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Clinical Development of the E75 Vaccine in Breast Cancer

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Summary

E75 is an immunogenic peptide derived from the human epidermal growth factor receptor 2 (HER2) protein. A large amount of preclinical work evaluated the immunogenicity of E75, after which phase I trials investigated using E75 mixed with an immunoadjuvant as a vaccine. Those studies showed the vaccine to be safe and capable of stimulating an antigen-specific immune response. Subsequent to that, our group conducted trials evaluating E75 + granulocyte macrophage colony-stimulating factor (GM-CSF) in the adjuvant setting. The studies enrolled node-positive and high-risk node-negative breast cancer patients, with the goal being to determine if vaccination could decrease the recurrence risk. The studies included 187 evaluable patients: 108 vaccinated ones and 79 controls. The 5-year disease-free survival for the vaccinated patients was 89.7% compared to 80.2% for the control patients, a 48% reduction in relative risk of recurrence. Based on these data, E75 + GM-CSF, now known as NeuVax™, is being evaluated in a phase III trial. In this article, we review preclinical data and results of the early-phase trials and provide an update on the ongoing phase III study. We also present additional strategies for employing the vaccine to be included as a component of combination immunotherapy as well as in the setting of ductal carcinoma in situ as an initial step towards primary prevention.

Introduction

Since its discovery over 30 years ago, human epidermal growth factor receptor 2 (HER2) has been one of the most studied oncogenes in cancer [1, 2]. It is a membrane-bound tyrosine kinase that is overexpressed in approximately 20% of breast cancers [3]. HER2 activation in these tumors creates a cascade of downstream signaling that drives proliferation, angiogenesis, invasion, and survival [4, 5]. Left untreated, HER2-positive tumors are highly aggressive. Fortunately, the development of HER2-targeted therapy has improved the prognosis for patients with HER2-positive breast cancer. Trastuzumab, a fully humanized immunoglobulin G1 (IgG1) monoclonal antibody that targets HER2, has improved the prognosis in HER2-positive breast cancer in both early and advanced disease [6, 7]. A second monoclonal antibody, pertuzumab, which blocks HER2 signaling by binding at a different site from trastuzumab, has been shown to improve survival when used concurrently with trastuzumab and docetaxel in HER2-positive metastatic breast cancer [8]. The addition of pertuzumab to trastuzumab and docetaxel administered in the neoadjuvant setting resulted in increased rates of pathologic complete response [9].

The monoclonal antibodies trastuzumab and pertuzumab are forms of passive immunotherapy. In addition to targeting HER2 with these antibodies, there has been significant interest in investigating HER2 as a target for active immunotherapy, specifically, vaccines. Cancer vaccines aim to stimulate an individual's immune system to recognize tumor-associated antigens (TAAs) that are unique to or overexpressed in cancer cells. Stimulated T cells can then recognize and attack tumor cells, resulting in tumor cell destruction which exposes the immune effector cells to additional TAAs unique to that tumor, leading to a broader antitumor immune response [10]. Cancer vaccines are an appealing therapeutic strategy because they are specific with minimal toxicity. HER2-

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targeted cancer vaccination strategies have included single- and multiple-peptide vaccines, dendritic cell vaccines, and, more recently, a modified whole-tumor cell vaccine [11–14]. The vaccine that is furthest along in development is NeuVax™, which is a major histocompatibility complex (MHC) class I vaccine that consists of the HER2-derived peptide E75 (nelipepimut-S) combined with the immunoadjuvant granulocyte macrophage colony-stimulating factor (GM-CSF). NeuVax™ is currently being evaluated in a phase III registration trial.

This review will focus on the clinical development of NeuVax™, highlighting the early clinical trials, updating the current status of ongoing studies, and discussing promising future directions.

Peptide-Based Breast Cancer Vaccines

Preclinical Evaluation of E75

E75 (HER2/neu 369–377: KIFGSLAFL) is a 9-amino acid peptide derived from the extracellular domain of the HER2 protein. It was identified as the immunodominant HER2 epitope due to predicted binding to human leukocyte antigen (HLA)-A2. Several studies confirmed that E75 stably binds to HLA-A2 and can stimulate T cells in vitro to lyse HER2-expressing cancer cells [15–17]. In vivo experiments confirmed that E75-pulsed cytotoxic T lymphocytes (CTL) were able to lyse HER2-expressing colon carcinoma and renal cell carcinoma cells in murine models [18, 19]. Furthermore, dendritic cells derived from healthy HLA-A2+ volunteers stimulated with E75 were shown to be capable of priming autologous peripheral blood mononuclear cells (PBMCs) to generate measurable E75-specific T cells [11, 20]. Additional preclinical work identified innate immune responses against E75 in tumor-associated lymphocytes and circulating lymphocytes in patients with a variety of tumor types [17, 19, 21–24]. Based on these preclinical data, there was significant interest in investigating E75 as a cancer vaccine.

