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A PHASE I TRIAL OF THE SAFETY AND IMMUNOGENICITY OF A DNA PLASMID BASED VACCINE (WOKVAC) ENCODING EPITOPES DERIVED FROM THREE BREAST CANCER ANTIGENS (IGFBP-2, HER2, AND IGF-1R) IN PATIENTS WITH BREAST CANCER

Consortium Name: University of Wisconsin Chemoprevention Consortium
Name of Consortium Principal Investigator: Howard Bailey, MD
University of Wisconsin Carbone Cancer Center
600 Highland Ave.
CSC K4/654
Madison, WI 53792-6164
Tel: 608-263-8624
hhb@medicine.wisc.edu

Organization Name: University of Washington
Protocol Principal Investigator: Mary L. (Nora) Disis, MD
Oncology
850 Republican Street
Box 358050
Seattle, WA 98195-8050
Tel: 206-616-1823
Fax: 206-685-3128
ndisis@medicine.washington.edu

Site: Providence Cancer Institute
Investigator: Sasha Stanton, MD, PhD
Cancer Immunoprevention Laboratory
4805 NE Glisan St.
Suite 2N103
Portland, OR 97213
Tel: 503-215-2597

Sasha.Stanton@providence.org

Site: University of Washington
Investigator: Andrew Coveler, MD
Oncology
825 Eastlake Ave. E, G4-830
Seattle, WA 98195-8050
Tel: 206-288-7509
Fax: 206-288-2042
acoveler@u.washington.edu

Site: University of Washington
Investigator: John Liao, MD
Gynecology
850 Republican Street
Box 358050
Seattle, WA 98195-8050
Tel: 206-543-3669
Fax: 206-685-3128
johnliao@uw.edu

Site: University of Washington
Investigator: William Gwin, MD
Oncology
850 Republican Street
Box 358050
Seattle, WA 98195-8050
Tel: 206-221-5956
Fax: 206-685-3128
wrgwin@medicine.washington.edu

Site University of Wisconsin
Lead Investigator: Kari Wisinski, MD
Hematology/Oncology
Wisconsin Institute Medical Research
1111 Highland Avenue
Madison, WI 53705
Tel: 608-262-2876
Fax: 608-265-6905
kbwisinski@medicine.wisc.edu

Site Investigator University of Wisconsin
Amye Tevaarwerk, MD
Hematology/Oncology
Wisconsin Institute Medical Research
1111 Highland Avenue
Madison, WI 53705
Tel: 608-262-2837
Fax: 608-265-6905
At4@medicine.wisc.edu

Site Investigator University of Wisconsin
Mark Burkard, MD
Hematology/Oncology
Wisconsin Institute Medical Research
1111 Highland Avenue
Madison, WI 53705
Tel: 608-262-2876
Fax: 608-265-6905
meburkard@medicine.wisc.edu

Site Investigator University of Wisconsin

Ruth O'Regan, MD
Hematology/Oncology
Clinical Science Center
600 Highland Avenue
Madison, WI 53792-0001
Tel: 608-265-9701
Fax: 608-265-8133
roregan@medicine.wisc.edu

Organization Name:	
Consortium Statistician:	KyungMann Kim, PhD Department of Biostatistics & Medical Informatics University of Wisconsin Madison 610 Walnut St. WARF 256J Madison, WI 53726-2336 Tel: 608-265-6380 Fax: 608-265-9768 kyungmann.kim@ wisc.edu
IND Sponsor:	University of Washington Mary L. (Nora) Disis, MD
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SCHEMA

A Phase I Trial of the Safety and Immunogenicity of a DNA Plasmid Based Vaccine (WOKVAC) Encoding Epitopes Derived From Three Breast Cancer Antigens (IGFBP-2, HER2, and IGF-1R) in Patients with Breast Cancer

Eligibility Assessment:
Patients with non-metastatic, node positive, HER2 negative breast cancer that is in remission and defined as no evidence of disease (NED). Patients must have a good performance status, be at least 28 days from last cytotoxic chemotherapy and/or radiotherapy and 28 days from any use of systemic steroids.

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Baseline Visit:
Physical examination, baseline symptom assessments, ECHO/MUGA, and Clinical Labs

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Sequential assignment to one of three dose arms:
Arm 1: WOKVAC (150 mcg) with Sargramostim (rhuGM-CSF) (100 mcg)
Arm 2: WOKVAC (300 mcg) with Sargramostim (rhuGM-CSF) (100 mcg)
Arm 3: WOKVAC (600 mcg) with Sargramostim (rhuGM-CSF) (100 mcg)

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First Vaccination Visit:
Clinical labs, immune monitoring labs, research blood for immunologic monitoring, Tetanus diphtheria (Td) vaccination (if applicable), and intradermal (id) vaccination with assigned dose

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Monthly Vaccination (vaccine 2 and 3):
Physical examination, symptom/toxicity assessments, clinical labs, immune monitoring labs, ECHO/MUGA (prior to Vaccine 2), and
intradermal (i.d.) vaccination with assigned dose.

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1 and 6 Months after the Final Vaccination:
28(+7) days and 168 (±14) days following the last vaccine
Physical examination, symptom/toxicity assessment, clinical labs, immune monitoring labs, research blood for immunologic monitoring, and ECHO/MUGA (at 1 month post 3rd vaccine only).

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Long-Term Follow-Up Period
Annually from enrollment to complete 5 years
Obtain clinical notes, laboratory values and imaging reports from patient's primary oncologist or primary care physician

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Primary Endpoint: Safety as assessed by NCI CTCAE v. 4.0.
Secondary Endpoints:
Immunogenicity of WOKVAC (Th1 and Th2 immunity); autoantibody immunity to WOKVAC; persistent T cell memory; modulation of regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSC) with vaccination

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1. OBJECTIVES

1.1 Primary Objectives

1. To assess the safety of 3 escalating doses of a deoxyribonucleic acid (DNA) plasmid based vaccine encoding three breast cancer antigens (IGFBP-2, HER2, and IGF-1R) in patients with breast cancer.

1.2 Secondary Objectives

1. To determine the immunogenicity of WOKVAC T helper cells (Th) polyepitope plasmid based vaccine in patients with breast cancer at 3 escalating doses.
2. To determine whether a WOKVAC Th polyepitope plasmid based vaccine elicits a persistent memory T cell response.
3. To evaluate whether WOKVAC vaccination modulates T regulatory cells (Treg) and myeloid derived suppressor cells (MDSC).
4. To evaluate antibody immunity to further define Th2 immune response.
5. To determine a recommended phase 2 WOKVAC dose for further breast cancer prevention studies.
6. To assess the long-term effects of 3 escalating doses of a deoxyribonucleic acid (DNA) plasmid based vaccine encoding three breast cancer antigens (IGFBP-2, HER2, and IGF-1R) in patients with breast cancer, for 5 years from enrollment to satisfy the evaluation requirements for the Food and Drug Administration (FDA)

2. BACKGROUND

2.1 Breast Cancer

According to the American Cancer Society, breast cancer is the most common cancer of women in the United States, the second most common cause of cancer death in women (after lung cancer). This study describes a Phase I trial of pUMVC3-IGFBP2-HER2-IGF1R(WOKVAC), a tri-antigen vaccine targeting insulin like growth factor binding protein 2 (IGFBP-2), human epidermal growth factor receptor 2 (HER2), and insulin like growth factor receptor-1 (IGF-1R) in patients with non-metastatic, node positive, HER2 negative breast cancer. These patients have been rendered free of disease and would simulate women who had not yet developed breast cancer. The evaluation of patients free of disease is necessary when contemplating a prophylactic vaccine. Residual disease can act as an antigen reservoir, thus, influencing the development of T cell memory via chronic antigenic stimulation. Moreover, residual disease may influence the maintenance of T helper cells (Th) to a more suppressive phenotype by virtue of existing tumor environmental cells secreting pro-angiogenic and anti-inflammatory cytokines. Immunization of patients who have no evidence of disease will allow for a more accurate assessment of the development of memory, persistence of immunity, and role of pre-existing regulatory cells in influencing vaccine priming.

2.2 WOKVAC

IGFBP-2, HER2, and IGF-1R are all cancer related proteins that are overexpressed in tumors of patients with breast cancer. These three proteins when used as immunogens provide broad antigenic coverage to all molecular breast cancer subtypes. The three antigens are also up-regulated in pre-invasive and high risk breast lesions and are associated with progression to invasive cancer. Therefore, generating protective immunity against these antigens could have the result of preventing cancer development in high risk patients.

This is a dose finding study. Patients will be assigned sequentially to receive three monthly vaccines of one of three doses: Arm 1 (150 mcg), Arm 2 (300 mcg), and Arm 3 (600 mcg).Our hypothesis is that aberrant expression of these proteins is what would trigger the immune response and studies from our group

demonstrate that up-regulation of a tumor associated protein, e.g. HER2, is an independent predictor of immunogenicity.[1]

The rationale for the minimally active dose was based on our previous dose escalation clinical trial using a HER-2/neu intracellular domain (ICD) plasmid-based vaccine (pNGVL3-hICD). The average HER-2/neu ICD immune responses in both 100 mcg and 500 mcg arms were greater than baseline responses to 10 mcg arm without any significant differences in toxicity. Therefore, we selected 100 mcg as the minimum active biologic dose. Since we did not observe any increased toxicities with the 500mcg dose of pNGVL3-hICD, we increased the concentration of the highest dose of WOKVAC to 600 mcg (Arm3). Several clinical trials have immunized patients targeting tumor associated self-proteins using more than over 1 g plasmid DNA with no significant toxicities observed. However the induction of tolerance against self-antigens has been seen with doses greater than 1g.[2, 3]

We have experience immunizing with two of the three components of WOKVAC in our clinical plasmid; HER2 and IGFBP-2. We have completed a 66 patient study of pUMVC3-HER2 ICD. Immunizing advanced stage breast cancer patients against pUMVC3-HER2 ICD did not result in significant toxicity. A total of 990 adverse events (AEs) were recorded. Ninety-nine percent of collected AEs were grades 1 and 2, but only 52% of those recorded were possibly, probably or definitely related to vaccination. The most common AEs were reaction at the injection site, and flu-like syndrome. All reactions at the injection site were attributable to the vaccine; however, only 82% and 78% of the fatigue and flu-like syndrome, respectively, were attributable to the vaccine. pUMVC3-HER2 ICD also did not induce clinically significant autoimmunity. We evaluated for the development of serologic autoimmunity, only four patients exhibited decreased complement C3, 2 patients had a positive antinuclear antibody (ANA) defined by titers of ≥ 1:160 by immunofluorescence (IF) and no patient developed clinical evidence of autoimmune disease. Lymphocytes from study patients were evaluated for presence of vector pre- and 1 month post-vaccination. If persistent DNA was detected at 1 month, serial assessment for vector presence was continued at 3, 6, and 12 months after the last vaccine, or until DNA was no longer detected. There was no detection of DNA plasmid in lymphocytes from patients immunized. No significant vaccine induced cardiac toxicity was observed and all cardiac toxicities observed on the study were either Grade 1 or Grade 2. In addition, those patients receiving concurrent trastuzumab during vaccination (n=31) across all arms, the median LVEF pre- and post-vaccination was 61% and 60%, respectively.

