Clinical Cancer Research

Efficacy and Safety Analysis of Nelipepimut-S Vaccine to Prevent Breast Cancer Recurrence: A Randomized, Multicenter, Phase III Clinical Trial

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Abstract

Purpose: In phase I/II studies, nelipepimut-S (NP-S) plus GM-CSF vaccine was well tolerated and effectively raised HER2-specific immunity in patients with breast cancer. Results from a prespecified interim analysis of a phase III trial assessing NP-S + GM-CSF are reported.

Patients and Methods: This multicenter, randomized, double-blind phase III study enrolled females ≥18 years with T1–T3, HER2 low–expressing (IHC 1+/2+), node-positive breast cancer in the adjuvant setting. Patients received 1,000 μg NP-S + 250 μg GM-CSF or placebo + GM-CSF monthly for 6 months, then every 6 months through 36 months. The primary objective was disease-free survival (DFS). Protocol-specified imaging occurred annually. New abnormalities were categorized as recurrence events; biopsy confirmation was not mandated. The interim analysis was conducted as specified in the protocol after 73 DFS events.

Results: A total of 758 patients (mean age 51.8 years) were randomized. Adverse events were similar between groups; most common were injection-associated: erythema (84.3%), induration (55.8%), and pruritus (54.9%). There was no significant between-arms difference in DFS events at interim analysis at median follow-up (16.8 months). In the NP-S arm, imaging detected 54.1% of recurrence events in asymptomatic patients versus 29.2% in the placebo arm ($P = 0.069$).

Conclusions: NP-S was well tolerated. There was no significant difference in DFS events between NP-S and placebo. Use of mandated annual scans and image-detected recurrence events hastened the interim analysis contributing to early trial termination.

Introduction

HER2 is a target for mAb or receptor tyrosine kinase inhibitor therapy (1–6). In addition, HER2 has been evaluated as a tumor-associated antigen to be targeted by vaccination. Several HER2-derived peptides have been shown to elicit specific T cell and humoral immunity (7–13). The most studied of these is nelipepimut-S [NeuVax; HER2:aa369-377; E75 (NP-S)], which has been used in multiple vaccine formulations (7–9, 12, 13).

In a phase I/II adjuvant trial enrolling patients with node-positive and high-risk node-negative breast cancer with tumors expressing any level of HER2 (IHC 1+/2+/3+), NP-S was well tolerated, with the majority of patients experiencing only grade 1 local and systemic toxicity, including injection site erythema or pruritus, as well as bone pain, influenza-like symptoms, and fatigue. All patients completed the vaccination series, and there were no grade 4 or 5 toxicities (12). The majority of patients developed HER2-specific immunity, as assessed by delayed-type hypersensitivity reactions and dimer assays performed to measure peptide-specific cytotoxic T lymphocytes stimulated by vaccination (12, 14, 15). On the basis of the safety and immunologic data, the monthly intradermal dose of 1,000 μg NP-S and 250 μg of GM-CSF for 6 months was determined to be optimal (12). The disease-free survival (DFS) rate at 5 years for optimally dosed vaccinated patients was 94.6% versus 87.1% for unvaccinated controls ($P = 0.05$; ref. 12).

Given these promising results, a phase III adjuvant trial was undertaken in disease-free patients with T1–T3, node-positive breast cancer with IHC 1+/2+ HER2 expression. The target patient population with HER2 IHC 1+/2+ tumors was chosen because: (i) data from the early trials suggested robust immune responses to vaccination in these patients (16); (ii) there are no approved agents specifically for patients with HER2 low–expressing tumors; and (iii) multiple trials were investigating HER2-targeted therapies for patients with HER2-overexpressing tumors. The trial randomized patients to receive NP-S or placebo. GM-CSF immunoadjuvant was administered in both arms. Here, we report the results of the prespecified interim analysis for safety and futility performed after occurrence of 50% of the required DFS events.

