AWARD NUMBER: W81XWH-21-1-0460

TITLE: Proteogenomic Analysis of Responders Versus Nonresponders in a Phase 1 Trial of Th17-Inducing Dendritic Cell Vaccination for Advanced-Stage Ovarian Cancer

PRINCIPAL INVESTIGATOR: Martin J Cannon

CONTRACTING ORGANIZATION: University of Arkansas for Medical Sciences
Little Rock, AR

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

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Proteogenomic Analysis of Responders Versus Nonresponders in a Phase 1 Trial of Th17-Inducing Dendritic Cell Vaccination for Advanced-Stage Ovarian Cancer

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**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

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**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

University of Arkansas for Medical Sciences
4301 West Markham
Little Rock, AR 72205

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U.S. Army Medical Research and Development Command
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**14. ABSTRACT**

We recently completed a phase I clinical trial of Th17-inducing dendritic cell (DC) vaccination for stage IIIC– IV ovarian cancer patients. Of 18 evaluable patients, 39% remain recurrence-free with a median follow-up of 49.2 months' post-enrollment, and overall survival was 55%. The DC vaccine trial revealed a clear dichotomy between those patients that suffered from early recurrent disease (within 12-18 months), and those that enjoyed prolonged recurrence-free survival (4-5 years), thus posing the question of whether we can identify biomarkers of response versus non-response. This proposal will use state-of-the-art proteogenomic approaches to identify candidate biomarkers of responsiveness to Th17- inducing DC vaccination. The goals are (i) to identify markers associated with clinical response, and (ii) to identify markers associated with immune response. We have collated clinical metadata and conducted detailed feasibility analysis on tumor samples preparatory to in depth proteogenomic analysis. During Year 2 of funding, we have completed sample processing and preparation, and data acquisition is either completed or in progress for both proteomic and genomic approaches. We have also continued development of a bioinformatic proteogenomic pipeline for data analysis. We anticipate that studies will be completed during the no-cost extension for this award.

**15. SUBJECT TERMS**
None listed.

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
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**Standard Form 298 (Rev. 8-98)**
**Prescribed by ANSI Std. Z39.18**

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## 1. INTRODUCTION

We recently completed a phase I clinical trial of Th17-inducing dendritic cell (DC) vaccination for stage IIIC– IV ovarian cancer patients following initial surgery and chemotherapy. DC vaccination induced durable Th1/Th17 T cell and antibody responses to FRα target antigen, and of 18 evaluable patients, 39% remain recurrence-free with a median follow-up of 49.2 months' post-enrollment, and overall survival was 55%. With the caveat that our enrollment is limited, the recurrence-free survival rate is markedly better than recently reported for maintenance PARP inhibitor treatment in patients without BRCA mutation or deficiencies in homologous recombination. The DC vaccine trial revealed a clear dichotomy between those patients that suffered from early recurrent disease (within 12-18 months), and those that enjoyed prolonged recurrence-free survival (>5 years), thus posing the question of whether we can identify biomarkers of response versus nonresponse. This study will use state-of-the-art proteogenomic approaches to identify candidate biomarkers of responsiveness to Th17- inducing DC vaccination. The goals are (i) to identify markers associated with clinical response, and (ii) to identify markers associated with immune response.

Treatment-naïve tissue samples and matching time course plasma sample sets have been collected from patients ultimately showing durable objective responses and from patients with progressive disease (nonresponders) following Th17-DC vaccination. The approach embodied laser microdissection (LMD) to enrich the tumor epithelium region from each of the tissue specimens. LMD-enriched specimens have been subjected to DNA, mRNA, and protein extraction. To identify tumor transcriptional signatures and pathways correlating to vaccine responsiveness, we are in the process of performing RNAseq. To identify tumor protein biomarkers, protein pathways, and phosphosignaling pathways correlating to vaccine responsiveness, quantitative mass spectrometry has been performed. By performing whole exome sequencing (WES), we will be able to determine whether our transcriptomic and proteomic signatures correlate to chromosome instabilities or mutations. This first-in-kind proposal uniquely leverages state-of-the-art resources to identify biomarkers of clinical response to DC vaccination. Proteogenomic profiling of ovarian cancer patients treated on the DC vaccine trial may also identify druggable targets that can improve responsiveness to DC vaccination and other immunotherapies.

