"Vaccination of High Risk Breast Cancer Patients"

A Combined Phase I/II Feasibility-and-Efficacy Study of a Carbohydrate Mimotope-Based Vaccine with MONTANIDE™ ISA 51 VG STERILE Combined with Neoadjuvant Chemotherapy

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1. ABBREVIATIONS

AC	Doxorubicin and Cyclophosphamide Combination Chemotherapy
ACLS	Advanced Cardiac Life Support
ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
AE	Adverse Event
AJCC	American Joint Commission on Cancer
ALT	Alanine Aminotransferase Test
AST	Aspartate Aminotransferase Test
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CCT	Cancer Clinical Trials
CD	Cluster of Differentiation
CDC	Complement Dependent Cytotoxicity
CFR	Code of Federal Regulations
Chemistry	BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium
CMPs	Carbohydrate Mimetic Peptides
CRA	Clinical Research Associate
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTL	Cytotoxic T Lymphocyte
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
CY	Cyclophosphamide
dL	Deciliter
ECOG score	Eastern Cooperative Oncology Group performance status
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked Immunospot Assay
ER	Estrogen-Receptor
FBS	Fetal Bovine Serum
FDA	United States Food and Drug Administration
FITC	Fluorescein Isothiocyanate
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HER2	Human Epidermal Growth Factor Receptor 2
HLA-DR	Human Leukocyte Antigen - antigen D Related
HOG	Highlands Oncology Group
HRP	Horseradish Peroxidase
ICH	International Council for Harmonisation
IFA	Incomplete Freund's Adjuvant
IFN-γ	Interferon Gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IND	Investigational New Drug Application
IRB	Institutional Review Board
IUDs	Intrauterine Device
IUL	Institutional Upper Limit
LDH	Lactate Dehydrogenase
LeY	Lewis Y antigen
µg	Microgram

mg	Milligram
mL	Milliliter
mm	Millimeter
MDSC	Myeloid-Derived Suppressor Cells
NCI	National Cancer Institute
NIH	National Institutes of Health
NK	Natural Killer
P10s-MAP	P10s-Multivalent-Antigen Peptide
PBMC	Peripheral Blood Mononuclear Cell
PD-1	Programmed cell death protein
PE	Physical Examination
PRMC	Protocol Review and Monitoring Committee
pCR	pathological Complete Response
SAE	Serious Adverse Event
SC	Subcutaneous
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic-Pyruvic Transaminase
SI	Stimulation Index
SoC	Standard/Standard of Care
STn	sialosyl-TN
TACA	Tumor-Associated Carbohydrate Antigen
TBAPS	Tissue Biorepository and Procurement Service
Tregs	Regulatory T cells
UAMS	University of Arkansas for Medical Sciences
UPIRTSO	Unanticipated Problem Involving Risks to Subjects or Others
WBC	White Blood Cell
WPRCI	Winthrop P. Rockefeller Cancer Institute

2. PROTOCOL SUMMARY

The purpose of this study is to evaluate an investigational agent, P10s-PADRE, a peptide mimotope-based vaccine, in combination with SoC neoadjuvant chemotherapy in subjects with clinical stage I, II or III estrogen-receptor (ER)-positive breast cancer. This combination of vaccine with chemotherapy will be called "Chemovax". P10s-PADRE will be administered with MONTANIDE™ ISA 51 VG as adjuvant. Human breast cancers that express Tumor-Associated Carbohydrate Antigens (TACAs) can be immunogenic, and enhancing the anti-TACA antibodies and immune effector function already present may augment the cytotoxic effects of SoC therapies.

A single-arm trial is designed with the goal being to evaluate the feasibility and efficacy of combining vaccination of the P10s-PADRE formulation with neoadjuvant chemotherapy. Feasibility will be based on the safety, tolerability, and immune-response adequacy of P10s-PADRE when it is administered in combination with neoadjuvant chemotherapy. Once a feasible schedule of Chemovax has been identified, efficacy will be based on the rate of pathologic Complete Response (pCR) observed among ER-positive breast-cancer subjects treated with the combination.

The feasibility of schedule and clinical response to the vaccine will be assessed as follows:

Primary Objectives:

1) **Identify a feasible schedule of vaccination relative to SoC neoadjuvant chemotherapy when the two are administered concurrently. Feasibility will be evaluated in terms of:**
   a) Generation of a sufficiently high anti-P10s immunoglobulin-G response
   b) Safety and tolerability of the combination of vaccine and chemotherapy

2) **Demonstration of clinical response:**
   Determine if the Chemovax regimen in ER-positive breast cancer would lead to a significantly higher rate of pCR in breast and axillary lymph nodes at time of definitive surgery compared to the corresponding rate reported by von Minckwitz et al (1, 2).

Secondary Objectives:

1) **Determine Short-term Immune Responses Induced by Chemovax:**
   a) Determine the functional humoral response as defined by:
      i. P10s-MAP-reactive immunoglobulin-G endpoint titers in serum.
      ii. Cytotoxicity of serum and plasma samples against two TACA-expressing breast cancer cell lines following Chemovax therapy.
   b) Determine the activation profile of NK cells before and after Chemovax therapy.
   c) Determine T-cell subpopulations and evaluate the effects of Chemovax therapy on the numbers of circulating T cells.
   d) Perform functional T-cell immune analysis by performing T-cell proliferation, ELISPOT, cytokine release and ADCC assays.
   e) Determine expression of biomarkers of efficacy and response on tumor tissues and characterize tumor infiltrating lymphocytes and their role in response to therapy.

2) **Determine Long-term Immune Responses Induced by Chemovax:**
   a) Determine antibody titers against P10s at approximately 6 and 12 months following the initial response evaluation.
   b) Measure anti-TACA cell-binding levels at approximately 6 and 12 months following the initial response evaluation.
   c) Determine the cytotoxic activity against TACA-expressing cell lines at approximately 6 and 12 months following the initial response evaluation.
   d) Evaluate the functionality of T and NK cells.

Study Population: We plan to screen up to 81 women in order to enroll approximately 61 women, 18 years of age and older, from the breast cancer clinics (Medical Oncology and Ladies Oncology Clinics) at the Winthrop P. Rockefeller Cancer Institute (WPRCI) at the University of Arkansas for Medical Sciences (UAMS) campus and the clinics of the Highlands Oncology Group (HOG) in Northwest Arkansas.

Summary of Inclusion Criteria: Females, 18 years of age or older, with clinical stage I, II or III ER-positive, HER2-negative breast cancer who qualify for SoC neoadjuvant treatment will be invited to participate.

Summary of Exclusion Criteria: Females with ER-negative, HER2-positive, inflammatory, or metastatic breast cancer; women who are pregnant, breast-feeding, have autoimmune disease or are immunosuppressed or receiving systemic corticosteroids (except from corticosteroids received as antiemetics or for hypersensitivity reactions related to chemotherapy) will be excluded from the study.

Investigational product: P10s-PADRE administered with the adjuvant MONTANIDE™ ISA 51 VG STERILE

Study Treatment: Chemovax - P10s-PADRE/MONTANIDE™ ISA 51 VG in combination with SoC neoadjuvant chemotherapy

Study Design: This combined Phase I/II feasibility-and-efficacy study will have three parts. Its first part will be a Phase I evaluation of the safety, tolerability, and feasibility of eliciting adequate IgG response with P10s-PADRE when administered in combination with SoC neoadjuvant chemotherapy, and will require 25 subjects. The timing of vaccination relative to chemotherapy will be evaluated to identify a Chemovax schedule in which a sufficiently high proportion of subjects achieve an adequate IgG response. Once such a schedule is identified, the study will proceed with its second and third parts. The study's second and third parts will respectively constitute Stages 1 and 2 of the Phase II primary-efficacy evaluation of Chemovax using a Simon optimal two-stage design, and will require a minimum of 19 subjects (5 subjects from Part 1 + an additional 14 subjects) and a maximum of 41 subjects (19 subjects from Part 1 & 2 + an additional 22 subjects). The total sample size in the entire study will have a theoretical minimum of 25 subjects, a theoretical maximum of 61 subjects, and a most likely value of 41 subjects based on demonstrating both immune-response feasibility and clinical efficacy.

To conduct this study, eligible subjects of all races, aged 18 years or older, will be enrolled and immunized by SC administration of P10s-PADRE vaccine on each of 3 separate occasions over a three-week period at a dose level of 500 micrograms (μg) per immunization. Blood will be collected from each subject once before and 4 times after the first immunization. Follow-up blood samples will also be taken approximately 6 and 12 months after treatment evaluation to assess durations of the anti-P10s titer and anti-breast-cancer cytotoxicity. Tissue samples will be obtained from excess tissue collected as part of standard biopsy and/or surgery. These samples will be used for histology and immune cell isolation to identify immune cell infiltrates. Safety and tolerability of Chemovax will be evaluated throughout all parts/phases of the study. The primary efficacy endpoint is pCR and will be used to determine if Chemovax treatment in ER-positive breast cancer subjects will improve pCR at time of the definitive surgery to a rate significantly higher than the corresponding rate reported by von Minckwitz *et al*. (1).

3. BACKGROUND

Anticipated anti-cancer impact of carbohydrate-targeted vaccines: The potential impact of vaccines that induce responses to tumor-associated carbohydrate antigens (TACAs) is demonstrated by clinical trials where subject survival significantly correlates with carbohydrate-reactive IgM levels (3). Such results suggest that TACA-targeting vaccines might have a beneficial effect on the course of malignant disease. Vaccine-induced anti-TACA antibodies can trigger cancer cell death and augmentation of cellular immune response through cross presentation of tumor antigens, leading to shrinkage of metastatic lesions and further improving survival. TACA-induced responses could augment naturally occurring carbohydrate-reactive IgM antibodies that trigger apoptosis of tumor cells (4). TACAs are attractive targets because the majority of cell-surface proteins and lipids are glycosylated, and the glycosyl moiety is fundamental to the biological functions of these molecules in cancer cells (5, 6). A unique advantage in targeting TACAs is that multiple proteins and lipids on the cancer cell can be modified with the same carbohydrate structure. Thus, targeting the carbohydrate antigen broadens the spectrum of antigens recognized by the immune response, thereby lowering the risk of developing resistant tumors due to the loss of any one antigen (7). In addition, antibodies that recognize glycolipids are more apt to mediate complement-dependent cytotoxicity (CDC) and may, therefore, be more cytotoxic to tumor cells than antibodies that recognize protein antigens (8).

Approaches to augment immune responses to TACA: A variety of approaches are being taken to generate responses to TACAs. Because TACAs are T-cell-independent antigens and self-antigens, conjugation to immunologic carrier proteins is perceived to be essential to recruit T-cell help in antibody generation. Conjugation does not, however, assure an increase in immunogenicity because conjugation strategies do not uniformly enhance carbohydrate immunogenicity (9, 10). Furthermore, even with conjugation, the lack of induction of cellular immune responses that would amplify TACA-reactive humoral responses necessitates constant boosting with vaccine. Representative examples of carbohydrate-based conjugate vaccines in clinical development include those directed toward gangliosides (11-14), polysialic acid (8), Globo-H (15), LeY (16), and the sialosyl-TN (STn) antigen (17).

An approach predicted to facilitate cellular responses exploits the molecular mimicry of TACAs by protein surrogates, as they are T-cell-dependent antigens. Clinical characterizations of anti-idiotypic antibodies that mimic the GD3 ganglioside antigen (18) and GD2 (19) have been described. Carbohydrate mimetic peptides (CMPs) are alternatives to anti-idiotypic antibodies. The characterization of CMPs is at present limited to preclinical studies. CMPs that induce immune responses cross-reactive with TACA are also referred to as peptide mimotopes. Peptide mimotopes have been described for GD2 (20-22), GD3 (23), sialylated Lewis a/x (24) and Lewis Y (LeY) antigens (22, 25). Importantly, in preclinical prophylactic and therapeutic vaccination studies, peptide mimotopes were efficacious in eliciting immune responses that reduced tumor burden and inhibited metastatic outgrowth (25-27). Thus, peptide mimotopes of TACAs represent a new and very promising tool to overcome T-cell independence and to increase the efficiency of the immune response to glycan antigens.

Benefits of peptide mimotope immunization: There are several benefits to vaccination strategies that employ peptide mimotopes of TACA. First, peptide mimotopes function as xenoantigens and, consequently, provide an advantage to overcome tolerance to carbohydrate self-antigens. Antibodies induced by peptide mimotopes are thought to have low affinities for TACA; specific targeting of tumor cells is due in part to over-expression of the carbohydrate antigen on tumor cells, which compensates for the low affinity of the carbohydrate cross-reactive antibodies (28). In addition, mimotope-induced antibodies preferentially recognize the terminal residues of the TACA oligosaccharides, which are often structurally distinct from those found on normal cells (29). Thus, potential immunopathology due to destruction of normal tissue is minimized (30, 31).

Second, peptide mimotopes have the potential to overcome immune deficiencies that suppress vaccine-induced carbohydrate-directed responses (32). Unlike carbohydrate antigens and carbohydrate-conjugate vaccines, peptide mimotopes also prime B- and T-cells for subsequent memory of carbohydrate antigens, facilitating long-term surveillance through recall of carbohydrate immune responses (7). This effect may minimize the need for constant boosting. In addition, they can functionally emulate conserved structures of TACA, inducing antibodies that recognize multiple TACA, and therefore functioning like a TACA multivalent vaccine.

