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PROTOCOL TITLE:	Combination immunotherapy with Herceptin and the HER2 vaccine E75 in low and intermediate HER2-expressing breast cancer patients to prevent recurrence
STUDY DRUGS:	HERCEPTIN® (Trastuzumab) E75 peptide (KIFGSLAFL, HER2/*neu*, 369-377) GM-CSF (Leukine®, Berlex)
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1. INTRODUCTION

1.1 DISEASE BACKGROUND

Breast cancer is, by far, the most commonly diagnosed malignancy in women (excluding cancers of the skin), and it is the second leading cause of cancer mortality among women. The American Cancer Society estimated that there were over 200,000 new cases of breast malignancy diagnosed in 2009, with an estimated 40,000 women expected to die from this disease each year (American Cancer Society 2009).

While nodal status is still the most important prognostic factor in breast cancer, other parameters such as HER2 expression are known to impact disease recurrence and ultimately survival (O'Shaughnessy et al. 2006). HER2 is expressed in approximately 70-80% of breast cancers and over-expressed in 20-30% of breast cancers (Slamon et al. 1989).

In addition to traditional therapies such as surgery, chemotherapy, radiation, and hormonal therapy, novel approaches to the management of this disease have recently shown promise. One such approach has been to use the body's own immune mechanisms to target and kill tumor cells. Trastuzumab (Herceptin), a monoclonal antibody (mAb) that binds the HER2 protein product, is designed to do just that, and recently published reports indicate that it affords a significant disease-free survival (DFS) benefit to high-risk node-negative (NN) and node-positive (NP) breast cancer patients (Piccart-Gebhart et al. 2005, Romond et al. 2005). In both settings, Herceptin has been shown to decrease the recurrence rate by approximately 50%. Unfortunately, Herceptin can only be used in a minority of breast cancer patients, specifically those that over-express the HER2 protein. These results have served to fuel ongoing research into immunologic approaches to cancer treatment ranging from the administration of mAbs, such as Herceptin, to the development of anti-cancer vaccines.

Advances in the understanding of the immune response to cancer have led to the genesis of other immunotherapeutic approaches as well. Specifically, the development of anti-cancer vaccines holds promise as an adjuvant and preventive therapy for breast cancer patients at high risk for recurrence after primary therapy (Khoo et al. 2006). HER2 has also been shown to be the source of multiple immunogenic peptides to include E75, GP2, and the wild type AE37 (Fisk et al. 1995, Peoples et al. 1995, Mittendorf et al. 2009). AE37 includes a four amino-acid modification that increases its effectiveness (Humphreys et al. 2000, Kallinteris et al. 2006). E75, GP2, and AE37 peptides have all been utilized as the basis of separate anti-cancer vaccines combined with the immunoadjuvant, granulocyte macrophage colony-stimulating factor (GM-CSF), and tested by our group in Phase I/II trials. E75 has been shown to reduce clinical recurrences in the adjuvant setting similar in magnitude to the benefit proven with Herceptin (Peoples et al. 2008)

While Herceptin has previously been reserved for HER2 3+ expressing tumors, emerging evidence suggests a potential benefit may exist for HER2 2+ expressing tumors as well

(Paik et al. 2008). In our vaccine trials, we have shown that HER2-derived, CD8-eliciting, peptide-based vaccines used in the adjuvant setting may decrease the recurrence rate in breast cancer patients with tumors expressing any level of HER2, especially HER2 1+ and 2+ tumors (Benavides et al. 2009). Finally, in ongoing Phase II trials of our HER2 vaccines, 30 patients with HER2 3+ tumors treated with standard of care Herceptin followed by HER2 vaccination, have had no breast cancer recurrences in 48 months of follow up (Sears et al. 2011). Given this potential of patients with HER2 1+ and 2+ expressing tumors to respond to both Herceptin and HER2-derived peptide vaccines, we believe that the combination of Herceptin and a HER2-derived, CD8-eliciting peptide vaccine could potentially show a dramatic improvement in preventing breast cancer recurrence.

1.2 HER2 AND BREAST CANCER

Growth factors and their receptors play critical roles in development, cell growth, differentiation, and apoptosis (Cross et al. 1991). Such receptors span the cell membrane, with the extracellular domain binding specific growth factors and the intracellular domain transmitting growth signals. Interaction of the extracellular domain with its cognate ligand often results in intracellular activation of tyrosine kinase activity. Overexpression of human epidermal growth factor receptor 2 (HER2, also known as *erb*B2, neu, and p185HER2) is observed in approximately 25-30% of human breast cancers (Slamon et al. 1987). HER2 overexpression has been reported to only rarely occur in the absence of gene amplification (Kallioniemi et al. 1992; Pauletti et al. 1996). High level of HER2 expression has been correlated with poor clinical outcome (Slamon et al. 1987).

Several lines of evidence support a direct role for HER2 overexpression in the pathogenesis and poor clinical course of human tumors (Hynes 1993). When the mutated gene is transfected into murine fibroblast (NIH 3T3) cells, it causes transformation, and the resulting cells are tumorigenic in the nude mice (Di Fiore et al. 1987; Hudziak et al. 1987). Additionally, transgenic mice that overexpress the rodent homolog of the human HER2 gene develop breast cancer (Guy et al. 1992). Finally, specific antibodies to the extracellular domain of HER2 inhibit the experimental growth of tumors that overexpress the gene (Drebin et al. 1985, 1988; Fendly et al. 1990). These data suggest a direct role for HER2 in both malignant transformation and enhanced tumorigenicity. Therefore, a strategy to antagonize the abnormal function of overexpressed HER2 was developed to improve the course of patients with HER2-overexpressing tumors. Monoclonal antibodies directed against the HER2 protein were developed and humanized to minimize the likelihood of immunogenicity. One of these antibodies (Herceptin) was very effective in inhibiting both in vitro and in vivo proliferation of human breast cancer tumor cells overexpressing the HER2 protein and in mediating antibody-dependent cellular cytotoxicity in the presence of human effector cells (Jurianz et al. 1999).

There is substantial preclinical evidence that inhibition of signal transduction pathways can potentiate the cytotoxic activity of chemotherapeutic drugs. Indeed, Herceptin has been shown to have synergy, in vitro and in vivo, with several chemotherapeutic drugs including cisplatin, doxorubicin, thiotepa, etoposide, vinorelbine, and taxanes (Pegram et al. 2000; Pietras et al. 1994; Arteaga et al. 1994; Hancock et al. 1991; Baselga et al. 1998; Pegram

et al. 1997). Given this promising preclinical data, Herceptin was tested in the clinic both as a single agent and in combination with chemotherapy.

1.3 HERCEPTIN CLINICAL EXPERIENCE

The clinical benefit of Herceptin in women with metastatic breast cancer has been demonstrated in two pivotal studies.

A large Phase II trial (H0649g) assessed the activity of Herceptin as a single agent in 222 women with HER2 overexpressing metastatic breast cancer with progressive disease after one or more chemotherapy regimens (Cobleigh et al. 1999). A blinded, independent response evaluation committee identified 8 complete and 26 partial responses, for an objective response rate of 15% in the intent-to-treat population (95% confidence interval, 11% to 21%). The median duration of response was 9.1 months, and the median duration of survival was 13 months. The most common adverse events, which occurred in approximately 40% of patients, were mild to moderate infusion-associated fever and/or chills. These symptoms usually occurred only during the first infusion. The most clinically significant event was cardiac dysfunction, which occurred in 4.7% of patients.

A large, open-label, randomized Phase III study (H0648g) in 469 patients with HER2-positive metastatic breast cancer was conducted to evaluate the efficacy of Herceptin in combination with chemotherapy as first-line treatment. Patients who were anthracycline-naïve were randomized to receive either anthracycline plus cyclophosphamide (AC) or Herceptin plus AC. Patients who had received prior anthracyclines in the adjuvant setting were randomized to receive either paclitaxel or Herceptin plus paclitaxel. Patients randomized to Herceptin and chemotherapy measurably benefited in comparison to patients treated with chemotherapy alone in terms of time to disease progression, overall response rate, median duration of response, and survival. As determined by an independent Response Evaluation Committee (REC), Herceptin prolonged median time to disease progression from 4.6 months to 7.4 months ($p<0.001$), improved the overall response rate (complete and partial responses) from 32% to 50% ($p<0.001$), and increased median duration of response from 6.1 to 9.1 months ($p<0.001$). Compared to chemotherapy alone, the addition of Herceptin significantly lowered the incidence of death at one year from 33% to 22% ($p=0.008$) and increased median overall survival 24% from 20.3 months to 25.1 months ($p=0.046$). The observed survival advantage remained despite crossover of 66% of patients initially randomized to chemotherapy alone who elected to receive Herceptin upon disease progression (Tripathy et al. 2000). Fever/chills were observed with the initial Herceptin infusion in approximately 25% of patients. Class III or IV cardiac dysfunction was observed in 16% of the Herceptin + AC subgroup; increasing age was an associated risk factor for the development of cardiotoxicity in this treatment cohort.

Based on these data, Herceptin was approved by the U.S. Food and Drug Administration (FDA) for use in HER2-overexpressing metastatic breast cancer in combination with paclitaxel for first-line treatment and as a single agent for patients failing prior chemotherapy for metastatic disease. However, current usage patterns of Herceptin indicate that the drug is now being used in a broader array of circumstances than in the

pivotal clinical trials. Since initiation of the pivotal clinical trials, docetaxel has become a commonly used taxane in the treatment of metastatic breast cancer (Chevallier et al. 1995) and new data have emerged on the weekly use of paclitaxel (Akerley et al. 1997). Herceptin has been studied in combination with paclitaxel and docetaxel using a variety of doses and schedules with promising results (Seidman et al. 1999; Nicholson et al. 2000; Kuzur et al. 2000). In addition, the combination of Herceptin with vinorelbine has recently been studied (Burstein et al. 2001). In this study, 30 of 40 women treated with Herceptin (4 mg/kg x 1, 2 mg/kg weekly thereafter) and vinorelbine (25 mg/m^2 weekly, with dose adjusted each week for neutrophil count) responded to therapy, for an overall response rate of 75% (95% confidence interval 57% to 89%). Neutropenia was the only grade IV toxicity. No patients had symptomatic heart failure. Grade 2 cardiotoxicity was observed in 3 patients; prior cumulative doxorubicin dose in excess of 240 mg/m^2 and borderline pre-existing cardiac function were associated with this toxicity.

1.4 SAFETY

Experience with Herceptin administration has shown that the drug is relatively safe. The most significant safety signal observed during clinical trials was cardiac dysfunction (principally clinically significant congestive heart failure [CHF]), particularly when Herceptin was given in combination with an anthracycline-containing regimen. Much of the cardiac dysfunction was reversible on discontinuation of Herceptin.

In addition, during the first infusion with Herceptin, a symptom complex most commonly consisting of fever and/or chills was observed in approximately 40% of patients. The symptoms were usually mild to moderate in severity and controlled with acetaminophen, diphenhydramine, or meperidine. These symptoms were uncommon with subsequent infusions. However, in the post-approval setting, more severe adverse reactions to Herceptin have been reported. These have been categorized as hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events. Rarely, these severe reactions culminated in a fatal outcome.

There are no adequate or well-controlled studies in pregnant women, and animal reproduction studies are not always predictive of human response. Therefore, Herceptin should be used during pregnancy only if the potential benefit to the mother outweighs the potential risk to the fetus. In the post-marketing setting, oligohydramnios (decreased amniotic fluid) has been reported in women who received Herceptin during pregnancy, either in combination with chemotherapy or as a single agent. Given the limited number of reported cases, the high background rate of occurrence of oligohydramnios, the lack of clear temporal relationships between drug use and clinical findings, and the lack of supportive findings in animal studies, an association between Herceptin and oligohydramnios has not been established.

Herceptin appears to be relatively nonimmunogenic. Only 1 of 903 patients evaluated developed neutralizing antibodies to Herceptin. The development of anti-Herceptin antibodies in this patient was not associated with clinical signs or symptoms.

1.5 CLINCAL PHARMACOKINETICS OF TRASTUZUMAB

A Phase I single dose study (H0407g) of intravenous trastuzumab infusions ranging from 10-500 mg resulted in dose-dependent pharmacokinetics (PK) with serum clearance of trastuzumab decreasing with an increasing dose at doses <250 mg. PK modeling of trastuzumab concentration-time data from 7 patients that were administered doses of 250 mg and 500 mg had in a mean half-life of 5.8 days (range 1-32 days). Additionally, PK modeling showed that weekly trastuzumab doses ≥250 mg resulted in serum trough levels of >20 μg/mL that was above the minimum effective concentration observed in preclinical xenograft studies in tumor-bearing mice. The Phase I data supported the weekly dosing schedule that was implemented in all subsequent Phase II and Phase III clinical trials. A weight-based dose schedule was adopted after two Phase II trials (H0551g and H0552g) suggested that inter-subject variability in trastuzumab PK was related to body weight. These findings resulted in a trastuzumab dose schedule of a 4 mg/kg loading dose followed by a weekly 2 mg/kg maintenance dose utilized in the two pivotal Phase III trials (H0648g and H0649g) that were the basis of the BLA filing and subsequent FDA approval of trastuzumab for HER2+ metastatic breast cancer.

The trastuzumab PK data from studies H0407g (Phase I), H0551g (Phase II), and H0649 (pivotal) have been subsequently reanalyzed by a population PK approach using nonlinear mixed effect modeling (NONMEM) {ref: Lu et al.}. A linear two-compartment model best described the concentration-time data, and accounted for the accumulation of trastuzumab serum concentrations seen in the Phase II and Phase III clinical studies. A covariate analysis was conducted using the subjects from these single agent studies to evaluate the effect of pathophysiologic covariates (e.g. age, weight, shed antigen) on the PK parameter estimates. The covariates, that significantly influenced clearance, were the level of shed antigen and the number of metastatic sites. Volume of distribution was significantly influenced by weight and shed antigen level. Additionally, data from the Phase III study, H0648g, were added to assess the influence of concomitant chemotherapy on trastuzumab PK. Importantly, chemotherapy (AC or paclitaxel) did not significantly alter trastuzumab PK. The estimated half-life of trastuzumab based on the final model was 28.5 days.

Analysis of data obtained from two Phase II studies which utilized a loading dose of 8 mg/kg trastuzumab followed by a 6 mg/kg maintenance dose administered every 3 weeks (q3 week) as a single-agent (Baselga et al. 1998), and in combination with paclitaxel (175 mg/m^2) (Leyland-Jones et al. 2000), confirmed that a two-compartment model best describes the PK of trastuzumab. Model-independent analysis of the of the data obtained in these studies gives comparable PK parameter estimates to those obtained by the population PK model, thus confirming the validity of the population PK model. In addition, the population PK model adequately predicted trastuzumab serum concentrations obtained independently in these studies. After two treatment cycles, trastuzumab exposure were similar to those measured in the once weekly dosing regimen used in the pivotal trials. Trough levels were in excess of the targeted serum concentrations established from preclinical xenograft models, and as expected, peak levels were greater than those observed upon weekly administration. The apparent half-life of trastuzumab in these

studies was determined to be approximately 21 days, and the PK was supportive of a q3 week dosing schedule.

The efficacy and safety results from these Phase II studies with q3 week dosing do not appear to be different from those with weekly dose-schedules (Slamon et al. 2001; Cobleigh et al. 1999). In the trastuzumab q3 weekly monotherapy study (Baselga et al. 1998), 105 patients with HER2+ metastatic breast cancer were treated, with an objective response rate of 19% (23% in patients with measurable centrally confirmed HER2+ disease). The median baseline LVEF was 63%, which did not significantly change during the course of the study. One patient experienced symptomatic CHF, which resolved with medical treatment for CHF and discontinuation of trastuzumab. In the study of q3 weekly trastuzumab and paclitaxel (Leyland-Jones et al. 2000), 32 patients were treated with an investigator-assessed response rate of 59%. Ten patients had a decrease in LVEF of 15% or greater. One patient experienced symptomatic CHF, which improved symptomatically after medical therapy for CHF and discontinuation of trastuzumab.

1.6 E75 BACKGROUND

E75 is a nine amino-acid peptide derived from the HER2/*neu* protein (369-377) (Fisk et al. 1995) and is the most studied CTL epitope from this tumor-associated antigen both in vitro and in vivo. Zaks and Rosenberg, in an early study, looked at vaccinating patients with the E75 peptide in an effort to determine its efficacy (Zaks et al. 1998). In this study, metastatic breast, ovarian, and colorectal cancer patients were given 1 mg of peptide mixed with incomplete Freud's adjuvant subcutaneously every 3-4 weeks for a total of four doses. Three of four patients demonstrated peptide-specific CTL responses; however, these cells did not lyse HER2-positive tumor cells. No clinical response data was disclosed.

Knutson and Disis also investigated the E75 peptide but mixed with GM-CSF which was chosen as an adjunct to stimulate an immune response based on previous animal studies (Disis et al. 1996). It is thought to potentiate the immunologic response by promoting the transformation of dermally present Langerhans cells into mature antigen-presenting dendritic cells (DC). The E75 + GM-CSF vaccine was given to six metastatic breast and ovarian cancer patients (Knutson et al. 2002). The vaccine resulted in E75-specific CTL responses in two of four evaluable patients. No clinical response information was given.

Murray et al then used the same E75 epitope combined with GM-CSF as an adjuvant in a Phase I trial to examine the toxicity of this formulation as well as its ability to promote a detectable CTL-mediated E75-specific immune response (Murray et al. 2003). A total of 14 patients with stage IV breast or ovarian carcinoma underwent vaccination with escalating doses of the peptide combined with 250 mcg GM-CSF. The formulation was found to be safe with no grade 3 or 4 toxicity reported. Eight of those receiving the vaccine were evaluated for CTL activity directed against the E75 peptide, and four of the eight patients showed CTL-mediated lytic activity. Furthermore, these CTL were shown to remain for between one and 12 months following completion of the vaccine. Of interest, these advanced stage patients continued to undergo disease surveillance at short intervals

throughout the study. Despite the promising immunologic response, no clinical response was noted, but there was a trend toward delayed time to progression.

All of the previously described studies were performed primarily in patients with metastatic disease and known residual tumor burden. While they demonstrate that the desired immune response could be elicited in the majority of patients, minimal clinical results were obtained or reported. This is likely related to the advanced stage of disease in the patients in whom these trials were conducted. Our approach has shifted focus from the treatment of late-stage disease to disease prevention among breast cancer patients considered to be at high risk for recurrence. E75 peptide admixed with GM-CSF has been previously shown to be a safe formulation which can induce an immune response. Because of its effectiveness and simplicity of design, this vaccine strategy was investigated as a possible adjunct after standard therapy to help reduce the risk of recurrence.

A total of 53 patients with NP breast cancer were reported initially in our first adjuvant vaccine study (Peoples et al. 2005). All patients had previously undergone traditional therapy including surgery, chemotherapy, and/or radiation therapy as appropriate. At time of enrollment, all patients were without any evidence of disease. Because of the HLA-A2 restriction of the E75 peptide, the patients were HLA typed after enrollment and then placed in either the treatment arm (HLA-$A2^+$) or the observation group (HLA-$A2^-$). Interestingly, there seemed to be some differences in the two groups with HLA-$A2^+$ patients having generally worse prognostic tumor characteristics (larger, higher histologic grade, less hormonal sensitivity). As a result of these differences, the treatment arm tended to have less hormonal therapy (a treatment known to reduce recurrence). These patients received the E75 peptide admixed with GM-CSF according to a two-stage design with escalating doses in the first stage and alterations in the vaccine schedule in the second stage. Again, this vaccination formula was shown to be safe and well tolerated. Clonal expansion of E75-specific CTL was noted along with CTL-mediated E75-directed lysis of HER2/*neu*-expressing tumors. Perhaps most striking, however, were the clinical results as related to disease recurrence. Keeping in mind the generally worse prognostic variables of the treatment arm, there were no deaths among those vaccinated versus two in the observation group. Furthermore, only two of 24 (8%) vaccinated patients developed recurrence versus six of 29 (21%) in the observation group (p=0.19). At a median follow-up of 22 months, the DFS among those vaccinated was 85.7% in contrast to 59.5% for those in the control group. These early results are compelling and indicate a tendency for this vaccination strategy to prevent recurrence among high-risk patients.

