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VADIS Trial: Phase II trial of Nelipepimut-S Peptide Vaccine in Women with DCIS of the Breast

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Agent(s)/Supplier: NeuVax ™ (nelipepimut-S) supplied by SELLAS, Inc.; GM-CSF (rhuGM-CSF; Leukine® [sargramostim]) supplied by MRI Global

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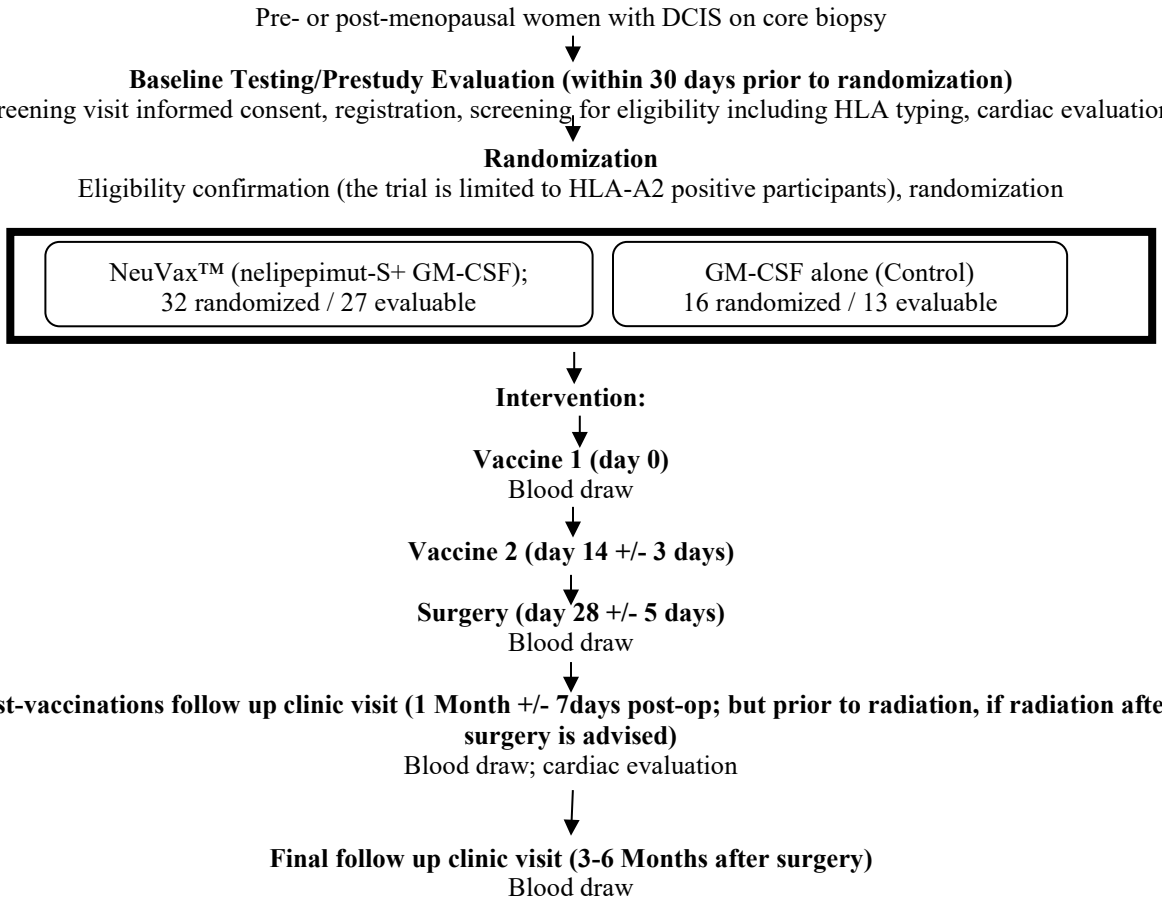
SCHEMA

VADIS Trial: Phase II trial of the Nelipepimut-S Peptide Vaccine in Women with DCIS of the Breast



Endpoints

Primary: Evaluate for nelipepimut-S-specific cytotoxic T lymphocyte (CTL; CD8+ T cell) response in vaccinated participants compared to participants receiving GM-CSF alone.

Secondary:

a) Toxicity profile and frequency of adverse events in women with DCIS of the breast receiving nelipepimut-S vaccine as compared to women receiving GM-CSF alone.
b) Presence of DCIS at resection;
c) Difference in HER2 expression in the biopsy and the surgical specimen excised post-vaccination;
d) Histologic responses:
    a. Degree of lymphocyte infiltration as determined on H&E stained slides and immune infiltration as determined by multiplex immunofluorescence staining for markers including but not limited to CD3, CD4 and CD8,
e) Immune infiltrates in normal tissue maximally distant from the tumor (in mastectomy samples).

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Evaluate for nelipepimut-S-specific cytotoxic T lymphocyte (CTL; $CD8^+$ T cell) response in participants receiving NeuVax (nelipepimut-S plus GM-CSF) compared to participants receiving GM-CSF alone (Control).

1.2 Secondary Objectives

1.2.1 Toxicity profile and frequency of adverse events in women with DCIS of the breast receiving nelipepimut-S vaccine as compared to women receiving GM-CSF alone;

1.2.2 Presence of DCIS at resection;

1.2.3 Difference in HER2 expression in the biopsy and the surgical specimen excised post-vaccination;

1.2.4 Histologic responses:

1.2.4.1 Degree of lymphocyte infiltration determined on H&E stained slides and immune infiltration as determined by multiplex immunofluorescence staining for markers including but not limited to CD3, CD4 and CD8;

1.2.4.2 Immune infiltrates in normal tissue maximally distant from the tumor (in mastectomy samples).

2. BACKGROUND

2.1 DCIS of the Breast

Breast cancer accounts for approximately 41% of all cancers in women in the U.S [1]. It is further estimated that, in 2014 alone, new cases of cancer in U.S. women will include 232,670 diagnoses of invasive breast cancer and 51,933 diagnoses of ductal carcinoma *in situ* [2]. Current treatment guidelines for DCIS recommend local therapy to include either lumpectomy without lymph node surgery plus whole breast radiation therapy or total mastectomy with or without sentinel lymph node biopsy. For patients that have ER+ DCIS and undergo breast conserving therapy with lumpectomy and radiation, tamoxifen may be considered for 5 years to reduce the risk of an ipsilateral breast recurrence. Despite high cure rates with this current standard of care for DCIS, there are still patients that recur and ultimately succumb to breast cancer. A recent observational study reported on 108,196 women who had been diagnosed with DCIS from 1988 to 2011 and were captured in the Surveillance, Epidemiology, and End Results (SEER) program [3]. The mean age of diagnosis was 54 years of age. For patients undergoing lumpectomy, the risk of an ipsilateral invasive recurrence at 10 years was 4.9% which was decreased to 2.5% if radiation was administered. At 20 years, the breast cancer-specific mortality was 3.3% (95% CI, 3.0%-3.6%). Importantly, the risk of dying of breast cancer was increased after an ipsilateral invasive breast cancer recurrence (HR 18, 95% CI: 14-24, $p<.001$). Interestingly, there were also patients (n=517) that died of breast cancer following a diagnosis of DCIS that did not experience a known in-breast invasive cancer recurrence. Given these data, there is a need for more effective prevention strategies. Our group has been investigating immune-based prevention strategies in breast cancer and this protocol represents one of these efforts specifically for patients with DCIS.

Little is known regarding the endogenous immune response to DCIS or the potential role of targeting DCIS with immunotherapy. In a study of 69 participants performed at the MD Anderson Cancer Center looking at the biologic and immunologic effects of pre-operative trastuzumab (monoclonal antibody targeting HER2), it was shown that a single dose of trastuzumab administered to participants with HER2+ DCIS does not result in histologic, antiproliferative or apopototic changes but did result in the ability to mount antibody-dependent cell mediated cytotoxicity in all participants [4]. Czerniecki et al. conducted a trial evaluating a vaccine that consisted of dendritic cells (DC) pulsed with HER2-derived major

histocompatibility class I and class II peptides. The trial enrolled participants with DCIS with HER2 overexpression (>2+ intensity) in at least 10% of cells as determined using HerceptTest [5-7]. The trial enrolled 29 participants who were administered the vaccine as four weekly intranodal injections administered prior to surgery. The vaccination strategy was shown to be feasible and safe. In addition, the investigators demonstrated an antigen specific immune response with induction of IFNγ producing CD4+ and CD8+ T cells in the peripheral blood as well as in increased lymphocyte infiltrate into the surgically excised tumors versus the biopsy specimens. Interestingly, in a small subset of participants, there was decreased HER2 expression in the surgically excised tumor compared to the pre-treatment biopsy in seven participants suggesting immunoediting of the HER2+ cells.

Our group has also been investigating vaccination strategies in breast cancer. Specifically, we have been investigating HER2-derived peptide vaccines. Compared to DC vaccines which require the patients to undergo leukapheresis followed by an *ex vivo* processing of the 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. One of the vaccines that we have studied is nelipepimut-S which is a major histocompatibility class (MHC) I vaccine that consists of the immunogenic peptide (nelipepimut-S) combined with the immunoadjuvant GM-CSF. Previous data from clinical trials has shown that the nelipepimut-S vaccine is safe, with minimal toxicity, and effective in stimulating an antigen-specific immune response in metastatic [8-10] and early-stage invasive [11-14] breast cancer. For patients with early stage cancer that have been rendered disease free with standard of care therapy, clinical trials have shown that the nelipepimut-S vaccine decreases the risk of recurrence [11-13]. Nelipepimut-S is currently being evaluated in a phase III registration trial (NCT01479244). In addition to its immunologic effects in breast cancer, the effect of nelipepimut-S treatment has been examined in the peripheral blood mononuclear cells (PBMCs) from healthy people. Following stimulation with nelipepimut-S, the PBMCs from 50% of healthy donors exhibited nelipepimut-S-specific cytolytic activity (2 responded at priming, 3 responded after 2 additional restimulations [15]. In addition, the study suggested that nelipepimut-S-precursory cytotoxic T lymphocytes (CTLs) were not tolerized in the majority of donors [15].

Based upon these positive results, we developed this study testing the Nelipepimut-S vaccine in women with DCIS of the breast to determine whether antigen-specific immunity is induced and whether the induced immune response suppresses the growth of DCIS cells. Completion of the current study will provide the necessary data to determine the next phase of study, specifically whether a subsequent, larger phase II will be conducted versus a phase III study.

2.2 NeuVax

Interest in the development of cancer vaccines increased after advances in the molecular characterization of human tumors led to the identification of tumor-associated antigens that can be recognized by T cells. Tumor-associated antigens expressed by tumors can elicit a very specific immune response; therefore, a vaccine is appealing in that it represents a nontoxic therapeutic modality with great specificity. HER2 is one such tumor-associated antigen. Several peptides capable of inducing CTL have been described from the HER2 protein including nelipepimut-S, a 9 amino acid peptide derived from the HER2 protein's extracellular domain (aa: 369-377:KIFGSLAFL), which is the most studied in laboratory and clinical investigation (reviewed by Mittendorf EA, et al. [16]).

The vaccine consists of the nelipepimut-S peptide (1000 μg) mixed with 250 μg GM-CSF (significantly less than the GM-CSF dose of 250 $\mu g/m^2$ administered daily either IV or SC for most clinical indications for GM-CSF). This dose was determined to be the optimal dose in early phase clinical trials [17]. It has

been shown to be safe and capable of stimulating an antigen-specific immune response. Nelipepimut-S has been licensed to SELLAS, Inc. (SELLAS).

2.3 Rationale

Preclinical studies investigating nelipepimut-S both *in vitro* and in an *in vivo* animal model showed that it was capable of inducing a peptide-specific, CTL-mediated immune response [18]. This then led to early clinical trials in patients with metastatic breast cancer where the vaccine, which consisted of nelipepimut-S and the immunoadjuvant GM-CSF, was shown to be safe and capable of eliciting an antigen-specific immune response [8-10]. The therapeutic benefit of the vaccine was then evaluated in phase I/II studies conducted by our group in the adjuvant setting in earlier stages of breast cancer [11-13, 19]. The initial trial, which enrolled node-positive breast cancer patients with tumors expressing any degree of HER2 (immunohistochemistry [IHC] 1+, 2+ or 3+), was a phase I two-stage safety trial designed with escalating doses of nelipepimut-S peptide in the initial stage followed by schedule alterations in the second stage [12]. A trial enrolling high-risk node-negative patients was designed to delineate optimal biologic dosing. Both trials transitioned to phase II with disease recurrence as the primary efficacy endpoint. Patients in both studies had received standard therapy including surgery, chemotherapy, and radiation therapy as appropriate and were without evidence of disease at the time of enrollment. Because of the human leukocyte antigen (HLA)-restriction of the nelipepimut-S peptide, patients were HLA typed after enrollment and then placed in either the treatment arm (HLA-$A2^+$ or $A3^+$ patients) or the observation group (HLA-$A2^-$ or $A3^-$ patients). The goals of these trials were to document safety, immunogenicity and clinical efficacy of the nelipepimut-S vaccine. Because the node-positive and node-negative trials were run concurrently and the protocols were identical except for overlapping doses/schedules, the results were merged for analysis [13].

