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TITLE: Targeting the Mevalonate Pathway to Reduce Mortality from Ovarian Cancer

PRINCIPAL INVESTIGATOR: Kala Visvanathan, MD MHS

CONTRACTING ORGANIZATION: Johns Hopkins University
Baltimore, MD 21205

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TARGETING THE MEVALONATE PATHWAY TO REDUCE MORTALITY FROM OVARIAN CANCER

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14. ABSTRACT
The primary purpose is to evaluate whether statins, a well-known cholesterol-lowering agent, will improve survival in women with epithelial ovarian cancer. Aim 1: a) prospectively examine whether statin use reduces both cancer specific and overall mortality among approximately 7886 women with epithelial ovarian cancer after adjustment for stage, grade, treatment, histologic subtype, co-medication use, type of surgery and type of hospital. Statin use will be compared to non-users as well as users of other lipid lowering agents; and b) test whether the association is modified by: i) dose and duration, ii) timing of the intervention (pre-diagnosis versus post diagnosis use), iii) histologic subtype and iv) degree of adherence. Aim 2:a) assess the anti-tumor effect of lovastatin, (a commonly prescribed statin) alone or in combination with carboplatin/paclitaxel in a mouse orthotopic tumor xenograft model bearing luciferase-expressing OVCAR3, SKOV3, and A2780 cells and; b) determine the molecular mechanism by which lovastatin inhibits tumor growth. The clinical and translational impact of this project are substantial because unlike other potential new drug treatments, statins are already in wide use, have been shown to have very low toxicity and could therefore be put quickly into practice (if clinical trials confirm their efficacy) and at low cost.

15. SUBJECT TERMS: cancer mortality, cholesterol-lowering drugs, disease progression, epithelial ovarian cancer, lovastatin, Mevalonate Pathway, prescription drugs, statins, survival, transgenic mouse tumor model
Section I – INTRODUCTION:

This project is evaluating whether the addition of statins, a well-known cholesterol-lowering agent, will improve survival in women with epithelial ovarian cancer. We hypothesize that both pre-diagnostic and post diagnostic statin use will improve survival among women with epithelial ovarian cancer and that a higher dose or longer duration of use will be associated with a greater reduction in overall mortality. We also hypothesize that the anti-tumor effects of statin is mediated by a pathway that included enzymes that are involved in farnesylation. To test our hypothesis, we proposed a multidisciplinary approach that includes conducting both a large epidemiological study and a number of preclinical studies. In Aim 2 we demonstrated that lovastatin significantly reduced the development of STICs in mogp-TAg mice and inhibited ovarian tumor growth in the mouse xenograft model. Knockdown of prenylation enzymes in the mevalonate pathway recapitulated the lovastatin-induced antiproliferative phenotype. We are now evaluating a similar hypothesis in a national dataset from Finland of over 12000 ovarian cancer cases. Despite some unavoidable delays we are confident that we will be able to complete Aim 1 during the one year NCE that was recently approved.

Section II – KEYWORDS:

- Cancer mortality
- Carboplatin/paclitaxel
- Cholesterol-lowering drugs
- Disease progression
- Drug exposure
- Epithelial Ovarian Cancer
- Finnish Cancer Registry
- Lovastatin
- Mevalonate pathway
- Prescription drugs
- Social Insurance Institute of Finland
- Statins
- Survival
- Trangenic mouse tumor model
- Bisphosphonates
Section III – ACCOMPLISHMENTS:

A. What were the major goals of the project?

**SPECIFIC AIM 1: 1a)** examine whether statin use, reduces both cancer specific and overall mortality among approximately 7886 women with epithelial ovarian cancer; **1b)** test whether the association is modified by dose and duration, timing of the intervention (pre vs. post diagnosis use), histologic subtype, and patterns of adherence after adjusting for prespecified covariates.

