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14. ABSTRACT
A serous carcinogenesis sequence has been described in the distal fallopian tube but a significant proportion of extra uterine high-grade serous carcinomas (HGSCs) are not associated with an "early" carcinoma in the fimbria. To explain this discrepancy, a hypothesis of "precursor escape" has been proposed, by which exfoliated cells from early serous precursors (ESPs)escape the tube and later undergo malignant transformation in the peritoneal cavity, leading to "primary peritoneal" serous carcinomas. The aims of this proposal were to identify mutations in exfoliated epithelial cells from peritoneal washings, further link HGSCs to ESPs by shared mutations and establish lineage between ESPs and HGSCs by whole exome sequencing. Flow cytometric enrichment of washings for epithelial cells generally yielded small numbers of cells but a TP53 mutation was identified by sequence analysis in one, supporting a model by which mutated epithelial cells escaped into the peritoneal cavity. Further sequence analysis of ESPs and paired HGSCs identified shared TP53 mutations, although there were technical limitations imposed by small samples. Additional cases material was analyzed following laser capture microdissection and the results are pending, as are whole exome sequencing studies.

15. SUBJECT TERMS
None listed.

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1. **Introduction**

The purpose of this research project was to further clarify the nature and pathway of early precursors development in the pathogenesis of extrauterine high grade serous carcinoma and address the hypothesis that "precursor escape" from the fallopian tube was a viable mechanism of tumor development. In this model, high-grade carcinomas would emerge from benign-appearing but genetically altered (p53 mutation) cells in the fallopian tube. The cells would escape the tube and later undergo malignant transformation in the peritoneal fluid.

2. Keywords:

High grade serous carcinoma, TP53, precancer, peritoneum, serous tubal intraepithelial carcinoma, serous tubal intraepithelial lesion, fallopian tube.

3. Accomplishments:

This summary is data-rich to simply underscore the effort made in addressing the AIMs. **The salient findings are highlighted in bold text.**

AIM 1.

1.1. *TP53* sequencing of peritoneal fluid samples, including unsorted ThinPrep Samples

Twelve ThinPrep samples from peritoneal washings were obtained (sample details below). DNA was extracted using the QIAamp DNA Mini Kit, and purified using the QIAQuick spin columns. The samples underwent deep, targeted *TP53* gene sequencing (CovarianceBio).

Case ID	Relevant Info	*TP53* gene sequencing results
02994	HGSC	Not informative
13190	RRSO, *BRCA2* germline mutation	Not informative
15733	RRSO, *BRCA1* germline mutation	Not informative
03605	HGSC, *BRCA2* germline mutation	Not informative
08832	Serous cystadenofibroma	Not informative
13128	RRSO, *BRCA1* germline mutation	Not informative
29050	RRSO with STIL, *BRCA2* germline mutation	Not informative
29363	RRSO with STIC, *BRCA2* germline mutation	Not informative
32348	Completion surgery for STIC	Not informative
32182	RRSO, *BRCA2* germline mutation	Not informative
28562	RRSO, *PALB2* germline mutation	Not informative
28563	RRSO, breast cancer history	Not informative

HGSC: high-grade serous carcinoma; STIL: serous tubal intraepithelial lesion; STIC: serous tubal intraepithelial carcinoma
RRSO: risk-reducing salpingo-oophorectomy

After manual review of the sequencing data, all samples were assessed to be non-informative; the different variants detected by the bioinformatics pipeline could not be reliably distinguished from noise. The obtained results also could not distinguish between *TP53* mutations in epithelial cells *vs.* other lineages, including leukocytes that may represent clonal hematopoiesis with *TP53* mutations. Decision was made to examine flow cytometry-sorted samples.

1.2. Flow cytometry-sorted fresh fluid samples.

Two unprocessed, fresh fluid samples were obtained from two patients undergoing RRSO. The two samples were used for a pilot of flow cytometry. A trial of flow cytometric sorting was performed using a panel of 3 antibodies – anti-EpCAM, anti-podoplanin, and anti-CD45--a sample of malignant peritoneal fluid (with HGSC) was washed in staining buffer, and stained for 1 hour at room temperature. The stained sample was sorted using the BD FACSAria II cell sorter. The obtained results were notable for high background, potentially attributable to unlysed, residual erythrocytes.