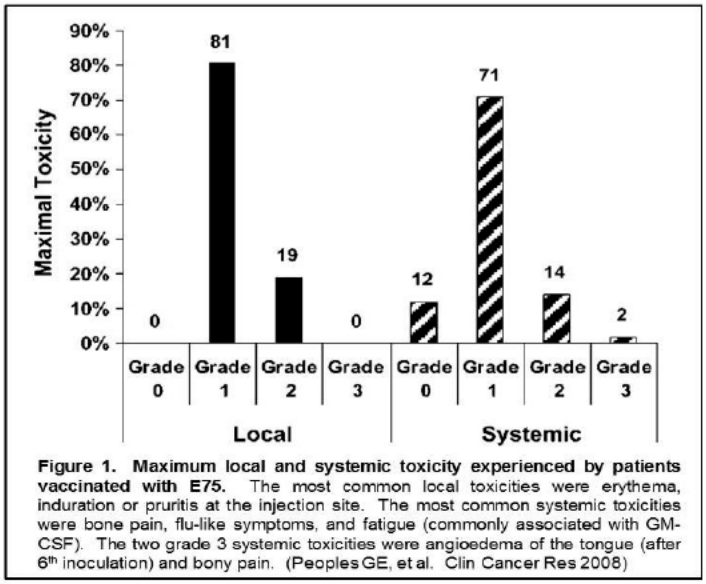

Figure 1. Maximum local and systemic toxicity experienced by patients vaccinated with E75. The most common local toxicities were erythema, induration or pruritis at the injection site. The most common systemic toxicities were bone pain, flu-like symptoms, and fatigue (commonly associated with GM-CSF). The two grade 3 systemic toxicities were angioedema of the tongue (after 6th inoculation) and bony pain. (Peoples GE, et al. Clin Cancer Res 2008)

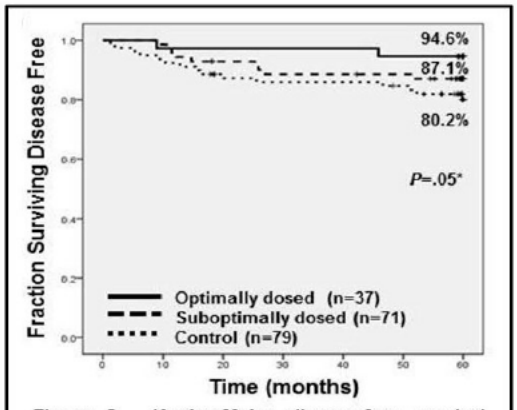

Figure 2. Kaplan-Meier disease-free survival curves evaluated by dosing group after 60 month follow-up for patients enrolled on phase I/II E75 trial. For 187 enrolled patients, the DFS rate in patients vaccinated with what was determined to be the optimal dose of the vaccine was 95% versus 87% for patients vaccinated with less than the optimal dose and 80% for unvaccinated controls.

At the time of the initial planned analysis of the combined results of the two concurrent nelipepimut-S +GM-CSF phase II trials in node-positive and node-negative disease-free breast cancer participants described above [12, 13], a total of 171 participants were enrolled; 90 participants received the nelipepimut-S vaccine while 81 were in the observation arm [13]. Toxicity was minimal with local reactions being grade 1 (in 81% of participants) and grade 2 (19%). Systemic toxicity was grade 0 (12%), 1 (71%), 2 (14%), and 3 (2%) (**Figure 1**). The most common systemic toxicities were bone pain, flu-like

symptoms and fatigue which are attributable to the GM-CSF. There were no grade 4 or 5 toxicities. All participants demonstrated *in vitro* immunologic responses and *in vivo* delayed type hypersensitivity (DTH) responses post-vaccination. With respect to *in vitro* immune responses, they were assessed using a dimer assay which enumerates the number of epitope-specific CTL (i.e. nelipepimut-S-CTL). A recurring pattern of clonal expansion was observed among vaccinated participants with an increasing percentage of CD8+ nelipepimut-S-CTL with successive vaccinations that peak during the vaccination series and then contract and plateau by the completion of the series. The peak clonal expansion most commonly occurred after the third inoculation [12, 13]. At a median follow-up of 20 months, the recurrence rate seen in the vaccinated participants was 6% compared to 15% for participants in the observation group (p=0.04). The level of nelipepimut-S-specific CTL generated in response to vaccination was assessed in HLA-A2+ participants enrolled on the study and 35% of participants were found to have a nelipepimut-S-CTL level above the mean with the recurrence rate among those participants being 3%. For participants with a nelipepimut-S-CTL level below the mean, the recurrence rate was 14% [20].

In light of these encouraging data, the trial was continued and follow-up was extended to five years. With additional follow-up, late recurrences were seen in the vaccination arms of the trials which correlated with decreased levels of nelipepimut-S-specific CTL. A voluntary booster program was initiated and vaccinated patients ≥ 6 months from completion of their primary vaccination series were eligible for participation. Participants enrolled onto the trial after initiation of the booster program were consented prospectively to receive booster inoculations. An initial report of the booster inoculations showed them to be safe and effective in stimulating nelipepimut-S-specific immunity in participants who had waning levels of nelipepimut-S -specific CTL six months following completion of their primary vaccination series [21]. When the length of follow-up for the trials was extended, additional analyses were incorporated to include evaluation of DFS at 24 and 60 months. With 60-month follow-up completed in all patients, we recently reported the final 5-year follow-up results [11]. Among the 187 participants (108 HLA-A2/A3^{+} vaccinated; 79 HLA-A2/3^{-} controls), the 60-month DFS rates were 90% for vaccinated participants versus 80% for controls (p=0.08), a 50% reduction in the relative risk of recurrence [11]. Because the trials began as dose- and schedule-finding trials, not all participants received the dose that was eventually determined to be optimal (1000µg nelipepimut-S + 250µg GM-CSF). When outcomes were evaluated by dosing, the DFS rate was 95% for those who received the optimal dose (p=.05 versus unvaccinated controls) (**Figure 2**). When evaluated by whether participants received a booster inoculation, the DFS rate in the 53 boosted participants was 96% versus 83% in vaccinated participants that did not receive a booster inoculation (p=.04).

Taken together, these data suggested clinical benefit [11]. Nelipepimut-S was recently evaluated in a multicenter, multinational, prospective, randomized, double-blind, controlled phase III study (NCT01479244; PI: Mittendorf) [22]. The trial randomized 758 lymph node-positive breast cancer participants with HER2 1+/2+ tumors in the adjuvant setting. Randomization was completed in April 2015. In June 2016, the Independent Data Monitoring Committee (IDMC) met to complete a planned safety and futility interim analysis that was triggered after 70 qualifying DFS events were reached. At that time, the IDMC recommended that the trial be stopped due to futility. The AEs observed in the Phase III study as of the date of the last data cutoff 27 June 2016 were consistent with the reactions observed in previous studies. AEs reported for ≥ 10% of participants (includes both arm of the trial) in the pivotal study were limited to local injection site reactions, including induration (56%), swelling (46%), pain (40%), pruritus (55%), and edema (17%). AEs reported for <20% of participants have included headache, fatigue, back pain and nausea. AEs have been generally mild in severity, with no safety trends identified for Grade 3 or higher AEs or SAEs/deaths. Two participants in the pivotal phase III study reported AEs related to study drug hypersensitivity including 1 patient with non-serious, Grade 2 angioedema and 1 participant with a Grade 4 SAE of anaphylactic reaction, both of which were considered definitely related to study drug, resulted in discontinuation of treatment, and were noted to have resolved. In addition, one participant in the early phase trials reported Grade 3 angioedema assessed

as related to rhGM-CSF. No other allergic- or anaphylactic-type reactions to E75 plus the rhGM-CSF adjuvant have been reported.

Having demonstrated clinical efficacy in the adjuvant setting to prevent disease recurrence, the next logical step evaluating the nelipepimut-S peptide vaccine is to look at it in patients with DCIS of the breast. DCIS of the breast represents premalignant disease and vaccinating in this setting is ideal for initial testing of the efficacy and safety of prophylactic vaccines. Giving a vaccine to a patient with a premalignant lesion would boost preexisting immunity to one or more tumor antigens against which the patient has already generated an adaptive immune response. In an analogous setting, Kimura et al. vaccinated participants with colonic adenomas and showed that vaccination was effective in inducing immune responses and immune memory in approximately 50% of participants [23]. This result led to Dr. Olivera Finn, a recognized expert in the field, to suggest that the next step in developing a preventive vaccine would be to evaluate vaccines known to generate an antigen-specific immune response in patients with premalignant disease [24].

The current study will randomize participants to vaccine (NeuVax™; nelipepimut-S+GM-CSF) or GM-CSF alone. The use of GM-CSF alone as a control arm will allow us to investigate whether the nelipepimut-S -specific CTL response generated after inoculation is secondary to the vaccine (nelipepimut-S + GM-CSF), versus GM-CSF alone. Because the local toxicity (erythema, pruritus) seen in our phase I/II trials is attributable to the GM-CSF, use of GM-CSF alone as a control arm will allow for participants to be blinded to their treatment allocation. Similarly, the use of GM-CSF alone as a control arm will allow us to investigate the effects of vaccination with the immunizing peptide (nelipepimut-S) versus GM-CSF for the protocols secondary endpoints. The participants will receive two inoculations (either vaccine or GM-CSF alone) prior to surgery. In the completed phase I/II trials evaluating NeuVax™, we have seen no interactions between vaccination and endocrine therapy. For the phase III trial evaluating NeuVax™, evaluations regarding interactions between vaccination and endocrine therapy are ongoing. That phase III trial is allowing for concomitant administration of the vaccine with endocrine therapy; therefore, for the current trial, for participants recommended to take endocrine therapy, the timing of beginning endocrine therapy will be at the discretion of the participant's treating oncologist and will not impact timing of vaccination. As part of the recent Phase III study the FDA required cardiac monitoring every 3 months during vaccination then every 6 months until 36 months and annually thereafter. Following a meeting in June 2015, the IDMC recommended routine cardiac monitoring by echocardiogram (ECHO) or multiple-gated acquisition (MUGA) scans could be reduced and that such a reduction was justified and consistent with the pre-specified Cardiac Toxicity Monitoring Stopping Rules defined in the study protocol. The reduced monitoring included a baseline assessment, 6 months, 12 months, 24 month, 36 months, and end of the study. Based on the safety data obtained from the phase III study, cardiac monitoring at baseline and at the post vaccinations follow-up visit will be conducted to ensure participant safety.

Data from our phase I/II trials evaluating the nelipepimut-S vaccine show a correlation between clinical efficacy and *in vitro* immune responses (specifically, enumeration of nelipepimut-S-specific CTL) [20] hence we will be looking at immunologic responses as the primary endpoint in this trial. The ability of the generated immune response to traffic to the tumor will be investigated as a secondary endpoint, specifically, we will be determining the degree of lymphocyte infiltration into the tumor in the resected surgical specimen. In order to determine the functional impact of the lymphocytes infiltrating the tumor, we will evaluate HER2 expression (*i.e.*, will evaluate HER2 expression in the pre-vaccination biopsy and the post-vaccination surgical specimen to determine if the antigen-specific immune response can eliminate the HER2+ clones in the tumor).

Completion of this study will determine the safety and immunologic efficacy of vaccination with NeuVax in patients with DCIS thereby providing necessary data to support the conduct of a larger study in DCIS as well as to build the foundation for a breast cancer prevention trial.

3. SUMMARY OF STUDY PLAN

A maximum of 48 participants will be accrued and randomized 2:1 to NeuVax or GM-CSF alone groups. Accounting for 10% attrition rate while on study and for approx. 5% non-evaluable sample rate, we expect to have 40 evaluable participants, 27 in vaccine and 13 in GM-CSF alone group, that have all baseline, post-vaccination (pre-surgery), and post-surgery measures of nelipepimut-S-specific CTL. Assuming a screening rate of approximately 3-4 participants per month and an accrual rate of approximately 1-2 participants per month, we expect the study to complete accrual within 30 months (2.5 years).

Women whose diagnostic biopsies show DCIS will be invited to participate in this trial. The tissue obtained from this biopsy will be used for the pre-treatment biomarker analysis. Participants with HER2 0, 1+, 2+ and 3+ will be eligible. Participants with confirmed DCIS will be HLA-typed. Because nelipepimut-S is an HLA-restricted peptide, the trial is limited to patients who are HLA-A2 positive.

HLA-A2+ participants who meet all other eligibility criteria will be randomized to receive NeuVax™ (1000 μg of nelipepimut-S and 250 μg of GM-CSF) or GM-CSF alone (Control). Participants randomized to the nelipepimut-S + GM-CSF arm will receive two vaccinations of nelipepimut-S (1000 μg) and GM-CSF (250 μg) administered **intradermally** two weeks apart prior to surgery. Participants randomized to the GM-CSF alone arm will receive inoculations of GM-CSF (250 μg) administered in an identical manner to those receiving nelipepimut-S/GM-CSF. Participants will be blinded as to whether they are receiving nelipepimut-S + GM-CSF or GM-CSF alone (Cotnrol). One post-vaccination clinic visit will occur 1 month (+/- 7 days) after surgery and before initiation of radiation, if post-operative radiation is advised; blood for biomarkers will be collected at this visit. The final follow up visit will occur 3-6 months post-surgery.

The following measurements will be taken to meet study objectives. Blood will be drawn for HLA typing at baseline. Blood for immunologic assays will be drawn at the time of the 1st vaccination, surgery, at the post-vaccination follow up visit and at the final follow up visit. Research tissue will be obtained from the surgical specimen.

Each participant will be on study for up to 8 months from randomization to the final follow up visit.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Participants must be female ≥18 years of age, pre- or post-menopausal. The minimum age of 18 was chosen as breast cancer is extremely rare in women less than 18 years. Because no dosing or adverse event data are currently available on the use of NeuVax in participants <18 years of age, children are excluded from this study but will be eligible for future pediatric trials, if applicable.

