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A Phase II Randomized Study of Safety and Efficacy of a Multiple Antigen Vaccine (STEMVAC) in Non-Small-Cell Lung Cancer Patients

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Protocol Signature Page

Site Principal Investigator's Agreement

I have read "A Phase II Randomized Study of Safety and Efficacy of a Multiple Antigen Vaccine (STEMVAC) in Non-Small-Cell Lung Cancer Patients".

I have fully discussed the objectives of this trial and the contents of this protocol with the Sponsor representative.

I agree to conduct the study as outlined herein and in accordance with International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP), with applicable Food and Drug Administration (FDA) regulations set forth in 21 Code of Federal Regulations (CFR) Parts 50,54, and 312, and all other applicable regulatory requirements.

Principal Investigator Name: _____

_____ _____
Signature Date

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STUDY SYNOPSIS

Title

A Phase II Randomized Study of Safety and Efficacy of a Multiple Antigen Vaccine (STEMVAC) in Non-Small-Cell Lung Cancer Patients

Study Population

Patients with histologically-confirmed diagnosis of stage IV non-small cell lung cancer (NSCLC), that are not eligible for approved targeted therapies, have completed 3 - 4 cycles of chemoimmunotherapy (induction treatment), have achieved responsive or stable measurable disease and are receiving or are candidates to continue maintenance treatment with pembrolizumab +/- pemetrexed; the treatment would be dependent on histology. Patients will be enrolled after induction chemoimmunotherapy, and will have received no more than 2 cycles of maintenance pembrolizumab +/- pemetrexed. Patients will be randomized to one of two research treatments given concurrently with maintenance therapy.

Rationale

Immunotherapy is a significant treatment advance for NSCLC patients and the only therapy that can lead to a long term survival, although this occurs in less than 20%[1]. The current standard of care for patients with NSCLC is chemoimmunotherapy with carboplatin, pemetrexed/paclitaxel and pembrolizumab. These regimens were studied in two Phase III trial, which randomized patients to either chemotherapy plus immunotherapy (pembrolizumab) vs. chemotherapy alone. In patients with non-squamous histology the combination arm showed a clear survival advantage with a median overall survival of 22 months vs 10.7 months for those who received chemotherapy alone (HR 0.56 [95% CI 0.45-0.70], P < .00001)[2]. In patients with squamous histology the combination arm showed a survival advantage with a median overall survival of 17.1 months versus 11.6 for those patients treated with chemotherapy alone (HR, 0.71 (95% CI: 0.58–0.88)[50]. However, it is also clear that the majority of patients will have a limited response and die from their disease within 2 years. New therapies are needed to stimulate an immune response against the cancer that could in turn lead to long term responses in all patients.

Vaccines are able to induce tumor specific immunity and increase CD8 tumor infiltrating T-cells (TIL). High levels of TIL predicts response to immune checkpoint inhibitors (ICI) therapy in animal models and lung cancer patients[3, 4]. Unfortunately, high levels of TIL, defined as >50% of tumor stromal lymphocytes, are found in less than 10% of patients and less than a quarter of NSCLC patients have any evidence of TIL. Vaccine induced TIL could “jump start” the immune system to stimulate further clinical responses with concurrent pembrolizumab. Vaccines targeting biologically relevant antigens could recognize and eliminate lung cancer cells which have undergone epithelial to mesenchymal transition (EMT); a process which promotes metastases in lung cancer.

We developed a multi-antigen vaccine, STEMVAC, that targets proteins in the EMT pathway. We have identified epitopes within the sequence of non-mutated-tumor antigens that selectively induce Type 1 Th1 immunity which supports generation of CD8 cytolytic T-cells (CTL)[5]. Th1 selective epitopes significantly inhibit cancer growth by causing a marked influx of CD8 TIL[6, 7].

We hypothesize that immunizing patients with advanced NSCLC with measurable disease after induction chemoimmunotherapy while receiving immune checkpoint inhibitors will generate tumor tracking Type I T-cells resulting in increased activated CD8 TIL. To further study this hypothesis, we propose a randomized Phase 2 study in which patients with NSCLC that have completed 3 - 4 cycles of induction treatment will be randomized to treatment with STEMVAC+GM-CSF or GM-CSF alone concurrently with pembrolizumab +/- pemetrexed.

Objectives

Primary

1. To determine whether intradermal (ID) injection of STEMVAC+GM-CSF vaccination increases the percentage of CD8+ TIL in patients with advanced NSCLC compared to patients who receive ID GM-CSF alone.
2. To evaluate safety of STEMVAC immunization and concurrent pembrolizumab +/- pemetrexed maintenance therapy in patients with advanced NSCLC.

Secondary

1. To evaluate the magnitude of the immune response generated to STEMVAC when given in combination with pemetrexed and pembrolizumab maintenance therapy.
2. To determine whether vaccine induced T-cells traffic to tumor and can eliminate cancer cells which have undergone EMT.
3. To evaluate potential clinical efficacy of STEMVAC immunization by comparing overall response rate between both arms.
4. To determine whether vaccination increases CD8 T-cell activation markers (GZB, CD127 and PD1) and Type I immune cells (CD4 Th1) in the tumor.

Study Design

This is a randomized Phase II study in patients with advanced NSCLC who have completed induction therapy without progression and have measurable disease.

Patients receiving maintenance pembrolizumab +/- pemetrexed (per standard of care) will be randomized to one of two treatments:

ARM 1: ID administration of 300 mcg/dose STEMVAC admixed with 100 mcg/dose of GM-CSF

ARM 2: ID administration of 100 mcg/dose GM-CSF alone

Patients will undergo a core biopsy (fresh tissue biopsy is preferred; archival tissue can be used if fresh tissue core is not feasible (only if there sufficient archival tissue) of their tumor prior to the assigned immunization arm and again three weeks after their third vaccine. Patients may also receive up to 1 additional booster vaccine (with the same treatment arm originally assigned) approximately 9 weeks after the third vaccine.

Number of Patients

The study will accrue up to 40 patients with at least 20 patients per arm. Replacements are possible.

Outcome Measures

Primary Endpoints

1. Percentage of CD8+ TIL in patients between the two arms. Immunohistochemical (IHC) staining for CD3, CD4, and CD8 will be performed on the biopsies collected pre-treatment and post 3 vaccine administration.
2. Safety will be assessed per Common Terminology Criteria for Adverse Events (CTCAE) v5.0, physical exam and laboratory tests.

Secondary Endpoints

1. STEMVAC induced immunity in combination with pembrolizumab +/- pemetrexed. Chemoimmunotherapy might impact the immune response generated to STEMVAC. We will measure the magnitude of the Th1 STEMVAC specific immune response using IFN-g ELISPOT.
2. T-cells traffic to tumor and can eliminate cancer cells which have undergone EMT. We will assess TCR-beta (TCRb) gene usage in both T-cell lines expanded from peripheral blood and in the tumor biopsy, and the expression of EMT related genes in the tumor after vaccination with STMEVAC+GM-CSF or GM-CSF alone.
3. Clinical Efficacy of the vaccine. We will evaluate clinical response three weeks after the 3rd vaccine using RECIST 1.1. Although the study is not powered to definitively address overall response rate (ORR), data generated may give some indication of clinical utility.
4. T-cell activation and Type I lymphocyte markers will be evaluated by IHC staining in pre- and post-vaccine biopsies. We will evaluate $CD4^{+}Tbet^{+}$ (Th1), $CD4^{+}GATA3^{+}$ (Th2) and

activation markers (GZB, CD127, and PD-1) on CD8 T-cells in tumor biopsies.

List of Appendices

Abbreviations

AE	Adverse Event
ANA	Antinuclear antibodies
ANC	Absolute neutrophil count
APC	Antigen presenting cells
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CNS	Central Nervous System
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTL	Cytolytic T-cell
Co-PI	Co - Principal Investigator
DSMP	Data and Safety Monitoring Plan
EDTA	Ethylenediaminetetraacetic acid
EMT	Epithelial to mesenchymal transformation
FH	Fred Hutchinson
FH/UW CC IRB	Fred Hutchinson /University of Washington Cancer Consortium Institutional Review Board
HLA	Human leukocyte antigen
HRPO	Human Research Protections Office
ICI	Immune checkpoint inhibitor
ID	Intradermal
IFN-g	Interferon-gamma
IHC	Immunohistochemistry
IND	Investigational New Drug
MDSC	Myeloid Derived Suppressor Cells

Abbreviations, continued

NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NIH	National Institutes of Health
NIH OBA	National Institutes of Health Office of Biotechnology Activities
NSCLC	Non-small cell lung cancer
ORP	Office of Research Protections
rhuGM-CSF	recombinant human granulocyte-macrophage colony stimulating factor; GM-CSF; LEUKINE® (sargramostim)
SAE	Serious Adverse Event
Th	T helper cells
Th1	T helper 1 cells
Th2	T helper 2 cells
TIL	Tumor infiltrating lymphocytes
Treg	T regulatory cell
ULN	Upper limit of normal
UPN	Unique patient number
USAMRMC	United States Army Medical Research and Material Command
UW	University of Washington
UW IBC	University of Washington Institutional Biosafety Committee
UW TRU	University of Washington Translational Research Unit
UW IDS	University of Washington Investigational Drug Pharmacy
UW IT	University of Washington Information Technology

1. INTRODUCTION

Lung cancer remains the leading cause of cancer mortality within the Veterans Affairs Health Administration[8] and the United States accounting for 24% of all cancer deaths[9]. In 2020 it is estimated that there will be 228,820 new cases and 135,720 deaths within the United States. Lung cancer is composed of different histologies of which non-squamous makes the preponderance of cases. The majority of patients present with metastatic disease for which treatment is offered to alleviate symptoms and prolong life but is not curative, and most patients die from their disease within 2 years of diagnosis[2].

Immunotherapy through immune checkpoint inhibition has dramatically changed the paradigm of how we treat this disease and it's the only therapy that has been shown to provide a subset of patients with long term outcomes. Surviving more than 5 years with NSCLC was extremely rare before the era of immunotherapy, now although difficult to estimate given the novelty of this therapy, it appears that close to 20% patients treated with checkpoint inhibitors are alive 5 years after diagnosis[1]. However, the great majority of patients experience only a small benefit and ultimately die from their disease.

Vaccines developed for the treatment of cancer have been in therapeutic human clinical trials for several years and are, at last, showing some success in randomized Phase III studies[10, 11]. To be effective, vaccines must arm the immune system to target and destroy the disease-causing agent. The mechanisms of immune evasion by non-small cell lung cancer (NSCLC) are currently poorly understood. In an effort to provide more patients with therapies that can achieve long term survival, development of new immunotherapeutic agents, such as vaccines, that can overcome cancer resistance is of paramount importance. We have developed a multi-antigen polyepitope vaccine, STEMVAC, targeting immunogenic proteins involved in epithelial to mesenchymal transition (EMT); a process which promotes metastases in lung cancer. We hypothesize that immunizing patients with advanced NSCLC, after an initial response to chemo-immunotherapy will generate tumor trafficking Type I T-cells resulting in increased activated CD8 tumor infiltrating T-cells (TIL).

Recent evidence indicates that Type I immunity, associated with the production of interferon-gamma (IFN-g), is needed for cancer eradication. Type I immunity enhances cross priming at the site of cancer initiation by activating local antigen presenting cells (APC) to more efficiently present immunogenic proteins or tumor antigens to T cells. Cross priming is the primary method by which immunity is generated against cancer as tumor cells do not express the recognition molecules needed for immune activation. IFN-g is primarily secreted by CD4+ T-helper 1 cells (Th1). Vaccine strategies designed to elicit tumor antigen specific Th1 immunity have the potential to generate epitope spreading (a broadening of immunity to additional antigens), concurrently stimulate antigen specific cytotoxic CD8+ T cells, and establish immunologic memory. Immunologic memory will ensure that the destructive immune response will deploy when the antigen is expressed in the future.

We have identified 5 stem cell/EMT proteins (CDH3, CD105, YB-1, MDM2 and SOX2) that are overexpressed in lung cancer and associated with poor survival. We have created a vaccine, STEMVAC, composed of extended Th1 epitopes derived from these proteins. Vaccination in lung and breast mouse models has shown immunization with STEMVAC antigen sequences to be safe and inhibit tumor growth. We have recently completed a Phase I study where we enrolled 32 patients to determine the safety and potential efficacy of up to 3 escalating dose-levels of STEMVAC. All 3 doses had an acceptable safety profile with the majority of adverse events being grades 1 and 2.

We propose a Phase II randomized clinical trial administering one of two treatment arms (1) STEMVAC+GM-CSF or (2) GM-CSF alone in patients with advanced non-squamous NSCLC who have completed induction

therapy without progression, have measurable disease and continue on pemetrexed/pembrolizumab maintenance (per standard of care).

