

“Vaccination of High Risk Breast Cancer Patients”
Phase 1 Safety Study
Of
A Carbohydrate Mimotope Based Vaccine with MONTANIDE™ ISA 51
VG Adjuvant

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APPENDIX

APPENDIX A – NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0

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1. Abbreviations

ACLS – advanced cardiac life support
 AE – adverse event
 AJCC – American Joint Commission on Cancer
 ANA – anti-nuclear antibody
 AST – aspartate aminotransferase test
 BUN – blood urea nitrogen
 CBC – complete blood count
 CDC – complement dependent cytotoxicity
 CMPs – carbohydrate mimetic peptides
 Co-PI – co-principal investigator/co-investigator
 CCTO- Cancer Clinical Trials Office
 CRA – Clinical Research Associate
 CRF – case report form
 CTC – circulating tumor cell
 CTCAE – Common Terminology Criteria for Adverse Events
 CTEP – Cancer Therapy Evaluation Program
 CTL – cytotoxic T lymphocyte
 dl – deciliter
 DLT – dose limiting toxicity
 DOD – Department of Defense
 DTH – delayed type hypersensitivity
 ELISA – enzyme-linked immunosorbent assay
 FACS – fluorescence activated cell sorting
 FDA – food and drug administration
 GCP – good clinical practice
 GGT – gamma-glutamyl transferase test
 GMP – good manufacturing practice
 id – intradermally
 IgG – immunoglobulin g
 IgM – immunoglobulin m
 IND - investigational new drug

IRB - institutional review board
 IUL – institutional upper limit
 kD – kiloDalton
 KLH – keyhole limpet hemocyanin
 LDH – lactate dehydrogenase
 LeY – Lewis Y antigen
 MFI – mean fluorescence intensity
 mg – milligram
 mL – milliliter
 mm – millimeter
 MTD – maximum tolerated dose
 µg – microgram
 NCI – National Cancer Institute
 NK – natural killer
 PBS – phosphate buffered saline
 PI – principal investigator
 PRMC – protocol review and monitoring committee
 PT – prothrombin time
 PTT – partial thromboplastin time
 RARE™ - RosetteSep-Applied imaging Rare Event (™StemCell Technologies, Vancouver)
 RSC – UAMS Research Support Center
 SAE – serious adverse event
 SC - subcutaneous
 SGOT – serum glutamic-oxaloacetic transaminase
 SLE – systemic lupus erythematosus
 SOP – standard operating procedure
 STn antigen – sialosyl Tn antigen
 TACAs - tumor associated carbohydrate antigens
 TSH – thyroid stimulating hormone
 UAMS - University of Arkansas for Medical Sciences
 WBC – white blood cell

2. Protocol Summary

Primary Objective – Safety: Determine the safety and tolerability of a peptide mimotope-based vaccine upon immunization of breast cancer subjects.

Secondary Objectives – Immune Response:

- 1) Determine whether immunization with the vaccine generates a humoral response against Tumor Associated Carbohydrate Antigens.
- 2) Determine the delayed-type hypersensitivity (DTH) response to the immunizing mimotope
- 3) Determine the effect of a late booster immunization on the humoral response against TACAs

Exploratory Objective: Determine the effect of immunizing mimotope on circulating tumor cells (CTCs)

Study Population: We plan to consent approximately 24 subjects with hope of reaching our enrollment goal of 18 women, ages 18 and older from the breast cancer clinics (Medical Oncology and Ladies Oncology Clinics) at the Winthrop P. Rockefeller Cancer Institute at the University of Arkansas for Medical Sciences (UAMS) campus.

Inclusion Criteria: Females with histologically or cytologically confirmed stage IV breast cancer (newly diagnosed metastatic or relapsed after primary or adjunctive therapy, which has not required a treatment change for 2 months) will be invited to participate.

Exclusion Criteria: Women who are pregnant, breast-feeding, have autoimmune disease or are immunosuppressed or receiving systemic corticosteroids will be excluded from the study.

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

Study Design: After signing IRB approved consent, cohorts of 3-6 stage IV breast cancer subjects will be enrolled into the study. The vaccine doses will be prepared and dispensed by the UAMS Pharmacy following the manufacturer's instructions. Subjects will receive up to 2.0 mL subcutaneous (SC) injections of the vaccine on 5 separate occasions during Weeks 1, 2, 3, 7, and 19. The first cohort will begin with the 300 µg dose, and then the subsequent cohorts will escalate to 500 µg or de-escalate to 100 µg as determined by the toxicity criteria shown in Table 1 of Section 7. The immunization at week 19 is considered a booster immunization. The vaccine will be administered at rotating sites on the limbs or abdomen by nurses in the Infusion Center at the Cancer Institute, using a dose volume of up to 2.0 mL per injection. Based on a series of criteria measuring tolerance and immune response in the first cohort on Week 9 (Section 7, Table 1), the P10s-PADRE dose will either be increased to 500 µg/mL or decreased to 100 µg/mL for the second cohort of subjects. The study will last for approximately 12 – 24 months.

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 patient survival significantly correlates with carbohydrate-reactive IgM levels (2). Such results suggest that TACA-targeting vaccines might have a beneficial effect on the course of malignant disease. TACA-induced responses could augment naturally occurring carbohydrate-reactive IgM antibodies that trigger apoptosis of tumor cells (3). 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 (4,5). 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 (6). 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 (7). Furthermore, preclinical studies support the hypothesis that vaccine-induced responses against TACAs might have their greatest impact in the adjuvant setting, as such responses inhibit tumor outgrowth in metastatic models (8,9).

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 (10,11). 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 (12-14), polysialic acid (15), Globo-H (16), Lewis Y (LeY) (1), 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 the GD2 (20-22), GD3 (23), sialylated Lewis a/x (24) and Lewis Y (LeY) antigens (20, 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 (8, 25, 26). 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.

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 (27-29). 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 (30, 31). 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 (32). The abundant gangliosides include GM3, GM2, GM1, and GD2, GD3 and GT3 (33). 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 (1). 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 (25, 34). Our *in vivo* studies demonstrate that the peptide mimotopes induce sustained immunity to these antigens (8, 25, 26). Collectively, these data provide the experimental foundation for evaluating peptide mimotopes as potential cancer vaccines in subjects with breast cancer.

Preclinical studies supporting the P10s-PADRE vaccine as a viable candidate for preventing breast cancer recurrence: The desired effect of a cancer vaccine is to modify the clinical outcome of the patient population of interest. Genetic studies have resurrected the concept that the adaptive and innate immune systems play roles in tumor surveillance. Cellular immunity, in which cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells are main effector cells, plays an important role in the antitumor defense mechanism. Tumors over express TACAs, which are reactive with B cells, but the use of TACAs as immunogens is restricted by a lack of B cell cross-talk with T cells. Consequently, this inherent limitation of plain polysaccharide vaccines includes limited duration of immunity, the potential for hyporesponsiveness with repeated vaccinations, and ineffectiveness in stimulating a cellular response. To circumvent this drawback we have developed CMPs with overlapping B- and T-cell epitopes to link TACAs' reactive humoral responses with anti-tumor cellular responses. Among the CMPs we have developed are a series that contain the amino acids Trp-Arg-Tyr as a centralized motif. CMPs with this motif display an ability to induce antibodies cross-reactive with tumor cells, induce cellular responses to tumor cells and induce or activate NK cells with anti-tumor activity.

P10s-PADRE immunization in prophylactic and therapeutic models of murine cancer suggest that the vaccine promotes tumor growth inhibition. The safety and efficacy of P10s-PADRE in humans remains to be determined. In many clinical trials, study subjects receive peptide vaccines in combination with a certain adjuvant, such as incomplete Freund's Adjuvant, for the purpose of enhancing immune responses against cancer. Water-in-oil or water-in-oil-in-water emulsions such as *MONTANIDE*[™] ISA 51 VG are very well characterized adjuvants and used for more than 50 years in pre-clinical studies and in human clinical trials. It is known that these types of adjuvant can induce strong Th2-type of antibody responses because they prolong the lifetime of antigens in vivo, enhance phagocytoses of antigens by professional Antigen Presenting Cells (APC), and induce transient expansion of CD4 positive Th cells which might also expand Th1 responses.