## 2. KEYWORDS

**Ovarian cancer, dendritic cell vaccination, immunotherapy, proteomics, WES, RNAseq, clinical trial, biomarkers**

## 3. ACCOMPLISHMENTS

**What were the major goals of the project?**

| Specific Aim (specified in proposal) | Timeline (months) | Site 1 (UAMS) |
|---|---|---|
| **Major Task 1:** Use state-of-the-art proteogenomic approaches to identify candidate biomarkers of responsiveness to Th17-inducing DC vaccination. | 1-24 | In progress |
| Subtask 1: Completion of DOD regulatory requirements | 1-6 | Done |
| Subtask 2: Provision of tumor samples, blood/plasma samples | 3-12 | Mayo Clinic Biospecimens Core, Done |
| Subtask 3: RNAseq of patient samples | 9-15 | In progress |
| Subtask 4: Whole exome sequencing of patient samples | 9-15 | In progress |
| Subtask 5: Quantitative phosphoproteomics of patient samples | 6-15 | In progress |
| Subtask 6: Plasma proteomics of patient samples | 15-24 | Pending |
| Subtask 7: Proteogenomic data integration | 6-24 | Pending |
| Milestones achieved: (i) Identification of candidate biomarkers of clinical responsiveness to Th17-inducing DC vaccination, and (ii) identification of biomarkers associated with immune response | 1-24 | Pending |
| | | **Site 2 (WHIRC)** |
| Subtask 1: Quantitative phosphoproteomics of patient samples | 6-15 | In progress |
| Subtask 2: Global proteome and phosphoproteome data acquisition and analysis | 6-24 | In progress |
| Milestone achieved: Cross-site validation of sample sets | 1-24 | Pending |

**What was accomplished under these goals?**

During Year 2 of funding, we have completed sample processing and preparation, and data acquisition is either completed or in progress for both proteomic and genomic approaches. We have also continued development of a bioinformatic proteogenomic pipeline for data analysis. For the outlined studies, we have curated all formalin-fixed, paraffin-embedded (FFPE) patient samples which were then processed by Dr. Conrads' laboratory at the Women's Health Integrated Research Center (WHIRC; Site 2) using laser microdissection. After tissue microdissection protein, mRNA, and DNA fractions were isolated from samples, aliquoted for transfer, then shipped to the University of Arkansas for Medical Sciences (UAMS; Site 1) for RNA-sequencing, WES, and TMT-based quantitative proteomics. Parallel quantitative proteomic approaches were also performed on a portion of the sample material retained by Dr. Conrads' group. For Year 2 nucleic acid and protein isolation are completed and both RNAseq and WES are in progress while protein mass spectrometry approaches at Sites 1 and 2 are either completed or are in progress. Global proteomic analysis of laser microdissection-harvested tumor epithelial samples have been completed at both Site 1 and Site 2. The results of these parallel proteomics projects have passed QC criteria (Table 1) and will be combined with our genomics data to generate a proteogenomic profile of responsiveness to the Th17-inducing DC vaccination.

| Quality control criteria | Site 1 | Site 2 |
|---|---|---|
| Input PSMs | 153051 | 277168 |
| Multi-mapper PSMs | 11650 | 21925 |
| Unique Multi-mapper PSMs by AnnSeq/Mod/PA | 3508 | 4420 |
| Single-mapper PSMs | 141401 | 255243 |
| Unique proteins in single-mapper PSMs | 8758 | 9893 |
| Unique multi-mapper PSMs mapped | 3178 | 4084 |
| Multi-mapper PSMs mapped | 10134 | 19928 |
| Total PSMs mapped | 151535 | 275171 |
| Proteins quantified | 7008 | 8149 |

**Table. 1.** Overview of global proteomics results at Site 1 (UAMS) and Site 2 (WHIRC). PSMs; peptide-spectrum matches.

In anticipation of the WES, RNAseq, and proteomics data, we have focused time during Years 1 and 2 of funding to develop a proteogenomics data analysis workflow. We have leveraged data collected in our laboratory on an independent project to develop the proteogenomics data analysis pipeline that will be used for the ovarian cancer samples. This independent project is a PDX model of NRAS mutant melanoma with acquired resistance to MAPK inhibitor treatment for which we have whole exome sequencing, RNAseq, and protein mass spectrometry data – identical to the dataset to be collected in the current project on ovarian cancer. This pipeline will allow for the development of custom gene and protein databases from patient-specific whole exome sequencing information that will be used for analyzing RNAseq and proteomics data. Using these custom databases, we can correlate transcriptomic and proteomic data to look for unique transcript and/or protein features in our ovarian cancer samples. Our new proteogenomics data analysis tool is publicly accessible on protocols.io (DOI: dx.doi.org/10.17504/protocols.io.36wgq7d83vk5/v1); and we have this work in peer review at *PLOS One*.

**What opportunities for training and professional development has the project provided?**

*Nothing to report*

**How were the results disseminated to communities of interest?**

*Nothing to report*

**What do you plan to do during the next reporting period to accomplish the goals?**

Our plan for the remainder of the reporting period is to complete the phosphoproteomics and the WES and RNAseq profiling analysis of these tissue samples, then to integrate the omics data to characterize

responsiveness to the TH17-inducing DC vaccine. For the genomics approaches, libraries have been constructed for both the WES and RNAseq projects, which are currently undergoing QC before sequencing. Once all the data has been collected, it will be transferred to the UAMS (Site 1) Bioinformatics core of integration and analysis, which will enable identification of biomarkers associated with clinical response to this therapy.

## 4. IMPACT

The clinical results revealed a clear dichotomy between those patients that suffered from early recurrent disease (within 12-18 months), and those that enjoyed prolonged recurrence-free survival (4-5 years). In this study, we propose that analysis of primary tumor tissue and plasma samples will reveal biomarkers of clinical responsiveness versus non-responsiveness in ovarian cancer patients enrolled in the clinical trial of Th17-inducing DC vaccination. This project has exceptional impact at multiple levels. First, the identification of biomarkers associated with clinical response to Th17-inducing DC vaccination may enable the identification of ovarian cancer patients most likely to benefit from DC vaccination in future clinical trials. Second, knowledge of biomarkers associated with multiple immune response parameters (e.g., Th1 or Th17 CD4+ T cell responses, FRα-specific antibody responses, which may in turn correlate with clinical response) may allow identification of druggable targets or pathways that enhance immune and clinical responses to DC vaccination.

## 5. CHANGES/PROBLEMS

**Changes in approach and reasons for change**
*Nothing to report*

**Actual or anticipated problems or delays and actions or plans to resolve them**
*Nothing to report*

**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**
*No significant changes*

**Significant changes in use or care of human subjects**
*Not applicable*

**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 AND 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:** | ***0.6*** |
| **Contribution to Project:** | ***Oversight of the project*** |
| **Funding Support:** | ***DOD OC200521*** |

| | |
|---|---|
| **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 OC200521*** |

| | |
|---|---|
| **Name:** | ***Alan Tackett*** |
| **Project Role:** | ***Co-Investigator*** |
| **Researcher Identifier (e.g. ORCID ID):** | |
| **Nearest person month worked:** | ***0.3*** |
| **Contribution to Project:** | ***Proteogenomics*** |
| **Funding Support:** | ***DOD OC200521*** |

| | |
|---|---|
| **Name:** | ***Nathan Avaritt*** |
| **Project Role:** | ***Co-Investigator*** |
| **Researcher Identifier (e.g. ORCID ID):** | |
| **Nearest person month worked:** | ***1.8*** |
| **Contribution to Project:** | ***Proteogenomics*** |
| **Funding Support:** | ***DOD OC200521*** |

| | |
|---|---|
| **Name:** | ***Thomas Conrads*** |
| **Project Role:** | ***Co-Investigator, WHIRC*** |
| **Researcher Identifier (e.g. ORCID ID):** | |
| **Nearest person month worked:** | ***0.3*** |

| | |
|---|---|
| **Contribution to Project:** | ***laser microdissection, proteogenomics*** |
| **Funding Support:** | ***DOD OC200521*** |

| | |
|---|---|
| **Name:** | ***Ming Zhou*** |
| **Project Role:** | ***Senior Scientist, WHIRC*** |
| **Researcher Identifier (e.g. ORCID ID):** | |
| **Nearest person month worked:** | ***0.6*** |
| **Contribution to Project:** | ***LC-MS analysis, bioinformatics*** |
| **Funding Support:** | ***DOD OC200521*** |

**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:

**Arkansas Breast Cancer Research Program Investigator Award:** Th17-inducing dendritic cell vaccination, immune checkpoint inhibition and PARP inhibition for treatment of triple-negative breast cancer (M.J. Cannon, P.I.)

**Funding period:** 09/01/2022 -08/31/2024

**Funding level:**

**Project Goals:** The major goal of this proposal is to explore the therapeutic potential of an innovative Th17-inducing dendritic cell vaccine in a mouse model of TNBC, either as a stand-alone immunotherapy, or combined with olaparib and anti-PD-1 immune checkpoint inhibition.

**Aims:** 1) Determine the mechanisms by which combined DC vaccination and anti-PD-1 induces effective and durable anti-tumor immunity, 2) Determine whether PARP inhibitors enhance the efficacy of Th17-DC/anti-PD-1 immunotherapy in TNBC.

**Time Commitment:** 10%

**Contracting Officer:** Nia Indelicato (NLIndelicato@uams.edu)

**Overlap:** None

**What other organizations were involved as partners?**

1) Women's Health Integrated Research Center, Inova Health System, Fairfax, VA

2) Mayo Clinic, Rochester, MN

## 8. SPECIAL REPORTING REQUIREMENTS

*Not applicable*

## 9. APPENDICES

*None*