4.1.2 Participants must have a diagnosis of DCIS made by core needle biopsy

4.1.3 Participants must be HLA-A2 positive.

4.1.4 ECOG performance status must be 0 or 1 (Karnofsky ≥ 60%; see **Appendix A**).

4.1.5 Clinical chemistry less than 2x normal upper limit of normal range; adequate kidney and liver function as measured by creatinine, bilirubin, and liver enzymes (see below):

- Platelets ≥100,000/mm^3
- Hemoglobin ≥10 g/dL
- Blood Urea Nitrogen <2 x ULN
- Alkaline Phosphatase <2 x ULN
- Lactate Dehydrogenase <2 x ULN
- Creatinine <2 x ULN
- Bilirubin <2 x ULN
- AST (SGOT)/ALT (SGPT) <2 x ULN

(ULN = upper limit of normal as defined by the participating institutions laboratory).

4.1.6 A normal ejection fraction, as defined by the participant's institution (see footnote 1 below).

4.1.7 Willingness to comply with all study interventions and follow-up procedures.

4.1.8 The ability to understand and willingness to sign a written informed consent document.

[1] Only limited ECHOs will be used as cardiac evaluation. No other tests are allowed. ECHO is to be done only in HLA-A2 positive participants. If ECHO has been done within 30 days prior to randomization and results showing a normal ejection fraction have been obtained prior to randomization, an additional ECHO is not needed at Baseline.

4.2 Exclusion Criteria

4.2.1 Invasive breast cancer. Areas of microinvasion or suspicious for microinvasion on the core biopsy is allowed.

4.2.2 History of prior breast cancer treated within the past two years. Patients completing all breast cancer-specific treatment over two years prior to the current diagnosis are eligible.

4.2.3 History of prior ductal carcinoma in situ (DCIS) treated within the past two years. Patients completing all treatment for a previous diagnosis of DCIS over two years prior to the current diagnosis are eligible.

4.2.4 Prior lobular carcinoma in situ (LCIS) is allowed.

4.2.5 Pregnant, unwilling to use adequate contraception during study treatment duration or breastfeeding (see footnote 1 below).

4.2.6 Any autoimmune disease or other medical condition that, in the opinion of the investigator, would compromise the subject's safety.

4.2.7 Immune deficiency diseases such as immunoglobulin deficiency or immunosuppressive therapy that might interfere with appropriate immune response.

4.2.8 Known history of or known active infection with human immunodeficiency virus (HIV), hepatitis B or hepatitis C.

4.2.9 Patients on chronic steroid therapy or other immunosuppressive therapy except for topical or inhaled steroids known to have low systemic absorption.

4.2.10 Patients with a known hypersensitivity to GM-CSF, yeast-derived products, or any component of the GM-CSF product (*e.g.*, mannitol).

4.2.11 Concurrent treatment with other investigational agent.

4.2.12 History of non-breast malignancy within 5 years prior to randomization, except curatively treated superficial bladder cancer, carcinoma in situ of the cervix (Stage 0 – 1), and basal cell or squamous cell carcinoma of the skin.

4.2.13 History of allergic reactions attributed to compounds of similar chemical or biologic composition to NeuVax.

4.2.14. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.15. No recent or planned immunotherapy.

[1] The effects of NeuVax on the developing human fetus are unknown. For this reason and because NeuVax may be teratogenic, pregnant women will be excluded. All heterosexually active women who may become pregnant must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation OR be post-menopausal defined as any one of the following 1) prior hysterectomy, 2) absence of menstrual period for 1 year in the absence of prior chemotherapy or 3) absence of menstrual period for 2 years in women with a prior history of chemotherapy exposure who were pre-menopausal prior to chemotherapy. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.3 Inclusion of Women and Minorities

Participants will be adult women of all races and ethnic groups who are at least 18 years old. Breast cancer is extremely rare in men and men have limited amounts of normal breast tissue; therefore, male participants will not be recruited to this trial. Children will not be recruited to the trial because breast cancer is not relevant to the child population.

Our goal is to ensure that the study is available to women of all races and ethnic groups. To ensure enrollment of minorities we will work with the minority community and social network groups available at each accruing site within the US, such as Black Women's Health Imperative, Sisters Network, Army of Women, Cancer Forward, Breast Health Collaborative, Asian Cancer Council (Indian American Cancer Network, Filipino Cancer Network, Light and Salt) and Susan G. Komen Breast Cancer Foundation.

Although minority groups will be targeted in recruitment, the HLA-A2 haplotype is less common in minorities than in Caucasians. In our previous studies close to 77% of HLA-A2 positive participants were Caucasian. This gives us grounds to expect that although we will approach all racial groups, the majority of participants who will test HLA-A2 positive during eligibility verification will be Caucasian.

4.4 Recruitment and Retention Plan

Participants will be recruited for enrollment on this trial from the primary clinics of the Investigators and/or surgical collaborators. Efforts will be made to enroll women from a diversity of ethnic and socio-economic backgrounds. There will be no payments made to participants or physicians for participation in this study. Prior to enrollment on the study, the physician will discuss the study protocol in detail with the patient, including possible toxicities. The informed consent document will be reviewed by the physician with the patient. To facilitate accrual, information about the trial will be advertised, for example, on the websites of participating institutions.

Please refer to the study-specific Recruitment and Retention Plan for more details.

5. AGENT ADMINISTRATION

Vaccine and GM-CSF alone inoculations will be administered in the clinic.

5.1 Dose Regimen and Dose Groups: Vaccine dosage and preparation

- *Agents:* The NeuVax vaccine (nelipepimut-S; as 1.5 mg/mL nelipepimut-S acetate solution) will be supplied to the NCI Repository by SELLAS. GM-CSF (as 250 μg lyophilized Leukine) will be supplied by the NCI Repository.
- *Administration:* Using aseptic technique, 0.8 mL of nelipepimut-S acetate solution (1136 μg nelipepimut-S peptide) is added to the re-constituted Leukine. Taking into account vial losses, this procedure will yield a total dose of approximately 1010 μg of nelipepimut-S peptide in a volume of 1.6 mL. Divide 1.6 mL into 4 equally divided 0.4 mL syringes for intradermal injection. The detailed guidance for mixing nelipepimut-S peptide mixed with lyophilized Leukine® (sargramostim, GM-CSF) or Control (sterile water for injection) with Leukine is presented in **Appendix B**.
- *Duration:* Participants randomized to the nelipepimut-S/GM-CSF arm will receive two vaccinations of nelipepimut-S (1000 μg) and GM-CSF (250 μg) administered **intradermally** two weeks apart prior to surgery. Participants randomized to the GM-CSF alone arm will receive inoculations of GM-CSF (250 μg) administered in an identical manner to those receiving nelipepimut-S+GM-CSF.

5.2 NeuVax Inoculation Series Administration

Subjects will receive 2 vaccinations each consisting of 4 intradermal inoculations on the anterior or medial side of the same thigh. The general area of inoculation will be at a location midway between the inguinal ligament and the knee.

The 1.6 mL by volume vaccine (or GM-CSF alone) will be administered **intradermally** in four equal inoculums at four different sites in a square configuration 5 cm from each other. Inoculations will be administered in the same lymph node draining area (same leg). Vaccinations will be made in the same thigh each time (Vaccines #1 through 2) to optimize the immune response by using the same draining lymph node each time.

Participants randomized to the nelipepimut-S/GM-CSF arm will receive two vaccinations of nelipepimut-S (1000 μg) and GM-CSF (250 μg) administered intradermally two weeks apart prior to surgery. Participants randomized to the GM-CSF alone arm will receive inoculations of GM-CSF (250 μg) administered in an identical manner to those receiving nelipepimut-S/GM-CSF.

A healthcare provider approved at each institution to deliver inoculations will administer the inoculations sterilely in the clinical facility at each study site. Post inoculation vital signs will be assessed at 30 +/- 5 minutes after inoculation by the research nurse / or coordinator. For female participants with childbearing potential, a urine pregnancy test will be performed before each inoculation. If this test is positive at any time, the participants will be discontinued from the study.

5.3 Run-in Procedures

This trial will not include a run-in period.

5.4 Contraindications

Drug interactions between NeuVax and other agents have not been studied.

Leukine is contraindicated:

1. in patients with excessive leukemic myeloid blasts in the bone marrow or peripheral blood (≥ 10%);
2. in patients with known hypersensitivity to GM-CSF, yeast-derived products or any component of the product;
3. for concomitant use with chemotherapy and radiotherapy.

Due to the potential sensitivity of rapidly dividing hematopoietic progenitor cells, Leukine should not be administered simultaneously with cytotoxic chemotherapy or radiotherapy or within 24 hours preceding or following chemotherapy or radiotherapy. In one controlled study, participants with small cell lung cancer received Leukine and concurrent thoracic radiotherapy and chemotherapy or the identical radiotherapy and chemotherapy without Leukine. The participants randomized to Leukine had significantly higher incidence of adverse events, including higher mortality and a higher incidence of grade 3 and 4 infections and grade 3 and 4 thrombocytopenia. Refer to the rhGM-CSF Leukine® [sargramostim] package insert for information on drug interactions with rhGM-CSF Leukine® [sargramostim].

5.5 Concomitant Medications

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication.

Participants experiencing a local or systemic reaction to vaccination may be advised to take Tylenol. For significant pruritis at the injection site, participants may use an over the counter oral anti-histamine. Steroids are prohibited unless, in the determination of the Investigator, the participant is experiencing systemic hypersensitivity or an allergic reaction.

5.6 Dose Modification

In our previous trials evaluating Nelipepimut-S + GM-CSF, approximately 18% of patients experienced robust local reactions (>100mm) or greater than or equal to grade 3 local or systemic reactions or greater than or equal to grade 2 hypersensitivity reactions [26]. For a description of how local reaction will be

measured, refer to section 7.3.1. To avoid higher grade toxicities, for participants meeting any of these criteria, we will dose-reduce the GM-CSF component of the vaccine as outlined below. Site PIs cannot make the decision to dose-reduce without consulting with Dr. Mittendorf. The decision to dose-reduce the GM-CSF will be made by the Protocol Principal Investigator, Dr. Mittendorf, who will communicate the need for dose-reduction to the individual site PI and site study research nurse / or coordinator who will be generating the orders for the pharmacy for preparation of vaccine. Adverse events that do not respond to either dose modification or clinical management of the reaction will result in taking the participant off agent but followed for study endpoints (refer to Section 8.3: Off-Agent Criteria).

Inoculations will be immediately halted if any serious adverse reactions occur to include: death, life-threatening adverse drug experience (i.e., severe anaphylactic reaction immediately after vaccine administration), inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect, or other important medical events that may not result in death, be life-threatening, or require hospitalization but which, when based upon appropriate judgment of the PI, be determined to jeopardize the participant or require medical or surgical intervention to prevent an outcome listed above.

Participants experiencing Grade 4 toxicities of any attribution will be taken off agent and followed (refer to Section 8.3: Off-Agent Criteria). Any participant with Grade 3 systemic toxicities possibly, probably or definitely attributable to study drug will be taken off agent and followed, except for participants with skin toxicities. Any participant experiencing Grade 3 skin toxicities will continue to receive inoculations as long as their skin toxicity has resolved prior to the next scheduled inoculation. Participants with Grade 3 toxicities not related or unlikely related to study agent will continue on study.

Dose Reductions of Vaccine. Toxicities observed with the vaccine are attributable to the GM-CSF. Although the toxicity profile of GM-CSF is the same for a 250 µg dose as for a 125 µg dose, the extent of the toxicity will vary between participants. Therefore, the provision for dose reductions of the GM-CSF component of the vaccine is a safety issue for an individual participant. For participants experiencing robust local reactions (>100mm x 100mm); grade 3 local reactions; or greater than or equal to grade 2 hypersensitivity reactions the dose of GM-CSF will be reduced for the subsequent inoculation as outlined in Table 1 below. This dose reduction will be done to decrease the likelihood that the participant will experience the same degree of reaction. Management of hypersensitivity reactions will be at the discretion of the Investigator or an appropriately qualified member of the study team designated by the Investigator.

Dose Reduction of GM-CSF.

Table 1. Dose Reduction Using Reconstituted Lyophilized Leukine

	Amount of reconstituted Leukine® solution removed prior to addition of E75 acetate solution	**Amount of WFI diluent added back to E75 acetate solution**	**Dose of rhGM-CSF**
No dose reduction	0 mL	0 mL	250 µg
1st dose reduction	0.5 mL	0.5 mL	125 µg

In our prior trial, approximately 5% of patients developed delayed urticarial reactions (generalized urticaria 10-14 days after booster inoculation). This was not seen during the primary vaccination series.

The majority of these patients were readily treated with anti-histamines or oral steroids and most continued with subsequent booster inoculations. If dose reduction is performed for hypersensitivity reactions (generalized urticaria), over the counter antihistamines may be used before or after subsequent inoculations at the discretion of the Investigator or an appropriately qualified member of the study team designated by the Investigator. If urticaria persists with the use of over the counter anti-histamines, then prescription anti-histamines will be prescribed. If urticarial persists with the use of prescription anti-histamines, the participant will be give oral steroids (Medrol Dosepak). If the urticarial continues to persist then the participant will be given intravenous steroids. Use of intravenous steroids has been required in <1% of participants on prior trials evaluating this vaccine. If intravenous steroids or hospitalization are required, or in the opinion of the Principal Investigator, further inoculations will be discontinued, the participant will be taken off agent but followed for study endpoints (refer to Section 8.3: Off-Agent Criteria).

5.7 Adherence/Compliance

- We plan to enroll 40 evaluable participants. Adjusting for the 5% non-evaluable sample rate and 10% attrition rate, our goal is to randomize a total of 48 participants to yield at least 40 evaluable participants.
- Compliance is defined as 100% adherence to the vaccination schedule.
- For the primary endpoint of change in the number of nelipepimut-S-CTL for an individual participants between the pre-vaccination timepoint and the 1 month (+/- 7 days) after surgery timepoint, "evaluable" will be defined as completing all pre-surgical vaccinations and the visit at 1 month (+/- 7 days) after surgery.
- For the safety endpoint, participants who receive at least one vaccination will be included in the safety evaluation.
- For all secondary endpoints, all non-compliant participants will be analyzed according to the intent-to-treat principle. All levels of compliance will be included in the full evaluation of endpoints if paired biopsies are available.