E75 for Advanced Disease

One early trial evaluating the E75 peptide for its ability to stimulate an antitumor immune response involved a dendritic cell vaccine formulation. The trial enrolled 6 patients with metastatic breast and ovarian cancer and treated them with autologous dendritic cells pulsed with E75 as well as GP2, a second HER2-derived immunogenic epitope. Vaccination produced a lytic CTL response, particularly to E75, and epitope spreading, providing evidence of a broader immunologic response to other TAAs not included in the vaccine formulation [11].

Other early studies evaluating E75 as a cancer vaccine investigated a simpler approach combining the peptide with incomplete Freund's adjuvant. These trials enrolled patients with metastatic breast, ovarian, and colorectal cancer. While vaccinated patients expanded their levels of circulating E75-specific CTLs, further testing showed that these cells displayed an anergic phenotype [24]. Subsequent studies have raised the possibility that incomplete Freund's adjuvant may cause CTL overstimulation, which could have contributed to these results [25].

E75 combined with GM-CSF as an alternative immunoadjuvant was also evaluated in a small trial that enrolled 6 women with advanced breast and ovarian cancer. Inoculations were administered as 6-monthly intradermal injections of 500 μg E75 with 125 μg GM-CSF. An immunologic response, defined as the induction of HER2 peptide-specific interferon-gamma (IFN-γ)-producing CD8+ T cells, was detected in 2 of 4 evaluable patients; however, the responses were of low magnitude and were short lived, i.e. no longer detectable 5 months after completing vaccination [26]. E75 + GM-CSF was tested in a second phase I dose escalation study in 14 patients with pretreated metastatic breast (n = 13) or ovarian (n = 1) cancer. Patients received 100, 500, or 1000 μg E75 with 250 μg GM-CSF immunoadjuvant, administered by intradermal injection weekly for 4 weeks, followed by monthly inoculations for a total of 10 inoculations. The inoculations were well tolerated without dose-limiting toxicity. The majority of vaccinated patients demonstrated an in vivo immunologic response, as measured using a delayed-type hypersensitivity reaction and an in vitro immune response determined by the enumeration of IFN-γ-producing CD8+ T cells in peripheral blood samples. Clinical outcomes were not reported [27].

E75 for Secondary Prevention

While these initial clinical trials evaluating E75 vaccines showed favorable safety and immunologic results, clinical responses to vaccination were either not reported or not seen in these heavily pretreated metastatic patients. This is consistent with a report from the National Cancer Institute detailing their early experience with inoculating patients with metastatic melanoma or metastatic ovarian cancer with single-epitope vaccines. In a series of studies that enrolled approximately 380 patients in total, the reported objective response rate was approximately 3% [28]. There is now a robust body of literature showing that the metastatic microenvironment is immunosuppressive, providing a possible rationale for the lack of efficacy for peptide vaccines in that setting. Given this, subsequent development of the E75 peptide vaccine focused on secondary prevention, i.e. immunizing patients in the adjuvant setting, with the goal being to prevent disease recurrence.

Phase I/II Trials

Our group subsequently initiated 2 early-phase clinical trials evaluating the safety and efficacy of E75 + GM-CSF administered in the adjuvant setting. The first study, which enrolled node-positive breast cancer patients, was designed as a standard dose escalation study. A second trial, which was conducted as a dose and schedule optimization study, enrolled high-risk node-negative patients. In both trials, patients with tumors expressing any degree of HER2 (1+ to 3+ by immunohistochemistry (IHC)) could participate. All patients were disease free at the time of enrollment, having completed

standard-of-care therapy including surgery, chemotherapy, and, when indicated, radiation. Patients on endocrine therapy continued on that treatment. Upon enrollment, patients had their HLA-A2 status determined and those who were HLA-A2+ were vaccinated while HLA-A2– patients were followed prospectively as controls. Patients were given escalating doses of E75 with GM-CSF immunoadjuvant monthly for 4 or 6 months. The vaccine series was well tolerated at all dose levels, with minimal toxicity [29]. Both trials showed that the vaccine can stimulate an antigen-specific immune response, and there was encouraging efficacy data; therefore, the studies were transitioned into phase II trials.

