Lynch Syndrome Screening for Endometrial Cancer: Basic Concepts
Hi, my name is Sarah Kerr. I’m a pathologist at Mayo Clinic, where I participate in our high volume Lynch syndrome tumor testing practice. Today I hope to cover some of the basics needed to understand this testing, which is increasingly a routine part of pathology practices in both community and academic settings.

Our speaker for this program is Dr. Sarah Kerr, Assistant Professor of Laboratory Medicine and Pathology and a Consultant in Anatomic Pathology and Laboratory Genetics at Mayo Clinic in Rochester, Minnesota.
Disclosures

• None

I have no relevant disclosures related to this presentation.
At the end of this presentation, the audience should be familiar with the following: First, you should be able to discuss the difference between a mismatch repair defect and microsatellite instability, terms that are easily confused with one another. Second, you should be able to explain why \textit{BRAF} mutation testing in the context of Lynch Syndrome screening is not useful for endometrial cancer. Third, you will learn the most common patterns of mismatch repair protein staining by immunohistochemistry as well as the most common causes of these patterns.
I will illustrate these concepts through a classic endometrial cancer case. A 55-year-old woman is found to have an early endometrial cancer. As part of your Universal Lynch Syndrome screening protocol, you perform mismatch repair (MMR) immunohistochemistry (IHC) per your Universal Screening Protocol.
Here is an H & E-stained slide for your reference when interpreting the immunohistochemical stains.
Immunohistochemistry shows loss of MLH1 and PMS2 protein expression and retention of MSH2 and MSH6 staining. The endometrial stroma and scant tumor infiltrating lymphocytes serve as internal controls.

In the case of this patient, the most common cause of this immunohistochemistry pattern is a genetic alteration affecting the \textit{MLH1} gene only, specifically somatic hypermethylation of the \textit{MLH1} gene promoter.
Here are some key concepts that you must know to understand Lynch syndrome tumor screening.

First, a mismatch repair defect refers to an alteration affecting a mismatch repair gene, resulting in abnormal protein function. A germline mutation in a mismatch repair gene defines Lynch syndrome. Tumors with mismatch repair defects usually show abnormal mismatch repair protein immunohistochemistry. There are exceptions. For example, some patients with Lynch syndrome have missense changes in an MMR gene that alters protein function, but allows for normal protein expression. It is important to realize that all MMR defects can potentially be either germline (or heritable) or can be somatic (which means occurring only sporadically in the tumor).

The second concept is that microsatellite instability (MSI) describes a phenotype that results from a defect in mismatch repair. Tumors that are deficient in mismatch repair protein function tend to have more mutations than MMR proficient tumors due to the lack of functioning repair of errors that occur during DNA replication. This lack of repair can be measured by PCR-based testing looking for variability in repetitive regions of DNA that are particularly sensitive to mutation and lack of repair in the setting of MMR deficiency. This testing will be shown in more detail on the slides that follow.
Note that neither test, MMR immunohistochemistry nor MSI testing, is 100% sensitive. Therefore, consideration should be given to performing both tests when the suspicion for Lynch syndrome is high, such as in young patients or those with a personal or family history of multiple Lynch syndrome-associated cancers.
Microsatellite instability testing requires extracted tumor DNA as well as extracted DNA from normal tissue or blood for comparison. This photograph shows a technologist scraping an unstained section of tumor in the area marked on the underlying H & E-stained template slide. Normal tissue can be obtained from the same block or a different block depending on the section taken.
Testing for MSI can then be performed using the extracted DNA. PCR primers flank multiple DNA microsatellites, or repetitive elements in the DNA. The test I am showing uses mononucleotide repeats, which are repeats of a single type of base. Shown here is a string of Ts. The length of this repeat of Ts can be measured on a gel. We use capillary electrophoresis, which is a more modern type of fragment separation technique.
Shown here is a capillary electropherogram typical of MSI-high endometrial cancer. The shift in the mononucleotide repeat fragment sizes is usually more subtle in endometrial cancer than it is in colorectal cancer. Here you can see the shifts more easily in the blue peaks and the black peaks, but there are also subtle differences in the microsatellite repeat lengths for the green markers in the tumor versus the normal tissue from this patient.
Here is an easier electropherogram to interpret. This case is from a colorectal cancer. Notice the more dramatic shifts in the microsatellite marker fragment sizes here.
Your patient has an MSI-high tumor with loss of MLH1 and PMS2 by immunohistochemistry. What should you order next for this patient? MLH1 promoter hypermethylation testing or BRAF mutation testing?

