AWARD NUMBER: W81XWH-17-1-0141

TITLE: Epigenomic Priming as an Immunotherapy Enhancer in Ovarian Cancer

PRINCIPAL INVESTIGATOR: Daniela Matei, MD

RECIPIENT: Northwestern University
Evanston, IL 60208

REPORT DATE: May 2019

TYPE OF REPORT: Annual Report

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)*
May 2019	Annual	1 May 2018 - 30 Apr 2019

4. TITLE AND SUBTITLE
Epigenomic Priming as an Immunotherapy Enhancer in Ovarian Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0141

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Daniela Matei, MD
Bin Zhang, MD, PhD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
NORTHWESTERN UNIVERSITY
633 CLARK ST
EVANSTON IL 60208

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel 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
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
unclassified	unclassified	unclassified		29	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.

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	37 of 35 patients enrolled, but of those, only 30 patients were evaluable for response (completed 2 cycles), thus the protocol was amended to allow enrollment of 6 more patients, funding was obtained for this and all regulatory approvals were obtained to extend enrollment. Because of this, the trial was closed for 4-5 months, and re-opened for enrollment 2 months ago. Two patients were recently registered.
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	Samples sent for analysis to EpigenDx, results not available
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 17 slides were stained. Staining will be complete by the end of June 2018 on all existing specimens. We are in the process of optimizing analysis of mIHC using a new software.

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 — This was not started because of the time needed to optimize mIHC

Subtask 15: IHC interpretation — In process

Subtask 16: Measure NY-ESO-1–specific $CD8_+$ response. — This was done on 3 ascites specimens collected that were HLA2 positive.

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.

1) Major activities and results

a) Clinical activities: The clinical trial was activated and 37 patients were enrolled to date, of which only 30 were evaluable for response. Enrollment was expanded to allow 7 more patients to be enrolled and reach 35 patients evaluable. All regulatory approvals were obtained to allow this expansion.

--Preliminary results:

-Efficacy: Milestones for first stage of accrual were met, allowing full enrollment to the trial. Analysis of efficacy endpoints is not complete, but we had several patients with response and prolonged stable disease (one for 2.5 years).

-Toxicity: The regimen was generally well tolerated, with myelosupression as the main toxicity. Use of growth factors was recommended and mitigated this toxicity. 19 SAEs have been reported, of which 7 were considered related (3 colitis, 1 arthritis, 2 grade 3 neutropenia, 1 infection and allergic reaction). Of those, 5 events represent immune mediated toxicity. The toxicity is in line with what was expected.

b) Nucleic acid extraction from all tumor biopsies collected from the protocol completed for the available specimens. 352 specimens were obtained from PBMCs and 97 specimens were obtained from tumors (DNA and RNA together) and were analyzed or are in the process of analysis. Year 1-2

c) FFPE slides obtained from the enrolled patients. Staining is in progress, multiplex IHC was standardized and 17 specimens were stained, with additional staining in progress right now. Year 2

d) Evaluation of gene expression changes was obtained by RNA Sequencing (and by QRT PCR for specific genes. Year 1

e) Development of multiplex IHC and staining of tumor biopsies before and after treatment (17 slides stained, analysis not complete). Year 2

f) Measurement of cytotoxic activity in T cells collected from ascites, before and after treatment. Year 2

g) Characterization of PBMCs by flow cytometry and CyTOF with the goal of identifying predictive markers of response. Year 2

Significant results:
Year 2:
Development of multiplex IHC

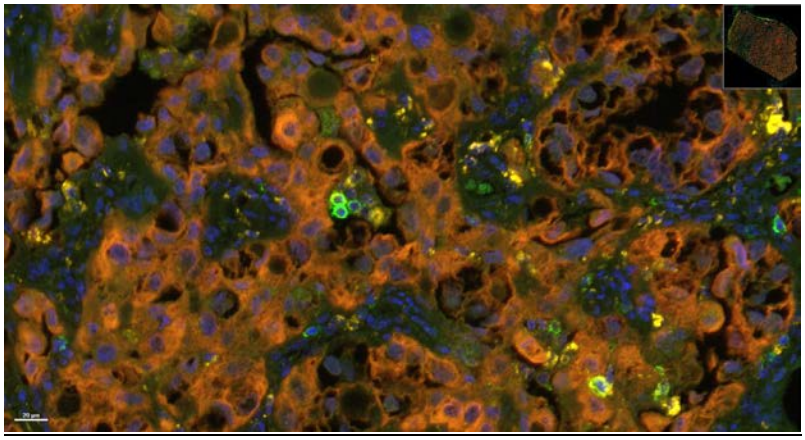


Figure 1: Multiplex IHC evaluates 7 markers on one specimen. Shown is an ovarian cancer specimen stained for CD3 (Green, 520); CD8 (yellow, 540); CD20 (yellow, 570); CD68 (orange, 620); FoxP3 (red, 650), PanCK (red, 690) and DAPI (Blue).

Characterization of PBMCs by mass cytometry (CyTOF) which permits high resolution definition of immune cell subpopulations.

