Results of a Randomized Phase IIb Trial of Nelipepimut-S + Trastuzumab versus Trastuzumab to Prevent Recurrences in Patients with High-Risk HER2 Low-Expressing Breast Cancer

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ABSTRACT

Purpose: Preclinical data provide evidence for synergism between HER2-targeted peptide vaccines and trastuzumab. The efficacy of this combination was evaluated in patients with HER2 low-expressing breast cancer in the adjuvant setting.

Patients and Methods: A phase IIb, multicenter, randomized, single-blinded, controlled trial enrolled disease-free patients after standard therapy completion (NCT01570036). Eligible patients were HLA-A2, A3, A24, and/or A26+, and had HER2 IHC 1+/2+, FISH nonamplified breast cancer, that was node positive and/or hormone receptor–negative [triple-negative breast cancer (TNBC)]. Patients received trastuzumab for 1 year and were randomized to placebo (GM-CSF, control) or nelipepimut-S (NPS) with GM-CSF. Primary outcome was 24-month disease-free survival (DFS). Secondary outcomes were 36-month DFS, safety, and immunologic response.

Results: Overall, 275 patients were randomized; 136 received NPS with GM-CSF, and 139 received placebo with GM-CSF. There were no clinicopathologic differences between groups. Concurrent trastuzumab and NPS with GM-CSF was safe with no additional overall or cardiac toxicity compared with control. At median follow-up of 25.7 (interquartile range, 18.4–32.7) months, estimated DFS did not significantly differ between NPS and control [HR, 0.62; 95% confidence interval (CI), 0.31–1.25; $P = 0.18$]. In a planned exploratory analysis of patients with TNBC, DFS was improved for NPS versus control (HR, 0.26; 95% CI, 0.08–0.81, $P = 0.01$).

Conclusions: The combination of NPS with trastuzumab is safe. In HER2 low-expressing breast cancer, no significant difference in DFS was seen in the intention-to-treat analysis; however, significant clinical benefit was seen in patients with TNBC. These findings warrant further investigation in a phase III randomized trial.

Introduction

Trastuzumab, a monoclonal antibody targeting the HER2 protein, has been the backbone of therapy for women with HER2-overexpressing (3+ by IHC or ISH amplified) breast cancer for two decades (1–3). Although only 15% to 20% of all patients with breast cancer have HER2-overexpressing tumors, the majority have tumors that express HER2 to a lesser degree (4). Retrospective data supporting the use of trastuzumab in HER2 IHC 1+ or 2+ breast cancer led to the conduct of the NSABP B-47 adjuvant therapy trial, which randomized patients with HER2 low-expressing tumors (1–2+ by IHC and ISH HER2: CEP17 <2.0 or HER2 gene copy number < 4 per nucleus) to trastuzumab versus placebo (5). At a median follow-up of 46 months, the 5-year invasive disease-free survival (DFS) rate did not differ between groups: 89.6% for patients receiving trastuzumab in addition to chemotherapy versus 89.2% for those receiving chemotherapy alone ($P = 0.90$).

Although passive, monoclonal antibody–based HER2-directed therapy alone has not shown clinical activity in patients with HER2 1+ or 2+ tumors, there may be benefit to HER2-targeted active immunotherapy in patients with HER2 low-expressing breast cancer. Our group has developed HER2-derived peptide vaccines, including nelipepimut-S (NPS), which are combined with GM-CSF as an immunoadjuvant. Promising initial clinical work with this $CD8^+$ T-cell–eliciting vaccine led to a phase II trial, which showed a significant improvement in DFS for vaccinated patients versus unvaccinated controls (6). Based on these results, the phase III PRESENT trial was conducted, randomizing patients with node-positive, HER2 1+ or 2+ breast cancer to NPS with GM-CSF versus

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Translational Relevance

This study is the first randomized trial evaluating the combination of trastuzumab with vaccination in the adjuvant setting. It demonstrates that the combination is safe with no added toxicity with the two HER2-targeted therapies given concurrently. In line with previous studies, no additional cardiac toxicity is seen with the combination of HER2-targeted vaccination together with trastuzumab. Together, these data should alleviate concerns about combining active immunotherapy with monoclonal antibodies targeting HER2. This study, taken together with evidence from single-arm trials in the metastatic setting, points to synergistic action in combining active specific immunotherapy (vaccines) with trastuzumab leading to clinical benefit. In addition, this is the first trial evaluating trastuzumab with vaccination in patients with HER2 low-expressing disease—a group who would not otherwise benefit from HER2-directed therapy. Importantly, it shows potential benefit from the combination in patients with triple-negative breast cancer.

GM-CSF alone (7). At the time of a planned interim analysis, this trial was terminated for futility due to lack of benefit from the vaccine.

Although phase III trials evaluating either trastuzumab or NPS as monotherapy failed to show benefit for patients with HER2 1+ or 2+ breast cancer, preclinical work suggests potential synergy through combining these two therapies (8–10). To further investigate this promising combination, a multicenter, prospective, randomized, single-blinded, placebo-controlled phase IIb trial of trastuzumab and NPS with GM-CSF versus trastuzumab and placebo with GM-CSF was conducted. Here, we report the final safety, immunologic response, and efficacy results of this trial.

Patients and Methods

Study design and patient eligibility

This was a multicenter, randomized, single-blind, placebo-controlled phase IIb trial enrolling at 26 centers in the United States comparing trastuzumab with concurrent NPS with GM-CSF to placebo with GM-CSF. Eligible patients had to be 18 years or older, have histologically confirmed invasive breast cancer that was HER2 1+ or 2+ (for IHC 2+ tumors, ISH nonamplified), be node positive or estrogen and progesterone receptor negative [triple-negative breast cancer (TNBC)], and be clinically disease-free after receiving surgery, chemotherapy, and radiotherapy administered as guideline concordant care. Patients receiving neoadjuvant chemotherapy were eligible based on their pretreatment clinical stage or final pathologic stage; patient staging was based on the American Joint Committee on Cancer 7th edition. Patients began study treatment 3 to 12 weeks after completion of their breast cancer treatment with the estrogen and/or progesterone receptor–positive patients who initiated endocrine therapy remaining on that treatment per standard practice. Patients were excluded for a history of prior trastuzumab therapy, New York Heart Association stage 3 or 4 cardiac disease, left ventricular ejection fraction (LVEF) by echocardiogram or radionuclide angiography (MUGA) less than 50% or below the lower limit of normal for the testing institution, active immunosuppression, or autoimmune diseases.

