Protocol Title:	Phase II Trial of Combination Immunotherapy with nelipepimut-S + GM-CSF (NeuVax™) and Trastuzumab in high-risk HER2+ Breast Cancer Patients
Study Drugs:	Nelipepimut-S + GM-CSF (NeuVax™)
Financial Sponsor:	Galena Biopharma (to provide NeuVax™)
Director:	Cancer Insight, LLC
IND:	BB-IND#14919
Principal Investigator (PI):	Elizabeth A. Mittendorf, MD, PhD Associate Professor Department of Surgical Oncology The University of Texas MD Anderson Cancer Center 1400 Pressler St Unit 1484 Houston, TX 77030
Medical Monitor:	**John Renshaw, MD** Department of Medicine: Hematology/Oncology Services Brooke Army Medical Center 3551 Roger Brooke Drive Fort Sam Houston, TX 78234
IND Sponsor/Investigator Program Director:	George E. Peoples, MD Cancer Insight, LLC 110 E. Houston Street, Floor 7 San Antonio, TX 78205
Protocol Version/Date:	2.1/11 April 2016
Protocol Number:	2014-0443

Table of Contents

1.0 INTRODUCTION

1.1 Disease background

Breast cancer is the most commonly diagnosed malignancy in women (excluding cancers of the skin) and is the second leading cause of cancer mortality**.** The American Cancer Society estimated that there will be over 230,000 new cases of breast cancer diagnosed in 2013, with an estimated 40,000 women expected to die from this disease annually. [1] Patients with breast cancer are treated with a combination of therapies to include surgery, chemotherapy, endocrine therapy, targeted monoclonal antibody (mAb) therapy and radiation as dictated by characteristics of their tumor and disease stage. [2] Patients with hormone receptor (HR) positive tumors are routinely administered endocrine therapy and patients with HER2-positive tumors receive trastuzumab, a humanized mAb targeting HER2, as a component of their standard of care therapy. Despite advances in treatment, approximately 10% of women with breast cancer will recur and succumb to their disease by the five-year mark; a number that increases to 17% at ten years. [1]

1.2 HER2-positive breast cancer

HER2 is a member of the epidermal growth factor receptor family of receptor tyrosine kinases. It is expressed in approximately 70-80% of invasive breast cancers and amplified and/or overexpressed (= HER2-positive) in approximately 20%. [3,4] HER2-positive breast cancers are more aggressive and susceptible to recurrence than HER2-normal cancers. HER2 is routinely assessed on breast tumors using immunohistochemistry (IHC) to detect protein expression or in situ hybridization (ISH) to identify gene amplification. IHC results are scored as 0, 1+, 2+ or 3+. ISH reports a ratio of the number of *HER2* gene copies to chromosome 17 copies. According to the 2013 update of the American Society of Clinical Oncology (ASCO) and College of American Pathologist (CAP) guidelines, a tumor is considered HER2-positive if any of the following criteria are met: [5]

- IHC 3+
- Dual-probe ISH ratio (*HER2*/CEP17) $\geq$ 2.0 with *HER2* copy number of $\geq$4.0/cell
- Dual-probe ISH ratio (*HER2*/CEP17) $\geq$ 2.0 with *HER2* copy number of <4.0/cell
- $\leq$ 2.0 overall ratio with *HER2* copy number of $\geq$ 6.0/cell
- Single probe tests average *HER2* copy number of $\geq$ 6.0/cell

Patients with HER2-positive tumors are treated with trastuzumab, a mAb that targets the extracellular portion of the HER2 protein. Trastuzumab was first evaluated in the metastatic setting where it was shown to provide significant clinical benefit as monotherapy and in combination with chemotherapy as first- or

second-line therapy. [6-11] Benefit of trastuzumab in the adjuvant setting for early-stage breast cancer has also been demonstrated. Four large trials, and several smaller studies, conducted in the adjuvant setting showed significant improvements in disease-free survival (DFS) (36%-52% reduction in DFS events) and overall survival (OS) (33% to 37% reduction in deaths), independent of patient age, tumor size, nodal status, or hormone receptor status. [12-16] Two of these trials, the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 and North Central Cancer Treatment Group (NCCTG) N9831 trials were conducted in North America. In these two studies, patients with HER2-positive breast cancer were randomized to adriamycin and cyclophosphamide followed by paclitaxel with or without trastuzumab. Because they were similar in design, the trials were analyzed jointly. At the most recent report, after 3.9 years of median follow-up, women in the trastuzumab arm had significantly increased DFS ($p<.001$; stratified hazard ratio [HR], 0.52; 95% CI, 0.45 to 0.60) and OS ($p<.001$; stratified hazard ratio [HR], 0.61; 95% CI, 0.50 to 0.75) compared with women in the control arm. [12] On the basis of these data and the data from the other adjuvant trials, trastuzumab has become a critical component of standard therapy for HER2-positive early breast cancer.

More recently, the efficacy of trastuzumab administered in combination with chemotherapy in the neoadjuvant (preoperative) setting has been evaluated. In the first randomized trial to evaluate this approach, Buzdar et al. reported a 67% pathologic complete response (pCR) rate, defined as no residual invasive disease in the breast or axilla, among patients receiving neoadjuvant chemotherapy plus trastuzumab versus a pCR rate of 25% among patients receiving noadjuvant chemotherapy alone ($p=.02$).[17] Subsequent to this, the GeparQuattro,[18] NOAH,[19] NeoALTTO,[20] NeoSphere[21], American College of Surgeons Oncology Group (ACOSOG) Z1041 [22] and Cancer and Leukemia Group B (CALGB) 40601 [23] trials, have also demonstrated improved pCR rates among patients receiving trastuzumab in

Table 1. Pathologic complete response rates in trials evaluating chemotherapy plus trastuzumab in the neoadjuvant setting.

Trial	pCR Rates		
	HER2+ Patients		HER2- Patients
	Chemo + Trastuzumab	Chemo Alone	Chemo Alone
MD Anderson (2005)	67%	25%	-
GeparQuattro (2010)	32%	-	17%
NOAH (2010)	38%	19%	16%
NeoALTTO (2012)	30%	-	-
NeoSphere (2012)	29%	-	-
CALGB 40601 (2013)	40%	-	-
ACOSOG Z1041* (2013)	51% (arm 1) 49% (arm 2)	-	-

*The ACOSOG Z1041 trial evaluated the timing of initiation of trastuzumab with neoadjuvant chemotherapy. Arm 1 received FEC followed by paclitaxel plus trastuzumab. Arm 2 received paclitaxel plus trastuzumab followed by FEC plus trastuzumab

addition to chemotherapy (table 1). These trials all used different chemotherapy backbones but are consistent in demonstrating high pCR rates in HER2-positive patients receiving neoadjuvant chemotherapy plus trastuzumab.

There has been recent interest in using dual HER2-targeted therapy to increase pCR rates. In the NeoSphere trial, in addition to the arm adding trastuzumab to docetaxel where the pCr rate was 29% (31 out of 107), there was another arm that added pertuzumab, a monoclonal antibody that binds to HER2 and inhibits dimerization of HER2 with other members of the EGFR family of receptors. The addition of pertuzumab to trastuzumab and docetaxel increased the pCR rate to 46% (49 out of 107). [21] The addition of pertuzumab did not significantly increase the rate of grade 3 or higher adverse events (AE). The NeoALTTO and CALGB trials evaluated the addition of lapatinib, a HER2-targeted receptor tyrosine kinase inhibitor. [20,23] In the NeoALTTO trial, the pCR rate in patients receiving trastuzumab plus paclitaxel was 30%; a rate that increased to 51% with the addition of lapatinib. [20] In the CALGB trial, the pCR rate for patients receiving paclitaxel plus trastuzumab was 40% versus 51% when lapatinib was added. [23] In both trials, the addition of lapatinib led to increased greater than grade 3 toxicities including diarrhea, neutropenia and liver enzyme alterations. [20,23]

In an initial report from MD Anderson, it was shown that patients achieving a pCR have improved outcomes when compared to patients not achieving a pCR after neoadjuvant chemotherapy plus trastuzumab. In this study, which included 142 patients with HER2-positive breast cancer that received neoadjuvant chemotherapy with a taxane, anthracycline and concomitant trastuzumab, a pCR was achieved in 72 (51%). The 3-year Kaplan-Meier estimates of recurrence-free survival (RFS) were 96% (95% CI, 91% to 100%) for patients achieving a pCR versus 80% (95% CI, 70.8% to 90.5%) for those not achieving a pCR (p=.02). [24]

In a subsequent report from MD Anderson that evaluated 229 patients with HER2-positive breast cancer, a pCR was achieved in 114 (50%). Compared to patients achieving < pCR, those with pCR had higher five-year rates of local recurrence-free survival (100% versus 95%, *P*=0.011), distant metastasis (96% versus 80%, *P*<0.001), RFS (96% versus 79%, *P*<0.001), and OS (95% versus 84%, *P*=0.006). Improvements in RFS and OS were seen with decreasing post-treatment stage. Failure to achieve a pCR was the strongest independent predictor of recurrence (HR=4.09, 95% CI, 1.67-10.04, *P*=0.002) and death (HR=4.15, 95% CI, 1.39-12.38, *P*=0.011). [25] These data are consistent with those reported from the TECHNO trial, a phase II non-randomized study of 217 patients with HER2-positive breast cancer who received 6 months of neoadjuvant epirubicin and paclitaxel-based chemotherapy and trastuzumab. [26] In this study, 39% of patients achieved a pCR. Three-year DFS was 88% for patients achieving a pCR versus 73% for patients not achieving a pCR (p=.01). Three-year OS rates were 96% for patients achieving a pCR versus 86% for patients

not achieving a pCR (p=.03). pCR was the strongest prognostic factor for relapse and death. Disease-free survival outcomes for patients failing to achieve a pCR are summarized in table 2. Taken together, these data show that achieving a pCR is a strong predictor of long-term outcome and that novel therapeutic strategies are needed for patients failing to achieve a pCR.

Also shown in table 2 are survival outcomes for patients enrolled on the BCIRG-006 trial that evaluated trastuzumab in the adjuvant setting. These data demonstrate that patients who undergo surgery as an initial intervention and are found to have pathologically node-positive disease also represent a high-risk population. Data from the combined analyses of the NSABP B-31 and N9831 adjuvant trastuzumab trials showed that the 5-year recurrence free-survival rates were higher for those patients with hormone receptor-positive patients (89.4%) compared to those with hormone receptor-negative patients (81.6%).[27]

Taken together, these data suggest that patients with HER2-positive breast cancer that receive neoadjuvant chemotherapy with HER2-targeted therapy as well as those that undergo upfront surgery and are found to have ≥ 4 positive lymph nodes or 1-3 positive lymph nodes and are hormone receptor negative are a high-risk group in which novel therapeutic strategies are needed.

Table 2. Disease-free survival in high-risk HER2+ breast cancer

Neoadjuvant Therapy						
Study	N	Regimen	Results			
TECHNO	217	EC→taxol + trastuzumab	3-yr DFS from randomization pCR = 88% no pCR = 73%			
MD Anderson (2009)	142	Included a taxane, anthracycline and concomitant trastuzumab	3-yr RFS from date of diagnosis pCR = 96% no pCR = 80%			
MD Anderson (2013) (update of experience, includes patients from earlier study)	229	Included a taxane, anthracycline and concomitant trastuzumab	5-yr RFS from date of diagnosis pCR = 96% no pCR = 79%			
Adjuvant Therapy						
BCIRG-006	3222	Randomized to: AC-T AC-T plus trastuzumab (AC-TH) TCH	5-yr DFS			
				NN	NP	≥4+ LN
			AC-TH	93%	80%	73%
			TCH	90%	78%	72%

Abbreviations: EC = epirubicin, cyclophosphamide; DFS = disease-free survival; pCR = pathologic complete response; RFS = recurrence-free survival; AC-T = Adriamycin, cyclophosphamide, Taxotere; TCH = Taxotere, carboplatin, Herceptin; NN = node negative; NP = node positive; LN = lymph node

1.3 Nelipepimut-S

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 (TAA) that can be recognized by T lymphocytes. TAAs 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 TAA. Several peptides capable of inducing $CD8^+$ T cells (also referred to as CTL) have been described from the HER2 protein including E75 (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.[28]).

Initial studies investigating E75 in vitro and in an in vivo animal model showed that it was capable of inducing a peptide-specific, CTL-mediated immune response.[29,30] This then led to a number of clinical trials including phase I/II studies conducted by our group evaluating E75+GM-CSF in the adjuvant setting.