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<td>Subtask 3: Develop MTA- Johns Hopkins &amp;Tampere University, Finland.</td>
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<th>Major Task 2: Request for data variables to be studied.</th>
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<th>Major Task 3: Clean data, generate study specific variables and analyze dataset on statin use and mortality among OV CA cases as well as associations/ modifications factors such as dose, duration, pre/post-diagnosis use, histologic subtype and patterns of adherence.</th>
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<td>Subtask 1: Data preparation which includes cleaning, extracting medication use from prescription records, recoding data, generating new study specific variables.</td>
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<td>Subtask 2: Conduct primary data analysis regarding statins and mortality among OV CA cases &amp; interpret results (aim 1a).</td>
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<td>Subtask 3: Conduct secondary data analysis regarding associations or modification of statin use &amp; adherence and a number of sensitivity analyses (aim 1b).</td>
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<td>Subtask 4: Prepare and submit an abstract to a national professional meeting (either AACR or ASCO).</td>
<td>14-20</td>
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<td>Subtask 4: Prepare and submit manuscript for journal submission and begin developing subsequent study based on results.</td>
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<td>2. Secondary analysis completed.</td>
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<td>3. Presentation of data at national professional meeting (either AACR or ASCO).</td>
<td>14-20</td>
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<td>4.</td>
<td>Journal manuscript submitted (i.e. Cancer Research or JCO).</td>
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<tr>
<td>5.</td>
<td>Data used in support of applying for further funding to study new agents in the mevalonate pathway.</td>
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**SPECIFIC AIM 2: 2a)** access anti-tumor effects of lovastatin alone or in combination with carboplatin/paclitaxel in a mouse orthotopic tumor xenograft model bearing luciferase-expressing OVCAR3, SKOV3, OVCAR5 cells; **2b)** determine molecular mechanism by which lovastatin inhibits tumor growth.

**Start date 9/30/14**

**Major Task 1:** Obtain approvals from Johns Hopkins Animal Care and Use Committee (IACUC) and the DoD Animal Care and Use Review Office (ACURO).

| Subtask 1: Develop and submit proposal to JH IACUC for review. | 0-4 | Completed Year 1 |
| Subtask 2: Submit documents for DoD ACURO for review. | 0-4 | Completed Year 1 |
| 1. Local JH IACUC Approval & DOD ACURO Approval. | 2-4 | Completed Year 1 |

**Major Task 2:** In vivo anti-tumor study.

| Subtask 1: Establish ovarian cancer cell lines with luciferase-expressing constructs (OVCAR3, SKOV3, & OVCAR5 cells.) Cell line source: ATCC | 0-4 | Completed Year 1 |
| Subtask 2: Purchase athymic nu/nu nude mice (n=120) and set ready for mouse tumor model. Mouse: purchased from Harlan Laboratories | 5,13 | Completed Year 2 |
| Subtask 3: Perform viable surgery by orthotopic injection ovarian cancer cells into mouse ovarian bursa. | 5,13 | N/A |
| Subtask 4: Treat the mice with lovastatin or vehicle control and monitor tumor load using live imaging system. | 6-8, 14-16 | Completed Year 2 |
| Subtask 5: Perform immunohistochemistry to study the expression of proliferation, autophagy, apoptosis markers, as well as other markers identified in our pilot study. | 9-12, 17-20 | Completed Year 2 |
| 1. Determine the anti-tumor effect of lovastatin alone or combination with conventional chemotherapeutic agents. | 5-20 | Completed Year 2 |
| 2. Identify biomarkers associated with statin treatment in cancer cells. | 5-20 | Completed Year 2 |

**Major Task 3:** Conduct gene knockdown and enzyme inhibitor study to determine which sub pathway(s) is mainly responsible for the anti-tumor effects of lovastatin.

