AWARD NUMBER: W81XWH-18-1-0132

TITLE: Phase 1 Safety and Feasibility Study of a Personal Neoantigen-Targeting Vaccine in Combination with Immune Checkpoint Blockade in Ovarian Cancer

PRINCIPAL INVESTIGATOR: Panagiotis A. Konstantinopoulos, MD, PhD

CONTRACTING ORGANIZATION: Dana-Farber Cancer Institute

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Fort Detrick, Maryland 21702-5012

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Phase 1 Safety and Feasibility Study of a Personal Neoantigen-Targeting Vaccine in Combination with Immune Checkpoint Blockade in Ovarian Cancer

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5b. GRANT NUMBER
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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Dana-Farber Cancer Institute
450 Brookline Avenue,
Boston, MA 02215-5450

8. PERFORMING ORGANIZATION REPORT NUMBER

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

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Although immune checkpoint blockade (CPB) therapy have demonstrated potent clinical activity across multiple tumor types, only modest effectiveness in ovarian cancer has been observed. In this application, we have proposed a novel treatment approach in epithelial ovarian cancer (EOC) which consists of administration of neoantigen-targeting vaccines in combination with CPB therapy. We hypothesized that applying this approach to patients with EOC will effectively expand existing tumor-reactive T-cells and broaden the T-cell repertoire to include new tumor-specific T-cells thereby generating highly specific anti-tumor immunity with fewer autoimmune side effects. Throughout the timeline of the award we have proposed to: i) conduct a clinical trial to determine if Neovax/Nivolumab (anti-PD-1 antibody) is feasible and safe in newly diagnosed or recurrent platinum sensitive EOC, and is associated with evidence of clinical activity; ii) define the evolution of T cell specificity, state and repertoire following standard of care (SOC) chemotherapy and after Neovax/Nivolumab and iii) to characterize the genetic evolution of patient tumor cells and of tumor microenvironment following standard-of-care chemotherapy and after Neovax/Nivolumab. We have now obtained HRPO approval and our clinical trial has activated. Relevant to analyses for Aims 2 and 3, we continue to characterize T cell responses and changes in immunogenicity through the course of standard of care chemotherapy. We have already found that standard of care platinum and taxane chemotherapy used in ovarian cancer: i) does not deplete T cells (although it does deplete granulocytes), ii) does not impact the ability of T cells to respond to antigens and iii) leads to an increase in TCR diversity.

15. SUBJECT TERMS Epithelial Ovarian Cancer, Neoantigens, Neoantigen Vaccines, Standard of Care chemotherapy, TCR diversity, PD-1 inhibition, Nivolumab					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
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Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

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

Although immune checkpoint blockade (CPB) therapy have demonstrated potent clinical activity across multiple tumor types, only modest effectiveness in ovarian cancer has been observed. In this application, we have proposed a novel treatment approach in epithelial ovarian cancer (EOC) which consists of administration of neoantigen-targeting vaccines in combination with CPB therapy. We hypothesized that applying this approach to patients with EOC will effectively expand existing tumor-reactive T-cells and broaden the T-cell repertoire to include new tumor-specific T-cells thereby generating highly specific anti-tumor immunity with fewer autoimmune side effects. Throughout the timeline of the award we have proposed to: i) conduct a clinical trial to determine if Neovax/Nivolumab (anti-PD-1 antibody) is feasible and safe in newly diagnosed or recurrent platinum sensitive EOC, and is associated with evidence of clinical activity; ii) define the evolution of T cell specificity, state and repertoire following standard of care (SOC) chemotherapy and after Neovax/Nivolumab and iii) to characterize the genetic evolution of patient tumor cells and of tumor microenvironment following standard-of-care chemotherapy and after Neovax/Nivolumab.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Epithelial Ovarian Cancer, Neoantigens, Neoantigen Vaccines, Standard of Care chemotherapy, TCR diversity, PD-1 inhibition, Nivolumab

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.

Throughout the timeline of the award the following goals have been proposed:
Major Task 1: Conduct a clinical trial to determine if Neovax/Nivolumab is feasible and safe in newly diagnosed or recurrent platinum sensitive epithelial ovarian cancer (EOC), and is associated with evidence of clinical activity.
Major Task 2: Define the evolution of T cell specificity, state and repertoire following Neovax/Nivolumab.
Major Task 3: Major Task 3: Characterize the genetic evolution of patient tumor cells and of tumor microenvironment following standard-of-care (SOC) chemotherapy, Neovax/Nivolumab, and upon disease progression.

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.