The E75 vaccine was also evaluated in NN breast cancer patients in a separate but almost identical trial. In an analysis of the combined results of the two E75 trials to determine the safety and effectiveness of the E75 vaccine (Peoples et al. 2008), a total of 171 patients were enrolled with 90 in the NP trial and 81 in the NN trial. During this trial, the E75 peptide was determined to bind to the HLA-A3 molecule in addition to HLA-A2. As such, the vaccine was extended to HLA-$A3^+$ patients as well. A total of 90 patients received the E75 vaccine (45 NP and 45 NN) while 81 were in the prospectively followed observation arm of the studies (45 NP and 36 NN). Combined toxicity was minimal with local reactions being grade 1 (86%) and grade 2 (14%). Systemic toxicity was grade 0 (16%), 1 (70%), 2 (13%),

and 3 (1%). All patients demonstrated in vitro immunologic responses and in vivo delayed type hypersensitivity (DTH) responses post-vaccination, though variable. For all 171 patients, the clinical recurrence rate for the vaccinated patients was 5.6% (5/90) compared to 14.8% (12/81) for the observation patients (p=0.04) at a median of 20 months. These combined results suggest that the E75 vaccine can be administered safely with minimal local or systemic toxicity. In vitro functional assays and in vivo DTH reactions indicate good immunologic responses to the vaccine, but most importantly, the E75 vaccine appears to reduce recurrences in disease-free, conventionally treated breast cancer patients. These results are similar to the Herceptin results with an approximate 50% reduction in recurrence rates in vaccinated NP and high-risk NN breast cancer patients. However, the vaccine was used in patients with all levels of HER2/*neu* expression, not only those with over-expression.

Recently we have reported updated results from these two trials (Clifton et al. 2010). A total of 188 patients have been enrolled with 109 (58%) HLA-$A2^{+}/A3^{+}$ patients in the vaccine arm and 79 (42%) HLA-$A2/A3^{-}$ patients in the control arm. A 24-month landmark analysis revealed a recurrence rate of 6.5% in vaccinated patients compared to a recurrence rate of 14.5% in control patients (p=0.08). This benefit also appears to be durable with a persistent 35% relative risk reduction in breast cancer recurrences in vaccinated patients at 57 months of median follow-up. In subset analyses, patients found to derive the most benefit from vaccination included HER2 low or intermediate expressors (IHC 1+ or 2+), low grade tumors (grade 1 or 2), patients optimally dosed, and patients who underwent booster inoculations.

The E75 vaccine will next be tested in a large, randomized multi-center Phase III industry-sponsored clinical trial.

With the initial success of E75 in phase II trials and the successful extension of the vaccine population from HLA-A2+ patients only to include HLA-A3+ patients (described above), other preclinical work has been done with E75 and additional common HLA alleles. In fact, Sotiropoulou et al have confirmed CTL precursor frequencies from cancer patients responding to E75 in not only HLA-A2+ and HLA-A3+ patients, but also HLA-A26+ patients. Furthermore, their lab as well as ours has confirmed the algorithm predictions from both the SYFPEITHI & BIMAS systems that E75 binds to HLA-A24 as well. These data suggest that just as the E75 benefit was extended to HLA-A3+ patients, it may also extend to HLA-A24+ and 26+ patients. These two alleles are particularly prevalent in the Asian population. Therefore, we intend to also collect clinical data on the use of the vaccine in these two new patient populations.

1.7 HER2-DIRECTED THERAPIES IN HER2 LOW-EXPRESSING TUMORS

While Herceptin has shown significant clinical benefit in the adjuvant setting for breast cancer patients with HER2 3+ expressing tumors, there has been some suggestion that patients with HER2 2+ expressing tumors may benefit as well from adjuvant Herceptin. In an exploratory analysis of the NSABP trial B-31 (Romond et al. 2005), 174 patients who

were subsequently found to be HER2-negative (negative on FISH and less than IHC 3+) may have derived a benefit from adjuvant Herceptin therapy with a hazard ratio for disease recurrence of 0.34 (95% CI, 0.14 to 0.80; p=0.014) (Paik et al, 2008). This finding has resulted in the initiation of the NSABP trial B-47 to evaluate the efficacy of Herceptin in HER2 low-expressing tumors.

HER2-derived, CD8-eliciting, peptide-based vaccines also appear to have benefit in patients with HER2 low-expressing tumors. Patients from our E75 vaccine trials were stratified by HER2 expression levels (Benavides et al. 2009). Patients with HER2 low-expressing tumors (IHC 1+, IHC 2+ or FISH<2.0) (n=100; 56 vaccine, 44 control) were compared with HER2 overexpressors (n=51; 29 vaccine, 22 control). Low-expressors had larger maximum immunological responses to E75 vaccination than over-expressors (p=0.04). This analysis also found that vaccinated IHC 1+ patients had increased long-term immunity (p=0.08). In addition to improved immunity compared to over-expressors, patients with low-expressing tumors had a mortality benefit compared to low-expressing control patients (p=0.08), a benefit present even in IHC 1+ vaccinated patients (p=0.05).

1.8 COMBINED HER2-DIRECTED THERAPY

While both Herceptin and E75 each have been shown to have clinical benefit in HER2-expressing breast cancer, the novel concept of combining these two immunotherapies is appealing and worthy of investigation. While Herceptin and E75 share the same immunologic target, the specific immunologic mechanisms of action for these therapies are different. This complementary approach to immune-mediated therapy in breast cancer may provide a clinically beneficial synergism.

The combination of Herceptin with HER2-targeted vaccines has been evaluated in preclinical testing (Mittendorf et al. 2006). HER2-expressing tumor cells were incubated overnight with Herceptin in concentrations of 10 mcg/mL and 50 mcg/mL. Cytotoxicity of both pretreated and untreated tumor cells was measured using peripheral blood mononuclear cells from healthy, HLA-$A2^+$ donors as well as from patients vaccinated with E75. Cytotoxicity by peptide-specific cytotoxic T lymphocytes from non-vaccinated patients was increased by 5.6% (10 mcg/mL) and 15.3% (50 mcg/mL) (p=0.002) in pretreated cells over untreated cells. Using peripheral blood mononuclear cells from E75-vaccinated patients, peptide-specific cytotoxicity was 34.2% in untreated cells compared to 40.6% (10 mcg/mL) (p=0.035) and 40.7% (50 mcg/mL) (p=0.0005) in pretreated cells. These findings suggest a synergism may exist with the combination of Herceptin and HER2 vaccination.

The safety of the combination of Herceptin 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 metastatic breast cancer patients over-expressing HER2 (n=22) was found to be safe (Disis et al. 2009). Over 99% of toxicity was grade 1 or 2. Median LVEF was unchanged pre- to post-treatment, with three patients (15%) experiencing asymptomatic decreases in LVEF; no patient experienced symptoms of left ventricular dysfunction. Experience in our Phase I trial evaluating the combination of

trastuzumab and a cytotoxic T cell-eliciting HER2 peptide vaccine in the adjuvant setting has not shown any added cardiac toxicity with combination therapy during vaccine dose escalation. Finally, in preliminary Phase II trial results of the combination of Herceptin and either HER2 $CD4^+$ helper or $CD8^+$ T cell-eliciting vaccines conducted by our group, no additive cardiac toxicity has been found (Mittendorf et al. 2010).

These early clinical trials have shown 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 (Disis et al. 2004). Finally, we have investigated sequential therapy with Herceptin followed by HER2 vaccination in the adjuvant setting in our ongoing Phase II trials. Of 62 patients who received standard of care Herceptin, the 32 who received no vaccine have experienced a 12.5% breast cancer recurrence rate (4/32) – comparable with reported rates of similarly staged and treated patients. In contrast, of the 30 patients who were vaccinated with either E75+GM-CSF (n=12) or GP2+GM-CSF (n=18) after completing Herceptin therapy, the recurrence rate is 0% (0/30) (p=0.065) (Sears et al. 2011).

The emerging evidence suggesting that patients with low-expressing HER2 breast cancer respond to Herceptin or HER2 vaccination combined with the encouraging results suggesting synergism in combination therapy are intriguing and are worthy of further investigation. Therefore, we believe that HER2 1+ and 2+ breast cancer patients, who have already shown a response to vaccination and possibly to Herceptin alone, could potentially show a dramatic response to the combination of Herceptin and E75 with the immunoadjuvant GM-CSF and that the combination can be delivered safely.

2. OBJECTIVES

In this study, we intend to assess the ability of the combination of Herceptin and the HER2 vaccine E75 (administered with the immunoadjuvant GM-CSF) given in the adjuvant setting to prevent recurrences in NP (or NN if negative for both estrogen (ER) and progesterone (PR) receptors) breast cancer patients with tumors that express low (1+) or intermediate (2+) levels of HER2. Enrolled patients will be randomized to receive Herceptin and E75 + GM-CSF or Herceptin with GM-CSF alone (no E75). The safety of the combination therapy will be documented, specifically to ensure that no additive cardiac toxicity results from combination HER2-directed therapy. Efficacy will be documented by comparing the DFS and immunological responses between treatment groups.

2.1 PRIMARY OBJECTIVES

The primary efficacy endpoint is to compare DFS at 24 months between treatment groups. The primary safety issue is to prove there is no additive cardiac toxicity with combination HER2-directed therapy.

2.2 SECONDARY OBJECTIVES

A secondary endpoint of the trial is to compare DFS at 36 months. Immunologic responses to the vaccine will also be documented and correlated to clinical benefit.

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

The study will be a multi-center, prospective, randomized, single-blinded, placebo-controlled Phase II trial of Herceptin + E75/GM-CSF versus Herceptin + GM-CSF alone. The vaccine to be used in this study, E75, is investigational and will be used in combination with Herceptin 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 NP (or NN if negative for both ER and PR) breast cancer patients with HER2 1+ and 2+ expressing tumors who are disease-free after standard of care therapy. Disease-free subjects after or during standard of care multi-modality therapy will be screened and HLA-typed (consent #1). E75 is a CD8-eliciting peptide vaccine that is restricted to HLA-$A2^+$ or HLA-$A3^+$ patients (approximately two-thirds of the US population).

HLA-$A2^+$/$A3^+$/$A24^+$/or $A26^+$ patients who meet all other eligibility criteria will be randomized to receive Herceptin + E75/GM-CSF or Herceptin + GM-CSF alone (consent #2). For both groups, Herceptin will be given every three weeks as monotherapy for one year, to be given upon completion of standard of care chemotherapy/radiotherapy (Appendix A). The first Herceptin infusion must be given no sooner than three weeks and no later than 12 weeks after completion of standard of care therapy. Herceptin will be dosed at the recommended initial loading dose of 8 mg/kg and at recommended maintenance doses of 6 mg/kg q3wk. Herceptin will be administered as described in Section 4.3. Patients randomized to the E75/GM-CSF arm will receive vaccinations of E75 (1000 mcg) and GM-CSF (250 mcg) administered intradermally every three weeks for six total vaccinations, 30 -120 minutes after completion of Herceptin infusion. With prior approval from the Principal Investigator, the vaccine may be administered 15 minutes after completion of Herceptin infusion. The E75/GM-CSF vaccine series will begin in conjunction with the third Herceptin infusion. Starting the vaccine series after two rounds of Herceptin alone will allow for evaluation and observation for toxicities related specifically to Herceptin. In extenuating circumstances, the first vaccination may be delayed to the fourth or fifth Herceptin infusion with prior approval from the Principal Investigator. Those patients randomized to the GM-CSF alone arm will receive vaccinations of GM-CSF (250 mcg) administered in an identical manner to those receiving E75/GM-CSF. Patients will be blinded as to whether they are receiving E75/GM-CSF or GM-CSF alone.

Upon completion of the vaccination series, booster inoculations (same dose and route) will be administered every six months x4 for total combination (Herceptin and vaccine) treatment duration of 30 months (Appendix A). The first booster inoculation will occur with the final Herceptin infusion, with subsequent boosters timed every six months from the first booster. Booster inoculations will occur for patients randomized to receive E75/GM-CSF as well as patients randomized to receive GM-CSF alone, and will consist of the same treatment drugs and dosing (i.e., E75/GM-CSF patients will be boosted with E75/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 be assessed 48-72 hours after each inoculation for reaction to the inoculation as well as documentation of any adverse effects experienced (Appendix A). 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 300 patients (150 in each treatment arm) at a planned accrual rate of 10-12 patients per month (approximately 0.5-1 enrollment per study site per month). With accrual beginning in approximately April 2013, enrollment of the last patient would be expected in approximately August 2017 followed by a three-year follow-up period. The duration of the trial is expected to be seven years.

3.2 RATIONALE FOR STUDY DESIGN

The recurrence rate among NP breast cancer patients with HER2 1+ and 2+ expressing tumors after standard of care therapy is reported to be 20% at 24 months. Herceptin alone (with GM-CSF) may have some benefit and decrease the rate to 15% (Baselga et al. 1998, Burstein et al. 2001, Cobleigh et al. 1999, Kuzur et al. 2000, Nicholson et al. 2000, Paik et al. 2008, Pegram et al. 2000, Pegram et al. 1997, Piccart-Gebhart et al. 2005, Romond et al. 2005, Seidman et al. 1999, Seidman et al. 2001). However, based on preliminary data, the combination of Herceptin + E75/GM-CSF virtually eliminated recurrences. For planning purposes, if the combination reduces the recurrence rate to 5%, then in order to show a statistical difference between 15% vs. 5%, a sample size of 150 patients per arm would be required or a study size of 300 patients (see statistical plan).

3.3 OUTCOME MEASURES

3.3.1 Primary Outcome Measure

The primary clinical outcome of DFS will be determined by patients' own physicians in the medical and surgical and/or the hematology/oncology clinics 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 and radiographic surveillance (e.g., CT, bone, and mammographic scans) as indicated. 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 after initiation of Herceptin therapy. The recurrence rate and time to recurrence from date of initial Herceptin treatment for the patients randomized to receive Herceptin + E75/GM-CSF will be compared to the cohort of patients randomized to receive Herceptin + GM-CSF alone. The primary objective of the trial is DFS at 24 months with a secondary objective of DFS at 36 months.

3.3.2 Secondary Outcome Measures

Secondary outcome measures include immunologic responses and safety of combination HER2 therapy. Immune responses will be primarily documented using the DTH reaction but also may be measured using the dimer assay and ELISPOT assay. Each of these measurements would be performed regardless of randomization. Detailed descriptions of these assays/tests are described in Section 4.4.4 – 4.4.9. Dimer and ELISPOT measurements may be performed prior to initiating the vaccination series as well as prior to the fourth inoculation, and at one month after completion of the primary series. Additionally, these assays may be performed pre- and post- each booster and at completion of the study. Alternatively, these assayed timepoints may also be performed all at once on frozen cells and may be collected from selected sites only. DTH reactions will be measured prior to initiation of the vaccination series, 3-4 weeks after completion of the primary series, and 3-4 weeks after the second and fourth boosters (Appendix A). Immunologic responses in the patients randomized to receive Herceptin + E75/GM-CSF will be compared to the cohort of patients randomized to receive Herceptin + GM-CSF alone.

Cardiac toxicity of combination therapy will also be assessed. Each patient, regardless of randomization, will undergo cardiac assessment (ejection fraction) at baseline (MUGA preferred, ECHO allowed, consistency required) and at 3, 6, 12, and 24 months. Cardiac assessment will continue every six months if a patient experiences a greater than 10% reduction from baseline for the duration of the trial or until resolution.

3.3.3 Ancillary Safety Outcome Measures

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 B). For both the regular inoculations and the booster inoculations, patients will be monitored for 30 minutes after the inoculation with vital signs taken as needed. Patients will either return to the vaccine clinic at their respective 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 adverse events will be reported as described in Section 5.

3.4 SAFETY PLAN

Patients will be evaluated at each study visit for the duration of their participation in the study (Appendix C.1, C.2).

Specific potential safety issues for this trial are outlined below.

3.4.1 Cardiac Dysfunction

Signs and symptoms of cardiac dysfunction were observed in a number of women who received Herceptin alone or in combination with chemotherapy, most often anthracycline-based treatment. Cardiac dysfunction was observed most frequently among patients who received Herceptin plus AC chemotherapy (28%), compared with those who received AC alone (7%), Herceptin plus paclitaxel (11%), paclitaxel alone (1%), or Herceptin alone (7%). Severe disability or fatal outcome due to cardiac dysfunction was observed in ~1% of all patients.

The nature of the observed cardiac dysfunction was similar to the syndrome of anthracycline-induced cardiomyopathy. The signs and symptoms of cardiac dysfunction usually responded to treatment. Complete and partial responses were observed among patients with cardiac dysfunction. The risk appears to be independent of tumor response to therapy. Analysis of the clinical database for predictors of cardiac dysfunction revealed only advanced age and exposure to an anthracycline as possible risk factors. In the clinical trials, most patients with cardiac dysfunction responded to appropriate medical therapy, often including discontinuation of Herceptin. In many cases, patients were able to resume treatment with Herceptin. In a subsequent study using weekly paclitaxel and Herceptin as first-line treatment for metastatic breast cancer, the observed incidence of serious cardiac dysfunction was 3% (n=95) (Seidmen et al. 2001). Since the occurrence of cardiac dysfunction in the Herceptin plus chemotherapy trial was an unexpected observation, no information is available regarding the most appropriate method for monitoring cardiac function in patients receiving Herceptin. Significant advances in the understanding and treatment of CHF have been made in the past several years, with many of the new drugs demonstrating the ability to normalize cardiac function. Patients who develop symptoms of CHF while on Herceptin should be treated according to the HFSA guidelines (Appendix D).

3.4.2 Management of Cardiac Safety

3.4.2.1 Adjuvant Breast Cancer Protocols

All patients must have a cardiac assessment at baseline (MUGA preferred, ECHO allowed, consistency required), and on a regular schedule throughout the course of the study. Investigators are strongly urged to schedule cardiac assessment studies at the same radiology facility where the patient's baseline assessment was done whenever possible. Cardiac assessment studies are required at protocol-specified time points and after any patient has any of the following: discontinuation of protocol therapy, CHF, breast cancer recurrence, or a second primary cancer.

Post-surgical radiation therapy may be required in patients at risk for recurrence. Whenever possible, irradiation to the internal mammary nodes should be avoided because of the concern for possible additional cardiotoxicity from the combination of Herceptin and radiation therapy. Efforts should be taken to ensure that the volume of the heart irradiated is minimal. Investigators are encouraged to discuss cardiac toxicity concerns with their radiation oncologists to ensure careful planning of the ports of ***left-sided*** lesions.

Cardiac Safety Criteria for initiation of Herceptin

Asymptomatic Patients

If a patient does not have significant symptoms related to left ventricular (LV) dysfunction, administration of Herceptin will depend on the absolute change in LVEF between baseline and follow-up assessments.

Herceptin should be initiated in an asymptomatic patient if:

a) The LVEF increased or stayed the same

b) The LVEF decreased by ≤15 percentage points but is still at or above the lower limit of normal for the radiology facility

Herceptin is ***PROHIBITED*** in an asymptomatic patient if:

a) The LVEF decreased ≤15 percentage points and is **below** the limit of normal for the radiology facility

b) The LVEF decreased by 16 percentage points or more (regardless of lower limits of normal for the radiology facility)

Symptomatic Patients

If a patient has significant symptoms related to LV dysfunction, cardiac ischemia, or arrhythmia, initiation of Herceptin is prohibited.

Management of Symptomatic Cardiac Changes. Patients who develop signs and symptoms of CHF will be taken off treatment, but continued in the study for disease follow-up. They will receive treatment for CHF as prescribed by the HFSA (e.g., ACE inhibitors, angiotensin-II receptor blockers, β-blockers, diuretics, and cardiac glycosides, as needed; see Appendix D for HFSA guidelines). Consideration should be given to obtaining a cardiac consultation. The patient will be followed until resolution as per treating physician and reported upon resolution of symptoms.