In our studies in mice an adjuvant was added with the P10s-PADRE vaccine; as administered with Stimulon[™]QS-21 (Antigenics Inc.) and in limited instance *MONTANIDE*[™] ISA 51 VG. In this proposed Phase I study of P10s-PADRE, study subjects will receive P10s-PADRE dissolved in physiological saline admixed with *MONTANIDE*[™] ISA 51 VG. We have chosen to use *MONTANIDE*[™] ISA 51 VG over QS-21 for two reasons. Antigenics, Inc., the provider of QS-21, imposed new restrictions upon the further supply of QS-21. These new restrictions contravene the stated mission of the University of Arkansas for Medical Sciences. Therefore, QS-21 is no longer available for use. Second, even if no serious adverse effects in an extensive non-clinical safety study as performed in our GLP study with QS-21 was observed, it cannot be guaranteed that the vaccine/adjuvant formulation presents no risks to vaccinees and unexpected events can occur. Unpredictability of adjuvant effects in humans results from a complex interplay between such factors as route of administration, antigen dose, and nature of the antigen. For this reason a final safety evaluation can only be conducted on the basis of a clinical trial. *MONTANIDE*[™] ISA 51 VG has been used in Phase I and II clinical trials for vaccines against malaria, HIV, and importantly various cancers (see Investigational brochure for *MONTANIDE*[™] ISA 51 VG. *MONTANIDE*[™] ISA 51 VG, has been tested in AIDS and cancer vaccine trials which represent more than 10,000 patients and around 100,000 injections. A survey of ongoing clinical trials listed in ClinicalTrials.gov revealed 36 trials currently accruing patients that are using the olive-derived *MONTANIDE*[™] ISA 51 VG. The formulation is generally well tolerated and induces transient local reactions. Some transient general reactions such as flu-like symptoms can also be noticed. The results suggest that numerous repeated vaccine doses with *MONTANIDE*[™] ISA 51 VG can be safely administered.

Dose Justification: Immunologic testing in mice indicates that there is no difference in antibody responses to P10s in mice between the 300 µg and 500 µg dose (GLP Report: “Determination of the Safety and Tolerability of Immunization with LeY Peptide Mimotope Vaccine in Mice”). Dose escalation is meant to test the safety of the preparation in terms of its non-immunological toxicity. In this case the assumption is that the putative adverse effects increase monotonically with the dose, and exploring the higher-than-necessary doses ensures safety of the lower ones. As for the immunological effects, they are characteristically non-monotonic with an optimal effect in a specific dose range, and weaker effects and even induction of tolerance for doses much higher or lower than the optimal one. Thus, the classical dose escalation trial may or may not detect the immunological effects, including adverse autoimmune phenomena. A dose-escalation algorithm better suited for determining the immunological impact should include both increasing and decreasing arms, and should be terminated when doses beyond the one that leads to optimal immunological effect are reached, provided they are tolerated otherwise. When increasing immunological effect is detected in one of the arms, the other one may be discontinued, if not necessary for the toxicology study.

4. Trial Objectives

a. Primary Objective – Safety: The safety and tolerability of the P10s-PADRE/ MONTANIDE™ ISA 51 VG vaccine will be determined by toxicity assessments throughout the duration of the study. Subjects will be evaluated for toxicity using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. A toxicity of Grade 3 or higher will be considered a dose limiting toxicity (DLT).

b. Secondary Objectives – Immune Response:

- 1) The ability of the P10s-PADRE/ MONTANIDE™ ISA 51 VG vaccine to generate a humoral response against TACAs will be determined in the investigator’s research laboratory by titrating anti-TACA serum IgG and IgM antibodies measured at pre-study and on weeks 2, 3, 4, 7, and 9 from study participant blood samples. IgM and IgG titers to TACAs will be evaluated by enzyme-linked immunosorbent assay (ELISA) of TACAs adhered to plates and by fluorescence-activated cell sorting (FACS) of TACAs adhered to liposomes. Both approaches will be validated prior to examination of research study samples. Titer will be defined as the highest serum dilution yielding an OD₄₀₅ ≥ 0.15, in accordance with previous studies (1) or a mean fluorescence intensity (MFI) two standard deviations higher than background. A positive TACA-directed immune response will be defined as an anti-TACA serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a ≥ 4-fold increase for a baseline titer > 0 (1).
- 2) Delayed type hypersensitivity (DTH) responses to the immunizing mimotope and control antigens will be determined by the amount of induration surrounding the injection site at 48- 72hrs post-injection. An induration diameter of > 5 mm at 48- 72 hrs post injection will be considered a positive response. Control agents to be tested in addition to the P10s-PADRE/ MONTANIDE™ ISA 51 VG vaccine include Trichophyton Antigen and the Candida antigen.
- 3) The effect of a late booster immunization (week 19 immunization) of P10s-PADRE/ MONTANIDE™ ISA 51 VG on the humoral response against TACAs will be determined by titrating anti-TACA serum IgG and IgM antibodies measured on weeks 19 and 21 with the methodology described above.

- 4) As part of the standard of care, disease assessments will be performed by PET CT or CT CAP at 8 to 12 week intervals. Enrollment will be done after one such assessment.

c. Exploratory Objective

- 1) The effect of immunizing mimotope on the presence of circulating tumor cells (CTCs) will be determined by subjecting pre-and post-vaccination blood (drawn on weeks 0 and 21, respectively) to the RARE™ technique – a single-step negative enrichment process with a bispecific antibody directed against erythrocytes and CD45-positive cells (StemCell Technologies) (35-41). Determining the presence of CTCs prior to and following mimotope administration could provide a potential indicator for clinical disease (Müller V, Hayes DF, Pantel K. Breast Cancer Res. 2006;8(5):110). Though previous efforts to assay CTCs have had technical difficulties, we will use a simple protocol that requires only 7.5ml of blood, thereby decreasing the likelihood of technical complications.

5. Patient Population

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

- 1) Female subjects of all races with histologically or cytologically confirmed stage IV breast cancer are eligible. The cancer may be 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 are allowed. The chemotherapy regimen cannot contain steroids in the pre or post supportive care medications. If a subject is on an investigational drug, the drug must be cleared from the body over a period of 4 weeks.
- 2) Disease staging will be done according to the American Joint Commission on Cancer (AJCC), sixth edition. The breast cancer staging information can be found at the following address:
<http://www.cancerstaging.org/education/tnmschema/breast.ppt>
- 3) Age 18 years and older of all races and ethnicity.
- 4) ECOG Performance Status 0 or 1.
- 5) Subjects must not have an active infection requiring treatment with antibiotics.
- 6) Subjects must not have other significant medical, surgical or psychiatric conditions, or require any medication or treatment, which may interfere with compliance of the treatment regimen.
- 7) Subjects must not have a diagnosis or evidence of organic brain syndrome, significant impairment of basal cognitive function or any psychiatric disorder that might preclude participation in the full protocol.
- 8) Subjects must have no 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.

