Overview
A contemporary understanding of cancer for a diagnostic pathologist consists of two elements:
1. Understanding the current role of pathologic variables in guiding the surgeon’s management decisions.
2. Understanding contemporary diagnostic classification schemes and tools for evaluating those variables and their diagnostic mimics.

In short, our goal as pathologists is to evaluate and report endometrial cancer such that the surgeon is equipped to select the best available management options. This syllabus will focus on that mission, including recent advances that are of practical relevance in daily surgical pathology practice.

Outline of Syllabus
1. Role of diagnostic pathology in guiding management of endometrial cancer
2. Subtyping endometrial cancer
3. Grading endometrial cancer
4. Staging endometrial cancer (including lymphovascular invasion (LVI))
5. Screening endometrial cancer patients for Lynch syndrome

Rather than present the standard approach of organizing the discussion by specific types of endometrial cancer, this syllabus will present an integrated, practice-based approach that highlights issues as they would appear in the daily experience of evaluating uterine cancer. Emphasis will be placed on:

- Clinically relevant elements of endometrial cancer pathology
- A pattern based approach to subtyping uterine cancer
- Problems and controversies in rules/criteria/guidelines for typing, grading, and staging
- Practical recommendations to manage scenarios not addressed by existing rules/criteria/guidelines.

Role of pathologic variables in management of endometrial cancer
There are four major decisions in the management of a patient with a biopsy diagnosis of endometrial cancer. Pathologic tumor variables, along with patient age and clinical/radiologic staging, play a central role in these decisions. In general, surgeons broadly categorize endometrial cancer into low versus high risk behavior in order to navigate these management options. High risk behavior is defined as a cancer that is likely to present at advanced stage, to be chemo-resistant, to recur locally (vaginal cuff, pelvis), to metastasize, or to progress to death. High risk cancer can be defined by histologic sub-type (all grade 3 cancers) and by higher stages (involvement of outer myometrium, lymphovascular spaces, cervix, adnexa, or extra-pelvic sites). Low risk cancer includes grade 1 endometrioid adenocarcinoma confined to endometrium. The behavior of grade 1 and grade 2 endometrioid cancers with myometrial invasion or LVI is difficult to estimate and therefore these scenarios constitute an intermediate risk zone. To demonstrate the specific role of pathology, the general concepts of management currently used at UCSF are discussed below (kindly summarized by my colleague Lee-may Chen MD, Gynecologic Oncology Division).

Decision #1: Option for trial of hormonal treatment. Data source = biopsy diagnosis.

Surgery is the main treatment of endometrial carcinoma, however, an option of a trial of hormonal treatment can be considered in two scenarios: 1.) A premenopausal woman who desires future child-bearing and who has a grade 1 endometrioid adenocarcinoma in a biopsy or 2.) a woman who, for medical reasons, is not a good operative candidate and who has a grade 1 endometrioid adenocarcinoma in a biopsy. Either patient may be offered a trial of hormonal therapy (high dose progestins) and about two-thirds to three-quarters of women will completely respond to hormonal therapy without need for surgery. This approach will not work in grade 2 endometrioid adenocarcinoma or “high risk” cancers.

Decision #2: Extent of surgery. Data sources = biopsy and intra-operative diagnosis.

Total hysterectomy with pelvic lymph node dissection is appropriate for "low risk" cancer but not for "high risk" cancers with a predilection for spread. Lymphovascular spread to the para-aortic lymph nodes is a risk in any tumor with LVI, high stage or grade 3 histologic type. Therefore, para-aortic lymph node dissection is added for grade 3 endometrioid adenocarcinoma, clear cell carcinoma, any tumor with LVI, or some cases with
myometrial/cervical/adnexal involvement. Because serous carcinoma and carcinosarcoma may also exhibit trans-peritoneal spread in addition to lymphovascular spread, omentectomy is additionally performed for these two tumor types.

Since a biopsy cannot evaluate involvement of myometrium, cervix, adnexa or lymphatics, intra-operative evaluation of these variables is needed if the biopsy contains "low/intermediate risk disease" (i.e. grade 1 or grade 2 endometrioid adenocarcinoma). In theory, intra-operative evaluation is not essential if the biopsy shows grade 3 endometrioid, serous, clear cell or carcinosarcoma since those histologic types alone define the surgical plan.

**Decision #3: Selection of role of adjuvant therapy. Data source = final hysterectomy diagnosis.**

No adjuvant treatment is generally needed for grade 1, stage IA (non-myoinvasive) endometrioid adenocarcinoma without LVI. Platinum-based chemotherapy is generally considered for all grade 3 adenocarcinomas and some grade 2 endometrioid cancers depending on age, LVI and myometrial invasion. Special chemotherapy is additionally considered for some tumor types, such as ifosfamide for carcinosarcoma containing rhabdomyosarcomatous elements or etoposide for neuroendocrine carcinoma. Radiation is often considered for clear cell carcinoma, which can be chemoresistant, as well as for some other types depending on stage.

**Decision #4: Selection to screen for Lynch syndrome. Data source: final hysterectomy diagnosis.**

In hospital settings prepared to screen, counsel, test, and treat uterine cancer patients found to have Lynch syndrome, the genetic syndrome predisposing a patient and her family to uterine and colon cancer, among other cancers, there is a role for the pathology of the cancer to play in helping deciding whether risk assessment is appropriate. In the appropriate clinical setting, the presence of tumor morphologic features associated with microsatellite instability (MSI) should trigger testing for MSI and/or mismatch repair protein immunohistochemistry. Those results are used by genetic counselors, along with personal and family cancer history, to determine whether formal genetic testing for the syndrome is warranted.

By understanding the general concepts of which pathology variables influence these four management decisions, it becomes clear that we as pathologists should place emphasis on the following elements of endometrial cancer pathology:

- Histologic sub-typing of endometrial cancer
- Grading
- Staging, LVI
- Criteria for triggering Lynch syndrome screening tests

Therefore, this list defines the outline of the syllabus.
Overview
Tumor sub-type is one the key pathologic variables that defines prognosis and management. The current WHO classification is the preferred system. It recognizes the following subtypes below. Tumors are usually one pure type but if a second, separate type is present and makes up at least 10% of the tumor, then a mixed type can be assigned.

WHO Classification of Endometrial Carcinoma
- Endometrioid adenocarcinoma
- Serous carcinoma
- Clear cell carcinoma
- Transitional cell carcinoma
- Mucinous carcinoma
- Small cell carcinoma
- Squamous cell carcinoma
- Undifferentiated carcinoma
- Carcinosarcoma
- Mixed type

Although not specified within the WHO system, each sub-type may exhibit myriad growth patterns, many of which overlap with each other, leading to problems in sub-typing. Resolving these problems will be the focus of this section.

Approach to sub-typing cancer in a biopsy or curettage
As discussed earlier, there are two key management decision forks that hinge on the pre-operative biopsy diagnosis:

Decision #1: Hormonal treatment option may be considered instead of surgery as first line treatment of grade 1 endometrioid adenocarcinoma (EmoidCA) in the appropriate clinical setting.

Decision #2: Grade 3 EmoidCA, clear cell carcinoma (CCC), serous carcinoma and carcinosarcoma need addition of para-aortic node dissection. In addition, omentectomy is needed if serous carcinoma or carcinosarcoma.

Practical recommendations: If there is difficulty in sub-typing a tumor in a biopsy/curettage, but the differential diagnosis includes only grade 3 types, then it is reasonable to report it as “High grade adenocarcinoma” and discuss the differential diagnosis in a comment. This approach will still result in the surgeon proceeding with the correct surgery. Final sub-typing can be done on the hysterectomy without any compromise to the surgical management. Other experts also condone this approach.

If there is difficulty in distinguishing grade 2 versus grade 3 EmoidCA, it is reasonable to provide a descriptive comment rather than be definitive about grade 2, which may lead to under-management. Comment: “The amount of solid growth in this EmoidCA is at the cusp between grade 2 and grade 3. Overall grading is deferred to review of the hysterectomy specimen”. This approach will allow the surgeon to tailor the surgical plan with other variables of the patient, including intra-operative evaluation for invasion of myometrium, lymphatics, or cervix, which would prompt more surgery.

Patterns That Cause Clinically Significant Problems in Sub-typing Endometrial Cancer
There are a few tumor growth patterns that commonly cause problems in sub-typing the cancer, as recognized by others as well as by our own personal experience and consultation experience. Many of these typing problems may lead to over/under treatment surgically; incorrect choice of adjuvant therapy; and/or missed opportunity to screen for Lynch syndrome. Pattern-based differential diagnosis can help minimize errors in sub-typing. Some errors occur because the differential diagnosis is not well-recognized; some errors occur because the diagnostic criteria are difficult to apply. The most common problematic patterns are as follows:

1. Glandular pattern
   - Pseudo-glandular serous carcinoma
   - Grade 1 endometrioid adenocarcinoma

2. Papillary pattern
   - Papillary endometrioid adenocarcinoma
   - Serous carcinoma

3. Solid pattern with high grade nuclei
   - Solid serous carcinoma
   - Grade 3 endometrioid adenocarcinoma
4. Spindled pattern
- Undifferentiated carcinoma
- Neuroendocrine carcinoma

4. Spindled pattern
- Endometrioid adenocarcinoma with spindle cells, with cored and hyalinized pattern, or with extensive progestin treatment effect.
- Carcinosarcoma

5. Clear cell-rich pattern
- Endometrioid adenocarcinoma with clear cells
- Clear cell carcinoma

6. Hobnail / papillary / clear cell pattern
- Clear cell carcinoma
- Serous carcinoma

7. Mucinous pattern
- Endometrioid adenocarcinoma with mucinous cells or microglandular hyperplasia-like features
- Endocervical adenocarcinoma

8. Necrosis or desmoplasia in a biopsy of tumor with low grade architecture
- Myoinvasive grade 1 endometrioid adenocarcinoma
- Myoinvasive pseudo-glandular serous carcinoma
- Low grade component of under-sampled dedifferentiated carcinoma
- Low grade component of under-sampled high grade endometrioid adenocarcinoma
- Low grade component of under-sampled carcinosarcoma

General Approach to Resolving Problematic Patterns

Since a single tumor sub-type can exhibit a range of morphologic patterns, it is helpful to briefly review the key defining features that are common to given sub-type regardless of the specific pattern it is displaying. These core features form the basis for resolving problematic patterns. Unfortunately not all tumor sub-types have a single pathognomonic feature so it is often a constellation of features that must be weighed. Clinical parameters, other than age, are not particularly helpful in sub-typing; morphology remains king.

Endometrioid Adenocarcinoma (EmoidCA): core features

Morphology:
- Architecture resembles endometrial glands: tubuloglandular growth
- Cells resemble endometrial glands: columnar cells.
- Nuclear pleomorphism/atypical mitoses restricted to highest end of grade 3.
- Squamous differentiation is common.

WHO criteria for squamous differentiation\(^{(109)}\) (any one criteria is sufficient)
- Keratinization
- Intracellular bridges
- Constellation of 3 or more of these:
  - Sheet-like growth without glands or palisading
  - Sharp cell margins
  - Eosinophilic and glassy cytoplasm
- Decreased N/C ratio compared to glandular component

Precursor morphology
- Complex hyperplasia with atypia (or EIN) often present in adjacent proliferative-pattern endometrium

WHO criteria for atypical hyperplasia\(^{(93, 109)}\)
- Round / oval nuclei
- Loss of polarization (jumbled stratification of nuclei)
- Nucleoli
- Vesicular chromatia

Endometrial intraepithelial neoplasia (EIN)\(^{(109)}\)
- Crowded glands occupy 50% or more of area
- Nuclei look different than adjacent normal nuclei
- Crowding is > 1mm in span
- Metaplasia excluded

Immunophenotype
- No pathognomonic positive marker of “endometrioid” differentiation
- Most express ER, PR, HNF-1
- p16 may be negative or focal/patchy in lower grades; increasing positivity in higher grades.
- p53 usually weak/focal but may be positive in high grade
- PTEN loss occurs in about half to three-quarters of cases (tricky stain to interpret)

Serous Carcinoma: core features

Morphology:
- Architecture often mixed: papillary, micropapillary, semi-solid with slit-like branching spaces
- Pleomorphic, high grade tumor cells; irregular/polygonal shape, rarely columnar shape.
- High nuclear: cytoplasmic ratio, minimal cytoplasm, nuclear enlargement, coarse chromatin, macro-nucleoli
- Occasional scattered tumor cells with bizarre nuclei (enlarged, bizarre shapes, multilobed)
- Brisk and atypical mitoses
Precursor morphology
- Serous endometrial intraepithelial carcinoma (EIC) in an atrophic background

**Serous EIC criteria**: replacement of existing epithelium of endometrial surface, glands, or polyp with malignant cells
- cells have cytologic appearance of typical high grade serous carcinoma
- diffuse/strong p16 and either diffuse/strong p53 or entirely absent p53

Immunophenotype
- Diffuse strong p16
- p53 either diffuse/strong or entirely negative (so-called “all or none" pattern)
- ER/PR is variable
- HNF-1 is negative
- PTEN loss is rare (tricky stain to interpret).

Other core features
- May colonize polyps
- Older age / post-menopausal

**Clear cell carcinoma**: core features
Morphology:
- Architecture is mixed: hobnail, papillary, tubuloglandular, solid
- Stromal core hyalinization; stromal core edema; myxoid stromal cores
- Floating “open tumor rings”
- Polygonal cells with well-defined cell membranes, clear cytoplasm, central irregular nuclei
- Hyaline globules in cytoplasm or extracytoplasmic
- Nuclear atypia usually moderate rather than severe

Precursor morphology
- Not well recognized. Background endometrium is often atrophic

Immunophenotype
- HNF-1 positive
- ER/PR: most are negative or focal/weak
- p53 usually weak to moderate and patchy or negative

**Carcinosarcoma**: core features
Morphology:
- Architecture shows distinct compartments of carcinoma versus sarcoma
- Carcinoma is usually serous carcinoma, sometimes high grade endometrioid
- Sarcoma cells are pleomorphic spindled cells, briskly mitotic
- Heterologous elements are common and are an adverse prognostic factor.

Precursor morphology
- Not well recognized.

Immunophenotype
- Carcinoma component is concordant with its pure counterpart. Usually p53/p16 positive
- Sarcoma component is keratin negative, p53/p16 positive.

**Undifferentiated uterine carcinoma (UUC) / dedifferentiated uterine carcinoma**: core features
(this tumor is described in full detail in a later section)

Morphology:
- Architecture is sheets of non-cohesive monomorphic tumor cells without gland formation
- Necrosis is common
- Monomorphic round/polygonal tumor cells with moderate atypia and mitoses
- Minimal cytoplasm but some show rhabdoid cytology

Precursor morphology
- Not well recognized

Immunophenotype
- Keratin/EMA is negative or focal (10%). CK18 yields best results, though still focal.
- ER/PR: conflicting reports of negative versus frequent positivity
- p16 commonly diffuse positive
- p53 not studied.

**Neuroendocrine carcinoma: core features**

**Morphology:**
- Same as for neuroendocrine carcinomas outside the uterus. Must have classical features.
- Large cell neuroendocrine carcinoma, small cell carcinoma, rarely carcinoid

**Precursor morphology**
- Not well recognized

**Immunophenotype**
- Diffusely positive synaptophysin, chromogranin, CD56

- Practical recommendation: If the tumor fits any of the sub-types other than neuroendocrine carcinoma (e.g. endometrioid or serous), do not order neuroendocrine stains. The results do not affect prognosis or management and may only serve to confuse the situation.

**An Important Addition to the Family of Endometrial Cancer Subtypes: UUC and DDUC**

One clinically significant advance in endometrial cancer subtyping that deserves special attention is undifferentiated uterine carcinoma and dedifferentiated carcinoma.

**Undifferentiated Uterine Carcinoma (UUC) and De-differentiated Carcinoma (DDUC)**

The current WHO system recognizes "undifferentiated carcinoma" as one that lacks "any evidence of differentiation" but no further commentary is provided about the epidemiology, gross / microscopic / immunohistochemical pathology, behavior, management or differential diagnosis. Thus, it is possible that the WHO term has been applied to a heterogeneous group of tumors (some cases of grade 3 endometrioid adenocarcinoma, serous carcinoma, or high grade neuroendocrine carcinoma), rather than representing a unique type of tumor.

In 2005, the term "undifferentiated uterine carcinoma" (UUC) was used to specifically define an uncommon type of carcinoma without differentiation that behaved worse than grade 3 endometrioid adenocarcinoma. That group and others have since observed that some UUC are associated with low grade endometrioid adenocarcinoma (so-called dedifferentiated uterine carcinoma (DDUC), yet still behave as UUC. This tumor has also been reported in the ovary. Recent work also suggests that UUC might be a risk factor for tumor microsatellite instability and therefore potentially linked to Lynch syndrome. It can be misdiagnosed as a variety of other neoplasms. Thus pathologists should be aware of this entity for 3 reasons:

- UUC should be reported as grade 3, regardless of how little is present when mixed with lower grade areas.
- UUC may warrant screening for Lynch syndrome
- UUC is a diagnosis of exclusion; a broad array of other malignancies resemble UUC

**Clinical presentation**

About 9% of all uterine cancer is UUC or DDUC. Average age at presentation of UUC is in the 5th to 6th decades (range 21-72 years) and for DDUC, about 50 years (range 21 to 82). The presenting symptoms are typical for uterine cancer: vaginal bleeding and/or pelvic pain. About half present at high stage (III or IV). Some cases of UUC present as a recurrence following an endometrial endometrioid adenocarcinoma or ovarian endometrioid adenocarcinoma; the interval to such recurrence is about 3 years. In one study 5 of 32 patients with UUC had first degree family members with a Lynch syndrome associated tumor.

**Gross pathology**

UUC tend to be large polypoid tumors extensively involving the endometrium and frequently extending to the cervix. Necrosis is commonly visible. Ovarian equivalent of UUC presents as a necrotic fleshy mass.

**Microscopic pathology**

[5, 98, 107]
The definition of UUC is solid patternless sheet-like growth of medium-sized relatively monomorphic round or polygonal epithelial cells that lack glandular differentiation and do not express any neuroendocrine immunohistochemical markers (or do so only focally). Tumor cells are frequently non-cohesive, resulting in a lymphoma-like appearance. Vague cords may be seen in some cases. Rarely, micro-foci of abrupt keratinization are found surrounded by tumor cells. Necrosis is common. Tumor cells contain minimal cytoplasm, though focal rhabdoid cells or cells with clear cytoplasm may be seen in some cases. Chromatin may be vesicular; prominent eosinophilic nucleoli may be present. Mitoses are brisk. The stroma may be somewhat myxoid. The majority of cases exhibit relatively monomorphic tumor cells but a minority may contain areas of marked nuclear pleomorphism. Tumor infiltrating lymphocytes are common.

