217 Gynecologic Familial Cancer Syndromes: What the Practicing Pathologist Needs to Know

Teri Longacre MD
Ann Folkins MD

2011 Annual Meeting – Las Vegas, NV

AMERICAN SOCIETY FOR CLINICAL PATHOLOGY
33 W. Monroe, Ste. 1600
Chicago, IL 60603

217 Gynecologic Familial Cancer Syndromes: What the Practicing Pathologist Needs to Know

This session provides the key tools to interpret morphologic, immunohistochemical, and molecular diagnostic tests in the assessment of gynecologic specimens associated with BRCA1/2 germline mutations and Lynch (Hereditary Nonpolyposis Colorectal Cancer) syndrome. By the end of the session, the participant will be able to identify key components in hereditary ovarian and endometrial cancer screening and risk reduction programs, including indications for testing, chemoprevention, surveillance, and risk reducing surgery. Cases will demonstrate appropriate site-specific screening algorithms. There will be emphasis on interpretation of immunohistochemical mismatch repair protein deficiency, microsatellite instability, MLH1 methylation, and BRAF mutation analysis. The participant will be able to implement proper gross prosection techniques for risk-reducing salpingo-oophorectomy and hysterectomy specimens, recognize gross and clinically inapparent tubal lesions, and reproducibly diagnose microscopic intraepithelial tubal, ovarian, peritoneal, and uterine lesions. The participant will also be able to list and recognize the common mimics of early tubal carcinoma and recognize other, less common, hereditary syndromes of the gynecologic tract.

- Actively participate in ovarian and endometrial cancer screening and risk reduction programs, including indications for testing, chemoprevention, surveillance, and risk reducing surgery.
- Assess for hereditary ovarian cancer risk; this will include utilization of proper prosection techniques for risk-reducing salpingo-oophorectomy specimens, ability to recognize gross and clinically inapparent tubal lesions, and reproducibly diagnose microscopic intraepithelial lesions. The participant will also be able to list and recognize the common mimics of early tubal carcinoma.
- Understand and interpret current morphologic, immunohistochemical, and molecular diagnostic tests to assess Lynch syndrome risk in patients with endometrial cancer using appropriate site-specific algorithms.

FACULTY:

Teri Longacre MD
Ann Folkins MD

Practicing Pathologists
Surgical Pathology
Surgical Pathology (Derm, Gyn, Etc.)
2.0 CME/CMLE Credits

Accreditation Statement: The American Society for Clinical Pathology (ASCP) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education (CME) for physicians. This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME).

Credit Designation: The ASCP designates this enduring material for a maximum of *2 AMA PRA Category 1 Credits*™. Physicians should only claim credit commensurate with the extent of their participation in the activity. ASCP continuing education activities are accepted by California, Florida, and many other states for relicensure of clinical laboratory personnel. ASCP designates these activities for the indicated number of Continuing Medical Laboratory Education (CMLE) credit hours. ASCP CMLE credit hours are acceptable to meet the continuing education requirements for the ASCP Board of Registry Certification Maintenance Program. All ASCP CMLE programs are conducted at intermediate to advanced levels of learning. Continuing medical education (CME) activities offered by ASCP are acceptable for the American Board of Pathology's Maintenance of Certification Program.

GYNECOLOGIC FAMILIAL CANCER SYNDROMES: WHAT DOES THE PRACTICING PATHOLOGIST NEED TO KNOW?

Ann K Folkins, M.D. [1], Karuna Garg, M.D.[2], Teri A Longacre, M.D. [1]

[1] Stanford University

[2] Memorial Sloan Kettering Cancer Center

The authors declare no conflict of interest.

Introduction

There have been significant advances in our understanding of female genital tract tumors in the last two decades. The discovery of *BRCA1* and *BRCA2* genes in breast cancer and the mismatch repair genes in colorectal and endometrial carcinoma has revolutionized our approach to the diagnosis and screening of women for ovarian and uterine cancers. As a result, the pathologist cannot fully function as a member of the health care team without a basic working knowledge of these two genetic diseases. This handout will discuss the epidemiology, basic molecular pathology, risk for cancer, type(s) of cancer, current screening techniques, risk-reducing procedures, treatment, and pathology that is associated with germline mutations in these genes. As this is an evolving field, some aspects of tumorigenesis are not as well understood as others; however current "state of the art" knowledge is incorporated whenever possible.

Breast Ovarian Cancer Syndrome

Epidemiology of BRCA-associated Female Genital Tract Cancers

Germline mutations in *BRCA1* and *BRCA2* are responsible for ovarian cancers associated with the breast ovarian cancer syndrome (BOCS) and the site-specific ovarian cancer syndrome (SSOCS). In the United States, the lifetime risk of developing ovarian cancer is approximately 1.8%, but germline mutations in *BRCA1* and *BCRA2* genes raises this risk to 20-50% (18, 53). Mutations in *BRCA1* and *BRCA2* do not appear to confer the same level of increased risk. *BRCA1* mutations are thought to result in a 40-50% lifetime risk, while *BRCA2* mutations are associated with a 20-30% risk (1, 7). At least 10% of epithelial ovarian cancers are hereditary, with most of these accounted for by mutations in *BRCA* genes (7).

The overall rate of germline mutations in *BRCA1* and *BRCA2* genes is relatively low, at <0.3%, but the rate of carriers is significantly affected by ethnic background (2). Mutation rates are probably highest in women of Ashkenazi

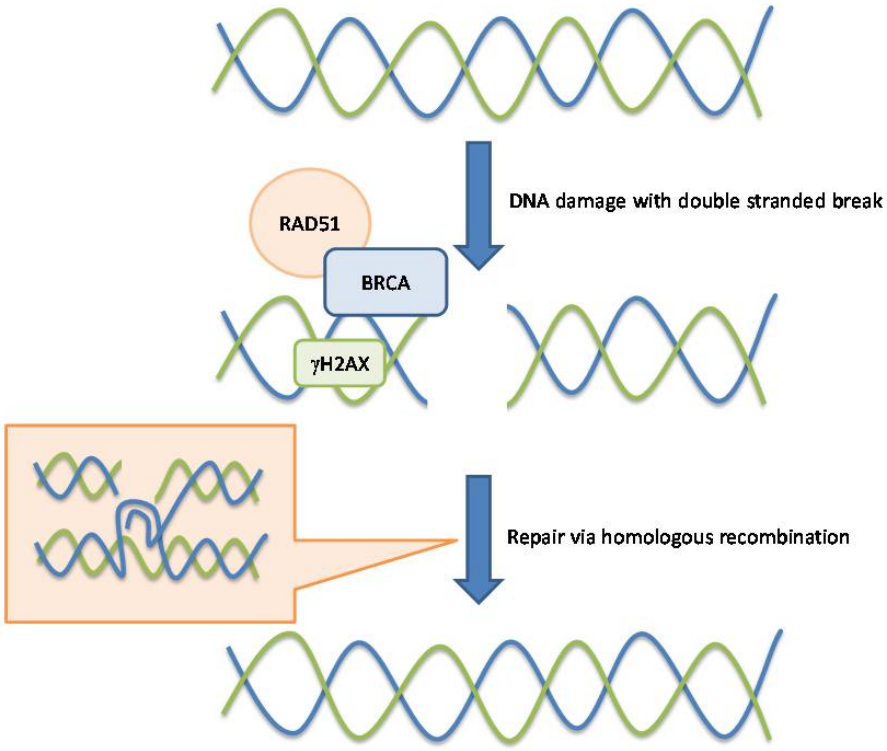
(Eastern European) Jewish descent, where the carrier rate of the most common mutations is approximately 2% (53). Founder mutations conferring higher carrier rates have also been described in Iceland, the Netherlands, and Sweden. Environmental factors may affect the development of hereditary ovarian cancers, just as they are thought to play a role in sporadic cancers. Most risk factors have been shown to be parallel in BRCA and sporadic cancer, with the exception of parity (38).

Pelvic cancers associated with hereditary mutations in *BRCA* occur at a younger mean age than sporadic tumors (53 years versus 63 years) (7). The majority of symptomatic ovarian malignancies diagnosed in *BRCA* mutation carriers are high-grade serous carcinomas at an advanced stage. The advent of risk-reducing salpingo-oophorectomy for known mutation carriers has allowed for the detection of tumors at a lower stage, as will be discussed in detail later. These lesions, whether they are in situ or invasive, are also usually high grade serous carcinomas.

Functions of *BRCA* Genes

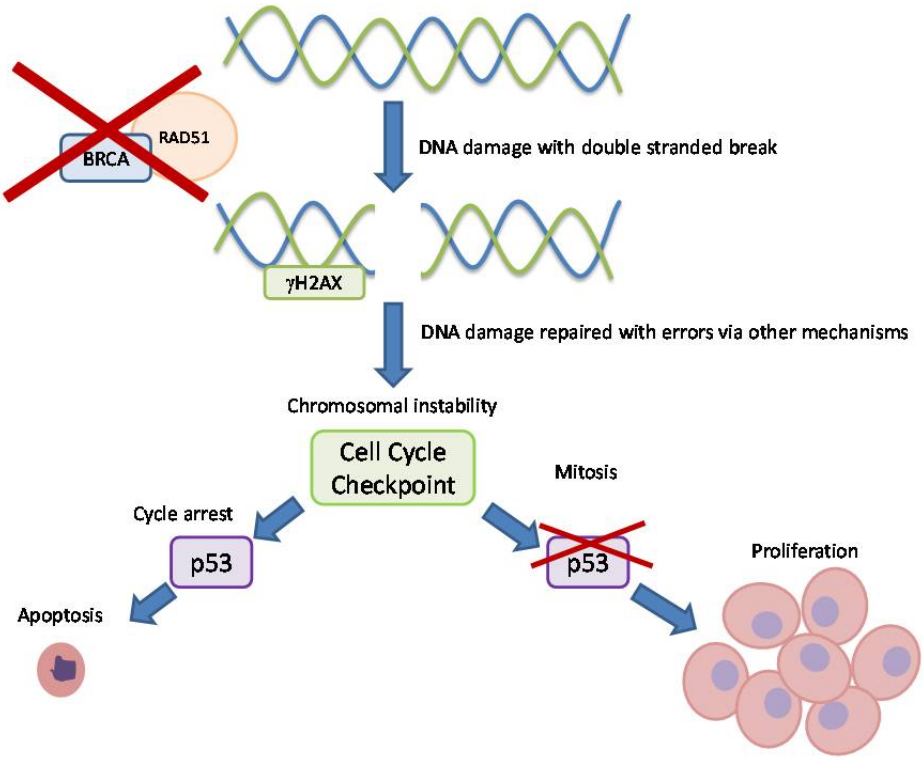
BRCA1 and *BRCA2* are classified as tumor suppressor genes; however, their roles in DNA repair and cell cycle control are complex and incompletely understood. *BRCA1* is located on chromosome 17, and *BRCA2* is on chromosome 13. The genes are autosomal recessive but have an autosomal dominant phenotype with incomplete penetrance. Both genes encode nuclear proteins that participate to some extent in DNA repair. A schematic of the role of the BRCA proteins in DNA damage repair is shown in Figure 1.

Figure 1: Function of BRCA in DNA damage repair



In response to double stranded breaks in DNA, BRCA1 co-localizes with RAD51 to sites of damage. BRCA appears to function in repair of such damage via non-error prone homologous recombination, which uses the template of the sister chromosome to replicate the lost segment of DNA (37). Germline mutations in *BRCA* affect only one inherited allele, and it appears that one functioning allele is sufficient for adequate function of this repair mechanism. When individuals carrying an inherited mutation suffer a second mutation in the normal allele (loss of heterozygosity) in an individual cell via somatic mutation, this renders the cell without functioning BRCA proteins. A schematic illustrating the impact of non-functional BRCA on DNA damage repair is shown in Figure 2.