| Subtask 1: Purchased siRNAs targeting the enzymes belonging to the three subpathways in the mevalonate metabolism. | 3-4 | Completed Year 1 |
| Subtask 2: Purchase small molecule enzyme inhibitors of the mevalonate subpathways. | 3-4 | Completed Year 1 |
| Subtask 3: Perform gene knockdown using siRNA in ovarian cancer cell lines (same as described in Task 2). Determine the effects of lovastatin on cellular proliferation, autophagy, and apoptosis as well as the expression of new markers identified in our pilot study. | 4-16 | Completed Year 1 |
| Subtask 4: Determine the effects of inhibitors on the phenotypes described in Subtask 3 in the same panel of | 4-16 | Completed Year 1 |
ovarian cancer cell lines. Compare the alterations of phenotypes between 3 and subtask 4.

1. Demonstrate the effects of knockdown in those genes regulating mevalonate pathway.
2. Demonstrate the effects of enzyme inhibitors for those proteins regulating the mevalonate pathway.
3. Demonstrate that mevalonate pathway is essential for cellular survival and growth in ovarian cancer cells.

Subtask 1: Analyze and summarize data to support or refute our hypothesis.
Subtask 2: Prepare manuscript(s) for journal submission.
1. Presentation of data in meetings or conferences such as AACR annual meeting and others.
2. Publication of data.
3. Generating research findings that can be used in support of applying for further funding opportunity.

Major Task 4: Final phase: data analysis, summary, and publication.

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<tr>
<td>3-16</td>
<td></td>
<td>Completed Year 2</td>
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B. What was accomplished under these goals?

**Aim 1:**

Aim 1a) examine whether statin use, reduces both cancer specific and overall mortality among approximately 7886 women with epithelial ovarian cancer; 1b) test whether the association is modified by dose and duration, timing of the intervention (pre vs. post diagnosis use), histologic subtype, and patterns of adherence after adjusting for prespecified covariates.

**Major Task 1:** Obtain approvals from Johns Hopkins SPH IRB, DoD HRPO, MTA and USAMRAA.

Task 1-1: Protocol development and submission to Johns Hopkins SPH IRB for review and approval.

**Progress:** Completed Year 1 A protocol was submitted and reviewed by the Johns Hopkins SPH IRB and determined that the proposed activity described in the protocol involved secondary data analysis of existing de-identified/de-linked, not publicly available datasets, and that we were not involved in the original data collection. As such, the proposed activity did not qualify as human subjects research as defined by DHHS regulations 45 CFR 46.102, and does not require IRB oversight.

Task 1-2: Submission of Johns Hopkins IRB approval and related material for DoD’s HRPO review and approval.

**Progress:** Completed Year 1 HRPO reviewed the Johns Hopkins SPH IRB Determination Letter and concurred and sent a “Research Not Involving Human Subjects Determination Memorandum” on 5/28/14 stating the project may proceed with no further requirement for review by the HRPO.

Task 1-3: Develop MTA- Johns Hopkins & Tampere University, Finland
**Progress: Completed Year 1** A fully executed MTA/Data Use Agreement was put in place 1/5/15. Additionally we have a signed Commission Decision C (2010) 593 Standard Contractual Clause (processors).

**Major Task 2:** Request for data variables to be studied.

Task 2-1: Submit proposal that includes a specific data request to Finnish Cancer Registry so they can identify variables.

**Progress: Completed Year 1** We submitted and obtained approval 12/23/14 from the Finland National Institute for Health & Welfare (THL) to obtain data for ovarian cancer cases registered in the Finnish Cancer Registry (FCR) during 1995-2013. Information on the cancer case’s children and 1st degree female relatives, in addition to information on emigrations and possible death was requested from the Finnish Population Register for linkage to FCR to obtain information on the 1st degree relatives’ breast and ovarian cancer diagnoses. This was to be merged with lifetime information on drug purchases and chronic diseases from the Social Insurance Institute (SII) of Finland. Additionally, information on co-morbidity from the hospital discharge (HILMO) will be linked to the data.