- 9) Subjects must not have autoimmune disorders or conditions of immunosuppression. This includes, but is not limited to being treated with corticosteroids, including oral steroids (i.e. prednisone, dexamethasone), continuous use of topical steroid creams or ointments or any steroid-containing inhalers. Subjects who have been on systemic steroids will require a 6-week washout period. 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.
- 10) Women of childbearing potential must not be pregnant (negative serum pregnancy test must be done 48 hours prior to receiving the first dose of study drug) or breast-feeding, due to the unknown effects of peptide/mimotope vaccines on a fetus or infant.
- 11) Women of childbearing potential 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 include oral contraceptives, barrier method, IUDs, and abstinence.
- 12) Subjects must have obtained a white blood cell (WBC) count $\geq 3,000/\text{mm}^3$ and platelet count $\geq 100,000/\text{mm}^3$ within 2 weeks prior to registration.
- 13) Subjects must have a serum glutamic-oxaloacetic transaminase (SGOT)/aspartate aminotransferase test (AST) and bilirubin $\leq 2 \times$ institutional upper limit (IUL) of normal and serum creatinine $\leq 1.8 \text{ mg/dl}$, all obtained within 2 weeks prior to registration.
- 14) Subjects must be immunocompetent as measured by responsiveness to one recall antigens by skin testing.
- 15) All subjects who wish to participate in the study must sign an informed consent approved by the UAMS Institutional Review Board (IRB).
- 16) Laboratory tests must be completed within 2 weeks before the first dose.
- 17) Subjects must not have a diagnosis or evidence of Central Nervous System metastases.

6. Investigational New Drug – P10S-PADRE/ MONTANIDE™ ISA 51 VG

- 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—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 29842, USA) will synthesize Mimotope P10s covalently linked with PADRE as a sterile lyophilized powder manufactured under GMP conditions. Mimotope P10s-PADRE will be manufactured in facilities registered with the FDA (FDA registration #3006446551). Once P10s-PADRE is manufactured by AmbioPharm the lyophilized powder will be shipped to NextPharma Technologies (FDA registration # 2027352, 5340 Eastgate Mall, San Diego, CA 92121) for sterile packaging in appropriate sized glass vials under GMP conditions, in quantities of 500 µg/vial. Advantar Laboratories, Inc. (3030 Bunker Hill Dr., Suite 102, San Diego, CA 92109) will perform product release and stability testing on the P10s-PADRE study drug. The study drug will be stored at $\leq -20^{\circ}\text{C}$ for maximum stability.

MONTANIDE™ ISA 51 VG will be supplied by SEPPIC, Inc. (30 Two Bridges Road, Suite 20, Fairfield, NJ 07004-1530). See investigator's brochure for formulation and storage conditions.

c. Vaccine Preparation:

The following doses of P10s-PADRE and MONTANIDE™ ISA 51 VG vaccine will be administered to subjects subcutaneously in rotating injection sites in the abdomen or extremities. Subjects will receive up to 2.0 ml subcutaneous (SC) injections of the vaccine on 5 separate occasions (Weeks 1, 2, 3, 7, and 19).

- **500 µg P10s-PADRE/ MONTANIDE™ ISA 51 VG Vaccine Dose preparation:** The UAMS pharmacist will prepare the vaccine dose following the manufacturer's instructions. (See CMC section of the IND).
- **300 µg P10s-PADRE/ MONTANIDE™ ISA 51 VG Vaccine Dose preparation:** The UAMS pharmacist will prepare the vaccine dose following the manufacturer's instructions. (See CMC section of the IND).

d. DTH Preparation:

- Triychophyton: per insert instructions.
- Candida: per insert instructions.
- 100 µg P10s-PADRE: The UAMS pharmacist will prepare the DTH dose following the manufacturer's instructions. (See CMC section of the IND).

e. Dosing Administration:

The DTH dose will be injected SC on the back of the patient by clinic staff.

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

- g. Agent Ordering:** All drug study supplies (P10s-PADRE vials and MONTANIDE™ ISA 51 VG 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 vials will be shipped directly to UAMS Research Pharmacy, 4301 W. Markham Street, # 547-10, Little Rock, AR 72205.

- h. Agent Accountability:** P10s-PADRE and MONTANIDE™ ISA 51 VG will be stored in the UAMS 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 NCI accountability logs will be used.

7. Treatment Plan

- a. **On-study Evaluation:** After signing the IRB-approved informed consent form, research participants will be assigned to a cohort at the time of consent by a clinical research associate (CRA) in the Cancer Clinical Trials Office (CCTO) in the Cancer Institute. All research participants will receive the Mimotope P10s-PADRE/ MONTANIDE™ ISA 51 VG vaccine via subcutaneous (SC) injection following the schedule on the Study Calendar in Section 9.
- b. **Dose Assignment:** The decision to escalate or de-escalate the dose, expand the cohort or terminate the study will be based on assessment for DLT, which will require 9 weeks per subject. The time to assess a cohort of 3 for DLTs and immune responses is thus anticipated to be 13 weeks based on an accrual rate of 2 eligible Stage IV subjects per month.
- c. **Specimen Handling:** A trained phlebotomist or registered nurse will draw the blood required for clinical and research purposes. Research specimens will be delivered to Dr. Kieber-Emmons' research laboratory.
- d. **Visit Breakdown:**

Pre-study Visit

- Complete medical history and physical examination
- Complete blood count with differential
- Other blood tests include:
 - Collection of serum samples used to measure the immune response to the vaccine.
 - SGOT (Serum glutamic-oxaloacetic transaminase)
 - Alkaline Phosphatase Level
 - LDH (lactate dehydrogenase)
 - GGT (gamma-glutamyl transferase)
 - Creatinine
 - Calcium
 - Albumin
 - Amylase
 - TSH (thyroid stimulating hormone)
 - T₄
 - ANA (Anti-nuclear antibody)
 - Bilirubin
 - PT/PTT
- Concomitant medications will be collected from registration to end of treatment.
- Serum pregnancy test for women of childbearing potential.
- Study lab samples. Up to 40 mLs of blood will be collected in red and purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays.

- DTH Skin Test to test the subjects' immunocompetency. Trichophyton Antigen and the Candida antigen will be administered intradermally (id) as control antigens at separate locations on the subject's back, and the resulting induration will be read 48– 72 hours later.
- CTC (circulating tumor cells) response to the vaccine will be measured in serum samples drawn to determine the presences of CTCs pre- and post-mimotope administration.

48 – 72 hours after Pre-Study Visit

- DTH Skin Test Reading

48 hours prior to dosing

- Serum pregnancy test for women of childbearing potential.

Week 1

- Administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine. The vaccine will be administered subcutaneously (SC) by nurses in the Infusion Center at the Cancer Institute.
- Post vaccine administration, the following vital signs will be monitored every 15 (+/- 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Complete medical history and physical examination
- Toxicity evaluations using the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) Version 4.0. Toxicities to be assessed are 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 prestudy or on the baseline evaluation. Special attention will be paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.
- Concomitant medications will be collected from registration to end of treatment.

Week 2

- Administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine. The vaccine will be administered subcutaneously (SC) by nurses in the Infusion Center at the Cancer Institute.
- Post vaccine administration, the following vital signs will be monitored every 15 (+/- 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Complete medical history and physical examination
- Complete blood count with differential
- Toxicity evaluations using the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) Version 4.0. Toxicities to be assessed are 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 prestudy or on the baseline evaluation. Special attention will be paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.
- Concomitant medications will be collected from registration to end of treatment.
- Collection of serum samples used to measure the immune response to the vaccine. If vaccine is given on these days, the study labs will be drawn before the vaccine is administered.

- Study lab samples. Up to 40 mLs of blood will be collected in red and purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays.