Eligible patients underwent a two-stage consent process. The initial consent allowed for screening HLA type. Patients positive for HLA-A2, A3, A24, and/or A26 were then consented for trial enrollment and randomized after completion of standard therapies. Patients were randomized 1:1 between treatment arms using a central computer-generated randomization table with an institutional balancing algorithm. Patients were blinded to their treatment arm. The study was approved by the Institutional Review Board for each participating institution and conducted in accordance with the Federal Policy for the Protection of Human Subjects. An independent data safety monitoring board (DSMB) monitored the study and reviewed safety data and efficacy endpoints at predefined milestones.

In December 2017, the NSABP B-47 was reported signifying no benefit to single-agent trastuzumab in HER2 1+ or 2+ breast cancer, the same regimen being given in the control arm of the current study (5). Based on potential risk to control arm patients without demonstrated benefit, the DSMB recommended stopping randomization with 25 enrolled patients pending randomization. The interim analysis to assess both safety and efficacy occurred as per the statistical analysis plan in March 2018, 6 months after the last patient was enrolled in September 2017. This interim analysis showed no benefit to vaccination in the intention-to-treat population, but there was a benefit to vaccination seen in an exploratory analysis performed in patients with TNBC (11). After review of both the data from the NSABP B-47 trial and the planned interim analysis by the independent DSMB, the decision was made to close the trial and complete the final analysis, which is presented here.

Treatment procedures

NPS (E75, HER2 369-377, KIFGLSAFL) is a 9-amino acid, MHC class I peptide produced by solid-phase peptide synthesis (Oso Biopharmaceuticals Manufacturing). NPS or placebo inoculations were administered as four 0.5-mL intradermal injections in the anterior thigh. Inoculations contained 1,000 μg of NPS combined with 250 μg of GM-CSF (vaccine arm) or 250 μg of GM-CSF (control arm). GM-CSF was used in the control arm to maintain blinding due to the resultant local reaction to inoculations.

Both vaccine and control arms received trastuzumab, dosed with an 8 mg/kg loading dose and 6 mg/kg maintenance doses every 3 weeks for 1 year. The primary vaccine series began as concurrent inoculations with the third trastuzumab infusion and continued, along with trastuzumab, every 3 weeks for a total of six inoculations. Booster inoculations were given once every 6 months for four doses; the first booster inoculation was administered with the final trastuzumab infusion. Treatment with trastuzumab in combination with vaccine or placebo inoculations was stopped if the patient experienced disease recurrence.

Toxicity monitoring

Patients were monitored for adverse events for 30 minutes following inoculations and were assessed again in person or by phone 48 to 72 hours later. The LVEF was evaluated with MUGA or echocardiogram at baseline, 3, 6, 12, and 24 months or more frequently as clinically indicated by symptoms or prior results. When LVEF was reported as a range, the lowest point in the range was recorded for trial purposes. Patients with a symptomatic or an asymptomatic decline in LVEF to below the upper limit of normal for the testing institution or a decline of ≥20% (even if above the upper limit of normal) were taken off treatment but remained in the study for disease follow-up. Local and systemic toxicity information was graded per the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 with the following exception: an asymptomatic decline in LVEF was recorded as grade 2 "left ventricular systolic dysfunction."

Toxicities were classified as unrelated if they were determined to be "unlikely" or "unrelated" to the study treatment by the site primary investigator and reviewed by the study medical monitor.

Immunologic testing

All patients were assessed for baseline immunologic *in vivo* response to NPS with delayed type hypersensitivity reaction (DTH) prior to initiating the primary vaccine series (DTH No. 1), 1 month after completion of the primary vaccine series (DTH No. 2), after the second booster inoculation (DTH No. 3), and after the final booster inoculation (DTH No. 4). To determine the DTH, 100 μg of NPS without GM-CSF was administered intradermally on the opposite thigh from the primary vaccine series, and the reaction was measured using the sensitive ballpoint pen test 48 to 72 hours later (12).

Patients were assessed for evidence of immunologic response by quantification of E75-specific $CD8^+$ T cells using a dextramer assay. Staining was performed on blood obtained prior to vaccination (R0), 1 month after completion of the primary vaccination series (R6), 1 month after the first booster vaccination (RB1), and 1 month after the third booster vaccination (RB3). Briefly, peripheral blood mononuclear cells were isolated using standard histopaque gradient centrifugation and stained with the following antibodies: CD8 APC-H7 (BD Biosciences), CD3 APC (BD Biosciences), E75-PE –conjugated dextramer (Immudex); the following Pacific Blue–conjugated lineage (lin) antibodies: CD4, CD14, CD16, CD19, CD56 (Biolegend); and Ghost Violet 510 Viability Dye live dead stain (TONBO Biosciences). Cells were then analyzed on a LSRFortessa Analyzer (BD Biosciences). The frequency of E75-specific $CD8^+$ T cells was determined as the percentage of cells that were alive, $lin^-/CD3^+/CD8^+$/E75-$dextramer^+$, and Flu and Negative HLA-A2 dextramer were used as a positive and negative controls in each sample.

