# Phase II Trial of Nelipepimut-S Peptide Vaccine in Women with Ductal Carcinoma *In Situ*

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## ABSTRACT

NeuVax is a vaccine comprised of the HER2-derived MHC class I peptide E75 (nelipepimut-S, NPS) combined with GM-CSF. We completed a randomized trial of preoperative vaccination with NeuVax versus GM-CSF alone in patients with ductal carcinoma *in situ* (DCIS). The primary objective was to evaluate for NPS-specific cytotoxic T lymphocyte (CTL) responses. Patients with human leukocyte antigen (HLA)-A2-positive DCIS were enrolled and randomized 2:1 to NeuVax versus GM-CSF alone and received two inoculations prior to surgery. The number of NPS-specific CTL was measured pre-vaccination, at surgery, and 1 and 3 to 6 months post-operation by dextramer assay. Differences in CTL responses between groups and between pre-vaccination and 1-month post-operation were analyzed using a two-sample *t* test or Wilcoxon rank sum test. The incidence and severity of adverse events were compared between groups. Overall, 45 patients were registered; 20 patients were HLA-A2 negative, 7 declined participation, 1 withdrew, and 4 failed screening for other reasons. The remaining 13 were randomized to NeuVax ($n = 9$) or GM-CSF alone ($n = 4$). Vaccination was well-tolerated with similar treatment-related toxicity between groups with the majority (>89%) of adverse events being grade 1. The percentage of NPS-specific CTLs increased in both arms between baseline (pre-vaccination) and 1-month post-operation. The increase was numerically greater in the NPS+GM-CSF arm, but the difference was not statistically significant. NPS+GM-CSF is safe and well-tolerated when given preoperatively to patients with DCIS. In patients with HLA-A2-positive DCIS, two inoculations with NPS+GM-CSF can induce *in vivo* immunity and a continued antigen-specific T-cell response 1-month postsurgery.

**Prevention Relevance:** This trial showed that vaccination of patients with HLA-A2-positive DCIS with NeuVax in the preoperative setting can induce a sustained antigen-specific T-cell response. This provides proof of principle that vaccination in the preoperative or adjuvant setting may stimulate an adaptive immune response that could potentially prevent disease recurrence.

## Introduction

Ductal carcinoma *in situ* (DCIS) is a pre-invasive malignancy accounting for approximately 15% to 20% of radiographically diagnosed breast neoplasms (1, 2). Current treatment of DCIS consists of various combinations of surgical resection, radiation therapy, and endocrine therapy. Treatment results in high cure rates with overall survival approaching 100% (3). Therefore, an important goal of these treatments is to prevent disease recurrence. However, there are both short-term and long-term potential morbidities with these therapies, including poor cosmesis, persistent pain, reconstruction complications, radiation toxicities, and side effects of hormonal therapy. Given the prevalence of DCIS, more effective strategies are needed to minimize disease recurrence while simultaneously limiting treatment morbidity. Immunotherapy is one such preventive strategy that may work by stimulating an adaptive immune response against the malignancy thereby increasing treatment options while decreasing morbidity. Importantly, an immunotherapy strategy such as vaccination may be most efficacious in pre-invasive malignancies like DCIS, where systemic and

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Cancer Prev Res 2023;16:333–42

**doi:** 10.1158/1940-6207.CAPR-22-0388

tumor microenvironmental immune suppression are less profound (4).

HER2 has been identified as a target antigen for cancer vaccines and immunotherapy because of its overexpression and oncogenicity in a subset of breast cancers. In DCIS, HER2 overexpressing phenotypes account for approximately 20% to 56% of all lesions, and are associated with increased rates of invasive foci and invasive recurrence when compared with other subtypes (5–8). The ability of HER2-based dendritic cell (DC) vaccines to induce an antigen-specific immune response in patients with DCIS has been previously demonstrated in a pilot clinical trial (9–11). Compared with DC vaccines, which require patients to undergo leukapheresis followed by an *ex vivo* priming of monocyte precursors to generate the DC product, peptide vaccines represent a simple, "off the shelf" strategy that combines a peptide with an immunoadjuvant that can be administered as an intradermal inoculation. Nelipepimut-S (NPS, E75, HER2 369–377, KIFGLSAFL), a nine amino acid human leukocyte antigen (HLA) restricted peptide from the extracellular domain of the HER2 protein, represents one such peptide vaccine. Prior clinical trials have established that the NeuVax (NPS + GM-CSF) vaccine is safe and effective in stimulating an antigen-specific immune response in metastatic and early-stage invasive breast cancer (12–18).

Given the known ability of HER2-based DC vaccines to induce antigen-specific responses in DCIS, the favorable immunologic results of NeuVax in invasive breast cancer, and the theory that immunotherapy is likely more effective in pre-invasive malignancies, we performed a multicenter, prospective, randomized, single-blind trial of NeuVax in patients with DCIS. Our primary objective was to evaluate for NPS-specific cytotoxic T lymphocyte (CTL) response 1-month postsurgical resection.

