Breast Cancer: Targets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

Progress in the development of a therapeutic vaccine for breast cancer

This article was published in the following Dove Press journal:
Breast Cancer: Targets and Therapy
3 June 2010
Number of times this article has been viewed

Andrew L Coveler
Nicole E Bates
Mary L Disis

Tumor Vaccine Group, Center for Translational Medicine in Women's Health, University of Washington, Seattle WA, USA

Abstract: Various human malignancies are immunogenic and recent cancer vaccine trials have demonstrated potential survival benefit. Breast cancer is immunogenic and there are several tumor associated antigens for which breast cancer vaccines have been developed. Breast cancer vaccines are designed to stimulate the immune response at various steps in the native antigen processing pathway for immunosurveillance. Human epidermal growth factor receptor 2 (HER-2/neu), mucin 1 (MUC-1), and human telomerase reverse transcriptase (hTERT) are some of the most studied antigens actively being targeted for vaccination in breast cancer patients. These vaccines are designed to elicit cytotoxic and/or helper T cell responses. Over the last several years, there has been reported progress in human clinical trials for these antigens. Cancer vaccines have repeatedly been shown to be safe with production of minimal toxicity. Recent clinical advances in the development of cancer vaccines demonstrate the potential clinical benefit that cancer vaccines hold.

Keywords: breast, cancer, vaccine, HER-2/neu, MUC-1, hTERT

Introduction

A 31-year-old patient with round celled sarcoma of the neck had 5 operations within 3 years. At the last operation it was impossible to remove the entire tumor. Two weeks after the operation, a severe erysipelas infection occurred, during which time "the sarcoma entirely disappeared".[1] Seven years later, the patient remained disease free. This case study, published in 1893, is one of the first reports linking immunity with cancer regression. Over the subsequent 100 years we have gained insight into the immune system and its interaction with tumor associated antigens (TAAs). It was postulated in the late 1950s that tumor growth is immunologically controlled and the theory of immunosurveillance was proposed in 1967.[2,3] Subsequent studies indicated adaptive immunity is the primary mediator of tumor regression and control.[4] The immune system has the ability to recognize TAAs that are presented by the tumor itself or, more likely, by antigen presenting cells (APCs) via cross priming. APCs, such as macrophages and in particular dendritic cells (DCs), are essential for priming naïve T cells and activating the immune response.[5] Antigen is processed into peptides and then presented by the major histocompatibility complex (MHC) and displayed at the cell surface for recognition by T lymphocytes.

Vaccines have been developed to stimulate the immune response at different steps in this pathway. Peptide based vaccines are designed to bind MHC molecules directly, and thus there may be no need for further endogenous processing after uptake. Conversely, protein-based vaccines are designed for uptake into APCs and require native

Correspondence: Andrew L Coveler
Tumor Vaccine Group, Center for Translational Medicine in Women's Health, University of Washington, 815 Mercer Street, Box 358050, Seattle, WA 98109, USA
Tel +1 206 288 7509
Fax +1 206 685 3128
Email acoveler@u.washington.edu

processing into peptides. Deoxyribonucleic acid (DNA) vaccines are designed to "infect" APCs to produce protein which is then processed as above. Finally, DC vaccines are designed to provide the most effective APC possible for presenting antigen to T cells. After reviewing recent clinical advances in the development of cancer vaccines as a whole, we review a select repertoire of breast cancer antigens before discussing specific breast cancer vaccine trials as examples to the progress being made in the development of targeting these antigens.

Cancer vaccine progress

Vaccines for protection against infectious agents have proven one of medicine's most successful interventions. However, almost no vaccines are utilized to treat ongoing infections. Similarly, cancer vaccines have had little success in eradicating rapidly growing tumors. Recent clinical trials have demonstrated that choosing the proper setting for cancer vaccines plays a large role in determining these vaccines' clinical success.

Prostate cancer is the most common (excluding non-melanoma skin) cancer in American men. The natural progression of prostate cancer means lengthy clinical trials must be performed to study vaccines in early stage disease. For this reason, trials are often performed in the metastatic setting. Recently, sipuleucel-T (Provenge®, Dendreon) an autologous cellular immunotherapy product that stimulates an immune response against prostate cancer, has been shown to impart potential clinical benefit.[6] Sipuleucel-T is made by first removing autologous peripheral blood mononuclear cells, including APCs, from the patient via leukapheresis. These cells are then co-cultured with a recombinant fusion protein containing prostatic acid phosphatase linked to granulocyte-macrophage colony stimulating factor (GM-CSF), an immune stimulatory molecule.[7] The resultant activated APCs are then infused into the patient to stimulate a prostate specific T cell response. A randomized phase III trial was performed in men with metastatic, androgen independent prostatic adenocarcinoma with overall survival (OS) as the primary end point and time to progression (TTP) as the secondary end point.[8] Five hundred and twelve patients were randomized 2:1 to receive either 3 infusions of vaccine every 2 weeks or a placebo. As expected from previous studies[6,7] an OS benefit was suggested. The median survival benefit was 4.1 months ($P = 0.032$) and the hazard ratio (HR) was 0.775 (95% confidence interval [CI]: 0.614–0.979). This benefit was seen, or a trend for benefit was seen, in all patients regardless of bisphosphonate use, Gleason score, number of metastases and age. This vaccine has now shown an OS benefit of 4 months (3.3–4.5) in 3 randomized phase III studies.[6–8] At 3 years approximately 10% more patients are alive in the group who received vaccine compared to those who did not. This is the first active immunotherapy to demonstrate improvement in OS for advanced prostate cancer.

Follicular lymphoma is the second most common lymphoma in the United States and is treatable but incurable with standard cytotoxic chemotherapy and/or monoclonal antibody based therapy. The disease is associated with 5- and 10-year survivals ranging from 91% and 71% for low risk disease to 52% and 36% for high risk disease.[9] Follicular lymphoma is marked by a clonal population of lymph node cells, which express patient specific surface immunoglobulins. Idiotype determinants (Id) are the portion of an immunoglobulin molecule that confers the molecule's unique character, most often including its antigen-binding site. Anti-idiotype vaccines can be constructed for each patient's specific idiotype. One such vaccine consists of antibody to tumor via patient specific immunoglobulin bound to keyhole limpet hemocyanin (KLH) as a carrier molecule and immune stimulant (BiovaxID®, Biovest International), and is given with GM-CSF concurrently. The BV301 phase III clinical trial of this approach was presented at the American Society of Clinical Oncology (ASCO) 2009 plenary session.[10] The trial's primary objective was to determine if Id-KLH/GM-CSF prolonged disease free survival (DFS) after patients obtained a complete response (CR) with chemotherapy. Two hundred and thirty-four untreated stage IIx, III and IV patients were enrolled, and 177 achieved a CR or CR unconfirmed (CRu) and were then randomized 2:1 to Id-KLH/GM-CSF or control (KLH/GM-CSF) vaccination. There was a 6- to 12-month period between completing chemotherapy and administration of the first vaccine, in order to permit time for vaccine construction and immunologic recovery after chemotherapy. Prespecified efficacy analysis consisted of intention to treat (ITT) and a modified-ITT (mITT) analysis based on patients who remained in CR and received vaccine or control vaccine. The mITT, ie, analysis of patients who remained in CR, demonstrated at a median follow-up of 56.6 months a median DFS of 44.2 vs 30.6 months. The Cox proportional hazard was 0.62 (0.39–0.99) with a significant P value of 0.047.