Outcomes

The primary endpoint of the trial was 24-month DFS, which was calculated from trastuzumab therapy initiation to the time of breast cancer recurrence. The patients were followed by their treating team for recurrent breast cancer with clinical exams, laboratory, and radiographic surveillance as per standard guidelines (13). Patients were considered to have recurrent breast cancer with histologic confirmation or highly suggestive radiographic and clinical findings that led to initiation of treatment for recurrence. Secondary endpoints included 36-month DFS, safety, and immunologic response. Exploratory analyses were performed to identify subgroups that may benefit from combination therapy.

Statistical analysis

An initial sample size of 300 patients was calculated to determine a 10% absolute improvement in DFS between treatment arms, assuming a 15% recurrence rate in the control group at 24 months with a 5% probability of a type 1 error and 80% power. Clinicopathologic variables were compared using χ^2 for categorical variables and Wilcoxon rank sum for age. Toxicity frequencies were compared using χ^2. Cardiac LVEF results were compared at prespecified time points using a *t* test and at all time points using a linear mixed regression model and included fixed effects for group, time, and the group time interaction. DTH reactions were compared between arms using the Kruskal–Wallis test and between time points using the Wilcoxon signed rank test. E75 dextramer analysis was evaluated using a mixed effects regression model examining treatment arms by time point (R0, R6, RB1, RB3), main effects, and interactions on E75-specific T-cell response. Kaplan–Meier-estimated DFS was compared by log-rank, and Cox regression models were used to determine the HR. IBM SPSS Statistics 22.0 was used for all statistical analyses. The safety/modified intention-to-treat (mITT) group consisted of any patients who received NPS with GM-CSF or placebo with GM-CSF inoculations. This study was registered with ClinicalTrials.gov (NCT01570036).

Results

Patients enrolled from May 2013 through September 2017. Of the 587 patients who underwent HLA testing, 96 (16.4%) were not HLA-A2, A3, A24, or A26 positive. Of those patients who were HLA-A2, A3, A24, or A26 positive, 300 patients were enrolled, and 275 patients were randomized: 136 were randomly assigned to receive NPS with GM-CSF, and 139 were assigned to receive placebo with GM-CSF (**Fig. 1**). There were no significant clinicopathologic differences between the treatment arms among randomized patients (**Table 1**).

Toxicity

At least one adverse event occurred in 246 of 261 (94.3%) patients who received at least one dose of NPS and GM-CSF or placebo and GM-CSF (mITT/safety population). There was an average of 2.4 adverse events and 1.9 related adverse events per inoculation in patients receiving the vaccine, and 2.3 adverse events and 1.8 related adverse events per inoculation in patients receiving placebo ($P = 0.65$ and 0.17, respectively). There were no differences in the distribution or severity of related toxicities (Supplementary Fig. S1A) or in the distribution and severity of the maximum toxicity experienced by each patient (Supplementary Fig. S1B). Similarly, there were no differences in the individual local or systemic toxicities patients experienced except more grade 1 and 2 localized pruritus and injection site pain in the vaccine group (Supplementary Table S1).

Cardiac toxicity

There were no significant differences in the rates of cardiac-related adverse events between the treatment arms (Supplementary Table S1). The mean LVEF decreased from baseline slightly in both groups at the prespecified 3-, 6-, and 12-month evaluations ($P = 0.02$) but did not differ after cessation of trastuzumab at 24 months ($P = 0.58$). Evaluating LVEF at all time points with a linear mixed regression model, there were no significant differences in cardiac ejection fraction change over time ($P = 0.65$), between randomization arm ($P = 0.91$), or between the arms over time ($P = 0.81$, **Fig. 2**).

Immunologic response

The DTH reaction was equivalent at baseline between patients receiving the vaccine and those receiving placebo ($P = 0.10$). Although the median DTH value remained at 0 mm for both groups over each time point measured, there was a significant change in the distribution in vaccinated patients at DTH No. 2 and DTH No. 3 (both $P < 0.05$, **Fig. 3A**). There was no change in the DTH distribution from baseline in patients receiving placebo.

The *in vitro* E75 dextramer analysis was performed on 117 patients and found that the placebo ($n = 51$) and vaccine ($n = 66$) group both changed differently with respect to E75-specific T-cell responses over time ($P = 0.04$, **Fig. 3B**). The simple main effect of time revealed that the placebo group did not change over time ($P = 0.27$), whereas the vaccine group increased E75-specific T-cell response over time ($P < 0.01$). The simple main effect of treatment arm indicated that the two groups were not significantly different at the first three time points (R0, R6, RB1), but the vaccine group had significantly more E75-specific T-cell response at the final time point (RB3) as compared with placebo

