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STATE OF THE ART

Breast Cancer Immunotherapy

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ABSTRACT

Although unlikely to replace current standard of care therapies, immunotherapy is emerging as a critical component of breast cancer treatment. Despite initial setbacks, clinical benefit is now evident through immunomodulation and cancer vaccines. Over the past decade, passive immunotherapeutic strategies such as anti-HER2 monoclonal antibody (mAb) therapy have significantly improved the prognosis in HER2 overexpressing breast cancers. Novel active immunotherapeutic strategies include checkpoint blockade modifiers, also a mAb therapy. Although non-specific, checkpoint blockade modifiers show great promise in clinical trials. A form of active and specific immunotherapy, cancer vaccines may be used alone or in conjunction with these aforementioned mAb therapies. While there is significant initial promise, the complexities of the host immune system-tumor interaction and the vast array of potential immune targets require the field of cancer immunotherapy to be further developed. Here, we briefly discuss the field of breast immunotherapy to date and its implications for the future of breast cancer care.

INTRODUCTION

Cancer immunotherapy has emerged as an important treatment modality in addition to surgery, chemotherapy, radiotherapy, and hormonal treatment. Whereas the expectations for improvement in survival advantage and toxicity reduction of current chemotherapies are relatively modest, immunotherapy holds substantial potential as a non-toxic, sustainable therapeutic option personalized to patients' specific tumor characteristics. As a result, most funding and academic interest over the past decade has been dedicated to targeted therapies. The clinical success of Herceptin (Genentech, Inc., San Francisco, CA), or trastuzumab, in metastatic breast cancer was largely instrumental in creating this enthusiasm (1). More recent advances in molecular oncology and immunotherapeutics include the recent FDA approvals of sipuleucel-T and ipilimumab (2,3).

It is well known that tumor cells activate compensatory signaling to make them resistant to particular therapies. The allure of immunotherapy is the ability to target and eliminate micrometastases based on the tumor's specific protein expression and with minimal toxicity. The addition of immunotherapy to a treatment repertoire is intended to combat this threat of tumor evasion, again with minimal to no additional toxicity to the patient.

Profiling breast cancers at a molecular level has revealed their immunoregulatory nature (4) where patients with more immunogenic tu-

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mors boast a better prognosis (5). We now know that breast cancer is a heterogeneous collection of tumor biologies. As a result, enhancing the immune system to recognize and destroy tumor cells via personalized immunotherapy is an increasingly intriguing possibility in the treatment of breast cancer. While nodal status remains the most important prognostic factor in breast cancer, HER2/*neu* (HER2) expression is known to impact breast cancer recurrence and ultimately survival (6). Anti-HER2-specific immunity has been associated with high levels of both cellular and humoral immunity (7,8) and is utilized with both passive and active immunotherapeutic strategies. HER2 is expressed at some level in 70-80% (IHC 1-3+) of breast cancers and overexpressed (IHC 3+, FISH >2.2) in 20-30% of breast cancers (9). As a result, HER2 is an attractive target for breast cancer immunotherapy.

Use of anti-HER2 monoclonal antibodies (mAbs) directed at HER2-overexpressing breast tumors has been shown to improve clinical outcome (1,10). The mAb, trastuzumab, is the most successful breast cancer therapy developed in the past decade. In the adjuvant setting, trastuzumab has been shown to significantly reduce the risk of recurrence and death (11-13). Unfortunately, as a passive immunotherapy, therapeutic effects purportedly last only as long as exogenous antibodies are administered. In contrast, active specific immunotherapy strategies, specifically cancer vaccines, utilize the host's own immune system with potential for long-term immune activation where the host's immune system can fight cancer long after treatment has been completed. Although passive immunotherapy is now standard of care, active specific immunotherapy continues to be investigated and developed given the potential for long-term, sustained immune benefit. The ideal therapy will likely be a combination of both passive and active specific immunotherapies. ❑

