AWARD NUMBER: W81XWH-19-1-0226

TITLE: Molecular Changes in Circulating Cell-Free DNA from BRCA1 and BRCA2 Mutation Carriers with Tubal Precursor Lesions and Occult Early High-Grade Serous Ovarian Cancer at Risk-Reducing Surgery

PRINCIPAL INVESTIGATOR: Theodore J. Brown, PhD

CONTRACTING ORGANIZATION: Sinai Health System

REPORT DATE: July 2021

TYPE OF REPORT: Annual report

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 2021	Annual	01Jul2020 – 30Jun2021

4. TITLE AND SUBTITLE
Molecular Changes in Circulating Cell-Free DNA from BRCA1 and BRCA2 Mutation Carriers with Tubal Precursor Lesions and Occult Early High-Grade Serous Ovarian Cancer at Risk-Reducing Surgery

5a. CONTR-ACT NUMBER
W81XWH-19-1-0226

5b. GRANT NUMBER
OC180325

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Theodore Brown
Gabrielle Ene

E-Mail: brown@lunenfeld.ca
Gabrielle.Ene@uhnresearch.ca

5d. PROJECT NUMBER
0011309564–0001

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Sinai Health System
600 University Ave
Toronto, Ontario, Toronto,
M5G 1X5

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

High grade serous ovarian cancer typically presents at advanced stage with a median survival of 44 months. Small precursors to this cancer are found in the fallopian tube and likely seed the ovary and peritoneum simultaneously. Early detection is urgently needed and ideally would detect precursor lesions. This award will determine if DNA methylation patterns exhibited in circulating cell-free DNA could be used to detect precursor lesions. During the first year of the award, application for province-wide (Ontario) research ethics approval was submitted to umbrella all 4 participating hospitals. This umbrella approval was obtained; however, we are awaiting final approval from 2 hospitals, which was delayed by the covid-19 shutdown. All research activities were halted (Covid-19 research being the exception) and department staff were redeployed to other hospital activities, causing a back-up of research-related activity. All centres are currently reopening (in stage 2) and study approval should be forthcoming. The Material Transfer Agreements have been started by Sinai Health System and once final REB/IRB approval has been obtained, these will be finalized across the all institutions. Patient samples for use in this study as identified have been secured and we are poised to complete the study once approvals are finalized.

15. SUBJECT TERMS
Ethics board consent, material transfer agreement, tissue identification

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	Unclassified	8	19b. TELEPHONE NUMBER *(include area code)*

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

TABLE OF CONTENTS

1. Introduction

Epithelial ovarian cancers, which constitute 90% of ovarian cancers, are the most lethal and include multiple types with distinct histologic appearance, characteristic genetic alterations and molecular signatures, cell of origin, and clinical course. The most common histotype is high-grade serous carcinoma (HGSC), which accounts for 70% of ovarian cancer cases overall and 90% of cases diagnosed at an advanced stage. Women with HGSC typically present once pelvic or more distant seeding has occurred, partly due to the fact that there are no clear symptoms of earlier stage disease or biomarkers capable of revealing early stage disease. Despite being initially responsive to platinum- and taxol-based chemotherapy, 80-90% of women with HGSC will die of their disease, with a median survival of 4 years. Efforts to identify diagnostic biomarkers for ovarian cancer screening have largely utilized tissues from patients with advanced stage disease and have thus far been disappointing. Cancer antigen 125 (CA125), which is widely used to monitor patients for chemotherapy response or recurrent disease, lacks sufficient specificity necessary to be predictive of an initial ovarian malignancy and, importantly, is detected in only 50% of stage I epithelial ovarian cancers. What is critically needed is a test capable of detecting asymptomatic ovarian cancer, when surgical approaches have the greatest chance of being curative. Women with a mutation in breast cancer susceptibility genes, BRCA1 or BRCA2, are at a very high risk of developing ovarian cancer. Due to this risk, it is recommended that these women undergo bilateral salpingo-oophorectomy once childbearing is completed. Upon close histological examination of the removed fallopian tubes, a small number of tpatients are found to have small occult (hidden) cancers or a lesion that is thought to precede and progress to these small cancers. These are the earliest stages of HGSC. Our goal is to develop a blood test to detect such lesions or small cancers while still within the fallopian tube. Our center has been collecting research blood samples from all women undergoing removal of their fallopian tubes for reduction of risk for HGSC, and we have identified some of these women who were subsequently discovered to have precursor lesions or small cancers. In this pilot project grant, we are determining if we can identify characteristic changes in DNA methylation in small early HGSCs or precursor lesions (serous tubal intraepithelial cancer, STIC) and whether we can detect these changes in DNA circulating in the blood. These findings could form the basis of a blood test that would enable detection of HGSC at its earliest stages when surgery would be most effective. The availability of small early stage HGSC also affords us the ability to assess whether emerging immune-based treatment approaches to early stage ovarian cancer might be effective. When detected at an early stage, approximately 10% of HGSC recur. Our findings may support the exploration and use of new immune checkpoint inhibition for the treatment of early stage HGSC as a targeted approach with less side effects than currently used conventional toxic chemotherapy. The ability to effectively screen and detect early stage HGSC and to efficiently treat it would greatly impact the survival of this lethal disease, thereby benefiting women in general as well as those who are part of the armed forces service community.