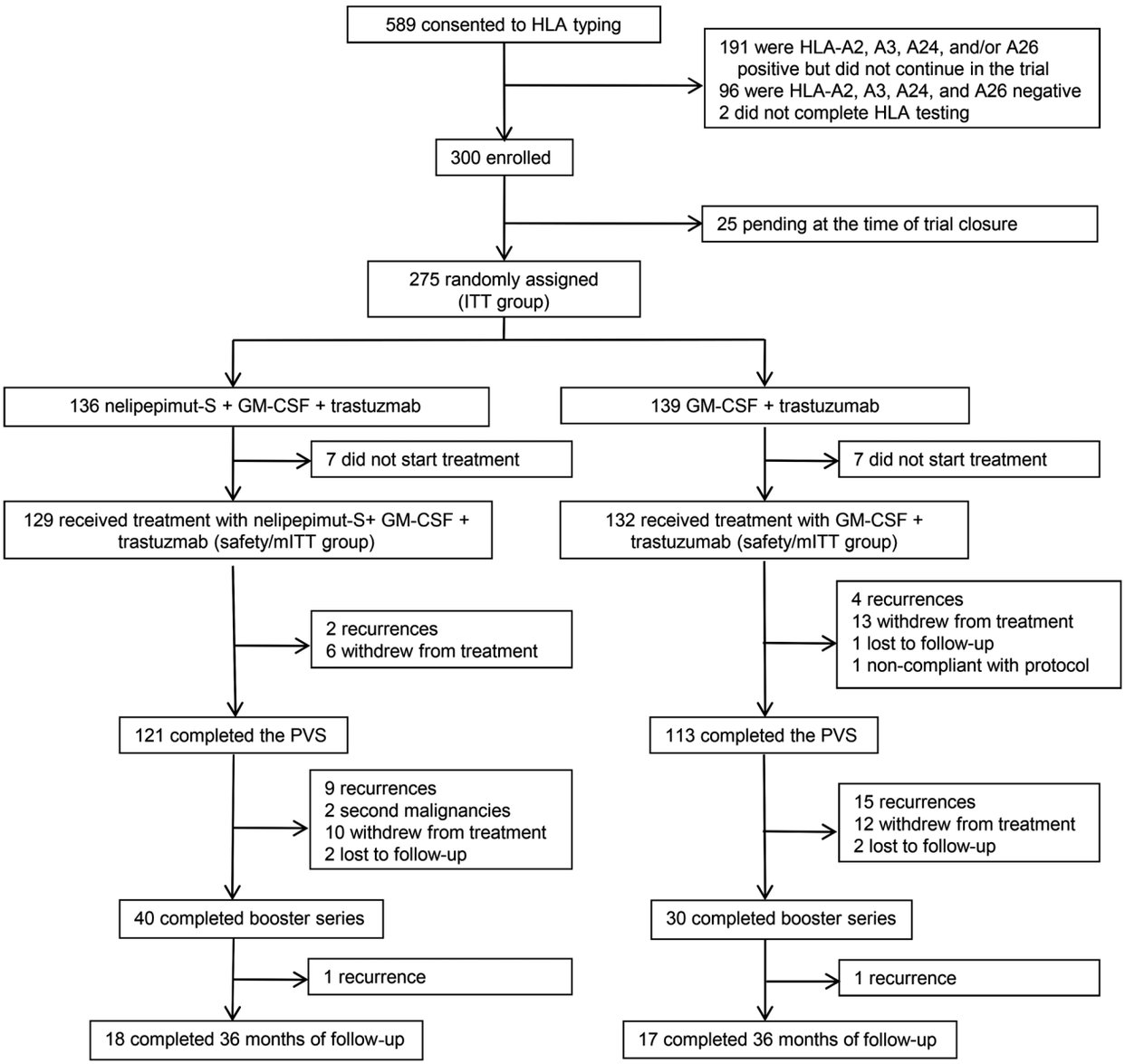


Figure 1.

CONSORT diagram. The final analysis was performed on all patients as randomized. The safety/mITT population included all patients that received inoculation of NPS or placebo and was used for comparing rates of adverse events. PVS, primary vaccine series.

($P = 0.01$). Subset analyses were performed in TNBC ($n = 40$ placebo, $n = 51$ NPS; **Fig. 3C**) and hormone receptor–positive patients ($n = 11$ placebo, $n = 15$ NPS; **Fig. 3D**). The TNBC vaccine group had a significant increase in E75-specific response over time ($P < 0.01$) and significantly different from placebo at the final time point (RB3, $P = 0.02$, **Fig. 3C**). Whereas, the hormone receptor–positive placebo group had a significant decrease in E75-specific response over time ($P = 0.04$), and there was no difference between groups at any time point (**Fig. 3D**).

Disease-free survival

After a median follow-up of 25.7 (interquartile range, IQR, 18.7–32.7) months, the estimated DFS did not significantly differ in the ITT population between the vaccine and placebo patients [HR, 0.62; 95% confidence interval (CI), 0.31–1.25]. The Kaplan–Meier-estimated 24-month DFS was 89.8% for vaccinated and 83.8% for control patients ($P = 0.18$, **Fig. 4A**). In the mITT population, there again was no significant difference in DFS (HR, 0.57; 95% CI, 0.28–1.17) with an estimated 24-month DFS of 89.3% in vaccinated patients and 82.3% in control patients ($P = 0.12$, **Fig. 4B**).

Exploratory analyses

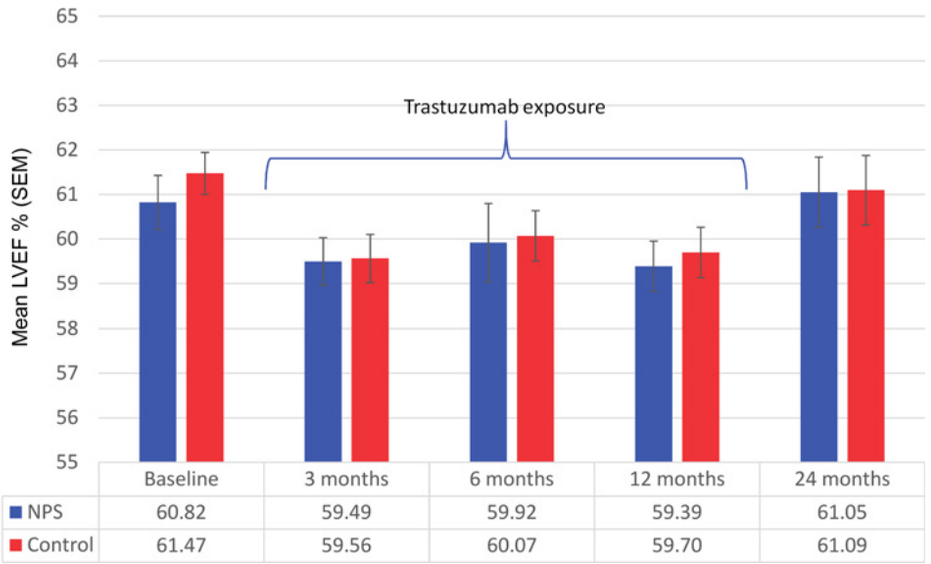
In planned exploratory analyses, outcomes were evaluated by hormone receptor status and nodal status. Among the patients with TNBC, there were no clinicopathologic differences between groups (Supplementary Table S2). The median follow-up for patients with

Table 1. Clinicopathologic characteristics of all randomized patients.