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Translational Relevance

Nelipepimut-S (NP-S), the most studied HER2-derived peptide, represents a promising target for vaccination in patients with breast cancer. A randomized phase III trial was undertaken exploring the safety and efficacy of the NP-S vaccine versus placebo in preventing disease recurrence when administered in the adjuvant setting to patients with node-positive breast cancer expressing low or intermediate HER2. The results of the prespecified interim analysis are reported. The NP-S vaccine was safe and tolerable. At 16.8 months' median follow-up, there was no difference in disease-free survival between NP-S and placebo ($P = 0.07$), and the trial was stopped for futility in accordance with the prespecified futility criterion; that is, lower bound of the two-sided 95% confidence interval for the DFS HR was >0.9. Protocol-specified annual imaging detected the majority of recurrence events, which were not biopsy confirmed. Given vaccine-mediated mechanism of action, future adjuvant trials should emphasize clinical confirmation of recurrence.

Patients and Methods

Study design

PRESENT (Prevention of Recurrence in Early-Stage, Node-Positive Breast Cancer with Low to Intermediate HER2 Expression With NeuVax Treatment) was a multicenter, prospective, randomized, double-blind, placebo-controlled phase III trial. An independent data monitoring committee (IDMC) was responsible for assessing safety and efficacy, as well as monitoring overall study conduct. The primary objective was DFS. The primary endpoint, as agreed upon with the FDA, was to compare DFS upon reaching 141 DFS events, or after 36 months of follow-up after enrollment of the last patient, whichever was longer. DFS was defined by breast cancer recurrence identified clinically or as a new radiographic abnormality by protocol-specified imaging, occurrence of another cancer, or death by any cause.

Patient eligibility

Eligible patients included females ≥18 years old with a breast cancer diagnosis; tumor stage T1–T3 at diagnosis; node positivity; low (IHC 1+) or intermediate (IHC 2+) HER2 expression; and FISH negativity, defined as a *HER2*/CEP17 ratio < 2.0. Patients received National Comprehensive Cancer Network guideline–concordant systemic therapy, including chemotherapy administered in the neoadjuvant or adjuvant setting, and were confirmed to be disease free prior to randomization. Exclusions included bilateral breast cancer; inflammatory breast cancer; breast cancer history, ductal or lobular carcinoma *in situ*; prior trastuzumab therapy; and concurrent treatment with another investigational agent.

The study was approved by an institutional review board and was conducted in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences). Informed written consent was obtained from each patient.

Stratification and randomization

The trial involved a two-stage screening process, the first for eligibility, the second for HLA-A2 and HLA-A3 determination and central HER2 review to confirm HER2 expression levels using the Bond Oracle HER2 IHC System III (Leica Biosystems). FISH testing was performed using the PathVysion HER-2 DNA Probe Kit (Abbott Laboratories) in HER2 IHC 2+ cases. Eligible patients were randomized 1:1 to NP-S plus GM-CSF or placebo plus GM-CSF. Randomization, by interactive voice response system, was stratified by stage, type of surgery (lumpectomy vs. mastectomy), hormone receptor status, and menopausal status.

Vaccination

NP-S was supplied in 1 mL water for injection (WFI) at a concentration of 1.5 mg/mL. The placebo was WFI. Both were masked and not known to patients or site personnel. Prior to administration, these were mixed with lyophilized GM-CSF (sargramostim, Leukine; Sanofi-Aventis US LLC). Dosing was one intradermal dose monthly for 6 months, then booster inoculations every 6 months for a total of 11 doses over 36 months. Each active dose consisted of 1,000 μg NP-S and 250 μg GM-CSF. Each placebo dose consisted of WFI and 250 μg GM-CSF.

Assessments

Safety assessments included adverse events (AE), laboratory data (hematology, serum chemistry, urinalysis, and complete blood count with differential and platelet count), physical exams/assessment, electrocardiogram, and vital signs. In addition, a cardiac toxicity monitoring protocol evaluated left ventricular ejection fraction (LVEF) by multigated acquisition scans or echocardiograms. If cardiac dysfunction or a cardiac event (defined as >15% decrease in LVEF or 10%–15% decrease in LVEF to less than the institutional lower limit of normal) was observed in any patient during the period of study drug administration, the drug was discontinued and the patient underwent appropriate follow-up.