The definition of DDUC is the co-existence of UUC with a low grade endometrioid adenocarcinoma. The two components may be adjacent to each other but they remain distinct compartments of tumor rather than merging together as seen in the relationship between solid and glandular growth in a grade 2 or 3 endometrioid adenocarcinoma. There is a sharp, well-defined interface between the two components. The amount of UUC within the tumor may be as little as 20% or as high as 90%.

In one study of 32 cases with UUC, about two-thirds are pure (i.e. UUC) and one third are admixed with lower grade adenocarcinoma (i.e. DDUC).

Some patients with UUC or DDUC may have concurrent tumor in the endometrium and in the ovary but the undifferentiated component is not always in both sites.

**Immunohistochemistry**

**Keratin / EMA:** Most UUC show only focal (<10%) keratin/EMA positivity. Rare cases show diffuse positivity. Cases with larger more eosinophilic tumor cells tend to be the cells with the most expression of these markers.\(^{107}\) CK18 is frequently the most positive keratin.

**Synaptophysin / chromogranin:** Most UUC are negative and a minority show focal (<10%) positivity. The latter cases do not exhibit any morphologic characteristics of neuroendocrine differentiation (such as molding or speckled chromatin).

**p16:** About half of UUC are diffusely/strongly positive for p16.\(^{89}\)

**Estrogen Receptor / Progesterone Receptor:** There is conflicting data: one study reported that all but one of 9 cases tested were negative.\(^{107}\) Another study reported that three-quarters of UUC are ER/PR positive.\(^{89}\)

**Mismatch repair proteins:** One study reported that nearly half of UUC had loss of at least one MMR protein; most being loss of MLH1/PMS2.\(^{107}\)

**BAF-47 (INI-1):** This protein is lost in extrarenal malignant rhabdoid tumor, which is characterized by deletion/mutation of INI-1 gene.\(^{42}\) This tumor is in the differential diagnosis, given the presence of rhabdoid cells in UUC. However, nuclear INI-1 expression is maintained in UUC.\(^{107}\)

**Pathogenesis:** Because this is a newly reported subset of tumor, there are no published studies on this yet.

**Recurrent DDUC:** In patients with DDUC, the recurrent tumor is almost always UUC.

**Behavior and Management**

UUC progresses rapidly to death. A study of 16 UUC reported that 75% died of disease, compared to 39% among 33 cases of grade 3 endometrioid adenocarcinoma. More of the deaths among UUC cases occurred within 5 years while the converse was observed for grade 3 endometrioid adenocarcinoma. Two studies of UUC and DDUC (32 patients, 25 patients) reported 41%-60% died, with median survival about 6 months (range 1 to 60 months).

In cases of DDUC, the amount of UUC does not appear to affect survival. Even as little as 20% to 30% UUC can be associated with advanced stage and death within months to 2 years.\(^{89}\) This is an important finding because if the UUC component in such cases was instead interpreted as solid growth of endometrioid tumor, 20%-30% would result in a diagnosis of grade 2 endometrioid adenocarcinoma, which does not behave like these cases did. In cases of concurrent ovarian and endometrial tumors, behavior does not appear related to whether the undifferentiated component is in one or the other site.
Currently, there are no management differences between grade 3 endometrioid adenocarcinoma and UUC or DDUC. The clinical significance of DDUC is that it should not be diagnosed as grade 2 endometrioid adenocarcinoma or else the patient could be under treated. Furthermore, UUC and DDUC should be considered for Lynch syndrome screening tests (mismatch repair protein immunohistochemistry and/or microsatellite instability testing).

**Differential Diagnosis**

UUC is best viewed as a diagnosis of exclusion.

**Grade 3 endometrioid adenocarcinoma:** Typically there is at least a focal clue to endometrioid differentiation, even in tumors that are nearly entirely solid grade 3 endometrioid adenocarcinoma, such as: a hint of tubuloglandular growth, abortive squamous whorls (immature squamous differentiation), columnar cell shapes. In the absence of any trace of endometrioid differentiation, it may be best to document diffuse keratin/EMA expression before labeling the tumor grade 3 endometrioid adenocarcinoma. If multiple keratin stains are negative or focal, UUC should be considered.

**Solid pattern serous carcinoma:** Typically there are focal features of papillary / micropapillary growth, compressed branching slit-like spaces, and severe nuclear pleomorphism in even a predominantly solid serous carcinoma. Such features are not seen in UUC. Although most serous carcinoma are p53 positive, there are no studies of p53 in UUC so its value is unknown. Diffuse keratin/EMA is expected in serous carcinoma.

**Undifferentiated endometrial sarcoma (UES):** UES frequently display a higher degree of pleomorphism than UUC, which have somewhat more monomorphic tumor cells. Keratin expression may also favor UUC.

**Other tumors to exclude with IHC before diagnosis of UUC:**
- Leiomyosarcoma, in particular epithelioid type if rhabdoid cells are prominent
- Lymphoma
- Melanoma
- Rhabdomyosarcoma

**Grade 2 endometrioid adenocarcinoma versus DDUC**

This is the most important differential diagnosis to be aware of since the outcome and management are so different.

<table>
<thead>
<tr>
<th>Grade 2 endometrioid cancer (solid component)</th>
<th>DDUC (solid component)</th>
</tr>
</thead>
<tbody>
<tr>
<td>diffuse sheet-like growth</td>
<td>no</td>
</tr>
<tr>
<td>intermingled glands</td>
<td>yes</td>
</tr>
<tr>
<td>squamous differentiation</td>
<td>yes</td>
</tr>
<tr>
<td>dyshesive cells</td>
<td>no</td>
</tr>
<tr>
<td>rhabdoid cytoplasm</td>
<td>no</td>
</tr>
</tbody>
</table>
| nuclei similar to lower grade area           | yes
| abundant necrosis                           | no                     |
| keratin/EMA                                 | diffuse                |

**Carcinosarcoma versus DDUC**

The UC component of DDUC could raise concern for a sarcomatous component of carcinosarcoma. The latter, however, tends to exhibit pleomorphic, sarcomatoid (i.e. spindled) features in contrast to the more monomorphic round appearance of UC. In many carcinosarcomas, the sarcomatous component infiltrates around gland tubules and along papillary, polypoid branching of glands, while still composing a discrete separate tumor compartment. Presence of heterologous elements also favors carcinosarcoma. Finally, it is not common that a low grade endometrioid adenocarcinoma would make up the carcinoma component of carcinosarcoma, which is typically high grade serous-like. Carcinosarcoma tends to show diffuse/strong p53/p16. p53 in DDUC or UUC has not been studied in the literature but our anecdotal experience is that it can be weak/patchy; studies need to be done to address the role of p53.

<table>
<thead>
<tr>
<th>Carcinosarcoma</th>
<th>DDUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Carcinoma component</td>
<td>High grade serous-like</td>
</tr>
<tr>
<td>-Non-cohesive cells</td>
<td>No</td>
</tr>
<tr>
<td>-Nuclear atypia</td>
<td>Pleomorphic spindle cells</td>
</tr>
<tr>
<td>-Heterologous elements</td>
<td>Common</td>
</tr>
<tr>
<td>-p53</td>
<td>Strong in spindle cells</td>
</tr>
<tr>
<td></td>
<td>Low grade endometrioid</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Monotonous round / polygonal cells</td>
</tr>
<tr>
<td></td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td>May be weak/patchy (anecdotal experience / not published)</td>
</tr>
</tbody>
</table>
Problematic Patterns for Subtyping:

1. Glandular Pattern:
*Pseudo-glandular serous carcinoma versus grade 1 EmoidCA*

Serous carcinoma rarely may grow in a simple tubulo-glandular pattern without any accompanying characteristic patterns such as papillary, micropapillary, or solid with compressed slit like branching spaces. The glands may show branching that looks like branching in EmoidCA but not the characteristic branching of conventional serous carcinoma. At low magnification the pattern may resemble complex atypical hyperplasia (CAH) or grade 1 EmoidCA. When involving a polyp, pseudo-glandular serous carcinoma may also resemble CAH or grade 1 EmoidCA. Myoinvasion also resembles grade 1 EmoidCA and in some cases the simple shapes of the tubules may even simulate stroma-poor adenomyosis. At high magnification, however, the cytology is that of conventional high grade serous carcinoma. If this is mis-classified as grade 1 EmoidCA, the patient will not undergo full surgical staging nor consideration of adjuvant therapy. The key observation is the discordance between the architectural pattern, which would be grade 1 if the tumor were of Emoid type, and the cytology, which is grade 3 in serous carcinoma. Conversely, both architecture and cytology are concordant (low) in grade 1 EmoidCA. Another clue is that the background endometrium is usually atrophic in pseudo-glandular serous carcinoma rather than proliferative.22

<table>
<thead>
<tr>
<th>Pseudo-glandular serous carcinoma</th>
<th>Grade 1 EmoidCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architectural grade</td>
<td>Low</td>
</tr>
<tr>
<td>Cytologic grade</td>
<td>High</td>
</tr>
</tbody>
</table>

Discordant architecture: cytology should prompt consideration of pseudo-glandular serous carcinoma and p53/p16/ER immunostaining. If the diagnosis is confirmed as serous carcinoma and there is apparent adenomyosis present, careful evaluation is warranted to exclude that the adenomyosis is not myoinvasive pseudo-glandular serous carcinoma.

2. Papillary pattern:
*Papillary variant EmoidCA versus serous carcinoma*

Papillary variants of EmoidCA are probably under recognized and at risk for mis-classification as serous carcinoma.18 The variants include villoglandular type, small non-villous papillary type, and artifactual pseudo-papillae due to sloughing. Villoglandular EmoidCA shows long thin finger-like villi of tumor cells arranged on narrow, delicate fibrovascular stalks. The tumor cells are columnar with basal low grade nuclei. Villoglandular features tend to be more superficially located, with more conventional EmoidCA architecture in the deeper tumor. Small non-villous papillae consist of bulbous proliferations of tumor cells that emanate within glands or from tumor surfaces, creating small like bud-like structures; fibrovascular cores are absent. The tumor cells are polygonal with loss of nuclear polarity. Degenerating EmoidCA may have detached bud-like clusters of tumor cells floating at the tumor surface. In all three papillary variants, the key findings are that 1.) the cytology matches that of the adjacent foci of non-papillary EmoidCA; 2.) the papillary morphology blends seamlessly with the non-papillary EmoidCA components and 3.) the nuclei are not severely atypical, briskly mitotic or show increased N/C ratio, as seen in serous carcinoma. These variants are almost always grade 1 or grade 2. Squamous differentiation in the papillary areas is also a clue to EmoidCA. These should be ER/PR positive, weak patchy p53 positive and lack strong p16.

3. Solid Pattern with High Grade Nuclei:
*Solid serous carcinoma versus grade 3 EmoidCA versus UUC versus neuroendocrine carcinoma*

Rarely, a tumor with high grade nuclei may show only solid pattern growth. Each of the 4 types above should be considered. Morphologically, grade 3 EmoidCA is favored by finding focal areas of squamous differentiation, focal gland formation, or columnar cells with nuclear polarity. Although nuclei may be severely atypical, the chromatin often remains vescicular rather than the coarse, inky-black hyperchromasia of serous carcinoma. UUC is favored by sheet-like growth of non-cohesive monomorphic cells with rhabdoid cytology and micro-foci of abrupt keratinization. Serous carcinoma is favored by any hint of compressed slit like branching spaces, diffuse severe nuclear pleomorphism, atypical mitoses, scattered bizarre nuclei, and atrophic endometrium. Nuclei are much more coarse and hyperchromatic than in other tumors. Papillary/micropapillary architecture of tumor in lymphatics or lymph nodes favors serous carcinoma. Neuroendocrine carcinoma should only be considered if the morphology matches what would be called small cell carcinoma or large cell neuroendocrine carcinoma in a more common anatomic location.

By IHC, UUC can be identified by absent or focal keratin/EMA. Diffuse/strong p53/p16 or diffuse strong p16 with entirely absent p53 favor serous carcinoma, however some grade 3 EmoidCA also show diffuse p53/p16, though the intensity and distribution is not to the degree of serous carcinoma. Conversely, ER/PR expression is greater in
grade 3 EmoidCA than in serous carcinoma. PTEN loss favors grade 3 EmoidCA. Not all cases can be resolved by IHC and reliance on morphologic clues becomes important. Even still, a minority defy classification. Such rare cases may reasonably be labeled as “High grade adenocarcinoma” with a comment. Some authors use the term “morphologically ambiguous” in this setting.

Is neuroendocrine IHC sufficient to designate a carcinoma as neuroendocrine type? No. Focal expression of neuroendocrine IHC markers does not affect the behavior of what is otherwise an endometrioid adenocarcinoma. Classification of endometrial neuroendocrine carcinoma should be restricted to cases that show clear cut morphology of conventional small cell carcinoma or large cell neuroendocrine carcinoma. Thus, neuroendocrine IHC should only be ordered as confirmation on cases that have specific neuroendocrine morphology.

4. Spindle Cell pattern:
EmoidCA with spindle cells / cored and hyalinized pattern / progestin effect versus carcinosarcoma

A few variants of grade 1 or grade 2 EmoidCA contain spindled cells that may be mis-classified as the sarcomatous component of a carcinosarcoma. First is EmoidCA with foci of solid spindled cells; some authors believe this represents a form of immature, incomplete squamous differentiation. Second is the cored and hyalinized variant of EmoidCA (CHEC) in which sex cord like or trabecular arrangements of oval, epithelioid and spindle tumor cells are embedded in a hyalinized matrix in between conventional neoplastic glands. Third, some patients initially treated with hormonal therapy for grade 1 EmoidCA do not respond and require hysterectomy. The stroma adjacent to cancer may exhibit a variety of pseudo-decidualization effects, one of which may result in spindled or stellate cells that may simulate the sarcomatous compartment of a carcinosarcoma. The two key features of endometrioid differentiation are 1.) the low nuclear grade and 2.) the spindled/cored component blends seamlessly with adjacent conventional EmoidCA growth. Areas of more typical stromal pseudo-decidualization may also be a clue. In contrast, in carcinosarcoma 1.) the carcinoma component is almost always a high grade serous carcinoma and 2.) there is distinct compartmentalization of the carcinoma versus sarcoma components (i.e. the cells do not blend or merge together). Presence of heterologous rhabdomyosarcomatous differentiation favors carcinosarcoma. Loss of keratin/EMA in the spindle cell component favors carcinosarcoma but presence of keratin/EMA in the spindle cell component favors carcinosarcoma but presence of keratin/EMA in the spindle cell component favors carcinosarcoma but presence of keratin/EMA in the spindle cell component favors carcinosarcoma. Conversely, diffuse strong p53/p16 favors carcinosarcoma.

5. Clear Cell-Rich pattern
Emoid CA with clear cell change versus clear cell carcinoma (CCC)

This is a difficult and controversial issue; there are no gold standard objective methods to distinguish the two and there can be significant variation in observer opinion. It is accepted that some EmoidCA may contain cells with clear cytoplasm, sometimes due to glycogenation, sometimes referred to as secretory variant because the cells resemble those of normal early secretory phase. In contrast to bona-fide CCC, the clear cells of EmoidCA tend to have 1.) columnar shape, 2) basally located nuclei, 3.) minimal atypia, 4.) absence of hyaline globules; the architecture lacks tubulocystic, papillary, or hobnail patterns; and there is no stromal core hyalinization, myxoid change or edema. Not all cases can be resolved with these criteria, however.

IHC is of minimal value. ER/PR in CCC tends to be weak or absent in contrast to strong expression in EmoidCA. Conversely, p16 can be positive in around half of CCC but is generally weak in most lower grade EmoidCA. p53 patterns are not reliably different between these tumors. Although HNF1 is highly sensitive for CCC in both uterus and ovary, its specificity with respect to EmoidCA in the uterus remains to be fully established.

6. Hobnail / Papillary / Clear Cell pattern
Serous carcinoma versus clear cell carcinoma

Both tumors can exhibit papillary and hobnail architecture; clear cells; atypia; psammoma bodies; and present in atrophic endometrium. Features favoring CCC include stromal hyalinization/edema; floating open tumor rings; hyaline globules; and more extensive presence of clear cytoplasm throughout all tumor cells. Features favoring serous carcinoma are complex papillary, micropapillary architecture, high N/C ratio and minimal cytoplasm in most of the tumor; and more extensive presence of cells with severe atypia/brisk mitoses. Generally the presence of clear cells in serous carcinoma is focal and this should not affect the subtyping as serous carcinoma.

Diffuse strong p53/p16 favors serous carcinoma, whereas these are weak and patchy in CCC. Conversely, HNF1 is positive in CCC but not serous carcinoma. ER/PR are not helpful since both may show variable weak positivity.

7. Mucinous Pattern
Lower grade EmoidCA with mucinous cells versus primary endocervical adenocarcinoma
Around three-quarters of primary endocervical adenocarcinoma are of the mucinous type, so called because most tumor cells resemble normal mucin containing endocervical cells. Thus, a mucin-rich adenocarcinoma in an endometrial sampling may raise concern that it represents a primary endocervical origin. However there are also occasional lower grade Endometrioid that may contain mucinous cells (locally or extensively, so-called mucinous adenocarcinoma) or even mucinous features resembling microglandular endocervical hyperplasia. Morphologic features that favor Endometrioid with such mucinous differentiation include 1.) foci of squamous differentiation, 2.) foci of conventional endometrioid differentiation, 3.) foci of atypical endometrial hyperplasia, 4.) foamy cell aggregates in stroma. Morphologic features favoring primary endocervical adenocarcinoma are 1.) notable apoptic debris in the basal cytoplasm, 2.) “floating” mitoses in the apical cytoplasm, 3.) small diameter cribriform growth, 4.) strips of cervical tissue showing adenocarcinoma in situ or severe squamous dysplasia. In the absence of those findings, it is reasonable to take further steps before diagnosing Endometrioid with mucinous differentiation. These steps include correlation with clinical/radiologic location of the tumor and IHC, which can be helpful but does not perfectly always correlate with clinical/radiologic findings. Endometrial origin is favored by ER and basolateral vimentin expression and lack of diffuse p16 or monoclonal CEA expression. If HPV in situ testing is available, that is highly useful if high risk HPV is detected.