PASSIVE IMMUNOTHERAPY

Passive immunotherapy has provided several successful treatments for breast cancer. The current mainstay of passive immunotherapy includes trastuzumab, pertuzumab, and trastuzumab emtansine (T-DM1). In fact, clinical success of trastuzumab and pertuzumab validated HER2 as a bonafide target in breast cancer immunotherapy. Utilized in both the metastatic and adjuvant setting, trastuzumab, confers considerable benefit to both node-positive and high risk node-negative patients breast cancer patients (11,12). In the adjuvant setting, trastuzumab reduces breast cancer recurrence by 50% in HER2 overexpressing patients and is now the standard of care. The antibody pertuzumab has gained approval as a combination therapy with trastuzumab. As a second line treatment, T-DM1, a combination of trastuzumab antibody and cytotoxic agent, exhibits superiority to standard of care chemotherapy (14) and has been approved for metastatic cancer no longer responsive to trastuzumab (14).

Combination therapy of multiple mAbs intends to take advantage of their synergistic properties. Researchers believe pertuzumab binds an alternate epitope from tratuzumab with complementary properties. Phase II trials revealed pertzumab/tratuzumab combination therapy to be well-tolerated and with a 24.2% overall response rate (15). The subsequent phase III CLEOPATRA study examined trastuzumab and docetaxel chemotherapy with or without pertuzumab. The study population of HER2 overexpressing breast cancer patients boasted a 18.5 months progression free survival versus 12.4 months in the control group ($p<0.001$) with overall survival rates favoring the pertuzumab/trastuzumab treatment arm (10). FDA approval of pertuzumab in combination with tratuzumab and docetaxel for HER2 overexpressing breast cancer followed this study.

Despite the success of trastuzumab, overcoming inherent and acquired tumor resistance remains a major obstacle in cancer care (16). T-DM1 is a single agent drug that combines the antitumor properties of trastuzumab with the cytotoxic microtubule-inhibitory agent, mertansine (DM1) (17). Combining an antibody with a cytotoxic agent maximizes efficacy while minimizing exposure to normal tissues (18). Interestingly, phase II trials revealed an objective response rate of 25.9% with a tolerable toxicity profile without dose-limiting cardiotoxicity in patients with tumor progression after HER2 targeted therapy (19). The phase III EMILIA trial was recently completed supporting both efficacy and safety of T-DM1 in HER2-overexpressing breast cancers (14).

Active Immunotherapy: Checkpoint Blockade Agents

Immunostimulatory mAbs exert their effect through the reactivation or elicitation of the immune response to tumor cells. Another immunotherapeutic strategy of great potential is blockade of inhibitory receptors such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death (PD-1). Whereas cytotoxic T lymphocytes (CTLs) can recognize and destroy tumor cells, inhibitory mechanisms exist that interrupt this mechanism. Modulating such regulators of immunity such as regulatory T cells (Tregs) and immune checkpoint pathways are novel methods of active immunotherapy with great therapeutic potential.

Use of mAbs to block CTLA-4, present on both CD8+ T cells and regulatory T lymphocytes (Tregs) is a promising approach in specific immunotherapy where Tregs can be functionally inhibited (20). CTLA-4 acts to inhibit CD8+ T cells and enhance inhibitory function of Tregs. Ultimately, the magnitude of the immune response is the summation of negative and positive signals presented by immune checkpoint molecules (21). By inhibiting CTLA-4 on Tregs, their inhibitory activity can be downmodulated and immune responses enhanced (22). The mAb, ipilimumab, inhibits binding to its natural ligand CD80/86, thus blocking the negative activity of the CTLA-4 molecule and has received FDA approval for treatment in metastatic melanoma patients (2,20). Unfortunately, ipilimumab, as well as other CTLA-4 blockade agents, carry a high risk of life-threatening autoimmunity. Ipilimumab is specifically associated with adverse events of the gastro-intestinal tract in addition to autoimmune hypophysitis, hepatitis, and thyroiditis. However, these autoimmune events do appear to correlate with the clinical efficacy of this agent.