6. PHARMACEUTICAL INFORMATION

6.1 Study Agent (IND # [redacted], IND Sponsor NCI DCP)

Physical description of agents:
NeuVax™ appearance: Clear, colorless solution, essentially free from visible particulates;
GM-CSF (Leukine®, sargramostim) appearance: The liquid Leukine presentation is formulated as a sterile, preserved, injectable solution in a vial. Lyophilized Leukine is a sterile, white, preservative-free powder that requires reconstitution with 1 mL Sterile Water for Injection.

Formulation to be used in this study and excipients:
The vaccine NeuVax ™, also known as nelipepimut-S, is a nine amino acid peptide (KIFGSLAFL) derived from the HER2 protein that is overexpressed in many breast cancer patients (reviewed in [16]). Vaccines containing the nelipepimut-S peptide mixed with an immunoadjuvant have proven to be efficacious in early phase (phase I/II clinical trials) conducted in the adjuvant setting to prevent recurrence of breast cancer [11].

The NeuVax vaccine (nelipepimut-S; as 1.5 mg/mL nelipepimut-S acetate solution) will be supplied by SELLAS. GM-CSF (as 250 μg lyophilized Leukine) will be supplied by NCI Repository, MRI Global.

GM-CSF is a potent cytokine. Recombinant human GM-CSF is a 14.6 kDa globular protein consisting of 128 amino acids containing 2 intramolecular disulfide bonds and 2 potential N-linked glycosylation sites. Known adverse effects of GM-CSF include bone pain, allergic reactions, lethargy, malaise, anorexia, skin rashes, flushing, fever, and chills. At doses higher than planned in this study, weight gain may be seen along with breathing difficulties, blood clots, and collections of fluid around the heart or lungs, dyspnea, thromboembolic phenomena, and pleural and pericardial effusion.

Leukine® (sargramostim) is a recombinant human granulocyte-macrophage colony stimulating factor (rhu GM-CSF) produced by recombinant DNA technology in a yeast (S. cerevisiae) expression system. Biological potency is expressed in International Units (IU) as tested against the World Health Organization (WHO) First International Reference Standard. The specific activity of GM-CSF is approximately 1.4 x 10^6 IU/mL. See GM-CSF (Leukine® (sargramostim)) package insert and labeling for additional details.

The nelipepimut-S vaccine acetate drug substance is a 9-amino acid peptide produced by solid-phase peptide synthesis. Nelipepimut-S acetate drug product is manufactured by Oso Biopharmaceuticals Manufacturing, LLC, Albuquerque, NM as a 1.5 mg/mL solution in 1 mL sterile water for injection (SWFI) in a 2-mL glass vial. Each mg of nelipepimut-S acetate is equivalent to 0.94 mg of nelipepimut S peptide. Hence, the vaccine concentration is equivalent to 1.42 mg/mL nelipepimut-S peptide. The control is SWFI.

Prior to administration, 1,136 μg (0.8 mL) of the nelipepimut-S acetate or control is mixed by the pharmacist or pharmacy technician under the supervision of the pharmacist with 1 mL of reconstituted lyophilized Leukine® GM-CSF (reconstituted as 250 μg/mL in solution). Immediately after mixing, the study drug is withdrawn into four 1 mL syringes and within 2 hours of mixing, a total of 1.6 mL is administered as 4 intradermal injections to deliver a total of 1,000 μg of nelipepimut-S peptide vaccine (1,010 μg of nelipepimut-S peptide). The control vial contains 1 mL SWFI. The detailed guidance for mixing nelipepimut-S (or control) solution from lyophilized Leukine® 250 μg and test drug (nelipepimut-S solution or control) is stated in **Appendix B**.

6.2 Reported Adverse Events and Potential Risks

Phase I trials addressing the immunogenicity and toxicity of escalating nelipepimut-S peptide doses mixed with GM-CSF as adjuvant were conducted in patients with metastatic breast and ovarian cancer.[22] Of the 14 subjects enrolled, a majority experienced mild pain at the injection site. Low-grade fever, nausea, fatigue, myalgia, itching at the injection site, and back and abdominal pain were also noted. One subject reported grade 2 chills, headache and ulceration at the injection site. No grade 3 AEs were reported. The side effects observed were not dose-dependent.

Two concurrent Phase II trials in both node-positive and node-negative breast cancer patients disease-free after standard treatment were recently completed [13, 27] (CCRE08140797). These trials were designed to address the ability of the vaccine to prevent disease recurrence. Subjects were observed for an hour after injection for any signs of hypersensitivity, and asked to return 48–72 hours later to monitor local/systemic reactions to injection. In a total of 187 subjects, local reactions included grade 1 erythema and induration, pain and soreness at the injection site (81% of vaccinated subjects) and grade 2 pruritus (19%). Systemic reactions included grade 1 headache and fatigue (71%); grade 2 pain, flu like symptoms, and fatigue (14%); and grade 3 angioedema of tongue and bone pain (2%). No grade 4 or 5 events were observed. Because the inoculations are very well tolerated with few immediate adverse events, only post inoculation vital signs will be assessed at 30 +/- 5 minutes after inoculation by the research nurse or coordinator.

The same vaccine (nelipepimut-S+GM-CSF) was evaluated in prostate cancer patients expressing varying levels of HER2/*neu* protein [25]. Of the 40 enrolled subjects, grade 1 local reactions were observed in all; systemic reactions were observed in 38.1% (grade 1) and 9.5% (grade 2).

These effects are likely attributable to GM-CSF. In studies utilizing this adjuvant, patients sometimes complained of mild to moderate flu-like symptoms (fever, chills, achiness, and fatigue) for 1–2 days after vaccination. In subsequent studies evaluating the AE37 vaccine (AE37 + GM-CSF), the toxicity profile was similar in patients randomized to receive the vaccine and those randomized to receive GM-CSF only (unpublished data).

Nelipepimut-S was recently evaluated in a multicenter, multinational, prospective, randomized, double-blind, controlled phase III study (NCT01479244; PI: Mittendorf) The trial randomized 758 lymph node-positive breast cancer patients with HER2 1+/2+ tumors in the adjuvant setting. Randomization was completed in April 2015. In June 2016, the Independent Data Monitoring Committee (IDMC) met to complete a planned safety and futility interim analysis that was triggered after 70 qualifying DFS events were reached. At that time, the IDMC recommended that the trial be stopped due to futility. The AEs observed in the Phase III study as of the date of the last data cutoff (16 Jan 2015) were consistent with the reactions observed in previous studies. AEs reported for ≥ 10% of patients in the pivotal study have been limited to local injection site reactions, including induration (56%), swelling (46%), pain (40%), pruritus (55%), and edema (17%). AEs reported for <20% of patients have included headache, fatigue, back pain and nausea. AEs have been generally mild in severity, with no safety trends identified for Grade 3 or higher AEs or SAEs/deaths. Two patients in the pivotal phase III study reported AEs related to study drug hypersensitivity including 1 patient with non-serious, Grade 2 angioedema and 1 patient with a Grade 4 SAE of anaphylactic reaction, both of which were considered definitely related to study drug, resulted in discontinuation of treatment, and were noted to have resolved. In addition, one patient in the early phase trials reported Grade 3 angioedema assessed as related to rhGM-CSF. No other allergic- or anaphylactic-type reactions to E75 plus the rhGM-CSF adjuvant have been reported.

6.3 Availability

NeuVax ™ is provided to the NCI under a Clinical Trials Agreement (CTA) between SELLAS and the DCP, NCI (see §12.7).

6.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of IRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or their authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form (NIH-986) (to include complete shipping contact information) and faxing or mailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 360-3805
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF) or an institutionally-approved accountability system. The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed and administered to each participant. Drs. Mittendorf or Garber, Bedrosian and Berger or their representatives will be responsible for study agent accountability for participants at Dana-Farber Cancer Center, UT MD Anderson Cancer Center, and Thomas Jefferson University, respectively.

6.6 Packaging and Labeling

NeuVax ™ will be provided by SELLAS and will be distributed to the clinical sites by MRI Global. GM-CSF (as 250 µg lyophilized Leukine) will be supplied to the clinical sites by NCI Repository, MRI Global.

6.7 Storage

For detailed storage instructions of the components of the vaccine (nelipepimut-S acetate and GM-CSF) please follow the instructions in the Investigator's Brochure. The components of the active study drug and the control will be stored separately in the research pharmacy and reconstituted when needed. The components will be stored as per appropriate storage temperature designated on clinical carton label. All study drug components (nelipepimut-S acetate and GM-CSF) will be stored under refrigerated conditions (5°C ± 3°C).

6.8 Registration/Randomization

Screening and Registration into the DMI Database:
Once informed consent has been signed, participants will be registered into the DMI database. The DMI database will assign a participant's PID upon completion of the registration process.

Randomization:
Participants will be assigned a randomization number once the following has been accomplished: eligibility has been verified at the site level, eligibility has been confirmed by the site PI, and eligibility CRF has been entered into the DMI web application. The randomization number will be generated by the database and assigned to the participant. Refer to **Section 13.2** for details of randomization.

Screening/Registration/Randomization into site-specific databases:
The DMI is the database of record for the study. Registration and randomization should occur per the procedures outlined above. If the site staff need to enter study data into site-specific electronic databases per their institutional requirements, they should do so in accordance with their institutional policies and procedures.

Appropriate CRFs must be completed for any participant who signs an informed consent. If a consented participant is a screen failure and deemed ineligible, the following CRFs must be completed: 1) the Registration CRF; 2) the Randomization CRF with the eligibility box checked "no", 3) the Inclusion and Exclusion CRFs showing why the participant is ineligible, 4) the Off-Study CRF, 5) the Adverse Event CRF, 6) the Concomitant Medication CRF and 7) the Verification CRF. If no Adverse Event and/or Concomitant Medications were assessed by the time the participant is deemed ineligible, the "NONE" box will be checked to complete both CRFs. All participants who sign an informed consent must formally go off study. All participant registration information will be entered into DMI. If a participant experiences a serious Adverse Event during the screening process, a Serious Adverse Event (SAE) form must be completed.

6.9 Blinding and Unblinding Methods

6.9.1 Participants will be blinded to NeuVax ™ or GM-CSF alone arm assignment.

6.9.2 The Statistician, Site Study Pharmacist and other investigators will not be blinded to NeuVax ™ or GM-CSF alone arm assignment.

6.9.3 Participants randomized to the nelipepimut-S/GM-CSF arm will receive two vaccinations of nelipepimut-S (1000 μg) and GM-CSF (250 μg) consisting of 4 intradermal inoculations two weeks apart prior to surgery. Participants randomized to the GM-CSF alone arm will receive inoculations of GM-CSF (250 μg) administered in an identical manner to those receiving nelipepimut-S/GM-CSF.

6.9.4 At the end of the study participants will be unblinded to their assignment.

6.9.5 Study agents may be unblinded during the course of the study if deemed medically necessary in the event of a serious adverse event after discussion with the NCI medical monitor. Unblinding will be conducted as follows:

1) The Site PI contacts the Protocol Principal Investigator (Elizabeth Mittendorf, MD, PhD) and requests the participant's treatment status be unblinded.
2) The Protocol Principal Investigator (Elizabeth Mittendorf, MD, PhD) contacts the NCI DCP Medical Monitor (Margaret (Malgorzata) Wojtowicz, MD) and requests the participant's treatment status be unblinded. The Protocol Principal Investigator then conveys the Medical Monitor's decision to the Site PI. The Site PI then proceeds with unblinding as written out below.
3) If the NCI Medical Monitor cannot be reached and the participant requires emergency care, the Protocol Principal Investigator (Dr. Mittendorf) may authorize the site PI to break the blind.
4) If the Site PI is unable to reach the Protocol Principal Investigator and the participant requires emergency care, then the Site PI must proceed with unblinding as written out below.
5) The Site PI officially unblinds the participant and takes the participant off-study.
6) The date and reason for breaking the blind must be submitted by the Site PI to the Protocol Principal Investigator, Elizabeth Mittendorf, MD, PhD as soon as possible.

7) It is the responsibility of the Protocol Principal Investigator to report the date and reason for breaking the blind to the NCI Medical Monitor**,** Margaret (Malgorzata) Wojtowicz, MD as soon as possible after receiving this information from the Site PI.

   NCI Medical Monitor:
   Margaret (Malgorzata) Wojtowicz, MD
   NCI/Division of Cancer Prevention
   Room 5E104
   9609 Medical Center Drive
   MSC 9781
   Bethesda, MD 20892
   Phone: (240) 276-7012; Fax: (240) 276-7848
   wojtowim@mail.nih.gov

8) The date and reason for breaking the blind must be submitted by the Protocol Principal Investigator to the MD Anderson Consortium Principal Investigator, Powel H. Brown, MD, PhD, or designee as soon as possible via email to phbrown@mdanderson.org.
9) The date and reason for breaking the blind will be reported by Dr. Brown or designee to the MD Anderson DSMB as soon as possible.

6.10 Agent Destruction/Disposal

At the completion of the study, all unused study agent will be returned to NCI, DCP Repository according to the DCP "Guidelines for AGENT RETURNS" and using the DCP form "Return Drug List".