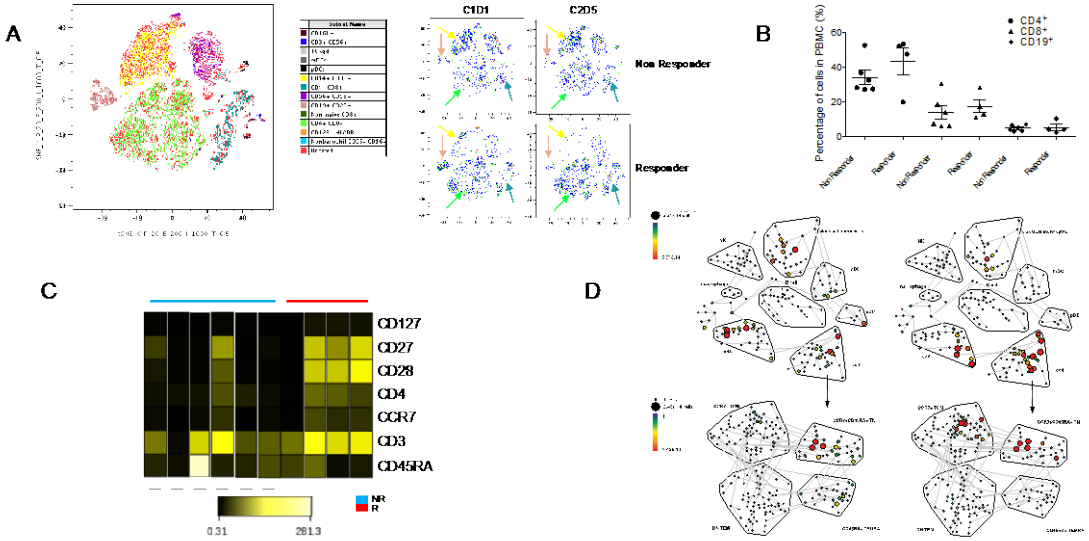


Figure 2. **Identification of differences in PBMCs from responders and non-responders to treatment with guadecitabine and pembrolizumab using mass cytometry.** (A). Exemplified tSNE visualization of overlaid events from nonresponder and responder. (B). Cell population composition in the PBMCs of, nonresponders (NR, n = 6) and responders (R, n = 4) before the initiation of therapy (C1D1). (C). (C). The heat map represents the median indicated marker expression within the live intact cells from non-responders (left) and responders (right). (D). SPADE analysis of different immune cell populations in the PBMCs of non-responders (upper left) and responders (upper right); as well as the subsets among total $CD4^+$ T cells of non-responders (lower left) and responders (lower right) at C1D1.

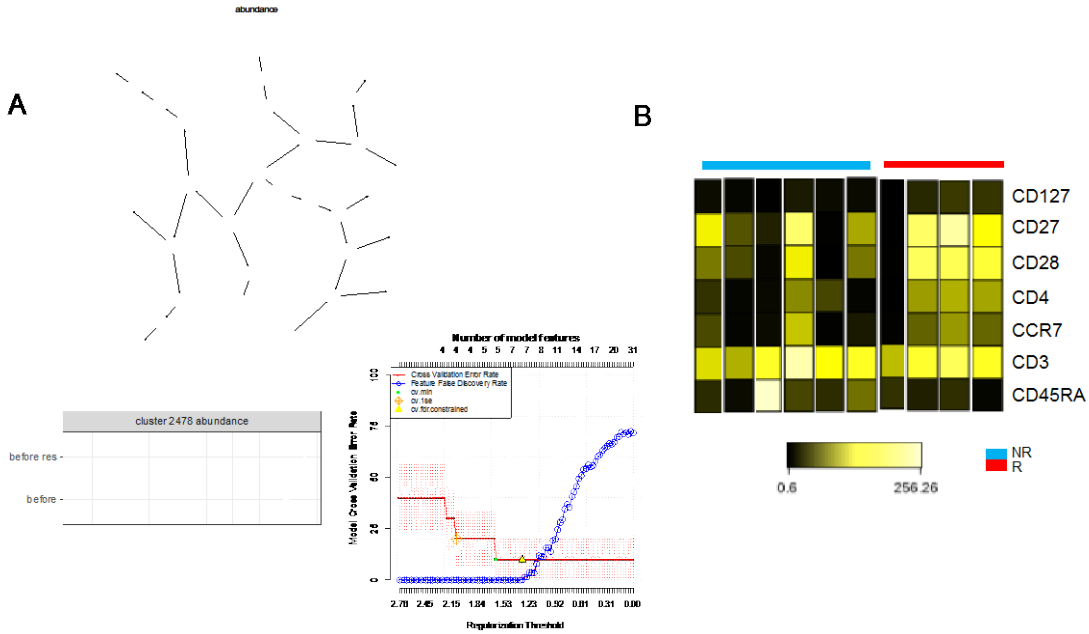


Figure 3. **Identification of clusters with significant difference between responders and non-responders before treatment with guadecitabine and pembrolizumab.** (A). Citrus analysis of cluster abundance identified multiple clusters that are non-responders or responders biased. (B). The heat map represents the median indicated marker expression within the cluster of 2478 from Citrus analysis from non-responders (left) and responders (right).

<u>Characterization of immune cell populations in ascites before and after treatment by mass cytometry (CyTOF). Characterization of cytotoxic tumor-specific activity of CD8 cells by ELISPOT in ascites.</u>