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 E75 peptide in the initial stage followed by schedule alterations in the second stage.[31] 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 E75 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 E75 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.[32]

At the time of an initial planned analysis of the combined results of the trials, a total of 171 patients were enrolled; 90 patients received the E75 vaccine while 81 were in the observation arms.[32] Combined toxicity was minimal with local reactions being grade 1 (81%) and grade 2 (19%). Systemic toxicity was grade 0 (12%), 1 (71%), 2 (14%), and 3 (2%) (Figure1). 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 patients demonstrated in vitro immunologic responses and in vivo delayed type hypersensitivity (DTH) responses post-vaccination. At a median follow-up of 20 month, the recurrence rate seen in the vaccinated patients was 6% compared to 15% for patients in the observation group (p=0.04). 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 E75-specific CTL. A voluntary booster program was initiated and vaccinated patients ≥ 6 months from completion of their primary vaccination series (PVS) were eligible for participation. Patients enrolled onto the trial after initiation of the booster program were consented prospectively to

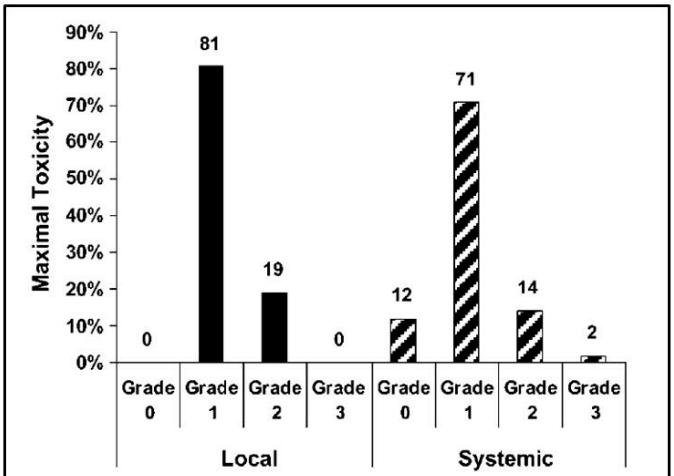


Figure 1. Maximum local and systemic toxicity experienced by patients vaccinated with E75. The most common local toxicities were erythema, induration or pruritis at the injection site. The most common systemic toxicities were bone pain, flu-like symptoms, and fatigue (commonly associated with GM-CSF). 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)

receive booster inoculations. An initial report of the booster inoculations showed them to be safe and effective in stimulating E75-specific immunity in patients who had waning levels of E75-specific CTL six months following completion of their PVS.[33] 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. Among the 187 patients (108 HLA-A2/A3^{+} vaccinated; 79 HLA-A2/3^{-} controls), the 60-month DFS were 90% for vaccinated patients versus 80% for controls (p=.08), a 50% reduction in the relative risk of recurrence.[34] Because the trials began as dose- and schedule-finding trials, not all patients received the dose that was eventually determined to be optimal (1000µg E75 + 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 patients received a booster inoculation, the DFS rate in the 53 boosted patients was 96% versus 83% in vaccinated patients that did not receive a booster inoculation (p=.04).

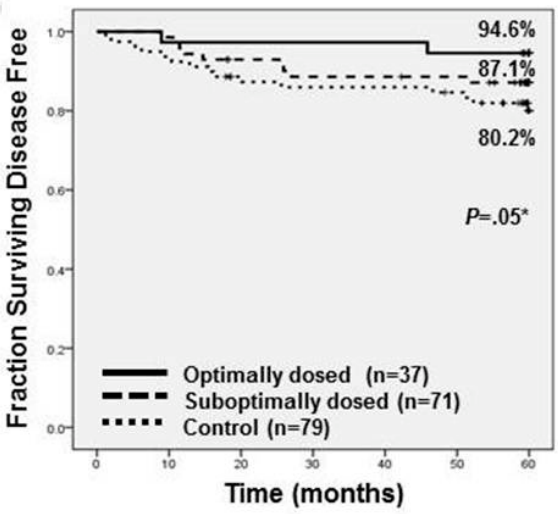


Figure 2. Kaplan-Meier disease-free survival curves evaluated by dosing group after 60 month follow-up for patients enrolled on phase I/II E75 trial. For 187 enrolled patients, the DFS rate in patients vaccinated with what was determined to be the optimal dose of the vaccine was 95% versus 87% for patients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

E75 has been licensed to Galena Biopharma which has secured the nonproprietary drug name nelipepimut-S. NeuVax (nelipepimut-S + GM-CSF) is currently being evaluated in a multicenter, multinational, prospective, randomized, double-blind, controlled phase III study (NCT01479244; PI: Mittendorf). [35] The trial is enrolling NP breast cancer patients with HER2 1+/2+ tumors that are HLA-A2/3+ and are disease free after standard of care therapy. Patients are randomized to receive nelipepimut-S+GM-CSF or GM-CSF alone. The primary endpoint is 36-month DFS.

Subsequently, E75 has also been found to bind to HLA-A24 and HLA-A26. Patients expressing these additional alleles have been included in another ongoing trial of NeuVax. Comparative clinical results between A2 and/or A3-positive patients and A24 and/or A26-positive patients has not been completed yet.

1.4 Combined HER2-directed therapy

Nelipepimut-S and trastuzumab have been shown to have clinical benefit in patients with HER2-positive breast cancer. Although these agents share the same target, their mechanisms of action are different; therefore, the concept of combining these two immunotherapies is novel and worthy of investigation. Nelipepimut-S works by stimulating a HER2-specific CTL response. There have been multiple mechanisms of action described for trastuzumab including decreased signaling by prevention of HER2 dimerization, increased endocytic destruction of the HER2 receptor, and inhibition of extracellular domain shedding.[36] In addition, multiple immune-mediated mechanisms of action have been described. Trastuzumab is a human IgG antibody with a conserved Fc portion. Early in vitro and in vivo reports support an important role for antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by natural killer cells which have an Fc receptor (FcR) that binds the Fc portion of trastuzumab.[37-39] More recently, an important role for adaptive immunity in mediating anti-HER2 therapy effects has been shown. Using a Rag-$1^{-/-}$ mouse that lacks T cells and B cells but does have FcR^{+} innate cells, Park et al. showed that HER2-targeted antibody treatment had minimal impact on tumor growth, suggesting that T cells are required for antibody activity.[40] Consistent with this, when wild-type mice were administered a CD8-depleting antibody, the mice showed minimal initial responses to antibody therapy then relapsed quickly. In a small study of patients with HER2-positive breast cancer receiving trastuzumab, Taylor et al. demonstrated generation of a HER2-specific $CD4^{+}$ T cell response in 6 of 10 evaluable patients.[41] This T cell response is in part due to increased antigen uptake through FcR on dendritic cells present in the tumor microenvironment resulting in increased antigen presentation for recognition by T cells. In that same study, investigators showed anti-HER2 antibodies in approximately 60% of 27 evaluable patients during treatment and found that these anti-HER2 humoral responses significantly increased during therapy and were associated with improvements in clinical response.[41] Data presented by Knutson et al. at the 2013 annual meeting of the American Society of Clinical Oncology showed that patients treated on the trastuzumab arm of the NCCTG 9831 trial developed HER2-specific antibody responses.[42] Cox modeling suggested that larger increases in antibody responses were associated with improved DFS (HR=0.23, p=.04). There is also data demonstrating an inverse relationship between HER2 and HLA class I expression suggesting that down-regulation of HER2 may increase antigen presentation by upregulating HLA molecules.[43] Thus a complementary approach to immune-mediated therapy in breast cancer may provide synergism resulting in improved clinical outcomes.

We have completed preclinical testing of the combination of trastuzumab with HER2-targeted vaccines.[44] HER2-expressing tumor cells were incubated overnight with trastuzumab in concentrations of 10μg/mL and 50μg/mL.

Cytotoxicity of both pretreated and untreated tumor cells was measured using peripheral blood mononuclear cells (PBMC) from healthy HLA-$A2^+$ donors as well as from patients vaccinated with nelipepimut-S. Cytotoxicity by peptide-specific CTL from non-vaccinated patients was increased by 5.6% (10μg /mL) and 15.3% (50μg /mL) (p=.002) in pretreated cells over untreated cells. Using PBMC from nelipepimut-S-vaccinated patients, peptide-specific cytotoxicity was 34% in untreated cells compared to 40% (10μg /mL) (p=.04) and 41% (50μg /mL) (p<.05) in pretreated cells. These findings suggest a synergism may exist with the combination of trastuzumab and vaccination.

The safety of the combination of trastuzumab and HER2-derived peptide vaccines has been evaluated in early clinical trials. The administration of trastuzumab concurrently with a HER2 $CD4^+$ helper T cell-eliciting vaccine composed of multiple peptides (one of which contains the E75 epitope) to patients with HER2-positive breast cancer (n=22) was found to be safe with over 99% of toxicities limited to grade 1 or 2.[45] Median left ventricular ejection fraction (LVEF) was unchanged pre- to post-treatment, with three patients (15%) experiencing asymptomatic decreases in LVEF. Our group recently completed enrollment to a phase I trial evaluating the combination of trastuzumab and a CTL-eliciting HER2 peptide vaccine administered concurrently in the adjuvant setting to patients with HER2-positive breast cancer. All patients (n=18) have completed their vaccination series and we have not seen any cardiac toxicity with combination therapy during vaccine dose escalation. Finally, in preliminary results from our group's ongoing phase II trial evaluating AE37 (HER2-derived $CD4^+$ helper T cell eliciting vaccine) or GP2 (HER2-derived CTL-eliciting vaccine), no cardiac toxicity has been found in HER2-positive patients randomized to receive the vaccine after trastuzumab.[46]

These early clinical trials have shown the promise of efficacy in combination therapy as well. Disis and colleagues demonstrated prolonged increases in peptide-specific immunity after vaccination, as well as epitope-spreading within the HER2 protein and to other non-HER2 tumor antigens.[47] In our studies, we investigated sequential therapy with trastuzumab followed by HER2 vaccination in the adjuvant setting. In our phase I/II studies of nelipepimut-S that enrolled patients with any degree of HER2 expression, 11 patients with HER2-positive tumors were vaccinated after standard of care trastuzumab. In our ongoing phase II study evaluating a second CTL-eliciting HER2-derived vaccine (GP2+GM-CSF) which has the same mechanism of action as nelipepimut-S, we have enrolled 78 HER2-positive patients who received standard of care trastuzumab; 44 were vaccinated after standard of care trastuzumab, 34 were randomized to no vaccine. Combining the patients from these two studies, after a median follow-up of 36 months, the DFS rate is 84% in the 34 patients randomized to no vaccine – comparable with reported rates of similarly staged and treated patients. In contrast, for the 55 patients who were vaccinated after completing trastuzumab therapy, the DFS rate is 100% (p=.012; unpublished

data) (Figure 3).

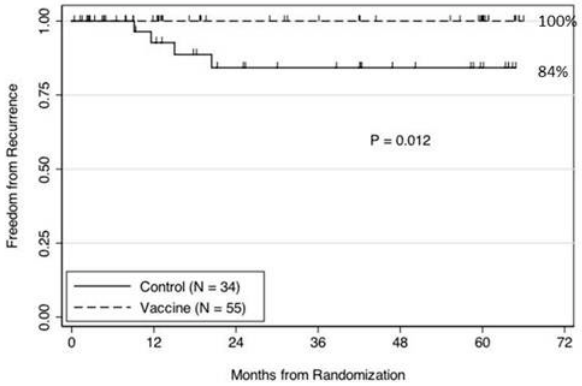


Figure 3. Disease-free survival in HER2+ patients enrolled on phase II trials evaluating CTL-eliciting HER2-derived peptide vaccine. After a median follow-up of 36 months, patients who received trastuzumab followed by GM-CSF only (control) had a DFS rate of 84%. Patients who received trastuzumab followed by vaccine had a DFS rate of 100% (p=0.012).

These encouraging data suggest that synergism between trastuzumab and HER2 CTL-eliciting vaccines warrant further investigation. We believe that the patients with high-risk HER2-positive breast cancer defined as those who receive a neoadjuvant chemotherapy regimen that contains trastuzumab and who fail to achieve a pCR or those that undergo upfront surgery and are found to be node positive (≥ 4 positive lymph nodes regardless of hormone receptor status or 1-3 positive lymph nodes and are hormone receptor negative) represent the ideal population in which to further investigate this strategy.

2.0 OBJECTIVES

In this study, we intend to assess the ability of the combination of trastuzumab and the HER2 vaccine nelipepimut-S (administered with the immunoadjuvant GM-CSF) given in the adjuvant setting to prevent recurrences in patients with high-risk HER2-positive breast cancer. High-risk is defined as those patients that do not achieve a pCR after neoadjuvant therapy with an approved regimen that includes trastuzumab and at least four cycles (12 weeks) of taxane-containing chemotherapy or those who undergo upfront surgery and are found to have ≥ 4 positive lymph nodes regardless of hormone receptor status or 1-3 positive lymph nodes and are hormone receptor negative.

2.1 Primary objective

The primary objective is to compare invasive DFS between the two treatment groups from time of initiation of trastuzumab maintenance therapy (trastuzumab monotherapy) to time of invasive local, regional or distant recurrence, new primary, or death due to any cause.

2.2 Secondary objectives

- Distant recurrence-free survival (DRFS). Time from initiation of trastuzumab maintenance therapy to distant recurrence, or death due to any cause (continuing follow-up on patients with local or regional

recurrence)
- Assess local and systemic toxicities using standard NCI-CTCAE criteria V 4.03
- Evaluate in vivo and in vitro immune responses

3.0 STUDY DESIGN

3.1 Description of study

This will be a multi-center, prospective, randomized, single-blinded, placebo-controlled phase II trial of trastuzumab + nelipepimut-S/GM-CSF versus trastuzumab + GM-CSF alone (Appendix A). The vaccine to be used in this study, nelipepimut-S, is investigational and will be used in combination with trastuzumab under the Investigational New Drug (IND) application BB-IND #14919. The Sponsor/Investigator of this IND is Dr. George E. Peoples.