A Phase I study of pUMVC3-IGFBP-2 is currently underway in 25 patients with ovarian cancer. 208 AEs have been collected to date (representative of 86 vaccine administrations in 25 patients) and 96% of the AEs were grade 1 or grade 2. Similar to the HER2 ICD vaccine, injection site reaction and fatigue, each comprising 12% of all AEs, were the most common complaints. All injection site reactions and 79% of reports of fatigue were considered related to vaccination. Mild changes in immune serologies, including one patient with a transient decreased complement C3 and one patient with a positive ANA defined by titers of ≥ 1:160 by IF, have occurred with no associated symptoms. There was no significant vaccine induced cardiac toxicity in this study. One patient reported Grade 1 palpitations that lasted for 2 days and was recorded as possibly related.

Studies performed to date evaluating immunization to IGF-1R in a genetically engineered model of human breast cancer in mice (TgMMTV-neu) show no evidence of histologic immunity or changes in blood chemistries or blood counts. These data are similar to what we have generated previously in the preclinical development of HER2 and IGFBP-2 as vaccine candidates.

Our pre-clinical studies of WOKVAC in mice have not demonstrated clinical signs of toxicity. Specifically, complete blood counts, electrolytes, glucose and liver function test remained within normal range and there was no evidence of autoimmunity by pathologic survey of organs. Regardless, all patients on study will be evaluated for acute autoimmune toxicity prior to each vaccination and at 1 and 6 months after the last

vaccine with (1) CBC with differential, serum electrolytes, creatinine, BUN, liver function tests to evaluate for liver, renal and marrow dysfunction, (2) ANA, anti-dsDNA, C3 serologies, and TSH (3) physical examination and symptom/toxicity assessment. No cardiac toxicities have been found in either the mice vaccinated with WOKVAC or the previous vaccine trials that enrolled HER2-positive (HER2+) patients that were treated with a monoclonal antibody (i.e. trastuzumab). However, we will be evaluating the effect of the WOKVAC vaccine on the heart by measuring left ventricular ejection fraction (LVEF) with a MUGA or Echocardiogram (ECHO) pre vaccine, one month after the first vaccine, and 1 month after completion of the vaccination series. Long-term monitoring will include review of patient clinical notes, lab values, and imaging reports obtained annually from the patient's primary oncologist in long term follow up.

A potential toxicity of the WOKVAC is hyperglycemia. Additionally, there is the potential for worsening the glycemic control of subjects with pre-existing but controlled diabetes. Thus, patients with a history of diabetes mellitus will be excluded from the study. In the mouse studies, we did not see any statistical differences in glucose levels between the mice treated with WOKVAC and empty vector suggesting there was no insulin receptor expressing cell cytotoxicity. Furthermore, IGFBP-2 is also in the insulin growth receptor family and in our previous clinical trials we did not see any changes in glucose levels with this vaccine.

We anticipate WOKVAC will demonstrate a safety profile consistent with what has been observed with the individual component vaccines.

2.3 Rationale

Our long-term goal is to develop WOKVAC as a prophylactic vaccine to be used to prevent the development of invasive breast cancer in high-risk women. The aim of the current study is to assess the safety and immunogenicity of WOKVAC in an immune competent population of breast cancer patients.

Vaccination targeting immunogenic proteins expressed by pre-invasive or proliferative breast lesions offers a unique approach to breast cancer prevention. Vaccines can elicit memory T-cells that remain in lymph nodes until exposed to the target antigen. After stimulation, T-cells migrate to the site of antigen expressing lesions regardless of location and will proliferate and destroy those lesions. If successful, a prophylactic breast cancer vaccine could eradicate high risk and pre-invasive breast tumors before progression to invasive cancer. IGFBP-2, HER2, and IGF-1R are overexpressed in multiple types of breast cancer and are associated with a poorer prognosis.[4-7] Likewise, these proteins are immunogenic and elicit both humoral and cellular immunity in breast cancer patients.[8, 9] Moreover, all three proteins are up-regulated in high risk and pre-invasive breast lesions and expression of each is associated with increased risk of developing invasive breast cancer. Indeed, these three proteins provide broad antigen coverage to both estrogen receptor (ER)+ and ER- pre-invasive high-risk breast disease.

2.3.1. Antigens in pre-invasive cancer and high-risk lesions Development of a prophylactic vaccine for the prevention of breast cancer is possible due to the inherent immunogenicity of breast cancer as well as the identification of defined high-risk populations which would allow testing of the approach. Vaccine development has been hampered, however, by a lack of characterized antigens that are prevalent in high-risk lesions and pre-invasive disease. Few immunogenic proteins have been identified for pre-invasive or high-risk breast lesions. HER2, one of the most commonly studied breast cancer antigens, has been shown to be expressed in ductal carcinoma in situ (DCIS) and expression of the protein has been associated with a higher risk of the development of subsequent invasive disease.[10, 11] In addition, HER2+ DCIS is more likely to be ER+.[12] Investigators have immunized patients with HER2+ DCIS against HER2, prior to definitive resection, and demonstrated some resolution of lesions at the time of surgery.[13] Remaining lesions, however, were antigen negative either due to the generated immune response eliminating antigen expressing cells or immunity having no effect on cells which were HER2 negative. To mitigate the limited

efficacy of single antigen vaccination we focused on both extending antigenic coverage and formulating a vaccine to stimulate $CD4^+$ T-cells.[14]

The insulin like growth factor (IGF) family of proteins has been shown to play a role in the progression of normal mammary tissue to preneoplasia.[15] We have previously published that IGFBP-2 is immunogenic in breast cancer patients and over 40% of patients have an increased level of expression of IGFBP-2 in their tumors.[6, 9] IGFBP-2 has also been shown to have increased expression in atypical hyperplasia and DCIS as compared to normal glandular cells.[16] Increased expression of another IGF family member, IGF-1R, in normal breast tissue has been linked with a greater risk of developing breast cancer.[17, 18] High levels of cytoplasmic IGF-1R in normal ductal lobular cells was associated with an overall risk of developing breast cancer of 15.9 (95% CI 3.6-69.8) as compared with women who had little or no expression of the protein in their breast tissue. Moreover, increased IGF-1R expression is correlated with non-invasive high risk breast lesions that are hormonally driven such as atypical ductal hyperplasia.[19] Similar to IGFBP-2 we have recently demonstrated that breast cancer patients have pre-existent immunity to IGF-1R.[20] We reasoned a vaccine targeting both HER2 and IGF family members may give broad phenotypic coverage of estrogen receptor positive (ER+) and estrogen receptor negative (ER-) pre-invasive and high risk lesions.

WOKVAC has been designed to include extended sequences of the immunizing antigens that are predominantly associated with eliciting Type I immune responses in population based studies. Vaccines designed to stimulate $CD4^+$ T-cells or T helper cells (Th) may also function to enhance antigenic coverage. Type I cytokine secretion by Th, particularly IFN-gamma (g), upregulates MHC class I expression on tumor cells, as well as antigen presenting cells (APC), facilitating tumor recognition by $CD8^+$ T-cells.[21] Tumor localized Th, secreting interferon-gamma (IFN-g)(Th1), will enable cross-presentation of tumor associated antigens by APC or dendritic cells to both $CD4^+$ and $CD8^+$ T-cells.[22] Clinical trials, in advanced stage patients, have shown that increased levels of Th1 potentially modulate the immune suppressive tumor environment as evidenced by an inverse relationship between the magnitude of HER2 specific vaccine induced Th1 and serum TGF-β) levels after immunization in advanced stage breast cancer patients.[23] The ability of vaccine induced Th1 to modulate immune suppression may have important consequences for the prophylaxis of breast cancer. A Type II T-cell (Th2) signature has been reported in DCIS.[24] Studies indicate Th2 may dominate the immune infiltration in breast cancer, secreting cytokines such as IL-13 and IL-4, which are conducive to breast cancer progression.[25]

2.3.2. Prevention of breast cancer in genetically engineered mice. The FVB/N-TgN (MMTVneu)-202Mul mouse (TgMMTV-neu) develop spontaneous mammary cancer. The tumors arising in this and other genetically engineered mice, share significant similarities with human breast cancer at both the molecular and protein level.[26] Indeed, spontaneously arising mammary cancers in these animals express HER2, IGF-1R and IGFBP-2 and investigations have shown that growth of syngeneic implanted tumors can be significantly delayed by vaccinating mice with peptides, derived from each of these proteins, designed to elicit antigen specific $CD4^+$ T-cells.[9,20,27]TgMMTV-neu is a stringent model for assessing prophylactic vaccination. Mechanisms of both central and peripheral tolerance significantly limit the ability to successfully immunize these mice.[28] The mice are tolerant to their tumors and T-cells derived from tumor draining lymph nodes do not secrete IFN-g when challenged with antigen.[29] The lack of Type I IFN-g secreting tumor antigen specific T-cells has also been well described in breast cancer patients.[30] IFN-g is needed for tumor recognition through activating antigen presenting cells (APC) to engage T-cells. If T-cells are induced by vaccination in this model, the response is short lived unless vaccine formulations include molecules to significantly enhance co-stimulation, thus, potentially increasing the risk for autoimmune toxicity.[31] Several prophylactic vaccine approaches have been evaluated in the TgMMTV-neu. The extent of protection in these studies was variable and vaccine efficacy decreased significantly with age at immunization.[32-34]

We immunized 18 week old mice (15 mice/group) to model the clinical translation of such an approach to mature adult women, with high risk of developing breast cancer, who would likely be the target population for prophylactic vaccination. The mouse homologues of all three proteins, IGFBP-2, IGF-1R and HER2, demonstrated expression in hyperplastic mammary lesions in TgMMTV-neu mice. Fifty percent of multi-antigen immunized mice did not develop breast cancer as compared with 0% of controls ($p<0.0001$) at approximately 1 year. The median disease-free-survival (DFS) of vaccinated animals was significantly longer than those mice receiving adjuvant alone, 46 vs. 30 weeks (HR 3.959, CI 2.018 to 7.768). At experiment termination, overall survival (OS) was also significant between the two groups ($p<0.0002$). The median OS of the vaccinated animals had not been reached while the median OS of the control group was 40 weeks (HR 4.335, CI 1.886-9.962). The use of a multi-antigen vaccine was significantly more effective in improving DFS than immunizing with the individual antigens alone. In a repeat experiment, mice immunized with a HER2-specific vaccine demonstrated a median DFS of 38 weeks as compared to 28 weeks in adjuvant controls ($p=0.251$ between groups). The DFS was 38 weeks for IGF-1R immunized mice ($p=0.367$) and 37 weeks for IGFBP-2 vaccinated animals ($p=0.251$) compared to adjuvant controls. In the multi-antigen vaccinated cohort, 80% of the mice remained tumor free and the median DFS for the multi-antigen vaccinated mice was not reached at the time of experiment termination ($p=0.0013$ compared to control). In addition, while the DFS of the single antigen vaccinated animals was not different from each other ($p=0.965$) the multi-antigen vaccine significantly prolonged DFS compared to any of the single antigen approaches ($p=0.018$). To evaluate whether antigen coverage provided by the vaccine would effectively inhibit the growth of additional mammary cancer phenotypes, TgC3(I)-Tag mice, a model of basal breast cancer, were immunized and then challenged with syngeneic tumor. Tumor growth was significantly inhibited ($p<0.00001$) in the multi-antigen vaccinated mice as compared to the adjuvant immunized controls. Indeed, tumor inhibition occurred despite the fact spontaneous tumors in these mice show expression of only IGF-1R and IGFBP-2 but not HER2[35].