		Trastuzumab + NPS + GM-CSF ($n = 136$)	Trastuzumab + GM-CSF ($n = 139$)	*P* value
Age (median)		52.2	50.5	0.38
	IQR	43.7–60.8	42.0–59.0	
Race	White	109 (80.1%)	97 (69.8%)	0.20
	Asian	2 (1.5%)	9 (6.5%)	
	Black	13 (9.6%)	20 (13.4%)	
	Hispanic	10 (7.4%)	9 (6.5%)	
	Unknown	2 (1.5%)	4 (2.9%)	
Nottingham modified, Scarff–Bloom–Richardson grade	Grade 1	8 (5.9%)	14 (10.1%)	0.31
	Grade 2	57 (41.9%)	56 (40.3%)	
	Grade 3	69 (50.7%)	69 (49.6%)	
Hormone receptor status	ER positive	81 (59.6%)	95 (68.3%)	0.20
	PR positive	77 (56.6%)	83 (59.7%)	0.13
	TNBC	53 (39.0%)	44 (31.7%)	0.60
HER2 IHC	1+	89 (65.4%)	89 (64.0%)	0.81
	2+	47 (34.6%)	50 (36.0%)	
Breast surgery	BCT	39 (28.7%)	34 (24.5%)	0.93
	Mastectomy	97 (71.4)	104 (74.9%)	
	None	0 (0.0%)	1 (0.7%)	
Chemotherapy	Neoadjuvant	72 (52.9%)	76 (54.7%)	0.85
	Adjuvant	59 (43.4%)	57 (41.0%)	
	None	5 (3.7%)	6 (4.3%)	
Radiotherapy with BCT	Adjuvant	39 (100.0%)	34 (100.0%)	1.00
	None	0 (0.0%)	0 (0.0%)	
Radiotherapy with mastectomy	Adjuvant	76 (78.4%)	89 (85.6%)	0.20
	Neoadjuvant	2 (2.1%)	0 (0.0%)	
	None	19 (21.6%)	15 (14.4%)	
Axillary surgery	Axillary dissection	81 (59.6%)	88 (63.3%)	0.29
	SLN biopsy	55 (40.4%)	48 (34.5%)	
	None	0 (0.0%)	3 (2.2%)	
Clinical stage (for patients receiving neoadjuvant chemotherapy)	Unknown	1 (1.4%)	2 (2.6%)	0.39
	0	0 (0%)	1 (2.6%)	
	I	4 (5.6%)	3 (3.9%)	
	IIA	16 (22.2%)	13 (17.1%)	
	IIB	19 (26.4%)	18 (23.7%)	
	IIIA	14 (19.4%)	25 (32.9%)	
	IIIB	7 (9.7%)	2 (2.6%)	
	IIIC	10 (13.9%)	13 (17.1%)	
	IV[a]	1 (1.4%)	0 (0%)	
Pathologic stage (for patients receiving neoadjuvant chemotherapy)	Unknown	1 (1.4%)	0 (0.0%)	0.77
	0	5 (6.9%)	4 (5.3%)	
	I	11 (15.3%)	9 (11.8%)	
	IIA	16 (22.2%)	15 (19.7%)	
	IIB	12 (16.7%)	11 (14.5%)	
	IIIA	11 (15.3%)	20 (26.3%)	
	IIIB	4 (5.6%)	3 (3.9%)	
	IIIC	12 (16.7%)	14 (18.4%)	
Pathologic stage (for patients not receiving neoadjuvant chemotherapy)	I	10 (15.6%)	9 (14.3%)	0.98
	IIA	11 (17.2%)	12 (19.0%)	
	IIB	14 (21.9%)	14 (22.2%)	
	IIIA	21 (32.8%)	18 (19.0%)	
	IIIB	0 (0%)	0 (0%)	
	IIIC	8 (12.5%)	10 (15.9%)	

Abbreviations: BCT, breast-conserving therapy; ER, estrogen receptor; PR, progesterone receptor; SLN, sentinel lymph node.
[a]One patient with pathologic stage IIB after completion of chemotherapy was enrolled and vaccinated. It was later discovered that the patient had metastatic disease prior to initiation chemotherapy and was enrolled in violation of the protocol. This patient was excluded from efficacy analyses due to ineligibility.

TNBC was 26.1 months (IQR, 19.9–31.9). There was significantly improved DFS in patients receiving the vaccine versus placebo (HR, 0.26; 95% CI, 0.08–0.81). The 24-month Kaplan–Meier-estimated DFS was 92.6% in vaccinated patients compared with 70.2% in control patients ($P = 0.01$, **Fig. 5A**). In hormone receptor–positive patients, node-positive, and node-negative patients, there was no significant difference in DFS between those receiving the vaccine versus placebo ($P > 0.05$; **Fig. 5B–D**).



	Baseline	3 months	6 months	12 months	24 months
NPS	60.82	59.49	59.92	59.39	61.05
Control	61.47	59.56	60.07	59.70	61.09

Figure 2.
Left ventricular cardiac ejection fraction over time. The cardiac ejection fraction was similar between patients receiving NPS and placebo.

Discussion

In patients with HER2 1+ or 2+ breast cancer at high risk of recurrence, the combination of trastuzumab with NPS is safe with no additional toxicity over trastuzumab alone. The combination did not significantly improve DFS in the ITT population; however, in a planned exploratory analysis, patients with TNBC experienced a significant improvement in DFS with the addition of NPS to trastuzumab compared with trastuzumab alone.

