AWARD NUMBER: W81XWH-20-1-0642

TITLE: Enhancement of Th17-Inducing DC Vaccination for Ovarian Cancer Through PARPi-Mediated Activation of Innate Immunity in the Tumor Microenvironment

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REPORT DATE: August 2022

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
August 2022	Annual	01Aug2021-31Jul2022

4. TITLE AND SUBTITLE

Enhancement of Th17-Inducing DC Vaccination for Ovarian Cancer Through PARPi-Mediated Activation of Innate Immunity in the Tumor Microenvironment

5a. CONTRACT NUMBER
W81XWH-20-1-0642

5b. GRANT NUMBER
OC190464

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)

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

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This proposal seeks to address the hypothesis that PARP inhibitors enhance the efficacy of active immunotherapy with Th17-inducing DC vaccination against ovarian cancer. The proposed mechanism is that PARP inhibition will lead to DNA damage and innate immune sensing of cytosol DNA fragments, principally through the cGAS-STING pathway. We further propose that induction of deficiencies in homologous recombination (HR) will (i) sensitize ovarian tumors to PARP inhibitors, and (ii) trigger innate inflammation via DNA sensing and STING activation. In year 2 of this project, we have shown that treatment with prexasertib, a CHK1 inhibitor that promotes HR deficiency, triggers DNA damage, activates STING-related inflammatory pathways and alleviates tumor-associated immune suppression when combined with olaparib treatment. We further show that direct STING activation on ovarian tumor-associated macrophages alleviates macrophage-mediated suppression of antigen-specific T cell responses. In vivo studies to test the therapeutic efficacy of Th17-DC vaccination combined with olaparib and agents that promote HR deficiency are in progress.

15. SUBJECT TERMS
None listed.

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

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION

$CD4^+$ Th17 T cell infiltration in ovarian cancer is strongly associated with improved patient outcomes and prolonged survival, prompting the question of whether Th17 cells could be induced or expanded to therapeutic advantage. Our recently completed a Phase I trial of Th17-DC vaccination in patients with stage IIIC/IV ovarian cancer showed that Th17-DC vaccination induced antigen-specific Th1/Th17 T cell responses and antibody responses in the majority of patients, and 39% of patients with stage IIIC-IV ovarian cancer enjoyed progression-free survival over a median of 49 months following initiation of Th17-DC vaccination. These results are encouraging, but immune suppression in the tumor microenvironment may operate to blunt the efficacy of Th17-DC vaccination. Recent studies have shown that PARP inhibitors can activate innate and adaptive immune responses in ovarian cancer through the cGAS-STING pathway, thus providing a link between DNA damage, inflammatory responses and antitumor immunity. These observations present a compelling case for the combination of PARP inhibitors and Th17-DC vaccination. We will test the hypothesis that PARP inhibitors trigger an inflammatory response in the ovarian tumor microenvironment and alleviate myeloid cell-associated immune suppression, thereby boosting therapeutic responses to Th17-DC vaccination. The 1st Aim will test the ability of olaparib to inhibit tumor progression in mouse models of ovarian cancer, as monotherapy, or as adjuvant treatment in combination with cisplatin and Th17-DC vaccination. The 2nd Aim will determine whether PARP inhibitors activate innate immunity and Type I IFN responses via the cGAS-STING pathway in ovarian tumor-associated myeloid cells. We will further determine whether STING activation in myeloid cells alleviates suppression of Th17-DC-stimulated antitumor T cell responses.

