AWARD NUMBER: W81XWH-14-1-0504

TITLE: The Oviduct and Serous Cancer Risk Assessment

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REPORT DATE: June 2017

TYPE OF REPORT: Final

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
June 2017	Final	30 Sep 2014 - 31 Mar 2017

4. TITLE AND SUBTITLE
The Oviduct and Serous Cancer Risk Assessment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0504

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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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

The proposal was designed to address three issues. First could we identify stem cells from the fallopian tube, including from patients with high grade serous cancer (HGSC). Second, could we link the molecular abnormalities in cancer associated stem cells and validate them in pathologic material, specifically in what we feel are stem cell outgrowths or SCOUTs and third could we identify molecular alterations that would place the oviduct or the patient at risk for HGSC. In essence we wished to drill down to the cell of origin and link it to cancer risk, identifying an assay that could predict the presence of cancer by analyzing lower genital tract fluids or other samples. All of these aims have been addressed and our studies have reinforced the likelihood that novel pathways to ovarian cancer exist, but evidence points to more than one mechanism and site of origin.

15. SUBJECT TERMS
HGSC = high grade serous cancer; Fallopian tube; BRCA; Tp53, SCOUT = stem cell outgrowth; STIC = serous tubal intraepithelial carcinoma; STIN = serous tubal intraepithelial neoplasia.

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	30	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

This report is identical to the progress report following year 2 with the inclusion of a statement at the end of accomplishments which addresses the work completed during the 6 month extension that terminated in April 2017. These additions and additional publications are underlined. The original proposal was designed to address three issues. First could we identify stem cells from the fallopian tube, including from patients with high grade serous cancer (HGSC). Second, could we link the molecular abnormalities in cancer associated stem cells and validate them in pathologic material, specifically in what we feel are stem cell outgrowths or SCOUTs and third could we identify molecular alterations that would place the oviduct or the patient at risk for HGSC. In essence, we wished to drill down to the cell of origin and link it to cancer risk, identifying an assay that could predict the presence of cancer by analyzing lower genital tract fluids or other samples. All of these aims have been addressed and our studies have reinforced the likelihood that novel pathways to ovarian cancer exist, but evidence points to more than one mechanism and site of origin.

2. KEY WORDS

Ovarian Cancer
Fallopian tube
High grade serous carcinoma
Stem cell
Serous tubal intraepithelial carcinoma
Secretory cell outgrowth
Serous tubal intraepithelial lesion

3. ACCOMPLISHMENTS

Major goals.

AIM 1: To isolate, grow in culture, and compare stem cells from the fallopian tubes of patients with and without malignancy.
AIM 2: To link and validate the molecular disturbances observed in cancer associated stem cells in pathologic material, specifically in an entity we have described called the stem cell outgrowth or SCOUT.
AIM 3: To exploit the molecular alterations discerned to make molecular probes that will detect those alterations that place women at risk for the disease, either in the fallopian tubes or lower genital tract fluids.

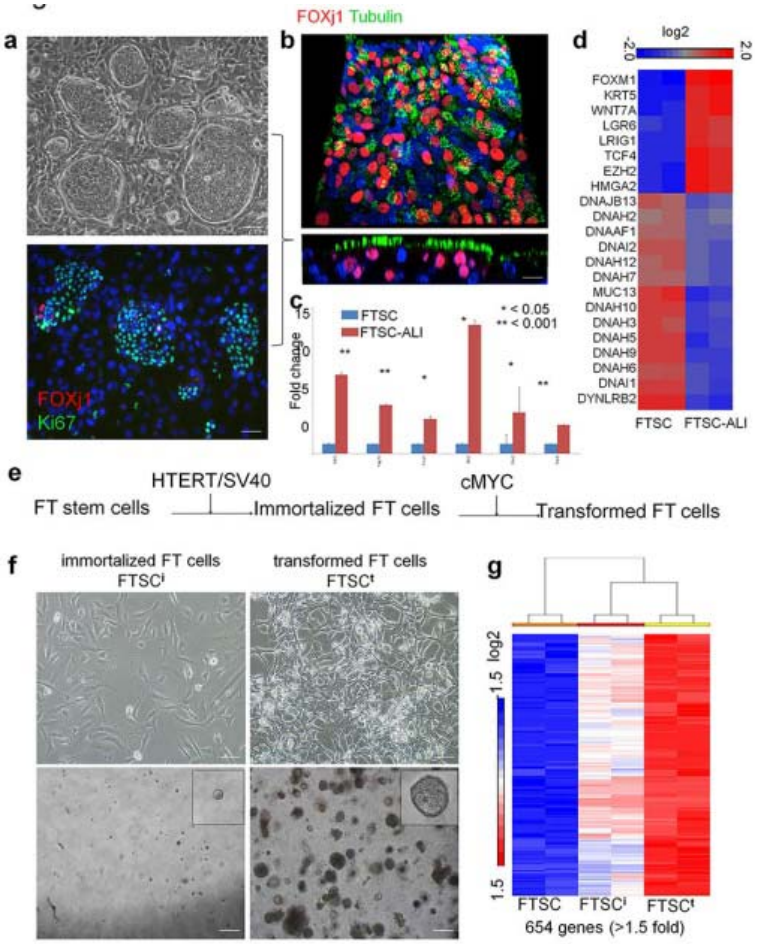

Figure 1. Upper panels show differences in expression between putative stem cells and cells undergoing in vitro ciliated differentiation. Lower panels depict progressive changes in gene expression with immortal and transformed cells

What was accomplished

AIM 1: 1) Major activity: isolate and grow fallopian tube stem cells in culture. 2) The specific objective was to identify stem cell characteristics that distinguished tumor associated (but normal appearing) stem cells from normal controls. 3) Significant results: a) We successfully cloned stem cells from normal fallopian tubes and showed that these cells were capable of both ciliated and squamous differentiation, in parallel with the histology of the fallopian tube (Figure 1). b) We generated a "stem cell" specific signature by comparing gene expression between undifferentiated stem cells

and those grown on an air-liquid interface, which permitted ciliated differentiation. This was the first ever successful cloning, propagation and maturation of fallopian tube stem cells [1]. What we have not accomplished is to show that stem cells from normal tubal epithelium in cancers can be distinguished from epithelium from normal controls. b) An additional achievement, however, was to identify potential stem cell markers that were novel and might be used to unearth potential stem cells in the general pool of non-ciliated tubal epithelium[2]. Moreover, we showed remarkable parallels between stem cells and putative stem cell outgrowths as well as HGSC precursors. c) Another added achievement was to demonstrate a parallel between immortalized and transformed stem cells and precursor and malignant HGSCs in the fallopian tube (Figures 2&3). D) Still another added achievement was a successful experiment focusing on propagating potential cancer stem cells. In this study we identified subpopulations that were Taxane resistant and were able to identify the same cells in Taxane naive cell cultures (Ning et al, submitted). This suggests that there is a small population of chemo-resistant cells that is inborn and not created by chemotherapy per se.

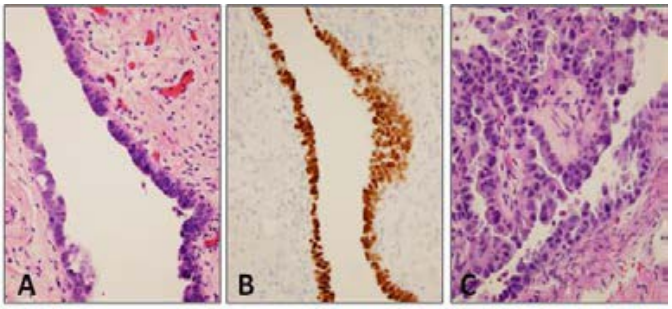

Figure 2. A mild atypia is the only abnormality in the tubal mucosa (A) of a patient with widespread metastatic HGSC (C) and stains strongly for p53 (B). Both lesions shared an identical p53 mutation following sequence analysis, supporting the concept of "precursor escape" (Soong R, Howitt BE, Crum CP unpublished).

AIM 2 1) Major activity: Translating the in vitro findings to histopathology. 2) Objective : To link the disturbances observed in isolated stem cells to stem cell outgrowths (SCOUTs) and serous cancer precursors (STICs). This was shown in the papers by Ning et al and Yamamoto et al[1,2]. Additional achievements: During these studies we took the opportunity to address an issue fundamental to the aims, which is the origin of HGSC. Because a high grade precancerous process (or STIC) can only be uncovered in subset of cancers, we felt it important to address this issue, the goal being to better understand the potential origins of these neoplasms. This was done in a series of studies. First, we showed a potential dualistic model for HGSC with a lower association with STIC seen for tumors with certain morphologic features[3] (Howitt 2015). This suggests that there could be more than one pathway to HGSC including one where STIC is not the primary predecessor. Second, we showed that certain histologic patterns were associated with specific gene mutations (Ritterhouse 2016) again suggesting more than one pathogenetic route to HGSC. Third, we recently sequenced cases with bilateral STICs and have shown them to contain identical p53 mutations. This raises the critical question that not all STICs develop de novo but may signify mucosal metastases from either the opposite tube or another site (Meserve et al in preparation). Fourth, we have concluded the preliminary phase of an ambitious project that has exhaustively analyzed fallopian tubes of women with HGSC but no STIC. In these tubes we have seen non-cancerous epithelium with p53 mutations. On comparing the p53 mutations status between these non-cancerous epithelia and the associated (and physically removed) HGSCs we have discovered identical mutations. This suggests the possibility that pelvic HGSCs could be derived from minor atypias within genetically altered stem cell proliferations (Figure 2). This has potentially profound implications in that it suggests that the serous carcinogenic sequence can initiate in the tube but continue beyond the confines of the oviduct at an unknown pelvic location (Soong et al manuscript in preparation).

AIM3: 1) Major activity: To develop a means to detect the presence of biomarkers unique to serous cancer or serous cancer risk in the uterus or lower genital tract. Objective: To employ deep sequencing to identify p53 mutations in the lower genital tract tissues or fluids that would indicate the presence of an upper genital tract neoplasm. Significant results: We decided to use a novel approach to this problem by first identifying cases of HGSC and then searching the archive for prior formalin-fixed, paraffin embedded endometrial specimens from diagnostic procedures. DNA from serial sections of this material was extracted and then analyzed on a platform targeting p53 mutations. These mutations were compared to those found in the tumors at a later date. We identified 5 samples in which information was available. In two (40%) we detected p53 mutation in prior endometrial samples that matched those found in the subsequent tumor. The intervals from detecting the mutations to the diagnosis of the tumors were 2 weeks and 2 months.

A six-month extension was granted to continue the study from October 2016 through March 2017. This period was devoted to completing two ongoing projects. The first was the comparison of p53 sequences between potential precursors in the tube and concurrent HGSCs by Dr. Soong to address the possibility that HGSCs could originate via "precursor escape". This study was completed and the manuscript is nearing completion for submission. The second was the analysis of Tumor-Specific TP53 Signatures from Archival Endometrial Biopsies Obtained Prior to the Diagnosis of High-Grade Serous Carcinoma. This work has been completed and has been accepted for presentation at the upcoming USCAP meeting in March 2018.

What opportunities for training and professional development has the project provided?