Management of Asymptomatic Decreases in LVEF. Herceptin may be continued in patients experiencing an asymptomatic absolute decrease in LVEF of <20 percentage points from baseline, when the ejection fraction remains within the imaging center's range of normal limits. Repeat measures of LVEF should be obtained using the methodology selected at baseline. Close follow-up of such patients is recommended. Patients with an asymptomatic absolute 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. They will be considered for treatment of incipient CHF as prescribed by the HFSA (e.g., ACE inhibitors, angiotensin-II receptor blockers, β-blockers, diuretics, and cardiac glycosides, as needed; see

Appendix D for HFSA guidelines). In light of the variability inherent in the assessment of ejection fraction, consideration should be given to repeating the study to confirm an observed decline. Repeat measures of LVEF should be obtained using the same methodology selected at baseline. If Herceptin has been discontinued for an asymptomatic decline in LVEF, a repeat measure of LVEF will be obtained in one month to determine if the decline has resolved.

Infusion-Associated Symptoms. During the first infusion with Herceptin, a symptom complex consisting of chills and/or fever is observed in approximately 40% of patients. Other signs and/or symptoms may include nausea, vomiting, pain, rigors, headache, cough, dizziness, rash, and asthenia. These symptoms are usually mild to moderate in severity, and occur infrequently with subsequent Herceptin infusions. These symptoms can be treated with an analgesic/antipyretic such as meperidine or paracetamol, or an antihistamine such as diphenhydramine.

Serious Infusion-Associated Events. Serious adverse reactions to Herceptin infusion including dyspnea, hypotension, wheezing, bronchospasm, tachycardia, reduced oxygen saturation and respiratory distress have been reported infrequently. In rare cases (4 per 10,000), these events were associated with a clinical course culminating in a fatal outcome. Serious reactions have been treated with supportive therapy such as oxygen, beta-agonists, corticosteroids and withdrawal of Herceptin as indicated.

Hematologic Toxicity and Neutropenic Infections. In the clinical trials, an increased incidence of anemia was observed in patients receiving Herceptin 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 Herceptin therapy.

In the clinical trials, the per-patient incidences of moderate to severe neutropenia and of febrile neutropenia were higher in patients receiving Herceptin 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 Herceptin 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; the effect of Herceptin on the pharmacokinetics of chemotherapeutic agents has not been fully evaluated.

Secondary acute leukemia or myelodysplastic syndrome has been reported in 4 of approximately 1200 patients who participated in Herceptin clinical trials. Patients treated with chemotherapeutic agents are known to be at increased risk for secondary leukemia. The observed incidence of leukemia among Herceptin 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 Herceptin 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.

Please refer to the HERCEPTIN® Investigator Brochure for a detailed description of the safety profile of Herceptin.

See Section 5 for complete details of the safety evaluation for this study.

3.5 Dose Reductions of Vaccine Component

For patients experiencing robust local reactions (>100 mm x 100 mm) or greater than or equal to grade 3 local or systemic reactions or greater than or equal to grade 2 hypersensitivity reactions (as defined by NCI CTCAE version 4.03 graded toxicity scale (Appendix B)), the dose of GM-CSF will be reduced by 50% on 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 E75 peptide dose (if applicable) will then be reduced by 50%.

In our prior trial, approximately 10% of patients developed delayed hypersensitivity reactions (generalized urticaria 10-14 days after booster inoculation). 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.

Dose reductions will continue for booster inoculations in the same manner as performed in the primary vaccination series, 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.

3.6 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 and U.S. Army ethical and legal requirements.

4. MATERIALS AND METHODS

4.1 SUBJECTS

Female patients (n=300) over the age of 18 years with a diagnosis of NP (or NN if negative for both ER and PR) breast cancer who have HER2 tumors which are 1+ or 2+ by IHC will be targeted. All patients will have completed primary surgical and medical therapies. Patients will be recruited from the medical and surgical oncology and/or the hematology/oncology clinics at the individual study sites. All patients will be properly consented.

4.1.1 Subject Selection

Potentially eligible patients, specifically NP (or NN if negative for both ER and PR) breast cancer patients with HER2 1+ or 2+ expressing tumors will be identified by staff in the medical and surgical and/or the hematology/oncology clinics at the individual study sites. IHC testing of primary tumor is necessary for trial inclusion. To be eligible for enrollment, patients must have HER2 1+ or 2+ tumors. For those with HER2 2+ tumors, FISH or Dual-ISH testing must also be performed. FISH or Dual-ISH results must be non-amplified (or ≤2.0). HER2 2+ patients with FISH or Dual-ISH amplification (>2.0) will not be eligible for this trial.

Patients who undergo neoadjuvant treatment regimens will be permitted in this trial. For patients who undergo pretreatment, eligibility will be determined from pretreatment clinical staging (or final pathologic staging if patient is up-staged).

NP and NN patients must ultimately complete standard of care chemotherapy treatment per the National Comprehensive Cancer Network (NCCN) guidelines (Appendix E). NN patients with ER/PR-negative tumors must undergo chemotherapy as dictated by standard of care for inclusion. NN patients in whom chemotherapy is not administered will not be eligible for enrollment.

A research nurse coordinator and/or study coordinator will approach these patients about being in the trial and will introduce the trial to the prospective volunteer. If the volunteer is interested and appears eligible, the nurse will arrange an appointment 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. This screening process may begin prior to the patient completing their standard of care therapy.

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 research nurse coordinator and/or study coordinator, or Principal Investigator (PI), 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 (FACS analysis performed in the Cancer Vaccine Development Laboratory [CVDL], Department of Surgery, Uniformed Services University of the Health Sciences [USUHS], Bethesda, MD. Alternatively, the analysis will be performed at M.D. Anderson Cancer Center (MDACC) by Dr. Beth Mittendorf. If a patient submits a prior HLA-2/A3/A24/A26 positive typing report from an outside CLIA certified lab, this will be accepted for screening. Only HLA-A2, A3, A24, or 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:

- Women 18 years or older
- Node-positive breast cancer (AJCC N1, N2, or N3)
- Node-negative breast cancer if negative for both estrogen (ER) and progesterone (PR) receptors and have received chemotherapy as standard of care
- Clinically cancer-free (no evidence of disease) after standard of care therapy (surgery, chemotherapy, radiation therapy as directed by NCCN guidelines). Hormonal therapy will continue per standard of care. Neoadjuvant chemotherapy is allowed.
- Recovery from any toxicity(ies) associated with prior adjuvant therapy.
- HER2 expression of 1+ or 2+ by IHC. FISH or Dual-ish testing must be performed on IHC 2+ tumors and shown to be non-amplified by FISH (≤2.0) or by Dual-ish (≤2.0).
- HLA-A2, A3, A24, or A26 positive
- LVEF ≥50%, or an LVEF within the normal limits of the institution's specific testing (MUGA or ECHO)
- ECOG 0,1
- Signed informed consent
- Adequate birth control (abstinence, hysterectomy, bilateral oophorectomy, bilateral tubal ligation, oral contraception, IUD, or use of condoms or diaphragms)
- Must start study treatment (receive first Herceptin infusion) between 3-12 weeks from completion of standard of care therapy.

4.1.3 Exclusion Criteria

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

- Node-negative breast cancer (AJCC N0 or N0(i+)) unless negative for both estrogen (ER) and progesterone (PR) receptors and has received chemotherapy as standard of care
- Clinical or radiographic evidence of distant or residual breast cancer
- HER2 negative (IHC 0) or HER2 3+ or FISH/Dual-ish amplified (FISH >2.0; Dual-ish >2.0)
- HLA-A2, A3, A24 and A26 negative
- History of prior Herceptin therapy
- NYHA stage 3 or 4 cardiac disease
- LVEF <50%, or less than the normal limits of the institution's specific testing (MUGA or ECHO)
- Immune deficiency disease or HIV, HBV, HCV
- Receiving immunosuppressive therapy including chemotherapy, chronic steroids, methotrexate, or other known immunosuppressive agents
- ECOG ≥2
- Tbili >1.8, creatinine>2, hemoglobin<10, platelets<50,000, WBC<2,000
- 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
- Involved in other experimental protocols (except with permission of the other study PI)

4.2 METHOD OF TREATMENT ASSIGNMENT

If volunteers 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 designated Cancer Insight staff using a computer-generated randomization table to receive Herceptin + E75/GM-CSF or Herceptin + GM-CSF alone. Randomization between the two treatment groups will occur in a 1:1 allocation ratio using an institutional balancing algorithm. Randomization will be stratified by HER2 status (1+ or 2+) and nodal staging (N0 or N1 vs, N2 or N3) (Appendix F). Patients will be blinded as to whether they are receiving Herceptin + E75/GM-CSF or Herceptin + GM-CSF alone.

4.3. STUDY TREATMENT

Commercial Herceptin drug will be used and provided free of charge by Genentech. The Sponsor/Investigator of the study will ensure maintenance of complete and accurate records of the receipt, dispensation, and disposal or return of all study drugs in accordance

with 21 Code of Federal Regulations (C.F.R.), Part 312.57 and 312.62 and Genentech requirements.

4.3.1 Herceptin Formulation

Herceptin is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. Each vial of Herceptin contains 400 mg of tratuzumab, 9.9 mg of L-histidine HCl, 6.4 mg of L-histadine, 400 mg of α,α-trehalose dihydrate, and 1.8 mg of polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI) USP, containing 1.1% benzyl alcohol as a preservative, yields 21 mL of a multidose solution containing 21 mg/mL trastuzumab, at a pH of ~6.

4.3.2 Herceptin Dosage, Preparation, Administration, and Storage

a. Dosage

The recommended initial loading dose is 8 mg/kg (for q3wk dosing schedules) Herceptin administered as a 90-minute infusion. The recommended maintenance Herceptin dose is 6 mg/kg q3wk and can be administered as a 30-minute infusion if the initial loading dose was well tolerated. Herceptin may be administered in an outpatient setting. DO NOT ADMINISTER AS AN IV PUSH OR BOLUS (see ADMINISTRATION). If different from above the specific dose and regimen of Herceptin to be used should be described here. For this study, a window of +/- 3 days is permitted on the every 3 week dosing schedule.

b. Preparation

Use appropriate aseptic technique. Each vial of Herceptin should be reconstituted with 20 mL of BWFI, USP, 1.1% benzyl alcohol preserved, as supplied, to yield a multidose solution containing 21 mg/mL Herceptin. Immediately upon reconstitution with BWFI, the vial of Herceptin must be labeled in the area marked “Do not use after” with the future date that is 28 days from the date of reconstitution.

If the patient has known hypersensitivity to benzyl alcohol, Herceptin must be reconstituted with Sterile Water for Injection (SWFI) (see PRECAUTIONS). Herceptin which has been reconstituted with SWFI must be used immediately and any unused portion discarded. Use of other reconstitution diluents should be avoided.

Determine the dose of Herceptin needed, based on a loading dose of 8 mg Herceptin/kg body weight or a maintenance dose of 6 mg Herceptin/kg body weight for q3wk dosing schedules. Calculate the correct dose using 21 mg/mL Herceptin solution. Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% sodium chloride, USP. **DEXTROSE (5%) SOLUTION SHOULD NOT BE USED.** Gently invert the bag to mix the solution. The reconstituted preparation results in a colorless to pale yellow transparent solution. Parenteral drug

products should be inspected visually for particulates and discoloration prior to administration.

No incompatibilities between Herceptin and polyvinylchloride or polyethylene bags have been observed.

c. Administration

Treatment may be administered in an outpatient setting by administration of a 8 mg/kg Herceptin loading dose by IV infusion over 90 minutes. **DO NOT ADMINISTER AS AN IV PUSH OR BOLUS.** If Herceptin is being administered concomitantly with chemotherapy, Herceptin administration should precede chemotherapy administration. Patients should be observed for fever and chills or other infusion-associated symptoms (see ADVERSE REACTIONS). If prior infusions are well tolerated subsequent doses of 6 mg/kg Herceptin q3wk (a window of +/- 3 days is permitted) may be administered over 30 minutes.

Table 1

Herceptin Infusion Time and Post-Infusion Observation Period

Herceptin Dose		Infusion Time (minutes)	Post-Infusion Observation Period (minutes)
First infusion	8 mg/kg	90	60
Second infusion	6 mg/kg	30 [a]	30 [a]
Third and subsequent infusions	6 mg/kg	30 [a]	None [a]

[a] Only if previous dose was well tolerated.

Herceptin should not be mixed or diluted with other drugs. Herceptin infusions should not be administered or mixed with Dextrose solutions.

d. Storage

Vials of Herceptin are stable at 2°C–8°C (36°F–46°F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of Herceptin reconstituted with BWFI, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2°C–8°C (36°F–46°F), and the solution is preserved for multiple use. Discard any remaining multi-dose reconstituted solution after 28 days. If unpreserved SWFI (not supplied) is used, the reconstituted Herceptin solution should be used immediately and any unused portion must be discarded. DO NOT FREEZE HERCEPTIN THAT HAS BEEN RECONSTITUTED.

The solution of Herceptin for infusion diluted in polyvinylchloride or polyethylene bags containing 0.9% sodium chloride for injection, USP, may be stored at 2°C–8°C

(36°F–46°F) for up to 24 hours prior to use. Diluted Herceptin has been shown to be stable for up to 24 hours at room temperature 15°C–25°C; however, since diluted Herceptin contains no effective preservative the reconstituted and diluted solution should be stored refrigerated (2°C–8°C).

4.3.3 Herceptin Dosage Modification

Dose modification of Herceptin is not permitted.

4.3.4 Herceptin Overdosage

There is no experience with overdosage in human clinical trials. Single doses higher than 500 mg have not been tested.

4.3.5 Vaccine

a. Dosage and Preparation

The E75 vaccine (HER2/neu p369-377) will be supplied by Galena Biopharma. The E75 acetate drug substance is a 9-amino acid peptide produced by solid-phase peptide synthesis. E75 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. The control is Water For Injection (WFI). Prior to administration, 0.8 mL of the E75 acetate or control is mixed by the pharmacist or research nurse with 1 mL of reconstituted lyophilized Leukine® (250 µg GM-CSF/mL in solution). Immediately after mixing, the study drug is withdrawn into four 1 mL syringes and within 6 hours, a total of 1.6 mL is administered as 4 intradermal injections. The control vial contains 1 mL WFI. The guidance for mixing E75 (or control) solution from lyophilized Leukine® 250 µg and test drug (E75 solution or control) is stated in Appendix G. Galena Biopharma, Inc. will supply both agents, the E75 and the GM-CSF.

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 H) for additional details.

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.

In the United States, GM-CSF is commercially available as Leukine® (sargramostim,expressed in yeast). In some countries, GM-CSF is commercially available as molgramostim (expressed in bacteria). For this study, Leukine® will be provided and exclusively used. The Package Insert for Leukine® is enclosed in this Protocol as Appendix H.

Allow the E75 acetate or control to warm to room temperature and the Leukine® to warm to room temperature. Using aseptic technique with a 1 mL syringe and provided needle, withdraw 0.8 mL from the test drug vial (E75 or control) and inject the syringe containing 0.8 mL of test drug into the reconstituted Leukine. Keeping the vial upright, slowly roll the Leukine/Test Drug vial between your hands. Allow any foaming to dissipate before continuing. Do not invert the vial. This assures the complete mixing of the E75 acetate or control and Leukine® solutions (see detailed guidance in Appendix G for vaccine and DTH preparation). Discard the mixing syringe and needle.

Once E75 or the control have been mixed, the four provided 1 mL syringes are used to withdraw equal volumes into each, and thus a total of 1.6 mL of the solution is withdrawn for administration. The syringes are now ready to be used for inoculation and **must be used within 6 hours of mixing**.

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.

b. 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.

4.3.6 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.

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 Herceptin treatment will occur as outlined in Section 3.1 (see also Appendix A). 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.

4.3.7 Booster Inoculations

After completion of the six-inoculation primary vaccine series, patients will then receive a total of four booster inoculations to be administered, every 6 months x4 for total combination (Herceptin and/or Vaccine) treatment duration of 30 months (Appendix A). As discussed in Section 3.1, the first booster inoculation will occur at the time of the final Herceptin infusion with subsequent boosters timed every 6 months (± 2 weeks) from the first booster and should be 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.

All patients will undergo a cardiac assessment at baseline (MUGA preferred, ECHO allowed, consistency require) and at 3, 6, 12, and 24 months. Cardiac assessment will continue every six months if a patient experiences a greater than 10% reduction from baseline for the duration of the trial or until resolution. There will be an allowable window of +/- 2 weeks for each study interval assessment test, beginning from the baseline MUGA or ECHO.

All patients must have a Complete Blood Count (CBC) and Comprehensive Metabolic Panel (CMP) (20 cc of blood) within two months of trial initiation (date of enrollment for full participation) and, for female patients, a urine pregnancy test (upon consent to study), for screening and immediately prior to any vaccine inoculations or DTH. Urine pregnancy tests will not be required for Herceptin infusions alone. If the pregnancy test is positive, the patient will be excluded from the study. Women who have had a hysterectomy, bilateral oopherectomy, 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. 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 I).

To ensure eligibility, each patient will be HLA-typed. They will be asked for 6-8 cc of blood for HLA-A2/A3/A24/A26 screening (FACS analysis performed in the CVDL at USUHS in Bethesda, MD or alternatively at UT M.D. Anderson as described in Section 4.4.5).

All patients regardless of randomization may be assessed for baseline immunologic responses prior to receiving the first dose of Herceptin and prior to the first inoculation. This may occur at selected sites. Specimen handling, processing, and assays are described in Sections 4.4.3, 4.4.4, 4.4.6 and 4.4.7.

All patients will be tested for pre-inoculation DTH reaction against the E75 vaccine peptide as described in Section 4.4.9. Reactions will be read 48-72 hours after placement of the peptide.

4.4.1 Assessments during Treatment

All patients will undergo cardiac assessment as per Section 3.4 at 0, 3, 6, 12, and 24 months, +/- a 2 week window at each interval test, beginning from the baseline study. Cardiac assessment will continue every six months if a patient experiences a greater than 10% reduction from baseline for the duration of the trial. The safety assessments will be evaluated by the site-specific study investigators. They will be responsible for assessment of patients with any toxicity reported to, or identified by, the study coordinators. The coordinators will assess the patients prior to and after Herceptin infusion, prior to and after vaccine inoculation, and will assess the patient 48-72 hours after vaccine inoculation, and on an "as needed" basis. The investigators will review all ECHO or MUGA results done at protocol time points, as well as any abnormal laboratory or image findings.

For both the regular inoculations and booster series of shots, the patients will be monitored for 30 minutes following inoculation and 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 the 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 B) will be utilized to assess local and systemic toxicity.

Blood samples will be obtained from all patients, regardless of randomization, to determine in vivo induction of peptide-specific immune responses as described in Sections 4.4.3, 4.4.4, 4.4.6 and 4.4.7. This may occur at selected sites.

4.4.2 Follow-Up Assessments

The clinical endpoint of recurrence will be determined by patients' own physicians in the medical and surgical and/or the hematology/oncology clinics at the individual study sites during routine follow-up screening of all enrolled patients, regardless of randomization, every three months for the first 24 months after completion of primary therapies and every six months thereafter with clinical exam, laboratory and radiographic surveillance, e.g., CT, bone, and mammographic scans as indicated. This follow-up strategy is standard of care for these patients. The determination of recurrence will be per standard of care by the treating/referring 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 treatment for the recurrence is initiated by the treating physician. Documentation of the latter will be obtained. Disease free survival (DFS) as described by the NCI is the length of time after treatment for a specific disease during which a patient survives with no sign of the disease. For the purposes of this trial, the DFS will be calculated from initiation of Herceptin therapy until date of last follow-up. 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 Herceptin therapy. This data will be added to the patient database.

4.4.3 Human Biological Specimens

In order to determine in vitro induction of peptide-specific immune responses in the vaccinated patients and GM-CSF-specific responses in the immunoadjuvant only patients, 50 cc of blood will be drawn prior to the first Herceptin dose, prior to first DTH #1 inoculation in the series, and before the fourth inoculation for immunologic studies to be performed in the CVDL at USUHS or at MDACC (Appendix A). In addition, 50 cc of blood will be drawn at 3-4 weeks after completion of the primary inoculation series for immunologic studies. Thus, a total of 200 cc of blood will be drawn related to the primary inoculation series, at selected sites. De-identified patient blood samples showing only the unique study ID number are sent from study sites via overnight delivery to the CVDL/MDACC where immunologic response assays (see Sections 4.4.4, 4.4.6 and 4.4.7) are performed. At no point will CVDL/MDACC personnel have access to patient identifiers. All assays performed at

the CVDL/MDACC are for research purposes only. These functions are conducted under a separate protocol approved by the USUHS Research Office as constituting non-human patients research or similar protocol at MDACC. Fifty cc of blood will also be drawn before administration of each booster inoculation and as well as at 3-4 weeks post-inoculation to record the immunologic response to each of 4 booster inoculations for a total of 400 cc of blood. Therefore, a maximum of up to 600 cc of blood will be drawn from all patients regardless of randomization over 36 months for immunologic response assessment. This blood will be in addition to the clinical laboratory tests (CBC and CMP) needed for enrollment screening. The clinical tests will require a total of 20 cc. The HLA typing requires 6-8 cc. Therefore, a total of 628 cc of blood will be required for the entire study.

As noted above, de-identified blood tubes for research purposes will be labeled only with the patients' unique study number and sent via overnight delivery to the CVDL/MDACC. At no point will CVDL/MDACC personnel have access to patient identifiers. Excess blood not used in assays will be frozen and stored under the unique protocol number and identifier for up to five years from collection for additional immunologic studies as needed. No genetic testing will be performed on this material, and it will not be made available to any other unrelated investigator. Peripheral blood mononuclear cells (PBMC) can be isolated by ficoll gradient separation. A small number may be tested immediately for peptide recognition. Some can be utilized to expand peripheral blood lymphocytes (PBL) in culture in low dose IL-2 and stimulated as necessary under different conditions. Evidence of immunologic recognition of the vaccinated peptide may be assessed by clonal expansion (dimer or tetramer assays) and functional assays such as cytokine release/production (ELISPOT). Other immunologic assays may be selectively assessed as well (e.g., antibody production). Additionally, excess blood may also be utilized to assess new generations of vaccines.

4.4.4 Specimen Processing

Peripheral blood from patients are collected into BD Vacutainer CPT Cell Preparation Tubes (BD, Franklin Lakes, NJ) 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. For the propagation of cultures the PBMC will be suspended at 1-2 x 10^6 cells/ml with or without 50 IU/ml of IL-2 (R&D Systems, Minneapolis, MN) being added at the beginning or at a later time. The cell cultures are placed in a humidified incubator at 37ºC in 5% CO_2 and maintained with the addition of media as needed and/or IL-2 every 2-3 days depending on the growth kinetics.

4.4.5 HLA-A2/A3/A24/A26 Testing

The expression of HLA-A2 by the patients is confirmed by staining peripheral blood mononuclear cells (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-phycoerythrin. After incubation the cells are washed and analyzed on a FACSCanto II flow cytometer (BD Biosciences, San Diego, CA).

4.4.6 Dimer Assay

Fresh PBMC can be isolated and stained with HLA-A2:Ig DimerX reagent (Dimer) (BD PharMingen). The HLA-A2:Ig DimerX is a recombinant fusion protein of two extracellular MHC Class I HLA-A2 domains that are fused together with the VH regions of mouse IgG1. The Dimer will be "loaded" with the E75 or control peptides (E37 and Flu-M) and then incubated with fresh PBMC. The PBMC will then be stained with FITC-labeled anti-mouse IgG1 and anti-CD8 PE and analyzed with two-color flow cytometry to determine the percentage of $CD8^+$ T cells which specifically recognize the E75 peptide.

4.4.7 ELISPOT Assay

Freshly isolated PBMC are cultured/stimulated overnight in complete medium (RPMI + 5 %FCS + PSG) supplemented with IL-7 (20 ng/mL) with the individual peptides at 25 µg/mL (E37, E75, GP2, Flu-M) or (AEN, AE36, AE37, tetanus toxoid) or PMA + Ionomycin in flat-bottom anti-human IFN-γ ELISPOT plates (BD PharMingen) at 5 x 10^5 cells/well/200 µL in duplicate wells. The plate is incubated at 37ºC overnight after which the wells will be washed and incubated with the biotinylated-anti-IFN-γ mAb for two hours. The wells will be washed again and incubated with streptavidin-conjugated HRP for one hour. After a final wash the AEC-substrate solution will be added to the wells and allowed to develop for approximately 5-10 minutes at which time the wells will be washed with deionized water to stop the reaction. The number of spots present in each well will be enumerated using the CTL ELISPOT analyzer (CTL Analyzers LLC, Cleveland, OH).

4.4.8 Local Reaction

Patients will also be assessed for evidence of in vivo immunologic response by evaluation of the injection site 48-72 hours after each inoculation (Appendix A). 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.

4.4.9 DTH Reaction

Regardless of randomization, a pre- and post-inoculation series DTH response (Appendix A) will be assessed of all patients with 100 mcg of E75 (without GM-CSF) injected intradermally at a site on the back or anterior thigh (opposite side from the vaccination site). See detailed guidance (Appendix G) for vaccine and DTH preparation. 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, DTH responses will also be assessed 3-4 weeks after the second and fourth booster. The low dose of peptide used in the DTH test is not expected to induce a long-term immune response; 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-SPECIFIED THERAPY

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

- Progressive disease
- Unacceptable toxicity as described in the protocol.
    - Please refer to sections 3.4-3.5 (Safety Plan) for the management of cardiac issues (symptomatic and asymptomatic), infusion-associated events, hematologic toxicity and neutropenic infections, as well as delayed hypersensitivity 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 (Subject 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 SUBJECT 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 to be directly 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.

Per 3.5 (Dose Reductions of Vaccine Component (paragraph one), 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.

In the event of cardiac toxicities, the determination for discontinuation is described in section 3.4.2. Although prior history of vaccine therapy (with or without Herceptin TM) in Phase I and II studies has not shown evidence of cardiac toxicities, potential overlapping of study treatment will be monitored by ECHO or MUGA scans done every three months during vaccine therapy. Any symptoms of CHF reported by patients, or observed by study coordinators, will be documented and reported to the investigators for a determination on relatedness to either Herceptin™ or the vaccine component. While this attribution will be difficult on a per patient basis, the incidence between the Herceptin only arm will be compared to the Herceptin + vaccine arm to assess for additive toxicities. The Principal Investigator will determine if safety permits continuation in study regardless of attribution, based on the parameters under Section 3.4 (Safety Plan). If LVEF drops >20% or is below facility limits of normal or patient is symptomatic of CHF and requires treatment, the patient will be discontinued from treatment but remain on the study for disease follow-up. Patients will be followed until the resolution of the toxicity.

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 age 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 oopherectomy, 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 as described in Section 4.4.2 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 (Appendix J).

4.7 STUDY DISCONTINUATION

Genentech Study Center, Galena Biopharma, the Data Safety Monitoring Board, Sponsor/Investigator 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 adverse events in this or other studies indicates a potential health hazard to patients
- Subject enrollment is unsatisfactory
- Data recording are inaccurate or incomplete
- Study protocol not followed

4.8 STATISTICAL METHODS

4.8.1 Analysis of the Conduct of the Study

Safety Population

The population of patients evaluable for safety will include all patients who receive at least one study inoculation

Intention-to-Treat Population

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)

Per-Protocol Population

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 primary vaccine series
- Receiving alternative disease-directed therapy without evidence of recurrence
- Major deviation from prescribed vaccination scheduled (to include boosters)

4.8.2 Analysis of Treatment Group Comparability

Demographic characteristics of all patients in the ITT and PP populations will be tabulated including age, race, country/region, disease histology, tumor size, ER/PR status, grade, HER2 status, nodal status, AJCC staging, surgery extent, and prior disease-directed therapies to include chemotherapy, radiation therapy, hormonal therapy, and immunotherapy.

Unless otherwise noted, 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.

Comparability between treatment groups for categorical variables will be assessed using a 2-sided Fisher's Exact test or a singly-ordered exact test (e.g. ER/PR status, stage), and 2-sample t-tests for continuous variables.

4.8.3 Efficacy Analysis

a. Primary Endpoint

The primary efficacy endpoint of the trial is DFS at 24 months, compared between the two treatment arms (Herceptin + E75/GM-CSF versus Herceptin + GM-CSF alone). Time to recurrence will be measured from the date of the first Herceptin infusion.

b. Secondary Endpoints

Secondary endpoints of the trial are:

- DFS at 36 months compared between the two treatment arms

- Immunologic responses to vaccination between treatment arms will be measured and compared using the DTH reaction, secondary comparisons may be made using the dimer and ELISPOT assays.

- Comparison of safety measures (MUGA or ECHO) related to combination therapy

Efficacy parameters will be analyzed and summarized for both the ITT and PP populations. Patients who entered the trial but did not meet inclusion criteria, or who did meet exclusion criteria will be excluded from efficacy analysis.

Withdrawal

Patients may withdraw or be discontinued from the study as discussed in Section 4.7. Patients who do withdraw or are discontinued will be included in the efficacy analyses unless a subject withdraws consent to participate. In the instance of a subject withdrawing consent, any data collected will be excluded from analysis.

Primary Efficacy

The primary efficacy endpoint analysis of 24-month DFS will be compared between the two treatment arms using a proportional hazard model including the stratification factors to estimate the hazard ratio. Hazard ratios and 95% two-sided confidence intervals will be presented. The model will also examine two-way treatment interactions with the baseline stratification factors. Kaplan-Meier curves will be generated to display time to events for each treatment group.

The null and alternative hypotheses are:

H0: $hV / hC = 1$

Ha: $hV / hC \neq 1$

where hV is the constant Herceptin + E75/GM-CSF hazard rate and hC is the constant Herceptin + GM-CSF alone hazard rate. Herceptin + E75/GM-CSF efficacy is demonstrated when $hV / hC < 1$; the alternative hypothesis is a 0.35 hazard ratio.

A difference in the recurrence rate of 5% in patients treated with Herceptin + E75/GM-CSF versus 15% in those treated with Herceptin and GM-CSF alone will be considered significant.

The PP population analyses will be the primary while ITT analyses will be confirmatory.

The stratification factors are the following:

- Nodal status (N0 or N1 versus N2 or N3)
- HER2 1+ versus HER2 2+

Stratification will be performed to balance the treatment arms; however, the trial is designed to detect vaccine efficacy as measured by DFS at 24 months between the two treatment groups. The study is not powered for subgroup analysis.

Confirmatory analyses will be performed using a stratified log rank test for each of the stratification factors.

To explore the possibility of a treatment by investigative site interaction, the primary analysis will also be conducted by site for the ITT and PP populations. Sites that have enrolled large numbers of patients relative to other trial centers will be identified to see if the results obtained at those investigative sites are influential. 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.

Secondary Efficacy

Secondary efficacy objectives include the same analysis (DFS) at 36 months and comparison of the immunologic responses to the target antigen in both the treatment and control groups with correlation to clinical outcomes.

The immunologic recognition by T-cells of the E75 peptide may be evaluated using a 2-sample t-test to evaluate the mean increase in cell populations from the dimer assay and peptide-specific IFN-gamma release from pre-vaccination (R0) to each of the following time points: before the 4th inoculation (R3), one month after completion of the six inoculation vaccine series (R6), six months after completion of the primary vaccine series (RC6), 12 months after completion of the primary vaccine series (RC12), 18 months after completion of the primary vaccine series (RC18), 24 months after completion (RC24), and 30 months after completion (RC30) (Appendix A).

Booster inoculations will be administered after the RC6, RC12, RC18, and RC24 blood samples have been drawn. The response to the booster inoculations may be measured one month after each successive booster inoculation: Month 13 (RB1), Month 19 (RB2), Month 25 (RB3), and Month 31 (RB4) (Appendix A).

The R3, R6, RC6, RC12, RC18, RC24, and RC30 assessments can be compared to pre-vaccination (R0) levels thereby using the patients as their own controls. RB1, RB2, RB3, and RB4 can be compared to the pre-booster responses. Evidence of in vivo immunologic response will also be assessed with the DTH reaction for each treatment group. The treatment groups will be compared with respect to the percentage with a positive post-vaccination DTH as well as absolute DTH reaction sizes within the groups. Expression percents will be compared using a two-sided Fisher Exact test while continuous outcomes such as mean DTH response will be compared using a paired t-test (using the patients as their own controls) and using an unpaired t-test to compare treatment groups for post-inoculation outcomes.

All of the above analyses will also be performed comparing safety, immunologic response and clinical outcomes between study patients based on HLA type. The HLA-A2 and/or HLA-A3 positive patients are the primary study population. The HLA-A24 and/or HLA-26 positive patients are an exploratory study population. If these patient populations respond comparably to the vaccine, then all vaccinated patients will be included in the final analysis.

4.8.4 Safety Analysis

Safety parameters will be analyzed and summarized for the Safety Population.

Adverse events will be classified and graded using the NCI CTCAE version 4.03 for both local and systemic toxicities. Only treatment-emergent AEs will be tabulated. All AEs will be included in the data listings, including onset timing (timing relative to study treatment administration), duration, severity, relationship, and resolution.

The incidence and frequency of treatment-emergent: AEs, SAEs, severe AEs, AEs related to study drug (See Appendix N for causality assessment guidelines) , and AEs which lead to study discontinuation will be presented by treatment group.

The incidence and frequency of treatment-emergent AEs will be summarized by treatment group using counts and percentages:

- by system organ class and preferred term
- by system organ class, preferred term, and maximum severity
- by system organ class, preferred term, and strongest relationship to study drug.

For the incidence, if a subject experiences the same event more than once, the event with the highest severity will be included in the severity tabulation, and the event with the highest relationship will be included in the relationship tabulation. For the frequency, all AEs will be tabulated.

A 2-sided Fisher Exact test will be used to compare the incidence of any expected AEs between the 2 treatment groups.

Adverse events origination will be attributed to prior breast cancer therapy, study therapy, or surgery or shared as most appropriate. The results will be tabulated be treatment group.

Kaplan-Meier life tables will be used to compare the time to onset and the duration of any AE with >10% incidence.

A singly ordered Kruskal-Wallis test will be used to compare the total number of AEs, SAEs, and related AEs per treatment group.

The incidence of clinically significant CHF will be estimated for patients in pre-specified treatment groups. Symptomatic CHF is defined as the occurrence of objective findings on clinical examination (e.g., rales, S3, elevated jugular venous pressure) and confirmed by chest X-ray and either MUGA or ECHO. Difference in risk based on prior anthracycline exposure as well as risk factors such as ECHO performance status and smoking status will be explored.

4.8.5 Planned Analyses

There are four formal analyses planned for this trial. The primary analysis of the trial will be conducted 24 months after the last patient is enrolled. The secondary analysis will occur 36 months after the last patient is enrolled. There are two interim analyses planned. The first will be conducted when the 150th patient is randomized to assess for safety. This interim analysis will look at the overall safety and specifically the cardiac safety measures between treatment arms. If any significant safety concern is noted by the DSMB, then trial modifications may be warranted.

Additionally, the DSMB has the authority to stop the trial early for either safety concerns or efficacy. Due to the long enrollment period, the second interim analysis will be conducted 6 months after the last patient is enrolled and will assess both safety and efficacy. The stopping boundary for the latter analysis has been set with a p value = 0.005 resulting in a final p value = 0.048 for the primary analysis (O'Brien-Fleming).

4.8.6 Missing Data

Every reasonable attempt will be made to recover any missing data. If any data remains missing that data point will be excluded from analysis for that patient. Subsequently measured data for a patient with missing data will be analyzed.

4.8.7 Determination of Sample Size

The primary endpoint of DFS at 24 months is used to determine the sample size and follow-up determination, based on the following background.

The previously described Phase 2 NeuVax study (USMCI Clinical Trials Group Study I-01 and I-02) (n=188) has reported 26 DFS events and 8 deaths with a 60-month median follow-up; this translates into an 88% DFS for vaccinated patients versus 78% for controls. In 24-month landmark analysis, the vaccinated patients had a 93.5% DFS compared to 85.5% in the control group. The DFS hazard ratio was 0.54 at 60 months and 0.45 at 24 months. In this study, the 24 months was measured from enrollment which was on average 6 months from completion of all standard of care therapy or approximately one year from surgery. This analysis equates to 3-year analysis in studies that enroll at initiation of chemotherapy such as the Herceptin studies. Unlike the Herceptin studies, this study enrolled both NP and high-risk NN patients which decreased the overall recurrence rate.

The NSABP adjuvant Herceptin studies (n=3351 combined) reported an 87% DFS rate at three years, a 90% distant recurrence-free rate at three years, and a 91% OS rate at four years for the combined Herceptin groups (plus adjuvant chemotherapy) versus a 75% DFS rate at three years, an 82% distant recurrence-free rate at three years, and a 87% OS rate at four years for the combined chemotherapy control; the DFS hazard ratio was 0.48 ($p<0.0001$) while the OS hazard ratio was 0.67 ($p=0.015$).

The adjuvant Herceptin (HERA) study (n=5081 total) reported an 86% DFS rate at two years, a 91% distant recurrence free rate at two years, and a 96% OS rate at two years for the combined Herceptin groups (plus adjuvant chemotherapy) versus a 77% DFS rate at two years, an 83% distant recurrence free rate at 2 years, and a 85% OS rate at two years for the combined chemotherapy control; the DFS hazard ratio was 0.54 ($p<0.0001$) while the OS hazard ratio was 0.76 ($p=0.26$). The recurrence rate among NP breast cancer patients with HER2 1+ and 2+ expressing tumors after standard of care therapy is reported to be 20% at 24 months, which is comparable to the two-year DFS of 77% observed in the HERA trial.

As described above, the 3-year DFS in the NSABP trial is equivalent to the 24-month DFS we intend to analyze as our primary endpoint in this trial based on differences in time of enrollment.

Based on this data, we expect Herceptin alone (with GM-CSF) may have some benefit and decrease the recurrence rate to 15%, similar to that seen in the NSABP trial. However, the combination of Herceptin + E75/GM-CSF based on preliminary data virtually eliminated recurrences (Sears et al, 2011). For planning purposes, if the combination reduces the recurrence rate to 5%, then in order to show a statistical differences between recurrence rates of 15% versus 5%, a sample size of 150 patients per arm would be required or a study size of 300 patients.

The overall type 1 error rate of 5% will be preserved for the rejection of the null hypothesis in favor of the alternative hypothesis that Herceptin + E75/GM-CSF will prolong DFS over that of Herceptin + GM-CSF alone. With this sample size, the power to detect our primary endpoint is 80%.

4.9 DATA QUALITY ASSURANCE

Accurate, consistent, and reliable data will be ensured through the use of standard practices and procedures. Data collection procedures are described in Section 6.6. Data monitoring and assurance procedures are described in the Data Safety Monitoring Plan (Appendix K).

5. REPORTING OF ADVERSE EVENTS

Reporting of adverse events will be performed in accordance with the Data Safety Monitoring Plan (Appendix K) 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 subject. 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 Herceptin or the vaccine components. Elective surgeries resulting in hospitalization and unrelated to the study agents will not be reported as SAEs (e.g., breast reconstruction, etc.). All events meeting the outlined criteria will be reported for the time period beginning with any amount of exposure to Herceptin or 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 financial sponsors, Genentech Drug Safety and Galena Biopharma. The reporting requirements/instructions of AEs from IND Sponsor/Investigator and/or representative to Genentech, Inc. are stated in Appendix N. Serious criteria, definitions, and guidance for reporting follow.

An **adverse event (AE)** is any untoward medical occurrence in a subject 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 adverse events 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 subject 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 AE required at least a 24-hour inpatient hospitalization or prolonged a hospitalization beyond the expected length of stay; hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAEs by this criterion)

Is **disabling** (i.e., the AE resulted in a substantial disruption of the subject's ability to carry out normal life functions)

Is a **congenital anomaly/birth defect** (i.e., an adverse outcome in a child or fetus of a subject exposed to the study drug prior to conception or during pregnancy)

Does not meet any of the above serious criteria but **may jeopardize the subject** and **may require medical or surgical intervention** to prevent one of the outcomes listed above.