## Materials and Methods

### Study design

This was a multicenter, prospective, randomized, single-blind phase II trial (www.clinicaltrials.gov, NCT02636582). HLA-A2-positive women with DCIS identified on core needle biopsy (CNB) were enrolled and randomized 2:1 to receive the NeuVax vaccine or GM-CSF alone. The primary objective was to evaluate for NPS-specific CTL responses in patients receiving NeuVax compared with patients receiving GM-CSF alone (control). The primary endpoint was change in mean percentage of NPS-specific CTLs from baseline to 1-month postsurgical resection. Secondary endpoints included toxicity profile and frequency of adverse events (AE), presence of DCIS at resection, difference in HER2 expression between biopsy specimen and surgical specimen, and degree of lymphocyte infiltration in surgically resected specimens. The trial was designed to have 40 evaluable patients, 27 in the vaccine group and 13 in the control group, to have 82% power to detect an effect size of one on the change of NPS-specific CTLs between a baseline level acquired prior to first vaccination and the 1-month postoperative follow-up. This would correspond to a change of 0.05% ± 0.05% in the vaccination group and 0% ± 0.05% in the control group using a two-sided $t$ test with a significance level of 0.05. Therefore, target accrual was set at 48 patients to allow for a 10% attrition rate while on study and for an approximately 5% nonevaluable sample rate.

### Patient eligibility and randomization

Eligible patients included females ≥18 years old with DCIS identified on CNB and an area of radiographic abnormality measuring at least 1 cm. As NeuVax is an HLA-restricted peptide vaccine, only patients with the HLA-A2 allele were eligible for randomization. HER2-positivity was not required for eligibility because HER2 is not routinely assessed at time of DCIS diagnosis as standard clinical practice. In addition, previous clinical trials enrolling women with invasive breast cancer have shown the ability of NeuVax to induce a NPS-specific CTL response in patients with tumors having any degree of HER2 expression (15–17). To be eligible, patients were also required to have an Eastern Cooperative Oncology Group performance status of 1 or 2, adequate liver and kidney function on clinical laboratory evaluation, and a normal left ventricular ejection fraction (LVEF) assessed by echocardiogram. Exclusion criteria included bilateral DCIS, invasive breast cancer, history of prior breast cancer or DCIS, or concurrent pregnancy or breastfeeding.

Forty-five patients were assessed for eligibility. Twenty patients were excluded for negative HLA-A2, seven declined to participate, one withdrew to undergo surgical resection at an external facility, and four failed screening for other reasons. A total of 13 patients were randomized 2:1 to receive NPS+GM-CSF vaccine or GM-CSF alone (**Fig. 1**). The trial was closed to new patient recruitment prior to achieving full accrual secondary to protracted study recruitment and drug expiration. The study was approved by the NCI Central Institutional Review Board with local acknowledgement required at each participating site and informed written consent obtained from each participant. The study was conducted in accordance with the CONSORT study guidelines and the ethical guidelines of the U.S. Common Rule.

### Vaccination protocol

NPS, a nine-amino acid, MHC class I peptide, is manufactured as a 1.5 mg/mL solution in 1.0 mL of sterile water for injection (Oso Biopharmaceuticals Manufacturing). Each vaccine dose consisted of 1,000 μg of NPS + 250 μg of GM-CSF. Patients randomized to the GM-CSF alone group received 250 μg of GM-CSF reconstituted in sterile water for injection. Inoculations were administered intradermally as four 0.4 mL aliquots in the anterior thigh 4 weeks and then again at 2 weeks prior to surgical resection. Both vaccine and control doses were masked and unknown to patient but not to site personnel.

### Safety assessments

Pre-vaccination safety assessments included physical examinations, laboratory data monitoring, and echocardiograms. Patients were assessed for AEs at the time of each study

