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PEARLS OF LABORATORY MEDICINE

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TITLE: Hereditary Endometrial Carcinoma (With focus on Lynch syndrome)

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Slide 1: Greeting

Hello, my name is Shabnam Zarei. I am a molecular genetic pathology fellow at the department of Laboratory Genomics and Genetics at Mayo Clinic in Rochester, MN. Welcome to this Pearl of Laboratory Medicine on "**Hereditary Endometrial Carcinoma, with focus on Lynch syndrome**."

Slide 2: Overview

First, we will start with a definition of Lynch syndrome, review current screening guidelines and testing, mention available diagnostic tools, overview the molecular basis and then end with a few words on the role of genetic counseling in management of patients with Lynch syndrome.

Slide 3: Introduction

Lynch syndrome or Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC) is a cancer predisposition syndrome associated with an increased risk of carcinoma in different organs, most commonly colorectal and endometrial carcinomas. It is associated with younger age at the time of cancer diagnosis, more than one organ involved by cancer, involvement of multiple family members and an overall better survival. Lynch syndrome is by far the most common cause of hereditary endometrial carcinoma.

Slide 4: Other Forms of Hereditary Endometrial Carcinoma

The second most common cause for hereditary endometrial carcinoma is *PTEN* germline mutation responsible for Cowden syndrome which is associated with increased risk for thyroid,

breast and endometrial carcinoma. The lifetime risk for developing endometrial carcinoma in patients harboring a germline *PTEN* mutation is about 28%. Other less common hereditary causes of endometrial carcinoma include *EPCAM* gene deletions (which inactivate *MSH2*), *POLE* and *POLD1* germline mutations. However, today we will be focusing on Lynch syndrome.

Slide 5: Screening guidelines

In the past two decades several screening guidelines including Bethesda, Amsterdam and their revisions were introduced to aid with identifying patients at risk for Lynch syndrome. For example, the revised Bethesda criteria recommends testing for Lynch syndrome in cases of colon cancer diagnosed in patients younger than 50 year old, presence of synchronous or metachronous Lynch associated carcinomas (including colorectal, endometrium, small bowel, ovary, stomach, and pancreas), specific histology of the tumor in a patient younger than 60 year old, colorectal cancer diagnosed in >1 first-degree relatives with at least one cancer diagnosed before age 50, or colorectal cancer diagnosed in >=2 first or second degree relatives with Lynch syndrome, regardless of age. Due to a low sensitivity and specificity of these criteria for screening patients at risk for Lynch syndrome, the newest screening guideline by National Comprehensive Cancer Network (NCCN) and American Cancer Society (ACS) recommends screening testing in ANY patient, especially those younger than 50 years old with newly diagnosed endometrial or colorectal carcinomas.

Slide 6: Genes and pattern of inheritance

Four major genes located on three different chromosomes (3, 7 and 2) are involved in the pathogenesis of Lynch syndrome: *MLH1, PMS2, MSH2* and *MSH6*. Lynch syndrome has an autosomal dominant pattern of inheritance with variable degree of mutation penetrance. About 3-5% of endometrial carcinomas diagnosed each year are associated with Lynch syndrome. On the other hand, patients with Lynch syndrome have 40-60% lifetime risk of developing endometrial and similar risk for colorectal carcinoma and lesser risk for developing ovarian, stomach, small bowel, ureter and renal pelvis carcinomas. The risk for cancer predisposition is higher with *MLH1* and *MSH2* mutations and it has the least association with *PMS2* mutations. Therefore, the chance of cancer predisposition is different based on different genes.

Slide 7: Function of MMR proteins

Mistakes can happen during DNA replication specifically in the repetitive regions of the genome. Bubble and loop formation and slippage are the most common errors that are naturally removed by the normal product of these four genes, called mismatch repair enzyme proteins that function as protein dimers: *MLH1* with *PMS2* and *MSH2* with *MSH6*. In the absence of normal gene or normal protein function, variation in the size of repetitive regions throughout the genome will happen. This phenomenon is called microsatellite instability and can be detected by microsatellite instability or MSI testing. The majority of Lynch syndrome cases (up to 90%) are due to germline mutations in the *MLH1* or *MSH2* genes.