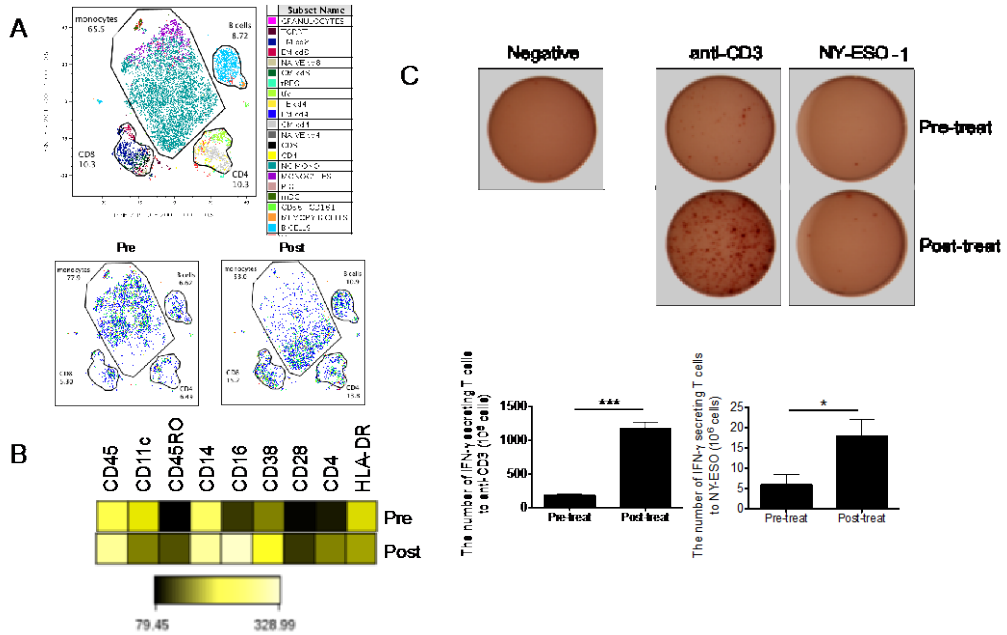
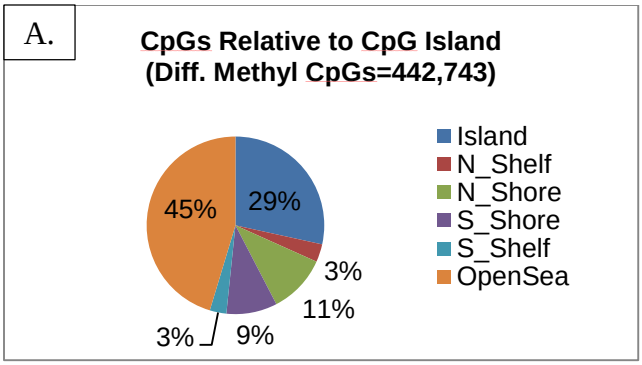
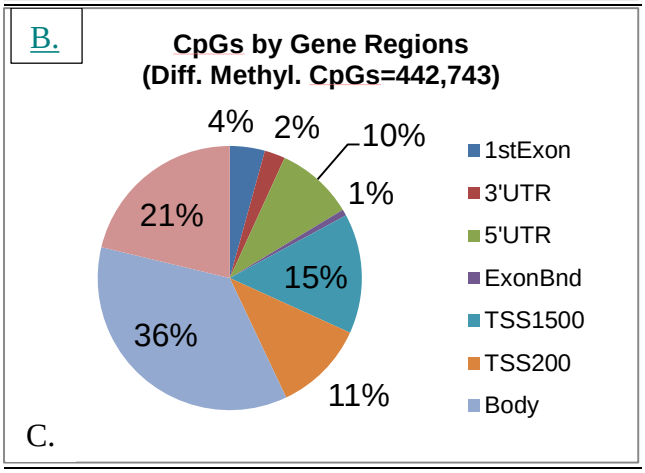


Figure 4. **Identification of differences in ascites from responder before and after treatment with guadecitabine and pembrolizumab.** (A). Exemplified tSNE visualization of overlaid events from responder before and after treatment. (B). The heat map represents the median indicated marker expression within CD45^{+} cells from responder ascites. (C). ELISPOT analysis of IFN-γ secreting T cells from tumor cell depleted ascites treated as indicated in the absence (negative) and presence of anti-CD3 or NY-ESO-1 peptides. The total number of spots were counted. *p < 0.05, ***p < 0.001.

A.



B.



C.

Top Canonical Pathways

Name	p-value	Overlap
CTLA4 Signaling in Cytotoxic T Lymphocytes	6.38E-05	12.8 % 12/94
T Cell Receptor Signaling	5.26E-03	8.6 % 10/116
Role of NFAT in Regulation of the Immune Response	7.17E-03	7.2 % 13/180
Fatty Acid Activation	8.11E-03	23.1 % 3/13
Mitochondrial L-carnitine Shuttle Pathway	1.48E-02	18.8 % 3/16

Figure 5: Methylation analyses of paired tumor biopsies (C1D1 vs. C2D5) reveal differential methylation occurring at >400,000 CpG sites (n=7 paired tumor biopsies). **A and B**: Location of differentially methylated CpG sites; C IPA analysis of top differentially hypomethylated sites associated with gene promoters identifies immune response and metabolic pathways as being most affected by methylation in tumor specimens.

Year 1:

RNA sequencing analysis of paired samples demonstrates upregulation of immune response pathways, specifically induction of IFNγ and Granzyme-A. However we also note up-regulation of IDO1, LAG3 and PD1, which might cause a feedback break on the anti-tumor response elicited.

Figure 1: Upregulation of immune related genes (mRNA level), as measured by RNA sequencing in RNA extracted from tumor biopsies obtained on C1D1 (baseline) and C2D2 (post-treatment).