Distinction of other histologic subtypes of primary endocervical adenocarcinoma from primary endometrial adenocarcinoma can be similarly challenging. The same principles discussed above for mucinous tumors applies to these subtypes as well.

<table>
<thead>
<tr>
<th>Primary Cervical Origin</th>
<th>Primary Endometrial Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucinous type endocervical adenocarcinoma</td>
<td>EmoidCA with mucinous differentiation</td>
</tr>
<tr>
<td>Endometrioid type endocervical adenocarcinoma</td>
<td>EmoidCA, typical endometrioid type</td>
</tr>
<tr>
<td>Villoglandular endocervical adenocarcinoma</td>
<td>Villoglandular EmoidCA</td>
</tr>
<tr>
<td>Serous carcinoma of endocervix</td>
<td>Uterine serous carcinoma</td>
</tr>
<tr>
<td>Clear cell carcinoma of endocervix</td>
<td>Uterine SCC</td>
</tr>
<tr>
<td>Squamous cell carcinoma of cervix</td>
<td>EmoidCA with squamous differentiation, Uterine SCC</td>
</tr>
<tr>
<td>Adenosquamous carcinoma of cervix</td>
<td>EmoidCA with squamous differentiation</td>
</tr>
</tbody>
</table>

Some experts also use the following observational guidelines in this setting:

- Extensive endometrial involvement is more likely to be due to a primary endometrial origin.
- Serous, clear cell, and mucinous cancer in an older patient is more likely to be primary endometrial origin.
- Poorly differentiated tumor in the cervix of a young patient is more likely to be primary endocervical origin.

Additional criteria need to be applied when considering a diagnosis of primary endometrial squamous cell carcinoma, which is an exceedingly rare entity. Endometrial origin is acceptable only by documenting 1.) absence of squamous dysplasia or carcinoma, 2.) absence of contiguous growth from endometrial tumor to ectocervical mucosa, 3.) absence of concurrent endometrial adenocarcinoma.

8. Necrosis or desmoplasia in a biopsy of a tumor with low grade architecture

**Under-sampled high grade type of carcinoma versus myoinvasive grade 1 endometrioid adenocarcinoma**

A biopsy/curettage containing necrosis or desmoplastic stroma along with a tumor exhibiting low grade architecture should raise concern. Although necrosis may simply reflect degeneration of the tumor, it could also indicate either myoinvasion or a high grade cancer sub-type. Desmoplasia often indicates myoinvasion. Although the low grade architecture may be consistent with grade 1 endometrioid adenocarcinoma, such a tumor is rarely extensively myoinvasive. Therefore, necrosis and/or desmoplasia should raise suspicion that the tumor is pseudo-glandular serous carcinoma or that the tumor is a component of an under-sampled tumor such as dedifferentiated uterine carcinoma, carcinosarcoma, or grade 2 or 3 endometrioid adenocarcinoma. If pseudo-glandular serous carcinoma can be excluded, it is best to be descriptive about tumor subtyping rather than flat-out label it as grade 1 endometrioid adenocarcinoma, which may result in the wrong type of surgery. It is reasonable to report: “Adenocarcinoma with extensive necrosis /desmoplasia” and then comment that while the tumor shows features of low grade endometrioid adenocarcinoma, the necrosis/desmoplasia are concerning for myoinvasion; for an unsampled high grade carcinoma; or alternatively may just represent degeneration of the tumor.
GRADING UTERINE CANCER

The goal of grading is to provide a way to stratify prognosis within a given stage of tumor; this may in turn allow for more fine-tuned decisions about management.

Although grading uterine cancer is relatively straightforward in most cases, there are a few controversies and a few pitfalls that merit attention. In terms of controversies, not all histologic permutations of uterine cancer are explicitly addressed by the current grading systems and so, in the absence of rules, a practical approach must be taken by individual pathologists on certain cases. In terms of pitfalls, there are histologic permutations that are prone to mis-grading and such errors may result in over treatment or under treatment. Familiarity with how the current grading system evolved is helpful in order to appreciate its details, its limitations and controversial issues. This section will begin with the definitions of the grading systems and then discuss pitfalls and controversies.

Definitions of Grading Systems
- FIGO system for endometrioid adenocarcinoma and GOG modification
- FIGO system for clear cell, serous and carcinosarcoma

Pitfalls of under-grading:
- Confusing high grade pseudo-glandular serous carcinoma as grade 1 endometrioid cancer
- Assigning de-differentiated uterine carcinoma as grade 2 endometrioid cancer
- Grading a mixed epithelial carcinoma based on the histologic sub-type with the largest volume than on the one with the highest grade.

Pitfalls of over-grading
- Upgrading corded/hyalinized, sertoliform, spindle cell or immature squamous variants of endometrioid cancer
- Upgrading an endometrioid cancer in which only a minority shows grade 3 nuclear atypia

Controversial grading scenarios
- No FIGO criteria to define “notable nuclear atypia”
- No FIGO rules for grading variants:
  - Dedifferentiated carcinoma
  - Corded/hyalinized, spindle cell, sertoliform variant of endometrioid adenocarcinoma
- No FIGO rule for grading mixed type carcinomas when higher grade tumor is <50% of overall tumor.

Evolution of Grading Systems for Uterine Cancer

Originally, the earliest uterine cancer grading system was proposed by the pathology committee of the Gynecologic Oncology Group (GOG) in the 1980’s. It was arbitrarily defined as an architecturally based system, using the percentage of tumor surface area that was glandular. The cut-off points of <5%, 5% to 50% and >50% were arbitrarily defined as grade 1, 2 and 3. Areas of solid squamous differentiation were excluded from evaluation. Though these definitions were not evidence-based, the system was in fact subsequently found to be clinically meaningful. Among stage I and II patients, 5-year survival for grades 1, 2, and 3 were 93%, 85%, and 69%, respectively; the grading also was prognostic for stage III disease, with survival rates of 70%, 63%, and 40%, respectively.

In 1988, FIGO adopted the GOG grading system and added three further rules:
- notable nuclear atypia, inappropriate for the architectural grade, bumps the overall grade by 1 level higher
- nuclei of areas of squamous differentiation are not graded; only the glandular nuclei
- nuclear grading of serous, clear cell and squamous cell carcinoma defines the overall grade

The GOG subsequently formally evaluated the first two rules added by FIGO. The role of nuclear grade was confirmed but only if strict definitions were used. The FIGO phrase “notable atypia” is not specific enough to carry clinical significance. The role, or lack thereof, of squamous differentiation in grading was also confirmed. The value of grading serous, clear cell or pure squamous carcinoma remains to be shown since all these tumor exhibit aggressive behavior independent of architectural or nuclear features. Thus, the currently used system is in part the GOG’s modification of the FIGO’s modification of the original GOG grading scheme.

Current Grading System for Uterine Cancer

Currently in the United States, most pathologists follow the AJCC, which has adopted the FIGO grading system. It is specific to the histologic subtype of the cancer. Since the FIGO rules do not address all histologic permutations
of cancer, the UCSF recommendations (based on GOG and other literature) are also listed below (these are explained further later on).

FIGO Rules:

- **If pure endometrioid adenocarcinoma:**
  - Grade 1* = <5% solid growth (not counting squamous differentiation)
  - Grade 2* = 5% to 50% solid growth (not counting squamous differentiation)
  - Grade 3 = >50% solid growth (not counting squamous differentiation)

  *Upgrade to grade 3 if there is notable nuclear atypia inappropriate for the architectural grade

- **If serous, clear cell, or carcinosarcoma:** All are grade 3

UCSF Practical Recommendations for Scenarios Not Addressed By FIGO Rules

- **Definition of notable nuclear atypia for endometrioid adenocarcinoma is the GOG definition:**
  - Majority (>50%) of the nuclei must show:
    - Large and pleomorphic nuclei
    - Coarse chromatin
    - Large, irregular nucleoli
  - B. If architecture is grade 2, upgrade to grade 3 if this definition of atypia is met.
  - C. If architecture is grade 1 and this definition of atypia is met:
    - First, exclude possibility of pseudoglandular high grade serous carcinoma
    - Upgrade to grade 2 overall, not grade 3 (based on GOG recommendations).

- **If mixed type of adenocarcinoma, the presence of any amount >10% of serous, clear cell, or carcinosarcoma is overall grade 3**

- **If undifferentiated carcinoma or any component of dedifferentiated carcinoma:** Overall grade 3

- **Areas of corded/hyalinized growth, sertoliform growth, spindled growth are not evaluated for grading**

Alternative Grading Schemes

While the FIGO grading system is the current system of choice in the United States, it is not without its limitations. One of the problematic elements is estimation of solid growth, leading to blurring of the prognostic value of grade 1 versus grade 2 cancers. As an example of interobserver variability, one recent study demonstrated this upgrading by comparing results of grading from a central expert group. Results were as follows:

<table>
<thead>
<tr>
<th>FIGO</th>
<th>FIGO</th>
<th>FIGO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>Grade 2</td>
<td>Grade 3</td>
</tr>
<tr>
<td>Original pathologist</td>
<td>21 %</td>
<td>57 %</td>
</tr>
<tr>
<td>Central expert review</td>
<td>67 %</td>
<td>8 %</td>
</tr>
</tbody>
</table>

In this study, the 5 year survival of grade 1 versus grade 2 was blurred (92% versus 94%, respectively), leading the authors to conclude that a two-tiered system was more appropriate than the three-tiered system.

Alternative grading schemes have been proposed, using some combination of architecture, grade or other variables. Some are 2-tiered systems and others 3-tiered. While the reproducibility of each alternative system appears reasonable, it remains to be demonstrated whether any of these alternatives are significantly better than the current FIGO approach, in terms of prognostic value, reproducibility or ease of use.

Piftalls and Controversies in Grading Uterine Cancer

Although the FIGO definitions appear straightforward, there are several potential pitfalls that may lead to over-grading or under-grading a tumor. Awareness of these issues will avoid over treatment or under treatment of patients.

Underestimation of grade:
- Confusing high grade tubulo-glandular serous carcinoma for grade 1 endometrioid cancer
- Assigning de-differentiated uterine carcinoma as grade 2 endometrioid cancer
- Grading a mixed epithelial carcinoma based on the largest tumor component rather than on the highest grade component

Overestimation of grade
- Upgrading corded/hyalinized, spindled or immature squamous variants of endometrioid cancer
- Upgrading an endometrioid cancer in which only a minority shows grade 3 nuclear atypia
Pitfall: confusing high grade tubulo-glandular serous carcinoma for grade 1 endometrioid cancer
As discussed in the section on histologic subtyping of uterine cancer, there are occasional examples of high grade serous carcinoma that grow in a simple tubulo-glandular pattern without solid or papillary architecture. At low magnification, this pattern may resemble grade 1 endometrioid cancer. However, at high magnification, the cytologic features of serous carcinoma and the grade 3 nuclei should be evident.

Practical recommendation:
- Other tips to prevent this error include being aware of the patient age. While grade 1 endometrioid cancer can arise in elderly women, this is the prime age range for serous carcinoma. Thus, elderly age should prompt re-evaluation before classifying a cancer as grade 1 endometrioid subtype.
- Similarly, the background endometrium should match the histologic subtype of tumor. An endometrioid adenocarcinoma generally should be accompanied by atypical hyperplasia or proliferative endometrium. In contrast, the presence of atrophy or an atrophic polyp, particularly in an elderly patient, should prompt re-evaluation for the possibility of high grade serous carcinoma.
- Even if the grade 3 nuclei are appreciated within an architecturally low grade cancer, there still remains the possible pitfall of classifying the tumor as endometrioid subtype, assigning an architectural grade of 1, then bumping to grade 2 overall based on the nuclear features. Such a tumor is most likely a high grade serous carcinoma. Although this is not grading pitfall, but a tumor typing pitfall, it is worth mentioning in the discussion of tumor grading.

Pitfall: assigning de-differentiated uterine carcinoma as grade 2 endometrioid cancer
When undifferentiated uterine carcinoma is admixed with grade 1 endometrioid adenocarcinoma, the term de-differentiated uterine carcinoma is used. This is a recently described tumor type. The behavior is aggressive, regardless of the relative amount of undifferentiated carcinoma in the overall tumor. In one study, among the 8 patients whose tumors contained less than 50% undifferentiated carcinoma (and therefore would be assigned FIGO grade 2), 7/8 presented with stage III or IV disease; 4 died of disease and 3 are alive with progressive disease. A second study has also documented that the relative proportion of grade 1 cancer versus undifferentiated cancer does not affect prognosis. This behavior is not consistent with that predicted for grade 2 endometrioid adenocarcinoma.

Controversy: The current FIGO grading system does not address this scenario because it is a recently described tumor and the literature is scant. Future modifications should provide guidance on this tumor type.

Practical recommendation:
- In the meantime, it is probably best to comment in the pathology report that the solid tumor components are not solid endometrioid adenocarcinoma but are components of undifferentiated carcinoma. From a practical perspective, it makes most sense to assign an overall grade of grade 3, regardless of the percentage involvement by undifferentiated carcinoma.

Pitfall: grading mixed type carcinoma based on the majority component rather than on the highest grade component
In the setting of a mixed type uterine cancer, such as endometrioid adenocarcinoma and serous carcinoma, there are no explicit FIGO or GOG rules on assigning overall grade. As discussed in the section on histologic subtyping, a secondary component of tumor must occupy at least 10% of the overall tumor to qualify as a mixed type carcinoma. Limited data suggests that as little as 25% component of serous carcinoma may result in the same poor outcome as cases with larger amounts of serous carcinoma. This suggests that if a component of grade 3 carcinoma is present in a mixed type carcinoma, its presence should result in an overall grade 3, regardless of its proportion (as long as it is above 10%). Conversely, it would not seem appropriate to assign such a case as grade 1 or grade 2 based on the larger component of endometrioid adenocarcinoma.

Controversy: The current FIGO grading system does not address this scenario. Future modifications should provide guidance on this tumor type. In their Cancer Protocol for the endometrium, the Cancer Committee of the College of American Pathologists does advise using the highest grade present as the overall grade.

Practical recommendation:
- Our practice at UCSF is to assign a mixed type adenocarcinoma as overall grade 3 if any serous carcinoma, clear cell carcinoma, or carcinosarcoma is present and makes up at least 10% or more of the total tumor. This is also the recommendation of CAP.
Pitfall: upgrading endometrioid carcinoma with corded/hyalinized, sertoliform, spindled, or immature squamous patterns

“We are a long way from having a grading system for [variants] in the clinicopathologic sense”.

There are several uncommon variants of endometrioid adenocarcinoma that grow in unusual patterns that could be interpreted as solid for grading: hyalinized/corded variant; sertoliform variant; spindled variant; and tumors with immature squamous differentiation. In the largest study of the corded/hyalinized variant of endometrioid adenocarcinoma, 83% of the patients were alive without disease; the majority had low stage disease; and all were either grade 1 or grade 2 tumors, though the authors did not explain whether the hyalinized/corded areas were counted in grading. The authors concluded that the corded/hyalinized variant should not be viewed as an aggressive tumor such as carcinosarcoma but as a variant of endometrioid adenocarcinoma associated with a good prognosis. The sertoliform variant of endometrioid adenocarcinoma most likely falls into the same category. Though much more rare and therefore not well-studied, these may exhibit a solid appearance that is in contrast to their low stage of presentation. Prominent spindle cell growth is another unusual variant that can be seen in endometrioid cancer of the endometrium, ovary or fallopian tube. The nuclear grade of the spindle cell component resembles that of the glandular component and these tumors do not follow a behavior worse than otherwise predicted for an endometrioid adenocarcinoma, leading many authors to only evaluate the conventional component of endometrioid growth. Circumferential whorls of spindled or epithelioid tumor cells in endometrioid adenocarcinoma have been interpreted by some authors as foci of immature squamous differentiation or abortive squamous differentiation even though there is no overt finding of squamous differentiation such as keratin pearls or glassy eosinophilic cytoplasm. These whorls may be seen in tumors that also show features of corded/hyalinized growth or spindle cell growth. The nuclear atypia and mitotic activity in these foci is low. Though these foci may resemble solid tumor growth at low magnification, it is not apparent from the behavior that they should count in tumor grading.

Controversy: The current FIGO grading system does not address these variants of endometrioid adenocarcinoma. Future modifications should provide guidance on tumors with these patterns.

**Practical recommendation:**

- In the meantime, our practice at UCSF is to only evaluate the conventional patterns of endometrioid differentiation in these variants for the purpose of grading.
STAGING UTERINE CANCER

“Correct staging is a cornerstone in cancer treatment” 119

Understanding the origins of today’s gynecologic cancer staging system is helpful in order to use it properly and in order to understand the periodic modifications to the system. Uterine cancer is staged using the FIGO system, which has been adopted by the AJCC system. Although great care has gone into the definitions of the staging rules, there are limitations to applying the rules in every day practice. Some rules are not defined as clearly as they could be while some case scenarios that occur are not addressed by any rule. Furthermore, in 2009, four major changes were made to FIGO uterine cancer staging criteria; three of these changes actually make life easier for the pathologist by eliminating elements that often can be challenging to evaluate. After briefly reviewing the history if FIGO staging, the following issues will be addressed:

- Origins of FIGO staging
- Challenging issues in staging:
  --defining myometrial invasion and recognizing unusual patterns of myoinvasion
  --defining cervical stromal invasion
  --recognizing artifacts and lesions that mimic spread of cancer
- 2009 updates to FIGO staging
  --new definitions for staging myometrial invasion
  --new definition for staging cervical invasion
  --elimination of peritoneal washings from staging criteria
  --sub-staging of nodal metastases

Purpose of FIGO staging of uterine cancer
One of major driving forces behind development of a cancer staging system was to provide a way of conveying clinical experience to others for the purpose of comparing treatment methods. Although cancer staging of an individual patient may help in deciding individual treatment plans and in estimating the behavior of an individual patient’s cancer, the larger goal is to facilitate the evaluation of new treatment modalities. Thus, the ideal cancer staging system has international acceptance and is valid, reliable, and practical.

Origins of the FIGO staging of uterine cancer
As summarized by Odicino et al.,79 the origins of today’s FIGO system trace back to 1928 when the Radiological Sub-Commission of the Cancer Commission of the Health Organization of the League of Nations was charged with the task of studying the effects of radiation treatment of cervical cancer. The commission recognized the need for accurate cancer statistics in order to address this task. As a result, an international classification system for grouping cervical cancer patients was designed and published in 1929 as the League of Nations Classification for Cervical Cancer. Using this system, the Health Organization of the League of Nations began in 1937 to publish an Annual Report of outcome of radiation treatment of cervical cancer. To facilitate uniformity of staging data, the “Atlas on Cervical Cancer Staging” was published in 1938 with definitions, staging diagrams and text, in English, French, and German. This atlas represents one of the earliest staging manuals for gynecologic cancer.