Our target study population is high-risk HER2-positive breast cancer patients. High-risk HER2-positive breast cancer patients are defined as:

- Those with HER2-positive breast cancer, regardless of hormone receptor status, who receive neoadjuvant therapy with an approved regimen that includes trastuzumab and at least four cycles (12 weeks) of taxane-containing chemotherapy, and fail to achieve a pCR.
- Those with HER2-positive breast cancer, regardless of hormone receptor status, who undergo surgery as a first intervention and are found to have ≥ 4 positive lymph nodes.
- Those with HER2-positive, hormone receptor negative breast cancer who undergo surgery as a first intervention and are found to have 1-3 positive lymph nodes.

Following surgery, patients will be screened and HLA-typed (consent #1). Nelipepimut-S is a CD8-eliciting peptide vaccine that is restricted to HLA-$A2^+$ or HLA-$A3^+$ or HLA-$A24^+$ or HLA-$A26^+$ patients (approximately 80% of the US population). HLA-A2+/ /A3+/A24+/orA26+ patients who meet all other eligibility criteria will be randomized to receive trastuzumab + nelipepimut-S/GM-CSF or trastuzumab + GM-CSF alone (consent #2). The trastuzumab will be administered to all patients consistent with current standard of care. Patients randomized to the nelipepimut-S/GM-CSF arm will receive vaccinations of nelipepimut-S (1000 µg) and GM-CSF (250 µg) administered intradermally every three weeks for six total vaccinations, 30-120 minutes after completion of trastuzumab infusion. With prior approval from the Principal Investigator, the vaccine may be administered 15 minutes after completion of the trastuzumab

infusion. The first vaccination will be given with the third dose of maintenance trastuzumab administered as monotherapy (Appendix B) optimally, but may be given with later maintenance doses of trastuzumab, provided there are at least six remaining doses of trastuzumab to overlap with the primary vaccine series (PVS). Patients 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. Patients will be blinded as to whether they are receiving nelipepimut-S/GM-CSF or GM-CSF alone.

Upon completion of the PVS, booster inoculations (same dose and route) will be administered every six months x 4 (Appendix B). The first booster inoculation will occur 6 months ± 2 weeks after the completion of the PVS with subsequent boosters timed every six months ± 2 weeks. Boosters will therefore occur at the following time points after completion of the PVS: 6 months ± 2 weeks, 12 months ± 2 weeks, 18 months ± 2 weeks and 24 months ± 2 weeks. Booster inoculations will occur for patients randomized to receive nelipepimut-S/GM-CSF as well as patients randomized to receive GM-CSF alone, and will consist of the same treatment drugs and dosing (i.e. nelipepimut-S/GM-CSF patients will be boosted with nelipepimut-S/GM-CSF while GM-CSF alone patients will be boosted with GM-CSF alone). Patient blinding will be maintained throughout the study.

Patients will be followed for safety issues, immunologic response and clinical recurrence. Patients will either return to their study site or be contacted by phone for questioning regarding any systemic and local toxicity during the initial 48-72 hours after inoculation. If they return to their study site, the local reaction at the inoculation sites, will be examined and measured. For patients that do not return to the study site, a tool will be given and instructions provided to measure the local reaction. Immunologic response will be monitored primarily by in vivo delayed type hypersensitivity (DTH) reactions but also may be documented by other immunologic assays. All patients will be followed for a total of 36 months to document disease-free status.

We plan to enroll 100 patients (50 in each treatment arm randomized 1:1) at a planned accrual rate of 5 patients per month (approximately 0.5-1enrollment per study site per month). With accrual beginning in approximately September 2014, enrollment of the last patient would be expected in approximately September 2016 followed by a three-year follow-up period. The duration of the trial is expected to be five years.

3.2 Rationale for study design

In HER2-positive breast cancer patients who receive neoadjuvant chemotherapy plus trastuzumab, the 3-year Kaplan-Meier estimates of RFS are 95.7% for patients that achieve a pCR versus 80.1% for patients that do not achieve a pCR

(p=.02). [24] For HER2-positive breast cancer patients that undergo surgery as a first intervention and are found to be node-positive, 5-year DFS rates are 72% for those with 4 or more positive lymph nodes and 78% for those with 1-3 positive lymph nodes. [15] Based on preliminary data, the combination of trastuzumab plus a CTL-eliciting HER2-derived peptide vaccine virtually eliminated recurrences. This current study will add to these data by investigating the combination of trastuzumab and nelipepimut-S+GM-CSF in an adequately powered randomized clinical trial.

3.3 Outcome measures

3.3.1 Primary outcome measure

Disease state will be determined by the patients' own physicians at the individual study sites during their routine follow-up screening. This will occur for all enrolled patients, regardless of randomization, approximately every three months for the first 24 months after completion of primary therapies and every six months thereafter with clinical exam, laboratory (CBC and CMP) and radiographic surveillance as indicated. Evaluation of the patients' labs will be performed approximately every 3 months during the primary series and approximately every 6 months during the booster phase to monitor for potential toxicity from the study drug combination. This follow-up strategy is standard of care for these patients. If records are not available, patients, or their referring physicians, will be contacted to discern their disease status. All enrolled patients, regardless of randomization will be followed for clinical recurrence for three years from the date of initiation of trastuzumab maintenance therapy. The primary outcome measure of the trial is invasive DFS.

3.3.2 Secondary outcome measures

Secondary outcome measures include determining DRFS from initiation of trastuzumab maintenance therapy, assessing local and systemic toxicities, and evaluating immune responses.

Distant recurrence free survival will be assessed as part of the patient's disease state determined by their physician at the individual study sites during routine follow-up screening (see 3.3.1 Primary outcome measure). Determination of DRFS will allow for continued follow-up on patients with local or regional recurrence.

Standard local and systemic toxicities will be collected and graded per the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 graded toxicity scale (Appendix C). For both the inoculations during the PVS and the booster inoculations, the patient will be monitored for 30 minutes with vital signs taken as needed. Patients will either return to their study site or be contacted by

phone for questioning regarding any systemic and local toxicity during the initial 48-72 hours after inoculation. If they return to their study site, the local reaction at the inoculation sites will be examined and measured. For patients that do not return to the study site, a tool will be given and instructions provided to measure the local reaction. Serious AEs will be reported as described in Section 5.

Immune responses will be primarily documented using the delayed type hypersensitivity (DTH) reaction and using the dextramer assay to enumerate peptide-specific CTL. Each of these measurements will be performed regardless of randomization. Detailed descriptions of these assays/tests are described in Sections 4.4.6 and 4.4.8. DTH reactions will be measured prior to initiation of the vaccination series, one month ± 1 week after completion of the PVS, and one month ± 1 week after the final booster inoculation. Dextramer measurements will be performed prior to initiating the vaccination series as well as one month ± 1 week after completion of the PVS. Additionally, these assays may be performed pre- and post-each booster. Alternatively, these assayed time points may also be performed all at once on frozen and banked cells.

3.4 Safety considerations

3.4.1 Trastuzumab

If trastuzumab is discontinued by the treating oncologist for any indication to include those listed below, the patient will be taken off treatment, but continued in the study for disease follow-up.

Cardiac. Cardiac evaluation with MUGA or ECHO will be performed per standard of care dictated by the treating oncologist.

> Management of symptomatic cardiac changes. Patients who develop signs and symptoms of congestive heart failure will be taken off treatment, but continued in the study for disease follow-up.
>
> Management of asymptomatic decreases in LVEF. Patients experiencing asymptomatic absolute decrease in LVEF < 20 percentage points from baseline, will continue on study as long as 1) the LVEF remains within the normal limits of the institution, and 2) their treating oncologist continues trastuzumab treatment. Patients with an asymptomatic decrease in LVEF of ≥ 20 percentage points (even if within the normal limits of the institution) or an ejection fraction below the range of normal limits, will be taken off treatment, but remain in the study for disease follow-up.

Hematologic Toxicity and Neutropenic Infections. In prior clinical trials, an increased incidence of anemia was observed in patients receiving trastuzumab plus chemotherapy compared with patients receiving chemotherapy alone. The

majority of these anemia events were mild or moderate in intensity and reversible; none resulted in discontinuation of trastuzumab therapy.

In the clinical trials, the per-patient incidences of moderate to severe neutropenia and of febrile neutropenia were higher in patients receiving trastuzumab in combination with myelosuppressive chemotherapy as compared to those who received chemotherapy alone. In the post-marketing setting, deaths due to sepsis in patients with severe neutropenia have been reported in patients receiving trastuzumab and myelosuppressive chemotherapy, although in controlled clinical trials (pre- and post-marketing), the incidence of septic deaths was not significantly increased. The pathophysiologic basis for exacerbation of neutropenia has not been determined.

Secondary acute leukemia or myelodysplastic syndrome has been reported in 4 of approximately 1200 patients who participated in trastuzumab clinical trials. Patients treated with chemotherapeutic agents are known to be at increased risk for secondary leukemia. The observed incidence of leukemia among trastuzumab-treated patients appears to be consistent with the expected incidence of leukemia among patients treated with chemotherapy for metastatic breast cancer. Therefore, the contribution of trastuzumab to the etiology of acute leukemia or myelodysplastic syndrome in these cases is unclear.

> Management of Hematologic Toxicities. Care should be taken to carefully monitor the patient's hematologic status throughout the course of the trial. Use of hematopoietic growth factors to ameliorate hematologic toxicity is at the discretion of the physician investigator and should be in accordance with the American Society of Clinical Oncologists (ASCO) guidelines.

3.4.2 Nelipepimut-S and GM-CSF

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 greater than or equal to grade 2 hypersensitivity reactions. [32]

> Dose Reductions of Vaccine. Toxicities observed with the vaccine are attributable to the GM-CSF. For patients experiencing robust local reactions (>100mm x 100mm) or greater than or equal to grade 3 local or systemic reactions greater than or equal to grade 2 hypersensitivity reactions, the dose of GM-CSF will be reduced by 50% for subsequent inoculations. If subsequent reactions occur necessitating dose reduction, the GM-CSF dose will continue to be reduced serially by 50%. For example, GM-CSF dose would first be reduced from 250 mcg to 125 mcg, with subsequent reductions to 62.5 mcg, then 30 mcg and finally 0 mcg. If

dose reduction of GM-CSF is inadequate in limiting reactions, the nelipepimut-S dose (if applicable) will then be reduced by 50%.

Dose reductions will continue for booster inoculations in the same manner as performed in the PVS, except for patients who underwent multiple dose reductions in the primary vaccine series, who will begin booster inoculations with an initial GM-CSF dose of 125 mcg.

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 PVS. 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), antihistamines may be used before or after subsequent inoculations. If urticaria persists with the use of anti-histamines and oral steroids, or intravenous steroids are required, or hospitalization is required, or in the opinion of the Principal Investigator, further inoculations will be discontinued.

3.5 Compliance with laws and regulations

This study will be conducted in accordance with current U.S. Food and Drug Administration (FDA) Good Clinical Practices (GCPs), and local ethical and legal requirements.

4.0 MATERIALS AND METHODS

4.1 Patients

Patients over the age of 18 years with a diagnosis of HER2-positive breast cancer who are receiving neoadjuvant therapy with an approved regimen that includes trastuzumab and do not achieve a pathologic complete response or who undergo upfront surgery and are found to be pathologically node-positive will be targeted. Patients treated with neoadjuvant therapy must receive an approved regimen that includes trastuzumab and at least four cycles (12 weeks) of taxane-containing chemotherapy. These patients will be approached after their surgical pathology report is available and confirms residual disease. For patients who undergo upfront surgery, they will be approached after their surgical pathology report confirms node-positive disease. For clarity, standard of care pertuzumab is allowed.. Patients will be recruited from medical and surgical oncology and/or hematology/oncology clinics at the individual study sites. All patients will be properly consented.

4.1.1 Patient selection

Potentially eligible patients, specifically patients with high-risk HER2-positive breast cancer will be identified by staff in the medical and surgical and/or the hematology/oncology clinics at the individual study sites. High-risk HER2-positive is defined as those that receive neoadjuvant therapy with an approved regimen that includes trastuzumab and at least four cycles (12 weeks) of taxane-containing chemotherapy and fail to achieve a pCR or those who undergo surgery as a first intervention and are found to be pathologically node-positive. For clarity, standard of care pertuzumab is allowed.

A research nurse and/or study coordinator will approach these patients about being in the trial and will introduce the trial to the prospective patient. If the patient is interested and appears eligible, the nurse will arrange to counsel the patient. The nurse will thoroughly screen the patient for inclusion and exclusion eligibility criteria. If the patient remains interested and eligible, informed consent will be obtained.

Written informed consent will be obtained from all study participants. Prospective participants will be provided with a copy of the consent form to read. The Principal Investigator (PI) or individuals who are authorized by their institution to obtain informed consent and who are familiar with the study, will explain the study and review the consent form with the patient. Patients will be given ample time to ask and have all questions answered prior to signing the consent form.

Once written informed consent is obtained, the patient will be HLA-typed. They will be asked for 6-8 cc of blood for HLA-A2/A3/A24/A26 screening to be performed in a CLIA-certified laboratory at MD Anderson. Only HLA-A2/ HLA-A3/HLA-A24 or HLA-A26 positive patients will be enrolled in the trial. Those patients who are not HLA-A2,A3, A24, or A26 positive will be excluded. Patients will be counseled prior to obtaining consent that lack of HLA-A2,A3, A24, or A26 positivity will result in exclusion.