Slide 8: Screening testing for Lynch syndrome

Different tests are available for screening of Lynch syndrome in endometrial carcinoma including immunohistochemistry (IHC) to look for mismatch repair protein expression, molecular testing for microsatellite instability to look into the repetitive regions of the genome, and *MLH1* hypermethylation testing which is predominantly a somatic alteration and it is absent in Lynch syndrome. *BRAF* V600E mutation is another somatic alteration that if present with *MLH1* promoter hypermethylation in colorectal carcinoma, makes the diagnosis of Lynch syndrome much less likely. The frequency of *BRAF* V600E mutation in sporadic endometrial carcinoma is very low therefore only *MLH1* promoter methylation status is typically used in combination with MMR by IHC and MSI by molecular testing. After identifying patients with positive screening tests by either MMR IHC or MSI, the next step is to do germline mutation analysis targeted for the genes that showed loss of protein expression by IHC. These include: Sanger sequencing, MLPA for gene dosage and more.

Slide 9: Algorithmic testing for Lynch syndrome

Here we see an algorithmic approach for diagnosis of Lynch syndrome which begins by IHC and MSI screening testing and ends with gene sequencing. For example if the IHC shows loss of expression of *MLH1*/*PMS2* and MSI testing shows microsatellite instability, it is recommended to perform *MLH1* hypermethylation testing in patients with endometrial carcinoma to rule out *MLH1* promoter hypermethylation which is seen mostly in patients with sporadic endometrial carcinoma. The absence of *MLH1* hypermethylation is supportive of diagnosis of Lynch syndrome. In cases of colorectal carcinoma, *BRAF* V600E testing is also performed with *MLH1* hypermethylation. Germline mutations in MMR genes do not typically co-

exist with *MLH1* hypermetheylation and/or *BRAF* V600E mutations. A positive MSI and negative *MLH1* hypermethylation result in a patient with endometrial carcinoma should prompt germline testing. This test is done on patient's blood sample and through the use of molecular methods, the exact molecular alteration may be identified and the diagnosis of Lynch syndrome confirmed.

Slide 10: MSI testing

Next, let's take a closer look at how microsatellite instability testing is performed. Microsatellites are short tandem repeats of DNA sequences, scattered through the entire genome, each 1-3 or more nucleotides in size. The germline mutations will result in abnormal or absent mismatch repair protein function which can be detected by microsatellite instability (MSI) testing which is the consequence of mismatch repair enzyme deficiency. It is recommended to use 5 markers including both mono and di-nucleotide markers for detection of microsatellite instability. DNA is extracted from formalin fixed paraffin embedded (FFPE) tissue from tumor and normal areas. A PCR based assay using capillary electrophoresis for fragment analysis is used and based on the number of unstable markers, a final call will be made: If 30% or more (meaning 2 out of 5) markers show instability, it's called MSI-high, if only 1 out of 5 markers shows instability, this is an MSI-low and if none of 5 markers shows instability that would be a case of microsatellite stable (or MSS).

Slide 11: Example case, H&E

Here we see a case of endometrial adenocarcinoma mostly present in the top part of this photomicrograph with few residual benign endometrial glands in the lower part of the photomicrograph. You can also appreciate tumor infiltrating lymphocytes present in this photo.

Slide 12: Example case, IHC

Here we see that in this case, by immunohistochemistry, the *MLH1/PMS2* protein expression is lost whereas the *MSH2* and *MSH6* nuclear expression is retained. Please note the retained protein expression in the normal glands and stroma highlighted by black circles and in the tumor infiltrating lymphocytes marked by black arrow head. This pattern of staining is suggestive of mismatch repair enzyme deficiency in the *MLH1/PMS2* complex. Since the immunohistochemistry is not 100% sensitive or specific for detection of Lynch syndrome,

microsatellite instability (MSI) testing should also be performed. There are cases that show intact protein expression by IHC and MSI-H by MSI testing and vice versa. Such discordant results can be explained by the fact that the immunohistochemical staining only shows the presence or absence of mismatch repair protein expression, and cannot distinguish between functional and non-functional proteins. In cases of discordant results, it is recommended to proceed with germline testing.