An additional trial, presented at ASCO 2009, evaluated patients with metastatic melanoma. Metastatic melanoma is an incurable disease for the vast majority of patients. The only systemic treatment with a potential cure is high-dose bolus recombinant interleukin-2 (IL-2), and a complete and durable response is achieved in less than 10% of patients.[11]

A phase II study of peptide vaccination and high-dose IL-2 demonstrated an increase in response rate to 42% compared to the 17% response rate seen in prior studies of high-dose IL-2 alone.[12] Subsequently, 185 patients with locally advanced stage III or IV cutaneous melanoma were randomized in a phase III study to high-dose IL-2 with or without a peptide vaccine targeting the gp100 protein.[13] The vaccine consisted of a human leukocyte antigen type 2 (HLA-A2) synthetic peptide (gp100:209-17[210M]) and incomplete Freund's adjuvant (IFA). The primary objective was clinical response and the secondary objectives were toxicity, DFS/progression free survival (PFS), immunologic response and quality of life. At the time of analysis, the centrally assessed response rate was 18.6% vs 6.5% ($P = 0.022$) in favor of those who received vaccination. The complete response rate was 14% vs 2.2% for vaccination plus IL-2 compared to IL-2 alone. PFS and OS improved as well: PFS significantly increased to 2.9 months vs 1.6 months ($P = 0.010$), and OS increased, albeit not significantly, to 17.6 months vs 12.8 months ($P = 0.096$). Furthermore, vaccination added minimally to toxicity during the first 2 cycles of therapy. Patients who were vaccinated received more IL-2 therapy than those who did not, and thus the increase in laboratory abnormalities and neurologic toxicities seen in the vaccination group is attributed to the greater number of cycles of IL-2 treatment.

The studies described above are encouraging in that each vaccine targeted only a single tumor specific antigen, yet those patients who were vaccinated appeared to derive benefit. Moreover, in the case of prostate cancer and melanoma, patients were immunized in the face of advanced stage disease and still demonstrated clinical response. The success of these studies underscores several important aspects of vaccine clinical trial design. First, the primary endpoint of the study must be clearly defined, whether OS, DFS, or TTP. Secondly, integration of a vaccine in upfront therapy may require initial consideration of relapse prior to vaccination, if there is to be a delay between completion of therapy and initiation of vaccine administration. Prespecified ITT and mITT, which prospectively allow for disease progression prior to vaccination, as seen in the BV301 trial, may be an important step in realizing the clinical benefit for a subset of patients. Lastly, vaccines, when integrated into standard regimens, such as IL-2, may have significant synergy. Clearly there is evidence that the integration of vaccination with chemotherapy may be beneficial.[14]

Breast cancer antigens

Breast cancer has been shown to be immunogenic.[15] A National Cancer Institute (NCI) workshop ranked 100 vaccine antigens based on various components including immunogenicity, specificity, oncogenicity, expression level, stem cell expression, number of epitopes, cellular location of expression and number of patients with antigen positive cancers. Eleven of the top 25 ranked antigens have substantial expression in some breast cancers (Table 1).[16] The promise of cancer vaccines lies in the exquisitely targeted nature, minimal known toxicity and potential for lasting immunologic memory, which can possibly eradicate cancer at a distant time from vaccination. Notwithstanding these potential benefits, there are no commercial breast cancer vaccines approved in the United States, despite the more than sixty companies and untold number of academic labs involved in breast cancer vaccine development. Human epidermal growth factor receptor 2 (HER-2/neu), mucin 1 (MUC-1), and human telomerase reverse transcriptase (hTERT) are some of the most studied antigens actively being targeted for vaccination in breast cancer patients and there are recent clinical trials demonstrating encouraging progress over the last several years.

The HER-2/neu protein is a member of the epidermal growth factor receptor family.[17,18] During adult life, the HER-2/neu protein is weakly detectable in the epithelial cells of most normal tissue, but is expressed significantly during human fetal development.[19] Alterations in the structure, copy number, or expression of epidermal growth factor receptor genes play a role in the pathogenesis in a variety of human malignancies, including breast cancer.[20] Over expression by immunohistocytochemistry (IHC) and/or amplification of the gene by fluorescent *in situ* hybridization (FISH) has been identified in many cancers such as breast, ovarian,

Table 1 Eleven of the top 25 ranked antigens

Breast cancer vaccine top 11 ranked antigens
CEA
EphA2
HER2/neu
MAGE-A3
MUC-1
NY-ESO-1
p53
p53 mutated
survivin
hTERT
WT1

Abbreviations: CEA, carcinoembryonic antigen; EphA2, Ephrin type-A receptor 2; HER2/neu, human epidermal growth factor receptor 2; MUC-1, mucin 1; p53, protein 53; hTERT, human telomerase reverse transcriptase; MAGE-A3, melanoma-associated antigen 3; WT1, Wilm's Tumor 1.

and gastric. In breast cancer, HER-2/neu amplification was associated with a worse prognosis prior to the development of HER-2/neu directed therapy with trastuzumab. The HER-2/neu oncogenic protein is also a tumor antigen.[21] HER-2/neu has been found to be an excellent target for immunotherapy, including monoclonal antibody therapy which has demonstrated clinical benefit in both the adjuvant and metastatic setting.[22–24]

MUC-1 (episialin, epithelial membrane antigen, CA15-3 antigen) is a highly O-glycosylated mucin-like transmembrane glycoprotein encoded on chromosome 1.[25] In most normal glandular epithelia cells, MUC-1 is expressed on the apical surface.[26] Over expression of an under glycosylated form of MUC-1 occurs in nearly all breast carcinomas. In a recent study, abnormal MUC-1 expression was seen in approximately 93% of 237 cases.[27] The prognostic significance of MUC-1 overexpression is unclear, with studies reporting better, no bearing, and worse prognosis in overexpressing patients. Using a new monoclonal antibody directed at the protein backbone, and thus not dependent on glycosylation, different membrane staining patterns were evaluated. MUC-1 staining patterns were correlated with relapse free survival (RFS) and OS and demonstrated that apical and diffuse cytoplasmic patterns predicted better RFS and OS, while entire membrane, focal cytoplasmic and inside-out patterns had no significant correlation with RFS and OS. Though not reliably prognostic, the abnormal expression of MUC-1 in 93% of breast cancers yields an almost universal target for immunotherapy in breast cancer patients. Importantly, a patient with an anti-MUC-1 immune response to her malignancy at diagnosis, whether this response is a cellular response or antibody-mediated, may live longer than a patient without an immune response to her cancer.[28,29]