2. KEYWORDS

Ovarian cancer, dendritic cells, T cells, immunosuppression, macrophages, interleukin-10, chemokines, cytokines, immunotherapy, olaparib

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1(specified in proposal)	Timeline (months)	Progress to completion
Major Task 1: Test the ability of olaparib to inhibit tumor progression in mouse models of ovarian cancer, as monotherapy, or as adjuvant treatment in combination with cisplatin and Th17-DC vaccination.	1-12	Done
Subtask 1: Regulatory review and approval by the USAMRMC Animal Care and Use Review Office and local IRB	1-3	Done
Subtask 2: Identification of study subjects; provision of tumor and ascites samples, blood draws	3-18	Ongoing: continuous process
Subtask 3: Assessment of tumor progression in BRCA-competent and BRCA-deficient mouse models of ovarian cancer following treatment with cisplatin, olaparib and Th17-DC vaccination	3-12	Done
Subtask 4: Immunological analysis of ovarian tumor-bearing mice following therapies described in Subtask 3	6-18	Not done

Subtask 5: Determination of the role of cGAS/STING in the efficacy of PARP inhibition	12-18	Not done
Subtask 6: Determination of the contribution of host antigen-presenting cells in the efficacy of olaparib/Th17-DC therapy.	12-18	Not done
Milestone achieved: Determination of the efficacy of olaparib and Th17-DC vaccination. Determination of underlying mechanisms. Statistical analysis of results.	1-18	Done
Specific Aim 2 (specified in proposal)		
Major Task 2: Determine whether PARP inhibitors activate innate immune signaling and Type I IFN responses via the cGAS-STING pathway in ovarian tumor-associated myeloid cells.		In progress
Subtask 1: Determine whether olaparib treatment of patient-derived primary ovarian tumor cells contributes to STING activation and an inflammatory signature	12-18	Done
Subtask 2: Determine whether olaparib treatment of patient-derived primary ovarian tumor ascites myeloid cells induces STING activation and an inflammatory signature	12-18	Done
Subtask 3: Determine whether coculture of olaparib-treated ovarian tumor cells with tumor-associated myeloid cells is required for STING activation in the myeloid compartment	18-24	Done
Subtask 4: Determine whether olaparib treatment and STING activation alleviates myeloid cell suppression of Th17-DC-stimulated tumor antigen-specific T cell responses	18-24	In progress
Subtask 5: Summation of results, statistical analysis, and determination of future directions	18-24	In progress
Milestone achieved: Determination of the impact of olaparib on ovarian tumor-associated myeloid cells	12-24	Not done

What was accomplished under these goals?

In Year 1 of this project, we showed that olaparib combined with Th17-DC vaccination improved the survival of mice engrafted with BRCA-deficient ID8 ovarian tumor cells, but found that olaparib was predictably ineffective against BRCA wildtype tumors. Consequently we explored strategies for rendering BRCA wild-type tumors sensitive to PARP inhibitors through induction of homologous recombination deficiency (HRD) with PG545 (Pixatimod), a heparan sulfate mimetic that increases DNA breaks and inhibits HR repair. Although we found some evidence that PG545 could induce a signature of HR deficiency through phosphorylation of γH2AX, and that PG545 could enhance phosphorylation of IRF3 (downstream of STING activation), there was limited evidence that PG545 could alleviate tumor-associated immune suppression. We also found limited evidence that

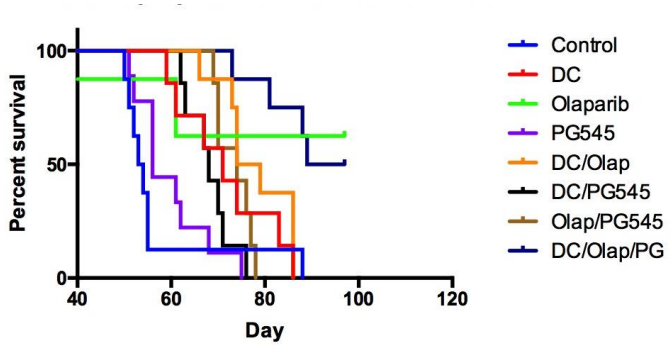

Figure 1. Kaplan-Meier curves for ID8 ovarian tumor-bearing mice treated with Th17-DC vaccination, olaparib and PG545.

PG545 could enhance the efficacy of olaparib and/or Th17-DC vaccination in the ID8 mouse model of ovarian cancer (Fig. 1). The combination of Th17-DC/olaparib/PG545 was significantly more effective than treatment with Th17-DC/olaparib, Th17-DC/PG545 or olaparib PG545, respectively, but the data do not present a compelling case for this approach.

