AWARD NUMBER: W81XWH-21-1-0271

TITLE: EMT Targeting Vaccination, Concurrent with Chemoimmunotherapy, in Advanced NSCLC

PRINCIPAL INVESTIGATOR: Dr. Shaveta Vinayak

CONTRACTING ORGANIZATION: University of Washington
Seattle, Washington
98109-4714

REPORT DATE: MAY-2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
MAY 2023	Annual	01-MAY-2022 TO 30-APR-2023

4. TITLE AND SUBTITLE

EMT Targeting Vaccination, Concurrent with Chemoimmunotherapy, in Advanced NSCLC

5a. CONTRACT NUMBER
W81XWH-21-1-0271

5b. GRANT NUMBER
LC200586

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Shaveta Vinayak

Laura Riolobos PhD E-Mail: lriolobo@uw.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Washington
850 Republican Street
Box 358050
Seattle, WA, 98109-4714

8. PERFORMING ORGANIZATION REPORT NUMBER

001

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Immunotherapy is a significant treatment advance for non-small cell lung cancer (NSCLC) and the only therapy that can lead to long term survival, although this occurs in less than 20% of patients. Patients receiving chemo-immunotherapy vs chemotherapy alone demonstrated a significantly longer survival. Despite this improvement, the majority of patients die within 2 years of diagnosis. High levels of tumor infiltrating lymphocytes (TIL) predict response to immune checkpoint inhibitor (ICI) therapy in animal models and lung cancer patients. Unfortunately, high levels of TIL are found in less than 10% of patients and a quarter of NSCLC patients have no evidence of TIL. Presence of CD8+ TIL is an independent prognostic variable in NSCLC and is associated with improved clinical outcomes. Strategies, such as vaccines, to increase CD8+ TIL could synergize ICI and improve survival in all NSCLC patients with advanced disease. Vaccines are able to induce tumor specific immunity and increase CD8 TIL in animal models.

Using novel techniques, we have developed STEMVAC, a multi-antigen vaccine targeting proteins in the epithelial to mesenchymal transition (EMT) pathway. EMT is the process by which anchorage dependent cancer cells develop the capacity to metastasize. We have identified upregulated proteins on the EMT pathway that are immunogenic and have defined in these proteins Type I CD4+ Th1 selective epitopes. CD4 Th1 immunity supports the generation of CD8+ cytotoxic T-cells. In animal models, STEMVAC profoundly inhibits cancer growth and results in the elimination of cells expressing EMT proteins. A Phase I trial has shown STEMVAC to be safe and immunogenic, generating high levels of peripheral blood antigen specific T-cells; similar to the levels reported with mutation-based vaccines suggesting Th1 selective epitopes act as neo-antigens.

Objective/Hypothesis. We hypothesize immunizing patients with advanced NSCLC, with measurable disease on maintenance immune checkpoint inhibitors (ICI), will generate tumor trafficking Type I T-cells resulting in increased activated CD8+ TIL. Vaccine induced TIL could "jump start" the immune system to stimulate further clinical responses with concurrent pemetrexed/pembrolizumab and eliminate lung cancer cells which have undergone epithelial to mesenchymal transition (EMT).

Specific Aims. (1) Determine whether intradermal (ID) 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) Evaluate safety and potential clinical efficacy of STEMVAC immunization and concurrent pemetrexed/pembrolizumab maintenance therapy in patients with advanced NSCLC, and (3) Determine whether vaccine induced T-cells traffic to tumor and can eliminate cancer cells which have undergone EMT.