Our primary endpoints are to assess whether intradermal (ID) injection of STEMVAC+GM-CSF vaccination increases the percentage of CD8+ TIL in patients with advanced NSCLC compared to patients who receive ID GM-CSF alone and the safety of immunization with concurrent pemetrexed/pembrolizumab maintenance therapy in patients with advanced NSCLC. The secondary objectives are to evaluate the magnitude of the response induced by STEMVAC concurrent with pemetrexed/pembrolizumab maintenance therapy, determine whether vaccine induced T-cells traffic to tumor and can eliminate cancer cells which have undergone EMT, to evaluate the clinical efficacy of the vaccine by comparing overall response in both arms, and determine whether vaccination with STEMVAC increases T-cell activation markers and Type I immune cells.

2. BACKGROUND

2.A. Role of immunotherapy in non-small cell lung cancer (NSCLC) treatment. Lung cancer is the leading cause of death from cancer, accounting for almost one third of all cancer deaths. The most common type of lung cancer is NSCLC that constitutes 85% of all lung cancer cases. Most patients are diagnosed at late-stage, when the treatments have a limited efficacy and treatment is palliative. For many years, approaches to improve outcome and survival focused on decreasing smoking rates, identification of early biomarkers and development of new imaging techniques for early detection, although without much success. Immunotherapy launched a new era in the treatment of NSCLC. Immune checkpoint inhibitor (ICI) monoclonal antibodies have changed the standard of care for metastatic NSCLC. The combination of chemotherapy and pembrolizumab as first line therapy for advanced PD-L1 positive NSCLC patients has significantly increased progression free and overall survival as compared to chemotherapy[2]. The overall response rate to combination immuno-chemotherapy is 48% as compared to 19% for chemotherapy alone in patients with non-squamous lung cancer and 62.6% vs 38.4% in patient with squamous NSCLC. Unfortunately, most clinical responses are transient and although patients are living longer, by 20 months after the initiation of treatment less than a quarter of patients are free of disease and the majority eventually succumb to NSCLC[2,50]. Our aim is to improve the success of immunotherapy to all patients.

2.B. Vaccines to elicit tumor specific Type I T-cells in NSCLC. There has been intense interest in defining the predictors of response to ICI in NSCLC in an attempt to optimize immunotherapy for all patients with the disease. Three markers predict outcome: (1) tumor mutational burden, (2) PD-L1 expression in the tumor, and (3) CD8+ tumor infiltrating lymphocytes (TILs). Tumors with high mutational burden have a higher likelihood of processing and presenting those mutations in class I MHC molecules for the generation of CD8 cytotoxic T-cells (CTL). Mutations can appear foreign to the immune system and elicit a more “viral-like” response. About 50% of all NSCLC have the level of mutations needed to potentially stimulate recognition of mutation related antigens[12]. Patients with PD-L1 expression of or above 50% of the tumor cells have increased rate of response to treatment with ICI, although responses are usually partial and most of them stop responding and relapse. Population based studies of tumor immune infiltrates have defined the type of immune response needed for clinically effective anti-tumor immunity; a high density of Type I T-cells, both CD4+ (Th1) and CD8+ that have trafficked to tumor, have penetrated the stroma, and are of a memory phenotype[13, 14]. Investigators studying factors that predict response to ICI in NSCLC have found Type I immunity, especially CD8 TIL, present in the tumor at the time of treatment indicates responders. The reality, however, is that high levels of TIL, defined as >50% of tumor stromal lymphocytes, are found in less than 10% of patients and about a quarter of NSCLC patients have no evidence of TIL[15, 16]. The tumor microenvironment in many NSCLC patients is Type II (Th2), characterized by T regulatory (Treg) cells, neutrophils and type 2 macrophages that prevent an efficient Type I response and contribute to escape from the immune system[17, 18].

In order to increase the response rate to ICI we need strategies to generate Type I T-cells, especially CD8 T-cells, in patients with NSCLC who have not completely responded to immuno-chemotherapy. Methods to increase Type I TIL at the time of maintenance pemetrexed/pembrolizumab in patients with measurable disease may stimulate further clinical responses as T-cells are elicited. Vaccines able to induce a strong Type I immune response could increase the CD8 T-cells infiltrating the tumor and improve responses to ICI.

2.C. T-helper Type I (Th1) vaccines as a vehicle for lung cancer treatment. What antigens should we target in a lung cancer vaccine? Lung cancer has one of the highest mutation rates of all types of cancer. Mutations can elicit immune response, as they look foreign to the immune system. However, mutations in NSCLC are highly heterogeneous not only between patients but also within the tumor, which makes difficult to define a vaccine based on mutated antigens that could succeed in most of the patients. In contrast, overexpressed non-mutated proteins in NSCLC are commonly shared between patients, as overexpression respond to the requirements of the tumor cells to grow and divide. Non-mutated tumor antigens have long been studied in cancer vaccines. Overexpression of the protein alters the manner in which immunogenic epitopes are displayed in MHC molecules as compared to the protein expressed at a basal level thus making immune recognition tumor specific[19]. Unfortunately, a barrier to the successful clinical application of vaccines targeting non-mutated tumor associated proteins is the finding that specific regions (epitopes) of these antigens induce T-regulatory (Treg) cells which are expanded when the epitopes are included in a vaccine formulation. The result is a suppression of therapeutically effective immunity[6, 20, 21].

Our group has focused on the development of vaccines specifically designed to elicit CD4+ T-cells for several reasons; (1) Type I CD4+ T-cell responses, Th1, can significantly alter the immune microenvironment to support a dominant cytotoxic T-cell response and epitope spreading which is a broadening of the immune response to multiple antigens in the tumor through enhanced cross-priming by cytokine activated antigen presenting cells (APC), (2) CD4 T-cell epitopes can be identified which bind with high affinity across multiple HLA-DR alleles (promiscuous epitopes) to create a universal vaccine which can be processed by diverse HLA and (3) appropriately primed CD4 T-cells establish immunologic memory which is essential for anti-tumor efficacy[22, 23]. We have developed a novel method of Class II (CD4) epitope identification that included functional screening to identify Th2 or Treg inducing epitopes with the aim of editing out those sequences to allow unfettered Type I immunity to be generated. Our model antigen was Insulin Growth Factor Binding Protein (IGFBP)-2 which we had defined as immunogenic in patients with cancer and a protein associated with increased metastases[6]. We identified putative Class II interacting sequences via a multi-algorithm approach to ensure responsiveness across diverse HLA alleles [24]. Secondly, we conducted population-based screening of predicted IGFBP-2 epitopes for Type I and II cytokine secretion to determine sequences that elicit Th2 or Treg using samples reflective of diverse HLA types (>95% population coverage (n=40)). We found that there were sequences of IGFBP-2 that, across most individuals studied, elicited primarily IFN-gamma (g) (Th1) or IL-10 (Th2) responses. IGFBP-2 is highly homologous between mouse and man (82%). Mice immunized with Th1 selective or Th2 selective sequences of IGFBP-2 developed a similar restricted phenotype of response as we observed in human T-cells in in vitro culture. Immunization with the Th1 selective vaccine significantly inhibited the growth of an implanted syngeneic epithelial tumor ($p<0.01$), while immunization with the Th2 selective epitopes had no effect on tumor growth ($p>0.05$ to control). When equal concentrations of the two vaccines were admixed and then used to immunize, the Th2 inducing vaccine completely abrogated the anti-tumor effect of the Th1 selective vaccine [6]. We have subsequently shown our Th1 selective vaccines induce tumor specific immunity and increase CD8+ TIL in animal models[7]. These data demonstrate that unless regulatory sequences are removed from vaccines targeting non-mutated tumor associated proteins effective immunity cannot be generated due to the immune-dominance of self-regulatory peptides.

2.D. STEMVAC: a Th1 vaccine targeting the epithelial to mesenchymal transition (EMT). EMT is a process by which anchorage dependent cancer cells develop the capacity to metastasize and become drug resistant as a stem cell-like phenotype emerges[25]. Metastases and the development of drug resistance are two of the main factors leading to relapse and death in NSCLC. EMT is a common process in metastatic NSCLC. Evidence of circulating tumor cells which have undergone EMT are present in most patients at this stage [26]. Tumor EMT signatures are associated with a lack of T-cell infiltration in NSCLC[27]. The presence of EMT increases expression of multiple immunosuppressive cytokines, including IL-10 and TGF-beta and the upregulation of numerous secondary immune checkpoint proteins[27]. Elimination of NSCLC cells that have undergone EMT may both limit metastasis and augment immunity.

We have identified several proteins on the EMT pathway, CDH3, CD105, YB-1, MDM2, and SOX2, which are important in lung cancer progression and are overexpressed in NSCLC as compared to normal lung tissue. CDH3 is highly expressed in more than 60% of NSCLC tumors[28]. High CDH3 expression has been significantly associated with metastasis, stage, shorter disease-free survival (DFS) and overall survival (OS) compared with low expression. CD105 upregulation correlates with neo-vascularization and angiogenesis and is highly expressed during EMT[29]. CD105 overexpression may define EMT in NSCLC. YB-1 is a protein involved in transcription and translation and is overexpressed in 50% of advanced NSCLC. YB-1 over-expression in lung cancer is associated with the development of metastasis and inferior OS[30]. MDM2 is also overexpressed in about 50% of NSCLC[31]. As a mediator of drug resistance, MDM2 overexpression is associated with reduced DFS and OS in multivariate analysis ($p<0.01$ for both comparisons)[32]. SOX2 overexpression is found in more than a third of NSCLC and is an independent predictor of poor prognosis[33]. Inhibition of SOX2 expression inhibits the migration and invasion of cancer cells in culture[34]. We hypothesize that proteins overexpressed in stem cells or in EMT can serve as vaccine candidates. We have used the Th screening method described before to identify epitopes that preferentially elicit Type I but not Type II cytokines for each of the 5 antigens included in the vaccine. These Th1 inducing epitopes are encoded in STEMVAC.

2.E. STEMVAC is immunogenic and safe in a Phase I clinical trial. We have performed a Phase I dose escalation study of 150mcg, 300mcg, and 600mcg of intradermal (ID) STEMVAC with 100mcg GM-CSF in patients with advanced stage breast cancer (NCT02157051). Vaccines were given once a month for 3 immunizations followed by 2 booster immunizations at 6 and 9 months. Thirty patients were enrolled (10/arm) and the primary objectives of the study were to determine the safety and optimal immunogenic dose of STEMVAC. Secondary objectives were to determine whether booster vaccines enhanced the level of immunity achieved and whether Treg cells or myeloid derived suppressor cells (MDSC) were elicited or impacted vaccine outcomes. Immunity was assessed by IFN-g and IL-10 ELISPOT. STEMVAC was immunogenic and we defined the 300mcg dose as optimal. IFN-g STEMVAC specific immune responses were detected at all dose levels and no increase in IL-10 secreting STEMVAC specific T-cells was observed. Flow cytometry evaluation revealed no increase in Treg levels, MDSC, or increase in PD-1 expression on either CD4 or CD8 T-cells. The 150mcg dose was the least immunogenic, while he 300mcg and 600mcg doses were equally immunogenic. STEMVAC immunization was safe, 98% of all adverse events (AE) were grade 1 or 2. Of the possibly, probably, or definitely related AEs the most common included injection site reaction (30%), flu-like symptoms after vaccination (18%), transient leukopenia (18%), transient lymphopenia (9%), arthralgia (9%) and fatigue (9%). There were 4 grade 3 events that consisted of transient lymphopenia temporally related to vaccination. Further, there was no clinical evidence of the development of autoimmunity. Several measures assessing the potential of subclinical autoimmunity were made evaluating serum antinuclear antibodies (ANA), C3, anti-dsDNA, and TSH at each time point. Only one grade 1 autoimmune AE was recorded on the study; a conversion of an ANA from 0 to a titer of 1:40 which corrected spontaneously on subsequent testing.

These data suggest that plasmid-based DNA vaccination, using sequences derived from tumor associated proteins such as those included in STEMVAC, which contain numerous Th1 epitopes, can be safely administered and result in long lived immunity.

2.F. GM-CSF as an immunotherapeutic in lung cancer and a vaccine adjuvant. We included a GM-CSF alone control arm due to the potential anti-tumor activity of GM-CSF alone in NSCLC. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine secreted by multiple cells including macrophages, T cells, mast cells, natural killer cells, endothelial cells and fibroblasts. This potent cytokine facilitates the maturation and migration of dendritic cells to lymph nodes and enables the presentation of tumor-associated antigens to generate a T-cell response. GM-CSF has been evaluated alone or in combination with radio- or chemo-therapy in several malignancies[35, 36]. NSCLC patients treated with an EGFR inhibitor in combination with radiotherapy and GM-CSF showed a significantly increased objective response (ORR 95.2% vs. 71.4%, $p<0.05$) compared to those patients treated with the EGFR inhibitor alone. Treatment with radiotherapy and GM-CSF significantly increased CD3+ and CD4+ T-cells ($p<0.05$) in blood, and improved one-year progression free survival ($p=0.047$)[37]. Combination of GM-CSF with radiotherapy has shown to induced immune mediated tumor regression at metastatic sites distant to the irradiated field (abscopal effect). In a phase II trial to evaluate abscopal response induced by radiotherapy + GM-CSF in patients with solid metastatic tumors, including lung cancer, 27% of patients presented abscopal responses, defined as a reduction of at least 30% in any measurable non-irradiated lesion >1cm. Those patients with abscopal response also showed improved overall survival (20.98 vs 8.33 months)[38].