Week 3

- Administration of the study vaccine. The vaccine will be administered subcutaneously (SC) by nurses in the Infusion Center at the Cancer Institute.
- Post vaccine administration, the following vital signs will be monitored every 15 (+/- 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Complete medical history and physical examination
- Complete blood count with differential
- Other blood tests include:
 - Collection of serum samples used to measure the immune response to the vaccine. If vaccine is given on these days, the study labs will be drawn before the vaccine is administered.
 - SGOT (Serum glutamic-oxaloacetic transaminase)
 - Alkaline Phosphatase Level
 - LDH (lactate dehydrogenase)
 - GGT (gamma-glutamyl transferase)
 - Creatinine
 - Calcium
 - Albumin
 - Amylase
 - TSH (thyroid stimulating hormone)
 - T₄
 - ANA (Anti-nuclear antibody)
 - PT/PTT
 - Bilirubin
- Toxicity evaluations using the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) Version 4.0. Toxicities to be assessed are 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 prestudy or on the baseline evaluation. Special attention will be paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.
- Concomitant medications will be collected from registration to end of treatment.
- Study lab samples. Up to 40 mLs of blood will be collected in red and purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays.

Week 4

- Complete blood count with differential
- Collection of serum samples used to measure the immune response to the vaccine. If vaccine is given on these days, the study labs will be drawn before the vaccine is administered.
- Study lab samples. Up to 40 mLs of blood will be collected in red and purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays.

Week 5

- Complete blood count with differential
- DTH Skin Test to test the subjects' immunocompetency. Trichophyton Antigen and the Candida antigen will be administered intradermally (id) as control antigens at separate locations on the subject's back, and the resulting induration will be read 48– 72 hours later; 100 µg Mimotope P10s-PADRE will be given id alongside the DTH control antigens to determine subjects' DTH response to vaccine.

48 – 72 hours later

- DTH Skin test reading.

Week 6

- Complete blood count with differential

Week 7

- Administration of the P10s-PADRE/ MONTANIDE™ ISA 51 VG vaccine. The vaccine will be administered subcutaneously (SC) by nurses in the Infusion Center at the Cancer Institute.
- Post vaccine administration, the following vital signs will be monitored every 15 (+/- 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Complete medical history and physical examination
- Complete blood count with differential
- Other blood tests include:
 - Collection of serum samples used to measure the immune response to the vaccine. If vaccine is given on these days, the study labs will be drawn before the vaccine is administered.
 - SGOT (Serum glutamic-oxaloacetic transaminase)
 - Alkaline Phosphatase Level
 - LDH (lactate dehydrogenase)
 - GGT (gamma-glutamyl transferase)
 - Creatinine
 - Calcium
 - Albumin
 - Amylase
 - TSH (thyroid stimulating hormone)
 - T₄
 - ANA (Anti-nuclear antibody)
 - PT/PTT
 - Bilirubin
- Toxicity evaluations using the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) Version 4.0. Toxicities to be assessed are 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 prestudy or on the baseline evaluation. Special attention will be paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.
- Concomitant medications will be collected from registration to end of treatment.

- Study lab samples. Up to 40 mLs of blood will be collected in red and purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays.

Week 8

- Complete blood count with differential

Week 9

- Complete medical history and physical examination
- Complete blood count with differential
- Other blood tests include:
 - Collection of serum samples used to measure the immune response to the vaccine. If vaccine is given on these days, the study labs will be drawn before the vaccine is administered.
 - SGOT (Serum glutamic-oxaloacetic transaminase)
 - Alkaline Phosphatase Level
 - LDH (lactate dehydrogenase)
 - GGT (gamma-glutamyl transferase)
 - Creatinine
 - Calcium
 - Albumin
 - Amylase
 - TSH (thyroid stimulating hormone)
 - T₄
 - ANA (Anti-nuclear antibody)
 - PT/PTT
 - Bilirubin
- Toxicity evaluations using the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) Version 4.0. Toxicities to be assessed are 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 prestudy or on the baseline evaluation. Special attention will be paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.
- Concomitant medications will be collected from registration to end of treatment.
- DTH Skin Test to test the subjects' immunocompetency. Trichophyton Antigen and the Candida antigen will be administered intradermally (id) as control antigens at separate locations on the subject's back, and the resulting induration will be read 48– 72 hours later; 100 µg Mimotope P10s-PADRE will be given id alongside the control antigens to determine subjects' DTH response to vaccine.
- Study lab samples. Up to 40 mLs of blood will be collected in red and purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays.

48 – 72 hours later

- DTH Skin test reading.

Week 19

- Administration of the P10s-PADRE/MONTANIDE™ ISA 51 VG vaccine. The vaccine will be administered subcutaneously (SC) by nurses in the Infusion Center at the Cancer Institute.
- Post vaccine administration, the following vital signs will be monitored every 15 (+/- 5 minutes) minutes for one hour: blood pressure, temperature, and pulse.
- Complete medical history and physical examination
- Complete blood count with differential
- Other blood tests include:
 - Collection of serum samples used to measure the immune response to the vaccine. If vaccine is given on these days, the study labs will be drawn before the vaccine is administered.
 - SGOT (Serum glutamic-oxaloacetic transaminase)
 - Alkaline Phosphatase Level
 - LDH (lactate dehydrogenase)
 - GGT (gamma-glutamyl transferase)
 - Creatinine
 - Calcium
 - Albumin
 - Amylase
 - TSH (thyroid stimulating hormone)
 - T₄
 - ANA (Anti-nuclear antibody)
 - PT/PTT
 - Bilirubin
- Toxicity evaluations using the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) Version 4.0. Toxicities to be assessed are 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 prestudy or on the baseline evaluation. Special attention will be paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.
- Concomitant medications will be collected from registration to end of treatment.
- Study lab samples. Up to 40 mLs of blood will be collected in red and purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays.

Week 20

- Complete blood count with differential

Week 21

- Complete medical history and physical examination
- Complete blood count with differential
- Other blood tests include:
 - Collection of serum samples used to measure the immune response to the vaccine. If vaccine is given on these days, the study labs will be drawn before the vaccine is administered.
 - SGOT (Serum glutamic-oxaloacetic transaminase)
 - Alkaline Phosphatase Level
 - LDH (lactate dehydrogenase)

- GGT (gamma-glutamyl transferase)
- Creatinine
- Calcium
- Albumin
- Amylase
- TSH (thyroid stimulating hormone)
- T₄
- ANA (Anti-nuclear antibody)
- PT/PTT
- Bilirubin
- Toxicity evaluations using the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) Version 4.0. Toxicities to be assessed are 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 prestudy or on the baseline evaluation. Special attention will be paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.
- Concomitant medications will be collected from registration to end of treatment.
- DTH Skin Test to test the subjects' immunocompetency. Trichophyton Antigen and the Candida antigen will be administered intradermally (id) as control antigens at separate locations on the subject's back, and the resulting induration will be read 48– 72 hours later; 100 µg Mimotope P10s-PADRE will be given id alongside the control antigens to determine subjects' DTH response to vaccine.
- Study lab samples. Up to 40 mLs of blood will be collected in red and purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays.
- CTC (circulating tumor cells) response to the vaccine will be measured in serum samples drawn to determine the presences of CTCs pre- and post-mimotope administration.

48 – 72 hours later

- DTH Skin test reading.