Regarding Major Task 1 (AIM 1), we are very excited to report that we have now received HRPO approval for the clinical trial which is now activated in our institution. The subject protocol (version 3 dated 19 February 2020) was approved by the Dana-Farber Cancer Institute Institutional Review Board (IRB) on 8 September 2020. The U.S. Army Medical Research and Development Command (USAMRDC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) reviewed the protocol and found that it complies with applicable DOD, U.S. Army, and USAMRDC human subjects protection requirements.

Relevant to analyses for Aims 2 and 3 (Major Tasks 2 and 3) we have been characterizing T cell responses, changes in the somatic mutation landscape and changes in immunogenicity through the course of standard of care chemotherapy. Below are some key results of these analyses through the second year. We are very close to reporting these findings in a major oncology conference and subsequently publishing these in a major oncology journal.

Specifically, we have performed analyses of samples collected during administration of standard of care platinum and taxane chemotherapy (Figure 1) and specific studies have been performed and are being performed as highlighted in the Figure.

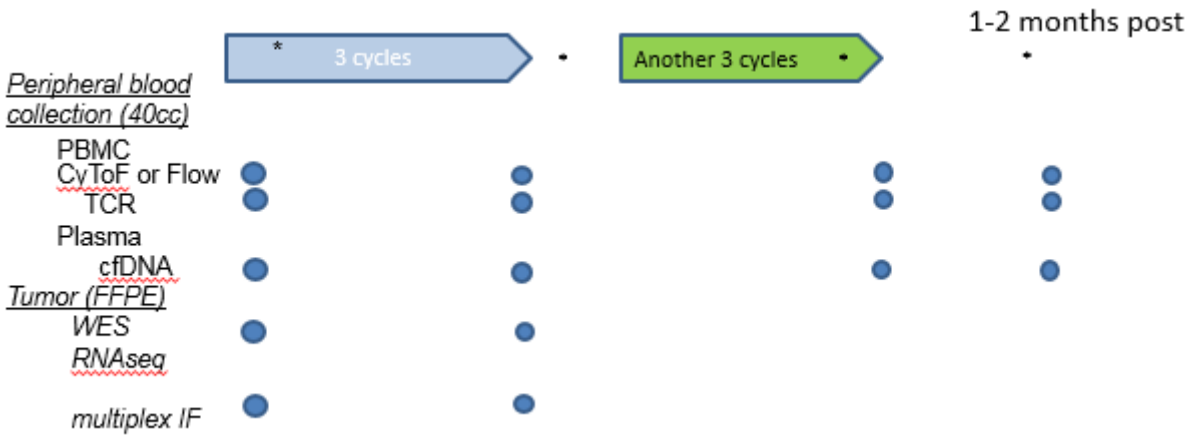

Figure 1. Timepoints and assays

Our findings thus far are:

i) Standard of Care chemotherapy does not deplete T cells

Figure 2 below highlights flow cytometric analysis of cell populations at paired samples from baseline, after C3, after C6 and 1-2 months after completion of chemotherapy. As evident in Figure 2, there is no change in the CD4+ and CD8+ T cells from baseline, throughout chemotherapy and at the end of chemotherapy. Similar analyses have been done with additional paired samples and demonstrated no depletion of T cells as a result of chemotherapy.

