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Initial safety analysis of a randomized phase II trial of nelipepimut-S + GM-CSF and trastuzumab compared to trastuzumab alone to prevent recurrence in breast cancer patients with HER2 low-expressing tumors

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ABSTRACT

The development of HER2-targeted therapy has decreased recurrence rates and improved survival, transforming the natural history of HER2-positive breast cancer. However only a minority of breast cancer patients benefit as these agents are not used in patients with tumors expressing low levels of HER2. Preclinical data suggests a synergistic action of HER2-targeted vaccination with trastuzumab. We report the initial safety interim analysis of a phase II trial that enrolled patients with HER2 low-expressing (IHC 1 + /2 +) breast cancer who were clinically disease-free after standard therapy. Patients were randomized to receive the HER2-peptide vaccine nelipepimut-S + GM-CSF with trastuzumab (vaccine arm) or trastuzumab + GM-CSF (control arm) and were followed for recurrence. A planned analysis that occurred after enrollment of 150 patients showed no significant differences in toxicity between the two arms, including cardiac toxicity. The clinical efficacy of this combination will be reported 6 months after the final patient was enrolled.

1. Introduction

The FDA approval of trastuzumab 20 years ago transformed the treatment and prognosis of HER2 over-expressing breast cancer. Trastuzumab improves survival in metastatic breast cancer and, in the adjuvant setting, decreases recurrence rates and improves survival in patients with earlier stage disease [1–3]. Although HER2 is expressed at some detectable level by immunohistochemistry (IHC) in approximately 70–80% of breast cancers [4], trastuzumab is currently only approved for the approximately 15–20% of patients who have HER2 positive tumors [5]. HER2 positivity has recently been redefined as 3 + by IHC staining, 2 + by IHC staining with single probe ISH average HER2 copy number > 6.0 signals per cell, or 2 + by IHC staining with dual probe staining ISH HER2/CEP17 ratio > 2.0 and average HER2

Abbreviations: IHC, immunohistochemistry; DFS, Disease-free survival; NSABP, the National Surgical Breast and Bowel Project; GM-CSF, granulocyte-macrophage colony-stimulating factor; ISH, in-situ hybridization; CEP17, chromosome enumeration probe 17; CTL, cytotoxic T lymphocytes; LVEF, left ventricular ejection fraction; ECHO, echocardiogram; MUGA, multigated acquisition scan; AE, adverse events; SAE, significant adverse events; PBMC, peripheral blood mononuclear cells

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copy number > 4.0 signals per cell [6]. Thus, the majority of patients with breast cancer have tumors that express some level of HER2, but do not meet the criteria for receipt of HER2-directed therapy. Patients with lower levels of HER2 expression who also do not express hormone receptors (triple negative breast cancer patients) also do not receive endocrine therapy and have a worse prognosis overall.

Retrospective evidence from two early trials of trastuzumab showed possible benefit of treatment in patients with tumors expressing low levels of HER2 (IHC 1+ or 2+), leading to a large phase III trial conducted by the National Surgical Breast and Bowel Project (NSABP) of trastuzumab in these patients [7,8]. The results of this study were recently presented and showed no benefit for the addition of trastuzumab to chemotherapy for breast cancer patients with low HER2-expressing tumors defined as IHC 1+/2 and ISH ratio of HER2:CEP17 < 2.0 or HER2 gene copy number < 4/nucleus [9]. The trial results have not yet been published however it is generally accepted that these results support current practice to not administer trastuzumab to patients with low HER2-exprssing tumors.

Cancer vaccines have the potential of offering an additional targeted option in breast cancer. Various approaches of targeting HER2 with active immunotherapy have been and are currently under investigation [10]. Our group has developed multiple immunogenic peptides derived from the HER2 protein as vaccines to be administered in combination with an immunoadjuvant to patients in the adjuvant setting with the goal being to reduce the risk of recurrence. One of these peptides, nelipepimut-S, when combined with the adjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF; Leukine®) has been shown to be safe and effective in stimulating, a robust immunologic response particularly in patients with HER2 low-expressing tumors [11,12]. Data from early phase trials showed promise for nelipepimut-S + GM-CSF in HER2 low-expressing patients in the adjuvant setting [13]. Unfortunately, a recently completed large phase III trial failed to show a benefit to vaccination as monotherapy in this setting.