Figure 2: Loss of functional BRCA proteins and implications for DNA repair

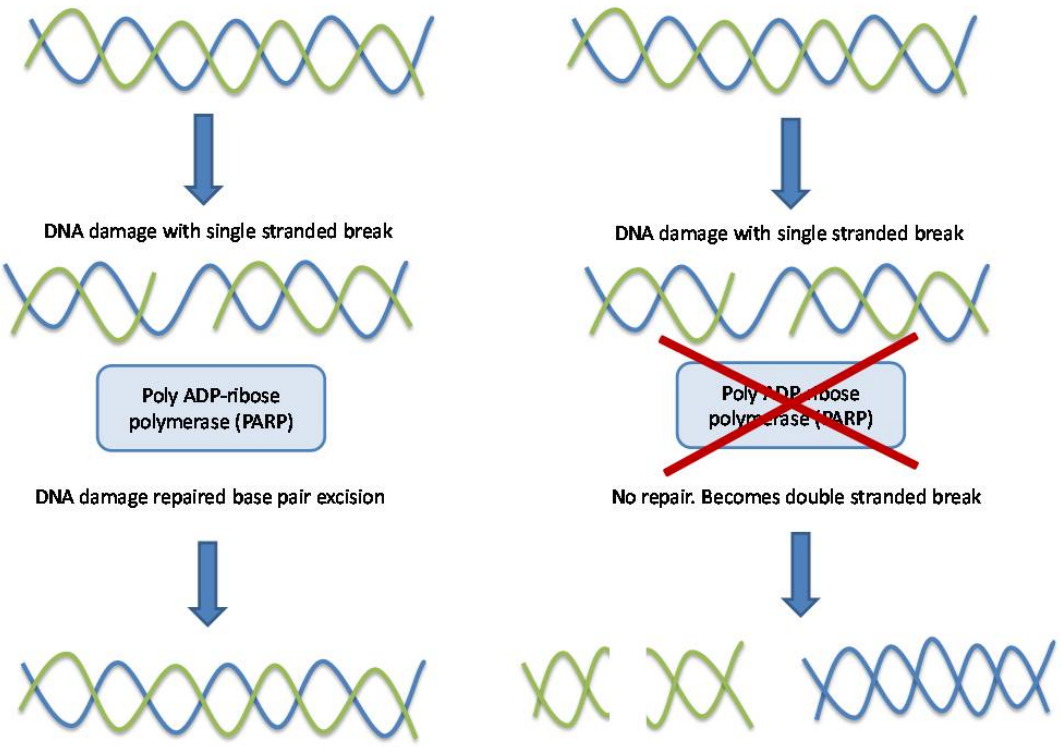


Without functional BRCA, the cell is forced to repair double stranded DNA breaks via other mechanisms, which are error prone and create instability and mutations (54). If significant errors are incorporated into the DNA, the cell will undergo cell cycle arrest and eventually apoptosis, as long as gatekeeper proteins, such as p53 are still functional. If the cell also acquires a mutation in p53, errors would be allowed to persist and proliferate, potentially leading to tumorigenesis. The loss of BRCA function and increase in errors from damage repair likely exerts a selective pressure for p53 mutations by overloading its capacity to initiate cell cycle arrest. Given this proposed interaction of BRCA and p53, it is not surprising that most high grade serous carcinomas, which are the predominate tumor type seen in hereditary ovarian carcinoma, harbor mutations in *p53*. While this model is appealing and probably accurate to some extent, it does not explain the full scope of the BRCA proteins' role in the cell. Research

has shown that BRCA likely regulates gene expression at the transcriptional level, particularly the expression of p53 and its downstream proteins.

Research into the efficacy of poly-ADP ribose polymerase (PARP) inhibitors in tumors with *BRCA* mutations has highlighted this complexity. The simultaneous loss of BRCA and PARP in a cell has been termed “synthetic lethal”, meaning that the loss of either functional protein by itself does not confer cell death but the loss of both proteins is not compatible with cell survival (34). PARP is thought to participate in repair of single stranded DNA breaks via base pair excision. Inhibition of PARP appears to selectively kill cells which lack functional BRCA. Initially, it was proposed that inhibition of PARP caused a failure of base excision repair of single stranded breaks, which were allowed to persist. At replication, the single stranded breaks would become double stranded breaks, which could not be repaired via homologous recombination in the absence of functional BRCA (Figure 3).

Figure 3: Initial proposed function of PARP



This theory, however, did not help to explain why these cells would undergo apoptosis. The double stranded breaks might be repaired again via other mechanisms, and if there was presumably no p53, the cells would be able to survive and proliferate. Further research highlighted that single stranded breaks do not actually increase after PARP inhibition. Instead, the synthetic lethality of PARP inhibitors and defective BRCA is probably more complex. It has been proposed the PARP inhibitors may trap PRAP on single stranded breaks during repair, preventing replication and requiring homologous recombination (using BRCA) to process. PARP and homologous recombination are both active at stalled replication forks, as well, so knock-out of both processes would prevent further replication.

High-grade "Ovarian" Serous Carcinomas: Lessons Learned

The traditional view of ovarian cancer is that it begins in the ovary and undergoes progressive transformation from a well to a poorly differentiated tumor, which then spreads to abdominal sites. It is therefore assumed that the outcomes for this disease could be improved with detection of the tumor when it is confined to the ovary. However, no randomized trails to date of early screening methods have shown any decrease in mortality. This failure may in part be to our lack of understanding of the natural development of at least some of these tumors. Serous cancers account for 60-80% of ovarian carcinomas. Recent work by multiple groups has suggested that epithelial ovarian cancer may develop in separate pathways (50, 51). Type I tumors are slow growing low-grade serous carcinomas as well as their putative precursors, serous borderline tumors; they often demonstrate mutations in K-RAS or B-RAF. Type II tumors, exemplified by high-grade serous carcinoma, are rapidly growing, highly aggressive neoplasms with no well defined precursor lesions because they are usually discovered at an advanced stage. They tumors usually harbor mutations in the tumor suppressor gene, *p53*. Therefore, a more realistic goal for early detection may be size rather than stage, since it is unclear if the ovary is always the "primary site".

Although the convention has been to assume that all these epithelial tumors arise primarily from the ovary, there have been conflicting reports regarding the existence of ovarian serous dysplasia and accumulations of p53 protein in cortical inclusion cysts and on the ovarian surface epithelium (see Table 1). The ovarian surface epithelium was the favored cell of origin for epithelial malignancies based on the traditional model of ovarian cancer. Since high grade serous carcinoma is the most common malignancy found in *BRCA* gene mutation carriers, it was presumed that examination of prophylactic ovaries would reveal a precursor lesion. The results of studies examining prophylactically removed ovaries from high risk women (including *BRCA* gene mutation carriers), however, have failed to identify any reproducible precursor lesions (see Table 1).

Table 1: Evidence for Serous Precursors in the Ovary

Study	Ovarian dysplasia	P53 staining in ovary
Bell 1994 (6)	Yes	N.A.
Hutson 1995 (25)	N.A.	Yes
Salazar 1996 (45)*	Yes	N.A.
Stratton 1999 (52)*	No	N.A
Sherman 1999 (49)*	Yes	N.A.
Deligdisch 1999 (17)*	Yes	N.A.
Barakat 2000 (4)*	No	No
Casey 2000 (13)*	No	N.A.
Schlosshauer 2003 (46)*	Yes	Yes
Piek 2003 (39)*	No	No
Kerner 2005 (28)*	N.A.	Yes
Zhang 2007 (56)*	N.A.	Yes
Folkins 2008 (20)*	N.A.	Rare

N.A., not available; * Prophylactically removed ovaries

Rather than revealing early serous carcinomas on the ovarian surface or within cortical inclusion cysts, evaluation of prophylactic salpingo-oophorectomies in women with *BRCA* mutations has provided support for the fallopian tube as a major source of high grade serous carcinomas. “Primary” fallopian tube carcinomas have an estimated incidence of 0.41 per 100,000 (versus 15 per 100,000 for ovarian) based on the traditional pathologic classification of site of origin. Likewise, most symptomatic serous cancers in women with *BRCA* mutations have been classified as ovarian. However, with the increase in prophylactic salpingo-oophorectomies, an increasing number of serous malignancies were found to involve the fallopian tube at an early stage. For example, in 2001, Piek described “dysplastic changes” of the fallopian tube mucosa which stained positive for p53 (6/12 high risk ovaries) (39). Carcangiu found in situ carcinoma and atypical hyperplasia in the tubes of BRCA1 individuals and not control subjects (11). Based on the available literature, early serous carcinomas in these specimens occur in about 10% of cases and involve that fallopian tube mucosa in 50-100% (see Table 2).

Table 2: Risk-reducing (Prophylactic) Salpingo-oophorectomy in Women with *BRCA* Germline Mutations: Evidence for Serous Precursors in the Fallopian Tube

Author	Number of cases	Tumor (%)	Tubal involvement (%)
Leeper 2002 (33)	30	5 (17)	3 (60)
Powell 2005 (40)	67	7 (10)	4 (57)
Finch 2006 (19)	159	7 (4)	6 (86)
Carcangiu 2006 (10)	50	6 (12)	4 (67)
Callahan 2007 (9)	100	7 (7)	7 (100)
Rabban 2009 (43)	108	7 (7)	6 (86)
Hirst 2009 (24)	45	4 (9)	4 (100)

The evaluation of prophylactic specimens has provided a potential precursor lesion to pelvic serous carcinoma in the fallopian tube and has changed the paradigm of the origin of these tumors. The finding of early serous carcinomas in the fallopian tubes of women with hereditary *BRCA* mutations endorsed the concept that these are potentially the most common early malignancy in this population. The next question was whether the same could be true of women of average genetic risk. This was addressed by an important study in 2007 done by Kindelberger el al (29). Of 55 cases of serous carcinoma, 41 (75%) involved the fallopian tube to some degree. Serous tubal intraepithelial carcinoma (STIC) was present in 29 of these cases. They tested the STIC and coexistent ovarian carcinoma in five cases, and all five (100%) showed identical *p53* mutations. Similarly, in a study by Carlson et al. of so called primary peritoneal serous carcinomas, approximately half of the cases showed a STIC (12).

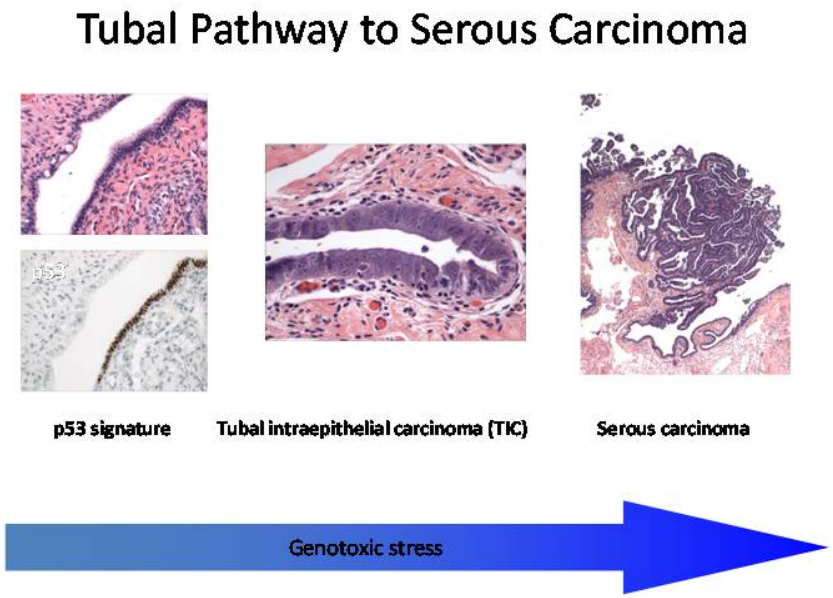
There is controversy over whether STIC actually represents the earliest manifestation of serous carcinoma in that it could represent spread from the invasive tumor. There is substantial evidence to support that STIC is the precursor lesion. One, STIC is the earliest lesion seen in BRCA prophylactic salpingo-oophorectomies. Two, although tubal mucosa may harbor metastatic lesions, this is less common than is observed in the ovary. Three, STIC is uncommon in advanced uterine serous carcinoma. Four, STIC shares *p53* mutations with serous cancer. Five, earlier precursor (pre-precursor) lesions have been identified in the tubal mucosa (p53 signatures, see later discussion). Moreover, the fact that STIC is required to support the diagnosis of primary fallopian tube carcinoma in the traditional scheme seems to support that most assume STIC to be evidence of origin in that tissue.

The paper by Meideiros et al. was the first to report strongly p53 positive areas of benign appearing fallopian tube epithelium, which were later termed “p53 signatures” by Lee et al (32, 36). The p53 signature is defined as benign appearing epithelium showing p53 positivity by immunohistochemical staining in at least 12 secretory cells. These secretory cells can be contiguous or interrupted

by some remaining ciliated cells, but the lesion should have a low proliferative index, as measured by MIB-1 staining (<20%). Of the 14 p53 signatures sequenced for p53 mutations in Lee and colleagues' study, 8 (56%) showed p53 mutations (32). There was one case in which the p53 signature and the STIC showed identical mutations. P53 signatures are common (30-40%) in the fallopian tubes of women with *BRCA* mutations and fallopian tubes from control populations, suggesting that their presence is independent of BRCA status and that most of these lesions do not progress to carcinoma (20, 48).