This grant has provided opportunities to several young investigators both at BWH and at collaborating institutions, including the following:

1) Collaboration in year 1 with the Xian laboratory at the Jackson laboratory (Farmington CT). This collaboration centered on sharing of samples with the Xian laboratory, primarily examining fallopian tubes for stem cells and characterizing putative stem cell in the fallopian tube. Young investigators Yamamoto and Ning were first authors on publications coming from this collaboration.
2) Career development for Dr. Xian (collaborator in the first year). Dr. Xian has recently obtained a Teal award stemming in part from opportunities created during this collaboration.
3) Career development for Brooke Howitt at BWH. Dr. Howitt is a young faculty member at BWH who was involved in several projects including genomic analysis of ovarian cancers, and was the lead author on a study proposing a dualistic model of high grade serous carcinogenesis. She is currently applying for funding to expand her protected time for ovarian cancer research.
4) Career development for visiting scholar Jan Brouwer. Mr. Brouwer is an MD PhD candidate from the Netherlands who worked for 6 months on a project dissecting the immunophenotype of putative stem cells in the fallopian tube.
5) Career development for Kyle Strickland, Thing Rinda Soong, and Lauren Ritterhouse. These trainees have been involved in projects supported by this grant. Dr. Strickland will be taking a position at Duke in gynecologic pathology and cancer research, Dr. Ritterhouse will be joining the Pathology Department at the University of Chicago Medical Center in Molecular Diagnostics and Dr. Soong is scheduled to spend the next year in clinical and research in breast and gynecologic neoplasia.
6) Support of colleagues. In addition to the above, we have supported colleagues at the Dana Farber Cancer Institute, including Dr. Alan D'Andrea's laboratory.

How were the results disseminated to the communities of interest?

Results were published in the pathology and gynecology journals and presented at yearly meetings (see Products below)

How do you plan during the next reporting period to accomplish these goals?

This is the final report.

4. IMPACT

What was the impact on the development of the principal discipline of the project?

The purpose of this project was broaden our understanding of the cells involved in the pathogenesis of high grade serous cancer. It is currently assumed by many that the fallopian tube is the only source of these tumors - prompting efforts to prevent cancer by salpingectomy alone - yet we cannot prove this based on the pathologic evidence. The stem cell work has elucidated immuno-phenotypes that bear further study as cancer stem cells that can be searched for in extra tubal site such as the ovary. Moreover, the studies of fallopian tubes from women with cancer raise the intriguing question that earlier precursors might escape to produce tumors

elsewhere. The identification of mutations in lower genital tract samples has raised the hope that a molecular Pap smear will be possible. However, our identification of p53 mutations in normal tubal mucosa raises concerns about the specificity of such a test, which is becoming more obvious.

What was the impact on other disciplines?

These studies are highly relevant to the epidemiology of ovarian cancer and expectations from surgical approaches.

What was the impact on technology transfer?

The discovery of genes deregulated in putative stem cells (Yamamoto et al) has been made public.

What was the impact on society beyond science and technology?

The fallopian tube and its role in ovarian cancer has had broad impact on women in general including those with or without genetic predisposition. The publications from this group have always addressed the strengths and limitations of the fallopian tube hypothesis with an eye on patient care.

5. CHANGES/PROBLEMS:
We encountered no major challenges in completing this work.

6. PRODUCTS

Publications

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Books or non-periodical publications

Berek JS, Crum C, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum. Int J Gynaecol Obstet. 2015 Oct;131 Suppl 2:S111-22.

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Crum CP. Preventing Ovarian Cancer. J Clin Oncol. 2016;34:198-9.

Presentations, conference publications

Past presentations

Jelena Mirkovic, Amy DiVasta, Stacey Missmer, Brooke Howitt, Christopher Crum, Marc Laufer, Sara Vargas. The Histologic Spectrum of Adolescent Endometriosis. USCAP meeting, Boston, March 2015

Andre Pinto, Brooke Howitt, Christopher Crum. The Variable Spectrum of Tubal Intraepithelial Neoplasia in Women with High Grade Serous Carcinoma. USCAP meeting, Boston, March 2015

Lauren Ritterhouse, Christopher Crum, Lynette Sholl, Neal Lindeman, Brooke Howitt. Morphologic and Molecular Evaluation of Extra-Uterine Mullerian Carcinoma. USCAP meeting, Boston, March 2015

Presentations at the USCAP 2016 meeting

Emily E Meserve, Jelena Mirkovic, James R Conner, Eric Yang, Brooke E Howitt, Christopher P Crum. Detection of serous tubal intraepithelial carcinoma (STIC) in incidentally removed fallopian tubes from low-risk women.

Thing Rinda Soong, Christopher P Crum, Brooke E Howitt. Serial Sectioning of Distal Fallopian Tubes and its Role in the Discovery of Occult Serous Tubal Intraepithelial Carcinoma in Women with High Grade Ovarian Serous Carcinoma.

Presentations at the USCAP 2017 meeting

Brooke E Howitt, Elizabeth Garcia, Lynette M Sholl, Neal I Lindeman, Laura E MacConaill, Fei Dong, Michelle S Hirsch, Marisa R Nucci, Christopher P Crum, W Glenn McCluggage, Jelena Mirkovic. Targeted Genomic Profiling of Female Adnexal Tumors of Probable Wolffian Origin.

T Rinda Soong, Lynette M Sholl, Michele T Baltay, Bradley J Quade, Michelle S Hirsch, Christopher P Crum, Marisa R Nucci, Brooke E Howitt. Targeted Genomic Profiling of Ovarian and Peritoneal Low- Grade Serous Carcinomas (LGSC) with Clinicopathologic Correlation

Presentations accepted for the USCAP 2018 meeting

Kolin D, Cramer D, Crum, CP. High Grade Serous Carcinoma: Is there a Correlation Between Putative Site of Origin, Hormonal Status and Talc Usage?

Strickland K, Howitt BE, Crum CP. Detection of Tumor-Specific TP53 Signatures from Archival Endometrial Biopsies Obtained Prior to the Diagnosis of High-Grade Serous Carcinoma.

Brouwer J, Strickland K, Kolin D, Crum CP, Howitt BE. Evidence for a Unique Endometrioid Carcinogenic Sequence in the Fallopian Tube Associated with a type II (Endometrioid) Secretory Cell Outgrowth (SCOUT).

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Name:	*Wa Xian, PhD*
Project Role:	*Co-investigator, Assistant Professor, Center for Stem Cell & Regenerative Medicine CPRIT Scholar in Cancer Research, University of Texas Health Sciences Center,*
Researcher Identifier	*NA*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Xian collaborated on the fallopian stem cell culture and stem cell studies.*
Funding Support:	*Currently recipient of a Teal Award*

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES

Selected manuscripts published since January 2017

Meserve E, Mirkovic J, et al, 2017
Meserve E, Brouwer J, Crum CP, 2017

Modern Pathology (2017), 1–12

Serous tubal intraepithelial neoplasia: the concept and its application

Emily EK Meserve[1], Jan Brouwer[2] and Christopher P Crum[1]

[1]*Division of Women's and Perinatal Pathology, Department of Pathology, Brigham and Women's Hospital, Boston, MA USA and* [2]*Department of Gynecologic Oncology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*

In recent years it has become clear that many extra-uterine (pelvic) high-grade serous carcinomas (serous carcinomas) are preceded by a precursor lesion in the distal fallopian tube. Precursors range from small self-limited 'p53 signatures' to expansile serous tubal intraepithelial neoplasms that include both serous tubal epithelial proliferations (or lesions) of uncertain significance and serous tubal intraepithelial carcinomas. These precursors can be considered from three perspectives. The first is biologic underpinnings, which are multifactorial, and include the intersection of DNA damage with *Tp53* mutations and disturbances in transcriptional regulation that increase with age. The second perspective is the morphologic discovery and classification of intraepithelial neoplasms that are intercepted early in their natural history, either incidentally or in risk-reduction surgeries for germline mutations. For the practicing pathologist, as well as the investigators, a distinction between a primary intraepithelial neoplasm and an intramucosal carcinoma must be made to avoid misinterpreting (or underestimating) the significance of these proliferations. The third perspective is the application of this information to intervention, devising strategies that will actually lower the ovarian cancer death rate by opportunistic salpingectomy, widespread comprehensive genetic screening and early detection. Central to this issue are the questions of (1) whether some STICs are metastatic, (2) whether lower-grade epithelial proliferations can invade prior to evolving into intraepithelial carcinoma, or (3) metastasize and become malignant elsewhere ('precursor escape'). An important caveat is the persistent and unsettling reality that many high-grade serous carcinomas are not associated with an obvious point of initiation in the fallopian tube. The pathologist sits squarely in the midst of all of these issues, and has a pivotal role in managing expectations for stemming the death rate from this lethal disease.
Modern Pathology (2017) 00, 1–12. doi:10.1038/modpathol.2016.238

The past two decades have witnessed a revolution in the field of ovarian cancer research. It began with the discovery of the *BRCA1* and *BRCA2* tumor suppressor genes, followed by the emergence of the fallopian tube as a potential source of at least some serous carcinomas and culminating in a body of evidence that has identified the fallopian tube as a major participant in the pathogenesis of this lethal disease.[1–8] As with many paradigm shifts, much of the data in support of this change in attention from ovary to fallopian tube was rooted in histopathologic study. Painstaking analysis of ovaries and fallopian tubes of healthy women undergoing risk-reduction bilateral salpingo-oophorectomy for mutations in *BRCA1* and *BRCA2* (BRCA+) has led to the identification of epithelial atypias or early carcinomas in the fallopian tube. The introduction of the SEE-FIM protocol for routine fallopian tube analysis in 2005 was followed by a widespread appreciation of early serous cancer of the tubes, both in BRCA+ women and those with no known genetic predisposition.[8] A serous carcinogenic sequence in the fallopian tubes, alluded to early on by Zweemer and colleagues, was described further, crystalizing the concept of a tubal origin.[2,4,9,10] The particularly high likelihood of discovering an early high-grade serous carcinoma in the fallopian tubes rather than elsewhere in the pelvis has raised the possibility that a significant percentage of these tumors start in the oviduct.[11,12] This has fueled hopes that opportunistic salpingectomy will not only benefit the general population but also be a viable alternative to salpingo-oophorectomy for BRCA+ women seeking

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Received 14 July 2016; revised 4 December 2016; accepted 5 December 2016

Table 1 Evidence supporting a tubal origin for extra-uterine high-grade serous carcinoma, with caveats

Supporting a tubal origin
Serous tubal intraepithelial carcinoma is the most common early malignancy in asymptomatic BRCA+ women.
Low-volume HGSCs found in women with or without germline BRCA mutations through increased surveillance are usually tubal in origin.
Intraepithelial carcinoma is associated with from 19–60% of advanced high-grade serous carcinomas.
Latent precursors with p53 mutations (p53 signatures) have been isolated in the fallopian tube, localize to the same (fimbria) region as intraepithelial carcinoma, are seen in continuity with intraepithelial carcinoma and share identical p53 mutations or other genomic changes with cancer.
Lesions intermediate between p53 signatures and intraepithelial carcinoma (tubal intraepithelial lesions) have been identified in the fallopian tube.
A spectrum of accumulated genomic disturbances link p53 signatures, intraepithelial carcinomas, and invasive/metastatic carcinomas.
Animal models of fallopian tube carcinoma have been constructed.
Fallopian tube secretory cells can be transformed, producing serous carcinomas.
'Precursor escape' could occur, whereby populations of non-malignant p53 mutated epithelial cells spread beyond the tube and eventually evolve into a pelvic carcinoma.
Caveats
In a significant number of high-grade serous carcinomas in which the fallopian tubes can be evaluated a tubal precursor or obvious tubal mucosal origin cannot be identified (see text for caveats).
Some subsets of carcinoma (pseudoendometrioid) are less likely to be associated with STIC.
In a ~~significant~~ percentage of carcinomas the intraepithelial carcinoma is particularly high-grade without an adjacent lower grade precursor, leaving open the possibility some of these lesions are mucosal metastases.
Some bilateral intraepithelial carcinomas share the same p53 mutation, suggesting one or both may not be a primary lesion (EK Meserve, K Strickland, B Howitt, C Crum, unpublished data).
Tubal intraepithelial carcinomas and tumors of the uterus share the same p53 mutations, leaving open the possibility that some intraepithelial carcinomas are metastatic deposits.

protection from serous carcinoma while retaining their ovarian function.[13] These hopes are countered by the vagaries inherent in assumptions made based on seemingly obvious data, unanswered questions and paradoxes that must be resolved by further investigation.