Week 24

- Complete medical history and physical examination
- Complete blood count with differential
- Other blood tests include:
 - Collection of serum samples used to measure the immune response to the vaccine. If vaccine is given on these days, the study labs will be drawn before the vaccine is administered.
 - SGOT (Serum glutamic-oxaloacetic transaminase)
 - Alkaline Phosphatase Level
 - LDH (lactate dehydrogenase)
 - GGT (gamma-glutamyl transferase)
 - Creatinine
 - Calcium
 - Albumin
 - Amylase
 - TSH (thyroid stimulating hormone)
 - T₄

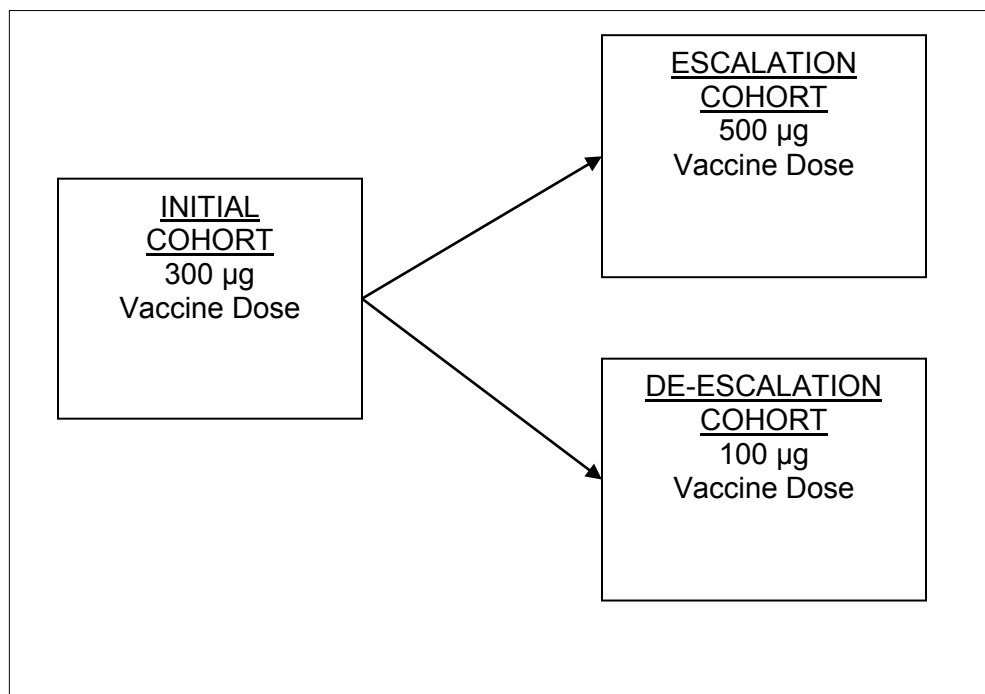
- ANA (Anti-nuclear antibody)
- PT/PTT
- Bilirubin
- Toxicity evaluations using the NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) Version 4.0. Toxicities to be assessed are the laboratory parameters listed in the study calendar, as well as any sign or symptom found during the history and physical examination not noted at prestudy or on the baseline evaluation. Special attention will be paid to signs and symptoms related to injection reactions, injection site reactions or symptoms or laboratory findings indicating autoimmune toxicities.
- Concomitant medications will be collected from registration to end of treatment. Study lab samples. Up to 40 mLs of blood will be collected in red and purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays.

Follow up

Follow up will occur at next scheduled standard of care visit every six months for five years and then yearly until death.

- Collection of serum samples used to measure the immune response to the vaccine.

- e. **Prohibited Medications:** Systemic steroids are prohibited. If a subject wishing to participate in the study has been on systemic steroids, a 6-week clearing of the drug will be required prior to participation in the study.
- f. **Dose Limiting Criteria:** Subjects who develop any grade 3 or greater toxicity as per NCI CTCAE v. 4 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 be removed from the study and will be followed as per protocol defined monitoring and/or until resolution of toxicity.
- g. **Dose Assignment:** Subjects will be treated with vaccine admixed with MONTANIDE™ ISA 51 VG on weeks 1, 2, 3, 7 and 19 in cohorts according to the following dosing diagram:



DLT and the immune-response endpoints are defined in Section 4, "Trial Objectives". Upon evaluation of all subjects in a cohort (3 or 6 per dose level), the decision whether to escalate, de-escalate or stop will proceed according to the cohort-appropriate schedule shown in **Tables 1, 2, and 3**.

Table 1: Toxicity Decision Rules for Initial Cohort (300 µg Vaccine Dose)	
<i>DLTs/ Cohort¹</i>	<i>Action</i>
0/3	Begin accrual to Escalation Cohort
1/3	Expand Initial Cohort to 6 subjects
1/6	Begin accrual to Escalation Cohort
2/6, 3/6, or 4/6	Begin Accrual to De-Escalation Cohort
2/3 or 3/3	Begin Accrual to De-Escalation Cohort
¹ DLT = Dose-Limiting Toxicity	

Table 2: Toxicity Decision Rules for Escalation Cohort (500 µg Vaccine Dose)	
DLTs/ Cohort¹	Action
0/3 or 1/3	Expand cohort to 6 subjects
1/6	Stop: Declare escalation dose to be MTD ²
2/6, 3/6, or 4/6	Stop: Previous dose level is MTD ²
2/3 or 3/3	Stop: Previous dose level is MTD ²
¹ DLT = Dose-Limiting Toxicity	
² MTD = Maximum Tolerated Dose	

Table 3: Toxicity Decision Rules for De-Escalation Cohort (100 µg Vaccine Dose)	
DLTs/ Cohort¹	Action
0/3 or 1/3	Expand cohort to 6 subjects.
1/6	Stop: De-escalation dose level is the MTD ²
2/6, 3/6, or 4/6	Stop: De-escalation dose level is above MTD ²
2/3 or 3/3	Stop: De-escalation dose level is above MTD ²
¹ DLT = Dose-Limiting Toxicity	
² MTD = Maximum Tolerated Dose	

If the initial-cohort dose of 300 µg P10s-PADRE is declared to be the MTD and only 3 subjects were enrolled into the initial cohort, then this cohort will be expanded to 6 to assure that 6 subjects are treated at the MTD. The CRA for the trial will assign the subjects to cohorts and communicate the assignments to the research nurse, who will communicate them to the investigator. Only one cohort will be open to enrollment at a time. After the last non-boost dose in each cohort, the study will have a 3-week hold pending the cohort's DLT evaluation. Staggered dosing for the first two patients within each cohort will allow an interval of one week observation for toxicity following the first injection in the previous patient. The research pharmacist will be notified by orders of the registration and cohort dose. Expansion of the cohort to 6, or enrollment to the next cohort, may start once the previous 3 subjects complete their week-9 serology and are evaluated for DLT. Subjects who withdraw from the dose-escalation study without a DLT will be replaced. Subjects who withdraw from the dose- de-escalation study without a DLT will be replaced. Subjects who withdraw with a DLT will be considered evaluable for MTD determination.

8. 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	<p>Qualified health care provider to evaluate potential subject</p> <p>Research records are kept in a locked area accessible only by study personnel.</p>

Procedure	Risks	Measures to Minimize Risks
Administration of study vaccine	<p>Experimental agent may be toxic or harmful.</p> <p>First time use in humans</p> <p>Risk of local reactions (i.e. swelling, redness, tenderness, itching, extravasations)</p> <p>Potential for side effects ranging from hematologic toxicities and hypersensitivity reactions to anaphylaxis</p> <p>Unanticipated risks</p> <p>Unknown risks</p>	<p>Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems</p> <p>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.</p> <p>Frequent laboratory tests including complete blood count (CBC) with differential, liver function tests, etc.</p> <p>Close and frequent monitoring of subjects by qualified staff</p> <p>DTH assay to monitor for hypersensitivity reactions</p> <p>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.</p> <p>The Medical Monitor will review all toxicities on a regular basis and will be available to aid subjects as needed.</p> <p>The study drug may be discontinued.</p> <p>This research is being conducted at an experienced clinical research center.</p> <p>Reporting and monitoring mechanisms are in place for AEs, serious adverse events (SAEs) and unanticipated problems.</p>
Administration of MONTANIDE™ ISA 51 VG	<p>Dermatology/Skin: local erythema, rash, pruritis</p> <p>Gastrointestinal: diarrhea, anorexia, nausea, vomiting, abnormal taste</p> <p>Hepatic: elevated</p>	<p>Careful monitoring by clinic visits and 24 hour, 7 days per week physicians on call for unexpected problems</p> <p>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.</p>