While both trastuzumab and nelipepimut-S + GM-CSF have failed to demonstrate clinical benefit in breast cancer patients when administered as monotherapy, preclinical data suggests that combining these two may result in a synergistic antitumor effect. One mechanism of action of trastuzumab is immune-mediated antibody-dependent cellular cytotoxicity [14]. In preclinical testing of trastuzumab combined with HER2-targeted vaccines, trastuzumab increased the uptake and cross presentation of HER2-specific epitopes by dendritic cells. The increased presentation led to greater expansion of peptide-specific cytotoxic T lymphocytes (CTLs) and greater cytotoxicity in tumors treated with the combination [15,16]. Additionally, in phase II trials of our HER2 vaccines, 55 patients with HER2 over-expressing tumors were treated with standard of care trastuzumab followed by vaccination with HER2-derived CD8 T cell-eliciting vaccines and there were no recurrences in these patients after 36 months of follow up [17].

Given the need for novel approaches in patients with HER2 low-expressing tumors and the potential synergism of trastuzumab and CD8 T cell-eliciting vaccines, we initiated a phase II adjuvant therapy trial enrolling high-risk, disease-free breast cancer patients with HER2 low-expressing tumors comparing the combination of nelipepimut-S + GM-CSF and trastuzumab to trastuzumab + GM-CSF. The endpoints of this trial include disease-free survival (DFS), immunologic responses and safety evaluation with a final analysis after 36 months of follow up. A preplanned interim safety analysis with a focus on cardiac toxicity was planned to occur following randomization of 150 patients. The results of that analysis are reported here.

2. Materials and methods

2.1. Patient characteristics

This study is an ongoing multi-center, prospective, randomized, single-blinded, placebo-controlled phase II trial of nelipepimut-S + GM-CSF and trastuzumab versus trastuzumab and GM-CSF alone. It was approved by a central Institutional Review Board and at each of the participating sites. To be eligible, patients had to have tumors that were HER2 1+ or 2+ by IHC (ISH ratio of HER2:CEP17 ≤ 2.0 if IHC 2+), and been rendered clinically disease-free by standard therapy to include surgery, neoadjuvant or adjuvant chemotherapy and radiation therapy if indicated. Patients with hormone receptor positive tumors had to be node positive while those with hormone receptor negative tumors could be node positive or node negative. Additional inclusion criteria included a left ventricular ejection fraction (LVEF) > 50% or within the normal limits of the institution's specific testing (by MUGA or ECHO), and an ECOG performance status of 0 or 1. Because the vaccine is human leukocyte allele (HLA)-restricted, all patients were confirmed to be HLA-A2, HLA-A3, HLA-A24 or HLA-A26 positive. Study treatment commenced between 3 and 12 weeks from completion of standard therapy for breast cancer with the exception that endocrine therapy for patients with hormone receptor positive tumors continued during their trial participation.

2.2. Vaccine preparation

Nelipepimut-S (E75, HER2 369-377, KIFGLSAFL) is a 9-amino acid peptide produced by solid-phase peptide synthesis. E75 acetate drug product was manufactured by Oso Biopharmaceuticals Manufacturing, Albuquerque, NM as a 1.5 mg/mL solution in 1 mL of water. Prior to administration, 1000 μg of the E75 acetate or an equal volume control (water for injection only) is mixed with 1 mL of reconstituted lyophilized Leukine® (250 μg /mL in solution). Immediately after mixing, equal amounts of the study drug were withdrawn into four syringes and, within 6 h, administered as four intradermal injections on the anterior medial thigh.