The principles of ideal cancer staging were developed further by several expert groups over the ensuing years. In 1949 it was advised that the ideal staging rules:

1. use simple and precise definitions
2. be easily interpretable
3. be applicable by clinicians who are not experts and who do not need specialized diagnostic procedures
4. capture the full range of permutations of cancer presentation

After modifications by several expert groups, a joint proposal in 1950 was made to A.) name the system “The International Classification of the Stages of Carcinoma of the Uterine Cervix” and B.) recommend that all organizations studying cervical cancer adopt this system. Uterine cancer treatment statistics were added to the Annual Report in 1953; vaginal cancer statistics in 1964; ovarian cancer statistics in 1973; and vulvar cancer statistics in 1979.

The International Federation of Gynecology and Obstetrics (FIGO) was given the leadership of the Annual Report in 1961. The “Annual Report on the Results of Treatment in Gynecological Cancer” has been issued every 3 years, coinciding with the FIGO World Congress. Originally based on data from 6 European medical centers, there were 108 centers contributing over 34,000 cases in the 2006 Annual Report.

Relationship of FIGO to other Cancer Groups
The vast experience of FIGO has informed many groups independent of FIGO that study cancer. Currently, modifications to the FIGO staging system are made with input and approval from UICC, AJCC and the World Health Organization such that the staging systems from all these groups are essentially identical.

International Union Against Cancer (UICC) In 1954, the UICC began to develop the tumor-node-metastasis (TNM) classification system to classify malignancies and treatment results. The 1966 cervical cancer staging rules drew much from the FIGO system.

American Joint Committee for Cancer Staging (AJCC) The AJCC was established in 1959 to develop cancer staging for use in the United States. The FIGO system was accepted by AJCC in 1976.

College of American Pathologists (CAP) The Cancer Committee of the CAP produces Cancer Protocols for the major types of cancer, including endometrial cancer. These guidelines offer recommendations for specimen dissection and evaluation, reporting terminology, and standards for cancer report content. For endometrial cancer staging, the CAP advises using the AJCC/FIGO criteria.¹

Current FIGO/AJCC Staging

<table>
<thead>
<tr>
<th>AJCC</th>
<th>FIGO</th>
</tr>
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<tbody>
<tr>
<td>pT1a</td>
<td>IA</td>
</tr>
<tr>
<td>Tumor confined to endometrium or invades less than half of myometrium</td>
<td></td>
</tr>
<tr>
<td>pT1b</td>
<td>IB</td>
</tr>
<tr>
<td>Tumor invades outer half of myometrium</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>II</td>
</tr>
<tr>
<td>Tumor invades stroma of cervix</td>
<td></td>
</tr>
<tr>
<td>pT3a</td>
<td>IIIA</td>
</tr>
<tr>
<td>Tumor involves uterine serosa or adnexa</td>
<td></td>
</tr>
<tr>
<td>pT3b</td>
<td>IIIB</td>
</tr>
<tr>
<td>Tumor involves vagina or parametrium</td>
<td></td>
</tr>
<tr>
<td>pT4</td>
<td>IVA</td>
</tr>
<tr>
<td>Tumor involves bladder mucosa or bowel mucosa</td>
<td></td>
</tr>
<tr>
<td>pN1</td>
<td>IIIC1</td>
</tr>
<tr>
<td>Pelvic lymph node metastasis</td>
<td></td>
</tr>
<tr>
<td>pN2</td>
<td>IIIC2</td>
</tr>
<tr>
<td>Para-aortic lymph node metastasis</td>
<td></td>
</tr>
<tr>
<td>pM1</td>
<td>IVB</td>
</tr>
<tr>
<td>Distant metastasis including omentum, inguinal lymph nodes. Excludes pelvic sites/serosa.</td>
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</tbody>
</table>

MYOMETRIAL INVASION

Pathologic evaluation for myometrial invasion by uterine carcinoma became a much easier task with the modifications of the 2009 FIGO staging system. By eliminating the need to distinguish non-myoinvasive cancer from inner-myometrial invasive cancer, one of the common sources of diagnostic problems in uterine cancer pathology was eliminated. However, there are still several scenarios pertaining to myometrial invasion that remain challenging. These include:

- Distinguishing true myoinvasion by cancer versus
  - non-invasive cancer involving an undulating endomyometrial junction
  - non-invasive cancer involving adenomyosis
- Pitfalls in measuring myoinvasion
  - over-estimation due to smooth muscle/fibroblastic metaplasia of endometrial stroma
  - over-estimation due to exophytic tumor growth
- Recognizing unusual patterns of myometrial invasion
  - Pushing pattern
  - MELF pattern
  - Diffusely infiltrating (adenoma malignum) pattern
  - Pseudo-glandular serous carcinoma with myoinvasion (mimicking adenomyosis)

**Defining the endomyometrial junction**

The normal endomyometrial junction may exhibit two features that result in difficulty in identifying whether an endometrial tumor is in the endometrium or in the superficial myometrium:

- The endomyometrial junction is not always a flat, linear junction but may irregularly undulate. In some cases, the undulation may result in rounded tongues of endometrium that dip significantly into the myometrium. If these rounded tongues of endometrium are entirely replaced by tumor, they could be misinterpreted as foci of myoinvasion. In such cases where this is suspected, it is helpful to assess the adjacent non-neoplastic endomyometrial junction. If there is no undulation, then the tongues of tumor likely represent true myoinvasion. If the adjacent non-neoplastic junction shows undulation, then caution
is advised before diagnosing myometrial invasion by the adjacent tumor and other evidence for myoinvasion should be sought such as:

- irregular, jagged contour of tumor nests
- single or clustered tumor cells in myometrium adjacent to suspicious tumor nests
- stromal desmoplasia
- absence of normal endometrial glands/stroma between contour of tumor nest and myometrium
- deep location of tumor nests, in proximity to large thick wall vessels of outer myometrium

Conversely, if even a few rare normal endometrial glands are found in between tumor and myometrium at the leading edge of a tongue of suspected myoinvasion, caution is advised against diagnosing myoinvasion unless other compelling supportive features are present.

➢ The stroma of the basalis layer of endometrium may be more spindled than the stroma of the functionalis and may contain smooth muscle cells. These changes can be exuberant and result in large zones or bands of endometrial stroma that look like myometrium or fibroblastic stroma; these changes have been referred to as endometrial stromal metaplasia, either fibroblastic metaplasia or smooth muscle metaplasia. Therefore, the distinct appearance of the endometrial stroma of the functionalis should not always be expected at the endomyometrial junction. Lack of awareness of the histology of the stroma of the basalis layer and of metaplastic changes may lead to misinterpretation when cancer is present. Thus, it is possible that the non-myoinvasive cancer is over-diagnosed as myoinvasive or that the depth of myoinvasion is over-estimated. Such errors have been demonstrated in the literature.

Grossing a uterine cancer to evaluate for myoinvasion
Proper grossing technique is essential to evaluate for myoinvasion by uterine cancer and to measure the depth for the purpose of tumor staging. The ideal approach is to bivalve the uterus by slicing along the lateral walls. To ensure that the bivalving results in opening the endometrial cavity, one method is to first insert a pair of extra-long forceps into the unopened uterus via the cervical os, all the way into the endometrial cavity. A long knife is then placed in between the arms of the forceps and the slicing is done using the forceps arms as a guide to ensure that the knife always is on track to properly open the endometrial cavity.

After bivalving, the lower uterine segment and uterine body are breadloafed in parallel slices running along the medial/lateral axis. The thinner the slice thickness, the better chance of assessing for myoinvasion.

The gross appearance of myoinvasion is not always straightforward. Infiltrating tumor may not exhibit distinct borders. Superficial myoinvasion may not even be grossly visible. Conversely, overestimation of myoinvasion may occur if there is extensive adenomyosis, which can exhibit a trabecular or even dense, solid but vaguely defined gross appearance.

Representative sections of the deepest foci of suspected gross myoinvasion should be submitted. If there are no gross features of myoinvasion, a few representative sections of tumor at the endomyometrial junction are advised in order to evaluate for microscopic features of superficial myoinvasion.

The shape and orientation of the tissue sections is essential. Ideally, the lateral edges of the tissue section should be straight and parallel to each other and should be perpendicular to the serosa. If the lateral edges are curved or irregular and are not parallel to each other, it makes calculating depth of myoinvasion difficult. If the thickness of the uterine wall and the tumor is too big to fit a section in a single cassette, the section should be blocked into several cassettes in a way that will allow reconstruction of the sections in the resulting glass slides for the purpose of measuring overall depth of invasion. In most cases this can be accomplished by dividing a full section of tumor and uterine wall into two cassettes: one with the endometrium and inner myometrium and one with the outer myometrium and serosa. Rarely, three or more cassettes may be needed. Here again, parallel and straight lateral edges become important, as well as clear documentation in the cassette codes of the orientation of each section.

Finally, representative sections of the upper endocervix / lower uterine segment in a section parallel to the endocervical canal are advised in order to systemically evaluate for stage II endocervical stromal involvement.

Modifications of 2009 FIGO staging of myometrial invasion
Outcome analysis of over 42,000 uterine cancer patients led to Volume 26 of the FIGO Annual Report which documented that the behavior of inner-half myoinvasive uterine cancer (grade 1 or 2) was not significantly different from that of non-invasive uterine cancer (grade 1 or 2). The 5 year survival for these groups was as follows
1988 FIGO stages  |  5 year survival
--- | ---
stage IA, grade 1 | 93.4%
stage IA, grade 2 | 91.3%
stage IB, grade 1 | 91.6%
stage IB, grade 2 | 93.4%

These findings led the FIGO committee to collapse the 1988 FIGO stages of IA and IB into a single category of IA, which includes both non-invasion and inner-half myoinvasive carcinoma. The 2009 FIGO stage IB now refers to outer-half myoinvasive carcinoma.

Although these modifications make it easier for pathologists by eliminating the struggle of distinguishing non-myoinvasive cancer from superficial myoinvasion, it is still important to accurately make the distinction in individual cases. There will always be a minority of cases that fall near the threshold of 50% depth of invasion and in these cases it is important to accurately and reproducibly apply criteria for measurement. Thus, making an effort to correctly evaluate for myoinvasion in every case is good for maintaining diagnostic skills, even if it does not result in a difference in staging.

**Practical recommendation:**
- As mentioned earlier in the section on gross dissection, if the gross findings suggest that there may be myoinvasion near the 50% depth threshold, care should be taken in preparing tissue sections so that the depth can be measured as accurately as possible. This means 1.) ensuring that the lateral edges of the tissue sections are trimmed to be parallel to each other and, if possible, perpendicular to the serosa. 2.) if the uterine wall is too thick to fit in a single cassette, a full cross section of the wall is blocked into two or more cassettes, with attention to describing the precise relationship of each cassette to each other and orienting the sections so that they can be reconstructed for measuring depth of myoinvasion.

**Problems in measuring depth of myometrial invasion**
Surprisingly there are no AJCC, FIGO or GOG rules defining the method of measuring myometrial invasion. In most cases, it is a straightforward process of identifying the deepest point of myoinvasive cancer and measuring from that point to the nearest endomyometrial junction. That measurement is then divided by the overall thickness of the myometrium (endomyometrial junction to serosa) at that part of the uterus. Less than 50% depth of myoinvasion is stage IA and more than 50% depth of myoinvasion is stage IB.

Not all cases are straightforward. Problematic scenarios that lack formal rules by FIGO, AJCC of GOG include:

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A significant amount of the common problems in measuring depth of myoinvasion was eliminating by the 2009 FIGO staging modification that eliminated the distinction of inner half myoinvasion from non-myoinvasive cancers. This eliminated much of the prior concern about superficial limited myoinvasion and also negated the clinical effect of overestimating non-myoinvasive cancers as superficially myoinvasive. Never the less, these problems still merit discussion.

The Cancer Committee of the College of American Pathologists (CAP) has adopted some guidelines for defining how to measure invasion; the guidelines are not evidence based but are based on recommendations from a single study by one of the authors of the Cancer Committee. The guidelines are illustrated in the CAP Cancer Protocol for the Endometrium. Though not evidence-based, the guidelines are for the most part in line with what most pathologists would do anyway, and in the absence of evidence-based literature, are worth adhering to for the purpose of attempting to achieve standardization and reproducibility among all patients.

**Practical recommendations:** (these are in line with the CAP recommendations)
- **Exophytic cancers** Many uterine cancers have a significant exophytic component. The exophytic component is not considered myoinvasive and should not be measured. If there is a myoinvasive component, then only that component should be measured. Difficulty may arise 1.) if there is no visible endomyometrial junction or 2.) if there is prominent smooth muscle metaplasia of the endometrial stroma in the exophytic component. In the first situation, it may be necessary to provide a best estimate of the location of the endomyometrial junction based on other information from other slides and/or from the gross data (including re-examination of the gross specimen). In the second situation, the key is to find the true
endomyometrial junction in order to avoid misinterpreting smooth muscle metaplasia of endometrial stroma as true myometrium.

- **Smooth muscle metaplasia of the endometrial stroma around cancer** This process receives little attention but is in fact not uncommon. Endometrial stroma is well known to undergo metaplastic changes in a variety of settings including fibroblastic-like changes in stromal desmoplasia and adenomyofibromatous polyps or myoid differentiation in endometrial stromal tumors and adenomyomatous polyps. Either myoid or fibroblastic metaplasia can also occur in the stroma surrounding endometrial cancer. When this process is exuberant, the metaplastic stroma may be misinterpreted as myometrium, thus leading to a misinterpretation of myoinvasion or overestimation of depth of true myoinvasion.

  Suggested features to recognize smooth muscle or fibroblastic metaplasia of the endometrial stroma include a fibrillary, loosely organized pattern of the stroma, in contrast to well-developed fascicular pattern of myometrium and the imperceptible merging of these areas with more conventional looking endometrial stroma. Immunostains are not useful (and could be problematic if misinterpreted) because the metaplastic endometrial stroma may express myoid markers in addition to CD10. CD10 can also be present around myoinvasion endometrial cancer.

- **Myoinvasion in a uterus with an irregular endomyometrial junction** The first step is to establish that there is myoinvasion present, using convention criteria (see later discussion). Once the presence of myoinvasion is established, the depth of invasion is measured using the deepest undulation of endomyometrial junction. Other authors also advocate this approach.

- **Myoinvasion when cancer also involves adenomyosis** This is discussed later in the section on adenomyosis.

**Conventional pattern of myoinvasion**

Myoinvasion is generally straightforward to recognize microscopically. Usually a constellation of the following features are present:

- irregular, jagged contours of the neoplastic glands
- single tumor cells or clusters in the myometrium
- desmoplastic stroma (edematous stroma with reactive nuclear changes and inflammation)
- absence of normal endometrial glands or stroma between leading edge of tumor and myometrium
- haphazard distribution of neoplastic glands in myometrium
- deep location of neoplastic glands, in proximity to large thick vessels of outer myometrium

**Pushing pattern of myoinvasion**

Rarely, endometrial cancer may invade the myometrium with a broad, smooth pushing leading edge rather than with an irregular jagged edge. Other conventional features of myoinvasion may also be absent. This has been referred to as pushing pattern or expansile pattern invasion. In such cases, the tumor/myometrial junction may even appear as a flat linear junction parallel to the serosa; this is a potential pitfall that may be misinterpreted as non-myoinvasive. The key to avoiding this pitfall is to always correlate the microscopic findings with the gross findings. The pushing pattern of myoinvasion often can be suspected on gross examination even though the tumor/myometrial junction may be smooth and flat/linear; a broad front of tumor may take over much of the wall. Thus, it is good practice to look at the gross description (or a gross photograph) of a uterine tumor before making a microscopic assessment that there is no myoinvasion.

Microscopic clues to pushing pattern myoinvasion are few. It is really a diagnosis that heavily rests on correlating with the gross appearance. Superficial myoinvasion in this pattern may be challenging, if not impossible, to diagnose. Deeper extents of myoinvasion (i.e. more than about 1/3 into the myometrium) are progressively easier to recognize. At these deeper levels of myoinvasion, low magnification evaluation will show that the uterine wall thickness is overtaken by tumor. If tumor is seen in proximity to large caliber, thick wall vessels, this is a strong clue that the tumor is deep in the myometrium since such vessels are less likely to be present in the superficial myometrium. Since calcification usually involves deeper myometrial vessels, tumor adjacent to heavily calcified vessels should also be a clue to pushing invasion.

**MELF pattern of myoinvasion**

A distinctive yet deceptive pattern of myoinvasion called MELF (an acronym for Microcystic / Elongated / Fragmented) merits attention for two reasons:
1. MELF is easy to overlook at low magnification yet it may be the only pattern of myoinvasion present.
2. MELF is easy to misinterpret as lymphovascular invasion or adenomyosis.

This pattern is found in up to 15% of uterine cancer cases. It appears to be seen only in endometrioid adenocarcinomas and, more specifically, grade 1 endometrioid adenocarcinomas that often contain some degree of mucinous differentiation. It is defined as a combination of changes in the tumor epithelium and in the adjacent stroma. The neoplastic glands exhibit a degenerative change accompanied by adjacent stromal desmoplasia and inflammation. The glands are usually single glands that either project off from a focus of tumor into the myometrium or are single detached glands sitting within myometrium. In general, the glands look like they have pinched-off from the main tumor. The shape of the gland may be either a microcyst; an elongated compressed tubule; an endothelial-like structure; or clusters of detached tumor cells within edematous myxoid tissue. The tumor cells in these structures may have eosinophilic cytoplasm that resembles squamous differentiation or, conversely, the tumor cells may be flattened/attenuated, resembling endothelial cells. Microcystic glands may contain intraluminal tufts of cells and/or inflammatory cells (usually neutrophils).

The degree of attenuation of the cells lining compressed endothelial-like structures may cause the gland itself to resemble a lymphovascular space. If such structures also contain intraluminal tufting or clusters of tumor cells, there is a risk of misinterpreting this to be lymphovascular invasion. The clue to avoid this pitfall is to recognize the adjacent desmoplastic stroma, which would not be expected adjacent to true lymphovascular invasion. Immunohistochemistry for keratin versus the lymphatic endothelial marker D240 can help resolve difficult cases. If the endothelial-like lining is keratin positive and D240 negative, then the structure in question represents MELF. If the converse staining results are found, then the structure represents a true lymphatic space.