Two peritoneal wash samples were processed as above, with the addition of an erythrocyte lysis step (RBC lysis buffer, eBioScience/Thermo Fisher Scientific), and stained using the same antibody cocktail as above. CD45+, PDPN+, and even rare EpCAM+ events were detected by flow cytometry. One sample (MSB) was found to contain ~80 flow cytometric events (i.e. intact, viable cells) with the expected surface immunophenotype of epithelial cells. A small fraction was of the samples were dropped onto charged slides, fixed in ThinPrep, and stained using the Papanicolau protocol. Unfortunately, cytologic examination was unsuccessful, related to very small number of cells present. Remainder of the samples were used for whole genomic DNA extraction (QIAamp spin column), and all of the eluted volumes (~20 μL) were sent for deep *TP53* gene sequencing. Cell-free DNA was extracted using the Qiagen Circulating cell-free DNA extraction kit. ~50 mL of cell-free fluid was used.

From the manual review of the data, deep *TP53* sequencing showed c.295T>C in the epithelial fraction. Other fractions were non-informative, lacking a clear *TP53* variant. We considered this an important preliminary finding. It supports a more comprehensive study of peritoneal fluid samples from women undergoing risk reduction surgery with the goal of linking TP53 mutations to epithelial cell populations that would contain putative HGSC precursors.

1.3. ThinPrep flow cytometry

Aligning the timing of sample acquisition, availability of samples, and booking of the flow cytometric sorting machine posed a significant challenge in forging ahead with more fresh samples being analyzed by flow cytometry. To address these logistic hurdles, 9 ThinPrep samples were acquired.

While rare papers were identified in the literature, flow cytometry is not routinely employed for ThinPrep specimens. A round of pilot flow cytometry was tried using two samples (15692, 13978, which were negative and positive for malignant cells, respectively), using the same staining protocol as above, including the antibody dilutions (1:200 for anti-EpCAM, 1:100 for others). While significant noise was encountered, the staining protocol was deemed sufficient, and, in total, 9 samples were analyzed as below.

ThinPrep flow cytometry – events per population

Case	Relevant info/Diagnosis	EpCAM+ (events)	PDPN+	CD45+
1	RRSO, *BRCA1*	2	4	11
2	Seromucinous cystadenoma	11	42	102
3	Serous cyst-adenofibroma	9	12	4
4	RRSO, *BRCA2*	508	1,531	810
5	Endometrioma	56	264	354
6	RRSO, breast CA	21	52	65
7	RRSO, *BRCA1*	11	615	3,239
8	Endometrioma	2	2	1,741
9	HGSC*	212	103	2,019

*Fluid was positive for malignant cells

Background noise, likely reflecting auto-fluorescence was significant, and most flow cytometric events could not be identified as true signals. In particular, forward and side scatter (FSC, SSC, respectively) voltage settings were required to be lowered (*vs.* sorting of live cells). Nonetheless, small number of events were assessed to be

likely candidates for cellular events. In two samples with more events (i.e. 13978 & 3672), 25 µL (I.e. ½) of the sorted samples were spun onto slides using CytoSpin3 (800 RPM, 3 minutes) and stored in ThinPrep solution for future analysis by immunofluorescence.

In the remainder of the specimens, gDNA was extracted using the QIAamp spin column method (i.e. FFPE protocol, without the deparaffinization step), eluted in 20 µL of ATE. For 4 of the samples, unsorted pellets were available. DNAs extracted from the 4 unsorted samples were quantified using NanoDrop, yielding from 4-400 ng/ul depending on cellularity.

Analysis of the nine cases for evidence of p53+ early serous precursors in the tube yielded 6. The goal is to analyze each for evidence of TP53 mutations.