Incorporation of adjuvants to vaccines increases their immunogenicity. As a vaccine adjuvant, GM-CSF upregulates co-stimulatory (CD80 and CD86) and MHC class II molecules, activating dendritic cells (DCs), CD4+ and CD8+ T-cells[39]. GM-CSF has been tested in numerous animal and human trials as vaccine adjuvant for anti-tumor immunotherapy in prostate, skin, breast and lung cancer[40, 41]. GM-CSF drives the activation of macrophages to an M1-like pro-inflammatory phenotype in vitro[42-44], producing chemokines for leukocyte recruitment[45] and pro-inflammatory cytokines[42]. These molecular signals contribute to the role of GM-CSF in the differentiation and activation of T helper (Th) type 1 and Th17 cells, further promoting pro-inflammatory events[46]. The use of GM-CSF as an adjuvant to the plasmid-based STMEVAC vaccine, which contain numerous Th1 epitopes, could potentiate the T helper (Th) type 1 immunity.

3. OBJECTIVES

3.A. Primary objectives

1. To determine whether intradermal (ID) injection of STEMVAC+GM-CSF vaccination increases the percentage of CD8+ TIL in patients with advanced NSCLC compared to patients who receive ID GM-CSF alone.

2. To evaluate safety of STEMVAC immunization and concurrent pembrolizumab and/or pemetrexed maintenance therapy in patients with advanced NSCLC.

3.B. Secondary objectives

1. To evaluate the magnitude of the immune response to STEMVAC when given in combination with pembrolizumab and/or pemetrexed maintenance therapy.
2. To determine whether vaccine induced T-cells traffic to tumor and can eliminate cancer cells which have undergone EMT.
    a. To evaluate by TCR beta (TCRb) sequencing in matched peripheral blood mononuclear cells (PBMC) of patients and in biopsy TILs whether the same TCRb clones in blood are found in abundance in tumor.

b. Biopsies before and after vaccination will be compared to determine if vaccination modulates the EMT gene signature in the tumor.

3. To evaluate potential clinical efficacy of STEMVAC immunization by comparing overall response rate between both arms.
4. To determine whether vaccination increases CD8 T-cell activation markers (GZB, CD127 and PD1) and Type I immune cells (CD4 Th1) in the tumor.

4. AGENT INFORMATION

4.A. Multi-antigen vaccine

The plasmid-based DNA vaccine we will use clinically is called STEMVAC (IND 16249, Sponsor: Mary L. Disis, M.D.). The STEMVAC fusion protein is 352 amino acids in length and contains epitopes derived from the CD105, Yb-1, SOX2, CDH3, and MDM2 proteins. The epitopes range from 32 to 92 amino acids in length.

4.A.1. Synthesis and characterization

STEMVAC for clinical use has been amplified, quantified and vialed by the Biologics Production Facility at the Fred Hutchinson under Good Manufacturing Practice laboratory conditions.

4.A.2 Formulation and stability

The vaccine is vialed as a single dose containing DNA suspended in tromethamine/EDTA (TE) as a stabilizing buffer. 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. Vials will be subjected to microbial, sterility and stability testing to ensure safety and stored at -20 ± 5ºC until use.

4.A.3. GM-CSF

Recombinant human granulocyte-macrophage colony stimulating factor (rhuGM-CSF; Sargramostatin, LEUKINE) will be used as adjuvant admixed with STEMVAC or administered alone and will be obtained by commercial suppliers and stored at their specifications.

GM-CSF is a growth factor that supports the survival, clonal expansion, and differentiation of hematopoietic progenitor cells including dendritic APCs. In general, the use of GM-CSF and rhuGM-CSF is associated with little toxicity when administered intravenous or subcutaneous injection. It is generally well tolerated at doses ranging from 50-500 mcg/m^2/day. Severe toxicity is extremely rare in patients treated with rhuGM-CSF. For the current study, rhuGM-CSF will be used at a total injection dose of 100 mcg.

Administration of rhuGM-CSF may aggravate fluid retention in patients with pre-existent edema, capillary leak syndrome, or pleural or pericardial effusions. In some patients with pre-existing renal or hepatic dysfunction, elevation of the serum creatinine or bilirubin and hepatic enzymes has occurred during rhuGM-CSF administration. Dose reduction or interruption of rhuGM-CSF administration has resulted in a decrease to pretreatment values. Occasional transient and reversible supraventricular arrhythmia has been reported in uncontrolled studies, particularly in patients with a previous history of cardiac arrhythmia. Stimulation of marrow precursors with rhuGM-CSF may result in a rapid rise in white blood cell count. If rhuGM-CSF is being used for the purpose of hematopoietic reconstitution, dosing should be stopped if the absolute neutrophil count (ANC) exceeds 20,000/cm^3. The dose being used in this study should not have an effect on ANC levels. RhuGM-CSF may stimulate the growth of myeloid malignancies; therefore caution must be exercised in its use in these malignancies or myelodysplastic syndromes. Our group has given over 1100 vaccine injections mixed with 100 mcg GM-CSF to patients on previous studies. During those studies patients sometimes complained of

mild to moderate flu like symptoms (fever, chills, achy, fatigue) for 1 – 2 days following vaccination which may be related to the use of GM-CSF.

Patients with contraindications to receiving rhuGM-CSF will not be eligible for study as noted in Section 6.B.3. Contraindications will include: (1) previous allergic reactions to GM-CSF, (2) laboratory values outside of an adequate range as described in Section 6.A.11 and (3) cardiac conditions as described in Section 6.B.1.

RhuGM-CSF is a sterile, white, preservative-free lyophilized powder in 250 mcg vials. Reconstituted rhuGM-CSF (Sargramostim, LEUKINE) will be used as adjuvant admixed with STEMVAC plasmid-based vaccine or given alone at the 100 mcg/dose. This will be administered intradermally.

4.A.4. Availability

STEMVAC for clinical use has been vialed by the Biologics Production Facility at the Fred Hutchinson 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. Each pharmacy will order this per their own procedures.

The STEMVAC vaccine is supplied as frozen vials and each vial carries a label bearing the drug identification and conditions for storage. The site pharmacy will prepare and dispense the vaccine for administration per a standard operating procedure established by the University of Washington Investigational Drug Service (UW IDS) by pulling a single vial vaccine from the freezer and thawing it slowly at room temperature. The rhuGM-CSF (Sargramostim, LEUKINE) will be reconstituted in sterile preservative free water. To admix vaccine with GM-CSF, it 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 3 Tuberculin Safety Syringes or 3 1 mL syringe with .01mL graduation with a 27 gauge, ½ inch needle for a total of 3 intradermal injections (2 syringes x 0.33 mL and 1 syringe x 0.34) each for intradermal injection. For rhuGM-CSF alone, it will be reconstituted and divided into two Tuberculin Safety Syringes or divided into two 1 mL syringe with .01mL graduation with 0.20ml each as described above.

4.A.5. Agent distribution

STEMVAC will be managed by the University of Washington Investigational Drug Service (UW IDS) as delegated by the IND Sponsor. 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 VA Puget Sound will each obtain the adjuvant, rhuGM-CSF (Sargramostim, LEUKINE) directly.

The prepared vaccine is stored at the University of Washington, Gene and Cell Therapy Laboratory (UW GCTL), a GMP facility, until it is transported to the UW IDS where it will be dispensed to UW patients or shipped/transported to VA Puget Sound for administration. Vaccine shipped/transported to VA Puget Sound will be sent/transported overnight on dry ice. Once received at VA Puget Sound the chain of custody the vaccine stored in the research freezer per label specifications and documentation should be completed. We are planning 2 shipments over the study, but if an additional request needs to be made VA Puget Sound should contact UW IDS using the contact information below:

Investigational Drug Services
Reference Study Number: RG1013946
FAX: 206-598-4901
Phone: 206-598-6054

4.A.6. Agent accountability
The Principal Investigator, Shaveta Vinayak, or a responsible party designated by the co-PI, must maintain a record of the inventory and disposition of all agents received. The PI 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.

4.A.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 UW GCTL and site pharmacies at temperature -20°C ± 5°C.

The GM-CSF will be stored per package insert. It should not be frozen or shaken.

4.B. Concurrent pembrolizumab +/- pemetrexed maintenance therapy
In patients with non-squamous disease pemetrexed will be administered IV every 3 weeks, at 500 mg/m2. This is part of the standard of care, administered by the patient's own oncologist. Commercial supply will be used, and the administration of this agent and the supportive care needed (i.e. steroids, folate, vitamin b12 and anti-nausea medications) will follow institutional guidelines. Steroid use should not exceed ≥ 7 days.

Pembrolizumab will be administered IV every 3 weeks, at a fixed dose of 200 mg. This is part of the standard of care, administered by the patient's own oncologist, and commercial supply will be used, and the administration of this agent will follow institutional guidelines.

5. VACCINE ADMINISTRATION
Vaccines will be administered on an outpatient basis at the University of Washington Translational Research Unit (UW TRU) or the VA Puget Sound Health Care System (VA Puget Sound). The vaccine will be administered on day 14 (+ 3 days) after maintenance therapy.

5.A. Final vaccine preparation
The STEMVAC vaccine is supplied in single-use vials as a sterile, frozen solution. Each single-use vial has a final concentration of 1mg/mL or 0.5 mg of DNA / 1 mL TE. Each STEMVAC vaccine will carry a label bearing the drug identification and conditions for storage. For Arm 1 we will reconstitute recombinant human GM-CSF (rhuGM-CSF, Sargramostim) will be used as adjuvant admixed with the STEMVAC plasmid based vaccine (see table below for details of preparation).

ARM 1: **STEMVAC Vaccine Admixed with GM-CSF** (nominal strength STEMVAC 300 mcg + 100 mcg GMCSF)	**STEMVAC Vaccine Vial Dosage**	**Number of ID Injections per dose**
Original/First Batch: 302.2 mcg STEMVAC + 99.4 mcg GM-CSF	1 mg/1 mL	**2 ID** injections x 0.35 mL
New/Second Batch Lot: 301.3 mcg STEMVAC + 99.3 mcg GM-CSF	0.5 mg/1 mL	**3 ID** injections (2 x 0.33 mL and 1 x 0.34 mL)

ARM 2: 100 mcg of GM-CSF

5.B. Vaccine 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 as 2 intradermal injections for the 1mg/1mL vial dosage 3 intradermal (ID) injections for the 0.5mg/1mL vial dosage. 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. Trained medical staff 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 the same general area approximately every three weeks along with a with a booster vaccine. 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. Additionally, anaphylactic reactions to vaccines should they occur, usually appear within 5 to 60 minutes.

5.C. Contraindications

Patients should not be pregnant or become pregnant throughout the remainder of the study. Patients should not concurrently enroll in any other treatment study. Participants scheduling a non-study-related vaccination (COVID-19, flu, shingrix, etc.) should ensure a minimum of 14 days between the vaccine dose and any STEMVAC vaccination.

5.D. Concomitant Medications

Throughout the duration of the study patients should not be on other cytotoxic chemotherapy aside from pemetrexed, systemic steroids (aside from those prescribed with pemetrexed or for immune related adverse events), monoclonal antibodies (with the exception of pembrolizumab), and/or other biologic therapy.

Systemic steroid therapy of $\geq$ 7 days is considered chronic use for this protocol. If a patient needs to be on systemic steroids $\geq$ 7 days please consult the IND sponsor before withdrawing the patient from the study. Topical, ocular, intra-articular, intranasal, inhalational corticosteroids (with minimal systemic absorption) and treatment for an immune-related adverse event are allowed (Section 10.A).

Bisphosphonates, such as denosumab, are permitted. 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 and may include: start and stop dates, dose, route of administration, and indication. Medications taken for a study procedure (e.g., biopsy) should also be included.

5.E. Dose Modifications/Delay

5.E.1. Vaccine

There are no dose modifications for the vaccine as part of this protocol. There may be allowances for the timing of the administration of vaccine to accommodate for special circumstances (i.e. illness). There may be vaccine administration interruptions due to holding of pemetrexed and/or pembrolizumab. If there are immune related adverse events (irAE) that are treated with systemic steroids at the time of scheduled vaccine, the vaccine administration should also be delayed. Any delay of vaccine, will be pre-approved by a PI, study physician, physician extender and/or IND sponsor and documented in the patient study chart.

The study team and the patient's oncology team will communicate any changes in the patient's treatment plan. Changes will be discussed with the study physician, PI/Co-PI, physician extender and/or IND sponsor in regards to vaccine administration.

5.E.2. Pemetrexed

After the first administration pemetrexed may be reduced, interrupted, or discontinued at the patient's clinicians' discretion per the approved product labels and local regulations. If pemetrexed is interrupted or discontinued, pembrolizumab may be continued.

5.E.3. Pembrolizumab

Based on existing clinical study data, most immune related adverse events (irAEs) were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids, and/or other supportive care. When an irAE is suspected every effort should be made to discard other possible etiologies that are non-immune related. The ASCO/NCCN guidelines should be followed to manage irAE[33] at the patient's oncologists' discretion.