Because the involved glands are small, compressed, usually single, resemble lymphovascular structures, and tend to be sparsely distributed in the myometrium, this pattern of myoinvasion frequently goes undetected at first glance at low magnification. The diagnostic clue is the presence of edematous, reactive (i.e. desmoplastic) stroma surrounding these glandular elements.

The term MELF derives from the observation that the glands may be Microcystic or may be Elongated or Fragmented. MELF pattern myoinvasion may accompany conventional patterns of myoinvasion or may represent the only pattern of myoinvasion.

The clinical significance of MELF requires further study. One study reported that the presence of MELF had no effect on survival.75 Another study showed that lymphovascular invasion was much higher in the presence of MELF (63%) than in its absence (25%).105 A third study showed that nodal metastasis was higher in the presence of MELF (54%) than in its absence (7%).82

Diffusely infiltrative pattern ("adenoma malignum" pattern) of myoinvasion
Infrequently, a pattern of myoinvasion called diffuse infiltration or adenoma malignum-like pattern may occur in low grade endometrioid adenocarcinoma.52, 58, 63, 65, 70, 108 It merits awareness from a diagnostic standpoint because it may be:

1. difficult to distinguish from non-invasive cancer in the setting of an undulating endomyometrial junction
2. difficult to distinguish from involvement of stroma-poor adenomyosis.
3. difficult to measure true depth of invasion
4. associated with a similar subtle pattern of cervical invasion

The pattern is defined as well-differentiated tumor glands scattered throughout the myometrium but, characteristically, lacking any other conventional features of invasion, particularly stromal response and irregular/jagged gland contour. In many instances the invasive foci are single round glands or aggregates of glands without significant architectural atypia or cytologic atypia, yet they are located deeply and haphazardly within myometrium. The distribution resembles that of adenoma malignum (minimal deviation type endocervical adenocarcinoma) of the endocervix. Some authors use the term "melting" to describe the subtle pattern of single glands descending from the tumor in the endometrium down into the myometrium. The myometrium adjacent to the invasive foci typically lacks any significant edema, reactive changes, or inflammation (i.e. there is no stromal desmoplasia). There is no adjacent endometrial stroma (which would otherwise indicate that the glands represented adenomyosis rather than invasive tumor). Rarely, there may be myometrial hyperplasia or concentric cuffing of myometrium around the invasive glands.

This pattern of myoinvasion may be associated with a similar subtle pattern of cervical stromal invasion, also referred to as “burrowing pattern” or “adenoma malignum-like” invasion of the endocervix.52, 58, 108 As discussed in
the section on cervical invasion, this pattern may resemble a variety of benign endocervical alterations, including cervical endometriosis, tubal metaplasia, or endocervical glandular hyperplasia.

Distinction from adenomyosis is primarily based on evaluating whether or not there is endometrial stroma surrounding the suspicious glands in the myometrium and whether there are any benign endometrial glands immediately adjacent to the suspicious glands. The presence of either should raise consideration of adenomyosis. More problematic is the issue of distinction from stroma-poor adenomyosis; this may not be possible in some cases in which the nuclear features of the tumor are bland. Features favoring true myoinvasion include the lack of any convincing adenomyosis elsewhere in the specimen, the presence of any nuclear atypia, stromal desmoplasia, or lymphovascular invasion. There are some cases in which carcinoma involves both adenomyosis and myometrium; as discussed elsewhere in this section, some of these cases of true myoinvasion co-existing with adenomyosis can be identified by convention features, such as stroma desmoplasia, irregular gland contours, absence of endometrial stroma. In reality, it may be impossible to resolve the issue of stroma-poor adenomyosis versus [or co-existing with] myoinvasion in a minority of tumors displaying the adenoma malignum like pattern of invasion.

The clinical significance of this pattern is not well established because the literature is limited. In one of the larger studies, there was no association with lymphovascular invasion or overall survival.63 That study also reported that cases with co-existing cancer involvement of deep adenomyosis plus foci of true myoinvasion did not appear to have worse prognosis. Other case series have reported association with cervical involvement but such reports were not designed to test the statistical significance of this association.52, 58, 108

Cancer involvement of adenomyosis versus true myoinvasion
There is a significant behavioral difference between uterine cancer with myoinvasion and uterine cancer that involves adenomyosis without myoinvasion.36, 40, 44, 71 The latter scenario is prognostically equivalent to non-myoinvasive endometrial cancer and is not staged as myoinvasive tumor. Therefore, it is important to distinguish the two. Most of the time, the distinction is straightforward, using conventional diagnostic criteria for myoinvasion.

A common diagnostic problem arises, however, when there is significant adenomyosis in a hysterectomy containing endometrioid adenocarcinoma. There are three issues with this problem:

1. distinguishing myoinvasion versus adenomyosis when the cancer is low grade
2. determining if true myoinvasion is also present when cancer is involving adenomyosis
3. measuring depth of myoinvasion when present along with cancer involving adenomyosis

In general, the conventional criteria for myoinvasion should be applied in this setting. In evaluating areas of adenomyosis that contain cancer, there may be foci at the interface with myometrium which are suspicious for invasion beyond adenomyosis. In this setting, the conventional criteria for myoinvasion should be applied. The suspicious foci should be evaluated for:

- irregular, jagged contours
- desmoplastic stroma (edematous stroma with reactive nuclear changes and inflammation)
- absence of normal endometrial glands or stroma between leading edge of tumor and myometrium

Conceptually, the absence of endometrial stroma around the suspicious glands should be, on its own, diagnostic for myoinvasion. The problem with this concept is two-fold. First, in areas around the endomyometrial junction, the normal endometrial stroma of the basalis layer may contain a more spindled, sometimes myoid population of stromal cells. This appearance may not be recognized as true endometrial stroma, leading to over-diagnosis of myoinvasion. Second, there are rare examples of stroma-poor adenomyosis, which may also lead to over-diagnosis of myoinvasion. Thus, it is best to look for multiple lines of evidence of myoinvasion when possible rather than relying alone on absence of endometrial stroma. In some cases, though, that variable may be the only evidence of myoinvasion.

In reality, it may be that small foci of myoinvasion that emanate from deep adenomyosis are not likely to affect overall behavior, as suggested by some studies.38, 63 Thus, the inability to reliably detect small foci of myoinvasion associated with cancer-involved adenomyosis may not necessarily result in under-treatment.

Can CD10 immunohistochemistry resolve this?
No. Immunohistochemical staining for the endometrial stroma marker CD10 has been studied in this context. The concept for its use is simply an extension of the concept of looking for endometrial stroma around suspicious glands in the myometrium using conventional H&E staining. Therefore the use of CD10 is subject to the same limitations discussed above for the H&E approach.
Two studies demonstrated that in bona fide myoinvasive endometrioid adenocarcinoma, CD10 is frequently expressed in the stroma immediately adjacent to the cancer.\textsuperscript{76, 103} By H&E stain, those CD10 positive stromal cells looked either like smooth muscle cells or like desmoplastic stromal cells but not like endometrial stromal cells. Therefore the studies concluded that CD10 positivity cannot be equated to the presence of endometrial stromal cells. Equally important was the observation that CD10 positivity in bona fide adenomyosis frequently could be patchy. Therefore, the absence of CD10 positive stroma does not necessarily mean that endometrial stromal cells are missing. In summary, using CD10 in this context can lead to erroneous over-diagnosis and under-diagnosis of myometrial invasion. In our practice at UCSF we generally do not use CD10 for this purpose but rely upon morphologic criteria seen on H&E stain.

As an aside, the explanation for the CD10 positive stroma around myoinvasive cancer in those two studies remains a puzzle. In most cases, those cells were negative for the myoid markers desmin and caldesmon. Prior studies of CD10 expression have shown that some smooth muscle tumors may be CD10 positive and conversely, some endometrial stromal tumors may be positive for myoid markers. Some authors have suggested that the altered immunophenotype is a result of some cancer-associated underlying transformation of the myoid stroma adjacent to uterine cancer.\textsuperscript{76}

**How should depth of invasion be measured in the setting of co-existing cancer-involved adenomyosis?**

- **Definition of adenomyosis:** Even the very definition of benign adenomyosis is subject to observer variation, particularly when trying to distinguish an irregular undulating endomyometrial junction from superficial adenomyosis. A commonly used practice is to require a minimum distance of one low power magnification field in between the endomyometrial junction and suspected focus of adenomyosis; any glands and stroma located closer than this distance are simply interpreted to be part of the endometrium showing an irregular endomyometrial contour. Other authors\textsuperscript{29} recommend that the foci be at least at a depth of 25\% of the uterine wall while others\textsuperscript{11} recommend a quantitative definition of at least 3 millimeters beneath the endomyometrial junction. Variation in these definitions can produce variations in the incidence of adenomyosis, with 10\% representing the more rigid definitions and nearly 20\% using less strict criteria.

**Practical recommendation:** In daily practice, a reasonable approach to defining adenomyosis is to use one’s judgment, since the orientation of tissue sections can be variable; using some combination of criteria of either a low power field (if the uterine wall is thick enough) or presence at least 25\% deep into the wall seem to be a practical rule of thumb. Most of the time this is not a clinically consequential issue.

- **Measuring depth of myoinvasion when adenomyosis is also involved by cancer:** Theoretically it is possible that the only foci of myoinvasive cancer are ones that emanate off of deep foci of involved adenomyosis without any myoinvasive foci that emanate from the endomyometrial junction. A recent study found no examples of such a scenario however the authors did propose that they would have measured such foci from the deepest point of the adjacent adenomyosis-myometrium interface.\textsuperscript{3} Two studies suggest that this rare scenario does not result in adverse outcome, therefore suggesting that it would be inappropriate to measure the depth from the endomyometrial junction (thus resulting in stage IB).\textsuperscript{38, 63} Neither FIGO nor AJCC provide any guidance.

**Practical recommendation:** The nearest endomyometrial junction should be used for measuring most cases of myoinvasion. In the rare event that the only foci appear to emanate from deep adenomyosis, it is reasonable to provide two measurements, one from the endomyometrial junction (which may result in stage IB) and one from the deepest interface of adenomyosis and myometrium; then provide a comment describing the lack of rules to guide measurement in this scenario and cite the references that suggest the latter method of measurement may be more prognostically accurate.\textsuperscript{38, 63} Other authors also advocate this approach, which is also advised by the CAP.\textsuperscript{1, 3}

**Pseudo-glandular serous carcinoma with myoinvasion**

Rarely, the pseudo-glandular variant of uterine serous carcinoma may invade the myometrium in a pattern that mimics adenomyosis at low magnification. The tumor is present as ectatic, irregularly dilated but otherwise architecturally simple glands (i.e. no intraglandular stratification, tufting, branching or papillary growth) sparsely distributed in the myometrium. At low magnification the glands may resemble adenomyosis, particularly if there is stromal desmoplasia that is mis-interpreted as endometrial stroma. The correct diagnosis is made by high magnification examination, which will reveal the cytologic features of high grade serous carcinoma.
LYMPHOVASCULAR INVASION

Although lymphovascular invasion (LVI) is not part of the AJCC or FIGO staging system, it is an important adverse prognostic factor that affects treatment decisions. The biggest effect of LVI on treatment is in the setting of grade 1, stage IA endometrioid adenocarcinoma; typically no adjuvant treatment is needed but GOG study 99 showed that LVI, particularly in older women, need radiation treatment. Thus, recognition of subtle forms of LVI and mimics of LVI is essential. Because the pathologic evaluation for LVI involves concepts that are involved in evaluation for myometrial invasion, it is appropriate to discuss LVI in this section of tumor staging. Generally the diagnosis of LVI is straightforward but there are a few pitfalls, particularly related to laparoscopic hysterectomy, and diagnostic clues that merit attention:

Definition of LVI by uterine cancer
- Histologic definition
- Immunohistochemical confirmation
- Diagnostic clues for LVI: (peri-vessel lymphoid aggregates, MELF pattern myoinvasion)
- Staging rules and LVI

Artifactual “pseudo”-LVI
- Artifacts due to surgical technique

Mimics of LVI
- Intravascular endometrium due to menses, adenomyosis or endometriosis
- Intravascular leiomyomatosis or endometrial stromal sarcoma
- MELF pattern myoinvasion

Definition of LVI

There are no AJCC, FIGO, or GOG definitions of LVI. Generally, for most organs, most pathologists define LVI as tumor cells present in an endothelial lined space. Therefore, endothelial cells should be visible lining the space, in particular the endothelial nuclei should be visible, often slightly bulging into the space. Distinguishing true LVI from retraction artifact is sometimes a problem that is encountered in any organ, including the uterus. When the presence of endothelium is uncertain, immunohistochemical confirmation can be helpful. D240 marks lymphatic endothelium while CD34, CD31 and Factor VIII mark vascular endothelium. LVI by uterine cancer tends to be lymphatic so D240 is the first choice, but if the morphology could be a vein or small artery, then CD34 or CD31 could be added as well.

Evaluation for LVI is in theory straightforward. However, there are many problematic scenarios, including artifacts and other lesions that simulate LVI. These are discussed below.

Practical recommendation:
- As a rule of thumb, if the tumor is low grade, is endometrioid subtype, and has limited or no myoinvasion, then the interpretation of LVI should be approached with caution and consideration of artifact or a mimic is advised, using the approaches described below.

Artifactual LVI due to Surgical Technique (Laparoscopic hysterectomy, with or without robotic assistance)

Technical advances in laparoscopic hysterectomy have made it the first line approach for many (but not all) clinical scenarios of uterine cancer management since, in the appropriate patient population, it is as effective as abdominal hysterectomy but with shorter hospital stays, complications and recovery time. One of the downsides of laparoscopic hysterectomy is that it is a likely source of artifacts in the hysterectomy specimen that may lead to over-diagnosis, or at least difficulties in diagnosis, of LVI, myometrial invasion, tubal spread, and peritoneal washing cytology. The reasons for this are due to a technique of manipulation of the uterus during surgery using a device called a uterine manipulator. This is an instrument that helps the surgeon better manipulate the position of the uterus, particularly for the purpose of avoiding injury to the ureters during dissection along the paracervical margins. The uterine manipulator consists of three elements: a vaginal expander, which may include a pneumo-occlusive component; an intra-uterine inflatable balloon which keeps the manipulator in place; and the uterine manipulator device itself which is manually controlled outside the vagina. Studies of LVI, tubal involvement by tumor and positive peritoneal washings as a function of surgical technique (laparoscopic approach, use of a uterine manipulator, use of robotic assistance) strongly suggest an artifactual effect of uterine manipulation by these techniques on the tumor.

Positive peritoneal cytology in laparoscopic hysterectomy has been reported with a higher incidence in many studies but not all. Though controversial, these findings have led some surgeons now to clamp or ligate the fallopian tubes prior to introducing the uterine manipulator.
Intraluminal tumor within fallopian tubes in laparoscopic hysterectomy has been reported with higher incidence (12% to 22% versus 2% to 4% comparing laparoscopic versus non-laparoscopic technique) in some studies, suggesting artifactual displacement of tumor rather than true biologic spread.

LVI in laparoscopic hysterectomy has been reported with higher incidence in many studies, leading many authors to suspect that some of the increased incidence is not biologically true LVI but represents artifactual displacement of tumor into lymphovascular spaces.

Criteria to distinguish true LVI from artifactual LVI have been proposed by the authors of one study.

**True LVI:**
- Smooth bordered, cohesive clusters of tumor cells
- Contours of tumor clusters conform to the vascular space
- Change in tumor morphology, often more eosinophilic cytoplasm
- Present in lymphatics adjacent to larger vessels

**Artifactual LVI**
- Disaggregated tumor cells
- Tumor cells intermixed with inflammation
- Tumor cell freely floating vascular space
- Tumor cells resemble main tumor mass
- Present in spaces immediately adjacent to invasive tumor with retraction artifact
- Present only in superficial vascular spaces

The criteria are not evidence-based; they are difficult to apply and there is significant interobserver variation. Never the less, using these criteria, one study reported artifactual LVI in 20% versus 4% of hysterectomies with versus without a uterine manipulator and in 49% versus 7% of malignant hysterectomies with versus without robotic assistance. There was no difference in the incidence of true LVI or nodal metastasis. Even in cases without any malignancy, endometrial glands were identified in LVI of a few cases performed with a uterine manipulator.

Multiple hypotheses have been offered to explain how uterine manipulation causes these artifactual findings. 1.) the increased intra-uterine pressure created by the inflated intra-uterine balloon may dislodge or disrupt endometrial tumor, particularly if it is an exophytic or highly papillary branching tumor, and push it into the lymphovascular system or extrude cells through the fallopian tubes; 2.) the mechanical motion of the uterine manipulator itself throughout the procedure may similarly disrupt and dislodge tumor cells into lymphovascular spaces or fallopian tubes; or 3.) disruption of the tumor makes it more prone to be dislodged during specimen grossing (so-called knife artifact). More studies are need to understand the basis for these artifacts.

From a diagnostic standpoint, it is clear that hysterectomies performed with uterine manipulation or with laparoscopic technique are likely to contain foci of tumor in lymphovascular spaces that raise concern for being artifact. Such foci are less likely to be seen in conventional abdominal hysterectomy. How to recognize artifact versus true LVI remains unresolved at the current time.

**Practical recommendations:**

In the absence of evidence based criteria for distinguishing true LVI from procedural artifact, using the published proposed criteria (listed above) is a reasonable approach. A discussion of the diagnostic problem in the report is merited in order to avoid conveying a sense of absolute certainty if in fact the interpretation in a given case remains somewhat uncertain.

- If the tumor is low grade, endometrioid histology and not significantly myoinvasive, the likelihood of true LVI is minimal and any intravascular tumor should be viewed suspiciously as potential artifact. In this setting, applying the proposed criteria for artifactual LVI seems reasonable.
- If the tumor is high grade endometrioid cancer or serous/clear cell carcinoma or deeply myoinvasive, then true LVI is a real possibility. Some, or all, of the suspicious foci may still represent artifact.
- If peri-lymphatic or peri-vascular aggregates of lymphocytes or mixed inflammatory cells are present, this gives more weight to an interpretation as true LVI versus artifactual LVI. Peri-vascular inflammation is a strong predictor of LVI. Two studies show that this finding is present in the majority of cases of LVI. Conversely, its absence also strongly predicted the lack of LVI.
- If MELF pattern myoinvasion is present, this also gives more weight to an interpretation as true LVI versus artifactual LVI since MELF is often accompanied by LVI. One study showed LVI in 63% of cases with MELF versus 25% without MELF. As discussed earlier in the section on myoinvasion, however, MELF...
itself can also be confused with LVI, so awareness of this special variant of myoinvasion is merited in order to avoid over-diagnosis of LVI in this setting.