2.3.3. Development of Th1 T cell immunity and persistent memory. Our immunologic analysis will focus on analyzing Type I immune responses via IFN-g ELISPOT as Type I immunity has been associated with beneficial clinical outcome in breast cancer.[36] We will, however, analyze both Th1 and Th2 responses to the WOKVAC antigens prior to and after active immunization. We have performed extensive screening of immunity across multiple breast cancer antigens and have found subtle difference between endogenous immune responses identified in breast cancer patients compared to age matched volunteer women. In population based screening studies of 49 class I epitopes derived from 6 breast cancer associated antigens immune responses directed against those antigens were found equally in breast cancer patients (n=20) and volunteer controls (n=20). In general, median incidence of IFN-g epitope specific responses were low with a median of 40% of volunteer donors demonstrating no response (range 5-58%) and a median of 33% of breast cancer patients demonstrating no response to a particular antigen (range 10-55%). The magnitude of IFN-g responses was greater in the volunteers compared to patients although the difference did not reach statistical significance ($p=0.45$). When IL-10 responses for each patient were evaluated in the context of the IFN-g response, significantly ($p< 0.0001$) higher IFN-g/IL-10 ratios were observed among volunteer control patients compared to breast cancer patients. We have identified that some epitopes of self-antigen elicit primarily Th2 immunity and if such epitopes are included in a vaccine will inhibit the development of Th1 response and vaccine efficacy.[37]For this reason we will explore the evolution of the Th1/Th2 ratio for the vaccine components during the course of active immunization. Maintenance of a Th1 dominance and the development of immunologic memory after cancer vaccination have been shown to be associated with a positive clinical outcome.[38] We will evaluate whether there is antibody development by ELISA to support the vaccine as being predominantly Th1.

Both MDSC and Tregs have been found to be elevated in the peripheral blood of some volunteer donors as well as cancer patients. Recent data suggests that pre-existing levels of MDSC and Treg influence the immunologic efficacy of vaccination and as an objective we will explore the association of levels of these

two immunosuppressive cells types with the development of a robust tri-antigen immune response. Therefore, we will assess levels of both of these cell types throughout vaccination.

2.3.4 Mechanism of action and advantages of plasmid based vaccines. The WOKVAC plasmid produces one single polypeptide of 70 kDa expressing the three extended epitope segments. It is given as an intradermal (i.d.) injection and is transiently transfected into local differentiated keratinocytes which then process the plasmid and present the epitopes to local antigen presenting cells.[39] Immune responses initiated after plasmid-based DNA vaccination are thought to be mediated by either direct transfection of APC in vivo or uptake of antigen via "cross presentation" by phagocytosis of non-APC transfected cells. Studies in mouse models have shown that keratinocytes and Langerhans cells (LC) constitute the major cell types transfected by plasmid DNA after i.d. injection.[40] Studies of plasmid vaccination alone demonstrate that approximately 1-5% of keratinocytes within the inoculation site are transfected and express antigen after inoculation.[41] Although maximal antigen production occurs within 3 days, transfected keratinocytes can continue to produce antigens for longer periods.[42] From previous clinical trials with the HER2 ICD plasmid, we have seen IFN-g ELISPOT results from PBMC showing an immune response to vaccination up to 1 year after receiving the vaccine. The plasmid itself is intradermal so does not get into the systemic circulation. We have previously biopsied the sites of vaccination with the HER2 vaccine and found that DNA persistence occurs in more than 50% of immunized patients.

Encoding epitopes in a DNA plasmid rather than delivering chemical peptides enhances the development of immunologic memory as the antigen is persistent at the vaccine site and not degraded as quickly in the skin as soluble peptides. The clinical application of plasmid based vaccines has several advantages over the use of synthetic peptides. Plasmid DNA can produce antigen inside a cell, enhancing the generation of a cytotoxic T lymphocytes response via antigen uptake in the class I processing pathway. Unlike peptides which are quickly degraded in the skin, plasmid DNA can stably transfect skin cells and result in a depot of antigen which may generate persistent immunity.[43] Despite safety concerns for persistence of the plasmid in keratinocytes, we have long term follow up data from two previous plasmid vaccine trials that have shown no long term toxicity with persistence of the plasmid. With the HER2 ICD plasmid, which is the same plasmid encoded in WOKVAC, we have long term follow up data since 2005 that has shown no new skin toxicities or autoimmune complications associated with the vaccine. We also have further long term follow up data on the IGFBP2 vaccine, which is encoded in the same plasmid from 2012 which again have shown no complications associated with the plasmid. Furthermore in the literature, DNA vaccination may be considered safer than other platforms, such as the use of viral or bacterial vectors, since it is neither infectious nor inherently immunogenic.[44] Additionally, our previous studies in both mice and humans have shown that the pUMVC3 plasmid remains localized to the dermis and is not distributed systemically; and thus, it should not be able to confer antibiotic resistance to the body. The kanamycin resistance gene used in the plasmid is neomycin phosphotransferase II [NPT II/Neo]) which confers resistance to kanamycin. This kanamycin resistance gene is encoded for selection and amplification in bacterial cultures. Only the bacteria that have successfully taken up the kanamycin resistance gene become resistant and will grow under these conditions. Moreover, this kanamycin resistance gene is rarely found in clinical isolates and therefore is not known to confer resistance to Amikacin.

Data generated in the this Phase I study will allow further evaluation of the safety of the approach as well as a delineation of the immunogenicity of WOKVAC in the absence of endogenous antigen. These data will lay the foundation of further evaluation of WOKVAC in patients at high risk who have not yet developed breast cancer.

3. SUMMARY OF STUDY PLAN

This will be a Phase I, non-randomized, dose escalation study in patients with node positive non-metastatic HER2 negative breast cancer that are in remission and defined as no evidence of disease (NED). The study will accrue 30 evaluable patients with up to 12 patients in each dose arm.

Patients will be assigned sequentially to one of three arms defined by vaccine dose. Patients will be accrued to Arm 1 first, then Arm 2, then Arm 3. Each dose arm will have a staggered enrollment. There will be one week between the first patient's vaccination 1 visit and second patient's vaccination 1 visit to each dose arm to ensure no toxicities. Furthermore, three patients will initially be enrolled to a dose arm, receive the first vaccine, and be evaluated for toxicity prior to the second vaccine (1 month of monitoring) before the remaining patients will be enrolled in that dose arm.

If there is sufficient evidence (please refer to Section 8.5.1"Accrual and Criterion for Premature Study termination".) to suggest the Arm 1 dose is safe (Section 13.1), then the Arm 2 cohort of up to 12 patients will be enrolled. If the Arm 2 dose is also sufficiently safe then enrollment into the Arm 3 dose may move forward. If the Arm 3 dose appears safe, the immunologic efficacy among three dose groups will be examined to determine the recommended phase 2 vaccine dose for further cancer prevention studies. Immunologic efficacy of the 3 WOKVAC doses, will be evaluated by an assessment of the generation of cellular and humoral response to IGFBP-2, HER2, and IGF-1R, development of persistent T cell memory; and modulation of Tregs and MDSC with vaccination as described in Section 13.5.

We will be evaluating the effect of the WOKVAC vaccine on the heart by measuring left ventricular ejection fraction (LVEF) with a MUGA or ECHO before vaccination, one month after the first vaccine, and one month after the vaccination series.

Clinical and autoimmune labs will be drawn prior to each vaccine and 1 month and 6 months following the last vaccine to evaluate for safety. Patients are monitored for the development of toxicities by assessing adverse events (AE's) with serum chemistries, liver function studies, and complete blood counts. Adverse events for all systems are graded on a scale of 1-5 using the Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.0). We will be using the Cancer Therapy Evaluation Program (CTEP), National Cancer Institute (NCI) Guidelines for reporting adverse events assigning attribution (unrelated, unlikely, possible, probable, definite) Blood samples to test the effect on the immune system will be drawn at baseline, and 1 month (28+7 days) and 6 months (168±14 days) following the last vaccine. The specific measurements being done to evaluate immune response are memory T cell response, modulation of Treg, and MDSC.

The duration of the study will be 5 years, which includes annual follow-up from enrollment, with the subject's primary oncologist or primary care physician. Long-term follow up data, beyond the 6 month follow-up visit, will be kept and managed by the IND Sponsor at the University of Washington.

4. PATIENT SELECTION

4.1 Inclusion Criteria

4.1.1. Patients with non-metastatic, node positive, HER2 negative breast cancer, confirmed by pathology report, who are in remission and defined as having no evidence of disease (NED). HER2 negative is defined as

a. 0-1+ HER2 expression by immunohistochemistry (IHC) OR
b. Fluorescence in situ hybridization (FISH) negative OR
c. HER2 2+ and FISH negative

4.1.2. Patients must be at least 28 days post cytotoxic chemotherapy, radiotherapy, monoclonal antibody and/or other biologic therapy, prior to enrollment. Patients on bisphosphonates, denosumab, and/or

endocrine therapy administered during the study are eligible and may continue throughout duration of study.

4.1.3. Patients must be at least 28 days post systemic steroids prior to enrollment.

4.1.4. Patients must be at least 18 years of age.

4.1.5. Patients must have Eastern Cooperative Oncology Group (ECOG) Performance Status Score of ≤ 2.

4.1.6. Adequate laboratory values within 90 days of enrollment defined as follows:
a. White blood cell (WBC) ≥ 3000/mm^3
b. Hemoglobin (Hgb) ≥ 10 g/dL
c. Lymphocyte count ≥ 800/mm^3
d. Platelet count ≥ 75,000/mm^3
e. Serum creatinine ≤ 2.0 mg/dL or creatinine clearance > 60 ml/min
f. Total bilirubin ≤ 1.5 mg/dL
g. Aspartate aminotransferase (AST)/Serum glutamic oxaloacetic transaminase (SGOT) ≤ 2 times upper limit of normal (ULN)
h. HbA1c <5.7%

4.1.7. Patients must have recovered from major infections and/or surgical procedures, and in the opinion of the investigator, not have any significant active concurrent medical illnesses precluding protocol treatment.

4.1.8. The effects of WOKVAC on the developing human fetus are unknown. For this reason, patients who are having sex that can lead to pregnancy must agree to use adequate contraception (hormonal, barrier method of birth control, or abstinence) for the duration of study participation. Should a woman become pregnant while participating in the study, she should inform her study doctor immediately and will not receive any more study treatment.

4.1.9. LVEF results must be ≥ lower limit of normal (LLN) for institution performing based on results from the MUGA or ECHO done at baseline.

4.1.10. Willing to not undergo any elective surgical procedure with general anesthesia or conscious sedation through the 1 month post-vaccination visit.

