AWARD NUMBER:	W81XWH-19-1-0346
TITLE:	"Making the CASE: Chemopreventive use of Aspirin for Ovarian Cancer- Integrating Epidemiological Data to Evaluate Population Subgroups and Tumor Expression"
PRINCIPAL INVESTIGATOR:	Dr. Britton Trabert
CONTRACTING ORGANIZATION:	The Geneva Foundation, Tacoma, WA
REPORT DATE:	October 2021
TYPE OF REPORT:	Annual Report
PREPARED FOR:	U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012
DISTRIBUTION STATEMENT:	Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2021	Annual Report	15Sep2020-14Sep2021

4. TITLE AND SUBTITLE

Making the CASE: Chemopreventive use of Aspirin for Ovarian Cancer- Integrating Epidemiological Data to Evaluate Population Subgroups and Tumor Expression

5a. CONTRACT NUMBER
W81XWH-19-1-0346

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Britton Trabert

britton.trabert@nih.gov
britton.trabert@hci.utah.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Geneva Foundation
917 Pacific Ave. # 600
Tacoma, WA 98402

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel 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

Objective: We hypothesize that the use of updated exposure information in cohort analyses will clarify and refine the ovarian cancer risk reduction associated with aspirin, that there are subgroups of women who will derive the most benefit from daily aspirin use with respect to ovarian cancer chemoprevention, and that aspirin will preferentially reduce risk for ovarian cancers dependent on the tumor immune microenvironment.

Impact: The proposed research directly addresses the OCRP vision – to eliminate ovarian cancer, by addressing critical questions related to the prevention of ovarian cancer. This research also addresses OCRP research objectives related to cancer etiology, primary prevention, and understanding the mechanism(s) by which aspirin can prevent ovarian cancer. By leveraging and expanding upon the OC3 infrastructure through collection of updated exposure information and tumor tissue this well-powered investigation of aspirin use with ovarian cancer risk will address key questions needed to develop recommendations for aspirin-based chemoprevention. The identification of women who will derive the most benefit from aspirin for ovarian cancer chemoprevention will guide future clinical trials in high-risk populations. Further, our examination of potential biologic mechanisms using tumor tissue expression of COX-1/2 and immune/inflammation markers will help strengthen the causal link between daily aspirin use and ovarian cancer development and inform potential co-testing of immune-modulators and daily aspirin use to improve cancer prognosis and/or progression-free survival. Since aspirin generally has few side effects, the potential for public health impact is substantial, particularly if risk reductions are identified among women at moderate to high risk of ovarian cancer. Ultimately, this innovative application combines epidemiologic and tumor tissue data to improve both the mechanistic understanding of ovarian carcinogenesis and the ability to make recommendations regarding the prevention of this fatal disease that will benefit all women, including military Service members, their families, and other military beneficiaries.

15. SUBJECT TERMS
Oncology, Pharmacology, Women's Health, Cancer, Ovarian Cancer, Prevention, Force Health Protection

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
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

1. INTRODUCTION:

The research conducted as part of this project aims to address the unresolved questions related to the potential chemoprevention of ovarian cancer associated with frequent aspirin use and provide mechanistic insight by collecting updated analgesic exposure information in cohort studies to refine risk assessment and clarify associations, combining cohort and case-control study data to evaluate the ability of aspirin to reduce ovarian cancer risk among high-risk subgroups of women, and to create/evaluate tumor tissue microarrays (TMAs) from cohort studies to explore possible mechanisms by which aspirin may reduce ovarian cancer risk.

2. KEYWORDS:

KEYWORDS: Ovarian cancer, chemoprevention, aspirin, mechanism, epidemiology, etiology, tumor tissue

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aims: The aims of the study are to:
1) Evaluate the relationship of daily aspirin use over the life course, including updated information on dose, duration, and frequency post-baseline, and ovarian cancer risk using data from 12 studies in the Ovarian Cancer Cohort Consortium (OC3);
2) Identify subgroups of women who could most benefit from aspirin chemoprevention in a well-powered study using harmonized case-control data from Ovarian Cancer Association Consortium (OCAC) and cohort data from OC3;
3) Explore mechanisms by which aspirin reduces ovarian cancer risk by utilizing ovarian tumor tissue from seven OC3 cohorts.

