AWARD NUMBER: W81XWH-17-1-0141

TITLE: Epigenomic Priming as an Immunotherapy Enhancer in Ovarian Cancer

PRINCIPAL INVESTIGATOR: Daniela Matei, MD

RECIPIENT: Northwestern University

REPORT DATE: August 2021

TYPE OF REPORT: FINAL

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
AUGUST 2021	FINAL	05/01/2017 - 04/30/2021

4. TITLE AND SUBTITLE

Epigenomic Priming as an Immunotherapy Enhancer in Ovarian Cancer

5a. CONTRACT NUMBER
W81XWH-17-1-0141

5b. GRANT NUMBER
OC160260

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)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
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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
New immunologic approaches targeting immune checkpoint pathways, such as the programmed cell death protein-1 (PD-1) are under clinical development for solid tumors,including ovarian cancer (OC). Anti-PD1 strategies prevent T-cell exhaustion, augmenting immune anti-tumor responses. The focus of this application is to develop a combination regimen that enhances the activity of PD1-targeted immunotherapy in a clinical trial designed for women with recurrent ovarian cancer. We speculate that an important mechanism of immune evasion in OC is represented by epigenetic silencing of tumor antigens. One of the mechanisms of transcriptional repression of tumor antigens.

15. SUBJECT TERMS
Ovarian cancer, DNA methylation, immune checkpoint inhibitors, tumor neoantigen

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	14	19b. TELEPHONE NUMBER *(Include area code)*

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

The purpose of the project is to analyze tumor biopsies and PBMCs collected as part of a clinical trial for women with platinum resistant ovarian cancer treated with epigenetic priming (guadecitabine) and pembrolizumab. The hypothesis is that epigenomic priming will enhance anti-tumor immunity and synergize with immune checkpoint inhibitors.

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

Ovarian cancer, DNA methylation, immune checkpoint inhibitors, tumor neoantigen

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.

Major Task 1: Measure tumor antigens in specimens collected from clinical trial

Subtask 1: Clinical trial enrollment and treatment	Completed; 48 patients enrolled, 43 patients treated and 33 patients evaluable. Clinical trial analysis is completed.
Subtask 2: Tumor biopsies, PBMC and plasma collection and storage	samples from 35 patients collected (325 PBMC specimens and 48 tumor biopsies and 8 ascites specimens)
Subtask 3: Extract DNA and RNA from tumor biopsies	100% completed for the patients enrolled
Subtask 4: Extract DNA from PBMC	100% completed for all patients enrolled
Subtask 5: LINE 1 and tumor antigen pyrosequencing	Completed for all specimens
Subtask 6: Tumor neoantigen measurement	3 paired samples completed
Subtask 8: Q-RT-PCR in tumor biopsies—tumor antigens	Completed in samples where RNA was sufficient
Subtask 9: Erv transcript assessment via PCR	Completed on existing specimens

Major Task 2: Measure immune response in specimens collected from clinical trial:

Subtask 11: FFPE tissue sections — Completed for all core biopsies collected to date

Subtask 12: IHC for CD3, CD8, CD4, granzyme B — Because the technology advanced since the time of the grant submission and the scant material available from the biopsies had to be prioritized, we developed multiplex IHC that allows evaluation of 7 markers on the same tissue (cytokeratin, CD3, CD8, CD20, CD68, FoxP3 and DAPI). The conditions for mIHC were optimized and slides were stained. Analysis has been completed on responders vs. non responders.

Subtask 13: Flow cytometry ascites and PBMC — Flow cytometry was performed on few ascites specimens. Additionally, because the technology advanced, we had access to CyTOF which permits much deeper characterization of immune cell subsets and performed CyTOF on several paired PBMC and ascites specimens, with very important results.

Subtask 14: Double IHC for TA and CD8 — Done on 20 specimens using mIHC

Subtask 15: IHC interpretation — Completed and analyzed

Subtask 16: Measure NY-ESO-1–specific $CD8^+$ response. — This was performed on 3 ascites specimens collected that were HLA2 positive.

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.

George Hutchins, undergraduate student, CURE Program, summer 2017
Natalia Rombell, high school student, spring 2018
Guangyuan Zhao, PhD student, fall 2017, spring 2018
Yaqi Zhang, PdD student, spring 2018
Azza Mohamed, Master student, spring-fall 2018
Nikita Lavanya Mani PhD student, fall 2017
Renqiang Ma, MD Visiting Scholar, spring 2018
Gaoxiang Wang MD Visiting Scholar, spring 2018
George Hutchins, undergraduate student, CURE Program, summer 2018
Natalia Rombell, undergraduate student, spring 2018
Hanna Kubo, PhD student, fall 2018
Matthew Cowan, DO, fall-winter 2018, spring 2019
Sonal Khare, PhD Postdoc, spring 2019
Ping Xie, Post doc fall 2019, winter, spring, summer 2020, fall, winter, spring 2021
Russel Keathley, graduate student, fall, winter 2020, spring, summer 2021

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.