**Major Task 3:** Clean data, generate study specific variables and analyze dataset on statin use and mortality among OV CA cases as well as associations/ modifications factors such as dose, duration, pre/post-diagnosis use, histologic subtype and patterns of adherence.

Task 3-1: **Almost completed** Data preparation that includes cleaning, extracting medication use from prescription records, recoding data, and generating new study specific variables.

**Progress:** The data from various national organizations have been merged together. The data consists of materials combined from the following health care register sources: 1) the Finnish Cancer Registry (information on ovarian cases); 2) the Social Insurance Institution of Finland (information on drug purchases and special reimbursements); 3) Finnish Population Register Center (information on born children and 1st level female siblings, emigration and death); and 4) patient register, HILMO (information on co-morbidity).

We have approximately 3000 more ovarian cancer cases than we initially estimated which will enable us to conduct more robust sub-analyses. Our total number of cases is now 12,122 compared to 7886, which was originally projected. These women were diagnosed with ovarian cancer between 1/1/1995 and 12/31/2015.

We also have prescription data for those same years. In our cohort women appear to have used 8 different types of statins based on distinct ATC drug codes. The total number of prescriptions for statin drugs is 81,572. Breaking this down further 3389 cases (28%) has filled at least one prescription for statins. We estimated that the prevalence of statin use would be 15% and therefore we should have more than adequate power to complete these analyses.

Task 3-2: Conduct primary data analysis regarding stains and mortality among OV CA cases and interpret the results (aim 1a).

**Progress:** Pending

Task 3-3: Conduct secondary data analysis regarding associations or modification of statin use & adherence and a number of sensitivity analyses (aim 1b).
**Progress:** Pending

Task 3-4: Prepare and submit an abstract to a national professional meeting (either AACR or ASCO).

**Progress:** Pending

Task 3-5: Prepare and submit manuscript for journal submission and begin developing subsequent study based on results.

**Progress:** Pending

**Aim 2:**

Aim 2 a) To assess the anti-tumor effects of lovastatin alone or in combination with carboplatin/paclitaxel in a mouse orthotopic tumor xenograft model bearing luciferase-expressing OVCAR3, SKOV3 and OVCAR5 cells, b) To determine the molecular mechanism by which lovastatin inhibits tumor growth.

**Major Task 1:** Obtain approvals from Johns Hopkins Animal Care and Use Committee (IACUC) and the DoD Animal Care and Use Review Office (ACURO).

**Progress: Completed Year 1** The approvals to conduct the proposed animal studies have been obtained.

**Major Task 2:** In vivo anti-tumor study.

Task 2-1: Establish ovarian cancer cell lines with luciferase-expressing constructs (OVCAR3, SKOV3, & OVCAR5 cells).

**Progress: The proposed study was completed Year 2.** In Fig. 1A, we demonstrated the luciferase activity measured in cultures of ovarian cancer cell lines, OVCAR3-luc, SKOV3-luc, and OVCAR8-luc. All these three cell lines are tumorigenic in athymic nu/nu mice and their growth was monitored luciferase activity using a quantitative bioluminescence imaging system (IVIS Lumina imaging) (Fig. 1B).

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**Fig. 1. Establishment of ovarian cancer cells with stable luciferase expression.** (A) SKOV3, OVCAR3 and OVCAR8 cancer cell lines were infected with a lentiviral CMV-GFP-T2A-Luciferase vector. Clones with stable expression of luciferase were selected by serial dilution method. Representative images of ovarian cancer cells expressing luciferase or parental control cells. (B) Athymic nu/nu mice were injected with luciferase-expressing ovarian cancer cells into the right leg or neck. Bioluminescent luciferase activity (RLU) was measured using IVIS imaging system 3 weeks after injection.
To assess drug interactions between lovastatin and paclitaxel and between lovastatin and carboplatin, we measured combination index for both combinations in SKOV3 and OVCAR5 cell lines. Both treatment combinations had a synergistic effect, with CI < 1. Both cell lines were more sensitive to the lovastatin/carboplatin combination treatment (Fig. 2).