Case	Relevant info/Diagnosis	p53 IHC	Sections for normal
1	RRSO, *BRCA1*	Signature (B6)	A6
2	Seromucinous cystadenoma	STIL (A4)	B4
3	Serous cyst-adenofibroma	Signature (A16, C1)	C1 (dissected)
4	RRSO, *BRCA2*	Signature (A3)	B3
5	Endometrioma	Signature (C5)	B1
6	RRSO, breast CA	Signature (C4)	B4
7	RRSO, *BRCA1*		
8	Endometrioma		
9	HGSC*		

This AIM was partially met given the technical constraints imposed. We succeeded in 1) segregating epithelial cells from peritoneal fluid; 2) identifying a TP53 mutation in one sample; 3) identifying early serous precursors in corresponding fallopian tubes. However, we have not yet linked TP53 mutations in suspended epithelial cells with a corresponding mutation in the fallopian tubes. However, in the context of a model for precursor escape, separating epithelial from non-epithelial cells in the peritoneal fluid is critical prior to genomic analysis. Moreover, isolating useful information from fixed material (which is far more easily obtained) is important to this goal. Dr. Ju Yoon Yoon, who performed these analyses, is pursuing this goal in a separate project.

AIM 2.

***TP53* variant delineation between signatures and HGSC**

Case selection was performed based on PowerPath and EPIC chart review, identifying recent cases of HGSC patients who are treatment-naïve (no neoadjuvant chemotherapy). Six patients were identified. Serial sections of the tube were correlated with p53 immunohistochemistry to identified early serous lesions. Several of the patients had undergone somatic sequenced (OncoPanel at the BWH). The corresponding areas in unstained sections were macrodissected, and gDNA was extracted using the QiaAMP FFPE kit. NanoDrop quantification results are below.

Case ID	Early lesion	HGSC	Normal
ESP-A	23.6 ng/µL	15.8 ng/µL	7.7 ng/uL
ESP10	8.3 ng/µL	1,079.1 ng/µL	5.5 ng/µL
ESP14	4.7 ng/µL	NA	8.7 ng/µL
3394	20.6 ng/µL	NA	undetectable
3451	70.1 ng/µL	1,013.2 ng/µL	26.2 ng/µL

NA = not available (case previously sequenced)

Case ID	Early lesion	HGSC	Normal
ESP-A	D2 (STIL): c.659A>G; p.Y220C (2.2%)	c.659A>G; p.Y220C (3.1%)	none
ESP10	D3 (STIC): c.499C>T; p.Q167* (13.1%)	c.499C>T; p.Q167* (37.2%)	none
ESP14	B1 (STIL): c.517G>T; p.V173L (0.1%#) c.452C>A; p.P151H (2.3%)	c.517G>T; p.V173L (89%)	c.517G>T; p.V173L (0.8%#)
ESP18	B1 (STIL): c.833C>G; p.P278R (0.3%#)	c.833C>G; p.P278R	c.833C>G; p.P278R (0.8%#)
ESP19	A16 (STIC): c.745A>T; p.R249W (2.0%)	c.745A>T; p.R249W (50.6%)	none

(variant allele fraction)
variants detected below reporting threshold

In total, 5/6 cases showed matching *TP53* variants between the early serous lesion and the HGSC. However, the low VAF seen in early lesions suggested that significant contamination from normal, surrounding tissue. In one sample (3394), the VAF was at the level of noise, and the same variant was seen at a comparable VAF in the normal control tissue. It was thus decided that more precise sample acquisition is needed, in form of laser capture microdissection (LCM).

2.2. LCM and DNA extraction

In order to examine the whole genomes, laser capture microdissection (LCM) was needed to better enrich for the lesional DNA. This entailed training sessions, troubleshooting, additional expense and refinements in extraction to meet DNA quality control, in addition to required pre-amplification. In order to control the final elution volume, QiaAMP FFPE kit was used for DNA extraction as below.