Roughly 80% of patents with breast cancer do not have HER2-positive tumors as demonstrated by IHC (3+) or FISH showing amplification and, therefore, do not qualify for trastuzumab as part of their standard therapy. The majority of these patients have some HER2 expression (1–2+ by IHC). Within the large phase III NSABP B-31 trial that evaluated the addition of trastuzumab to adjuvant chemotherapy among patients found locally to have HER2-postive breast cancer, 174 (9.7%) patients were found on central review to actually have HER2 1+ or 2+, ISH nonamplified disease (3). The clinical course of these HER2 low-expressing patients (92 in control arm and 82 in the treatment arm) was analyzed separately, and the analyses suggested that trastuzumab may also benefit these patients (HR, 0.34; 95% CI, 0.14–0.80; $P = 0.014$ for disease progression; ref. 14). This led to the large phase III NSABP B-47 trial, which treated patients with HER2 1+ or 2+ tumors with trastuzumab versus placebo (5). Despite the promising subset analysis from the NSAPB B-31 trial, the results from NSABP B-47, which have been presented in abstract form but not published at the time of this writing, revealed no benefit to trastuzumab in this population (5).

This same population, patients with HER2 low-expressing breast cancer, is potentially treatable with HER2-directed vaccination. The phase II trial of the NPS peptide vaccine enrolled patients with any level of HER2 expression (HER2 1–3+ by IHC; ref. 6). The final analysis of that trial showed improved DFS for all optimally dosed patients in the vaccine group (94.6% in vaccinated patients compared with 80.2% in the control arm, $P = 0.05$) and in patients with HER2 1+ or 2+ disease. This finding correlated with more robust immunologic responses to vaccination seen in HER2 low-expressing patients (15). The results of the phase II trial of NPS led to the phase III PRESENT trial (6, 7), which specifically targeted HER2 1+ or 2+ patients based on the observed increase in efficacy in this population. This trial, however, did not confirm this benefit of vaccination alone and was stopped early by the Independent Data Monitoring Committee for futility (7).

Although neither trastuzumab nor the NPS vaccine as monotherapy was shown to have a significant effect in large phase III trials, there is strong rationale that the combination will be synergistic. Preclinical work from our group showed that trastuzumab facilitates Fc receptor–mediated uptake and cross-presentation of soluble HER2 antigens by dendritic cells (8). This led to priming of naïve cytotoxic T cells and increased generation of NPS-specific cytotoxic T cells. Thus, patients who are treated with trastuzumab likely have some endogenous immune response to HER2-derived peptides. This response can then be augmented by vaccination with NPS. In line with the preclinical work suggesting synergy between vaccine and trastuzumab, in our experience with two HER2-directed, $CD8^+$ T-cell eliciting peptide vaccines, we have seen no breast cancer recurrences in 60 patients with HER2-postive breast cancer vaccinated after receiving trastuzumab on our previous trials (8, 16). In the phase II trial of NPS, 12 patients with HER2-postive breast cancer were vaccinated after receiving trastuzumab, and there were no recurrences after 60 months of follow-up. In a phase II trial evaluating GP2, a second MHC class I HER2-derived peptide vaccine, 48 patients with HER2-positive breast cancer received both trastuzumab and the vaccine sequentially with no recurrences observed at a median follow-up of 34 months compared with a recurrence rate of 11% in 50 HER2-positive control patients who received trastuzumab alone (8, 16).

Although the overall efficacy results of the current trial did not show a statistically significant difference between groups, there is a suggestion of potential benefit in patients with TNBC. Although the trial was not adequately powered for subgroup analysis, this finding is potentially important because we know from studies of patients with TNBC receiving neoadjuvant chemotherapy that the approximately 50% of patients with chemotherapy-insensitive TNBC have a poor prognosis (17). Data from the CREATE-X trial conducted in Japan and Korea showed improved outcomes in this population with the addition of adjuvant capecitabine (18). An ongoing trial being conducted by the Southwest Oncology Group is looking at adding adjuvant immunotherapy with the anti–PD-1 antibody pembrolizumab for patients with TNBC with residual disease after neoadjuvant chemotherapy (19).