Third, peptide mimotopes can be manipulated in ways that TACA cannot. Peptide mimotopes can be engineered to induce CD8+ T cells cross-reactive with tumor-associated glycopeptides and/or to induce CD4+ T cells that benefit the further expansion of CD8+ T cells and B-cells (7, 27). The ability to induce a humoral carbohydrate cross-reactive response, a CD4+ T helper response, and a CD8+ cytotoxic T lymphocyte (CTL) response with one simple inoculation is a novel approach to vaccination. Therefore, peptide mimotopes hold the potential to generate a multifaceted TACA-reactive immune response.

Target carbohydrate antigens expressed on breast cancer cells: Tumors expressing high levels of certain types of TACAs exhibit greater metastasis than those expressing low levels of these antigens, and this negatively impacts prognosis (33-35). In breast cancer, the LeY, STn, and KH-1 antigens, as well as selected gangliosides, glycosphingolipids and Globo-H carbohydrate antigens, are considered prime vaccine candidates because of their tissue distribution (36, 37). In particular, LeY has long been recognized as a potential target for immunotherapy because it is expressed in 70–90% of tumors of epithelial origin (38). The abundant gangliosides include GM3, GM2, GM1, and GD2, GD3 and GT3 (39). Antibodies to TACAs mediate a variety of effector functions and might lend to cross-presentation of tumor antigens to stimulate anti-tumor cellular responses. At present, LeY-conjugate vaccines appear to have only a limited ability to induce anti-LeY immune responses in humans. Our *in vitro* studies demonstrate that peptide mimotopes of LeY and gangliosides induce serum antibodies in mice that recognize the appropriate carbohydrate antigens on human or murine breast cancer cell lines. Our *in vivo* studies demonstrate that the peptide mimotopes induce sustained immunity to these antigens. Collectively, these data provide the experimental foundation for evaluating peptide mimotopes as potential cancer vaccines in subjects with breast cancer.

Role of Natural Killer (NK) cells in P10s mediated anti-tumor activity: In preclinical studies we observed a role for NK cells in tumor-growth inhibition upon immunization with the P10s-PADRE vaccine. NK cells are known to have potent anti-tumor activity and thus play an important role in metastasis clearance. In light of the finding of increased NK activation in similar preclinical setting by Kawashima et *al.* (40), we hypothesize that the anti-tumor effect observed in mice immunized with P10s could be due to interactions between NK cells and the anti-tumor B cell response that has been boosted by the immunization (40). Human subjects immunized with P10s-PADRE in the Phase I safety study of P10s-PADRE displayed elevated activation of their NK cells. This finding provides an expectation of benefit upon immunization with P10s-PADRE.

Role of B and T cells: Chemotherapy is assumed to be immunosuppressive. However, each drug might have differing affects when used in combination with specific immunotherapy. Thus, diverse effects may have been achieved depending on the time and the dose of administration. For example, high dose doxorubicin and cyclophosphamide (CY), while not beneficial for T-cell response, have been noted to enhance the immunogen-specific antibody-forming cells (41). Early reports indicated that low dose CY treatment might facilitate adaptive T cell immunity by eliminating “suppressor” T cells. In addition to its role on T lymphocytes, CY has been shown to switch cytokine production of tumor-infiltrating-macrophages from IL-10 to IFN-γ (42) and polarize Th1/Th2 responses (43). Our own studies indicate that immunization with a CMP followed by CY treatment can facilitate inhibition of tumor growth (44), including P10s-PADRE (data unpublished). The administration of docetaxel to patients with metastatic prostate or breast cancer, as well as that of cisplatin plus vinorelbine to non-

small cell lung cancer patients, appears to significantly increase the ratio between effector T cells and regulatory T cells (Tregs) and to reduce the immunosuppressive activity of the latter in the majority of patients (45). Such studies provide the rationale for the selective use of active immunotherapy regimens in combination with specific SoC therapies to achieve the most beneficial clinical outcome among carcinoma patients. Developing the time and dose schedules therefore may increase the effectiveness of combining chemotherapy and immunotherapy. Recently introduced immunotherapies, such as anti-PD-1 or anti-CTLA-4 therapeutic antibodies, need the presence of anti-tumor immune cells to be effective. A targeted vaccination strategy may activate cellular response synergizing the impact of immune checkpoint-based therapies (46-48).

Potential for benefit with combination therapy: Mechanisms by which chemotherapy enhances vaccine-induced humoral immunity and vice versa remain largely unknown. It is possible that antibodies can sensitize tumor cells for more efficient killing by chemotherapeutics. In a Phase I trial with the P10s-PADRE vaccine, we observed that antibodies induced by P10s are cytotoxic to human breast cancer cell lines including a Trastuzumab-resistant one (49). TACA expression is part of signaling pathways that confer death resistance. Cells resistant to death are those that cause treatment failure and can colonize distant organs and vice versa. Admixing serum from P10s-PADRE immunized subject with docetaxel was shown to lower the LC_{50} value for the drug in killing a human tumor cell line. Consequently, immunization with P10s-PADRE followed by chemotherapy might augment the chemotherapy.

Summary of results from the Phase I trial with P10s-PADRE: A Phase I dose-escalation trial was designed to determine a maximum tolerated dose of vaccine plus adjuvant in two cohorts of stage IV breast cancer subjects. The study design was the rule-based “traditional” 3+3 design (50), with 3 subjects at each dose if no toxicities were observed. This dose-escalation Phase I trial was approved by the UAMS Institutional Review Board (IRB) and was completed.

Female subjects 18 years of age or older, of all races with histologically or cytologically confirmed Stage IV breast cancer were eligible, and subjects were enrolled after providing written, informed consent. The following eligibility criteria were used: The cancer must have been newly diagnosed metastatic or relapsed after primary or adjunctive therapy and must not have required a treatment change for 2 months. Treatments with anti-estrogen therapy or chemotherapy were allowed. The chemotherapy regimen could not contain steroids in the pre or post supportive care medications. Disease staging was done according to the American Joint Commission on Cancer (AJCC), sixth edition; no expectation of response to other therapies; an Eastern Cooperative Oncology Group performance status of 0–1; no prior therapy within 4 weeks and adequate organ function (white blood cell count ≥3,000/ mm^3, hemoglobin ≥8.0 g/dL, platelets ≥100,000/mm^3 within 2 weeks prior to registration, total bilirubin ≤3.0 mg/dL, aspartate aminotransferase ≤200 IU/L, alanine aminotransferase ≤200 IU/L, and serum creatinine ≤1.5 mg/dL). Subjects had to be immunocompetent as measured by responsiveness to two recall antigens by skin testing. The following exclusion criteria were applied: massive ascites; known brain metastasis; pregnancy or lactation; known history of HIV infection; clinically serious infection; severe cardiac insufficiency; other active malignancy; history of organ allograft; immunodeficiency or history of splenectomy; concurrent treatment with steroids or immunosuppressive agents; and unsuitability for the trial, based on clinical judgment.

The peptide vaccine was administered in liquid form, emulsified with incomplete Freund's adjuvant (IFA; Montanide ISA 51 VG SEPPIC), by subcutaneous (SC) injections on 5 separate occasions during Weeks 1, 2, 3, 7, and 19. The first cohort began with a 300 µg dose, and then the subsequent cohort received a 500 µg dose of P10s-PADRE. The peptides and IFA were synthesized according to Good Manufacturing Practice (GMP) guidelines. The primary endpoint was the safety of P10s-PADRE vaccination. The secondary endpoints were immunologic responses, clinical outcomes, and determination of the optimal dose of peptide for further clinical trials. The trial is registered on NIH’s ClinicalTrial.gov results database (number NCT01390064).

Subjects were evaluated for signs of toxicity during and after vaccination. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 4.0. Toxicities assessed were the laboratory parameters listed in the study calendar, as well as any sign or symptom found during the history and physical examination not noted at pre-study or on the baseline evaluation. Special attention was paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.

Sixteen subjects were consented, but only 6 met the eligibility criteria. Six subjects consequently received the P10s-PADRE vaccine. P10s vaccination was well-tolerated. All 6 subjects displayed a persistent IgG response to P10s after vaccination, and induced antibodies in serum and plasma displayed cross-reactivity to TACA-expressing human breast cancer cell lines. Antibody induced by P10s displayed cytotoxic functionality to human breast cancer cell lines, including a Trastuzumab-resistant one, which established an expectation of therapeutic benefit (expectation of efficacy). In summary, P10s vaccination was well-tolerated with injection site irritation as the only repetitive AE, and measurable immune responses and anti-cancer-cell efficacy were noted. This is the first study to show that CMP vaccination is safe and can induce functional antibodies that might have therapeutic benefit in subjects immunized with P10s.

Neoadjuvant treatment in breast cancer: The treatment of breast cancer includes the treatment of local disease with surgery, radiation therapy, or both, and the treatment of systematic disease with cytotoxic chemotherapy, endocrine therapy, biologic therapy (such as Trastuzumab in HER2 positive disease), or combination of these. The need for and selection of various local or systemic therapies are based on several prognostic and predictive factors that include tumor histology, clinical and pathologic characteristics of the primary tumor, axillary lymph node status, tumor hormone receptor content, tumor HER2 status, multi-gene testing, presence or absence of detectable metastatic disease, patient comorbidities, patient age, and menopausal status.

Neoadjuvant chemotherapy has become commonly used based on results from randomized trials suggesting no difference in relapse-free or overall survival whether chemotherapy is administered before or after surgery (51). A major benefit of neoadjuvant chemotherapy is the increase in the rate of breast conserving surgery. Tumor response to neoadjuvant chemotherapy has proven to be a good surrogate end-point for survival; the achievement of pCR is strongly associated with a favorable long-term survival rates (75% to 85% at 10 to 15 years) (51-55). Thus, improving pCR has become a valid strategy in the development of new treatment regimens. Specifically, the rate of pCR achieved in ER-positive breast-cancer subjects following vaccination with P10s-PADRE administered concurrently with neoadjuvant chemotherapy will be compared with the corresponding pCR rate from a pooled analysis of seven randomized trials published in the Journal of Clinical Oncology (1) in which 287 of 3,771 ER-positive breast-cancer subjects (approximately 8%) achieved the “ypT0 ypN0” definition of pCR following neoadjuvant chemotherapy.

Definition of Pathological Complete Response: Pathological Complete Response will be defined as “the absence of any residual invasive cancer on hematoxylin and eosin evaluation of the resected breast specimen and all sampled ipsilateral lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0/Tis ypN0 in the current AJCC staging system).” (56)

Rationale for using P10s-PADRE in the neoadjuvant setting: The traditional drug development process based on large phase III trials to show statistical incremental benefit from SoC therapy is slow, expensive, and inefficient. In contrast, the neoadjuvant trial model provides rapid assessment of short-term drug efficacy and triage utilizing pCR as the primary endpoint. Thus, improving pCR has become a valid strategy in the development of new treatment regimens.

This issue is perhaps best exemplified in the development of HER2-directed therapies, such as lapatinib (NeoALTTO trial) (57), and pertuzumab (NeoSphere trial) 58). Recognizing the importance of neoadjuvant trials for drug development, the FDA recently announced the consideration of neoadjuvant randomized trials for accelerated drug approval in early breast cancer (59). In line with this approach, in September 2013 the FDA granted accelerated approval of pertuzumab for neoadjuvant treatment of women with early-stage HER2-positive breast cancer.

In our Phase I study, we were able to demonstrate that P10s vaccination was well-tolerated, and measurable immune responses and anti-cancer-cell efficacy were noted. Our previous data suggest that both anti-peptide IgM and IgG titers rose sharply by week 7 (four weeks after the third immunization) of the study (when additional boost was administered) in most subjects. IgM titers were short-lived and returned to baseline by week 19, whereas IgG titers remained elevated through at least week 21. Based on the above data, we expect that three doses of the vaccine will be sufficient to achieve a significant immune response, but no previous data are available on the immunogenicity of the investigational agent when administered concurrently with chemotherapy and steroids (used as antiemetics). Because of the above concerns, our study will begin by assessing the feasibility of achieving adequate immune response with P10s-PADRE when it is administered in combination with neoadjuvant chemotherapy. To this end, we have defined five different Chemovax schedules, and named them A, B, C, D, and E (see *8. Chemovax Schedules* for the details of each schedule). There will be five subjects in each cohort enrolled onto a Chemovax schedule. Schedules will be evaluated for immune-response adequacy.