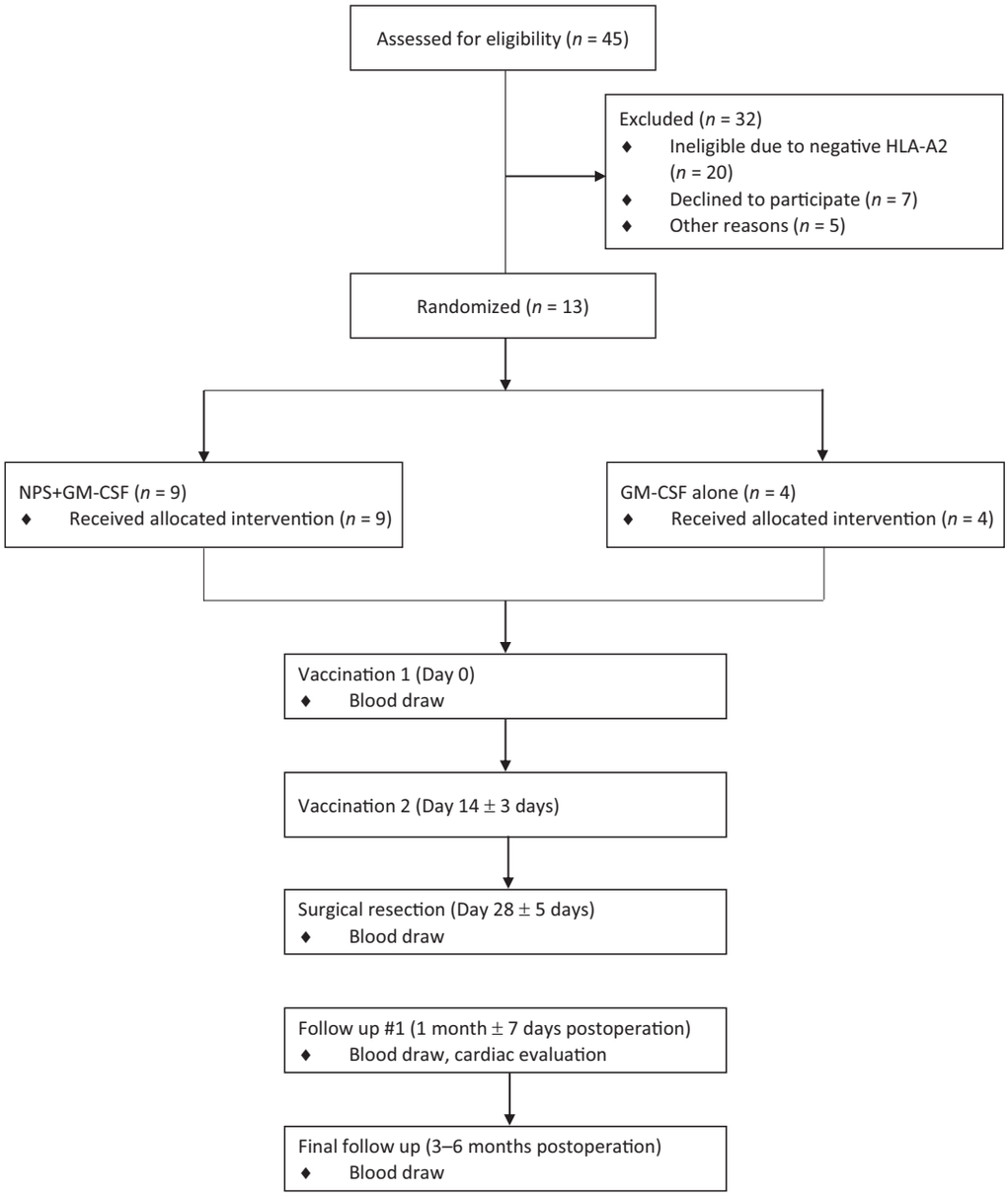


**Figure 1.**
CONSORT flow diagram for VADIS trial. Consort flow diagram showing participant flow through each stage of the randomized trial (eligibility assessment, randomization, treatment, follow-up and biospecimen collection).

vaccination, prior to surgery and at both the first follow-up (1 month ± 7 days postoperatively) and final follow-up (3–6 months postoperatively) appointments. Routine laboratory monitoring was performed at the time of the first vaccination and at the time of surgical resection. Cardiac toxicity monitoring was performed at first follow-up by evaluation of LVEF with echocardiogram. Local and systemic toxicities were identified using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Toxicities were classified as related if they were deemed "definite," "possible," or "probable" by the site primary investigator and as not related if they were deemed "unlikely" or "unrelated" to the study treatment by the site primary investigator.

## Dextramer NPS flow cytometry assay

Patients were assessed for evidence of immunologic response by quantification of NPS-specific CTL (=$CD8^+$ T cells) using a dextramer assay. Staining was performed on blood obtained pre-vaccination, at the time of surgical resection, 1 month after surgical resection, and at final follow-up (3–6 months post-surgical resection). Peripheral blood mononuclear cells (PBMC) 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 LSR Fortessa Analyzer (BD Biosciences). The frequency of NPS-specific CTL was determined as the percentage of live cells gated on $lin^-/CD3^+/CD8^+/E75\text{-}dextramer^+$. Flu and negative HLA-A2 dextramer were used as positive and negative controls, respectively, in each sample (19).

### IHC

Standard IHC techniques were utilized to measure expression of specific biomarkers in tissue samples from both the initial breast CNB and the subsequent surgical excision specimen. H&E slides as well as additional unstained slides for biomarker assessment were prepared. The presence or absence of DCIS or invasive breast cancer, the grade of tumor, and the presence or absence of necrosis was assessed. Standard antibody recognition techniques were used to assess the presence of the following specific biomarkers: HER2, CD3, CD4, and CD8.