From this research has emerged a new model for the origin and development of high grade serous carcinoma, both in women with *BRCA* mutations and women of average risk (see Figure 4). P53 signatures are apparently common in the fallopian tube, regardless of BRCA status. Some small proportion of these p53 signatures undergoes additional mutation to form STICs. In women with *BRCA* mutations who acquire an additional loss of the functional *BRCA* allele, there is presumably more unrepaired DNA due to lack of homologous recombination, so more cells need to activate p53 at the cell cycle checkpoint. The relationship between the load of DNA damage on the cell and the development of *p53* mutations is unknown; however, there is a theory that continual DNA damage exerts pressure on the p53 pathways, leading to the selective development of mutations to allow proliferation of the damaged cells.

Figure 4: New paradigm for development of high grade serous carcinoma



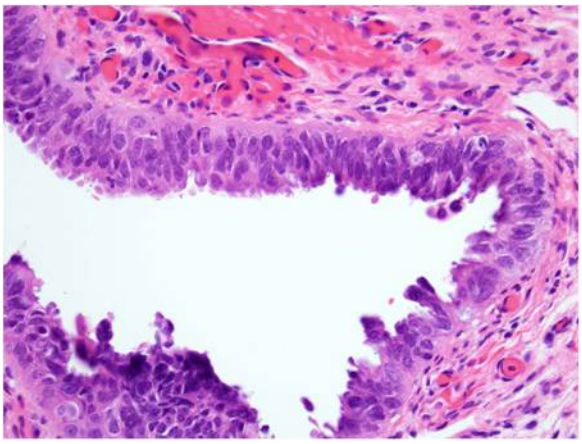
Pathology of BRCA-associated Pelvic Tumors: Serous Tubal Intraepithelial Carcinoma (STIC) and High-grade Serous Carcinoma

A serous tubal intraepithelial carcinomas (STIC) is defined as a morphologically distinct population of cells in the tubal epithelial demonstrating atypia, a secretory cell phenotype, and an increased proliferative index, with or without intense p53 staining by immunohistochemistry. Atypia in this context is can be reflected by an increased nuclear to cytoplasmic ratio, loss of cell polarity, prominent nucleoli, pleomorphism, stratification, and exfoliation. Although p53 immunohistochemical staining is seen in the majority of STICs, it is not required for the diagnosis. Most of the mutations in *p53* are frameshift or nonsense mutations that increase the stability of the altered and truncated protein, leading to accumulation detectable by immunohistochemistry. However, sometimes the mutations are insertions, deletions, or stop codons leading to lack of p53 production. In the recent series by Shaw et al, only 79% of the STICs showed p53 overexpression (48). Therefore, p53 positive staining is not required to make a diagnosis of STIC. Immunohistochemistry for MIB-1 should stain >40% of

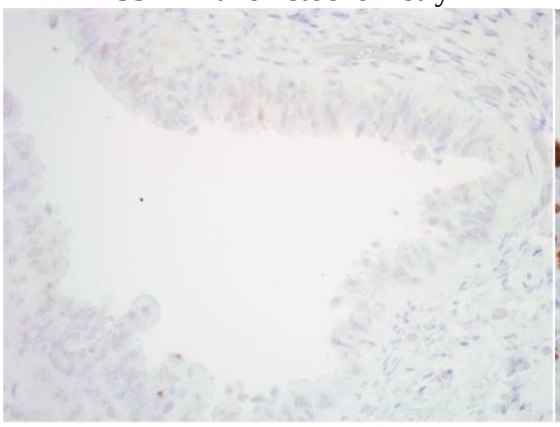
the cells in the lesion. Figure 5 illustrates the typical morphology of at STIC, which happens to be negative for p53.

Figure 5: Examples of p53 negative STIC

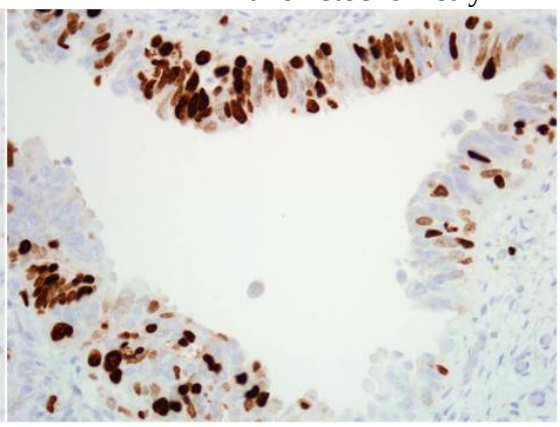
H&E



P53 immunohistochemistry



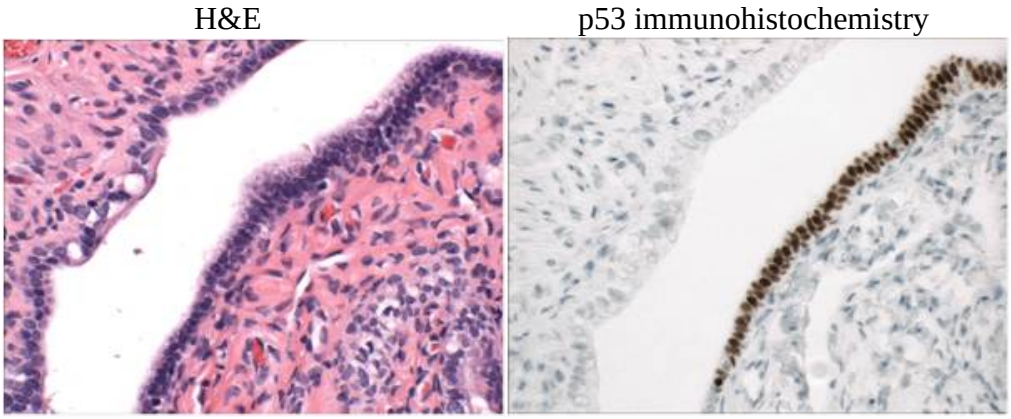
MIB-1 immunohistochemistry



Interpretation of p53 immunohistochemical staining in the fallopian tube should be made with caution, as the accumulation of mutant p53 does not mean that the lesion is neoplastic. As was discussed earlier in the section on the origin of high grade serous carcinomas, multiple groups have now confirmed the presence of p53 signatures as a relatively common occurrence in fallopian tubes, regardless of BRCA status. The p53 signature is defined as benign appearing epithelium showing p53 positivity by immunohistochemical staining in at least 12 secretory cells, with a low proliferative index (MIB-1 <20%) (see Figure 6).

Therefore, routine staining of fallopian tubes with p53, which we do not recommend as standard practice, could disclosure areas of strong p53 positivity. We do not use the term p53 signature as a clinical diagnosis.

Figure 6: p53 signature



It is really in the area of transition from the p53 signature to the STIC that many diagnostic dilemmas arise. When we see areas of tubal epithelium that show increased nuclear atypia, we routinely order p53 and MIB-1 immunohistochemistry to aid in the classification of the lesion. In general, there should be both significant nuclear atypia and a high proliferative rate in order to diagnose the lesion as a STIC. As was stated before, p53 positivity is not required for the diagnosis of STIC, but it can often confirm that the morphologic area of atypia does in fact harbor alterations in p53. Lesions which either lack enough atypia or have an unexpectedly low proliferative rate, are termed atypical tubal epithelial proliferations and mentioned in the diagnostic line with a comment that it does not fulfill criteria for a diagnosis of STIC. These atypical lesions have been referred to as serous tubal intraepithelial lesions in transition (STILTs) by some to describe their position in the model of tubal serous carcinogenesis, but again, this is not a diagnosis that is used in clinical practice.

The differential diagnosis of STICs usually involves distinguishing it from other tubal secretory proliferations (see Table 3) but also includes tangentially sectioned tubal epithelium, transitional cell metaplasia, tubal mucosal

hyperplasia, metastatic breast cancer, reactive epithelial changes, and Arias-Stella effect. Transitional metaplasia consists of small uniform fusiform cells and will not show the atypia that is typical of STICs. The incidence of metastatic breast cancer in prophylactic salpingo-oophorectomies is exceedingly rare at about 2 in 1000 cases (42). Acute salpingitis can induce florid hyperplasia of the fallopian tube epithelium. Architecturally, this reactive phenomenon often exhibits a cribriform pattern but the cells retain a low nuclear to cytoplasmic ratio. The presence of abundant acute inflammation should be a clue to this diagnosis. Arias-Stella effect in the fallopian tube can demonstrate marked atypia; however, the cells usually have abundant eosinophilic cytoplasm, imparting a lower nuclear to cytoplasmic ratio. The morphology of high grade serous carcinomas occurring in women with *BRCA* mutations is indistinguishable from that of sporadically occurring cancers and will not be discussed in depth here.

Table 3: Histologic & Immunohistologic Features of Serous Precursor Lesions [adapted from (16)]

Feature	P53 signature	Atypical serous proliferation	STIC
N/C ratio	Variable	Variable	High
Thickness	Variable	Variable	High
Nucleoli	Occasional	Occasional	Common
Molding	Occasional	Occasional	Common
Cell shape	Round/oblong	Round/oblong	Round
Unpolarized	No	No	Yes
Exfoliation	No	No	Common
Intraepithelial fractures	No	No	Common
P53 staining	Yes	Yes	Usually yes
MIB-1	<20%	20-50%	40-90%

Although much has been written regarding the STIC lesions, there has been limited data concerning the morphologic features of fully developed carcinoma in women harboring *BRCA* germline mutations. Not surprisingly, most BRCA-associated carcinomas are high-grade serous tumors (31, 55), but undifferentiated tumors may also be seen. The tumors often harbor markedly anaplastic nuclei with giant, bizarre nuclei, abundant mitotic figures, and prominent intraepithelial lymphocytes (defined as >40 per high power field) (22). More importantly, absence of this phenotype has a negative predictive value >95%. Low grade serous carcinoma and serous borderline tumors are not associated with *BRCA* mutations. Primary ovarian mucinous tumors, which tend to occur in younger women, are also not associated with BRCA status. Rarely, women with a known *BRCA* germline mutation develop clear cell carcinoma, but it is not clear whether this is directly associated with the mutation or a chance occurrence.

Screening For Breast Ovarian Cancer Syndrome

The American College of Obstetrics and Gynecology (ACOG) has published guidelines to aid in the selection of women who should be referred for genetic testing for *BRCA1* and *BRCA2* mutations (1). In general, genetic testing is recommended when there is an estimated 20-25% risk of having an inherited mutation, as assessed by personal and family history. Women who fulfill the following criteria should be tested: personal history of both breast and ovarian cancer; personal history of ovarian cancer and a close relative with ovarian cancer or premenopausal breast cancer; personal history of ovarian cancer with Ashkenazi Jewish ancestry; personal history of breast cancer at age 50 years or younger and a close relative with ovarian cancer or male breast cancer at any age; personal history of breast cancer at less than age 40 with Ashkenazi Jewish; and close relative with a known *BRCA1* or *BRCA2* mutation.

One of the greatest successes in gynecologic cancer prevention has been the use of the Pap smear and more recently the vaccine to reduce the incidence of cervical cancer. The success of this intervention was based on two observations: cervical cancers are preceded by precursors that can remain non-

invasive for as long as 20 years and these cancers and their precursor lesions are linked to HPV infection. The prevention of ovarian cancer is more complicated and serologic biomarkers as well as other screening techniques have not been as fruitful. It is reasonable to assume that Type I tumors might remain localized for a sufficient length of time so that early detection could prevent deaths. The success of early detection in serous carcinomas, however, is more controversial, in that they probably spread throughout the peritoneum very early, even when there is a low volume of disease. We do not have a good model for the progression of serous carcinoma.

The information available from risk-reducing salpingo-oophorectomy specimens has been used to develop a model for the progression of serous cancer in order to define a window of opportunity for early detection and prevention of disease. The ideal approach would be to observe the natural history of precursor lesions and cancer over time, but this is not feasible. Using the estimated prevalence of early cancers in prophylactic salpingo-oophorectomies and the incidence of serous cancers in *BRCA1* mutation carriers, one group has estimated progression using tumor size at PBSO, occult period, and growth rate estimates (8). This model estimates that most serous carcinoma progress to advanced stage (III, IV) by a median of 0.8 years (CI 0.4-1.9 y) before detection. Occult early stage serous ovarian cancers in BRCA1 women have a median diameter less than 0.3 cm, and 90% of the duration of the window of opportunity is spent at a diameter less than 0.9 cm. Tumor size, along with the inaccessible location of the fallopian tubes and ovaries, provides perhaps the biggest challenge to early detection. Although biomarkers continue to be investigated, none have been sufficiently sensitive to detect these low volume tumors.