Slide 13: Example case, MSI

Here we see that all 4 of 4 markers show instability in the form of increase in the size of the tandem repeat region seen in the tumor compared to normal. This is compatible with microsatellite unstable or MSI-H status. Since, microsatellite instability can also be due to *MLH1* hypermethylation which is predominantly a somatic alteration, *MLH1* hypermethylation testing is performed which showed no *MLH1* hypermethylation (not shown in here). As mentioned earlier, *BRAF* V600E somatic mutation testing can also be done in conjunction with *MLH1* hypermethylation in colorectal carcinomas, and the absence of *BRAF* V600E mutation is similarly supportive of diagnosis of Lynch syndrome. Per the algorithm that we discussed earlier, this case was sent for further genetic testing. The *MLH1* gene sequencing showed a c.940delG deleterious alteration, confirming the diagnosis of Lynch syndrome. Identifying the exact genetic alteration in this case will be a guide for possible testing of the patients' family members to identify those at risk for Lynch syndrome.

Slide 14: Histomorphology and prognosis

Some histologic findings are found to be more associated with Lynch syndrome, especially in colorectal carcinomas. These findings include: mucinous or signet cell morphology, increased intra-epithelial infiltrating lymphocytes, a Crohn's like response at the edge of the tumor and medullary carcinoma like morphology. In endometrial carcinomas, Lynch syndrome is associated with tumors arising in the lower uterine segment, more peritumoral and intratumoral lymphocytes and tumor heterogeneity (mixed morphology including endometrioid and serous carcinoma). Patients with MSI-H colorectal carcinomas have a better prognosis. The data on prognosis in MSI-H endometrial carcinomas remains controversial. Despite being described and incorporated in some screening guidelines, these morphologic findings are not 100% specific for Lynch associated endometrial or colorectal carcinomas.

Slide 15: Genetic counseling

Patients with Lynch syndrome should be identified clinically as they are at higher risk for developing multiple primary malignancies. These patients will benefit from closer clinical follow-ups and/or prophylactic surgeries if applicable. Usually the genetic counselor will meet with the patient after screening testing showed abnormal IHC and/or MSI results. The genetic counselor will explain the findings and go over risk associated with developing other malignancies. He or she will also talk about the risk for the family members and will facilitate further specific germline genetic testing of the patient and in case of positive germline testing result in the patient, will recommend testing of the family members for the same known genetic alteration.

Slide 16: Summary

In summary, Lynch syndrome is a cancer predisposition syndrome associated with an increased risk of developing multiple cancers most importantly endometrial and colorectal carcinomas. It has an autosomal dominant pattern of inheritance with variable degree of penetrance. Most patients with Lynch syndrome have a detectable germline mutation in one of the four major genes responsible for DNA mismatch repair. *MLH1* promoter hypermethylation results in gene silencing, and is predominantly a somatic alteration which also causes microsatellite instability. *MLH1* promoter hypermethylation should be ruled out by molecular testing to support diagnosis of Lynch syndrome. Screening guidelines will help identify the patients and their relatives who are at risk for developing cancer in different organs. Earlier detection of cancer will improve patients' prognosis and bring better quality of care for patients and their families.

Slide 17: References

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Slide 17: Disclosures

None.

Slide 18: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Hereditary Endometrial Cancer"**

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PEARLS OF LABORATORY MEDICINE

Pearl Title: Hereditary endometrial carcinoma (with a focus on Lynch syndrome)

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DOI:

CCTC Clinical Chemistry Trainee Council

Overview

- Definition of Lynch syndrome
- Recommended screening guidelines
- Screening tools: IHC, MSI testing and more
- Diagnostic tools: gene sequencing and dosage
- Underlying molecular mechanism
- Role of genetic counseling
- Summary

Lynch Syndrome

- Another name for Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC)
- Associated with carcinoma of different organs most importantly colorectal and endometrial and less commonly gastric, ovarian, small bowel and pancreatic
- Younger age at time of cancer diagnosis, multiple cancer types and multiple family member involvement

Other Forms of Hereditary Endometrial Carcinoma

- *PTEN* germline mutation – Cowden syndrome
  - Second most common cause of hereditary endometrial carcinoma
  - Associated with increased risk for thyroid, breast and endometrial carcinoma
  - Patients have a 28% life time increased risk for developing endometrial carcinoma
- Other causes: *EPCAM* gene deletions (causing MSH2 inactivation), *POLE* and *POLD1* germline mutations