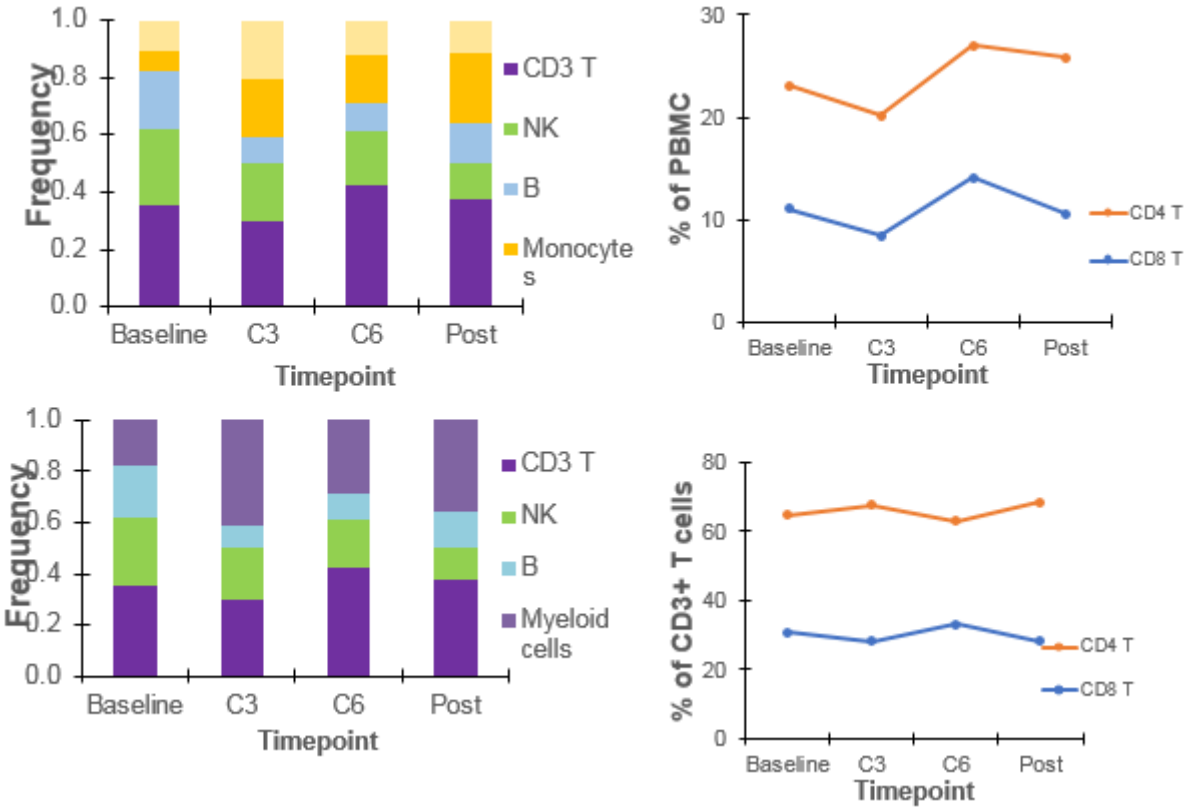

Figure 2. Flowcytometric analyses of cell populations during chemotherapy.

ii) Standard of care chemotherapy does not impact the ability of T cells to respond to antigens

To assess whether chemotherapy impacts the functional capacity of T cells, we assessed the reactivity of T cells to specific antigens using ELISpot assays at baseline, during chemotherapy and 1-2 months after completion of chemotherapy.

As shown in Figure 3 below, the ability of T cells to respond to various antigens (FLU-A, CEF, OVA) is not affected by chemotherapy and even leads to increased reactivity to CEF. Similar analyses have been done with additional paired samples and demonstrated no depletion of T cell ability to respond to antigens.

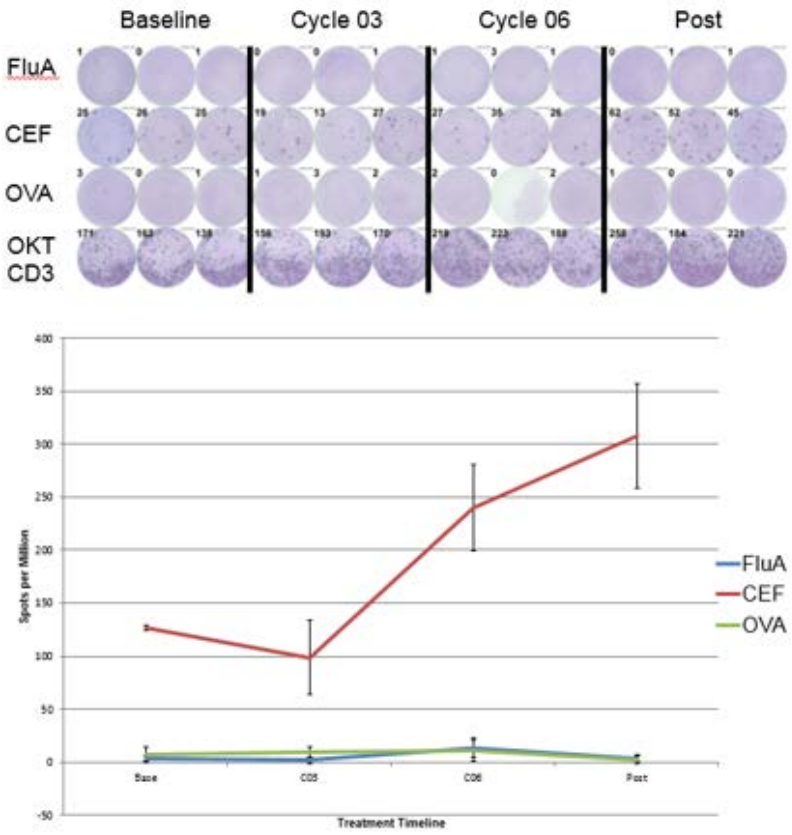

Figure 3. ELISpot assays (above) and quantification (below) of reactivity of T cells for specific antigens (FLU-A, CEF and OVA) at baseline, after 3 cycles, after 6 cycles and 1-2 months after completion of chemotherapy.

iii) Standard of care chemotherapy leads to an increase in TCR diversity

To assess whether chemotherapy impacts T cell receptor (TCR) diversity, we performed TCR sequencing (TCRalpha and beta sequencing) from bulk RNA at baseline, after 3 cycles, after 6 cycles and 1-2 months after completion of chemotherapy.