The degree of lymphocyte infiltration was determined in both biopsy and postsurgical excision specimens. Intratumoral tumor-infiltrating lymphocytes (iTIL) were defined as those within the basement membrane. Stromal TILs were defined as those in the periductal/lobular stroma including the intralobular stromal infiltrate. Cells in the interlobular stromal inflammatory infiltrate were excluded. All mononuclear cells were scored with polymorphonuclear leukocytes being excluded. Intratumoral and stromal TILs were scored as a continuous variable and the percentage of TILs in the surgical specimen was compared with that in the pre-vaccination diagnostic biopsy.

### HER2 expression

HER2 expression was not used for patient eligibility for this trial. However, HER2 was measured in the pre- and post-vaccination tissue samples to determine whether there was any immune-mediated change in HER2 expression induced by the vaccine. Differences in HER2 expression in the pre-vaccination biopsy specimen and the post-vaccination surgical specimen were evaluated. HER2 scoring was determined according to the American Society of Clinical Oncology/College of American Pathologists clinical guidelines (20). Positive cases were defined as IHC score 3+. Equivocal or indeterminate cases were those with IHC 2+. Negative cases were defined as those with IHC score 0–1+. Pre-vaccination and post-vaccination specimens from individual patients were compared.

### Statistical analysis

Data were summarized either as median and interquartile range (IQR) or count (*n*) and percentage and compared using either a Wilcoxon rank-sum nonparametric test (continuous) or Fishers exact test (categorical) where appropriate. Differences in NPS-specific CTLs between groups were evaluated by two sample *t* test or Wilcoxon rank sum test where appropriate. Across all analyses, statistical significance was set at $P < 0.05$. Stata (Release 16; StataCorp LLC) was used for all calculations.

### Data availability

The data from the trial are available on the Cancer Data Access System at: https://cdas.cancer.gov/learn/eppt/browse/mda2014–04–02/.

## Results

### Demographics

Overall, most patients included in the study were postmenopausal and Caucasian. When compared with the NeuVax treatment group, patients enrolled in the GM-CSF alone control group were slightly older (median age 57 vs. 55) with a higher proportion of black/African American patients [50.0% (2 of 4) vs. 22.2% (2 of 9)], and Hispanic or Latino patients [25.0% (1 of 4) vs. 11.1% (1 of 9)]. Despite the HLA-A2 haplotype being less prevalent in minorities than in Caucasians (21), 31% of patients (4 of 13) included in the study were African American and 15% (2 of 13) were Hispanic or Latino (Supplementary Table S1).

### NPS-specific CTL responses

The trial's primary endpoint was change in mean percentage of NPS-specific CTLs from baseline to 1-month postsurgical resection. Both groups experienced an increase in NPS-specific CTLs (**Fig. 2**). The relative increase was numerically higher for the NeuVax group (11-fold; $0.01 \pm 0.02\%$ vs. $0.11 \pm 0.12\%$) than the GM-CSF alone control group (2.25-fold; $0.04 \pm 0.07\%$ vs. $0.09 \pm 0.15\%$; **Fig. 2**). The elevation in mean percentage of NPS-specific CTL in both groups was sustained at final follow up 3 to 6 months post-operatively (NeuVax: $0.14 \pm 0.12\%$, GM-CSF alone: $0.15 \pm 0.06\%$; **Fig. 2**). Patients in the NeuVax treatment group experienced a numerically larger but not statistically significantly different increase in median percentage of NPS-specific CTL during the 1-month time period when compared with the GM-CSF alone control group (0.09 vs. 0.02, $P = 0.71$; Supplementary Table S2).

### Safety and tolerability

Vaccination was well-tolerated with similar treatment-related toxicity profiles in both the treatment and control groups. No grade 3 or higher treatment-related AEs were experienced in either group. In the NeuVax treatment group, there were 45 treatment-related AEs reported [42 (93.3%) grade 1, 3 (6.7%) grade 2] with all patients experiencing at least one treatment-related AE. When evaluated relative to the total number of patients in each group, the GM-CSF alone control group experienced a similar rate of treatment-related AEs [29 treatment-related AEs reported in 4 (100%) patients], with 89.7% of treatment-related AEs considered grade 1 and 10.3% considered grade 2. Injection site reaction was the most prevalent AE overall, comprising 68.9% of all AEs experienced by the NeuVax treatment group and 58.6% of all AEs