In year 2, we explored alternative approaches to promoting HRD and activating innate inflammation. We focused on prexasertib, a CHK1 inhibitor that has shown clinical activity in patients with BRCA wildtype ovarian cancer. CHK1 plays an important role in HR DNA repair, and thus inhibition of CHK1 may promote HRD and sensitivity to olaparib, in turn leading to activation of the STING pathway through cytoplasmic DNA sensing.

Treatment of human ovarian tumor cells with olaparib and prexasertib induces phosphorylation of γH2AX

We found that treatment of OvCa26 patient-derived ovarian tumor cells with olaparib or prexasertib as single

ned treatment

of olaparib to

overall benefit

γH2AX phosphorylation as a marker of DNA damage and homologous recombination deficiency in OvCa26 tumor cells treated with olaparib and the CHK1 inhibitor prexaser b

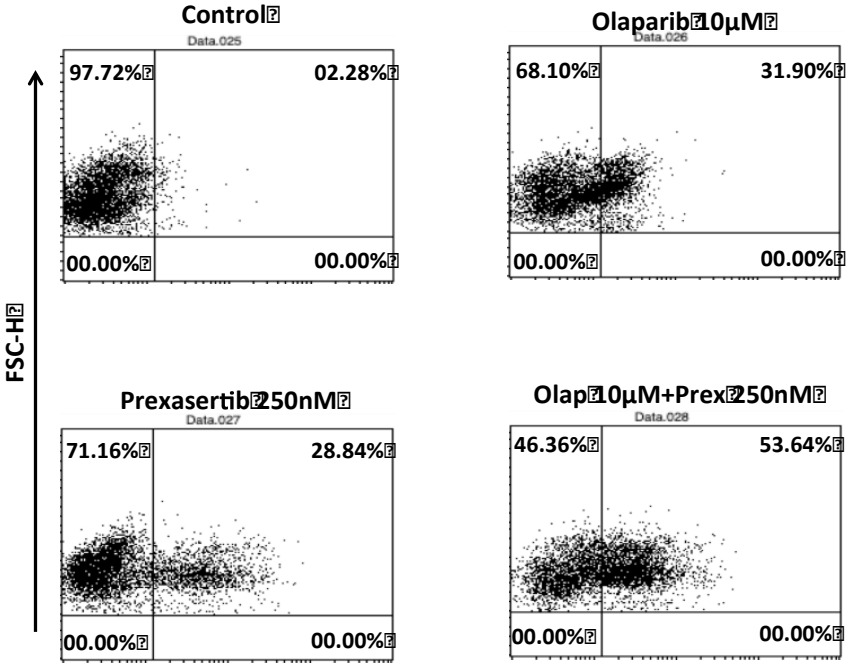

Figure 2. Phosphorylation of γH2AX following treatment of OvCa-26 ovarian tumor cells with olaparib and prexasertib.