Justification for 500ug vaccine dose: Whereas medications work via distribution through the bloodstream, the immune response generated by vaccination typically occurs at or near the injection site. In the Phase I study we observed an increase in IgG binding to the P10s peptide after immunization as measured by ELISA assay. Anti-P10s serum reactivity surged dramatically from week 4 to week 7 of the study in most subjects except in subject #6, in whom it was already surging at week 4. The increases at week 7 compared to pre-immune ranged from 31 fold (in subject #2) to 256 fold (in subjects #5 and #6), and the titers in subsequent weeks showed little change from their week-7 values. The cohort immunized with the higher dose of vaccine (500 µg dose) displayed higher normalized endpoint titers. In nonparametric repeated-measures analysis, the main effects of dose and week were statistically significant (both *P* values <.0001), but the dose×week interaction was not (*P*=0.40). While we did not observe dose-limiting toxicity in this study, it is difficult to determine the maximum tolerated dose of the vaccine because of the potential of forming granulomas due to the nature of the adjuvant. P10s is admixed with MONTANIDE™ ISA 51 VG, an adjuvant known to form granulomas.

4. TRIAL OBJECTIVES

Primary Objectives

1) **Identify a feasible schedule of vaccination relative to SoC neoadjuvant chemotherapy when the two are administered concurrently. Feasibility will be evaluated in terms of:**
   a) Generation of a sufficiently high anti-P10s immunoglobulin-G response.
   b) Safety and tolerability of the vaccine-chemotherapy combination.

2) **Demonstration of clinical response:**
   Determine if the Chemovax regimen in ER-positive breast cancer would lead to a significantly higher rate of pCR in breast and axillary nodes at time of definitive surgery compared to the pCR rate of 8% observed among 3,771 ER-positive breast-cancer subjects in the pooled analysis of seven randomized clinical trials reported by von Minckwitz *et al* (1-2).

Secondary Objectives

1) **Determine Short-term Immune Responses Induced by Chemovax:**
   a) Determine the functional humoral response as defined by:
      i. P10s-MAP-reactive immunoglobulin-G endpoint titers in serum.
      ii. Cytotoxicity of serum and plasma samples against two TACA-expressing breast cancer cell lines following Chemovax therapy.
   b) Determine the activation profile of NK cells before and after Chemovax therapy.
   c) Determine T-cell subpopulations and evaluate the effects of Chemovax therapy on the numbers of circulating T and B cells as determined by flow cytometry.
   d) Perform functional T-cell immune analysis by performing T-cell proliferation, ELISPOT, cytokine release, and ADCC assays.
   e) Determine expression of biomarkers of efficacy and response on tumor tissues and perform characterization of tumor infiltrating lymphocytes and their role in tumor response.

2) **Determine Long-term Immune Responses Induced by Chemovax:**
   a) Determine antibody titers against P10s at approximately 6 and 12 months following the initial response evaluation.
   b) Measure anti-TACA cell-binding levels at approximately 6 and 12 months following the initial response evaluation.
   c) Determine the cytotoxic activity against TACA-expressing cell lines at approximately 6 and 12 months following the initial response evaluation.
   d) Evaluate the functionality of T and NK cells.

5. PATIENT POPULATION

Eligibility Criteria: Subjects are eligible for the vaccine study if the following inclusion and exclusion criteria are met:

Inclusion Criteria:

1. Females of all races with clinical stage I, II, or III ER-positive, HER2 negative breast cancer who will undergo SoC neoadjuvant treatment. Age 18 years and older.
3. ECOG Performance Status 0 or 1.
4. White blood cell (WBC) count ≥ 3,000/mm^3 within 3 weeks prior to registration.
5. Platelet count ≥ 100,000/mm^3 within 3 weeks prior to registration.
6. Bilirubin ≤ 2 x institutional upper limit (IUL) of normal obtained within 3 weeks prior to registration.
7. Serum glutamic-oxaloacetic transaminase (SGOT) or aspartate aminotransferase test (AST) ≤ 2 x IUL of normal obtained within 3 weeks prior to registration.
8. Serum glutamic-pyruvic transaminase (SGPT) or alanine aminotransferase test (ALT) ≤ 2 x IUL of normal obtained within 3 weeks prior to registration.
9. Serum creatinine ≤ 1.8 mg/dL obtained within 3 weeks prior to registration.
10. Must sign an informed consent document approved by the UAMS IRB.

Exclusion Criteria:

1. ER-negative, HER2-positive, inflammatory, metastatic, stage IV or recurrent breast cancer
2. Active infection requiring treatment with antibiotics.
3. Existing diagnosis or history of organic brain syndrome that might preclude participation in the full protocol.
4. Existing diagnosis or history of significant impairment of basal cognitive function that might preclude participation in the full protocol.
5. Other current malignancies. Subjects with prior history at any time of any in situ cancer, including lobular carcinoma of the breast in situ, cervical cancer in situ, atypical melanocytic hyperplasia or Clark I melanoma in situ or basal or squamous skin cancer are eligible, provided they are disease-

free at the time of registration. Subjects with other malignancies are eligible if they have been continuously disease free for ≥ 5 years prior to the time of registration.

6. Active autoimmune disorders or conditions of immunosuppression; Existing diagnosis or history of autoimmune disorders or conditions of immunosuppression that have been in remission for less than 6 months
7. Treatment with corticosteroids, including oral steroids (i.e. prednisone, dexamethasone [except when used as an antiemetic in SoC therapy]), continuous use of topical steroid creams or ointments or any steroid-containing inhalers. Subjects who discontinue the use of these classes of medication for at least 6 weeks prior to registration are eligible if, in the judgment of the treating physician, the subject is not likely to require these classes of drugs during the treatment period. Replacement doses of steroids for subjects with adrenal insufficiency are allowed.
8. Pregnancy or breastfeeding (due to the unknown effects of peptide/mimotope vaccines on a fetus or infant). Women of childbearing potential must have a negative urine pregnancy test within 72 hours prior to starting week 1 and must be counseled to use an accepted and effective method of contraception (including abstinence) while on treatment and for a period of 18 months after completing or discontinuing treatment. Accepted methods of contraception include tubal ligation, oral contraceptives, barrier methods, IUDs, and abstinence.
9. Any other significant medical or psychiatric conditions, which, in the opinion of the enrolling investigator, may interfere with consent or compliance of the treatment regimen.
10. Enrollment in any other clinical trial using investigational drug products or devices prior to first post-surgery study lab. Concurrent enrollment in observational studies is allowed.

6. INVESTIGATIONAL PRODUCT – P10S-PADRE/ MONTANIDE™ ISA 51 VG STERILE

a. General Description: P10s-PADRE is a short peptide (P10s) coupled to PADRE, a synthetic, non-natural peptide that binds with high or intermediate affinity to 15 of 16 of the most common HLA-DR types tested to date. Both components contribute to the efficacy of this molecule in stimulating an immune response. Because of its binding promiscuity, PADRE should overcome the problems posed by the extreme polymorphism of HLA-DR molecules in the human population. Furthermore, the PADRE peptide was specifically engineered as an antigen-presenting molecule for use in humans. Carbohydrate moieties, such as TACAs, typically do not induce T-cell responses. Thus, P10s, a TACA peptide mimotope, was developed. By coupling P10s to PADRE, the likelihood of generating an immune response increases, including T-cell "help" in the vaccine construct designed for human use.

MONTANIDE™ ISA 51 VG STERILE is defined as an oil adjuvant containing surfactant based mannide monooleate and oil, which is of white mineral oil origin.

b. Vaccine Manufacturing and Formulation: AmbioPharm, Inc. (North Augusta, SC) will synthesize Mimotope P10s covalently linked with PADRE as a powder manufactured under GMP conditions in facilities registered with the FDA. Once P10s-PADRE has been manufactured by AmbioPharm, the powder will be shipped to Bioserv Corporation, Inc. (San Diego, CA) for sterile packaging as a lyophilized powder in quantities of 500 µg/vial in appropriately sized glass vials under GMP conditions,. Advantar Laboratories, Inc. (San Diego, CA) will perform product release and stability testing on the P10s-PADRE study drug. The study drug will be stored per label instructions.

MONTANIDE™ ISA 51 VG STERILE will be supplied by SEPPIC, Inc. (Fairfield, NJ). See Investigator's Brochure for formulation and storage conditions.

c. Vaccine Preparation: The following doses of P10s-PADRE and MONTANIDE™ ISA 51 VG STERILE vaccine will be administered to subjects subcutaneously in rotating injection sites in the abdomen. Subjects will receive 2 mL SC injections of the vaccine on each of 3 separate occasions according to each study schedule.

- **500 µg P10s-PADRE/ MONTANIDE™ ISA 51 VG STERILE Vaccine Dose preparation:** Allow P10s-PADRE to come to room temperature before reconstitution (15 to 30 min). At the same time, remove Montanide™ ISA 51 VG STERILE vial from refrigerator. Reconstitute P10s-PADRE with 1.1 mL sterile water (final concentration 500 µg/mL). Use 1 mL of the peptide solution and mix with an equal amount of Montanide ISA 51 VG STERILE to a final volume of 2 mL. Mix as instructed by Seppic until emulsification is complete. The pharmacist will prepare the vaccine dose following the manufacturer's instructions.

d. Label Information: The vaccine drug supply (both P10s-PADRE and MONTANIDE™ ISA 51 VG STERILE will be labeled with the following statement: "Caution: New Drug – Limited by Federal Law to Investigational Use."

e. Agent Ordering: All study drug supplies (P10s-PADRE vials and MONTANIDE™ ISA 51 VG STERILE vials) will be sent to the attention of the UAMS Research Pharmacy staff. Initial drug supply order will be placed by primary investigator and both P10s-PADRE vials and MONTANIDE™ ISA 51 VG STERILE vials will be shipped directly to Cancer Institute Pharmacy, Room 5132, 4301 W. Markham Street, Slot 721-11, Little Rock, AR 72205-7199. The UAMS Research Pharmacy will be the primary pharmacy for this study. All drug ordering and shipment to other sites will be described in the Pharmacy Instructions binder.

f. Agent Accountability: P10s-PADRE and MONTANIDE™ ISA 51 VG STERILE will be stored in the UAMS Research Pharmacy under the supervision of the research pharmacist who will be responsible for maintaining the supply according to the manufacturer's specifications, dispensing the drug for administration and maintaining all accountability logs. Standard UAMS accountability logs will be used.

7. STUDY DESIGN

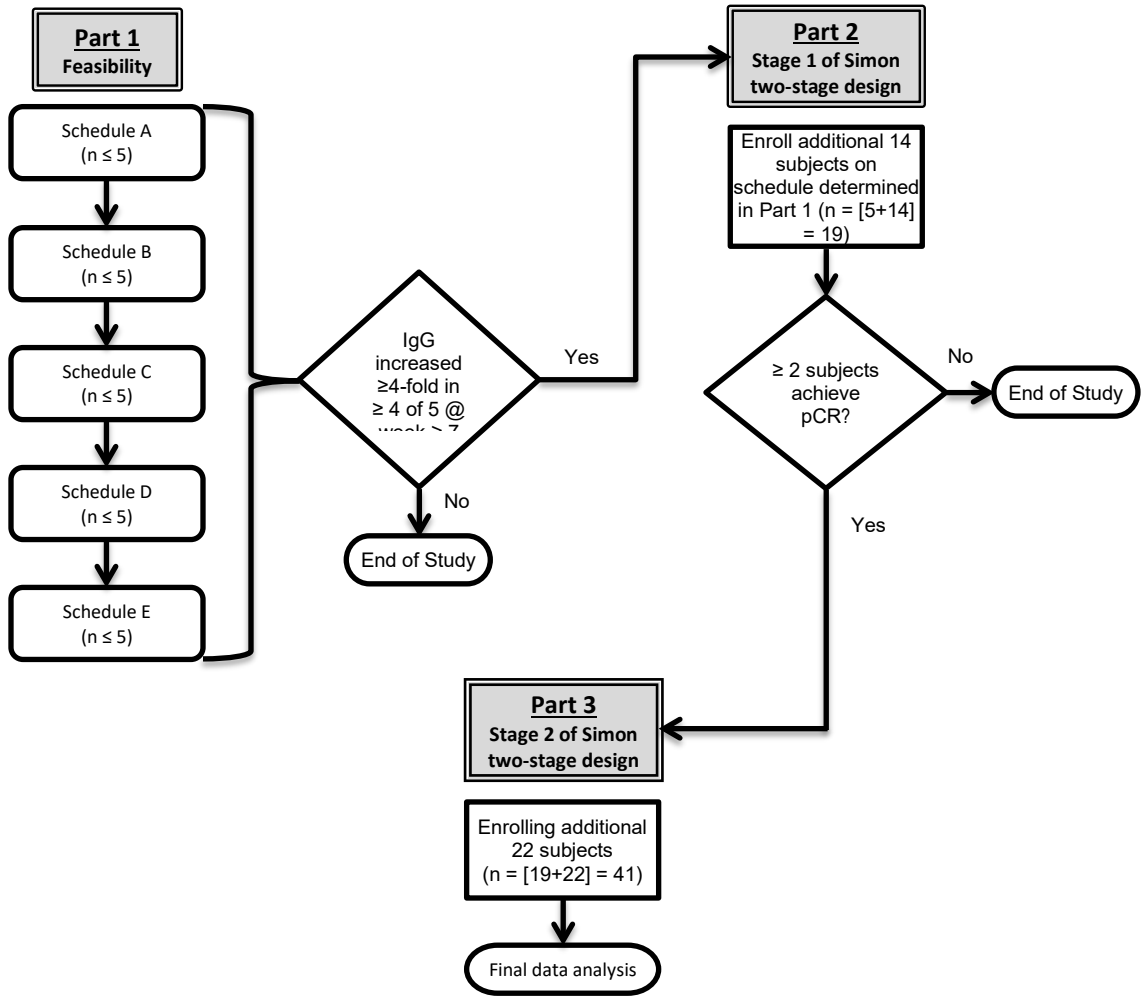
This is a single-arm, multi-site Phase I/II study designed with the two goals being (1) to evaluate the feasibility of combining vaccination with the P10s-PADRE formulation with neoadjuvant chemotherapy and (2) to determine if the pCR rate among ER-positive breast-cancer subjects treated with the combination is significantly higher than the 8% rate observed among ER-positive breast-cancer subjects in a pooled analysis of seven randomized clinical trials (1). P10s-PADRE vaccine with MONTANIDE™ ISA 51 VG as adjuvant will be given in combination with neoadjuvant chemotherapy in female subjects with clinical stage I, II or III ER-positive breast cancer.