6. PATIENT SELECTION

6.A. Inclusion criteria

1. Histologically-confirmed diagnosis of stage IV non-squamous or squamous NSCLC.
2. Have measurable disease based on RECIST 1.1 within one month of first vaccine. Target lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.
3. Have completed 3 - 4 cycles of chemoimmunotherapy, without evidence of progressive disease. Pembrolizumab has to be included in at least 3 of these cycles.
4. Have not received more than 2 cycles of maintenance pembrolizumab and/or pemetrexed and be a candidate for continuation of this therapy.
5. At least 1 site of disease that could be biopsied during treatment. This site should not be a site that is used to determine measurable disease for efficacy purposes. Lesions that will be biopsied should not be on a previously irradiated area unless progression has been demonstrated in such lesions.
6. Patients must be at least 28 days post systemic steroids prior to enrollment, unless this is a steroid administered concurrently with chemotherapy or used as part of prophylaxis to prevent IV contrast reactions.
7. Patients must have ECOG Performance Status Score of 0 or 1 (Appendix A).
8. Patients must have recovered from major infections and/or surgical procedures, and in the opinion of a PI/Co-PI/study physician/physician extender, not have any significant active concurrent medical illnesses precluding protocol treatment.
9. Willing to undergo up to two serial biopsies while on study.
10. Estimated life expectancy of more than 6 months.
11. Adequate laboratory values within 60 days of first vaccination defined as follows:
    a. WBC $\geq 3000/mm^3$
    b. Lymphocyte count $\geq 800/mm^3$
    c. Platelet count $\geq 75{,}000/mm^3$
    d. Hgb ≥ 9 g/dl
    e. Serum creatinine ≤ 1.2 mg/dl or creatinine clearance > 50 ml/min
    f. Total bilirubin ≤ 1.5 mg/dl.
    g. AST/SGOT ≤ 2 times upper limit of normal (ULN) or SGOT ≤ 5 times upper limit of normal (ULN) in the presence of liver metastasis

12. If female of childbearing potential has a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of study medication.
13. All patients who are having sex that can lead to pregnancy must agree to contraception for the duration of study.
14. Patients must be at least 18 years of age.

6.B. Exclusion criteria

1. Patients with any of the following cardiac conditions:
   a. Symptomatic restrictive cardiomyopathy
   b. Unstable angina within 4 months prior to enrollment
   c. New York Heart Association functional class III-IV heart failure on active treatment
   d. Symptomatic pericardial effusion
2. Patients with central nervous system (CNS) metastasis that have not been treated. Patients with previously treated brain metastases may participate provided they are clinically stable for at least 4 weeks and, have no evidence of new or enlarging brain metastases and also are off steroids for 2 weeks prior to dosing with study medication.
3. Patients with any contraindication to receiving rhuGM-CSF based products.
4. Patients with any clinically significant autoimmune disease that requires active treatment with immunosuppressants. Replacement therapy (e.g., thyroxine, insulin) is not considered a form of systemic treatment. Administration of systemic steroids (i.e., for allergic reactions, CT scans, or the management of irAEs) is allowed.
5. Has a known history of another prior invasive malignancy within 2 years, except subjects with early stage cancer that has undergone potentially curative therapy with no evidence of that disease recurrence for 2 years since initiation of that therapy.
6. Patients who are simultaneously enrolled in any other treatment study.
7. Patients who are pregnant or breastfeeding.
8. Patients with genetic driver alterations (e.g EGFR, ALK, ROS1, BRAF, MET ex 14, RET) for which targeted treatment exist and are FDA approved, except if the subject is not eligible or has progressed through those therapies.

7. EXPERIMENTAL DESIGN

7.A. Study design

This is a Phase II randomized study to examine the safety and efficacy of in patients with advanced NSCLC who have completed induction therapy without progressive disease and have measurable disease and are continuing on maintenance pembrolizumab +/- pemetrexed.

Patients receiving concurrent pembrolizumab +/- pemetrexed (per standard of care) will be randomized to either:

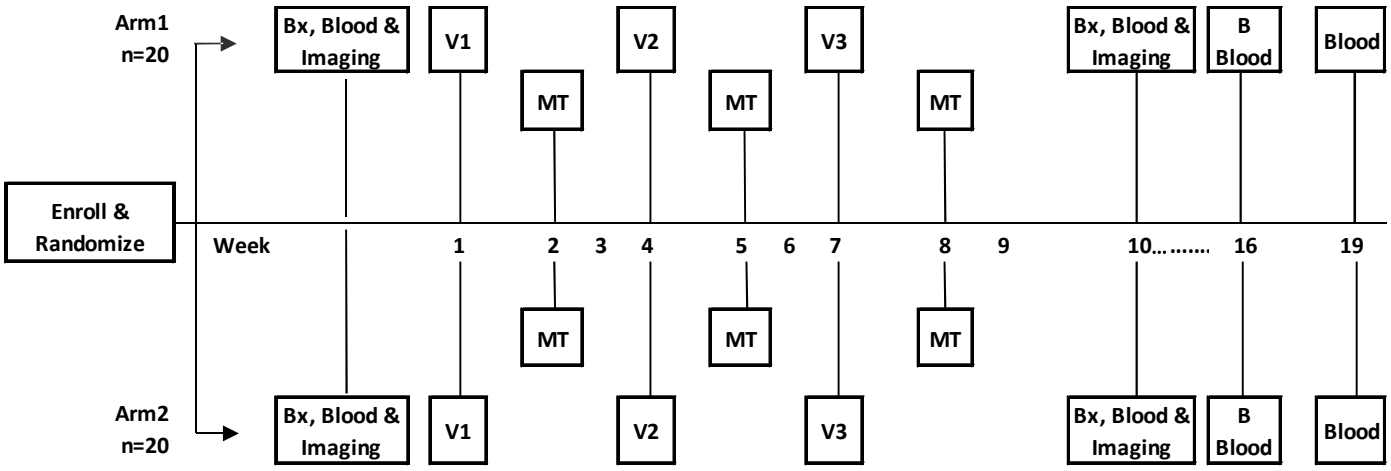
ARM 1: STEMVAC 301.59 mcg + GMCSF 99.2 mcg (nominal strength STEMVAC 300 mcg + 100 mcg GMCSF) administered at day 14 (+3 days) of the 21-day cycle for a series of 3 vaccines and a booster vaccine.

ARM 2: ID administration of GM-CSF alone, administered at day 14 (+3 days) of the 21- day cycle for a series of 3 vaccines and a booster vaccine.

Twenty patients will be enrolled per arm, STEMVAC and GM-CSF or GM-CSF alone. First vaccine will be administered approximately two weeks after pembrolizumab +/- pemetrexed infusion. The next two vaccines

approximately two weeks after each infusion. Imaging and biopsy will be done prior to the first vaccination (archived tissue may be used at the discretion of the Clinical PI) and approximately three weeks after the 3rd vaccine. One additional booster will be given approximately 9 weeks after last vaccine. Blood for immunologic monitoring will be done prior or at vaccine 1, and at evaluation 1 (three weeks after 3rd vaccine, week 10), evaluation 2/second booster vaccine (week 16) and post booster evaluation (week 19). Those booster patients will have an additional visit and blood draw. Toxicity evaluations will be performed at each patient visit.

The study schema is shown below:



Arm 1 -Vaccine (STEMVAC + GM-CSF)
Arm 2 - GM-CSF Alone
V - Vaccine
MT - Maintenance Treatment
Bx - Biopsy
B- Booster

7.B. Sample size

The study will accrue at least 40 patients who will be randomized sequentially to one of the two arms (20 patients/arm). Additional patients may be enrolled to replace patients who fail to complete the study in order to meet the target accrual of 40 patients.

7.C. Outcome measures

Primary endpoints:

1. Percentage of CD8+ TIL will be evaluated by immunohistochemistry (IHC) on tumor biopsies pre- and post-vaccine. Immunohistochemical (IHC) staining for CD3, CD4, and CD8 will be performed on the biopsies collected pre-treatment and post 3 vaccine administrations. The mean of the number of CD8+ T-cells from an evaluation of 4 high powered fields will be determined for each sample and reported as a % of total CD3+ cells. Tissue staining and cell quantification will be performed by qualified personnel blinded to the study randomization and clinical outcome.

2. Safety of STEMVAC immunization and concurrent pembrolizumab and/or pemetrexed maintenance therapy in patients with NSCLC will be evaluated using the modified NCI toxicity criteria. Toxicity evaluation will be based on Common Terminology Criteria for Adverse Events (CTCAE) v5.0, physical exam and laboratory tests and documented for each patient visit. Complete physical examination will be performed at

each vaccine visit. Laboratory tests including renal function tests, uric acid, blood counts, serum glucose, and liver function tests will be evaluated. ANA, anti-C3, ds-DNA antibodies, and a thyroid panel will be evaluated at each time-point. Laboratory tests will be done prior to vaccination and at 3, 9 and 12 weeks after the third vaccine (12 weeks or 3 weeks post booster for those receiving the booster vaccine).

Secondary endpoints:

1. To determine the magnitude of the immune response to STEMVAC when given in combination with pemetrexed and pembrolizumab maintenance therapy. Chemoimmunotherapy might impact the immune response generated to STEMVAC. We will measure the magnitude of the Th1 STEMVAC specific immune response using IFN-g ELISPOT. The magnitude of Th1 response will be defined for each antigen in STEMVAC for each patient as the value of the corrected spots per well (CSPW) (CSPW= [(mean of spots in the antigen stimulated wells) – (mean of antigens for the no-antigen negative control wells)] for the same time point). The antigens to be evaluated are: 1ug/mL protein antigens (recombinant proteins are available on all of the EMT associated proteins), human myoglobin (negative control), 0.5U/mL tetanus toxoid (positive control), and the epitopes encompassed within the vaccine at 10ug/mL.

2. To evaluate if vaccine induced T-cells traffic to tumor and eliminate tumor cells that have undergone EMT transformation.
    a. TCR sequencing will be performed in T-cell lines specific for STEMVAC antigens generated from peripheral blood mononuclear cells (PBMC) of patients and in biopsy TILs. We will evaluate matched T-cells from PBMCs and biopsies from the patients to determine whether the same TCR-beta (TCRb) clones in blood are found in abundance in tumor.
    b. Expression of EMT related genes will be analyzed by qPCR using the Human EMT RT^2 Profiler PCR Array, which includes 84 genes related to the EMT process. Gene expression profiles from biopsies before and after vaccination will be compared to determine if vaccination modulates the EMT gene signature in the tumor.

3. To evaluate the clinical efficacy of the vaccine. We will evaluate clinical response approximately one month after the 3rd vaccine using RECIST 1.1. Although the study is not powered to definitively address overall response rate (ORR), data generated may give some indication of clinical utility.

4. To determine whether vaccination increases CD8 T-cell activation markers (GZB, CD127 and PD-1) and Type I (CD4 Th1) immune cells. Tumor microenvironment in NSCLC patients is frequently Type II, characterized by T regulatory (Treg) cells, neutrophils and type 2 macrophages that prevent an efficient Type I response and contribute to escape from the immune system. T-cell activation markers and Type I immune cells will be evaluated by IHC staining in pre- and post-vaccine biopsies. We will evaluate CD4+Tbet+ (Th1), CD4+GATA3+ (Th2) and activation markers (GZB, CD127, and PD-1) on CD8 T-cells. The % of change in these populations will be described similarly as CD8+ TIL.

8. PLAN OF TREATMENT (SEE APPENDIX B)

Standard-of-care laboratory evaluations done prior to signing consent may qualify for baseline evaluations if they have been conducted within 60 days prior to first vaccine. In addition, we can use CT scans within 60 days. All baseline evaluations must be completed before study agent is dispensed.

8.A. Initial/baseline evaluation (may be performed up to 60 days prior to vaccine visit)

1. Sign consent form before initiation of study procedures
2. Medical history and complete physical examination which includes weight, vital signs, baseline symptom assessment and ECOG scoring (Appendix A)

3. CT of chest and abdomen (per standard of care) and read by RECIST 1.1, pelvic CT scans are required if there is evidence of disease there.
4. CT or ultrasound guided core biopsy of tumor – to be done prior to receiving vaccine (archived tissue may be used at the discretion of the Clinical PI provided there is sufficient tissue to evaluate the primary endpoints of the trial.)
5. Clinical labs
   a. Complete blood count with differential and platelet count
   b. Comprehensive metabolic panel which includes electrolytes, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, and total bilirubin

8.B. Chemoimmunotherapy (administered per standard of care by the patient's oncologist)

Pembrolizumab at a fixed dose of 200 mg +/- pemetrexed at a dose of 500 mg/m2 will be administered every 21 days. Rate of administration and pre-medications such as folic acid, vitamin b12, antiemetics and steroids will be administered under local custom. Patients should have a physical examination (which includes weight, vital signs,), laboratory evaluation (including a complete blood count with differential and platelet count, comprehensive metabolic panel which includes electrolytes, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, and total bilirubin) before each chemotherapy administration.