Telomerase is a ribonucleoprotein complex that maintains chromosomal integrity by protecting telomeric DNA for continuous cell proliferation. The complex contains telomerase reverse transcriptase (TERT) and a ribonucleic acid (RNA) template.[30] hTERT is a large protein of 1132 amino acid residues that has broad expression in greater than 85% of all human cancers, with little or no expression in normal somatic cells.[31] Peptides of hTERT degradation are presented on the tumor cell surface as antigens by the MHC class I and II pathways.[30] Patients in remission from their malignancy often have high levels of CD8+ reactive T cells towards hTERT peptide I540, whereas patients with active disease often have lower levels of reactive T cells.[32] Given that hTERT is differentially expressed in cancer with little to no expression in somatic cells, that its inhibition *in vitro* leads to growth arrest, and that it appears to play an important role in carcinogenesis, this antigen is a potential therapeutic target for several malignancies, including breast cancer.

Although many more proteins have been shown to be immunogenic in breast cancer, HER-2/neu, MUC-1, and hTERT are actively being studied as vaccine immunogens in human clinical trials. Moreover, a variety of vaccine constructs have been created to target these antigens, thus providing examples of the different approaches available for immunizing against breast cancer.

Breast cancer vaccines in clinical trials

The adaptive immune response to breast cancer antigens can be biased towards CD4+ or CD8+ T cell responses. By choosing peptides that bind different MHC molecules, which come in two classes and bind different-size peptides, an immune response directed towards class I CD8+ T cell responses or class II CD4+ T cell responses can be preferentially generated. In an attempt to generate both a class I and class II response, autologous dendritic cell-protein based- and DNA-based vaccines are also being studied (Table 2). In all of the studies reviewed below, the vaccines were found to be safe, with patients experiencing minimal toxicity attributed to vaccination.

Peptide vaccines: class I

The HER-2/neu E75 vaccine is a peptide vaccine consisting of amino acids 369–377. It has been most thoroughly studied as a single peptide vaccine combined with various adjuvants to stimulate class I cytotoxic CD8 T cell responses, and it demonstrates both HLA-A2 and later discovered A3 specificity.[33] Recently published are the combined data from

Table 2 Dendritic cell-protein based- and DNA-based vaccines

Classes of breast cancer vaccines
Antigen vaccines
Peptide
Class I[a]
Class II[a]
Protein[a]
Tumor cell vaccines
Autologous
Allogenic
Dendritic cell vaccines
Peptide pulsed[a]
Tumor cell fused
Vector-based
DNA[a]

[a]Examples of these vaccines are in the text.
Abbreviation: DNA, deoxyribonucleic acid.

2 clinical studies of the E75 vaccine given to node positive and node negative breast cancer patients.[34] Patients were vaccinated after undergoing the appropriate standard therapies of surgery, chemotherapy and radiation therapy as applicable. Hormonal therapy as appropriate was administered to patients on trial. The vast majority of patients, approximately 95%, did not receive adjuvant trastuzumab, as this was not the standard of care at the time of enrollment. Patients were HLA typed and those that were HLA-A2+, approximately 50% of the population, received the vaccine, while HLA-A2– patients were prospectively observed. The node positive patients received 4- to 6-monthly injections of 100, 500 or 1,000 μg of E75 in a typical dose escalation fashion with 3 to 6 patients per group. The node negative patients were not required to have HER-2/neu expressing tumors in an attempt to study the feasibility of vaccination in antigen-naïve hosts. These patients received 3- to 6-monthly vaccines consisting of either 500 or 1,000 μg of peptide with 125 or 250 μg of GM-CSF. Investigators enrolled 95 and 91 patients in the node positive and node negative study, respectively. In the node positive study, 49 patients tested HLA-A2 or A3 positive and were subsequently vaccinated. In the node negative study, 52 patients were vaccinated. Overall the patients were well balanced, but notably more patients were hormone receptor negative in the vaccinated group. Primary analysis was performed at an 18-month median follow-up after 171 patients had been enrolled. The recurrence rate in the vaccinated group was 5.6%, compared with 14.2% in the observation group ($P = 0.04$). Cytotoxic T lymphocyte (CTL) dimer assay was performed and demonstrated a pattern of increased CD8+ E75-specific CTL during vaccination, peaking and then receding to plateau.[33] In long term follow-up, immunity appeared to wane over 5 years of extended follow-up. Investigators extended their analysis and, with a median follow-up of 26 months, found the recurrence rates were 8.3% and 14.8% respectively; however, the results were no longer significant ($P = 0.15$). Moreover, there was no significant difference in survival between vaccinated and unvaccinated patients. It is unknown whether the loss of immunity over time was associated with relapse.

An evaluation of the impact of HER-2/neu expression levels on the response to the E75 vaccines demonstrated low-expressor patients, defined as IHC 1+, 2+, or FISH $<$ 2.0, had significantly higher maximum immune responses compared with overexpressors, IHC 3+ or FISH $\geq$ 2.0.[35] Vaccinated patients in this study had fewer recurrences compared with control patients, with a trend toward decreased mortality. This data has resulted in an ongoing phase III trial to evaluate the E75 vaccine further. One question is whether any clinical difference seen is secondary to an inherent difference in outcomes related to HLA-A2 status. Furthermore, HER-2/neu vaccines will now need to be administered with trastuzumab in most cases. There are several proposed mechanisms for trastuzumab benefit: HER-2/neu receptor internalization and degradation, inhibition of HER-2/neu signaling via its signaling cascade, inhibition of DNA repair and immune effects via stimulation of natural killer cells and antibody dependent cellular cytotoxicity (ADCC); all of which require overexpression.[36] Evaluation of patients who received the E75 vaccine at baseline indicated that some HER-2/neu negative patients had pre-existent immunity to this antigen. This observation may imply that some HER-2/neu negative patients potentially had, at a time point prior to presentation, a HER-2/neu positive malignancy that a successful immune response prevented; this is a process called immunoediting.[37] Immunoediting may slow tumor growth initially, but in the long run, a more aggressive phenotype may escape the immune response. The use of a vaccine in HER-2/neu negative patients, or in patients with HER-2/neu overexpressing premalignant conditions, eg, DCIS, may benefit from targeted immunoediting with vaccination in an attempt to prevent the aggressive HER-2/neu positive invasive malignant phenotype.[38] In this way a HER-2/neu negative patient may benefit from HER-2/neu immunity, and thus HER-2/neu immunotherapy.