Lesions that Mimic LVI
A few lesions that mimic LVI can be present in a uterus containing cancer and can be cause for over-diagnosis of LVI. Though uncommon, they merit attention.

- **Intravascular endometrium due to menses, adenomyosis or endometriosis** Benign endometrial tissue can be found within lymphovascular spaces of the uterus or within parametrial vessels on rare occasion, either following menses or in the setting of adenomyosis or endometriosis. About 10% of uteri with adenomyosis may contain endometrial glands and stroma; glands only; or stoma only within lymphovascular spaces of the uterine wall or within parametrial vessels. The epithelium may be condensed into compact clusters and, if menstrual, the stroma may also be compact and clustered. The etiology of this finding during menstruation may be related to disruption of endomyometrial veins during menstruation. The etiology of this finding in association with adenomyosis is unclear but may have some relationship to the proposed development of adenomyosis from cells associated with myometrial blood vessels. Distinction from cancer with LVI rests on recognizing the cytologically bland epithelium or stroma.

- **Intravascular leiomyomatosis and intralymphatic endometrial stromal sarcoma** Extension of leiomyoma into the myometrial veins adjacent to the leiomyoma may be seen on rare occasion, particularly in highly cellular leiomyomas; this is referred to as intravenous leiomyomatosis (IVL). If the involved vessels are within the leiomyoma itself, it is referred to as leiomyoma with vascular invasion. The clinical significance of IVL is that worm-like extensions of the tumor may grow beyond the uterine vasculature and enter the inferior vena cava, leading to potential embolic or occlusive events in the right heart and pulmonary vasculature. Diagnostically, IVL should not be confused with LVI due to uterine cancer if it happens that IVL co-exists with uterine cancer. This is not likely to occur and the cytologic features should enable the correct diagnosis yet it is worth mentioning. Similarly, intralymphatic spread of endometrial stromal sarcoma should not be misinterpreted as LVI if an endometrial stromal sarcoma co-exists with uterine cancer. Again this is an unlikely scenario yet worth mentioning.

- **MELF pattern myoinvasion of uterine cancer** Some foci of MELF may look like LVI. This is discussed in detail in the section of myometrial invasion patterns.

Staging rules and LVI
LVI within the uterus does not contribute to FIGO staging. It should still be reported, however, because it carries adverse prognostic value and may contribute to additional adjuvant therapy.

Controversy exists though regarding LVI outside of the uterus, specifically LVI in the para-ovarian, para-tubal, tubal or ovarian lymphovascular spaces. There are no rules to guide whether LVI in those sites qualifies as stage IIIA in the absence of tumor in the parenchyma/connective tissue/serosa. Such a scenario is rare.

Practical recommendation:

- In the absence of specific rules by FIGO/AJCC, it is reasonable to clearly report the presence of LVI in the adnexa as part of the final diagnosis/synoptic comment but not upstage to IIIA if the adnexal involvement is only in the form of LVI.

CERVICAL INVASION
About 13% to 29% of endometrial cancer patients have cervical involvement. This is defined in the current staging system as uterine tumor growth within the cervical stroma. While this sounds like a straightforward rule to apply, several challenges may arise in actual practice, including distinguishing:

- Endocervix versus lower uterine segment involvement.
- Glandular versus stromal involvement
- Exophytic mucosal growth versus stromal involvement
- Uterine cancer involving an endocervical polyp
- Polypoid uterine cancer prolapsing into endocervical canal
- Minimal deviation pattern ("burrowing" pattern) of cervical invasion by uterine cancer
- Uterine cancer involving cervical lymphatic spaces but not stroma
- Stage II uterine cancer versus stage 1 endocervical cancer
- Benign mimics
These are problems that are posed not only to general surgical pathologists but also to gynecologic pathologists, as demonstrated in a recent study.68

**Grossing technique for evaluating the cervix for stage II uterine cancer**

Attention to gross specimen evaluation and dissection of the lower uterine segment and cervix is important in hysterectomies containing uterine cancer. As discussed in detail below, the anatomic boundary between the lower uterine segment and upper endocervix is not well-defined in all patients. As a general rule of thumb, in evaluating a bivalved uterus, the endocervix can be defined as the zone in which the endocervical canal has parallel walls. The point at which those parallel walls flare outward at an angle can be defined as the boundary with the lower uterine segment. This is an idealized definition. Not all cases are easy to evaluate and many are distorted by the presence of leiomyomas, endocervical cysts, or the tumor itself.

The location of the endometrial tumor relative to the lower uterine segment and endocervix should be described with care. There are some exophytic tumors which may be confined to the uterine body but which may prolapse into the endocervical canal. This can be recognized by noting that the tumor is not actually attached to the endocervix itself.

Even if there is no gross extension of the endometrial tumor into the cervix, there may be microscopic involvement. The likelihood is higher the closer the gross tumor approaches the endocervix. At a minimum, one full cross section of the ectocervix/endocervix/lower uterine segment should be taken from the anterior and from the posterior half of the bivalved uterus. If the uterus is large, the full cross section may not fit into a single cassette. In such cases, the section should be blocked into two or three cassettes, with appropriate steps to preserve the orientation so that the anatomy can be reconstructed during microscopic evaluation. In some cases, using ink to orient sections that are blocked in over several cassettes may be helpful.

If there is gross extension of the endometrial tumor into the cervix, additional sections are advised to fully evaluate for cervical invasion.

A final note regarding specimen inking and margin evaluation. There are two surgical margins for a hysterectomy: the paracervical (or “radial” or “deep”) margin and the cervical/vaginal cuff margin. The latter margin is more relevant for hysterectomies for cervical squamous lesions such as dysplasia or invasive squamous cell carcinoma but it is still advised to ink the cuff and evaluate/report. Some hysterectomies will contain substantial vaginal cuff tissue while others may have minimal or absent vaginal cuff, in which case that margin is represented by the ectocervical outer periphery of the cervix. The paracervical/radial cervical margin is grossly identifiable as the non-peritonealized rough outer surface around the endocervix. Although this margin can be appreciated grossly in distinction from the peritonealized uterine serosa, it may not be so easy to appreciate microscopically. Therefore, inking the paracervical margin is advised. In our tumor synoptic template, we report the status of both margins as either positive (ink on tumor cells) or negative (and report the distance away from tumor).

**Endocervical involvement (stage II) versus lower uterine segment involvement (stage I)**

Anatomically, the endocervix is defined as the region between the external os and the internal os of the cervix. The lower uterine segment begins at the internal os and gradually becomes the uterine body. In reality, these anatomic definitions do not translate to clear cut landmarks grossly or microscopically. The internal os does not present as a discrete structure on gross examination. At the interface of the upper endocervix and lower uterine cervix, there is usually a zone in which the glands of the endocervix and lower uterine segment co-mingle. Similarly, the stroma of the endocervical wall, (predominantly fibrous with occasional bands of smooth muscle) and of the lower uterine segment wall (well-developed intersecting fascicles of smooth muscle) gradually merge together.

In individual cases, this may lead to difficulty in determining whether tumor is present within the lower uterine segment or within the endocervix. There is no gold standard method to resolve such a difficulty.

**Practical recommendations:**

1. Define location based on adjacent normal glands:
   - If there are unequivocal normal endocervical glands (without nearby endometrial glands) immediately adjacent to or infiltrated by the tumor, then the tumor is most likely in the endocervix. Conversely, if there are unequivocal normal endometrial glands (without nearby endocervical glands) immediately adjacent to the tumor, then the tumor is in the lower uterine segment. If there are both types of glands present, then it remains uncertain as to whether endocervix is involved and in this scenario, my recommendation is to reserve a designation of stage II uterine cancer to cases in which the findings are unequivocal. Thus, if
both endometrial and endocervical glands co-exist around the tumor, it does not seem prudent to designate it as stage II.

- **Define location based on appearance of stroma of uterine wall:**
  If the uterine wall involved by tumor is well-developed intersecting fascicles of smooth muscle, the tumor is within the lower uterine segment. If the wall does not contain well-developed smooth muscle but is fibrous, then the slide may represent endocervix. The glands in the overlying mucosa should be endocervical.

- **Correlate with other prognostic features of the tumor:**
  The majority of true stage II uterine cancers also exhibit other poor prognostic features such as higher grade, outer myometrial invasion, and LVI. Some authors question whether the outcome of stage II tumors is really due to these other prognostic features rather than the independent effect of cervical invasion. Therefore, if there is uncertainty about uterine cancer in the lower uterine segment versus endocervix, and the tumor lacks these additional poor prognostic features (i.e. the tumor is low grade and confined to endometrium), then caution is advised before assigning the tumor as stage II. It probably is not really an example of the prototypical stage II tumor and likely will not behave as such.

These practical approaches may not resolve every case, even when correlating with the gross findings. In the absence of any other helpful tools, in my view it is better to maintain stringent criteria for designating a tumor as stage II and withhold that designation unless the evidence is unequivocal. Otherwise, it seems most prudent to report such cases as stage I, with a description of the staging difficulty.

**Cervical stromal versus glandular involvement by uterine cancer**
The 2009 FIGO staging system defines stage II uterine cancer as tumor within the cervical stroma, meaning that tumor involving only the endocervical glands or surface does not qualify as stage II but remains as stage I. In most cases of cervical involvement, this distinction is straightforward. On occasion, however, it may not be so simple. FIGO does not provide a detailed definition of how to distinguish glandular versus stromal involvement.

Problems in distinguishing glandular versus stromal invasion are mostly in the setting of lower grade endometrioid adenocarcinomas because 1.) the architecture and the cytology may resemble that of the native endocervical glands and 2.) stromal desmoplasia is less likely to be present when there is stromal invasion, as compared to the frequent presence of stromal desmoplasia in higher grade tumors. The first of these two issues may result in over-diagnosis of stromal invasion and may explain why some studies comparing 1988 FIGO stage IIA versus IIB cancers did not detect any clinical outcome difference while others showed worse outcome for stage IIB. An even more difficult scenario occurs if the uterine tumor colonizes the endocervical glands and begins to expand them, particularly at the deep base of the glands; recognizing the point at which such expansion qualifies as early stromal invasion may not be possible in some cases. This scenario is analogous to the definition of early, limited expansile type invasion of endocervical adenocarcinoma arising from adenocarcinoma in situ.

The difficulty of diagnosing stage II uterine cancer is nicely summarized by a GOG pathology specialist:

"Neither the prognostic importance nor the reproducibility of the diagnosis of stage II (and substages IIA and IIB tumors) has been rigorously evaluated, but reproducibility seems to be poor and prognostic importance weak."  

**Practical recommendations:**

- Low magnification examination may be the most helpful way to distinguish colonization of endocervical glands from stromal invasion. By appreciating the architecture and depth of growth of the adjacent uninvolved endocervix, it may be possible to determine whether the tumor growth pattern is simply colonization of the underlying gland architecture or whether it is expanding and proliferating beyond that underlying footprint.
- Irregular, jagged tumor nest contours, desmoplastic stroma, single invasive tumor cells/clusters and deep location of the tumor nests are all features that strongly suggest stromal invasion versus glandular involvement.

**Exophytic growth of uterine cancer along endocervical mucosal surface**
Some uterine cancers may extend from the lower uterine segment into the endocervical canal by growing along the mucosal surface, without invading into the underlying endocervical stroma. Regardless of how bulky or extensive this mucosal surface growth may be, it does not qualify as stage II growth using the 2009 FIGO staging rules unless there is also a component of unequivocal stromal invasion of the underlying cervical wall.

The same approach applies to polypoid uterine cancer that prolapses through the internal cervical os and sits within the endocervical canal. Such a scenario is not stage II cancer.
Endocervical polyp involvement by uterine cancer
In the rare circumstance that uterine cancer involves an endocervical polyp but does not involve the stroma of the cervical wall, this does not fulfill criteria for stage II. Some pathologists may propose that the uterine cancer is invading the stroma of the polyp and therefore assign stage II, however the FIGO rules refer only to stroma of the cervical wall and they do not mention stroma of a cervical polyp.

Prolapsed uterine cancer
Uncommonly, uterine cancer can grow in a polypoid manner and prolapse into the endocervical canal. Clinically and radiologically this may simulate primary cervical cancer origin. However, careful radiologic analysis and careful specimen dissection will reveal that the tumor is based in the uterine body and has a polypoid growth pattern, including a prominent stalk, that results in prolapse. No cervical stromal invasion is present and therefore this is not stage II uterine cancer. Awareness of the possibility and careful gross specimen evaluation and documentation will avoid up-staging.

Minimal deviation pattern (“burrowing pattern”) cervical invasion by uterine cancer
As discussed in the section on variations of uterine cancer myoinvasion, there is pattern of endometrioid adenocarcinoma myoinvasion referred to by the following terms: minimal deviation pattern; diffusely infiltrative pattern; or adenoma malignum-like pattern. These names reflect the resemblance of the deceptive growth pattern to minimal deviation type endocervical adenocarcinoma. The pattern consists of simple glands or clusters scattered in the deep myometrium but lacking the conventional myoinvasive features of stromal desmoplasia, jagged gland contours, detached single or clustered tumor cells within myometrium or high nuclear grade.

Cervical invasion of this same pattern can be seen with this type of myoinvasion. It is deceptive because the tumor glands may resemble endocervical adenocarcinoma in situ or may resemble any of a number of benign cervical glandular lesions such as tunnel clusters, endocervical glandular hyperplasia (either the diffuse laminar type or the lobular type), cervical endometriosis or mesonephric glands/mesonephric hyperplasia.

Distinguishing this burrowing type of cervical invasion from the diagnostic mimics rests primarily on comparison of the glands in question to the primary endometrial tumor. They should appear morphologically similar. There should also be a smooth continuous transition between the myoinvasive glands and the cervical invasion component. Additional sampling of the lower uterine segment in continuity with the upper endocervical canal may be needed to demonstrate this.

Uterine cancer in cervical lymphatic spaces but not in stroma
Uncommonly, a uterine cancer may involve the lymphatic spaces of the cervix without stromal invasion. This rare event is more likely in high grade tumors such as uterine serous carcinoma. The FIGO rules do not address this scenario. Therefore this finding alone in the cervix does not qualify for stage II. However, as some authors point out, this finding likely is an adverse finding but this has not been formally studied. Thus, the finding should be clearly documented in the pathology report but cannot be used to assign the tumor as stage II.

Stage II uterine cancer versus stage I endocervical cancer
The site of origin may be challenging to determine if a carcinoma sits at the interface of the endocervix and lower uterine segment. The distinction is particularly important pre-operatively because the surgical plan is more extensive for primary endocervical adenocarcinoma (radical hysterectomy is generally performed).

In a hysterectomy specimen, both the gross and the microscopic findings may be helpful to distinguish primary endometrial versus primary endocervical adenocarcinoma, although it often requires interpreting a constellation of variables, including immunostains, to arrive at a decision.

In a biopsy specimen from the endocervix, the pathologist does not always have the benefit of clinical and radiologic data that may help guide interpretation of site of origin. Furthermore, architectural features that can be seen in sections from a hysterectomy, such as stromal desmoplasia and relationship of tumor to native glands, are not present in a biopsy, or they are difficult to interpret.

Grossly, the site of the bulk of tumor mass often correlates with primary site of origin. Primary endocervical adenocarcinoma can cause a so-called barrel-shaped cervix, distended and expanding the cervical wall. Lower uterine segment based tumors can be seen in the setting of tumor microsatellite instability, which is associated with Lynch syndrome.

Microscopically, the presence of precursor lesions can be helpful, such as endocervical adenocarcinoma in situ or atypical endometrial hyperplasia. Features favoring endometrioid adenocarcinoma include squamous
differentiation (including mature squamous differentiation, keratinization, abortive squamous whorls, or immature squamous differentiation), tall columnar cells and columnar nuclei, and stromal foamy histiocytes. Features favoring endocervical adenocarcinoma include intracytoplasmic mucin, goblet cells, apoptotic bodies in the basal cytoplasm, floating mitoses in the apical cytoplasm, cribriform growth, extensive stromal desmoplasia and co-existing high grade squamous intraepithelial lesion or adenocarcinoma in situ. It is important to be aware that these variables are not pathognomonic and they may sometimes be seen in tumors of either origin.

Immunohistochemistry has received much attention in this context. The bottom line is that there are no perfect markers to determine site of origin. A panel approach may be helpful, but does not always resolve every case, and the results need to be interpreted in the context of the clinical, gross and histologic findings. Endometrioid adenocarcinoma tends to exhibit basolateral membrane staining for vimentin and strong staining for estrogen receptors. Conversely, endocervical adenocarcinoma tends to show diffuse strong p16 staining and apical membrane staining for monoclonal CEA. Many cases will exhibit the expected phenotype for all 4 markers but occasional cases will exhibit puzzling combinations of results. Identification of high risk HPV also favors a primary endocervical origin, however HPV testing is not available in most practices; p16 is a reasonable surrogate marker since it is overexpressed in cells infected by high risk HPV. Not all cases can be resolved easily and it may become a matter of judgment when the findings are equivocal on site of origin.

Practical recommendations:

- In endocervical biopsy specimens containing adenocarcinoma: Before assuming that adenocarcinoma in an endocervical/cervical biopsy is of primary cervical origin, it is a good idea:
  - to verify that the clinical and radiologic context are consistent with primary cervical cancer.
  - to verify that there are no endometrioid histologic features such as squamous differentiation, tall columnar nuclei, or foamy stromal histiocytes.

Any uncertainty should prompt consideration of a stage II endometrial cancer and consideration of immunohistochemical confirmation. Because the origin cannot be resolved confidently in all cases, it is best to acknowledge uncertainty when the morphology and immunohistochemistry do not match up or do not correlate with the clinical/radiologic context. Communicating this uncertainty will allow the gynecologic oncologist to make the final decision regarding whether the patient should receive management for cervical adenocarcinoma or for stage II endometrial cancer.