Assessments for recurrence were performed via protocol-specified imaging at the 12-, 24-, and 36-month follow-up visits and compared with mandatory baseline images. These imaging assessments included mammogram (except when bilateral mastectomy had been performed), CT (chest, abdomen, and pelvis), and bone scan. MRI was used if CT scan was not feasible, and positron emission tomography was performed if bone scan was not feasible. In addition, patients underwent routine clinical follow-up and possible disease recurrences suggested by clinical signs or symptoms were assessed using standard imaging modalities, including plain radiography, CT, MRI, or bone scan as recommended by the treating physician. Biopsies were not mandated for suspected recurrences.

The endpoint assessment committee (EAC) of two oncologists and a radiologist reviewed investigators' recurrence determinations. The EAC provided final adjudicated results, agreed upon by at least two reviewers.

Statistical analysis

DFS was compared using a proportional hazards model including four stratification factors (disease stage, type of surgery, hormone receptor status, and menopausal status). All patients were included in the DFS analysis. DFS by treatment arm was

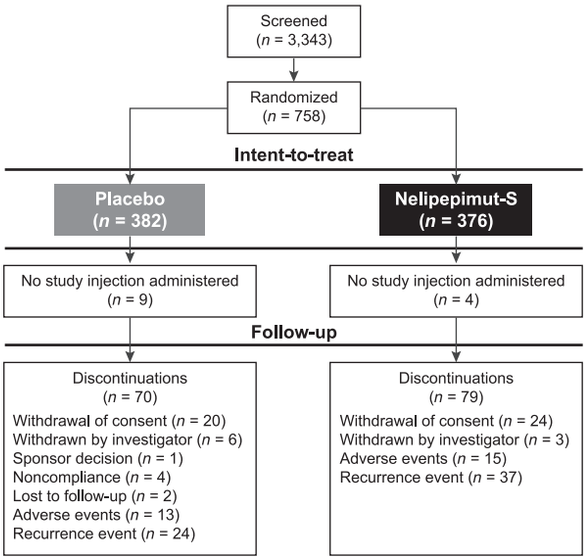

Figure 1.
CONSORT diagram illustrating the flow of patients through the study.

estimated via the Kaplan–Meier method. By prespecified criteria, the study was considered futile at the time of interim analysis if 95% two-sided lower confidence interval (CI) for the DFS HR was >0.9. This was a fixed criterion to be applied regardless of the number of events and subjects included as part of the analysis; no spending function boundary methodology was used to derive the futility criterion.

The study was designed to detect a 0.62 HR corresponding to an improvement in 3-year DFS from 77% for placebo plus GM-CSF to 85% for NP-S plus GM-CSF. The calculated total sample size for the trial was 709 patients to be enrolled over 33 months. A sample size of 332 subjects per group (allowing for dropout) had 80% power to detect the difference at a two-sided alpha level of 0.05 using a log-rank test for equality of survival curves. The total number of events needed to achieve the specified power was 141.

The interim analysis was prespecified to occur upon reaching 50% of the required DFS events, and occurred after 73 DFS events.

Results

Patient disposition

A total of 758 patients were enrolled (December 2011 to April 2015) and randomized, 376 to NP-S and 382 to placebo (Fig. 1). Patient characteristics are shown in Table 1. Median duration of time on trial was comparable between treatment arms (508 days for NP-S vs. 510 days for placebo). Median follow-up, to September 2016, was 16.8 months.

Safety and toxicity

Thirteen (3.5%) patients in the NP-S and 15 (3.9%) in the placebo group discontinued study participation due to AEs. In the NP-S group, AEs leading to discontinuation of more than 1 patient were urticaria in 4, and erythema and pruritus in 2 patients each. Treatment-emergent AEs described by the investigator as related to the study drug are summarized in Table 2. These were generally similar between groups, with the most common being those associated with injections, such as erythema (84.3%), induration (55.8%), and pruritus (54.9%). Severe AEs related to treatment occurring in 3 or more patients in the NP-S group were: injection site erythema (7); arthralgia (4); and neutropenia, increased alanine aminotransferase, increased aspartate aminotransferase, urticaria, and weight increase (3 each). Serious AEs in the NP-S group recorded in more than 1 patient included: ischemic stroke and endometrial hyperplasia in 3 patients each; and pulmonary embolism, depression, uterine leiomyoma, and postoperative wound infection in 2 patients each. No serious AEs were determined to be related to the vaccine. Three deaths were recorded: acute B-cell precursor lymphoblastic leukemia and pulmonary embolism (NP-S), and asthmatic crisis (placebo). These were considered not related to treatment.

