observation group (P = .04) at a median follow-up of 20 months. The disease-free survival rates in the vaccinated and control groups were 92.5% and 77%, respectively.

These data provide proof of principle that cancer vaccines used in the minimally residual disease state can reduce recurrences. However, whether this reduction in recurrence is due to the E75 vaccine, the GM-CSF adjuvant, or both remains unclear. This is because the disease burden and cancer-related immunosuppression are low in the adjuvant setting. A significant finding was that only 48% of the vaccinated patients in the above-mentioned trial had positive dimer responses at 6 months, indicating a lack of durability in the CTL response to the E75 HER2 vaccine. A different HER2 epitope, AE37, which stimulates HTLs instead of CTLs, is currently being tested for its ability to elicit protective anti-HER2 responses in a clinical trial. To prolong the protective immune response, it is likely that both CTLs and HTLs need to be stimulated simultaneously and booster vaccines will be required in an efficacy trial.

Several advantages are associated with peptide vaccines: target antigens can be targeted with more specificity, large amounts of pharmacologic grade peptides can be produced easily, patients do not need to undergo any additional procedures, and the peptides are not pharmacologically active and so have few toxicities, if any. A key disadvantage of peptides is immunologic compatibility only in patients with specific HLA subtypes (ie, HLA-A2+ patients), depending on the epitope chosen. This can make accrual to a trial more difficult, as a significant portion of patients may not be included due to HLA incompatibility. Some epitopes, such as E75, demonstrate the ability to bind to multiple HLA subtypes, thereby increasing the number of patients who may be treated with that specific peptide. Also, since only one or two antigens are targeted by peptide vaccines, the risk of immune escape by tumor cells is higher as resistant clones with lower expression of those antigens can emerge. Additional peptides can be added, but the cost and complexity of the vaccine will increase substantially. Overall, properly designed peptide vaccines paired with active adjuvants provide an easily scalable, effective method to vaccinate large numbers of HLA-compatible patients.

**Whole-Cell Vaccines**

Whole-cell vaccines are a fourth method for providing target antigens. Some vaccines can be from autologous cells prepared from a patient’s own tumor samples or allogeneic cancer cell lines. Since tumor cells are not very immunogenic, either the cancer cells or other bystander cells included in the vaccine are transfected with vectors containing genes that express potent immunostimulating proteins or cytokines. These immunostimulating proteins include B7.1 (CD80) and, more recently, CCL21. APCs express B7.1, and this binds CD28 on effector T cells, leading to their activation. The chemotaxis signal provided by the cytokine CCL21 allows the recruitment of naive T cells to an injection site, in essence forming an outsourced lymph node. Additional compounds such as GM-CSF can be added as an adjuvant to stimulate APCs. A hybrid approach that has been clinically evaluated in metastatic breast cancer has involved fusing patients’ tumor...
cells to their own leukapheresed DCs ex vivo and then reinjecting the fusion cells into the patients.40 The specific allogeneic cancer cell lines for the desired tumor type are selected on the basis of many factors. Some of the criteria involve the effectiveness of growing cells in culture, the expression of desired antigens, its availability as a clinical grade cell line, and patent issues. The advantages of using whole cells is that a broad array of TAAs is represented, thereby minimizing immune escape; thus, because the whole proteins are present, there are no HLA restrictions on who can receive the vaccine. An example of this approach in breast cancer involved the use of MDA-MB-231 breast cancer cells lipofected with cDNA expressing the costimulatory molecule CD80 and administered with GM-CSF in 30 women with metastatic breast cancer.58 The results of this trial were disappointing, with only 6 patients developing antibodies to HER2 and MUC-1 antigens expressed on the cancer cells. No clinical responses were noted, but this is often the case in patients with advanced disease. This approach is currently being scaled up for larger-scale production using complex cell line banking facilities to generate large batches of desired cell lines for production. However, it remains to be seen what combination of cell lines, cell modifications to express costimulatory molecules, and adjuvant compounds provides the most effective approach.

**Overcoming Tumor-Related Immunosuppression**

To allow tumor vaccines to deliver greater clinical benefits, especially in the metastatic setting, overcoming tumor-related immunosuppression is of paramount importance. Stimulated effector T cells may be appropriately primed to attack a TAA outside of the tumor microenvironment, but the tumor often renders these infiltrating cells inert once they arrive to attack the malignant cells.41,42 Developing strategies to overcome these immunosuppressive mechanisms and combining them with vaccines may allow these stimulated effector T cells to attack malignant cells with greater efficiency.