Major Task 1: Update data use/data transfer agreements to include aims and sign Material Transfer Agreements for transfer of biologic specimens for assays.
Major Task 2: Submission of institutions IRB approval and related materials for DOD's HRPO approval.
Major Task 3: Collect and harmonize questionnaire data from non-baseline time points across 12 cohorts, conduct analyses of analgesic use with ovarian cancer risk using updated follow-up data.
Major Task 4: Obtain OCAC dataset and conduct study-specific analyses. Conduct meta-analyses to evaluate aspirin-ovarian cancer associations by risk factors.
Major Task 5: Collect Tissue microarray (TMA) slides and create TMAs for studies with tumor tissue only. Complete molecular analysis of TMAs.
Major Task 6: Integrate TMA expression data with OC3 dataset. Analyze tumor expression data to evaluate heterogeneity in aspirin association by tumor markers.

What was accomplished under these goals?

For this reporting period we completed major tasks 1, 2, and 4 and made substantial progress toward completed major tasks 3 and 5. Progress on major task 6 will begin in the next annual reporting period as planned. All stated goals for this annual reporting period were met.

Accomplishments:

For Major Task 1, Dr. Trabert completed and received IRB approval from her new institution (University of Utah) for the project. Dr. Tworoger and colleagues at Moffitt Cancer Center with the assistance of Dr. Trabert updated all necessary information at the Moffitt Cancer Center related to Dr. Trabert's change in institution.

For Major Task 2, Dr Trabert submitted a revised DOD HRPO submission with the updated IRB approval from the University of Utah and the updated SOW that added University of Utah as an additional research site. The HRPO concurrence correspondence (E01116.1b - HRPO Concurrence Memorandum (IRB Study Number 00043994, Proposal Number OC180339, Award Number W81XWH-19-1-0346).) was received on July 16, 2021.

For Major task 4, (NCI, Drs. Trabert and Hurwitz) completed the manuscript evaluating frequent aspirin use and ovarian cancer risk by strata of defined effect modifiers in both OCAC and OC3.

Subtask 1 involved requesting the OCAC data and Dr. Trabert did that directly through the OCAC data coordinating center at Duke University. Dr. Trabert and Hurwitz then conducted study-specific unconditional logistic regression with adjustment for harmonized confounders to estimate odds ratios and 95% confidence intervals of the frequent aspirin use exposure with ovarian cancer risk by strata of ovarian cancer risk factors (i.e., age, endometriosis, family history of breast and/or ovarian cancer, body mass index, oral contraceptive use, parity, and tubal ligation status. Dr. Trabert accessed the baseline OC3 data and completed analyses using study-specific cox proportional hazards models with adjustment for harmonized confounders to estimate hazards ratios and 95% confidence intervals of frequent aspirin use exposure and ovarian risk by strata of ovarian cancer risk factors. Individual effect estimates and standard errors for the aspirin-ovarian cancer association by strata of risk factor and study were then transferred to Stata and meta-analyses were conducted by study design and overall. We tested for heterogeneity in the findings across the strata of risk factors overall and by study design. To complete the analyses by histologic subtype we first estimated associations in individual studies, however, given the rarity of many of the ovarian cancer subtypes, we conducted analyses by study design (case-control and cohort) and pooled the study design specific estimates using meta-analysis. We reviewed results from these analyses 3 times on the OC3 bi-weekly programming conference call to solicit feedback and input on the methods and results

and received input from all co-authors/collaborating studies and incorporated that feedback into the manuscript.