4.1.11. Ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1. Patients with any of the following cardiac conditions:
a. Symptomatic restrictive cardiomyopathy
b. Dilated cardiomyopathy
c. Unstable angina within 4 months prior to enrollment
d. New York Heart Association functional class III-IV heart failure on active treatment
e. Symptomatic pericardial effusion

4.2.2. Patients may not be receiving any other investigational agents.

4.2.3. History of allergic reactions attributed to compounds of similar chemical or biologic composition to WOKVAC.

4.2.4. Patients with any contraindication or known hypersensitivity to receiving rhuGM-CSF or other yeast based products.

4.2.5. Pregnant women are excluded from this study because WOKVAC is a vaccine agent with unknown potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with WOKVAC, breastfeeding should be discontinued if the mother is treated with this vaccine.

4.2.6. History of diabetes.

4.2.7. Known history of human immunodeficiency virus (HIV) infection, hepatitis B, or hepatitis C.

4.2.8. History of autoimmunity that has not been controlled with treatment in the last 12 months.

4.3 Inclusion of Women and Minorities

Both men and women (as applicable) and members of all races and ethnic groups are eligible for this trial.

Men develop breast cancer at 1%, thus, the study population will be primarily female. These estimates are based on the following: the population pool from which patients will be drawn in Washington state is 83.8% Caucasian, 7.0% Asian American, 3.9% African American, 1.8% American Indian or Alaska Native and 0.5% Native Hawaiian or Other Pacific Islander. The population pool from which patients will be drawn at the University of Wisconsin is 93% Caucasian, 2% Asian American, 3% African American, 1% American Indian or Alaska Native, 0% Native Hawaiian or other Pacific Islander and 1% other/unknown. This would give a total average between Wisconsin and Washington of 88.4% Caucasian, 4.5% Asian American, 3.45% African American, 1.4% American Indian or Alaska Native, and 0.25% other/unknown.

5. AGENT ADMINISTRATION

Vaccines will be administered on an outpatient basis either at the University of Washington Clinical Research Center (UW CRC) or the University of Wisconsin Clinical Research Unit (UWI CRU).

Reported adverse events (AEs) and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

There will be three treatment groups for this trial:

Arm 1: WOKVAC (150 mcg) with Sargramostim (rhuGM-CSF) (100 mcg); administered as 1 i.d. injection every month for 3 total doses.

Arm 2: WOKVAC (300 mcg) with Sargramostim (rhuGM-CSF) (100 mcg); administered as 2 i.d. injections every month for 3 total doses.

Arm 3: WOKVAC (600 mcg) with Sargramostim (rhuGM-CSF) (100 mcg); administered as 3 i.d. injections every month for 3 total doses.

5.2 WOKVAC Administration

Standard precautions should be taken when handling the vaccine/GM-CSF. Gloves and lab coats should be worn per the standard of the clinic administering the vaccine. Caution should be taken when handling the vaccine that is prepared by the pharmacy in syringes. Sharps containers should be readily available. The vaccine is administered intradermal (i.d.). It should be injected slowly to avoid any vaccine leaking

out from under the skin. Standard reporting procedures should be implemented as needed per the standard of the clinic.

Patients with axillary lymph node dissection (ALND) will have vaccine administered to the contralateral arm. Patients with bilateral ALND will have vaccine administered in the thigh. As much as possible each vaccine dose will be given within the same draining lymph node site.

Study clinicians will administer vaccines using professional standards of medication administration that include:

- Right patient, right vaccine, right dose, right route, right site.
- Personnel who will administer vaccines will have received training and education on vaccine administration and disposal before providing vaccines to patients.

One dose of the vaccine may be given in several injections in the same general area once a month for three months. Patients will be monitored for a minimum of 60 minutes post vaccine administration. Acute hypersensitivity and/or anaphylactic reactions are very rare with reported rates ranging from 0.22 - .065 per 100,000 doses of vaccinations. [45] Additionally, anaphylactic reactions to vaccines should they occur, usually appear within 5 to 60 minutes.

5.3 Run-in Procedures

There is no run-in phase for this study.

5.4 Contraindications

Patients should not be pregnant or become pregnant through the 6 month follow-up visit. Patients should not concurrently enroll in any other treatment study.

5.5 Concomitant Medications

Throughout the duration of the study patients should not be on cytotoxic chemotherapy, systemic steroids, monoclonal antibody, and/or other biologic therapy. Exceptions are bisphosphonates, denosumab, and endocrine-based therapy. All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the patient will be documented in their study chart through the 6 month follow-up visit and may include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included.

5.6 Dose Modification

There are no dose modifications as part of this protocol. There may be allowances for the timing of the administration of vaccine to accommodate for special circumstances including repeated blood glucose testing. Subsequent vaccinations will be scheduled 28 (+ 7) days from when the previous vaccine was actually administered, not when it should have been administered.

5.7 Adherence/Compliance

5.7.1. All patients who receive any vaccinations will be evaluated for primary endpoint of safety.
5.7.2. Patients who have baseline and at least one post vaccine research blood sample will be evaluated for immune response.
5.7.3. Compliance will be assessed by tracking visit dates and completion of protocol procedures.

6. PHARMACEUTICAL INFORMATION

6.1 WOKVAC
(IND#16870), IND Sponsor: Mary L. (Nora) Disis, MD, University of Washington, Seattle, WA)

WOKVAC Vaccine
The plasmid we will use clinically is pUMVC3-IGFBP2-HER2-IGF1R(WOKVAC) and contains a total of 5947 base pairs. A DNA insert encoding the N-terminal 163 amino acids of IGFBP2, HER2 amino acids 686-994/1036-1051/1156-1197 and IGF1R amino acids 1196-1261/1323-1360 has been inserted between the Kpn1 (at base 1125) and Not1 restriction sites.

The WOKVAC vaccine is supplied in single-use vials as a sterile, frozen solution. Each single use vial has a final concentration of 1 mg of DNA/ 1mL tromethamine/EDTA (TE). Each WOKVAC vaccine will carry a label bearing the drug identification and conditions for storage.

GM-CSF
Reconstituted recombinant human GM-CSF (rhuGM-CSF, Sargramostim) as a 100 mcg dose will be used as adjuvant admixed with WOKVAC plasmid based vaccine.

RhuGM-CSF is a sterile, white, preservative-free lyophilized powder in 250 mcg vials.

6.2 Reported Adverse Events and Potential Risks
This is the first time that this investigational vaccine has been used in humans. Although there were no serious side effects seen in mice given the vaccine, it is possible that humans will have a different response than the mice.

Risk of Study Vaccine
UW has given over 1100 peptide/DNA plasmid vaccine injections in previous studies, and below are some possible risks:

Likely	Less likely	Rare, some may be serious
Pain and discomfort during vaccine administration Redness and tenderness at injection site (this usually goes away in 1-2 days) Itching at vaccine site Fatigue Headache	Flu-like syndrome Muscle pain Nausea Chills Diarrhea	Allergic reaction, including shortness of breath, dizziness, a feeling of fainting, hives, and difficulty breathing caused by swelling of the mouth, face, tongue or throat Severe allergic reaction to the vaccine may require medication, lead to hospitalization, and may result in death Hyperglycemia

Risk of Granulocyte-Macrophage Colony Stimulating Factor (rhuGM-CSF, Sargramostim)

GM-CSF is a man-made protein that is almost identical to a protein the body makes, and will be mixed and injected in very small amounts with the vaccine. GM-CSF is also known as an adjuvant. In our previous vaccine studies that used GM-CSF, patients sometimes complained of mild to moderate flu like symptoms (fever, chills, achiness, and fatigue) for 1-2 days after vaccination that may be related to the use of GM-CSF. The possible risks listed below are for a larger dose of GM-CSF than will be given in this study; patients will be getting a fraction of the regular GM-CSF dose.

Likely	Less likely, some may be serious	Rare, some may be serious
Local reactions at the site of the injection Low grade fever (less than 100.5°F) Chills Pain in the bones, muscles, chest, abdomen, or joints Nausea Vomiting Diarrhea Flu-like symptoms including fatigue, weakness, headache Decreased appetite Increased white blood cell count	Kidney and liver problems Local reactions at the site of injection Rashes Liver enlargement Low blood pressure	Fluid retention (including fluid in lungs or around the heart) Blood clotting, including blood clots in the leg veins that can break loose and go to the lung Increased platelets, low albumin, increase of liver enzymes Rapid or irregular heartbeat or other heart problems Allergic reaction, including shortness of breath, dizziness, a feeling of fainting, hives, and difficulty breathing caused by swelling of the mouth, face, tongue or throat Worsening of pre-existing fluid accumulation in arms and legs, in the lungs and around the heart that may result in breathing problems and heart failure Neurologic syndrome called Guillain-Barré syndrome, where a person's own immune system damages their nerve cells, causing muscle weakness and sometimes paralysis Temporary loss of consciousness

Risk of Generation of an Immune Response to Normal Cells

It is unknown whether generating an immune response to specific proteins will have any effect on normal cells.

- We will be monitoring closely for signs of immune damage to normal cells. We will be looking for diarrhea, development of any unusual rashes and changes in liver, kidney, and blood function through blood tests.
- Medicine may be administered if patients develop any autoimmune symptoms.

Likely	Less likely	Rare, some may be serious
None	Skin rashes Diarrhea	A severe autoimmune reaction which could cause death

Allergic Reaction Monitoring

Severe allergic reactions are not common, but they do occur. If they occur, they tend to happen within an hour of exposure to the substance causing the allergy. Because of this rare risk, patients will be observed in the clinic for a minimum of one hour after each vaccine to make sure there are no immediate side effects or allergic reactions. While other rare systemic allergic reactions (i.e., generalized skin rash) may occur

within 24 hours they are rarely associated with life-threatening adverse events that would require emergency treatment. To date, aside from the expected and transient vaccine-related side effects of injection site redness, warmth, edema, induration with or without tenderness and occasionally malaise, low-grade fever, and arthralgia we have not observed any allergic-type or anaphylactic reactions. Furthermore, in our recently completed phase I DNA vaccine study of 66 patients, who were immunized with the same plasmid construct, proposed in this study, there were no related grade 3 or 4 AEs observed within the first 24 hours.

Should an allergic reaction occur, treatment per standard of care will be implemented. If symptoms of a mild vaccine-related reaction occur, such as a skin rash or itching, the study doctor may treat the patient with acetaminophen or diphenhydramine if clinically indicated. Vital signs will be taken at 60 minutes, with a window of between 55 and 75 minutes, post immunization and as indicated.

6.3 Availability

WOKVAC for clinical use will be amplified, quantified and vialed by the Biologics Production Facility at the Fred Hutchinson Cancer Research Center under good manufacturing practice (GMP) laboratory conditions.

RhuGM-CSF is a sterile, white, preservative-free lyophilized powder in 250 mcg vials and is obtained by commercial suppliers and stored at their specifications.

The vaccine will be supplied as frozen vials and each vial will carry a label bearing the drug identification and conditions for storage. The site pharmacy will prepare and dispense the drug for administration per a standard operating procedure established by the UW Investigational Drug Services (IDS).

