AWARD NUMBER: W81XWH-21-1-0346

TITLE: A Novel Platform for Ovarian Cancer Diagnosis and Screening

PRINCIPAL INVESTIGATOR: Ronald Buckanovich, MD

CONTRACTING ORGANIZATION: Magee-Womens Research Institute and Foundation, Pittsburgh, PA

REPORT DATE: July 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**
July 2022	Annual	01Jun2021 – 31May2022

4. TITLE AND SUBTITLE

A Novel Platform for Ovarian Cancer Diagnosis and Screening

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-21-1-0346

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Dr. Ronald Buckanovich

E-Mail: buckanovichrj@mwri.magee.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

MAGEE-WOMENS RESEARCH
INSTITUTE AND FOUNDATION
3240 Craft place Pittsburgh, PA 15213
(new)

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

The goal of this project is to use a special marker of this circulating tumor DNA, called methylation, to make a highly sensitive and specific test that could be used for screening and early diagnosis of ovarian cancer. This work will involve collecting blood samples from women without cancer, with cancer, with non-cancerous ovarian masses and those with a genetic predisposition to cancer to develop and refine the test so it can differentiate between these different groups. If successful, this test could revolutionize about ability to detect and treat ovarian cancer.

15. SUBJECT TERMS
cfDNA, ovarian cancer, biomarker, cancer associated mesenchymal stem cell

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	20	USAMRDC

TABLE OF CONTENTS

Abstract:
Epithelial ovarian cancer (OvCa) has the third highest incidence to mortality ratio of all cancers. Cure rates are high for patients with early stage disease, but early stage disease is typically asymptomatic and there is no effective screening test. Consequently, over 75% of women are diagnosed with late stage disease with a 5-year survival rate of 30%. Therefore, there is a clear need for a screening strategy that can diagnose disease early and improve survival. Our preliminary data indicate that the ovarian cancer stroma has a unique DNA methylation signature and that stromal markers, as opposed to epithelial cancer cell markers, can be used to identify patients with cancer. Unfortunately, plasma sample DNA methylation samples need to be collected with special We propose to collect specimen methods. We therefore propose to prospectively collect plasma for cfDNA methylation study and develop a diagnostic algorithm. With this award to date we have prospectively collected plasma samples from ~90 patients. We have isolated and sequences methylated cfDNA from ~60 patients and have begun to develop a diagnostic algorithm. Current studies generated a an AUC of 0.71. However, we have not yet applied the stromal specific signature or patient specific attributes needed to fine tune the algorithm.

1. INTRODUCTION:

Epithelial ovarian cancer (OvCa) has the third highest incidence to mortality ratio of all cancers. Cure rates are high for patients with early stage disease, but early stage disease is typically asymptomatic and there is no effective screening test. Consequently, over 75% of women are diagnosed with late stage disease with a 5-year survival rate of 30%. Therefore, there is a clear need for a screening strategy that can diagnose disease early and improve survival. Our preliminary data indicate that the ovarian cancer stroma has a unique DNA methylation signature and that stromal markers, as opposed to epithelial cancer cell markers, can be used to identify patients with cancer. Unfortunately, plasma sample DNA methylation samples need to be collected with special We propose to collect specimen methods. We therefore propose to prospectively collect plasma for cfDNA methylation study and develop a diagnostic algorithm.

2. KEYWORDS:

cfDNA, ovarian cancer, biomarker, cancer associated mesenchymal stem cell

3. ACCOMPLISHMENTS:

What was accomplished under these goals?

Major Task 1: Prospectively collect plasma samples for cfDNA methylation analysis

Subtask 1: Collect sample from healthy (benign gynecologic conditions) controls

Subtask 2: Collect sample from healthy women with hereditary genetic predisposition without known cancer

Subtask 3: Collect preoperative samples from women with early stage ovarian cancer

Milestone(s) Achieved

Local IRB/IACUC Approval

Milestone Achieved: HRPO

To date we have collected samples from 65 patients. This includes 29 'control' samples with no evidence of cancer. Of these patients, 11 patients were BRCA mutation carriers for exploratory analysis. 36 were patients with cancer, 6 of these were excluded from analysis due to the presence of a second cancer (breast cancer, endometrial cancer) or excluded histology (borderline tumor, etc).

Specific Aim 2: Develop a cfDNA TME specific methylation ovarian cancer diagnostic signature

Major Task 2

Subtask 1: Develop a cfDNA TME specific methylation signature that differentiates early stage ovarian cancer from controls.

Subtask 2: Perform exploratory analysis in BRCA carriers to determine if a cfDNA methylation signature can distinguish patients with STIC lesions from patients without STIC lesions

Specific Aim 1.

During this period of study, we bisulfite-sequenced 43 plasma libraries from 19 control patients, 23 ovarian cancer patients, and 1 breast cancer patient. The bisulfite sequencing data were aligned and processed using the Bismark program to extract methylation status for the covered CpG sites.