Screening guidelines

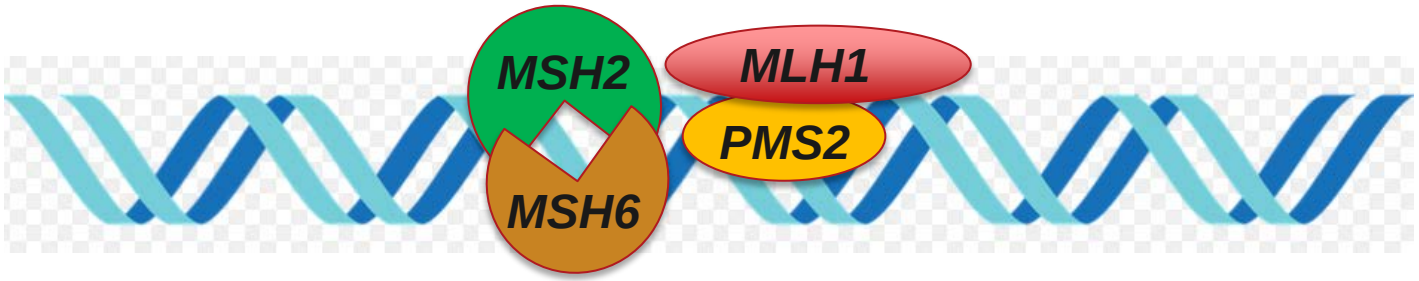
- Revised Bethesda criteria, 2004:
  - At least three relatives with any Lynch associated cancer (colorectal, endometrium, small bowel, ovary, stomach, pancreas) with at least one relationship being first degree relative
  - At least two successive generations should be affected
  - At least one family member with cancer diagnosis before age of 50
  - Familial Adenomatous Polyposis (FAP) should be excluded
- Universal screening: recent guidelines by NCCN and ACS: ANY patient, especially those younger than 50 years old, with a new diagnosis of endometrial and/or colorectal carcinoma should be screened for Lynch syndrome

Genes and pattern of inheritance

- Four major genes located on chromosomes 3, 7 and 2
- Genes: *MLH1*, *PMS2*, *MSH2* and *MSH6*
- Autosomal dominant, variable penetrance
- Lynch syndrome patients have a 40-60% lifetime chance for endometrial and similar risk for developing colorectal carcinoma
- Lynch syndrome patients have higher chance of cancer with *MLH1* and *MSH2* mutations and the lowest chance with *PMS2* mutations