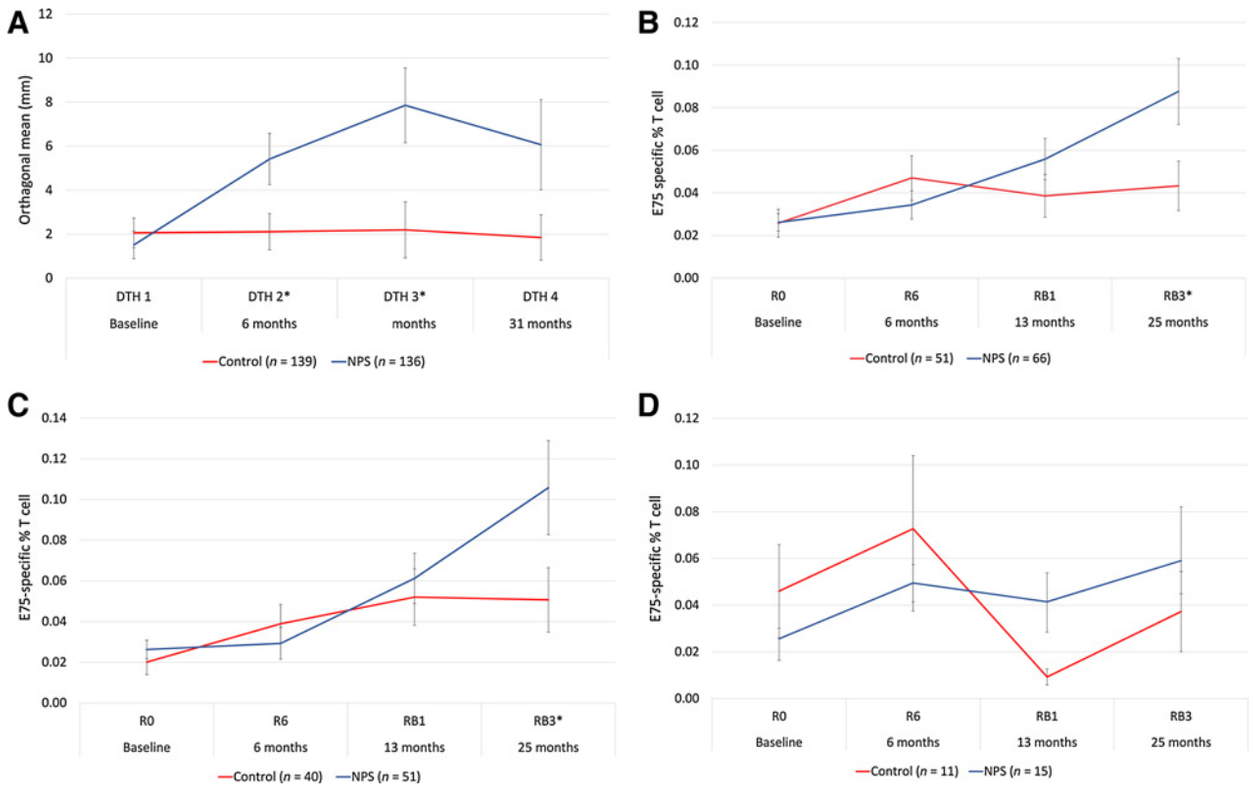


Figure 3.

A, *In vivo* immunologic response. **B,** *In vitro* immunologic response. **C,** TNBC *in vitro* immunologic response. **D,** hormone receptor–positive *in vitro* immunologic response. **A,** DTH reaction was measured prior to initiating the primary vaccine series (DTH No. 1), and 1 month after completion of the primary vaccine series (DTH No. 2), after the second booster inoculation (DTH No. 3), and after the fourth (final) booster inoculation (DTH No. 4). **B,** The E75-specific T-cell response was measured on 117 patients and within subsets of (**C**) TNBC and (**D**) hormone receptor–positive patients prior to initiating the primary vaccine series (R0), 1 month after completion of primary vaccine series (R6), 1 month after first booster vaccination (RB1), and 1 month after third booster vaccination (RB3). *Time points where the treatment arms were significantly different, $P < 0.05$.

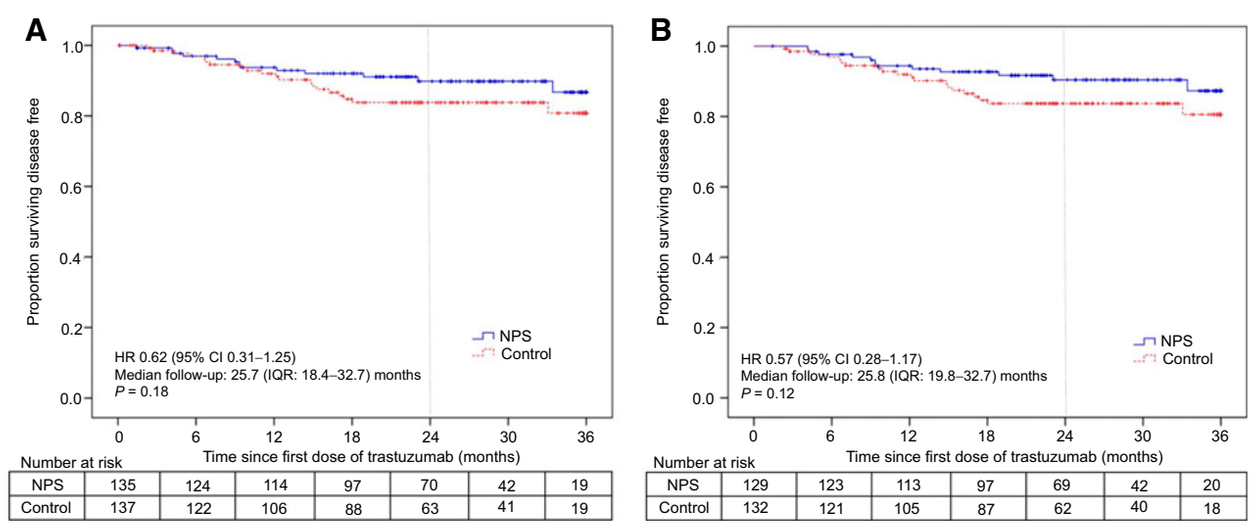


Figure 4.

A and **B,** DFS. (**A**) Overall population as randomized and (**B**) the mITT population consisting of only patients who received NPS or placebo. Dashed line indicated primary outcome of 24-month DFS.