Similar in function to CTLA-4 blockade, PD-1 blockade was initially tested in metastatic melanoma, colorectal cancer, castrate-resistant prostate cancer, non-small-cell lung cancer, and renal cell carcinoma with evidence of clinical efficacy and an acceptable safety profile (23). These results were again validated in a trial examining additional antibodies specific to PD-1 in patients with advanced non-small-cell lung cancer, renal carcinoma, melanoma, ovarian cancer, and breast cancer. Again, the PD-1 blockade resulted in clinical response and a tolerable toxicity profile that appears to be better than ipilimumab (24).

Active Specific Immunotherapy: Cancer Vaccines

Advances in techniques of passive cancer immunotherapy and early success in active immunotherapies have led to the development of cancer vaccines, an active and specific immunotherapeutic approach. Provenge (sipuleucel-T), the first true cancer vaccine, gained FDA approval for the treatment of hormone-resistance metastatic prostate cancer in 2010 (25). No active specific immunotherapy, or cancer vaccine, is currently approved for the treatment of breast cancer.

Cancer vaccines hold promise as an adjuvant and preventive therapy in patients at high risk for cancer recurrence after initial standard of care therapy (26). Cancer vaccines target immunogenic cancer-related antigens. The goal is to target cancer-related epitopes over-expressed on malignant tissue but distinct from normal tissue to stimulate the human body's own immune system to recognize and destroy tumor cells specifically.

Administration of cancer vaccines as an active specific immunotherapeutic approach offers several theoretical advantages. First, vaccines offer tumor specificity by eliciting an immune response against antigens specifically expressed on tumor cells. These beneficial effects may be perpetuated by the secondary release of antigens and cytokines following tumor lysis, thus potentiating the effect of the vaccine. The key advantage of vaccines is the potential for a specific, sustained immune response long after completion of treatment. This possibility of immunologic memory negates the requirement for the multiple treatments and infusions typical of passive immunotherapeutic strategies (27).

An ideal vaccine should induce activation and proliferation of specific lymphocytes to stimulate both humoral and cellular immunity. The vaccine should induce immunologic memory while being safe, simple to administer, and widely exportable to the eligible population.

HER2 Vaccine Development

Clinical evaluation of HER2 targeting breast cancer vaccine platforms has included peptide,

protein, plasma DNA, and dendritic based vaccines. The earliest trials of single epitope peptide vaccinations occurred in the 1990s. Initial formulation strategies included emulsification with immunoadjuvant or pulsation with dendritic cells. More recent trials are utilizing longer and/or multiple peptide sequences aimed to produce a more effective, complete immune response in the adjuvant setting. Additional challenges include the identification of the ideal tumor associated antigen (TAA), the basis of peptide vaccination, given the complex mechanisms of immune escape and tolerance following malignancy. The ultimate goal is to create personalized vaccine therapy specific to the individual's tumor biology.

Nelipepimut-S

HER2 has been shown to be the source of multiple immunogenic peptides to include E75, GP2, and AE37 (7,28,29). Nelipepimut-S (E75), a nine amino-acid peptide (HER2/*neu* 369-377, KIFGSLAFL) from the extracellular domain of HER2 (28), is characterized by HLA-A2 restriction. Nelipepimut-S has the appeal of a peptide-based strategy, an attractive option for its simplicity of use and monitoring in addition to the ability to use the immunodominant epitope in combination with other antigens or alone.

Preclinical studies confirmed nelipepimut-S-specific tumor immunity. Two independent studies confirmed the presentation of nelipepimut-S on HLA-A2 in ovarian cancer cells as well as recognition by ovarian cancer associated CD8+ T cell clones (28,30). High percentages of breast, ovarian, lung and colorectal cancer patients have been found to have pre-existing immunity to nelipepimut-S (31). Lymphocytes from the draining lymph nodes of breast cancer patients of any level of HER2 expression (IHC 1-3+) mounted a T helper type 1 cytokine response after exposure to nelipepimut-S and other HER2 derived peptides. Of note, blunted proliferative response occurred in nodes with metastatic cancer compared to unaffected nodes of the same patient (32,33).

Phase I trials of nelipepimut-S were conducted in combination with different immunoadjuvants. These trials demonstrated the peptide to be safe and capable of eliciting a peptide-specific CTL immune response (32,34-35) in metastatic breast, ovarian, and colorectal cancer patients.