You should order MLH1 promoter hypermethylation testing. MLH1 promoter hypermethylation is the most common mechanism of mismatch repair deficiency and resulting microsatellite instability in endometrial cancer. A positive result makes a germline mutation very unlikely. It is important to note, however, that rare Lynch syndrome families are explained by germline MLH1 promoter hypermethylation, and so blood testing for methylation should be considered in women whose endometrial tumors show MLH1 methylation. Also remember here that BRAF testing is not helpful in endometrial cancer like it is in colorectal cancer. While BRAF mutations in colorectal cancer are strongly inversely correlated with Lynch syndrome, the same is not true for endometrial cancer. This is because BRAF V600E mutations are extremely uncommon in endometrial cancer.
MLH1 promoter methylation testing is performed by using methylation-specific PCR. This method takes advantage of bisulfite treatment of the extracted DNA, which differentially modifies methylated versus unmethylated regions. Following bisulfite treatment, primers targeting the modified and unmodified DNA sequences can be used to detect whether methylation of the target region is present.

Methylation-Specific PCR

- Sodium bisulfite deaminates unmethylated cytosine (C) to uracil (U)
  - When PCR amplified, uracil is converted to thymine (U to T)
- Methylated C’s are “protected”
- Sodium bisulfite treatment can be used to detect methylated DNA by using primers specific to the methylated sequence with C’s and the unmethylated sequence with T’s.
Like MSI testing, amplified DNA fragments in MLH1 promoter hypermethylation testing are detected by capillary electrophoresis. Shown here, your patient has a blue peak in the expected region for the fragment amplified by the methylation-specific primer. Therefore, her tumor demonstrates MLH1 promoter hypermethylation, which is usually the result of a somatic, rather than a germline, event. This result makes Lynch syndrome less likely, but does not entirely exclude it. In other words, this patient is unlikely to have a germline MLH1 mutation, but MLH1 germline promoter methylation testing should be considered to exclude germline MLH1 promoter methylation.
Here are a few more words on BRAF. BRAF V600E mutation testing is useful in the setting of colorectal cancer. This is because colorectal cancers with MLH1 loss and BRAF mutations are almost exclusively seen in patients that do not have Lynch syndrome. BRAF mutation testing has not been sufficiently tested in other tumor types, and should not be used to exclude Lynch syndrome in tumors other than colorectal cancers with MLH1 loss. Specifically, do not use BRAF testing in endometrial cancer.
Finally, it is useful to know the typical mismatch repair patterns seen by immunohistochemistry. Loss can occur in pairs like MLH1 and PMS2 or MSH2 and MSH6. When there is loss of MLH1 and PMS2, for example, the MLH1 gene is usually affected, while PMS2 is lost secondarily due to instability of the protein in the absence of MLH1. The same concept is true for MSH2 and MSH6. On the other hand, when PMS2 or MSH6 loss are seen alone, this usually indicates a germline mutation in the gene. Less commonly, somatic mutations in that gene are present, so it is important that such patients are managed by a medical geneticist to determine appropriate follow-up testing.

Also note that unusual patterns outside of those listed occur. If you are uncertain of the implications of the pattern you are seeing, seek the help of an expert.
Shown here is an unusual pattern that I see from time to time in endometrial cancer. Some of the tumor shows loss of MLH1 and PMS2 in the same glands, while other areas show normal expression. This phenomenon is almost always due to MLH1-promoter hypermethylation. In order for follow-up testing to be accurate in this setting, the pathologist must mark the area showing abnormal loss of staining for DNA extraction for MLH1-promoter hypermethylation testing. Failure to do so could lead to the incorrect conclusion that the tumor is unmethylated.
This concludes the presentation. I hope you can now define the difference between a mismatch repair defect and microsatellite instability. You should now know that BRAF testing is not appropriate for endometrial cancer, and you should be familiar with the most common patterns of mismatch repair protein staining.
I hope you will join us for the upcoming Diagnostic Molecular Pathology Conference in Chicago, Illinois in April 2017.
Reference

Thank you for your attention.