4.1.2 Inclusion criteria

Patients will be included in the study based on the following criteria:

- 18 years or older
- Eastern Cooperative Oncology Group (ECOG) performance status 0,1
- AJCC stage I – III non-inflammatory, HER2-positive (according to ASCO-CAP guidelines [5]) breast cancer
- Completed neoadjuvant therapy with an approved regimen that includes trastuzumab and at least four cycles (12 weeks) of taxane-containing chemotherapy and underwent surgery with final pathology showing evidence of residual disease in the breast or axilla (residual

ductal carcinoma in situ or microinvasive disease not eligible) or underwent surgery as a first intervention and was found to be pathologically node-positive: ≥ 4 positive lymph nodes (pN2 or pN3) regardless of hormone receptor status or 1-3 positive lymph nodes (pN1) if hormone receptor negative. Patients with micrometastases (pN1mi) are not eligible. For clarity, standard of care pertuzumab is allowed.

- Completed an approved regimen of neoadjuvant or adjuvant therapy with an approved regimen that includes trastuzumab and at least four cycles (12 weeks) of taxane-containing chemotherapy with plan for completion of one year of trastuzumab therapy. For clarity, standard of care pertuzumab is allowed.
- Completed appropriate surgical therapy to include:
    - Total mastectomy and axillary staging with sentinel lymph node dissection or axillary lymph node dissection (level I/II). Patients with a positive sentinel lymph node must have undergone a completion axillary lymph node dissection.
    - Breast conserving surgery (BCS) and axillary staging with sentinel lymph node dissection or axillary lymph node dissection. Patients undergoing surgery as a first intervention with a positive sentinel lymph node must have undergone a completion axillary dissection level I/II unless they had clinically node negative T1-T2 tumors and fewer than 3 involved lymph nodes. Patients receiving neoadjuvant chemotherapy that have a positive sentinel lymph node must have undergone a completion axillary lymph node dissection.
- Completed or receiving appropriate radiation therapy if indicated:
    - For patients undergoing total mastectomy surgery as a first intervention, post-mastectomy radiation to the chest wall, infraclavicular and supraclavicular areas is required for patients with ≥ 4 positive lymph nodes. Radiation to the internal mammary lymph nodes is not required per protocol but is allowed at the discretion of the patient's treating radiation oncologist. For patients with 1-3 positive lymph nodes, post-mastectomy radiation to the chest wall, infraclavicular, supraclavicular, and internal mammary areas is not required per protocol but is allowed at the discretion of the patient's treating radiation oncologist.
    - For patients undergoing breast conserving surgery (BCS) as a first intervention, whole breast irradiation with or without a boost, and radiation to the infraclavicular and supraclavicular areas is required for patients with ≥ 4 positive lymph nodes. Radiation to the internal

mammary lymph nodes is not required but is allowed at the discretion of the patient's treating radiation oncologist. For patients with 1-3 positive lymph nodes, whole breast irradiation with or without a boost is required. Radiation to the infraclavicular, supraclavicular, and internal mammary areas is not required per protocol but is allowed at the discretion of the patient's treating medical oncologist.

- For patient's undergoing mastectomy after neoadjuvant chemotherapy post-mastectomy radiation to the chest wall, infraclavicular and supraclavicular areas is required for patients presenting with clinical N2 or N3 disease or with ≥ 4 positive lymph nodes identified pathologically at the time of surgery. Radiation to the internal mammary lymph nodes is not required per protocol but is allowed at the discretion of the patient's treating radiation oncologist. For patients with 0-3 positive lymph nodes identified pathologically, post-mastectomy radiation to the chest wall, infraclavicular, supraclavicular and internal mammary areas is not required per protocol but is allowed at the discretion of the patient's treating radiation oncologist.
- For patient's undergoing BCS after neoadjuvant chemotherapy, whole breast irradiation with or without a boost is required. For patients with clinical N2 or N3 disease or with ≥ 4 positive lymph nodes identified pathologically at the time of surgery, radiation to the infraclavicular and supraclavicular areas is required. Radiation to the internal mammary lymph nodes is not required per protocol but is allowed at the discretion of the patient's treating radiation oncologist. For patients with 0-3 positive lymph nodes identified pathologically, radiation to the infraclavicular, supraclavicular and internal mammary areas is not required per protocol but is allowed at the discretion of the patient's treating radiation oncologist.

- HLA-A2A3, A24, or A26 positive
- LVEF ≥50%, or an LVEF within the normal limits of the institution's specific testing (MUGA or ECHO)
- Adequate organ function as determined by the following laboratory values:
  - ANC ≥ 1,000/μL
  - Platelets ≥ 75,000/μL
  - Hgb ≥ 9 g/dL

- Creatinine ≤ 1.5 x upper limit of normal (ULN) of institution's range or Creatinine clearance ≥ 50%
- Total bilirubin ≤ 1.5 ULN of institution's range
- ALT and AST ≤ 1.5 ULN of institution's range

- For women of child-bearing potential, agreement to use adequate birth control (abstinence, hysterectomy, bilateral oophorectomy, bilateral tubal ligation, oral contraception, IUD, or use of condoms or diaphragms)

- Signed informed consent

4.1.3 Exclusion criteria

Patients will be excluded from the study based on the following criteria:

- AJCC Stage IV breast cancer
- NYHA stage 3 or 4 congestive heart failure
- Immune deficiency disease or known history of HIV, HBV, HCV
- Receiving immunosuppressive therapy including chronic steroids, methotrexate, or other known immunosuppressive agents
- Pregnancy (assessed by urine HCG)
- Breast feeding
- Any active autoimmune disease requiring treatment, with the exception of vitiligo
- Active pulmonary disease requiring medication to include multiple inhalers (≥3 inhalers including one containing steroids)
- Involved in other experimental protocols

4.2 Method of treatment assignment

If patients meet all inclusion criteria and none of the exclusion criteria and agree to participate, they will continue in the study. Patients will be randomly assigned by a designated Cancer Insight staff member using a computer-generated randomization table to receive trastuzumab + nelipepimut-S/GM-CSF or trastuzumab + GM-CSF alone. Randomization between the two treatment groups will occur in a 1:1 allocation ratio using an institutional balancing algorithm. Patients will be blinded as to whether they are receiving trastuzumab + nelipepimut-S/GM-CSF or trastuzumab + GM-CSF alone.

4.3 Study treatment

4.3.1 Trastuzumab

All patients will receive trastuzumab as per current standard practice. Trastuzumab will be administered as per institutional protocol.

4.3.2 Vaccine

4.3.2.1 Dosage and preparation

The E75 vaccine (nelipepimut-S; as 1.5 mg/mL E75 acetate solution) and GM-CSF (as 250 mcg lyophilized Leukine) will be supplied by Galena Biopharma. Galena Biopharma also provides DTH skin test kits, each of which contains 1.5 mg/mL E75 acetate solution.

GM-CSF is a potent cytokine that stimulates granulocytes (neutrophils, eosinophils and basophils) and monocytes, but not lymphocytes, erythrocytes, or platelets. 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 2.8 x 106 IU/mL. See GM-CSF (Leukine® (sargramostim)) package insert and labeling (Appendix E) for additional details. The E75 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, Albuquerque, NM as a 1.5 mg/mL solution in 1 mL water for injection (WFI) 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 1.41 mg/mL Nelipepimut-S peptide. The control is WFI.

Prior to administration, 1,065 mcg (0.8 mL) of the E75 acetate or control is mixed by the pharmacist or research nurse with 1 mL of reconstituted lyophilized Leukine® GM-CSF (reconstituted as 250 mcg/mL in solution). Immediately after mixing, the study drug is withdrawn into four 1 mL syringes and within 6 hours of

mixing, a total of 1.6 mL is administered as 4 intradermal injections to deliver a total of 1,000 mcg of E75 peptide vaccine (1,065 mcg of E75 acetate). The control vial contains 1 mL WFI. The detailed guidance for mixing E75 (or control) solution from lyophilized Leukine® 250µg and test drug (E75 solution or control) is stated in Appendix D.

For DTH skin test, E75 acetate solution (1.5mg/1mL vial) is supplied in DTH skin test kit. Detailed mixing instruction for DTH skin test prep is stated in Appendix D. If dose reduction of GM-CSF is required based on >100 mm x 100 mm induration, and/or ≥Grade 3 toxicity at the injection site after the previous dose, the liquid Leukine® vial is diluted with an additional 1.0 mL WFI, using aseptic technique, and mixed gently by rolling the Leukine vial between your hands. Allow any foaming to dissipate before continuing. Do not invert the vial. Subsequently, 1 mL is withdrawn and discarded and the remaining 1 mL is used for mixing following the same methodology above to make a 1.6 mL Leukine + E75 mixture. Further dilutions (approximately 1:1) may be required if >10 cm erythema and induration and/or ≥ Grade 3 toxicity are observed on subsequent doses, the details of which will be provided in study instructions.

4.3.2.2 Storage

For detailed storage instruction of the vaccine and its components (E75 acetate in WFI and GM-CSF) please follow the instructions in the Investigator's Brochure. Stability data support storage of E75 acetate in WFI and control to E75 acetate in WFI at -20°C (± 5°C) or 5°C (± 3°C). Store study drug (E75 acetate in WFI, control to E75 acetate in WFI and GM-CSF) as per appropriate storage temperature designated on clinical carton label.

4.3.3 Inoculation series – administration

Patients will receive 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 preferably, but may be given in the arm.

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 given every three weeks and will be administered in the same lymph node draining area (same arm or leg). Timing of the inoculation series with trastuzumab treatment will occur as outlined in Section 3.1 (see also Appendix B). The dose will be 1000 mcg of E75 peptide and 250 mcg of GM-CSF. The research nurse coordinator will administer the inoculations sterilely in the vaccine or clinical laboratory facility located at each study site. For female patients with childbearing potential, a urine

pregnancy test will be performed before each inoculation. If this test is positive at any time, the patient will be discontinued from the study.

4.3.4 Booster inoculations

After completion of the six-inoculation PVS, patients will then receive a total of four booster inoculations to be administered, respectively, at 6, 12, 18, and 24, months +/- 2 weeks from the date of completion of the PVS (Appendix B). One booster inoculation will be administered at each time point noted above ± two weeks and administered in the same extremity as the primary series. Booster inoculations will consist of the same intervention as each patient received during their regular inoculation series. Patients enrolled to the E75/GM-CSF vaccine arm of the trial will receive four consecutive booster inoculations of 1000 mcg E75 peptide + 250 mcg GM-CSF, and patients enrolled to the GM-CSF alone arm will receive four consecutive booster inoculations of 250 mcg GM-CSF only.

For patients with childbearing potential, a urine pregnancy test will be performed before each booster inoculation. If this test is positive at any time, the patient will be discontinued from the study.

4.4 Study assessments

Signed, IRB-approved informed consent must be obtained from patients prior to any pretreatment assessments.

4.4.1 Pretreatment assessments

All patients must have a Complete Blood Count (CBC) and Comprehensive Metabolic Panel (CMP) within two months of trial initiation and, for female patients, a urine pregnancy test (upon consent to study), for screening and immediately prior to any vaccine inoculations or DTH. If the pregnancy test is positive, the patient will be excluded from the study. Women who have had a hysterectomy, bilateral oophorectomy, tubal ligation, documented absence of menses for two years, or FSH hormonal laboratory results that verify menopause, will not be required to have pregnancy testing. For patients who have a complete metastatic evaluation (CBC, LFTs, CXR, chest and abdomen CT, bone scan, and PET scan) available, all studies will be screened, but these studies are not required for enrollment. Results of cardiac evaluation with MUGA or ECHO will also be screened. Disease-free status will be assured by the patients' primary treating/referring physician. Overall health screen will be assessed utilizing the ECOG performance status grading system (Appendix F).

To ensure eligibility, each patient will be HLA-typed as described in Section 4.4.5). They will be asked for 6-8 cc of blood for HLA-A2/A3/A24/A26 screening. Analysis will be performed at a CLIA certified laboratory at MD Anderson.

All patients regardless of randomization may be assessed for baseline immunologic responses prior to the first inoculation. Specimen handling, processing, and assays are described in Sections 4.4.4 and 4.4.6.

All patients will be tested for pre-inoculation DTH reaction against nelipepimut-S as described in Section 4.4.8. Reactions will be read 48-72 hours after placement of the peptide.

4.4.2 Assessments during treatment

All patients will have cardiac assessment performed according to standard practice (approximately every 3 months) as dictated by their treating oncologist. Either MUGA or ECHO is acceptable but should be consistently used for any given patient. Results of cardiac testing will be reviewed by the research nurses or study coordinators and any abnormal results will be reviewed by the study investigators. Per standard practice, all patients will have CBC and CMP monitoring approximately every 3 months during the primary series and approximately every 6 months during the booster phase. Any laboratory evaluations or imaging studies ordered by the treating oncologist as part of standard practice will be reviewed by the research nurses or study coordinators and any abnormal results will be reviewed by the study investigators.

For both the PVS and the booster inoculations, the patient will be monitored for 30 minutes after the inoculation with vital signs taken as needed.

Patients will either return to their study site or be contacted by phone for questioning regarding any systemic and local toxicity during the initial 48-72 hours after inoculation. If they return to their study site, the local reaction at the inoculation sites will be examined and measured. For patients that do not return to the study site, a tool will be given and instructions provided to measure the local reaction.

The NCI CTCAE version 4.03 graded toxicity scale (Appendix C) will be utilized to assess local and systemic toxicity.

Blood samples will be obtained from all patients, regardless of randomization, to determine induction of peptide-specific immune responses as described in Sections 4.4.4 and 4.4.6.