Cardiac events were recorded in 15 of 372 NP-S patients and 10 of 373 placebo patients in the safety population. Eight (2.2%) NP-S patients and 7 (1.9%) placebo patients experienced changes in LVEF. One (0.3%) NP-S patient and 3 (0.8%) placebo patients experienced a new onset or sustained rhythm or conduction abnormality, and 6 (1.6%) NP-S and 2 (0.5%) placebo patients experienced a thromboembolic disorder. Two NP-S patients experienced new onset or exacerbation of acute coronary syndrome. There was 1 discontinuation in the NP-S group, due to hypertensive crisis.

Efficacy

Over an 18-month period, the EAC reviewed 81 cases, of which 73 (90.1%) were determined to be DFS events. There were 61 cases of suspected disease recurrence, 9 cases of new primary breast or second cancers, and 3 deaths (Table 3). A detailed distribution of cancers contributing to DFS events is shown in Supplementary Table S1. Median time to any DFS event was 319 days. The difference in DFS between groups was not significant ($P = 0.07$), based on a log-rank test stratified by disease stage, type of surgery, hormone receptor status, and menopausal status (Fig. 2). The estimated HR was 1.564 (95% CI, 0.960–2.549) from a stratified Cox proportional hazards model with stage, type of surgery, hormone receptor status, and menopausal status as stratification factors. The 3-year Kaplan–Meier estimated DFS rates were 77.1% in the NP-S group versus 77.5% in the placebo group. On the basis of these data, the IDMC recommended the study be stopped for futility.

Timing of DFS events is shown in Supplementary Fig. S1. Recurrence events were identified at 12 months in 17 patients in the NP-S arm and 9 patients in the placebo arm. Recurrence events were identified at 24 months in 6 patients in the NP-S arm and 1 patient in the placebo arm. Patient charts were reviewed to determine whether recurrence events revealed by protocol-specified imaging on scheduled annual scan visits corresponded temporally with the presence of clinical symptoms of recurrence. Forty-four percent (27/61) of all recurrence events were detected by imaging without associated clinical symptoms (Table 3; Supplementary Table S2). The number of recurrence events found by protocol-specified imaging in the NP-S arm (20 patients, 54.1%) was higher than in the placebo arm (7 patients, 29.2%), whereas the percentage diagnosed clinically in the NP-S arm was lower than in the placebo arm (45.9% vs. 70.8%). Overall, of all image-only detected events, 20 of 27 (74%) occurred in the NP-S arm.

Discussion

Here, we report the interim analysis of the PRESENT trial that investigated the NP-S vaccine administered in the adjuvant setting to patients with T1–T3, node-positive breast cancer with low to intermediate HER2 expression. The NP-S safety profile was not significantly different from the placebo population, confirming vaccine safety. In contrast to the phase I/II study, where vaccination resulted in an approximately 50% decrease in clinical

Table 1. Patient characteristics (ITT population)