**Fig. 2.** Combination index plot of lovastatin and paclitaxel and lovastatin and carboplatin in SKOV3 (A) and OVCAR5 (B) cell lines. The Y-axis represents the percent of inhibition for lovastatin, and the X-axis represents the percent of inhibition for either paclitaxel or carboplatin. Combination index is less than 1 for all plots, indicating a synergistic relationship. CI is larger for lovastatin/carboplatin combination in both cell lines, indicating greater sensitivity.

**Major Task 3:** Conduct gene knockdown and enzyme inhibitor study to determine which subpathway(s) is mainly responsible for the anti-tumor effects of lovastatin.

**Progress: Completed Year 2** Tasks 3-1 to 3-4 have been accomplished and the results were published in *Clinical Cancer Research* (PMID: 26109099). See Year 1 Annual Report for a copy of the full manuscript.

**Summary Results:**

Two ovarian cancer mouse models were employed to determine the effect of statins on tumor growth in ovarian cancer. The first one was a genetically engineered model, mogp-TAg, in which the promoter of oviduct glycoprotein-1 was used to drive the expression of SV40 T-antigen in gynecologic tissues. These mice spontaneously develop serous tubal intraepithelial carcinomas (STICs), which are known as ovarian cancer precursor lesions. The second model was a xenograft tumor model in which human ovarian cancer cells were inoculated into immunocompromised mice. Mice in both models were treated with lovastatin, and effects on tumor growth were monitored. Lovastatin significantly reduced the development of STICs in mogp-TAg mice and inhibited ovarian tumor growth in the mouse xenograft model. Knockdown of prenylation enzymes in the mevalonate pathway (such as PGGT1B and RABGGTB) recapitulated the lovastatin-induced anti-proliferative phenotype, and addition of pathway metabolite geranylgeranyl pyrophosphate (GGPP) reverted the anti-proliferative effects of lovastatin. Transcriptome analysis indicated that lovastatin affected the expression of genes associated with DNA replication, Rho/PLC signaling, glycolysis, and cholesterol biosynthesis pathways, suggesting that statins have pleiotropic effects on tumor cells. Overall, the results suggest that repurposing statin drugs for ovarian cancer may provide a promising strategy to prevent and manage this devastating disease.

*Clin Cancer Res. 2015 Oct 15;21(20):4652-62*
Task 3-1: Purchased siRNAs targeting the enzymes belonging to the three sub pathways in the mevalonate metabolism.

Progress: Completed Year 1

Task 3-2: Purchase small molecule enzyme inhibitors of the mevalonate subpathways.

Progress: Completed Year 2

Task 3-3: Perform gene knockdown using siRNA in ovarian cancer cell lines (same as described in Task 2). Determine the effects of lovastatin on cellular proliferation, autophagy, and apoptosis as well as the expression of new markers identified in our pilot study.

Progress: Completed Year 2

Task 3-4: Determine the effects of inhibitors on the phenotypes described in Subtask 3 in the same panel of ovarian cancer cell lines. Compare the alterations of phenotypes between 3 and subtask 4.

Progress: Completed Year 1

Major Task 4: Final phase: data analysis, summary, and publication

Task 4-1: Analyze and summarize data to support or refute our hypothesis. Completed
Task 4-2: Prepare manuscript(s) for journal submission. Completed

Progress: Completed Year 2 As described above, we have recently completed Task 3 and the results were published.

C. What opportunities for training and professional development have the project provided?

Aim 1 has provided the opportunity for staff and faculty to gain experience in the selection, merging and data management of a large international dataset that included extensive prescription data and inpatient data.

Aim 2 of the study provided an opportunity for a graduate student, JC Kuan, to obtain knowledge and skills in animal models of ovarian cancer and molecular and biochemical techniques for cancer biology studies. JC is a student in the epidemiology doctoral program, and this project provides him with cross-disciplinary training.