This revolution in our understanding of ovarian cancer—and the questions that remain—can be viewed from three perspectives that inform future strategies of management and prevention. The first is the intersection of a series of biologic events that give rise to cancer risk in the fallopian tube, which highlights the profound need to understand the sequence of events involved in tubal carcinogenesis. The second is the interception of serous carcinoma by examination of the tube, which addresses the detection, its etiologic significance, proper classification, and ascertainment of outcome risk. The third is intervention, which draws from the existing knowledge base and pertains to the promise of prophylactic salpingectomy and potential pitfalls. In this review, we summarize each of these three perspectives with attention to what has been accomplished and what remains to be clarified.

In this review we accept the fact that the level of certainty regarding the origin of serous tubal intraepithelial carcinoma is governed by certain variables. The likelihood that the lesion arose in the tube is greatest when (1) a spectrum of atypia from low to high grade is present, (2) the lesion is unilateral, and (3) advanced disease is absent, as seen in risk reducing salpingo-oophorectomies for women with germline *BRCA1* or *BRCA2* mutations. In contrast, the likelihood that the intraepithelial carcinoma arose from the tube is less when (1) a spectrum of atypia is absent—that is, the intraepithelial neoplasm is composed exclusively of cells with marked atypia -, (2) the neoplasm is seen in both fallopian tubes, and (3) advanced disease (including endosalpingeal tumor) is present, the possibility that one or both tubal intraepithelial neoplasms is a metastasis must be considered. The purpose of making these distinctions is to foster a critical estimate of the potential origin for each intramucosal carcinoma in the fallopian tube.

Intersection

Serous tubal intraepithelial carcinomas have been recognized in the fallopian tube for decades but the link between these lesions and ovarian carcinomas in general has gone undeveloped until recently.[14] Several observations have clarified this relationship, some of which are summarized in Table 1. A series of serous tubal intraepithelial carcinomas is illustrated in Figures 1a–d.

BRCA1 or BRCA2 Mutation-Associated Atypia

The presence of epithelial atypia was appreciated in fallopian tubes of women with *BRCA* mutations (BRCA+). The discovery of the *BRCA* genes accelerated programs targeting the ovaries and fallopian tubes with the onset of the risk reducing salpingo-oophorectomy.[1] Beginning in 2000, several groups

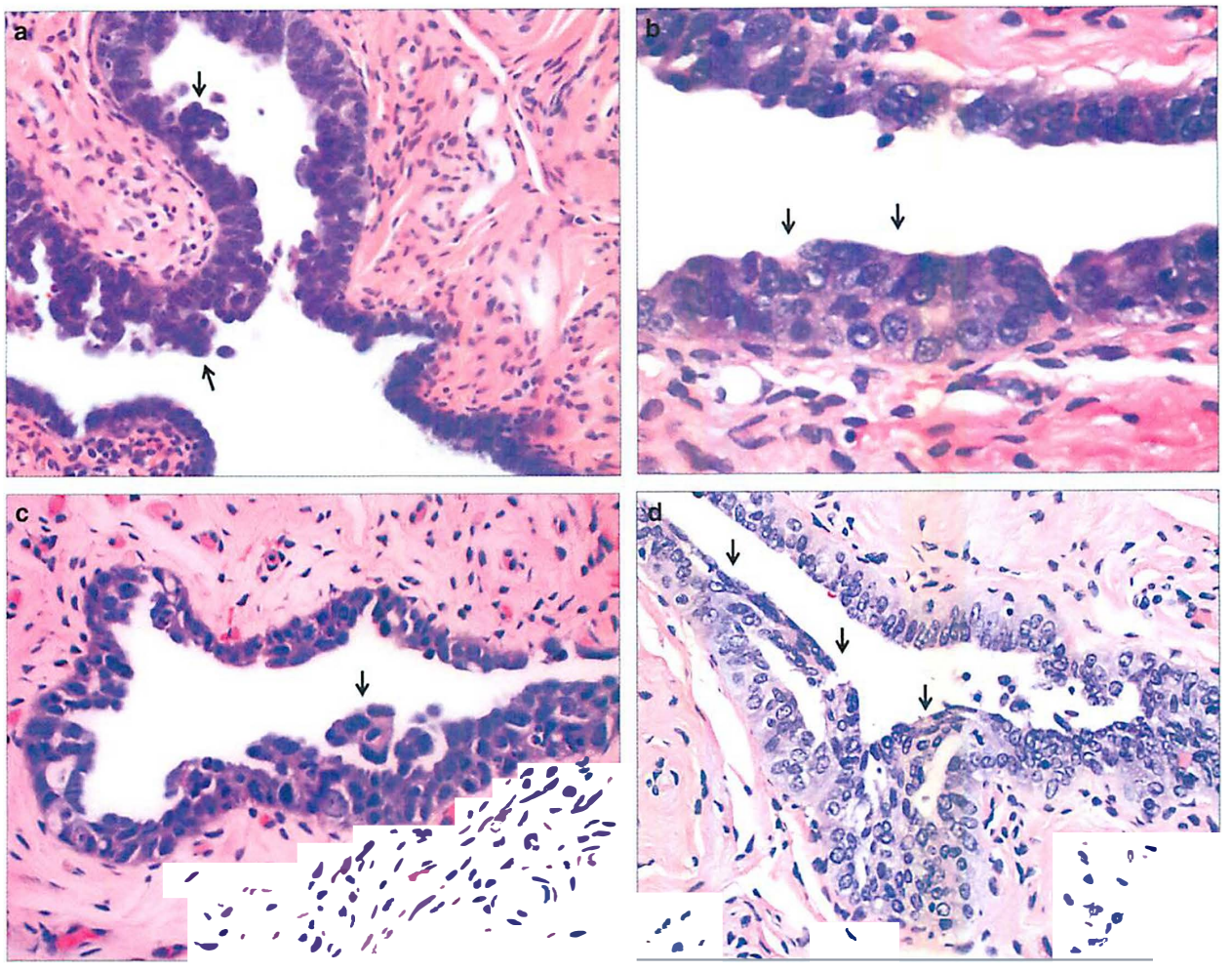

Figure 1 Examples of serous tubal intraepithelial carcinoma. Note the conspicuous loss of normal epithelial maturation, with loss of polarity. Arrows denote irregular nuclear stacking (**a–d**), small detached papillary clusters (**a**), horizontally oriented surface nuclei (**d**), and intraepithelial fractures (**a**, **c**, and **d**).

noted the potential role of the fallopian tube in the genesis of serous carcinoma in BRCA+ women.[2–4,6] These studies identified serous tubal intraepithelial neoplasms in women at risk and in some cases linked them genetically to serous carcinomas.

The Distal Fallopian Tube

There was a predilection for serous carcinomas (in the form of serous tubal intraepithelial carcinomas) to emerge from the distal fallopian tube. Cass *et al* noted in 2005 that the majority of tubal cancers arose from the distal or fimbriated end.[5] In January 2005, Brigham and Women's Hospital initiated the SEE-FIM protocol for the evaluation of women at genetic (BRCA+) or historical risk (strong family history of breast and/or ovarian cancer) for serous carcinoma. As a result of this protocol, it was possible to corroborate the observation that most early tubal malignancies arose in the fimbria or distal one-third of the oviduct.[8]

Association with Advanced Serous Carcinoma

The frequency of serous tubal intraepithelial carcinoma is high in women with symptomatic serous carcinoma. To determine whether serous carcinomas in general had a high frequency of serous tubal intraepithelial carcinoma, Kindelberger *et al* applied the SEE-FIM protocol to a consecutive series of women with high-grade serous carcinoma.[15] They found that ~45% had a coexisting STIC. The precise percentage was somewhat difficult to ascertain given the fact that the distal tube was frequently obliterated or not amenable to thorough examination. Subsequent estimates have linked STICs to high-grade serous carinomas in from < 20 to 60 percent.[16] This broad figure contrasted with the frequency of STIC detection when high-grade serous carcinomas were detected incidentally or early. In BRCA+ women undergoing risk-reduction surgery, early high-grade serous carcinomas were discovered on examination of the tubes and ovaries in from 5 to 10%.

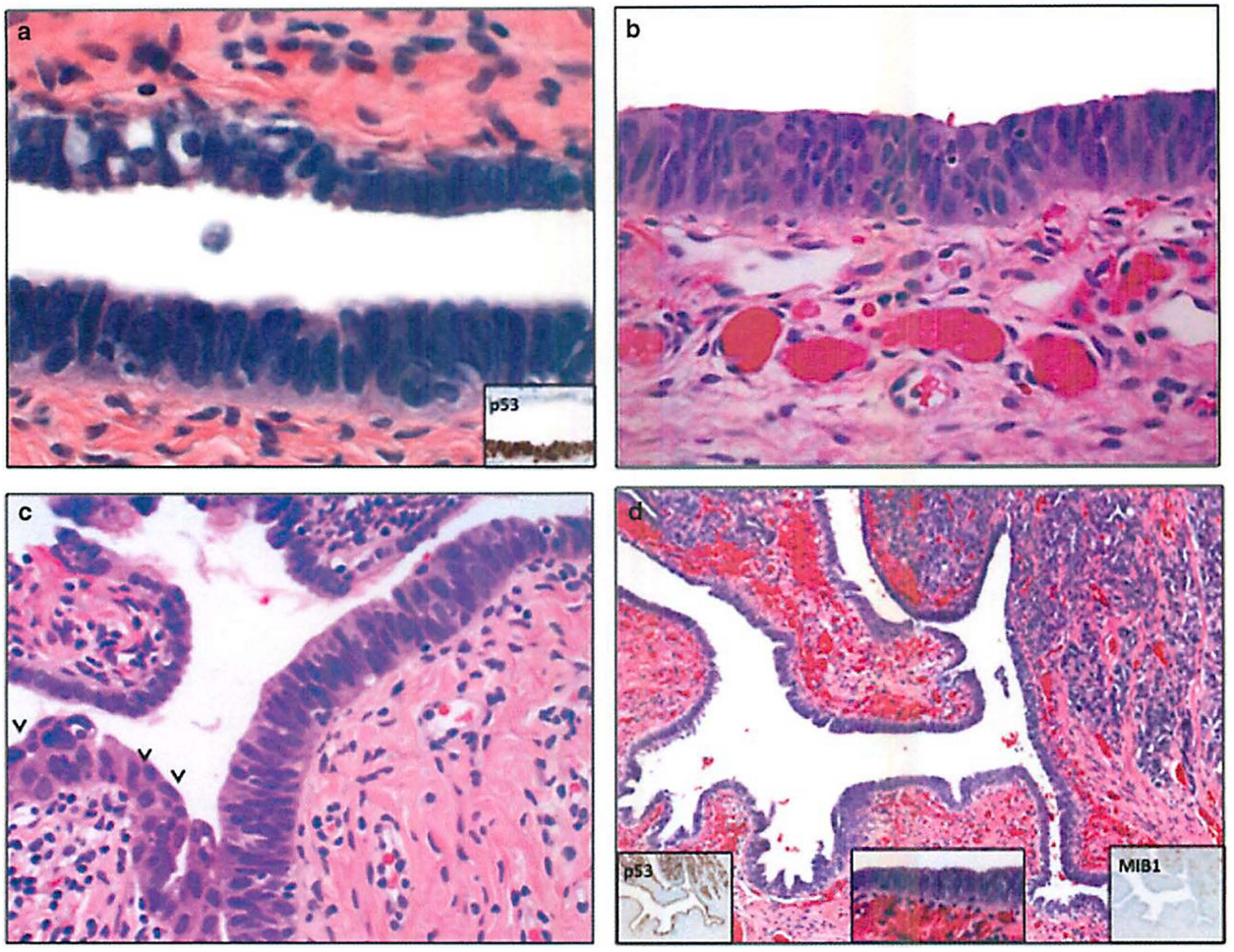

Figure 2 (a) p53 signature (lower) with inconspicuous atypia and strong p53 staining (inset). (b) Serous tubal epithelial proliferation (lesion) of uncertain significance, exhibiting multilayered cohesive epithelium with preserved polarity. We would not classify this as a serous tubal intraepithelial carcinoma but the reader is advised that there is considerable inter-observer variability in interpretation of such proliferations. (c) Serous tubal intraepithelial proliferation (right) merges with a serous tubal intraepithelial carcinoma (left, arrows). (d) Serous tubal intraepithelial proliferation (left) with adjacent invasive carcinoma (right). Note there is no intervening tubal intraepithelial carcinoma. Immunostains and the epithelium in question at greater magnification (center) are in the insets.