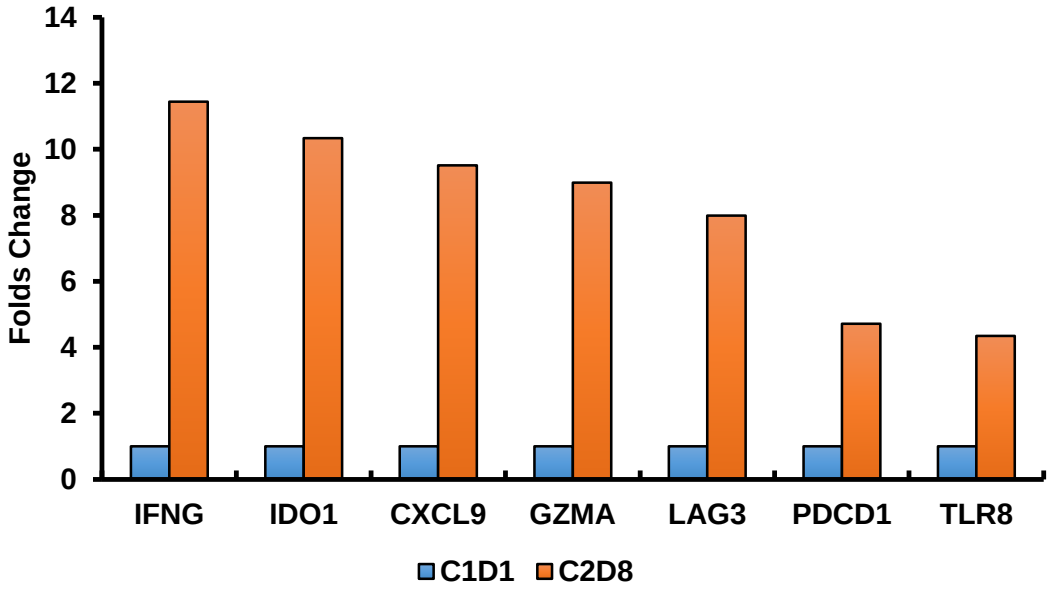
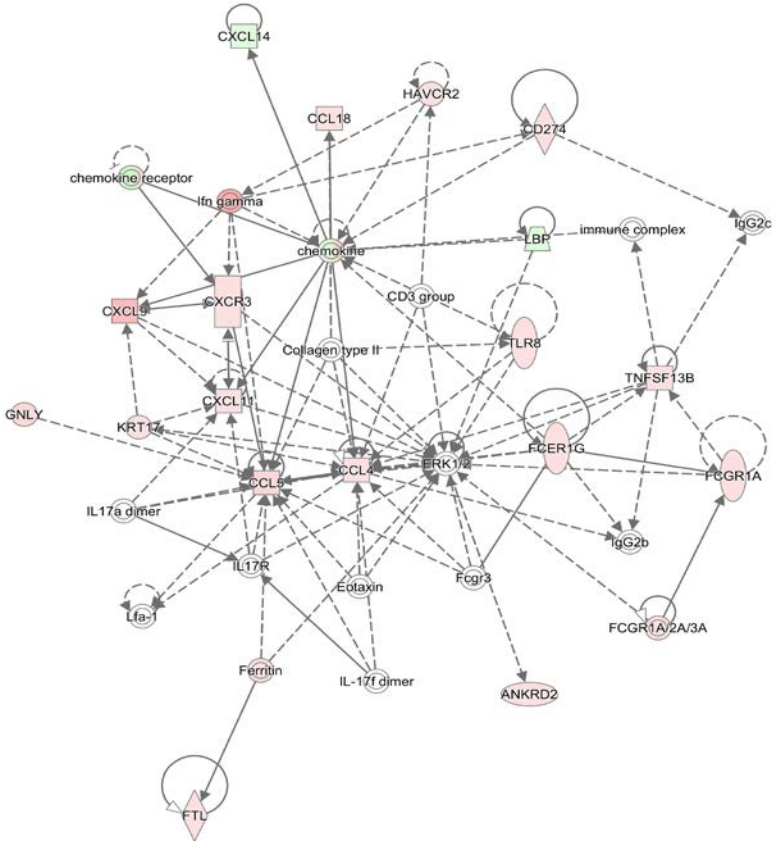


Table 1: Top 30 upregulated genes as measured by RNA sequencing in RNA extracted from tumor biopsies obtained on C1D1 (baseline) and C2D2 (post-treatment).

Gene Symbol	Fold Change	pvalue
TLR8	4.3	7.43E-05
CALHM6	4.5	7.99E-06
BIRC7	4.7	0.000150151
PDCD1	4.7	3.51E-05
EOMES	4.8	0.000174714
MT1L	5.0	0.000259089
GNLY	5.3	7.78E-05
HTRA4	5.4	7.20E-10
JAKMIP1	5.6	0.000406371
SIGLEC8	5.6	5.86E-05
CCL5	5.7	2.78E-05
TMIGD3	5.8	8.48E-09
GZMK	5.8	0.000112385

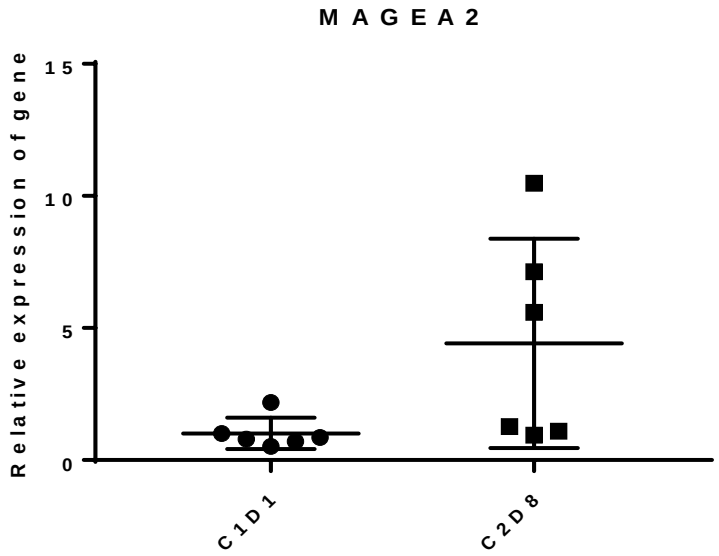
GBP5	5.9	6.64E-08
CD8A	6.3	6.31E-06
CXCR6	6.4	1.01E-05
GZMH	6.5	1.78E-07
NKG7	8.0	6.72E-07
LAG3	8.0	3.03E-08
GZMA	9.0	3.09E-07
CXCL9	9.5	1.75E-09
MT1M	10.1	2.89E-06
IDO1	10.3	1.19E-07
IFNG	11.4	9.83E-05
ACOD1	11.5	0.000120561
AC106865.1	17.9	3.05E-05
ELF5	22.3	7.56E-05
HBG1	23.0	8.62E-06
MT1H	28.7	2.66E-05
MT1G	29.0	2.90E-12

Figure 2: IPA analysis identifies immune cell trafficking as one of the most significant pathways altered by treatment. These results are highly consistent with our hypothesis.