4.4.3 Follow-up assessments

The clinical endpoint of recurrence will be determined by patients' own physicians at the individual study sites during routine follow-up. This will include

physical examination every 3-6 months for all patients and yearly mammogram for patients undergoing breast conserving therapy (per ASCO guidelines). [48] Additional laboratory and radiographic surveillance will be performed as indicated and directed by the patients treating physicians. The determination of recurrence will be per standard practice by the treating physicians and communicated to the study investigators on a routine basis. Documentation of the recurrence will be obtained. Whenever possible, pathologic confirmation of recurrence will be obtained. However, a patient will also be considered to have a recurrence if highly suggested on radiographic evaluation and oncologic treatment for the recurrence is initiated by the treating physician. Documentation of the latter will be obtained. For the purposes of this trial, the invasive DFS will be calculated from the date of initiation of trastuzumab maintenance therapy until date of last follow-up or recurrence. If records are not available, patients, or their referring physicians, will be contacted to discern their disease status and every effort will be made to obtain documentation. All enrolled patients, regardless of randomization, will be followed for clinical recurrence for up to three years from the date of initiation of trastuzumab maintenance therapy.

4.4.4 Blood collection and processing

Multiple blood draws will be required for this trial. All blood tubes will be labeled only with the patients' unique study number. Approximately 6-8 cc will be drawn for HLA-typing and approximately 70cc for each Immunologic assessment (pre DTH #1 and #2, prior to each booster and 1 month ± 1 week after each booster and at study completion 36 months after initiation of trastuzumab maintenance therapy). Thus a total of approximately 800cc of blood will be drawn over the three year course of the study. De-identified patient blood samples showing only the unique study ID number will be sent from study sites via overnight delivery to the laboratory of the Principal Investigator, Dr. Elizabeth Mittendorf, at MD Anderson where immunologic response assays (Section 4.6) will be performed. At no point will MD Anderson personnel have access to patient identifiers. Blood not used to perform the dextramer assay (Section 4.6) will be frozen and stored in the laboratory of Dr. Elizabeth Mittendorf under the unique protocol number and identifier for up to five years for additional immunologic studies related to this protocol (for example, to repeat assays or perform new immunologic assays that do not exist at present but may become available) as needed and then destroyed. 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 at any time. Additionally, any stored blood may also be utilized to assess new generations of vaccines.

For blood draws performed for immunologic assessment, 10cc 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 1cc vials and frozen. The remaining 60cc 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.

4.4.5 HLA-A2/A3/A24/A26 testing

HLA testing will be performed at the CLIA certified human flow cytometry lab at MD Anderson. The expression of HLA-A2 by the patients is confirmed by staining PBMC with anti-HLA-A2 monoclonal antibody, (clone BB7.2), directly conjugated to phycoerythrin (PE) or fluorescein-isothiocyanate (FITC) (BD Biosciences) at 4ºC for 60 minutes. The expression of HLA-A3, A24, and A26 by the patients is confirmed by staining PBMC with the appropriate biotinylated-anti HLA-allele monoclonal antibody (One Lambda Inc.) for 45 minutes after which the cells are washed and incubated for an additional 15 minutes with streptavidin-PE. After incubation the cells are washed and analyzed on a flow cytometer.

4.4.6 Dextramer assay

Fresh PBMC will be stained with aqua live/dead stain (Invitrogen) and the following antibodies: CD8 APC-H7 (BD Biosciences), CD3 PE Cy7 (BD Biosciences), E75-APC-conjugated dextramer (Immudex), and the following pacific blue conjugated lineage antibodies: CD14 (BD Biosciences), CD16 (BD Biosciences), and CD19 (Biolegend). Cells will be analyzed on a Canto flow cytometer (BD Biosciences). The frequency of E75-specific CD8+ T cells will be determined as the percentage of cells that are alive, lineage-, CD3+ CD8+ and E75-dextramer+.

4.4.7 Local reaction

Patients 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 if the patient returns to the study site. A digital photograph of the local reaction may be taken to document the reaction and for future reporting purposes. It will in no way be visually identifiable as that patient. Photographs will be labeled with the patient's study number and will not include the face or distinguishing birthmarks or tattoos. They will be electronically stored on a password protected computer in a locked office in the vaccine or clinical laboratory facility at each site. Per

FDA regulations, each patient's photograph will be kept for two years after submission of a New Drug Application (NDA) and then destroyed. If the patient does not return to the study site, they will be contacted by phone 48-72 hours after inoculation for questioning regarding the reaction at the injection sites.

4.4.8 DTH reaction

Regardless of randomization, a pre--inoculation series DTH response will be assessed of all patients with 100 mcg of nelipepimut-S (without GM-CSF) injected intradermally at a site on the back or anterior thigh (opposite side from the vaccination site). A post-inoculation series DTH response will be assessed one month ± 1 week after completion of the PVS. The DTH reaction will be measured at 48-72 hours using the sensitive ball point pen method and compared between pre-inoculation and post-inoculation time points. Additionally, a DTH responses will be assessed 1 month ± 1 week after the fourth booster. The low dose of peptide used in the DTH test is not expected to induce a long-term immune response in control patients; however, any response that is induced is expected to be transitory in nature. A digital photograph of the local reaction will be taken to document the reaction and for future reporting purposes. It will be labeled with the patient's study number, will not visually identify the patient, and will not include the face or distinguishing birthmarks or tattoos. Photographs will be electronically stored on a password protected computer in a locked office in the vaccine or clinical laboratory facility at each site. As per FDA regulations, each patient's photograph will be kept for two years after submission of a NDA and then destroyed. These results will also be recorded in the immune response database.

4.5 Discontinuation of protocol-specific therapy

Protocol-specific therapy may be discontinued for any of the following reasons:

- Progressive disease
- Unacceptable toxicity as described in the protocol
    - Please refer to sections 3.4 (Safety Considerations) for the management of cardiac issues (symptomatic and asymptomatic), hematologic toxicity and neutropenic infections, as well as robust local reactions and delayed urticarial reactions. Adverse events that do not respond to either dose modification (as described) or clinical management of the reaction will result in discontinuation from the study.
    - Please refer to section 4.6 (Patient Discontinuation) for a list of severe adverse reactions warranting discontinuation from the study.

- Patient election to discontinue therapy (for any reason)
- Physician's judgment

4.6 Patient discontinuation

Those patients who display significant reactions (i.e. severe anaphylactic reaction immediately after vaccine administration) or toxicities will be discontinued from the study as determined by the PI. They will be followed by the study investigator until resolution of the adverse event.

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 patient or require medical or surgical intervention to prevent an outcome listed above. Any death or grade 4 adverse drug experience found at least possibly related to the experimental vaccine will result in suspension of patient enrollment to the study. If the SAE is determined to be unrelated to the study drug, inoculations may be re-started if the patient desires to continue and it is safe for them to do so as determined by the PI and Sponsor.

For robust local, systemic > Grade 3, and > Grade 2 hypersensitivity reactions, as evaluated under CTCAE v4.03, will result in dose reduction or discontinuation as discussed (section 3.4.2).

Patients may withdraw from the study at any time and for any reason. A patient may be asked to withdraw from the study by the PI if they are not compliant with the timing of the inoculation series, observation period, or return visits to monitor for study-associated toxicities. Additionally, if the PI determines that it is no longer safe for a patient to continue in the trial for any reason, they may be withdrawn.

Because it is not known whether these inoculations might harm an unborn child, patients who are pregnant, plan on becoming pregnant, or who are breast-feeding will not be enrolled into the study. Women of childbearing potential will take a urine pregnancy test before starting this study and prior to each inoculation; a positive test result will terminate the patient's participation in the study. Patients will be counseled to avoid becoming pregnant while participating in this study, and that in order to prevent pregnancy they should either have no sexual relations or use a reliable type of birth control. They will be counseled

that with the exception of hysterectomy, bilateral oophorectomy, or tubal ligation, birth control methods are not totally effective in preventing pregnancy, and that the only ways to completely avoid the risk of the vaccine or immunoadjuvant alone to an unborn baby are (1) avoid becoming pregnant, or (2) do not receive these inoculations. Patients will be counseled to avoid becoming pregnant for at least 6 months after receiving the inoculations as pregnancy within this time after inoculation administration may be a risk to an unborn baby.

If a patient is discontinued from the study for an adverse event or pregnancy, they will continue to be followed for resolution of adverse event and clinical recurrences unless the patient withdraws consent for further study evaluation. The reason for any premature discontinuation of a patient from the study will be recorded on the appropriate Case Report Form.

4.7 Study discontinuation

The IND sponsor Dr. George E. Peoples, the financial sponsor Galena Biopharma, the Data Safety Monitoring Board, and the Principal Investigator have the right to terminate this study at any time. Reasons for terminating the study may include the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to patients. While vaccine related toxicity has been shown to be low, and the safety profile of Herceptin is known, the safety of the concurrent use is yet to be proven. As such, toxicities will be carefully monitored per treatment group in real time. If at any point, the level of toxicity, particularly cardiac toxicity, becomes statistically higher in the vaccinated group, the study will be stopped.
- Patient enrollment is unsatisfactory
- Data recording are inaccurate or incomplete
- Study protocol not followed

4.8 Data collection

Basic demographic, pathologic, and relevant clinical information will be gathered on each patient and entered into an Electronic Data Capture (EDC) database. The URL for the EDC system is https://cancerinsight.eclinicalhosting.com/OpenClinica. It can be accessed from any web browser. User name and password will be generated by the Data Manager of Cancer Insight and sent via email to appropriate site personnel prior to enrolling the first patient. Clinical nurses at the site will be provided with Source Document Flow Sheets to capture data at enrollment and for each study

visit. Although some data fields on the Flow Sheets will not be entered into EDC, they must be captured on the Flow Sheets for monitoring purposes.

All data .must be submitted within 72 hours of the data collection visit. Data entry will begin with the patient's randomization with enrollment date to be date of signed informed consent. Edit checks will fire in real time as data is being entered in the EDC system to ensure quality data is provided. In addition to edit checks, queries will be generated as Discrepancy Notes from the Data Manager and Monitors. Sites will have ten business days to update a query. It is the responsibility of the coordinator at each site to ensure that data has been submitted. Cancer Insight quality assurance personnel or the sponsor's representative will perform an audit of the site-specific Flow Sheets and will match them against source documents to ensure the quality of data coming to the Data Manager in the EDC per the internal monitoring plan.

The database, hosted by OpenClinica, resides in a SAS 70 Type II data center and meets ISO 17799 standards for information security. The EDC system is HIPAA and 21 CFR part 11 compliant with robust audit logs, controlled user access and electronic signature/password management. In addition to the site user, the Data Manager, the Monitors, the study PI and Sponsor/Sponsor representative have access to the database. Each user is assigned a role which grants limited access and functionalities dependent upon that specific role.

4.9 Statistical methods

4.9.1 Rationale for study design

In patients with HER2-positive breast cancer who receive neoadjuvant chemotherapy plus trastuzumab, the 3-year Kaplan-Meier estimates of RFS are 95.7% for patients that achieve a pCR versus 80.1% for patients that do not achieve a pCR (p=.02).[24] For HER2-positive breast cancer patients that undergo surgery as a first intervention and are found to be node-positive, 5-year DFS rates are 72% for those with 4 or more positive lymph nodes and 78% for those with 1-3 positive lymph nodes. [15] Based on preliminary data, the combination of trastuzumab plus a CTL-eliciting HER2-derived peptide vaccine virtually eliminated recurrences. This study will investigate the combination of trastuzumab and nelipepimult-S+GM-CSF in an adequately powered randomized clinical trial.

4.9.2 Sample size determination

The primary objective of this study is to compare DFS between the two treatment groups. The study will have a 3 year follow-up period. We anticipate a 3-year DFS of 80% in patients randomized to receive trastuzumab without the vaccine versus 95% in patients randomized to receive trastuzumab + nelipepimut-S+GM-

CSF (estimates are based on data from previous studies, section 1.2 and table 2).[24-26] In order to detect a DFS difference of 0.15 using a one-sided alpha level of 0.05 with 80% power, assuming an accrual time of 2 years, follow-up time of 3 years and 5% loss to follow-up (2.5% in each group), 100 patients (50 in each arm) are required. The power computation was determined using PASS software package version 2005.

4.9.3 Data analysis

The intention-to-treat (ITT) population will include all patients who were randomly assigned. ITT analysis will be performed with each randomized patient evaluated in the treatment arm to which randomized, regardless of actual treatment received. The per-protocol (PP) population is a subset of the ITT population. Patients may be excluded from the PP population for the following reasons and others as determined prior to database lock: violations of eligibility criteria, development of other malignancies, early recurrences occurring prior to completion of the entire PVS, receiving alternative disease-directed therapy without evidence of recurrence, or major deviation from prescribed vaccination schedule (to include boosters). Additionally, with the inclusion of HLA-A24 and/or A26-positive patients, the ITT and PP analysis will be performed with and without these patients.

4.9.3.1 Study patient characteristics

Demographic characteristics of all patients in the ITT and PP populations will be summarized including age, race, disease histology, tumor size, estrogen receptor/progesterone receptor status, grade, nodal status, AJCC clinical stage, surgery extent, and other disease-directed therapies to include chemotherapy, radiation therapy, and hormonal therapy. Continuous variables will be summarized using the number of patients, mean, standard deviation, median, minimum, and maximum; and categorical variables will be summarized using the frequency count and the percentage of patients in each category. Differences between treatment groups will be determined using a chi-square test for categorical variables and a t-test for continuous variables.