8.C. Immunizations

1. A physical examination will be done prior to each vaccination which will include weight, vital signs, vaccine symptom/toxicity assessment. May use initial evaluation results prior to vaccine 1.
2. Females who are having sex that can lead to pregnancy must have a negative urine pregnancy test prior to vaccination. This excludes patients who have undergone permanent sterilization (i.e., tubal ligation, hysterectomy or menopause as defined per the NCCN Guidelines. See Appendix D).
3. Clinical labs prior to vaccine (may use initial evaluation results prior to vaccine 1)
   a. Complete blood count with differential and platelet count (initial evaluation labs may be used prior to vaccine 1)
   b. Comprehensive metabolic panel which includes electrolytes, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, and total bilirubin (eligibility labs, if available may be used prior to vaccine 1)
   c. Autoimmune labs: ANA, C3, anti-dsDNA, and thyroid function tests
4. Tetanus diphtheria (Td) immunization if one has not been administered within six months-prior to first vaccine only. 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.
5. Research blood
   a. Prior to first vaccine, approximately 200 ml for immunologic monitoring as described in Section 8.H.
6. Vaccinations will be administered ID on Day 14 (+3 days) post pembrolizumab +/- pemetrexed infusion.
   a. All vaccines will be administered in the outpatient setting at the University of Washington Translational Research Unit (UW TRU) and VA Puget Sound
   b. Post-immunization monitoring: Patients will be observed for a minimum of 60 minutes post immunization and post-vaccine vital signs will be documented (See Section 8.H. below)

8.D. First follow up post vaccine (approximately 3 weeks post third vaccine) – Evaluation 1

1. A physical examination will be performed which will include weight, vital signs, symptom/toxicity assessment as well as:
   a. Complete blood count with differential and platelet count
   b. Comprehensive metabolic panel which includes electrolytes, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, and total bilirubin
   c. Immunotherapy labs: ANA, C3, anti-dsDNA, and thyroid function tests

2. Research blood
    a. Approximately 200 ml for immunologic monitoring as described in Section 8.H.
3. CT or Ultrasound guided core biopsy of tumor
4. CT of chest and abdomen (and pelvis if there is evidence of disease there) performed per standard of care and read by RECIST (shortly prior to or shortly after this visit).
    a. Patients without progressive disease will continue receiving chemoimmunotherapy treatment under the care of their treating physician with further restaging under their guidance.

8.E. Booster vaccine - second follow up post vaccine (approximately 9 weeks post third vaccine) – Evaluation 2

1. A physical examination will be performed which will include weight, vital signs, symptom/toxicity assessment as well as:
    a. Complete blood count with differential and platelet count
    b. Comprehensive metabolic panel which includes electrolytes, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, and total bilirubin
    c. Autoimmune labs: ANA, C3, anti-dsDNA, and thyroid function tests
2. Females who are having sex, that can lead to pregnancy, must have a negative urine pregnancy test
3. Research blood draw (for immunologic monitoring)
    a. Approximately 200 ml of blood will be collected as described in Section 8.H. below
4. This will be the last study visit for those not receiving the booster vaccine (for those off-study treatment)
5. Booster vaccine administration for those patients still on study treatment
    a. This vaccine will be the same as the one administered in Section 8.C
    b. As much as possible each dose of vaccine will be administered within the same draining lymph node site
    c. All vaccines will be administered in the outpatient setting at the UW TRU and VA Puget Sound
    d. Post-immunization monitoring: Patients will be observed for a minimum of 60 minutes post immunization and post-vaccine vital signs will be documented (See Section 8.H. below)

8.F. Post Booster vaccine evaluation (approximately 3 weeks post booster vaccine)

1.A physical examination will be performed which will include weight, vital signs, symptom/toxicity assessment as well as:
    a. Complete blood count with differential and platelet count
    b. Comprehensive metabolic panel which includes electrolytes, glucose, creatinine, BUN, AST, ALT, alkaline phosphatase, and total bilirubin
    c. Autoimmune labs: ANA, C3, anti-dsDNA, and thyroid function tests
2. Research blood draw (immunologic monitoring)
    a. Approximately 200 ml of blood will be collected as described in Section 8.H. below

8.G. Long-Term Follow-Up

A request for records will be sent to the patient's primary oncologist twice yearly from end of study visits for a total of 5 years. This information will be put in the study chart which is kept in a locked filing cabinet in a secured building. Information requested will include:

a. Most recent laboratory evaluation (CBC, CMP)
b. Patient's disease free and overall survival status
c. Recent clinical notes which may include history and physical exam notes
    - Notes will be reviewed by study team for vaccine toxicity and followed up with the patient's primary physician if indicated.

Study charts are kept for 30 years after the close of the study per University of Washington policy. VA Puget Sound study will be utilizing both electronic and paper research charts which are kept for at least 6 years after the fiscal year in which the study was closed at VA in accordance with RCS 10-1.

We will be looking for possible autoimmune symptoms (e.g. unexplained rash, dry eyes, unexplained diarrhea) within the above collected records.

We will encourage patients to notify us if they become pregnant after they complete study visits.

8.H. Management of potential study risks

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 and monitoring of vital signs for a minimum of 60 minutes post-vaccination.

Large volume research blood draws (up to 200 ml) are required at designated study visits for immunologic monitoring. The amount of blood obtained for immunologic monitoring assays at designated time points is in strict adherence with guidelines set by the BloodWorks NW and are associated with minimal risks. However, as with any large volume blood draw patients should be well hydrated prior to the blood 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. As part of the study we will be evaluating the laboratory values, such as hematocrit. If the patient's prior hematocrit value was ≥ 30 we will proceed with the large volume blood draw at the subsequent visit.

Patients will receive two biopsies which are not part of regular treatment. A biopsy can result in adverse events including pain, discomfort, bleeding, swelling, scarring, bruising, and infection, dependent on the site of disease. We will attempt to minimize risk by closely evaluating accessible lesions. Patients will be given a separate surgical consent form for this hospital procedure.

8.I. Storing 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. The VA Puget Sound will not be participating in sample storage for future testing. At the end of the study all samples provided by VA Puget Sound will need to be documented as destroyed by the University of Washington and that documentation will be keep in the subject's research chart and recorded on the appropriate VA Research and Development (R&D) sub-Committee closure paperwork.

8.J. Randomization

Patients will be randomized by the University of Washington staff delegated by Co-PI, Shaveta Vinayak. Patients will be randomized using block randomization method to ensure a balance in sample size with two treatment groups over time. Specifically, with a block size of 4, we have six possible sequences; these are AABB, ABAB, ABBA, BAAB, BABA, and BBAA, where A and B stands for Arm 1 or 2, respectively. A list of 10 random numbers will be generated within the range of 1 to 6. Each number refers to one of the sequences allocations in each block.

9. WITHDRAW FROM STUDY TREATMENT

9.A. Patient Withdrawal

Patients may decide to withdraw from the study treatment at any time and for any reason, at which time all research-related treatment procedures will then cease. Patients, who have persistent grade 3 or 4 toxicity (of a two week or greater duration) related to the vaccine, at time of withdrawal from study treatment, will continue to be followed until toxicity resolves or returns to baseline for that patient. We will continue to follow them for disease status as conducted by their primary physician according to conventional practice standards. If the patient is willing, we will continue to draw research blood per protocol timelines. Prior to blood draw, labs will be reviewed by study physician/physician extender for patients receiving cytotoxic chemotherapy or immunosuppressive agents. Study physicians/physician extender will assess if blood draw would be safe for these patients, and what amount may be drawn (not to exceed 200 ml). Once patients have withdrawn from treatment, they will continue to be followed in Long Term Follow Up unless they have withdrawn their consent.

Withdrawal will be reported in the annual renewal report to all appropriate agencies. We will report the reason for the withdrawal if known and whether it was related to the study.

9.B. Study Physician Withdrawal

A PI/Co-PI/Study Physician/Physician Extender, or IND sponsor may deem it appropriate to remove a patient from study participation for toxicity (as described in Section 11- Accrual and Criteria for Premature Study Termination), recurrent disease, study non-compliance, current illnesses or therapy, or other reasons. 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.

Patients may continue to be followed for disease status as conducted by their primary physician according to conventional practice standards. If applicable, patients who are no longer being vaccinated may continue to be followed for immunity and research blood may be requested as described in above. If a co-PI/Study Physician/Physician Extender withdraws the patient from study, it will be reported in the annual renewal report to all appropriate agencies. We will report the reason for the withdrawal and whether it was related to the study.

9.C. Discontinuation of Study Treatment

The study may be discontinued if the toxicity rate criteria, as described in Section 11 – Accrual and Criteria for Premature Study Termination, are met. If the study is discontinued for toxicity all research-related treatment procedures will cease. We will continue to monitor clinically significant toxicity until the toxicity resolves or returns to an individual's baseline.

If patients are willing, we will continue to follow them for disease status and/or research blood to follow immunity as described in Section 8.G. above. We will report the study termination to the Fred Hutchinson /University of Washington Cancer Consortium Institutional Review Board and VA Puget Sound Health Care System IRB #1 at the time we become aware of the discontinuation (with 10 days).

10. EVALUATION AND MANAGEMENT OF TOXICITY RELATED TO THE STUDY TREATMENT

Patients will be asked to report local injection site reactions and systemic reactions. Toxicity will be evaluated according to the CTEP CTCAE v5.0 and the Data and Safety Monitoring Plan (DSMP) as described in Appendix C. At each visit, evaluation for toxicity will include, but is not limited to: complete blood count (CBC), serum electrolytes, creatinine, BUN, liver function tests, and a physical examination. Study physicians/physician extender will attribute adverse events and its relatedness. Adverse events may be related to the STEMVAC, GM-CSF, pemetrexed and/or pembrolizumab or all of them or neither of them.

For grade 1 or 2 vaccine-related reactions, patients may be treated with acetaminophen or Benadryl as clinically indicated at the discretion of the study clinician. For grade 3 or greater adverse events, using the CTEP CTCAE v5.0, that are considered definitely, probably, or possibly related to the vaccine (other than those listed below in Table 3), we have defined a stopping boundary rule as described in Section 11 – Accrual and Criteria for Premature Study Termination.

We will begin to evaluate and record systemic reactions from the time of confirmed eligibility through End of Treatment visit.

10.A. Vaccines

Risk of STEMVAC Vaccine

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

Table 1: STEMVAC Expected Adverse Events

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**[a, b] Headache	Flu-like symptoms Muscle pain **Nausea**[a, b] Chills **Diarrhea**[a, b]	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 Seizures Severe allergic reaction to the vaccine may require medication, lead to hospitalization, and may result in death

[a] Also common and expected in pemetrexed and/or pembrolizumab.
[b] Also common and expected with pembrolizumab

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 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 and more frequent doses of GM-CSF than will be given in this study.

Table 2: GM-CSF Expected Adverse Events

Likely	Less likely	Rare, some may be serious
Local reactions at the site of the injection **Low grade fever (< 100.5°F)[a, b]** Chills Pain in the bones, muscles, chest, abdomen, chest, or joints **Nausea[a, b]** **Vomiting[a, b]** **Diarrhea[a, b]** Flu-like symptoms including **fatigue[a]**, weakness, headache **Decreased appetite[a, b]** Increased white blood cell count	Kidney and liver problems Local reaction 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 Temporary loss of consciousness

[a] Also common and expected in pemetrexed and/or pembrolizumab.
[b] Also common and expected with pembrolizumab

Based on our previous Phase I study with STEMVAC, Grade 3 or higher adverse events occurred in 2% of the vaccinated patients. Table 3 shows those Grade 3 or higher adverse events that are expected and allowable in this Phase II study.

Table 3. Vaccine related expected and allowable Grade 3 and 4 Related Toxicities (CTEP CTCAE v5.0) that are not included in the stopping rule.

Category	Toxicity/AE	Allowable Duration
General disorders and administration site conditions	Flu-like symptoms	1 week
Musculoskeletal and connective tissue disorders	Arthralgia	1 week
	Myalgia	1 week
Investigations	Lymphocyte count decreased	2 weeks
	Thrombocytopenia*	1 weeks
Blood and lymphatic system disorders	**Anemia***	1 weeks

Category	Toxicity/AE	Allowable Duration
Investigations	White blood cells decreased	2 weeks

***Also common and expected in pemetrexed and/or pembrolizumab.**

We 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 [47]. Examples of such immune related AEs include; rash, diarrhea/colitis, and hepatitis. The following dose schedule may be used:

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

10.B. Pemetrexed

Pemetrexed is a type of chemotherapy called an anti-folate. As a chemotherapy, it is believed to help to kill or slow the growth of cancer cells. Table 4 provides expected adverse events related to the combination of pembrolizumab +/- pemetrexed.[2, 48]

10.C. Pembrolizumab

Pembrolizumab is an immunotherapy and it works with your immune system to help fight certain cancers by blocking the PD-1 pathway and to help prevent cancer cells from hiding. Pembrolizumab helps the immune system do what it was meant to do: detect and fight cancer cells.