Testing For *BRCA* Germline Mutations

Given that women with high grade serous carcinoma may have a higher rate of underlying germline mutations in *BRCA* than unselected ovarian tumors, a case can be made for testing all women with this type of tumor. However, routine testing for *BRCA1* germline mutation is not without costs. Genetic testing is

expensive and the frequency of *BRCA1* mutation carriers in the general population is extremely low (0.0006), with only 5-7% of all breast cancer and 10-12% of all ovarian cancer attributable to *BRCA1* germline mutations (15, 44). Given the low incidence of the mutation, the current false positive rate, while acceptable under current testing procedures, is too high if testing were offered to all women. These criteria are mostly used by gynecologic oncologists; however, the pathologist should be aware of the criteria so that he or she may alert the referring physician in cases that appear to be high risk. Testing for mutations in *BRCA* remains proprietary and can only be performed at Myriad Genetics. Full sequencing is recommended as the initial test of choice for the first family member, as more than 250 mutations can occur in the two genes (41). If a specific mutation is identified, more tailored testing can be performed for other family members.

Evaluation of Risk-Reducing (Prophylactic) Salpingo-oophorectomies

The presence of early serous carcinomas in the fallopian tube mucosa of prophylactic salpingo-oophorectomies necessitates a grossing procedure aimed at maximizing visualization of tubal mucosal surface area, especially in the fimbriated end of the tube (35). Prior to the discovery of STICs in the tube, it was thought that tubal carcinoma was relatively rare and that microscopic evaluation of the tube was not as vital as evaluation of the ovary itself. As discussed previously, this paradigm has changed, and it is now equally important to evaluation the tubal as well as the ovarian surface area. Given that the majority of early serous cancers in prophylactic specimens that involve the tube are 1) grossly invisible and 2) present in the fimbriated end of the tube, Medeiros et al. developed the SEE-FIM protocol (sectioning and extensively examining the fimbriated end) (36). In this prosection technique, the fimbriated end is first amputated from the end of the tubes. Next, the fimbriated end is sectioned longitudinally into four pieces. This method maximizes the amount of fimbrial mucosa that will be examined microscopically. The remainder of the tube is serially sectioned at 2-3 mm intervals, and the entire tube is submitted for

examination. Likewise, both ovaries should be entirely submitted for histologic examination, following serially cross-sectioning at 2-3 mm intervals.

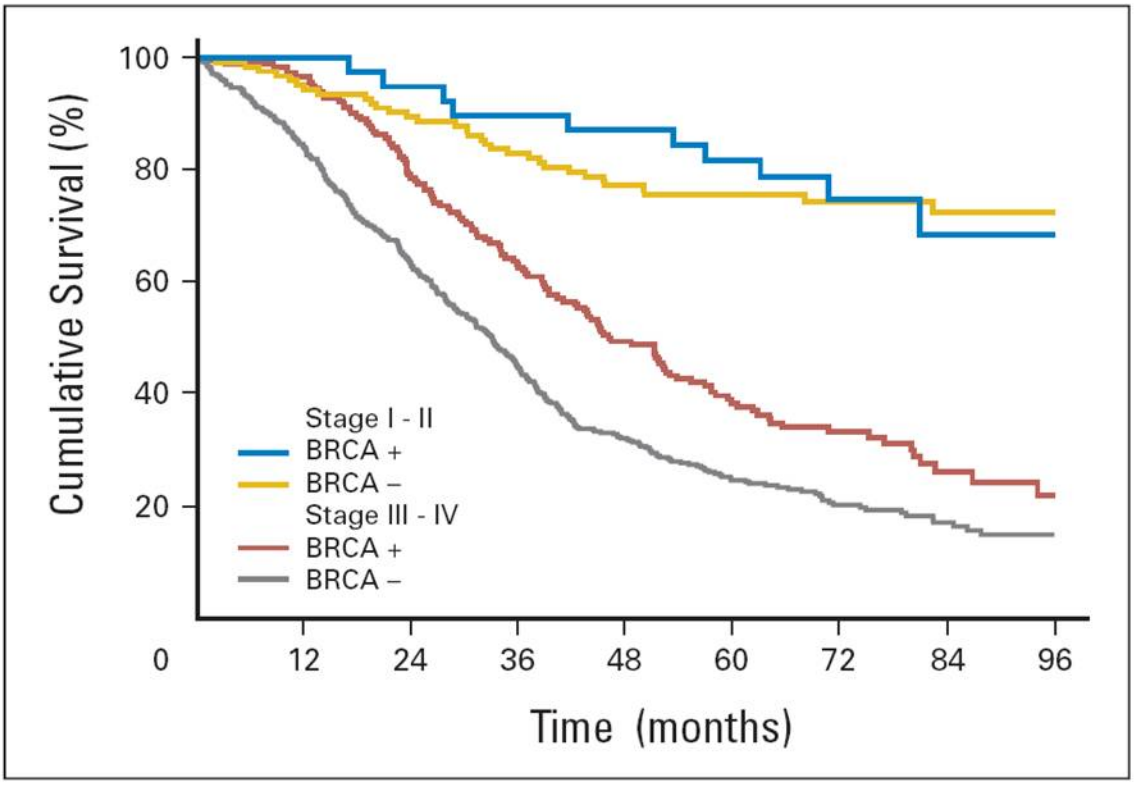
There is no real data on the rate of occult tumors of the fallopian tube in routine specimens. The distal tube is not always sampled and certainly not always completely examined. Given the that multiple studies have suggested that serous tumors may arise in the fallopian tubes regardless of BRCA status, it seems reasonable to extrapolate that there should be some routine evaluation of the fallopian tube in routine salpingo-oophorectomies for both benign and malignant disorders. After implementing routine examination of the fimbria in all cases at Brigham and Women's Hospital, they reported a 2 mm invasive serous carcinoma with adjacent STIC in the fimbria of a patient with an ovarian fibrothecoma (47). We recommend submission of entire fimbria and infundibulum of the fallopian tube (distal 2 cm) and a single central cross section in all salpingectomy specimens. Given that the fimbria is the most likely source of occult cancer, and it can be submitted in one block, it makes sense to have it be the representative sections of the fallopian tubes in cases where no gross lesion is visualized. Early cancers are small and may be missed with representative sampling of fallopian tube cross sections. Detection of early cancers in women with or without *BRCA* mutations has implications for intervention and surveillance. Moreover, finding an early cancer may impact a decision to pursue testing for hereditary *BRCA* mutations.

Treatment of BRCA-associated Pelvic (Non-uterine) Mullerian Serous Carcinoma

The treatment of BRCA-related serous carcinomas is essentially the same as for ovarian carcinoma in general, although evidence is emerging that more tailored chemotherapy could be designed to exploit the DNA repair defects inherent to *BRCA* mutated tumors. The standard treatment is surgery, followed by chemotherapy, usually with carboplatin and paclitaxel for six cycles. Women with *BRCA* mutations tend to have a better overall prognosis than stage matched sporadic tumors (see Figure 7), and this is thought to relate, at least in part, to a better response to chemotherapy (14, 23, 30). This survival advantage appears

to be present for both *BRCA* mutation carriers of Ashkenazi Jewish background and those with other heritage.

Figure 7: Survival of epithelial ovarian cancer patients by stage and BRCA1/2 mutation stage (from Chetrit A et al. *Effect of BRCA1/2 Mutations on Long-Term Survival of Patients With Invasive Ovarian Cancer: The National Israeli Study of Ovarian Cancer*. Clin Oncol 2008)



The enhanced sensitivity of BRCA-associated tumors to platinum based chemotherapy has been explained by the fact that these tumors have lost the ability to cope with large amounts of double stranded DNA breaks, without functional homologous recombination (26). Interestingly, this is also the rational proposed for the potential efficacy of PARP inhibitors in *BRCA* mutated tumors, as was discussed previously. Phase 1 and Phase 2 clinical trials have shown the potential selective efficacy of these drugs in BRCA-associated hereditary breast cancers, and research is beginning in the possibility of PARP inhibitors in ovarian cancers (3, 21). Early trials are showing promising activity of PARP inhibitors in

hereditary ovarian cancer (3, 21), and clinical trials are beginning using PARP inhibitors as chemoprophylaxis.

Risk-reducing (prophylactic) salpingo-oophorectomy is currently the standard treatment for women found to be carriers of *BRCA* mutations. The risk for the development of subsequent pelvic cancer in carriers is reduced as much as 80-90% with this intervention, but the timing of the surgery, especially in women of childbearing years, is critical (5, 27). It is recommended that prophylactic surgery be offered by age 40 or when child bearing is complete (1). If chemoprevention, with an agent selective for *BRCA*-mutated tumor cells and little toxicity to normal cells, were available, if could allow delay in prophylactic surgery with subsequently more years of potential childbearing and later onset menopause. Currently, no such intervention exists, although small molecule inhibitor drugs, like PARP inhibitors, may hold some promise.

The optimal treatment of early serous cancers (STICs) remains unclear. Although based on a few cases, there is evidence that removal of early serous cancers in BRCA women is effective (12) and that there is a low risk of recurrence (see Figure 8). The use of adjuvant chemotherapy for in situ lesions appears to vary by institution, with oncologists generally more likely to give chemotherapy if the abdominal washings are positive.

Figure 8: Reported Outcomes of Serous Tubal Intraepithelial Carcinoma (stage 0) in BRCA-Positive Women (from Carlson JW et al. Serous tubal intraepithelial carcinoma: its potential role in primary peritoneal serous carcinoma and serous cancer prevention. J Clin Oncol 2008 Sep 1;26(25):4160-5)

Table 3. Reported Outcomes of Serous Tubal Intraepithelial Carcinoma (stage 0) in *BRCA*-Positive Women

First Author	Age (years)	*BRCA*	Washings	Chemotherapy	Follow-Up (months)
Agoff[16]	65	2558inA *BRCA2*	Positive	Yes	NED, 36
Finch[8]	64	*BRCA1*	Negative	UK	NED, 4
Carcangiu[17]	49	*BRCA1*	Negative	None	NED, 87
	61	*BRCA1*	Negative	None	NED, 38
	48	*BRCA1*	Negative	None	NED, 7
Paley[18]	65	*BRCA2*.2558insA	Positive	No	NED, 36
	47	*BRCA1*.2800delAA	Positive	Yes	NED, 48
Callahan[9]	44	*BRCA2*.W2598X	Positive	Yes	NED, 36
	66	*BRCA1*.5301insA	Negative	Yes	NED, 36
	44	*BRCA1*.1294del40	Negative	Yes	NED, 36

Abbreviations: *BRCA*, heterozygous *BRCA* mutation (1 or 2); NED, no evidence of disease; UK, unknown.

Summary

- Germline mutations in *BRCA1* and *BCRA2* genes confer a 20-50% lifetime risk of ovarian cancer.
- In general, genetic testing is recommended when there is an estimated 20-25% risk of having an inherited mutation, as assessed by personal and family history.
- *BRCA* genes function as tumor suppressor genes and participate in repair of double stranded DNA breaks via homologous recombination.
- Most ovarian carcinomas in women with germline *BRCA* mutations are high-grade serous carcinomas.
- Women with *BRCA* mutations tend to have a better overall prognosis than stage matched sporadic tumors.
- Emerging evidence suggests that a large proportion of high-grade serous carcinomas actually arise in the fallopian tube, rather than the ovary.
- Risk-reducing (prophylactic) salpingo-oophorectomy is currently the standard treatment for women found to be carriers of *BRCA* mutations.
- Between 5-10% of prophylactic salpingo-oophorectomies in this group will contain an early tubal carcinoma.
- Evaluation of prophylactically removed fallopian tubes and ovaries from women with *BRCA* mutations should include the SEE-FIM protocol for examination of the fallopian tubes.
- The diagnosis of serous tubal intraepithelial carcinoma (STIC) requires the presence of significant nuclear atypia and a high proliferative rate. Positive staining for p53 is helpful but is not required for the diagnosis.
- Treatment of BRCA mutation associated serous carcinomas is currently the same as for non-hereditary serous carcinomas, but research is underway investigating the use of novel drugs, such as PARP inhibitors, that may selectively target BRCA mutated cancers.