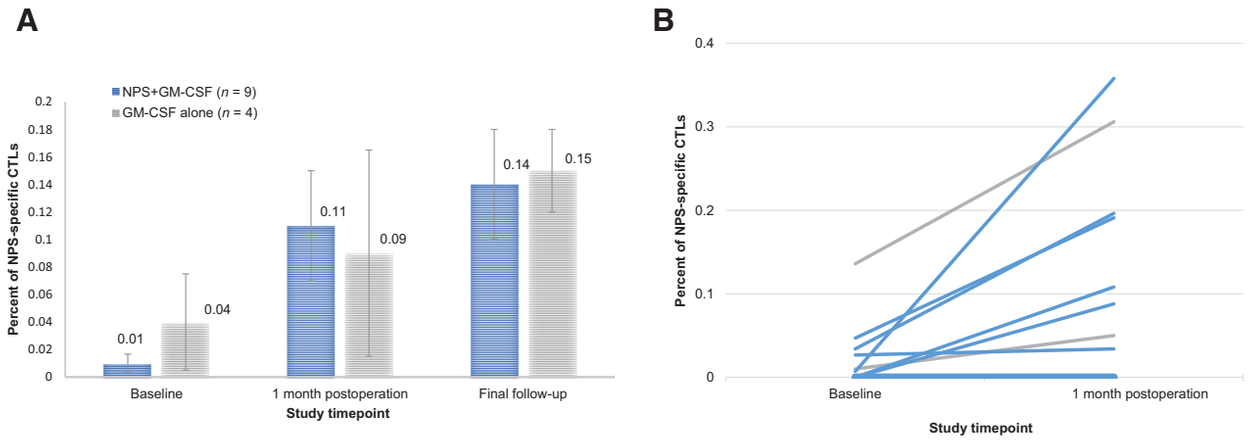


**Figure 2.**

NPS-specific CTL responses. **A,** Mean percent and SD (error bars) of NPS-specific CTL responses as determined by flow cytometry-based dextramer assay presented by treatment group at each study time point. The trial's primary endpoint was the change in mean percentage of NPS-specific CTL from baseline to 1-month postsurgical resection. Both groups experienced an increase in NPS-specific CTLs with the increase in the NeuVax group being numerically higher but not statistically significantly so as determined by the Wilcoxon rank sum test. The elevation in the mean percentage of NPS-CTL in both groups was sustained at long term follow up which occurred 3 to 6 months postoperatively. **B,** Baseline to 1-month postsurgical resection levels of NPS-specific CTL as a percentage of total lymphocytes for each patient. The line along the x-axis showing no change (from 0% to 0%) comprises five patients; three receiving NeuVax and two in the control arm receiving GM-CSF only.

experienced by the GM-CSF alone control group. A full list of treatment-related AEs is shown in **Table 1**.

One AE met the criteria of a serious AE (SAE). This involved a patient who had her baseline and 1-month postoperative LVEF assessed using two different modalities; a multigated acquisition scan (MUGA) at baseline had an LVEF of 67% and an echocardiogram (ECHO) after surgery of 57%. There was a ≥10% absolute difference in LVEF reading between these two testing points, therefore, per protocol requirement, this was reported as an SAE. The patient remained asymptomatic and per the treating physician, following discussion with a consulting cardiologist, the difference in LVEF was attributed to the two different modalities used to assess. It is noted that the protocol specified that ECHO should be performed to assess

**Table 1.** Study drug-related[a] treatment-emergent AEs.

| Grade 1: CTCAE term | NeuVax, *n* (%)[b] | Patients experiencing AE, *n* (%)[c] | GM-CSF alone, *n* (%) | Patients experiencing AE, *n* (%) | Total |
|---|---|---|---|---|---|
| Injection site reaction, *n* (%)[c] | 31 (68.9) | 8 (88.9) | 17 (58.6) | 4 (100.0) | 48 (64.9) |
| Arthralgia | 2 (4.4) | 1 (11.1) | 0 (0.0) | 0 (0.0) | 2 (2.7) |
| Bruising | 0 (0.0) | 0 (0.0) | 2 (6.9) | 1 (25.0) | 2 (2.7) |
| Fatigue | 2 (4.4) | 2 (22.2) | 0 (0.0) | 0 (0.0) | 2 (2.7) |
| Headache | 1 (2.2) | 1 (11.1) | 2 (6.9) | 1 (25.0) | 3 (4.1) |
| Myalgia | 3 (6.7) | 2 (22.2) | 1 (3.4) | 1 (25.0) | 4 (5.4) |
| Nausea | 0 (0.0) | 0 (0.0) | 1 (3.4) | 1 (25.0) | 1 (1.4) |
| Pain | 0 (0.0) | 0 (0.0) | 1 (3.4) | 1 (25.0) | 1 (1.4) |
| Pruritus | 2 (4.4) | 2 (22.2) | 1 (3.4) | 1 (25.0) | 3 (4.1) |
| Sinus tachycardia | 1 (2.2) | 1 (11.1) | 0 (0.0) | 0 (0.0) | 1 (1.4) |
| Skin hyperpigmentation | 0 (0.0) | 0 (0.0) | 1 (3.4) | 1 (25.0) | 1 (1.4) |
| Grade 1 total | 42 (93.3) | 8 (88.9) | 26 (89.7) | 4 (100.0) | 68 (91.9) |
| **Grade 2: CTCAE term** | **NeuVax, *n* (%)** | **Patients experiencing AE, *n* (%)** | **GM-CSF alone, *n* (%)** | **Patients experiencing AE, *n* (%)** | **Total** |
| Injection site reaction | 0 (0.0) | 0 (0.0) | 3 (10.3) | 1 (25.0) | 3 (4.1) |
| Cardiac disorders—other[d] | 1 (2.2) | 1 (11.1) | 0 (0.0) | 0 (0.0) | 1 (1.4) |
| Fatigue | 1 (2.2) | 1 (11.1) | 0 (0.0) | 0 (0.0) | 1 (1.4) |
| Hypertension | 1 (2.2) | 1 (11.1) | 0 (0.0) | 0 (0.0) | 1 (1.4) |
| Grade 2 total | 3 (6.7) | 3 (33.3) | 3 (10.3) | 1 (25.0) | 6 (8.1) |
| Overall total | 45 | 9 (100.0) | 29 | 4 (100.0) | 74 (100.0) |