The trials ultimately enrolled 195 patients (100 node-positive, 95 node-negative patients) who were followed for 60 months, with the primary endpoint of disease-free survival (DFS). At the time of study completion, there were 187 evaluable patients: 108 patients who were vaccinated and 79 followed as unvaccinated controls. The vaccine and control groups were well matched for demographic and prognostic features, with the exception that the vaccinated group had a higher percentage of hormone receptor-negative patients [14]. Because hormone receptor-negative patients are not administered endocrine therapy, which we know decreases the risk of recurrence, it has been suggested that this actually may have biased against the vaccine. This larger trial confirmed that the vaccine is well tolerated, with generally mild toxicities. Local toxicities, most commonly injection site erythema and pruritus, were all of grade 1 (83.3%) and grade 2 (16.7%). Systemic toxicities, most commonly bone pain, influenza-like symptoms, and fatigue, were mild. Only 1.9% experienced grade 3 toxicities, and no grade 4 or 5 toxicities were observed. The 5-year DFS for vaccinated patients was 89.7% compared to 80.2% for control patients ($p = 0.08$), giving the vaccine a 48% reduction in relative risk of recurrence [14].

During the conduct of the trials, several modifications were made to the protocols, based on new data that became available both from planned interim analyses and from additional preclinical work. First, the optimal biologic dose was determined to be the maximal dose administered (1000 μg E75 with 250 μg GM-CSF). Higher doses of peptide could not be solubilized for intradermal administration. Patients at this dosing level had similar toxicity profiles with improved immunologic response to vaccination when compared to patients receiving a lower dose of the vaccine [30]. Additionally, based on preclinical data suggesting that E75 can bind to HLA-A3 and that HLA-A3+ cancer patients have high rates of immunity to E75, HLA-A3+ patients were enrolled. The inclusion of HLA-A3+ patients expanded the percentage of eligible patients to 60–75% of the population [23]. In total, 13 HLA-A3+ patients were included in the vaccine group while HLA-A2/A3– patients continued to be followed as controls. The vaccinated HLA-A3+ patients experienced similar toxicities to HLA-A2+ patients and developed comparable immunologic responses, as assessed by delayed-type hypersensitivity reactions. HLA-A3+ patients also had a similar 5-year DFS (92.3%) as HLA-A2+ vaccinated patients [31]. Finally, with longer follow-up, recurrences were noted several years after completion of the vaccine series, corresponding with waning immunity in some patients. A program of voluntary booster inoculations every 6 months after completion of the primary vaccine series was therefore initiated. The booster inoculations were well tolerated and effective in maintaining immunity [30].

Predefined subgroups that benefited most from vaccination included the 37 patients who received the optimal biologic dose (5-year DFS 94.6%, $p = 0.05$ compared to DFS = 80.2% in unvaccinated controls), and the 21 patients who received boosted inoculations starting 6 months after completing the primary vaccine series (5-year DFS 95.2%, $p = 0.11$) [14]. These findings suggest that using the optimal dose of vaccine to promote greater E75 immunity over a longer period may lead to greater clinical benefit. Another subgroup that derived greater benefit from vaccination comprised those patients with low-grade (grade 1 or 2) breast cancer. The 5-year DFS rate was 96.7% (59/61) in low-grade vaccinated patients, compared with 80.9% in low-grade control patients, a relative risk reduction of 84% ($p = 0.01$) [32]. This finding provides evidence that active immunotherapy may be most effective in less aggressive cancer subtypes [33].

An important aspect of this trial is that it enrolled patients with any level of HER2 expression (IHC 1+, 2+, and 3+). Interestingly, patients with low-HER2-expressing tumors (IHC 1+ or 2+) had the most robust immune responses [34]. They also appeared to derive benefit from vaccination, with an 88.1% 5-year DFS in vaccinated patients compared to 77.5% in well-matched controls ($p = 0.16$), a relative risk reduction of 48% [14].

Phase III Trial

Based on the encouraging early-phase trial data, E75 + GM-CSF is currently being evaluated in a phase III registration trial. The PRESENT trial (NCT01479244), which completed accrual in April 2015, enrolled HLA-A2+/A3+, node-positive, HER2 IHC 1+, 2+ breast cancer patients who were clinically disease free after completion of standard therapy. Patients have been randomized to receive the E75 + GM-CSF combination or GM-CSF alone. All patients will receive a 6-inoculation primary series followed by booster inoculations every 6 months through 3 years. The trial's primary endpoint is 3-year DFS.