The resulting manuscript was then circulated to Drs. Penny Webb and Shelley Tworoger for completed technical and scientific review and then circulated to all OCAC and OC3 study authors. Once the manuscript revision process was completed, the manuscript was submitted through clearance at the National Cancer Institute and through cohort specific clearance processes (Nurses' Health Study). The manuscript is currently under review at a high impact journal (IF >40) and we are awaiting the status of that review. This is a major accomplishment as we have completed Aim 2 of the proposes study. The submitted manuscript is attached to this report. We included 9 cohort studies from the Ovarian Cancer Cohort Consortium (OC3) and 8 case-control studies from the Ovarian Cancer Association Consortium (OCAC). We used Cox proportional hazards regression and logistic regression to assess associations between frequent aspirin use and ovarian cancer within each study. All models were adjusted for age, number of full-term births, duration of oral contraception use, duration of menopausal hormone therapy use, and body mass index (BMI). Study-specific effect estimates were then pooled using random effects meta-analysis. We conducted analyses for all women and within subgroups defined by history of endometriosis, obese BMI (>30 kg/m^2), family history of breast/ovarian cancer, parity, oral contraceptive use, and tubal ligation. Heterogeneity across study design and subgroup was assessed using Cochran's Q test. Overall, frequent aspirin use was associated with a 13% reduction in ovarian cancer risk (RR: 0.87, 95% CI: 0.80-0.94), with no significant heterogeneity by study design (p=0.48). Though no association was observed among women with endometriosis (RR: 1.15, 95% CI: 0.80-1.65), consistent risk reductions were observed across all other subgroups defined by ovarian cancer risk factors (RRs ranging from 0.79 to 0.93, all p-heterogeneity >0.05). Among women with two or more ovarian cancer risk factors, frequent aspirin use was associated with a 19% reduced risk of ovarian cancer (95% CI: 0.73-0.90). Importantly, this study is the largest to-date on aspirin use and ovarian cancer and provides evidence that frequent aspirin use reduces ovarian cancer risk, including risk of high-grade serous cancers, regardless of the presence of other ovarian cancer risk factors. Risk reductions were also retained among women with multiple ovarian cancer risk factors, providing proof-of-principle that chemoprevention programs for frequent aspirin use could target higher-risk subgroups.

Progress towards completing Major tasks 3 and 5:

The progress we have made towards completing Major Task 3 is as follows: Dr. Trabert created a data request form with data abstracting instructions for cohorts and data requests were formally submitted to the cohorts in Jan 2020 and at the time of this progress report (Oct 2021), data had been received from 12 cohorts of the 12 cohorts requested. As part of subtask 2, we are continuing to use the data capture and processing architecture as provided in the Quad chart (upper right-hand corner) to harmonize the data and have solicited support from the Biostatistics Core at Moffitt Cancer Center to develop a macro to generate the harmonized variables necessary and format the data for the planned pooled logistic regression analyses to update the follow-up data at 2-year time windows. We continue to have biweekly conference calls with a group of ~12 individuals to review data and discuss progress and troubleshoot any issues we are having. Most recently this has included presentations from biostatistical experts to answer questions related to the pooled logistic regression data structure and analysis. The

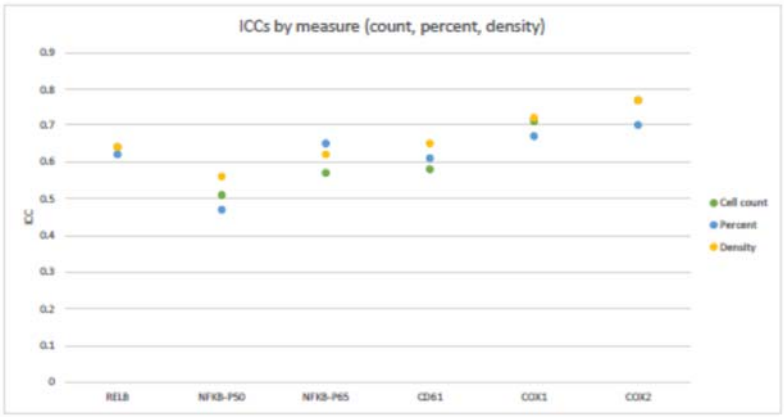
data harmonization process so far is running smoothly, and we have harmonized approximately 40 variables for 9 of the 12 submitted cohorts and are working towards completing the final harmonization for the analysis. Even with delays related to the COVID19 pandemic and work from home requirements we met our goal of receiving all data for aim 1 by the end of the 3rd quarter of CY21. We anticipate that harmonization will be completed by the end of the calendar year. Our analysis will be delayed slightly as we anticipate starting to conduct analyses in the first quarter of CY22. With the resulting manuscript being written and submitted before our next progress report.