This combined Phase I/II feasibility-and-efficacy study will have three parts. Its first part will be a Phase I evaluation of the safety, tolerability, and feasibility of eliciting adequate IgG response with P10s-PADRE when administered in combination with SoC neoadjuvant chemotherapy, and will require 25 subjects. The timing of vaccination relative to chemotherapy will be evaluated to identify a Chemovax schedule in which a sufficiently high proportion of subjects achieve an adequate IgG response. Once such a schedule is identified, the study will proceed with its second and third parts. The study's second and third parts will respectively constitute Stages 1 and 2 of the Phase II primary-efficacy evaluation of Chemovax using a Simon optimal two-stage design, and will require a minimum of 19 subjects (5 subjects from Part 1 + an additional 14 subjects) and a maximum of 41 subjects (19 subjects from Part 1 & 2 + an additional 22 subjects). The total sample size in the entire study will have a theoretical minimum of 25 subjects, a theoretical maximum of 61 subjects, and a most likely value of 41 subjects based on demonstrating both immune-response feasibility and clinical efficacy.

To evaluate the feasibility of eliciting adequate immune response with P10s-PADRE when it is administered in combination with neoadjuvant chemotherapy, we will sequentially evaluate different schedules of vaccination relative to chemotherapy, and stop evaluating as soon as we have identified a feasible schedule. To this end, we have defined five different Chemovax schedules, and named them A, B, C, D, and E; see *Chemovax Schedules* for the details of each schedule. From one to five cohorts of subjects will be enrolled sequentially and used to evaluate the schedules in alphabetical order, beginning with Schedule A. There will be five subjects in each cohort enrolled onto a Chemovax schedule. A schedule that has a cohort enrolling onto it will be evaluated for immune-response adequacy.

The best immune response with the least toxicity will be the Chemovax schedule that will be expanded and used to initiate the primary efficacy evaluation of Chemovax using a Simon optimal two-stage design (60, 61).

Figure 1: Study Schema



Following Part 1 (feasibility), Cohort C and E displayed a significant average fold increase in anti-peptide titers after immunization. Cohort C had a higher overall average fold increase and a higher number of responders. Therefore, Cohort C was identified as the best schedule for Part 2 (primary efficacy). Cohort C was expanded with an additional 14 subjects to test primary efficacy with a goal of at least 2 subjects from Cohort C achieving pCR. A total of 4 subjects obtained pCR. Cohort C will be expanded for Part 3 (expanded efficacy).

8. TREATMENT PLAN

a. On-study Plan: After signing the IRB-approved informed consent form, research participants with eligibility confirmed will be registered and assigned a study number by a clinical research associate (CRA) or Study Coordinator. All research participants will receive the Mimotope P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine via SC injection as well as SoC neoadjuvant chemotherapy according to the schedule on which they are enrolled (see Schedules below).

b. Chemotherapy: For all subjects, doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) (AC) will be administered concurrently every three weeks for four cycles followed by docetaxel (75 mg/m^2) every three weeks for four cycles, with growth factor at physician's discretion. Subjects will be monitored for chemotherapy-related toxicities and, if clinically necessary, chemotherapy dosing may be modified per the Chemotherapy Dose Adjustment table.

c. Chemotherapy Dose Adjustment: Any or all of the SoC neoadjuvant chemotherapies may be dose-reduced, as necessary, according to the table below. If docetaxel is not tolerated, paclitaxel may be used in its place. Chemotherapy dosing may be re-escalated to the starting dose at the treating physician's discretion and per SoC. Subjects who require different chemotherapy agents the first post-surgery study lab at Week 49 (Schedule C) will be followed for 30 days after the last vaccination and then be withdrawn from the study.

Chemotherapy	Starting Dose	Reduction 1	Reduction 2	Reduction 3
Doxorubicin	60 mg/m^2	-20%	-10%	Discontinue
Cyclophosphamide	600 mg/m^2	-20%	-10%	Discontinue
Docetaxel	75 mg/m^2	-20%	-10%	Discontinue
Paclitaxel*	175mg/m^2	-20%	-10%	Discontinue

* in case of docetaxel substitution only

SoC neoadjuvant chemotherapy dosing may be delayed up to +7 days due to AEs (e.g. anemia, neutropenia, leukopenia, etc.). For delays longer than 7 days, the Medical Monitor and Sponsor should be contacted. Any delay outside the allotted time window will be a deviation. The remainder of the study visits should be adjusted accordingly with a note-to-file. Each initial shift in the study calendar would be a deviation due to medical necessity and the following visits would be recalibrated without deviation.

Subjects who require dose modifications, treatment delays, or are unable to resume their SoC neoadjuvant chemotherapy (e.g. due to chemotherapy-related toxicities) will remain in the study to continue with assessments per the study calendar, including collection of study labs.

d. Chemovax Schedules:

- SCHEDULE A: Subjects will receive the first cycle of chemotherapy along with the first dose of vaccine on week 1, the subsequent two doses of vaccine one week apart (week 2 and 3), second cycle of chemotherapy on week 4, and subsequent cycles of chemotherapy every 21 days (week 7, 10, 13, 16, 19, 22).

- SCHEDULE B: Subjects will receive the first cycle of chemotherapy on week 1, the first dose of vaccine on week 2, the subsequent two doses of vaccine one week apart (week 3 and 4), second cycle of chemotherapy on week 4 (along with second vaccine dose) and subsequent cycles of chemotherapy every 21 days (week 7, 10, 13, 16, 19, 22).

- SCHEDULE C: Subjects will receive three weekly doses of vaccine (week 1, 2, 3), then first cycle of chemotherapy (week 4), and subsequent cycles of chemotherapy every 21 days (week 7, 10, 13, 16, 19, 22, 25).

- SCHEDULE D: Subjects will receive the first dose of vaccine on week 1, the subsequent two doses of vaccine one week apart (week 2 and 3), the first cycle of chemotherapy on week 2 (along with second vaccine dose) and subsequent cycles of chemotherapy every 21 days (week 5, 8, 11, 14, 17, 20, 23).

- SCHEDULE E: Subjects will receive the first dose of vaccine on week 1, the subsequent two doses of vaccine one week apart (week 2 and 3), the first cycle of chemotherapy on week 3 (along with third vaccine dose) and subsequent cycles of chemotherapy every 21 days (week 6, 9, 12, 15, 18, 21, 24).

e. Prohibited Medications: Immunosuppressant medications are not allowed including inhaled and topical steroids, except for those subjects on chemotherapy as prescribed to reduce chemo symptoms by attending oncologist or for subjects with an adrenal insufficiency. All other medications are allowed.

Concurrent enrollment in another clinical trial using drugs or devices may be allowed only after the first post-surgery study lab at Week 49 (Schedule C). Concurrent enrollment in observational trials is allowed throughout the study.

f. Dose Limiting Criteria: This dose of investigational agent was well tolerated when used in our Phase I study in breast cancer subjects. However, if a subject does not tolerate the vaccine, judged by having any of the dose-limiting criteria below, their vaccination treatment will be discontinued.

- Any grade 3 or greater toxicity as per NCI CTCAE v4 toxicity criteria
- Grade 2 or greater autoimmune toxicity, with the exception of vitiligo
- Grade 2 or greater hypersensitivity reactions

Subjects who develop any grade 3 or greater toxicity per NCI CTCAE v4.0 toxicity criteria, Grade 2 or greater autoimmune toxicity with the exception of vitiligo, or Grade 2 or greater hypersensitivity reactions will be treated and referred for additional care as indicated with systemic steroids, topical steroids, epinephrine or Benadryl. These subjects will no longer receive the vaccine but will continue with assessments per the study calendar, including collection of study labs.

g. Study Pausing Rules:

- Death (other than due to disease progression or motor vehicle accident or to other events determined to be not related to the vaccine)
- Two Grade 4 toxicity events that are possibly/probably related to the vaccine

9. STUDY CALENDARS

See following pages.

9.1. Study Calendar – Schedule A – Retired (Used only in Part 1)

Procedures	Pre-study/ Screening[a]	Wk 1	Wk 2	Wk 3	Wk 4	Wk 7	Wk 10	Wk 13	Wk 16	Wk 19	Wk 22	Surgery (4-8 weeks after chemo)	Wk 46 (± 4 wks)	Wk 70 (± 4 wks)
Informed Consent	X													
Vaccination with Mimotope P10s-PADRE/ MONTANIDE™ ISA 51 VG		X[b]	X[b]	X[b]										
Cyclophosphamide[cd]		X			X	X	X							
Doxorubicin[cd]		X			X	X	X							
Docetaxel[cd]								X	X	X	X			
AEs		X	X	X	X	X	X	X	X	X	X		X	X
Concomitant Medications	X	X			X	X	X	X	X	X	X		X	X
History/PE/Vital Signs[ef]	X	X			X	X	X	X	X	X	X		X	X
CBC with Diff	X				X	X	X	X	X	X	X		X	X
Total Bilirubin	X													
SGOT/AST	X				X	X	X	X	X	X	X		X	X
SGPT/ALT	X													
Alk Phos	X				X	X	X	X	X	X	X		X	X
LDH	X												X	X
Creatinine	X												X	X
Calcium	X												X	X
Urine Pregnancy Test	X	X[g]												
Registration		X[h]												
Study Lab[i]		X[h]			X	X	X	X			X		X	X
Surgery[j]												X		

[a] Pre-study/Screening should be completed within 21 days prior to registration/week 1.
[b] Post vaccine administration, the following vital signs will be monitored every 15 minutes (± 5 minutes) for one hour: blood pressure, temperature and pulse.
[c] Chemotherapy administration date may be delayed up to 10 days at investigator discretion.
[d] If clinically necessary, chemotherapy dosing may be modified per the Chemotherapy Dose Adjustment in Section 8c.
[e] Vital signs to include blood pressure, temperature, pulse and weight as described in the visit breakdown section. Height is only required at Pre-study/Screening.
[f] Tumor Assessments will be performed by physical exam according to standard practice.
[g] To be completed prior to vaccine dose
[h] Registration occurs after subject eligibility has been confirmed. Note: The registration process, as outlined in Section 15, may occur prior to Week 1.
[i] Study lab: Up to 100 mLs of blood may be collected. Red Vacutainer tubes: Approximately 50 mL collected at all study lab timepoints. Lavender vacutainer tubes: Approximately 50 mL will be collected at Weeks 1, 4, and 7 with the amount decreasing to 25 mL for Weeks 10, 13, 22, 46, and 70.
[j] Surgery will be performed according to standard practice unless they are considered inoperable or if surgery is medically contraindicated.

9.2. Study Calendar – Schedule B – Retired (Used only in Part 1)

Procedures	Pre-study/ Screening[a]	Wk 1	Wk 2	Wk 3	Wk 4	Wk 7	Wk 10	Wk 13	Wk 16	Wk 19	Wk 22	Surgery (4-8 weeks after chemo)	Wk 46 (± 4 wks)	Wk 70 (± 4 wks)
Informed Consent	X													
Vaccination with Mimotope P10s-PADRE/ MONTANIDE™ ISA 51 VG			X[b]	X[b]	X[b]									
Cyclophosphamide[cd]		X			X	X	X							
Doxorubicin[cd]		X			X	X	X							
Docetaxel[cd]								X	X	X	X			
AEs		X	X	X	X	X	X	X	X	X	X		X	X
Concomitant Medications	X	X			X	X	X	X	X	X	X		X	X
History/PE/Vital Signs [ef]	X	X			X	X	X	X	X	X	X		X	X
CBC with Diff	X				X	X	X	X	X	X	X		X	X
Total Bilirubin	X													
SGOT/AST	X				X	X	X	X	X	X	X		X	X
SGPT/ALT	X													
Alk Phos	X				X	X	X	X	X	X	X		X	X
LDH	X												X	X
Creatinine	X												X	X
Calcium	X												X	X
Urine Pregnancy Test	X		X[g]											
Registration		X[h]												
Study Lab[i]		X[h]				X	X	X	X		X		X	X
Surgery[j]												X		

[a] Pre-study/Screening should be completed within 21 days prior to registration/week 1.
[b] Post vaccine administration, the following vital signs will be monitored every 15 minutes (± 5 minutes) for one hour: blood pressure, temperature and pulse.
[c] Chemotherapy administration date may be delayed up to 10 days at investigator discretion.
[d] If clinically necessary, chemotherapy dosing may be modified per the Chemotherapy Dose Adjustment in Section 8c.
[e] Vital signs to include blood pressure, temperature, pulse and weight as described in the visit breakdown section. Height is only required at Pre-study/Screening.
[f] Tumor Assessments will be performed by physical exam according to standard practice.
[g] To be completed prior to vaccine dose
[h] Registration occurs after subject eligibility has been confirmed. Note: The registration process, as outlined in Section 15, may occur prior to Week 1.
[i] Study lab: Up to 100 mLs of blood may be collected. Red Vacutainer tubes: Approximately 50 mL collected at all study lab timepoints. Lavender vacutainer tubes: Approximately 50 mL will be collected at Weeks 1, 7, and 10 with the amount decreasing to 25 mL for Weeks 13, 16, 22, 46, and 70.
[j] Surgery will be performed according to standard practice unless they are considered inoperable or if surgery is medically contraindicated.