15. SUBJECT TERMS

Non-small cell lung cancer, NSCLC, STEMVAC, cancer vaccines

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	12	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Immunotherapy with immune checkpoint inhibitors (ICI) is a significant treatment advance for non-small cell lung cancer (NSCLC) and the only therapy that can lead to long term survival, although this occurs in less than 20% of patients. Despite this improvement, the majority of patients die within 2 years of diagnosis. Our challenge is to make immunotherapy work for all patients with lung cancer. Once T-cells are appropriately activated, the cells will home to any site of disease, migrate from the blood stream into the tissue, and kill the cancer cells until none remain. Furthermore, if "memory" is generated tumor specific T-cells will remain in a resting state able to re-expand if the cancer returns. ICI therapy is most effective in patients who have started their own immune response before treatment. Studies have found that patients with higher levels of CD8+ T-cells in their tumors (TIL) prior to treatment respond the best to ICI. Unfortunately, most patients with NSCLC have minimum levels of TIL. Our objective, in this randomized Phase II clinical trial is to evaluate whether a multiple antigen vaccine, STEMVAC, given with GM-CSF as an adjuvant, and used to immunize NSCLC patients who are not responding to immuno-chemotherapy, increases the level of CD8 T-cells in the tumor. STEMVAC might "jump start" the immune system and lead to more clinical responses.

We aim to give STEMVAC to patients with metastatic NSCLC who have gone through immuno-chemotherapy and still have measurable disease. While the patients are on a maintenance therapy with a drug called pemetrexed and an ICI called pembrolizumab, we will vaccinate patients with STEMVAC and the immune stimulator GM-CSF or with GM-CSF alone as control group. GM-CSF has been shown to have anti-tumor effects so it is important to have two arms to the study to determine which effects are due to STEMVAC and which are due to GM-CSF. Our study is designed to evaluate whether the vaccine increases CD8+ T-cells in the tumor, is safe to use in combination with maintenance immune-chemotherapy, and will result in further clinical responses. We will also test whether vaccine induced T-cells home to the tumor and eliminate cells that have undergone epithelial to mesenchymal transition (EMT), which is a process involved in metastasis.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*
Non-Small Cell Lung Cancer; NSCLC; STEMVAC; Vaccine; Cancer Vaccine; Pembrolizumab; Pemetrexed

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aims. (1) Determine whether intradermal (ID) 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) Evaluate safety and potential clinical efficacy of STEMVAC immunization and concurrent pemetrexed/pembrolizumab maintenance therapy in patients with advanced NSCLC, and (3) Determine whether vaccine induced T-cells traffic to tumor and can eliminate cancer cells which have undergone EMT

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Task 0: Design, initiate and enroll a randomized Phase II study of ID STEMVAC administered with GM-CSF as a vaccine adjuvant vs. ID GM-CSF alone in 40 advanced stage non-squamous NSCLC patients with measurable disease receiving maintenance pemetrexed and pembrolizumab (20 patients/arm).

Subtask: Finalize protocol and obtain all required approvals

- Milestone Achieved: Local IRB and Military HRPO approval obtained at UW – **COMPLETED**
- Milestone Achieved: Protocol open to enrollment at UW– **COMPLETED**
- Milestone Achieved: VA added as a site by the UW local IRB - **COMPLETED**
- Milestone Pending: VA Puget Sound Health Care System site submission for IRB approval. - **ONGOING**

  The UW is currently negotiating the Data Use Agreement (DUA) with the VA. The DUA is the last document pending for the VA IRB submission, all the other documents have been prepared for submission to the VA IRB.

Subtask: Recruit, consent, screen and enroll all subjects - **ONGOING**

- Study is open to enrollment. We have identified physicians and clinics that are willing to refer patients to this study.
  - We have screened a total of 12 patients, we are currently following 2 patients that should complete their initial standard treatment and then will be screened for eligibility to this study.
  - Subjects enrolled – 1
  - Subjects on-trial – 1

Major Task 1: Determine whether ID STEMVAC+GM-CSF vaccination increases the percentage of CD8+ TIL in patients with advanced NSCLC compared to patients who receive ID GM-CSF alone.
We had maintained meetings with the radiologists that will perform the biopsies, the lab that will process the tissue and the pathologist that will analyze the samples to make sure everything is ready the trial. We have also stablished a Standard Operating Protocol (SOP) to define every step for processing the biopsies.

Blood samples and biopsies have already been obtained for the first patient enrolled in the trial and the samples are currently stored in the Cancer Vaccine Institute biorepository until they are analyzed. Biopsies were fixed in formalin for 24 hours and subsequently embedded in paraffin by the UW Histology Core. Formalin fixed paraffin embedded (FFPE) biopsy tissue will be analyzed to determine the percentage of CD8+ TIL in NSCLC patients before and after vaccination (Task 1) as well as the percentage of tumor cells that have undergone epithelial to mesenchymal transformation (EMT) (Task 3). Blood has been processed to isolate peripheral mononuclear blood cells (PBMC) and it is currently frozen until further analysis. PBMC will be used to confirm immune response to the vaccine.