The site pharmacy will prepare and dispense the drug for administration per a standard operating procedure established by the UW IDS by pulling a single vial vaccine from the freezer and thawing it slowly at room temperature. The GM-CSF (rhuGMCSF, Sargramostim) will be reconstituted in sterile preservative free water. The GM-CSF (rhuGMCSF, Sargramostim) will be added to an empty vial and then the vaccine is added to that. The vial is rotated gently to mix. The dose will be divided into Tuberculin Safety Syringes with a 27 gauge, ½ inch needle with no more than 0.33ml each for intradermal injection.

6.4 Agent Distribution

WOKVAC will be managed by the University of Washington Investigational Drug Service (UW IDS). UW IDS will be responsible for the study drug disposition (drug receipt, transfer or return) and shall be documented on the Investigational Drug Accountability Record. UW and UWI will each obtain the adjuvant, GM-CSF (rhuGM-CSF; Sargramostim;) directly.

The prepared vaccine will be stored at the FHCRC Biologics Facility until it is transported to the University of Washington Investigational Drugs Services (IDS) where it will either be dispensed to UW patients or shipped to UWI.

Vaccine shipped to UWI will be sent overnight on dry ice. Once received at UWI the chain of custody documentation should be completed and the vaccine stored in the freezer per label specifications. We are planning 2 shipments over the study, but if an additional request needs to be made UWI should contact UW IDS using the contact information below:

Investigational Drug Services
Reference Study Number: CRC# 9983
FAX: 206-598-4901
Phone: 206-598-6054

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a record of the inventory and disposition of all agents received. The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to UW IDS as well as dispensing records by the site pharmacy. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed.

6.6 Packaging and Labeling

WOKVAC will be packaged by the Biologics Production Facility at the Fred Hutchinson Cancer Research Center under GMP laboratory conditions. The vaccine will be vialed as single doses containing DNA suspended in tromethamine/EDTA (TE) as a stabilizing buffer of each of the three study doses. The vaccine formulation is labeled to accurately reflect the product identity, concentration, lot number and fill date. These labels are attached to vials immediately post-fill.

6.7 Storage

Vials of the vaccine will be subjected to microbial, sterility and stability testing to ensure safety and stored at -20ºC +/- 5ºC until use. The product will be stored in freezers with alarmed temperature controls at both the FHCRC Biologics Facility and site pharmacies at temperature -20°C ± 5°C.
The GM-CSF (rhuGMCSF, Sargramostim) will be stored per package insert. It should be stored at 2-8°C (36-46°F). It should not be frozen or shaken.

6.8 Registration

All patients who sign an informed consent form are assigned a patient identification number (PID). These numbers are assigned sequentially and designate the recruitment site, the study and the patient. These numbers will be provided to the site in a Registration Log provided by the Consortium Lead Organization (CLO-UWI) at the time of study initiation.

University of Washington: Once screening is complete, and the patient is determined to be eligible, UW will fax (608-807-4061) or email (prevention@uwcarbone.wisc.edu) to the UWI during normal business hours (M-F 8:00 am - 4:15 pm Central Time), the following information:

Signed Informed Consent Form	Eligibility Questionnaire
Baseline Visit Guide	Physical Exam and Vital Sign Form
Med/Surg History Assessment	Baseline Symptom Assessment
Concomitant Medication Worksheet	Hematology and Chemistry Lab Results
Prior Breast Cancer History	

The CLO-University of Wisconsin will confirm eligibility, the registration number and the dose level. UWI will email a confirmation of the registration to the Consortium PI, Study PI's, Co-Investigators and Study Coordinators.

University of Wisconsin: Once screening is complete, and the patient is determined to be eligible, the University of Wisconsin will email a confirmation of the registration to the Consortium PI, Study PI's, Co-Investigators and Study Coordinators.

The Study Coordinator at the CLO-UWI will email the study coordinator at UW once/week for an update on screening efforts for UW and to provide information about screening efforts at UWI.

6.9 Blinding and Un-blinding Methods

This is not a blinded study.

6.10 Agent Destruction/Disposal

UW IDS will be responsible for the study drug disposition and shall be documented on the Investigational Drug Accountability Record. The disposal of research trial materials that are in UW IDS pharmacy will be incinerated under a state contract through an Environmental Protection Agency (EPA) licensed facility.

UWI should return empty and unused vaccine to the UW IDS for destruction and disposal. This will also serve as a double check of the product inventory.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Evaluation/ Procedure	Baseline[1]	1st vaccination	2nd, 3rd vaccination[10]	Post-vaccination Follow-up Visits[11]	Long-term Follow-up[14]
Informed Consent	X				
Assess Eligibility	X				
Medical History	X				
Baseline Symptom Assessment	X				
Physical Exam[2]	X		X	X	
Vital Signs/ and Weight	X	X	X	X	
Concomitant Medications	X	X	X	X	
Assign dose arm	X				
Urine Pregnancy Test[3]	X	X	X		
CBC[4]	X	X[5]	X	X	
CMP[6]	X	X	X	X	
HbA1c	X			X	
GAD65		X		X	
ANA, anti-dsDNA, C3 and TSH		X	X	X	
Research Blood[7]		X		X	
Tetanus- diphtheria Immunization[8]		X			
MUGA/ECHO[9]	X		X	X	
Symptom Assessment/ Adverse Events		X	X	X	
Dispense Study Vaccine		X	X		
60 minutes post vaccine vitals[13]		X	X		
Medical Record Chart review					X[12]

[1]Must occur within 3 weeks (21 days) of 1st vaccination with the exception of CMP, CBC, HbA1c and ECHO/MUGA which must be in acceptable range within 90 days of enrollment

[2]Physical Exam includes: Weight, vital signs, symptom/toxicity assessment, and ECOG scoring

[3]Performed on females who are having sex that could lead to pregnancy.

[4]Complete blood count (CBC) includes differential and platelets.

[5]Complete blood count (CBC) with differential and platelets does not need to be drawn if completed within 90 days of enrollment.

[6] Comprehensive Metabolic Panel (CMP) includes serum electrolytes, creatinine, BUN, AST, ALT, alkaline phosphatase, and total bilirubin. For vaccine visits follow glucose monitoring per section 8.5.1.1.

[7]During the consent process, and at subsequent visits, patients will be instructed to hydrate sufficiently prior to visits requiring large volume blood draws.

[8]Tetanus-diphtheria immunization only given if patient has not received one within 6 months.

[9]MUGA or ECHO to determine LVEF will be performed at baseline (if not completed within 90 days of prior to enrollment), prior to 2nd vaccination and 1 month post study treatment. Once a patient has had the baseline LVEF results, the same monitoring modality should be used for all subsequent LVEF evaluations. The MUGA/ECHO prior to 2nd vaccination and at 1 month post can be performed within 1 week of their scheduled visit.

[10]Vaccines are scheduled 28 (+7) days from the previous vaccine.

[11]The 1-month post vaccine visit should occur 28 (+7) days following the last vaccine. The 6-month post vaccine visit should occur 168 (± 14) days following the last vaccine.

[12]Follow-up includes requesting MD notes, clinical labs and imaging reports.

[13] 60 minutes Post vaccine vitals may be collected 55-75 minutes after vaccination.

[14] Long-term follow up data, beyond the 6 month follow-up visit, will be kept and managed by the IND Sponsor (University of Washington).

7.2 Baseline Visit (may be performed up to 3 weeks prior to 1st vaccination)

Baseline evaluations determine if the patient is an eligible candidate for study participation and establish the patient's health status at the time of study entry. Standard-of-care laboratory evaluations done prior to signing consent will qualify for baseline evaluations if they have been conducted within 90 days prior to enrollment. Standard-of-care MUGA or ECHO done prior to signing consent will qualify for baseline evaluations if conducted within 90 days prior to enrollment. All baseline evaluations must be completed before study agent is dispensed. If a study patient is found to be ineligible any blood collected for research purposes will be destroyed. The destruction of the blood will be clearly documented in the documented study records.

7.2.1. Sign informed consent prior to any study-specific baseline testing

7.2.2. Medical history and complete physical examination which includes weight, vital signs, baseline symptom assessment, review of concomitant medications, and ECOG scoring (Appendix A)

7.2.3. MUGA or ECHO
   a. LVEF must be ≥LLN per institution performing the test, to allow the patient to continue with vaccination

7.2.4. Patients will have the following tests and procedures completed prior to the first vaccine.
   a. Clinical labs
      i. Complete blood count (CBC) with WBC differential, hemoglobin and platelet count
      ii. Comprehensive metabolic panel (CMP) which includes electrolytes, glucose, creatinine, blood urea nitrogen (BUN), AST (SGOT), ALT, alkaline phosphatase, and total bilirubin
      iii. HbA1c

7.2.5. Urine pregnancy testing will be performed on female patients who are having sex that could lead to pregnancy. If a patient is found to be pregnant, she will not be able to participate in the study.

7.3 First Vaccination

7.3.1. Patients will have the following tests and procedures completed prior to the first vaccine:
   a. Vital signs
   b. Complete blood count (CBC) with WBC differential, hemoglobin and platelet count if not completed within 90 days of enrollment

c. Comprehensive metabolic panel (CMP) which includes electrolytes, glucose, creatinine, blood urea nitrogen (BUN), AST (SGOT), ALT, alkaline phosphatase, and total bilirubin (within 2 days of visit is allowable)
    i. Review non-fasting glucose value prior to vaccine administration then follow Section 8.5.1.1 to determine whether vaccine can be administered
d. ANA, anti-ds DNA, C3, TSH
e. GAD65
f. Urine pregnancy testing if applicable

7.3.2. Approximately 200 ml of blood (about 1 cup) will be collected for immunologic monitoring, prior to the first vaccine. It is possible that this volume of blood loss could cause the patient to feel lightheaded or dizzy. If clinically indicated, IV fluids will be administered

7.3.3. Tetanus diphtheria (Td) immunization if one has not been administered within six months and used as a positive control for immune responses. If patient has a history of an allergic reaction to the Td immunization, one will not be given and patient can still continue to be in the study as there are other positive controls used for immune assays.

7.3.4. WOKVAC vaccine given intradermally

a. Patients with axillary lymph node dissection (ALND) will have vaccine administered to the contralateral arm. Patients with bilateral ALND will have vaccine administered in the thigh. For 300mcg and 600mcg doses where multiple injections are given, they should each be given in the same area of the arm or leg.

b. Vaccines will be administered in the outpatient setting at the UW CRC or at the UWI CRU.
c. 60 minutes post vaccine observation including vital signs (collection between 55-75 minutes post vaccination is allowed) (see section 6.2)

7.4 Second and Third Vaccination

7.4.1. Clinical labs prior (within 2 days of visit is allowable):

a. CBC
b. CMP
    i. Review non-fasting glucose value prior to vaccine administration then follow Section 8.5.1.1 to determine whether vaccine can be administered.
c. ANA, C3, anti-dsDNA, TSH

7.4.2. MUGA or ECHO prior to the second vaccine only (within 1 week of the scheduled visit)

a. LVEF must be ≥LLN per institution performing the test, to allow the patient to continue with vaccination
b. The same monitoring modality used at baseline must be used

7.4.3. A physical examination will be done that will include weight, vital signs, symptom/toxicity assessment, and ECOG scoring.