9.3. Study Calendar – Schedule C – Optimal Schedule (To be used in Parts 1, 2 & 3)

Procedures	Pre-study/ Screening[a]	Wk 1 (± 2 days)	Wk 2 (± 2 days)	Wk 3 (± 2 days)	Wk 4 (± 2 days)	Wk 7 (± 2 days)	Wk 10 (± 2 days)	Wk 13 (± 2 days)	Wk 16 (± 2 days)	Wk 19 (± 2 days)	Wk 22 (± 2 days)	Wk 25 (± 2 days)	Surgery (4-8 weeks after chemo)	Wk 49 (± 4 wks)	Wk 73 (± 4 wks)
Informed Consent	X														
Vaccination with Mimotope P10s-PADRE/ MONTANIDE™ ISA 51 VG		X[b]	X[b]	X[b]											
AC Chemotherapy[cd]					X	X	X	X							
Docetaxel (75 mg/m^2)[d]									X	X	X	X			
AEs		X	X	X	X	X	X	X	X	X	X	X		X	X
Concomitant Medications	X	X			X	X	X	X	X	X	X	X		X	X
History/PE/Vital Signs [ef]	X	X	X	X	X	X	X	X	X	X	X	X		X	X
CBC with Diff	X				X	X	X	X	X	X	X	X		X	X
Total Bilirubin	X														
SGOT/AST	X				X	X	X	X	X	X	X	X		X	X
SGPT/ALT	X				X	X	X	X	X	X	X	X		X	X
Alk Phos	X				X	X	X	X	X	X	X	X		X	X
LDH	X													X	X
Chemistry[g]	X				X	X	X	X	X	X	X	X		X	X
Urine Pregnancy Test	X	X[h]													
Registration		X[i]													
Study Lab[j]		X				X	X	X	X			X		X	X
Surgery[k]													X		

[a] Pre-study/Screening should be completed within 21 days prior to registration/week 1.
[b] Post vaccine administration, the following vital signs will be monitored every 15 minutes (± 5 minutes) for one hour: blood pressure, temperature and pulse.
[c] Cyclophosphamide (600 mg/m^2) and Doxorubicin (60 mg/m^2) (AC) to be administered concurrently every three weeks for four cycles
[d] SoC growth factor may be given at physician's discretion. Chemotherapy administration date may be delayed up to 7 days at investigator discretion. If clinically necessary, chemotherapy dosing may be modified per the Chemotherapy Dose Adjustment in Section 8c.
[e] Vital signs to include blood pressure, temperature, pulse and weight as described in the visit breakdown section. Height is only required at Pre-study/Screening.
[f] Tumor Assessments will be performed by physical exam according to standard practice.
[g] Chemistry (OP Chem 7) includes blood urea nitrogen (BUN), carbon dioxide, creatinine, calcium, chloride, potassium, sodium.
[h] To be completed prior to vaccine dose. Week 1 test may be omitted if screening urine pregnancy test is performed within the 72 hours prior to first vaccine dose.
[i] Registration occurs after subject eligibility has been confirmed. Note: The registration process, as outlined in Section 15, may occur prior to Week 1.
[j] Study lab for Week 1 should be completed prior to the vaccine. Study lab: Up to 100 mLs of blood may be collected. Red Vacutainer tubes: Approximately 50 mL collected at all study lab timepoints. Lavender vacutainer tubes: Approximately 50 mL will be collected at Weeks 1, 7, and 10 with the amount decreasing to 25 mL for Weeks 13, 16, 25, 49, and 73.
[k] Surgery will be performed according to standard practice unless they are considered inoperable or if surgery is medically contraindicated.

9.4. Study Calendar – Schedule D – Retired (Used only in Part 1)

Procedures	Pre-study/ Screening[a]	Wk 1	Wk 2	Wk 3	Wk 5	Wk 8	Wk 11	Wk 14	Wk 17	Wk 20	Wk 23	Surgery (4-8 weeks after chemo)	Wk 47 (± 4 wks)	Wk 71 (± 4 wks)
Informed Consent	X													
Vaccination with Mimotope P10s-PADRE/ MONTANIDE™ ISA 51 VG		X[b]	X[b]	X[b]										
Cyclophosphamide[cd]			X		X	X	X							
Doxorubicin[cd]			X		X	X	X							
Docetaxel[cd]								X	X	X	X			
AEs		X	X	X	X	X	X	X	X	X	X		X	X
Concomitant Medications	X	X	X		X	X	X	X	X	X	X		X	X
History/PE/Vital Signs [ef]	X	X	X		X	X	X	X	X	X	X		X	X
CBC with Diff	X		X		X	X	X	X	X	X	X		X	X
Total Bilirubin	X													
SGOT/AST	X		X		X	X	X	X	X	X	X		X	X
SGPT/ALT	X													
Alk Phos	X		X		X	X	X	X	X	X	X		X	X
LDH	X												X	X
Creatinine	X												X	X
Calcium	X												X	X
Urine Pregnancy Test	X	X[g]												
Registration		X[h]												
Study Lab[i]		X[h]			X	X	X	X			X		X	X
Surgery[j]												X		

[a] Pre-study/Screening should be completed within 21 days prior to registration/week 1.
[b] Post vaccine administration, the following vital signs will be monitored every 15 minutes (± 5 minutes) for one hour: blood pressure, temperature and pulse.
[c] Chemotherapy administration date may be delayed up to 10 days at investigator discretion.
[d] If clinically necessary, chemotherapy dosing may be modified per the Chemotherapy Dose Adjustment in Section 8c.
[e] Vital signs to include blood pressure, temperature, pulse and weight as described in the visit breakdown section. Height is only required at Pre-study/Screening.
[f] Tumor Assessments will be performed by physical exam according to standard practice.
[g] To be completed prior to vaccine dose
[h] Registration occurs after subject eligibility has been confirmed. Note: The registration process, as outlined in Section 15, may occur prior to Week 1.
[i] Study lab: Up to 100 mLs of blood may be collected. Red Vacutainer tubes: Approximately 50 mL collected at all study lab timepoints. Lavender vacutainer tubes: Approximately 50 mL will be collected at Weeks 1, 5, and 8 with the amount decreasing to 25 mL for Weeks 11, 14, 23, 47, and 71.
[j] Surgery will be performed according to standard practice unless they are considered inoperable or if surgery is medically contraindicated.

9.5. Study Calendar – Schedule E – Retired (Used only in Part 1)

Procedures	Pre-study/ Screening[a]	Wk 1	Wk 2	Wk 3	Wk 6	Wk 9	Wk 12	Wk 15	Wk 18	Wk 21	Wk 24	Surgery (4-8 weeks after chemo)	Wk 48 (± 4 wks)	Wk 72 (± 4 wks)
Informed Consent	X													
Vaccination with Mimotope P10s-PADRE/ MONTANIDE™ ISA 51 VG		X[b]	X[b]	X[b]										
Cyclophosphamide[cd]				X	X	X	X							
Doxorubicin[cd]				X	X	X	X							
Docetaxel[cd]								X	X	X	X			
AEs		X	X	X	X	X	X	X	X	X	X		X	X
Concomitant Medications	X	X		X	X	X	X	X	X	X	X		X	X
History/PE/Vital Signs [ef]	X	X		X	X	X	X	X	X	X	X		X	X
CBC with Diff	X			X	X	X	X	X	X	X	X		X	X
Total Bilirubin	X													
SGOT/AST	X			X	X	X	X	X	X	X	X		X	X
SGPT/ALT	X													
Alk Phos	X			X	X	X	X	X	X	X	X		X	X
LDH	X												X	X
Creatinine	X												X	X
Calcium	X												X	X
Urine Pregnancy Test	X	X[g]												
Registration		X[h]												
Study Lab[i]		X[h]			X	X	X	X			X		X	X
Surgery[j]												X		

[a] Pre-study/Screening should be completed within 21 days prior to registration/week 1.
[b] Post vaccine administration, the following vital signs will be monitored every 15 minutes (± 5 minutes) for one hour: blood pressure, temperature and pulse.
[c] Chemotherapy administration date may be delayed up to 10 days at investigator discretion.
[d] If clinically necessary, chemotherapy dosing may be modified per the Chemotherapy Dose Adjustment (Section 8c).
[e] Vital signs to include blood pressure, temperature, pulse and weight as described in the visit breakdown section. Height is only required at Pre-study/Screening.
[f] Tumor Assessments will be performed by physical exam according to standard practice.
[g] To be completed prior to vaccine dose
[h] Registration occurs after subject eligibility has been confirmed. Note: The registration process, as outlined in Section 15, may occur prior to Week 1.
[i] Study lab: Up to 100 mLs of blood may be collected. Red Vacutainer tubes: Approximately 50 mL collected at all study lab timepoints. Lavender vacutainer tubes: Approximately 50 mL will be collected at Weeks 1, 6, and 9 with the amount decreasing to 25 mL for Weeks 12, 15, 24, 48, and 72.
[j] Surgery will be performed according to standard practice unless they are considered inoperable or if surgery is medically contraindicated.

10. VISIT BREAKDOWN

10.1. Visit Breakdown – Schedule A – Retired (Used only in Part 1)

Pre-study/Screening (within 21 days prior to initiating study treatment)

- Complete medical history and physical examination: vital signs to include height, weight, blood pressure, temperature and pulse.
- Informed Consent
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - Total Bilirubin
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Urine pregnancy test for women of childbearing potential

Week 1

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse
- Urine pregnancy test for women of childbearing potential (within 72 hours prior to dosing)
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 minutes (± 5 minutes) for one hour: blood pressure, temperature, and pulse
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0
- Concomitant medications
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 2

- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.

Week 3

- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.

Week 4

- Complete medical history and physical examination: vital signs to include weight, blood pressure temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 7

- Complete medical history and physical examination: vital signs to include weight, blood pressure temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 10

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 13

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 16

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 19

- Complete medical history and physical examination: vital signs to include: weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 22

- Complete medical history and physical examination: vital signs to include: weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2

- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Breast cancer surgery

- Definitive surgery will be performed according to standard practice within 4-8 weeks after completion of chemotherapy. All subjects should undergo appropriate surgical management unless they are inoperable or if surgery is medically contraindicated. This surgery is SoC.

Week 46 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 70 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium

- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

10.2. Visit Breakdown – Schedule B – Retired (Used only in Part 1)

Pre-study/Screening (within 21 days prior to initiating study treatment)

- Informed Consent
- Complete medical history and physical examination: vital signs to include: height, weight, blood pressure, temperature and pulse.
- Concomitant medications will be collected from consent to end of treatment
- Blood Tests to include the following:
  - Complete blood count with differential
  - Total Bilirubin
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Urine pregnancy test for women of childbearing potential

Week 1

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 2

- Urine pregnancy test for women of childbearing potential (within 72 hours prior to dosing)
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.

Week 3

- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.

Week 4

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine. Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 7

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 10

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 13

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 16

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 19

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications

- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 22

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Breast cancer surgery

- Definitive surgery will be performed according to standard practice within 4-8 weeks after completion of chemotherapy. All subjects should undergo appropriate surgical management unless they are inoperable or if surgery is medically contraindicated. This surgery is SoC.

Week 46 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 70 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.

- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

10.3. Visit Breakdown – Schedule C – Optimal Schedule (To be used in Parts 1, 2 & 3)

Pre-study/Screening (within 21 days prior to initiating study treatment)

- Informed Consent
- Complete medical history and physical examination: vital signs to include height, weight, blood pressure, temperature and pulse.
- Concomitant medications will be collected from consent to end of treatment.
- Blood Tests to include the following:
  - Complete blood count with differential
  - Total Bilirubin
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)
- Urine pregnancy test for women of childbearing potential.

Week 1 (± 2 days)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Urine pregnancy test for women of childbearing potential (within 72 hours prior to dosing). Test not required if screening pregnancy test is performed within 72 hours prior to first vaccine.
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 2 (± 2 days)

- Vital signs prior to vaccine administration to include blood pressure, temperature, and pulse
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.

Week 3 (± 2 days)

- Vital signs prior to vaccine administration to include blood pressure, temperature, and pulse
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.