Function of MMR genes

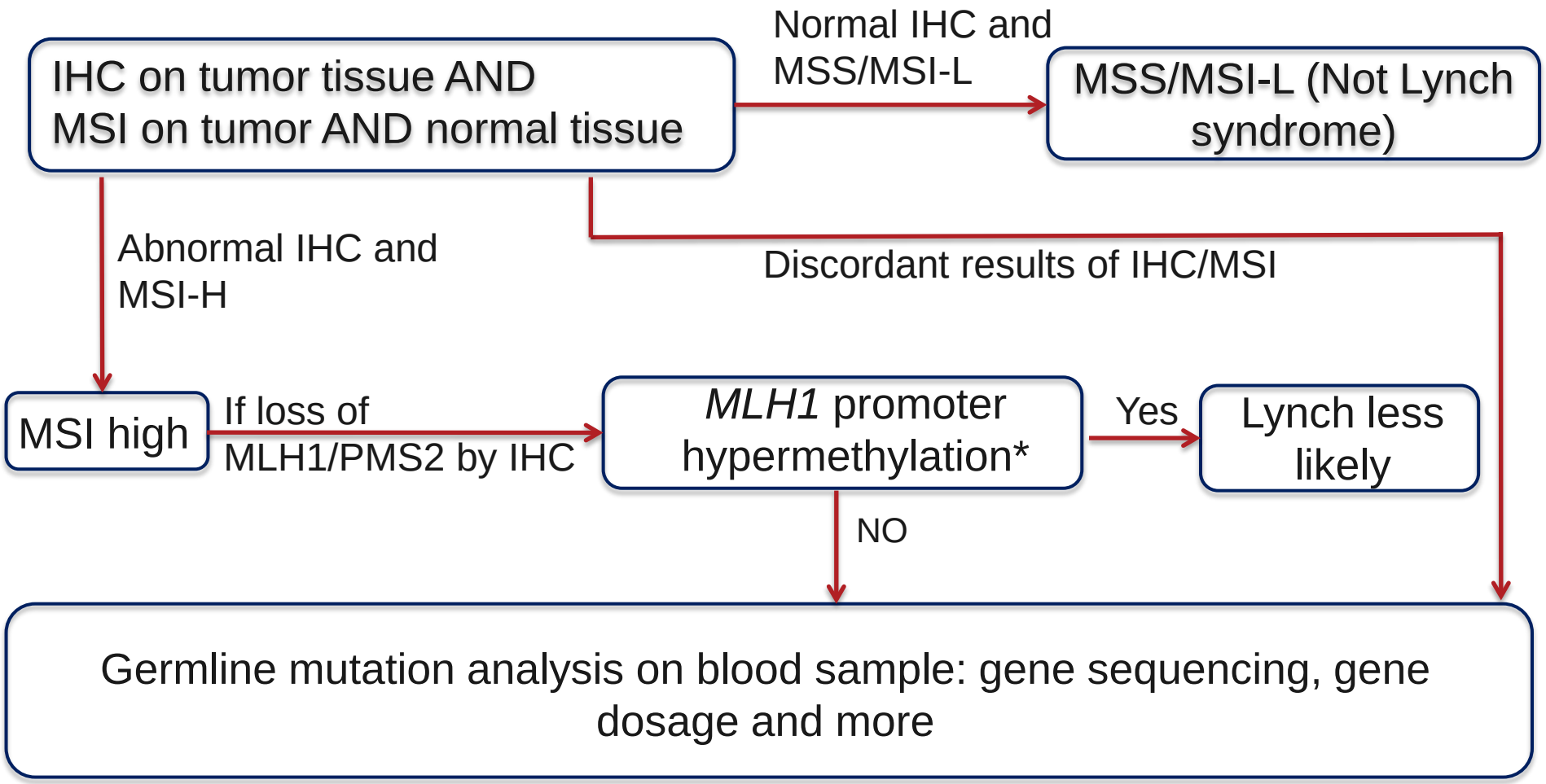
- DNA replication at the repetitive regions can be associated with slip and bubbles/loops formations
- These mistakes (bubbles, loops) are corrected by MMR enzymes
- *MLH1/PMS2* and *MSH2/MSH6* are two protein dimers involved in mismatch repair during DNA synthesis



Screening testing for Lynch syndrome

- IHC for MMR gene defects: Look at protein expression of *MLH1*, *PMS2*, *MSH2* and *MSH6* by using specific antibodies directed against each
- MSI (using short tandem repeat markers): Often using 5 molecular markers (combination of mono- and di-nucleotide repeat markers)
- *MLH1* hypermethylation testing: rule out somatic alteration resulting in *MLH1* silencing
- Positive screening test → do germline genetic testing, using sequencing and gene dosage