A phase I study of hTERT peptide vaccination was performed in HLA-A2+ patients with metastatic breast cancer who were refractory to one conventional therapy. The patients were given 4 vaccines, 1 every other week, as induction and then received monthly vaccination until unacceptable toxicity or clinically significant disease progression occurred.[32] Patients were randomized to hTERT or cytomegalovirus (CMV) peptide admixed with IFA and granulocyte colony-stimulating factor (G-CSF). Nineteen patients with metastatic breast cancer were enrolled and the vaccine was found to be safe at all dose levels. As a marker of potential vaccine activity, tumor infiltration of CD8+ T cells was evaluated in 6 patients before and after vaccination. At baseline, no infiltration of hTERT specific CD8+ T cells was found. However, following vaccination, hTERT specific CD8+ T cells were observed in 50% of these patients' tumors. Despite demonstrated tumor infiltration by CD8+ T cells, no objective clinical response as measured by Response Evaluation Criteria in Solid Tumors[39] (RECIST) was seen.

To assess the association between the induction of hTERT I540 specific immunity and OS, a landmark survival analysis was performed. Median OS from vaccination four was greater in high-responders compared to non/low responders (32.2 months vs 17.5 months, $P = 0.03$). The hypothesis that vaccine induced tumor infiltrating lymphocytes (TIL) may improve survival, as seen with native TIL in ovarian cancer patients, is supported by this finding.[28] This study underscores the importance of assessing whether vaccine induced T cells are capable of homing to the site of tumor and infiltrating the tumor stroma. This migratory ability is necessary to induce tumor destruction. While this study established the presence of TIL immediately following vaccination, it did not evaluate long-term persistence of the cytotoxic T cell response. One of the pitfalls of vaccines designed only to induce CD8+ T cells is the lack of persistence of those cells *in vivo*, as described above. Without CD4+ T cell help, antigen specific CD8+ T cells are only transitory. This observation has led to the generation of Class II peptide based vaccines, which are capable of inducing both memory as well as CD8 antigen specific T cells.

Peptide vaccines: class II

Our group has evaluated HER-2/neu vaccines containing longer peptide mixtures toward the intracellular domain (ICD) and extracellular domain (ECD) of HER-2/neu. The vaccines are HLA unrestricted, and therefore, could potentially benefit any patient regardless of HLA type. The vaccines are designed to elicit a predominant CD4+ class II antigen specific response. CD4+ T cells activate and expand CTL from naïve T cell pools. In addition, CD4+ T helper cells are required for reactivation of memory CTL.[40] These vaccines contain potential helper epitopes, which are predicted by computer modeling and empiric testing to be immunogenic.[41,42] In early studies, patients with stage III or IV HER-2+ breast, ovarian or non-small-cell lung cancer were eligible for study if they had received prior treatment so their disease was not detectable or was stable on hormonal therapy.[41] The vaccination series consisted of 6 monthly intradermal injections of peptide with GM-CSF as adjuvant. Sixty-four patients were enrolled on study, and 38 completed all 6 vaccines. Of the 38 patients, 31 had breast cancer, 5 had ovarian cancer and 2 had lung cancer. 92% of patients who completed all 6 vaccinations developed T-cell immunity. The probability of detecting an immune response by the third vaccination was 82%, thus demonstrating an immune response can be generated with a limited number of vaccines.[43] Epitope spreading, whereby the patient develops an immune response to peptides other than those administered during vaccination, is one of the hallmarks of endogenous immunity.[44] The majority of patients (84%) who completed all six immunizations developed epitope spreading, and this was associated with the development of a HER-2/neu protein-specific immune response. In a long term follow-up study, 52 patients (37 stage IV, 15 stage III) were identified and 21/52 patients (12 stage IV, 9 stage III) were determined to be living. The median follow-up time for the 21 study patients still alive was 112 (range, 104 months–126 months). Blood samples were collected in 10/21 subjects, and 6/8 evaluable patients (75%) had persistent T cell immunity to immunizing HER-2/neu peptides; in addition, 7/8 patients (88%) had T cell immunity specific for HER-2/neu protein and peptides not contained in their immunizing mixture, ie, epitope spreading. In a multivariate analysis, the number of chemotherapy regimens prior to vaccination (HR = 5.7 [CI 95%, 1.5–23; $P < 0.001$]), and the development of epitope spreading after HER-2/neu vaccination (HR = 0.34 [CI 95%, 0.12–1.0; $P = 0.05$]) were independent predictors of OS. Median OS for subjects (n = 33) who developed epitope spreading was 84 months vs 25 months for 16 subjects who did not develop epitope spreading.[45]

The identification of HER-2/neu as a potential tumor antigen marked the beginning of efforts to develop targeted HER-2/neu immunotherapy, including vaccines and monoclonal antibody therapy.[20,44] With the approval of trastuzumab and the discovery of cardiac toxicity secondary to an unknown mechanism, assessing the safety of HER-2/neu vaccination given concurrently with trastuzumab was an important next step in safety analysis. Our group conducted a phase I/II study of HER-2/neu class II peptides with GM-CSF given as 6 monthly administrations to patients with stage IV HER-2/neu positive breast cancer while receiving trastuzumab. Additional toxicities were minimal, with the majority of toxicities characterized as grade 1 or 2 (99%). Most importantly, HER-2/neu vaccination with concurrent trastuzumab did not result in additional cardiac toxicity. Three (15%) patients had a decrease in left ventricular ejection fraction (LVEF) to less than normal, but none developed symptomatic left ventricular dysfunction. Vaccination was found to augment or induce new HER-2/neu immunity in 90% of patients. In addition, not only was intramolecular epitope spreading seen, but intermolecular spreading to other antigens such as IGFBP-2, p53 and topoisomerase-II-alpha was seen as well. This study was not designed to address a clinical endpoint, but PFS and OS were assessed to gather additional data on the potential therapeutic efficacy.

Whereas the median PFS for patients receiving trastuzumab alone in this setting ranges from 7 months to 12 months, in this study population it was 17.7 months. The median OS has not yet been reached. The combination therapy of HER2/neu specific vaccination with trastuzumab needs to be, and will be, further studied.[46]

HER-2/neu vaccines designed to stimulate class II responses have been shown to be safe and potentially effective. If cancer vaccines are to play a role in the treatment of patients, they will be used in an adjuvant or adjuvant-like setting. Integration of a HER-2/neu specific vaccine into standard of care will mean the co-administration of vaccine during the use of common adjuvant therapies such as trastuzumab, bisphosphonates and hormonal therapy. In a retrospective analysis based on the usage of these agents, common adjuvant therapies were found to not have an impact on the patient's ability to develop an immune response; nor were they found to have an impact on the magnitude of immune response specific for HER-2/neu peptides.[47] Vaccines in the adjuvant setting are reasonable, and importantly, remain able to stimulate an immune response when given with a monoclonal antibody directed to the same target.