DNA extraction (material amount used, with A260/A280 ratios)

	Early lesion	HGSC	Normal
ESP10	A2 (12 caps): 20 µL total**	A20 (4 sections): 119.5 ng/µL (2.389/1.187 = 2.01)	E1 (4 sections): 76.3 ng/µL (1.527/0.776 = 1.97)
ESP14	B1 (11 caps): 20 µL total**	B5 (3 sections): 163.6 ng/µL (3.271/1.637 = 2.00)	C1 (3 sections): 197.7 ng/µL (3.954/2.004 = 1.97)
ESP15	N/A	D5 (4 sections): 203.9 ng/µL (4.079/2.057 = 1.98)	A1 – not done
ESP18	B1 (12 caps): 20 µL total**	D4 (3 sections): 306.7 ng/µL (6.134/3.116 = 1.97)	H1 (4 sections): 165.3 ng/µL (3.306/1.635 = 2.02)
ESP19	A16-1 (10 caps): 20 µL total** A16-2 (5 caps): 20 µL total** A16-3 (5 caps): 20 µL total**	A1 (4 sections): 38.6 ng/µL (0.773/0.381 = 2.03)	A3 (2 sections): 66.8 ng/µL (1.337/0.65 = 2.06)
ESP32	B7 (10 caps): 20 µL total**	B3 (8 sections): 57.7 ng/µL (1.154/0.58 = 1.99)	D2 (4 sections): 244.8 ng/µL (4.895/2.478 = 1.98)

*ESP14/15 (same patient)
**concentration not measured

In order to address the small amount of DNA available in the early lesions, several different methods for DNA sequencing were explored through literature review, and communication with company sales representatives/tech experts, and CAMD personnel. Methods included SNP microarray-based methodology (OncoScan plus), as well as whole genome amplification. The OncoScan methodology was found to be unsuitable for the research aims.

Based on the unsuccessful, previous lab experience with Sigma (GenomePlex) whole genome amplification, a decision was made to attempt WGA using the Qiagen REPLI-G FFPE kit. The kit is suitable for WGA using either FFPE materials, or purified genomic DNA. A hybrid protocol was developed to overcome obstacles imposed by LCM cap membranes and sufficient DNA samples were quantified using NanoDrop as below:

	Early lesion	HGSC	Normal
ESP10	A2: 2,441.2 ng/µL (48.824/26.572)	A20: 2,429.2 ng/µL (48.584/26.839 = 1.81)	E1: 2,382.7 ng/µL (47.674/26.046)
ESP14/15	B1: 2,538.4 ng/µL (50.768/27.682)	B5: 2,506.4 ng/µL (50.128/27.698 = 1.81)	C1: 2,292.3 ng/µL (45.846/25.156)
ESP14/15	D2: 3,140.6 ng/µL (62.811/33.626 = 1.87)	D5 – not done	A1 – not done
ESP18	B1: 2,531 ng/µL (50.62/26.1 = 19.4)	B4: 1,693.6 ng/µL (33.872/17.699 = 1.91)	H1: 1,852.7 ng/µL (37.053/19.992 = 1.85)
ESP19	A16-1: 2,461.3 ng/µL (49.226/26.442) A16-2: 2,320.5 ng/µL (46.409/24.892) A16-3: 2,451.7 ng/µL (49.033/26.284)	A1: 2,555.2 ng/µL (51.104/28.461 = 1.80)	A3: 1,418.6 ng/µL (48.372/26.587)
ESP32	B7: 2,403 ng/µL (48.06/26.063 = 1.84)	B3: 2,548.8 ng/µL (50.975/27.63 = 1.84)	D2: 2,421 ng/µL (48.42/26.514 = 1.83)

Elution volume = 50 µL

Because the post-WGA samples contain polymerases, along with dNTPs and various solutes, the samples required post-WGA clean up. Following this, the results were as follows:

Case ID	Early lesion	HGSC	Normal
0458 (ESP10)	A2: 76.5 ng/µL (1.529/0.821 = 1.86)	A20: 43.3 ng/µL (0.855/0.456 = 1.9)	E1: 56.3 ng/µL (1.127/0.609)
2548* (ESP15)	B1: 102.5 ng/µL (2.05/1.087 = 1.89)	B5: 62.3 ng/µL (1.246/0.674 = 1.85)	C1: 37.9 ng/µL (0.759/0.396)
2055* (ESP14)	D2: 459.2 ng/µL (9.184/4.906 = 1.87)	D5 – not done	A1 – not done
3394 (ESP18)	B1: 280.1 ng/µL (5.6/3 = 1.87)	B4: 155.3 ng/µL (3.106/1.664 = 1.87)	H1: 125.6 ng/µL (2.513/1.348 = 1.86)
3451 (ESP19)	A16-1: 86.9 ng/µL (1.738/0.922 = 1.89) A16-2: 113.4 ng/µL (2.269/1.215 = 1.87) A16-3: 94.7 ng/µL (1.895/1.007 = 1.88)	A1: 58.6 ng/µL (1.172/0.613 = 1.91)	A3: 51.4 ng/µL (1.028/0.559)
1990 (ESP32)	B7 (10 caps): 47.7 ng/µL (0.954/0.52 = 1.84)	B3 (8 sections): 85.4 ng/µL (1.708/0.894 = 1.91)	D2: 65.6 ng/µL (1.312/0.701)

Elution volume = 50 µL

The samples have now been shipped to GeneWiz for whole genome sequencing, and we await result.

2.3. Immunostains for early carcinogenesis events

Beside the genetic events, we also looked for other biologic events that may be contributing to the carcinogenic sequence. Firstly, in looking at the origin of the cells, we performed immunostains for two potential stem/progenitor cell markers, namely LGR5 and HPGD. Also, we examined epigenetic events, looking for global dysregulation in histone 2A lysine 118/119 (H2AK) mono-ubiquitination, and histone 3 methylation at lysine 4 (H3K4me), lysine 9 (H3K9me2), and lysine 27 (H3K27me3). The results are currently being analyzed.

AIM 3.
Specifically, we examined for early stage disease (FIGO stage I or II), with available positive peritoneal fluid samples. No appropriate case was identified for investigation. The table below highlights the challenges faced in attempting to identify early stage disease that will permit gleaning meaningful data in terms of "directionality" of tumor spread. This is an analysis of 99 consecutive surgical specimens (it includes women treated with neo-adjuvant therapy. We have not compelling data that the detection of STIC is influenced to any meaningful degree by prior therapy).

Distribution	STIC	Endosalpinx	Tube not seen	Serosa only	Normal
(%)	18	39	5	22	15

As has been discussed previously, very early HGSCs almost always present as STIC, supporting a model where cancer begins as STIC and then spreads beyond the fallopian tube. These are typically in risk reduction surgeries where incidental tumors are most likely to be encountered. However, most HGSCs are encountered following dissemination, in symptomatic women, usually without a STIC. Less than 20% of cases in this group exhibited a STIC. The largest group presented with involvement of the endosalpinx, which is held synonymous with a tubal origin by current pathology guidelines. However, in a significant number, both STICs and Endosalpingeal carcinomas were observed in both tubes, a perplexing finding given that the great majority of HGSCs have the same genetic identifier (TP53 mutation). Moreover, 37% of the cases in the above group did not have involvement of the endosalpinx, manifesting with either serosal involvement only or no visible disease in the fallopian tubes. This is the basis for the argument that the tumor emerges elsewhere but assessing directionality was beyond the scope of this project given the challenges faced addressing the preceding aims.

4. Impact

The potential impact of this project is two-fold. First, the fundamental problems in sequencing small samples will not go away but in the opinion of the study investigators, the disparity between the observed distribution of tumor and putative early cancers (STICs) associated with HGSC require critical appraisal, in as much as they bear on the strategies that target cancer prevention. The observed distribution of HGSC highlighted in the above table combined with the recent UKCTOCS screening trial (which noted that early detection with combined CA125 screening with ultrasound down-staged HGSCs somewhat but did not impact mortality) suggests that the direction of spread of HGSC is not strictly from tube to peritoneum. This underscores the importance of developing markers that will link tumor and precursor at the *cellular* level. Based on our experience in this project, we are investigating avenues of research that allow the detection of mutations *in situ*, permitting us to scan fallopian tube and endometrial lining epithelium for cancer specific mutations that highlight early events more decisively. This effort is currently underway as a follow-up to this project.
A second initiative of importance is the retrieval of small numbers of epithelial cells from washings by flow sorting methods to ensure that the mutations being identified in the peritoneal cavity are derived from exfoliated epithelial cells. This part of the study is being investigated further by one of the participants (J.Y. Yoon).