Algorithmic testing in Lynch syndrome

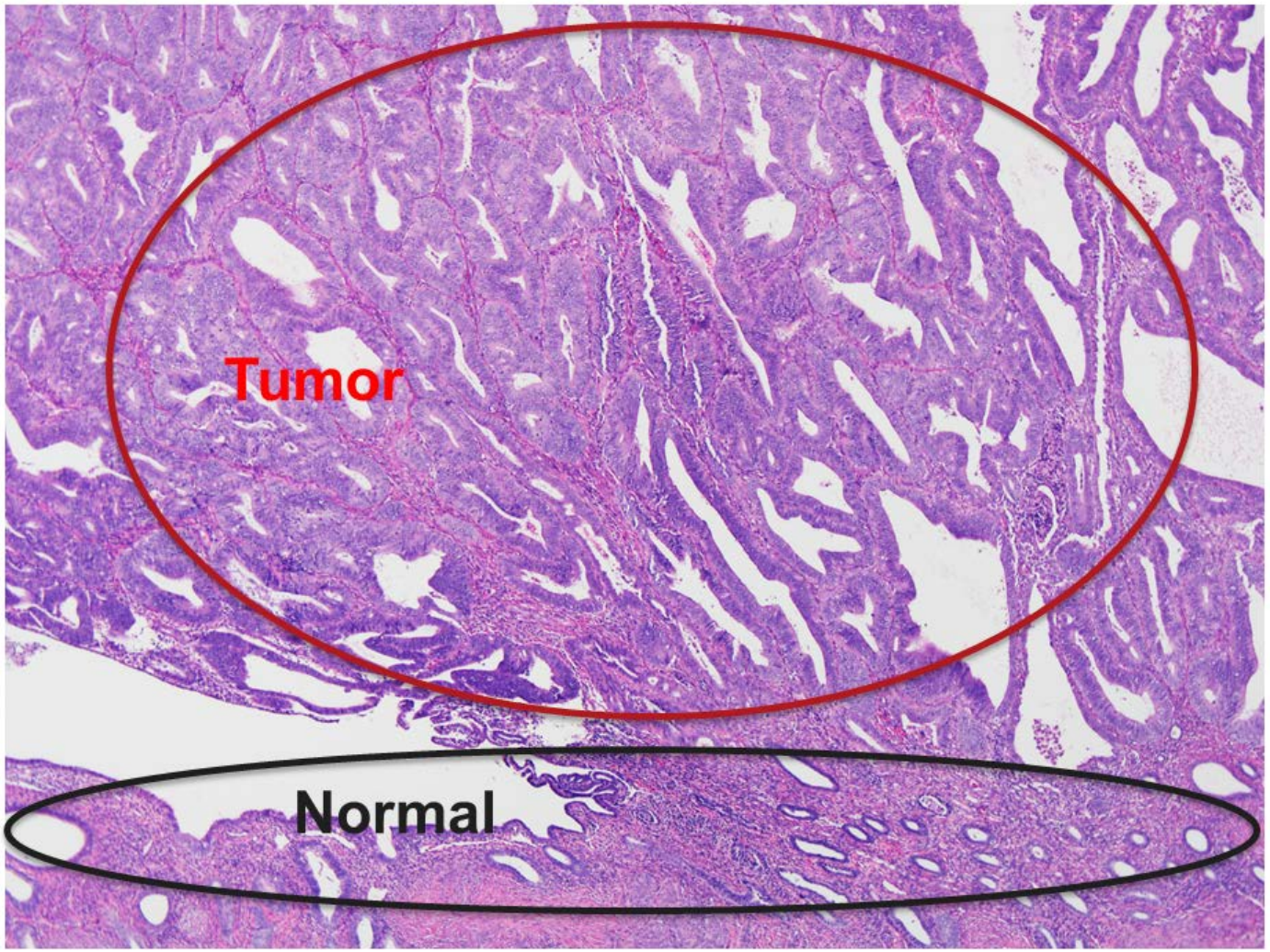


* and *BRAF* V600E mutation in colorectal carcinoma

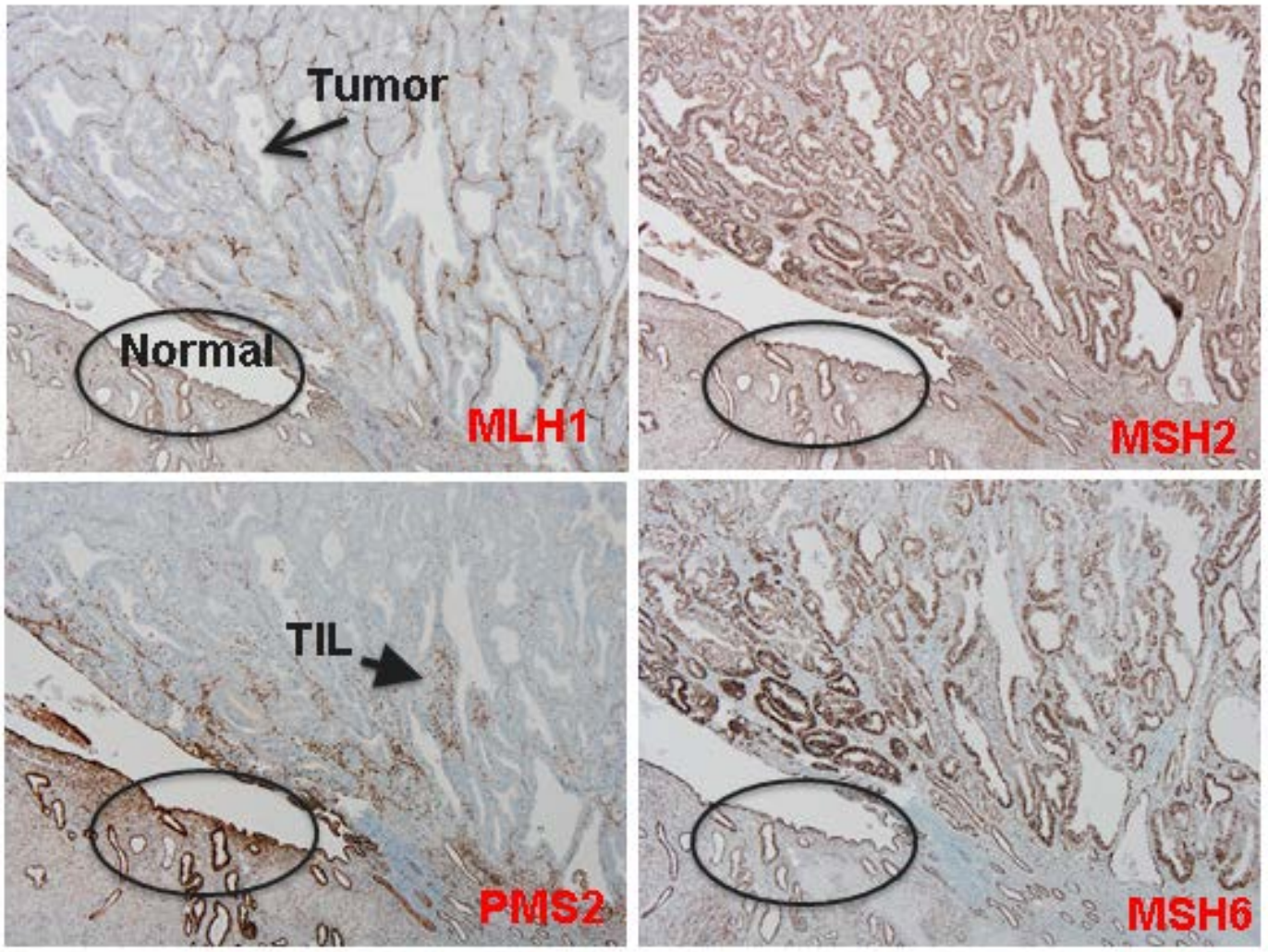
MSI testing

- Microsatellites are short tandem repeats of DNA scattered through the entire human genome, 1-3 or more nucleotides in size
- Abnormal or non-functioning MMR proteins (due to germline or somatic mutations) will cause shifts in microsatellite size in the repetitive regions of genome
- DNA extraction from normal and tumor area → capillary EP of PCR products
- It is recommended to use 5 markers (mono- and di-N):
  - Instability in >=2/5 (>30% of markers): MSI-H
  - Instability in 1/5 markers (1-29%): MSI-L
  - Instability in 0/5 markers (<1%): MSS