The guidelines and the form are available on the DCP website.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Evaluation/ Procedure	Baseline Testing/Pre study Evaluation [#]	Randomization	Vacc #1	Vacc #2	Surgery	Post-Vacc Follow-Up Visit)	Final Follow-Up Visit
	Within 30 days prior to randomization		Day 0	Day 14 ±3 days	Day 28 ±5 days	1 month (30 days) ±7 days post-op	3-6 months post-op
Informed Consent	X						
Assess Eligibility	X						
Confirm Eligibility		X					
Registration	X						
Randomization		X					
Medical History	X						
Physical Exam	X						X
Vital Signs/ Height and Weight	X						X
Laboratory Tests	X				X[6]		
Cardiac evaluation [1]	X					X	
HLA blood draw for typing	X						
Immunological Samples			X		X[6]	X	X
Vital Signs Prior to Inoculation			X	X			
Agent Administration (Inoculation) [2]			X[2]	X[2]			
Pregnancy Test	X		X	X			
Research Tissue		X[4]			X		
Concomitant Medications	X		X	X	X[7]	X[5]	X
Adverse Events/Symptom Assessment			X	X	X[7]	X[5]	X
Local Reaction[3]			X[3]	X[3]			
Telephone Contact or Study Nurse Visit			X	X			

[#] Baseline testing may require multiple visits.

[1] Only limited ECHOs will be used as cardiac evaluation. No other tests are allowed. ECHO is to be done only in HLA-A2 positive participants (i.e., participants with confirmed DCIS will be HLA-typed prior to conducting ECHO). If ECHO has been done within 30 days prior to randomization and results showing a normal ejection fraction have been obtained prior to randomization, an additional ECHO is not needed at Baseline.

[2] Post inoculation vital signs will be assessed at 30 +/- 5 minutes post inoculation.

[3] Participants will be assessed for evidence of *in vivo* immunologic response by evaluation of the injection site 48-72 hours after each inoculation. Participants will either return to their study site or be contacted by phone for questioning regarding any systemic toxicity during the initial 48-72 hrs after inoculation. If they return to their study site, the local reaction at the inoculation sites will be examined and measured. For participants that do not return to their study site, a tool will be given, and instructions provided to measure the local reaction.

[4] An H&E stained slide and 10 unstained slides (or 11 unstained slides) from the initial breast biopsy should be sent to the laboratory of Dr. Gheath Al-Atrash at the address provided in **Section 10.3**. The biopsy slides should be sent within 6 weeks of randomization.

[5] May be assessed on the phone.

[6] May be performed up to 5 days prior to surgery relative to the visit target date.
[7] May be assessed the day prior to surgery in clinic or on the phone.

7.2 Baseline Testing/Prestudy Evaluation (Within 30 days prior to randomization)

Baseline Testing/Prestudy Evaluation interventions will be conducted within 30 days of randomization, may require multiple visits and will consist of the following procedures:

- Informed consent must be obtained prior to starting any further study procedures.
- Registration: Once informed consent has been signed, participants will be registered into the DMI database. The DMI database will assign a participant's PID upon completion of the registration process. Participants will also be registered into site-specific registry databases as applicable.
- Baseline medical history, to include a review of breast cancer history and previous medical history, previous surgery (*e.g.*, lumpectomy or mastectomy), chemotherapy and radiation therapy history, reproductive history, family history, demographic information, including age and race.
- Diagnosis of ductal carcinoma in situ by core needle biopsy; must be confirmed by review of the pathology report.
- Participants with confirmed DCIS will be HLA-typed. Because nelipepimut-S is an HLA-restricted peptide, the trial is limited to patients who are HLA-A2 positive (approximately 45% of the US population). HLA typing will be done locally by each site. Approximately 6-8 mL of blood will be drawn for HLA-typing (or as directed by the site HLA typing facility).
- Use of concomitant medications will be reviewed.
- A physical examination will be done that will include vital signs (temperature, blood pressure, heart rate, respiratory rate).
- Height, weight.
- Pre-study routine laboratory evaluations will include the following:
    - Platelets
    - Hemoglobin
    - Blood Urea Nitrogen
    - Alkaline Phosphatase
    - Lactate Dehydrogenase
    - Creatinine
    - Bilirubin
    - AST (SGOT)/ALT (SGPT)
- In women of child bearing potential a pregnancy test must be done within 30 days prior to Randomization. The pregnancy test will be repeated prior to each vaccination. Results must be known before vaccination. Either a serum or urine pregnancy test may be performed. If a urine pregnancy test is performed, the test must be done in the clinic.
    - If the pregnancy test result is positive at Baseline (any time before randomization), the participant is a Screen Failure.
    - If the pregnancy test result is positive prior to any Vaccination, do not vaccinate, take the participant off-study, complete the Off-Study Case Report Form (CRF), follow the pregnancy to term and complete the Outcome of Pregnancy Case Report Form (CRF).
- Cardiac evaluation: limited ECHOs to determine the ejection fraction. A normal ejection fraction, as defined by the participant's institution is required for randomization. Only limited ECHOs will be used as cardiac evaluation. No other tests are allowed. ECHO is to be done only in HLA-A2 positive participants (i.e., participants with confirmed DCIS will be HLA-typed prior to conducting ECHO). If ECHO has been done within 30 days prior to

randomization and results showing a normal ejection fraction have been obtained prior to randomization, an additional ECHO is not needed at Baseline.
- Confirm eligibility: At the completion of the screening period eligibility must be confirmed. Only after eligibility is confirmed, can eligible participants be randomized.

Randomization
- Once registration is complete, eligibility has been confirmed and eligibility CRF is entered into the web application, participants will be assigned a randomization number pre-generated by the database. Participants will be randomized to receive either NeuVax™ (nelipepimut-S; 1000 μg and 250 μg of GM-CSF) or GM-CSF alone. Participants will be blinded as to whether they are receiving nelipepimut-S/GM-CSF or GM-CSF alone.
- An H&E stained slide and 10 unstained slides (or 11 unstained slides) from the initial breast biopsy should be sent to Dr. Al-Atrash's laboratory at the address provided in **Section 10.3**. The biopsy slides should be sent within 6 weeks of randomization.
- Randomization and Vaccination #1 may occur on the same day.

7.3 Evaluation During Study Intervention

7.3.1 Vaccination #1 (Day 0 +/-3 days):

Pre-Vaccination:
- Pregnancy test prior to vaccination. Negative pregnancy result must be confirmed prior to vaccination:
  - If the pregnancy test result is positive prior to any Vaccination, do not vaccinate, take the participant off-study, complete the Off-Study Case Report Form (CRF), follow the pregnancy to term and complete the Outcome of Pregnancy Case Report Form (CRF).
- Blood for immunologic assays.
- One set of vital signs prior to inoculation.
- Adverse Events.
- Concomitant medications.
- Symptom assessment.

Vaccination
- Agent administration: inoculation.
- Post inoculation vital signs will be assessed at 30 +/- 5 minutes after inoculation.

Post-Vaccination (48-72 hrs after inoculation):
- Telephone contact with research nurse /or coordinator or nurse visit during the initial 48-72 hrs after inoculation: Participants will either return to their study site or be contacted by phone for questioning regarding any systemic toxicity during the initial 48-72 hrs after inoculation. If they return to their study site, the local reaction at the inoculation sites will be examined and measured. For participants who do not return to their study site, a tool will be given, and instructions provided to measure the local reaction. The NCI CTCAE version 4.03 graded toxicity scale will be utilized to assess local and systemic toxicity.
- Local reaction (for participants who return to the study site after inoculation):
  - Participants will be assessed for evidence of *in vivo* immunologic response by evaluation of the injection site 48-72 hours after each inoculation. The injection site reaction will be measured using the sensitive ball point pen method.
- Local reaction (for participants who do not return to the study site after inoculation):

- For participants that do not return to their study site, a self-assessment tool will be given, and instructions provided to measure the local reaction. This self-assessment tool is currently being utilized in the phase III trial evaluating NeuVax as well as two ongoing phase II trials evaluating NeuVax in combination with trastuzumab. The tool has been well received by the participants who appreciate its ease of use (the tool consists of a transparent piece of lightweight plastic with concentric circles depicted on it to allow for measurement) and the fact that it reduces the need to return to the study site for injection site assessment. To date, at MD Anderson, we have had 100% compliance with the use of the tool.

7.3.2 Vaccination #2 (Day 14 +/-3 days);

- Pregnancy test prior to vaccination. Negative pregnancy result must be confirmed prior to vaccination:
  - If the pregnancy test result is positive prior to any Vaccination, do not vaccinate, take the participant off-study, complete the Off-Study Case Report Form (CRF), follow the pregnancy to term and complete the Outcome of Pregnancy Case Report Form (CRF).
- One set of vital signs prior to inoculation.
- Post inoculation vital signs will be assessed at 30 +/- 5 minutes after inoculation.
- Adverse Events.
- Concomitant medications.
- Symptom assessment.
- Local reaction (during the initial 48-72 hrs after inoculation).
- Telephone contact with research nurse /or coordinator or nurse visit during the initial 48-72 hrs after inoculation.

7.3.3 Day of Surgery (Day 28 +/-5 days):

- Blood for immunologic assays will be drawn prior to the surgical procedure.
- Blood will be drawn for laboratory evaluations prior to surgery. Pre-surgery laboratory evaluations will include the following:
  - Platelets
  - Hemoglobin
  - Alkaline Phosphatase
  - Lactate Dehydrogenase
  - Bilirubin
  - AST (SGOT)/ALT (SGPT)
- Research tissue for tissue biomarkers (from the surgical specimen). For patients undergoing mastectomy, a representative sample of normal tissue maximally distant from the tumor will be obtained to assess for immune infiltrates.
- Adverse Events.
- Concomitant medications.
- Symptom assessment.

7.4 Evaluation at Completion of Study Intervention

7.4.1 Post Vaccinations Follow-Up Visit (Day +30 post op +/-7 days): the post vaccinations follow-up visit will be conducted on Day +30 (+/- 7 days) post op and prior to initiating radiation, if post-op radiation is advised.
The following will be assessed:

- Adverse Events. May be assessed on the phone.
- Concomitant medications. May be assessed on the phone.
- Symptom assessment. May be assessed on the phone.
- Blood for immunologic assays.

Cardiac evaluation: limited ECHOs to determine the ejection fraction. Decreases of > 10% in the ejection fraction from baseline will be reported as an SAE and the participant experiencing the SAE will be managed according to the best clinical practice. Only limited ECHOs will be used as cardiac evaluation. No other tests are allowed.

7.5 Post-intervention Follow-up Period

The Final Follow-Up Visit (3-6 Months post op): the Final Follow-Up Visit will be conducted 3-6 Months post-surgery.
The following will be assessed:

- Physical exam.
- Vital signs.
- Adverse Events.
- Concomitant medications.
- Symptom assessment.
- Blood for immunologic assays.

7.6 Methods for Clinical Procedures

7.6.1 Local reaction assessment. The local reaction will be measured by either the research nurse /or coordinator (if the participant returns to clinic) or by the participant using the local reaction assessment tool, 48-72 hours after inoculation. The local reaction will be measured and reported in two dimensions.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

Primary Endpoint: Enumeration of nelipepimut-S-specific CTL
The primary endpoint of the trial is to evaluate for nelipepimut-S-specific CTL response in vaccinated participants compared to participants receiving GM-CSF alone. The primary biomarker therefore is nelipepimut-S-specific CTL, which will be detected using a dextramer assay, which is a flow cytometry based assay. The number of nelipepimut-S-CTL will be determined in all participants, regardless of randomization to vaccine or GM-CSF alone, at the following time points: pre-vaccination, at the time of surgery (after two preoperative vaccinations), 1 month (+/- 7 days) after surgery, and 3-6 months at the time of the final study visit. The change in the number of nelipepimut-S-CTL for an individual participant between the pre-vaccination timepoint and the 1 month (+/- 7 days) after surgery timepoint will be used as the primary endpoint measure for this study. Planned subset analyses will be performed based on the extent of HER2 expression in the biopsy specimen (HER2 3+ vs HER2 0, 1+ or 2+), hormone receptor status, and menopausal status.

8.2 Secondary Endpoints

Secondary endpoints include:

8.2.1 Toxicity profile and frequency of adverse events in women with DCIS of the breast receiving nelipepimut-S vaccine as compared to women receiving GM-CSF alone.

8.2.2 Presence of DCIS at resection. The histologic data will be presented in tabular form to examine whether the vaccine treatment is associated with the absence of DCIS, induction of necrosis, reduction in histologic grade, or a reduction in the size of DCIS. These data will be descriptive only. The data will be obtained from the standard surgical pathology report generated as part of the patient's clinical care. Formal comparisons of these parameters would likely require a larger sample size to see a statistically significant difference in presence of DCIS, induction of necrosis, or reduction in size of DCIS.

8.2.3 Difference in HER2 expression in the biopsy and the surgical specimen excised post-vaccination. HER2 scoring will be determined according to the American Society of Clinical Oncology/College of American Pathologists clinical guidelines [7]. Positive cases are those with circumferential membrane staining that is complete, intense, and within >10% of tumor cells (Score 3+). Negative cases are defined as those with no observable staining, or membrane staining that is incomplete and is faint/barely perceptible and within less than or equal to 10% of tumor cells (Score 0) or incomplete membrane staining that is faint/barely perceptible and within >10% of tumor cells (Score 1+). Equivocal or indeterminate cases are those with circumferential membrane staining that is incomplete and or weak/moderate and within >10% of tumor cells or complete and circumferential membrane staining that is intense and within less than or equal to 10% of tumor cells (Score 2+). Pre-vaccination and post-vaccination specimens will be compared.