Procedure	Risks	Measures to Minimize Risks
	<p>hepatic enzymes, hypo-albuminemia with prolonged treatment</p> <p>Neurology: confusion, neuropathies Possible CNS toxicity in those with known metastatic disease to brain</p> <p>Pulmonary: dyspnea (due to fluid retention and capillary leak syndrome), pleuritis</p> <p>Cardiovascular: hypertension, cardiac arrhythmias, atrial fibrillation, pericarditis</p> <p>Pain: headache, arthralgias, bone pain, abdominal pain, chest pain, myalgia</p> <p>Fever, flu-like syndrome (chills, rigors, myalgias), fatigue, headache, abnormal labs including blood urea nitrogen and albumin</p>	<p>Thorough review of scans used for disease assessment performed prior to participation in study, including brain imaging.</p> <p>Frequent laboratory tests including CBC with differential, liver function tests, etc.</p> <p>Close and frequent monitoring of subjects by qualified staff.</p> <p>DTH assay to monitor for hypersensitivity reactions</p> <p>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.</p> <p>The Medical Monitor will review all toxicities on a regular basis and will be available to aid subjects as needed.</p> <p>The study drug may be discontinued.</p> <p>This research is being conducted at an experienced clinical research center in the cancer institute.</p> <p>Reporting and monitoring mechanisms are in place for AEs, SAEs and unanticipated problems.</p>
Collection of blood samples	<p>Pain, bruising at the injection site and rarely infection</p> <p>Discovery of previously unknown conditions</p> <p>Possible breach of</p>	<p>Experienced personnel will perform the phlebotomies using approved techniques.</p> <p>Pressure and dressings will be used to minimize pain, bruising and infection.</p> <p>Research records are kept in a locked area accessible only by study personnel.</p> <p>Subject study numbers will be used for</p>

Procedure	Risks	Measures to Minimize Risks
	confidentiality	identification of samples so that they may be retained for future research and confidentiality is ensured.
Serum 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 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.
Skin test and DTH assay – performed at various locations on subjects' backs	Pain, bruising at the injection site, and rarely infection Potential for allergic reaction including anaphylaxis Discovery of previously unknown conditions	Experienced personnel will perform the injections using approved techniques. Pressure and dressings will be used to minimize pain, bruising and infection. 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. This research is being conducted at an experienced clinical research center. Reporting and monitoring mechanisms are in AEs, SAEs and unanticipated problems. 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. Subjects will only be identified by study numbers on all research documents. Investigators will provide certification of completion of human subject protection training course.

Procedure	Risks	Measures to Minimize Risks
		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 is closed and discontinued with the Food and Drug Administration (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

Subjects will receive up to 5 planned vaccine doses unless they withdraw from the study or develop a dose limiting event as stated above, at which time they will discontinue the injections and be removed from the study. There will be no dose modifications for toxicity. Special attention will be given to toxicities mediated by autoimmune mechanisms, such as colitis, thyroiditis or systemic lupus erythematosus (SLE), as well as to injection-site local reactions or allergic reactions. 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 or viewed in **APPENDIX A**. All appropriate treatment areas have access to a copy of the CTCAE Version 4.0.

Any subject may voluntarily revoke consent and withdraw from the study at any time. A subject may be terminated early 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, (iv) for toxicity as determined by the DLT criteria, or (v) for any reason that the investigator feels it is not in the subject's best interest to continue.

- b. Benefits:** As this is the first time Mimotope P10s-PADRE will be administered to humans, there are no clearly defined benefits to subjects of this study. However, this vaccine may potentiate an immune response which could improve median progression-free survival and overall survival of cancer patients.
- c. Injury:** For DOD-funded research, participants can receive medical care at an Army hospital or clinic free of charge. If participants pay out-of-pocket for medical care elsewhere for injuries caused by this research study, they should contact the Principal Investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221

9. Specimen Handling

Samples will be stored in the UAMS Tissue Procurement Facility, located in room B.018 of the hospital. Cryotubes that contain specimens will be labeled with barcodes. The coded number is linked to patient identification and time of blood draw. Specimens will be stored frozen at -80°C

or lower. Samples will be kept for maximum of 5 years. At the end of year 5, any unused specimen will be mixed with bleach and disposed in a sink. All specimen containers (tubes and containers) will be disposed into biohazard red plastic bags according to UAMS Occupational Health & Safety's policy. Red bag waste will be picked up by occupational Health & Safety for final disposal.

10. Study Calendar

TEST/EVENT		WEEK ⁷																			
		Prestudy ¹	48 hrs later	48 hours prior to dosing	1	2	3	4	5	48 hrs later	6	7	8	9	48 hrs later	19	20	21	48 hrs later	24	Follow up ¹⁰
Informed Consent		x																			
Vaccination with Mimotope P10s-PADRE/ MONTANIDE™ ISA 51 VG					X ⁹	X ⁹	X ⁹					X ⁹				X ⁹					
Adverse Events					x	x	x					x		x		x		x		x	
Concomitant medications ⁸		x			x	x	x					x		x		x		x		x	
History/Physical Exam/		x			x	x	x					x		x		x		x		x	
CBC with Differential		x				x	x	x	x		x	x	x	x		x	x	x		x	
SGOT		x					x					x		x		x		x		x	
Bilirubin		x					x					x		x		x		x		x	
Alkaline Phosphatase		x					x					x		x		x		x		x	
LDH (lactate dehydrogenase)		x					x					x		x		x		x		x	
GGT (gamma-glutamyl transferase test)		x					x					x		x		x		x		x	
Creatinine		x					x					x		x		x		x		x	
Calcium		x					x					x		x		x		x		x	
Albumin		x					x					x		x		x		x		x	
Amylase		x					x					x		x		x		x		x	
TSH (thyroid stimulating hormone)		x					x					x		x		x		x		x	
T ₄		x					x					x		x		x		x		x	
Anti-nuclear Antibody (ANA)		x					x					x		x		x		x		x	
PT/PTT		x					x					x		x		x		x		x	
Serum Pregnancy Test ²		x		x																	
Study Lab ³		x ⁴				x	x	x				x		x		x		x ⁴		x ⁴	x
DTH Skin Test ^{5,6}		x ⁵							x ⁶					x ⁶				x ⁶			
Read DTH Skin Test ^{5,6}			x ⁵							x ⁶					x ⁶				x ⁶		

¹Prestudy is to be completed within 14 days before beginning injections.

²For women of child bearing potential, a serum pregnancy test must be done at prestudy and within 48 hours prior to dosing. One test may suffice for both

³ Study lab samples. Up to 40 mLs of blood will be collected in purple tubes for circulating tumor cell evaluation, TACA (tumor associated carbohydrate antigens) assessment, and immune effector assays. Specimens will be picked up by Dr. Kieber-Emmons or a member of his research staff. Call 526-5930 for pick-up.

⁴An additional 6.0 ml of blood will be taken during these study labs (Pre-Study, Week 21, and Week 24) to assay circulating tumor cells (CTCs).

⁵The DTH skin test at prestudy will test the subjects' immunocompetency. Control antigens, Trichophyton Antigen and Candida antigen, will be administered id and the resulting induration will be ready at 48 - 72 hours post injection.

⁶ The DTH skin tests on weeks 5, 9 and 21 will test the DTH response to Mimotope P10s-PADRE. Trichophyton Antigen and Candida antigen will be administered id alongside Mimotope P10s-PADRE as control antigens to assess immunocompetency. DTH skin test indurations will be read at 48 – 72 hours post injection.

⁷ Visits must occur at set time points mentioned in the study calendar (+/- 3 days) with the exception of reading the skin test with must occur 48-72 hours post administration.

⁸ Concomitant medications will be collected from registration to end of treatment.

⁹ Post vaccine administration, the following vital signs will be monitored every 15 minutes (+/- 5 minutes) for one hour: blood pressure, temperature, and pulse.

¹⁰ Follow up visits shall begin with the next scheduled standard of care appointment and then shall occur every six months for five years and then yearly until death.