D. How were the results disseminated to the communities of interest?

At the completion of Aim 1 Dr. Visvanathan plans to present our results at either the ASCO or the AACR Annual meeting. In addition she expects to publish the results in a high impact journal and disseminate the data through academic talks.

To date there has been one publication from Aim 2 and Dr. Wang presented the results of this research to the Division of Cancer Prevention at the National Cancer Institute (NCI) on 11/17/14. The lecture was entitled “Development of new strategies for ovarian cancer prevention and treatment.” Fellow cancer researchers were thereby informed of the potential new treatment and preventative modalities in patients with ovarian cancer.

E. What do you plan to do during the next report period to accomplish the goals?

Aim1: We are currently completing our data management that includes both data cleaning and formulating appropriate variables for our specific analysis from this large data set. We plan to start on the analysis very soon and expect to have a manuscript submitted by the end of this no cost extension year.

This data will provide the foundation for future funding opportunities to apply to study new agents in the mevalonate pathway.

Section IV – IMPACT:

A. What was the impact on development of the principal disciplines(s) of the project?

Our first publication from Aim 2 provides proof-of-principal evidence for an overarching question: whether persistent intake of statins can protect from gynecologic malignancies, including ovarian cancer. Our work clearly demonstrated that statin intake led to a delay in tumor progression in both genetically engineered mouse and xenograft tumor models of ovarian cancer. The result provides a biological basis for explaining the cancer protection effects of statin intake observed in epidemiology studies. We are now looking at examining lovastatin with some cox based anti-inflammatory therapies.

We expect the future publication from Aim 1 to also have impact on the field.
B. What was the impact on other disciplines?

The publication from Aim 2 provides a biological basis for explaining the cancer protection effects of statin intake which we will examine further now in Aim 1, our epidemiological cohort.

We are hopeful that the data from Aim 1 which will evaluate the relationship between statin intake and ovarian cancer by subtype and by type of statin use will lead to more directed experimental studies.

C. What was the impact on technology transfer?

Nothing to Report at this time.

D. What was the impact on society beyond science and technology?

Nothing to Report at this time.

Section V – CHANGES/PROBLEMS:

A. Changes in approach and reasons for change

Nothing to Report at this time.

B. Actual or anticipated problems or delays and actions or plans to resolve

Nothing to Report at this time.

C. Changes that had a significant impact on expenditures

Nothing to Report at this time.

D. Significant changes in use of care of human subjects

Nothing to Report at this time.

E. Significant changes in use of care of vertebrate animals

Nothing to Report at this time.

F. Significant changes in use of biohazards and / or select agents

Nothing to Report at this time.

Section VI – PRODUCTS:

A. Publications, conferences, and presentations

1. Journal publications

   Nothing to Report at this time.

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

   Nothing to Report at this time.
3. Other publications, conference papers, and presentations

Nothing to Report at this time.

B. Website(s) or other internet site(s)

Nothing to Report at this time.

C. Technologies or techniques

Nothing to Report at this time.

D. Inventions, patent applications, and/or license

Nothing to Report at this time.

E. Other Products

Nothing to Report at this time.

Section VII – PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

A. What individuals have worked on the project?

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<tr>
<th>Name</th>
<th>Kala Visvanathan, MD</th>
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<td>Principal Investigator</td>
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<td>Nearest person month worked:</td>
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<td>Contribution to Project:</td>
<td>Provided overall project oversight to ensure aims met according to the proposed timeline. Responsible for direct oversight conduct of Aim 1</td>
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<tr>
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<tr>
<td>Name:</td>
<td>Yusuke Kobayash, MD, PhD</td>
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<td>Oversee data acquisition, linkage of registry data and anonymization</td>
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<td>Research Identifier (e.g. ORCID ID):</td>
<td>NA</td>
</tr>
<tr>
<td>Nearest person month worked:</td>
<td>0.13</td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Support with IRB, MTA, Data Application, Research &amp; Confidentiality Agreement, and Invoicing</td>
</tr>
<tr>
<td>Funding Support:</td>
<td>Supported by other grants under Dr. Kala Visvanathan</td>
</tr>
<tr>
<td>(other than this award)</td>
<td></td>
</tr>
</tbody>
</table>