7.4.4. Urine Pregnancy Test if applicable

7.4.5. Vaccines may be scheduled every 28 (+7) days from previous vaccine.

a. Vaccines will be administered i.d. (see 7.3.4.a). As much as possible each vaccine dose should be given in the same area of the arm or leg as the first vaccine.
b. Vaccines will be administered in the outpatient setting at the UW CRC or at the UWI CRU
c. 60 minutes post vaccine observation including vital signs (collection between 55-75

minutes post vaccination is allowed)
i. See 6.2 regarding allergic reactions

7.5 Evaluation at Completion of Study Intervention

(The 1-month post vaccine visit must occur 28(+7) days following the last vaccine. The 6-month post vaccine visit must occur 168 (±14) days following the last vaccine.)

7.5.1. MUGA or ECHO (completed at 1-month post vaccine visit) using the same imaging modality used at baseline

7.5.2. A physical examination will be performed which will include weight, vital signs, symptom/toxicity assessment and ECOG scoring.

a. Clinical labs:
b. CBC
c. CMP
d. ANA, C3, anti-dsDNA, TSH
e. HbA1c and GAD65

7.5.3. Research blood:
Approximately 200mls of blood will be collected for immunologic monitoring. It is possible that this volume of blood loss could cause the patient to feel lightheaded or dizzy. If clinically indicated, IV fluids will be administered.

7.6 Long-Term Follow-up (LTFU) Period

7.6.1. Information on patient's health status will be requested from the patient's primary oncologist or primary care physician annually from enrollment to complete 5 years. The following will be requested and reviewed:
a. Clinical notes
b. Laboratory values
c. Imaging reports

Notes will be reviewed by study team for vaccine toxicity such as site reaction and possible autoimmune symptoms (examples - unexplained rash, dry eyes, unexplained diarrhea) and overall relevant health status within the above collected records.

7.7 Methods for Clinical Procedures

Although patients have a small chance of experiencing an allergic-type reaction to the vaccine, if a reaction were to happen, it would usually occur within one hour of the vaccination. For this reason, all patients will undergo observation for a minimum of 60 minutes post-vaccination, after which time vital signs will be performed.

Large volume blood draws (up to 200mls) are required at designated study visits for immunologic monitoring. The amount of blood obtained for immunologic monitoring assays is in strict adherence with guidelines set by the Puget Sound Blood Center and are associated with minimal risks. However, as with any large volume blood draw patients should be well hydrated prior to the draw. Thus, during the consent process, and at subsequent visits, patients will be instructed to hydrate sufficiently prior to visits requiring large volume blood draws. If it is clinically indicated, IV fluids will be administered.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

A primary endpoint of the study is safety. Safety of the three escalating dose of WOKVAC will be assessed by adverse events as per CTCAE v4.0.

8.2 Secondary Endpoints

Secondary endpoints of the study include the immunogenicity of WOKVAC Th polyepitope plasmid based vaccine, antibody response to the three WOKVAC targets, memory T cell response, modulation of (Treg), and MDSC in patients with breast cancer at three escalating doses.

These secondary endpoints will be used to determine whether an immune response is observed between the three doses and to determine a recommended phase 2 dose for further cancer prevention studies as detailed in Section 13.5. Determining a phase 2 dose will be performed by key study personnel and NCI/DCP staff. The recommendation will be based on dose level safety/tolerance, immunogenicity, memory T cell response, T cell response, Treg and MDSC data.

8.3 Off-Agent Criteria

Patients may discontinue vaccines for the following reasons: completed the protocol-prescribed intervention, pregnancy, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, or medical contraindication. Patients may continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. Patients, who have persistent grade 3 or 4 toxicity (of a two week or greater duration), at time of withdrawal from study treatment, will continue to be followed until toxicity resolves or returns to baseline for that patient.

8.4 Off-Study Criteria

Participants may go "off study" for the following reasons: the protocol intervention follow-up period is completed including long term follow-up, adverse event/serious adverse event, lost to follow-up, noncompliance, concomitant medication, medical contraindication, development of cancer of the breast or other site, withdrawal of consent, death, determination of ineligibility (including screen failure), or pregnancy.

8.5 Study Termination

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

8.5.1. Criteria for Premature Study Termination

Benchmarks for the safety of a dose level will be that there are no attributable (definitely, probably and possibly-related to study drug) grade 4 toxicity and <30% incidence of attributable Grade 3 toxicity (definitely, probably and possibly related to the study agent except as noted in the table below). If the observed rate of each of these toxicities is consistent with these benchmarks, escalation to the next higher dose level will be allowed.

Escalation will not be allowed if the observed toxicity rate exceeds the above. Other participants at the same dose level without toxicities will be allowed to continue at that dose level.

For grade 1 or 2 vaccine-related reactions, patients may be treated with acetaminophen or diphenhydramine as clinically indicated at the discretion of the study clinician and continue to receive vaccinations. For this prevention study ≥ grade 3 attributable toxicity (other than those listed below; CTEP CTCAE v4.0) is considered dose-limiting for the patient. If grade 3 toxicity that is at least possibly related to the vaccine (except as described below) is observed, no further immunizations will be administered to that patient.

If ≥ 3 participants experience a grade 3 attributable toxicity, except as noted in the table below, or if any participant experiences a grade 4 attributable toxicity within any dose level, further accrual at that dose level will stop and accrual to the study will be terminated.

Category	Grade 3 Attributable Toxicity/AE	Allowable Duration
General disorders and administration	Flu-like symptoms	1 week
Musculoskeletal and connective tissue disorders	Arthralgia	1 week
	Myalgia	1 week
Investigations	Lymphocyte count decreased	2 weeks
	Platelet count decreased	1 week
	Hemoglobin decrease	1 week
	White blood cells decreased	2 weeks

We would consider a grade 3 or 4 autoimmune event a dose limiting toxicity and would consider steroid administration for grade 3 or 4 adverse immune related events that have been reported with other immune based therapies, i.e. ipilimumab. Examples of such immune related AEs include; rash, diarrhea/colitis, and hepatitis. The following dose schedule may be used:

Day 1-2: Intravenous Solu-Medrol at 1 mg/kg
Day 3-4: Prednisone at 30 mg BID PO q day
Day 4-5: Prednisone at 15 mg BID PO q day
Day 5-6: Prednisone at 10 mg BID PO q day
Day 6-7: Prednisone at 10 mg PO q day
Day 8-9: Prednisone at 5 mg PO q day

8.5.1.1 Blood Glucose Monitoring
If random blood glucose is ≥140 mg/dl

1. Vaccine will be held and fasting glucose should be performed within a week:
   a. If fasting glucose is ≥100 mg/dl patient should have a repeat fasting glucose done within 1 week
   b. If fasting glucose is <100 mg/dl patient can receive vaccine
   c. If this repeat fasting glucose remains ≥100 mg/dl patient will not receive additional vaccinations.

Patients who do not receive any more vaccines should continue to be followed for adverse events and immunogenicity.

For hyperglycemia with symptomology, please contact the IND Sponsor and DCP Medical Monitor.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

9.1.1. Immunogenicity of the 3 doses will be evaluated by an assessment of the generation of IGFBP-2,

HER2, and IGF-1R specific T cells (both Th1 and Th2).

Cellular immune response will be defined by the magnitude of the Th1 (IFN-gamma (g)) vs. Th2 (IL-10) antigen specific immune response using ELISPOT. Successful immunization will be defined by generating a protein specific IFN-g precursor frequency greater than 1:20,000 PBMC for each antigen. In patients with pre-existent immunity, responses must augment over 2 times baseline for immunization to be considered successful.

Exploratory analysis will assess whether patients maintain a Th1 dominant immune response to all three antigens over time.

Antibody immunity will be assessed as an additional measure of a Th2 response.
A humoral immune response will be measured by ELISA and serum antibody avidity for IGFBP-2, HER2, and IGF-1R (using ELISA) to determine an avidity index (AI) before and after vaccination. Low avidity antibodies will have an AI <30%, moderate avidity antibodies 30-50% and high-avidity antibodies 51-100%. Patients will be considered to have developed an antibody response if antigen specific IgG antibodies are both detectable and have moderate to high avidity.

9.1.2. To detect persistent T cell memory, flow cytometry panels will be used to evaluate antigen specific central and effector memory phenotypes prior to vaccination and at 1 and 6 months after immunizations.

9.1.3. To detect modulation of Tregs and MDSC with vaccination, levels over the course of immunization will be assessed by flow cytometry of PBMC.

9.2 Comparable Methods
N/A

10. SPECIMEN MANAGEMENT

10.1 Laboratories
The University of Washington Research Testing Service (UW RTS) will result the UW clinical labs.

The University of Washington Laboratory Medicine will result the STAT UW clinical labs.

The University of Wisconsin Hospital and Clinics Clinical Laboratories will result the clinical labs at UWI.

The University of Washington Immunological Monitoring Lab (UW IML) will perform immune analysis for each research specimen.

The UW IML uses the Biological Specimen Inventory II (BSI-II) system, which is an established software system for bio-specimen management that includes tracking of specimen shipments via a web interface. It provides freezer, acquisition, entry, report, and requisition modules in a validated secure software environment that includes a full audit trail and is CFR 21 Part 11-compliant. All specimens received and processed in this laboratory are tracked using this system. Rates include general maintenance, data back-up and hosting of the SQL database by Information Management Services.

10.2 Collection and Handling Procedures
Approximately 15 mls of blood will be drawn for clinical labs at each study visit.

Approximately 200 mls of blood for immunologic monitoring will be collected at the first vaccination visit, as well as, 1 month (28+7 days) and 6 months (168 ± 14 days) after the last vaccine. Research blood will be transported at ambient temperature until processed by the UW IML within approximately 34 hours of the blood draw. Specimens and plasma are then stored at approximately -80°C until use. Serum will be processed at each site and stored in a -80°C freezer and will be shipped to IML in batches. When whole blood is to be shipped overnight to the IML, it cannot be drawn on a Friday or a day prior to a holiday. Blood cannot be drawn or shipped on Friday or day prior to a holiday if it is being shipped overnight.

ELISPOT and FACS will be done on research samples in batches by the UW IML. Serum samples will be stored and analyzed at the end of the study.

Samples for future testing
If any research blood is left over after the study's immunologic monitoring, it may be stored for future research related to the development of other immunotherapies. The choice to store any leftover samples is up to the patient and whatever their decision, it will not affect their participation in this study.

10.3 Shipping Instructions
Research specimens will be tracked from their point of origin to the UW-IML using BSI-II. All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

Research specimens (blood) by the clinical team will be collected at UW and UWI. UW-collected specimens will be transported by the clinical team or a courier to the UW-IML for processing. UWI-collected specimens will be shipped via FedEx to the following location:

All research specimens should be shipped to the following location (UWI: via FedEx; UW will courier samples):

UW Immune Monitoring Laboratory
Attn: Yi Yang
University of Washington Tumor Vaccine Group
850 Republican Street
Brotman Building, Room 232
Seattle, WA 98109

To contact the UW IML Laboratory:
Email: uwiml@uw.edu
Telephone: 206-221-1469, Monday-Friday 8:00 AM - 4:30 PM PST
Before/after hours phone: 206-484-3544 (for emergencies only)

10.4 Tissue Banking
Tissue will not be banked for this study.

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

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on clinician's assessment are to be reported as AEs. Patients with a clinically significant clinical

laboratory AE ongoing at the last visit will be followed for up to 6 months or resolution to baseline, whichever is shorter.