Combination Immunotherapy: Trastuzumab + Vaccine

Preclinical data has indicated that there may be synergistic activity if passive immunotherapy (monoclonal antibodies) is combined with active immunotherapy (cancer vaccination). Jaffee and colleagues conducted a series of laboratory studies using a HER2/neu-transgenic mouse model where they showed that both cellular and humoral anti-neu immune responses are necessary to eliminate HER2/neu-expressing tumors [35–37]. Park et al. [38] confirmed in a murine model that anti-HER2 antibody-induced tumor regression is T cell dependent. Additionally, because trastuzumab is an IgG antibody with a conserved Fc portion, antibody-dependent cellular cytotoxicity mediated by natural killer cells is a known mechanism of action. Antibody-dependent cellular cytotoxicity

causes tumor cell lysis with subsequent release of antibody-coated tumor antigens, which are taken up by dendritic cells and presented on MHC class I molecules by a process known as cross-presentation. Trastuzumab therefore effectively turns the tumor into a vaccine [39].

Additional clinical evidence suggests potential synergy between trastuzumab and a CD8+ T cell-eliciting vaccine. In a small study of metastatic HER2-positive breast cancer patients receiving trastuzumab, Taylor et al. [40] showed the generation of HER2-specific CD4+ T cell responses as well as anti-HER2 antibody responses that increased significantly during therapy and were associated with improved clinical response. In a study that included patients treated on the trastuzumab arm of the North Central Cancer Treatment Group 9831 adjuvant therapy trial, Knutson et al. [41] showed HER2-specific antibody responses that again correlated with survival outcomes.

Potential synergy between trastuzumab and a CD8+ T cell-eliciting vaccine is further supported by observations from our early-stage E75 trials. While those trials were initiated before trastuzumab became standard-of-care therapy in the adjuvant setting, during the course of the study, data demonstrating benefit of trastuzumab in these patients was published and practice changed. Therefore, 12 HER2 IHC 3+ patients enrolled in the study received trastuzumab as part of their standard-of-care therapy, which was then followed by vaccination. After 5 years of follow-up, there were no recurrences in any of these patients [42]. Our group has also conducted phase I and II clinical trials evaluating GP2, a second MHC class I peptide derived from the HER2 protein that stimulates a CD8+ T cell response [43, 44]. In a per-treatment analysis of the randomized phase II trial comparing GP2 + GM-CSF-vaccinated patients to control patients inoculated with GM-CSF alone, HER2-overexpressing patients vaccinated after receiving trastuzumab (n = 48) had a 100% DFS compared to 89% DFS in similar control patients (n = 50) (p = 0.08) at a median follow-up of 34 months [44]. Based on these results, our group has begun investigating combination immunotherapy strategies administering the vaccine and trastuzumab concurrently. In a phase I study, we have shown the combination to be safe without cardiac toxicity in a phase I trial [45]. This is consistent with a study from Disis et al. [46] demonstrating that concurrent treatment with trastuzumab and a vaccine designed to elicit a HER2-specific helper T cell response was safe in patients with metastatic HER2-positive breast cancer.

Having demonstrated the safety of administering an MHC class I, CD8+ T cell-eliciting vaccine in combination with trastuzumab, we are currently evaluating this strategy in 2 phase II adjuvant therapy trials. One of these studies (NCT02297698) is enrolling high-risk HER2+ breast cancer patients and randomizing them to trastuzumab alone, which is standard of care, or trastuzumab plus the E75 + GM-CSF vaccine. For this study, 'high risk' is defined as patients with HER2+ breast cancer who receive neoadjuvant therapy including HER2-targeted therapy and who do not achieve a complete response, or, for those undergoing surgery as an initial intervention, those with nodal disease (any nodal disease for hormone receptor-negative tumors, ≥ 4 positive lymph nodes for patients with hormone receptor-positive tumors). The second study (NCT01570036) is enrolling patients with HER2 IHC 1+ or 2+ tumors, a group that does not receive HER2-targeted therapy as part of their standard treatment, and randomizing to trastuzumab versus trastuzumab plus the E75 + GM-CSF vaccine.