Alteration of amino acid metabolism appears to be one of the important methods by which tumors induce immunosuppression both systemically and in the tumor microenvironment. One such area of active clinical investigation is the effect of tryptophan metabolism through overexpression of the enzyme indoleamine 2,3-dioxygenase (IDO) by tumors. Certain events in tumor development, such as mutations in the tumor repressor Bin1, lead to IDO overexpression when tumor cells are exposed to cytokines such as interferon gamma.43 Increased IDO activity within the tumor microenvironment and draining lymph nodes causes local tryptophan depletion and production of catabolites such as kynurenine, which is toxic to infiltrating effector T cells and causes them to become anergic and die.44-46 Also, naïve T cells exposed to IDO expressing immature DCs become T regulatory cells that promote systemic anergy toward TAAs. The phase I solid tumor trial for a novel IDO inhibitor, 1-methyl-D-tryptophan (1-MT) is ongoing. However, data have shown that IDO inhibition can augment the response to DC vaccines in mice and synergize with chemotherapy agents such as taxanes, anthracyclines, and cyclophosphamide in regressing mouse MMTV-Neu breast tumors.45 Current trials are combining 1-MT with a DC-based p53 vaccine and also with taxane chemotherapy to test these combinations in patients with metastatic breast cancer.

A similar enzyme called arginase exerts similar immunosuppressive effects through a breakdown of L-arginine to ornithine. This process promotes tumor growth and causes decreased effector T-cell activity, as evidenced by downregulation of CD3-zeta expression.47,48 Agents that block arginase activity, such as 2(S)-amino-6-boronohexanoic acid, are currently in the early development stage for various conditions (eg, pulmonary arterial hypertension) and as cancer immunotherapy agents.49

The blockade of inhibitory signals that help to regulate the immune response has also been the subject of much research in various malignancies. CTLA-4 has been targeted using monoclonal antibodies such as ipilimumab and tremelimumab to block its inhibitory signal toward effector T cells. This approach has not been extensively clinically evaluated in breast cancer, but preclinical murine models suggest synergy with radiation.50,51 B7-H1 is another potential inhibitory signal expressed by APCs infiltrating high-risk breast cancer types such as high Ki-67 and triple-negative subtypes. This signal binds to programmed death-1 (PD-1) on the surface of tumor-infiltrating lymphocytes, causing them to become anergic and undergo apoptosis.52,53 Blockade of PD-1 using monoclonal antibodies currently in clinical development may allow tumor infiltrating lymphocytes to more efficiently kill tumor cells when stimulated with an appropriate target.

A comprehensive review of the various other immunosuppressive pathways involved is beyond the scope of this article. The main point is that pairing cancer vaccines with these immunomodulating agents will probably be required in order to improve the modest clinical efficacy observed so far in immunotherapy trials. Successfully testing these combinations can be challenging for several reasons. At a 2007 National Cancer Institute workshop on immunotherapy, the number of possible different combinations of cancer vaccines, adjuvants, and CTEP immunomodulating agents was calculated to be over 2,000.54,55 Finding the combination that yields the maximal efficacy in a given disease is a daunting task. Another challenge will be determining which immunomodulators can be combined with vaccines without resulting in excessive toxicity from autoimmune events such as severe colitis. Finally, overcoming the myriad of regulatory/intellectual property hurdles involved with combining multiple investigatory agents is not a trivial task. A concerted collaborative effort by the NCI, pharmaceutical companies, and regulators is required to prioritize combination immunotherapy strategies and facilitate their development.
Conclusions
Cancer vaccines have been fraught with many failures but have had a few recent successes. In order for this research to remain viable, major progress must be made to improve clinical outcomes in cancer immunotherapy. While there are no clear winners among the various strategies outlined, multiple approaches may be needed to provide a breast cancer vaccine to the majority of women due to differences in immunologic compatibility. The ultimate goal of breast cancer vaccines should be to reduce the risk of recurrence from minimally residual disease in patients with no evidence of disease following or in combination with endocrine therapy, chemotherapy, radiotherapy, and novel immunomodulators. While breast cancer vaccines may provide additional palliative benefits to patients with metastatic disease, this approach will likely need to be combined with chemotherapy and immunomodulators to improve outcomes significantly. Proper clinical trial design geared specifically toward cancer immunotherapy with rigorous immune monitoring methods and appropriate endpoints is essential in developing novel breast cancer vaccines. With intense collaboration, we may someday be able to stimulate the immune system's response to target specific cells as a means to cure breast cancer with improved precision, less toxicity to healthy cells, and minimal side effects.

Appreciation is expressed to Scott J. Antonia, MD, PhD, of Moffitt Cancer Center, to Howard Strehler, MD, of the National Cancer Institute’s Cancer Therapy Evaluation Program, and to Nicholas Vahanian, MD, of Neutlink Genetics Inc, for their input.

References
ondary lymphoid tissue chemokine in human dendritic cells augments chemotactic activities for lymphocytes and antigen presenting cells. Mol Cancer. 2003;2:35.