In most of the following analysis, we are focusing on the 42 samples from control and ovarian cancer patients. Figure 1 is a hierarchical clustering of the samples using the top 50% of CpG sites with highest variation across all samples. The Pearson's correlation coefficients were converted and used as the distance measure.

We performed logistic regression with correction for overdispersion to identify the set of CpGs with differential methylation between the ovarian cancer and control samples, using the R package "methylKit". In total we identified 505 differentially methylated (DM) CpG sites. Figure 2 is the heatmap plot of the 105 of these CpG sites with larger methylation level difference, with columns representing plasma samples, and rows representing individual CpG sites. Note that the samples with "ova" suffix in their names are ovarian cancer samples.

We compared the DM CpG sites with a recently published study on the methylation difference between carcinoma-associated mesenchymal stem cells (CA-MSCs) and normal mesenchymal stem cells (Fan et al, 2020). In that study, 103 differentially methylated regions (DMRs) between CA-MSCs and normal MSCs were identified. However, we were unable to find any overlap between the 505 DM CpG sites found in our study, and the 103 DMRs from Fan et al 2020.

We selected 7 ovarian cancer plasma samples from this study, and 8 control plasma samples from a previous project, to form a training data set. Based on these 15 samples, we performed similar differential methylation analysis to identify about 147379 CpG sites with highest and most consistent differential methylation between the ovarian cancer plasma and control. Based on these CpG sites, we designed an ovarian cancer risk score, and calculated this score for the other 35 plasma samples in this study. The plot of the risk scores and the ROC curve of using this score to classify ovarian cancer for the 35 plasma samples are presented in Figure 3.

From Figure 3, we can see that the risk scores for the control plasma samples are significantly lower than that for the ovarian cancer samples (p values <= 0.05 using both t test and Wilcoxon test), and that as a classifier, its area under ROC curve (AUC) is significantly above 0.5.

In the next step of this study, we plan to increase the training data to include more plasma samples from this study, so that we can identify more reliably the set of CpG sites differentially methylated between ovarian cancer and control plasma samples. These CpG sites will allow us to design a better risk score. We plan to perform cross-validation analysis to estimate the performance of the new risk score.

We also notice that during this report period, we have yet to incorporate the demographic information into the design of our risk score. The information we have collected includes age, race, BMI, and smoking habit. In the next step, we plan to include the demographic information in the design of risk score. In addition, we also plan to further classify the ovarian cancer samples into early (I & II) and later (III and IV) stages, and design risk scores for them respectively.

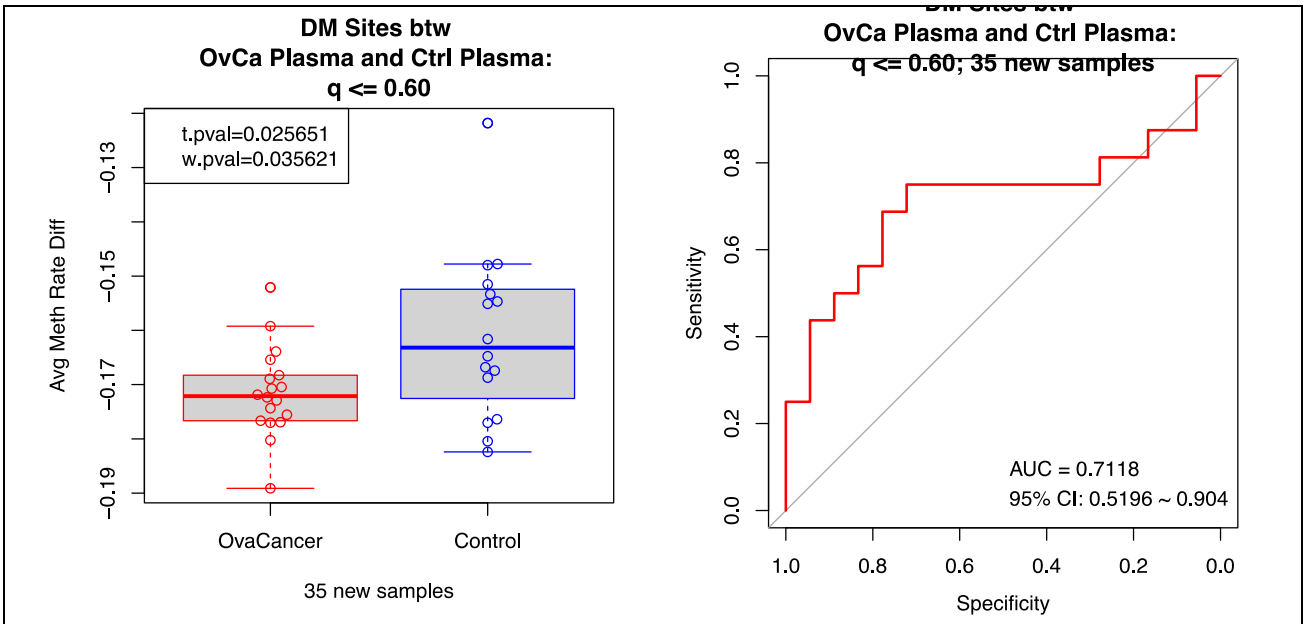

Figure 1. Boxplot (left) and ROC curve (right) for the ovarian score of 35 plasma samples.