11. Criteria for Evaluation

Primary Objective – Safety:

- **Determination of DLT:** See Section 8, “Risks and Toxicities to be Monitored”. Any event that meets the dose limiting criteria will be determined a dose limiting toxicity.

Secondary Objectives – Immune Response:

- **Immunological Evaluation:** Serum will be collected at the Study Lab time points indicated in the study calendar in Section 10. Samples will be collected and stored by the UAMS Cancer Institute Tissue Bank following established Tissue Bank SOPs. IgM and IgG titers to TACAs will be evaluated by ELISA on TACA adhered to plates and FACS analysis of TACA adhered to liposomes. A positive TACA-directed immune response will be defined as an anti-TACA serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a ≥ 4 -fold increase over baseline titer > 0 (1, 11). Subjects will be judged to have had an adequate immune response if they have a positive TACA-directed immune response at any one of the first five designated time points following vaccine administration (Weeks 2, 3, 4, 7 and 9). The value of a booster immunization will be determined by anti-TACA IgM and IgG titers obtained from study labs collected on week 21.
- **Determination of DTH Responses:** To evaluate DTH responses, study subjects will be skin-tested according to the time points in the study calendar against the Mimotope P10s-PADRE, Trichophyton antigen and Candida antigen. The latter 2 antigens serve as control antigens. DTH responses to the control antigens will be assessed at pre-study to determine immunocompetency of study subjects. All antigens will be administered id at separate locations on the subject’s back. Induration will be measured using calipers or a ruler and reported in mm across one diameter at 48 – 72 hours post injection. An induration > 5 mm across at least one diameter of 1 or more DTH antigens will be considered positive.

Exploratory Objective:

- **Determination of presence of CTCs:** To evaluate the presence of CTC’s pre- and post-mimotope administration, 1 EDTA 6.0 mL Vacutainer test tube for blood to evaluate circulating tumor cell markers. Multiparameter flow cytometry analysis will be used for reducing false positives from background noise. Multiple reagents grouped in conjugates with different fluorochromes including at least 1 for negative selection (CD45, may be also CD48). The positive identification will be on the basis of EpCAM and/or folate-Alexa488. Assay sensitivity will be determined beyond the scope of this protocol by spiking normal- non cancer patient- deidentified donor blood obtained from UAMS blood bank with known numbers of tumor cells (e.g. MDA 231) to determine the detection limit.

12. Statistical Considerations

- a. **Sample Size, Study Duration and General Considerations:** Because each subject will receive multiple vaccine injections at a constant dose over an extended period of time, the second and third subject of *the same* dose cohort will be enrolled, as available, before the first subject has finished all scheduled injections. However, if two or more subjects are enrolled on the same day, then their injection schedules will be staggered at least one week apart. The decision to escalate or de-escalate the dose, expand the cohort, or terminate the study will be based on assessment for DLTs, which will require 9 weeks per subject. Upon evaluation of all subjects in a cohort (3-6/dose), dose escalation will proceed according to the schedule shown in **Tables 1, 2, and 3**. Except for the fact that they allow for possible de-escalation from the initial dose of 300 µg, **Tables 1, 2, and 3** constitute the toxicity-based “traditional” design of Storer (43). The time to assess a cohort of 3 for DLTs is anticipated to be 13 weeks based on an accrual rate of 2 eligible Stage IV subjects per month. If no DLTs occur in the initial and final cohorts, then this study will require nine subjects. Subjects who withdraw from the dose-escalation study without a DLT will be replaced. Subjects who withdraw from the dose-de-escalation study without a DLT will be replaced. Subjects who withdraw with a DLT will be considered evaluable for MTD determination. Given the recruitment and immunization/evaluation schedule, we expect this study to be completed in a minimum of 12 and a maximum of 24 months.

It should be noted that the primary objective of this study is to ensure the safety of the mimotope vaccine. Because of sample size, these studies only provide a qualitative assessment of vaccine immunogenicity. However, our immunization schedule should favor the generation of antibodies (1, 11, 42). A positive TACA-directed immune response will be defined as an anti-TACA serum antibody titer of 1:40 for a baseline pre-vaccination titer of 0 or a ≥ 4 -fold increase for a baseline titer > 0 (1, 11). A subject will be judged to have had an adequate immune response if they have a positive TACA-directed immune response at any one of the first five indicated time points following vaccine administration on the study calendar (Section 10, Study Lab Weeks 2, 3, 4, 7 and 9). An additional immune-response endpoint will be the DTH response to Mimotope P10s-PADRE. The components of the DTH response, namely, the induration in mm across two diameters, will be tabulated by antigen (the mimotope vs. the two DTH controls) for each subject at the indicated time points on the study calendar. An induration of >5 mm in at least one diameter will be considered a positive DTH response.

- b. **Data Analysis Plan:** Toxicity will be graded according to the NCI CTCAE Version 4.0. All toxicities observed in a study subject will be enumerated and reported in terms of type (organ affected or laboratory determination such as absolute neutrophil count), severity (by NCI Common Toxicity Criteria (CTC) and nadir or maximum values for the laboratory measures), time of onset (i.e. dose number), duration and reversibility or outcome. A toxicity meeting the dose limiting criteria as stated above will be scored as a DLT. The number and proportion of subjects in each dose cohort who suffer a DLT will be reported. The number of DLTs observed will be reported by dose cohort, both overall and also broken down by CTCAE category.

To meet the secondary objective of determining the humoral response against TACAs, the anti-TACA serum titers measured from subjects’ blood samples will be collected at pre-study and on weeks 2, 3, 4, 7, and 9. IgM and IgG titers to TACAs will be evaluated by ELISA and

FACS analysis. Titer will be defined as the highest serum dilution yielding an $OD_{405} \geq 0.15$, in accordance with previous studies (1) or an MFI two standard deviations higher than background. A positive TACA-directed immune response will be defined as an anti-TACA serum antibody titer of 1:40 post-vaccination when the baseline pre-vaccination titer was 0, or a ≥ 4 -fold increase in titer post-vaccination when the baseline titer was greater than 0. The number and proportion of positive TACA-directed immune responses at each time point will be reported, both overall and by dose cohort. The number and proportion of subjects with at least one positive TACA-directed immune response will also be reported, both overall and by dose cohort. Medians and quartiles of titer will also be reported at each time point for any dose cohort of size 6.

To meet the secondary objective of analyzing the DTH response, we will measure the induration in mm across two diameters at the injection sites of the mimotope and control antigens. This will be done for each study subject at all indicated time points on the study calendar. An induration of >5 mm across at least one diameter will be considered a positive DTH response to that antigen at that time point. The number of subjects who show at least one positive DTH response to mimotope during the study will be reported as the number and proportion in each dose cohort. The number of positive DTH responses to mimotope at each time point will be reported as the number and proportion in each dose cohort. Positive DTH responses to the control antigens will be summarized the same way. Additionally, the two induration measurements per injection site, as well as their product (in mm^2), will be graphed as subject profiles over time, and summarized by antigen, dose group, and time as means and standard deviations.

To meet the secondary objective of determining sustainability of the immune response, the subject's TACA-directed immune titer at Week 19 will be compared to her TACA-directed immune titer at Week 9. The number and percentage of subjects with a sustained immune response will be reported in the aggregate and by dose cohort if more than one dose cohort is enrolled. In addition, the ratio of week-19 and week-9 titers will be plotted as dot plots and summarized as the mean, median and range.

To meet the secondary objective of determining the immune response to the week-19 booster immunization, the subject's TACA-directed immune titer at Week 21 will be expressed as a ratio relative to her TACA-directed immune titer at Week 19. This ratio will be summarized as the mean, median and range, and plotted as dot plots. Subjects in whom the week-21 titer is more than 2-fold higher than the week-19 titer will be considered as having shown a boosted response to the booster immunization. The number and percentage of subjects showing a boosted response will be reported in the aggregate and by dose cohort if more than one cohort is enrolled.