2.3. Randomization and treatment protocols

A computer-generated randomization table was used to randomize patients to either nelipepimut-S + GM-CSF and trastuzumab or trastuzumab and GM-CSF alone in a 1:1 allocation ratio. A site-balancing computer algorithm was used to balance the arms per site allocation by the completion of the study. Trastuzumab was dosed with an initial loading dose of 8 mg/kg and maintenance doses of 6 mg/kg every 3 weeks ± 3 days. Patients were blinded to which treatment they received. Trastuzumab was given every three weeks for a total of one year to patients in both arms. The first trastuzumab infusion was given no sooner than three weeks and no later than 12 weeks after completion of other standard therapy. The primary vaccine series for all patients began in conjunction with the third trastuzumab infusion and continued with one inoculation every three weeks, coinciding with the standard schedule of trastuzumab infusions, for a total of six intradermal inoculations. Vaccinations were given within two hours of completion of trastuzumab infusion. Upon completion of the primary vaccine series, booster inoculations were administered once every six months for four doses with the first booster inoculation administered with the final trastuzumab infusion.

2.4. Outcome and toxicity monitoring

The trial's primary endpoint is disease-free survival (DFS) with secondary objectives of immune responses measured by DTH reactions, dimer assays and ELISPOT assays. An additional secondary outcome is the safety of the combination of two HER2-targeted therapies, with specific emphasis on cardiac toxicity. Following trastuzumab infusion and vaccination, patients in both groups were monitored for toxicity and adverse events. Starting the vaccine series after two doses of trastuzumab alone allowed for evaluation of toxicities related specifically to trastuzumab prior to initiation of vaccine therapy. Each patient had cardiac ejection fraction evaluated with MUGA or ECHO at baseline,

then at 3, 6, 12, and 24 months or more frequently for patients who experienced a > 10% reduction in ejection fraction. The preferred test (MUGA or ECHO) was selected based on the treating physician and the institutional preferences but used consistently throughout study participation. Following inoculation, patients were monitored for 30 min following inoculation and then were contacted by phone or asked to return to the clinic 48–72 h after each inoculation for review of any local or systemic toxicities, which were collected and graded per the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 graded toxicity scale.

2.5. Statistical analysis

Demographic characteristics of all patients were recorded and tabulated. The incidence and frequency of adverse events (AEs), serious AEs (SAEs; AE ≥ grade 3, as defined by the Common Terminology Criteria for Adverse Events [CTCAE] v 4.03), AEs related to study drug, and AEs that led to study discontinuation were evaluated and determined by treatment group. A 2-sided Fisher Exact test was used to compare the incidence of any expected AEs between the 2 treatment groups. AE origination was attributed to prior breast cancer therapy, study therapy, surgery, or shared as most appropriate. A singly ordered Kruskal-Wallis test was used to compare the total number of AEs, SAEs, and related AEs per treatment group. The incidence of clinically significant congestive heart failure, defined as the occurrence of objective findings on clinical examination (e.g., rales, S3, elevated jugular venous pressure) and confirmed by chest X-ray and either MUGA or ECHO, was estimated for patients in pre-specified treatment groups. Differences in risk based on prior anthracycline exposure were evaluated. Cardiac ejection fraction results were compared over time between groups using a linear mixed regression model and included fixed effects for group, time, and the group time interaction.

3. Results

At the time of this pre-specified safety analysis, there were 81 patients randomized to the nelipepimut-S + GM-CSF and trastuzumab arm and 69 to the trastuzumab and GM-CSF control arm (Fig. 1). Clinicopathologic characteristics of enrolled patients are detailed in Table 1. Groups were similar except for more node positive patients in the control group (100% vs 90.1% in the vaccine group; $p = .01$; all other factors $p > .05$). The arms were not entirely balanced as the randomization process is on-going.