Characteristic	Placebo ($n = 382$)	NP-S ($n = 376$)	Total ($n = 758$)
Age, median (range), year	52.0 (24.0–75.0)	52.0 (26.0–76.0)	52.0 (24.0–76.0)
BMI, mean (SD)	28.0 (6.3)	28.0 (5.8)	28.0 (6.0)
Race, *n* (%)			
White	373 (97.6)	361 (96.0)	734 (96.8)
Non-white/missing	9 (2.4)	15 (4.0)	24 (3.2)
Menopausal status, *n* (%)			
Pre-/perimenopausal	161 (42.1)	157 (41.8)	318 (42.0)
Postmenopausal	221 (57.9)	219 (58.2)	440 (58.0)
Time since diagnosis, days			
Mean (SD)	290.3 (66.5)	292.5 (66.2)	291.4 (66.3)
Median	287.0	287.0	287.0
Disease stage, *n* (%)			
IIA	113 (29.6)	118 (31.5)	231 (30.5)
IIB	164 (42.9)	161 (42.9)	325 (42.9)
IIIA	105 (27.5)	96 (25.6)	201 (26.6)
Missing	0	1 (0.3)	1 (0.1)
Grade			
Low	32 (8.4)	30 (8.0)	62 (8.2)
Intermediate	184 (48.2)	164 (43.6)	348 (45.9)
High	93 (24.3)	101 (26.9)	194 (25.6)
Missing	73 (19.1)	81 (21.5)	154 (20.3)
Hormone receptor status[a], *n* (%)			
Positive	324 (84.8)	321 (85.4)	645 (85.1)
Negative	58 (15.2)	55 (14.6)	113 (14.9)
HER2 Status			
0	14 (3.7)	14 (3.7)	28 (3.7)
1+	261 (68.3)	267 (71.0)	528 (69.7)
2+	106 (27.7)	94 (25.0)	200 (26.4)
3+	0	1 (0.3)	1 (0.1)
Missing	1 (0.3)	0	1 (0.1)
T Stage, *n* (%)			
T1	131 (34.3)	127 (33.8)	258 (34.0)
T2	225 (58.9)	219 (58.2)	444 (58.6)
T3	26 (6.8)	29 (7.7)	55 (7.3)
Could not be assessed	0	1 (0.3)	1 (0.1)
Clinical nodal status[b], *n* (%)			
NX	7 (1.8)	8 (2.1)	15 (2.0)
N0	158 (41.5)	147 (39.1)	305 (40.3)
N1	208 (54.6)	213 (56.6)	421 (55.6)
N2	8 (2.1)	7 (1.9)	15 (2.0)
N3	0	1 (0.3)	1 (0.1)
Missing	1 (0.3)	0	1 (0.1)
Pathologic nodal status, *n* (%)			
pNX	1 (0.3)	1 (0.3)	2 (0.3)
pN0	9 (2.4)	8 (2.1)	17 (2.2)
pN1	280 (73.3)	279 (74.2)	559 (73.7)
pN2	91 (23.8)	86 (22.9)	177 (23.4)
pN3	1 (0.3)	2 (0.5)	3 (0.4)
ECOG Performance status, *n* (%)			
0	311 (81.6)	307 (82.1)	618 (81.9)
1	70 (18.4)	67 (17.9)	137 (18.1)
Missing	1 (0.3)	2 (0.5)	3 (0.4)
Surgery, *n* (%)			
Breast conservation	130 (34.1)	125 (33.2)	255 (33.7)
Mastectomy	251 (65.9)	251 (66.8)	502 (66.3)
Missing	1 (0.3)	0	1 (0.1)
Therapy, *n* (%)			
Chemotherapy	381 (99.7)	376 (100.0)	757 (99.9)
Hormonal	322 (84.3)	322 (85.6)	644 (85.0)
Radiotherapy	327 (85.6)	323 (85.9)	650 (85.8)
Other	2 (0.5)	2 (0.5)	4 (0.5)

Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; ITT, intent-to-treat.

[a]Patients were considered hormone receptor–positive if either estrogen receptor or progesterone receptor was positive.

[b]Node-positive disease was defined as clinically node–positive disease in patients who presented with a suspicious lymph node on physical examination or identified by radiologic imaging and confirmed by core or fine needle aspiration biopsy, or pathologically node-positive disease identified in the surgical specimen. Patients with clinically node–positive disease qualified even if final pathologic assessment of their lymph nodes was pN0 following neoadjuvant chemotherapy.