Major Task 2: Evaluate safety and potential clinical efficacy of STEMVAC immunization and concurrent pemetrexed/pembrolizumab maintenance therapy in patients with advanced NSCLC. – Not Applicable for this reporting period.

Major Task 3: Determine whether vaccine induced T-cells traffic to tumor and can eliminate cancer cells which have undergone EMT – Not Applicable for this reporting period.

Major Task 4: Disseminate findings – Not Applicable for this reporting period.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those

in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

- We are working with the VA Puget Sound Health Care System to open the study at their location.
- We have presented this study to local physicians who treat Non-Small-Cell Lung Cancer at the Seattle Cancer Care Alliance monthly meeting. This will help identify potential patients, at a specific time in their treatment, to determine if they are eligible to participate.
- Enrollment and treatment are ongoing according to the approved protocol.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report. We have not made any significant changes in our approach, objectives or scope that have not been reviewed by the HRPO.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We know that it takes time, once a study opens, to make physicians aware of the study for them to refer their patients. We are working with local physicians on a regular basis to promote this study.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No changes.

Significant changes in use or care of vertebrate animals

No changes.

Significant changes in use of biohazards and/or select agents

No changes.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to Report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	Laura Riolobos PhD
Project Role:	Project Principal Investigator
Research Identifier:	0000-0002-8876-2514
Nearest person month worked:	2.40
Contribution to Project:	Dr. Riolobos oversees all aspects of project design and implementation.
Name:	Mary (Nora) L Disis MD
Project Role:	Co-Investigator
Research Identifier:	0000-0001-7653-4648
Nearest person month worked:	0.48
Contribution to Project:	Dr. Disis provides medical expertise on the protocol.

Name: Yi Liu PhD
Project Role: Biostatistician
Research Identifier: N/A
Nearest person month worked: 0.84

Contribution to Project: Dr. Liu oversees statistical study design and statistical data analysis.

Name: Jennifer Childs
Project Role: Research Coordinator
Research Identifier: N/A
Nearest person month worked: 1.20

Contribution to Project: Ms. Childs is responsible for regulatory oversight. She assists in protocol development, IND application and IRB applications and will maintain all clinical trial regulatory documents and safety reporting as applicable.

Name: Kris Kauno RN
Project Role: Research Coordinator
Research Identifier: N/A
Nearest person month worked: 1.63

Contribution to Project: Ms. Kauno manages subject recruitment/screening, oversees all patient visits, and monitoring of data and adverse events.

Name: Katelyn Jones
Project Role: Research Coordinator
Research Identifier: N/A
Nearest person month worked: 0.53

Contribution to Project: Ms. Jones is responsible for subject recruitment/screening, and data entry relating to subject visits.

Name: Evangeline Chang
Project Role: Research Coordinator
Research Identifier: N/A
Nearest person month worked: 1.20

Contribution to Project: Ms. Chang is responsible for subject recruitment/screening, and assisting with patient visits.

Name: Yi Yang
Project Role: Research Scientist
Research Identifier: N/A
Nearest person month worked: 1.98

Contribution to Project: Mr. Yang is responsible for processing, documenting, storage, and control of all human specimens obtained for the life of the clinical trial

Name: Ryan Brennan
Project Role: Research Study Coordinator/Research Coordinator
Research Identifier: N/A

Nearest person month worked:	0.85
Contribution to Project:	Mr. Brennan is responsible for processing, documenting, storage, and control of all human specimens obtained for the life of the clinical trial.
Name:	Huiyun Shen
Project Role:	Research Scientist
Research Identifier:	N/A
Nearest person month worked:	0.60
Contribution to Project:	Ms. Shen is responsible for overseeing processing, documenting, and storage activities, and she will oversee all assay work relating to clinical specimens obtained during the trial.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*