Week 4 (± 2 days)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2 (SoC growth factor may be given at physician's discretion)
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)

Week 7 (± 2 days)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2 (SoC growth factor may be given at physician's discretion)
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 10 (± 2 days)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2 (SoC growth factor may be given at physician's discretion)
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 13 (± 2 days)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2 (SoC growth factor may be given at physician's discretion)
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 16 (± 2 days)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2 (SoC growth factor may be given at physician's discretion)
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT

  - Alkaline Phosphatase
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 19 (± 2 days)
- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2 (SoC growth factor may be given at physician's discretion)
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)

Week 22 (± 2 days)
- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2 (SoC growth factor may be given at physician's discretion)
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)

Week 25 (± 2 days)
- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2 (SoC growth factor may be given at physician's discretion)
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Breast cancer surgery

- Definitive surgery will be performed according to standard practice within 4-8 weeks after completion of chemotherapy. All subjects should undergo appropriate surgical management unless they are inoperable or if surgery is medically contraindicated. This surgery is SoC.

Week 49 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 73 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Chemistry (BUN, carbon dioxide, creatinine, calcium, chloride, potassium, sodium)
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

10.4. Visit Breakdown – Schedule D – Retired (Used only in Part 1)

Pre-study/Screening (within 21 days prior to initiating study treatment)

- Complete medical history and physical examination: vital signs to include height, weight, blood pressure, temperature and pulse.
- Informed Consent
- Concomitant medications will be collected from consent to end of treatment

- Blood Tests to include the following:
  - Complete blood count with differential
  - Total Bilirubin
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Urine pregnancy test for women of childbearing potential

Week 1

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Urine pregnancy test for women of childbearing potential (within 72 hours prior to dosing)
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 2

- Complete medical history and physical examination: vital signs to include: weight, blood pressure, temperature and pulse.
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 3

- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.

Week 5

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 8

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 11

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 14

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 17

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 20

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 23

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2

- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Breast cancer surgery

- Definitive surgery will be performed according to standard practice within 4-8 weeks after completion of chemotherapy. All subjects should undergo appropriate surgical management unless they are inoperable or if surgery is medically contraindicated. This surgery is SoC.

Week 47 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 71 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium

- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

10.5. Visit Breakdown – Schedule E – Retired (Used only in Part 1)

Pre-study/Screening (within 21 days prior to initiating study treatment)

- Informed Consent
- Complete medical history and physical examination: vital signs to include height, weight, blood pressure, temperature and pulse.
- Concomitant medications will be collected from consent to end of treatment.
- Blood Tests to include the following:
  - Complete blood count with differential
  - Total Bilirubin
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Urine pregnancy test for women of childbearing potential

Week 1

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Urine pregnancy test for women of childbearing potential (within 72 hours prior to dosing)
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 2

- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.

Week 3

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- SC administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine.
- Post vaccine administration, the following vital signs will be monitored every 15 (± 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 6

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 9

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma and PBMCs, and immune-cell characterization: Approximately 50 mL of blood will be collected in potassium EDTA tubes to collect plasma and PBMCs, and further

assess the peripheral-blood NK-cell activation markers pre- and post-vaccine administration. PBMCs will be used to characterize B-cell and T-cell sub-populations in pre and post-vaccination blood samples.

Week 12

- AC Chemotherapy - cyclophosphamide 600 mg/m^2 and doxorubicin 60 mg/m^2
- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 15

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 18

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 21

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications.
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase

Week 24

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Docetaxel 75 mg/m^2
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Breast cancer surgery

- Definitive surgery will be performed according to standard practice within 4-8 weeks after completion of chemotherapy. All subjects should undergo appropriate surgical management unless they are inoperable or if surgery is medically contraindicated. This surgery is SoC.

Week 48 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

Week 72 (±4 weeks)

- Complete medical history and physical examination: vital signs to include weight, blood pressure, temperature and pulse.
- Adverse Events: events will be monitored using the NCI CTCAE Version 4.0.
- Concomitant medications
- Blood Tests to include the following:
  - Complete blood count with differential
  - SGOT/AST
  - SGPT/ALT
  - Alkaline Phosphatase
  - LDH
  - Creatinine
  - Calcium
- Study Lab:
  - Serum preparation: Approximately 50 mL of blood will be collected in red tubes for serum collection to determine the presence of glycan-reactive antibodies through ELISA and flow-cytometry assays, and to determine cytotoxicity effect on breast cancer cells.
  - Isolation of plasma: Approximately 25 mL of blood will be collected in potassium EDTA tubes to collect plasma.

11. RISKS AND TOXICITIES TO BE MONITORED

a. Potential Toxicities, Risks and Precautions:

Procedure	Risks	Measures to Minimize Risks
Complete history and physical exam, including blood chemistries	- Identification of previously unknown condition	- Qualified health care provider to evaluate potential subject. - Research records are kept in a locked area accessible only by study personnel.

Administration of study vaccine - Mimotope P10s-PADRE/ MONTANIDE™ ISA 51 VG	- Refer to the Investigator's Brochures for P10s-PADRE and MONTANIDE™ ISA 51 VG for a comprehensive list of possible adverse reactions. - Experimental agent may be toxic or harmful. - Risk of local reactions (i.e. swelling, redness, tenderness, itching, extravasations) - Potential for side effects ranging from hematologic toxicities and hypersensitivity reactions to anaphylaxis - Unanticipated or unknown risks - Dermatology/Skin: local erythema, rash, pruritus - Gastrointestinal: diarrhea, anorexia, nausea, vomiting, abnormal taste - Hepatic: elevated hepatic enzymes, hypoalbuminemia with prolonged treatment - Neurology: confusion, neuropathies - Pulmonary: dyspnea (due to fluid retention and capillary leak syndrome), pleuritis - Cardiovascular: hypertension, cardiac arrhythmias, atrial fibrillation, pericarditis - Pain: headache, arthralgias, bone pain, abdominal pain, chest pain, myalgia - Fever, flu-like syndrome (chills, rigors, myalgias), fatigue, headache, abnormal labs including BUN and albumin	- Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems - Only non-pregnant, non-lactating females may participate. The use of contraception during the study and the use of contraception for 18 months post completion of the trial are required. - Frequent laboratory tests including complete blood count (CBC) with differential, liver function tests, etc. - Close and frequent monitoring of subjects by qualified staff - Emergency equipment including crash carts, advanced cardiac life support (ACLS) certified staff and rescue medications such as Benadryl, epinephrine, high dose steroids, etc. will be on-site during administration. - **The Medical Monitor will review all serious toxicities as they occur.** - The study drug may be discontinued if significant AE occurs or if subject chooses to discontinue. - Reporting and monitoring mechanisms are in place for AEs, serious adverse events (SAEs) and unanticipated problems.
Administration of SoC chemotherapy	- Refer to the approved Package Insert or Investigator's Brochure for each chemotherapy agent for a comprehensive list of possible adverse reactions. - The following is a list of common known possible adverse reactions: infections, neutropenia, anemia, febrile neutropenia, hypersensitivity, thrombocytopenia, anaphylactic reactions, dysgeusia, dyspnea, constipation, anorexia, nail disorders, fluid retention, asthenia, pain, nausea, diarrhea, vomiting, mucositis, alopecia, skin reactions, myalgia, arthralgia, fatigue, neuropathy, sensory neuropathy, peripheral neuropathy, leukopenia, urinary bladder, myeloproliferative or lymphoproliferative malignancies, hemorrhagic cystitis, cardiotoxicity.	- Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems - Only non-pregnant, non-lactating females may participate. The use of contraception during the study and the use of contraception for 18 months post completion of the trial are required. - Frequent laboratory tests including CBC with differential, liver function tests, etc. - Close and frequent monitoring of subjects by qualified staff - Emergency equipment including crash carts, ACLS certified staff and rescue medications such as Benadryl, epinephrine, high dose steroids, etc. will be on-site during administration. - **The Medical Monitor will review all serious toxicities as they occur.** - The study drug may be discontinued if significant AE occurs or if subject chooses to discontinue. - Reporting and monitoring mechanisms are in place for AEs, SAEs and unanticipated problems.

Collection of blood samples	- Pain, bruising at the injection site and rarely infection - Discovery of previously unknown conditions	- Experienced personnel will perform the phlebotomies using approved techniques. - Pressure and dressings will be used to minimize pain, bruising and infection. - Research records are kept in a locked area accessible only by study personnel.
Urine pregnancy testing	- Discovery of previously unknown conditions - Possible breach of confidentiality	- Research records are kept in a locked area accessible only by study personnel. - Subjects will only be identified by study numbers on all research documents.
Serum for immunologic evaluation	- Discovery of previously unknown conditions	- Research records are kept in a locked area accessible only by study personnel. - Subjects will only be identified by study numbers on all research documents.
Collection of data	- Possible breach of confidentiality	- Research records are kept in a locked area accessible only by study personnel. - Subject study numbers will be used for identification of samples so that they may be retained for future research and confidentiality is ensured. - Investigators will provide certification of completion of human subject protection training course. - UAMS shall retain the records and reports for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery, or for 2 years after the IND (Investigational New Drug Application) is closed and discontinued with the FDA. After such time all study records will be destroyed as well as the links between identifiers of the research subjects and their research study numbers according to UAMS' record destruction policy.

Any subject may voluntarily revoke consent and withdraw from the study at any time. A subject may be terminated early (completely withdrawn from the study) for the following conditions: (i) non-compliance, (ii) an unrelated intercurrent illness that may affect assessment or place the subject at risk for AEs or require systemic steroids, (iii) deterioration in performance status so as to make participation a hardship for the subject, or (iv) for any reason that the investigator feels it is not in the subject's best interest to continue (i.e. different chemotherapy regimen prior to the first post-surgery study lab (e.g. Week 49 for Schedule C), etc.). These subjects must be followed for at least 30 days after the last dose of study vaccine.

b. Benefits: There is no guarantee that subjects will experience direct benefit from participation in this study. However, it is expected that the rate of pCR following Chemovax will be at least as high as would be expected were the patient to receive only SoC neoadjuvant chemotherapy. Chemovax may potentiate an immune response, which could improve the rate of pCR compared to SoC therapy alone.

12. SPECIMEN HANDLING

A trained phlebotomist or registered nurse will draw the blood required for clinical and research purposes. Research staff will pick up specimens from the Cancer Institute Blood Draw or Infusion Center. Research staff will relabel specimens with a subject identifier, date of specimen, and name of visit. Specimens will then be delivered to the UAMS Tissue Biorepository and Procurement Service (TBAPS) in the Cancer Institute. The serum tubes will remain in the UAMS TBAPS and will be stored frozen at -65ºC or lower. The EDTA tubes will be delivered to Dr. Kieber-Emmons' research laboratory for processing and analysis. Samples will be kept for the duration of this study and may be used for future P10s-PADRE vaccine research. At the end of this cancer vaccine research, any unused specimen will be mixed with bleach and disposed in a sink.

Tissue specimens will be obtained from archival tissue collected as part of standard biopsy and/or surgery. Whenever available, a total of 4 H&E slides and 36 unstained slides (half from the original biopsy and half from tissues collected at surgery) per subject will be requested for all analyses (see *Immunohistochemistry* and *Characterization of tumor infiltrating lymphocytes (TILs)*).

All specimen containers (tubes and containers) will be disposed into biohazard red plastic bags according to the UAMS Occupational Health & Safety policy. Red bag waste will be picked up by occupational Health & Safety for final disposal.

HOG will have study specimens brought to UAMS by courier service. Study specimen will be delivered to the UAMS TBAPS the same day they are drawn following the same process above.

13. ENDPOINT DEFINITIONS, EVALUATION of IMMUNE-RESPONSE ENDPOINTS, and ENDPOINT-ASSAY METHODS

a. ***Primary Feasibility Endpoint: Adequate IgG Response***
This will be defined as a >4-fold increase in a subject's anti-P10s-MAP IgG titer at Week 7 or later (at least 4 weeks after the 3rd immunization) relative to her pre-immune titer.

b. ***Primary Efficacy Endpoint: Pathologic Complete Response (pCR)***
We will employ the definition of pCR proposed by the FDA in their May 2012 Draft Guidance for Industry, "Pathologic Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval". The FDA's proposed definition reads as follows:

> Pathological Complete Response is defined as the absence of any residual invasive cancer on hematoxylin and eosin evaluation of the resected breast specimen and all sampled ipsilateral lymph nodes following completion of neoadjuvant systemic therapy (i.e., ypT0/Tis ypN0 in the current AJCC staging system) (59).

c. ***Secondary Endpoints for determining short-term immune responses to Chemovax***

- P10s-MAP-reactive immunoglobulin titers: The anti-P10s binding level will be measured via ELISA method after incubation with a subject's serum or plasma sample.
  - The P10s-MAP-reactive immunoglobulin titers will be determined on each subject from serum and plasma samples drawn as specified in the study calendar.

- Anti-TACA cell-binding level: The anti-TACA cell-binding level will be measured via flow-cytometry methods as the median fluorescence intensity of TACA-expressing tumor cells obtained after incubation with a subject's serum or plasma sample.