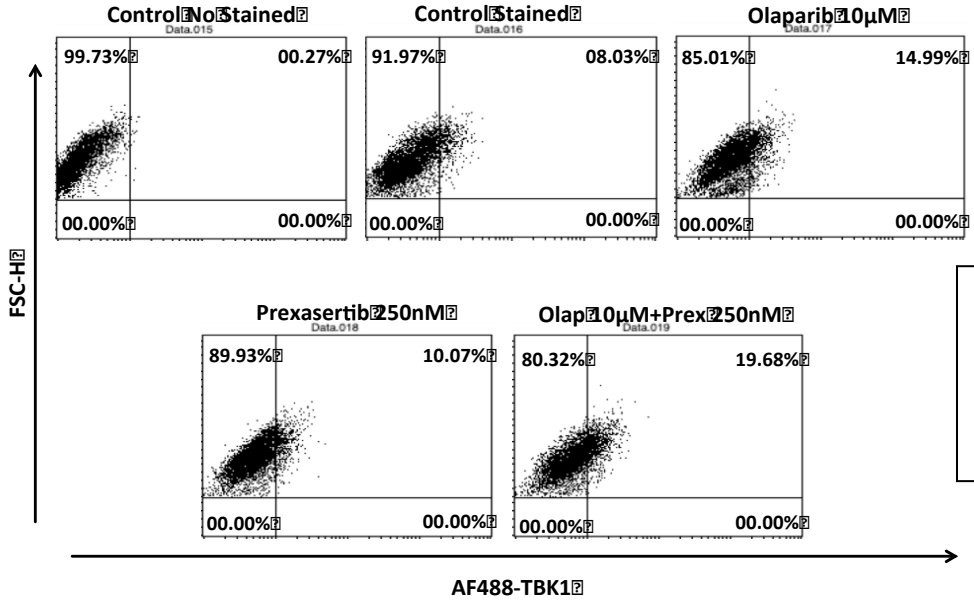

Figure 3. Phosphorylation of TBK1 following treatment of OvCa-26 ovarian tumor cells with olaparib and prexasertib.

Impact of olaparib/prexasertib or ADU-S100 STING agonist treatment on ovarian tumor cell suppression of antigen-specific CD4$^+$ T cell responses

We treated ovarian tumor cells with olaparib or prexasertib as single agents, or the combination of both drugs, or a direct STING agonist (ADU-S100) and tested whether these treatments would reverse tumor cell-mediated suppression of CD4$^+$ T cell responses in coculture assays. We found that olaparib or prexasertib monotherapy had negligible impact on T cell suppression, but combined olaparib/prexasertib drug treatment was completely effective in reversing tumor cell suppression of T cell responses, as measured by intracellular TNFα expression following antigen stimulation (Fig. 4). We also observed that treatment with the direct STING agonist ADU-S100 alleviated tumor cell-mediated suppression of T cell responses (Fig. 4).

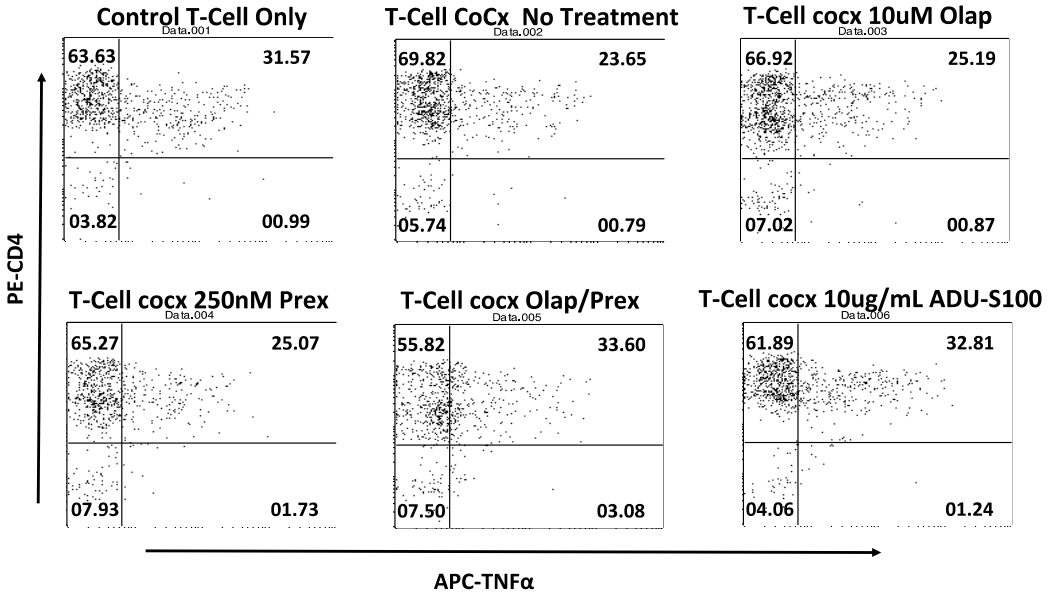

Figure 4. Coculture of ovarian tumor antigen-specific CD4$^+$ T cells with OvCa-2a ovarian tumor cells treated with olaparib, prexasertib, combined olaparib plus prexasertib, or the STING agonist AD-S100. T cell responses are measured by intracellular TNFα expression following antigen stimulation.