These analyses are ongoing, but as shown in Figure 4 below, after C6 of chemotherapy and 1-2 months after completion of chemotherapy, new clonotypes have emerged and the TCR diversity and repertoire has increased.

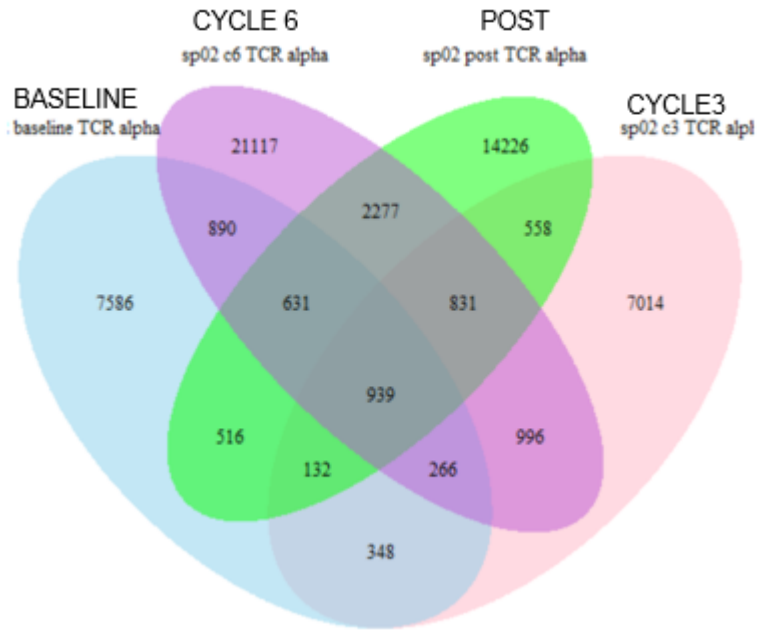

Figure 4. Bulk TCR alpha sequencing results at various time points (baseline, after C3, after C6 and 1-2 months after chemotherapy.

Additionally, as shown in Figure 5, TRAV gene usage analysis showed a high divergence between C6 and post chemotherapy and low divergence between baseline and C3 samples.