Expected adverse events are those adverse events that are **listed** or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those **not listed** in the Package Insert or current IB. This includes adverse events for which the specificity or severity is not consistent with the description in the Package Insert or IB. For example, under this definition, hepatic necrosis would be unexpected if the Package Insert or 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 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 Herceptin or the vaccine components, occurring in patients enrolled at their respective study site to the Internal Review Board (IRB) of their site (see Adverse Event Reporting Algorithm, Appendix L). This will be accomplished by submitting an adverse event 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 adverse event 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 an FDA Form 3500 (MedWatch) (Appendix L). 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 MedWatch 3500A form:

- 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 adverse event 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 subject identifiers (i.e., D.O.B. initial, subject 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, Genentech, or Galena Biopharma may contact the reporter for additional information, clarification, or current status of the subject for whom and adverse event was reported. For questions regarding SAE reporting, you may contact the Quality Assurance (QA) Officer or her designee.

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 Herceptin® or vaccine components if the Investigator believes:

- There is a clinically plausible time sequence between onset of the AE and Herceptin and/or vaccine components administration; and/or
- There is a biologically plausible mechanism for Herceptin and/or 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

Expedited IND Safety Reports:

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** adverse event that is **unexpected** and assessed by the investigator to be **possibly related** to the use of the investigational product. An unexpected adverse event is one that is not already described in the Package Insert or Investigator Brochure for Herceptin and Investigator Brochure for vaccine. Such events are to be reported by the sponsor to the FDA and Genentech 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 adverse event is one that is not already described in the Package Insert or Investigator Brochure for Herceptin and/or 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, Genentech Drug Safety, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500A Form but alternative formats are acceptable (e.g., summary letter).

6. INVESTIGATOR REQUIREMENTS

6.1 STUDY INITIATION

Before the start of this study, the following documents must be on file with the IND sponsor, or sponsor representative, Galena Biopharma, and Genentech or a Genentech 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 and Genentech 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 subject or the subject's legally authorized representative before his or her participation in the study. The case history for each subject 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 subject or the subject'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 subject'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 Genentech and 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, unexpected or expected, and reasonably or possibly related 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 Genentech and 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, Genentech, the U.S. FDA, and the respective national or local health authorities to inspect facilities and records relevant to this study.

6.6 DATA COLLECTION

Clinical nurses at each study site will be provided with Source Document Flow Sheets to capture data at enrollment and for each study visit (Appendix G). Data collected on the Flow Sheets will be entered into an Electronic Data Capture (EDC) system by the site. The URL for the EDC system is https://cancerinsight.eclinicalhosting.com/OpenClinica. It can be accessed with any web browser. User name and password will be generated by OpenClinica and sent via email to appropriate site personnel prior to enrolling the first patient.

Database Management

Data must be submitted within 72 hours of the data collection visit. Data entry will begin with the patient's second informed consent visit. Please see CRF guidelines for specific information.

Tracking data to ensure the highest quality is important. 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. Edit Checks will fire in real time as data is being entered in the EDC system to ensure quality data is complete, consistent, and clean. In addition to edit checks, queries will be generated as Discrepancy Notes by 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 all data has been submitted. The CRO QA Officer or 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 Database 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 Overall 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. Data extracted from the database may be accessed by other study collaborators and investigators at the discretion of the Overall Study Principal Investigator.

Data Safety Monitoring Plan

A Data Safety Monitoring Plan (DSMP) (Appendix K) 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

If the study drug will be provided by Genentech and 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 Genentech and Galena Biopharma.

6.8 DISCLOSURE OF DATA

Subject medical information obtained by this study is confidential, and disclosure to third parties other than those noted above is prohibited.

Upon the subject'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, Genentech, 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 THE 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.

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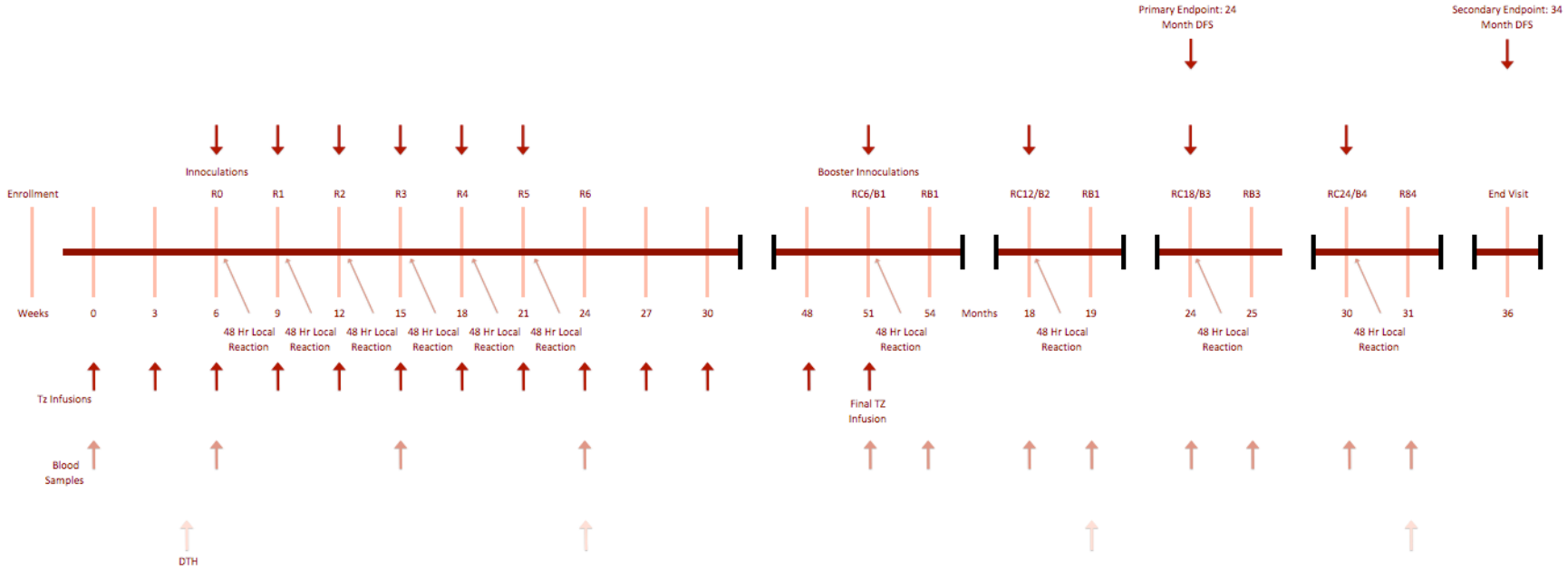
APPENDIX A
Herceptin and Vaccination Timeline



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APPENDIX B
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 C.1
Study Flowchart

	Days –60 to –1	Week 1 (Day 0)	DTH inoculations (4) [2]	Every 3 Weeks	Every 3 Months	Every 6 Months	If Clinically Indicated	Final Visit in 36th month
Hematology (CBC, diff., platelets)	X							
Chemistry panel (CMP)	X							
HLA blood draw for typing	During study screening process/patient identification process.							
Immunological Samples[1]		X[1]	X[1]	X[1]		X[1]		
Herceptin administration (q3 weeks x 18) [3]				X[3]				
DTH administration/Photos[2]			X[2]					
Vaccine administration[3]				X[3]				
Booster inoculations [4]						X[4]		
Post-Booster Immunological Samples[1]						X[1]		
Complete medical history (treating physician)	X						X	
Complete physical exam (treating physician, based on SOC)	X				X	X	X	
Clinical assessment-Study nurse visit[3,4,9]		X	X[3]	X[3]		X[4]		X[9]
Weight, height (treating physician)	X						X	
ECOG performance status		X						
Toxicity evaluation[8,9]			X[8]	X[8]		X[8]	X[8]	X[9]
Vital signs[8]			X[8]	X[8]		X[8]		
MUGA scan or ECHO, consistency required[5]	X[5]				X[5]	X[5]	X[5]	
Chest X-ray (AP, lateral)/EKG							X	
Urine pregnancy test (if applicable)[6]		X[6]	X[6]	X[6]		X[6]		

Tumor assessment (treating physician)	X[7]						X	
Disease status monitoring[7,9]	X[7]				X[7]	X[7]	X[7]	X[9]

[1]Immunological samples will be drawn , prior to initiation of Herceptin, prior to DTH #1, prior to inoculation #4, prior to DTH #2 and every six months prior to each of 4 boosters. Samples are also drawn 3-4 weeks post each booster.

[2]DTH #1 given prior to third Herceptin infusion, DTH #2 given 3-4 weeks after 6th vaccine inoculation, DTH #3 given 3-4 weeks after booster #2 and DTH#4 given 3-4 weeks after booster #4. The final two will be given after the collection of the post booster blood sample at the same visit. DTHs do not need to be scheduled on day of Herceptin infusion.

[3]Vaccine inoculation administered every 3 weeks for 6 inoculations during Herceptin treatment schedule. Vaccine inoculations to begin after 3rd Herceptin infusion, except with prior approval from Sponsor to begin at 4th or 5th infusion time point. 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 Herceptin infusion).

[4]Booster inoculations will be given after initial series, every 6 months x4, Booster #1 to be given with thefinal Herceptin infusion. Subsequent boosters will be given every 6 months (+/- 2 weeks) from Booster #1

[5]MUGA scans or ECHO, consistency required will be done at baseline prior to enrollment and at 3, 6, 12, and 24 months. If EF change > 10%, continue with MUGA or Echo every 6 months through trial.

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

[7]Must be free of disease “NED” prior to enrollment per physician. Disease status monitored every 3 months from consent for 18 months, then every 6 months until study completed by study coordinator/nurse

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

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

APPENDIX C.2
Patient Visit Chart

CLINIC VISIT				*PROCEDURES*									*TOTAL*	
Visit #	**Time Req (hr)**	**Time Point**	**Purpose**	**Herceptin IV Infusion**	**MUGA SCAN or EjCHO**	**Toxicity Eval**	**Nursing Visit**	**Urine HCG[1]**	**Blood Draws**		**Inoc**	**DTH Test**	**# of Shots This Visit**	**Time for Inoc + Follow-Up**
									Pre-Inoc	Post -Inoc				
1	2	Baseline	•Pre CBC/CMP Informed Consent Document • Gather baseline data. • Draw blood for HLA typing .		X		X	X	X				0	N/A
START HERCEPTIN INFUSIONS														
2	2	Starting Time Point	R0 (1) immunology sample then First Herceptin infusion	X					X				0	N/A
3	1	Approx q3 weeks	Herceptin infusion	X									0	
START PRE-INOCULATION BLOOD DRAWS AND INOCULATIONS AFTER HERCEPTIN INFUSIONS X6														
4	0.5	1-2 wks prior to Herceptin #3	• Draw blood R0 (2) to record immunologic activity before DTH skin test #1. • Administer DTH skin test #1.			X	X	X	X			X	1	0.5 hrs over 1 day
5	**0.25**	**48-72 hrs later**	**• Examine DTH skin test site for any local reaction and photograph.**			**X**	**X**							**0.25 hrs**
6[2]	2.5	Approx q3 weeks	• Administer inoculation #1 after Herceptin infusion #3[2]	X		X	X	X			X		4	2.75 hrs over 2-3 days
7[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
8	2.5	Approx q3 weeks	• Administer inoculation #2 after Herceptin infusion.	X		X	X	X			X		4	2.75 hrs over 2-3 days
9[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
10	2.5	Approx q3 weeks	• Administer inoculation #3 after Herceptin infusion.	X	X	X	X	X			X		4	2.75 hrs over 2-3 days
11[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
12	2.5	Approx q3 weeks	• Draw blood to record immunologic response to inoculation #3. • Administer inoculation #4 after Herceptin infusion.	X		X	X	X	X		X		4	2.75 hrs over 2-3 days
13[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
14	2.5	Approx q3 weeks	• Administer inoculation #5 after Herceptin infusion.	X		X	X	X			X		4	2.75 hrs over 2-3 days
15[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
16	2.5	Approx q3 weeks	• • Administer inoculation #6 after Herceptin infusion.	X		X	X	X			X		4	2.75 hrs over 2-3 days
17[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
18	1	Approx q3 weeks	• Draw blood to record immunologic response to #6. • Administer DTH skin test #2 after Herceptin infusion.	X	X	X	X		X			X	1	0.75 hrs over 2-3 days

19	0.25	48-72 hrs later	• Examine the DTH skin test site for any local reaction and photograph.			X	X						0	
20	1	Approx q3 weeks	Herceptin infusion	X									0	
21	1	Approx q3 weeks	Herceptin infusion	X									0	N/A
22	1	Approx q3 weeks	Herceptin infusion	X									0	
23	1	Approx q3 weeks	Herceptin infusion	X									0	
24	1	Approx q3 weeks	Herceptin infusion	X									0	
25	1	Approx q3 weeks	Herceptin infusion	X									0	
26	1	Approx q3 weeks	Herceptin infusion	X									0	
27	1	Approx q3 weeks	Herceptin infusion	X									0	
RESUME IMMUNOLOGY BLOOD DRAWS AND ADMINISTRATION OF BOOSTER INOCULATIONS														
28[3]	2.5	Month 12 from Starting Time Point (±2 weeks)	• Draw blood to record immunologic levels pre-Booster #1 inoculation. • Final Herceptin infusion (see comment 3 below). • Administer Booster #1 inoculation.	X	X	X	X	X	X		X		4	3.25 hrs over 4 weeks
29[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
30	0.5	3-4 weeks later	• Draw blood to record immunologic response to Booster #1 inoculation.				X			X			0	
31	1.5	Month 18 (±2 weeks)	• Draw blood to record immunologic levels pre-Booster #2 inoculation. • Administer Booster #2 inoculation.			X	X	X	X		X		4	3 hrs over 4 weeks
32[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
33	1	3-4 weeks later	• Draw blood to record immunologic response to Booster #2 inoculation then administer DTH #3.				X	X		X		X	1	
34	.25	48-72 hrs later	• **Examine DTH skin test site for any local reaction and photograph**				X						0	
35	1.5	Month 24 (±2 weeks)	• Draw blood to record immunologic levels pre-Booster #3 inoculation. • Administer Booster #3 inoculation.		X	X	X	X	X		X		4	2.25 hrs over 4 weeks
36[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
37	0.5	3-4 weeks later	• Draw blood to record immunologic response to Booster #3 inoculation.				X			X			0	
38	1.5	Month 30 (±2 weeks)	• Draw blood to record immunologic levels pre-Booster #4 inoculation. • Administer Booster #4 inoculation.		X*	X	X	X	X		X		4	3 hrs over 4 weeks
39[4]	0.25	48-72 hrs later	• Examine the inoculation site for a local reaction.[4]			X	X						0	
40	1	3-4 weeks later	• Draw blood to record immunologic response to Booster #4 inoculation then administer DTH#4.				X	X		X		X	1	
41	.25	48-72 hrs later	• **Examine DTH skin test site for any local reaction and photograph**				X						0	
42	.5-1	Month 36	• Final study assessment		X*	X	X						0	End Study

VISITS: 30-42[4] # INOCULATIONS: 48
BLOOD DRAWS: 12

1 Urine HCG if applicable per protocol
2 Herceptin/vaccine combination may begin at 4th or 5th infusion time point if clinically indicated with prior Sponsor approval
3 To allow PI flexibility in scheduling, total Herceptin infusions will be 17-18, approximately every three weeks
4 Patients have the option to 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.
[4]NOTE: If ejection fraction >10% reduction from baseline, then continue MUGA or ECHO every 6 months for duration of study

APPENDIX D
HFSA Guidelines
Recommendations for Pharmacological Therapy: Left Ventricular Systolic Dysfunction

ß-Adrenergic Receptor Blockers
Background for Recommendations

The single most significant addition to the pharmacological management of heart failure since the publication of previous guidelines involves the use of ß-receptor antagonists. This represents a noteworthy departure from traditional doctrine in which ß-blocking agents were classified as contraindicated in the setting of left ventricular systolic dysfunction. A solid foundation of both clinical and experimental evidence now firmly supports their use in heart failure with the aim of reducing both morbidity and mortality (16,22,23).

ß-Blocker therapy for heart failure has been advocated by some investigators since the 1970s (24). During the subsequent 2 decades, many small- to medium-sized placebo-controlled trials, which used a variety of agents, showed several common findings: 1) the use of ß-blockers in mild to moderate heart failure was generally safe when initiated at low doses and gradually uptitrated under close observation; 2) improvement in left ventricular ejection fraction was observed in all trials that lasted at least 3 months; and 3) there was wide variability in the effects of ßblockade on exercise tolerance but improvement in outcome and symptomatic benefits was noted in many studies. These generally positive findings stimulated additional, large-scale clinical trials that have provided an impressive body of evidence that supports the use of ß-blockers in patients with heart failure caused by left ventricular systolic dysfunction. The recommendations that follow are derived from nearly 2 decades of research that include basic science data, animal models, and clinical trial experience in over 10,000 patients (25,26).

Although this is a major advance in efficacy, identification of appropriate candidates for ßblocker therapy is essential to ensure safe and effective treatment. Prescribing physicians should understand the potential risks of ß-blocker therapy, as well as the benefits. The interested practitioner who is unfamiliar with ß-blocker initiation and titration may first seek further education and counsel from sources such as the Heart Failure Society of America or local and regional heart failure specialty centers.

> **Recommendation 1. ß-blocker therapy should be routinely administered to clinically stable patients with left ventricular systolic dysfunction (left ventricular ejection fraction less than or equal to 40%) and mild to moderate heart failure symptoms (ie, NYHA class II-III, AppendixD.1)**
> **who are on standard therapy, which typically includes ACE inhibitors, diuretics as needed to control fluid retention, and digoxin (Strength of Evidence = A).**

The most persuasive outcome in heart failure management remains all-cause mortality.

Combined endpoints, including mortality or hospitalization and mortality or hospitalization for heart failure, have also emerged as key outcomes. These latter endpoints reflect a more comprehensive assessment of the influence of therapy on quality of life and disease progression and are assuming more importance as mortality rates decline with treatment advances. The substantial beneficial effect of ß-blocker therapy on these endpoints has been well shown in clinical trials of symptomatic patients (NYHA class II - III) treated with carvedilol, bisoprolol, or metoprolol controlled release/extended release (CR/XL) (27-29). Trials with these agents encompass the combined, worldwide experience with ß-blocker therapy in patients with chronic
heart failure who were stable on background therapy, including ACE inhibitors (over 90%) and diuretics (over 90%). Digoxin was common as background therapy, particularly in studies conducted in the United States. Trial results indicate that both selective and nonselective ßblockers, with and without ancillary properties, have significant efficacy in heart failure. ß-Blocking agents with intrinsic sympathomimetic activity appear to have a negative impact on survival and should not be used in heart failure patients.

Metoprolol. The MDC Study was an early trial that included 383 patients with heart failure caused by nonischemic causes, NYHA class II-III symptoms, and a left ventricular ejection fraction of less than or equal to 40% (30). Patients with coronary artery disease were excluded. Study results showed a 34% reduction in risk in patients treated with metoprolol, although this strong trend toward benefit (P = .058) was entirely attributable to a reduction in the frequency of cardiac transplantation listing in the treatment group. In fact, the absolute number of deaths in the metoprolol group was higher than in the placebo group (23 *v* 19, P = .69).

The MERIT-HF Trial evaluated the effect of metoprolol CR/XL with all-cause mortality as the primary endpoint. The trial included 3,991 patients with NYHA class II-IV heart failure, although 96% of the study patients were functional class II or III (31). In this study, investigators were allowed to select the starting dose of metoprolol CR/XL. Seventy-nine percent chose 25 mg as the starting dose for class II patients, and 77% chose 12.5 mg for class III-IV patients. The target dose was 200 mg and doses were up-titrated over a period of 8 weeks. Premature discontinuation of blinded therapy occurred in 13.9% of those treated with metoprolol CR/XL and 15.3% of those in the placebo group (P = .90). The study results revealed a 34% reduction in mortality in the metoprolol group (relative risk of .66; 95% confidence interval [CI], .53 to .81; p=.0062 after adjustment for interim analyses), with annual mortality rates of 11% in the placebo and 7.2% in the metoprolol CR/XL group (29).