Treatment with olaparib, prexasertib and Th17-DC vaccination in the ID8 mouse model of ovarian cancer

In vitro studies showed that prexasertib combined with olaparib could induce a signature of HR deficiency, activate STING-related innate immune signaling and alleviate tumor-associated suppression of antigen-specific CD4$^+$ T cell responses. Based on these encouraging results, we tested combinatorial treatment of ID8 (BRCA wild-type) ovarian tumor-bearing mice with Th17-DC vaccination, olaparib and prexasertib. Combined treatment with Th17-DC, olaparib and prexasertib did not significantly improve survival relative to various control groups (Fig. 5).

While this is disappointing, the experiment may have been compromised by delays in treatment due to unexpected problems with IACUC review and approval (related to adoption of a new online management system for protocol review that resulted in a backlog in the review process). The practical consequence was that ID8 tumor growth in engrafted mice was further advanced than usual at the time we initiated treatment, and we found that more advanced tumors are more difficult to treat effectively with immunotherapy. The treatment schedule itself involves four weekly cycles of Th17-DC vaccination, preceded by olaparib/prexasertib treatment on four consecutive days before each round of Th17-DC vaccination. In this instance, we started treatment on day 28 after engraftment of ID8 tumor cells (rather than day 18-21, per our usual practice). Given that ID8 tumor growth is rapid and aggressive, with median survival in the 40-50 day range, we struggled to complete a four-week course of treatment starting as late as day 28.

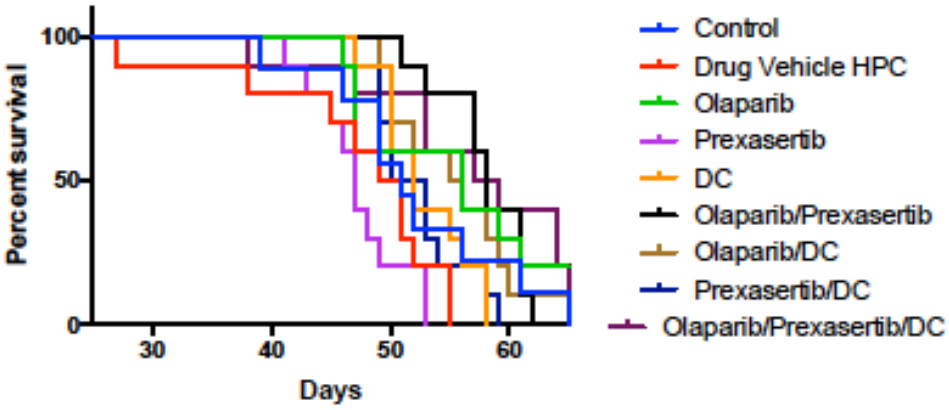

Figure 5. Kaplan-Meier curves for ID8 ovarian tumor-bearing mice treated with Th17-DC vaccination, olaparib, PG545, or prexasertib.

Impact of STING activation on immune suppression by ovarian tumor-associated CD14$^+$ macrophages

Ovarian tumor-associated macrophages play a major role in suppression of antitumor immunity. We have previously demonstrated that ovarian tumor ascites macrophages are powerful suppressors of Th17-DC-stimulated T cell responses. In these studies, we tested whether direct STING activation in primary patient-derived tumor ascites CD14$^+$ macrophages would alleviate suppression of tumor antigen-specific CD4$^+$ T cell responses. We found that pre-treatment of macrophages was totally effective in reversing macrophage-mediated T cell suppression (Fig. 6), suggesting that strategies to promote STING activation in tumor-associated macrophages may support the efficacy of Th17-DC vaccination against ovarian cancer. We will pursue in vivo studies targeting STING activation in the ID8 mouse model of ovarian cancer to determine whether these observations translate to in vivo benefit in limiting tumor progression.