5. Changes/Problems

We encountered considerable technical difficulty in the analysis of small tissue samples, either because the input DNA was limited by the size of the precursor or because precursor epithelium was contaminated with too much background DNA. The latter problem is compounded by inherent errors in the sequencing programs. We addressed this by laser capture micro-dissection in AIM2 and are awaiting the results.

6. Products

Manuscript under revision

J-Y Yoon, D Chapel, E Goebel, X Qian, MD, J Mito, N Horowitz, A Miron, T. R. Soong, W Xian, C P. Crum. Molecular catastrophe, the peritoneal cavity and ovarian cancer prevention. Journal of Pathology, in revisions.

Meeting presentations (abstracts)

D Chapel, C Robinson, E Goebel, T R Soong, D Kolin, C Crum. Landscape of Putative Precursors to High Grade Serous Carcinoma (HGSC) in the Female Genital Tract. Mod Pathol 2020; 100; Supplement 1; 1027

A F L Da Silva, C Crum, D Kolin. Primary Peritoneal High Grade Serous Carcinoma Revisited: Precursor Frequency and Implications. Mod Pathol 2020; 100; Supplement 1; 1053

E Goebel, X Qian, J Xie, D Chapel, S Hill, J Garber, W Xian, C Crum. Detection of TP53 Mutations in the Peritoneal Washings of Women with Germline Mutations in Ovarian Cancer Susceptibility Genes. Mod Pathol 2020; 100; Supplement 1; 1063

J Brouwer, J-Y Yoon, J Xie, S Hill, W Xian, C Crum. Tubal p53 Signatures in Li-Fraumeni Syndrome (LFS) are Geographically Unique, Multi-Clonal and Temporally Dynamic. Mod Pathol 2020; 100; Supplement 1; 1019

Oral presentation

C Crum. Killer on the loose? Precursor escape and ovarian carcinogenesis.2020 Maude Abbott Lecture. United States Canadian Academy of Pathology, Los Angeles, 2020.

7. Participants & Other Collaborating Organizations

Name:	Wa Xian, PhD
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	0000-0001-6315-8858
Nearest person month worked:	1
Contribution to Project:	Dr. Xian assisted in interpretation of sequencing data

8. Special reporting requirements

Nothing to report

9. Appendix

Award Expiration Transition Plan
Award Chart Template

OC180172: Early Precursor Escape and High-Grade Serous Carcinogenesis

PI: Christopher P. Crum, Brigham and Women's Hospital, Boston MA **Budget:** $425,039

Topic Area: DoD Ovarian Cancer Research Program **Mechanism: Year 2018 OCRP Pilot Award**

Research Area(s): SCS Coding **Award Status:** 01 July 2019 – 30 June 2021

Study Goals: 1) To strengthen the link between early serous cancer precursors (ESPs) and concurrent high grade serous carcinomas (HGSCs). 2) To identify pathways by which ESP eventuate in HGSCs.

Specific Aims: 1) To identify mutations in exfoliated epithelial cells and link them to concurrent ESPs.2) To identify markers confirming a lineage relationship between ESPs and HGSCs 3) To confirm a lineage direction from intraperitoneal HGSC and ovarian HGSC.

Key Accomplishments and Outcomes:

1) With EP-CAM labeling, epithelial cells were sorted from a peritoneal fluid and on sequencing, were found to contain a TP53 mutation. A link between the mutation and a precursor in the fallopian tube has not yet been established. 2) Sequencing of mutant TP53 sequences in ESPs and concurrent HGSCs yielded evidence of shared sequence specific mutations, further supporting the concept of "precursor escape". Data from whole exome sequencing is under analysis.