Longer peptide-based vaccines have also been developed for MUC-1. The first phase I clinical trial of a 105 amino acid synthetic MUC-1 peptide was performed in the 1990s, and in this trial, 63 patients with various malignancies (including nine with breast cancer) were vaccinated.[48] The primary goal was to evaluate safety, and the vaccine was found to have minimal toxicity. The secondary goal was to evaluate the delayed type hypersensitivity (DTH) response to mucin-specific peptides after vaccination, and only 3 patients of the 63 developed a strong DTH response. In a subsequent study, 16 patients with metastatic breast cancer were immunized with a 16 amino acid MUC-1 peptide conjugated to KLH and an adjuvant that consists of an oil droplet emulsion of monophosphoryl lipid A and mycobacterial cell wall skeleton (DETOX).[49] In this study, low dose cyclophosphamide was administered before the first and third vaccination. Boosters were offered to patients after 4 immunotherapy treatments if there was no disease progression. Despite all patients developing strong anti-KLH antibodies, only 3 patients developed anti-MUC-1 antibodies. Eleven patients had CD8 CTL activity as assessed by chromium release assay after vaccination, but the study did not assess CTL activity prior to immunization, so it is unknown how many patients developed the CTL response from vaccination. This study suggests that breast cancer antigens may vary greatly in their immunogenicity. Novel immunologic adjuvant and vaccine constructs may be needed to generate a robust immune response to such antigens.

Protein vaccines

Peptide based vaccines have a potential disadvantage in their necessity of preclinical modeling and prediction as to the most useful immune response desired.[50] Whole protein vaccines are composed of both MHC class I and class II epitopes and are not HLA specific. Our group studied a recombinant HER-2/neu ICD protein based vaccine in a phase I clinical trial, in which patients were vaccinated after optimal cytoreductive debulking.[51] Twenty-nine subjects were enrolled onto the trial at 3 escalating doses. The protein based vaccine was well tolerated at all doses. The majority, 89%, of patients who completed all 6 vaccines developed HER2/neu specific T cell immunity. Interestingly, the dose of protein in the vaccine did not predict the percentage of patients who developed immunity, nor did it predict the magnitude of immunity. However, higher doses were associated with more rapid development of detectable immunity. Duration of immunity was not dependent on dose and persisted equally across the different doses, with over half of the assessable patients at 1 year demonstrating continued T cell specific immunity.

A pilot randomized trial of MUC-1 vaccination with protein subunit vaccines has been performed in patients with early stage disease.[52] The vaccine consisted of glutathione-S-transferase fused to 3 MUC-1 variable number of tandem repeats (VNTR), which are 20 amino acid peptides from the ECD of the MUC-1 protein, plus 2 flanking homologous sequences. Thirty-one patients with stage II breast cancer, fewer than 4 involved lymph nodes, and no evidence of disease were enrolled in a pilot randomized, double-blinded study. Patients received 7 injections of vaccine or placebo at 2-week intervals with boosters at 6 and 9 months. Antibodies were induced in 9 out of 13 participants who received vaccination. The antibody responses were initially IgM and then seroconverted to IgG, and persisted for 12 months to 24 months after immunization. No detectable antibody responses were observed in the placebo group or the pre-treatment samples of immunized patients. MUC-1 VNTR interferon-gamma (IFN-g) secreting T cells were generated in 40% of patients immunized with the MUC-1 fusion protein vaccine. In non-immunized patients, no MUC-1 VNTR specific response was found. With a follow up period ranging from 60 months to 99 months, there were no recurrences in the vaccinated group and 4 recurrences in the placebo group.

Kaplan-Meier survival curves were compared for all patients enrolled and were found to be significantly different (P = 0.029). This randomized pilot study justified investigation of MUC-1 immunotherapy in a larger population, and that trial is currently underway.

Dendritic cell vaccines

Peptide and protein vaccines need to be taken up by and processed by APCs. DCs are the immune system's most potent APCs and stimulate T lymphocytes. Autologous DCs can be modified by either fusing cancer cells, usually autologous tumor, pulsing them with peptides or transfecting them with human tumor antigen. In addition, DCs can be modified further by the addition of costimulatory molecules.

In an attempt to generate both a class I and class II response autologous dendritic cells have been pulsed with HER-2/neu and MUC-1 peptides.[38] A recent clinical trial has attempted to generate both a class I and class II response through DC vaccination. Peripheral blood mononuclear cells (PBMCs) were isolated and cultured with HER-2/neu peptide (E75) or MUC-1.[53] Ten patients were enrolled; all had breast or ovarian cancer, had been heavily pretreated and had measurable disease. Prior to collection of autologous DCs, patients were off systemic treatments and immunosuppressive drugs, including steroids, for at least 4 weeks. The vaccine was administered subcutaneously close to the inguinal nodes on days 1, 14 and 28. On day 35, an evaluation for clinical response was performed, and booster vaccinations were given every 28 days if stable disease or tumor regression was seen. The booster vaccines were stopped when there was evidence of tumor progression. The DC injections were well tolerated with minimal to no side effects. Five of 10 patients demonstrated antigen-specific T-cell responses after 3 vaccinations. Of the 10 patients enrolled, 1 had regression of her disease, another patient had stable disease and 1 patient had a short period of stabilization before development of progressive disease. The authors are appropriately wary of attributing the regression to vaccination. Safety, not clinical response, was the primary endpoint. It is, however, the rare clinical responses that demonstrate how the immune system can achieve remission in advanced disease. Also evident in this vaccination series was epitope spreading, which was seen in the one patient who demonstrated a clinical response. Further study in the minimal residual or no evidence of disease state would potentially be more efficacious for producing a clinical benefit.

In another approach, autologous DCs can be modified by transfecting them with human tumor antigen DNA. A study of MUC-1 cDNA-transfected DCs was performed in 10 patients, of which 7 had mucin expressing breast cancer and most had undergone pretreatment with surgery, radiation and/or multiple cycles of chemotherapy.[54] Patients received one million transfected DCs subcutaneously 3 times: on days 1, 21 and 42. Two of the 10 patients received only 2 vaccinations, as they died secondary to the advanced stage of their disease. A positive DTH reaction was seen in 1 patient before and after vaccination and in 2 additional patients after vaccination. Four patients showed a 2- to 10-fold increase in the frequency of IFN-g secreting CD8+ T cells after stimulation with the mucin peptide. Vaccination was well tolerated in all patients and did not produce any side effects. Nine out of 10 patients had progression of disease within 3 months of beginning vaccination. One patient remained stable for 3 months after starting vaccination until she was transferred to a different type of therapy. This trial adequately demonstrated the feasibility and safety of vaccinating patients with autologous gene-transfected DCs and confirmed that an immunologic response could be induced even in patients with advanced disease. The lack of clinical benefit is not a surprise, and the authors appropriately concluded a future study would be preferentially used in the setting of minimal residual disease.