8.2.4 Degree of lymphocyte infiltration. We will define intra-tumoral TIL as those within the basement membrane. Stromal TIL will be defined as those in the periductal/lobular stroma including the intralobular stromal infiltrate. Cells in the interlobular stromal inflammatory infiltrate will be excluded. All mononuclear cells will be scored but polymorphonuclear leukocytes will be excluded. Intra-tumoral and stromal TILs will be scored as a continuous variable and the percentage of in the surgical specimen will be compared to that in the pre-vaccination diagnostic biopsy.

8.2.5

We will utilize tissue-based cyclic immunofluorescence (t-CyCIF), a novel, highly multiplexed imaging modality that allows for the imaging of formalin-fixed, paraffin embedded (FFPE) tissue sections at subcellular resolution across 20-60 distinct antigen channels.[4-6] Antibody panels that will be used include but may not be limited to an already optimized immune panel (Table 2).

Table 2. Optimized t-CyCIF immune panel.

Immune Panel:										
DAPI/Blue	Hoechst_1	Hoechst_2	Hoechst_3	Hoechst_4	Hoechst_5	Hoechst_6	Hoechst_7	Hoechst_8	Hoechst_9	Hoechst_10
FITC/Green	Background	inos (Rab)	GrB	PD-L1	CD11b	CD4	IBA1	CD163	CD15	Arg1
Cy3/White	Background	pRB (Gt)	pSTAT1	FOXP3	CK7	Lag3	CD1cZ	CD11cZ	CD56	CD45RO
Cy5/Red	Background	BDCA2 (Ms)	CD45	PD1	MHCI	CD8	MHCII	CD20	CD14	SMA

Table: Optimized t-CyCIF immune panel

ImageJ an Matlab computer software will be used to analyze single cells and yield high-dimensional data analysis including tSNE analysis, dot plot analysis, heatmap analysis and scatter plots. The total number of prioritized immune cell types (Table 3) per mm2 of tumor tissue and

the percent of specific types of immune cells of total cells will be assessed. Additionally, we will spatially resolve where cells are found and the proximity to other cell types.

Table 3. Prioritized human immune cell populations.

Prioritized Immune Cell Types		+/- PD-L1
Macrophages:	CD45, CD11b, CD68	+
Macrophages:	CD45, CD11b, CD68, CD163	+
M1 Macrophages:	CD45, CD68, iNOS	+
M1 Macrophages:	CD45, CD68, pSTAT1	+
M2 Macrophages:	CD45, CD163, Arg1	+
Macrophages:	CD45, CD68, PD-L1	+
MDSC - PMN:	CD45, CD14(neg), CD15	+
MDSC-monocytic:	CD45, CD14, CD15(neg)	+
Dendritic cells:	CD45, IBA1(neg), CD11b	+
NK cells:	CD45, CD56	-
T helper:	CD45, CD4	-
Treg:	CD45, CD3, CD4, FoxP3	-
CTL:	CD45, CD3, CD8, GrB	-
T effector memory:	CD45, CD3, CD8, CD45RO	-
T exhausted:	CD45, CD3, PD-1	-
T activated:	CD45, CD3, Ki67	-
B cells:	CD45, CD20	-

8.2.6 Immune infiltrates in normal tissue maximally distant from the tumor (in mastectomy samples). In the mastectomy samples only we will perform core needle biopsies of the normal tissue, maximally distant from the tumor (*ex vivo* after the mastectomy if performed) and store for future studies of immune infiltrates.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, medical contraindication, or pregnancy. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

8.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention is completed, AEs, SAEs, lost to follow-up, participant's refusal of follow up, participant withdrawal (*e.g.*, due to toxicity unacceptable to the participant or the investigator), physician decision to take the participant off study, other reason (*e.g.*, determination of ineligibility including screen failure, non-compliance, pregnancy or medical contraindication), abnormal results of ECHO at baseline (defined as an ejection fraction less than the institution's lower limit of normal), or death. Participants found to be ineligible during the screening phase and after signing the Informed Consent document and assigning the PID number, will be considered "screen failures". Such participants will be taken off study and the appropriate end of study CRFs will be completed for these participants. In cases of early termination, when possible, participants

will be asked to return to the clinic site prior to going off study for an early termination visit according to the Schedule of Events.

8.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

9.1.1 Enumeration of nelipepimut-S-specific CTL

Rationale. MHC class I vaccines such as nelipepimut-S+GM-CSF are designed to stimulate clonal expansion of antigen-specific CTL. In a subset of 86 patients from our phase I/II trials investigating nelipepimut-S+GM-CSF, we used a dimer assay to quantify nelipepimut-S-specific CTL and found that only 1 of 30 (3%) patients with -specific CTL levels above the mean recurred compared to 8 of 56 (14%) with levels below the mean [21]. This suggests that robust *in vitro* immunologic responses may identify participants in whom vaccination will be efficacious. The primary objective of this trial is to evaluate the nelipepimut-S-specific CTL response in participants receiving NeuVax versus control participants receiving GM-CSF alone.

9.1.2 Histologic Responses

Rationale. Because this trial will administer vaccines before surgical therapy, the effects of immunization will be assessed on residual tumor at the time of surgical resection. A previous trial investigating a HER2-targeting dendritic cell vaccine in participants with DCIS showed a decrease in the number of HER2-expressing tumor cells in residual DCIS lesions suggesting that vaccination had stimulated an immune response capable of eliminating the HER2+ clones within the tumor. Dendritic cell vaccines are limited by their complexity which requires leukapharesis to obtain an individual participant's blood from which dendritic cells can be isolated, matured, and stimulated with the antigen of interest at a central processing facility. Our peptide vaccine strategy is also capable of stimulating an antigen-specific immune response but has the obvious advantage of being much simpler as it is an "off the shelf" therapy. In order to determine the intratumoral immune response stimulated in response to vaccination, we will evaluate for: 1) HER2 expression, and 2) the degree of lymphocyte infiltration (determined on H&E stained slides as well as determined by multiplex immunofluorescence), .

9.2 Comparable Methods

The methodology described above are those previously used (see details above), and the resulting data will be able to be compared to existing data.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

We plan to evaluate biomarkers in both PBMC and tissue specimens. For our primary biomarker, nelipepimut-S-specific CTL, we will use a dextramer assay, which is a flow cytometry based assay, to enumerate the number of nelipepimut-S-specific CTL generated in response to vaccination with the nelipepimut-S peptide. Phase I/II studies of NeuVax (= nelipepimut-S + GM-CSF) showed a correlation between nelipepimut-S-specific CTL and disease free survival [20]. Our tissue based assays will be performed in a CLIA certified laboratory (HER2 [32] and lymphocyte infiltration [assessed on hematoxylin & eosin stained slides] [33] and multiplex immunofluorescence will be analyzed in the laboratory of our pathology collaborator using established protocols .

10.1.1 PBMC and serum samples

Dextramer Assay: to enumerate the number of nelipepimut-S-specific CTL	Gheath Al-Atrash, DO, PhD The University of Texas MD Anderson Cancer Center

10.1.2 Tissue samples

Histologic analysis (presence or absence of DCIS, invasive breast cancer, grade of tumor)	Breast Tumor Immunology Lab Dana Farber Cancer Institute
HER2	Breast Tumor Immunology Lab Dana Farber Cancer Institute
Multiplex Immunofluorescence	Laboratory of Systems Pharmacology Harvard Medical School PI: Peter Sorger, PhD
Immune infiltration in normal tissue maximally distant from the tumor (in mastectomy samples)	Undetermined* MD Anderson Cancer Center

* In the mastectomy samples we will perform core needle biopsies of the normal tissue, maximally distant from the tumor (*ex vivo* after the mastectomy if performed) and store for future studies of immune infiltrates.

10.2 Collection and Handling Procedures

Random, blinded, unique ID numbers will be assigned to specimens at each time point and will be distributed with the PID numbers. All specimens will be labeled with the unique specimen number (XXXX), date and time specimen collected and protocol # MDA2014-04-02, at a minimum. Laboratories receiving the specimens will be blinded from distinguishing participant information and visit point but this information will be kept available in a password protected electronic log at each site to indicate which specimen number belongs to which participant and at what visit. The master log of specimen IDs will be kept at the Coordinating Center.

Multiple blood draws will be required for immunologic assessments in this trial. Approximately 70 mL will be drawn at each time point when blood is drawn for immunologic assessments. Participant blood samples labeled as described above will be sent from study sites via overnight delivery to the laboratory of Gheath Al-Atrash, DO, PhD at MD Anderson where immunologic response assays will be performed. Assays will be run within 30 days of receipt of specimen. Blood not used to perform the dextramer assay will be frozen and stored in the laboratory of Gheath Al-Atrash, DO, PhD under the unique protocol number and identifier for up to five years for additional immunologic studies related to this protocol. No genetic testing will be performed on this material. Study participants will not be contacted in the future for additional use of these stored blood specimens. If study participants want their blood specimens removed from storage and destroyed, they may do so by contacting the PI or research nurse or/ coordinator at any time. Additionally, any stored blood may also be utilized to assess new generations of vaccines.

For blood draws performed for immunologic assessment, 10 mL are collected into a BD Vacutainer Rapid Serum tube (BD, Franklin Lakes, NJ) which contain a clot activator and silicone coated interior. After centrifugation, serum will be collected, aliquoted in 1 mL vials and frozen for batch analysis. The samples will undergo a single freeze-thaw. The remaining 60 mL are collected into BD Vacutainer CPT Cell Preparation tubes which contain an anticoagulant (sodium heparin or sodium citrate) with FICOLL HYPAQUE density gradient fluid and a polyester gel barrier. The density gradient fluid and the gel barrier allow for the separation of PBMC from the red blood cells by a single step centrifugation process. The PBMC fraction will be collected by centrifugation and suspended in RPMI-1640 (GIBCO, Invitrogen Corporation, Carlsbad, CA) with 10% FCS (Gemini Bio-Products, West Sacramento, CA) and antibiotics. Cells will be counted, aliquoted at $5x10^6$ cells in 1 mL vials and either used to perform the indicated immunologic assays or frozen.

10.2.1 Materials and Methods for nelipepimut-S-CTL Determination

PBMC will be isolated by ficoll gradient and stained with aqua live/dead stain (Invitrogen) and the following antibodies: CD8 APC-H7 (BD Biosciences), CD3 PE Cy7 (BD Biosciences), nelipepimut-S -APC-conjugated dextramer (Immudex), and the following pacific blue conjugated lineage antibodies: CD4 (BD Biosciences), CD14 (BD Biosciences), CD16 (BD Biosciences), and CD19 (Biolegend). Cells will be analyzed on a LSR Fortessa Analyzer (BD Biosciences) and the number of nelipepimut-S-specific CTL will be determined.

10.2.2 Immunohistochemistry (IHC)

We will utilize standard IHC techniques to measure expression of specific biomarkers in tissue samples. Tissue samples will be required from both the initial breast biopsy and the subsequent surgical excisional procedure. An H&E stained slide and 10 unstained slides (or 11 unstained slides) from the initial breast biopsy and an H&E and 10 unstained slides (or 11 unstained slides) from the subsequent surgical excisional procedure should be sent to the Breast Tumor Immunology Lab at the address provided in **Section 10.3** below. The biopsy slides should be sent within 6 weeks of randomization. The slides from the surgical procedure should be sent within 6 weeks of the surgery. All slides can be sent at room temperature. The study pathologist will review all of the H&E slides as well as subsequent slides prepared for biomarker assessment and mark the relevant areas for evaluation. Standard antibody recognition techniques will be used to assess expression of specific biomarkers of interest listed below. Both positive and negative controls will be included in each batch to ensure the functionality of the antibody. We plan to assess the following biomarkers: HER2 and lymphocyte infiltration. Multiplex

immunofluorescence will be analyzed in the laboratory of our collaborator using established protocols.

In the mastectomy samples only we will perform core needle biopsies of the normal tissue, maximally distant from the tumor (ex vivo after the mastectomy if performed) and store for future studies of immune infiltrates. These core needle biopsy samples should be formalin fixed and paraffin embedded. The block or 10 unstained slides should be sent to the Breast Tumor Immunology Lab with the surgical samples at the address provided in Section 10.3.

10.3 Shipping Instructions

Tissue samples will be shipped to:

Breast Tumor Immunology Lab (BTIL)
Elizabeth Mittendorf, MD, Ph.D.
Jennifer Guerriero, Ph.D.
Dana-Farber Cancer Institute
450 Brookline Avenue, Smith Building, **Room 930**
Boston, MA 02215

Blood samples will be shipped to:

Gheath Alatrash, DO PhD
Department of Stem Cell Transplantation and Cellular Therapy
c/o Na Qiao, PhD
The University of Texas MD Anderson Cancer Center
7435 Fannin St
Unit 0900
Houston, TX 77030
Telephone: (713) 563-3337
Fax: 713-794-5720

Notify the following via email:
emittendorf@bwh.harvard.edu
ibedrosian@mdanderson.org
nqiao@mdanderson.org
galatras@mdanderson.org

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

10.4 Tissue Banking

Biologic specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

An abnormal lab value should be deemed clinically significant if either of the following conditions is met:

- The abnormality suggests a disease and/or organ toxicity that is new or has worsened from baseline.
- The abnormality is of a degree that requires additional active management, e.g., change of dose, discontinuation of the drug, close observation, more frequent follow-up assessments, or further diagnostic investigation.

Therefore, a clinically significant lab value is one that indicates a new disease process, an exacerbation or worsening of an existing condition, or requires further action(s) to be taken.

A list of AEs that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in §6.2, Pharmaceutical Information, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

11.1.1.1 All AEs that occur after the informed consent is signed and baseline assessments are completed (including run-in) through the day preceding the day of surgery must be recorded on the AE CRFs (paper and/or electronic) whether or not related to study agent.