- The anti-TACA cell-binding level will be determined on each subject from serum and plasma samples drawn as specified in the study calendar.

- Anti-breast-cancer cytotoxicity: The cytotoxicity of a sample towards a breast cancer cell line will be measured as the percent decrease (relative to a control) in the cells' survival following incubation with pre- and postimmune serum and plasma.
  - The anti-breast-cancer cytotoxicity will be determined on each subject from serum and plasma samples drawn as specified in the study calendar.

- Frequencies of circulating T cells, B cells, NK cells, and regulatory T cells (Tregs): Peripheral blood mononuclear cells (PBMCs) will be isolated and the above populations of immune cells will be determined by flow cytometry. See Section *f* below for additional details.

- Activation profiles of NK cells: Activated-NK-cell profiles will be determined via flow cytometry as the expression levels of different activation markers on NK cells in the subject's blood sample. See Section *f* below for additional details.

- Functionality of T cells and NK cells: Effector functions will be determined through stimulation with anti-CD3-coated plates, phytohemagglutinin (PHA; Sigma) and tumor cell lysate, and production of cytokines will be measured. The effector function of NK cells will be measured by Antibody Dependent Cell Cytotoxicity (ADCC) assay monoclonal antibodies or using serum samples. See Section *f* below for additional details.

- Biomarkers of response and efficacy: The expression of glycan antigens and proliferation/apoptosis markers will be determined on tumor tissues.

- Characterize infiltrating lymphocytes: TILs will be characterized and frequency of subpopulation with efficacy will be correlated.

d. ***Secondary Endpoints for determining persistence of immune responses***

We will further determine the persistence of a subject's immune responses upon treatment with the Chemovax therapy.

- The anti-P10s antibody titer will be determined on each subject's serum and plasma samples drawn approximately 6 and 12 months, as specified in the study calendar.
- The anti-TACA cell-binding level will be determined on each subject's serum and plasma samples drawn approximately 6 and 12 months as specified in the study calendar.
- The anti-breast-cancer cytotoxicity will be determined on each subject's serum and plasma samples drawn approximately 6 and 12 months as specified in the study calendar.
- Functionality of T and NK cells will be evaluated.

e. ***Tolerability and Safety Endpoints***

Subjects will receive 3 planned vaccine doses unless they withdraw from the study or develop any grade 3 or greater toxicity as per NCI CTCAE v4.0 toxicity criteria, Grade 2 or greater autoimmune toxicity with the exception of vitiligo, or Grade 2 or greater hypersensitivity reactions. These subjects will be treated and referred for additional care as indicated with systemic steroids, topical steroids, epinephrine or Benadryl at which time they will discontinue the vaccine injections and will continue to have assessments per the study calendar defined in the protocol. Subjects will be followed until resolution of toxicity. There will be no dose modifications for toxicity.

The NCI CTCAE Version 4.0 will be used for toxicity and SAE reporting. A copy of the CTCAE Version 4.0 can be downloaded from the Cancer Therapy Evaluation Program (CTEP) home page. All appropriate treatment areas have access to a copy of the CTCAE Version 4.0.

The study will be paused upon Death (other than disease progression or motor vehicle accident) or two Grade 4 Toxicity events that are possibly/probably related to the study agent.

The endpoints used to monitor tolerability will mirror safety endpoints, and will be classified into reactogenicity endpoints and vaccine-related AEs, the most serious of which will be reported as SAEs. Reactogenicity endpoints will be further divided into: (1) systemic symptoms that include fever, malaise, and myalgia; and (2) local symptoms that include pain, tenderness, and induration at the injection site.

f. ***Assay methods for determining the endpoints will follow established SOPs***
The following are brief descriptions of the methods by which the study's endpoints will be determined.

- Sample cytotoxicity towards MDA-MB-231 and HCC1954: This assay will be conducted on both serum samples and plasma samples. Cells from each cell line will be grown in 24-well plates, and exposed in triplicate to the samples from each subject, and to fetal bovine serum (FBS) as a control. Cells will be incubated for 24, 48 and 72 hours, after which wells will be photographed and then dead cells and debris will be removed and viable cells will be fixed and stained. More pictures will be taken and the number of viable cells for each treatment will be counted. The number of alive cells in sample-treated wells will be expressed as a percentage of the number of cells in FBS-treated wells.

- Anti-P10s antibody titer: In each sample, the anti-peptide reactivity will be determined by ELISA methods using P10s-MAP adhered to 96-well microtiter plates, with secondary antibody being HRP-conjugated anti-human antibody. Endpoint titers will be determined from reactivities via the serial 2-fold dilution method. Both the IgG titer and the IgM titer will be determined.

- Binding of antibody to TACA-expressing tumor cells: From each sample, aliquots will be removed and used to incubate 1-to-2 x 10^5 cells from each of three human breast-cancer cell lines: MCF-7 (which expresses LeY), MDA-MB-231 (which expresses gangliosides), and HCC1954. Serum-incubated cells will be post-incubated with FITC-labeled goat antihuman antibody, then fed through the flow cytometer. The median fluorescence intensity of the treated cells will be determined and reported as the measure of binding of serum antibody to TACA-expressing tumor cells. The monoclonal antibodies BR55-2 and 14G2a will be used as positive controls.

- Determining NK-cell activation: To count the number of NK cells in a blood sample, PBMCs will be separated from blood using Histopaque® (Sigma), washed and stained with anti-CD3 and anti-CD56 (BD biosciences), and the number of cells that are determined to be $CD3^-/CD56^+$ will be counted and used as the total number of NK cells in the sample. To determine the expression levels of activation markers CD69, CD94, CD16, and NKp46, cells will be stained with a third color specific for each marker. The expression level of each activation marker will be determined in $CD3^-/CD56^+$ cells (NK cells) via flow cytometry as either the median or geometric mean of the marker's fluorescence-intensity histogram.

- Determine T-cell and B-cell subpopulations: Both lymphocytes and $CD3^+$ cells will be stained for CD4 and CD8 markers. The $CD19^+$ B cell subpopulations, including naive B cells (IgD^+ $CD27^-$), marginal zone-like/natural effector B cells (IgD^+ $CD27^+$), class-switched memory B cells (IgD^- $CD27^+$), transitional B cells (IgM^{++} $CD38^{++}$) and plasmablasts (IgM^- $CD383^+$) will be determined. Percentage of $CD40^+$, $CD69^+$, $CD83^+$ and IgG^+ CD19 B-cells will be determined.

- Determine T-cell subpopulations: Peripheral blood mononuclear cells (PBMCs) will be isolated by density gradient centrifugation using Ficoll-Paque™ solution. Lymphocytes will be stained and the expression levels of CD69, PD-1, and CTLA-4 among CD4 and CD8 T cells will be determined. The regulatory subpopulation of $CD4^+/CD25^{hi}/CD127^{-/lo}$ ($FOXP3^+$) and MDSC populations with granulocytic (Lin^- $HLA\text{-}DR^{-/lo}$ $CD15^+$ $CD33^+$ $CD11b^+$) and monocytic ($HLA\text{-}DR^{-/lo}$ $CD14^+$) phenotype will be determined. The expression of PD-L1 on monocytes will also be determined. The above cell populations will be assessed and assays performed in each subject's pre- and post-immune blood samples and comparisons will be made to understand whether vaccination affects immune cell profile and function.

- Proliferation assay and cytokine detection: The effector functions through stimulation of T cells with P10s and P10s-PADRE peptides, tumor cell lysate, and anti-CD3-coated plates will be conducted and production of cytokines will be measured. 2x10^5 PBMCs will be incubated in 200 µl of RPMI 1640 medium containing 5% heat inactivated fetal calf serum. Proliferation will be induced using P10s, P10s-PADRE, PHA (10 µg/ml), anti-CD3-coated plates (5 µg/ml) or lysate of a representative breast cancer cell line. After 24 (PHA or anti-CD3) to 72 (tumor lysate) hours of incubation 3H thymidine will be added and incubated for another 8 hours. Cells stimulated with lysate may need another round of stimulation (total of 6 days) before thymidine addition. A fraction of supernatant from each well will be collected before thymidine addition for detection of cytokines by ELISA. The cells will be harvested and 3H thymidine incorporation determined using a liquid scintillation counter (Perkin Elmer Inc.) and expressed as counts per minute (cpm). The stimulation index (SI) will be obtained by calculating total cpm/control cpm. PBMCs that had not been subjected to PHA addition will be used as controls. The collected supernatant will be frozen and kept at -80ºC. Supernatants will thawed and used for detection of cytokines (IFN-γ, IL-2, TNFα, IL-4, IL-10, and IL-13) by ELISA using kits from R&D Systems.

- ELISPOT assay: ELISPOT will be used to further confirm T-cell effector function. PBMCs will be stimulated with P10s, P10s-PADREE, tumor cell lysate, and anti-CD3 and cytokine secretion profile will be determined using human BD™ ELISPOT kit set following manufacturer's instructions.

- ADCC assay: ADCC assays will be performed to evaluate functionality of post-immune serum in inducing immune cell-mediated cell death and compare it to pre-immune serum for each subject, where breast cancer cell lines will be used as targets. Target cells will be labelled with calcein AM and 10^4 cells per well in 50 µl of medium will be added to 96 well microtiter plates and then 50 µl serum will be added and incubated for 30 minutes. Effector cells will be added in 100µl volume of medium to give serially diluted effector:target cell ratios (E:T) with maximum E:T of 50:1. Primarily, PBMCs from healthy donors or NK-92 cells will be used as effectors. After 4 hours of incubation imaging with Cytation™ 5 cell imaging reader (BioTek, Winooski, VT) will be performed to count live target cells in wells with and without effector cells and percent cytotoxicity will be calculated as [1-(calcein AM target cells with effector cells /calcein AM target cells without effector cell)]X100. In separate experiments, pre- and post-immune PBMCs from responder subjects will be compared using mAb BR55-2 (mediates ADCC on LeY-positive cells). This will show whether vaccination results in more potent effector cells.

- Immunohistochemistry: In those subjects with enough pathological specimens, tumor-tissue slides will be requested from tissue collected at both the original biopsy before treatment (2 H&E and 8 unstained slides) and at time of definitive surgery (2 H&E and 8 unstained slides). The tumor-tissue slides from the original biopsy will be compared to the tumor-tissue slides from surgery in order to learn more about the mechanism of action. These slides will be stained for anti-glycan mAbs (anti-LeY and GD2 mAb), to evaluate potential correlation of expression of LeY and GD2 gangliosides levels with clinical response. Caspase-3 and activated caspase-3 (Abcam Inc., MA) expression will also be determined. Ki67 staining will be performed and its expression determined. Besides pCR, potential tumor response by evaluating invasion burden and location will be evaluated. Staining of provided slides will be performed by the UAMS experimental Pathology Core Facility. To perform these analyses, a total of 4 H&E slides and 16 unstained will be requested, whenever available, for each procedure (half from original biopsy and half from tissues collected at surgery) per subject.

- Characterization of tumor infiltrating lymphocytes (TILs): The characterization of TILs in tumor tissues of subjects of this study is planned to help us to understand whether vaccination efficacy relate to pre-treatment immune infiltrates or whether vaccination affect lymphocyte infiltration and the composition of the infiltrates. We also seek to understand whether any response to vaccination is associated with immune infiltrates and their compositions. Therefore, we will first evaluate H&E and Ki67 expression in tumor specimens collected at original biopsy before treatment and tissues collected at surgery. Whenever available, tumor-tissue slides will be requested to perform staining to detect subpopulations of infiltrating immune cells. Staining will be performed with anti-CD3, -CD4, -CD8, -FOXP3, and -CD56 mAbs (two slides per antibody from both biopsy and surgery). In addition to the slides for immunohistochemistry, 20 unstained slides (half from original biopsy and half from tissues collected at surgery) will be requested per subject for TILs characterization. Staining will be performed through the UAMS Experimental Pathology Core Facility.

- Genome-wide gene expression and methylation analyses using plasma, immune and tumor cells: Genetic and epigenetic variation in plasma samples, immune cells and tumors before and after immunization will be explored to study the potential biomarkers of immune and tumor responses.

14. STATISTICAL CONSIDERATIONS

General Considerations: The primary objectives of this multi-center study are (1) to identify a Chemovax schedule that is “feasible” in the sense of being safe, tolerable, and capable of generating adequate immune response in enough subjects, and (2) determine if ER-positive breast-cancer subjects treated with the Chemovax regimen experience a significantly higher rate of pCR than ER-positive subjects treated with SoC neoadjuvant chemotherapy. The size of the study population was purposely chosen to provide adequate power (>90%) to properly evaluate the clinical efficacy of the vaccine-chemotherapy combination. In addition, the translational effectiveness of any cancer vaccine in a large population depends on both the level and the functionality of the resultant immune responses.

Analysis Plan for Primary Feasibility Endpoint: To evaluate the feasibility of eliciting adequate immune response with P10s-PADRE when it is administered in combination with neoadjuvant chemotherapy, we will sequentially evaluate different schedules of vaccination relative to chemotherapy, and stop evaluating as soon as we have identified a feasible schedule. Immune-response adequacy will be evaluated using the previously described Schedule Evaluation Rule and will be defined as four or more of the 5 subjects exhibit a ≥4-fold increase in anti-P10s serum IgG titer at Week 7 or later (at least 4 weeks after the third immunization) relative to pre-immune.