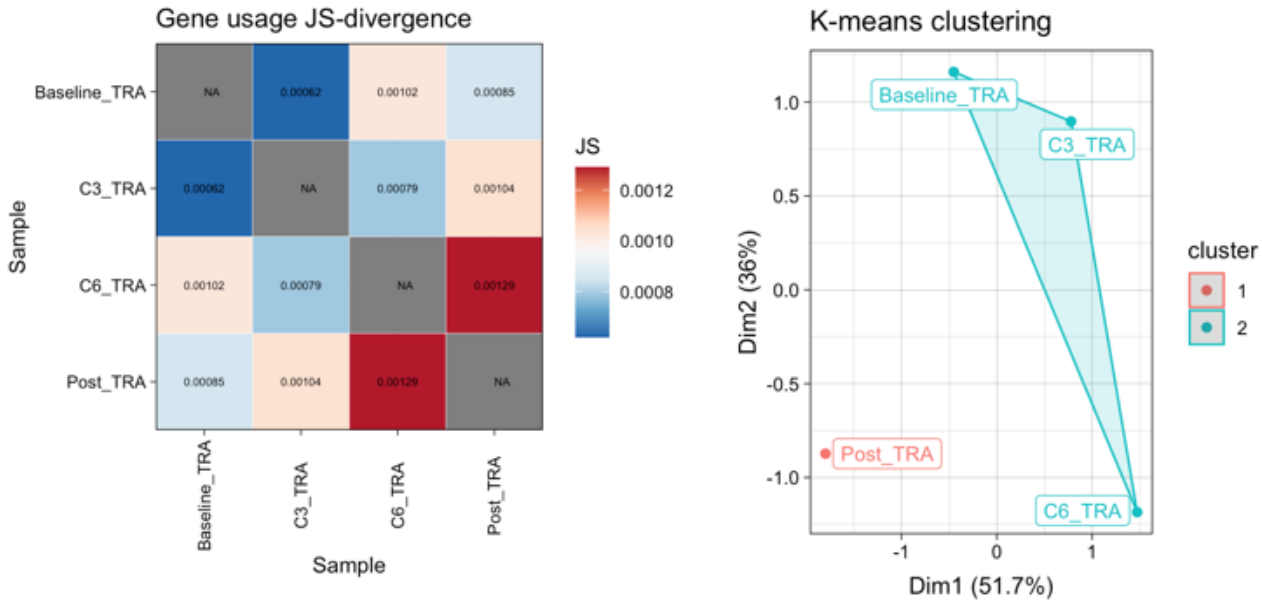

Figure 5. Divergence of T cell repertoire based on TRAV Gene Usage

In Figure 6 below, we present the Top 10 most abundant clonotypes that were more "stable" throughout chemotherapy and the 10 least abundant clonotypes which changed dramatically from baseline to post chemotherapy.

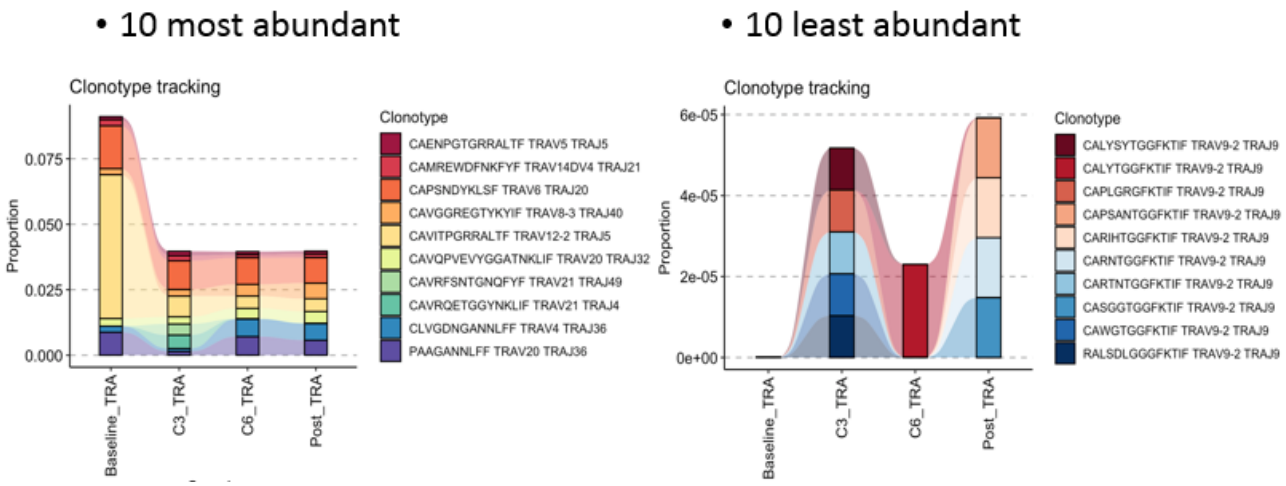

Figure 6. Clonotype tracking and annotation

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."

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

Regarding Major Task 1 (AIM 1), our clinical trial has received HRPO approval and is now activated. Regarding the remaining AIMS, further analyses are ongoing on the above data and we anticipate that we will be reporting these findings soon in a major oncology conference and subsequently publishing these in a major oncology journal. Finally, WES, RNAseq and multiplex IF studies are ongoing to determine if chemotherapy changes the tumor and tumor immunogenicity.

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:*

As discussed above, regarding Major Task 1 (AIM 1), our clinical trial has not been activated yet but we do expect this will happen in July-August 2020. The reason for that was that we had encountered problems initiating the study mainly because of an issue with the peptide manufacturing process for the neoantigen vaccine. These issues have been completely resolved and we are now ready to initiate the trial. There is significant patient interest in our study and we expect to enroll promptly when the study is activated.

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.

"Nothing to Report."

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

"Nothing to Report."

Significant changes in use or care of vertebrate animals

"Nothing to Report."

Significant changes in use of biohazards and/or select agents

"Nothing to Report."

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Panagiotis Konstantinopoulos
Project Role: PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Contribution to Project: Dr Konstantinopoulos is working on characterizing T cell responses and evolution of immunogenicity during standard of care chemotherapy

Name: Catherine Wu
Project Role: Co-I
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Contribution to Project: Dr Wu is working on characterizing T cell responses and evolution of immunogenicity during standard of care chemotherapy.

Name: Derin Keskin
Project Role: Staff Scientist
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 3
Contribution to Project: Dr Keskin is working on characterizing T cell responses and evolution of immunogenicity during standard of care chemotherapy.

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."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project 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."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

"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://ers.amedd.army.mil*** *for each unique award.*

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

None.

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.*

None.