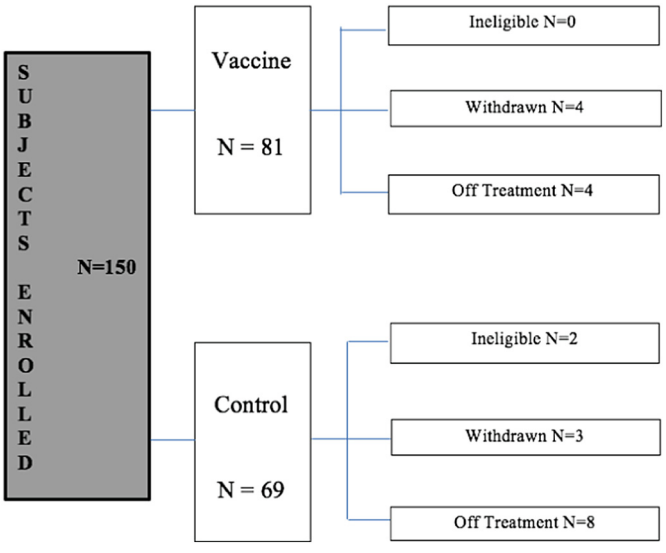

Fig. 1. Consort diagram: A total of 150 patients were enrolled at the time of this analysis. 81 patients were randomized to nelipepimut-S + GM-CSF and trastuzumab (vaccine) and 69 were randomized to trastuzumab and GM-CSF (control). In the vaccine arm, 8 withdrew or were off treatment. In the control arm, 13 withdrew, were off treatment, or were found to be ineligible after enrollment.

At the time of the analysis, 147 (98%) patients received at least one dose of trastuzumab (79 vaccine group patients, 68 control group patients) and 130 (86.7%) patients received at least one dose of nelipepimut-S + GM-CSF (67 patients) or GM-CSF (63 patients). The percentage of patients experiencing any AE was similar between groups (vaccine: 79% vs control: 84%, p = .43). There was no difference in the number of reported AEs per patient in each group (vaccine: 15.5 vs control: 13.3, $p = .57$). Similarly, there was no difference in the percent experiencing a related AE (72% vs 72%, p = .83). Most related toxicities were grade 1 or grade 2, and there was no difference between the groups with respect to graded toxicities ($p = .38$). There were no grade 4 or 5 toxicities. When accounting for AEs only in patients who received at least one dose of nelipepimut-S + GM-CSF or GM-CSF, there were again no significant differences in the frequency of any related AE types (Table 2).

There were nine SAEs in the treatment arm and eleven in the control arm. A total of six SAEs (all grade 3) were identified as being related to study treatment, four in the vaccine group and two in the control group. In the vaccine group, one patient had grade 3 injection site pain, one had grade 3 hypertension, and there were two incidences of grade 3 decreased LVEF In the control group, the related SAEs included one grade 3 pruritus, and one instance of grade 3 decreased LVEF.

Among the six patients who experienced a decrease in LVEF, five had received prior anthracycline-based chemotherapy. All patients had resolution by echocrardiogram or MUGA within six months of experiencing the AE. In the vaccine arm, one of the grade 3 decreases in LVEF occurred in response to trastuzumab after completion of the primary vaccine series. This patient had recovery of her LVEF after one month and continued on treatment. The vaccine patient with grade 3 decrease in LVEF was withdrawn from the study due to the adverse event. In the control arm, the patient with a grade 3 decrease in LVEF was withdrawn from the study. One additional control patient with a grade 2 decrease in LVEF voluntarily withdrew due to the toxicity. The only other patient who discontinued treatment due to an AE was a control patient who experienced a grade 2 allergic reaction. One grade 4 toxicity of increased creatinine was identified in the control group, but was determined to be unrelated to the study treatment.

Although there were four cardiac SAEs, there was no significant change in the mean LVEF over time in either group. In all patients and in those who received anthracyclines, there was again no significant difference in mean EF in either group at three, six, twelve, or twenty-four-month follow-up (Fig. 2a and b). The overall incidence of cardiac toxicity was 7.5% in the vaccine group and 5.7% in the control group (p = 1.0).