Benign mimics of stage II low grade endometrial endometrioid adenocarcinoma

A number of benign glandular lesions in the cervix may mimic stage II disease in a patient with low grade endometrial endometrioid adenocarcinoma. The resemblance is most problematic with endometrioid adenocarcinoma exhibiting extremely bland cytology and lacking any solid growth. The most helpful criteria common to all the following scenarios is evaluation of the low magnification distribution pattern of the glands in question. If it is well-circumscribed and orderly, then consideration of one of these benign entities is merited before diagnosing stage II uterine cancer:

- cervical endometriosis
- tubal or tubo-endometrioid metaplasia of normal endocervical glands
- mesonephric glands / mesonephric hyperplasia
- tunnel clusters
- endocervical glandular hyperplasia (diffuse laminar type or lobular type)

Cervical endometriosis is distinguished from uterine cancer by the presence of periglandular endometrial stroma. Usually, the glands of endometriosis in this site will be simple, without intraluminal proliferation, branching or cribriform growth. In contrast, stage II endometrial endometrioid adenocarcinoma lacks endometrial stroma and should display some sort of architectural complexity or proliferation that is not expected in endometriosis.

Tubal or tubo-endometrioid metaplasia of normal endocervical glands is a cytologic change in the gland lining. There is no proliferation of glands or glandular lining. The process simply alters the cytology of the existing glands, which otherwise should appear normal in terms of the architecture. In contrast, stage II endometrial endometrioid adenocarcinoma is an addition of glands to the endocervical wall. At low magnification, the mucosa consists not just of the native endocervical glands, but of additional glands (the neoplastic glands) within the stroma. Thus, the distinction from tubal/tubo-endometrioid metaplasia is most easily made by low magnification evaluation of the architecture of the endocervix. This is especially important when the uterine cancer is low grade and cytologically resembles metaplastic endocervical glands.

Mesonephric glands or hyperplasia typically involves the deep cervical wall and often the adjacent presence of an elongated mesonephric duct helps to establish the diagnosis. The low magnification pattern of distribution of mesonephric glands, even when hyperplastic, tends to be lobular, well-circumscribed, and arranged around a central duct. This pattern is in contrast to the haphazard infiltrative pattern of most stage II uterine cancer. The
cytologic appearance of mesonephric glands should also distinguish them from most stage II uterine cancer since the cells tend to be uniformly cuboidal with monotonous round nuclei and eosinophilic intraluminal contents. Low grade endometrial endometrioid adenocarcinoma tends to exhibit more of a columnar cell shape, elongated nuclei, and is not characterized by striking monotony of the cells.

Tunnel clusters of the endocervix are usually straightforward to recognize. They are mentioned here mostly for completeness sake. The constellation of radial, lobular growth pattern, attenuated cell lining, and amorphous grey-blue intraluminal secretion are characteristic features.

Endocervical glandular hyperplasia, either diffuse laminar type or lobular type, are more likely to cause diagnostic concern for primary endocervical adenocarcinoma. Both patterns have one key feature in common that distinguishes them adenocarcinoma: a sharp, well-defined interface between the proliferation and adjacent stroma. An additional feature is the lack of stromal desmoplasia. Stage II uterine cancer will exhibit a more haphazard, infiltrative distribution.

ADNEXAL and SEROSAL INVOLVEMENT

Uterine cancer spread to the tube, ovaries or serosa represents stage IIIA. There are a few potential diagnostic problems that arise in evaluating adnexal or serosal spread of uterine cancer:

Pitfalls in diagnosing stage IIIA uterine cancer
- Distinction from artifactual intraluminal tumor in the fallopian tube
- Distinction from peritoneal keratin granulomas
- Distinction from atypical endometriosis in the ovary, tube or peritoneum

Controversies in defining stage IIIA uterine cancer
- Staging of adnexal involvement restricted to lymphovascular spaces only
- Distinction from synchronous primary ovarian cancer

Pitfall: artifactual intraluminal tumor in the fallopian tube versus true stage IIIA disease

As discussed earlier in the section on artifacts induced by surgical technique (see the section on LVI), the presence of free-floating tumor clusters/cells within the fallopian tube lumen does not necessarily indicate biologic spread of the tumor but may represent artifactual contamination. If the latter, such findings on their own should not qualify for stage IIIA.

Generally, artifactual contamination of the fallopian tube occurs when the uterine tumor is highly fragmented (such as papillary morphology), poorly fixed, or removed via laparoscopic technique/robotic assisted technique/uterine manipulator. As discussed in the earlier section on this topic, this is an iatrogenic issue, not a biologic issue. The challenge to pathologists is distinguishing artifact from true involvement of the tube. There are no published guidelines to do so and the literature on this finding is scant, thus individual cases should be evaluated using judgment and common sense.

Practical recommendations:
- The following features favor artifactual contamination.
  - free floating tumor clusters or cells without attachment to the tubal mucosa
  - lack of tumor in the mucosa, wall or serosa of the tube
  - lack of LVI anywhere in the adnexa
  - disruption, fragmentation, or distortion of the free floating tumor cells
  - surgical technique is laparoscopic or used a uterine manipulator or used robotic assistance
  If a constellation of these are present, it seems reasonable to describe the intraluminal tumor in the report but not upstage to IIIA based solely on this finding; an explanation of the reasoning should also be provided.
- True involvement of the tube should consist of tumor growth in the mucosa, wall and/or serosa of the tube. At least one of these features should be present to qualify for stage IIIA; intraluminal tumor alone is not enough.

Pitfall: peritoneal keratin granulomas versus true stage IIIA disease
Peritoneal keratin granulomas should not be interpreted as malignant or as metastatic tumor deposits or as stage IIIA disease. These are simply foci of granulomatous peritonitis that arise in reaction to dislodged keratin or keratinized metaplastic squamous epithelium from endometrial origin endometrioid adenocarcinoma with squamous
differentiation. The granulomatous nodules can occur anywhere on the peritoneal lining, whether along the peritoneal walls, uterine serosa, adnexal serosa or omentum. They may be visible to the surgeon as nodules, and thus raise clinical concern for carcinomatosis or they may be occult microscopic findings. Histologically they consist of nodular aggregates of multinucleate giant cells and keratin, with or without anucleate squamous epithelium, and a variable degree of chronic inflammation and fibrosis. They are considered benign because they do not confer adverse prognosis. Thus, their presence does not qualify as stage IIIA. True stage IIIA disease involving the peritoneum/serosa requires the presence of malignant epithelium.

Pitfall: atypical forms of endometriosis versus true stage IIIA disease
Endometriosis may undergo the same spectrum of alterations (including metaplasias, hyperplasia, and neoplastic transformation) seen in the endometrium. Caution is advised in evaluating endometriosis of the adnexa or serosa in patients with endometrial endometrioid adenocarcinoma. Conventional criteria should be adhered to before diagnosing adenocarcinoma within endometriosis. Variations such as polypoid endometriosis or cytologically atypical endometriosis should not be classified as malignant and should not result, on their own, in designation as stage IIIA.

Controversy: staging of tumor restricted to adnexal lymphatic spaces
There are no FIGO/GOG rules regarding uterine cancer involving the adnexa only by involvement of the lymphatics of the ovary, tube, or para-tubal/para-ovarian connective tissue. It is a rare occurrence but is reported by some authors as well as in my own experience. There are not any studies to provide evidence for how such tumor should be staged.

Practical recommendation:
- In the absence of specific rules by FIGO/AJCC, it is reasonable to clearly report the presence of LVI in the adnexa as part of the final diagnosis/synoptic comment but not upstage to IIIA if the adnexal involvement is only in the form of LVI.

Controversy: distinguishing stage IIIA uterine cancer from synchronous primary ovarian cancer
Some patients present with concurrent tumor in the endometrium and in one or both ovaries. The problem of distinguishing metastatic uterine cancer to the ovary (or vice versa) from synchronous primary uterine and ovarian carcinoma is a longstanding problem. Despite much effort in the literature, there are no gold standard methods to resolve the dilemma using routinely available diagnostic tests. The short answer is that this remains controversial. There are no FIGO/AJCC rules. The practical recommendations for managing individual patients is based on consensus criteria in the literature and in textbooks, not evidence-based criteria. Much of the published recommendations are essentially derived from common sense. Whether such common sense can be clinically validated requires further studies. The approach also is dependent on the histologic subtype of the uterine cancer.

Concurrent endometrioid adenocarcinoma in uterus and ovary
Approximately 10% of women with endometrioid adenocarcinoma of the endometrium will also have concurrent endometrioid adenocarcinoma in one or both ovaries. If a histologic precursor to endometrioid adenocarcinoma can be identified in an organ, then it is reasonable (though not necessarily valid) to presume that the tumor originated in that organ. For the uterus, the precursor lesion is atypical hyperplasia. For the ovary, the precursor lesion is either endometriosis or endometrioid adenofibroma. The criteria proposed in the Armed Forces Institute of Pathology (AFIP) Atlas on Tumor Pathology are used by most pathologists and are similar to many of those proposed in other gynecologic pathology textbooks. The criteria for each of the three possible classifications are as follows. There is no rule on how many variables need to be present to choose one classification over another. It is more of a subjective judgment of the constellation of features (and therefore subject to observer variation):

**Primary Uterine Cancer with Metastasis to the Ovaries**
- Uterus contains atypical hyperplasia
- Uterine tumor has deep myoinvasion
- Uterine tumor has LVI
- Uterine tumor involves cervix
- Uterine tumor and ovarian tumor are similar appearance and grade
- Ovarian tumor has LVI
- Ovarian tumor is bilateral and/or multinodular
- Ovarian tumor lacks endometriosis or adenofibromatous background

**Primary Uterine Cancer and Primary Ovarian Cancer**
- Uterus contains atypical hyperplasia
- Uterine tumor has no or limited myoinvasion
- Uterine tumor lacks LVI
- Uterine tumor does not involve cervix
• Uterine tumor and ovarian tumor have different appearance and grade
• Ovarian tumor lacks LVI
• Ovarian tumor is unilateral and is a dominant mass (not multinodular)
• Ovarian tumor has endometriosis or adenofibromatous background

Primary Ovarian Cancer with Metastasis to Uterus (very rare!)
• Uterine tumor lack atypical hyperplasia or complex hyperplasia
• Uterine tumor is confined to endometrium or to a polyp
• Ovarian tumor is unilateral and large
• Ovarian tumor has endometriosis or adenofibromatous background

While these criteria appear straightforward, applying them can be difficult because all of the findings may not perfectly fit into one of the three categories. Some findings may fit in one category and other findings may fit it another category. Since these are not evidence-based criteria so there is no way to “correctly” resolve such problem scenarios.

Practical recommendation:
➢ Using a “majority-wins” approach to apply the AFIP criteria seems reasonable in individual cases of concurrent endometrioid adenocarcinoma of the uterus and ovary: use the classification category for which the majority of the findings support. For example, if a tumor has 5 features supporting primary uterine origin with metastasis to the ovary and 2 features supporting synchronous primary tumors, I would favor the former classification.
➢ Regardless of classification of tumor origin, the concurrent presence of endometrioid adenocarcinoma in the uterus and ovaries is a proposed risk factor for Lynch syndrome and therefore should trigger pathologic screening tests for Lynch syndrome (mismatch repair protein immunohistochemistry and/or microsatellite instability testing) if such screening is part of your routine pathology practice.
➢ For clear cell carcinoma and carcinosarcoma, the same approach can be used.

Concurrent serous carcinoma in uterus and ovaries

There are no gold standard criteria to resolve the primary site of origin in this scenario. The role of histologic precursors to serous carcinoma in assigning primary origin is not well understood, but may offer some practical guidance in individual cases. The histologic precursor to uterine serous carcinoma is endometrial intraepithelial carcinoma (EIC). EIC is defined as the replacement of existing endometrial gland epithelium with tumor cells that look like those of conventional high grade serous carcinoma. EIC typically is focal and confined to the surface endometrium or to surface of an endometrial polyp. EIC may arise in pure form or it may be present adjacent to conventional uterine serous carcinoma. Like the latter, most EIC exhibits strong diffuse p53 immunohistochemical expression or none at all. P16 and MIB-1 will be strongly positive. The vast majority of uterine serous carcinoma and EIC is WT-1 negative or weak/patchy. Conversely, diffuse/strong WT-1 is a hallmark of most primary ovarian, tubal or peritoneal serous carcinoma. Thus, the presence of EIC and absence of strong/diffuse WT-1 are features supporting a primary uterine origin.

The histologic precursor of high grade serous carcinoma in the ovary is a subject of recent developments and ongoing controversy. In short, the mucosa of the fimbriae of the fallopian tube is currently proposed to be the origin of a significant proportion of ovarian and peritoneal high grade serous carcinoma. The details behind these developments are beyond the scope of this syllabus but there are many review articles which offer useful summaries.

The tumor size does not seem to be associated with site of origin or risk of spread beyond site of origin. Studies of uterine serous carcinoma involving only an endometrial polyp show that there still can be extensive extra-uterine spread either concurrently or subsequently. Thus, in a patient with a small focus of serous carcinoma in an endometrial polyp and a large mass of cancer in the ovary or peritoneum, the relative sizes of tumor cannot be used to assign site of origin.

Unlike the setting of endometrioid adenocarcinoma, here are no well-recognized consensus criteria for assigning site of origin when multiple gynecologic organs are involved by high grade serous carcinoma. Much work is needed in this area.

Practical recommendations:
▪ In the absence of FIGO or consensus criteria, it seems reasonable to look for the following features: In the uterine tumor, the presence of EIC and the absence of WT-1 favors that the uterine tumor is primary uterine serous carcinoma. Conversely, strong/diffuse WT-1 favors an ovarian origin. In the ovarian tumor, the absence of fimbrial involvement by tumor and absence of WT-1 favors that it is metastatic uterine
serous cancer. The relative size of the tumor in the uterus versus adnexa does not appear to be a helpful criteria.

OMENTAL INVOLVEMENT / EXTRA-PELVIC INTRA-ABDOMINAL INVOLVEMENT

Uterine cancer involvement at intra-abdominal sites outside of the pelvis are staged as IVB. This is a straightforward rule. However, it merits attention because it is slightly different than the staging rules for primary ovarian cancer, in which case tumor in the omentum or other extra-pelvic peritoneal sites is stage III. Because the latter is encountered much more commonly than IVB uterine cancer, it is easy to forget about this difference in the rules and mistakenly provide the lower stage of III. Thus, this discussion is a reminder that the staging rules for distant disease are different between ovarian and uterine cancer.

PERITONEAL CYTOLOGY

The 2009 FIGO staging rules eliminated the role of peritoneal cytology in uterine cancer staging. The basis for this change presumably stems from the controversy in the literature regarding whether peritoneal cytology is an independent prognostic variable. There are studies which support that it is and there are studies which refute this. The details of the decision to eliminate this variable and the details of which studies formed the basis of the final decision are not published.

Whether this staging change means that surgeons will stop collecting peritoneal cytology remains to be seen. At my institution, peritoneal cytology is still collected in the same scenarios that it was before the 2009 FIGO change. Our department still reports the results in the uterine tumor synoptic.
SCREENING UTERINE CANCER PATIENTS FOR LYNCH SYNDROME

Overview
The pathologist now plays a critical role in the multi-disciplinary effort to identify which uterine cancer patients might have Lynch syndrome, an autosomal dominant syndrome that predisposes to uterine and colon cancer. Identifying such patients allows for early detection of other cancers in that patient as well as in her family. The pathologist’s role is in performing one or both of the screening tests for Lynch syndrome using tissue from the patient’s uterine cancer. The two tests are immunohistochemistry for mismatch repair (MMR) proteins and tumor microsatellite instability (MSI) testing. The test results are used to guide whether the patient should undergo further risk assessment by a genetics counselor and germline mutation.

Outline
- Rationale for screening uterine cancer patients for Lynch syndrome
- Current understanding of screening algorithms
- Mismatch repair protein immunohistochemistry
- Tumor microsatellite instability testing
- How to interpret and report the screening tests

Rationale for screening
Lynch syndrome (LS), an autosomal dominant cancer syndrome resulting from a germline mutation in one of several DNA mismatch repair genes, is responsible for about 2%-5% of all endometrial cancers. The lifetime risk for endometrial cancer in Lynch syndrome is about 40%-60% and for colon cancer is about 70%. Both cancers will arise in about 50% of patients and a third (urothelial carcinoma, ovarian carcinoma, small intestinal carcinoma) will arise in about 15%. In half of the women with Lynch syndrome, endometrial cancer is the first cancer. Thus, it would be valuable to know which women presenting with endometrial cancer might be at risk for developing colon cancer. The benefits extend to the patient's family as well since there are screening and preventive options for colorectal, endometrial and urothelial cancer. Family members are advised to begin colonoscopy screening at age 20, yearly endometrial biopsy at age 30 and urinalysis with cytology in the 2nd or 3rd decade. The role of surveillance pelvic/transvaginal ultrasound in affected women without cancer has not yet been established. Studies of chemoprophylaxis (anti-estrogen therapy) and surgical prophylaxis (risk reducing hysterectomy) are ongoing.

How is Lynch syndrome diagnosed
The definitive test for Lynch syndrome is germline analysis to detect a mutation in one of the four mismatch repair (MMR) genes (MLH-1, MSH-2, MSH-6, PMS-2). The test is only available in specialty molecular diagnostic laboratories and is expensive. At a population level, it is not feasible to test every patient who presents with uterine cancer. Thus, a screening approach is needed.

Screening uterine cancer patients for Lynch syndrome: the big picture
The optimal strategy for screening uterine cancer patient’s for Lynch syndrome remains to be defined. It is currently a subject of intense investigation. There are no FIGO, GOG, CAP or National Cancer Institute recommendations to date, unlike the recommendations for screening colon cancer patients for Lynch syndrome. The Society of Gynecologic Oncologists (SGO) does have a consensus statement that advises formal genetic risk assessment in uterine cancer patients who are at risk for Lynch syndrome. The SGO advises that assessment be performed if the estimated risk is greater than 20-25%; this risk is defined from a constellation of personal and family cancer history variables (see below). If the risk is greater than 5-10%, the language is that formal risk assessment “may be helpful”. The SGO also emphasizes that hereditary cancer risk assessment is a process rather than a test. The components, as defined in the consensus statement are as follows:

Hereditary cancer risk assessment is a process that:
- includes assessment of risk, education and counseling
- is conducted by a physician, genetic counselor or other provider with expertise in cancer genetics
- May include genetic testing if desired after appropriate counseling and consent has been obtained

Based on data available at that time (2007), the SGO defines estimated risks for Lynch syndrome as follows:

Greater than 20-25% chance that the uterine cancer is inherited:
- Patient meets revised Amsterdam criteria:
  - 3+ relatives with Lynch associated cancer in one lineage
  - 1 affected individual is a first degree relative of the other two
  - 2+ successive generations should be affected
  - 1+ Lynch associated cancer diagnosed <age 50
- Patient has synchronous or metachronous uterine/ovarian and colon cancer, one of which was diagnosed <age 50
- Patient has uterine cancer with loss of mismatch repair protein or microsatellite instability
- Patient has first or second degree relative with mismatch repair gene mutation.
Greater than 5-10% chance that the uterine cancer is inherited:

- Patient diagnosed < age 50
- Patient has synchronous or metachronous colon cancer / other Lynch associated tumor
- Patient has first degree relative with Lynch associated tumor diagnosed < age 50
- Patient has two or more first/second degree relatives with Lynch associated tumor
- Patient has first/second degree relative meeting above criteria.