Expression of specific antigens (MAGE) was measured by QRT-PCR in RNA from paired tumor biopsies (n=7). Mage A11 was detectable only in 3 post-treatment biopsies (not shown); MAGEA2 was increased in post- vs. pre-treatment biopsies. These analyses will be continued for other antigens .

Figure 3: MAGEA2 mRNA expression in RNA extracted from tumor biopsies obtained on C1D1 (baseline) and C2D2 (post-treatment).



Expression of retroviral transcripts was measured by QRT-PCR in RNA from paired tumor biopsies (n=7). There was high variability between specimens, precluding definitive conclusions. We will try to expand measurements to additional specimens as they are being collected and correlate change in expression of these transcripts with clinical response.

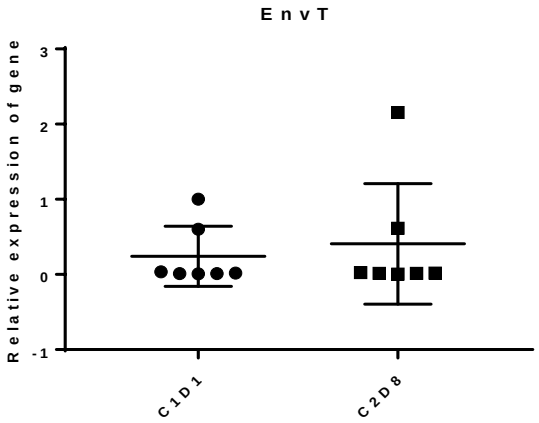


Figure 4: Expression of Env T in RNA extracted from tumor biopsies obtained on C1D1 (baseline) and C2D2 (post-treatment).

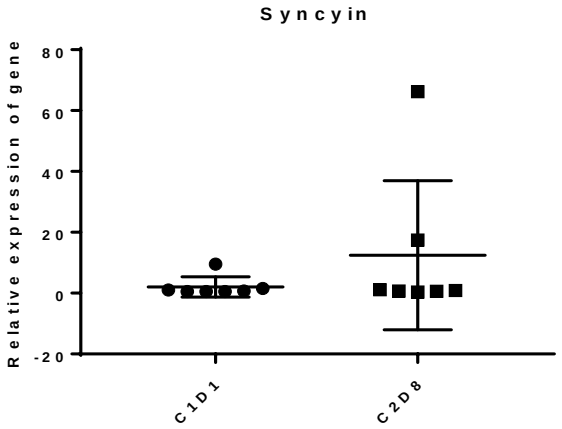


Figure 5: Expression of Syncyn in RNA extracted from tumor biopsies obtained on C1D1 (baseline) and C2D2 (post-treatment).

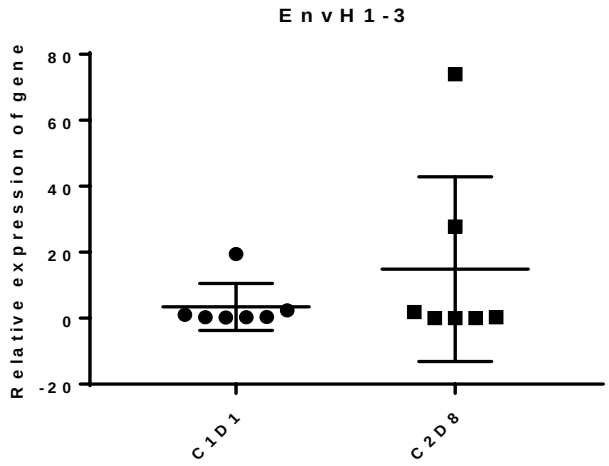


Figure 6: Expression of Env 1-3 in RNA extracted from tumor biopsies obtained on C1D1 (baseline) and C2D2 (post-treatment).

In conclusion, analyses are in progress and are proceeding according with proposed plan. We have extended our analyses to include multiplex IHC and CYTOF to characterize PBMCs in blood and immune cells in ascites, as the technologies have evolved.

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
Yakqi 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

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

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

We anticipate completing enrollment to the trial (enrollment was expanded to allow more evaluable patients, since some patients were discontinued from treatment before completing 2 cycles); completing pyrosequencing, and multiplex IHC analyses. Analysis of mIHC has to be completed. From the patients still being enrolled samples are still being collected and have to be processed as above. The staining for tumor antigen and CD8 has to be optimized.

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).

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.

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 will use RNA sequencing (instead of RT-PCR for multiple genes) for the specimens yielding sufficient amount of RNA. This will allow getting information on many genes, rather than on a small set of genes. Validation will be limited by the amount of RNA available for the specific specimens.
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 were enrolled, but were not evaluable due to early disease progression. These patients will have to be replaced in order to have sufficient numbers to reach the clinical objectives of the trial. The regulatory approvals slightly delayed completion of enrollment—which should complete by early fall. Research plans are proceeding as projected.

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.

There is a slight lag in expenditures, as collection and processing of specimens is ongoing.

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).*

  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. Per Lurie Cancer Center policy, preliminary results of a trial cannot be presented before enrollment and primary endpoints analysis is complete. However, we are planning shortly a manuscript describing one case report related to the trial. We anticipate submitting the rest of the data to AACR 2020 and publication shortly thereafter.