Clinical Efficacy of Nelipepimut-S

As stated earlier, the majority of early clinical work with this peptide vaccine targeted patients with advanced and metastatic disease, a strategy that proved disappointing given issues of immunosuppression and tolerance. With the hypothesis that peptide vaccines could work more effectively in an environment with minimal residual disease, and hence minimal tolerance, clinical trials of nelipepimut-S + GM-CSF (NeuVaxTM, Galena Biopharma, Portland, OR) began to focus outside the metastatic setting.

NeuVaxTM phase I trials in both node-positive and node-negative breast cancer patients at high risk of recurrence who were clinically disease-free were initiated. All patients had completed standard of care with surgery, chemotherapy, and radiation treatment as indicated. Patients were allowed concomitant hormonal therapy during the trial. HLA-A2 positive patients were vaccinated while HLA-A2 negative patients were followed as controls given HLA-A2 status has not been shown to impact prognosis in breast cancer. Importantly, patients with any level of HER2 expression were enrolled. Results showed the vaccine to be well-tolerated with encouraging immunologic results (36).

Given these encouraging results, NeuVaxTM phase II trials were again conducted as an adjuvant strategy given the hypothesis that a minimal tumor burden environment is more appropriate for a treatment developed to prevent, not treat, disease. The phase I/II studies were published as a combined analysis (36,37). Again, node positive and high-risk node negative breast cancer patients with any level of HER2 expression >1 by immunohistochemistry were given a vaccination series of 6 monthly inoculations. In the phase II trial, both HLA-A2/A3 positive patients were enrolled given preclinical data suggesting nelipepimut-S to be clinically effective in HLA-A3 patients as well (31). This additional inclusion criteria expanded applicability of vaccination to as much as 60-75% of the US and European population. In the largest adjuvant breast cancer vaccine study to date with 195 women enrolled, NeuVaxTM was shown again to be safe but more importantly effective with a significantly greater disease free survival (DFS) rate in the vaccinated group at 18 months (92.5 vs. 77%, $p=0.04$); although, this effect waned at 24 months (37).

Booster inoculations were enacted into the trial after initiation of the study protocol based on observations of waning immunity in trial patients a year or more after completion of the vaccine series. Following an amendment to the study protocol, trial patients were offered up to four booster inoculations every 6 months. A total of 53 (49%) patients received booster inoculations. The additional inoculations were well-tolerated and were effective in maintaining nelipepimut-S-specific immunity (38). In addition, a trend towards decreased disease recurrence occurred in the optimally boosted patients who received their initial booster inoculation less than 6 months after completion of the primary vaccine series (39). Also interesting was the observation of a more robust immunologic response in the HER2 expressing (IHC 1-2+) population (40). At 24 months follow-up, DFS was 94% versus 79.4% in the control population, p=0.0441.

The ability to confer clinical benefit to patients with all levels of HER2 expression (IHC 1+, 2+, 3+) is a critical benefit of the nelipepimut-S-HER2 directed vaccine. In addition, vaccines hold promise of immunologic memory whereas the therapeutic effectiveness of mAb therapy ends when the infusion is terminated. Given the benefit toward HER2 expressing (IHC 1-2+) subjects within the phase II trial , our group is currently focused on the adjuvant PRESENT trial, a prospective, randomize phase III clinical trial to test the NeuVaxTM vaccine (NCT 01479244) (42). Currently being conducted in the United States and internationally, this randomized study enrolls HLA-A2/A3 positive, node positive, HER2 expressing (IHC 1-2+) patients who are clinically free of disease after standard of care treatment. All patients will be optimally dosed and boosted. The primary endpoint is three years disease-free survival. Enrollment was completed in 2014.