Those labs determined to be of no clinical significance or of unknown clinical significance (per the clinician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

For blood glucose measurements: All fasting glucose measurements ≥ 100 mg/dL per Section 8.5.1.1 should be reported as an AE.
A list of expected AEs that we have seen in previous vaccines can be found in§6.2., Reported adverse events and potential risks, as well as, in the Pharmaceutical Information, or the Investigator's Brochure.

11.1 Adverse Events

11.1.1 Reportable AEs

AEs that occur after the informed consent is signed and baseline assessments are completed (including run-in) must be recorded on the AE Case Report Form (paper and/or electronic) whether or not related to study agent. AEs including Serious Adverse Events (SAEs) will be collected through the 6-month follow-up visit.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting:

- CTCAE (MedDRA) System Organ Class (SOC)
- NCI Common Terminology Criteria for Adverse Events v4.0 (CTCAE) AE term (MedDRA lowest level term)
- Event onset date and event ended date
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a serious adverse event (SAE)
- Whether or not the patient dropped due to the event
- AE verbatim term
- Action
- Outcome
- Treatment assignment code (TAC) at time of AE onset

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the NCI Common Terminology Criteria for Adverse Events CTCAE v4.0. The CTCAE v4.0. provides descriptive terminology (MedDRA lowest level term) and a grading scale for each adverse event listed. A copy of the CTCAE 4.0 can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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

CTCAE v.4.0 general severity guidelines:

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

*Instrumental activities of daily living (ADL) refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

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

11.1.4 Assessment/Attribution of relationship of AE to treatment.
The possibility that the adverse event is related to study agent will be classified as one of the following attributions: unrelated, unlikely, possible, probable, definite.

11.1.5 Follow-up of AE's
Clinically significant laboratory Adverse Events will be reported to the participant to have them follow up with their primary care physician. That conversation will be documented in the research chart. Grade 3 or 4 AEs including lab abnormalities, which in the opinion of the investigator, are clinically significant, will be followed until resolution to baseline or stable.

11.2 Serious Adverse Events

11.2.1. DEFINITION: Regulations at 21 CFR Section 312.32 (revised April 1, 2014) defines serious adverse events (SAEs)as any untoward medical occurrence that at any dose has one or more of the following outcomes:

a. Death.
b. A life threatening AE
c. Inpatient hospitalization or prolongation of existing hospitalization.
d. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
e. A congenital abnormality/birth defect.
f. Important medical events that may not be immediately life-threatening or result in death of hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require intervention to prevent one of the other outcomes.

11.2.2. Reporting Serious Adverse Events to Division of Cancer Prevention

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

11.2.2.2 Contact the DCP Medical Monitor by phone and email within 24 hours of knowledge of the event.

Medical Monitor - Division of Cancer Prevention
Malgorzata (Margaret) Wojtowicz, M.D.
Lung and Upper Aerodigestive Cancer Research Group
Division of Cancer Prevention, NCI, NIH
9609 Medical Center Drive, Rm 5E-104, MSC 9781
Bethesda, MD 20892 (For FedEx, use Rockville, MD 20850)
Phone: (240)276-7012

E-mail: wojtowim@mail.nih.gov

The following information will be included when calling the Medical Monitor:

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

University of Wisconsin will alert the University of Washington, IND sponsor by one of the methods below:

Mary L. (Nora) Disis, M.D.
University of Washington
IND Sponsor
ndisis@u.washington.edu
Phone: 206-616-1823

11.2.2.3 The Lead Organization and all Participating Organizations will email written SAE reports to DCP's Regulatory Contractor CCS Associates, Inc. (CCSA; phone: 650-691-4400) at safety@ccsainc.com within 48 hours of learning of the event using the fillable PDF SAE Report Form.

11.2.2.4The DCP Medical Monitor and UW IND Sponsor will determine which SAEs require Food and Drug Administration (FDA) and National Institutes of Health Office of Biotechnology Activities (NIH OBA) submissions.

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

11.2.3 Follow-up of SAE

Site staff will send follow-up reports as requested when additional information is available. Additional information will be entered on the DCP SAE form in appropriate format. Follow-up information will be sent to DCP as soon as available. SAEs will be followed until resolution to baseline or stable.

11.2.4 Reporting to other agencies
Procedure for reporting serious adverse events:

a. Identify the "System Organ Class" of the adverse event as defined above using CTCAE v4.0. Attribution must be assigned by the study doctor.
b. After appropriate medical intervention has been instituted, the PI or his/her designee will be notified within the first 24 hours of the event.
c. File appropriate reports immediately by phone/fax with appropriate agencies
d. Notify the patient's primary doctor or referring doctor within a medically appropriate timeframe, depending on the severity of the adverse event.
e. Submit written reports to appropriate agencies.
f. Document the adverse event in the patient's research file, using a progress note to describe the event and treatment, if appropriate.
g. File copies of all forms/correspondence relating to the adverse event in the patient's research file.

FDA – Serious adverse events will be reported by UW, Dr. Nora Disis, (IND Sponsor) per 21 CFR 312.32 using the narrative format and/or MedWatch Form 3500A.

NIH OBA - any SAE will be reported per Appendix M. SAEs undergoing expedited reporting to the FDA as IND safety reports will be reported to OBA in the same timeframe.

Institutional Biosafety Committee (IBC) – IBC should be notified at the same time as NIH OBA. This study will go through UW and UWI's IBC and each group will report their institution's committee using the documentation submitted to the FDA.

IRB/IEC–UW and UWI will follow the current AE reporting policy of the institutional and NCI central IRB.

UW CRC - The UW CRC will receive copies of the documents submitted to the FHCRC/UW CC IRB within 7-10 calendar days.

FHCRC Biologic Production - The FHCRC Biologic Production will receive copies of the documents submitted by Dr. Disis, IND Sponsor, using either the narrative format, the MedWatch Form 3500A, or using the Genetic Modification Clinical Research Information System within 7-15 calendar days.

UWI Chemoprevention Consortium Data Safety Monitoring Board (DSMB) – the UW will follow the UWI Consortium DSMB.

11.3 Long-Term Follow-Up (LTFU)

Long-term follow-up will continue through review of clinical notes, lab values, and imaging reports requested annually from the participant's primary oncologist or primary care provider.

Each site will be responsible for requesting the selected medical records from their participants' primary oncologist or primary care provider. The collected information will be abstracted to record any possible autoimmune reactions and relevant health status.

This information will be reported annually to the FDA in order to satisfy their LTFU reporting requirements.

12. STUDY MONITORING

12.1 Data Management

All of the procedures outlined in the University of Wisconsin Chemoprevention Consortium standardized Data Management Plan (approved 09/23/2019) will be followed in this protocol. Please refer to this document for additional details on data management procedures. This plan will be followed until all participants have completed the 6-month follow-up visit.

Long-term follow-up data will be kept and managed by University of Washington per their institutional policy. Systems are in place to ensure data integrity. Once notes are received, a University of Washington Clinical Research Staff Member, designated by the IND Sponsor, will review the summary and report relevant autoimmune and relevant health status data in the database.

12.2 Case Report Forms

All data entry until completion of the 6 month follow-up visit will be performed by the Consortium Lead Organization (or CLO, the University of Wisconsin) staff. Electronic CRF (eCRF) screens will be created in OnCore. CLO staff will enter data into OnCore and transfer to Federal Security Compliant formats for transmission to DCP according to pre-established DCP standards and procedures. Amended CRFs will be submitted to the DCP Protocol Information Office for review and approval.

This study will report clinical data using the CLO database OnCore which is a web-based clinical trials/database. OnCore will be the database of record for the protocol and subject to NCI and FDA audit. All OnCore data entry including the 6 month follow-up visit will be performed at the CLO where staff is trained in OnCore per our DMP and applicable regulatory requirements such as 21 CFR; Part 11.

Data collected for LTFU is entered into a database that is password protected, has an audit trail and only allows limited access to patient information. Only research staff that has been designated by the IND Sponsor will have access to the LTFU data in the database. The database is regularly backed-up on tape from our secured server. This server network is HIPAA compliant and backed up to a tape on a regular basis so we may recover data that is lost for any reason. This is all managed according to HIPAA regulations.

12.3 Source Documents

In order to standardize the collection of study data, the University of Wisconsin (CLO) and the University of Washington will work together to create Visit Guides and Source Document Worksheets to ensure that all required data elements are captured. These documents will be used to supplement data collected on primary clinical source documents. All data reported must be documented either on a separate source document found in the patient's medical record or on the Visit Guides and Source Document Worksheets. All source documents must be signed by the staff that collected or elicited the information in the source documents. Source documents will be submitted to the CLO (either by fax, or e-mail) for entry into the OnCore database. Primary clinical source documents will be de-identified and relabeled with the patient's PID number prior to submission. All data is due at the CLO within 10 business days of each study visit.

Source documents for annual long-term follow-up will be submitted to the University of Washington and entered into the database. All data will follow the above de-identification process.

12.4 Data and Safety Monitoring Plan

All of the procedures outlined in the University of Wisconsin Chemoprevention Consortium standardized Data and Safety Monitoring Plan (approved 03/20/2018) as well as the Master Data and Safety Monitoring Plan: Addendum #1 will be followed in this protocol. The UW Chemoprevention Consortia Data and Safety

Monitoring Committee meet every 6-12 months to review all data from ongoing consortium studies. Members review pooled, unblinded safety data to assess ongoing human subject's safety. Data from this study will be monitored through the end of the 6 month follow-up visit by UW Chemoprevention Consortia DSMB. Data collected as part of long term follow-up will be monitored by the University of Washington.

12.5 Sponsor or FDA Monitoring

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

12.6 Record Retention

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

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

N/A

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This is a phase I non-randomized, dose - escalation study of safety and immunogenicity of a triple antigen vaccine. The study population consists of relatively healthy and stable patients with history of breast cancer.

Patients will be assigned sequentially to one of three arms defined by vaccine dose. Patients will be accrued to Arm 1 first, then Arm 2, and Arm 3.

Arm 1: WOKVAC (150 mcg) with rhuGM-CSF (100 mcg); administered i.d. every month for 3 total doses.
Arm 2: WOKVAC (300 mcg) with rhuGM-CSF (100 mcg); administered i.d. every month for 3 total doses.
Arm 3: WOKVAC (600 mcg) with rhuGM-CSF (100 mcg); administered i.d. every month for 3 total doses.

If there is sufficient evidence (please see Section 8.5.1. Criteria for Premature Study Termination) to suggest the Arm 1 dose is safe; the Arm 2 cohort of up to 12 patients will be enrolled. If the Arm 2 dose is also regarded as sufficiently safe, the Arm 3 cohort of up to 12 patients will be enrolled. If the Arm 3 dose appears safe, the immunologic efficacy among three dose groups will be examined to determine the recommended phase 2 dose for further cancer prevention studies. For the phase 2 study, safety and immunologic efficacy decisions will be made on the evaluation of data from the three possible doses.

After the Arm 3 dose, key study personnel in concert with the NCI/DCP staff will review the immunologic efficacy data along with safety with other laboratory findings to make a decision on an appropriate phase 2 dose.