Publications: See Final Report
Patents: none to date
Funding Obtained: none to date

Transition Plan Questionnaire

Directions: Please answer all questions that apply for each product under development. Please fill out one document per product.
This is not an application for funding; however, answers will help us understand the outcomes and products from your award.

1. After the award closes, would you be willing to periodically provide voluntary information (via email) regarding the project status (i.e. where the research is headed)? **Yes** ☑ or **No** ☐
These responses will help CDMRP demonstrate the return on its investments and will help demonstrate that the CDMRP is a responsible and successful steward of federal research funding.

2. What **conclusion(s)** does your final data support?

Our data continues to support the concept that a significant subset of disseminated high-grade serous carcinomas (HGSCs) undergo malignant transformation not in the fallopian tube but in the peritoneal cavity from pre-malignant cells that previously shed from the fallopian tubes (so called precursor escape). We were able to support prior observations to some extent by identifying TP53 mutations in tubal serous cancer precursors (so called early serous precursors) that are shared with concurrent HGSCs. We also identified mutations in suspended epithelial cells in the peritoneal fluid, using flow cytometry to isolate ep-cam positive cells followed by sequencing. We were not successful in consistently generating mutation data from small precursors (necessary to maximize the connection to cancers, which are easily analyzed by virtue of large cell numbers) to link them to concurrent cancers, but I believe our continued pathologic analysis of HGSC distribution favors considering alternative pathways.

3. Will you/have you applied for/obtained follow-on-funding for this project? **If yes**, please list (a) funding organization, (b) total budget requested/obtained, and (c) title of the funded proposal. *This information will be recorded as an outcome to this award*.

We have funding left over from an OCRA project and some carry over from an NIH SPORE that will hold us for the next year while we address one aspect of the project. The fellow in our lab, Ju Yoon Yoon, is applying for a pilot award to investigate peritoneal fluids from which epithelial cells will be isolated for TP53 mutation assessment.

4. What will be **the next step(s)** for this project?

I am currently communicating with a commercial probe vendor to generate mutation specific labeled oligonucleotide probes. The use of such probes ideally would make it possible to identify at the cyto-histologic level potential precursors that share the same TP53 mutation with concurrent cancers. This would circumvent the struggles inherent in isolating cells by manual or laser capture micro-dissection and the inherent error rate of deep sequencing. We will select cases of HGSC that do not contain a visible malignancy in the tube or ovary, generate mutation specific probes following TP53 sequencing of the tumors, and interrogate the normal (or potential precursor) epithelium for evidence of the same mutations.

5. How would you classify your **lead candidate product**? Please choose best option
(a) Therapeutic (Small Molecule, Biologic, Cell/Gene Therapy): Please choose, if applicable
(b) Diagnostic
(c) Device
(d) Research Tool to Address a Research Bottleneck
(e) Knowledge Product (Non-material product such as a compound library, database, something that improves clinical practice, education, etc.)
(f) Other - Please Specify:

6. How does your candidate product aid the Warfighter, Veteran, Beneficiary, and/or General Population?

Results generated from this analysis would impact our understanding of HGSC development and could be relevant to cancer prevention strategies, such as risk reduction surgery, opportunistic salpingectomy and the role of the peritoneal cavity in precursor evolution.

7. Therapy / Product Development, Transition Strategies, and Intellectual Property

Describe the steps and relevant strategies required to move the candidate product (knowledge or tangible) to the next phase of development and/or commercialization. Please address any issues with intellectual property.

PIs are encouraged to explore the technical requirements and the current regulatory strategies involved in product development as well as to work with their organization's Technology Transfer Office (or equivalent regulatory/legal office), federal/international regulatory experts, to develop the transition plan and to explore developing relationships with industry, DoD advanced developers (e.g. USAMMDA), and/or other funding agencies to facilitate moving the product into the next phase.

If the hypothesis were to be correct, strategies to lower risk would be explored. It is premature at this point to expand this concept but this work would be directly relevant to the expectations from risk reducing surgery in preventing HGSC.