Table 4. Expected adverse events related to pemetrexed and/or pembrolizumab (Events occurring in ≥10% of patients being treated with pemetrexed and/or pembrolizumab with carboplatin).[2, 48]

Fatigue	Decreased appetite	Neutropenia	Peripheral edema
Fever	Vomiting	Asthenia	Dyspnea
Constipation	Cough	Rash	Dysgeusia
Diarrhea	Nausea	Pruritus	Anemia
Thrombocytopenia	Dizziness	Increased lacrimation	Increased blood creatinine
Increased alanine aminotransferase	Increased aspartate aminotransferase	Alopecia	Peripheral neuropathy

11. ACCRUAL AND CRITERIA FOR PREMATURE STUDY TERMINATION

We have completed a Phase I study where we evaluated the safety of the vaccine at 3 different doses. All three doses exhibited a satisfactory safety profile with the majority of adverse events were grade 1 and 2.

Although the chance is low, the trial will be stopped based on two continuous toxicity monitoring rules for adverse events of interest (Table 5). Current standard of care with pemetrexed/pembrolizumab therapy induces

a broad spectrum of adverse events as indicated in Table 4 above. Up to 99.8% of the patients treated with pemetrexed/pembrolizumab presented with adverse events, and 67.2% of them presented Grade 3 or higher adverse events[2]. We have defined a list of adverse events of interest (Table 5) as described in Gandhi et al.[2]. The adverse events of interest are defined as those with an immune-related cause and are considered regardless of attribution to a trial drug by a PI/Co-PI. Grade 3 or higher adverse events of interest were reported in 8.9% of patients treated with pemetrexed/pembrolizumab[2].

Table 5. Expected adverse events of interest

Hypothyroidism	Colitis	Hypophysitis	Thyroiditis
Pneumonitis	Severe skin reaction	Pancreatitis	Type 1 diabetes mellitus
Hyperthyroidism	Nephritis	Adrenal insufficiency	
Infusion reaction	Hepatitis	Myositis	

Two continuous monitoring rules for toxicity will be used to ensure stopping trial if the observed toxicity rate exceed a threshold. For the investigation arm with STEMVAC the two continuous toxicity monitoring rules assume that adverse event of interest (Table 5) probability is not more than 20% for grade 3 and no more than 10% for grade 4. The maximum number of grade 3 or 4 toxicity events of interest for every patient enrolled at a specific timepoint is shown in Tables 6 and 7. The trial will be stopped if the number of AE of interest is equal to or exceeds boundary (bn) out of n patients with completed follow-up. This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 0.05 when the rate of AE of interest is equal to the acceptable rate (<20% for grade 3 AE rate and <10% for grade 4 AE rate).[49]

Table 6. Toxicity monitoring for grade 3 AE of interest

Number of patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	-	3	4	4	4	5	5	5	6	6	6	7	7	7	8	8	8	9	9

Table 7. Toxicity monitoring for grade 4 AE of interest

Number of patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	2	3	3	3	3	4	4	4	4	4	4	5	5	5	5	5	6	6	6

12. STATISTICAL CONSIDERATIONS

12.A. Statistical analysis plan

This is a phase II randomized study of STEMVAC+GM-CSF or GM-CSF administered ID concurrently with pembrolizumab +/- pemetrexed maintenance therapy.

Primary endpoints

1. Determine whether ID STEMVAC+GM-CSF vaccination increases the percentage of CD8+ TIL in patients with advanced NSCLC compared to patient who receive ID GM-CSF alone by immunohistochemistry (IHC). The mean of the number of CD8 T-cells will be determined by IHC for each biopsy sample, at baseline and after the third vaccine, as a % of total CD3 cells. Two-sample Student's t-test with a 1-sided 0.05 significance level will be used to assess the difference. The distribution of the differences of CD8 T-cells between baseline and post treatment will be examined to check the normality assumption and outliers. If the outliers are substantial or the distribution is skewed, Wilcoxon nonparametric test will be used to assess the difference between the two treatment arms. Depending on the correlation between baseline and

post-treatment measures, alternative analytical method will be adopted using an ordinary least square regression for the post-treatment CD8 T-cells percentage while adjusting for the baseline measure.

2. Evaluate safety of STEMVAC immunization and concurrent pembrolizumab and/or pemetrexed maintenance therapy in patients with advanced NSCLC. Safety of STEMVAC immunization and concurrent pembrolizumab and/or pemetrexed maintenance therapy in patients with NSCLC will be evaluated using the modified NCI toxicity criteria. Toxicity evaluation will be based on Common Terminology Criteria for Adverse Events (CTCAE) v5.0, physical exam and laboratory tests. Complete physical examination will be performed at each vaccine visit and will include weight, vital signs, vaccine symptom/toxicity assessment. Laboratory tests including complete blood count with differential and platelet counts, and comprehensive metabolic panel for renal function, uric acid, serum glucose, and liver function will be evaluated. Anti-ANA, anti-C3, ds-DNA antibodies, and a thyroid panel will be also evaluated at each time-point. All clinically significant adverse events (including lab abnormalities) will be graded on a scale of 1-5 and documented.

Secondary endpoints

1. Determine the magnitude of the immune response to STEMVAC when given in combination with pemetrexed and pembrolizumab maintenance therapy. The Th1 STEMVAC antigen specific immune response will be determined by IFN-g ELISPOT. The magnitude of Th1 response will be defined for each antigen in STEMVAC for each patient as the value of the corrected spots per well (CSPW) (CSPW= [(mean of spots in the antigen stimulated wells) – (mean of antigens for the no-antigen negative control wells)] for the same time point). The antigens to be evaluated are: 1ug/mL protein antigens (recombinant proteins are available on all of the EMT associated proteins), human myoglobin (negative control), 0.5U/mL tetanus toxoid (positive control), and the epitopes encompassed within the vaccine at 10ug/mL. An increase in the number of peptide or protein specific IFN-g producing spots that is statistically different than no antigen and/or controls ($p<0.05$) will be taken as an indication of vaccinated immune response. T-test among the 2 groups (vaccine and adjuvant alone) will be conducted to evaluate immune response if skewness is not observed.

2. Evaluate if vaccine induced T-cells traffic to tumor and eliminate tumor cells that have undergone EMT transformation. Patients from the STEMVAC arm with the most robust antigen specific responses will undergo peripheral blood T-cell expansion and blood and tumor TCR sequencing. Shannon diversity index will be summarized and the Clopper-Pearson confidence interval will be computed for the rate of T-cell trafficking among the 10 patients subject to TCRb sequencing. If we observe antigen specific responses at high level in the patients receiving GM-CSF alone, Fisher exact test will be used to compare the rates of expansion of T-cell clones in PBMC from the vaccine arm and PBMC from the control arm. Logistic regression will be used to compare the rates between arms if adjusting for the baseline rates. The expression of EMT genes in tumor biopsies will be compared between all patients in the two arms, adjusting for their respective baseline levels, with multiple testing adjustment for declaring significance. For each EMT marker, the differences of gene expressions pre- and post-vaccination will be assessed for association with the differences of antigen-specific, pre- and post-vaccination CD8 TIL (or the presence of GZB positive cells), stratified by treatment arm. A significant negative association accounting for multiple testing will support the hypothesis that CD8 TIL eliminates cancer cells that have undergone EMT.

3. Evaluate potential clinical efficacy of STEMVAC immunization by comparing overall response rate between both arms. We will evaluate potential clinical response approximately one month after the 3rd vaccine using RECIST 1.1. In a Phase III trial the ORR observed with maintenance pemetrexed was 3% [57]. There is no clinical trial that has studied ORR due to pembrolizumab in the maintenance setting,

however, the median time to response when pembrolizumab is added to chemotherapy is 2.2 months [1]. Further response after the induction treatment is likely to be low. We would assume an ORR less than 5% would be attributed to maintenance therapy. An informal comparison will be conducted to compare the ORR between the two arms, with the Fisher exact test to account for small sample size. Additional analyses will be conducted to evaluate other efficacy endpoints, which include progression free survival (PFS) and overall survival (OS). Time-to-event variables are analyzed using the Kaplan-Meier method. Kaplan-Meier estimates of the survival function with 95% CIs at specific time points (using Greenwood's formula for the standard error) will be computed. Comparisons of PFS and OS in the two arms will be conducted by the log-rank test.

4. Determine whether vaccination increases CD8 T-cell activation markers (GZB, CD127 and PD1) and Type I immune cells (CD4 Th1) in the tumor. CD8 T-cell activation and Type I immune cells will be evaluated by IHC staining in pre- and post-vaccine biopsies. We will evaluate $CD4^{+}Tbet^{+}$ (Th1), $CD4^{+}GATA3^{+}$ (Th2) and activation markers (GZB, CD127, and PD-1) on CD8 T-cells. The % of change in these populations will be described similarly as CD8+ TIL. Similar analyses comparative to the CD8 in the primary objective will be conducted. In brief, two-sample T-tests or the Wilcoxon test will be utilized to compare the absolute change of T-cell activation markers and the Type I immune cells from baseline based on the normality of the data.

12.B. Sample size

Primary endpoints:

1. Determine whether intradermal (ID) injection of STEMVAC+GM-CSF vaccination increases the percentage of CD8+ TIL in patients with advanced NSCLC compared to patients who receive ID GM-CSF alone by IHC. Determine the percentage of CD8+ TIL in patients between the two arms. With a proposed sample size of 20 subjects per treatment arm (40 subjects total), the study has ≥ 0.80 power to detect a ≥ 0.80-standard-deviation difference in the maximum absolute change from baseline in % CD8 TIL in the experimental arm relative to the control arm.

2. Evaluate safety of STEMVAC immunization and concurrent pembrolizumab and/or pemetrexed maintenance therapy in patients with advanced NSCLC. Toxicity evaluation will be based on Common Terminology Criteria for Adverse Events (CTCAE) v5.0, physical exam and laboratory tests.

Secondary endpoints:

1. Determine the magnitude of the immune response to STEMVAC when given in combination with pemetrexed and pembrolizumab maintenance therapy. The Th1 STEMVAC antigen specific immune response will be determined by IFN-g ELISPOT. A proposed sample size of 20 patients per group provide 80% power to detect a statistically significant difference (at the one-sided level of 0.05) if the true effect size is one standard deviation, correcting the multiple comparisons using Bonferroni method. Effect size is defined as the difference in mean specific T-cell response level divided by the common standard deviation.

2. Evaluate if vaccine induced T-cells traffic to tumor and eliminate tumor cells that have undergone EMT transformation. We will assess TCR-beta (TCRb) gene usage in both T-cell lines expanded from peripheral blood and in the tumor biopsy, and the expression of EMT related genes in the tumor after vaccination with STMEVAC+GM-CSF or GM-CSF alone. We hypothesize a successful therapeutic immunization would be the ability to detect vaccine induced T-cells clones in the majority of immunized patients (80%) or at least a minimum of half the patients analyzed. With 10 patients included in this analysis, we will have a standard error of 0.13 if the true rate of T-cell clone trafficking to tumor is 80% and a standard error 0.16 if the true rate of trafficking is 0.5, the minimal rate we would expect for a clinically efficacious vaccine.

3. Evaluate potential clinical efficacy of STEMVAC immunization by comparing overall response rate between both arms. We will evaluate clinical response approximately one month after the 3rd vaccine using RECIST 1.1. Although the study is not powered to definitively address overall response rate (ORR), data generated may give some indication of clinical utility. In a Phase III trial the ORR observed with maintenance pemetrexed was 3% [57]. There is no clinical trial that has studied ORR due to pembrolizumab in the maintenance setting, however, the median time to response when pembrolizumab is added to chemotherapy is 2.2 months [1]. Further response after the induction treatment is likely to be low. We would assume an ORR less than 5% would be attributed to maintenance therapy. With 20 patients in the STEMVAC arm, we have >80% power to detect a significant difference if the response rate in the STEMVAC arm is > 23% compared to 5% response rate as the historical control rate.
4. Determine whether vaccination increases CD8 T-cell activation markers (GZB, CD127 and PD1) and Type I immune cells (CD4 Th1) in the tumor. CD8 T-cell activation markers and Type I immune cells will be evaluated by IHC staining in pre- and post-vaccine biopsies. With the proposed sample size of 20 patients per group, we have more than 80% power to detect a statistically significant difference (at the one-sided level of 0.05) in mean specific T cell immune response and in CD8 T-cell activation markers if the true effect size is one standard deviation. Multiple-testing will be accounted for using the Bonferroni procedure.

12.C. Projected gender and ethnic distribution

The ethnic and gender distribution chart below (Table 8) reflects estimates of race and gender of the population to be included in this study. The ethnic and gender distribution reflects estimates that are based on the Washington State Department of Health - Washington State Cancer Registry data released on March 2020. The data was filtered on incidence, Lung and Bronchus (how it is categorized in database), 4 year time period (2013-2017), and race. Based on this data we calculated the following: Hispanic (2.4%), White (88.1%), Asian (4.4%), Black or African American (2.4%), American Indian/Alaska Native (< 1%), and Native Hawaiian or Other Pacific Islander (1.1%).

Table 8. Distribution of ethnicity and gender.