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

Other Support Updates: Kala Visvanathan

**ACTIVE (NEW)**

(Visvanathan)

**Title:** Evaluation of breast cancer susceptibility and prevention strategies in women affected and unaffected with the disease

**Effort:** 1.20 calendar months (10%)

**Supporting Agency:** Breast Cancer Research Foundation

**Performance Period:** 10/01/2016-09/30/2017

**Level of Funding:** $250,000

**Project Goals:** Improve risk stratification of women at risk for breast cancer or breast cancer progression and/or other health outcomes across the continuum in various populations and to identify preventive strategies to alter the natural history.

**Role:** PI
Title: Biomarkers and breast cancer risk prediction in younger women  
**Effort:** 0.12 calendar months (1%)  
**Supporting Agency:** New York University  
**Name of Procuring Contracting/Grants Officer:** N/A  
**Address of Funding Agency:** N/A  
**Performance Period:** 09/09/2013-08/31/2016  
**Level of Funding:** $26,985  
**Project Goal:** This study will provide robust estimates of the relationship between MIS and breast cancer risk and of the performance of the Gail model 2 with the addition of biomarkers.  
**Role:** co-Investigator

1R01CA190428-01A1 (Epplein)  
**Title:** Helicobacter pylori protein-specific antibodies and colorectal cancer risk  
**Effort:** 0.60 calendar months (5%)  
**Supporting Agency:** NIH/NCI (Subaward-VUMC)  
**Name and Address of the Funding Agency’s Procuring Contracting/Grants Officer:**  
**Performance Period:** 04/01/2015 – 03/31/2019  
**Level of Funding:** $94,607 (subcontract only)  
**Project Goal:** To evaluate the novel association between Helicobacter pylori protein-specific infection and colorectal cancer risk, building the groundwork for significantly strengthening colorectal cancer prevention and screening strategies with a new risk biomarker, as well as the possibility of identifying and exposure that is proven to be modifiable through the use of radiation therapy.  
**Specific Aims:**  
Aim 1: Definitively evaluate the association of *H. pylori* protein antibody levels in pre-diagnostic blood samples, utilizing novel *H. pylori* multiplex serology, with colorectal cancer risk in 10 prospective cohorts.  
Aim 2: To investigate whether the association of *H. pylori* subtype-specific association with risk of colorectal cancer is modified by regular aspirin use.  
**Role:** co-Investigator

1R21CA194194-01A1 (Pollack)  
**Title:** The Impact of Provider Social Networks on Breast Cancer Screening  
**Effort:** 0.30 calendar months (2.5%)  
**Supporting Agency:** NIH/NCI  
**Name and Address of the Funding Agency’s Procuring Contracting/Grants Officer:**  
**Performance Period:** 12/01/2015 – 11/30/2017  
**Level of Funding:** $133,092  
**Project Goal:** The Impact of Provider Social Networks on Breast Cancer Screening. Through a national survey of primary care providers and gynecologists, this grant seeks to better understand how physician social networks influence recommendations for breast cancer screening. Parameterized by the survey, we will construct an agent based model to examine ways to alter current patterns.  
**Role:** co-Investigator
**Title:** Circulating Vitamin D Levels and Risk of Breast and Colorectal Cancer: A Pooled Analysis  
**Effort:** 0.60 CM, 5%  
**Supporting Agency:** NIH/NCI  
**Performance Period:** 08/01/2010-07/31/2015  
**Level of Funding:** $442,061  
**Project Goal:** We propose to conduct a prospective, pooled analysis of the association between circulating 25-hydroxyvitamin D [25(OH) D] levels and risk of breast and colorectal cancer within a consortium of 15 cohort studies.  
**Role:** co-Investigator, Lead Breast Writing Team