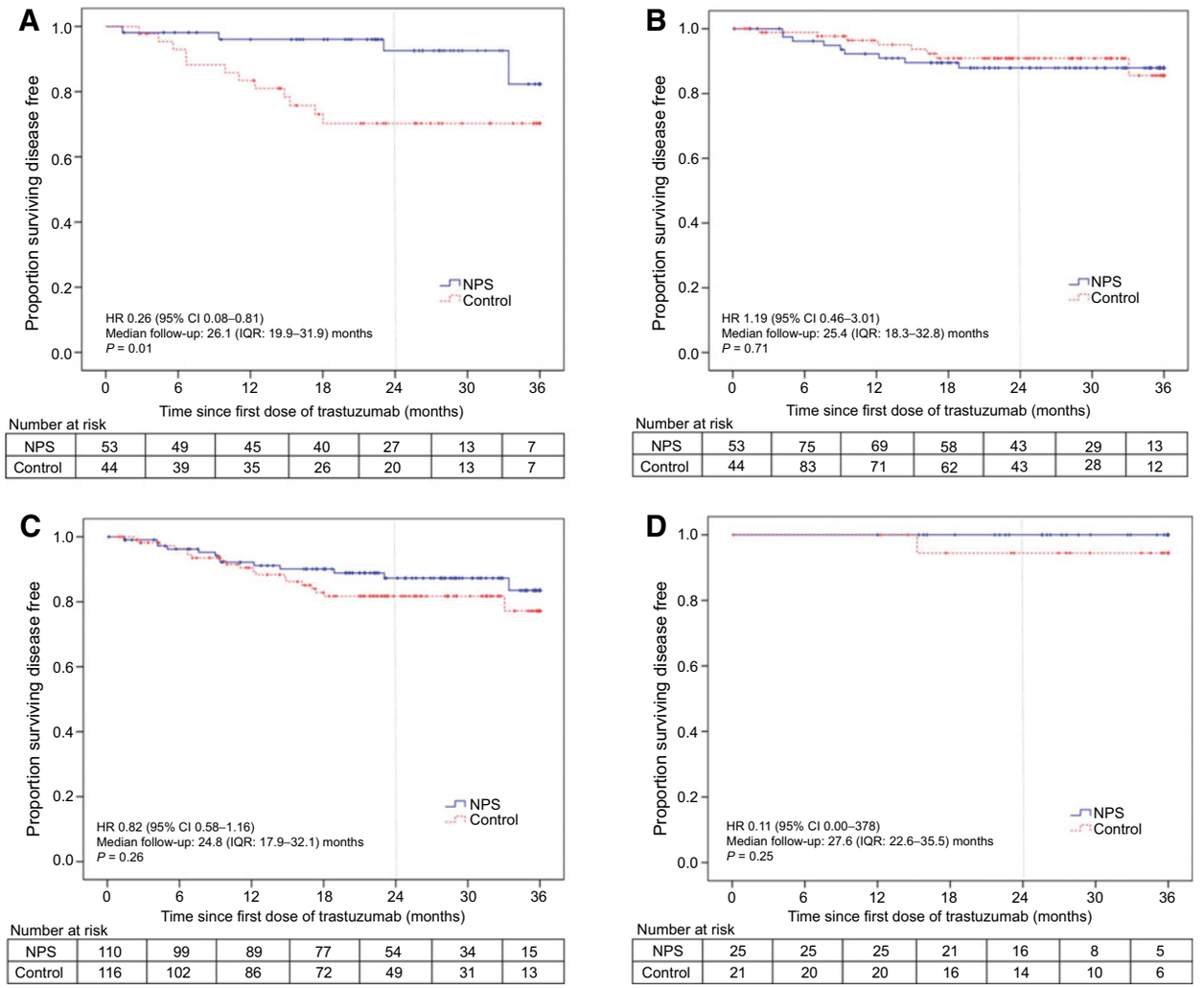


Figure 5.

A–D, DFS subsets. **A,** Patients with TNBC. **B,** Patients with hormone receptor (estrogen and/or progesterone receptor)–positive breast cancer. **C,** Patients with node-positive breast cancer. **D,** Patients with node-negative breast cancer. Dashed line indicated primary outcome of 24-month DFS.

Data from the current study suggest another potential approach for patients with TNBC that express a low level of HER2, specifically vaccination with NPS combined with trastuzumab. TNBC may be more responsive to a combination of active and passive immunotherapy due to increased levels of immune infiltrate in the tumor microenvironment relative to other breast cancer subtypes (20). This is further supported by improved progression-free survival seen in the IMpassion 130 trial that evaluated the anti–PD-L1 antibody atezolizumab combined with nab-paclitaxel in metastatic TNBC (21). Although other treatment options are emerging for TNBC, NPS combined with trastuzumab remains an attractive alternative, particularly in the adjuvant setting, due to low toxicity rates seen in this study.

The next step in the development of NPS is to confirm the efficacy in a phase III trial. The trial will enroll clinically disease-free patients with TNBC who are at high risk for recurrence due to the burden of residual cancer (residual cancer burden II or III; ref. 17) on their pathology after the completion of standard neoadjuvant chemotherapy and surgery. In order to evaluate the contribution of components of the combination of NPS and trastuzumab, the trial will randomize patients 1:1:2 to placebo (GM-CSF alone), NPS, or NPS with trastuzumab.

In conclusion, the combination of trastuzumab and NPS is safe with no additional toxicity over trastuzumab alone. This dual therapy was able to generate a specific and sustained immunologic response *in vitro* and *in vivo*. This combination did not show clinical benefit in the intention-to-treat population of patients with HER2 1+ or 2+ breast cancer. The exploratory analysis evaluating the TNBC subset found significant improvement in DFS. Based on these promising results, a phase III trial planned to enroll patients with TNBC with residual disease after neoadjuvant chemotherapy is being designed.

Disclosure of Potential Conflicts of Interest

J.K. Litton reports receiving commercial research grants from Genentech, Novartis, EMD-Serono, GlaxoSmithKline, Pfizer/Medivation, Zenith, Jounce, and AstraZeneca; reports receiving speakers bureau honoraria from Medlearning, Physician's Education Resource, Prime Oncology, Medscape, Medpage, and Clinical Care

Options; and is an unpaid consultant/advisory board member for Pfizer, AstraZeneca, and Ayala. R.K. Murthy is a paid consultant for Daiichi Sankyo, Genentech/Roche, Seattle Genetics, and Puma, and reports receiving other remuneration in the form of research support to their institution from Seattle Genetics, Genentech/Roche, EMD-Serono, Pfizer, and Daiichi Sankyo. G.E. Peoples is a paid consultant for Sellas Life Sciences Group; reports receiving commercial research grants from Sellas Life Sciences Group and Genentech; and is listed as a coinventor on a patent regarding the use of the NPS (E75) vaccine in the prevention of breast cancer recurrences that is owned by the U.S. government and licensed to Sellas Life Sciences Group. E.A. Mittendorf is a paid advisory board member for Merck, Genomic Health, Sellas Life Sciences, Genentech, and AstraZeneca, and reports receiving commercial research grants from GlaxoSmithKline. No potential conflicts of interest were disclosed by the other authors.

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