4. Discussion

In this analysis based on the first 150 patients enrolled, we have demonstrated that the combination of a HER2-directed vaccine, nelipepimut-S + GM-CSF, and trastuzumab therapy in breast cancer patients with HER2 low-expressing tumors is well tolerated. There is no difference in the incidence of local or systemic AEs between the vaccine and control groups and there are no cardiac safety concerns with the combinatorial strategy.

While HER2-targeted therapy has dramatically changed the prognosis for patients with HER2-positive breast cancer, those patients with low HER2-expressing tumors do not have clinically significant benefit from trastuzumab as recently reported in the large randomized NSABP B47 clinical trial [9]. Of note, the NSABP B47 trial showed similar findings between the two arms and similar hazard ratios were observed for a number of other prespecified endpoints including HER2 IHC level, extent of lymph node involvement, and hormone receptor status. Our

Table 1
Clinicopathologic features of patients enrolled.

	Nelipepimut-S + GM-CSF + trastuzumab	GM-CSF + trastuzumab	p value
	(n = 81)	(n = 69)	
Age (median)	52.8	51.7	0.868
IRQ 1	44.3	45.2	
IRQ 3	61.9	60.2	
Race			0.12
White	68 (84.0%)	48 (69.6%)	
Asian	0 (0%)	5 (7.2%)	
Black	9 (11.1%)	11 (15.9%)	
Hispanic	3 (3.7%)	3 (4.3%)	
Other	1 (1.2%)	2 (2.9%)	
ER/PR positive	51 (63.0%)	44 (63.8%)	0.919
ER positive	49 (60.5%)	44 (63.8%)	0.681
PR positive	48 (59.3%)	36 (52.2%)	0.384
HER2			0.728
HER2 1+	55 (67.9%)	45 (65.2%)	
HER2 2+	26 (32.1%)	24 (34.8%)	
Timing of chemotherapy			
Neoadjuvant	47 (58.0%)	39 (56.5%)	0.853
Adjuvant	34 (42.0%)	30 (43.5%)	
Clinical stage (for patients receiving neoadjuvant chemotherapy)[a]			0.777
0	0 (0%)	1 (2.6%)	
I	3 (6.4%)	2 (5.1%)	
IIA	9 (19.1%)	9 (23.1%)	
IIB	12 (25.5%)	13 (33.3%)	
IIIA	8 (17.0%)	7 (17.9%)	
IIIB	5 (10.6%)	2 (5.1%)	
IIIC	9 (19.1%)	5 (12.8%)	
IV[b]	1 (2.1%)	0 (0%)	
Pathologic stage (for patients receiving neoadjuvant chemotherapy)[a]			0.959
0	2 (4.2%)	2 (5.1%)	
I	8 (17.0%)	6 (15.4%)	
IIA	8 (17.0%)	10 (25.6%)	
IIB	8 (17.0%)	5 (12.8%)	
IIIA	8 (17.0%)	7 (17.9%)	
IIIB	3 (6.4%)	3 (7.7%)	
IIIC	9 (19.1%)	6 (15.4%)	
Pathologic stage (for patients not receiving neoadjuvant chemotherapy)[c]			0.919
I	4 (11.8%)	5 (16.7%)	
IIA	6 (17.6&)	5 (16.7%)	
IIB	8 (23.5%)	5 (16.7%)	
IIIA	12 (35.3%)	10 (33.0%)	
IIIB	0 (0%)	0 (0%)	
IIIC	4 (11.8%)	5 (16.7%)	

[a] Percentages listed are calculated as the percent of patients who received neoadjuvant chemotherapy.
[b] One patient who was clinically stage IV prior to chemotherapy was down-staged and then enrolled. The patient was deemed ineligible.
[c] Percentages listed are calculated as the percent of patients who received adjuvant chemotherapy.