[a]Possibly, probably, or definitely related.
[b]Percentages calculated from total number of treatment-related AEs within each treatment group.
[c]Percentages calculated from total number of patients within each treatment group.
[d]Decreased ejection fraction.

LVEF. The MUGA had been obtained in this patient around the time of study entry for a different clinical indication and since it was interpreted as a normal was used as the cardiac assessment for trial participation. A repeat MUGA performed 1 month after the ECHO showed a LVEF of 59%.

### Pathologic characteristics

One patient out of the 9 receiving NeuVax experienced complete response with no residual DCIS found on surgical specimen as compared with none out of the 4 patients in the control group. Only eight of 13 patients randomized had pathology data at both baseline and 1-month post-resection and were included in this analysis (NeuVax: $n = 5$, GM-CSF alone: $n = 3$). In the NeuVax treatment group, both baseline CNB and surgical specimens had higher proportions of high-grade DCIS when compared with the GM-CSF alone control group (CNB: 40% vs. 33.3%, $P = 1.0$; surgical: 60% vs. 33.3%, $P = 0.68$; Supplementary Table S3). In contrast, baseline CNB and surgical specimens in the GM-CSF alone control group demonstrated higher proportions of comedo necrosis (CNB: 66.7% vs. 40%, surgical: 66.7% vs. 20%, overall $P = 0.71$ and 0.68, respectively; Supplementary Table S3). The two groups had similar amounts of DCIS remaining in their surgical specimens (Supplementary Table S3). Differences in pathologic characteristics between treatment and control groups were not statistically significant (Supplementary Table S3).

### Lymphocyte infiltration

When comparing degree of lymphocytic infiltration, there was an average increase in the mean number of iTILs and decrease in the number of stromal TILs comparing baseline CNBs to surgical specimens in both the treatment and control groups. However, no significant statistical difference was noted between the two treatment groups (Supplementary Table S4).

### HER2 expression

Patients in the NeuVax treatment group exhibited a higher proportion of HER2 overexpression (HER2 3+) in baseline biopsy specimens when compared with the GM-CSF control group (33.3% vs. 0.0%, overall $P = 0.172$; Supplementary Table S5). However, both groups exhibited similar proportions of HER2 overexpression in the surgical resection specimens (22.2% vs. 25.0%, overall $P = 0.38$; Supplementary Table S5). In addition, 1 patient in the NeuVax group exhibited decreased HER2 expression on the surgical specimen as compared with none in the control group (11.1% vs. 0.0%). On comparing change in HER2 status between CNB specimens and surgical specimens against NPS-specific CTL responses, no correlation was noted (Supplementary Figs. S1 and S2).

## Discussion

Given challenges with accrual resulting in only 13 patients enrolling on this trial, definitive conclusions cannot be drawn. The primary endpoint of this study was change in mean percentage of NPS-specific CTLs from baseline to 1-month postsurgical resection. Therefore, because there was no difference in the change in NPS-specific CTLs between patients receiving NeuVax and those receiving GM-CSF only, this was a negative study. However, the trial did show that antigen-specific T-cell response can be induced in patients with DCIS 1-month postsurgical resection in HLA-A2-positive patients that is sustained through final follow-up which occurred 3 to 6 months after surgery. Interestingly, patients in both the NeuVax group and the control group saw an increase in their NPS-specific CTL that was sustained long-term. This finding was unexpected and indicates that the effect should be attributed to GM-CSF, an immunoadjuvant, stimulating an immune response against a strong tumor-associated antigen, HER2. Unfortunately low accrual into the trial left it underpowered to show differences between the groups as well as limited the ability to draw meaningful conclusions from additional correlative studies performed on specimens obtained from enrolled patients. However, it is noted that there was no significant difference in lymphocytic infiltration or HER2 expression between the NeuVax and control groups.