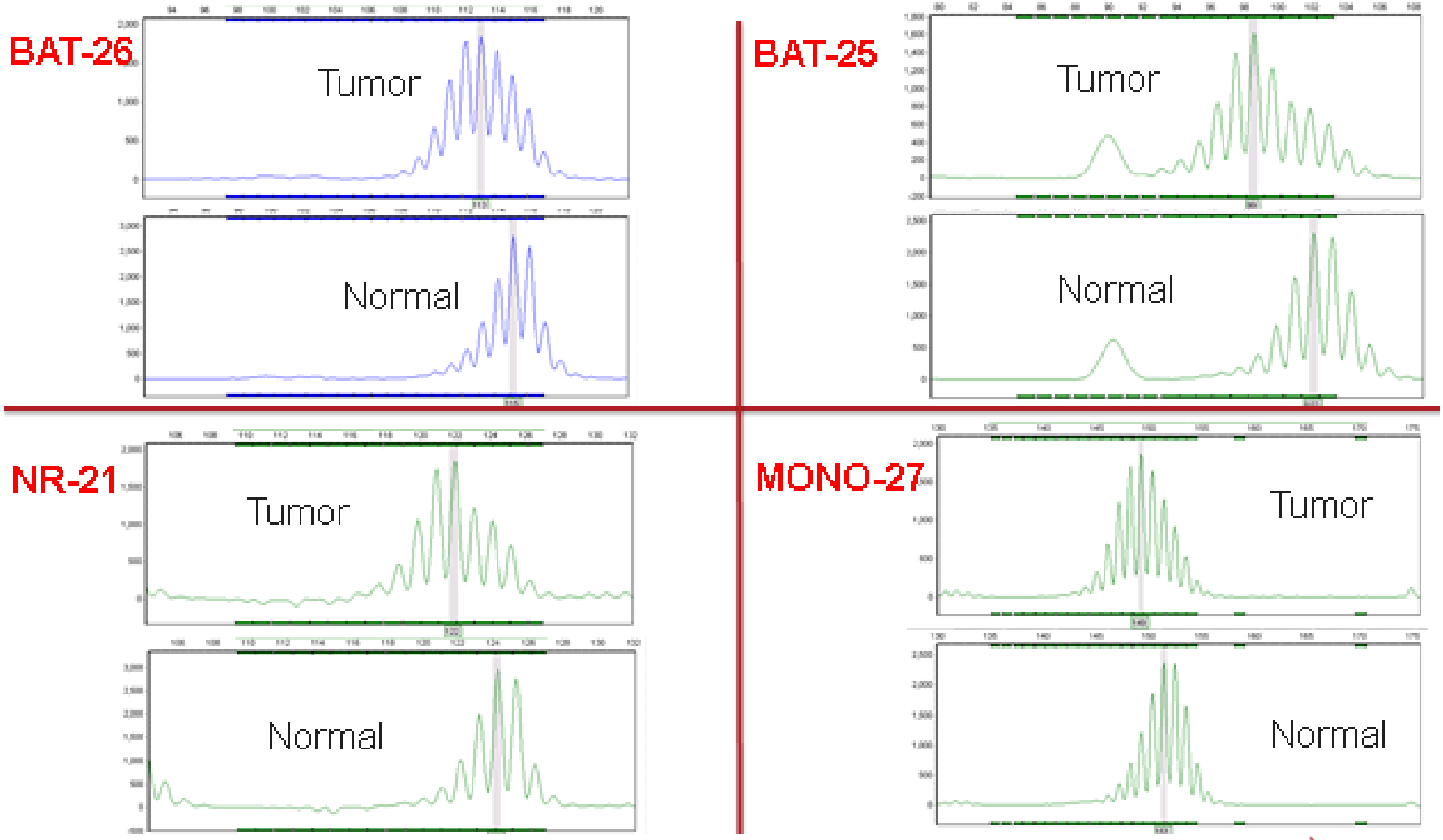
Example case- H&E



Example case- MMR IHC



Example Case- MSI



Histomorphology and prognosis

- Colorectal carcinoma with MSI-H: mucinous or signet ring cell morphology, increased intratumoral lymphocyte, poorly differentiated (medullary like) morphology and a Crohn's like response at the edge of the tumor

- Endometrial carcinoma with MSI-H: Less established, but associated with more peritumoral and intratumoral lymphocytes and tumor heterogeneity including different tumor histology (endometrioid and serous)

- Colorectal MSI-H carcinomas have better prognosis and data on MSI-H endometrial carcinomas is controversial

Genetic Counseling

- Patients with Lynch syndrome have higher risk for developing multiple primary malignancies and will benefit from closer monitoring
- Genetic counseling should be offered to both patients and their family members, usually after finding of abnormal IHC and/or MSI result
- Early detection of individual at risk for developing cancer is beneficial

Summary

- Cancer predisposition syndrome with increased risk of colorectal, endometrial, ovarian, small bowel, sebaceous, gastric and pancreatobiliary carcinoma
- Most patients with Lynch syndrome have a germline mutation in one of the 4 MMR genes, most commonly in *MLH1* or *MSH2* genes
- *MLH1* hypermethylation (predominantly a somatic alteration) should be ruled out
- Diagnostic and screening guidelines help identify patients and their family members who are at risk for developing Lynch syndrome related carcinomas

Figure/Table Title

- Figure1. Function of MMR genes, personal collection
- Figure 2. Algorithmic testing in Lynch syndrome, personal collection
- Figure2. H&E photomicrograph of an example, personal collection
- Figure3. Mismatch Repair protein expression by IHC, personal collection
- Figure4. MSI testing, personal collection

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- **Consultant or Advisory Role:**
- **Stock Ownership:**
- **Honoraria:**
- **Research Funding:**
- **Expert Testimony:**
- **Patents:**

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