To meet the exploratory objective of determining the vaccine's effect on the presence of CTCs at datable levels, the subject will be classified as Yes versus No for CTCs Detected, both at baseline and at Week 21, based on the outcomes of the assay described in paragraph d. of Section 9 above. The number and proportion of subjects with CTCs detected at each time will be reported in the form of a 2x2 contingency table for paired data, in order to facilitate the visualization of how many subjects change from Yes to No after completing the vaccination schedule.

Any deviations from the above analysis plan will be reported to the U.S. Army Medical

Research and Material Command Human Subjects Research Review Board (HSRRB), the Food and Drug Administration (FDA), and the UAMS Institutional Review Board

- c. **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.

13. Registration Guidelines

Screening logs will be maintained by the study nurses. Subject registration will occur after the IRB-approved consent is signed and eligibility has been confirmed. The subjects will be registered in the CCTO and assigned a study number by the CRA. The study number will be used for identification of the research subject during the study.

14. Data Submission Schedule

Data must be submitted according to protocol requirements for ALL subjects registered, whether or not assigned treatment is administered. This includes subjects deemed to be ineligible to participate in the study or for whom documentation is inadequate to determine eligibility. Data obtained during the study will be collected at each subject visit and entered into the protocol database. Subjects will be registered in C3PR, a cancer Biomedical Informatics Grid (*caBIG*®, NCI) application. Data will be entered into OpenClinica through electronic web-based case report forms (CRFs) which replicate the paper CRFs attached to this protocol. OpenClinica is a secure open source system for electronic data capture and clinical data management.

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 is closed and discontinued with the Food and Drug Administration (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.

15. Ethical and Regulatory Considerations

The following must be observed to comply with FDA regulations for the conduct and monitoring of clinical investigations. The following also represents sound research practice:

All study personnel must have completed training in good clinical practice (GCP) and protection of human subjects.

- a. **Recruitment and Informed Consent:** Research subjects will be recruited from the breast cancer clinics (Medical Oncology and Ladies Oncology Clinics) at the Winthrop P. Rockefeller Cancer Institute on the UAMS campus. The research subjects will be identified by the research nurse and/or physician. Prior to any research activities, the research 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 will follow with a detailed review of the informed consent document. The research subject will be encouraged to have family or friends participate in any or all of the process. The research 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. In general, registration and prestudy work will begin the next business day, allowing additional time for the research subject to reflect and request additional 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 research subject, and additional copies will be sent to the medical records department for distribution to the research pharmacy. The original informed consent will be filed with the subject file in CCTO. The consent process will occur in a private exam room or in the private office of the research nurse. 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.

- b. Institutional Review:** This study will be approved by the UAMS IRB 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 IRB and PRMC is required before the clinical trial can be activated. An active IND is also required before the clinical trial can commence.

Approval will also be obtained by the U.S. Army Medical Research and Materiel Command, Human Subjects Research Review Board (HSRRB) prior to implementation. A copy of all continuing review and final reports will be submitted to the HSRRB.

- c. Investigational Agent Accountability:** For each investigational drug, drug disposition (drug receipt, dispensing, transfer or return) will be maintained on the UAMS Investigational Agent Accountability Record. Drug supplies will be kept in a secure, limited access storage area under the recommended storage conditions in the research pharmacy in the Winthrop P. Rockefeller Cancer Institute 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.

16. Adverse Events

- 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) ICH E6 1.50 Partially IRB Handbook policy 10.3]

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

Any adverse experience that meets reporting guidelines for SAEs must be reported to the CRA for the study in the CCTO within 24 hours of knowledge of the event. The CRA will follow AE reporting plans per institutional policies and applicable regulations. All qualified AEs will be reported to the Investigators, U. S. Army Medical Research and Materiel Command Human Subjects Research Review Board (HSRRB), FDA, IRB, Medical Monitor, and the UAMS Research Support Center according to this plan.

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 study medications(s) (i.e., "probable", "possible" or "unrelated").

Unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and all subject deaths should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the U.S. Army Medical Research and Materiel Command, Human Subjects Research Review Board (HSRRB). A complete written report should follow the initial notification. In addition to the methods above, the complete report can be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-QH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

17. Monitoring

- a. Medical Monitor:** The Medical Monitor, Principal Investigator and study staff will meet to review safety data after each cohort has been enrolled and prior to enrollment of the next cohort. Any death will trigger an additional urgent meeting.
- b. Data Monitor:** UAMS is the IND Sponsor. One (or more) Data Monitor(s) will be appointed by the monitoring division of the UAMS Research Support Center (RSC) to assure that the rights and well-being of human subjects are protected, that the data are accurate, complete and verifiable from source documents and that the trial is conducted in compliance with

currently approved protocol/amendments, with GCP, and with the applicable regulatory requirements set forth in *21 CFR 312*.

The Data Monitor(s) will be familiar with the investigational products, the protocol, the informed consent form, any other information provided to the subjects, SOPs, GCP and applicable regulatory requirements.

Data Monitor(s) will have access to research subjects' medical records and other study-related records. The investigator agrees to cooperate with the study coordinator and Medical Monitor to ensure that any problems detected in the course of these monitoring visits are resolved. Personal contact between the Data Monitor, Medical Monitor, study staff and the investigator will be maintained throughout the clinical trial to assure that the investigator is fulfilling his/her obligations and that the facilities used in the clinical trial remain acceptable.

- 1) **Pre-investigation Site Visit:** A pre-investigation site visit will be performed by the Data Monitor in order to inspect the facility where the study is going to be conducted, and to assure that the investigator and his/her staff understand the protocol and agree to comply with the current regulations for clinical trial conduct in human subjects (*21 CFR 312, 21 CFR 50, 21 CFR 56, 21 CFR 11, 21 CFR 21*). The Data Monitor will document the IRB approval and generate a special report that will allow subject enrollment on the trial to begin.
- 2) **Periodic Site Visits:** The first visit of the Data Monitor will occur as soon as possible after the first research subject has enrolled in the study. Subsequent monitoring visits will take place approximately quarterly based on enrollment. The Data Monitor will review the CRFs, source data/documents and other trial-related records for accuracy, consistency and completeness. Enrollment of research subjects after meeting eligibility criteria and signing a consent form will be documented. Missing visits, withdrawals and subject recruitment rate will be monitored.
- 3) **Investigational Products:** The Data Monitor will verify that the storage conditions are appropriate and that the investigational drug is being dispensed to eligible subjects according to the study protocol. The Data Monitor will verify that there are accurate records of the receipt, use and return of the investigational product.
- 4) **Monitoring Report:** After each monitoring visit a separate monitoring report will be generated and submitted to the investigator, U. S. Army Medical Research and Material Command Human Subjects Research Review Board, research staff and the Medical Monitor. This report will include significant findings related to deficiencies and deviations from the protocol, SOPs, GCP and the applicable regulatory requirements and actions taken to prevent recurrence of the detected deviations. The report will make recommendations for actions to be taken to secure compliance. The study team, which includes the PI, subinvestigators, biostatistician, Medical Monitor and the study coordinator, will meet after each cohort completes the protocol to review AEs and review monitoring reports in order to make adjustments necessary to protect the research subjects and the integrity of the trial.
- 5) **Research Subject Safety and Stopping Rules:** If 3 SAEs occur with attribution to the study drug, the trial will be suspended until further review is completed by the

Medical Monitor, PI, sponsor and FDA. This will be accomplished by the study team, the PI, Co-PI, biostatistician, Medical Monitor and CRA, either at the regular meeting or a special meeting called by the Medical Monitor or the PI because of the SAEs. The study will be stopped upon Death not related to cancer or two Grade 4 Toxicities related to the study article.

- 6) Audits:** An audit by UAMS will be scheduled after the completion of the first cohort. The audit will follow UAMS' standard auditing procedure.

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