Early-phase studies of NeuVax in invasive breast cancer showed encouraging results. In a phase II clinical trial including patients with both node positive and node negative breast cancer with any degree of HER2 expression (IHC 1+ to 3+), vaccination with NeuVax was associated with lower recurrence rates at 18 months when compared with controls (5.6% vs. 14.2%, $P = 0.04$) and at 5 years, optimally vaccinated patients had higher disease-free survival (DFS) rates (94.6% vs. 87.1%, $P = 0.05$; refs. 15, 16). In a separate phase II trial evaluating trastuzumab and NeuVax versus trastuzumab and GM-CSF in patients with HER2 low-expressing (IHC 1+/2+) breast cancer at high risk of recurrence (node positive or estrogen receptor negative/progesterone receptor negative), patients with hormone receptor-negative, HER2 low-expressing breast cancer who received the vaccine in addition to the HER2-targeting mAb had significantly improved 24-month DFS when compared with control (92.6% vs. 70.2%, $P = 0.01$; ref. 19). However, the phase III PRESENT trial that evaluated the administration of the vaccine in the adjuvant setting for patients with HER2 low-expressing, T1-T3, node-positive breast cancer failed to demonstrate a DFS difference between NeuVax and GM-CSF groups at a median follow-up of 18.6 months (9.8% vs. 6.3%, $P = 0.07$; ref. 22). The study was terminated early due to lack of efficacy. Despite the limited effectiveness of the vaccine in the phase III trial, it is noted that NeuVax has been consistently safe and well-tolerated across all studies. The finding that the toxicities are comparable between patients receiving NeuVax and those receiving GM-CSF alone suggests that the toxicities are attributable to the GM-CSF immunoadjuvant.

We have hypothesized that the vaccine may be less effective in the setting of immunosuppression that develops in association with invasive disease. A more effective strategy may be to administer vaccines early, such as in patients with *in situ* cancer, to increase immunosurveillance and prevent progression to

invasive disease. Moving vaccination earlier in the disease process is a path towards creating a preventive vaccine. It is well-established that there are many different mechanisms of immune suppression present in advanced malignancy from increased numbers of myeloid-derived suppressor cells, tumor-associated macrophages, and regulatory T cells, to disturbance of cytokine networks, to increased production of amino-acid degrading enzymes, and establishment of a stromal barrier that limits infiltration of immune cells into the tumor (23–25). In contrast, early premalignant lesions have microenvironments that are permeated by cells of both the innate and adaptive immune systems with activated phenotypes as well as higher levels of effector cytokines (i.e., IFNγ) indicative of an ongoing and more robust antitumor immune response (4). HER2 is a well-established tumor-associated antigen that is overexpressed in a portion of DCIS. In addition, Datta and colleagues have demonstrated that HER2-directed $CD4^+$ T helper 1 cell ($CD4^+$ Th1) responses decrease with increasing tumorigenesis and are associated with poorer prognosis in HER2-positive DCIS and invasive cancer (26). These diminished responses have provided the impetus for investigation of neoadjuvant and adjuvant HER2-directed immunotherapy in DCIS to prevent invasive conversion or recurrence.

In 2012, Sharma and colleagues (9) published the results of a pilot trial evaluating a HER2 DC-based vaccine in patients with HER2 overexpressing DCIS. Twenty-seven patients received weekly injections of personalized *in vitro* matured DCs pulsed with HLA class I-binding and HLA class II-binding HER2-derived peptides in the 4 weeks prior to surgical resection. On pathology, 18.5% (5/27) had no residual DCIS, and of the 22 patients with evaluable specimens, 50% experienced a decrease in HER2 expression suggesting an active process of "immunoediting" in HER2-expressing tumor cells following vaccination (9). When compared with this autologous DC-based vaccine, our peptide-specific vaccine resulted in 1 of 9 (11.1%) patients without residual DCIS, but only 1 of 9 (11.1%) patients experienced a decrease in HER2 expression. This diminutive proportion of decreased HER2 expression may be a result of the paucity of IHC data available secondary to issues with slide retrieval and processing limiting the number of evaluable specimens. In addition, our study enrolled patients with DCIS regardless of the extent of HER2 expression in their CNB, which may also account for a discrepancy between the decreases in HER2 expression. Overall, no definitive conclusions can be reached regarding the ability of NeuVax to stimulate an antigen-specific response contributing to immunoediting of the in-breast DCIS.