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	1	1	2
Not Hispanic or Latino	19	19	38
Ethnic Category: Total of All Subjects *	20	20	40
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	1	2
White	18	18	36
Racial Categories: Total of All Subjects *	20	20	40

13. SAFETY REPORTING

For definitions of adverse events, reference Appendix C. Mary L. (Nora) Disis, M.D., UW CVI, is the IND sponsor of this trial. Any serious adverse event (SAEs) will need to be reported to Dr. Disis within 24 hours of learning of the event.

Mary L. (Nora) Disis, M.D.
University of Washington
IND Sponsor
ndisis@u.washington.edu
Phone: 206-616-1823
Fax: 206-221-5529

The following information will be included when calling Dr. Disis:

- 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

Mary L. (Nora) Disis, M.D.
University of Washington
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14. ADMINISTRATIVE CONSIDERATIONS

14.A. Institutional review board

In accordance with federal regulations, an Institutional Review Board that complies with the regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to initiation of the study.

14.B. Consent

A PI/Co-PI/Study Physician or their designated physician extender (Nurse Practitioner/Physician Assistant) or study coordinator per institutional policy, must explain verbally and in writing the nature and duration of the study and possible consequences of the treatment. Patients must also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. This should be documented by the consenting physician or physician extender in a consent note and signed.

14.C. Reporting adverse events related to the vaccine

Patients are monitored for the development of vaccine toxicities by assessing adverse events with serum chemistries, liver function studies, complete blood counts and physical exams. All clinically significant adverse events (including lab abnormalities) are graded on a scale of 1-5 and attribution is assigned as to its relation to the vaccines. We will begin to evaluate and record systemic adverse events from the time of confirmed eligibility through End of Treatment visit. We will record adverse events that are reported to us by the patient, caregivers, and clinical support staff.

Post visit AEs will be reported using the NCI's CTEP CTCAE v5.0. A copy of the CTEP CTCAE v5.0 can be downloaded from the CTEP homepage (http://ctep.info.nih.gov). Guidelines for AE reporting to the FDA and the NCI are described in the DSMP, Appendix C. The Monitoring Plan (as mandated by the National Institutes of Health (NIH)/National Cancer Institute (NCI)) Policy and Procedure for this phase II study is described in Appendix C.

SAEs are communicated to the PI/Co-PI, Fred Hutchinson/University of Washington Cancer Consortium (FH/UW CC IRB), IND Sponsor, FDA, Medical Monitor, University of Washington Institutional Biosafety Committee (UW IBC), UW TRU, and FH Biologic Production, VA Puget Sound Health Care System IRB#1 and U.S. Army Medical Research and Materiel Command Human Subjects Research Review Board (USAMRMC) Office of Research Protections (ORP) Human Research Protection Office (HRPO). A status report including accrual, AEs and death information will be reviewed by the PI/Co-PI every 6 months and the FH/University of Washington Cancer Consortium Data and Safety Monitoring Committee (DSMC) annually. In addition, the study will be monitored by the Clinical Research Support Services according to the FH /University of Washington– Cancer Consortium monitoring plan.

14.D. Confidentiality of patient records

All eligible patients will be assigned a unique patient number (UPN) that will not contain any personally identifying information, such as name, initials, medical record number, social security number, etc. To maintain confidentiality, we protect the link between the patients' personal identifying information and UPN number by limiting who has access to the patients' chart documentation. Only delegated clinical research staff has access to the data, which remain locked at all times when not in use or kept on secured servers with permission access to only approved clinical research staff.

All hard copy research records collected on potential and enrolled patients are stored in a locked filing cabinet in a locked office that can only be accessed by approved clinical research staff when not in use. This staff includes: co-PIs/study physician(s), physician extenders and designated clinical research staff. These are also the only people that have access to the link between the patients' personal identifying information and their assigned UPN codes. Any electronic VA research charts or data will be kept on VA secured servers and protected by VA IT in accordance with VA regulations and policies.

In terms of the protections and security of electronic clinical data, it is being performed by the University of Washington Information Technology (UW IT) Services. The UW and VA IT information security policy is to protect information and information systems. It also ensures compliance with UW/VA policy and state and federal regulations.

14.E. Study team roles and responsibilities

Drs. Vinayak (PI) and Santana-Davila, Co-PI in collaboration with Dr. Disis (IND Sponsor) will be responsible for the oversight of the study. Dr. Vinayak is responsible for the overall conduct of the study and Dr. Santana-Davila will be specifically responsible for the clinical evaluations for the study. The PI/Co-PI collaborate on the preparation and implementation of the protocol with the PI being responsible for PI study required signatures (i.e. adverse event log at end of study, etc.). The PI/Co-PI also analyze the results of the study data. Drs. Vinayak and Santana-Davila are responsible for ensuring that all information and documentation related to the conduct and safety of the study is disseminated to the proper agencies in the proper timeframe. Dr. Vinayak and Santana-Davila will delegate tasks to qualified research staff for this study as needed at UW. Dr. Graf will delegate tasks to the research staff at VA Puget Sound.

Research physicians/physician extenders per institutional policy will be delegated to perform physical exams, and collect, review, and attribute adverse events per protocol. In addition, they will be available to answer patient study related questions. They collaborate attentively with the research staff to ensure the safety and efficacy of the patient.

Research Nurses/Coordinators will be responsible for recruitment, initial screening, baseline event collection, coordination of clinical treatment, study visits and follow up. They may do medication/adverse event collection review with patients and ensure it is entered into the database in a timely manner, so as to have real time data to review and report any safety concerns or trends. They will be responsible for maintaining regulatory documentation to the various agencies involved with this research and assist with ensuring that all research team members are following the protocol and all regulations.

All clinical research staff are required to complete the following training:

1. Human Subjects Protections
2. HIPAA
3. Good Clinical Practice

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Appendix A. ECOG Performance Scale

Performance status: Patients will be assessed according to the current ECOG performance scale (Table 9).

Table 9. ECOG performance scale

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

As published in Am. J. Clin. Oncol: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: *Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group.* Am J Clin Oncol 5:649-655, 1982.

The ECOG/Zubrod Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

Appendix B. Calendar of Events

Table 10. Calendar of events

Visit Time Point	Procedures
Initial visit/Biopsy Some procedures may be done up to 60 days prior to the first vaccine	• Informed Consent • Medical history and complete physical examination • Vitals signs-including weight • Clinical labs[a, b] • Urine pregnancy test (if applicable) • CT or Ultrasound guided core needle biopsy of tumor (archived tissue may be used at the discretion of the Clinical PIs) • RECIST 1.1
VACCINE 1 [c] 14 days (+ 3) post pembrolizumab +/- pemetrexed infusion	• Complete physical examination[d] may use initial evaluation results prior to vaccine 1. • Vitals signs-including weight • Urine pregnancy test (if applicable) • Clinical labs[b] • ANA, C3, anti-dsDNA, thyroid function test • Research blood: approximately one cup • Tetanus diphtheria (Td) vaccine • Vaccine 1 (*STEMVAC+GM-CSF or GM-CSF alone*) • After vaccine monitoring for allergic reaction for a minimum of one hour
Maintenance treatment **Approx. 7 days after vaccine 1**	• Pembrolizumab +/- pemetrexed
VACCINE 2 [c] 14 days (+ 3) post pembrolizumab +/- pemetrexed infusion	• Complete physical examination ✓ Vitals signs-including weight ✓ Assess for any side effects • Urine pregnancy test (if applicable) • Clinical labs[b] • ANA, C3, anti-dsDNA, thyroid function test • Vaccine 2 (*STEMVAC+GM-CSF or GM-CSF alone*) • After vaccine monitoring for allergic reaction for a minimum of one hour
Maintenance treatment **Approx. 7 days after vaccine 2**	• Pembrolizumab +/- pemetrexed
VACCINE 3 [c] 14 days (+ 3) post pembrolizumab +/- pemetrexed infusion	• Complete physical examination ✓ Vitals signs-including weight ✓ Assess for any side effects • Urine pregnancy test (if applicable) • Clinical labs[b] • ANA, C3, anti-dsDNA, thyroid function test • Vaccine 3 (*STEMVAC+GM-CSF or GM-CSF alone*) • After vaccine monitoring for allergic reaction for a minimum of one hour

Visit Time Point	Procedures
Maintenance treatment **Approx. 7 days after vaccine 3**	• Pembrolizumab +/- pemetrexed
EVALUATION 1 – At least 3 weeks after THIRD vaccine	• Complete physical examination ✓ Vitals signs-including weight ✓ Assess for any side effects • Clinical labs[b] • ANA, C3, anti-dsDNA, thyroid function test • Research blood: approximately one cup • Imaging – CT scan per standard of care • RECIST 1.1 • CT or Ultrasound guided core biopsy of tumor Chest x-ray
BOOSTER VACCINE **EVALUATION 2 -** At least 9 weeks after THIRD vaccine	• Complete physical examination ✓ Vitals signs-including weight ✓ Assess for any side effects • Urine pregnancy test (if applicable and receiving booster vaccine) • Clinical labs[b] • ANA, C3, anti-dsDNA, thyroid function test • Research blood: approximately one cup • For those on study treatment: o Booster Vaccine (*STEMVAC+GM-CSF or GM-CSF alone*) o After vaccine monitoring for allergic reaction for a minimum of one hour
POST BOOSTER EVALUATION- At least 3 weeks after BOOSTER vaccine	• Complete physical examination ✓ Vitals signs-including weight ✓ Assess for any side effects • Clinical labs[b] • ANA, C3, anti-dsDNA, thyroid function test • Research blood: approximately one cup
LONG TERM FOLLOW-UP	• Twice yearly for 5 years after last visit • Request records from the patient's oncologist

[a]Can use standard of care laboratory evaluations if performed 60 days prior to prior to the vaccine visit

[b]CBC with differential: white blood cells, red blood cells, platelets; Comprehensive metabolic panel (CMP): sugar (glucose) level, electrolyte and fluid balance, kidney function, liver function

[c]Vaccine administered Day 14 (+3 days) after pembrolizumab and/or pemetrexed infusion

[d]May use physical exam that was done prior to vaccine 1

[e]Records may include: most recent laboratory evaluation, patient's disease status, and recent clinical notes with medical history/physical exam(s)

Appendix C. Data and Safety Monitoring Plan

A. PURPOSE

To ensure that the Cancer Vaccine Institute follows NIH/NCI/CTEP/FDA guidelines with respect to: (1) accurate assessment and timely reporting of adverse drug reactions associated with investigational drugs, (2) adherence to protocol, and (3) accurate reporting of data. The DSMP will be approved by FH/UW CC IRB in Seattle, Washington.

This is a multi-center clinical study including the FH/UW Consortium and the VA Puget Sound Health Care System. The PI for this project are responsible for every aspect of the design, delegating responsibility/authority, study conduct and final analysis of the protocol. The PI, or delegated qualified research staff, will document that each external performance site is qualified to conduct the trial and conforms to all relevant regulations and guidelines. The PI, or delegated trained research staff, will obtain copies of VA Puget Sound IRB approval and will have the responsibility for receiving the information required for adverse event reporting and safety monitoring, and disseminating that information to the appropriate FH/UW committees. Regulations defining the responsibilities for assessment and reporting of AEs, SAEs, and unexpected AEs are defined by the Code of Federal Regulations: 21 CFR 312.32 and CTEP CTCAE v5.0 published by CTEP, a division of the NCI/NIH. A matrix of reporting requirements and schedules is at the CTEP web-site at http://ctep.infi.nih/gov.

This clinical study will rely upon the monitoring of the trial by the PI in conjunction with a Research Nurse, a Statistician, an Independent Medical Monitor and an Independent Study Monitor assigned by the Clinical Research Support of the FH/UW CC IRB. The trial PI/Co-PI will establish and carry out procedures for assessing protocol compliance, data accuracy and completeness, and full and timely reporting of safety data. VA Puget Sound acknowledges their responsibilities for data and adverse event reporting. Study monitors will be required to complete either on-site or remote monitoring visits with the VA Puget Sound Health Care System study coordinator to review VA study data and VA medical records. VA Puget sound will provide the CRF source data to the UW study team by secured fax or secured email and all provided data is in accordance with applicable sub-award grant application provisions for the conduct of this study at the external participating site.

There is an Independent Medical Monitor for this study. The Medical Monitor, Cristina P. Rodriguez, will be informed that study enrollment has begun and become familiar with the study. The Medical Monitor will review the study approximately every 6 months throughout the study treatment period with a PI/Co-PI, Research MD(s), Research Nurse(s), Research Coordinator(s), and/or other related clinical research staff. The Medical Monitor will review patient recruitment and retention, adherence to protocol, follow-up, data quality, and participant risk versus benefit. The Medical Monitor has the authority to stop the research protocol in progress, remove individual human subjects from the research protocol, and take whatever steps necessary to protect the safety and well-being of human subjects and recommend actions that may need to be reported to the IRB.

If a SAE occurs the PI will be notified along with the FH/UW CC IRB, VA Puget Sound Health Care System IRB #1, IND Sponsor, FDA, Medical Monitor, UW IBC, UW TRU, FH Biologic Production, and U.S. Army Medical Research and Materiel Command Human Subjects Research Review Board (USAMRMC) Office of Research Protections (ORP) Human Research Protection Office (HRPO).