Table 2. Study drug–related[a] treatment-emergent AEs

Number of patients (%)	Placebo ($n = 373$)	NP-S ($n = 372$)	Total ($N = 745$)
Any related treatment-emergent AE	334 (89.5)	330 (88.7)	664 (89.1)
Any related treatment-emergent AE CTCAE grade 3–5	21 (5.6)	22 (5.9)	43 (5.8)
Grade 3	20 (5.4)	22 (5.9)	42 (5.6)
Grade 4	1 (0.3)	2 (0.5)	3 (0.4)
Grade 5	0 (0)	0 (0)	0 (0)
Related treatment-emergent AEs, ≥3% in the NP-S group			
Injection site			
Erythema	312 (83.6)	316 (84.9)	628 (84.3)
Induration	195 (52.3)	221 (59.4)	416 (55.8)
Pruritus	191 (51.2)	218 (58.6)	409 (54.9)
Swelling	158 (42.4)	181 (48.7)	339 (45.5)
Pain	135 (36.2)	154 (41.1)	289 (38.8)
Edema	55 (14.7)	73 (19.6)	128 (17.2)
Discomfort	36 (9.7)	35 (9.4)	71 (9.5)
Arthralgia	13 (3.5)	22 (5.9)	35 (4.7)
Influenza-like illness	16 (4.3)	20 (5.4)	36 (4.8)
Fatigue	21 (5.6)	17 (4.6)	38 (5.1)
Headache	18 (4.8)	17 (4.6)	35 (4.7)
Pyrexia	16 (4.3)	13 (3.5)	29 (3.9)
Back pain	13 (3.5)	13 (3.5)	26 (3.5)
Pruritus	7 (1.9)	11 (3.0)	18 (2.4)

Abbreviation: CTCAE, Common Terminology Criteria for Adverse Events (version 4.03).
[a]Possibly, probably, or definitely related.

recurrence risk (12), this interim analysis failed to demonstrate clinical benefit for vaccination. Although the active and placebo arms were well matched with respect to clinicopathologic characteristics, there were 37 (9.8%) recurrence events in the NP-S group versus 24 (6.3%) in the placebo group. However, the number of deaths, second cancers, or symptomatic recurrences contributing to the total number of recurrence events was not different between treatment groups. The number of recurrence events identified by protocol-specified imaging of asymptomatic patients, which accounted for approximately half of recurrence events, was disproportionate, with 74% occurring in the NP-S group. Given these findings, the study was stopped for futility after a median follow-up of 16.8 months.

Previous studies of NP-S vaccine showed encouraging results (12). The phase I/II clinical trials enrolled 187 patients, including 97 node-positive and 90 node-negative patients with tumors expressing any degree of HER2 (IHC 1+ to 3+). Per protocol design, the primary analysis of the combined trials was performed at 18 months' median follow-up. At that time, the recurrence rate was 5.6% in the vaccinated group compared with 14.2% in the control group ($P = 0.04$; ref. 15). In the PRESENT trial, at 16.8 months' median follow-up, recurrence rates in the vaccinated and placebo groups were 9.8% and 6.3%, respectively.

Table 3. Composition of the interim analysis DFS events

DFS event, *n* (%)	Placebo ($n = 382$)	NP-S ($n = 376$)
Recurrence events	24 (6.3)	37 (9.8)
Clinical recurrence events	17 (70.8)	17 (45.9)
Protocol-specified imaging-only event	7 (29.2)	20 (54.1)[a]
Second cancer[b]	4 (1.0)	5 (1.3)
Deaths not due to cancer	1 (0.3)	2 (0.5)

[a]$P = 0.069$ versus placebo via Fisher exact test.
[b]New breast cancer or new secondary cancer.

This low recurrence rate in the placebo group is notable, as the statistical plan for the PRESENT trial was based on a projected recurrence rate of 23% at 3 years in unvaccinated patients, derived from the recurrence rate in the earlier study. The PRESENT trial took longer than anticipated to be approved, fully activated, and accrue, and during that time, there were changes in standard practice that may have impacted recurrence rates. Specifically, the improvement seen in the control cohort could be due to the addition of taxanes as a standard component of adjuvant chemotherapy regimens (17). Of note, however, is that the estimated 3-year DFS rate for the control group in PRESENT was 77.5%, which is in line with the projected 23% recurrence rate used for statistical assumptions during trial planning.