Approximately 80–100% of these early high-grade serous carcinomas were associated with involvement of the fallopian tube in the form of a serous tubal intraepithelial carcinoma.[11,17,18] To reduce the likelihood that intraepithelial carcinoma was a product of metastatic spread, Kindelberger *et al* required that it be separated from the invasive component and conform to a pattern in keeping with an intraepithelial carcinoma.

A Morphologic Spectrum of Precursor Disease

Serous cancer precursors in the tube comprise a morphologic spectrum. The least atypical are small stretches of nearly normal appearing, mostly non-ciliated cells with evidence of *Tp53* mutations called p53 signatures (Figure 2a).[9] p53 signatures are encountered in ~50% of fallopian tubes of women irrespective of their level of genetic risk and are presumed to be emblematic of the early events on the pathway to serous carcinoma. Much less common are atypical serous tubal epithelial proliferations that are more conspicuous histologically and usually more extensive (Figures 2b–d).[17,19,20] The morphologic point at which a p53 signature transitions to such a intraepithelial lesion is somewhat arbitrary and is governed chiefly by the appreciation of more conspicuous cytologic changes, including not only loss of cilia but greater epithelial pseudo-stratification, often with some nuclear enlargement.[9,10]

Microenvironment of the Distal Fallopian Tube

It is important to stress that small foci of *Tp53* mutations (p53 signatures), whereas occurring in a

high percentage of tubes, are not abundant in a given tube, indicating that loss of p53 function is an uncommon event over the lifetime of a given individual. Precisely where the genotoxic insult leading to loss of p53 function comes from is not entirely clear. One possibility is oxidants in follicular fluid post ovulation, which might explain why fimbrial mucosa, which is identical biologically to that in the more proximal tube but closer to the ovarian surface, is more susceptible.[21,22] Logically, the cells most vulnerable to transformation are those less mature, so-called secretory (or non-ciliated) cells. These have also been shown to be most vulnerable to DNA damage *in vitro*.[23]

Another variable that may be geographically unique is so-called 'epigenetic reprogramming,' alterations in the methylome unique to the distal fallopian tube. This has been proposed recently and is another argument for the unique susceptibility of the distal tube to serous carcinoma.[24]

Why is the BRCA+ fallopian tube susceptible to malignancy? The simplest explanation is that the combined functional loss of *p53* and *BRCA* (or related pathway) is a fundamental driving force in the genesis of high-grade serous carcinoma.[25] Given that the tubal cells in the woman with germline *BRCA1/2* loss contain only one functional allele, they may be particularly vulnerable to loss of *BRCA* function via genotoxic stress, which must be substantial in a particular cell to inactivate the entire *p53* locus. Although loss of both *BRCA* alleles should lead to cell death if *p53* is intact, the more likely scenario is loss of p53 function (relatively common) followed by a second insult inactivating the *BRCA* locus.

What if the patient is uniquely prone to loss of p53 function? Such a scenario can be witnessed in the fallopian tubes of women with Li Fraumeni syndrome. In this unique population, the fallopian tubes contain abundant p53 signatures, most manifesting as very short stretches of p53-positive cells.[26] The simplest explanation for these prevalent foci of p53 staining is that a single functioning allele is more easily inactivated, requiring less genotoxic damage. Why these individuals do not have an elevated risk for serous carcinoma is not clear, but conceivably the events that lead to loss of the second *p53* allele are not sufficient to inactivate both *BRCA* alleles in these patients. Still, STICs have rarely been encountered in tubes from these individuals (Carinelli, personal written communication).

Ultimately *p53* mutations link signatures to both intraepithelial neoplasms of the tube and serous carcinomas. Shared *p53* mutations have been shown in these precursors and adjacent neoplasms.[9,15,27] Other abnormalities have also been documented including altered telomeres.[28,29]

It is possible to envision that the probability of small genotoxic insults—leading to p53 signatures—is high, whereas insults must either be severe or repetitive to lead to a significant loss of function with immediate neoplastic growth. Still both likely occur, leading to a range of atypias in the tubes with different transit times to malignancy and metastasis. It should be emphasized that the genotoxic stimulus may not be the same in all individuals. We have occasionally seen multiple precursors in the fallopian tubes of some women with early serous carcinoma, raising the possibility that certain fallopian tubes are exposed to more severe genotoxic insults.

Generic Stem Cell Vulnerability

There are other molecular events taking place in the tubal mucosa that appear independent of the process of cell exposure to reactive oxygen species. These proliferations are neither linked to *p53* mutations nor confined to the distal fallopian tube but may emerge from the same cell as serous cancer precursors.[30] We have termed these proliferations secretory or stem cell outgrowths (SCOUTs). They share some attributes with serous cancer precursors.[31,32] Type I SCOUTs, have conspicuous ciliated differentiation (Figure 3a). Type II SCOUTs are distinctly endometrioid in appearance, limited ciliated cells and a more pseudostratified growth pattern and evidence of disturbances in expression in the Wnt pathway (Figures 3b and c). SCOUTs appear to be more common after menopause and are increased in women with serous carcinoma although not directly related.[33] Another study examining a similar entity in the fallopian tube termed secretory cell expansions (SCEs) found a similar association with serous carcinoma.[34] A number of studies have focused on the role of stem cells in this process and why this population is particularly vulnerable.[35]

Interception

This segment addresses three issues of particular interest to the practitioner, including (1) protocols for detecting serous tubal intraepithelial neoplasms and their realistic expectations, (2) Terminology for this spectrum and criteria for diagnosis, and (3) Reporting these findings in a manner that will facilitate management.

Detection

The SEE-FIM protocol was designed to provide for a systematic examination of the distal fallopian tube in women undergoing risk-reduction surgery for *BRCA*1 or *BRCA2* mutations (BRCA+). At least 70% of tubal cancers arise in the fimbriated portion, and nearly all will be found in the distal one-third of the tube. The SEE-FIM protocol entails multiple sagittal sections of the distal tube to increase the surface area examined, combined with 2 mm-thick sections of the remainder. This detailed sectioning protocol is

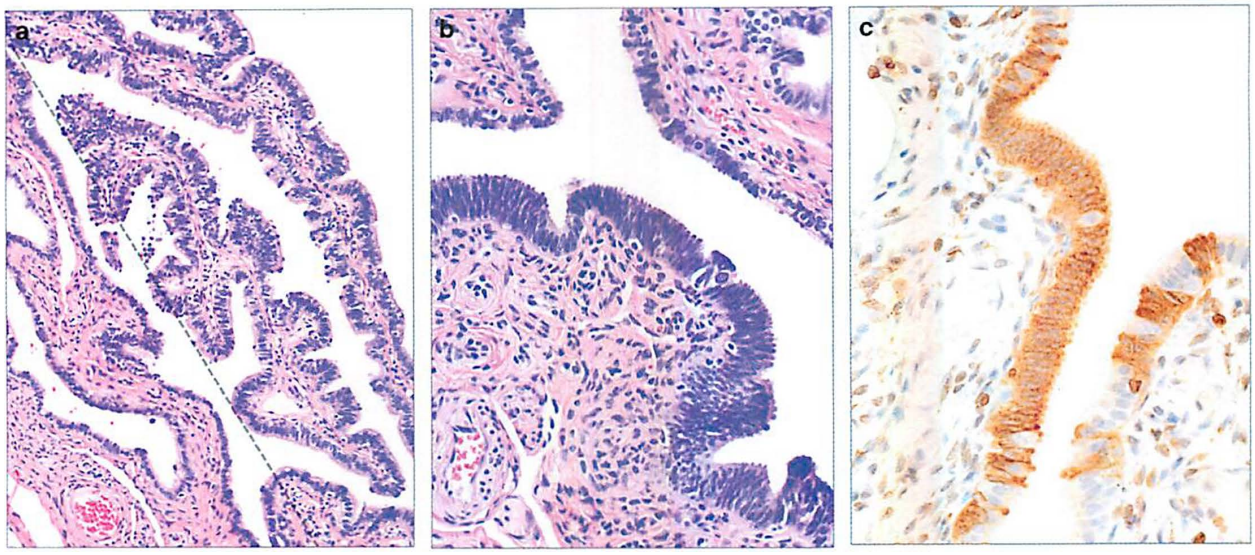

Figure 3 Stem cell outgrowths in the fallopian tube. (a) A type I stem cell outgrowth (right of dotted line) demonstrates a discrete pattern of ciliated differentiation. (b) A type II stem cell outgrowth has a distinctly endometrioid appearance. (c) A secretory cell expansion is similar and consists of a discrete linear population of secretory cells, highlighted here by immunostaining for BCL-2.

necessary to maximize the detection of early cancers in women at risk.[8] We also employ this protocol in all cases of uterine or ovarian cancer where the tubes can be identified, and this approach has identified early tubal carcinomas in women with both serous and endometrioid tumors in the uterus.[36–38]

Terminology and Its Implications

The term serous tubal intraepithelial carcinoma has been used in practice but there are several issues that remain in flux for both the practitioner and investigator. (1) The malignant potential (ie risk of serous cancer outcome) of incidentally discovered intraepithelial carcinoma is at this writing low, estimated a ~5% in BRCA+ women. (2) A series of incidentally discovered intraepithelial carcinomas and subsequent serous cancers have yet to be linked by a common mutation in *Tp53* to prove a temporal link between the two. (3) The malignant potential of tubal atypias falling short of intraepithelial carcinoma remains uncertain.[39] (4) Some serous tubal intraepithelial carcinomas discovered in the context of metastatic disease may not necessarily have originated from the fallopian tube. A tubal intraepithelial carcinoma found associated with serous carcinoma might be viewed as a *fait accompli*, assumed to be the origin of the tumor based simply on a shared mutation with the latter.[14,24] However, a similar sharing of mutations can be seen between serous carcinomas in the endometrium and tube, yet the direction of spread (from the tube or to the tube) is not entirely clear.[36,40] Moreover, the possibility that the tubal mucosa can be not only a source but also a destination of serous carcinoma is being increasingly recognized.[41] Thus when a diagnosis of intraepithelial carcinoma is made, the practitioner should view it in the context of whether the tubal lesion is isolated or a component of widespread disease.