Combination Therapy

HER2-directed vaccines exhibit immunogenicity, favorable safety profiles, and in the case of nelipepimut-S, clinical efficacy. Challenges of vaccine therapy include antigen variability and the complex immune escape mechanisms of cancer cells. The simplest approach to maximize vaccine efficacy is to explore options for a multi-epitope vaccine. It is now understood that eliciting the CD4+ helper T-cell response is important to maximizing tumor immunity by optimizing both humoral anti-tumor and CD8+ T-cell responses (43). MHC Class II-binding and promiscuous peptides are being investigated as possible adjunct therapies in conjunction with nelipepimut-S to elicit a CD4+ T cell response to sustain immunologic memory (44). A helper peptide combination utilizing longer MHC Class II peptides containing MHC Class I peptides (including nelipepimut-S), have been used to vaccinate HLA-A2+ patients with demonstration of post-inoculation immunity (44).

An additional approach is the combination of cancer vaccines with mAb therapies. Given that molecular immune checkpoints such as CTLA-4 and PD1/PD1L play key roles in maintaining self-tolerance and mechanisms by which tumors cells can escape immune attack, this combination therapy may enhance a vaccine approach. Additionally, vaccines may be used synergistically with passive immunotherapy directed against the same target antigen. A phase I/II study examining administration of a HER2-directed vaccine in combination with trastuzumab in stage IV breast cancer patients did not result in additional toxicity (45). Within the nelipepimut-S phase I/II trials, Benavides et al. reported that sequential administration of NeuVaxTM after trastuzumab among the HER2 overexpressing population to be safe as well as clinically and immunologically beneficial (40).

Our group is currently evaluating the combination therapy of nelipepimut-S with trastuzumab in HER2 expressing (IHC 1-2+), node positive or high-risk node negative patients who are clinically disease-free in a phase II clinical trial (NCT01570036) (46). Initiation of this study was supported by the preclinical and early clinical evidence of synergism of trastuzumab with active specific immunotherapy (45,47). Here, HER2 peptide presentation on MHC receptors is augmented by the increased internalization from proteolytic degradation of HER2 molecules by trastuzumab binding.

The ultimate goal of effective immunotherapy is to elicit potent T-cell responses given the association with a more favorable prognosis. New immunomodulatory agents and vaccines show promise in reversing the immunosuppression caused by established tumors. Both downregulating inhibitory response and boosting CTL immune activity are immunotherapeutic mechanisms being explored to prevent tumor recurrence. Combining these

novel agents with each other or other established treatments may boost efficacy and durability of clinical responses. ❑

CONCLUSION

Immunotherapy has become an important element in the treatment of breast cancer. HER2 targeted therapies are now an essential component of HER2 overexpressing breast cancer treatment. Trastuzumab, with the more recent additions of pertuzumab and TDM1, have significantly improved breast cancer prognosis. With multiple FDA approved antibody therapies utilized in both the adjuvant and metastatic settings, progress continues to be made in the field of passive, active, and active specific immunotherapies. Recent successes in targeted therapies, active specific immunotherapy in particular, hold promise for continued gains in overall survival within the adjuvant setting. The highly specific and targeted approach of vaccine therapy not only avoids the toxicities of current standard of care therapies, passive immunotherapies, and active immunotherapies such as ipilimumab; but offers therapeutic options beyond just the HER2-overexpressing population. Vaccines hold potential as an adjuvant in lower HER2 expressing (IHC 1-2+) and triple negative breast cancer patients, a currently underserved population.

As breast immunotherapy research continues, issues of the ideal epitope combination, adjuvant selection, and tumor immune-escape mechanisms remain relevant to the development of the optimal vaccine strategy. Although breast cancers vaccines have been largely unsuccessful in past clinical trials, the majority of these trials occurred in the setting of late-stage metastatic disease, an unfavorable environment for agents designed to prevent, as opposed to treat, disease. With current trials focused on the adjuvant settings, immunogenicity is now showing correlation with clinical response.

While vaccine administration offers key advantages, passive and active strategies should be appreciated for mutually beneficial biologic effects and synergistic potential. Integrative immunotherapy utilizing established standard of care therapies with novel approaches such as cancer vaccines and immune checkpoint blockades holds the greatest chance for sustained clinical response and quite possibly, a breast cancer cure. The future of breast immunotherapy and research will continue to be a dynamic field as understanding of the complexity of tumor progression grows.

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