- **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: 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: 5
Contribution: RT-PCR, library preparation and sequencing, methylomic analysis, data analysis

Name: Horacio Cardenas
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution: specimen collection and logging, sequencing analysis, data analysis, pyrosequencing

Name: Siqi Chen
Project Role: Co-Investigator
Nearest person month worked: 11
Contribution: postdoctoral fellow, IHC, flow cytometry, data analysis

Name: Mathew Cowan
Project Role: Fellow in training
Nearest person month worked: 6
Contribution: multiplex IHC

Name: Azza Mohammad
Project Role: Technician
Nearest person month worked: 2
Contribution: nucleic acid extraction, specimen collection and logging.

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.

MATEI, DANIELA E.

CHANGES
*Indicates New Award since last report
**All Pending new since last report
***Completed since last report

Active

R01 CA182832 (Matei) 02/14/14 - 01/31/20 1.20 Calendar Months
NIH/NCI $257,789 (NCE)
An Epigenetic Strategy for Restoring Carboplatin Sensitivity in Ovarian Cancer
This study will bring epigenetic interventions to the forefront of therapy for ovarian cancer impacting treatment strategies and outcomes for this deadly cancer. Successful completion of this study will move forward the field of epigenome-targeted therapy for solid tumors and will provide key information for biologically- directed future design of phase III trials.
Aims:
1. To measure DNMTI (SGI-110)-induced changes in the OC methylome by performing MethylCap-seq.
2: To determine if DNMT expression levels differ in recurrent vs. primary tumors and whether expression levels at enrollment or changes induced by DNMTIs correlate with clinical benefit and survival.
3: To determine whether specific genes methylation levels at enrollment and changes induced by DNMTIs correlate with clinical benefit and survival.
Agency Contact: Min-Kyung Song, songm@mail.nih.gov

***R01CA224275 (Matei) 08/01/2018 – 07/31/2023 1.80 Calendar Months**
NIH/NCI $421,380
Targeting Lipid Unsaturation in Ovarian Cancer Stem Cells

Objective: To analyze whether the balance between saturated and unsaturated lipids enhance the survival of drug-tolerant cells after chemotherapy. We will use SCD1 knock down and chemical inhibitors to eradicate drug-tolerant cells persisting after treatment with platinum in ovarian xenografts and patient derived xenografts (PDX).
Agency Contact: Arya Suresh Email: aryas@mail.nih.gov

Aim 1: Determine the mechanisms by which lipid unsaturation mediated by SCD1 promotes stemness.
Aim 2: Use label free chemical imaging to identify and characterize ovarian CSCs within the tumor microenvironment (TME).
Aim 3: Define anti-tumor and metabolic effects of SCD1 inhibition or knock down in vivo.

W81XWH-17-0141 (Matei) $200,000 0.60 Calendar
USAMRMC/CDMRP
Epigenomic Priming as an Immunotherapy Enhancer in Ovarian Cancer
Objective: To propose a clinical trial testing a novel strategy to enhance efficacy of immunotherapy in OC by using epigenetic priming. The ultimate goal of the project is to develop a new treatment that extends the lives of women with OC, while preserving the quality of life of the survivors.
Aim 1: Measure the antigen burden induced bytreatment with guadecitabine in human tumors in relationship to clinical response.
Aim 2: Determine the anti-tumor activity of CD8+ effector T cells induced by treatment with guadecitabine and pembrolizumab in relationship to clinical response.
Role: PI
Contact: Lisa Wells Roark, lisa.l.wellsroark.civ@mail.mil

I01 BX000792 (Matei) 10/01/10 - 12/31/19 (NCE) 4.20 Calendar Months

NIH/VA Merit Review Award $200,000
The Tissue Transglutaminase-Fibronectin Interaction in Ovarian Cancer Metastasis
We propose to elucidate the mechanism by which TG2/FN complex initiates oncogenic signaling leading to metastasis and to characterize pre-clinically the top hit identified during the screening process.

Aims: 1: Characterize the mechanisms by which formation of the TG2/FN/integrin complex activates EMT. 2: Define mechanisms engaged by TG2/FN/integrin complexes to promote stem cell signaling. 3: Characterize the effects of the lead TG2/FN inhibitor in an OC metastasis model. Agency Contact: Kenute Myrie, kenute.myrie@va.gov

Agreement 01/23/17 (Nephew) 01/01/17 - 02/01/20 0.60
Calendar Ovarian Cancer Research Fdn (IUPUI Subcontract) $68,182
Epigenetic Vulnerabilities of Ovarian Cancer Stem Cells

The goal is to identify how key pathways are epigenetically maintained and regulated in ovarian cancer stem cells and epigenetic vulnerabilities that can be targeted to switch off paths responsible for ovarian cancer stem cell survival after platinum therapy.
Aims are as stated in goals Role: Co-Investigator

Completed

#T2013-003B (Matei) 01/01/16-01/01/18 N/A
The V Foundation for Cancer Research $188,636
An Epigenetic Strategy for Restoring Carboplatin Sensitivity in Ovarian Cancer
This study will bring epigenetic interventions to the forefront of therapy for ovarian cancer impacting treatment strategies and outcomes for this deadly cancer. Successful completion of this study will move forward the field of epigenome-targeted therapy for solid tumors and will provide key information for biologically- directed future design of phase III trials
Aims:
1. To measure DNMTI (SGI-110)-induced changes in the OC methylome by performing MethylCap-seq.
2: To determine if DNMT expression levels differ in recurrent vs. primary tumors and whether expression levels at enrollment or changes induced by DNMTIs correlate with clinical benefit and survival.
3: To determine whether specific genes methylation levels at enrollment and changes induced by DNMTIs correlate with clinical benefit and survival.
Agency Contact: Carole Wegner, cwegner@jimmyv.org