11.1.1.2 AEs not related to surgery that occur from the day of surgery through the post vaccination follow-up visit (30 days±7 days post-op) must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.1.3 All SAEs, including all hospitalizations, during the study period will be reported as per DCP SAE reporting procedures, with the following exception:

> Hospitalization for planned surgery will not be reported. However, if this hospitalization event lasts longer than the usual period at the institution (as determined by the Site Principal Investigator or Sponsor), it will be reportable as an SAE. Adverse events (AEs) relevant to the prolongation of this hospitalization will also be collected and reported on AE CRFs.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting.

- AE verbatim term

- System Organ Class (SOC)
- Common Terminology Criteria for Adverse Events v4.0 (CTCAE) AE term
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a serious adverse event (SAE)
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the adverse event is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

Unresolved AEs with a "possibly", "probably" or "definitely" attribution to study agent at the post vaccination follow-up visit will be managed according to good medical practices and documented as such with a minimum of a telephone call to follow-up on resolution at 20-30 days after the post vaccination follow-up visit.

11.2 Serious Adverse Events

11.2.1 DEFINITION: Fed. Reg. 75, Sept. 29, 2010 defines SAEs as those events, occurring at any dose, which meet any of the following criteria:

- Results in death
- Is life threatening (*Note: the term life-threatening refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality/birth defect
- Important medical events that may not result in death, be life-threatening or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed.

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE form found at https://prevention.cancer.gov/clinical-trials/clinical-trials-management/protocol-information-office/pio-instructions-and-tools/2012-consortia.

11.2.2.2 Reporting within 24 hours of knowledge of the event.

11.2.2.2(A) Report to the NCI DCP Medical Monitor within 24 hours:
Contact the DCP Medical Monitor by email or phone within 24 hours of knowledge of the event.

Margaret (Malgorzata) Wojtowicz, MD
NCI/Division of Cancer Prevention
Room 5E104
9609 Medical Center Drive
MSC 9781
Bethesda, MD 20892
Phone: (240) 276-7012
wojtowim@mail.nih.gov

Include the following information when emailing / calling the Medical Monitor:

- Date and time of the SAE

- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug and expectedness

11.2.2.2(B) Report to the Consortium Lead Organization (CLO) (Dr. Powel Brown or designee) within 24 hours of knowledge of the event:
The same information reported to the DCP Medical Monitor should be provided to the Consortium PI, Dr. Powel Brown or the CLO Coordinator (Saba Abutaseh) via email, phone or fax within 24 hours of knowledge of the event. The contact details are provided on the cover page of this protocol.

11.2.2.3 Reporting within 48 hours of knowledge of the event:

11.2.2.3 (A) Email the written SAE reports to the DCP Medical Monitor within 48 hours of learning of the event using the paper SAE form. The SAE forms should be obtained at https://prevention.cancer.gov/sites/default/files/uploads/clinical_trial/SAE-Form_05-02-2016.pdf.

11.2.2.3 (B) The written SAE reports will also be sent to DCP's Regulatory Contractor, CCS Associates, at safety@ccsainc.com.

11.2.2.3 (C) The written SAE report will also be emailed to the CLO PI (Dr. Powel Brown), or faxed to (713) 792-4003.

It is the responsibility of the CLO to inform the Protocol Principal Investigator upon receipt of the report from the organization experiencing the event.

11.2.2.4 The DCP Medical Monitor and regulatory staff will determine which SAEs require FDA submission.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.2.6 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE form in the appropriate format. Follow-up information should be sent to DCP as soon as available. SAEs related to the study agent will be followed until resolved.

12. STUDY MONITORING

12.1 Data Management

This study will report clinical data using the Data Management Initiative (DMI) web-based application managed by the Consortium Biostatistics and Data Management Core. Data Management Initiative (DMI) infrastructure has been developed in the Division of Quantitative Sciences (DQS), MD Anderson Cancer Center. This infrastructure supplies integrated database and software services for web-based data collection, randomized treatment assignment, reporting, query, data download, and data quality management. The DMI will be the database of record for the protocol and subject to NCI and FDA audit. All DMI users will be trained to use the DMI system and will comply with the instructions in the protocol-specific "DMI User Manual" as well as applicable regulatory requirements such as 21 CFR; Part 11. Data management procedures for this protocol will adhere to the Data Management Plan (DMP) on file at the DCP for contract HHSN261201200034I.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used to create the electronic CRF (e-CRF) screens in the DMI application. Site staff will enter data into the e-CRF. Amended CRFs will be submitted to the DCP Protocol Information Office for review and approval. Approved changes will be programmed into the DMI database by the Consortium Biostatistics and Data Management Core.

12.3 Source Documents

Source documentation will include only those documents containing original forms of data, including clinic charts, shadow files, hospital charts, and physician notes. Data recorded directly on the CRFs designated as source documents (*i.e.*, no prior written or electronic record of data) will be considered source data. All other data recorded on the CRFs will not be considered source documentation.

12.4 Data and Safety Monitoring Plan

The Data and Safety Monitoring Plan for the MD Anderson Consortium is on file at the DCP. This study will be monitored by the MDACC Data and Safety Monitoring Board (DSMB), the data and safety monitoring board of record for this study. The DSMB reports to the President, or his designee, as the on-campus representative of The University of Texas Board of Regents. It oversees the data and patient safety issues for randomized clinical trials that originate at MD Anderson; that are coordinated or analyzed by MD Anderson and are not being monitored by any other DSMB; or have been designated as requiring DSMB monitoring at the request of the IRB, the CRC, or institution. The primary objectives of the DSMB are to ensure that patients' rights pertaining to participation in a research study are protected, and that patients' interests are prioritized over the interests of the scientific investigation. Responsibilities include:

(a) Review interim analyses of outcome data (prepared by the study statistician or other responsible person at the time points defined in the study) approved by the IRB and additional time points as determined by the DSMB, and to recommend, if necessary, whether the study needs to be changed or terminated based on these analyses;

(b) Determine whether, and to whom, outcome results should be released prior to the reporting of study results;

(c) Review interim toxicity data and efficacy of treatment;
(d) Review major research modifications proposed by the investigator or appropriate study committee prior to implementation (*e.g.*, termination, dropping an arm based on toxicity results from the study or results of other studies, increasing target sample size).

Refer to the Data and Safety Monitoring Plan for the MD Anderson Consortium on file at the DCP for further details.

12.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

The agent(s) supplied by DCP, NCI, used in this protocol, is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, MTRA) between SELLAS (hereinafter referred to as Collaborator(s)) and the NCI Division of Cancer Prevention. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator(s) contained within the terms of award, apply to the use of Agent(s) in this study:

12.7.1 The Research Material will only be used for research purposes by Recipient, its contractors, and agents and Investigator for the Research Project described in Attachment A, under suitable containment conditions. This Research Material will not be used for any other purpose including but not limited to commercial purposes for screening, production or sale, for which a commercialization license may be required. DCP and its Investigator shall not use the Research Material in assays for the identification of potential commercial products. At the end of the Research Project, Recipient and its Investigator will be responsible for ensuring the return of any unused Research Material to Sponsor, unless otherwise directed by Sponsor. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential information and should

not be shared or distributed without the permission of the NCI. If an individual participating on the study or participant's family member requests a copy of this protocol, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from the DCP website.

12.7.2 Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval, or commercialize its own investigational agent.

12.7.3 Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational agent.

12.7.4 Clinical Trial Data and Results and Raw Data developed under a collaborative agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate. All data made available will comply with HIPAA regulations.

12.7.5 When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators of Collaborator's wish to contact them.

12.7.6 Any abstract or manuscript reporting the results of this clinical trial must be provided to DCP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days (or as specified in the CTA) from the date of receipt for review. Press releases and other media presentations must also be forwarded to DCP prior to release. Each Party agrees to provide proposed press releases that reference or rely upon the work under this MTRA to the other party for review and comment at least seven business (7) days prior to publication. Copies of any manuscript, abstract, and/or press release/ media presentation should be sent to the Protocol Information Office at NCI_DCP_PIO@mail.nih.gov.

The Protocol Information Office will forward manuscripts to the DCP Project Officer for distribution to the Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This is a Phase II, randomized, single-blind study with 2 intervention arms. A total of 108 DCIS participants will be consented and screened for eligibility and, with a conservative estimate, 48 (45%) participants are expected to be HLA-A2 positive. These 48 participants will be randomized 2:1 to vaccine and GM-CSF alone groups. Accounting for 10% attrition rate while on study and for approx. 5% non-evaluable sample rate, we expect to have 40 evaluable participants, 27 in vaccine and 13 in GM-CSF alone group, that have all baseline, post-vaccination (pre-surgery), and post-surgery measures of nelipepimut-S-specific CTL. With 27 and 13 in vaccine and GM-CSF alone groups, respectively, we will have 82% power to detect an effect size of 1 on the change of nelipepimut-S-specific CTL ("1 month (+/- 7 days) after completion of the vaccination series timepoint" minus pre-1st vaccine) between the two groups, corresponding to change of 0.05% ± 0.05% in vaccine group and 0 ± 0.05% in control group, using two-sided t-test with a significance level of 0.05.

The decision rule for declaring the agent promising will be if we detect an effect size of 1 on the change of nelipepimut-S-specific CTL, and if there are not greater than 10% grade III AEs attributed to the active

study drug or IV AEs of any attribution. Completion of the current study will provide the necessary data to determine the next phase of study, specifically whether a subsequent, larger phase II will be conducted versus a phase III study.

13.2 Randomization/Stratification

Participants will be assigned a randomization number once the following has been accomplished: eligibility has been verified at the site level, eligibility has been confirmed by the site PI, and eligibility CRF has been entered into the DMI web application. The randomization number will be generated by the database and assigned to the participant.

A total of 48 eligible participants will be randomized 2:1 to vaccine and GM-CSF alone groups. Accounting for approx. 10% attrition rate and approx. 5% non-evaluable sample rate, we expect to achieve 40 evaluable participants, 27 in vaccination and 13 in GM-CSF alone group. We will stratify the randomization by site using random permutated block design with the block size of 6.

13.3 Accrual and Feasibility

We plan to consent and screen 108 potentially eligible subjects in the study over 2.5 years at four clinical centers. Our goal is to randomize 48 participants. The randomized participants will receive the intervention for approx. 4 weeks; and will be followed up for an additional approx. 3-6 months period. They will be followed closely to monitor the toxicity and drug compliance.

13.4 Primary Objective, Endpoint, Analysis Plan

The primary objective of this study is to evaluate the effect of the nelipepimut-S vaccine on nelipepimut-S-specific CTL measured in blood in DCIS participants that are HLA-A2 positive. We will use a dextramer assay, which is a flow cytometry based assay, to enumerate the number of nelipepimut-S-specific CTL generated in response to vaccination with the nelipepimut-S peptide. The number of nelipepimut-S-CTL will be determined in participants at the following time points: pre-vaccination, at the time of surgery (after two preoperative vaccinations), 1 month (+/- 7 days) after surgery, and 3-6 months after surgery at the time of the final study visit. The change in the number of nelipepimut-S-CTL for an individual participant between the pre-vaccination timepoint and the 1 month (+/- 7 days) after surgery timepoint will be used as the primary endpoint measure for this study.

At the end of the study, the change of nelipepimut-S-specific CTL at the 1 month (+/- 7 days) after completion of the vaccination series timepoint from baseline will be estimated for each group using mean, standard deviation, median, minimum and maximum. Two-sample t-test or Wilcoxon rank sum test, whichever appropriate, will be used to compare the change between the two groups. Nelipepimut-S-specific CTL will also be measured repeatedly through 3 months after surgery. Repeated measures analysis including mixed effects model will be performed to analyze the effect of treatment on nelipepimut-S-specific CTL change over time [35]. We will evaluate the results in the whole participant population as well as the participants with or without radiation treatment separately, when appropriate

13.5 Secondary Objectives, Endpoints, Analysis Plans

The secondary objectives of the study include evaluation of the toxicity profile of nelipepimut-S vaccine and to compare the toxicity profile between the two groups. Additional secondary objectives include presence of DCIS at resection, difference in HER2 expression in the biopsy and the surgical specimen

excised post-vaccination and histologic responses (degree of lymphocyte infiltration determined on H&E stained slides as well as immune infiltration as determined by multiplex immunofluorescence staining for markers including but not limited to CD3, CD4 and CD8). Comparisons of intraperson changes in the vaccination versus GM-CSF alone arms will be made.

Adverse events by grade and relationship will be summarized by tabulation for each group. Correlation among the biomarkers described above at baseline in each specimen and between different specimens will be assessed. The association among various continuous and discrete biomarkers or treatment groups will be assessed by the exploratory data analysis using scatter plot matrix, box plots, BLiP plot [10] and trellis plot, etc., and may be tested by t-test/ANOVA/Wilcoxon rank sum test/Kruskal-Wallis test, when appropriate. Correlation between continuous biomarkers will be examined by Pearson or Spearman rank correlation coefficients. The association between discrete biomarkers will be tested by chi-square or Fisher's exact test. Paired t-test/Wilcoxon rank sum test and McNemar's test may be used to test the change of a single continuous biomarker and discrete biomarker, respectively, over time within each treatment group. Repeated measures analysis including mixed effects model will be performed to analyze the effect of treatment on biomarkers change over time [35].

13.6 Reporting and Exclusions

Every reasonable attempt will be made to recover any missing data. If any data for the primary efficacy measure remains missing that data point will be excluded from analysis for that participant.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first vaccination. The toxicity profile in terms of type, attribute, and grade of toxicities will be described in each of the treatment arms.