Analysis Plan for Primary Efficacy Endpoint: If a feasible Chemovax schedule is identified, then that schedule's cohort will be expanded and used to initiate the primary efficacy evaluation of Chemovax using a Simon optimal two-stage design (60, 61).

The following is a Simon two-stage optimal design intended to test the null hypothesis that the pCR rate with Chemovax will be 8% or less versus the alternative that the pCR rate with Chemovax will be 23% or more. Nineteen subjects will be accrued, treated with Chemovax, and evaluated during the first stage of the primary efficacy evaluation. If one or none achieve pCR, then the trial will be terminated for futility; if two or more achieve pCR, then the second stage of the primary efficacy evaluation will open. If the second stage opens, it will accrue 22 additional subjects, so that a total of 41 subjects will be accrued, treated with Chemovax, and evaluated. If and only if the total number of subjects who achieve pCR is 6 or more, then the null hypothesis (that the pCR rate is ≤8%) will be rejected in favor of the alternative hypothesis (that the pCR rate is ≥23%). When the null hypothesis is true, this design will have a true Type I error rate of 9.6%, and an expected sample size of 29.03 based on a 54.4% probability of early termination. This design will attain 90.8% power to reject the null hypothesis in favor of the alternative when the true rate of pCR is 23%.

Determination of Sample Size: The primary efficacy evaluation just described will be preceded by an immune-response feasibility stage during which a sequence of up to five schedules of vaccination relative to chemotherapy will be evaluated. Each schedule will accrue a cohort of five subjects, and be evaluated using the previously described Schedule Evaluation Rule to identify a feasible Chemovax schedule that does not interfere with subjects' ability to mount an adequate IgG response. Therefore:

1. If a feasible Chemovax schedule is identified, then there could be from zero to 20 subjects who are accrued to unsuccessful schedules, in addition to the 19 or 41 subjects who are accrued to the successful schedule. Under this scenario, the final sample size of the entire study would range from 25 to 61 subjects.
2. If all five Chemovax schedules are evaluated and none of them are identified as feasible then none of the cohorts will be expanded, and the primary efficacy analysis will not take place. Under this scenario, the final sample size of the entire study would be 25.

Thus, the final sample size of the entire study will have a theoretical minimum of 25 subjects, and a theoretical maximum of 61 subjects.

Analysis Plans for Secondary Endpoints: To analyze the secondary endpoints, data will be summarized by collection time as means, standard deviations, medians, and ranges, and displayed graphically using profile plots and box plots. Residuals analysis will be used to examine adherence to distributional assumptions, and data transformations will be applied if warranted. Each endpoint will be analyzed longitudinally using either generalized estimating equations or mixed-models analysis. In particular, post-immune levels of each endpoint will be compared to its pre-immune level for (a) occurrence of a significant increase, and (b) duration of the significant increase if one occurred. In addition, subjects will be classified individually with respect to each endpoint as a "responder" if any of that endpoint's post-immune levels attains an increase of at least two folds relative to its pre-immune level, and the number and proportion of "responders" with respect to each endpoint will be reported. Finally, a mixed-models regression approach will be used to determine how much of the samples' cytotoxicity towards HCC1954 and MDA-MB-231 can be predicted by the secondary endpoints.

Missing, Unused and Spurious Data: Missing data will be treated as missing, and will not be imputed. All data collected will necessarily be reported to the FDA. Spurious data will be corrected at the source document. Any data documented as spurious that is unable to be corrected at the source will be treated as missing.

Data Safety Monitoring Plan: AEs and safety will be evaluated throughout the study by a Data Safety Monitoring Board (DSMB). The DSMB will meet at predetermined intervals set forth in the DSMB Charter to review toxicities and serious and/or unexpected AEs. The DSMB will primarily evaluate safety data; efficacy data will only be reviewed in the event of a serious safety concern. Minutes for all DSMB meetings will be generated.

15. REGISTRATION GUIDELINES

Screening logs will be maintained by research staff at UAMS and HOG.

Subject registration will occur in Clinical Trials Office (CTO) within the UAMS WPRCI after the IRB-approved consent is signed and eligibility has been confirmed. Subjects will be registered in the Clinical Trial Management Suite (CTMS) and will be assigned a study number by CTO. The study number will be used for identification of the research subject during the study.

HOG will send registrations to research staff at CTO. CTO will register the subject in the CTMS and will assign each subject a study number. Once registration has been completed, the information will be sent back to HOG.

16. DATA SUBMISSION SCHEDULE

Data must be submitted according to protocol requirements for ALL subjects registered to the treatment portion of this trial, whether or not assigned treatment is administered.

For subjects deemed to be ineligible to participate in the study or for whom documentation is inadequate to determine eligibility, only the eligibility case report form will be completed. Data obtained during the study will be collected at each subject visit and entered into the clinical trial management suite.. Data will be captured on the case report forms (CRFs).

UAMS and HOG shall retain the records and reports for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been notified. After such time all study records will be destroyed as well as links between identifiers of the research subjects and their study numbers according to UAMS' record destruction policy.

17. ETHICAL AND REGULATORY CONSIDERATIONS

a. Study Personnel: Study personnel must have completed training in Good Clinical Practice (GCP) and Protection of Human Subjects.

b. Recruitment and Informed Consent: Potential subjects will be recruited from the WPRCI on the UAMS campus and clinics at HOG. The potential subjects will be identified by the research physician or staff. Prior to any research activities, the potential subject will be approached for participation in the study by her physician, who will discuss the protocol along with the risks and potential benefits of participating in it. A clear statement will be made concerning the voluntary nature of her participation and that her decision will have no effect on her remaining care. The research nurse or research staff will follow with a detailed review of the informed consent document. The potential subject will be encouraged to have family or friends participate in any or all of the process. The potential subject will be given time to ask questions, will be questioned to be certain she understands the information, and if she agrees to proceed, will sign consent. Subjects will be allowed time to reflect, ask questions or withdraw. The consent process will be documented in the medical record. A copy of the informed consent document will be given to the

subject. The original informed consent will be filed with the subject file. The consent process will occur in a private exam room. There will be no additional recruitment materials. The principles of informed consent are described by Federal Regulatory Guidelines (21CFR50) and the Office for Human Research Protections: Protection of Human Subjects (Code of Federal Regulations 45CFR46). These principles must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

c. Institutional Review: This study will be approved by the sites' IRBs as defined by Federal Regulatory Guidelines 21CFR56 and the Office for Human Research Protections: Protection of Human Subjects 45CFR46. This study will also undergo scientific review by the Cancer Institute's Protocol Review and Monitoring Committee (PRMC). Approval by both the UAMS PRMC and the IRB of record for each institution is required before the clinical trial can be activated.

d. Investigational Agent Accountability: Documentation of drug disposition (drug receipt, dispensing, transfer or return) will be maintained on the UAMS Investigational Agent Accountability Record or its equivalent that is on file with FDA. Drug supplies (long-term storage) will be kept in a secure, limited access storage area under the recommended storage conditions in the research pharmacy at the WPRCI under the direction of the research pharmacist. During the course of the study, the following information will be noted on the Investigational Agent Accountability Record; the study number, the research subject's initials, the research subject's assigned number, the dose of drug, the date(s) and quantity of drug dispensed to the subject, the balance forward, the lot number and the recorder's initials. These Investigational Agent Accountability Records will be readily available for inspection and are open to FDA inspection at any time.

e. Dissemination of Data: The data collected in this study may be used for presentations, posters, and publications or uploaded into open or controlled-access databases, like NIH database of genotypes and phenotypes and Gene expression omnibus, upon request. The publications or data uploaded will not contain any identifiable information that could be linked to a participant. We are fully committed to the responsible data sharing and therefore, the de-identified data, including analyzed and raw, supporting the conclusions made in any publications will be made available to any qualified researcher upon legitimate request. The study will be listed on clinicaltrials.gov in accordance with FDA requirements.

18. ADVERSE EVENTS

Following consent, safety will be measured by assessment of adverse events through the duration of the study, which is approximately 16 months post first vaccination.

a. Adverse Event (AE): Any unfavorable and unintended sign, symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure. Each AE is a unique representation of a specific event used for medical documentation and scientific analysis. *[ICH E6 1.2]*

b. Serious Adverse Event (SAE): Any adverse drug experience occurring at any dose that results in any of the following outcomes: death, a life-threatening adverse drug experience, inpatient hospitalization, prolongation of existing hospitalization, a persistent or significant disability/incapacity or a congenital anomaly/birth defect. Important medical events that may not result in death, be life threatening or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. FDA requires IND sponsors to report qualified AEs and SAEs through the expedited reporting system. *[21CFR312.32 (a)]*

To avoid confusion, as the terms “serious” and “severe” are not synonymous, the following clarification is given: The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is *not* the same as “serious,” which is based on subject/event *outcome* or *action* usually associated with events that pose a threat to a subject’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. *[ICH-E2A(II)(B)]*

c. Related: An event is related if more likely than not it was caused by the research activity.

d. Unexpected: An event is unexpected when its specificity, nature, severity or incidence is not accurately reflected in the consent form, protocol, or investigator’s brochure previously reviewed and approved by the IRB. Examples include a lower rate of response to treatment or a side effect that is more severe than initially expected.

e. Monitoring, Recording and Reporting of AEs: All subjects will be monitored for AEs during the study. Assessments may include monitoring the subject’s clinical symptoms; laboratory, pathological, radiological, or surgical findings; physical examination; or other appropriate tests and procedures.

Any condition, laboratory abnormality, or physical finding with an onset date prior to the subject signing consent for study participation is considered to be pre-existing in nature and part of the subject’s medical history. All relevant historical medical conditions that are known/diagnosed prior to the administration of study vaccine are to be recorded. All AEs, which completely resolve and then recur, should be recorded as a new AE, regardless of whether it is related or not. In addition to new events, any increase in the severity or frequency of a pre-existing condition that occurs after the administration of the first vaccine is considered an AE.

All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed for up to 30 days to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately to the Sponsor.

All AEs and SAEs must be recorded in the appropriate section of the CRF. The report should include, whenever possible, the investigator’s written medical judgment as to the relationship of the AE/SAE to the vaccine and/or to the study (not the chemotherapy) (i.e., “definite”, “probable”, “possible” or “unrelated”).

Expedited Reporting: *Any instance of a Guillain-Barre Syndrome event will require expedited reporting to the FDA, IRB, and IND Sponsor.*

AEs that are serious, unanticipated/unexpected, and possibly related to the vaccine qualify for expedited reporting. Any adverse experience that meets expedited reporting guidelines for SAEs must be reported immediately to the Sponsor. The CRA will follow AE reporting plans per institutional policies and applicable regulations. All qualified AEs will be reported to the Investigators, FDA, IRB, Medical Monitor, and the UAMS Office of Research Regulatory Affairs (Sponsor) according to this plan. The Sponsor will report all events that meet expedited reporting requirements to FDA in accordance with 21CFR312. All other AEs will be reported to the Sponsor and FDA in the Annual Progress Report.

Only AEs meeting UPIRTSO (Unanticipated Problem Involving Risks to Subjects or Others) will be reported to the UAMS IRB within the required 10-day allotment of being notified of the event. UPIRTSO requires that an unanticipated problem meet the following qualifications: a) unanticipated or unexpected; b) related to the research; and c) involves new or increased risk to the subject(s). All other AEs should be recorded and reported to the UAMS IRB at continuing review.

Deaths that are related to research will be reported to the Sponsor immediately upon Investigator notification. A death due to a terminal condition of the research participant would be considered anticipated and not related to the research and will be reported at continuing review and the Annual Progress Report.

19. CLINICAL SITE MONITORING

Clinical site monitoring will be conducted by the UAMS Office of Research Regulatory Affairs (ORRA) to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable from source documents, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), ICH GCP, and applicable regulatory requirements.

Monitoring specialists from ORRA will conduct periodic on-site, comprehensive monitoring as determined by a protocol specific monitoring plan, which will be provided by the ORRA Monitoring Unit to the Investigator.

20. DEVIATIONS AND VIOLATIONS

a. Protocol Deviation: A deviation is any unintentional change, divergence, or departure from the study design or procedures defined in the protocol. Protocol deviations will be tracked and compiled in a Protocol Deviation Log. Deviations that potentially cause concern for the subject health, safety, or rights will be reported to the Sponsor as soon as possible for guidance on reporting.

b. Protocol Violation: A violation is a change to, or non-compliance with, the IRB-approved procedures without prior Sponsor and IRB approval (excluding changes made to eliminate apparent immediate hazard to subjects). A violation may affect health, safety, or rights of a subject. Any violation will be reported immediately to the Sponsor for guidance on reporting.

If the protocol deviation/protocol violation does not represent a significant alteration in the approved protocol and/or affect the safety or welfare of the subject, it will be reported to the UAMS IRB at the time of Continuing Review. If the protocol deviation/violation represents a significant alteration in the approved protocol and/or if it affects the safety or welfare of the subject, it must be reported to the Sponsor and UAMS IRB immediately.

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