Survive and Thrive, Chicago, September 2017 –Dr. Matei presented the clinical trial to a group of patients at Northwestern University
Stop Cancer, Bucharest April 2017—Dr. Matei presented design of the study to a group of physicians and scientists in Romania
MDACC, March 2018: Research Seminar, Houston Texas
Cleveland Clinic, December 2017: Research Seminar, Cleveland OH
Ohio State University, September 2018: Research Seminar including preliminary results from the trial
Oklahoma University, March 2019: Research Seminar including preliminary results from the trial
Stop Cancer, Bucharest, May 2019—Research seminar to physicians and scientists in Romania
Gynecology Oncology Showcase, Northwestern University May 2019—results presented to physicians from the Department of Obstetrics and Gynecology
ASCO 2020; poster presentation of clinical endpoints
AACR 2020; part of oral presentation in Educational Symposia
SGO 2021: Plenary session, oral presentation of translational endpoints by Matthew Cowan
Translational Bridge Symposium Lurie Cancer Center 2021: Matei presents final results of the study

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

Study and analysis are completed, we are finalizing the manuscript, which has not yet been submitted, but should be submitted in September/October 2021.

4. IMPACT: Describe 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).

1. The study is providing new information on the transcriptome and methylome of platinum resistant ovarian cancer. Data will be soon made publicly available in GEO.
2. The study is providing new information about the immune composition of the ovarian tumor microenvironment in platinum resistant ovarian cancer.
3. The study is providing new information on the impact of epigenetic priming to immunotherapy in ovarian cancer.
4. The study is proposing new biomarkers to predict response to immunotherapy in recurrent ovarian cancer.

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.

Because the tissue obtained through biopsies is scant, nucleic acids extracted for some specimens are in low amount and will have to be prioritized for use. To gain most knowledge from the specimens obtained, we used RNA sequencing (instead of RT-PCR for multiple genes) for the specimens yielding sufficient amount of RNA. This allowed getting information on many genes, rather than on a small set of genes. Likewise, for the IHC analyses proposed in Aim 2, we developed multi-channel IHC to allow examining multiple markers on the same specimen and maximize use of tissue. Additionally, we have used CyTOF to characterize PBMC populations, as this technology allows for higher resolution definition of immune cell populations. This is in line with the advancement of technology during the past 2 years and represents the current state of the art and does not change the scope of the research objectives proposed. The costs for these analyses are higher than what is originally proposed and we supplemented with additional internal sources of funding.

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.

Several patients enrolled were not evaluable due to early disease progression. These patients were replaced in order to have sufficient numbers to reach the clinical objectives of the trial. The regulatory approvals slightly delayed completion of enrollment. However, the trial completed enrollment in late fall 2019 and the analysis is now almost complete. One patient remains on treatment beyond 2 years with stable disease. All specimens were received and processed according to the proposal.

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.

Expenditures are now complete.

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

None

Significant changes in use or care of vertebrate animals

None

Significant changes in use of biohazards and/or select agents

None

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).*

One high impact manuscript is planned for this fall (Cancer Discovery or JCI) and a second smaller impact manuscript will be submitted in the winter (Cancer or Gynecologic Oncology).

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.*

Poster presentation at ASCO 2020, Oral Presentation at AACR 2020, Plenary presentation at SGO 2021. Two manuscripts are in preparation.

- **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.

Transcriptomic and methylomic data are being deposited in GEO.

- **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: Daniela Matei
Project Role: PD/PI
Nearest person month worked: 1
Contribution: oversees clinical trial activities, oversees research activities, organizes monthly meeting with co-Is, meets individually with co-Is at least quarterly, meets with research coordinators weekly, reviews results, organizes plan for analyses

Name: Bin Zhang
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution: responsible for completion of Aim 2, oversees one postdoctoral fellow, reviews results, organizes plan for analyses

Name: Hao Huang
Project Role: Co-Investigator
Nearest person month worked: 6
Contribution: RT-PCR, library preparation and sequencing, methylomic analysis, data analysis

Name: Horacio Cardenas
Project Role: Co-Investigator
Nearest person month worked: 6
Contribution: specimen collection and logging, sequencing analysis, data analysis, pyrosequencing

Name: Siqi Chen
Project Role: Co-Investigator
Nearest person month worked: 12
Contribution: postdoctoral fellow, IHC, flow cytometry, data analysis

Name: Azza Mohammad
Project Role: Technician
Nearest person month worked: 6
Contribution: nucleic acid extraction, specimen collection and logging.

Name: Mathew Cowan, MD
Project Role: Fellow
Nearest person month worked: 12
Contribution: multi-plex IHC and analysis.

Name: Ping Xie, PhD
Project Role: Post doctoral fellow
Nearest person month worked: 12
Contribution: multi-plex IHC and analysis.

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.

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: N/A