Several terms have been proposed to classify lesions that fall between p53 signatures and tubal intraepithelial carcinomas, including tubal intraepithelial lesions in transition, serous tubal intraepithelial lesions and tubal epithelial atypias. We asked a group of experienced gynecologic oncologists to advise us on their preference for terminologies in a report (see 'Acknowledgments' section). Diagnoses such as serous tubal epithelial proliferation or serous tubal intraepithelial lesion were considered suitable to many but the addition of 'of uncertain significance' was preferred by many as it clarified the uncertainty of this diagnosis (see Table 2).

There are four reasons for including both serous tubal epithelial proliferations (or lesions) of uncertain significance and serous tubal intraepithelial carcinomas within the category of tubal intraepithelial neoplasia, at least for the purposes of study and follow-up.

(1) Both are often found in continuity (Figure 2c).
(2) Both share a similar age distribution and association with germline *BRCA* mutations
(3) The two cannot always be distinguished with certainty.
(4) In our experience serous tubal epithelial proliferations can be found adjacent to invasive serous carcinoma without an intervening intraepithelial carcinoma (Figure 2d), implying that invasion might occur directly from the lesion.
(5) There is the possibility that tubal epithelial proliferations could initiate a sequence of biologic

Table 2 Categories of tubal intraepithelial proliferations

Definition and setting	*Criteria*	*Diagnostic terminology*	*Implications*
Non-ciliated or minimal ciliated differentiation. Commonly found (30–50%) in tubes from all women.	1. Evidence of p53 mutation by immunohistochemistry. 2. Mild nuclear enlargement.	Benign serous tubal epithelial proliferation/ lesion	1. An early (latent) phase of serous carcinogenesis. 2. No increased risk of recurrence or subsequent serous carcinoma.
Altered and expanded epithelium with p53 mutation and a spectrum of atypia with preservation of cell polarity (Smooth luminal border, cohesive cell population, cilia or terminal bars present, preservation of a pseudostratified nuclear arrangement with uniform vertical orientation, and absence of vertical nuclear stacking). Found incidentally in salpingectomy specimens, in RRSOs, and in fallopian tubes of women with carcinoma.	1. Evidence of p53 mutation by immunohistochemistry. 2. Nuclear enlargement, increased N/C ratio. 3. Polarity is preserved 4. Ki-67 index is variably increased.	Serous tubal epithelial proliferation/lesion of uncertain significance	1. A serous ~~cancer precursor originating in the fallopian tube~~. 2. Uncertain, presumed minimal risk of a serous carcinoma outcome. 3. Can be associated with intraepithelial carcinoma, therefore serial sectioning encouraged. 4. May be a direct precursor to invasive serous carcinoma. 5. Role as precursor to extra-tubal serous carcinoma is unknown.
Altered and expanded epithelium with p53 mutation and a spectrum of atypia including loss of cell polarity (Irregular luminal border, loss of pseudostratified nuclear orientation with irregular vertical nuclear stacking, intraepithelial fractures, exfoliation) and/or marked atypia in a portion of the lesion. Found incidentally in salpingectomy specimens, and in fallopian tubes of women with serous cancer.	1. Evidence of p53 mutation by immunohistochemistry. 2. Nuclear enlargement, increased N/C ratio. 3. Polarity is lost. 4. Ki-67 index is variably increased.	Serous tubal intraepithelial carcinoma	1. A serous ~~cancer precursor originating in the fallopian tube~~, 2. Detected in 5–10% of BRCA+ women. 3. Outcome risk of ~~HGSC averages around 5%~~, ranges from 1–10%. 4. Consider genetic counseling (BRCA) if found incidentally. 5. Most institutions will not treat in the absence of invasion or local spread. 6. Peritoneal washings and possibly further staging with biopsies may be considered as clinically appropriate.
Intramucosal serous carcinoma in the absence of a lower grade proliferation. Found typically in the setting of disseminated HGSC and rarely incidentally as an isolated finding.	1. p53 mutation (IMPOX) 2. Nuclear enlargement, increased N/C ratio. 3. Polarity is lost. 4. Ki-67 index is variably increased. 5. No evidence of lower grade proliferation.	Serous tubal intraepithelial carcinoma	1. Replacement of mucosa with ~~HGSC~~. 2. ~~Site of origin~~ unclear in the absence of a lower grade proliferation, ~~particularly~~ in the setting of disseminated ~~HGSC~~.

Abbreviation: RRSO, risk reducing salpingo-oophorectomy.

events culminating in a serous carcinoma outside of the fallopian tube. This process, which we term precursor escape, would entail an initial genotoxic insult in the fallopian tube leading to a p53 signature or intraepithelial lesion, the cells of which could escape and emerge later in the pelvis as a more advanced neoplasm. This concept is under investigation.

The Risk-Reduction Salpingo-Oophorectomy

From 5–10% of BRCA+ women undergoing risk-reduction surgery will harbor an asymptomatic early serous neoplasm, and at least 80% of those neoplasms will be an intraepithelial carcinoma with either early invasion or spread to adjacent peritoneal surfaces.[6,8,17] In this population, many will be found to have intraepithelial carcinoma alone. It is important to emphasize that women who undergo risk-reduction surgery for a history of breast or ovarian cancer who do not have germline *BRCA* mutation seem to have a very low risk of intraepithelial carcinoma, approaching that of the general population.

The Symptomatic Woman with Uterine or Pelvic Epithelial Cancer

Approximately 40–60 percent of women with an extra-uterine serous carcinoma will harbor an intraepithelial carcinoma in the fallopian tube.[15,16] The precise percentage varies considerably, and it is impossible to obtain accurate information because (1) in many cases the fallopian tube is obscured by a tumor mass, (2) the tumor might overrun an intraepithelial carcinoma, and (3) a tumor could arise on the ovary within adhesions or endosalpingiosis ultimately derived from the fallopian

tube. (4) It is also possible that a lesion might be missed that was present on deeper levels.[43] However, in a study employing exhaustive serial sectioning, we found a serous tubal intraepithelial carcinoma in only 2 of 35 fallopian tubes initially ruled negative from women with serous carcinoma. What is obvious is that identifying a clear-cut origin in the ovary for serous carcinoma is more difficult than in the fallopian tube. Some feel strongly that serous carcinomas can arise from the ovarian surface epithelium, defined as a specialized mesothelial covering.[44,45] Another, more visibly tangible argument is that serous carcinomas arise from endometriosis, endosalpingiosis or a similar nidus of ectopic mullerian epithelium (secondary Mullerian system) in the ovarian cortex.[46,47] However, such associations, including reports of serous carcinomas arising in benign or borderline cystadenomas, are uncommon.[48,49] It should be noted however, that precursor lesions with *Tp53* mutations are rarely found in the ovarian cortex despite some earlier reports.[50–52] Moreover, Bell and Scully reported only 13 cases of 'early' serous carcinoma in their review of a large consultation practice.[53]

The Woman who is not at Risk and Undergoing Routine Salpingo-Oophorectomy

There is little information on the likelihood of encountering a serous tubal intraepithelial carcinoma in a routine specimen. One study noted a frequency of ~1 in 150.[54] In an analysis of cases seen at Brigham and Women's hospital over the 10-year period 2006–2015, the overall frequency of encountering intraepithelial carcinoma in any tubal specimen associated with anything other than a serous carcinoma of the ovary or endometrium was ~1 in every 500 cases; however, these included incidental cases discovered in women with endometrioid adenocarcinomas as discussed above.[35]

Histologic Criteria for Serous Tubal Intraepithelial Carcinoma

The histologic criteria for intraepithelial carcinoma are on one hand empiric, ie, they are dictated by prior experience in the uterus (with serous endometrial intraepithelial carcinoma).[55] On the other hand, they are corroborated to some degree by several biomarkers that correlate with serous cancer, most notably, p53, p16, Stathmin, Cyclin E, Ki-67, and others.[9,19,56] However, a lack of precision in terms of both information on natural history and biomarker specificity hamper efforts to achieve a perfect separation of proliferations of unknown significance from intraepithelial carcinomas.

(1) The entire spectrum from p53 signature to intraepithelial carcinoma exhibits altered p53 expression.

(2) Biomarkers positive in intraepithelial carcinomas might also be positive in earlier precursors. STMN1 is sometimes up-regulated in some SCOUTs.[31] Proliferation (MIB1) indices between serous tubal epithelial proliferations and intraepithelial carcinomas overlap. Cyclin E and p16 are up-regulated in intraepithelial carcinomas but not always.[26] It is fair to say that: (a) pathologists cannot consistently separate an intraepithelial lesion from an intraepithelial carcinoma with biomarkers, (b) most of these lesions, whether they be intraepithelial proliferations of uncertain significance or intraepithelial carcinomas, will not be followed by a pelvic serous carcinoma.

The proliferative index is increased relative to the normal mucosa. Some have given an overall proliferative index of >10% to be frequently associated with intraepithelial carcinoma.[39] Another approach has been to record proliferative index based on the maximum proliferative index in a given portion of the lesion.[10] The purpose for doing this is the assumption that the most proliferative region carries the most biologic significance. However, this is unproven and irrespective of the approach used, a specific cutoff value does not exist as a stand-alone parameter to distinguish intraepithelial lesion from intraepithelial carcinoma. Strong staining with both stathmin 1 and p16 supports a diagnosis of STIC in the context of the above findings and may be helpful, but such staining patterns can be found in lower grade lesions.[31] Additional markers that have been proposed include Rsf-1, LAMC1, and fatty acid synthase.[57,58]

Examples of intraepithelial carcinomas are shown in Figures 1a–d and we pay attention to the following:

(1) Epithelial thickness: The epithelium is virtually always multilayered although the thickness can be highly variable.
(2) Loss of normal cell to cell orientation (polarity): in a pure non-ciliated population of neoplastic cells, loss of polarity may take the form of small micropapillae (Figures 1a and d, arrows) and un-oriented stratified cells with stacking of rounded nuclei in contrast to a more uniform interdigitated population of elongated nuclei (Figure 1b, arrows). This is in contrast to lesser proliferations (Figure 2c). Small clusters of cells near the epithelial surface may become separate from the group and in some instances a pronounced horizontal intraepithelial fracture is present (Figure 1d, arrows).[59]

In normal epithelium, ciliated differentiation can occur throughout the epithelium and can be associated with epithelial stratification and multi-layering, features that in a pure non-ciliated epithelium might be a cause for concern (Figures 4a and b). Uncommonly, intraepithelial lesions with loss of *Tp53* function will still manifest with ciliated differentiation, creating some difficulty in determine