The trial's small sample size limited the power and ability to draw conclusions regarding differences in NPS-specific CTL responses as well as the other correlative endpoints. There were multiple reasons cited by the study team for low accrual in the trial including: (i) HLA-A2 negativity (20 of 45 women screened), (ii) a reluctance by patients to delay their surgery for the administration of the study vaccine, (iii) a competing trial evaluating a strategy of "watchful waiting" versus surgery for DCIS patients, and (iv) during the conduct of this trial, the phase III study of NeuVax in patients with invasive breast cancer was stopped due to futility possibly reducing enthusiasm from participants and investigators. To improve accrual, the study team employed several strategies. To address the concern over surgery delay, the team adapted an existing "DCIS Fact Sheet" to inform women about the nature of DCIS and the safety of a short delay in surgery given what is known about DCIS progression. In addition, the study initially called for six inoculations and 9 months of follow-up. The study was revised to two inoculations and a 1-month follow-up aligned with the post-operational visit. In addition, "Talking Points" were created to help the study staff explain the study to best be able to inform potential participants. Unfortunately, it took time to revise the trial design and implement the tools described. That, combined with an issue of drug supply availability due in part to the vaccine being acquired by a company other than the one that had initially approved of this trial, forced us to close the study to accrual; therefore, we were unable to assess the impact of these changes.

In conclusion, despite being underpowered, this trial provides support for further testing of vaccines such as NeuVax in the neoadjuvant and adjuvant settings in an attempt to prevent invasive recurrence in DCIS and the challenges associated with accruing to these types of studies. The stimulation of a sustained NPS-specific CTL response in the peripheral blood suggests that this strategy may in fact stimulate an adaptive immune response that could potentially prevent disease recurrence. Additional studies would be required to determine the optimal number and timing of inoculations to stimulate an optimal antigen-specific immune response followed by trials randomizing patients to NeuVax or no therapy with disease recurrence as the primary endpoint. Such a study would require a large number of patients followed for a long period of time given the known rates of DCIS recurrence following current standard therapy.

## Authors' Disclosures

G.T. Clifton reports personal fees from Parthenon Therapeutics outside the submitted work. D. Weber reports grants from NCI during the conduct of the study. A. Husband reports nonfinancial support and other support from University of Texas MD Anderson Cancer Center, nonfinancial support from SELLAS, Inc. and MRI Global during the conduct of the study; other support from Dana-Farber Cancer Institute outside the submitted work. P.H. Brown reports other support from Genetex outside the submitted work. G.E. Peoples reports grants from Sellas during the conduct of the study; personal fees from Nighthawk Biosciences, Pelican Therapeutics, Emtora Biotherapeutics, Elios Therapeutics, and Orbis Health Solutions outside the submitted work; also has a patent for On E75 peptide issued, licensed, and with royalties paid from Sellas. E.A. Mittendorf reports grants from NIH and MD Anderson Cancer Center during the conduct of the study; personal fees from Merck, Genomic Health, and Roche/Genentech, and grants from Roche/Genentech outside the submitted work. No disclosures were reported by the other authors.

## Authors' Contributions

**A.E. O'Shea:** Data curation, writing–original draft, writing–review and editing. **G.T. Clifton:** Data curation, writing–original draft, writing–review

and editing. **N. Qiao:** Data curation, investigation, writing–review and editing. **B.M. Heckman-Stoddard:** Conceptualization, formal analysis, supervision, project administration, writing–review and editing. **M. Wojtowicz:** Formal analysis, supervision, writing–review and editing. **E. Dimond:** Supervision, project administration, writing–review and editing. **I. Bedrosian:** Investigation, writing–review and editing. **D. Weber:** Data curation, investigation, writing–review and editing. **J.E. Garber:** Investigation, writing–review and editing. **A. Husband:** Data curation, investigation, writing–review and editing. **R. Pastorello:** Data curation, writing–review and editing. **J. Lee:** Supervision, writing–review and editing. **M. Hernandez:** Data curation, writing–review and editing. **D.D. Liu:** Data curation, writing–review and editing. **L.A. Vornik:** Data curation, project administration, writing–review and editing. **P.H. Brown:** Conceptualization, resources, formal analysis, supervision, funding acquisition, project administration, writing–review and editing. **G. Alatrash:** Formal analysis, methodology, writing–review and editing. **G.E. Peoples:** Conceptualization, formal analysis, methodology, writing–review and editing. **E.A. Mittendorf:** Conceptualization, resources, formal analysis, supervision, funding acquisition, methodology, writing–original draft, project administration, writing–review and editing.

## Acknowledgments

This work was supported by the NCI, DCP Contracts HHSN261201200034I to the UT MD Anderson Early Phase Chemoprevention Consortium (to P.H. Brown) and by a grant from The University of Texas MD Anderson Cancer Center Duncan Family Institute for Cancer Prevention and Risk Assessment. Dr. Mittendorf acknowledges the Rob and Karen Hale Distinguished Chair in Surgical Oncology for support. E75 peptide was provided under a clinical trials agreement with the Division of Cancer Prevention.

The publication costs of this article were defrayed in part by the payment of publication fees. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

## Note

Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

Received August 29, 2022; revised January 31, 2023; accepted April 24, 2023; published first April 25, 2023.

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