B. OBJECTIVES

1. To ensure that the PI, Co-PIs and Clinical Research Staff follow federal and institutional regulatory guidelines with respect to timely reporting of adverse reactions associated with investigational drugs

2. To define classification of adverse drug reactions as expected or unexpected
3. To define classification of adverse drug reactions as serious or non-serious
4. To define monitoring and stopping rules based on adverse drug reactions reports
5. To ensure compliance and accuracy of documentation of adverse drug reactions reportable to: (1) FH/UW CC IRB, (2) IND Sponsor, (3) FDA, (4) Medical Monitor, (5) UW IBC, (6) UW TRU, (7) FH biological production, (8) VA Puget Sound Health Care System IRB #1, and (9) USAMRMC ORP HRPO.

C. ADVERSE EVENT REPORTING POLICY AND PROCEDURES

Evaluation of AEs: AEs are graded on a scale of 1-5 and attribution is assigned using the CTEP CTCAE v5.0 which uses descriptive terminology. Information pertaining to toxicity is recorded.

If a particular significant AE severity/intensity is not specifically graded by the CTEP CTCAE v5.0, the PI, Co-PI, study physicians and/or physician extenderare to revert to the general definitions of Grade 1 through Grade 5 and use his or her best medical judgment.

The 5 general grades are:

- **Grade 1:** Mild
- **Grade 2:** Moderate
- **Grade 3:** Severe
- **Grade 4:** Life-threatening or disabling
- **Grade 5:** Death related to AE

Patients are monitored for the development of toxicities by assessing AEs with physical examinations and serum chemistries, liver function studies, and complete blood counts at baseline and prior to each vaccine and at post vaccine evaluation visits. The development of connective tissue disorders and laboratory autoantibody responses will also be clinically assessed for the development of anti-DNA antibodies (ANA, C3, thyroid function tests, and anti-dsDNA).

Abnormal laboratory findings and other abnormal investigational findings may not be reported as AEs unless they are clinically significant, associated with clinical signs and symptoms, lead to treatment discontinuation or are considered otherwise medically important by a PI and/or Co-PI.

If an abnormality fulfills these criteria, the identified medical condition (e.g. anemia) should be reported as the AE rather than the abnormal value itself (e.g. low hemoglobin).

For patients who are hospitalized, due to their disease, a PI, Co-PI, study physician, physician extender or IND sponsor will review their records and determine what may relevant event(s), procedures, and medications shall be documented in the study record.

1. Definitions of AEs:
   a. *AE* - any unfavorable and unintended sign (including abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.
      An AE may include:
      - an exacerbation of a pre-existing illness
      - an increase in frequency or intensity of a pre-existing episodic event or condition
      - a condition detected or diagnosed after study drug administration
      - continuously persistent disease or symptoms that were present at baseline and worsen following the start of the study

An AE does not include:

- medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, or transfusion); however, the condition that leads to the procedure may be an AE
- pre-existing diseases, conditions, or laboratory abnormalities present or detected at the start of the study that do not worsen
- hospitalizations or procedures that are done for elective purposes not related to an untoward medical occurrence (e.g., hospitalizations for cosmetic or elective surgery or social/convenience admissions)
- the disease being studied or signs/symptoms associated with the disease unless more severe than expected for the patient's condition
- overdose of study drug without any clinical signs or symptoms

b. *Expected AE* - an event that may be reasonably anticipated to occur as a result of the study procedure and is described in the Investigator Brochure and/or consent form. Expected AEs for this study are listed in Section 10.A. Tables 1-5.
c. *Unexpected AE* - an AE that is not described in the Investigator Brochure and/or consent form and is unanticipated. An event that might have been anticipated but is more serious than expected or occurs more frequently than expected, would be considered an unexpected AE.
d. *Life-threatening AE* - the patient was at substantial risk of dying at the time of the AE or it is suspected that the use or continued use of the product would have resulted in the patient's death.
e. *Serious AE* - grade 4 or 5 toxicity or any AE occurring at any dose that results in any of the following outcomes: death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization due to the AE, a persistent or significant disability/incapacity or a congenital anomaly/birth defect.
f. Hospitalizations that do not meet these criteria are:
    - social reason in the absence of an AE
    - surgery or procedure planned prior to entry into the trial

    Note: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations; for example, important medical events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Any AE is considered a SAE if it is associated with clinical signs or symptoms judged by a PI and/or Co-PI to have a significant clinical impact.

    Note: Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

2. Scale of scoring AEs
    a. Grade 1 = mild AE
    b. Grade 2 = moderate AE
    c. Grade 3 = severe adverse event
    d. Grade 4 = life-threatening or disabling AE
    e. Grade 5 = death related to AE

3. Attribution of AE
    a. 5 (definite): the AE is clearly related to the investigational agent.
    b. 4 (probable): the AE is likely related to the investigational agent.
    c. 3 (possible): the AE may be related to the investigational agent.

d. 2 (unlikely): the AE is doubtfully related to the investigational agent.
e. 1 (unrelated): the AE is clearly NOT related to the investigational agent.

4. SAE event reporting
   a. This procedure is outlined below by regulatory agency (Table 11).

FH/UW CC IRB – We will follow the current AE reporting policy of our institutional IRB.

VA Puget Sound Health Care System IRB#1 – We will follow the current AE reporting policy of our institutional IRB.

FDA (for trials using an Investigational New Drug (IND)) – SAEs will be reported per 21 CFR 312.32 by telephone or facsimile transmission (using either the narrative format, the MedWatch Form 3500A as soon as possible but no later than seven calendar days after initial receipt of the information concerning the event. All unexpected, SAEs are reported in writing (using either the narrative format, the MedWatch Form 3500A to the FDA Center for Biologics within 15 calendar days after initial receipt of the information concerning the event. Yearly written progress reports to the FDA will summarize expected or non-serious unexpected AEs.

Independent Medical Monitor - At a minimum, the medical monitor should comment on the outcomes of SAEs and relationship of the SAE to the vaccine or prepare an unbiased written report of the event. They should also indicate whether they agree with the details of the report provided by the study PI and/or Co-PI. SAE's determined by either the PI/Co-PI or medical monitor to be possibly or definitely related to participation and reports of events resulting in death must be promptly forwarded to the USAMRMC ORP HRPO (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil).

UW IBC - We will follow the current AE reporting policy of the UW IBC.

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

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

USAMRMC ORP HRPO – although we will report expected and unexpected serious and unexpected serious/fatal events to the FHCRC/UW CC IRB, we will also report these unanticipated events to the USAMRMC ORP HRPO promptly by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), reported by telephone (301-619-2165), or by facsimile (301-619-7803) to the HRPO.

Follow-up reports will need to be completed until resolution of the unanticipated event that was not available at the time of initial reporting.

Table 11. AE reporting

	Expected	Expected	Unexpected	Unexpected
	Non-Serious	Serious	Non-Serious	Serious (including grade 4 & 5 toxicity)
	Attribution * (Possible, Probable, Definite)	Attribution * (Possible, Probable, Definite)	Attribution * (Possible, Probable, Definite)	Attribution * (Possible, Probable, Definite)
FH/UW CC IRB	Continuation Review Report	Continuation Review Report	Continuation Review Report	FH AE Expedited Reporting Form – ASAP but within 7 days
VA Puget Sound Health Care IRB #1	Continuation Review Report	Continuation Review Report	Continuation Review Report	FH AE Expedited Reporting Form – ASAP but within 7 days and VA UPIRTSO form within 5 days and if resulted in death within 24 hours.
FDA	Annual Progress Report	Annual Progress Report	Annual Progress Report	FDA Form 3500A or Narrative Format – ASAP but within 7 days)
Medical Monitor	Bi-annual Meeting (twice a year)	Bi-annual Meeting (twice a year)	Bi-annual Meeting	FH AE Expedited Reporting Form – ASAP but within 7 days
UW IBC	Routine Report, as appropriate	Routine Report, as appropriate	Routine Report, as appropriate	ASAP within 7 days – MedWatch Form 3500A or Narrative Format)
UW TRU	Copy of Continuation Review Report if requested	Copy of Continuation Review Report if requested	Copy of Continuation Review Report if requested	Copy of FH AE Expedited Reporting Form
FH biological production	Yearly FDA Progress Report if requested	Yearly FDA Progress Report if requested	Yearly FDA Progress Report if requested	ASAP within 7 days – MedWatch Form 3500A or Narrative Format)
USAMRMC	Annual Progress	Annual Progress	Annual Progress	By FAX once we learn of

	Expected	Expected	Unexpected	Unexpected
	Non-Serious	Serious	Non-Serious	Serious (including grade 4 & 5 toxicity)
	Attribution * (Possible, Probable, Definite)	Attribution * (Possible, Probable, Definite)	Attribution * (Possible, Probable, Definite)	Attribution * (Possible, Probable, Definite)
ORP HRPO	Report	Report	Report	the event

5. Procedure for reporting SAEs:
   a. Identify the classification/attribution of the AEs as defined above using the CTEP CTCAE v5.0.
   b. After appropriate medical intervention has been instituted, the PI or his/her designee will be notified within 24 hours.
   c. File appropriate reports immediately by phone/fax with appropriate agencies, as described in Table 11.
   d. Notify the patient's primary physician or referring physician within a medically appropriate timeframe, depending on the classification of the AE.
   e. Submit written reports to appropriate agencies.
   f. Document the AE 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 AE in the patient's research file.

D. CLINICAL TRIALS MONITORING, STOPPING RULES AND OPERATIONAL PROCEDURES

Adverse Events and subject safety will be reviewed continuously. The PI will ensure that the DSMP is followed and that the AEs are properly monitored and accurately reported to the appropriate regulatory agencies in a timely manner. A status report including accrual, AEs and death information will be reviewed by a PI every 6 months and the FH/University of Washington Cancer Consortium Data and Safety Monitoring Committee annually. In addition, the study will be monitored by the Clinical Research Support Services according to the FH /University of Washington– Cancer Consortium monitoring plan.

1. Clinical data documentation
   a. <u>Internal study monitoring</u>:
      Clinical labs are evaluated at each patient visit for the development of toxicity (AEs) related to the vaccine. Clinically significant abnormal lab values may be faxed to the patient's physician.

      A Research Physician, physician extender or a PI/Co-PI sees each patient at each visit with the following evaluations being completed: toxicity evaluation, physical assessment and AEs summary (these evaluations are part of the source documentation that is filled out at each visit). Grade 1 and 2 non-serious and expected AEs will be reviewed with the PI or designee regularly at clinical meetings. All other AEs will be reported to the PI or designee at the time they become known and reported as outlined above in Section C.

      Each patient research file is audited for completeness, legibility, and accuracy. Audits may be conducted by a designated clinical research staff member(s) on an ongoing basis.

   b. <u>Biannual review with the independent Medical Monitor:</u>

The Medical Monitor will meet/or conference call with the PI or designee, and other members of the clinical team, approximately every 6 months. All patients are reviewed for AEs. Conduct of the study is reviewed for any practice changes and documentation of proper notification of changes as appropriate to the IRB and FDA.

c. Biannual study audit:
Institutional support of trial monitoring will be in accordance with the Fred Hutch/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, Fred Hutch Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP. In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), Fred Hutch Scientific Review Committee (SRC), and the Fred Hutch/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study. The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state, and federal guidelines.

2. Data validity/integrity
Systems to insure data integrity have been put into place to provide multiple checks to data entry. Patient eligibility is initially reviewed by the Research Coordinator/Research Nurse and a PI or designee during the screening portion of the study. The chart is then reviewed by a study physician/physician extender after the consent conference and before study procedures start. It is then ultimately signed off on by a PI or designee. Additionally, research data will be reviewed biannually by the Independent Medical Monitor and Study Monitor.

Clinical laboratory monitoring data is reviewed by a qualified clinician (Research MD, physician extender, Research Nurse and/or PI/Co-PI) and any abnormalities are assessed. Patient records are kept in the patient's research file and reviewed at biannual monitoring visits. Data is taken from the patient's source documentation, the patient's research file, and entered into a database which links data to patient by a UPN and is accessible by password code only. Data entry is made by the Research Coordinator/designated clinical research staff member and is verified at end of study by the Research Nurse/designated clinical research staff member by reviewing source documents and case report forms and reconciling them to the study database. A percentage of all data entry is reviewed by a member of the clinical research team and the Independent Study Monitor.

The trial will be stopped based on two continuous toxicity monitoring rules for adverse events of interest as defined in Section 11 - Table 5. The two continuous toxicity monitoring rules assume that the adverse event of interest (Table 5) probability is not more than 20% for grade 3 and no more than 10% for grade 4. The threshold to stop the trial, defined as the maximum number of grade 3 or 4 toxicity events of interest for every patient enrolled at a specific timepoint is shown in Tables 6 and 7. The trial will be stopped if the number of AE of interest is equal to or exceeds boundary (bn) out of n patients with completed follow-up.

Appendix D. NCCN Guidelines for Menopause Status

The National Comprehensive Cancer Network (NCCN) defines menopause as "generally the permanent cessation of menses, 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; amenorrheic for 12 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, then 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.