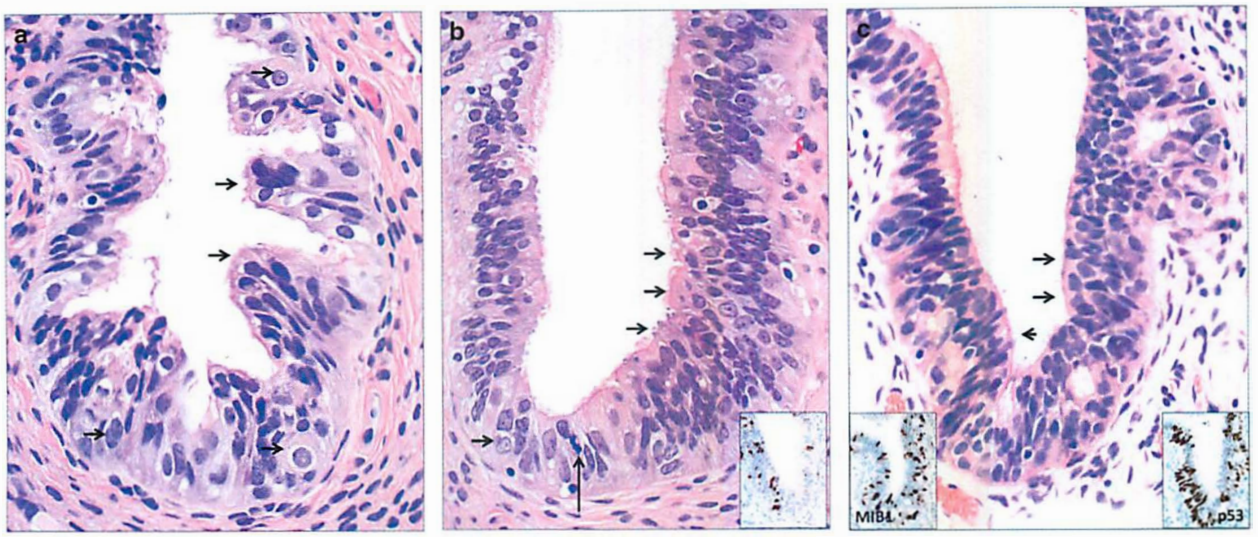

Figure 4 Potential difficulties in interpreting polarity in the context of multilayered epithelium. (a) Benign epithelium is multilayered but terminates in a smooth luminal border with ciliated differentiation (arrows). (b) An adjacent serous epithelial proliferation (or lesion) of uncertain significance with mild atypia and multilayered epithelium. This was positive for abnormal p53 expression. Note the presence of some nuclear enlargement and nucleoli and a mitotic figure (long arrow); however, the luminal surface is uniform with normal differentiation (cilia; arrows). (c) Another multilayered tubal epithelium with strong p53 staining and moderate MIB1 staining (insets). Note however, the abundant cilia on the hematoxylin and eosin stained section, corresponding to the absence of p53 immunostaining in the inset. We would classify this as a serous tubal epithelial proliferation (or lesion) of uncertain significance, with a comment that it does not fulfill the criteria for an intraepithelial carcinoma. Cases like this underscore the range and complexity of serous cancer precursors that can be encountered in the fallopian tube and the care that must be taken in classifying problematic proliferations.

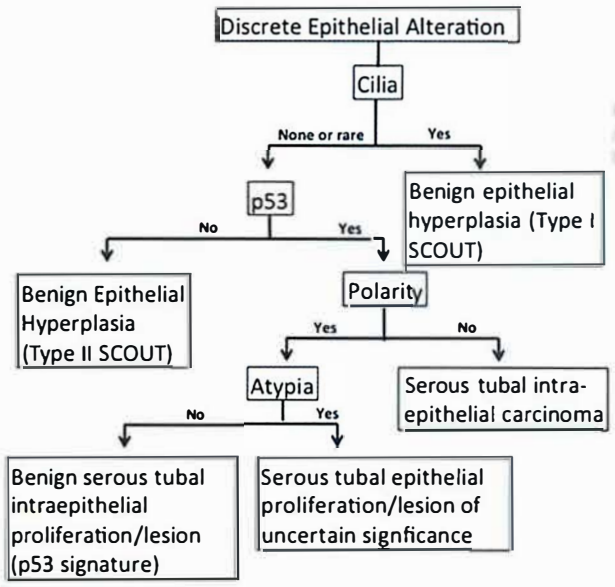

Figure 5 A decision tree for unusual tubal intraepithelial proliferations. *Altered epithelium is defined simply as epithelium that contrasts with the background tubal epithelium, such as discrete multilayered epithelium, monomorphic single and multilayered populations, or stretches of epithelium with loss of cilia etc. **Nuclear changes pertain to nuclear enlargement, nuclear molding, nuclear crowding etc. Note that given the morphologic 'plasticity' of the tubal epithelium, contrasting nuclear changes may still not by themselves signify a precursor or early serous carcinoma.

whether polarity is disrupted (Figure 4c). In general, cilia should be scarce in intraepithelial carcinomas because they signify differentiation. When encountered they must be carefully evaluated and their presence weighed against the other findings when considering a diagnosis of intraepithelial carcinoma. Ciliated cells will not stain for p53, however ciliated differentiation is sometimes seen in lower grade lesions. Because a lower grade lesion could immediately precede an invasive carcinoma (Figure 3d, arrows), careful sectioning is needed to ensure that invasion has not occurred.

Management

Once the pathologist has identified a tubal intraepithelial neoplasm, he or she must render a diagnosis and provide information helpful to the clinician. Table 2 provides a variety of terminologies that might be used in practice. A diagnosis of tubal intraepithelial carcinoma need not be accompanied by an estimate of recurrence risk but referral to a gynecologic oncologist is encouraged. A diagnosis of a tubal proliferation of undetermined significance should come with an assessment of the level of atypia and why it is not being classified as an intraepithelial carcinoma. If there is uncertainty as to the classification this should be conveyed as well.

One issue that remains unclear is the staging of serous carcinomas, specifically the role of the fallopian tube. Currently staging is done with the tube in mind but does not rely on defining the source, an approach the authors support.[60] Others have made an effort to assign probable origin based on pathologic parameters and estimate likelihood that the carcinomas originated in the fallopian tube

based on an algorithm. The relevance of this algorithm to management remains unclear at this point.[61]

The differential diagnosis of serous tubal intraepithelial carcinoma includes a range of proliferations (Figure 5). These are discussed in detail in a number of publications. An important feature distinguishing most is the presence of normal immunostaining for p53, which largely excludes a serous cancer precursor.[19,32,52]

Intervention

In the past 15 years pathologists have had a critical role in driving the shift in our understanding of the origins of serous carcinoma, mostly through the painstaking analysis of pathologic specimens and progressive attention paid to the distal fallopian tube. With the close attention to the distal fallopian tube in routine practice the pathologist creates the opportunity to intercept early serous carcinomas. The ultimate significance of this discovery for the individual patient is unclear, but when intraepithelial carcinomas are discovered, it is reasonable to assume that some of these individuals will harbor germline *BRCA* mutations. Thus, testing should be offered to these patients, and the chance discovery of a STIC could therefore benefit additional persons in the family of the affected patient. The more widespread benefit of these discoveries pertains to the use of 'opportunistic' salpingectomy, the removal of the fallopian tubes in women who are undergoing surgery for benign disorders.[12] Estimates based on retrospective studies project that the risk of ovarian cancer would be reduced by ~50% in those who have had their tubes removed.[62] The American College of Obstetricians and Gynecologists and Society of Gynecologic Oncologists as well as many international groups have now issued policy statements encouraging physicians to have a dialog with their patients about undergoing this procedure during routine surgery with the expectation that it will reduce their risk of serous carcinoma.[63,64] This approach has received more urgent attention as serologic screening programs have been deemed to be of no real value.[65] Additional studies are being planned that extend opportunistic salpingectomy to the BRCA+ population as a temporary measure to reduce risk while allowing for normal ovarian function for a period of time.[66] At this point this approach would only be offered to BRCA+ women who have refused oophorectomy despite counseling due to its undesirable side effects. It is supported by the fact that a very high percentage of early high-grade serous carcinomas are found in the fallopian tube(s).[11,12] The pervasive caveat is the fact that many advanced serous carcinomas do not have a coexisting intraepithelial carcinoma.[67] Other caveats, including the possibility that not all intraepithelial carcinomas are primary lesions, are listed in Table 1. This does not mean that salpingectomy will not be effective, but it does underscore the need to continually re-evaluate serous carcinomas from multiple perspectives, to identify subsets with different categories and to firmly establish their natural history and to determine if more than one pathway is involved.[68,69] Regardless, the progress achieved in this field in identifying site of origin in the past 20 years since the discovery of the *BRCA* genes has been transformative and will hopefully result in a significant reduction in the death rate from this disease.

Acknowledgments

We express our gratitude to the following for helpful discussions: T. Rinda Soong, MD, PhD, Jonathan S. Berek, MD, Ross S. Berkowitz MD, Kevin Elias, MD, Panagiotis Konstantinopoulus, MD PhD, Colleen Feltmate, MD, Barbara A. Goff, MD, Michael G. Muto, MD, William A. Peters III, MD. This work was funded by a grant from the Department of Defense (W81XWH-14-1-0504, to CPC).

Disclosure/conflict of interest

The authors declare no conflict of interest.

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Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com

Frequency of "incidental" serous tubal intraepithelial carcinoma (STIC) in women without a history of or genetic risk factor for high-grade serous carcinoma: A six-year study☆

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ARTICLE INFO

Article history:
Received 10 January 2017
Received in revised form 19 April 2017
Accepted 21 April 2017
Available online xxx

Keywords:
Serous carcinoma
Fallopian tube
Serous tubal intraepithelial carcinoma

ABSTRACT

Objective

The purpose of this study was to determine the prevalence of incidentally discovered serous tubal intraepithelial carcinoma in women without a genetic risk for or history of high grade serous carcinoma (HGSC) in the gynecologic tract.

Methods

All pathology reports at our institution that included bilateral salpingectomies from January 2006–December 2011 were examined in women > 50 years old in which the entire tube or the distal one-third was examined histologically with the complete (proximal and distal fallopian tube) or modified (distal one third of the tube) SEE-FIM protocol. Cases were divided into: Group 1, a history of or known risk factors (BRCA1 or BRCA2 mutations) for HGSC and Group 2, those without these attributes for whom a STIC would be unexpected (incidental). Women undergoing unspecified "risk-reducing" procedures were included in Group 1.

Results

Of 4051 identified total, 2268 had complete examination of the distal fallopian tube and were age 50 or above. Of these, 1747 were in group 2. Two STICs were identified (0.1%), one associated with a grade 2 endometrial endometrioid adenocarcinoma and one with a low-grade ovarian serous carcinoma in the setting of a serous borderline tumor.

Conclusions

Incidental STICs in women over age 50 are uncommon. However, the significance of lesser tubal atypias (0.3% in this study), risk of STIC in women with no epithelial pathology and the risk imposed by coexisting endometrioid neoplasia are unclear and require further study.

1. Introduction

Extra-uterine high grade serous carcinoma (HGSC) is the most common variant of pelvic cancer in women and over 95% of patients present with high stage (II or greater) disease, including the long-term survivors [1]. As a consequence, attention is now heavily focused on therapies and interventions aimed at prevention. Due to research on early serous carcinogenesis in the last 10–15 years, many cases of HGSC are now believed to arise from the distal fallopian tube in the form of serous tubal intraepithelial carcinoma (STIC) [2–5]. Thus, in addition to prophylactic salpingo-oophorectomy, which is a powerful risk-reducing intervention for women with germ line BRCA1 or BRCA2 mutations, [6,7] [8,9] the Society of Gynecologic Oncology (2013) and American College of Obstetricians and Gynecologists (2015) have suggested that opportunistic salpingectomy be considered at the time of hysterectomy for "average-risk" (alternatively "low-risk") women after the completion of child bearing to reduce the risk of a later HGSC [10,11]. The success of opportunistic salpingectomy is predicated on the idea that this surgery will prevent the development of an intraepithelial precursor to HGSC or intercept a precursor before spread outside of the tube.