In addition to the SGO recommendations, many individual institutions are currently using various screening strategies in clinical practice and are evaluating and publishing their experiences in an effort to eventually define an optimal screening approach. Most of this activity is done in a multi-disciplinary setting, integrating the work of pathologists, gynecologic oncologists and genetics counselors. A multi-disciplinary approach is essential because the proper implementation of the screening tests (to be discussed below), interpretation, reporting, and follow up with the patient requires coordination and communication between pathologists, gynecologic oncologists and genetics counselors. Because the results affect not only the patient but also her extended family, it is critical that test results and the implications be presented to patients in a manner that is easy to understand. Furthermore, they should receive appropriate psychosocial support not only to deal with a genetic disease diagnosis themselves but to convey it to their family along with the appropriate medical steps that the family members should take.

The following discussion of the screening tests for Lynch syndrome is designed for educational purposes only. Pathologists who are currently not performing these tests in their practice or whose institutions do not have providers with expertise in cancer genetics are not advised to begin performing these tests independently without first establishing a multi-disciplinary approach such that there is a clear strategy for appropriate education of patients and follow up with results.

Screening uterine cancer patients for Lynch syndrome: the detailed picture

Currently, the likelihood of Lynch syndrome in a uterine cancer patient is best predicted by three variables: young age, personal/family history of cancer, and pathology of the uterine cancer. To understand the screening tests, it is necessary to understand the definitive test for Lynch syndrome:

Detecting MMR gene mutations in GYN Cancer patients: Germline mutation in one of four MMR genes defines Lynch syndrome.\(^{114}\) The MMR genes are:

- MLH1 mutL homolog 1 gene
- MSH2 mutS homolog 2 gene
- MSH6 mutS homolog 6 gene
- PMS2 postmeiotic segregation increased 2 gene

Affected individuals carry one mutated copy of the gene in all cells of the body and either a somatic mutation or loss of the second copy in the tumor cells. The normal role of these proteins is to repair base pair mismatches in DNA. When this normal repair function is lost due to MMR gene mutation, DNA becomes unstable, particularly at locations called microsatellites (short tandem DNA repeat sequences). This is called microsatellite instability (MSI) and is a hallmark of Lynch syndrome (though not necessarily specific). To complicate matters, loss of MMR protein function can also occur by methylation of a MMR gene (MLH1), which results in silencing of transcription and an absence of protein. MLH1 methylation is not associated with a mutation in the MMR genes, and it has only been identified in sporadic tumors, rather than in those associated with Lynch syndrome. Importantly, the majority of MSI-H tumors are sporadic, apparently caused by MLH1 hypermethylation and not Lynch syndrome.

Why not test for the actual mutations if Lynch syndrome is suspected? Such testing is expensive and both labor and time-intensive. Therefore it is prohibitive to use in a general population. Instead, pre-selecting a group of high risk patients is necessary. Pre-selection can be done based on the revised Bethesda criteria and can also be done based on detecting abnormalities in the MMR proteins by immunohistochemistry and/or by detecting microsatellite instability (MSI test) in the tumor.

Pathologists' role in pre-selecting high risk patients: As surgical pathologists, we can help pre-select high risk patients who have endometrial cancer by assessing for immunohistochemical loss of MMR proteins and by ordering MSI testing using the cancer tissue block.

MMR Immunohistochemistry in Endometrial Cancer: Mutations in the MMR proteins can be detected by loss of expression in endometrial cancer cells. Antibodies for MLH1, MLH2, MLH6 and PMS2 are commercially available for immunohistochemical staining. Normal, non-neoplastic tissues show nuclear expression of all four stains and serve as internal control. The staining may be heterogeneous, though usually is diffusely positive. Loss of one of
the proteins suggests a germline mutation may be present. Based on a recent review of literature, the sensitivity of MMR immunohistochemistry (when using all 4 antibodies) for germline mutation is 92%; the specificity, however, is unknown because large scales studies to determine specificity do not exist. A single study reports 95% specificity using MLH1 and MSH2.

Using these 4 MMR antibodies, it is possible to observe a number of staining results:

If all 4 stains are expressed, it is interpreted that germline mutations are not likely present. It is possible, however, for a mutation to result in expression of an inactive but antigenic protein. Thus, despite a mutation present, the mutated protein will be detected by MMR immunohistochemistry. This partly explains why the sensitivity of immunostaining is not 100%.

At UCSF we use the following template to report such results:

MLH1 expression: Present.
MSH2 expression: Present.
MSH6 expression: Present.
PMS2 expression: Present.

Expression for all four markers, in most cases, indicates that the mismatch repair proteins are intact. This result should be correlated with the patient’s clinical presentation, family history, and findings from the microsatellite instability (MSI) test.

Immunohistochemistry for mismatch repair proteins (MMR) and polymerase chain reaction (PCR) for microsatellite instability (MSI) are offered at UCSF to evaluate whether a tumor shows mismatch repair deficiency. The results of the MSI test are issued directly from the Molecular Pathology Laboratory.

If one of the 4 antibodies shows complete absence of expression in tumor cells, the patient should be referred for further evaluation by formal genetics counseling. Before designating “loss of expression”, positive internal control should be verified. The normal cells (lymphocytes, myometrium) should be positive otherwise the assay is not working correctly. Also, pathologists should be aware that the expression pattern can be heterogenous/focal and therefore the entire section of tumor on the slide should be examined carefully before designating “loss of expression”. MSH6 is particularly reported to have focal staining, sometimes in less than 10% of tumor cells.

At UCSF we use the following template to report such results.

MLH1 expression: Absent.
MSH2 expression: Present.
MSH6 expression: Present.
PMS2 expression: Absent.

Absence of staining indicates that the tumor shows loss of one of the mismatch repair proteins. However, the immunohistochemical stain does not distinguish between loss due to promoter hypermethylation and loss because the patient is a carrier of a mismatch repair gene mutation associated with Lynch syndrome. This result should be correlated with the patient’s clinical presentation, family history, and findings from the microsatellite instability (MSI) test. Referral for clinical genetics evaluation and counseling should be considered to help distinguish between these possibilities for the patients and their family members.

Immunohistochemistry for mismatch repair proteins (MMR) and polymerase chain reaction (PCR) for microsatellite instability (MSI) are offered at UCSF to evaluate whether a tumor shows mismatch repair deficiency. The results of the MSI test are issued directly from the Molecular Pathology Laboratory.

It is possible for two of the antibodies to show complete absence of expression. This may occur because the proteins normally form heterodimers. MLH1 normally dimerizes with PMS2 to form the MutL-alpha functional protein complex while MSH2 normally dimerizes with MSH6 to form the MutS-alpha functional protein complex. MLH1 and MSH2 are considered the obligatory partners while PMS2 and MSH6 are considered secondary partners. Mutation in one of the obligatory heterodimer partners can result in degradation of the secondary partner protein. Thus, defects in MLH1 may cause PMS2 loss (both proteins would be “negative”). Similarly, defects in MSH2 may cause MSH6 loss (both proteins would be “negative”). The reverse does not necessarily occur. Loss of MSH6 may not result in loss of MSH2. Loss of PMS2 may not result in loss of MLH1.

One of the advantages of using immunohistochemistry over the MSI test is that it identifies which MMR gene should then be tested for mutation. Thus, it further increases the efficiency of the screening process.

The pathologist should select a block for immunostaining that contains tumor as well as adjacent normal tissue (i.e. myometrium) that can serve as internal positive control.
Is MMR immunohistochemistry reliable in a biopsy?: Although formal evidence is not available, it is possible that negative staining in a biopsy of endometrial cancer could be a false-negative result due to sampling and due to heterogenous staining pattern. The biopsy may contain the “negative” component of a tumor that elsewhere is truly “positive”. Therefore we and others advise against testing in biopsies; or at least re-confirming “negative” results in the hysterectomy specimen.

Is MMR protein loss diagnostic of Lynch syndrome?: This depends on the staining pattern seen. Most cases of MMR loss involve MLH1, and the majority of these cases are not associated with Lynch syndrome. Other staining patterns, such as MSH6 loss, are very highly associated with Lynch syndrome. However, sequencing of MMR genes remains the gold standard, and patients should not be diagnosed with Lynch syndrome solely on the basis of immunohistochemical staining.

MSI testing of Endometrial Cancers:

What are microsatellites and what does instability mean?: Distributed throughout the normal human genome, often in non-coding regions, there are short tandemly repeated DNA sequences of 1 to 6 bases. These are called microsatellites. Microsatellite instability (MSI) refers to insertions or deletions of these repeating units that result in changes in the length of the microsatellite. These insertions or deletions may occur during DNA replication. A proof-reading system involving DNA polymerase usually detects and corrects these errors. Those that go uncorrected are generally corrected by the MMR system. When the MMR system fails, due to defects in the MMR proteins, these insertions or deletions accumulate, creating genomic instability, referred to as MSI. Genes which contain microsatellites in their coding regions are then rendered susceptible to frameshift mutations as these microsatellites change in size. Instability in key regulatory genes may lead to dysregulation of cell proliferation and eventually contribute to evolution to cancer.

How are MSI and Lynch syndrome related?: Because the definition of the syndrome is mutation in one of the MMR genes, tumors in patients with Lynch syndrome are likely to contain MSI due to failure of their MMR system.

Testing for MSI: Various studies have evaluated which microsatellite regions are the best to analyze in this setting. In two of the commonly used tests, the Bethesda panel and the Microsatellite Instability Analysis System kit by Promega, there is some overlap in microsatellites targeted. The choice of type(s) of microsatellite to test (mononucleotide versus polynucleotide) affects sensitivity and specificity. Work is ongoing to build consensus on a standardized panel of microsatellites to test for Lynch syndrome. These are PCR-based (polymerase chain reaction) tests that amplify the target microsatellite DNA and then compare the lengths of the microsatellites in the tumor tissue to the lengths in the normal tissue. In both the Bethesda and Promega test, instability in more than one microsatellite is considered MSI-high while instability in only one microsatellite is considered MSI-low; MSI-low is not associated with Lynch syndrome. The test can be performed on formalin fixed, paraffin-embedded tissue. Tumor blocks need to contain normal tissue as well to serve as the reference control in the test. A number of commercial molecular diagnostic laboratories offer this test, as do many academic centers, including our own.

Is MSI diagnostic of Lynch syndrome?: No. MSI can be shown in up to 15-25% of sporadic colorectal carcinomas. Again, the mechanism here is thought to be silencing of MLH1 expression by hypermethylation, not by germline mutation in MHL1. Thus, MSI is not specific for Lynch syndrome.

Patients with MSI-high endometrial cancers are referred to geneticists for further evaluation for Lynch syndrome.

MMR immunohistochemistry versus MSI testing: There is an ongoing debate to determine how these tests should be best incorporated into an algorithm for screening. Various institutions are using various combinations of these tests and it remains to be worked out what algorithm optimizes screening of patients. At UCSF we currently perform both tests in patients who fit the screening criteria listed earlier.

Advantages of MMR immunostaining:
1. Can be performed in any lab that offers immunohistochemistry
2. Usually easy to interpret
3. Allows identification of the MMR gene of highest interest for mutation analysis

Disadvantages of MMR immunostaining
- Some mutations do not cause truncation (loss) of the protein but just functional inactivation and so false-positive staining (i.e., failure to recognize defective MMR proteins) can occur
- Limited staining incorrectly interpreted as negative
- Certain staining patterns (e.g., loss of MSH6) are very highly correlated with Lynch syndrome, and some may consider this a surrogate genetic test, for which the patient may not have given consent.

Advantages of MSI testing
Usually easy to interpret
Not affected by truncating MMR mutations that could lead to false positive immunostain results
May detect defects in novel MMR proteins which are not tested by immunostaining

Disadvantages
Requires specialized diagnostic lab to perform test
Does not indicate which gene is involved
MSI is not specific for Lynch syndrome

Selecting which endometrial cancers should get screening tests (MMR IHC / MSI testing)
It is unclear whether these screening tests should be performed universally on all patients with endometrial cancer or in a selected sub-group at increased risk. Three variables to stratify risk for Lynch syndrome have received the most attention: patient age, personal/family cancer pedigree, and tumor morphology. None of these are accurate on their own. Regarding age, the Bethesda guidelines recommend age under 50 as a trigger for evaluating colorectal patients for Lynch syndrome.\textsuperscript{87, 113} Though these guideline were not designed for endometrial cancer patients, this age cut off has been adopted by many gynecologic oncologists.\textsuperscript{57} While the average age of Lynch syndrome endometrial cancer (average age between 46 and 55 years)\textsuperscript{14, 35, 45, 115, 116} is indeed lower than for sporadic endometrial cancer, a significant proportion (between 40% to 60%)\textsuperscript{35, 37, 45, 116} of Lynch syndrome endometrial cancer presents after age 50, with some patients presenting in the 7\textsuperscript{th} decade. Regarding personal/family cancer pedigree, neither the Bethesda guidelines nor the Amsterdam criteria are sensitive enough to identify all Lynch syndrome endometrial cancer; some studies report the sensitivity of these criteria to be well under 50%.\textsuperscript{35, 37, 88, 117} Regarding tumor morphology, the literature is too limited to identify specific microscopic features that accurately separate Lynch syndrome versus sporadic endometrial cancer, however, there is a suggestion that Lynch syndrome should be suspected in a cancer arising from the lower uterine segment or exhibiting tumor infiltrating lymphocytes or peri-tumoral lymphocytes.\textsuperscript{14, 88, 115, 120} Tumor morphology has also been linked to the broader category of endometrial cancers with MSI and with abnormal MMR IHC status; these features include tumor infiltrating lymphocytes, peri-tumoral lymphocytes, undifferentiated tumor histology, lower uterine segment origin and synchronous ovarian and endometrial tumors.\textsuperscript{32, 33, 96, 115} A variety of screening algorithms have been proposed using one or more of these variables (age, cancer pedigree, tumor morphology) to determine when MSI and/or MMR IHC testing should be performed, while some authors have simply proposed universal testing of all endometrial cancer patients.\textsuperscript{32, 56, 57, 86, 88} An optimal strategy remains to be established.

UCSF Criteria for ordering MMR IHC / MSI testing:

At least one of these criteria must be present:
- Bethesda Guidelines criteria fulfilled (as indicated by the clinician to the pathologist)
- Age 50 or younger
- any one MMR Tumor Morphology criteria present:

MMR Tumor Morphology criteria are:
- Tumor infiltrating lymphocytes >40 per 10 hpf
- Peri-tumoral Crohn’s like lymphocytic infiltrate
- Lower uterine segment is primary origin of tumor
- Synchronous primary endometrial and ovarian adenocarcinoma
- Undifferentiated uterine carcinoma or dedifferentiated uterine carcinoma

Managing results of MMR IHC / MSI testing
As shown earlier, we use templates to report the results. The language of the templates has been developed jointly with our genetics counselors so it is clear to the patient with abnormal results that they are going to be contacted for further risk assessment. These screening tests should only be performed if the pathologist has a working relationship with genetics counselors who can follow through with abnormal results and provide appropriate counseling regarding the next step.
UCSF Uterine Carcinoma Synoptic Comment

Type of tumor: if mixed (each component >10%), list each component:

Pathologic criteria for mismatch repair protein/microsatellite instability combination PCR & IHC testing:
Check all that apply and order "MSI-Combo panel" if any criteria are present. Results reported by addendum.
☐ Age <50
☐ Prominent peri-tumoral lymphocytic infiltrates
☐ Tumor infiltrating lymphocytes >40 per 10 hpf within tumor epithelium
☐ Lower uterine segment is the primary site
☐ Synchronous primary ovarian and uterine adenocarcinoma
☐ Heterogeneous areas of well-differentiated endometrioid adenocarcinoma and undifferentiated carcinoma
☐ None. Do not order the MSI-Combo panel unless specifically requested by clinician.

FIGO grade:
1. FIGO grade = architectural grade; if grade 3 nuclei then raise grade by 1 for architectural grade 1 and 2 tumor
2. Areas of squamous differentiation are not included as "solid" growth; only the glandular component is graded
3. Serous, clear cell and carcinosarcoma are by definition grade 3

☐ 1 (< 5% solid growth)
☐ 2 (6-50% solid growth)
☐ 3 (> 50% solid growth)

Myometrial invasion:
☐ None, confined to endometrium
☐ Present. Depth of invasion: __________ cm.
  Thickness of myometrium: __________ cm.
  Percent of myometrium invaded: ______%
  Tumor directly extends from myometrium to involve serosa: ☐ No ☐ Yes

Tumor size: ______ cm.

Location of tumor in uterus:
☐ Anterior wall
☐ Posterior wall
☐ Both
☐ Cornua

Lymphatic/vascular invasion:
☐ None
☐ Present in lymphatics
☐ Present in blood vessels

Lymph node status:
☐ None present.
☐ Negative. Total number of nodes examined ______
☐ Positive. Total number of positive nodes ______
  Total number of nodes examined ______

Margins:
- Vaginal cuff / cervical cuff margin:
  ☐ Negative. Tumor is _____ cm away from margin. (list block # if less than 0.5 cm away)
  ☐ Positive (block # ____)
- Paracervical margin:
  ☐ Negative. Tumor is _____ cm away from margin. (list block # if less than 0.5 cm away)
  ☐ Positive (block # ____)

Pelvic/abdominal cytology:
☐ Not performed
☐ Benign. CoPath Case #__________
☐ Malignant. CoPath Case #__________

AJCC/UICC stage: pT_____N_____ (cM_____

Notes: TNM Descriptors such as ‘m’, ‘r’, ‘y’ should be added to the AJCC stage if applicable.
pM0 & pMx are no longer valid categories. Instead, please use cM0 or cM1 (if provided by the clinical information) in conjunction with pTpN to obtain the final AJCC anatomic stage. pM1 is still a valid category. Otherwise, please omit M information (AJCC Cancer Staging Manual, 7th Edition, p. 8, 11).

FIGO Stage: __________________