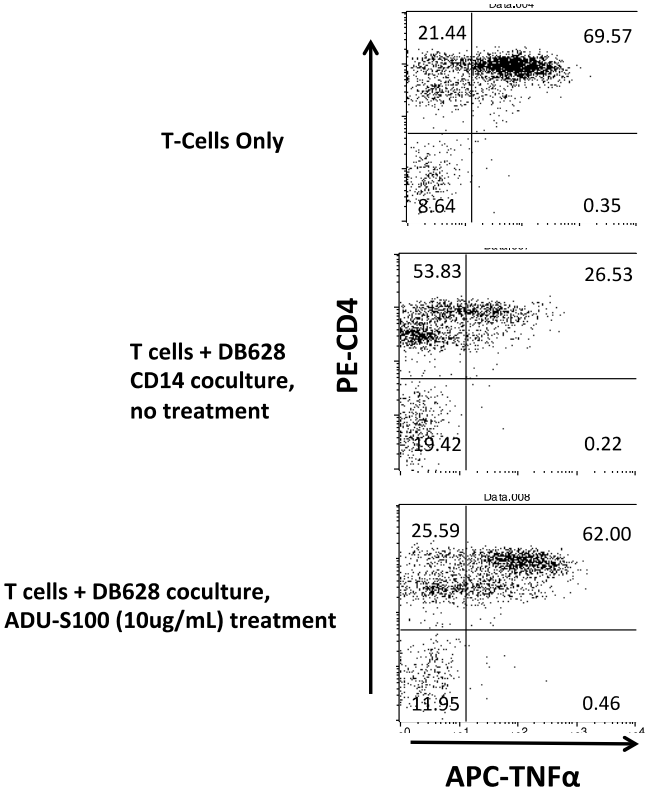

Figure 6. Coculture of ovarian tumor antigen-specific CD4$^+$ T cells with DB628 patient-derived ovarian tumor ascites CD14$^+$ macrophages. CD14$^+$ ascites macrophages were untreated, or treated with ADU-S100 for 24 hours prior to coculture with tumor antigen-specific CD4$^+$ T cells. T cell responses are measured by intracellular TNFα expression following antigen stimulation.

4. Impact

This project has evolved to focus on strategies that promote HR deficiency in ovarian cancer. The impact is two-fold. First, HR deficiency will favor sensitivity to PARP inhibitors. This is important because a large proportion of ovarian tumors are BRCA wild-type and do not have a signature of HR deficiency, and thus PARP inhibitors are relatively ineffective. Second, HR-deficiency may trigger innate inflammation through

DNA damage sensing. We (and others) propose that an inflammatory response within the tumor will enhance the efficacy of immunotherapies for ovarian cancer, including Th17-DC vaccination.

Collectively, this investigation may point to novel combinatorial strategies for treatment of ovarian cancer, involving inhibition of DNA repair, promotion of HR deficiency and triggering of innate inflammatory pathways in the tumor microenvironment (effectively turning a cold tumor into a hot tumor). We propose that this combinatorial approach will promote enhancement of responses to immunotherapy, including DC vaccination, but also possibly responses to immune checkpoint inhibitors.

5. Changes/Problems

Changes in approach and reasons for change
The overall approach and focus remains unchanged. We have undertaken additional studies to determine whether treatment with agents that promote HR deficiency will enhance the therapeutic benefit of targeting PARP with olaparib. We have also completed new mouse experiments, as described above. This project has been afforded a no-cost extension, in which we will continue to pursue new strategies for sensitization of ovarian tumor cells to treatment with olaparib.

Actual or anticipated problems or delays and actions or plans to resolve them
COVID-19-related supply chain problems have slowed progress. Internal changes in regulatory and administrative management (including purchasing) have also contributed to significant delays. We do not have control over issues of this nature, and we do not have an effective plan to resolve these difficulties, beyond the granted request for a no-cost extension of the project.

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

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

Publications, conference papers, and presentations
Nothing to report

Website(s) or other Internet site(s)
Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
Nothing to report

7. Participants & Other Collaborating Organizations

a. What individuals have worked on the project?