To have an impact on serous cancer incidence, opportunistic salpingectomy theoretically either removes precursors that would have progressed to HGSC or, by removing the fallopian tubes, preempts the tubal serous carcinogenic sequence entirely. Germane to both goals is the prevalence of STICs in women who are not considered at

☆ Supported by a grant from the Department of Defense (OC130500 to CPC).

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http://dx.doi.org/10.1016/j.ygyno.2017.04.015
0090-8258/

high risk for HGSC. The prevalence has not been established in large studies but at institutions where complete microscopic examination of the distal fallopian tube has been implemented (SEE-FIM protocol), it is estimated that the frequency of STIC in these women is between 0.6 and 1.1% [12–16].

The purpose of this study was to determine the frequency of STICs discovered incidentally in women not considered at high risk for HGSC and whose entire distal fallopian tubes had been submitted for pathologic examination. The study population encompassed a 6 year span from 2005 to 2011.

2. Materials & methods

This study was approved by the Institutional Review Board at Brigham and Women's Hospital. Anatomic pathology reports during the period January 2006 to December 2011 were screened to identify all cases with, at minimum, bilateral salpingectomies from women who were > 50 years old (n = 4051). This age cut-off was selected 1) to reflect a conservative estimate of the completion of childbearing and 2) to collect patients at a time when risk of HGSC begins to increase while excluding young women at extremely low-risk of HGSC.

Surgical pathology reports were manually reviewed for gross descriptions with documentation of the presence of the fallopian tubes and/or fimbriae and microscopic keys with sections specifically designated as entirely submitted fimbriae, distal one-third of the fallopian tube, or fallopian tube. Cases were retained in the study if bilateral distal fallopian tubes were grossly identified and entirely submitted for microscopic examination (n = 2286). Unilateral salpingectomy cases were excluded, even if the fallopian tube was completely examined, as absence of STIC in the contralateral fallopian tube could not be documented. Diagnoses were collected from surgical pathology reports and were used to classify cases into one of five categories: 1) benign (leiomyoma, uterine prolapse, etc.), 2) endometrioid neoplasia (of endometrium or ovary) including endometrial intraepithelial neoplasia, 3) HGSC of the endometrium, adnexa, or peritoneum, 4) other non-HGSC neoplasms (including low grade serous carcinoma), and 5) prophylactic procedures for high-risk women (RRS or RRSO).

For the purposes of this study, The subjects were divided into two groups. Group 1 consisted of categories 3 and 5 above, women at risk for HGSC or who had been diagnosed with HGSC. Group 2 consisted of groups 1, 2, and 4. In these patients the presence of STIC would normally be unexpected or "incidental".

All diagnoses of STIC and tubal epithelial atypia for which a diagnosis of STIC was considered but excluded were identified from pathology reports. A diagnosis of STIC required 1) a multilayered non-ciliated epithelium, 2) increased nuclear to cytoplasmic ratio, 3) loss of cell polarity with irregularly arranged nuclei in the vertical plane. Strong or completely absent p53 staining was present and the proliferative (MIB-1) index was increased, albeit variably. Additionally, tubal/fimbrial sections from 5% of women without a diagnosis of STIC were re-reviewed to confirm the original pathologic interpretation.

3. Results

3.1. Study population

During the 6-year study period, 4051 cases including bilateral salpingectomies from women > 50 years of age were identified; 2268 cases were over age 50 and had documented complete gross and microscopic examination of the distal fallopian tube and were retained in the study. Of these, 1747 (77.0%) cases were in Group 2 and had no history of risk for or a diagnosis of HGSC [Fig. 1].

STIC is only rarely encountered as an "incidental" finding in women without the presence of or risk for HGSC.

Eighty STICs were encountered in this study, 78 of which were identified either from risk reducing salpingo-oophorectomies or cases with advanced HGSC. Two STIC were identified incidentally in 1747 women in Group 2 (0.1 One was associated with a low grade serous carcinoma of the ovary and coexisting bilateral borderline serous tumors and the other with a grade 2 endometrial endometrioid adenocarcinoma. [Fig. 2].

The clinical management and follow-up of both LRW found to have incidental STIC during the six year study period was dictated by the concurrent neoplastic process (ie. low grade serous carcinoma or endometrial endometrioid adenocarcinoma). [Table 1] In neither case was an elevated CA125 documented prior to surgery. (See Table 2.)

4. Discussion

Beginning in 2000, a progressive accumulation of data has implicated the distal fallopian tube as a site of origin of HGSC by iden-

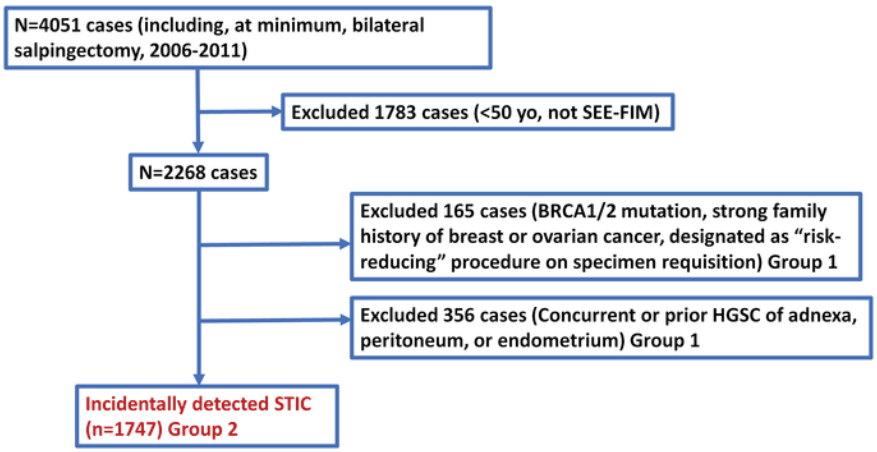

Fig. 1. Breakdown of cases and groupings. Group 1 cases were at increased risk for an associated STIC. STICs were not expected to be found with increased freqency in Group 2 cases and were designated as "incidental" in these patients.

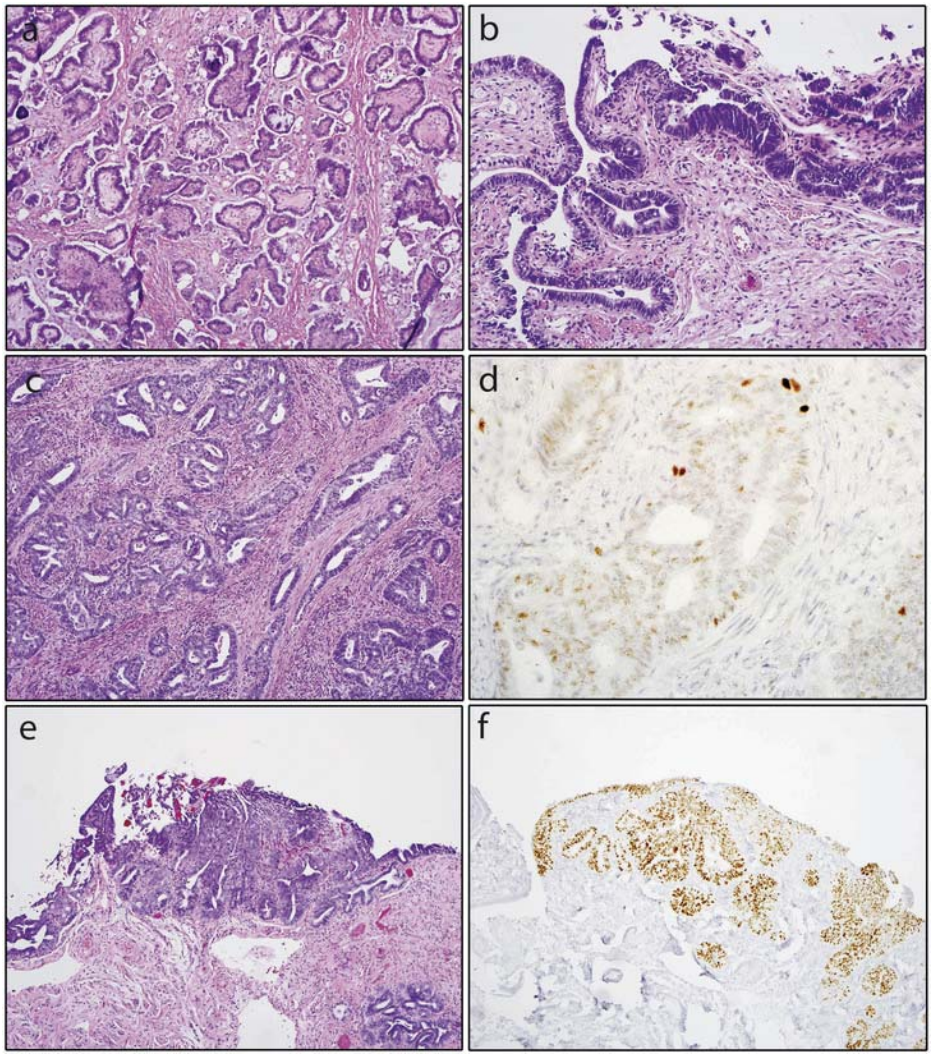

Fig. 2. a) Low grade serous carcinoma of the ovary associated with b) STIC of the distal fallopian tube. C) Endometrial endometrioid adenocarcinoma, grade 2, with d) weak, heterogenous staining for p53 by IHC. In contrast, e) a small focus of serous carcinoma associated with STIC in the distal fallopian tube showed f) strong, diffuse staining in keeping with p53 mutation.

Table 1
Clinical parameters of all patients with incidental tubal serous neoplasia. The two cases above the shaded bar were from the original study (2006–2011). Those below the bar were accrued later (2012–2015).

Age	Indication	Concurrent diagnosis	Serous lesion	Peritoneal cytology	Follow-up
51	Ovarian masses	Low-grade ovarian serous carcinoma	STIC	Negative	24 months NED
72	EMB: EMCA	G1 EMCA	STIC	Negative	42 months NED
67	EMB: EMCA	G1 EMCA	STIC	None	None
55	EMB: EMCA	G1 EMCA	STIC HGSC on ovary (stage IIa)	Suspicious for carcinoma	28 months NED.
71	Pelvic mass	Mucinous cystadenofibroma	R&L STIC, HGSC on ovaries (stage IIIa)	Positive (carcinoma)	10 months NED.
64	Pelvic mass	Mucinous cystadenoma	STIC	Negative	5 months NED

EMB = endometrial biopsy; EMCA = endometrioid endometrial adeocarcinoma; G1 = FIGO grade 1; STIC = serous tubal intraepithelial carcinoma; HGSC = high grade serous adenocarcinoma; NED = no evidence of disease.

Table 2
Clinical and pathologic data.

Age	Indication	Concurrent diagnosis	Serous lesion	Peritoneal cytology	Follow-up
51	Ovarian masses	Low-grade ovarian serous carcinoma	STIC	Negative	24 months NED
72	EMB: EMCA	G1 EMCA	STIC	Negative	42 months NED
67	EMB: EMCA	G 1 EMCA	STIC	None	None
55	EMB: EMCA	G 1 EMCA	STIC HGSC on ovary (stage IIa)	Suspicious for carcinoma	28 months NED.
71	Pelvic mass	Mucinous cystadenofibroma	R&L STIC, HGSC on ovaries (stage IIIa)	Positive (carcinoma)	10 months NED.
64	Pelvic mass	Mucinous cystadenoma	STIC	Negative	5 months NED

tification of precursors in tubal epithelium [2–5]. As a consequence, in addition to clinical management guidelines recommending salpingo-oophorectomy for women with germ-line mutations in BRCA1 or BRCA2, recommendations have been published for opportunistic salpingectomy as a cancer preventive in women who are not at increased risk for ovarian cancer [9–11].