In the first hTERT vaccination trial, seven HLA-A2 patients with advanced breast or prostate cancer were treated with autologous monocyte-derived DCs pulsed *ex vivo* with hTERT I540 peptide and KLH.[55] Patients underwent leukapheresis and PBMCs were isolated in the usual fashion before they were cultured and pulsed with peptides. The peptides included hTERT and other more immunogenic peptides, such as HIV RT-pol476 and influenza MP58, to boost the immune response. Eligible patients were administered autologous DCs every other week for up to 6 vaccinations. For each vaccination, 3 injections were given: DCs pulsed with I540 hTERT peptide, MP58 influenza peptide and RT-pol476 HIV peptide were administered with KLH and injected in distinct locations. Injections alternated, when possible, between upper and lower extremities. The 7 patients, of whom 2 had breast cancer, received a total of 34 vaccinations; the vaccination series was well tolerated. Among 6 evaluable patients, 1 mixed clinical response was observed in 1 of the 2 patients with metastatic breast cancer, whose disease was confined to multiple skin nodules on her chest wall. These lesions had, before vaccination, progressed despite chemotherapy, radiation therapy, and hormonal therapy. After vaccination,

partial tumor nodule regression was observed without the appearance of new nodules or new sites of disease. Bidimensional measurement of the largest lesion demonstrated a 60% reduction, but overall objective criteria for partial response were not met. Sequential biopsies of 1 lesion performed before and after vaccination demonstrated the induction of a predominant CD8+ lymphoid infiltrate into the tumor.

Construction of dendritic cell vaccines is a labor intensive method and not easily scaled to large production, as it is intrinsically a patient specific process. At this time, most breast cancer DC vaccine trials have been performed in patients with advanced stage disease, and they have shown that DC vaccines generate immune responses. These vaccines may have a greater role in earlier stage disease.

DNA vaccines

In an attempt to increase endogenous processing of target antigens, DNA vaccines are under study. The goal of a DNA vaccine is for the DNA plasmid to be taken up by APCs and translated into protein for endogenous presentation. This process eliminates the need to predict which peptides are most immunogenic, and yet still allows the vaccine to be stable and easily manufactured. A phase I trial of a HER2/neu specific DNA plasmid based vaccine encoding the HER2 ICD in stage III and IV breast cancer patients is currently ongoing.[56,57] This vaccine is composed of plasmid encoding the HER-2/neu ICD and GM-CSF as adjuvant. The vaccine is given intradermally monthly for a total of 3 administrations and 3 dose levels are being evaluated in this phase I study. To assess the immune response in a statistically significant way, 22 patients have been enrolled at each dose level instead of only 3 to 6 as in a typical phase I study. Forty-three subjects in the first 2 arms have been evaluated for toxicity; at the low and medium dose level, the vaccine has been found to have minimal toxicity. The majority, 62%, of patients in the low dose arm developed T-cell immunity during immunization. Analysis of the middle dose and enrollment at the high dose continues. Biopsies of the vaccine site, after completion of vaccination, demonstrated persistence of plasmid DNA in 32% of patients in the low dose arm. This persistence potentially allows a more chronic stimulation of the immune response after administration of vaccine is complete.

MUC-1 as a TAA is not limited to breast cancer, but is also overexpressed in gastrointestinal, lung and ovarian cancer. In the search for the most widely applicable, or universal, vaccine, MUC-1 has been incorporated into a vaccine consisting of MUC-1, CEA and poxviral costimulatory molecules. Each peptide by itself has been shown to be safe and immunogenic. In a pilot study of a MUC-1, CEA, poxviral based vaccine the vectors and proteins were modified to be as immunogenic as possible and were given with a triad of costimulatory molecules (TRICOM).[58] The vaccine series consisted of a prime boost regimen, in which the CEA-MUC-1-TRICOM is administered as prime vaccination engineered into vaccinia (PANVAC-V) and as a booster vaccination engineered into fowlpox (PANVAC-F). G-CSF 100 μg was administered on the day of vaccination and the subsequent three days. Twenty-five heavily pretreated patients were enrolled, all with heavily pretreated, CEA or MUC-1 expressing, progressive metastatic cancers. The vaccine was found to be safe and produced no significant systemic toxicities. Immune responses developed in 9 of 16 patients, and several patients had clinical responses. The trial was conducted in patients with a variety of malignancies, and further testing needs to be performed in disease-specific groups, including breast cancer patients.

DNA vaccines can potentially overcome the limitations of protein based vaccine stability, and yet yield a significant amount of protein *in vivo* to stimulate both CD4+ and CD8+ responses. DNA vaccines are designed to generate protein *in vivo* for processing into peptides and subsequent *in vivo* presentation. DNA vaccines are also a platform for incorporating future antigens. Furthermore, these vaccines allow for the integration of costimulatory molecules, antigens and growth factor to yield potentially more efficacious vaccines.

Conclusion

Initial cancer vaccine trials and some current clinical trials remain stymied by the classical notion of a phase I cancer trial being for patients with only advanced disease. It is apparent cancer vaccine immunotherapy requires time to induce an immune response. In addition, vaccine therapy has the greatest potential in disease where relapse is likely, but a minimal residual disease state can be achieved prior to vaccine administration. The immune system has the potential to slow cancer progression without inducing tumor regression or impacting response rate during the allotted time period for assessing response. As proliferation of substantial numbers of T cells takes time, responses may be delayed and occur over months, as has been seen with donor lymphocyte transfusions after failed transplants.[59] Modern targeted therapies, such as the tyrosine kinase inhibitors, have clearly shown TTP and OS can be improved without a RECIST response.[60] Monoclonal antibodies, a

well established immunotherapeutic modality, require the use of cytotoxic chemotherapy to achieve maximal tumor regression. However, once a state of no evidence of disease or minimal residual disease is achieved, the continuation of monoclonal antibody therapy, eg, trastuzumab and rituximab, does yield improvement in DFS.[61,62] Cancer vaccine therapy and breast cancer specific vaccines may advance more quickly if they are administered to patients in a similar manner, in the adjuvant setting. Furthermore, if a cancer vaccine is going to prevent invasive cancer, research is needed to study premalignant lesions such as DCIS, in order to discover what could be a potential advance in cancer treatment/prevention. A subset population of DCIS demonstrates HER-2/neu overexpression, and HER-2/neu vaccines are ready to be tried in less invasive stages of disease.[63] Given the overwhelming pattern of minimal toxicity seen in vaccine studies, it is appropriate to move cancer vaccines into clinical trials in patients with earlier stage disease. Cancer vaccines are making progress in human clinical trials, and we are encouraged they will one day be a standard component of breast cancer treatment.

Acknowledgment

Grant support from GSK and Hemispherex.

Disclosures

The University of Washington holds patents on the HER-2/neu vaccines on which I am the inventor, developed by MLD. Support for MLD by Gateway Foundation. Consulting for VentiRx.

References

1. Coley WB. The treatment of malignant tumors by repeated inoculations of Erysipelas: With a report of ten original cases. *Am J Med Sci*. 1893;105(5):487.
2. Goedegebuure PS, Watson MA, Viehl CT, Fleming TP. Mammaglobin-based strategies for treatment of breast cancer. *Curr Cancer Drug Targets*. 2004;4(6):531–542.
3. Burnet FM. Immunological aspects of malignant disease. *Lancet*. 1967;1(7501):1171–1174.
4. Shankaran V, Ikeda H, Bruce AT, et al. IFN gamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature*. 2001;410(6832):1107–1111. 10.1038/35074122.
5. Anderson KS. Tumor vaccines for breast cancer. *Cancer Invest*. 2009;27(4):361–368. 10.1080/07357900802574421.
6. Higano CS, Schellhammer PF, Small EJ, et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase III trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer*. 2009;115(16):3670–3679. 10.1002/cncr.24429.
7. Small EJ, Schellhammer PF, Higano CS, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J Clin Oncol*. 2006;24(19):3089–3094. 10.1200/JCO.2005.04.5252.
8. Schellhammer PF, Higano CS, Berger ER, et al. A randomized, double-blind, placebo-controlled, multi-center, phase III trial of sipuleucel-T in men with metastatic, androgen independent prostatic adenocarcinoma (AIPC). *American Urological Association, Annual Meeting*. 2009.
9. Solal-Celigny P, Roy P, Colombat P, et al. Follicular lymphoma international prognostic index. *Blood*. 2004;104(5):1258–1265. 10.1182/blood-2003-12-4434.
10. Schuster SJ, Neelapu SS, Gause BL, et al. Idiotype vaccine therapy (BiovaxID) in follicular lymphoma in first complete remission: phase III clinical trial results. *[Meeting Abstracts] J Clin Oncol*. 2009;27 Suppl 18:2.
11. Rosenberg SA, Yang JC, White DE, Steinberg SM. Durability of complete responses in patients with metastatic cancer treated with high-dose interleukin-2: identification of the antigens mediating response. *Ann Surg*. 1998;228(3):307–319.
12. Rosenberg SA, Yang JC, Schwartzentruber DJ, et al. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nat Med*. 1998;4(3):321–327.
13. Schwartzentruber DJ, Lawson D, Richards J, et al. A phase III multi-institutional randomized study of immunization with the gp100: 209–217(210M) peptide followed by high-dose IL-2 compared with high-dose IL-2 alone in patients with metastatic melanoma. *J Clin Oncol*. 2009;27 Suppl 18.
14. Emens LA, Asquith JM, Leatherman JM, et al. Timed sequential treatment with cyclophosphamide, doxorubicin, and an allogeneic granulocyte-macrophage colony-stimulating factor-secreting breast tumor vaccine: a chemotherapy dose-ranging factorial study of safety and immune activation [Breast Cancer]. *Journal of Clinical Oncology*. 2009;27(35):5911–5918. http://jco.ascopubs.org. 10.1200/JCO.2009.23.3494
15. Disis ML, Shiota FM, Cheever MA. Human HER-2/neu protein immunization circumvents tolerance to rat neu: a vaccine strategy for 'self' tumour antigens. *Immunology*. 1998;93(2):192–199.
16. Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res*. 2009;15(17):5323–5337. 10.1158/1078-0432.CCR-09-0737.
17. Bargmann CI, Hung MC, Weinberg RA. The neu oncogene encodes an epidermal growth factor receptor-related protein. *Nature*. 1986;319(6050):226–230. 10.1038/319226a0.
18. Coussens L, Yang-Feng TL, Liao YC, et al. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science*. 1985;230(4730):1132–1139.
19. Press MF, Cordon-Cardo C, Slamon DJ. Expression of the HER-2/neu proto-oncogene in normal human adult and fetal tissues. *Oncogene*. 1990;5(7):953–962.
20. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science*. 1989;244(4905):707–712.
21. Disis ML, Cheever MA. HER-2/neu protein: a target for antigen-specific immunotherapy of human cancer. *Adv Cancer Res*. 1997;71:343–371.
22. Vogel CL, Cobleigh MA, Tripathy D, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol*. 2002;20(3):719–726.
23. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med*. 2005;353(16):1673–1684. 10.1056/NEJMoa052122.
24. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med*. 2005;353(16):1659–1672. 10.1056/NEJMoa052306.
25. Gendler SJ, Cohen EP, Craston A, Duhig T, Johnstone G, Barnes D. The locus of the polymorphic epithelial mucin (PEM) tumour antigen on chromosome 1q21 shows a high frequency of alteration in primary human breast tumours. *Int J Cancer*. 1990;45(3):431–435.