References

1. Hereditary breast and ovarian cancer syndrome. ACOG Practice Bulletin No. 103. American College of Obstetricians and Gynecologists. *Obstet Gynecol* 2009;113:957-966.
2. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet* 2003;72:1117-1130.
3. Audeh MW, Carmichael J, Penson RT, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. *Lancet* 2010;376:245-251.
4. Barakat RR, Federici MG, Saigo PE, et al. Absence of premalignant histologic, molecular, or cell biologic alterations in prophylactic oophorectomy specimens from BRCA1 heterozygotes. *Cancer* 2000;89:383-390.
5. Barnes MN, Grizzle WE, Grubbs CJ, et al. Paradigms for primary prevention of ovarian carcinoma. *CA Cancer J Clin* 2002;52:216-225.
6. Bell DA, Scully RE. Early de novo ovarian carcinoma. A study of fourteen cases. *Cancer* 1994;73:1859-1864.
7. Boyd J. Specific keynote: hereditary ovarian cancer: what we know. *Gynecol Oncol* 2003;88:S8-10; discussion S11-13.
8. Brown PO, Palmer C. The preclinical natural history of serous ovarian cancer: defining the target for early detection. *PLoS Med* 2009;6:e1000114.
9. Callahan MJ, Crum CP, Medeiros F, et al. Primary fallopian tube malignancies in BRCA-positive women undergoing surgery for ovarian cancer risk reduction. *J Clin Oncol* 2007;25:3985-3990.
10. Carcangiu ML, Peissel B, Pasini B, et al. Incidental carcinomas in prophylactic specimens in BRCA1 and BRCA2 germ-line mutation carriers, with emphasis on fallopian tube lesions: report of 6 cases and review of the literature. *Am J Surg Pathol* 2006;30:1222-1230.
11. Carcangiu ML, Radice P, Manoukian S, et al. Atypical epithelial proliferation in fallopian tubes in prophylactic salpingo-oophorectomy specimens from BRCA1 and BRCA2 germline mutation carriers. *Int J Gynecol Pathol* 2004;23:35-40.
12. Carlson JW, Miron A, Jarboe EA, et al. Serous tubal intraepithelial carcinoma: its potential role in primary peritoneal serous carcinoma and serous cancer prevention. *J Clin Oncol* 2008;26:4160-4165.
13. Casey MJ, Bewtra C, Hoehne LL, et al. Histology of prophylactically removed ovaries from BRCA1 and BRCA2 mutation carriers compared with noncarriers in hereditary breast ovarian cancer syndrome kindreds. *Gynecol Oncol* 2000;78:278-287.
14. Chetrit A, Hirsh-Yechezkel G, Ben-David Y, et al. Effect of BRCA1/2 mutations on long-term survival of patients with invasive ovarian cancer: the national Israeli study of ovarian cancer. *J Clin Oncol* 2008;26:20-25.
15. Claus EB, Schildkraut JM, Thompson WD, et al. The genetic attributable risk of breast and ovarian cancer. *Cancer* 1996;77:2318-2324.

16. Crum C et al. *Diagnostic Gynecologic and Obstetric Pathology*. Philadelphia: Elsevier; 2011; p .
17. Deligdisch L, Gil J, Kerner H, et al. Ovarian dysplasia in prophylactic oophorectomy specimens: cytogenetic and morphometric correlations. *Cancer* 1999;86:1544-1550.
18. Easton DF, Ford D, Bishop DT. Breast and ovarian cancer incidence in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. *Am J Hum Genet* 1995;56:265-271.
19. Finch A, Metcalfe KA, Chiang JK, et al. The impact of prophylactic salpingo-oophorectomy on menopausal symptoms and sexual function in women who carry a BRCA mutation. *Gynecol Oncol*;121:163-168.
20. Folkins AK, Jarboe EA, Saleemuddin A, et al. A candidate precursor to pelvic serous cancer (p53 signature) and its prevalence in ovaries and fallopian tubes from women with BRCA mutations. *Gynecol Oncol* 2008;109:168-173.
21. Fong PC, Boss DS, Yap TA, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009;361:123-134.
22. Fujiwara M, Felberg A, Whittemore A, et al. Prediction of BRCA1 germline mutation status in women with ovarian cancer using morphology-based criteria: Identification of a BRCA1 ovarian cancer phenotype. *Mod Pathol* 2011.
23. Gallagher DJ, Konner JA, Bell-McGuinn KM, et al. Survival in epithelial ovarian cancer: a multivariate analysis incorporating BRCA mutation status and platinum sensitivity. *Ann Oncol* 2011;22:1127-1132.
24. Hirst JE, Gard GB, McIllroy K, et al. High rates of occult fallopian tube cancer diagnosed at prophylactic bilateral salpingo-oophorectomy. *Int J Gynecol Cancer* 2009;19:826-829.
25. Hutson R, Ramsdale J, Wells M. p53 protein expression in putative precursor lesions of epithelial ovarian cancer. *Histopathology* 1995;27:367-371.
26. Imyanitov EN, Moiseyenko VM. Drug therapy for hereditary cancers. *Hered Cancer Clin Pract* 2011;9:5.
27. Kauff ND, Satagopan JM, Robson ME, et al. Risk-reducing salpingo-oophorectomy in women with a BRCA1 or BRCA2 mutation. *N Engl J Med* 2002;346:1609-1615.
28. Kerner R, Sabo E, Gershoni-Baruch R, et al. Expression of cell cycle regulatory proteins in ovaries prophylactically removed from Jewish Ashkenazi BRCA1 and BRCA2 mutation carriers: correlation with histopathology. *Gynecol Oncol* 2005;99:367-375.
29. Kindelberger DW, Lee Y, Miron A, et al. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. *Am J Surg Pathol* 2007;31:161-169.
30. Lacour RA, Westin SN, Meyer LA, et al. Improved survival in non-Ashkenazi Jewish ovarian cancer patients with BRCA1 and BRCA2 gene mutations. *Gynecol Oncol* 2011;121:358-363.
31. Lakhani SR, Manek S, Penault-Llorca F, et al. Pathology of ovarian cancers in BRCA1 and BRCA2 carriers. *Clin Cancer Res* 2004;10:2473-2481.

32. Lee Y, Miron A, Drapkin R, et al. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J Pathol* 2007;211:26-35.
33. Leeper K, Garcia R, Swisher E, et al. Pathologic findings in prophylactic oophorectomy specimens in high-risk women. *Gynecol Oncol* 2002;87:52-56.
34. Livingston DM, Silver DP. Cancer: crossing over to drug resistance. *Nature* 2008;451:1066-1067.
35. Longacre TA, Oliva E, Soslow RA. Recommendations for the reporting of fallopian tube neoplasms. *Hum Pathol* 2007;38:1160-1163.
36. Medeiros F, Muto MG, Lee Y, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am J Surg Pathol* 2006;30:230-236.
37. Nagaraju G, Scully R. Minding the gap: the underground functions of BRCA1 and BRCA2 at stalled replication forks. *DNA Repair (Amst)* 2007;6:1018-1031.
38. Narod SA, Goldgar D, Cannon-Albright L, et al. Risk modifiers in carriers of BRCA1 mutations. *Int J Cancer* 1995;64:394-398.
39. Piek JM, van Diest PJ, Zweemer RP, et al. Dysplastic changes in prophylactically removed Fallopian tubes of women predisposed to developing ovarian cancer. *J Pathol* 2001;195:451-456.
40. Powell CB, Kenley E, Chen LM, et al. Risk-reducing salpingo-oophorectomy in BRCA mutation carriers: role of serial sectioning in the detection of occult malignancy. *J Clin Oncol* 2005;23:127-132.
41. Prat J, Ribe A, Gallardo A. Hereditary ovarian cancer. *Hum Pathol* 2005;36:861-870.
42. Rabban JT, Barnes M, Chen LM, et al. Ovarian pathology in risk-reducing salpingo-oophorectomies from women with BRCA mutations, emphasizing the differential diagnosis of occult primary and metastatic carcinoma. *Am J Surg Pathol* 2009;33:1125-1136.
43. Rabban JT, Krasik E, Chen LM, et al. Multistep level sections to detect occult fallopian tube carcinoma in risk-reducing salpingo-oophorectomies from women with BRCA mutations: implications for defining an optimal specimen dissection protocol. *Am J Surg Pathol* 2009;33:1878-1885.
44. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. *Am J Hum Genet* 2001;68:700-710.
45. Salazar H, Godwin AK, Daly MB, et al. Microscopic benign and invasive malignant neoplasms and a cancer-prone phenotype in prophylactic oophorectomies. *J Natl Cancer Inst* 1996;88:1810-1820.
46. Schlosshauer PW, Cohen CJ, Penault-Llorca F, et al. Prophylactic oophorectomy: a morphologic and immunohistochemical study. *Cancer* 2003;98:2599-2606.
47. Semmel DR, Folkins AK, Hirsch MS, et al. Intercepting early pelvic serous carcinoma by routine pathological examination of the fimbria. *Mod Pathol* 2009;22:985-988.

48. Shaw PA, Rouzbahman M, Pizer ES, et al. Candidate serous cancer precursors in fallopian tube epithelium of BRCA1/2 mutation carriers. *Mod Pathol* 2009;22:1133-1138.
49. Sherman ME, Lee JS, Burks RT, et al. Histopathologic features of ovaries at increased risk for carcinoma. A case-control analysis. *Int J Gynecol Pathol* 1999;18:151-157.
50. Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *Am J Pathol* 2004;164:1511-1518.
51. Singer G, Stohr R, Cope L, et al. Patterns of p53 mutations separate ovarian serous borderline tumors and low- and high-grade carcinomas and provide support for a new model of ovarian carcinogenesis: a mutational analysis with immunohistochemical correlation. *Am J Surg Pathol* 2005;29:218-224.
52. Stratton JF, Buckley CH, Lowe D, et al. Comparison of prophylactic oophorectomy specimens from carriers and noncarriers of a BRCA1 or BRCA2 gene mutation. United Kingdom Coordinating Committee on Cancer Research (UKCCCR) Familial Ovarian Cancer Study Group. *J Natl Cancer Inst* 1999;91:626-628.
53. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med* 1997;336:1401-1408.
54. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* 2002;108:171-182.
55. Werness BA, Ramus SJ, DiCioccio RA, et al. Histopathology, FIGO stage, and BRCA mutation status of ovarian cancers from the Gilda Radner Familial Ovarian Cancer Registry. *Int J Gynecol Pathol* 2004;23:29-34.
56. Zhang Z, Singh M, Davidson S, et al. Activation of BTAK expression in primary ovarian surface epithelial cells of prophylactic ovaries. *Mod Pathol* 2007;20:1078-1084.

Lynch syndrome

Epidemiology of MMR-associated Female Genital Tract Cancers

Lynch syndrome (Hereditary Non Polyposis Colorectal Carcinoma) is an autosomal dominant syndrome that increases risk for multiple cancers. This syndrome results from germline mutations in the DNA mismatch repair genes, most frequently *MLH1, MSH2* and *MSH6* and rarely *PMS2* (64). Patients with Lynch syndrome are at increased risk for multiple malignancies (Table 1) (3, 64, 67). While colon cancer and endometrial cancer are the most frequent, these families are also at increased risk for cancers of the ovary, stomach, urinary tract, hepatobiliary tract, small intestine and brain. Lynch syndrome has traditionally been perceived as a colorectal carcinoma dominated syndrome, and proposed screening strategies have focused almost exclusively on colon cancer. However, women with Lynch syndrome are at equal, if not higher, risk for development of gynecologic malignancies compared to their risk for colon cancer (3). Moreover, more than half of these patients present with a gynecologic malignancy as their sentinel cancer (37). The lifetime risk for development of endometrial carcinoma in these patients is 20 to 60% (3, 64). The frequency of LS associated germline mutations in endometrial carcinomas has been estimated at 1.8 to 2.1%, which is similar to that in colon cancer (26, 27, 44). In younger patients with endometrial carcinoma, the prevalence increases to approximately 9% (6, 38). A relationship to body mass index has been proposed. Most endometrioid adenocarcinomas in young patients are associated with estrogen excess and these patients are often obese or overweight. However, Lynch syndrome patients with endometrial carcinoma trend towards a low BMI (23, 38). Since, occasional overweight or obese young patients with Lynch syndrome have also been reported (23, 38), the strength of this association is unclear.

Lynch syndrome increases risk for epithelial ovarian cancers, with a reported lifetime risk of 4-12% (3, 8, 11, 15, 18, 24, 29, 31, 39, 66, 67). Lynch syndrome accounts for approximately 2% of all ovarian cancers (39). Risk for ovarian cancer appears to be particularly high for patients with *MSH2* and MSH6 mutations (29, 31). Patients with Lynch syndrome often present with ovarian

tumors at relatively younger ages, mean age of 40-48 years, unlike endometrial carcinoma in Lynch syndrome, most patients with ovarian cancer are younger than 50 years of age (31, 66).