Important aspects of the PRESENT trial are that protocol-specified imaging was required yearly, and that this annual imaging is not standard practice. Interestingly, approximately half of the recurrence events were detected only by this protocol-specified imaging. Clinical correlations, serial imaging, and/or biopsies were not required to define these events as recurrences. By including protocol-specified annual imaging, the study was different than other adjuvant therapy trials in which follow-up imaging is dictated by clinical assessment consistent with American Society of Clinical Oncology guidelines for routine follow-up of patients with early-stage breast cancer (18). The PRESENT trial did not require biopsy confirmation of recurrent disease; therefore, it is unknown whether these imaging-identified lesions were all clinically relevant.

When the two treatment groups were compared, there were more DFS events in the NP-S group than in the placebo group, but the deaths, second cancers, and clinical recurrences were similar at 16.8 months median follow-up. The difference between groups was exclusively related to protocol-specified annual imaging, which accounted for 54% of the recurrence events in the NP-S group compared with 29% in the placebo group. Because the two groups were comparable with respect to clinicopathologic characteristics and standard-of-practice therapies received, the near tripling (20 vs. seven) of radiographic findings seen in the vaccine arm compared with the placebo arm is likely related to NP-S treatment. This begs the question of whether the vaccine caused recurrences. The vaccine comprises a nine-amino acid peptide combined with GM-CSF. The placebo group received GM-CSF, suggesting the GM-CSF is not responsible for the differences. Data from previous peptide–GM-CSF versus GM-CSF trials suggest side effects, but not the immunologic activity, are attributable to GM-CSF (10, 11). The nine-amino acid peptide has no intrinsic biologic activity; therefore, it is difficult to construct a mechanism by which the vaccine can cause disease recurrence. An alternative hypothesis to explain the differences in image-detected recurrence is pseudoprogression. Briefly, pseudoprogression is a phenomenon where a cancer appears to show progression on radiographic imaging due to increased size from immune cell infiltration (19). While pseudoprogression has been described primarily in the context of trials investigating immune checkpoint blockade agents, it has also been described in studies evaluating cytokines and cancer vaccines, including a recombinant canarypox viral vaccine in patients with metastatic melanoma (20–22). Recognizing different response patterns observed with immune therapy, the iRECIST for immune-based therapeutics were proposed to account for apparent increases in tumor burden, including the appearance of new lesions, which may precede response to

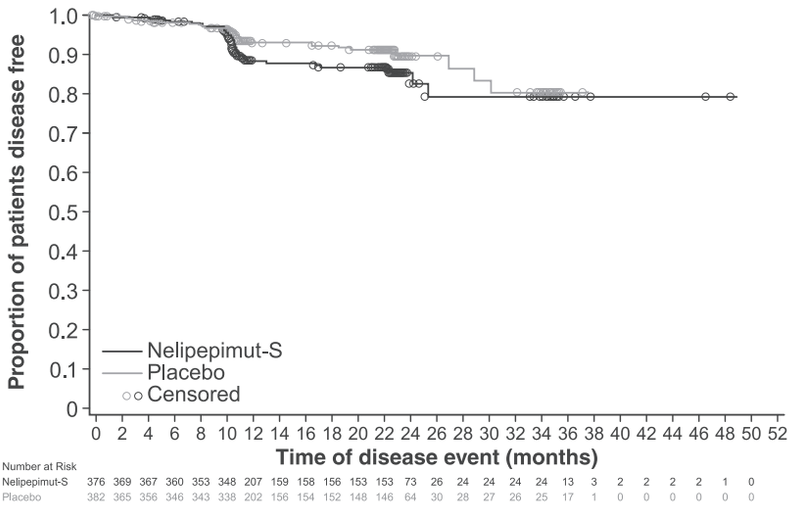

Figure 2.