The progress we have made towards completing major task 5 is as follows: We are in the process of finalizing the remaining cases that can be included in the TMA that will be established from the University of Iowa (Dr. Charles Lynch, IWHS samples). Creation of the TMA is ongoing at Moffitt (site 2). The University of Iowa identified almost ~300 ovarian cancer tumor blocks from within the Iowa Women's Health Study that they can send to Moffitt's Tissue Core to create the TMA for Aim 3. We are in the process of working with the new investigators from the Breakthrough Generations Study to get re-approval to include their existing TMAs in the project as planned. We will then initiate a material transfer agreement between their institution and Moffitt Cancer Center and finalize shipping details for the TMA sections. We are also working on the appropriate approvals with PLCO to obtain their TMA sections. In the interim we made progress on subtask 3 by evaluating preliminary results from the molecular analysis of quality control TMAs made of NHS study samples and finalized the molecular markers that will be evaluated on each panel. The panel that will include COX and NFkB markers has passed quality control testing and we are in the process of finalizing and testing the markers on the immune panel and the panel measuring tumor associated macrophages. We are slightly delayed in starting to run the molecular assays by the 4th quarter of CY21, but we anticipate that the first panel of samples on the in-house NHS/NHSII and IWHS TMAs will be run before the end of CY21, with the remaining assays being run in the first half of CY22.
Major task 5, subtask 3 assay QC: Below are the results of the preliminary analysis of the inflammatory marker panel: In summary, the ICCs for the markers on the panel were all above acceptable (0.47 and greater, mean 0.62).

Preliminary data, Distribution of immune cells (percent positive) in Tester TMA (204 cores), Inflammation-related tumor marker panel.

Cell type	Location	% >0	% >3%	% >5%	P0	P20	P40	P50	P60	P80	P100	Mean	ICC	ICC CI
RelB	Overall	98.0	76.5	70.1	0.0	2.3	10.0	17.7	28.2	52.3	99.7	27.4	0.62	(0.50, 0.73)
NFKB-p50	Overall	99.5	97.1	97.1	0.0	36.5	59.6	68.7	77.6	87.5	98.9	62.9	0.47	(0.33, 0.62)
NFKB-p65	Overall	97.6	95.1	94.6	0.0	42.7	67.9	75.9	81.8	92.6	99.7	66.4	0.65	(0.53, 0.75)
CD61	Overall	99.0	68.1	53.4	0.0	1.8	3.9	5.8	9.1	19.2	80.8	12.7	0.61	(0.49, 0.72)
COX1	Overall	93.6	56.4	43.0	0.0	0.3	2.5	4.4	9.4	33.9	90.4	16.5	0.67	(0.56, 0.76)
COX2	Overall	90.7	57.8	49.5	0.0	0.2	2.6	4.9	11.1	41.5	97.9	19.2	0.70	(0.60, 0.79)

Cell marker	Measure	ICC
RELB	Cell count	0.64
NFKB-P50	Cell count	0.51
NFKB-P65	Cell count	0.57
CD61	Cell count	0.58
COX1	Cell count	0.71
COX2	Cell count	0.77
RELB	Percent	0.62
NFKB-P50	Percent	0.47
NFKB-P65	Percent	0.65
CD61	Percent	0.61
COX1	Percent	0.67
COX2	Percent	0.7
RELB	Density	0.64
NFKB-P50	Density	0.56
NFKB-P65	Density	0.62
CD61	Density	0.65
COX1	Density	0.72
COX2	Density	0.77



What opportunities for training and professional development did the project provide?
How were the results disseminated to communities of interest?

Professional development opportunities have been provided to trainees at both Moffitt Cancer Center and NCI. Dr. Hurwitz worked one-on-one with her mentor Dr. Trabert to complete the analysis and manuscript for Aim 2 and submitted an abstract for that project to an internal award. Dr. Hurwitz reported the results of Aim 2 analyses to the Cancer Prevention Fellowship Program as part of her Fellows Research Meeting in February 2021, her talk was titled: "Aspirin for ovarian cancer chemoprevention: Building the epidemiologic evidence". She has also had the opportunity to lead data analysis calls and participate in dissemination of study results to collaborators and study PIs. She has also participated in media training at the NCI in anticipation of receiving media inquiries related to the Aim 2 paper that she and Dr. Trabert will address.

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 and objectives?

Over the next reporting period we will complete Aim 1 (Major Task 3) and submit the resulting manuscript to a journal for publication. We also anticipate that we will complete the molecular assays for Aim 3 (Major Tasks 5 and 6) during the next reporting period. We anticipate that the data analysis and manuscript preparation for Major Task 6 will extend beyond the end of the next reporting period.