Table 1. Lifetime Risk of Cancer Reported in Families with an Identified Mismatch Repair Gene Mutation

Colorectal cancer (men)	28-75%
Colorectal cancer (women)	24–52%
Endometrial cancer	27–71%
Ovarian cancer	4-12%
Gastric cancer	2-13%
Urinary tract cancer	1-12%
Brain tumors	1-4%
Bile duct/gallbladder cancer	2%
Small bowel cancer	4-17%

Since a substantial number of women with Lynch syndrome first present with a gynecologic cancer, it is extremely important for gynecologists and pathologists to be aware of this association. These patients and their family members are at considerable risk for synchronous and metachronous tumors, particularly colon cancer (2, 14). Lynch syndrome patients who first present with an endometrial or ovarian cancer may then go on to develop a colon cancer; the time to development of second cancer can vary with a median time of 11 years for patients with endometrial cancer and 5.5 years for patients first diagnosed with ovarian cancer (37). Timely detection of Lynch syndrome in these patients and their family members could lead to appropriate surveillance measures, and may prevent morbidity and mortality from a metachronous colon cancer.

Function of DNA Mismatch Repair Genes

Mutations in the DNA mismatch repair genes involved in Lynch syndrome most frequently lead to loss of function and to microsatellite instability (MSI) (46). Microsatellites are repetitive widely dispersed DNA sequences that are prone to

replication errors. Normally these errors are corrected by the DNA mismatch repair system (DNA-MMR). Deficiencies in the DNA-MMR therefore results in MSI (35). Microsatellite instability can be a consequence of both genetic (related to mismatch repair gene mutations or Lynch syndrome) and epigenetic changes (sporadic due to *MLH1* promoter methylation) (19). Therefore, Lynch syndrome and MSI should not be used synonymously. In fact, 20-25% of all endometrial carcinomas show MSI, the majority (75%) of which result from sporadic *MLH1* promoter methylation, and the minority are Lynch syndrome associated.

The mechanism of tumorigenesis in the setting of MSI appears to involve frameshift mutations of microsatellite repeats within coding regions of genes. Affected genes are well characterized in MSI-associated colorectal carcinomas, but not in endometrial carcinomas, although *PTEN* appears to be a candidate gene (25).

Pathology of Lynch Syndrome Female Genital Tract Tumors

Lynch syndrome endometrial cancers show a predilection for the lower uterine segment (this refers to tumors that arise in the lower uterine segment, and does not include fundic tumors that may involve the LUS). As many as one-third of tumors arising in the lower uterine segment (LUS) may be Lynch syndrome associated (68). The endometrial carcinomas can show a wide spectrum of histologic subtypes. Endometrioid carcinomas are the most common type, but non endometrioid carcinomas also occur, including serous carcinoma, clear cell carcinoma and carcinosarcomas, often at relatively younger ages (12, 16). Amongst endometrioid carcinomas, presence of certain histologic features have been shown to be suggestive of mismatch repair abnormalities (22, 56, 63). Although there is conflicting data regarding the utility of these features (28), they include the presence of prominent peritumoral lymphocytes (apparent at scanning magnification); increased tumor infiltrating lymphocytes i.e. lymphocytes located within the boundary of tumor cell nests or glands (TILs >42 per 10 high power fields); tumor heterogeneity defined as two morphologically distinct tumor populations juxtaposed but not intimately admixed, each

constituting at least 10% of the tumor volume; and presence of undifferentiated or dedifferentiated histology.

Undifferentiated endometrial carcinomas were first described by the group from M D Anderson as solid, dyshesive sheets of round or polygonal cells with vesicular nuclei and prominent nucleoli, without any evidence of gland formation (4). Some may exhibit foci with myxoid matrix and rhabdoid cells, and occasional cases can show lymphoepithelioma-like areas, defined as sheets of undifferentiated cells with a prominent lymphocytic infiltrate. When accompanied by a component of well to moderately differentiated endometrioid carcinoma, they may be referred to as dedifferentiated endometrial carcinomas (58). Undifferentiated/dedifferentiated endometrial carcinomas are associated with mismatch repair abnormalities (5, 61). Some are sporadic and associated with *MLH1* promoter methylation (12), while others are Lynch syndrome associated (22). Undifferentiated/dedifferentiated carcinomas appear to be particularly associated with abnormalities of MLH1/PMS2, both in the form of promoter methylation and germline mutations (21).

The spectrum of ovarian tumor types seen in Lynch syndrome differs from that of the general population. In contrast with hereditary breast and ovarian carcinoma, the majority of ovarian cancers in Lynch syndrome patients are non-serous histology; most are endometrioid, clear cell, or undifferentiated carcinomas (5, 31, 36). The endometrioid carcinomas are usually well to moderately differentiated, present at early stages and pursue favorable clinical outcomes (18, 31, 66). Ovarian clear cell carcinomas, particularly in younger patients, appear to be the most strongly associated with Lynch syndrome; 14-17% of ovarian clear cell carcinomas are associated with mismatch repair defects (8, 15, 18, 29, 31). In the study by Jensen et al, 10% of all ovarian carcinomas in patients 50 years of age or younger were associated with mismatch repair defects, and the majority (60%) of these were clear cell carcinomas (29), with the remainder showing undifferentiated or endometrioid histology.

Some studies have reported an association between Lynch syndrome and presence of synchronous endometrioid carcinomas of the ovary and endometrium (66), but others have not found this association (23, 54). Synchronous endometrioid carcinomas of the ovary and endometrium are relatively common, particularly in young patients with estrogen excess (59). Synchronous uterine endometrioid carcinoma and ovarian clear cell carcinoma has been seen in association with mismatch repair defects, but the numbers of reported cases are few (23, 29).

There is no association between Lynch syndrome and endocervical adenocarcinoma. Uterine and cervical mesenchymal tumors (leiomyosarcoma, endometrial stromal sarcoma, adenosarcoma) are also not considered to be LS-associated tumors.

Screening for Lynch Syndrome

There are currently no uniform screening guidelines for detection of Lynch syndrome in patients who present with gynecologic cancers. Various screening modalities that employ patient factors including age, personal and family history have been proposed and tested. The Amsterdam and Bethesda criteria focus primarily on colorectal carcinomas. The more recently proposed Society of Gynecologic Oncologists (SGO) guidelines for detection of LS in gynecologic cancer patients focus on gynecologic tumors.

Table 2. Amsterdam II Criteria (65)

Amsterdam II criteria:
- Three or more family members with LS/HNPCC-related cancers, one of whom is a first degree relative of the other two.
- Two successive affected generations.
- One or more of the LS/HNPCC-related cancers diagnosed under age 50 years
- Familial adenomatous polyposis (FAP) has been excluded.

Table 3. Revised Bethesda Guidelines (62).

Revised Bethesda guidelines:
- Diagnosed with colorectal cancer before the age of 50 years.
- Synchronous or metachronous colorectal or other LS/HNPCC-related tumours (which include stomach, bladder, ureter, renal pelvis, biliary tract, brain (glioblastoma), sebaceous gland adenomas, keratoacanthomas and carcinoma of the small bowel), regardless of age.
- Colorectal cancer with a high-microsatellite instability morphology that was diagnosed before the age of 60 years.
- Colorectal cancer with one or more first-degree relatives with colorectal cancer or other LS/HNPCC-related tumours. One of the cancers must have been diagnosed before the age of 50 years (this includes adenoma, which must have been diagnosed before the age of 40 years).
- Colorectal cancer with two or more relatives with colorectal cancer or other LS/HNPCC-related tumours, regardless of age.

Table 4. Society of Gynecologic Oncology (SGO) Guidelines (34).

SGO guidelines - Patients with greater than 20-25% chance of having an inherited predisposition to endometrial, colorectal and related cancers for whom genetic risk assessment may be helpful:
- Patients with endometrial or colorectal cancer who meet the revised Amsterdam criteria (as listed above)
- Patients with synchronous or metachronous endometrial and colorectal cancer with the first cancer diagnosed prior to age 50.
- Patients with synchronous or metachronous ovarian and colorectal cancer with the first cancer diagnosed prior to age 50.
- Patients with colorectal or endometrial cancer with evidence of a mismatch repair defect (i.e. microsatellite instability or immunohistochemical loss of expression of MLH1, MSH2, MSH6 or PMS2).
- Patients with first or second degree relative with a known mismatch repair

gene mutation.

SGO guidelines - Patients with greater than 5-10% chance of having an inherited predisposition to endometrial, colorectal and related cancers for whom genetic risk assessment may be helpful:
- Patients with endometrial or colorectal cancer diagnosed prior to age 50.
- Patient with endometrial or ovarian cancer with a synchronous or metachronous colon or other LS/HNPCC associated tumor at any age.
- Patients with endometrial or colorectal cancer and a first degree relative with LS/HNPCC associated tumor diagnosed prior to age 50.
- Patients with colorectal or endometrial carcinoma diagnosed at any age with two or more first or second degree relatives with LS/HNPCC associated tumors, regardless of age.

In general, Lynch syndrome should be a consideration in young (but >40 years) patients with gynecologic cancers (11). However, most women with LS present with EC at older ages, this is particularly true for those with *MSH6* mutations (11). In the study by Hampel et al, 6 of 10 Lynch syndrome patients with EC were older than 50 years of age (26). Similar to age, personal and/or family history of Lynch syndrome associated tumors is extremely useful, but it is not sufficiently sensitive. The majority of patients with Lynch syndrome do not meet the Amsterdam criteria or Bethesda guidelines and do not have personal or family history suggestive of Lynch syndrome. In the study by Hampel et al, 70% of patients with Lynch syndrome did not meet the Amsterdam criteria or Bethesda guidelines (26). In another study by Ryan et al, only 58% of the 76 endometrial carcinoma patients with Lynch syndrome met Amsterdam II criteria, while only 36% met revised Bethesda guidelines, 71% met the SGO 20-25% screening criteria and 93% met the SGO 5-10% criteria (50).

Testing patients of age <50 years with a first degree relative with LS-associated tumor(s) has also been proposed as an option (6). When tumor characteristics are incorporated in a screening algorithm along with age and

personal/family history, enhances detection of mismatch repair abnormalities in endometrial carcinoma patients by approximately 3-fold (22). Other studies have also found that utilizing both clinical and pathologic factors can be useful in detection of LS, but whether these are sufficiently sensitive remains uncertain (45, 51). In a study by Ryan et al, only 42% of endometrial carcinoma from Lynch syndrome patients demonstrated any of these 4 pathologic features: LUS origin, tumor heterogeneity including dedifferentiated histology, presence of peritumoral lymphocytes or tumor infiltrating lymphocytes (50).

Recently a recommendation has been made to offer mismatch repair deficiency testing to all patients newly diagnosed with endometrial cancer, irrespective of age and history (40). This proposal is similar to that recently proposed for colorectal cancer screening (1, 43). Both screening procedures could be accomplished in a cost effective model that incorporates the 2 mismatch repair protein antibody panel.

In a study that compared six criteria for Lynch syndrome testing in women with endometrial cancer (criteria includes all patients and those with variable combinations of age and/or family history), IHC triage of endometrial cancer patients with at least one first degree relative with a Lynch associated cancer, was found to have the best incremental cost-effectiveness ratio (33). IHC testing of all endometrial cancer patients detected the most mutations, but was not found to be cost effective.

Testing for Lynch syndrome

Mutational analysis of the DNA mismatch repair genes is the definitive test to establish a diagnosis of Lynch syndrome. However, mutation analysis is not an effective screening test, and should be utilized as a confirmatory test. Other tests including immunohistochemistry (IHC), MSI analysis and *MLH1* methylation studies may serve as better screening tests.

Immunohistochemistry (IHC) for DNA mismatch repair proteins

IHC for DNA mismatch repair proteins has been shown to be a sensitive and specific test for detection of mismatch repair abnormalities in endometrial carcinoma (41). When all 4 antibodies (MLH1, PMS2, MSH2 and MSH6) are

utilized, IHC has a sensitivity of 91% and specificity of 83% for detecting MSI-high (41). The lower specificity is likely due to mutations in *MSH6*, that can result in MSI-low or MS-stable tumors. In their functional state, the mismatch repair proteins form dimers, MLH1 dimerizes with PMS2 and MSH2 dimerizes with MSH6. MLH1 and MSH2 are the obligatory partners in these dimers, therefore mutations in MLH1 and MSH2 lead to concurrent loss of PMS2 and MSH6 respectively. Therefore *MLH1* promoter methylation or mutation will result in IHC loss of both MLH1 and PMS2. Similarly, mutations in *MSH2* will lead to IHC loss of both MSH2 and MSH6. However, isolated loss of PMS2 and MSH6 can occur due to *PMS2* and *MSH6* mutations respectively. Loss of more than 2 proteins or loss of mismatch repair proteins in other combinations is extremely rare and should be interpreted with caution.

IHC loss of MLH1 and PMS2 may be due to *MLH1* promoter methylation or germline mutations in MLH1 or PMS2, and further testing is required to differentiate between genetic versus epigenetic mechanisms of loss. Loss of MSH2 and/or MSH6 is virtually diagnostic of Lynch syndrome.

Recent data suggests that a two antibody panel (composed of PMS2 and MSH6) is as effective as the 4 antibody panel for detection of mismatch repair abnormalities in colonic and gynecologic tract carcinomas (42, 57).

IHC has numerous advantages as a screening test. It is simple, easily available and relatively inexpensive. Another advantage of IHC is that it can help direct gene sequencing to one or two specific genes, based on the pattern of loss. This algorithm of IHC followed by directed gene sequencing has been shown to be the most cost effective strategy for detection of LS in EC patients (49). IHC is particularly advantageous in tumors with *MSH6* mutations, since these can be MSI-low or MS-stable and may therefore be missed by MSI analysis alone.

The interpretation of immunohistochemistry for mismatch repair proteins can sometimes be difficult (55). There should be complete loss of staining in all tumor cells for an interpretation of IHC loss of a protein. Care should be taken to examine for presence of internal positive control (stroma, lymphocytes or normal

endometrium) which should show nuclear staining. Nuclear staining of tumor cells, even when focal and weak, should be interpreted as retained staining. MSH2 and PMS2 stains are usually straightforward and easy to interpret. However, MSH6 and MLH1 stains can sometimes be challenging. MSH6 expression can be heterogeneous with some areas appearing to lack expression, while others clearly demonstrate nuclear expression; hence small biopsies (e.g., tissue microarrays in research or small biopsies in clinical practice) may be insufficient to entirely rule in or rule out loss of MSH6 protein. Sometimes, tumor cells are negative for MLH1, but the surrounding stromal cells and lymphocytes are also negative. In other instances, tumor cells can show extremely weak and equivocal staining, and occasionally a speckled pattern of nuclear staining can be observed. In these situations, evaluation of PMS2 stain is helpful. If there is unequivocal loss of PMS2, equivocal MLH1 staining probably indicates staining loss. In difficult situations, the slide should be reviewed with another experienced pathologist or the stain may be repeated. If it continues to be problematic, the stain should be interpreted as equivocal or inconclusive, and alternative testing mechanisms should be pursued. Because many of these tumors harbor numerous intraepithelial lymphocytes, it may be difficult in some cases to distinguish nuclear expression in the infiltrating lymphocytes from that of the tumor cell nuclei, thus resulting in a false negative test (i.e., the tumor is interpreted as MMR proficient, when in fact it is MMR deficient). IHC fails to recognize occasional germline mutations, most often these are missense mutations (26).

Since IHC loss can be due to epigenetic *MLH1* promoter methylation, it is not considered a germline test. However, the issue of patient consent is important since it can indicate the presence of Lynch syndrome. In some hospitals, specific consent is required while in others, this is part of the general consent signed by the patient at time of surgery and no separate consent is needed. If no consent is required, it is important that the pathologists, surgeons and oncologists communicate with the genetic counselors to ensure that all

patients with an abnormal result receive appropriate counseling and further testing as required.

MSI analysis

MSI analysis by PCR uses dinucleotide and mononucleotide markers . DNA from tumor and normal tissue is isolated and tested, most commonly with 5 mononucleotide and dinucleotide microsatellite markers as recommended by NCI: (BAT25, BAT26, D2S123, D5S346 and D17S250) (10). More recently, a panel of 5 mononucleotide markers (BAT25, BAT26, NR21, NR24, and NR27) has been shown to be effective and reproducible and superior to the NCI panel (60). When a tumor shows MSI at 2 or more loci, it is considered MSI-high, instability at one locus is interpreted as MSI-low and if no instability is detected, it is considered MS-stable. The disadvantages of MSI analysis compared to IHC are that it is more expensive and requires a molecular laboratory set up and staff. Also, it may not detect carcinomas associated with *MSH6* mutations, which may be MSI-low or stable (26). MSI can result from *MLH1* promoter methylation or germline mutations, and this test cannot differentiate between these two mechanisms. However, some authors advocate a combination of both IHC and MSI analysis to maximize detection of mismatch repair abnormalities.

***MLH1* promoter methylation assay**

This test detects the presence of *MLH1* promoter methylation, which is an acquired phenomenon that results in inactivation of *MLH1* and resulting in loss of MLH1 protein by IHC. If a tumor shows *MLH1* promoter methylation, it is less likely to be Lynch syndrome associated (14, 69). In contrast, a tumor that shows loss of MLH1/PMS2 by IHC but no evidence of promoter methylation is more likely to be due to germline mutations.

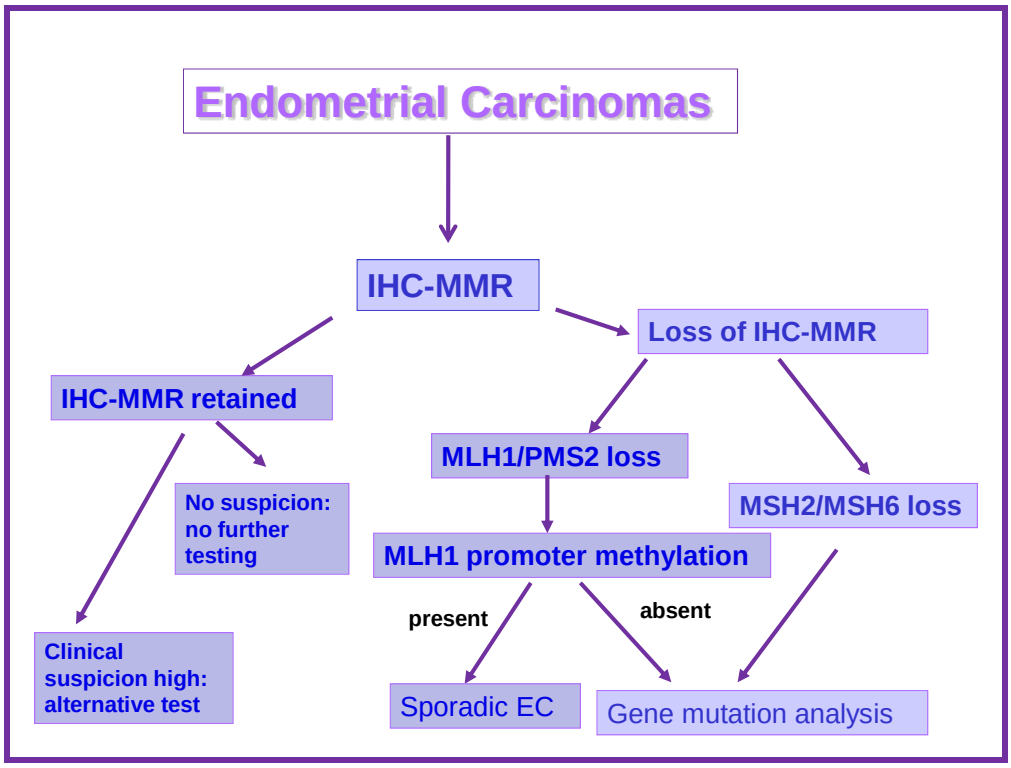
Sporadic colorectal carcinomas with *MLH1* promoter methylation frequently show *BRAF* mutations, and assessment for *BRAF* mutations is an effective and relatively inexpensive test (7). BRAF mutations, however, have not been detected in MLH1 deficient endometrial or ovarian carcinomas (30, 32).

DNA mismatch repair gene mutation analysis

This is a confirmatory test to establish a diagnosis of Lynch syndrome. It may be efficient and cost-effective to narrow the gene sequencing efforts to one or two genes by using immunohistochemical stains. Sometimes molecular analysis results in variants of uncertain significance (VUS), these are then further classified as likely mutations or not based on functional assays and segregation data.

All the above tests have their advantages and disadvantages and are best used in combination with each other. We advocate IHC as a preferred screening method, given the advantages listed above. If the IHC is abnormal, further testing depends on the pattern of IHC loss. In the event of MLH1/PMS2 loss, *MLH1* promoter methylation analysis can be the next step. If methylation is present, the tumor is likely sporadic. In the absence of methylation, *MLH1* mutation analysis should be pursued. *PMS2* mutations are rare, therefore *PMS2* mutation analysis should be reserved for patients with no detectable *MLH1* mutation. If there is IHC loss of MSH2 and MSH6, mutation analysis for *MSH2* should follow. With isolated MSH6 loss, *MSH6* gene mutation analysis should be pursued.

Algorithm for Testing Endometrial Carcinomas by IHC **



****Microsatellite instability by PCR assay is also utilized in association with IHC in most institutions**

Surveillance and risk reducing strategies for endometrial and ovarian carcinomas in Lynch syndrome

Gynecologic cancer surveillance measures in Lynch syndrome patients include annual pelvic exam with Pap smear, transvaginal ultrasound, pelvic ultrasound, and endometrial biopsy starting at age 25-35 years (13). However, these surveillance measures have not shown clinical benefits, and cases of interval endometrial carcinomas not detected by surveillance have been reported (48, 49). The effect of chemoprevention with oral contraceptives in the setting of Lynch syndrome is currently not known. Small studies have shown that prophylactic hysterectomy and bilateral salpingo-oophorectomy after age 35 years or once child bearing is complete can prevent development of endometrial and ovarian cancer in women with LS (53). Risk-reducing (prophylactic) surgery has also been shown to be a more effective and comparatively less expensive option compared to gynecologic surveillance in Lynch syndrome (47, 53). These

patients are at risk for having occult endometrial and/or ovarian cancer, therefore these women should be consented for staging should there be intraoperative evidence of carcinoma (17). Disadvantages of risk-reducing surgery include surgical complications and induction of surgical menopause. There are also occasional reported cases of primary peritoneal carcinoma in LS patients after hysterectomy and bilateral salpingo-oophorectomy, but it is not clear that these carcinomas were directly linked to mismatch repair deficiency (52).

Prognostic and therapeutic implications of mismatch repair abnormalities in endometrial & ovarian carcinoma

Whether mismatch repair status has any impact on prognosis and/or therapy is endometrial carcinomas is currently not known. The available data are controversial; some studies have found an association between mismatch repair defects and improved survival, while others have shown no association with survival or worse clinical outcomes (9, 20, 70).

Many studies have noted that LS associated endometrial carcinomas are often associated with adverse prognostic indicators, including non-endometrioid and undifferentiated histologies, higher FIGO grade, higher stage and more frequent lymphovascular invasion (12, 16, 22, 23, 28, 63). Larger studies with long term clinical follow up are required to definitively assess the impact of mismatch repair status on therapy and outcome in endometrial carcinoma patients.

Summary

- Women with Lynch syndrome are at equal or higher risk for gynecologic cancers compared to their risk for colon cancer.
- Their lifetime risk for endometrial cancer is 40-60% and risk for ovarian cancer is 4-12%.
- Women with Lynch syndrome often present with a gynecologic cancer as their first or sentinel malignancy.
- Lynch syndrome patients are at substantial risk for synchronous and metachronous tumors, identification of these patients has important ramifications for them and their family members.

- Available testing modalities for Lynch syndrome in gynecologic cancers include immunohistochemistry for DNA mismatch repair proteins, microsatellite instability analysis, *MLH1* promoter methylation assay and mismatch repair gene mutation analysis.
- Current screening recommendations for colorectal carcinoma are not sensitive for detection of Lynch syndrome in patients with gynecologic tumors.
- Proposed screening recommendations vary but include utilization of patient age and history, with or without incorporation of tumor pathology. Because each of these strategies misses a significant proportion of women with Lynch syndrome, a case can be made for testing all newly diagnosed endometrial cancer patients with IHC.
- Prognostic and therapeutic impact of mismatch repair status in endometrial and ovarian cancer is undefined.
- Current surveillance techniques for endometrial and ovarian cancer are not effective.
- Risk-reducing (prophylactic) hysterectomy and bilateral salpingo-oophorectomy may be considered in Lynch syndrome patients once childbearing is complete.
- The entire lower uterine segment should always be examined in hysterectomy specimens from Lynch syndrome patients.

References

1. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med.* 2009;11:35-41.
2. Aarnio M, Mecklin JP, Aaltonen LA, et al. Life-time risk of different cancers in hereditary non-polyposis colorectal cancer (HNPCC) syndrome. *Int J Cancer.* 1995;64:430-433.
3. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. *Int J Cancer.* 1999;81:214-218.
4. Altrabulsi B, Malpica A, Deavers MT, et al. Undifferentiated carcinoma of the endometrium. *Am J Surg Pathol.* 2005;29:1316-1321.