DFS. Kaplan–Meier estimate for DFS at the interim analysis conducted after 73 adjudicated recurrence events. Median follow-up = 16.8 months. There was no difference with respect to DFS between vaccinated patients and those patients receiving placebo ($P = 0.07$).

immunotherapy (23). Pseudoprogression has been described, and iRECIST used, in studies in patients with established, measurable metastatic disease. It is interesting to hypothesize that a phenomenon similar to pseudoprogression may occur in patients with minimal residual disease to include those treated in the adjuvant setting, such as in the current trial, reflecting initial signs of immune activity. Any future immunotherapy adjuvant trials where routine imaging for recurrence will be utilized should include clinical correlation, biopsy confirmation, and/or serial imaging as done in metastatic trials per iRECIST, to confirm recurrence.

The PRESENT trial enrolled patients with HER2 1+ or 2+ tumors. The NSABP B-47 trial also addressed this population of patients, and investigated whether adding trastuzumab to chemotherapy improved invasive DFS rates (24). The results of that study were recently reported. The 5-year invasive DFS rate was 89.2% for patients receiving chemotherapy alone versus 89.6% with the addition of trastuzumab ($P = 0.90$). While the PRESENT trial only enrolled patients with node-positive disease, the NSABP study enrolled patients with high-risk node-negative disease, and 20% of patients in the study had no nodal metastases, suggesting a slightly lower-risk population. While trastuzumab did not show benefit in the NSABP study, our group previously reported data suggesting potential synergy between trastuzumab and a $CD8^+$ T-cell–eliciting vaccine. Specifically, a study evaluating a $CD8^+$ T-cell–eliciting vaccine that enrolled patients with HER2-positive (IHC 3+) breast cancer showed a 100% DFS rate for patients vaccinated after trastuzumab ($n = 48$) compared with 89% in unvaccinated controls ($n = 50$), after a median follow-up of 34 months (10). Preclinical data suggest trastuzumab-enhanced antigen cross-presentation as a potential mechanism of synergy. Briefly, trastuzumab facilitates uptake of HER2 by dendritic cells for subsequent presentation of the HER2-derived epitope NP-S complexed with MHC class I molecules. This increased NP-S cross-presentation enables more efficient expansion of peptide-specific $CD8^+$ T cells. These data suggest that patients treated with trastuzumab may experience robust antitumor immune responses by restimulation of T cells by vaccination (25). On the basis of these data, our group is currently conducting a study of patients with HER2 1+ or 2+ breast cancer, randomizing them to trastuzumab or trastuzumab plus the NP-S vaccine to determine whether stimulation of a $CD8^+$ T-cell response can render HER2 1+ or 2+ breast cancer (or a specific subgroup thereof) susceptible to trastuzumab therapy. Trial accrual and randomization have been completed.

Conclusion

In conclusion, at the time of this prespecified interim analysis, there were no differences in DFS between arms. The use of mandated annual scans and image-only recurrence events hastened the interim analysis, and the increased number of events in the vaccine arm directly contributed to early trial termination. The clinical significance of these radiographic-only findings is unclear, but they were seen three times more frequently in the vaccine arm, suggesting a relationship with vaccine treatment. Whether this demonstrates pseudoprogression and vaccine activity cannot be known, as these patients were not followed after trial termination. Future studies of immunotherapeutic agents in the adjuvant setting should be conducted similarly to adjuvant trials of other chemotherapeutic or targeted therapies, with patients being followed per standard practice for clinical recurrence.

Disclosure of Potential Conflicts of Interest

E.A. Mittendorf is a consultant/advisory board member for Amgen, Peregrine Pharmaceuticals Inc., AstraZeneca, TapImmune, Merck, SELLAS Life Sciences, and Genetech/Roche. M. Melisko has ownership interests (including patents) at Merrimack, reports receiving speakers bureau honoraria from Genentech, Pfizer, and Agendia, and is a consultant/advisory board member for Eisai and Genetech. K. Petrakova reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Novartis, Pfizer, and Roche. G.E. Peoples is listed as a co-inventor on a patent on the use of nelipepimut-S as a vaccine to prevent breast cancer recurrence, owned by the US government and licensed to Sellas Life Sciences Group, and is a consultant/advisory board member for Galena Biopharma. No potential conflicts of interest were disclosed by the other authors.

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