***R21 CA198409 (Hurley) 07/01/15 - 06/30/18 0.96 Calendar Months
NIH/NCI $128,346
Targeting Ovarian Cancer Stem Cells Through Selective Inhibition of ALDH1A1
We propose to optimize and validate the lead inhibitor for the first time in a cancer model, focusing on inhibiting the functions of ALDH1A1+ ovarian CSCs. We will determine the lead inhibitor's target specificity and its cytotoxic activity in ALDH1A1+ ovarian cancer cells and will measure its anti-cancer activity in an animal model that replicates tumor recurrence after chemotherapy.
Aims: The aim is stated as the goal statement.
Role: co-PI
Agency Contact: Suresh Arya, aryas@mail.nih.gov

R01 EB016582 (Nolte) 05/01/13 - 04/30/18 1.00 Calendar Months
NIH/NIBIB $257,755
Tissue-dynamics Imaging for Therapeutic Efficacy in Ovarian Cancer
We propose that by exploiting the intracellular dynamical properties of ovarian tumors or metastatic implants

ex-vivo, this new technology can be adapted to overcome a problem of high clinical relevance for women with ovarian cancer. A commercial partner, Animated Dynamics LLC, will receive technology transfer and construct the first clinic-based TDI system.
Aims: The aim is stated as the goal statement.
Role: co-PI
Agency Contact: Behrouzb Shabestari, shabestb@mail.nih.gov

Pending

R01CA182832 (Matei) 07/01/2019 – 06/30/2024 1.20 Calendar Months
NIH/NCI $478,141
An Epigenetic Strategy for Restoring Carboplatin Sensitivity in Ovarian Cancer
Objective: The overall goal of this proposal is to identify patients that are most likely to benefit from epigenetic strategies. This proposal will continue our decade-long quest to use hypomethylating agents (HMA) as re-sensitizers to platinum in ovarian cancer. Our group has made important preclinical observations leading to bench-to-clinic therapeutic interventions targeting aberrant DNA methylation to re-sensitize ovarian tumors to platinum.
Agency Contact: Arya Suresh Email: aryas@mail.nih.gov

Aim 1: To test the hypothesis that methylation of specific CpG sites in PBMC is associated with acquired resistance to platinum and predicts survival and response to epigenetic therapy.
Aim 2: To investigate the effects of HMA treatment on patient blood DNA methylation changes and RNA profiles in correlation with survival.
Aim 3: To test the hypothesis that expression and activity of DNMT1 contribute to acquired platinum resistance and response to HMA.
Overlap: None

UG1 CA233320 (Benson) 04/01/19 - 03/31/25 1.20 Calendar
NCI $18,960
Northwestern University Lead Academic Participating Site
The goal of this project is the development of bioinformatics methods and user-friendly software which will provide useful tools to identify with high confidence a set of genes showing significant alternative isoform usage events associated with tumor development and/or progression for several tumor types. The resulting cancer-associated alternative splicing variants will provide new tools for the diagnosis and classification of cancers and could be the targets for innovative therapeutic interventions.

Aims are as stated in goals

Role: Principal Investigator
Agency Contact: WLODEK LOPACZYNSKI, lopacw@mail.nih.gov

OTHER SUPPORT
ZHANG, BIN

CHANGES
*Indicates New Award since submitted
**All Pending new since submitted
***Completed since submitted

Current Support

*Title: The role of GPSM3 in tumor-promoting emergency myelopoiesis
Time Commitments: 2.40 Calendar
Supporting Agency: NIH/NCI R01CA208354
9606 Medical Center Drive
Bethesda, MD 20892-9760
Grants Officer: Barbara Hodgkins, barb.hodgkins@nih.gov, (240) 276-6294
Performance Period: 03/01/2017 - 02/29/2022
Level of Funding: $1,805,410
Goals: The goal of this project is to determine the role of GPSM3 in regulating the cancer-driven myelopoiesis.
Specific Aims:
Aim 1: Define the role of GPSM3 in regulating the cancer-driven myelopoiesis.
Aim 2: Determine whether cytokine-induced GPSM3 regulates critical transcriptional mediators of cancer-associated myelopoiesis.
Aim 3: Study the role of GPSM3 in MDSC-mediated immune suppression and tumor promotion.
Role: PI

Title: Project #3 SNAs as Immunotherapeutic Agents for Prostate Cancer
Time Commitments: 1.20 Calendar
Supporting Agency: NIH/NCI U54CA199091 (Mirkin)
9606 Medical Center Drive
Bethesda, MD 20892-9760
Grants Officer: Barbara Hodgkins, barb.hodgkins@nih.gov, (240) 276-6294
Performance Period: 09/01/2015 - 08/31/2020
Level of Funding: $663,685
Goals: The goal of Project 3 is to develop SNAs that are capable of inducing an immune response that will destroy prostate tumors.
Specific Aims:
Aim 1: Design, synthesize and characterize IS-SNAs for activation of lymphocytes.
Aim 2: Analyze the immunostimulatory activity of IS-SNAs with a panel of standardized in vitro assays.
Aim 3: Assess and characterize IS-SNA activity in immunocompetent mouse models.
Aim 4: Development of combination therapies for an optimized cancer immunotherapy: Immunostimulation by IS-SNAs combined with modulation of the immunosuppression of solid tumors.
Role: Project 3 Co-Leader

*Title: CCNE Pilot Project: Engineered Spherical Nucleic Acids for Advanced Cellular Therapy
Time Commitments: 0.12 Calendar
Supporting Agency: NIH/NCI U54CA199091 (Mirkin)
9606 Medical Center Drive
Bethesda, MD 20892-9760
Grants Officer: Barbara Hodgkins, barb.hodgkins@nih.gov, (240) 276-6294
Performance Period: 09/01/2015 - 07/31/2020

Level of Funding: $40,000
Goals: To develop T cell chaperones with IS-SNAs for ACT is based on the properties and cellular interactions that are unique to SNAs and distinguish SNAs from other nanoparticle systems for cancer therapy: highly efficient uptake of SNAs into T-cells is in a controlled, ex vivo environment.