5. Azueta A, Gatius S, A. V, et al. Dedifferentiated carcinoma of the endometrium and ovary: A molecular study of 8 cases. *Mod Pathol.* 2011;24,supplement 1:236A.
6. Berends MJ, Wu Y, Sijmons RH, et al. Toward new strategies to select young endometrial cancer patients for mismatch repair gene mutation analysis. *J Clin Oncol.* 2003;21:4364-4370.
7. Bessa X, Balleste B, Andreu M, et al. A prospective, multicenter, population-based study of BRAF mutational analysis for Lynch syndrome screening. *Clin Gastroenterol Hepatol.* 2008;6:206-214.
8. Bewtra C, Watson P, Conway T, et al. Hereditary ovarian cancer: a clinicopathological study. *Int J Gynecol Pathol.* 1992;11:180-187.
9. Black D, Soslow RA, Levine DA, et al. Clinicopathologic significance of defective DNA mismatch repair in endometrial carcinoma. *J Clin Oncol.* 2006;24:1745-1753.
10. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* 1998;58:5248-5257.
11. Bonadona V, Bonaiti B, Olschwang S, et al. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. *JAMA.* 2011;305:2304-2310.
12. Broaddus RR, Lynch HT, Chen LM, et al. Pathologic features of endometrial carcinoma associated with HNPCC: a comparison with sporadic endometrial carcinoma. *Cancer.* 2006;106:87-94.
13. Burke W, Daly M, Garber J, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. BRCA1 and BRCA2. Cancer Genetics Studies Consortium. *JAMA.* 1997;277:997-1003.
14. Buttin BM, Powell MA, Mutch DG, et al. Increased risk for hereditary nonpolyposis colorectal cancer-associated synchronous and metachronous malignancies in patients with microsatellite instability-positive endometrial carcinoma lacking MLH1 promoter methylation. *Clin Cancer Res.* 2004;10:481-490.
15. Cai KQ, Albarracin C, Rosen D, et al. Microsatellite instability and alteration of the expression of hMLH1 and hMSH2 in ovarian clear cell carcinoma. *Hum Pathol.* 2004;35:552-559.
16. Carcangiu ML, Radice P, Casalini P, et al. Lynch syndrome--related endometrial carcinomas show a high frequency of nonendometrioid types and of high FIGO grade endometrioid types. *Int J Surg Pathol.* 2010;18:21-26.
17. Chung L, Broaddus R, Crozier M, et al. Unexpected endometrial cancer at prophylactic hysterectomy in a woman with hereditary nonpolyposis colon cancer. *Obstet Gynecol.* 2003;102:1152-1155.
18. Domanska K, Malander S, Masback A, et al. Ovarian cancer at young age: the contribution of mismatch-repair defects in a population-based series of epithelial ovarian cancer before age 40. *Int J Gynecol Cancer.* 2007;17:789-793.

19. Esteller M, Levine R, Baylin SB, et al. MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. *Oncogene.* 1998;17:2413-2417.
20. Fiumicino S, Ercoli A, Ferrandina G, et al. Microsatellite instability is an independent indicator of recurrence in sporadic stage I-II endometrial adenocarcinoma. *J Clin Oncol.* 2001;19:1008-1014.
21. Garg K, Kauff ND, Soslow RA. Endometrial carcinomas with DNA mismatch repair abnormalities: Genotypic phenotypic correlations. *Mod Pathol.* 2011;24,supplement 1:247A.
22. Garg K, Leitao MM, Jr., Kauff ND, et al. Selection of endometrial carcinomas for DNA mismatch repair protein immunohistochemistry using patient age and tumor morphology enhances detection of mismatch repair abnormalities. *Am J Surg Pathol.* 2009;33:925-933.
23. Garg K, Shih K, Barakat R, et al. Endometrial carcinomas in women aged 40 years and younger: tumors associated with loss of DNA mismatch repair proteins comprise a distinct clinicopathologic subset. *Am J Surg Pathol.* 2009;33:1869-1877.
24. Grindedal EM, Renkonen-Sinisalo L, Vasen H, et al. Survival in women with MMR mutations and ovarian cancer: a multicentre study in Lynch syndrome kindreds. *J Med Genet.* 2010;47:99-102.
25. Gurin CC, Federici MG, Kang L, et al. Causes and consequences of microsatellite instability in endometrial carcinoma. *Cancer Res.* 1999;59:462-466.
26. Hampel H, Frankel W, Panescu J, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. *Cancer Res.* 2006;66:7810-7817.
27. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med.* 2005;352:1851-1860.
28. Honore LH, Hanson J, Andrew SE. Microsatellite instability in endometrioid endometrial carcinoma: correlation with clinically relevant pathologic variables. *Int J Gynecol Cancer.* 2006;16:1386-1392.
29. Jensen KC, Mariappan MR, Putcha GV, et al. Microsatellite instability and mismatch repair protein defects in ovarian epithelial neoplasms in patients 50 years of age and younger. *Am J Surg Pathol.* 2008;32:1029-1037.
30. Kawaguchi M, Yanokura M, Banno K, et al. Analysis of a correlation between the BRAF V600E mutation and abnormal DNA mismatch repair in patients with sporadic endometrial cancer. *Int J Oncol.* 2009;34:1541-1547.
31. Ketabi Z, Bartuma K, Bernstein I, et al. Ovarian cancer linked to Lynch syndrome typically presents as early-onset, non-serous epithelial tumors. *Gynecol Oncol.* 2011;121:462-465.
32. Kitayama J, Longacre TA, Liou S, et al. MLH-1 deficient ovarian and endometrial carcinomas most often result from epigenetic silencing of MLH1 by promoter methylation and do not harbor BRAF V600E mutations: Implications for identifying patients with Lynch syndrome. *Mod Pathol.* 2011;24, supplement 1:252A.
33. Kwon JS, Scott JL, Gilks CB, et al. Testing women with endometrial cancer to detect Lynch syndrome. *J Clin Oncol.* 2011;29:2247-2252.

34. Lancaster JM, Powell CB, Kauff ND, et al. Society of Gynecologic Oncologists Education Committee statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecol Oncol.* 2007;107:159-162.
35. Loeb LA. Microsatellite instability: marker of a mutator phenotype in cancer. *Cancer Res.* 1994;54:5059-5063.
36. Lu FI, Pollett A, Ryan P, et al. Prevalence of DNA mismatch repair protein loss on 342 primary malignant epithelial ovarian tumors. *Mod Pathol.* 2011;24, supplement 1:258A.
37. Lu KH, Dinh M, Kohlmann W, et al. Gynecologic cancer as a "sentinel cancer" for women with hereditary nonpolyposis colorectal cancer syndrome. *Obstet Gynecol.* 2005;105:569-574.
38. Lu KH, Schorge JO, Rodabaugh KJ, et al. Prospective determination of prevalence of lynch syndrome in young women with endometrial cancer. *J Clin Oncol.* 2007;25:5158-5164.
39. Malander S, Rambech E, Kristoffersson U, et al. The contribution of the hereditary nonpolyposis colorectal cancer syndrome to the development of ovarian cancer. *Gynecol Oncol.* 2006;101:238-243.
40. Mills AM, Liou S, Ford JM, et al. Lynch syndrome screening should be considered for all patients with newly diagnosed endometrial cancer. *Mod Pathol.* 2011;24,supplement 1:260A.
41. Modica I, Soslow RA, Black D, et al. Utility of immunohistochemistry in predicting microsatellite instability in endometrial carcinoma. *Am J Surg Pathol.* 2007;31:744-751.
42. Mojtahed A, Schrijver I, Ford JM, et al. A two-antibody mismatch repair protein immunohistochemistry screening approach for colorectal carcinomas, skin sebaceous tumors, and gynecologic tract carcinomas. *Mod Pathol.* 2011;24:1004-1014.
43. Mvundura M, Grosse SD, Hampel H, et al. The cost-effectiveness of genetic testing strategies for Lynch syndrome among newly diagnosed patients with colorectal cancer. *Genet Med.* 2010;12:93-104.
44. Ollikainen M, Abdel-Rahman WM, Moisio AL, et al. Molecular analysis of familial endometrial carcinoma: a manifestation of hereditary nonpolyposis colorectal cancer or a separate syndrome? *J Clin Oncol.* 2005;23:4609-4616.
45. Palacios J, Alenda C, Lujan D, et al. Pathologic features of endometrial cancer in patients with Lynch syndrome. *Mod Pathol.* 2010;23,supplement 1:258A.
46. Peltomaki P, Lothe RA, Aaltonen LA, et al. Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. *Cancer Res.* 1993;53:5853-5855.
47. Pistorius S, Kruger S, Hohl R, et al. Occult endometrial cancer and decision making for prophylactic hysterectomy in hereditary nonpolyposis colorectal cancer patients. *Gynecol Oncol.* 2006;102:189-194.
48. Renkonen-Sinisalo L, Butzow R, Leminen A, et al. Surveillance for endometrial cancer in hereditary nonpolyposis colorectal cancer syndrome. *Int J Cancer.* 2007;120:821-824.

49. Resnick K, Straughn JM, Jr., Backes F, et al. Lynch syndrome screening strategies among newly diagnosed endometrial cancer patients. *Obstet Gynecol.* 2009;114:530-536.
50. Ryan P, Mulligan AM, Aronson M, et al. Comparison of clinical schemas and morphologic features in predicting Lynch syndrome in mutation-positive patients with endometrial cancer encountered in the context of familial gastrointestinal cancer registries. *Cancer.* 2011.
51. Ryan P, Mulligan AM, Aronson M, et al. Endometrial carcinoma in Lynch syndrome: Genotype-phenotype correlation. *Mod Pathol.* 2010;23,supplement 1:261A.
52. Schmeler KM, Daniels MS, Soliman PT, et al. Primary peritoneal cancer after bilateral salpingo-oophorectomy in two patients with Lynch syndrome. *Obstet Gynecol.* 2010;115:432-434.
53. Schmeler KM, Lynch HT, Chen LM, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. *N Engl J Med.* 2006;354:261-269.
54. Shannon C, Kirk J, Barnetson R, et al. Incidence of microsatellite instability in synchronous tumors of the ovary and endometrium. *Clin Cancer Res.* 2003;9:1387-1392.
55. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. *J Mol Diagn.* 2008;10:293-300.
56. Shia J, Black D, Hummer AJ, et al. Routinely assessed morphological features correlate with microsatellite instability status in endometrial cancer. *Hum Pathol.* 2008;39:116-125.
57. Shia J, Tang LH, Vakiani E, et al. Immunohistochemistry as first-line screening for detecting colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: a 2-antibody panel may be as predictive as a 4-antibody panel. *Am J Surg Pathol.* 2009;33:1639-1645.
58. Silva EG, Deavers MT, Bodurka DC, et al. Association of low-grade endometrioid carcinoma of the uterus and ovary with undifferentiated carcinoma: a new type of dedifferentiated carcinoma? *Int J Gynecol Pathol.* 2006;25:52-58.
59. Soliman PT, Slomovitz BM, Broaddus RR, et al. Synchronous primary cancers of the endometrium and ovary: a single institution review of 84 cases. *Gynecol Oncol.* 2004;94:456-462.
60. Suraweera N, Duval A, Reperant M, et al. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. *Gastroenterology.* 2002;123:1804-1811.
61. Tafe LJ, Garg K, Chew I, et al. Endometrial and ovarian carcinomas with undifferentiated components: clinically aggressive and frequently underrecognized neoplasms. *Mod Pathol.* 2010;23:781-789.
62. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst.* 2004;96:261-268.

63. van den Bos M, van den Hoven M, Jongejan E, et al. More differences between HNPCC-related and sporadic carcinomas from the endometrium as compared to the colon. *Am J Surg Pathol.* 2004;28:706-711.
64. Vasen HF, Offerhaus GJ, den Hartog Jager FC, et al. The tumour spectrum in hereditary non-polyposis colorectal cancer: a study of 24 kindreds in the Netherlands. *Int J Cancer.* 1990;46:31-34.
65. Vasen HF, Watson P, Mecklin JP, et al. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. *Gastroenterology.* 1999;116:1453-1456.
66. Watson P, Butzow R, Lynch HT, et al. The clinical features of ovarian cancer in hereditary nonpolyposis colorectal cancer. *Gynecol Oncol.* 2001;82:223-228.
67. Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. *Cancer.* 1993;71:677-685.
68. Westin SN, Lacour RA, Urbauer DL, et al. Carcinoma of the lower uterine segment: a newly described association with Lynch syndrome. *J Clin Oncol.* 2008;26:5965-5971.
69. Whelan AJ, Babb S, Mutch DG, et al. MSI in endometrial carcinoma: absence of MLH1 promoter methylation is associated with increased familial risk for cancers. *Int J Cancer.* 2002;99:697-704.
70. Zighelboim I, Goodfellow PJ, Gao F, et al. Microsatellite instability and epigenetic inactivation of MLH1 and outcome of patients with endometrial carcinomas of the endometrioid type. *J Clin Oncol.* 2007;25:2042-2048.