Bisoprolol. The CIBIS Study evaluated the effects of bisoprolol in 641 patients with left ventricular systolic dysfunction caused by ischemic or nonischemic causes and NYHA class III-IV heart failure (32). The primary endpoint was all-cause mortality, and

hospitalization for worsening heart failure was one of the secondary outcomes of interest. The initial bisoprolol dose was 1.25 mg/day, which was increased to a maximum dose of 5 mg/day. The trial found no significant reduction in all-cause mortality in patients treated with bisoprolol (20% reduction bisoprolol *v* placebo, $P = .22$) (32). The risk of hospitalization was significantly reduced by 34% (28% placebo group *v* 19% bisoprolol group, $P < .01$).

The favorable trends seen in CIBIS led to the larger CIBIS II Study, which ultimately was prematurely terminated as a result of a significant reduction in mortality in the bisoprolol arm (28). These results were obtained in 2,647 patients who were followed for an average of 1.3 years. Over 80% of the patients were judged to be NYHA class III at enrollment. Background therapy included ACE inhibitors in 96% and diuretic in 99% of the study patients, whereas 52% were taking digoxin. In contrast to the original CIBIS study, CIBIS II had a similar starting dose of 1.25 mg but had a greater target dose of 10 mg daily of bisoprolol. More stringent criteria for defining ischemic cardiomyopathy were used. Treatment with bisoprolol reduced the annual mortality rate by 34% (13.2% placebo *v* 8.8% bisoprolol; hazard ratio .66; 95% CI, .54 to .81; $P< .0001$). Hospitalizations for worsening heart failure were also decreased by 32% (18% placebo *v* 12% bisoprolol, hazard ratio .64; 95% CI, .53 to .79; $P< .0001$). Although a post hoc analysis of the CIBIS Study had suggested benefit might be consigned to patients without coronary disease, the survival benefit, with significant reductions apparent in both ischemic or nonischemic patients, was not influenced by disease origins.

Carvedilol. Carvedilol, a nonselective ß-blocker and a-blocker, has been extensively investigated for treatment of heart failure caused by left ventricular systolic dysfunction. In the United States carvedilol trials, 4 separate study populations were examined and the data from 1,094 patients were combined to evaluate the effect of carvedilol therapy on the clinical progression of heart failure (27). Clinical progression was defined as worsening heart failure leading to death, hospitalization, or, in one study, a sustained increase in background medications. Patients with a left ventricular ejection fraction of 35% or less and NYHA class II-IV were eligible if they tolerated 6.25 mg of carvedilol twice per day for a 2-week, open-label, run-in period. Although this run-in phase biased the ultimately randomized patient population, less than 8% of eligible patients failed the open-label challenge. Target dosages for the studies were 50 to 100 mg/day of carvedilol that were administered in divided doses twice daily. Patients completing the run-in period were randomized based on results from their 6- minute walk test into mild, moderate, or severe trials. These studies were prematurely terminated (median follow-up 6.5 months) by the Trial Data and Safety Monitoring Board because of reduced mortality across the 4 combined trials of patients treated with carvedilol.

Data from these combined trials indicated a substantial benefit from carvedilol treatment. The risk of mortality was 65% lower (7.8% placebo *v* 3.2% carvedilol; 95% CI, 39% to 80%; $P< .001$) and the combined risk of hospitalization or death was reduced by 38% (20% on placebo *v* 14% on carvedilol; 95% CI, 18% to 53%; $P< .001$). A significant mortality reduction was also noted when deaths that occurred in the run-in period were included in

the analysis. The statistical validity of the survival analysis across the trials has been questioned because mortality was not the primary endpoint, and only 1 of the 4 trials achieved a significant result when analyzed based on the primary endpoint. Nevertheless, the magnitude of the survival benefit and the reduction in hospitalization were impressive. The survival benefit was not influenced by the cause of disease, age, gender, or baseline ejection fraction. Overall, 7.8% of the placebo group and 5.7% of the carvedilol group discontinued study medication. Data from the individual trials, PRECISE and MOCHA, which evaluated patients with moderate to severe heart failure, found that carvedilol reduced the risk of the combined endpoint of mortality or heart failure hospitalization by 39% to 49% (33,34). The MOCHA Study provided strong evidence for increased benefit from higher dosages (25 mg twice per day) versus lower dosages (6.25 mg twice per day) of carvedilol, so uptitration of carvedilol dosages to 25 mg twice per day is generally recommended. However, favorable effects were noted at 6.25 mg twice per day, so intolerance of high doses should not be a reason for discontinuation of therapy.

The Australia-New Zealand Carvedilol Trial enrolled 415 patients with ischemic cardiomyopathy and a left ventricular ejection fraction of less than 45% (35). Although patients with NYHA functional classes I-III were eligible, the majority enrolled were NYHA functional class I (30%) or II (54%). ACE inhibitors were used in 86% of the participants, whereas 76% were on diuretic therapy, and 38% were on digoxin. This trial also had a run-in phase during which 6% of the patients discontinued ß-blocker therapy. During an average follow-up of 19 months, carvedilol decreased the combined risk of all-cause mortality or any hospitalization by 26% (relative risk .74; 95% CI, .57 to .95; *P*= .02). Overall mortality was 12.5% in the placebo group and 9.6% in the carvedilol group which was not statistically significant (relative risk .76; 95% CI, .42 to 1.36; *P* > .10).

Unreported or Ongoing Trials. Studies that are underway will provide additional data concerning specific aspects of the efficacy of ß-blocker therapy in heart failure. The effect of bucindolol on mortality and morbidity in patients with moderate to severe heart failure has been evaluated in the BEST Study. This study enrolled a substantial number of women so the potential influence of gender on the efficacy of ß-blocker therapy can be investigated. The trial has been stopped, and no results are available for analysis.

The COPERNICUS Trial is designed to assess the effect of carvedilol treatment on disease progression and survival in patients with advanced heart failure with symptoms at rest or on minimal exertion. The COMET protocol is a 3,000 patient study that directly compares the survival benefit of carvedilol versus metoprolol. This trial will provide important data concerning the relative efficacy of a selective ß-blocker versus a nonselective ß-blocker with ancillary properties.

Recommendation 2. ß-blocker therapy should be considered for patients with left ventricular systolic dysfunction (left ventricular ejection fraction less than or equal to 40%) who are asymptomatic

(ie, NYHA class I) and standard therapy, including ACE inhibitors (Strength of Evidence = C).

Data from the SOLVD Prevention Trial prospectively illustrated the efficacy of ACE inhibitors in delaying the onset of heart failure symptoms and the need for treatment or hospitalization for heart failure in asymptomatic patients with a left ventricular ejection fraction less than or equal to 35% (36). Similar controlled, clinical trial data that support the use of a ß-blocker in this clinical circumstance are not available. However, significant support for the use of ßblocker therapy in patients with asymptomatic left ventricular dysfunction can be derived from clinical trials in coronary artery disease and hypertension. Previous data indicate that ß-blocker therapy should be used in patients after myocardial infarction (MI) and in patients with myocardial revascularization who have good symptomatic and functional recovery but residual ventricular systolic dysfunction. Trials in hypertension indicate that ß-blocker therapy decreases the risk of developing heart failure. Given the potential of ß-blockers to retard disease progression and improve ventricular function, the risk to benefit ratio seems sufficiently low to support ß-blocker use in asymptomatic patients with left ventricular dysfunction, especially when the dysfunction is marked, and coronary artery disease is present.

Recommendation 3. To maximize patient safety, a period of clinical stability on standard therapy should occur before ß-blocker therapy is instituted. Initiation of ß-blocker therapy in patients with heart failure requires a careful baseline evaluation of clinical status (Strength of Evidence = B).

Initiation of ß-blocker therapy has the potential to worsen heart failure signs and symptoms. This risk increases with the underlying severity of the heart failure that is present. To minimize the likelihood of worsening failure, a period of treatment with standard therapy and evidence of clinical stability without acute decompensation or fluid overload is recommended before initiation of ß-blocker therapy. The majority of the large-scale, ß-blocker heart failure trials required that chronic heart failure be present 3 months or more before initiation of ß-blocker therapy. Patients enrolled in these trials were typically treated with ACE inhibitors (if tolerated), diuretic, and digoxin for at least 2 months and were observed to be clinically stable for 2 to 3 weeks before beginning ß-blocker therapy. Thus, many heart failure clinicians favor a minimum of 2 to 4 weeks of clinical stability on standard therapy before ß-blocker therapy is instituted. Likewise, most clinicians discourage the initiation of ß-blocker therapy in the hospital setting after treatment for new or decompensated heart failure (with or without associated inotrope administration). Some experienced clinicians initiate ß-blocker therapy in the hospital in selected patients who have responded well to inpatient treatment and who can be followed closely after discharge.

Recommendation 4. There is insufficient evidence to recommend the use of ß-blocker therapy for inpatients or outpatients with symptoms of heart failure at rest (ie, NYHA class IV) (Strength of Evidence = C).

ß-Blocker therapy cannot be routinely recommended for NYHA class IV patients because there are currently no clinical trial data to indicate favorable long-term efficacy and safety of ßblocker therapy in this patient population. A substantial body of observational data indicates that successful institution of ß-blocker therapy in patients with this degree of heart failure is problematic. If used, these agents may precipitate deterioration, and patients so treated should be monitored by a physician who has expertise in heart failure.

The number of patients with class IV heart failure at the time of ß-blocker initiation in controlled clinical trials is small. Available trials, which report data on patients with severe heart failure mostly labeled as NYHA class III, show the potential problems of ß-blocker therapy in this part of the heart failure spectrum. This experience is reflected in a 14-week study that evaluated the effects of ß-blocker therapy in 56 patients (51 NYHA class III and 5 NYHA class IV at randomization) with severe left ventricular dysfunction (average left ventricular ejection fraction of 16% ± 1% and left ventricular filling pressure of 24 mm Hg ± 1 mm Hg) (37). These patients had significant impairment of exercise capacity (mean VO2 max of 13.6 mL/kg/min ± 0.6 mL/kg/min) despite ACE-inhibitor, digoxin and diuretic therapy. Patients were believed to be clinically stable (requiring no medication adjustments) for a 2-week period before an open-label challenge was conducted. Seven patients (12%) failed to complete the open-label, run-in period, during which 5 died and 2 had nonfatal adverse reactions.

Clinical parameters did not distinguish these patients from those who were able to continue in the trial. Eighteen of the 49 patients (37%) completing the run-in period experienced worsened dyspnea or fluid retention during this phase. Also, 22% experienced dizziness and required medication adjustment, which delayed up-titration during the run-in. Subsequently, an additional 12% of the patients randomized to carvedilol withdrew from the blinded arm of the study. One of the United States carvedilol trials studied patients with severe left ventricular dysfunction who had markedly reduced exercise capacity as assessed by the 6-minute walk test (38). In this trial, 131 patients with a mean left ventricular ejection fraction of 22% and severe impairment in quality of life underwent a 2-week, open-label challenge phase of 6.25 mg of carvedilol twice per day. Ten of these 131 patients (8%) were unable to complete this run-in phase, most because of worsening heart failure, dyspnea, or dizziness. Subsequently, 11% of the patients randomized to carvedilol withdrew, as did a similar number of patients (11%) in the placebo group. In the recently completed large-scale BEST Trial, the mortality trend in NYHA class III-IV patients favored the ß-blocker bucindolol, but the difference from placebo was not significant. Further analysis of these preliminary findings is necessary, but the data suggest that the striking benefit of ß-blockers in mild-to-moderate heart failure may not be extrapolated to those with severe symptoms.

Recommendation 5. ß-Blocker therapy should be initiated at low doses and up-titrated slowly, generally no sooner than at 2-week intervals. Clinical reevaluation should occur at each titration point and with worsening of patient symptoms. Patients who develop worsening heart failure or other side effects after drug initiation or during titration require adjustment of concomitant medications. These patients may also require a reduction in ß-blocker dose and, in some cases, temporary or permanent withdrawal of this therapy (Strength of Evidence = B).

ß-Blocker therapy should be initiated at doses substantially less than target doses. Clinical trials required patient reassessment at up-titration of each dose. This careful evaluation by trained nurses and/or heart failure specialists likely contributed to the relatively low withdrawal rates and safety profiles observed in the clinical trials.

Treatment for symptomatic deterioration may be required during ß-blocker titration, but with appropriate adjustments in therapy, most patients can be maintained and generally achieve target doses. There is a risk of worsening heart failure, and vasodilatory side effects may occur with certain agents. Worsening heart failure is typically reflected by increasing fatigue, lower exercise tolerance, and weight gain. Increased diuretic doses may be required for signs and symptoms of worsened fluid retention. Treatment options also include temporary down-titration of the ß-blocker to the last tolerated dose. Abrupt withdrawal should be avoided. A minimum period of stability of 2 weeks should occur before further up-titration is attempted. Hypotensive side effects may often resolve with reduction in diuretic dose. Temporary reductions in ACE inhibitor dose may be helpful for symptomatic hypotension not obviated by staggering the schedule of vasoactive medications. Administration of carvedilol with food may alleviate vasodilatory side effects as well.

If ß-blocker treatment is interrupted for a period exceeding 72 hours and the patient is still judged a candidate for this therapy, drug treatment should be reinitiated at 50% of the previous dose. Subsequent up-titration should be conducted as previously described.

Recommendation 6. In general, patients who experience a deterioration in clinical status or symptomatic exacerbation of heart failure during chronic maintenance treatment should be continued on ß-blocker therapy (Strength of Evidence = C).

Clinical decompensation that occurs during stable maintenance therapy is less likely caused by chronic ß-blocker therapy than other factors (diet or medication noncompliance, ischemia, arrhythmia, comorbid disease, infection, or disease progression). In these situations, maintaining the current ß-blocker dose while relieving or compensating for the precipitating factor(s) is most often the best course. Data from patients randomized to continue or discontinue ß-blocker therapy in this setting are not currently available.

However, studies of the withdrawal of ß-blocker therapy in patients with persistent left ventricular systolic dysfunction but improved and stable clinical heart
failure have revealed a substantial risk of worsening heart failure and early death after discontinuation of ß-blocker therapy (39,40).

> **Recommendation 7. Patient education regarding early recognition of symptom exacerbation and side effects is considered important. If clinical uncertainty exists, consultation with clinicians who have expertise in heart failure and/or specialized programs with experience in ß-blocker use in patients with heart failure is recommended (Strength of Evidence = B).**

In certain patients, frequent return visits for dose-titration may be difficult to accommodate in a busy clinical practice. Trained personnel, including nurse practitioners, physicians' assistants, and pharmacists with physician supervision, may more efficiently perform patient education and reevaluation during up-titration. Heart failure specialty programs are more likely to have the resources to provide this follow-up and education (41). Consultation or referral may be particularly beneficial when the clinical heart failure status of the patient is uncertain or problems arise during initiation of therapy or dose-titration that may cause unwarranted discontinuation of therapy. Ideal patients for ß-blocker therapy should be compliant and have a good understanding of their disease and their overall treatment plan. Patients should be aware that symptomatic deterioration is possible early in therapy and that symptomatic improvement may be delayed for weeks to months.

Unresolved Therapeutic Issues

Combining ß-Blocking Agents With Amiodarone Therapy. Concomitant use of amiodarone was generally precluded in the trials evaluating carvedilol and most other ß-blockers. However, the use of this agent for rate control of atrial arrhythmia or for maintenance of sinus rhythm is common in heart failure patients. Drug interactions between ß-blockers and amiodarone are possible, including symptomatic bradycardia, and may limit the maximum tolerated dose of the ß-blocker. When the combination is used, the smallest effective dose of amiodarone should be employed. Given the lack of a clear survival benefit, amiodarone is not a substitute for ß-blocker therapy in heart failure patients who are candidates for this therapy.

Implantation of Cardiac Pacemakers. Given the strength of evidence that supports ß-blocker therapy in patients with symptomatic heart failure, some physicians would consider pacemaker implantation when symptomatic bradycardia or heart block occur during the initiation of this therapy, although no data are available to support such use. Consideration should be given, after weighing risks and benefits, to the withdrawal of other drugs that may have bradycardia effects.

Duration of Therapy. Whether patients experiencing marked improvement in left ventricular systolic dysfunction and heart failure symptoms during therapy can be

successfully withdrawn from ß-blocker therapy remains to be established. Concern continues that such patients would experience worsening after ß-blocker withdrawal, either in systolic function or symptoms, over a time period that is undefined. Until clinical trial data indicate otherwise, the duration of ßblocker therapy must be considered indefinite.

Digoxin
Background for Recommendations

Although little controversy exists as to the benefit of digoxin in patients with symptomatic left ventricular systolic dysfunction and concomitant atrial fibrillation, the debate continues over its current role in similar patients with normal sinus rhythm. Recent information regarding digoxin's mechanism of action and new analyses of clinical data from the DIG Trial and the combined PROVED and RADIANCE Trial databases provide additional evidence of favorable efficacy that was unavailable to previous guideline committees (42-47). In fact, this information has recently formed the basis of Food and Drug Administration (FDA) approval of digoxin for the treatment of mild to moderate heart failure (48). Digoxin, a drug that is inexpensive and can be given once daily, represents the only orally effective drug with positive inotropic effects approved for the management of heart failure. The committee's consensus is that digoxin, when used in combination with other standard therapy, will continue to play an important role in the symptomatic management of the majority of patients with heart failure.

The efficacy of digoxin for the treatment of heart failure caused by systolic dysfunction has traditionally been attributed to its relatively weak positive inotropic action that comes from inhibition of sodium-potassium adenosine triphosphatase (ATPase) that results in an increase in cardiac myocyte intracellular calcium. However, in addition to positive inotropy, digitalis has important, neurohormonal-modulating effects in patients with chronic heart failure, including a sympathoinhibitory effect that cannot be ascribed to its inotropic action (49,50). Digoxin also ameliorates autonomic dysfunction as evidenced by studies of heart rate variability, which indicates increased parasympathetic and baroreceptor sensitivity during therapy (51).

> **Recommendation 1. Digoxin should be considered for patients who have symptoms of heart failure (NYHA class II-III, Strength of Evidence = A and NYHA class IV, Strength of Evidence = C) caused by left ventricular systolic dysfunction while receiving standard therapy.**

Digoxin increases left ventricular ejection fraction and alleviates symptomatic heart failure as evidenced by drug-related improvement in exercise capacity and reductions in heart-failureassociated hospitalization and emergency room visits. Digoxin should be used in conjunction with other forms of standard heart failure therapy including ACE inhibitors, diuretics and ß-blockers.

The DIG Trial, a randomized, double-blind, placebo-controlled trial in over 7,000 patients

with heart failure, showed a neutral effect on the primary study endpoint and mortality from any cause during an average follow-up of approximately 3 years (42). In the main trial, 6,800 patients with left ventricular ejection fraction less than or equal to 45% were randomized to digoxin or placebo, in addition to diuretics and ACE inhibitors. A total of 1,181 deaths occurred on digoxin (34.8%) and 1,194 on placebo (35.1%) for a risk ratio of .99 (95% CI, .91 to 1.07; $P = .80$). These results differ from other oral agents with inotropic properties that have been associated with an adverse effect on mortality. In addition, the need for hospitalization and cointervention (defined as increasing the dose of diuretics and ACE inhibitors or adding new therapies for worsening heart failure) was significantly lower in the digoxin group, even in those patients who were not previously taking digoxin. Fewer patients on digoxin compared with placebo were hospitalized for worsening heart failure (26.8%*v* 34.7%; risk ratio .72; 95% CI, .66 to .79; $P < .001$). These long-term data are consistent with recent results obtained from an analysis of the combined PROVED and RADIANCE databases (45). In this analysis, patients who continued digoxin as part of triple therapy with diuretics and an ACE inhibitor were much less likely to develop worsening heart failure (4.7%) than those treated with a diuretic alone (39%, $P < .001$), diuretic plus digoxin (19%, $P = .009$) or diuretic plus an ACE inhibitor (25%, $P = .001$).