13.8 Evaluation of Response

All randomized and treated participants are assessed for response to intervention including participants with protocol deviations. For the primary endpoint of change in the number of nelipepimut-S-CTL for an individual participant between the pre-vaccination timepoint and the 1 month (+/- 7 days) after surgery timepoint, "evaluable" will be defined as completing all pre-surgical vaccinations and all post-surgical vaccinations up to the 1 month (+/- 7 days) timepoint. However, the response outcome for both the evaluable and the non-evaluable participants will be computed and compared. For the safety endpoint, participants who receive at least one vaccination will be included in the safety evaluation. For the biomarker (biologic efficacy) endpoints, all non-compliant participants will be analyzed according to the intent-to-treat principle. All levels of compliance will be included in the full evaluation of endpoints if paired biopsies are available.

The primary endpoint is defined as the change in the number of E75-CTL for an individual participant between the pre-vaccination timepoint and the 1 month (+/- 7 days) after surgery.

13.9 Interim Analysis

No formal statistical interim analysis and no early stopping rules have been planned for the study primary and secondary endpoints. However, we plan to evaluate HER2 expression of the baseline biopsy

specimens of the first 12 participants to ensure that accrual is balanced and the trial enrolls women with DCIS on core biopsy who are HER2 0, 1+, 2+ or 3+ approximately equally. If, on the other hand, we determine that the trials enrolls disproportionately more participants with a particular level of HER2 expression, we will re-design the trial to include HER2 testing at baseline and stratification based on HER2 expression.

13.10 Ancillary Studies

There are no plans for ancillary studies.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Signed and dated current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g.*, CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of training in "Protection of Human Research Subjects" for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of training in "Good Clinical Practice" for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.6 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.7 Signed Investigator's Brochure/Package Insert acknowledgement form.

14.2.8 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator and all study personnel listed on the form for each site.

14.2.9 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the CLO, and the IRB at each participating organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the CLO's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the CLO and reviewed for completeness and accuracy. Once the CLO has received complete and accurate documents from a participating organization, the CLO will forward the regulatory documents to the DCP Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates
1923 Landings Drive
Mountain View, CA 94043
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions:
regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the CLO for review, which will then be electronically forwarded to the DCP Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Participants will not be responsible for the costs of this study. Study agent will be provided at no cost to the subject. If, as a result of participation in this study, an individual experiences injury from known or unknown risks of the research procedures as described in the informed consent, immediate medical care and treatment, including hospitalization, if necessary, will be available. No monetary compensation is available for the costs of medical treatment for an injury, thus, the participant will be responsible for the costs of such medical treatment, either directly or through their medical insurance and/or other forms of medical coverage.

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Descriptions**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix B

Guidance for mixing NeuVax (or control) solution

Dosing Summary:
The study treatment is 1000 μg of nelipepimut-S peptide mixed with 250 μg of lyophilized Leukine® (sargramostim, GM-CSF) or control (sterile water for injection [SWFI]) mixed with Leukine, administered in four separate 0.4 mL intradermal injections.

The nelipepimut-S peptide is provided as 1.5 mg/mL of nelipepimut-S acetate (contains a 1.42 mg/mL solution of the nelipepimut-S peptide). Using aseptic technique, 0.8 mL of nelipepimut-S acetate solution (1136 μg nelipepimut-S peptide) is added to the re-constituted Leukine. Taking into account vial losses, this procedure will yield a total dose of approximately 1010 μg of nelipepimut-S peptide in a volume of 1.6 mL.

Stability:
Once prepared, administer the nelipepimut-S peptide/control and lyophilized Leukine mixture within 2 hours.

Materials:

1. Test Drug kit with Subject ID number. This kit contains a single 1.0 mL vial of nelipepimut-S acetate solution (1.5 mg/mL)
2. Lyophilized Leukine kit with Subject ID number. This kit contains a single vial of lyophilized Leukine (250 μg).
3. Sterile Water for Injection (SWFI)
4. Ancillary Supplies: 7-1.0 mL TB syringes, 5-3/8 inch 26G needles, 4-2 inch 21G needles, 2-3 mL luer lock syringes.

NeuVax Dose Preparation:

NOTE: DO NOT INVERT ANY STUDY DRUG VIALS DURING PREPARATION

1. Remove cap from vial of sterile water for injection (SWFI) and wipe septum with alcohol prep pad.
2. Remove cap from vial of lyophilized Leukine (250 μg) and wipe with alcohol prep pad.
3. Attach a 2 inch 21G needle to a 1.0 mL TB syringe and draw up 1.0 mL from the vial of SWFI.
4. Using the needle and syringe from step #3, insert the needle into the Leukine vial.
   - Angle the needle to the side of the Leukine vial and slowly expel 1.0 mL SWFI into the lyophilized Leukine.
   - DO NOT INVERT VIAL at any time. Roll the vial slowly between your hands to mix, taking care to be sure all powder is dissolved in the vial.
   - *Reconstitution of lyophilized Leukine should take approximately 2 minutes. DO NOT SHAKE OR VORTEX.*
5. Remove cap from vial of Test Drug (nelipepimut-S acetate, 1.5 mg/mL) and wipe septum with alcohol prep pad.
6. Attach a 2 inch 21G needle to a 1.0 mL TB syringe. Leaving vial upright (do not invert), insert the 2 inch needle all the way down to the bottom of the vial and withdraw 0.8 mL into the syringe.
7. Using the needle and syringe from step #6, slowly inject the 0.8 mL of Test Drug into the reconstituted Leukine vial.

8. Keeping the vial upright, slowly roll the Leukine/Test Drug vial between your hands. Allow any foaming to dissipate before continuing.
9. Attach a 2 inch 21G needle to a 3.0 mL syringe and draw 2.0 mL of air into the syringe. Inject 2.0 mL of air into the upright Leukine/Test Drug vial. DO NOT INVERT THE VIAL. Insert the 2 inch needle down to the bottom of the vial and slowly withdraw the entire contents of the vial making sure all liquid is removed.
10. Using the needle and syringe containing the Leukine/Test Drug from step #9, top-fill four 1.0 mL TB syringes with 0.4 mL of the Leukine/Test Drug in each syringe. [Note: Using the same needle from step #9 or changing the needle to a new one is allowed.]
11. Attach a new sterile 3/8 inch 26G needle to each syringe containing 0.4 mL of Leukine/Test Drug. Appropriately label each syringe and dispense syringes for intradermal administration of study drug. The syringes must be used within 2 hours of mixing.

Control Dose Preparation:

NOTE: DO NOT INVERT ANY STUDY DRUG VIALS DURING PREPARATION

1. Remove cap from vial of sterile water for injection (SWFI) and wipe septum with alcohol prep pad.
2. Remove cap from vial of lyophilized Leukine (250 μg) and wipe with alcohol prep pad.
3. Attach a 2 inch 21G needle to a 1.0 mL TB syringe and draw up 1.0 mL from the vial of SWFI.
4. Using the needle and syringe from step #3, insert the needle into the Leukine vial.
   - Angle the needle to the side of the Leukine vial and slowly expel 1.0 mL SWFI into the lyophilized Leukine.
   - DO NOT INVERT VIAL at any time. Roll the vial slowly between your hands to mix, taking care to be sure all powder is dissolved in the vial.
   - *Reconstitution of lyophilized Leukine should take approximately 2 minutes. DO NOT SHAKE OR VORTEX.*
5. Using the same vial of SWFI from step#1, attach a 2 inch 21G needle to a 1.0 mL TB syringe and draw up 0.8 mL from the vial of SWFI, slowly inject an additional 0.8 mL of SWFI into the reconstituted Leukine vial.
6. Keeping the vial upright, slowly roll the Leukine/Control vial between your hands. Allow any foaming to dissipate before continuing.
7. Attach a 2 inch 21G needle to a 3.0 mL syringe and draw 2.0 mL of air into the syringe. Inject 2.0 mL of air into the upright Leukine/Control vial. DO NOT INVERT THE VIAL. Insert the 2 inch needle down to the bottom of the vial and slowly withdraw the entire contents of the vial making sure all liquid is removed.
8. Using the needle and syringe containing the Leukine/Control from step #9, top-fill four 1.0 mL TB syringes with 0.4 mL of the Leukine/Control in each syringe. [Note: Using the same needle from step #7 or changing the needle to a new one is allowed.]
9. Attach a new sterile 3/8 inch 26G needle to each syringe containing 0.4 mL of Leukine/Control. Appropriately label each syringe and dispense syringes for intradermal administration of study drug. The syringes must be used within 2 hours of mixing.

NeuVax Dose Preparation for 1st Dose Reduction

NOTE: DO NOT INVERT ANY STUDY DRUG VIALS DURING PREPARATION

1. Remove cap from vial of sterile water for injection (SWFI) and wipe septum with alcohol prep pad.
2. Remove cap from vial of lyophilized Leukine (250 µg) and wipe with alcohol prep pad.
3. Attach a 2 inch 21G needle to a 1.0 mL TB syringe and draw up 1.0 mL from the vial of SWFI.
4. Using the needle and syringe from step #3, insert the needle into the Leukine vial.
   - Angle the needle to the side of the Leukine vial and slowly expel 1.0 mL SWFI into the lyophilized Leukine.
   - DO NOT INVERT VIAL at any time. Roll the vial slowly between your hands to mix, taking care to be sure all powder is dissolved in the vial.
   - *Reconstitution of lyophilized Leukine should take approximately 2 minutes. DO NOT SHAKE OR VORTEX.*
5. Using the needle and syringe from step #4, draw up 0.5 mL from the vial of reconstituted Leukine.
   - Dispose of the needle and syringe containing 0.5 mL of reconstituted Leukine.
6. Remove cap from vial of Test Drug (nelipepimut-S acetate, 1.5 mg/mL) and wipe septum with alcohol prep pad.
7. Attach a 2 inch 21G needle to a 1.0 mL TB syringe. Leaving vial upright (do not invert), insert the 2 inch needle all the way down to the bottom of the vial and withdraw 0.8 mL into the syringe.
8. Using the needle and syringe from step #7, slowly inject the 0.8 mL of Test Drug into the reconstituted Leukine vial.
10. Using the same vial of SWFI from step#1, using the needle and syringe from step #8, draw up 0.5 mL from the vial of SWFI.
    - Insert the needle into the Leukine/Test Drug vial.
    - Angle the needle to the side of the Leukine/Test Drug vial and slowly expel 0.5 mL SWFI into the Leukine/Test Drug.
    - Keeping the vial upright, slowly roll the Leukine/Test Drug vial between your hands. Allow any foaming to dissipate before continuing.
11. Attach a 2 inch 21G needle to a 3.0 mL syringe and draw 2.0 mL of air into the syringe. Inject 2.0 mL of air into the upright Leukine/Test Drug vial. DO NOT INVERT THE VIAL. Insert the 2 inch needle down to the bottom of the vial and slowly withdraw the entire contents of the vial making sure all liquid is removed.
12. Using the needle and syringe containing the Leukine/Test Drug from step #11, top-fill four 1.0 mL TB syringes with 0.4 mL of the Leukine/Test Drug in each syringe. [Note: Using the same needle from step #11 or changing the needle to a new one is allowed.]
13. Attach a new sterile 3/8 inch 26G needle to each syringe containing 0.4 mL of Leukine/Test Drug. Appropriately label each syringe and dispense syringes for intradermal administration of study drug. The syringes must be used within 2 hours of mixing.

Control Dose Preparation for 1st Dose Reduction

NOTE: DO NOT INVERT ANY STUDY DRUG VIALS DURING PREPARATION

1. Remove cap from vial of sterile water for injection (SWFI) and wipe septum with alcohol prep pad.
2. Remove cap from vial of lyophilized Leukine (250 µg) and wipe with alcohol prep pad.
3. Attach a 2 inch 21G needle to a 1.0 mL TB syringe and draw up 1.0 mL from the vial of SWFI.
4. Using the needle and syringe from step #3, insert the needle into the Leukine vial.

- Angle the needle to the side of the Leukine vial and slowly expel 1.0 mL SWFI into the lyophilized Leukine.
- DO NOT INVERT VIAL at any time. Roll the vial slowly between your hands to mix, taking care to be sure all powder is dissolved in the vial.
- *Reconstitution of lyophilized Leukine should take approximately 2 minutes. DO NOT SHAKE OR VORTEX.*

5. Using the needle and syringe from step #4, draw up 0.5 mL from the vial of reconstituted Leukine.
   - Dispose of the needle and syringe containing 0.5 mL of reconstituted Leukine.
6. Using the same vial of SWFI from step#1, attach a 2 inch 21G needle to a 1.0 mL TB syringe and draw up 0.8 mL from the vial of SWFI, slowly inject an additional 0.8 mL of SWFI into the reconstituted Leukine vial.
8. Using the same vial of SWFI from step#1, Using the needle and syringe from step #6, draw up 0.5 mL from the vial of SWFI.
   - Insert the needle into the Leukine/Control vial.
   - Angle the needle to the side of the Leukine/Control vial and slowly expel 0.5 mL SWFI into the Leukine/Test Drug.
   - Keeping the vial upright, slowly roll the Leukine/Control vial between your hands. Allow any foaming to dissipate before continuing.
9. Attach a 2 inch 21G needle to a 3.0 mL syringe and draw 2.0 mL of air into the syringe. Inject 2.0 mL of air into the upright Leukine/Control vial. DO NOT INVERT THE VIAL. Insert the 2 inch needle down to the bottom of the vial and slowly withdraw the entire contents of the vial making sure all liquid is removed.
10. Using the needle and syringe containing the Leukine/Control from step #9, top-fill four 1.0 mL TB syringes with 0.4 mL of the Leukine/Control in each syringe. [Note: Using the same needle from step #9 or changing the needle to a new one is allowed.]
11. Attach a new sterile 3/8 inch 26G needle to each syringe containing 0.4 mL of Leukine/Control. Appropriately label each syringe and dispense syringes for intradermal administration of study drug. The syringes must be used within 2 hours of mixing.