4.9.3.2 Primary efficacy analysis

The primary efficacy endpoint of the trial is invasive DFS. Invasive DFS will be defined as the time from the date of initiation of trastuzumab maintenance therapy to the date of invasive local, regional or distant recurrence, new primary, death due to any cause or last follow-up date if patient had no evidence of disease. Invasive DFS estimates will be determined using the Kaplan-Meier method and differences between treatment groups will be assessed using the

log-rank test. In addition, Cox proportional hazards regression models will be fit to determine the association between DFS and treatment group and covariates of interest. The primary efficacy analysis will be performed on the ITT population as primary and the PP population as secondary.

Because it is anticipated that 50% of patients will be enrolled at MD Anderson, the primary efficacy analysis will also be conducted by site for the ITT and PP populations in order to explore the possibility of a treatment by investigative site interaction. Investigative sites that individually represent fewer than ten patients will be combined for this exploratory analysis; geographic region will be substituted if the average number of patients per site is fewer than ten.

4.9.3.3 Secondary efficacy analysis

Safety

The incidence of treatment-emergent AEs, SAEs, severe AEs, AEs related to study drug (defined as possibly, probably, or definitely related to study drug), and AEs which lead to study discontinuation will be summarized by treatment group. Differences between treatment groups will be determined using Fisher's exact tests.

A formal interim analysis for safety will be performed after the midpoint (50^{th} patient) of enrollment and randomization.

Time to AE onset will be estimated using the Kaplan-Meier method and treatment differences will be determined using the log-rank test. In addition, duration of any AE with >10% incidence, total number of AEs, SAEs, and related AEs will be summarized and treatment group differences assessed using a t-test or Wilcoxon rank sum test, depending on the distribution of the data.

Immunologic response

Immunologic responses will be determined using DTH reactions (in vivo) and dextramer assays (in vitro). With respect to the in vivo immunologic response as assessed by DTH reaction, the treatment groups will be compared with respect to the percentage with a positive post-vaccination DTH (defined as greater than pre-vaccination DTH) as well as absolute DTH reaction sizes within the groups. Expression percentages will be compared using a chi-square test while continuous outcomes such as mean DTH response will be compared using a paired t-test (within treatment group) and a two-sample t-test (between treatment groups). For the in vitro response evaluated by the dextramer assay, the number of peptide-specific CTL will be determined at multiple time points (see Section 4.4.4) during the trial. Changes from pre-vaccination levels to

each time point will be computed and evaluated using a repeated measures mixed-effects model with terms for treatment group, time point, and treatment group-by-time point interaction. .

4.9.4 Withdrawal

Patients may withdraw or be discontinued from the study as discussed in Section 4.6. Patients who do withdraw or are discontinued will be included in the efficacy analyses unless a patient withdraws consent to participate. In the instance of a patient withdrawing consent, any data collected will be excluded from analysis.

4.9.5 Missing data

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 patient.

4.9.6 Interim analysis

No interim analysis is currently planned.

5.0 ADVERSE EVENTS

Reporting of adverse events will be performed in accordance with the Data Safety Monitoring Plan (Appendix G) and Sections 5.2 and 5.3 below.

5.1 Adverse event and reporting definitions

With the occurrence of an adverse event, the first concern will be for the safety of the patient. Investigators are required to report to the IRB, medical monitor, clinical research organization (CRO) Quality Assurance Officer or her designee and CRO Regulatory Affairs Officer any serious adverse event, whether expected or unexpected, and which is assessed by the investigator to be reasonably or possibly related to or caused by the vaccine components. Elective surgeries resulting in hospitalization and unrelated to the study agents will not be reported as SAEs, (breast reconstruction, etc.). All events meeting the outlined criteria will be reported for the time period beginning with any amount of exposure to vaccine components through the protocol-defined follow-up period. The Regulatory Affairs Officer will then report the event to the Sponsor/Investigator. The IND Sponsor/Investigator or Sponsor/Investigator's representative will report these to the FDA and Galena Biopharma. Serious criteria, definitions, and guidance for reporting follow.

An adverse event (AE) is any untoward medical occurrence in a patient participating in an investigational study or protocol regardless of causality

assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product.

Serious adverse events (SAE) are AEs occurring at any dose which meet one or more of the following serious criteria:

- Results in death (i.e. the AE caused or led to death)
- Is life-threatening (i.e. the AE placed the patient at immediate risk of death; it does not apply to an AE which hypothetically might have caused the death if it were more severe)
- Requires or prolongs inpatient hospitalization (i.e. the patient was admitted to the hospital or extended hospitalization due to the event)
- Is disabling (i.e. the AE resulted in a substantial disruption of the patient's ability to carry out normal life functions)
- Results in a congenital anomaly/birth defect (i.e., an adverse outcome in a child or fetus of a patient exposed to the study drug prior to conception or during pregnancy)
- Does not meet any of the above serious criteria but may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above

Expected AEs are those AEs that are listed or characterized in the current Investigator Brochure.

Unexpected AEs are those not listed in the current Investigator Brochure. This includes AEs for which the specificity or severity is not consistent with the description in the Investigator Brochure. For example, under this definition, hepatic necrosis would be unexpected if the Investigator Brochure only referred to elevated hepatic enzymes or hepatitis.

5.2 Reporting of serious adverse events associated with this study

Each site PI will within 24 hours of notification of the event report all related or unrelated serious AEs occurring in patients enrolled at their respective study site to the Internal Review Board (IRB) of their site (see AE Reporting Algorithm, Appendix H). This will be accomplished by submitting an AE report memorandum to the IRB per the IRB's site-specific standard operating procedures. The site PI will also, within 24 hours of notification of the event, forward a copy of the serious

adverse event report (CRF Form 3.1(g)) to the CRO Quality Assurance (QA) Officer or her designee, and to the CRO Regulatory Affairs Officer. In addition, the site PI will also follow the same reporting for all hypersensitivity events of grade 2 or greater in the primary series.

The QA Officer or her designee will then forward the report to the Sponsor/Investigator. The Sponsor/Investigator or sponsor's representative will review the serious AE to determine the need for expedited reporting to the FDA. If expedited reporting is required, the site nurse coordinator will be notified by the QA Officer or her designee to complete a FDA Form 3500 (MedWatch) (Appendix I). The Regulatory Affairs Officer will then submit the final report to the Sponsor/Investigator or Sponsor/Investigator's representative who will report them to the FDA.

5.2.1 FDA Form 3500A (MedWatch) reporting guidelines

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (Section 5) of the FDA Form 3500A form (MedWatch):

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the AE to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original FDA Form 3500A (MedWatch) report and submitting as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original FDA Form 3500A (MedWatch)
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B. initial, patient number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted. (The patient

identifiers are important so that the new information is added to the correct initial report.)

The CRO, IND sponsor or Galena Biopharma may contact the reporter for additional information, clarification, or current status of the patient for whom an AE was reported. For questions regarding SAE reporting, you may contact the Quality Assurance (QA) Officer.

Study Drug Relationship:

The Investigator will determine which events are associated with the use of study drug. For reporting purposes, an AE should be regarded as possibly related to the use of vaccine components if the Investigator believes:

- There is a clinically plausible time sequence between onset of the AE and administration of vaccine components; and/or
- There is a biologically plausible mechanism for vaccine components to cause or contribute to the AE; and
- The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.

5.3 Reporting requirements for IND holders

For Sponsored IND Studies, there are some additional reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR § 600.80. Events meeting the following criteria need to be submitted to the FDA as expedited IND Safety Reports according to the following guidance and timelines:

7 Calendar-Day Telephone or Fax Report: The Sponsor is required to notify the FDA of any fatal or life-threatening AE that is unexpected and assessed by the investigator to be possibly related to the use of the investigational product. An unexpected AE is one that is not already described in the Investigator Brochure for the vaccine. Such events are to be reported by the sponsor to the FDA and within 7 calendar days of first learning of the event.

15 Calendar-Day Written Report: The Sponsor is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of the study agents. An unexpected AE is one that is not already described in the Investigator Brochure for vaccine.

- Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports

previously filed with the IND concerning similar events should be analyzed and the significance of the new report in light of the previous, similar reports commented on.

- Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a FDA Form 3500A (MedWatch) but alternative formats are acceptable (e.g. summary letter).

6.0 INVESTIGATOR REQUIREMENTS

6.1 Study initiation

Before the start of this study, the following documents must be on file with the sponsor, Galena Biopharma, or a representative:

- Original U.S. FDA Form 1572 for each site (for all studies conducted under U.S. Investigational New Drug [IND] regulations), signed by the Principal Investigator. The names of any sub-investigators must appear on this form. Investigators must also complete all regulatory documentation as required by local and national regulations.
- Current *curriculum vitae* of the Principal Investigator
- Written documentation of IRB approval of protocol and informed consent document
- A copy of the IRB-approved informed consent document
- A signed Clinical Research Agreement

6.2 Study completion

The following materials are requested by Galena Biopharma when a study is considered complete or terminated:

- A summary, prepared by the Principal Investigator, of the study, and/or a study manuscript, and/or a study abstract submitted to scientific conferences.

6.3 Informed consent

An informed consent template will be provided, and the final IRB-approved document must be provided to the sponsor for regulatory purposes. The informed consent document must be signed by the patient or the patient's legally authorized representative before his or her participation in the study. The case history for each patient shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent document must be provided to the patient or the patient's legally authorized representative. If applicable, it will be provided in a certified translation of the local language. Signed consent forms must remain in each patient's study file and must be available for verification by study monitors at any time.

6.4 Institutional review board or ethics committee approval

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB for review and must be approved before the study is initiated. The study will be conducted in accordance with U.S. FDA, applicable national and local health authorities, and IRB requirements.

The Principal Investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case the IRB must be updated at least once a year. The Principal Investigator must also keep the IRB informed of any significant adverse events. Investigators are required to promptly notify their respective IRB of all adverse drug reactions that are both serious and unexpected. This generally refers to serious adverse events that are not already identified in the Investigator Brochure and that are considered possibly or probably related to the molecule or study drug by the investigator. Some IRBs may have other specific adverse event requirements that investigators are expected to adhere. Investigators must immediately forward to their IRB any written safety report or update provided by Galena Biopharma (e.g., IND safety report, Investigator Brochure, safety amendments and updates, etc.).

Ethics and Regulatory Considerations

The protocol will be reviewed and approved by the IRB or Independent Ethics Committee (IEC) of each participating center prior to study initiation. A list of IRB/IEC members should be obtained by the investigator and provided to the sponsor and sponsor representative. Any documents that the IRB/IEC may need to fulfill its responsibilities, such as protocol amendments and/or information from the sponsor will be submitted to the IRB/IEC. The IRB/IEC's written unconditional approval of the study protocol and the informed consent form will be in the possession of the investigator and the sponsor before the study is initiated. The IRB/IEC's unconditional approval statement will be transmitted by the investigator or designee to the sponsor/sponsor representative prior to shipment of study

drug supplies to the site. This approval document must refer to the study by exact protocol title and protocol version number/date and must identify the documents reviewed and the date of review.

Protocol modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazard to the patients. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and IRB/IEC acknowledgement/approval should be obtained and transmitted to the Sponsor/Investigator or Sponsor/Investigator's representative. The IRB/IEC must be informed by the principal investigator of any changes or revisions of informed consent form or other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study; any new information that may affect adversely the safety of the patients or the conduct of the study; annual updates and/or request for re-approval; and when the study has been completed.

6.5 Study monitoring requirements

Site visits may be conducted by authorized Sponsor representative or Galena Biopharma representatives to inspect study data, patient's medical records, and CRFs in accordance with current U.S. GCPs and the respective local and national government regulations and guidelines (if applicable).

The Principal Investigator will permit authorized representatives of Sponsor/Investigator, Galena Biopharma, the U.S. FDA, and the respective national or local health authorities to inspect facilities and records relevant to this study.

6.6 Data Safety Monitoring Plan

A Data Safety Monitoring Plan (DSMP) (Appendix F) describing the CRO internal monitoring plan includes data safety and integrity and site initiation/QA monitoring, as well as external monitoring plan - the Data Safety Monitoring Board (DSMB) charter and responsibilities.

6.7 Study medication accountability

The study drug will be provided by Galena Biopharma. The recipient will acknowledge receipt of the drug by returning the INDRR-1 form indicating shipment content and condition. Damaged supplies will be replaced. Accurate records of all study drug dispensed from and returned to the study site should be recorded by using the institution's drug inventory log or the NCI drug accountability log. All partially used or empty containers should be disposed of at the study site according to institutional standard operating procedure. Return

unopened, expired, or unused study drug with the Inventory of Returned Clinical Material form as directed by Galena Biopharma.

6.8 Disclosure of data

Patient medical information obtained by this study is confidential, and disclosure to third parties other than those noted above is prohibited. Upon the patient's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, national and local health authorities, Galena Biopharma, and the IRB for each study site, if appropriate.

6.9 Retention of records

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for two years after marketing application approval. If no application is filed, these records must be kept two years after the investigation is discontinued and the U.S. FDA and the applicable national and local health authorities are notified. The sponsor will notify the Principal Investigator of these events. For studies conducted outside the United States under a U.S. IND, the Principal Investigator must comply with U.S. FDA IND regulations and with the record retention policies of the relevant national and local health authorities.