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

NA

How were the results disseminated to communities of interest?

Results are still preliminary and being collected.

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

We will continue to collect specimens from patients to expand our analysis and improve the classifier. With new samples we will fine-tune our analysis and apply external data sets and add clinical information which can impact the cfDNA methylation score, such as age, smoking, BMI etc. We will also determine if a classifier can be developed to distinguish patients with STICs from controls.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

No current impact as the study is ongoing. If it appears that the classifier can improve diagnosis of early stage ovarian cancer, this could move into a clinical trial and ultimately save lives.

What was the impact on other disciplines?

No current impact as the study is ongoing. If it appears that the classifier can improve diagnosis of early stage ovarian cancer, this could move into a clinical trial and ultimately save lives.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Nothing to report

A

We had some delays in collecting samples related to COVID with collections temporarily suspended to reduce patients contacts.

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 of biohazards and/or select agents

NA

NA

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

NA

Books or other non-periodical, one-time publications.

NA

Other publications, conference papers and presentations.

NA

- **Website(s) or other Internet site(s)**

NA

- **Technologies or techniques**

Currently still being developed.

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Ronald Buckanovich
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Supervises project, collects/organizes samples, organizes team meetings.
Name:	Sarah Taylor
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Supervises CRC in patient sample collection.
Name:	Sarah Taylor
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Supervises CRC in patient sample collection.
Name:	David Peters
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Supervises Processing of patient samples for cfDNA isolation and sequencing of cfDNA
Name:	Tianjiao Chu
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Performs bioinformatic analysis to generated diagnostic algorithm
Name:	Stacy McGonigal
Project Role:	Laboratory Manager
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Coordinates, processes, stores all plasma samples for cfDNA methylation sequencing. Coordinates sample transfer to Peters lab
Name:	Elizabeth Lewis
Project Role:	Lab assistant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4
Contribution to Project:	Performs cfDNA capture and sequencing studies. Transfer data to Dr. Chu for analysis

Name:	Angela Laslavic
Project Role:	Clinical research coordinator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	coordinates clinical collection of biospecimens, coordinates collection of clinical data with honest broker.

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. Buckanovich

New support:

Title: Defining the impact of stromal aging on ovarian cancer initiation
Major Goals: The goal of this proposal, in direct response to the request for application, is to define the impact of aging on interactions between stromal cells and cancer initiating cells (CIC) that drive ovarian cancer formation.
Project Number: U01AG077923
Name of PD/PI: Lan Coffman, **Ronald Buckanovich**, Toren Finkel
Source of Support: NCI (University of Pittsburgh)
Primary Place of Performance: Magee-Womens Research Institute
Project/Proposal Start and End Date: 09/30/2021 – 05/31/2026
1.2 CM effort

Title: Target Tumor Desmoplasia to Enhance Immunotherapy
Major Goals: This collaborative project will develop key data on the importance of CA-MSC in the generation of the immunosuppressive OvCa TME.
Project Number: OCRFA-Renewal
Name of PD/PI: Ronald J. Buckanovich
Source of Support: Ovarian Cancer Research Fund Alliance
Primary Place of Performance: Magee-Womens Research Institute
Project/Proposal Start and End Date: 01/01/2022 – 12/31/2022
0.60 CM effort

Pending:

Title: DOD Omics Consortium to Study the Origins of Ovarian Cancer (DOCSOOC)

Major Goals: The goal of the project is to define alterations in the stromal microenvironment and their influences on the epithelial compartment of normal fallopian tube, precursor (STIC) lesions, and the influence on tumor initiation.

Status of Support: Pending (22-0013P)

Project Number: W81XWH-21-OCRP-OM

Name of PD/PI: Lan Coffman

Source of Support: DOD (University of Pittsburgh)

Primary Place of Performance: Magee-Womens Research Institute

Project/Proposal Start and End Date: 01/01/2022 – 12/31/2026

Dr. Bucknaovich – Co-I, 0.36 calendar months effort.

Grant Support that ended in the 06/01/21 – 05/31/2022 Period:

1. NIH - Using microfluidic single cell culture to characterize cancer cell asymmetric division - R01CA203810
2. NIH - Wang R21 Characterization of EPHA3 - 5R21 CA237964-02
3. NIH - Developing a Human in Mouse Cancer Model with a Completely Humanized Stroma - R01CA211913

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: