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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

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TYPE OF REPORT: Annual report

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

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July 2020	Annual	1 Jul 2019 – 30 Jun 2020

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. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-19-1-0226

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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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

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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	Unclassified	12	USAMRMC
U	U	U			19b. TELEPHONE NUMBER *(include area code)*

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 was 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 is needed for full IRB approval. Two of the four centres have 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. Since the step-wise reopening of research activity has begun (currently phase 2), all centres are currently working to speed their internal approval processes in order to have this study approved and started. The Material Transfer Agreements have been started by Sinai Health System and these are in the process of negotiation with the other centres. 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 is expected shortly.

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 ethics board approvals have been secured and material transfer agreements completed. We are on schedule for this task. We had hoped to be processing these tissues and are approximately 4-6 months behind schedule at this point.

Major Task 2: Extract DNA and cfDNA and perform methylome screen: We are awainting final approvals to begin this task. And maintain that this work can be completed within 5 months.

Major Task 3: Analyze Data: This task falls within year 2 of the award period.

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. We are awaiting final ethics approval.

Major task 2: Perform whole exome sequencing and RNAseq: These activities are waiting for tissue release and processing.

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 should be forthcoming and we will then quickly release the tisues to the labs for processing and analysis.

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

  There was one minor change made to processing stage 1a HGSC patient specimens to address a concern raised by the REB/IRB. The board indicated it was necessary to ensure that for these small tumors, residual tumor tissue be retained for potential future clinical use (i.e. diagnosis verification). To address this concern, we will cut 20 thin sections and if tumor tissue is not observed in the last section, all sections will be returned to Pathology. This change was accepted by the committee.

- Actual or anticipated problems or delays and actions or plans to resolve them

  We have encountered two sources of delay. The forst is the time it took to gain umbrella REB/IRB approval. This has accounted for 3 months of delay. The second is the Covid-19 pandemic that resulted in halting all non-essential research activity (covid-19 related) and the redeployment of hospital staff (including REB) to other areas of the hospitals. This has accounted for 6 months or more in delay.

- Changes that had a significant impact on expenditures

  No changes. We have not yet accessed any 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?

During the past year, Dr. Daniel DeCarvalho has been awarded a CIHR grant that does not present any overlap.

Project Title: Viral mimicry tolerance as a mechanism of early tumor immune invasion
Time Commitment: 20 hours/month
Funding Agency: Canadian Institutes of Health Research (CIHR) – Project Grant
Grants Officer: Renee Venne, Acting Manager. Canadian Institutes of Health Research, 160 Elgin Street, Ottawa, Ontario, Canada K1A 0W9
Total Award: 868275
Start Date: October 2019 End Date: September 2024
Project Goals: The main goal of our proposal is to inform approaches that will improve immunotherapy responses and cancer outcomes, particularly for ovarian cancer.
Specific Aims: To uncover novel mechanisms underlying how p53 regulates the repeatome. To assess how dsRNA signalling upon p53 inactivation promotes immune evasion. To assess how increased NMD upon p53 inactivation promotes tumor immune evasion.
Project Overlap: None

What other organizations were involved as partners?

Nothing to report in this period

8. Special Reporting Requirements

Not applicable

Appendices

1. Provincial REB/IRB approval
2. Sinai Health System Approval
3. Women's College Hospital Approval

MOUNT SINAI HOSPITAL
Joseph and Wolf Lebovic Health Complex
Bright Minds. Big Hearts. The Best Medicine.

Date: 25 November 2019

To: Professor Theodore Brown, Sinai Health Systems

CTO Project ID: 1777

Study 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

Sponsor Study ID: OC1803250

Study Sponsor: USAMRMC-CDMRP

Application Type: Observational Provincial Initial Application

Review Type: Delegated

Meeting Date: N/A

Date Approval Issued: 25/Nov/2019 12:07

Approval Expiry Date: 25/Nov/2020

Dear Provincial Applicant,

Thank you for submitting the above referenced study on behalf of all Ontario centres through the Clinical Trials Ontario Streamlined Research Ethics Review System. The Mount Sinai Hospital Research Ethics Board has reviewed the study and granted initial provincial approval as of the date noted above.

Provincial documents approved:

Document Name	Document Date	Document Version
OC1803250 Protocol	18/Nov/2019	

Note: Provincial REB approval does not confer ethics approval for participating centres. Each participating centre, including that of the Provincial Applicant, must submit the "Centre Initial Application" and receive approval from this REB prior to the conduct of the study at that centre. All other required institutional approvals must also be obtained prior to the conduct of the study.

No deviations from, or changes to, the protocol should be initiated without prior written approval from Mount Sinai Hospital Research Ethics Board, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial (such as a change in telephone number).

REB members involved in the research project do not participate in the review, discussion or decision.

Mount Sinai Hospital Research Ethics Board operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. Mount Sinai Hospital Research Ethics Board is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Please do not hesitate to contact us if you have any questions.

Sincerely,

Ronald Heslegrave, Ph.D., REB Chair

Mount Sinai Hospital Research Ethics Board

MOUNT SINAI HOSPITAL
Joseph and Wolf Lebovic Health Complex
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Date: 16 January 2020

To: Dr. Theodore Brown, Lunenfield Tanenbaum Research Institute

CTO Project ID: 1777

Study 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

Sponsor Study ID: OC1803250

Study Sponsor: USAMRMC-CDMRP

Application Type: Observational Centre Initial Application

Review Type: Delegated

Meeting Date: N/A

Date Approval Issued: 16/Jan/2020 15:22

Approval Expiry Date: 25/Nov/2020

Dear Principal Investigator,

The Mount Sinai Hospital Research Ethics Board has reviewed the application and granted approval for your centre to participate in this study. This approval is granted until the expiration date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study at this centre.

Provincial Documents approved:

Document Name	Document Date	Document Version
OC1803250_CTO Research Protocol	18/Nov/2019	

No deviations from, or changes to the protocol should be initiated without prior written approval of an appropriate amendment from Mount Sinai Hospital Research Ethics Board, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

Mount Sinai Hospital Research Ethics Board operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. Mount Sinai Hospital Research Ethics Board is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Please do not hesitate to contact us if you have any questions.

Sincerely,

Ronald Heslegrave, Ph.D., REB Chair

Mount Sinai Hospital Research Ethics Board

MOUNT SINAI HOSPITAL
Joseph and Wolf Lebovic Health Complex
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Date: 22 April 2020

To: Dr. Michelle Jacobson, Women's College Hospital

CTO Project ID: 1777

Study 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

Sponsor Study ID: OC1803250

Study Sponsor: USAMRMC-CDMRP

Application Type: Observational Centre Initial Application

Review Type: Delegated

Meeting Date: N/A

Date Approval Issued: 17/Apr/2020 15:47

Study Approval Expiry Date: 25/Nov/2020

Dear Principal Investigator,

The Mount Sinai Hospital Research Ethics Board has reviewed the application and granted approval for your centre to participate in this study. This approval is granted until the expiration date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study at this centre.

Provincial Documents approved:

Document Name	Document Date	Document Version
OC1803250_CTO Protocol	18/Nov/2019	

No deviations from, or changes to the protocol should be initiated without prior written approval of an appropriate amendment from Mount Sinai Hospital Research Ethics Board, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

Mount Sinai Hospital Research Ethics Board operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. Mount Sinai Hospital Research Ethics Board is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Please do not hesitate to contact us if you have any questions.

Sincerely,

Ronald Heslegrave, Ph.D., REB Chair

Mount Sinai Hospital Research Ethics Board