6.10 Publications

The investigator must agree to send to the Sponsor/Investigator or Sponsor/Investigator's representative, for review all manuscripts, abstracts and presentations using data from this study prior to their submission. The Sponsor/Investigator or Sponsor/Investigator's representative reserves the right to delete from such materials any part or parts deemed to be confidential or proprietary.

6.11 Changes to protocol

The protocol may not be modified without written approval of the Sponsor/Investigator or Sponsor/Investigator's representative, or the study director. All changes to the protocol must be submitted to the FDA, the overseeing IRB, and local IRB/IEC. Additionally, changes must be approved by overseeing IRB prior to their implementation. Documentation of IRB/IEC approval must be sent to the Sponsor/Investigator or Sponsor/Investigator's representative, and the Study Director immediately upon receipt. Any changes

and modifications to the informed consent language must be reviewed and approved by the Sponsor/Investigator or Sponsor/Investigator's representative, and the Study Director prior to submission to the local IRB.

REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2013, 2013

2. NCCN Clinical Practice Guidelines in Oncology: Breast Cancer. 3.2013, 2013

3. Slamon DJ, Clark GM, Wong SG, et al: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177-82, 1987

4. Slamon DJ, Godolphin W, Jones LA, et al: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707-12, 1989

5. Wolff AC, Hammond ME, Hicks DG, et al: Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 31:3997-4013, 2013

6. Cobleigh MA, Vogel CL, Tripathy D, et al: Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 17:2639-48, 1999

7. Marty M, Cognetti F, Maraninchi D, et al: Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. J Clin Oncol 23:4265-74, 2005

8. Perez EA: Impact, mechanisms, and novel chemotherapy strategies for overcoming resistance to anthracyclines and taxanes in metastatic breast cancer. Breast Cancer Res Treat 114:195-201, 2009

9. Slamon DJ, Leyland-Jones B, Shak S, et al: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344:783-92, 2001

10. Vogel CL, Cobleigh MA, Tripathy D, et al: Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 20:719-26, 2002

11. von Minckwitz G, du Bois A, Schmidt M, et al: Trastuzumab beyond progression in human epidermal growth factor receptor 2-positive advanced breast cancer: a german breast group 26/breast international group 03-05 study. J Clin Oncol 27:1999-2006, 2009

12. Perez EA, Romond EH, Suman VJ, et al: Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. J Clin Oncol 29:3366-73, 2011

13. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al: Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 353:1659-72, 2005

14. Romond EH, Perez EA, Bryant J, et al: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 353:1673-84, 2005

15. Slamon D, Eiermann W, Robert N, et al: Adjuvant Trastuzumab in HER2-positive breast cancer. N Engl J Med 365:1273-83, 2011.

16. Smith I, Procter M, Gelber RD, et al: 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. Lancet 369:29-36, 2007

17. Buzdar AU, Ibrahim NK, Francis D, et al: Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 23:3676-85, 2005

18. Untch M, Rezai M, Loibl S, et al: Neoadjuvant treatment with trastuzumab in HER2-positive breast cancer: results from the GeparQuattro study. J Clin Oncol 28:2024-31, 2010

19. Gianni L, Eiermann W, Semiglazov V, et al: Neoadjuvant chemotherapy with trastuzumab followed by adjuvant trastuzumab versus neoadjuvant chemotherapy alone, in patients with HER2-positive locally advanced breast cancer (the NOAH trial): a randomised controlled superiority trial with a parallel HER2-negative cohort. Lancet 375:377-84, 2010

20. Baselga J, Bradbury I, Eidtmann H, et al: Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. Lancet 379:633-40, 2012

21. Gianni L, Pienkowski T, Im YH, et al: Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 13:25-32, 2012

22. Buzdar AU, Suman VJ, Meric-Bernstam F, et al: ACOSOG Z1041

(Alliance): Definitive analysis of randomized neoadjuvant trial comparing FEC followed by paclitaxel plus trastuzumab (FEC → P+T) with paclitaxel plus trastuzumab followed by FEC plus trastuzumab (P+T → FEC+T) in HER2+ operable breast cancer. J Clin Oncol (Meeting Abstracts) 31, 2013

23. Carey LA, Berry DA, Ollila D, et al: Clinical and translational results of CALGB 40601: A neoadjuvant phase III trial of weekly paclitaxel and trastuzumab with or without lapatinib for HER2-positive breast cancer. J Clin Oncol (Meeting Abstracts) 31, 2013

24. Mittendorf EA, Wu Y, Scaltriti M, et al: Loss of HER2 amplification following trastuzumab-based neoadjuvant systemic therapy and survival outcomes. Clin Cancer Res 15:7381-8, 2009

25. Kim MM, Allen P, Gonzalez-Angulo AM, et al: Pathologic complete response to neoadjuvant chemotherapy with trastuzumab predicts for improved survival in women with HER2-overexpressing breast cancer. Ann Oncol 24:1999-2004, 2013

26. Untch M, Fasching PA, Konecny GE, et al: Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2-overexpressing breast cancer: results from the TECHNO trial of the AGO and GBG study groups. J Clin Oncol 29:3351-7, 2011

27. Norton N, Perez EA: How relevant is hormone receptor status in the context of outcome to HER2-positive breast cancer? Breast Cancer Res 15:101, 2013

28. Mittendorf EA, Holmes JP, Ponniah S, et al: The E75 HER2/neu peptide vaccine. Cancer Immunol Immunother 57:1511-21, 2008

29. Anderson BW, Peoples GE, Murray JL, et al: Peptide priming of cytolytic activity to HER-2 epitope 369-377 in healthy individuals. Clin Cancer Res 6:4192-200, 2000

30. Lustgarten J, Theobald M, Labadie C, et al: Identification of Her-2/Neu CTL epitopes using double transgenic mice expressing HLA-A2.1 and human CD.8. Hum Immunol 52:109-18, 1997

31. Peoples GE, Gurney JM, Hueman MT, et al: Clinical trial results of a HER2/neu (E75) vaccine to prevent recurrence in high-risk breast cancer patients. J Clin Oncol 23:7536-45, 2005

32. Peoples GE, Holmes JP, Hueman MT, et al: Combined clinical trial results of a HER2/neu (E75) vaccine for the prevention of recurrence in high-risk breast

cancer patients: U.S. Military Cancer Institute Clinical Trials Group Study I-01 and I-02. Clin Cancer Res 14:797-803, 2008

33. Holmes JP, Clifton GT, Patil R, et al: Use of booster inoculations to sustain the clinical effect of an adjuvant breast cancer vaccine: from US Military Cancer Institute Clinical Trials Group Study I-01 and I-02. Cancer 117:463-71, 2011

34. Vreeland TJ, Clifton GT, Hale DF, et al: Final Results of the Phase I/II Trials of the E75 Adjuvant Breast Cancer Vaccine. Cancer Research 72, 2012

35. Efficacy and Safety Study of NeuVax(TM)(Nelipepimut-S or E75) Vaccine to Prevent Breast Cancer Recurrence. , Galena Biopharma, Inc., 2013

36. Hudis CA: Trastuzumab--mechanism of action and use in clinical practice. N Engl J Med 357:39-51, 2007

37. Barok M, Isola J, Palyi-Krekk Z, et al: Trastuzumab causes antibody-dependent cellular cytotoxicity-mediated growth inhibition of submacroscopic JIMT-1 breast cancer xenografts despite intrinsic drug resistance. Mol Cancer Ther 6:2065-72, 2007

38. Clynes RA, Towers TL, Presta LG, et al: Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med 6:443-6, 2000

39. Musolino A, Naldi N, Bortesi B, et al: Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J Clin Oncol 26:1789-96, 2008

40. Park S, Jiang Z, Mortenson ED, et al: The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell 18:160-70, 2010

41. Taylor C, Hershman D, Shah N, et al: Augmented HER-2 specific immunity during treatment with trastuzumab and chemotherapy. Clin Cancer Res 13:5133-43, 2007

42. Knutson KL, Perez EA, Ballman KV, et al: Generation of adaptive HER2-specific immunity in HER2 breast cancer patients by addition of trastuzumab to chemotherapy in the adjuvant setting: NCCTG (Alliance) study N9831, J Clin Oncol (Meeting Abstracts), 2013, pp 522

43. Inoue M, Mimura K, Izawa S, et al: Expression of MHC Class I on breast cancer cells correlates inversely with HER2 expression. Oncoimmunology 1:1104-1110, 2012

44. Mittendorf EA, Storrer CE, Shriver CD, et al: Investigating the combination of trastuzumab and HER2/neu peptide vaccines for the treatment of breast cancer. Ann Surg Oncol 13:1085-98, 2006

45. Disis ML, Wallace DR, Gooley TA, et al: Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. J Clin Oncol 27:4685-92, 2009

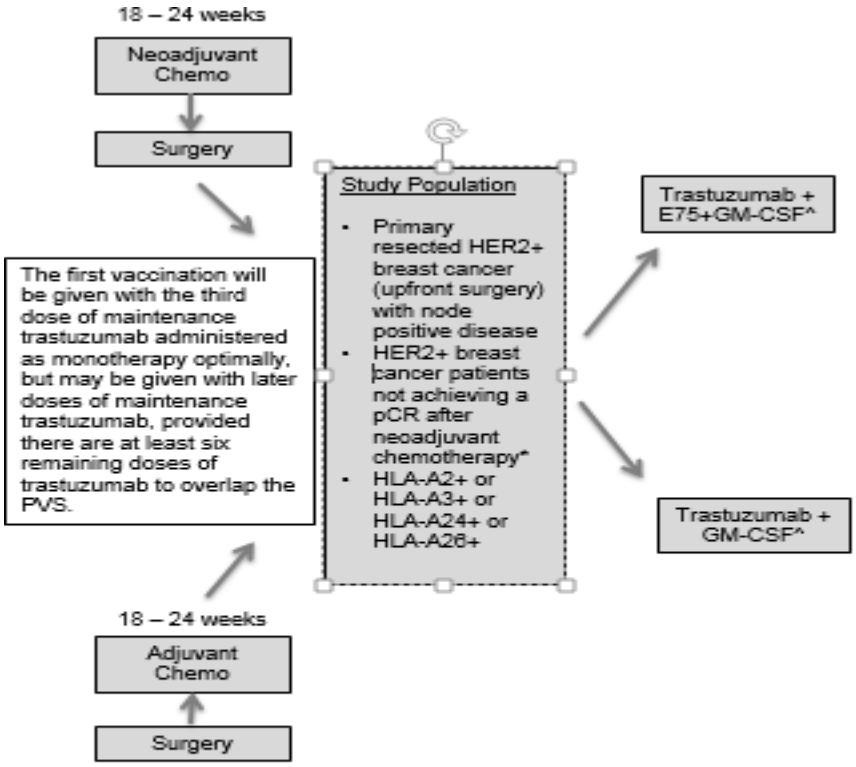
46. Mittendorf E, Perez S, Tzonis P, et al: Abstract P2-20-03: Combination Immunotherapy for Breast Cancer Patients: Safety and Immunologic Data from a Phase II Trial Administering a HER2/neu-Derived Peptide vaccine (AE37+GM-CSF) Sequentially or Concurrently with Trastuzumab in the Adjuvant Setting. Cancer Research 70:P2-20-03, 2011

47. Disis ML, Goodell V, Schiffman K, et al: Humoral epitope-spreading following immunization with a HER-2/neu peptide based vaccine in cancer patients. J Clin Immunol 24:571-8, 2004

48. Khatcheressian JL, Hurley P, Bantug E, et al: Breast cancer follow-up and management after primary treatment: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 31:961-5, 2013

Appendix A

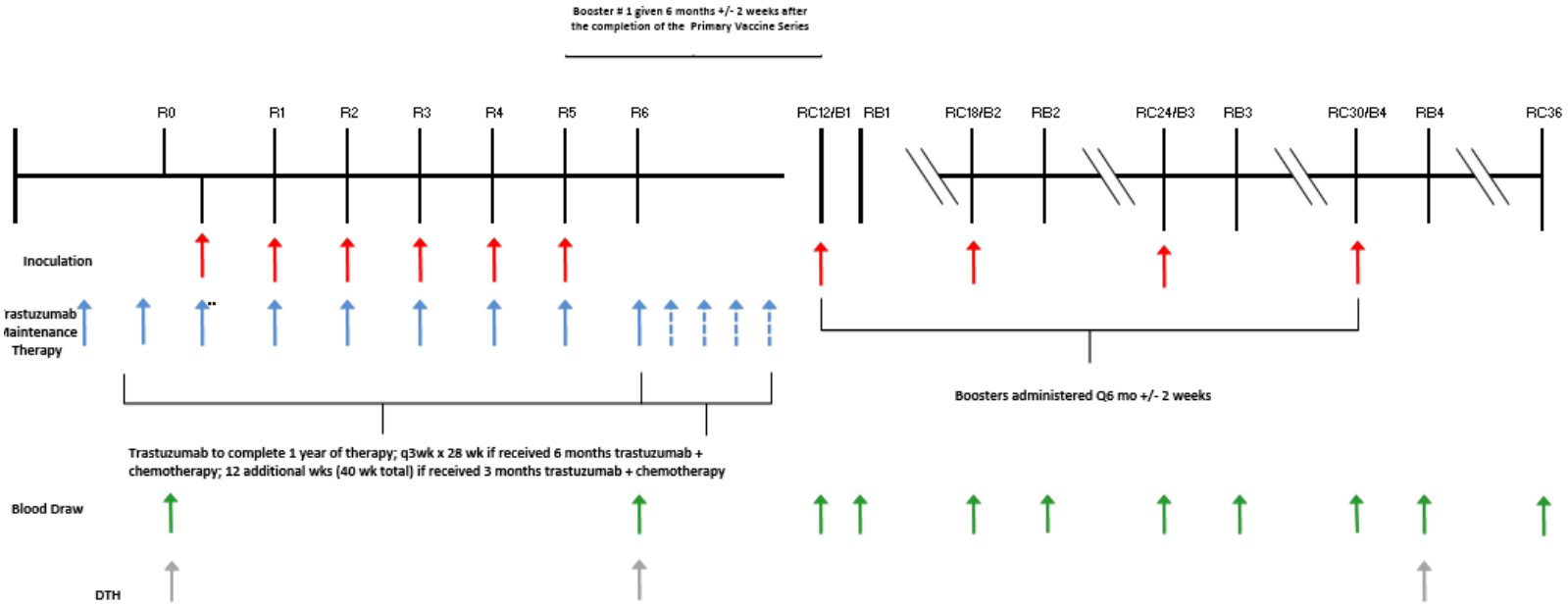
Trial Schema



* Neoadjuvant chemotherapy regimen must include trastuzumab and at least four cycles (12 weeks) of taxane-containing therapy
^ Inoculations to begin with 3rd dose of trastuzumab maintenance therapy optimally, but may be given with later maintenance doses provided at least six doses of trastuzumab remain to overlap the PVS

Appendix B

Vaccination Timeline



**"Vaccine inoculations to begin with 3rd dose of maintenance trastuzumab infusion optimally, but may be given with later maintenance doses of trastuzumab, provided there are at least six remaining doses of trastuzumab to overlap with the PVS

Appendix C

NCI Common Terminology Criteria for Adverse Events, v4.03

obtained from http://ctep.cancer.gov/forms/CTCAEv4.03.pdf
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06 14_QuickReference_8.5x11.pdf

Appendix D

GALENA BIOPHARMA

2014-0443

Phase II Trial of combination immunotherapy with nelipepimut-S + GM-CSF (NeuVax™) and trastuzumab in high-risk HER2+ breast cancer patients

Dosing Preparation Instructions

Dosing Summary:
The study treatment is 1000 mcg of E75 peptide (nelipepimut-S) mixed with 250 mcg of lyophilized Leukine® (sargramostim, GM-CSF) or placebo (water for injection) mixed with Leukine, administered in four separate 0.4 mL intradermal injections.

The E75 peptide is provided as 1.5 mg/mL of E75 acetate (contains a 1.42 mg/mL solution of the E75 peptide). Using aseptic technique, 0.8 mL of E75 acetate solution (1136 mcg E75 peptide) is added to the re-constituted Leukine. Taking into account vial losses, this procedure will yield a total dose of approximately 1010 mcg of E75 peptide in a volume of 1.6 mL.

Stability:
Once prepared, administer the E75 peptide/placebo and lyophilized Leukine mixture within 6 hours.

Materials:

1. Randomized Test Drug kit with Patient ID number. This kit contains either a single 1.0 mL vial of E75 acetate solution (1.5 mg/mL) or a single 1.0 mL vial of placebo.
2. Lyophilized Leukine kit with Patient ID number. This kit contains a single vial of lyophilized Leukine (250 μg).
3. Ancillary Supplies: 7-1mL TB syringes, 5-3/8 inch 26G needles, 4-2 inch 21G needles, 2-3 mL luer lock syringes.

Dose Preparation:

NOTE: DO NOT INVERT ANY STUDY DRUG VIALS DURING PREPARATION

1. Remove cap from vial of 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 (E75 acetate, 1.5 mg/mL or placebo) 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.
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 6 hours of mixing.

Guidance for DTH Preparation from E75 Acetate Solution 1.5mg/1ml Vial

To Prepare the DTH skin test, withdraw 0.07 mL of the 1.5mg/1.0mL E75 acetate solution.
The DTH skin test is administered intradermally using 0.1 mL (100 µg) of the E75 acetate solution.

Supplies: **All** DTH skin test kits will contain an active vial, which contains 1.0 mL of E75 acetate in WFI at 1.5mg/mL. Some DTH kits will contain a second vial, which is a control vial containing 1.0 mL of Water For Injection (WFI). If the DTH kit contains the WFI discard the WFI in the kit per your institution's policy.

E75 acetate solution preparation:

1. Remove cap from 1.5 mg/1mL E75 acetate solution and wipe with alcohol prep pad
2. Open 1 mL syringe with needle attached and uncap the needle
3. Draw > 0.07 mL air into the syringe and inject into the E75 acetate solution vial
4. Withdraw 0.07 mL of E75 acetate solution into the syringe
5. Remove the syringe and the needle from the vial
6. Drawback syringe plunger to capture hub volume into the syringe
7. Remove and discard needle
8. Attach a new, sterile needle to the syringe containing 0.07 mL (100 ug) E75 acetate solution for intradermal administration.

Appendix E

Leukine package insert

Appendix F

ECOG Performance Status Criteria

Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	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
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

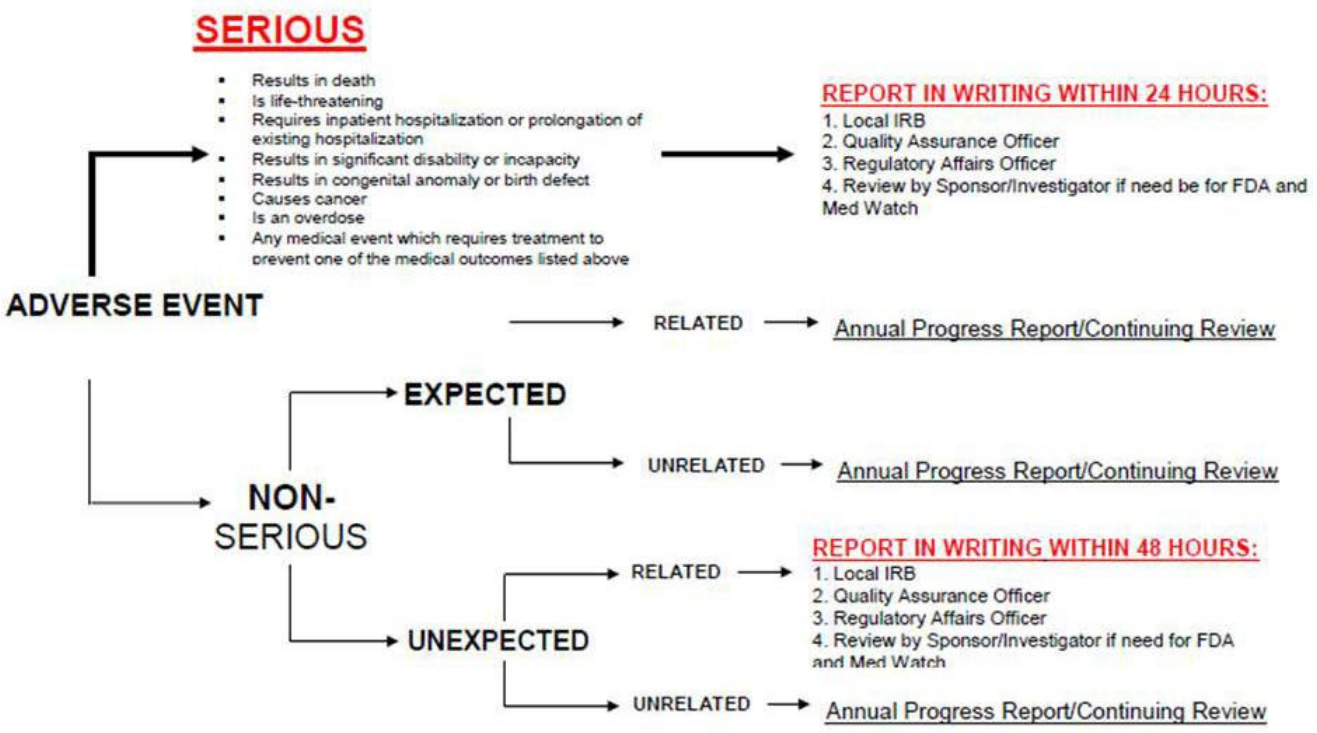
Appendix G

Data Safety Monitoring Plan

This is a free standing document

Appendix H

Adverse Events Reporting Algorithm



Appendix I

MedWatch FDA Form 3500 Link

http://www.fda.gov/downloads/Safety/MedWatch/DownloadForms/ucm082728.pdf

APPENDIX J

Study Flowchart

	Days –60 to –1	Week 1 (Day 0)	DTH Pre/Post (6) vaccines [2]	Every 3 Weeks	Every 3 Months	Every 6 Months	If Clinically Indicated	Final Visit in 36th month
Hematology (CBC, diff., platelets)[1]	X				x	X[1]		
Chemistry panel (CMP)	X				X	X[1]		
HLA blood draw for typing	During study screening process/patient identification process.							
Immunological Samples[2]			X[2]			X[2]		X[2]
Trastuzumab administration (q3 weeks)				X				
DTH administration[3]			1, 2[3]					
Vaccine administration[4]				X[4]				
Booster inoculations [5]						X[5]		
Post-Booster Immunological Samples[2]						X[2]		
Complete medical history (treating physician)	X						X	
Complete physical exam (treating physician, based on SOC)	X				X	X	X	
Clinical assessment-Study nurse visit[4,5,10]		X	X[4]	X[4]		X[5]		X[10]
Weight, height (treating physician)	X						X	
ECOG performance status		X						
Toxicity evaluation[9,10]			X[9]	X[9]		X[9]	X[9]	X[10]
Vital signs[9]			X[9]	X[9]		X[9]		
Cardiac Assessment (MUGA scan or Echo)[6]					X		X[6]	
Chest X-ray (AP, lateral)/EKG							X	
Urine pregnancy test (if applicable)[7]		X[7]	X[7]	X[7]		X[7]		
Tumor assessment (treating physician)[8]	X[8]						X	
Disease status monitoring[8,10]	X[8]				X[8]	X[8]	X[8]	X[10]

[1] Per standard practice, all patients will have CBC/CMP monitored approximately every 3 months during the primary series and approximately every 6 months during the booster phase.

[2]Immunological samples will be drawn pre DTH#1 and #2, prior to each of 4 boosters, 1 month (+/-1 week) after each booster, and at study completion 36 months after initiation of Trastuzumab maintenance therapy.

[3]DTH #1 given prior to initiation of primary vaccine series, one month (+/- 1 week) after completion of primary vaccine series, and one month (+/-1 week) after the final booster inoculation.

[4]Vaccine inoculation administered every 3 weeks for 6 inoculations during trastuzumab treatment schedule. Vaccine inoculations to begin with 3rd dose of maintenance trastuzumab infusion, optimally, but may be given with later maintenance doses of trastuzumab, provided there are at least six remaining doses of trastuzumab to overlap

with the PVS. Vaccine inoculations given within 30-120 minutes after completion of infusion. (With prior approval from the Principal Investigator, the vaccine may be administered 15 minutes after completion of trastuzumab infusion).

[5]Booster inoculations will be given after initial vaccine series, every 6 months x4, beginning 6 months (+/- 2 weeks) after the completion of the Primary Vaccine Series Subsequent boosters will be given at 12, 18, and 24 months (+/- 2 weeks), after the completion of the PVS.

[6]All patients will have cardiac assessment performed according to standard practice as dictated by their treating oncologist. Results of cardiac testing will be reviewed by the research nurses or study coordinators and any abnormal results will be reviewed by the study investigators.

[7]Urine HCG waived if hysterectomy, bilateral oopherectomy, tubal ligation, documented absence of menses for two years, or FSH lab results verifying menopause.

[8]Disease status will be determined by patients' own physicians at the individual study sites during routine follow-up. This will include physical examination every 3-6 months for all patients and yearly mammogram for patients undergoing breast conserving therapy (per ASCO guidelines)

[9]Toxicity evaluation done during 30 minutes observation after vaccine administration with vital signs taken as needed and during return visit or by phone call 48-72 hours after inoculation. Delayed adverse event reporting per protocol.

[10]Final study assessment in month 36 by study nurse with toxicity assessment and disease status update

Appendix K

PROTOCOL TITLE: Phase II Trial of Combination Immunotherapy with nelipepimut-S + GM-CSF (NeuVax™) and Trastuzumab in high-risk HER2+ Breast Cancer Patients (2014-0443)

STUDY DRUG: Nelipepimut-S + GM-CSF (NeuVax™)

PRINCIPAL INVESTIGATOR: Elizabeth A. Mittendorf, MD, PhD

PROGRAM DIRECTOR SPONSOR/INVESTIGATOR: George E. Peoples, MD, FACS

PROTOCOL VERSION/DATE: 2.1/11 April 2016

INVESTIGATOR'S AGREEMENT / INVESTIGATOR'S SIGNATURE PAGE

I have read the protocol described above. I have fully discussed the objectives of this trial and the content of this protocol with the Sponsor or Sponsor's representative. I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the ethical review of the trial, without written authorization from Cancer Insight. It is, however, permissible to provide information to a patient in order to obtain consent. I agree to conduct this trial according to the protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with all applicable regulations, and guidelines as stated in the protocol and other information supplied to me. I understand that the Sponsor may decide to suspend or prematurely terminate the trial at any time, for whatever reason. Such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the trial, I will communicate my intention immediately, in writing to the Sponsor.

Signed:_____ **Date:** _____

Investigator's Name and Address:

_____
_____
_____
_____