4. IMPACT:

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

Nothing to report

What was the impact on other disciplines?

Nothing to report

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:

Changes in approach and reasons for change

In the previous report we reported the change in Major task 5, instead of utilizing the NCI laboratory for creation and quantification of the TMA we have decided to go with our back up laboratory (Moffitt Cancer Center). The NCI laboratory is in underwent a major geographic relocation (entire lab moved facilities March 2020-September 2020) and as such they would not have been able to make progress on our project in a timely fashion. Rather than suffer major delays and down time, we utilized our contingency laboratory and are making progress on major task 5 as a result of this change. Due to the COVID19 pandemic, the NCI laboratory is still not fully operation and would have not been able to take on our project, we are fortunate that we had Moffitt Cancer Center as a contingency as they have been back in person since summer 2020 and have made substantial progress towards completing Major task 5.

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

We do not anticipate any problems or delays in our plans going forward. 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:

Nothing to report. At all institutions (NCI, Moffitt, and Univ. of Utah) our work is considered not human subjects research since it involves de-identified data and previously collected tumor specimens. There is no anticipated change to this status through the duration of the project.

Significant changes in use or care of human subjects:

Not applicable, Nothing to report.

Significant changes in use or care of vertebrate animals:

Not applicable, Nothing to report.

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

Not applicable, Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications:

Nothing to report

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

Nothing to report

Other publications, conference papers, and presentations:

Dr. Lauren Hurwitz presented results at the Cancer Prevention Fellowship

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:

- **follow-up data collection for all 12 prospective cohorts was completed during this CY.**

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name:	Britton Trabert
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-1539-6090
Nearest person month worked:	3.6 calendar months
Contribution to Project:	Project management
Funding Support:	Changed from NCI intramural research program to University of Utah, Huntsman Cancer Institute

Name:	Shelley Tworoger
Project Role:	Site PI/Co PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-6986-7046
Nearest person month worked:	NO CHANGE
Contribution to Project:	

Funding Support:	

Name:	Mary Townsend
Project Role:	Applied Research Scientist
Researcher Identifier (e.g. ORCID ID):	0000-0003-2452-4477
Nearest person month worked:	NO CHANGE
Contribution to Project: Contribution to Project:	
Funding Support:	

Name:	Lauren Hurwitz
Project Role:	Cancer Prevention Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	0000-0001-8932-5028
Nearest person month worked:	2.4 Calendar Months
Contribution to Project:	Data analysis, manuscript writing
Funding Support:	Division of Cancer Prevention, Fellowship, Intramural Research Program of the National Cancer Institute

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

Active support for PI Dr. Britton Trabert changed in April 2021 from the Intramural Research Program at the National Cancer Institute to start-up funds from the University of Utah and Hunstman Cancer Institute. These start-up funds cover her salary for 3 years starting in April 2021, and will extend beyond completion of this project.

Active support for co-PI Dr. Shelley Tworoger totals 7.59 calendar months and has changed as follows:

Remained active support (no change to effort): W81XWH1910346 (Trabert, 0.72 calendar), OC190330 (Tworoger, 0.6 calendar)

Additions to active support: NIH/NCI - 5U01CA164973-09 (Administrative Supplement: LeMarchand, 0.24 calendar), NCI 1R01CA258679-01 (Terry, 0.84 calendar), W81XWH-21-1-0914 (Merritt, 0.6 calendar), W81XWH2110326 (Kubzansky, 0.39 calendar)**,** NIH/NHLBI K01HL143034 (Huang, 0 calendar – in kind).

Changes to active support level of effort: 9JK02 (Tworoger, decreased from 2.4 calendar to 1.8); P30 CA076292 (Cleveland, increased from 0.6 to 2.4 calendar).

No longer active: P01 CA087969 (Eliassen), Q81XWH1910307 (Kaaks), U01CA200464 (Heine, Gillies, Schabath), OT123-407 (BMS), W81XWH-17-1-0153 (Kubzansky).

What other organizations were involved as partners?

Organization Name: University of Iowa
Location of Organization: (if foreign location list country) Iowa City, Iowa
Partner's contribution to the project (identify one or more) Other. Contribution of tumor tissue from Iowa Women's Health Study to create Tumor Tissue Microarray

8. SPECIAL REPORTING REQUIREMENTS:

QUAD CHARTS:

9. APPENDICES: