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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

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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 was to determine if DNA methylation patterns exhibited in circulating cell-free DNA could be used to detect precursor lesions. The work outlined in the application has been delayed due to restrictions imposed by the response to the covid-19 pandemic and loss of critical study personnel. No funds were expended during the tenure of the grant.

15. SUBJECT TERMS
None listed.

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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 patients 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

Unfortunately, we were not able to complete the study due to multiple factors. Primarily, the covid-19 slowdown significantly impacted our progress. Canada's response to the Covid-19 pandemic differed from that in the U.S. and its recovery lagged, initially due to vaccine shortages. The covid-19 mandated shutdown delayed us in ethics approvals and in securing Material Transfer Agreement signoff from our institutions. This delay also saw many of our co-principal investigators

and co-investigators leave the institution, resulting in delays and ultimately in our inability to begin the molecular work. Although we were poised to begin the actual work in September of 2022, further institutional delays in accessing the material at our sites prevented our being able to make progress. This prevented us from making sufficient progress necessary to support a last request for a no-cost extension. Moreover, due to the loss of key study personnel we would no longer be able to complete the study within the remaining time afforded by an 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 encountered further institutional delays in accessing tissues and loss of key research personnel.

• Changes that had a significant impact on expenditures
Nothing to report. No expenditures to report.

• Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

• Significant changes in use or care of human subjects
Nothing to report/not applicable

• Significant changes in use or care of vertebrate animals.
Nothing to report/not applicable

• Significant changes in use of biohazards and/or select agents
Nothing to report

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 – No change

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

What other organizations were involved as partners?
Nothing to report in this period

8. Special Reporting Requirements

Not applicable