2. Keywords

High-grade serous ovarian cancer, fallopian tube; BRCA1, BRCA2, STIC lesions, DNA methylation, circulating tumor cDNA, early detection

3. Accomplishments

Specific Aim 1: To perform global methylation sequencing on STIC lesions, occult HGSC, and plasma cfDNA. Three major tasks contribute to this Aim, preceded by research board and HRPO approvals.

REB/IRB approval: Our first goal is to perform global methylation sequencing on ovarian cancer precursor lesions, occult high grade serous ovarian cancers and plasma cell-free DNA. Our first tasks were to obtain IRB/REB approvals and submit these to the DoD for approval. Since this work involves multiple institutions, research ethics approval was submitted to a province-wide agency for Ontario, which includes all participating centres (University Health Network, Sunnybrook Health Sciences Centre, Women's College Hospital, and Sinai Health System). Womens College Hospital was added for the approval process as the clinical data associated with the patient samples has originated from that site. The provincial research ethics approval had been obtained, providing an umbrella approval and oversight. At the time of covid-19 mandated shutdown, we were in the process of securing signoff from each of the four institutions, which was needed for full IRB approval. Although significantly delayed because of the pandemic and protective measures put in place, all of the four centres have now completed this process. Importantly, due to COVID-19, research activities were paused at all centres (Covid-19 research being the exception) and department staff were redeployed to other hospital activities, causing a back-up of research-related activity. The Material Transfer Agreements are completed . We had allowed 3 months for REB approval in our SOW and are behind schedule on this task. However, the covid-19 pandemic has featured prominently in this delay. Full approval has been secured and HRPO approval has been obtained as well. We have just learned that we have been approved for a 12-month no cost extension, enabling us to complete the project.

Major Task 1: Tissue procurement: All patient samples for use in this study have been identified and been secured and for the study. These will be released to the lab once all material transfer agreements are complete. We had hoped to be processing these tissues and are approximately one year behind schedule at this point.

Major Task 2: Extract DNA and cfDNA and perform methylome screen: We are set to begin this task, which will occur immediately upon tissue transfer. This work can be completed within 5 months.

Major Task 3: Analyze Data: This task fell within year 2 of the award period; however, we will initiate this task in approximately 5 months.

Specific Aim 2: Determine the immune environment of stage 1a HGSC. Three major tasks contribute to this Aim.

Major task 1: Tissue procurement: We have identified the formalin fixed paraffin-embedded tissues that will be used in the study but have not initiated sectioning.

Major task 2: Perform whole exome sequencing and RNAseq: These activities are waiting tissue release and processing. We anticipate starting these within one month.

Major task 3: Assess tissues for TILS: These activities are waiting for tissue release and processing.

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

  Nothing to report in this period

- How were the results disseminated to communities of interest?

  Nothing to report in this period

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

Final institutional ethics approvals and transfer agreements as well as HRPO approval is now in place. Upon learning we have been granted a one-year extension, we are poised and will release the tissues to the labs for processing and analysis. We fully expect to have the project completed within the extension.

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?

  Nothing to report in this period

- What was the impact on other disciplines?

  Nothing to report in this period

- What was the impact on technology transfer?

  Nothing to report in this period

- What was the impact on society beyond science and technology?

  Nothing to report in this period

5. Changes/Problems

The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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
  Nothing to report

- Actual or anticipated problems or delays and actions or plans to resolve them
  We have been significantly delayed by measures implemented to contain the pandemic. These led to significant delays in obtaining REB approvals. We obtained approvals, including HRPO approval, just as our original funding period was closing. We submitted for a 12-month extension in May but only learned of approval of this request on August 27, 2021. We are now proceeding with tissue procurement and distribution to the research labs.

- Changes that had a significant impact on expenditures

  No changes. We have not yet accessed any of the research funds

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

None other than the change in processing stage 1a HGSC as indicated above.

- Significant changes in use or care of human subjects

  Not applicable

- Significant changes in use or care of vertebrate animals.

  Not applicable

- Significant changes in use of biohazards and/or select agents

  Not applicable

6. Products

Nothing to report

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

a. 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."

   1. *Theodore Brown*

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

Nothing to report in this period

What other organizations were involved as partners?

Nothing to report in this period

8. Special Reporting Requirements

Not applicable