Although there are no clinical trial data (level A evidence) for the efficacy of digoxin in patients with NYHA Class IV heart failure, there is evidence that digoxin works across the spectrum of left ventricular systolic dysfunction. A prespecified subgroup analysis of patients enrolled in the DIG Trial with evidence of severe heart failure (as manifested by left ventricular ejection fraction less than 25%, or cardiothoracic ratio [CTR] greater than .55) showed the benefit of digoxin (48). The following reductions in the combined endpoint of all-cause mortality or hospitalization were seen on digoxin compared with placebo: 16% reduction (95% CI, 7% to 24%) in patients with a left ventricular ejection fraction of less than 25%, and a 15% reduction (95% CI, 6% to 23%) in patients with a CTR of greater than .55 (43). Reductions in the risk of the combined endpoint of heart-failure related mortality or hospitalization were even more striking: 39% (95% CI, 29% to 47%) for patients with left ventricular ejection fraction less than 25%, and 35% (95% CI, 25% to 43%) for patients with a CTR greater than .55 (48).

Evidence for the efficacy of digoxin in patients with mild symptoms of heart failure has been provided by a recent retrospective, cohort analysis of the combined PROVED and RADIANCE data (52). The outcome of patients in these trials who were randomized to digoxin withdrawal or continuation was categorized by using a prospectively obtained heart failure score based on clinical signs and symptoms. Patients in the mild heart failure group (heart failure score of 2 or less) who were randomized to have digoxin withdrawn were at increased risk of treatment failure and had deterioration of exercise capacity and left ventricular ejection fraction compared with patients who continued digoxin (all $P < .01$). Patients in the moderate heart failure group who had digoxin withdrawn were significantly more likely to experience treatment failure than either patients in the mild heart failure group or patients who continued digoxin (both $P < .05$). These data suggest that patients with left

ventricular systolic dysfunction benefit from digoxin despite only mild clinical evidence of heart failure.

In summary, a large body of evidence supports the efficacy of digoxin in patients with symptomatic heart failure caused by left ventricular systolic dysfunction. Digoxin has been shown to decrease hospitalizations, as well as emergency room visits; decrease the need for cointervention; and improve exercise capacity (42-44,53,54). Taken as a whole, these clinical trial data provide support for digoxin's beneficial effect on morbidity and neutral effect on mortality (42).

Recommendation 2. In the majority of patients, the dosage of digoxin should be .125 mg to .25 mg daily (Strength of Evidence = C).

Recent data suggest that the target dose of digoxin therapy should be lower than traditionally assumed. Although higher doses may be necessary for maximal hemodynamic effects (55), beneficial neurohormonal and functional effects appear to be achieved at relatively low serum digoxin concentrations (SDC) typically associated with daily doses of .125 mg to .25 mg of digoxin (55-57). The utility of lower SDC is supported by recent clinical trial data; the mean SDC achieved in the RADIANCE Trial was 1.2 ng/mL and in the DIG Trial was 0.8 ng/mL (42,44). Recent retrospective, cohort analysis of the combined PROVED and RADIANCE databases indicates that patients with a low SDC (less than .9 ng/mL) were no more likely to experience worsening symptoms of heart failure on maintenance digoxin than those with a moderate (.9 to 1.2 ng/mL) or high (greater than 1.2 ng/mL) SDC (41). All SDC groups were significantly less likely to deteriorate during follow-up compared with patients withdrawn from digoxin.

Therefore, patients with left ventricular systolic dysfunction and normal sinus rhythm should be started on a maintenance dosage of digoxin (no loading dose) of .125 or .25 mg once daily based on ideal body weight, age, and renal function For patients with normal renal function, a dosage of digoxin of .25 mg/day will be typical. Many patients with heart failure have reduced renal function and should begin on .125 mg daily. In addition, patients with a baseline conduction abnormality, or who are small in stature or elderly, should be started at .125 mg/day, which can be up-titrated if necessary. Once dosing has continued for a sufficient period for serum concentration to reach steady state (typically in 2 to 3 weeks), some clinicians consider the measurement of a SDC, especially in elderly patients or those with impaired renal function in which the digoxin dose is often not predictive of SDC. SDC measurements may be considered when 1) a significant change in renal function occurs; 2) a potentially interacting drug (amiodarone, quinidine, or verapamil) is added or discontinued; or 3) confirmation of suspected digoxin toxicity is necessary in a patient with signs or symptoms and/or electrocardiographic changes consistent with this diagnosis. Samples for trough SDC should be drawn more than 6 hours after dosing. Otherwise, the result is difficult to interpret because the drug may not be fully distributed into tissues.

Recommendation 3. In patients with heart failure and atrial fibrillation with a rapid ventricular response, the administration of high doses of digoxin (greater than .25 mg) for the purpose of rate control is not recommended. When necessary, additional rate control should be achieved by the addition of ß-blocker therapy or amiodarone (Strength of Evidence = C).

Digoxin continues to be the drug of choice for patients with heart failure and atrial fibrillation. However, the traditional practice of arbitrarily increasing the dose (and SDC) of digoxin until ventricular response is controlled should be abandoned because the risk of digoxin toxicity increases as well. Digoxin alone is often inadequate to control ventricular response in patients with atrial fibrillation, and the SDC should not be used to guide dosing to achieve rate control. Therefore, digoxin should be dosed in the same manner as in a patient with heart failure and normal sinus rhythm.

Digoxin slows ventricular response to atrial fibrillation through enhancement of vagal tone. However, with exertion or other increases in sympathetic activity, vagal tone may decrease and ventricular rate accelerate. Addition of a ß-blocker or amiodarone 1) complements the pharmacological action of digoxin and provides more optimal rate control; 2) allows the beneficial clinical effects of digoxin to be maintained; and 3) limits the risk of toxicity that may occur if digoxin is dosed to achieve a high SDC (58). For patients who have a contraindication to ßblockers, amiodarone is a reasonable alternative. If amiodarone is added, the dose of digoxin should be reduced, and the SDC should be monitored so that the serum concentration can be maintained in the desired range. Some clinicians advocate the short-term, intravenous administration of diltiazem for the acute treatment of patients with very rapid ventricular response, especially those with hemodynamic compromise. This drug is not indicated for long-term management because its negative inotropic effects may worsen heart failure.

Unresolved Therapeutic Issues

Combination With ß-blockers. ß-Blocker therapy has become pivotal in the management of heart failure. However, the majority of patients enrolled in controlled clinical trials that study the efficacy of digoxin were not taking ß-blockers. Therefore, it is uncertain whether or not digoxin should be routinely included as part of a ß-blocker regimen for symptomatic heart failure caused by left ventricular systolic dysfunction. There are attractive features of combining digoxin with ß-blocker therapy in the treatment of heart failure. The majority of heart failure patients have coronary artery disease and may be at risk for transient episodes of myocardial ischemia that could cause catecholamine release and sudden cardiac death. Combining digoxin with a ß-blocker may preserve the beneficial effects of digoxin on the symptoms of heart failure while minimizing the potential detrimental effects of this therapy on catecholamine release in the setting of ischemia (47).

Combination with Diuretics. Non-potassium-sparing diuretics can produce electrolyte abnormalities such as hypokalemia and hypomagnesemia, which increases the risk of digoxin toxicity. The combination of digoxin with a potassium- sparing diuretic would be a potentially safer alternative. Further study will be necessary to carefully elucidate the efficacy and safety of combining digoxin with these agents.

Anticoagulation and Antiplatelet Drugs
Background for Recommendations

Patients with heart failure are recognized to be at increased risk for thromboembolic events that can be arterial or venous in origin. In addition to atrial fibrillation and poor ventricular function (which promote stasis and increase the risk of thrombus formation), patients with heart failure have other manifestations of hypercoagulability. Evidence of heightened platelet activation; increased plasma and blood viscosity; and increased plasma levels of fibrinopeptide A, ßthromboglobulin, D-dimer, and von Willebrand factor (59-61) have been found in many patients. Despite a predisposition, estimates regarding the incidence of thromboemboli in patients with heart failure vary substantially between 1.4 and 42 per 100 patient years (62-65). Although variability in the reported incidence likely results from differences in the populations studied and the methods used to identify these events, the consensus is that pulmonary and systemic emboli are not common in heart failure patients. Traditionally, the issue of anticoagulation in patients with heart failure centered on warfarin. Growing recognition of the importance of ischemic heart disease as a cause of heart failure suggests that the role of antiplatelet therapy must be considered in patients with this syndrome as well.

Previous guidelines have recommended warfarin anticoagulation in patients with heart failure complicated by atrial fibrillation and in heart failure patients with prior thromboembolic events (18,19). Warfarin anticoagulation specifically was not recommended in patients with heart failure in the absence of these indications. There have been no randomized, controlled trials of warfarin in patients with heart failure. Therefore,

recommendations regarding its use, in the absence of atrial fibrillation or clinically overt systemic or pulmonary thromboemboli, must be made on the basis of cohort data and expert opinion. The likely incidence of thromboembolic events and the possibility of averting them with warfarin are important considerations for any guideline recommendation. In addition, the potential beneficial effects of warfarin on coronary thrombotic events, independent of embolic phenomenon, must be taken into account. The
substantial clinical trial data that reflect the beneficial effects of antiplatelet therapy in patients with ischemic heart disease suggest that new guideline recommendations for heart failure should address the role of this form of therapy in patients with left ventricular dysfunction.

Anticoagulation

> **Recommendation 1. All patients with heart failure and atrial fibrillation should be treated with warfarin (goal, international normalized ratio (INR) 2.0 to 3.0) unless contraindicated (Strength of Evidence = A).**

The committee agrees with previous guideline recommendations that concern warfarin therapy in patients with heart failure complicated by atrial fibrillation. The benefit of warfarin anticoagulation in this setting is well established through several randomized trials (66). Patients with heart failure commonly have atrial fibrillation. Warfarin anticoagulation should be implemented in all of these patients unless clear contraindications exist.

> **Recommendation 2. Warfarin anticoagulation merits consideration for patients with left ventricular ejection fraction of 35% or less. Careful assessment of the risks and benefits of anticoagulation should be undertaken in individual patients (Strength of Evidence = B).**

Cohort analyses examining the relationship between warfarin use and noncoronary thromboembolism in patients with heart failure have not consistently yielded positive findings (62,63,65,67-69). It is possible that the lack of consistent benefit was related to the low incidence of identifiable embolic events in these populations. However, these studies do not make a convincing argument for the use of warfarin to prevent embolic events in the absence of atrial fibrillation or a previous thromboembolic episode.

In contrast, a recent cohort analysis of the SOLVD population focused on the relation between warfarin use and the risk of all-cause mortality rather than risk for embolic events (70). After adjustment for baseline differences, patients treated with warfarin at baseline had a significantly lower risk of mortality during follow-up (adjusted hazard ratio .76; 95% CI, .65 to .89, *P* = .0006). In addition to a mortality benefit, warfarin use was also associated with a significant reduction in the combined endpoint of death or hospitalization for heart failure (adjusted hazard ratio .82; 95% CI, .72 to .93, *P* = .002). In the SOLVD population, the benefit associated with warfarin use was not significantly influenced by 1) presence or absence of symptoms (treatment trial *v* prevention trial), 2) randomization to

enalapril or placebo, 3) gender, 4) presence or absence of atrial fibrillation; 5) age, 6) ejection fraction, 7) NYHA class, or 8) origins of disease.

The benefit associated with warfarin use in the cohort analysis of the SOLVD population was related to a reduction in cardiac mortality. Specifically, there was a significant reduction among warfarin users in deaths that were identified as sudden, in deaths associated with heart failure, and in fatal MI. In contrast (yet in agreement with previous cohort analyses), there was no significant difference in deaths considered cardiovascular but noncardiac, including pulmonary embolism and fatal stroke. Some caution is needed in consideration of this finding because the number of cardiovascular deaths that were noncardiac was far less than the number of cardiac deaths.

Reduction in ischemic events is one potential explanation for the apparent benefit from warfarin in the SOLVD Study. Warfarin users showed a reduced rate of hospitalization for unstable angina or nonfatal MI. Prior investigations of patients after acute MI showed that warfarin anticoagulation, when started within 4 weeks, reduces the incidence of fatal and nonfatal coronary events, as well as pulmonary embolus and stroke (71).

As with other post hoc, cohort analyses, it is possible that the findings from the SOLVD Study may result from differences between the treatment groups that were not identified and for which statistical correction could not adequately adjust. For this reason, evidence from any cohort study must be considered less powerful compared with evidence derived from randomized, controlled trials. Nevertheless, in the absence of randomized data, the SOLVD cohort analysis represents reasonable evidence to support more aggressive use of warfarin anticoagulation than previously recommended in patients with reduced left ventricular ejection fraction and sinus rhythm. The data from this analysis provide no information regarding the ideal warfarin dose in this patient population. Therefore, the dosing recommendation should likely conform to that derived from previous randomized trials performed in patients without mechanical prosthetic valves (INR 2.0 to 3.0).

Antiplatelet Drugs

> **Recommendation 1. With regard to the concomitant use of ACE inhibitors and acetylsalicylic acid (ASA), each medication should be considered on its own merit for individual patients. Currently, there is insufficient evidence concerning the potential negative therapeutic interaction between ASA and ACE inhibitors to warrant withholding either of these medications in which an indication exists (Strength of Evidence =** C).

Strong evidence supports the clinical benefit of aspirin in ischemic heart disease and atherosclerosis (72-75). However, recent post hoc analyses of large randomized trials involving ACE inhibitors in heart failure and post-MI suggest the possibility of an adverse drug interaction between ASA and ACE inhibitors (76-78). A retrospective cohort analysis of the SOLVD Study found that patients on antiplatelet therapy (assumed to be ASA in the great majority of patients) derived no additional survival benefit from the addition of

enalapril. Data from CONSENSUS II and GUSTO-1 in post-MI patients, suggest not only no additive benefit, but the possibility of a negative effect on mortality from the combination of ASA and ACE inhibition. In contrast, an unadjusted, retrospective registry study in patients with chronic coronary artery disease did not support an adverse interaction (79). Interestingly, in an adjusted analysis of the subset of patients with heart failure in this study, the beneficial effects of aspirin seemed less evident in patients taking ACE inhibitors. Despite these provocative post hoc findings, no prospective studies have yet been reported that concern the possible adverse interaction between ACE inhibitors and aspirin. To date, there is no clear evidence of harm from the combination of ASA and ACE inhibitors in patients with heart failure (76).

There is also some evidence that the potential interaction between ASA and ACE inhibitors may be dose related. A recent meta-analysis of all hypertension and heart failure patients who have received both ASA and ACE inhibitors suggests that ASA at doses equal to or less than 100 mg showed no interaction with ACE inhibitors (80). Any interaction, if observed, occurred at higher doses of aspirin.

A potential mechanism for the hypothesized adverse interaction between ASA and ACE inhibitors in patients with heart failure involves prostaglandin synthesis. ACE inhibition is believed to augment bradykinin which, in turn, stimulates the synthesis of various prostaglandins that may contribute vasodilatory and other salutary effects. In the presence of ASA, the bradykinin-induced increase in prostaglandins should be attenuated or blocked, which potentially reduces the benefits of ACE inhibition. Invasive hemodynamic monitoring has shown that the acute hemodynamic effect of enalapril is blunted by concomitant administration of aspirin (81). Another possibility is that ASA and ACE inhibitors act in a similar fashion in heart failure, therefore no added benefit is gained from the combination. ACE inhibitors appear to reduce ischemic events in heart failure patients possibly through antithrombotic effects, which could mimic those of antiplatelet agents. Recent study results that suggest ASA may have independent beneficial action on ventricular remodeling support the hypothesis of similar mechanisms of action for ACE inhibitors and ASA (82).

Development of the adenosine diphosphate (ADP) antagonists, ticlopidine and clopidogrel, provides alternative therapy for platelet inhibition that does not appear to influence prostaglandin synthesis (83). In direct comparison with aspirin, large-scale clinical trial results have established the efficacy of clopidogrel in the prevention of vascular events in patients with arteriosclerotic disease (84). Clinical data are limited with ADP antagonists in heart failure. However, hemodynamic evaluation found a similar reduction in systemic vascular resistance in heart failure patients treated with the combination of ACE inhibitors and ticlopidine versus ACE inhibitors alone, which suggests no adverse hemodynamic interaction with ACE inhibition with this type of antiplatelet compound (85). Definitive resolution of the therapeutic implications of the ASA/ACE inhibitor interaction and the appropriate alternative therapy, if any, in heart failure awaits the results of additional clinical research studies.

Angiotensin II Receptor Blockers

Background for Recommendations
Angiotensin ll (AT) receptor blockers (ARBs) differ in their mechanism of action compared with ACE inhibitors. Rather than inhibiting the production of AT by blockade of ACE, ARBs block the cell surface receptor for AT. ARBs that are currently available are selective and only effectively inhibit the AT1 subtype of this receptor. Theoretical benefits of ARBs include receptor blockade of AT produced by enzymes other than ACE and maintenance of ambient AT to maintain or increase stimulation of AT2 receptors. AT1 receptor antagonism is important because this receptor appears to mediate the classical adverse effects associated with AT in heart failure. In contrast, the AT2 receptor subtype appears to counterbalance AT1 receptor stimulation by causing vasodilation and inhibiting proliferative and hypertrophic responses (86). Thus, the selective receptor blockade of the current ARBs may be particularly advantageous. Theoretical concerns about ARB therapy include the potential deleterious effects of increased AT levels and AT2 receptor-mediated enhancement of apoptosis. Whether ARBs have beneficial effects similar to ACE inhibitors on the course of coronary artery disease remains to be determined. ARBs may or may not influence bradykinin concentrations, which are anticipated to rise with ACE inhibitor therapy and may contribute to their efficacy.

The hemodynamic actions of ARBs have, thus far, been similar to ACE inhibitors for reduction of blood pressure in hypertension and lowering of systemic vascular resistance in heart failure (87). ARBs have a similar mild-to-modest effect on exercise capacity and produce a comparable reduction in norepinephrine relative to ACE inhibitors (88).

> **Recommendation 1. ACE inhibitors rather than ARBs continue to be the agents of choice for blockade of the renin-angiotensin system in heart failure, and they remain the cornerstone of standard therapy for patients with left ventricular systolic dysfunction with or without symptomatic heart failure (Strength of Evidence = A).**

At present, it is not possible to predict where ARBs will ultimately reside among accepted therapies for heart failure. Although the initial small ELITE Trial suggested a greater benefit from a losartan dosage of 50 mg daily than from a captopril dosage of 50 mg 3 times daily on mortality in elderly patients with heart failure (89), the ELITE II Mortality Trial, which included more than 3,000 patients (90), showed no comparative benefit from losartan and a trend for a better outcome and fewer sudden deaths with captopril (91). This result provides no evidence that the low dose (50 mg) of losartan that was tested is better than an ACE inhibitor for treating heart failure, but it does not exclude the efficacy of a higher dose designed to provide continuous inhibition of the AT1 receptor. Tolerability of losartan was better than of captopril, primarily because of an ACEinhibitor cough. But the well-established efficacy of the ACE inhibitors on outcome in the post-MI period, in diabetes, in atherosclerosis, and in heart failure mandates that this drug group remains agents of choice for inhibiting the renin-angiotensin system in heart failure. The RESOLVD Trial suggested

no major differences in efficacy of candesartan and enalapril, with a trend favoring enalapril during the study period of 43 weeks (92). The OPTIMAAL and VALIANT Studies will provide information specifically about the role of ARBs versus ACE inhibitors in the post-MI population.

Currently, ACE inhibitors continue to be regarded as the therapy of choice to inhibit the renin-angiotensin system in patients with asymptomatic and symptomatic left ventricular dysfunction. There is no current rationale to recommend initiating ARBs in patients with new onset heart failure or for switching from a tolerated ACE-inhibitor regimen to an ARB in patients with chronic heart failure.

> **Recommendation 2. All efforts should be made to achieve ACE inhibitor use in patients with heart failure caused by left ventricular dysfunction. Patients who are truly intolerant to ACE inhibitors should be considered for treatment with the combination of hydralazine and isosorbide dinitrate (Hyd-ISDN) (Strength of Evidence = B) or an ARB (Strength of Evidence = C).**

Previous large-scale trials do not specifically address the role of ARB and Hyd-ISDN in patients who are intolerant to ACE inhibitors. One arm of the CHARM Study has been specifically designed to test the effectiveness of candesartan in patients with systolic dysfunction who are intolerant to ACE inhibitors. The primary endpoint in this study will be a composite of cardiovascular death and time until first hospitalization for heart failure. For now, ARBs offer a reasonable alternative in the heart failure or post-MI patient who is truly intolerant to ACE inhibition. Intolerance because of cough should always trigger a careful reevaluation for congestion. If congestion is present, cough should abate with increases in diuretic that should allow ACE-inhibitor use to continue (93). It should be emphasized that patients intolerant to ACE inhibitor because of renal dysfunction, hyperkalemia, or hypotension are often intolerant to ARBs as well. ACE inhibitor intolerance because of persistent symptomatic hypotension in advanced heart failure may represent severe dependence on the hemodynamic support of the renin-angiotensin system, which generally would predict hypotension with ARB use as well.

The combination of Hyd-ISDN has not been studied in the post-MI population, but sufficient experience exists to support its use in the ACE-inhibitor-intolerant patient with symptomatic heart failure. Hydralazine blocks the development of nitrate tolerance, which argues for the use of combination therapy. Although they were not studied alone in a heart failure mortality trial, oral nitrates represent another reasonable alternative for patients intolerant to both ACE inhibitors and hydralazine.

Unresolved Therapeutic Issues

Combination Therapy With ACE Inhibitors and ARBs. Interest has grown in the potential utility of combining ACE inhibitors and ARBs in patients with heart failure. Initial

data suggest that the combination yields more vasodilation and decreased blood pressure than either agent alone. The addition of losartan to an ACE inhibitor has been found to improve exercise capacity compared with an ACE inhibitor alone (94). Preliminary data from the RESOLVD Trial suggest that ventricular dilation and neuroendocrine activation may be best reduced with combination therapy, but other endpoints were not clearly affected. Trials are currently underway to determine the safety, as well as benefit, of more complete blockade of the renin-angiotensin system. The Val-HeFT Trial is a large-scale investigation of the effect of valsartan in addition to ACE inhibitors on morbidity and mortality in symptomatic patients with heart failure caused by systolic dysfunction. One arm of the CHARM Study will also examine the effect of the addition of candesartan in patients with symptomatic, systolic dysfunction treated with an ACE inhibitor. Preliminary data from the RESOLVD Trial suggest that combination therapy may be even more efficacious when used in conjunction with ß-blocker treatment. Results from Val-HeFT and CHARM in the subset of patients treated with ß-blocker therapy will provide more information concerning this strategy.

Combination therapy represents a rational option when treating severe hypertension or other vasoconstriction but cannot, at present, be recommended as routine therapy in the absence of a proven superiority to ACE-inhibitor therapy alone.

HFSA Guidelines
Appendix D.1
Criteria for NYHA functional classification for chronic heart failure patients, functional capacity (130)

CLASS 1	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea.
CLASS 2	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation or dyspnea.
CLASS 3	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation or dyspnea.
CLASS 4	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

Appendix D.2
Glossary of Clinical Trials

AVID	Antiarrhythmics Versus Implantable Defibrillators
BEST	Beta-blocker Evaluation of Survival Trial
CAMIAT	Canadian Amiodarone Myocardial Infarction Arrhythmia Trial
CAPRIE	Clopidogrel vs Aspirin in Patients at Risk of Ischemic Events
CASH	Cardiac Arrest Study HamburgCHF-STAT Congestive Heart Failure-Survival Trial of Antiarrhythmic Therapy
CHARM	Candesartan Cilexetil in Heart Failure Assessment of Reduction in Mortality and Morbidity
CIBIS	Cardiac Insufficiency BIsoprolol Study
CIBIS II	Cardiac Insufficiency Bisoprolol Study II
CIDS	Canadian Implantable Defibrillator Study
COMET	Carvedilol or Metoprolol European Trial
CONSENSUS	Cooperative North Scandinavian Enalapril Survival Study
CONSENSUS II	Cooperative New Scandinavian Enalapril Survival Study II
COPERNICUS	Carvedilol Prospective Randomized Cumulative Survival Trial
DEFINITE	Defibrillators in Nonischemic Cardiomyopathy Treatment Evaluation
DIAMOND	Danish Investigation of Arrhythmia and Mortality on Dofetilide
DIG	Digitalis Investigation Group
ELITE	Evaluation of Losartan In The Elderly
ELITE II	Losartan Heart Failure Survival Study - ELITE II
EMIAT	Infarction Amiodarone TrialGESICA Grupo de Estudio de Sobrevida en Insuficiencia Cardiaca en Argentina
GUSTO 1	Global Utilization of Streptokinase and TPA for Occluded coronary arteriesMADIT Multicenter Automatic Defibrillator Implantation Trial
MADITII	Multicenter Automatic Defibrillator Implantation Trial II
MDC	Metoprolol in Dilated Cardiomyopathy trial
MERIT-HF	Metoprolol CR/XL Randomized Intervention Trial in Heart Failure
MOCHA	Multicenter Oral Carvedilol in Heart-failure Assessment
MTT	Myocarditis Treatment Trial
OPTIMALL	Optimal Therapy in Myocardial Infarction with the Angiotensin II Antagonist Losartan
PRECISE	Prospective Randomized Evaluation of Carvedilol In Symptoms and Exercise
PROVED	Prospective Randomized study Of Ventricular failure and the Efficacy of Digoxin
RADIANCE	Randomized Assessment of Digoxin on Inhibitors of the Angiotensin Converting Enzyme
RALES	Randomized Aldactone Evaluation Study
RESOLVD	Randomized Evaluation of Strategies for Left Ventricular Dysfunction
SAVE	Survival And Ventricular Enlargement
SCD-HeFT	Sudden Cardiac Death in Heart Failure: Trial of prophylactic amiodarone versus implantable defibrillator therapy
SOLVD	Studies Of Left Ventricular Dysfunction
SWORD	Survival With Oral D-sotalol
ValHeFT	Valsartan Heart Failure Trial

APPENDIX E
NCCN Guidelines for Adjuvant Breast Cancer Treatment

http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

For hormone receptor positive, NP breast cancer see BINV-6 or BINV-9

For hormone receptor negative, NP breast cancer see BINV-8 or BINV-9

For hormone receptor negative, NN breast cancer see BINV-8 or BINV-9

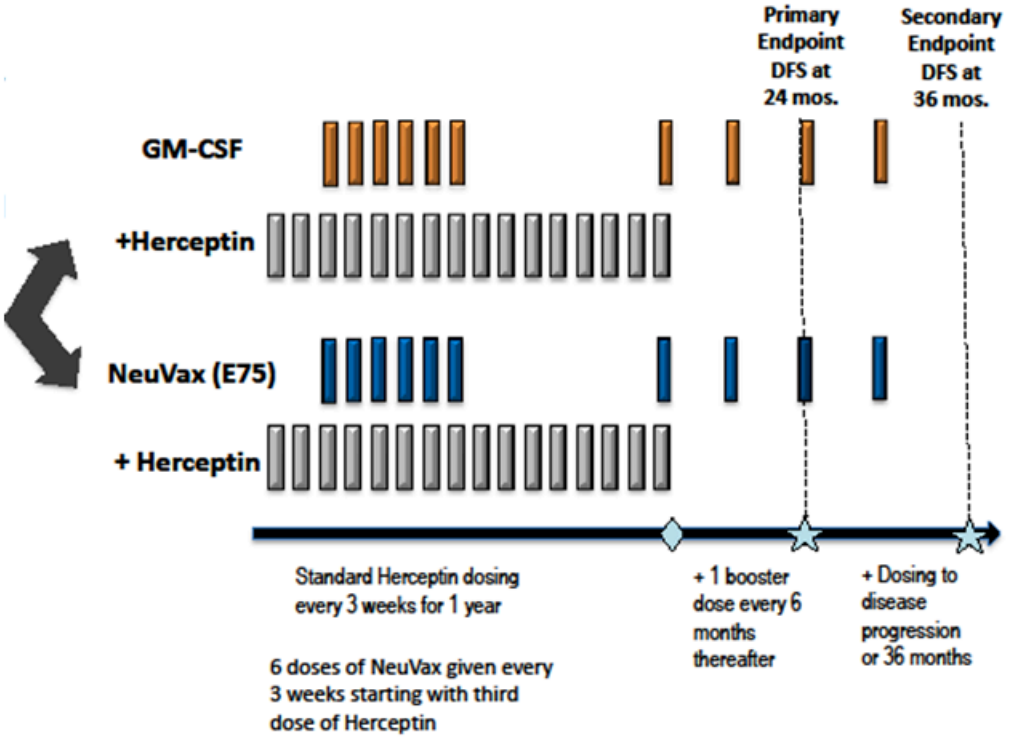
APPENDIX F
Study Randomization and Stratification Schema

Phase II: NeuVax (E75) + Herceptin v. Herceptin alone in HER2 IHC 1+/2+, early-stage breast cancer

Study Population

Adjuvant Breast cancer (BC) patients, n=300, randomized 1:1

- HLA A2+/A3+/A24+/A26+, low and intermediate HER2 (IHC 1+/2+) expression; node positive (HR+/-) or node negative (HR-)
- Stratified by nodal status and HER2 status
- Single dose level of Herceptin + NeuVax vs Herceptin + GM-CSF alone



APPENDIX G
E75 + GM-CSF Formulation Plan

GALENA BIOPHARMA

ML25749

Combination immunotherapy with Herceptin® and the HER2 vaccine E75 in low and intermediate HER2 expressing breast cancer patients to prevent recurrence

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 Subject 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 Subject ID number. This kit contains a single vial of lyophilized Leukine (250 µg).
3. Ancillary Supplies: 7-1.0 mL TB syringes, 5-3/8 inch 26G needles, 4-2 inch 21G needles, 2-3 mL luer lock syringes.

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 0.07mL of the 1.5mg/1.0mL E75 acetate solution. The DTH skin test is administered intradermally using 0.07 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 1.5 mg/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 of 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 syringe
7. Remove and discard needle
8. Attach a new, sterile needle to the syringe containing 0.07 mL (100 μg) E75 acetate solution for intradermal administration

APPENDIX H
Leukine® Package Insert

APPENDIX I
Performance Status Criteria

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.

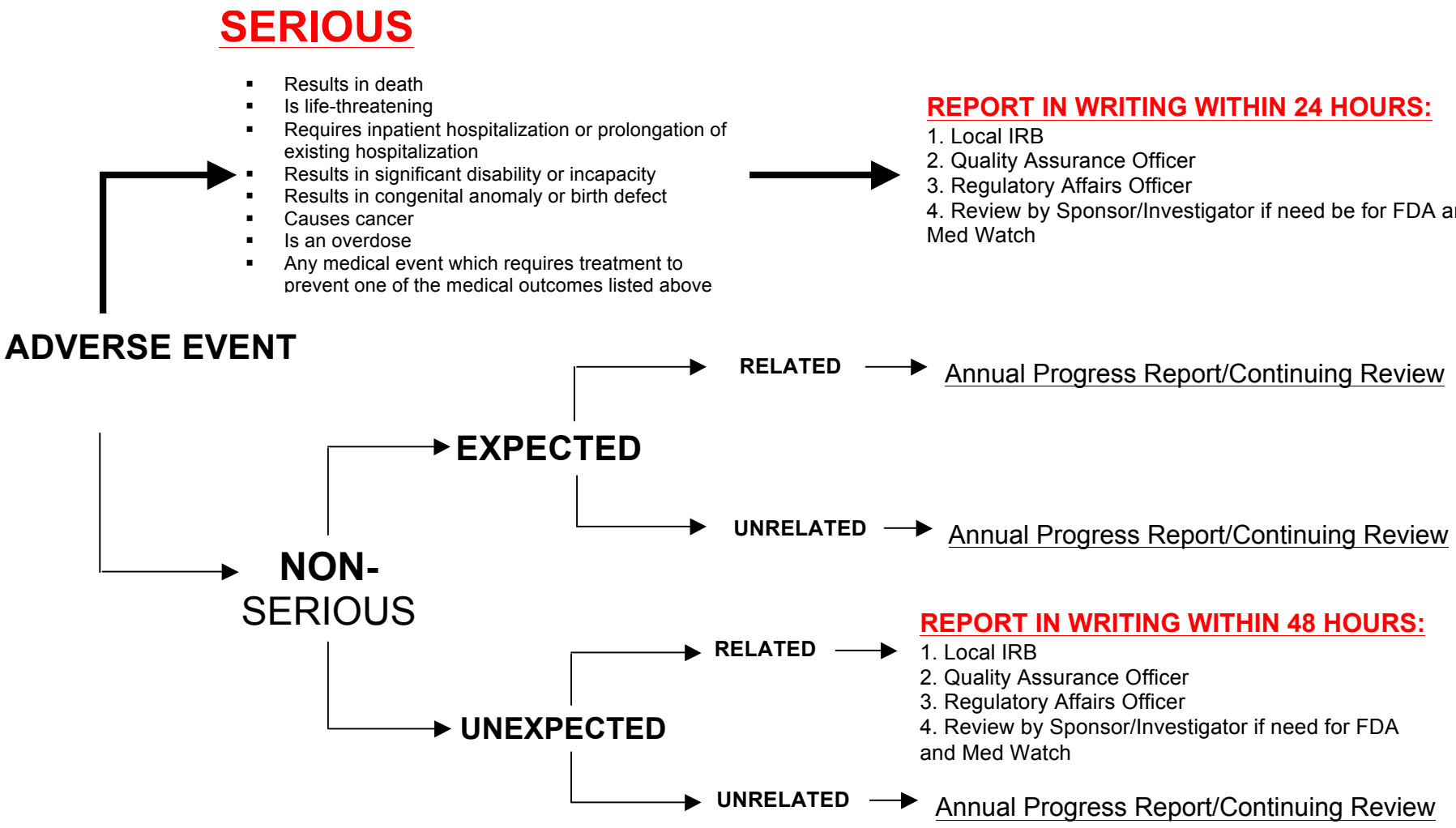
APPENDIX J
Case Report Forms

These are free standing electronic documents.

APPENDIX K
Data Safety Monitoring Plan

This is a free standing document

APPENDIX L
Adverse Event Reporting Algorithm



APPENDIX M
FDA MedWatch 3500A Form Link

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

APPENDIX N

SAFETY LANGUAGE INCLUDED IN THIS DOCUMENT IS MANDATORY AND MUST BE INCLUDED IN PROTOCOL

SAFETY REPORTING OF ADVERSE EVENTS

ASSESSMENT OF SAFETY

Specification of Safety Variables

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) that are considered related to {study drug}, all events of death, and any study specific issue of concern.

Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with breast cancer that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).

If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention.

Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

Serious Adverse Events

An AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

METHODS AND TIMING FOR ASSESSING AND RECORDING SAFETY VARIABLES

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, are collected and reported to the FDA, appropriate IRB(s), and Genentech, Inc. in accordance with CFR 312.32 (IND Safety Reports).

Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the {study drug} (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes

There is a plausible temporal relationship between the onset of the AE and administration of the {study drug}, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the {study drug}; and/or the AE abates or resolves upon discontinuation of the {study drug} or dose reduction and, if applicable, reappears upon re-challenge.

No

Evidence exists that the AE has an etiology other than the {study drug} (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to {study drug} administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

PROCEDURES FOR ELICITING, RECORDING, AND REPORTING ADVERSE EVENTS

Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation timepoints should be adopted. Examples of non-directive questions include:

"How have you felt since your last clinical visit?"
"Have you had any new or changed health problems since you were last here?"

Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section 5.1.2), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report “Unexplained Death”.

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

e. Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 6 months after receiving the inoculations or after the last dose of study drug, a report should be completed and expeditiously submitted to the Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the {study drug} should be reported as an SAE.

f. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior {study drug} exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

g. Reconciliation

The Sponsor agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange monthly line listings of cases received by the other party. If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

h. AEs of Special Interest (AESIs)

AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the Product

The Adverse Events of Special Interest are:

SAE Reporting

Investigators must report all SAEs to both Genentech and Galena within the timelines described below. The completed Medwatch/case report should be faxed immediately upon completion to Genentech Drug Safety at:

(650) 225-4682
OR
(650) 225-5288
AND
drugsafety@galenabiopoharma.com

Relevant follow-up information should be submitted to both Genentech and Galena Drug Safety as soon as it becomes available.
Serious AE reports that are related to the study drugs and AEs of Special Interest (regardless of causality) will be transmitted to both Genentech and Galena within fifteen (15) calendar days of the Awareness Date.
Serious AE reports that are unrelated to the study drugs will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
Additional Reporting Requirements to both Genentech and Galena include the following:
Any reports of pregnancy following the start of administration with the study drugs will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
All Non-serious Adverse Events originating from the Study will be forwarded in an annual report to both Genentech and Galena.

Note: Investigators should also report events to their IRB as required.

MedWatch 3500A 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 MedWatch 3500A form:
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 adverse event 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 MedWatch 3500A report and submitting it as follow-up
Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
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)

Occasionally Genentech may contact the reporter for additional information, clarification, or current status of the patient for whom and adverse event was reported. For questions regarding SAE reporting, you may contact the Genentech Drug Safety representative noted above or the MSL assigned to the study. Relevant

follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available and/or upon request.

MedWatch 3500A (Mandatory Reporting) form is available at
http://www.fda.gov/medwatch/getforms.html

Additional Reporting Requirements for IND Holders

For Investigator-Sponsored IND Studies, some additional reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR § 600.80.

Events meeting the following criteria need to be submitted to the Food and Drug Administration (FDA) as expedited IND Safety Reports according to the following guidance and timelines:

7 Calendar Day Telephone or Fax Report:

The Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of study drugs. An unexpected adverse event is one that is not already described in the study drugs Investigator Brochure. Such reports are to be telephoned or faxed to the FDA and Genentech within 7 calendar days of first learning of the event.

15 Calendar Day Written Report

The Investigator 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 study drugs. An unexpected adverse event is one that is not already described in the study drugs investigator brochure.

Written IND Safety reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the investigator 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, Genentech, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a Medwatch 3500 form, but alternative formats are acceptable (e.g., summary letter).

FDA fax number for IND Safety Reports:

Fax: 1 (800) FDA 0178

All written IND Safety Reports submitted to the FDA by the Investigator must also be faxed to Genentech Drug Safety:

Fax: (650) 225-4682 or (650) 225-5288

And will be submitted to the Site IRBs.

For questions related to safety reporting, please contact Genentech Drug Safety:

Tel: (888) 835-2555
Fax: (650) 225-4682 or (650) 225-5288

IND Annual Reports

Copies to Genentech:

All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. Copies of such reports should be faxed to Genentech Drug Safety:

Fax: (650) 225-4682 or (650) 225-5288

STUDY CLOSE-OUT

Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Genentech. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Genentech. Copies of such reports should be mailed to the assigned Clinical Operations contact for the study.

Genentech
A Member of the Roche Group

SAFETY REPORTING FAX COVER SHEET

Genentech Supported Research

AE / SAE FAX No: (650) 225-4682

Alternate Fax No: (650) 225-5288

Genentech Study Number	
Principal Investigator	
Site Name	
Reporter name	
Reporter Telephone #	
Reporter Fax #	

Initial Report Date	[DD] / [MON] / [YY]
Follow-up Report Date	[DD] / [MON] / [YY]

Subject Initials (Enter a dash if patient has no middle name)	[ ] - [ ] - [ ]

SAE or Safety Reporting questions, contact Genentech Safety: (888) 835-2555

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET

APPENDIX O

PROTOCOL TITLE:	Combination immunotherapy with Herceptin and the HER2 vaccine E75 in low and intermediate HER2-expressing breast cancer patients to prevent recurrence
STUDY DRUGS:	HERCEPTIN® (Trastuzumab) E75 peptide (KIFGSLAFL, HER2/*neu*, 369-377) GM-CSF (Leukine®, Berlex)
SPONSOR/INVESTIGATOR PROGRAM DIRECTOR	George E. Peoples, MD
PROTOCOL VERSION/DATE:	3.1 / 05 March 2017

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 Vaccine Development Program. 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:

_____

_____

_____

_____