Beginning in January of 2005, we instituted the SEE-FIM protocol at Brigham and Women's Hospital, based on the hypothesis that the distal fallopian tube was the most vulnerable site for the onset of precursors to HGSC [12]. Later that year, Cass and colleagues reported that most fallopian tube carcinomas arose in or near the fimbria [17]. Subsequently, Medeiros et al. and Callahan et al. reported that virtually all *incidentally discovered* HGSCs in RRSOs from women at genetic risk were found in the distal fallopian tube, and Kindelberger et al. showed that over 40% of women with HGSC had evidence of STIC in their distal fallopian tube [3,12,18]. Thus, in our practice, the SEE-FIM protocol or a protocol in which the entire distal fallopian tube was submitted is followed in all salpingectomy specimens.

The purpose of this study was to assess the prevalence of STIC in a setting where they are detected "incidentally", i.e. in specimens from patients where there was no expectation of an increased detection risk. In assessing the statistical information we emphasize several potential variables that would influence detection rate in this population. First, in every case in this study ($n = 1747$) at least the distal one-third of the fallopian tube was examined, often but not always with the entire proximal tube. It is conceivable that expansion of the sectioning protocol to include the entire proximal two-thirds of the fallopian tube segments in every case might have increased the odds of identifying incidental STICs. However, we emphasize that the likelihood of uncovering a significant number of STICs in the proximal segments would be low. Second, tubes were not exhaustively sectioned to uncover small lesions that might escape notice with routine sectioning. We have found that exhaustive sectioning of "negative" fallopian tubes from women with HGSC will uncover a STIC in 6% (Soong R, unpublished data). This will slightly increase the overall percentage with STIC but only a few percentage points. Third, we excluded women under age 50 years from the study for three reasons: 1) women under age 50 with no known increased risk have a very low likelihood of either HGSC or STIC and are effectively a different population in terms of risk, 2) in our consultation practice where we have received 52 cases of STIC or atypias for review in approximately 10 years; 10 were under age 50 and 6 of these were found to have mutations in BRCA1 or BRCA2, and thus would not have been included in the study group, and 3) in our surgical practice, no STIC has yet to be discovered in a women under age 50 who were not at high risk.

This study comprehensively examines the yield of 6 years of this protocol and represents the largest examined cohort of women who were not deemed high risk. The frequency of STIC in this population is estimated to be one per every 873 cases, which is lower than estimates from all previously published studies. We also performed a preliminary analysis of cases accessioned between January 2012 and December 2015 from women age 50 or over including bilateral salpingectomies by searching our pathology database and identified an additional 4 STICs. Based on the total case volume in this interval we estimate these 4 STICs occurred in an estimated cohort of *approximately* 1150 or 1 per 287 cases. The associated pathology included a mucinous cystadenoma, mucinous cystadenofibroma, and two cases of endometrial endometrioid adenocarcinoma. The estimated frequency of STICs and the circumstances under which they were discovered in this second group make two important points. First, the frequency will vary as a function of the time frames involved. Second, all of the cases of STIC identified in this study in women in group 2 were undergoing staging procedures for ovarian masses or concurrent epithelial neoplasia. *None were identified in women who would fall into the group commonly assumed to have undergone "opportunistic" salpingectomy* during a procedure for benign conditions. The absence of incidental STICs in women undergoing surgery for indications other than suspected neoplasia might be explained in part by two variables: 1) women who underwent morcellation procedures in which tubes could not be optimally examined and were excluded, and 2) referral bias, with a gynecologic surgery practice heavily weighted toward suspected oncology cases. Still, the frequency of incidental STIC in these women appears particularly low and remains to be further defined. Still, the question remains as to what percentage of women without any gynecologic epithelial neoplasia that can be expected to develop a tubal precursor. A related question is whether lesser atypias containing p53 mutations that do not fulfill the criteria for STIC (6/1747 or 0.3% from 2006 to 2011 in this study) impose any longitudinal risk of HGSC later in life.

The second observation (with caveat) was the finding of STICs in women with concurrent endometrioid neoplasia. Concurrent endometrioid and serous tumors are presumed to arise via independent molecular pathways and cells of origin [5,19]. Coexistence of these two tumor types is considered rare, confirmed in this study where STIC was discovered in only 0.2% of endometrioid neoplasms. However, other studies have suggested an association between STIC and endometrioid neoplasia [16,20]. Moreover, we have identified an association, albeit unclear, between benign proliferations with a "clonal" appearance (stem cell outgrowths (SCOUTs)) in the tube - some with endometrioid histology - and HGSC [21,22]. Thus it is conceivable that a small but unique subset of women have a vulnerability to both endometrioid and serous cancer precursor pathways or that both pathways share some yet to be clarified biologic underpinning. It does not appear to be via a mismatch repair defect based on the normal findings in two of the tumors.

In summary, the frequency of "incidentally" discovered STIC in is low in postmenopausal women, particularly in women with no gynecologic epithelial neoplasms. The extent to which this will inform our understanding of the efficacy of opportunistic salpingectomy will hinge on a clearer vision of how the serous carcinogenic sequence develops and the percentage of HGSCs that can be attributed to STICs.

Conflict of interest

The authors have no conflicts of interest.

Uncited references

[23–26]

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USAMRMC Office of Research Protections
Human Research Protection Office
Continuing Review Submission Form

Protocol Title	"Serous Cancer Precursor Initiation and Progression in the Distal Fallopian Tube"
Submitted By	Dr. Christopher P. Crum, Brigham and Women's Hospital, Boston, MA
Supporting Proposal	"The Oviduct and Serous Cancer Risk Assessment"
Submitted By	Dr. Christopher P. Crum, Brigham and Women's Hospital, Boston, MA
Proposal/Study Number	OC130500
Award Number	W81XWH-14-1-0504
HSRRB Log Number	A-18216

This form is required to be submitted with current continuing review documents to the U.S. Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO). Please complete the following questions to determine the appropriate documents to submit to HRPO.

1. Have any of the following study-related events occurred during the continuing review period AND have not been reported to HRPO:

   Y N

   _ N Major/Substantive modifications to the research protocol and any modifications that could potentially increase risk to volunteers, during the continuing review period.

   Note: The USAMRMC ORP HRPO defines a substantive modification as a change in Principal Investigator, change or addition of an institution, change to the IRB, elimination or alteration of the consent process, change to the study population that has regulatory implications (e.g. adding children, adding active duty population, etc), significant change in study design (i.e. would prompt additional scientific review) or a change that could potentially increase risks to subjects.

   _ N Suspensions or terminations of the research by the IRB, institution, Sponsor, or regulatory agencies.

   _ N Unanticipated problems involving risks to subjects or other (UPIRTSO). Copies of the event(s) description and documentation of IRB review if a UPIRTSO occurred and was not submitted to HRPO previously.

   Note: UPIRTSOs are defined as problems/events that are:

   - Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
   - Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
   - Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

2. If you checked "No" to all items in question 1:

   a. Submit a copy of the local IRB approval letter for the continuation of the study to HRPO along with this completed form.

b. If there was a lapse in approval of more than 1 day, explanation and confirmation that no study-related procedures occurred during the lapse.

c. If the research is conducted outside of the United States and/or involves children as a study population, please submit all documents listed under item number three.

d. Skip to number 4.

3. **If you check "Yes" to any of the items in question 1, submit the following additional documents and information to HRPO.**

_ Continuing review summary report that was submitted to you IRB.

_ Local IRB approval letter with next expiration date.

_ If there was a lapse in approval of more than 1 day, explanation and confirmation that no study-related procedures occurred during the lapse.

_ Current copy of protocol.

_ Current (stamped) consent form if applicable.

_ Total number of subjects enrolled in the study. Please indicate if there has been an increase in approved enrollment numbers.

_ Substantive Amendment(s). Copies of amendment submission forms, amendment approval letters, and all revised and tracked documents, if a substantive amendment was approved by the IRB and not submitted to HRPO previously.

_ Unanticipated problems involving risks to subjects or others (PIRTSO). Copies of the event(s) description and documentation of IRB review if a UPIRTSO occurred and was not submitted to HRPO previously.

_ Documentation of suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the sponsor, or regulatory agencies.

4. **Signature of Principal Investigator** 

5. **Date (DDMMYYYY)** 31102017

6. **Name of individual to contact with questions regarding this submission** Helen Wong

7. **Contact phone number** (857) 282-1708 **Email** hwong3@partners.org

PARTNERS HEALTHCARE
FOUNDED BY BRIGHAM AND WOMEN'S HOSPITAL AND MASSACHUSETTS GENERAL HOSPITAL

Partners Human Research Committee
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Continuing Review: Notification of IRB Approval/Activation
Protocol #: 2009P002281/PHS

Date: November 1, 2017

To: Christopher P Crum, MD
BWH
Pathology

From: Partners Human Research Committee
399 Revolution Drive, Suite 710
Somerville, MA 02145

Title of Protocol:	The oviduct and serous cancer risk assessment
Version Date:	10/26/2009
IRB Continuing Review #:	8
IRB Review Type:	Expedited
Expedited Category/ies:	(5)
IRB Approval Date:	10/31/2017
Approval Activation Date:	11/1/2017
IRB Expiration Date:	10/31/2019

This project has been reviewed by PHS IRB . During the review of this project, the IRB specifically considered (i) the risks and anticipated benefits, if any, to subjects; (ii) the selection of subjects; (iii) the procedures for obtaining and documenting informed consent; (iv) the safety of subjects; and (v) the privacy of subjects and confidentiality of the data.

Please note that if an IRB member had a conflict of interest with regard to the review of this project, consistent with IRB policies and procedures, the member was required to leave the room during the discussion and vote on this project except to provide information requested by the IRB.

Ongoing research limited to the use of excess human material and related information.

As Principal Investigator, you are responsible for ensuring that this project is conducted in compliance with all applicable federal, state and local laws and regulations, institutional policies, and requirements of the IRB, which include, but are not limited to, the following:

1. Submission of any and all proposed changes to this project (e.g., protocol, recruitment materials, consent form, status of the study, etc.) to the IRB for review and approval prior to initiation of the change(s), except where necessary to eliminate apparent immediate hazards to the subject(s). Changes made to eliminate apparent immediate hazards to subjects must be reported to the IRB as an unanticipated problem.
2. Submission of continuing review submissions for re-approval of the project prior to expiration of IRB approval and a final continuing review submission when the project has been completed.

Partners Human Research Committee
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3. Submission of any and all unanticipated problems, including adverse event(s) in accordance with the IRB's policy on reporting unanticipated problems including adverse events.
4. Obtaining informed consent from subjects or their legally authorized representative prior to initiation of research procedures when and as required by the IRB and, when applicable, documenting informed consent using the current IRB approved consent form(s)with the IRB-approval stamp in the document footer.
5. Informing all investigators and study staff listed on the project of changes and unanticipated problems, including adverse events, involving risks to subjects or others.
6. When investigator financial disclosure forms are required, updating your financial interests in Insight and for informing all site responsible investigators, co-investigators and any other members of the study staff identified by you as being responsible for the design, conduct, or reporting of this research study of their obligation to update their financial interest disclosures in Insight if (a) they have acquired new financial interests related to the study and/or (b) any of their previously reported financial interests related to the study have changed.

The IRB has the authority to terminate projects that are not in compliance with these requirements.

Questions related to this project may be directed to Georgia Washington, gwashington@partners.org, 857-282-1906.