Name:	***Martin Cannon***
Project Role:	***Principal Investigator***
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	***1.68***
Contribution to Project:	***Oversight of the project***
Funding Support:	***DOD OC190464***

Name:	***Eric Siegel***
Project Role:	***Biostatistician***
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	***0.6***
Contribution to Project:	***Statistical guidance, data analysis***
Funding Support:	***DOD OC190464***

Name:	***Richard Connor***
Project Role:	***Research Technician***
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	***12***
Contribution to Project:	***Technical support***
Funding Support:	***DOD OC190464***

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Cannon (PI) has the following new support:

Winthrop P. Rockefeller Team Science Award: Immunotherapy of hepatocellular carcinoma by live attenuated vaccine vectors (B.M. Nagalo, M.J. Cannon, A. Basnakian, Co-PIs)

Funding period: 05/01/2022 – 04/30/2023

Funding level:

Project goals: This project will evaluate the ability of a live attenuated viral vaccine to synergize with immune checkpoint inhibitors to provide durable anti-tumor immunity in an animal model of HCC.

Aims: 1. To investigate if intratumoral injections of MMR mediate strong and durable immunologic anti-tumor memory against primary and distant tumor cells. 2. To determine whether immune checkpoint

inhibitors (anti-PD-1/anti-CTLA-4) can potentiate the anti-tumor activity of MMR to provide a long-lasting anti-tumor effect.

Time Commitment: 5%

Contracting Officer: Petra Stephens, PStephens@uams.edu

Overlap: None

NIH R01 CA245083: Improvement of cellular immunotherapy during dysbiosis (R.P.M. Dings, P.I., M.J. Cannon, Co-I.)

Funding period: 09/15/2021 – 08/31/2026

Funding level:

Project goals: The overall goal is to define the systemic effects of antibiotic-induced dysbiosis on the distal tumor microenvironment and develop therapies to promote antitumor immunity. The major objective of this application is to overcome dysbiosis-induced ICAM-1 suppression and thereby enhance the effectiveness of cellular immunotherapy.

Aims: 1. Identify ABX primarily responsible for stromal immune suppression resulting in tumor progression. 2. Increase ICAM-1 on tumor-associated endothelial cells during dysbiosis. 3. Increase cellular immunotherapy efficacy in melanoma during dysbiosis.

Time Commitment: 5%

Contracting Officer: Connie Sommers, sommersc@mail.nih.gov

Overlap: None

NASA 21-EPSCoR2021-0022: Preventing immune system dysregulation during deep-space missions by Tocoflexol, a modified isomer of vitamin E (R. Pathak, P.I., M.J. Cannon, Co-I.)

Funding period: 08/15/2021 – 07/31/2024

Funding level:

Project goals: To compare the efficacy of Tocoflexol with the natural isomers of tocotrienol (γ- and δ-tocotrienol) in attenuating immune dysregulation in the primary and secondary lymphoid organs following coexposure to simulated microgravity and chronic irradiation.

Aims: 1. Characterize immune dysregulation after exposure to SMG or CIR alone and in combination. 2. Determine the efficacy of Tocoflexol in mitigating immune dysregulation after coexposure to SMG and CIR.

Time Commitment: 5%

Contracting Officer: Jeppie R. Compton, agency-epscor@mail.nasa.gov

Overlap: None

NIH/NIAID: U01AI170039-01, Platelets in radiation-induced immune dysregulation (R. Pathak, J.S. Ware, M.J. Cannon, Co-PIs)

Funding period: 07/25/2022 – 04/01/2027

Funding level:

Project Goals: The overall objectives of this project are to understand how platelet contribute to immune dysregulation after exposure to ionizing radiation (IR) and test platelet-centric strategies to mitigate IR-induced immune dysregulation using murine models.

Aims: 1. Determine whether selectively blocking the binding of GPIbα to Mac-1 exacerbates, while exogenous GPIbα administration mitigates, TBI-induced immune dysregulation. 2. Determine whether limiting PMP generation mitigates TBI-induced immune dysregulation.

Time Commitment: 15%

Contracting Officer: Thomas Winters, twinters@mail.nih.gov

Overlap: None

What other organizations were involved as partners?

Nothing to report

8. Special Reporting Requirements

Not applicable

9. Appendices

None