26. Patton S, Gendler SJ, Spicer AP. The epithelial mucin, MUC1, of milk, mammary gland and other tissues. *Biochim Biophys Acta*. 1995;1241(3):407–423.
27. van der Vegt B, de Roos MA, Peterse JL, et al. The expression pattern of MUC1 (EMA) is related to tumour characteristics and clinical outcome of invasive ductal breast carcinoma. *Histopathology*. 2007;51(3): 322–335. 10.1111/j.1365-2559.2007.02757.x.
28. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T Cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med*. 2003;348(3):203–213. 10.1056/NEJMoa020177.
29. von Mensdorff-Pouilly S, Verstraeten AA, Kenemans P, et al. Survival in early breast cancer patients is favorably influenced by a natural humoral immune response to polymorphic epithelial mucin. *J Clin Oncol*. 2000;18(3):574–583.
30. Liu J, Chen W, Schwarer AP, Li H. Telomerase in cancer immunotherapy. *Biochimica et Biophysica Acta (BBA) – Reviews on Cancer*. [In Press] DOI: 10.1016/j.bbcan.2009.09.001.
31. Kim N, Piatyszek M, Prowse K, et al. Specific association of human telomerase activity with immortal cells and cancer. *Science*. 1994;266(5193):2011–2015. 10.1126/science.7605428.
32. Domchek SM, Recio A, Mick R, et al. Telomerase-specific T-cell immunity in breast cancer: effect of vaccination on tumor immunosurveillance. *Cancer Res*. 2007;67(21):10546–10555. 10.1158/0008-5472.CAN-07-2765.
33. Peoples GE, Gurney JM, Hueman MT, et al. Clinical trial results of a HER2/neu (E75) vaccine to prevent recurrence in high-risk breast cancer patients. *J Clin Oncol*. 2005;23(30):7536–7545. 10.1200/JCO.2005.03.047.
34. Peoples GE, Holmes JP, Hueman MT, et al. Combined clinical trial results of a HER2/neu (E75) vaccine for the prevention of recurrence in high-risk breast cancer patients: U.S. Military cancer institute clinical trials group study I-01 and I-02. *Clin Cancer Res*. 2008;14(3):797–803. 10.1158/1078-0432.CCR-07-1448.
35. Benavides LC, Gates JD, Carmichael MG, et al. The impact of HER2/neu expression level on response to the E75 vaccine: from U.S. Military cancer institute clinical trials group study I-01 and I-02. *Clin Cancer Res*. 2009;15(8):2895–2904. 10.1158/1078-0432.CCR-08-1126.
36. Nahta R, Yu D, Hung M, Hortobagyi GN, Esteva FJ. Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. 2006;3(5):280. http://dx.doi.org/10.1038/ncponc0509
37. Koski GK, Cohen PA, Roses RE, Xu S, Czerniecki BJ. Reengineering dendritic cell-based anti-cancer vaccines. *Immunol Rev*. 2008;222: 256–276. 10.1111/j.1600-065X.2008.00617.x.
38. Czerniecki BJ, Koski GK, Koldovsky U, et al. Targeting HER-2/neu in early breast cancer development using dendritic cells with staged interleukin-12 burst secretion. *Cancer Res*. 2007;67(4):1842–1852. 10.1158/0008-5472.CAN-06-4038.
39. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst*. 2000;92(3):205–216. 10.1093/jnci/92.3.205.
40. Knutson KL, Schiffman K, Cheever MA, Disis ML. Immunization of cancer patients with a HER-2/neu, HLA-A2 peptide, p369-377, results in short-lived peptide-specific immunity. *Clin Cancer Res*. 2002;8(5):1014–1018.
41. Disis ML, Gooley TA, Rinn K, et al. Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. *J Clin Oncol*. 2002;20(11):2624–2632.
42. Disis ML, Cheever MA. HER-2/neu oncogenic protein: issues in vaccine development. *Crit Rev Immunol*. 1998;18(1–2):37–45.
43. Salazar LG, Coveler AL, Swensen RE, et al. Kinetics of tumor-specific T-cell response development after active immunization in patients with HER-2/neu overexpressing cancers. *Clin Immunol*. 2007;125(3):275–280. 10.1016/j.clim.2007.08.006.
44. Disis ML, Grabstein KH, Sleath PR, Cheever MA. Generation of immunity to the HER-2/neu oncogenic protein in patients with breast and ovarian cancer using a peptide-based vaccine. *Clin Cancer Res*. 1999;5(6):1289–1297.
45. Salazar LG, Goodell V, O'Meara M, et al. Persistent immunity and survival after immunization with a HER2/neu (HER2) vaccine. *J Clin Oncol (Meeting Abstracts)*. 2009;27(15S):3010.
46. Disis ML, Wallace DR, Gooley TA, et al. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. *J Clin Oncol*. 2009:27(28) 10.1200/JCO.2008.20.6789.
47. Coveler AL, Goodell V, Webster DJ, et al. Common adjuvant breast cancer therapies do not inhibit cancer vaccine induced T cell immunity. *Breast Cancer Res Treat*. 2009;113(1):95–100. 10.1007/s10549-008-9910-y.
48. Goydos JS, Elder E, Whiteside TL, Finn OJ, Lotze MT. A phase I trial of a synthetic mucin peptide vaccine: induction of specific immune reactivity in patients with adenocarcinoma. *J Surg Res*. 1996;63(1):298–304. DOI: 10.1006/jsre.1996.0264.
49. Reddish MA, MacLean GD, Koganty RR, et al. Anti-MUC-1 class I restricted CTLs in metastatic breast cancer patients immunized with a synthetic MUC-1 peptide. Int J Cancer 1998;76(6):817–823. http://dx.doi.org/10.1002/(SICI)1097-0215(19980610)76:6<817::AID-IJC9>3.0.CO;2-0. 10.1002/(SICI)1097-0215(19980610)76:6<817::AID-IJC9>3.0.CO;2-0
50. Disis ML, Knutson KL, McNeel DG, Davis D, Schiffman K. Clinical translation of peptide-based vaccine trials: the HER-2/neu model. *Crit Rev Immunol*. 2001;21(1–3):263–273.
51. Disis ML, Schiffman K, Guthrie K, et al. Effect of dose on immune response in patients vaccinated with an her-2/neu intracellular domain protein – based vaccine. *J Clin Oncol*. 2004;22(10):1916–1925. 10.1200/JCO.2004.09.005.
52. Apostolopoulos V, Pietersz GA, Tsibanis A, et al. Pilot phase III immunotherapy study in early-stage breast cancer patients using oxidized mannan-MUC-1 [ISRCTN71711835]. *Breast Cancer Res*. 2006;8(3):R27. 10.1186/bcr1505.
53. Brossart P, Wirths S, Stuhler G, Reichardt VL, Kanz L, Brugger W. Induction of cytotoxic T-lymphocyte responses in vivo after vaccinations with peptide-pulsed dendritic cells. *Blood*. 2000;96(9): 3102–3108.
54. Pecher G, Haring A, Kaiser L, Thiel E. Mucin gene (MUC1) transfected dendritic cells as vaccine: results of a phase I/II clinical trial. *Cancer Immunol Immunother*. 2002;51(11–12):669–673. 10.1007/s00262-002-0317-z.
55. Vonderheide RH, Domchek SM, Schultze JL, et al. Vaccination of cancer patients against telomerase induces functional antitumor CD8+ T lymphocytes. *Clin Cancer Res*. 2004;10(3):828–839.
56. Salazar LG, Slota M, Wallace D, et al. A phase I study of a DNA plasmid based vaccine encoding the HER2/neu (HER2) intracellular domain (ICD) in subjects with HER2+ breast cancer. *J Clin Oncol (Meeting Abstracts)*. 2009;27 Suppl 15:3054.
57. Salazar LG, Coveler A, Fintak PA, et al. Low dose of a plasmid DNA vaccine encoding the HER-2/neu (HER2) intracellular domain (ICD) effectively stimulate tumor specific immunity. *San Antonio Breast Cancer Symposium*. 2006;100 Suppl 1.
58. Gulley JL, Arlen PM, Tsang KY, et al. Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. *Clin Cancer Res*. 2008;14(10):3060–3069. 10.1158/1078-0432.CCR-08-0126.
59. Collins R Jr, Shpilberg O, Drobyski W, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol*. 1997;15(2):433–444. http://jco.ascopubs.org/cgi/content/abstract/15/2/433
60. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359(4):378–390. 10.1056/NEJMoa0708857.

61. Vidal L, Gafter-Gvili A, Leibovici L, et al. Rituximab Maintenance for the Treatment of Patients With Follicular Lymphoma: Systematic Review and Meta-analysis of Randomized Trials. *J Natl Cancer Inst*. 2009;101(4):248–255. 10.1093/jnci/djn478; http://jnci.oxfordjournals.org/cgi/content/abstract/jnci;101/4/248
62. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of Chemotherapy plus a Monoclonal Antibody against HER2 for Metastatic Breast Cancer That Overexpresses HER2. *N Engl J Med*. 2001;344(11):783–792. 10.1056/NEJM200103153441101.
63. Czerniecki BJ, Roses RE, Koski GK. Development of vaccines for high-risk ductal carcinoma in situ of the breast. *Cancer Res*. 2007; 67(14):6531–6534. 10.1158/0008-5472.CAN-07-0878.