13.2 Randomization/Stratification
N/A.

13.3 Accrual and Feasibility
The study will accrue 30 evaluable patients with up to 12 patients in each dose arm. We anticipate up to 15% of enrolled patients may fail to receive all three vaccinations and the 1 month post vaccine follow-up.

For information regarding the study population (including gender and minority considerations) please see Section 4.3 Inclusion of Women and Minorities.

In order to meet the primary objective of this study, AE information is collected at each of the study visits and reviewed by the study team on a regular basis. If patients are unable to complete to the one month post vaccination follow-up visit, AE information may be collected by phone if possible. For secondary objectives, the research samples will be batched after each cohort to achieve consistency in the assays.

When we batch the research samples we will run the baseline time point and post vaccine time point(s) for a patient (usually 4-5 patients at one time). With this we are looking at results of an individual (comparing their IFN-g response to their baseline) therefore the results can be analyzed together.

13.4 Primary Objective, Endpoint(s), Analysis Plan
A primary endpoint of the study is safety through the 6 month follow-up visit. Safety of the three escalating dose of WOKVAC will be assessed by adverse events as per CTCAE v4.0.

The type and grade of toxicities noted during the immunization regimen will be summarized. The duration of toxicities will also be summarized using descriptive statistics such as mean and standard deviation. All adverse events noted by the investigator will be tabulated according to the affected body system. The frequency and severity of adverse events will be summarized with a proportion and a 95% confidence interval. Despite lack of randomization, the incidence and severity of toxicity will be compared between successive dose groups using nonparametric test with the understanding that due to small sample size per dose group such test will have limited sensitivity to detect any small to moderate differences. These tests will be performed without adjustment for multiplicity of testing.

Demographic and baseline characteristics obtained at enrollment will be summarized in tabular or graphical formats with descriptive statistics.

13.5 Secondary Objectives, Endpoints, Analysis Plans
- To determine the immunogenicity of WOKVAC Th polyepitope plasmid based vaccine in patients with breast cancer at 3 escalating doses.
- To evaluate for the presence of a Th2 immune response by antibodies against the WOKVAC antigens
- To determine whether a WOKVAC Th polyepitope plasmid based vaccine elicits a persistent memory T cell response.
- To evaluate whether WOKVAC vaccination modulates T regulatory cells (Treg) and myeloid derived suppressor cells (MDSC).
- To determine a recommended phase 2 dose for further breast cancer prevention studies.
- To assess the long-term effects of 3 escalating doses of a deoxyribonucleic acid (DNA) plasmid

based vaccine encoding three breast cancer antigens (IGFBP-2, HER2, and IGF-1R) in patients with breast cancer, for 5 years from enrollment to satisfy the evaluation requirements for the Food and Drug Administration (FDA)

Immune responses as measured by IFN-g ELISPOT will be summarized with mean and standard deviation or median and range over time, the change over time will be summarized with graphs, and also analyzed using linear mixed-effects regression models with normalizing transformation if necessary. Each patient would be given a value at each immune evaluation that is the sum of the median response to HER2, IGFBP-2 or IGF1R, and a composite median would be calculated for each WOKVAC dose level.

Antibody immunity will be assessed as an additional measure of a Th2 response. A humoral immune response will be measured by ELISA and serum antibody avidity for IGFBP-2, HER2, and IGF-1R (using ELISA) to determine an avidity index (AI) before and after vaccination. Low avidity antibodies will have an AI <30%, moderate avidity antibodies 30-50% and high-avidity antibodies 51-100%. Patients will be considered to have developed an antibody response if antigen specific IgG antibodies are both detectable and have moderate to high avidity.
Memory T cell response will be summarized with proportion over time, and the change over time will be summarized with bar charts, and analyzed using generalized linear mixed effects regression models with suitable link function.

Treg cell, and MDSC levels will be summarized with mean and standard deviation or median and range over time, the change over time will be summarized with graphs, and analyzed again using linear mixed-effects regression models with normalizing transformation if necessary.

Analyses of the above secondary endpoints will be descriptive in nature by necessity. Despite lack of randomization, we will compare the immunologic efficacy between the 3 dose levels data using linear dose-response relationship with a regression analysis. Normalizing transformation will be used as well if necessary. The linear regression model will have a 0.80 power to detect an anticipated effect size (defined as $R_2/ (1-R_2)$) of 0.281 according to the level 0.05 F-test, given the sample size of 30.

Once the dose escalation is completed, key study personnel in concert with the NCI/DCP staff will review the toxicity, immunogenicity, antibody immune response, memory T cell response, Treg, and MDSC, and make a recommendation for a phase 2 dose. Statistical tests will only be advisory due to lack of randomization and limited sensitivity of such tests given the small sample size for each dose group. We may seek other experts in this deliberation.

13.6 Outcome measures

1. Primary endpoints: Safety through 6 month follow-up visit will be assessed per CTEP CTCAE v. 4.0.
2. Secondary endpoints:
    a. Immunogenicity will be evaluated via an assessment of the generation of IGFBP-2, HER2, and IGF-1R specific IFN-g and IL-10, IFN-g/IL-10 ratios, inducing T cells, Th1:Th2 ratios and IgG antibodies.
        (i) Cellular immune response will be defined by the magnitude of the Th1 (IFN-gamma (g)) antigen specific immune response using ELISPOT. Successful immunization will be defined by generating a protein specific IFN-g precursor frequency greater than 1:20,000 PBMC for each antigen. In patients with pre-existent immunity, responses must augment over 2 times baseline for immunization to be considered successful.
        (ii) Humoral immune response will be measured by ELISA and serum antibody avidity for IGFBP-2, HER2, and IGF-1R to determine an avidity index (AI) before and after vaccination. Low avidity antibodies will have an AI <30%, moderate avidity antibodies 30-50% and high-avidity

antibodies 51-100%. Patients will be considered to have developed an IGFBP-2 antibody response if antigen specific IgG antibodies are both detectable and have moderate avidity.

b. To detect persistent T cell memory, flow cytometry panels will be used to evaluate antigen specific central and effective memory phenotypes prior to and at 1 and 6, months after immunizations have ended for all 3 doses.
c. To detect modulation of Tregs and MDSC with vaccination, levels over the course of immunization will be assessed by flow cytometry of PBMC.
d. Long-term safety including vaccine toxicity and overall relevant health status.

13. 7 Reporting and Exclusions

If a patient drops from the study prior to becoming evaluable for immune responses, data will still be used to assess the safety of WOKVAC. In the event that an enrolled patient drops from the study for a reason other than a dose limiting toxicity (as defined in Section 8.5.1) prior to completing the 1-month post-vaccination follow up visit, then an additional patient will be enrolled onto the study, up to a maximum of 12 patients per dose level.

13.8 Evaluation of Toxicity

All patients who received WOKVAC on study will be evaluated for safety from the time of their first dose of WOKVAC. Primary safety endpoint will include data through the 6 month follow-up visit. Long term follow-up data will be collected to satisfy the evaluation requirements for the FDA.

We do not expect significant toxicity at any dose-level based on the safety demonstrated in infectious disease models using DNA plasmid based vaccines and results from previous vaccine trials from our group. These trials demonstrated the safety and low toxicity profile associated with development of immunity to HER2 or IGFBP-2 after vaccination to these antigens.

13.9 Evaluation of Response

The number of patients traditionally required to gather preliminary data on the safety of a dose in a phase I study is less than the number needed to evaluate immunologic responses and differences in the immunologic responses between cohorts of patients. Since Phase I tumor vaccine studies to date have proven to be relatively free of serious toxicity, the defining endpoint that would move a study forward to a more extensive phase II efficacy trial will likely be immunogenicity. In order to have meaningful immunologic data, the study will accrue 30 evaluable patients (10 patients per dose arm). When accrual is complete, we will: (1) determine if the WOKVAC vaccine elicits specific cellular and humoral immune responses for each antigen, and persistent antigen specific memory T cells can be detected and (2) which dose is most immunogenic. We expect that there may be differences in immune response between dose-levels. Moreover, the dose-level that is determined to be the most biologically relevant in terms of stimulating immunity may not be the highest dose.

Immune responses will be evaluated for patients who complete the vaccination series with both a baseline and a one month post vaccination series blood draw. Durability of generated immune responses will be evaluated in patients who complete the vaccination series with both a baseline and a 6 month post vaccination series blood draw.

13.10 Interim Analysis

There will be ongoing monitoring of toxicity data and the attribution as well as immunologic efficacy. The

secondary endpoints of immunogenicity, T-cell memory, Treg and MDSC levels will be analyzed after each dose level.

After each dose group, interim analysis of toxicity will be reviewed by key study personnel in consultation with NCI/DCP staff before proceeding to the next dose level. Dose escalation and accrual at current dose will follow the criteria described above in section 8.5.1 and statistical tests described above in sections 13.1 and 13.4.

13.11 Ancillary Studies
There are no ancillary components to this study.

14. ETHICAL AND REGULATORY CONSIDERATIONS

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

14.2 Other Required Documents

14.2.1 A current (within two years) curriculum vitae (CV) or bio-sketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

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

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

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

14.2.5 Documentation of Federal Wide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 IND will serve as the Investigator's Brochure.

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the PI for each site and initialed by all study personnel listed on the form.

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

14.3 Institutional Review Board Approval
Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO

according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation.

14.4 Informed Consent

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

Patients must be provided the option to allow the use of blood samples obtained during testing or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent.

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

14.5 Submission of Regulatory Documents

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

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

E-mail Submissions:
regulatory@ccsainc.com

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

Once all participants have entered long-term follow-up, regulatory documents will be handled by the IND Sponsor (University of Washington).

14.6 Other

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

15. FINANCING, EXPENSES, AND/OR INSURANCE

There is no payment for being in this study. Patients will be given the option of receiving pre-paid parking

vouchers for research visits to the UW CRC and the UWI CRU.

There is no charge for the medical costs directly related to the clinical and laboratory testing done as part of this study. The costs related to the preparation and administration of the vaccine will also be provided.

The patient or their insurer will be billed for treatment of problems that results from the patient's cancer or from standard clinical care.

If the patient has a research related injury or illness the patient or the patient's insurer will be billed for any additional costs.

There are no funds to pay the patient for loss of work or other costs, lost time, or pain to the patient or the patient's family.

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APPENDIX A

ECOG PERFORMANCE STATUS SCALE

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B

The National Comprehensive Cancer Network (NCCN) defines menopause as "generally the permanent cessation of menses, and as the term is utilized in breast cancer management, includes a profound and permanent decrease in ovarian estrogen synthesis." According to their guideline, the criteria for determining menopause are:

- Prior bilateral oophorectomy
- 60 or older
- Age less than 60 years; ammenorrheic for 12 months or more months in the absence of chemotherapy, tamoxifen, toremifene, or ovarian suppression; and follicle-stimulating hormone (FSH) and plasma estradiol in the postmenopausal range
- If taking tamoxifen or toremifene, and age is under 60 years, the FSH and plasma estradiol level should be in the postmenopausal range
- It is not possible to assign menopausal status to women who are receiving a leuteinizing hormone-releasing hormone agonist or antagonist. In women premenopausal at the time of adjuvant chemotherapy, amenorrhea is not a reliable indicator of menopausal status.