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**Title:** Multicenter combined Genetic, Epigenetic and Expression Analysis of DCIS outcome predictors.  
**Effort:** 0.72 calendar months (6%)  
**Supporting Agency:** NIH  
**Name of Procuring Contracting/Grants Officer:** Connie Murphy, Grants Management Officer National Cancer Institute  
**Address of Funding Agency:** 6120 Executive Boulevard, EPS Room 243 Bethesda, MD 20892 Phone: (301) 496-8657, Fax: (301) 496-8601 ca114e@nih.gov  
**Performance Period:** 04/01/2011-03/30/2016  
**Level of Funding:** $583,283  
**Project Goals:** Identify genetic and epigenetic markers predictive of disease progression among women with DCIS.  
**Specific Aims:**  
**Aim 1:** Perform a comprehensive genome-wide analysis of somatic genotypic and phenotypic changes in a multicenter, case-control study of institutional cohorts of DCIS patients with either progression to invasive breast cancer, or over 10 years of disease free survival. The objective is to obtain a comprehensive catalog of somatic change in DCIS in Caucasian populations, covering copy-number variation, loss of heterozygosity, altered CpG methylation patterns, and differential gene expression using state-of-the-art array platforms.  
**Aim 2:** Perform an integrated bioinformatic analysis identifying signatures that are specific for DCIS with the potential to progress to invasive breast cancer. The objective is to select small sets of array feature that together discriminate classes, while avoiding over-fitting. Our comprehensive approach, looking at CpG methylation and copy number, supplemented with expression data on the same samples, was developed to maximize our chances of finding informative genomic events, but has the added benefit of offering cross platform validation. We will assemble small, non-overlapping models for validation in subsequent aims.  
**Aim 3:** Develop a panel of multiplex PCR assays that can be used in minimal routine clinical material to predict long-term outcome in DCIS, and optimize performance on in-house DCIS samples. Candidate marker sets will be characterized biochemically and marker-specific assays applicable to high throughput analysis of clinical samples developed. Markers that perform well will be combined into multiplex quantitative PCR and MSP assays that can be tested for optimal prognostic performance on in-house tissue samples.  
**Aim 4:** Validate the results in independent, population-based test cohorts of DCIS patients with progressive disease vs. DCIS patients without recurrent disease obtained from the Surveillance Epidemiology End Results (SEER) repositories of the National Cancer Institute, using the newly developed multiplex PCR assays. Our objective is to prospectively test our DCIS assay on an independent test set in order to obtain a realistic assessment of its potential positive and negative predictive power. While this validation will require remaining within the same ethnic group to avoid confounding the initial analysis, we will also test a non-Caucasian cohort to determine how much of tests performance may depend on the ethnic and racial background.
C. What other organizations were involved as partners?

<table>
<thead>
<tr>
<th>Organization Name</th>
<th>Location</th>
<th>Contribution to the Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Tampere</td>
<td>Finland</td>
<td>Collaboration: Dr. Teemu Murtola is Co-Investigator and responsible for oversight of the data acquisition, linkage of registry data and anonymization and assist Johns Hopkins with the analysis</td>
</tr>
<tr>
<td>Finnish Cancer Registry</td>
<td>Finland</td>
<td>Other: provide linkage to health information (ovarian cancer case data) for project</td>
</tr>
<tr>
<td>Social Insurance Institution of Finland</td>
<td>Finland</td>
<td>Other: provide approval for access and linkage of medical and prescriptions reimbursement data for project</td>
</tr>
</tbody>
</table>

Section VIII – SPECIAL REPORTING REQUIREMENTS:

A. Collaborative Awards: Not applicable

B. Quad Charts: Not applicable

Section IX – APPENDICES: None