group has previously shown that patients with low HER2 expressing tumors have a robust immune response when vaccinated with a HER2-targeted CD8 T cell-eliciting vaccine [12]. In an analysis of the phase II trial of nelipepimut-S + GM-CSF comparing patients according to levels of HER2 expression in their tumors, patients with HER2 low-expressing tumors had larger maximum immunological responses to nelipepimut-S than those with HER2 over-expressing tumors (p = .04) [12]. These data were used to design the phase III PRESENT trial which evaluated nelipepimut-S in pathologically node positive breast cancer patients with HER2 low expressing tumors. This trial, which randomized 758 patients, was stopped after an interim analysis showed no benefit to vaccination.

While neither nelipepimut-S + GM-CSF nor trastuzumab alone seem to be effective in the adjuvant setting for patients with low HER2-expressing tumor, the combination of trastuzumab and a CD8 T cell-eliciting vaccine has shown promise in the preclinical setting. In one study, tumor cell lines with variable HER2 expression were incubated overnight with trastuzumab or without trastuzumab then co-cultured in cytotoxicity experiments with peripheral blood mononuclear cells (PBMC) from patients vaccinated with nelipepimut-S. Cytotoxicity of tumor targets by PBMCs from nelipepimut-S-vaccinated patients was significantly greater when tumor cells were treated with trastuzumab [15]. Further experiments showed cross-presentation as a potential mechanism for this potential synergy. Trastuzumab enhances uptake of soluble HER2 by dendritic cells and in both in vitro and in vivo models. Increased HER2 uptake results in increased cross-presentation of E75 peptide by dendritic cells, which, in turn leads to priming of an anti-tumor immune response with increased antigen-specific cytotoxic T lymphocyte generation [16]. This preclinical data showing synergy with trastuzumab, as well as an observation from previous vaccine studies showing no recurrences in 48 patients with HER2-positive breast cancer that were vaccinated after receipt of trastuzumab, prompted our current trial designed to determine the clinical utility of combining trastuzumab with a CD8 T cell eliciting vaccine [18].

There are, however, safety concerns associated with synergistically targeting the HER2 protein, which is expressed at some level on normal cells, including cardiac myocytes. In a large Phase III trial evaluating the combination of chemotherapy and trastuzumab in HER2-positive metastatic breast cancer, class III or IV cardiac dysfunction was observed in 16% of patients treated with the combination of trastuzumab, anthracycline, and cyclophosphamide [1]. Further studies revealed a lower risk of cardiac dysfunction in patients treated with trastuzumab

Table 2
Adverse events that occurred in > 5% of patients and all cardiac-related events.[a]

	Nelipepimut-S + GM-CSF + Trastuzumab (n = 67)		GM-CSF + Trastuzumab (n = 63)		p-Value
	Any grade (%)	Grade 3 (%)	Any grade (%)	Grade 3 (%)	
Local					
Injection erythema	48 (71.6%)	0 (0.0%)	40 (63.5%)	0 (0.0%)	0.35
Pruritus'	40 (59.7%)	0 (0.0%)	34 (54.0%)	1 (1.6%)	0.59
Skin induration	39 (58.2%)	0 (0.0%)	29 (46.0%)	0 (0.0%)	0.22
Pain at injection site	22 (32.8%)	1 (1.5%)	12 (19.0%)	0 (0.0%)	0.11
Systemic					
Fatigue	27 (40.3%)	0 (0.0%)	21 (33.3%)	0 (0.0%)	0.47
Headache	25 (37.3%)	0 (0.0%)	16 (25.4%)	0 (0.0%)	0.18
Arthralgia	16 (23.9%)	0 (0.0%)	11 (17.5%)	0 (0.0%)	0.40
Back pain	13 (19.4%)	0 (0.0%)	9 (14.3%)	0 (0.0%)	0.49
Nausea	12 (17.9%)	0 (0.0%)	10 (15.9%)	0 (0.0%)	0.82
Malaise	12 (17.9%)	0 (0.0%)	9 (14.3%)	0 (0.0%)	0.64
Myalgia	11 (16.4%)	0 (0.0%)	11 (17.5%)	0 (0.0%)	1.00
Chills	11 (16.4%)	0 (0.0%)	10 (15.9%)	0 (0.0%)	1.00
Bone pain	10 (14.9%)	0 (0.0%)	7 (11.1%)	0 (0.0%)	0.61
Diarrhea	8 (11.9%)	0 (0.0%)	4 (6.3%)	0 (0.0%)	0.37
Cardiac	5 (7.5%)	3 (4.5%)	4 (6.3%)	1 (1.6%)	0.82
Decrease LVEF	3 (4.5%)	2 (3.0%)	3 (4.8%)	1 (1.6%)	1.00
Palpitations	1 (1.5%)	0 (0.0%)	1 (1.6%)	0 (0.0%)	1.00
Hypertension	1 (1.5%)	1 (1.5%)	0 (0.0%)	0 (0.0%)	1.00

[a] All patients who received at least one dose of Nelipepimut-S + GM-CSF or GM-CSF (patients who received trastuzumab alone in the lead up to inoculations were excluded). Adverse events are reported per patient (multiple toxicities of the same type in a single patient were listed once). P-values compare adverse events of any grade.

alone (3–7%) or trastuzumab plus paclitaxel (13%) compared to trastuzumab and anthracycline plus cyclophosphamide (27%), indicating that this toxicity may be less of a concern in patients who are not receiving a combination of trastuzumab and anthracyclines [19]. In contrast, we have shown no concerns for cardiac related toxicity across multiple trials of HER2-directed vaccines [13,18,20].

The safety of combining trastuzumab and a HER2-derived peptide vaccine has been evaluated in early clinical trials. The administration of trastuzumab concurrently with a vaccine composed of multiple HER2-derived, CD4+ T cell-eliciting peptides (one of which contains the E75 epitope) to metastatic breast cancer patients was found to be safe with over 99% of the toxicity being grade 1 or 2. Median LVEF was unchanged pre- to post-treatment, with three patients (15%) experiencing asymptomatic decreases in LVEF, but no patients experiencing symptoms of left ventricular dysfunction [21]. Multiple patients in our phase II trials of both HER2-directed CD4+ and CD8+ T cell-eliciting vaccines received trastuzumab as part of standard therapy prior to vaccination and no additive cardiac toxicity has been found in these patients [13,18,20]. We have also conducted a phase I trial evaluating the combination of trastuzumab and a CD8 T cell-eliciting HER2-derived peptide vaccine in the adjuvant setting, and saw no added cardiac toxicity with combination therapy [22].

Given the potential for efficacy and the safety of this combination, our group has initiated two phase II trials comparing the combination of

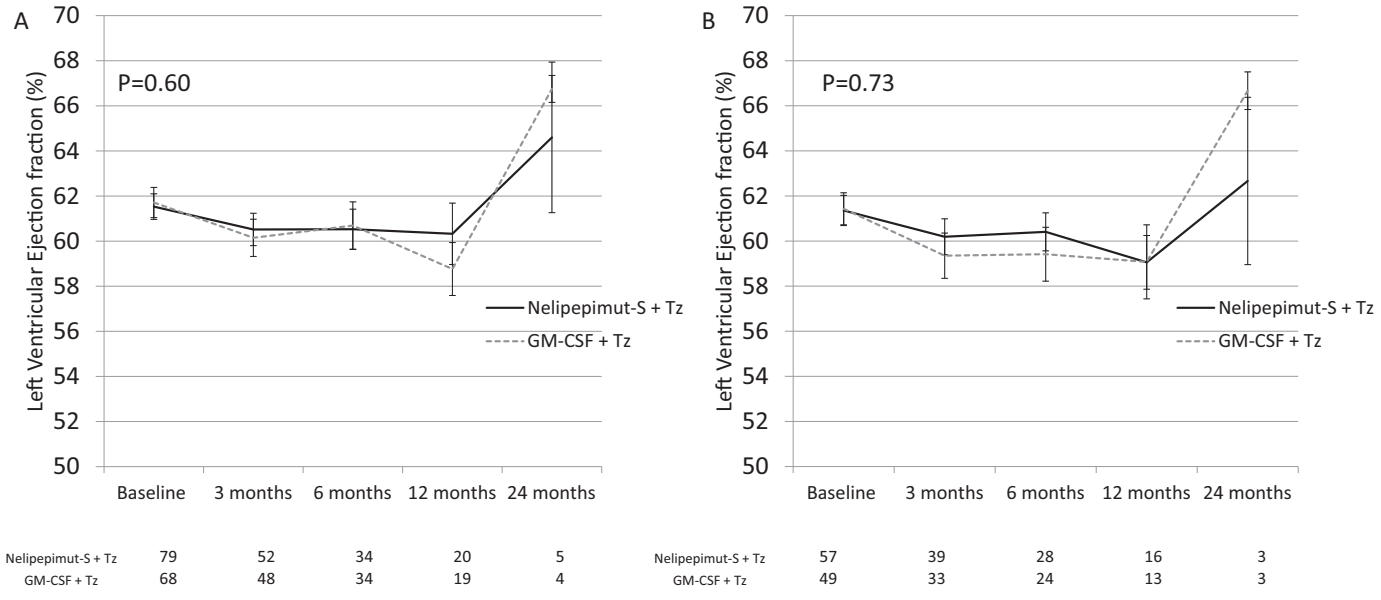

Fig. 2. Mean Left Ventricular Ejection Fraction in all patients (A) and patients who received prior anthracycline chemotherapy (B). Mean ejection fraction at baseline, 3, 6, 12, and 24 months after enrollment. There was no significant difference between patients who received nelipepimut-S + GM-CSF and Trastuzumab and those who received Trastuzumab and GM-CSF. The table below the x-axis lists the number of evaluable patients at each time point.

adjuvant nelipepimut-S + GM-CSF and trastuzumab to trastuzumab alone. In the current trial of HER2 low-expressing patients, we saw no difference in toxicity between vaccinated and control patients. Although roughly 80% of patients in each arm did experience some AE, the vast majority of these were grade 1 or 2 with only nine SAEs experienced across the entire trial. These rare events were evenly distributed between the groups. Given the known cardiac toxicity of trastuzumab, we focused especially on cardiac toxicity in this study. The incidence of decreased LVEF was under 5% in both groups, with no difference between the two groups. Finally, analysis limited to patients who received an anthracycline as part of their adjuvant therapy again confirmed no change in average LVEF and no difference between groups.

In addition to the trial in HER2 low-expressing patients that is the subject of this report, we are also conducting a similar trial in patients with HER2 over-expressing tumors who are already receiving trastuzumab as part of their standard of care adjuvant therapy. The initial safety evaluation from this trial was recently completed after enrollment of the first 50 patients. There were no related grade 4 or 5 toxicities and no differences in related toxicities between the vaccinated group and the control group. Importantly, we found no significant reduction in LVEF pre- to post-treatment in patients receiving the combination or those receiving trastuzumab alone and no difference in the overall change in LVEF between the two groups [23]. This further corroborates the results of the current trial in HER2 low-expressing patients, in which we have shown that the addition of nelipepimut-S + GM-CSF to trastuzumab does not increase cardiac toxicity.

5. Conclusions

This study has shown that the combination of nelipepimut-S + GM-CSF and trastuzumab in patients with HER2 low-expressing tumors is safe, with most related toxicities being grades 1 and 2. The combination did not have a deleterious effect on the LVEF of enrolled patients, even in those who had previously been treated with anthracyclines. Patients will continue to be monitored for cardiac toxicity as the trial progresses with a second interim analysis for safety and efficacy planned for 6 months after enrollment of the final patient.

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