*Title: Epigenomic Priming to Enhance Immunotherapy in Ovarian Cancer
Time Commitments: 0.60 Calendar
Supporting Agency: DOD/CDMRP W81XWH-17-1-0141
Performance Period: 05/01/2017 - 04/30/2020
Level of Funding: $945,000
Goals: The goal of this project is to test if treatment with a DNMT inhibitor increases the anti-tumor activity of PD-1 blockade by enhancing tumor cell recognition by CD8+ effector T cells in a phase II clinical trial.
Specific Aims:
Aim 1: Measure the antigen burden induced by treatment with guadecitabine in human tumors in relationship to clinical response.
Aim 2: Demonstrate that the combination of guadecitabine and pembrolizumab blockade increases anti-tumor efficacy of cytotoxic CD8+ T cells in vivo.
Role: Co-PI

*Title: Targeting chemokine signaling and MAPK/ERK pathway in advanced prostate cancer
Time Commitments: 0.36 Calendar
Supporting Agency: Prostate Cancer Foundation
Performance Period: 12/31/2017-12/31/2019
Level of Funding: $1,000,000
Goals: The goal of this project is to prove that CXCR7/MAPK/ERK signaling drives CRPC progression and resistance to AR-targeted therapies and that clinically available MAPK/ERK inhibitors might delay or overcome CRPC drug resistance.
Aim 1. To analyze CXCR7-MAPK-ERK pathway in PCa models and in mCRPC patient specimens.
Aim 2. To determine the functional importance of the CXCR7-MAPK-ERK pathway in CRPC progression and Enz resistance using preclinical models and in the tumor microenvironment using transgenic mice.
Aim 3. To test the efficacy of MAPK-ERK inhibitors in overcoming CRPC Enz resistance using preclinical and PDX models and to develop novel CXCR7 antagonists.

*Title: Spherical Nucleic Acids as Therapeutic Vaccines for the Treatment of Prostate Cancer
Time Commitments: 1.20 Calendar
Supporting Agency: Prostate Cancer Foundation
Grants Officer: Audrey Gardner agardner@pcf.org (310) 570-4792
1250 Fourth Street
Santa Monica, CA 90401
Performance Period: 09/01/2017 - 08/31/2019
Level of Funding: $236,148
Goals: To develop and test a novel nanoparticle-based therapeutic prostate cancer vaccine in preclinical models which may lead to a new immunotherapy for prostate cancer.
Role: Co-Investigator

*Title: PARP inhibition and tumor immunity
Time Commitments: 0.36 Calendar
Supporting Agency: AbbVie, Inc
Grants Officer: Eric Johnson
Performance Period: 09/29/2017 - 09/29/2019
Level of Funding: $350,000
Specific Aims
Aim 1: Define the role of PARPi in regulating the cancer-driven Myelopoiesis
Aim 2: Determine the molecular mechanism by which PARPi regulates MDSCs
Aim 3: Determine the immunoregulatory effect of PARPi on tumor cells
Role: PI

Title: Treating Breast Cancer by Novel WEE1 inhibitors
Time Commitments: 0.36 Calandar
Supporting Agency: NMG Lynn Sage
Grants Officer:
Performance Period: 9/1/2016- 8/31/2018
Level of Funding: $50,000
Goals: The Goal of this study is to explore the translation relevance of the use of FDA-approved WEE1 inhibitors MK-1775 as modulators of the antitumor immune response in breast cancer.
Specific Aims:

*Title: WEE1 inhibition and tumor immunity
Time Commitments: 1.20 Calendar
Supporting Agency: NIH/NCI R01CA222963
9606 Medical Center Drive
Bethesda, MD 20892-9760
Grants Officer: Barbara Hodgkins, barb.hodgkins@nih.gov, (240) 276-6294
Performance Period: 07/01/2018-06/30/2023
Level of Funding: $2,725,465
Goals: The goal of this project is to characterize the novel regulatory perspectives of WEE1-mediated crosstalk between tumor cells and host immune cells that should significantly forward the field. Our work will identify an unappreciated role of WEE1 inhibition in reversing tumor-induced immune suppression of Tregs, in addition to its direct cytotoxic activity.
Specific Aims:
Aim 1: To determine the intrinsic role of WEE1 in inducible Tregs for tumor promotion.
Aim 2: To determine the immunomodulatory effects of WEE1 expression in tumor cells.
Aim 3: To determine the therapeutic efficacy of WEE1 inhibition in combination with the anti-PD-1/PD-L1 immunotherapy.
Role: PI

*Title: Tumor microenvironment-induced natural killer cells (MINK) for cancer immunotherapy
Time Commitments: 0.60 Calendar
Supporting Agency: AbbVie Inc.
Grants Officer: Jesseca Rodgers
Performance Period: 12/13/18-12/12/20
Level of Funding: $958,000
Goal: The goal of this project is to evaluate a novel strategy for potentiating antitumor immune responses by engineering natural killer cells to sense and therapeutically respond to general features of the tumor microenvironment.
Specific Aim 1: To evaluate whether NK cells may be engineered to sense hypoxia in vitro and in vivo.
Specific Aim 2: To evaluate whether NK cells may be engineered to sense VEGF in vitro and in

vivoSpecific
Aim 3: To evaluate whether MINK can
therapeutically modulate tumor growth and local immune state.
Role: Co-PD/PI

OVERLAP
None

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: 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.

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.