E75 for Primary Prevention

The studies discussed above have been designed to determine the efficacy of the E75 + GM-CSF vaccine for secondary prevention, i.e. prevention of disease recurrence in those patients already diagnosed and treated. There is significant interest in further investigating cancer vaccines for primary prevention, a concept some have referred to as immunoprevention. It is accepted that the immune system, specifically tumor antigen-specific T cells, can eliminate nascent tumor cells through a process referred to as immunosurveillance. Tumor cells that escape detection and elimination become clinical disease [47]. Escape can be facilitated by many processes including the loss of MHC class I expression and T cell anergy, expression of T cell-inhibitory receptors, and immunosuppressive mechanisms within the tumor microenvironment including regulatory T cells and myeloid-derived suppressor cells [48–54]. Given this, it has been hypothesized that strengthening the immunosurveillance by vaccination prior to cancer occurrence would favor cancer elimination [48–54]. This model is comparable to that used for infectious disease. In fact, for tumors that are largely virally induced, such as cervical cancer caused by human papillomavirus infection and hepatocellular carcinoma caused by hepatitis B infection, there are Food and Drug Administration-approved vaccines that could potentially guard against the development of these cancers.

The majority of cancers are not virally induced. For those malignancies, vaccines against antigens on tumor cells targeted by the immune system should be developed. A strategy outlined by Olivera Finn is to initially evaluate prophylactic vaccines to boost or prime the immune response targeting premalignant lesions, to prevent recurrence or progression to cancer. As an example, her group has completed a trial in which patients with a recent diagnosis and removal of advanced colonic adenomas were inoculated with a vaccine containing the mucin-1 (MUC1) tumor antigen [55]. In that study, vaccination was successful in inducing immune responses in approximately 50% of patients. In breast cancer, ductal carcinoma in situ (DCIS) represents a premalignant lesion that could be targeted with vaccination. In a trial reported by Sharma et al. [56], patients with DCIS were vaccinated prior to surgical resection of their tumor with autologous dendritic cells that were pulsed with a combination of peptides, including 6 MHC class II HER2-derived peptides and, for HLA-A2-positive patients, E75 and a second HER2-derived MHC class I peptide. The study, which enrolled 27 patients, showed the vaccine to be safe and effective at stimulating an E75-specific CTL response. 5 of the patients had no evidence of disease in their resected specimen. In 11 of the remaining 22 patients, HER2 expression was eradicated in the resected specimen following vaccination.

Recognizing the complexity and challenges of a dendritic cell vaccine approach, our group has recently designed a study evaluating the E75 + GM-CSF peptide vaccine in DCIS. The study will enroll 48 patients who will be randomized 2:1 to vaccine or GM-CSF alone groups. Patients will receive 3 inoculations prior to surgery and then complete the final 3 inoculations of the 6-shot vaccination series in the post-operative period. The study's primary objective is to evaluate for E75-specific CTLs in vaccinated patients compared to patients receiving GM-CSF alone. There are several secondary objectives including evaluation of the extent of HER2 expression and the degree of lymphocyte infiltration in the surgically resected specimen. In addition, we will evaluate for epitope spreading by looking for the presence of CTLs specific for other HER2-derived epitopes as well as other tumor antigens in the patients' peripheral blood. Epitope spreading, which would represent a broadening of the immune response, is of particular interest as we have postulated that this is one reason that a simple strategy such as vaccinating with a single epitope may have clinical benefit. The trial is scheduled to begin accrual in the spring of 2016.

Conclusion

Because cancer vaccines offer the promise of a very specific long-term antitumor immune response, their development continues to be of great interest. The E75 + GM-CSF vaccine represents a simple approach combining an immunogenic peptide with an immunoadjuvant that could be administered as an off-the-shelf therapy. It is likely that this vaccine will be most effective in patients with minimal disease burden, and an ongoing phase III trial will determine its effectiveness when administered in the adjuvant setting to prevent disease recurrence. Ongoing studies will determine if the vaccine's efficacy can be enhanced by combining it with trastuzumab or, alternatively, if the effect of trastuzumab is enhanced by the T cell response generated by vaccination. Although not discussed in this review, one might hypothesize that vaccinating patients with E75 + GM-CSF prior to administering a checkpoint blockade agent, such as an anti-CTLA-4, anti-PD-1 or anti-PD-L1 antibody (CTLA-4 = cytotoxic T lymphocyte antigen 4, PD-1 = programmed cell death protein 1, PD-L1 = programmed death ligand 1), may induce the necessary T cell response to make those immunotherapeutic agents more effective. Evaluation of such a strategy in patients with metastatic disease is warranted. Similarly, evaluation of the vaccine in patients with premalignant disease as a next step towards a truly preventive vaccine is also warranted.

Disclosure Statement

Dr. Peoples has inventor rights to E75. This vaccine has been licensed for commercial development. He is entitled to financial proceeds associated with this license per federal policy. Dr. Peoples also consults in the development of the vaccine. All remaining authors have declared no conflicts of interest.

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