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Pathology Driven Management of Endometrial Cancer

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Learning Objectives

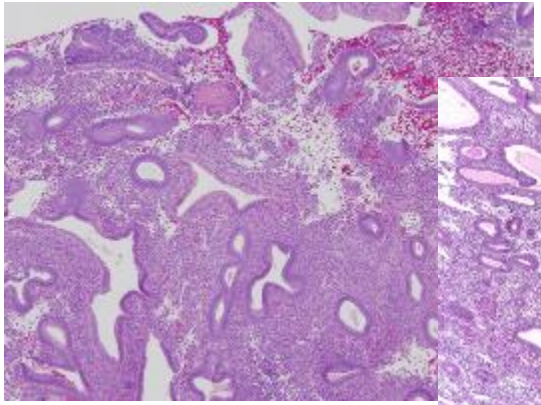
- Discuss staging challenges when evaluating depth of myometrial invasion in the setting of adenomyosis.
- Discuss the processing of sentinel lymph nodes.
- Discuss the interpretation of Mismatch Repair Protein (MMR) testing by immunohistochemistry.

Conflicts of interests to declare: none.

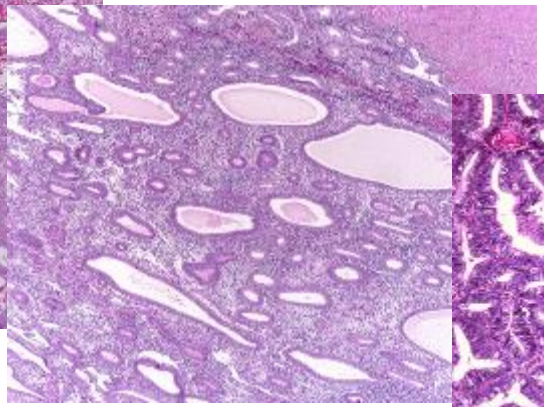
Depth of Invasion

In the context of adenomyosis

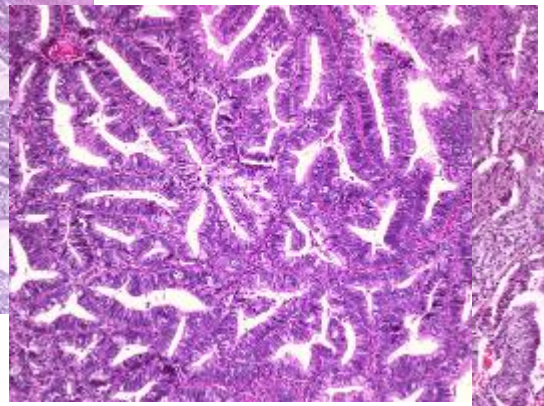
Endometrial cancer progression



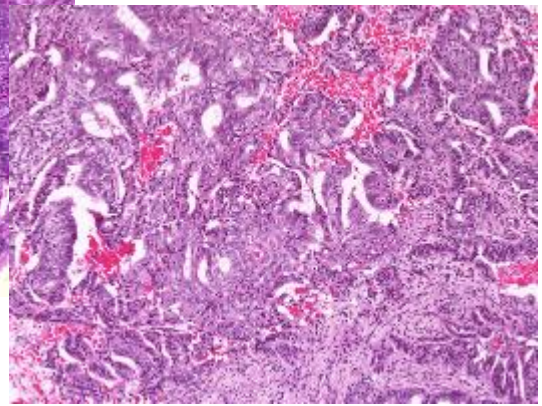
Disordered proliferative endometrium



Simple hyperplasia



Complex hyperplasia



Endometrioid carcinoma

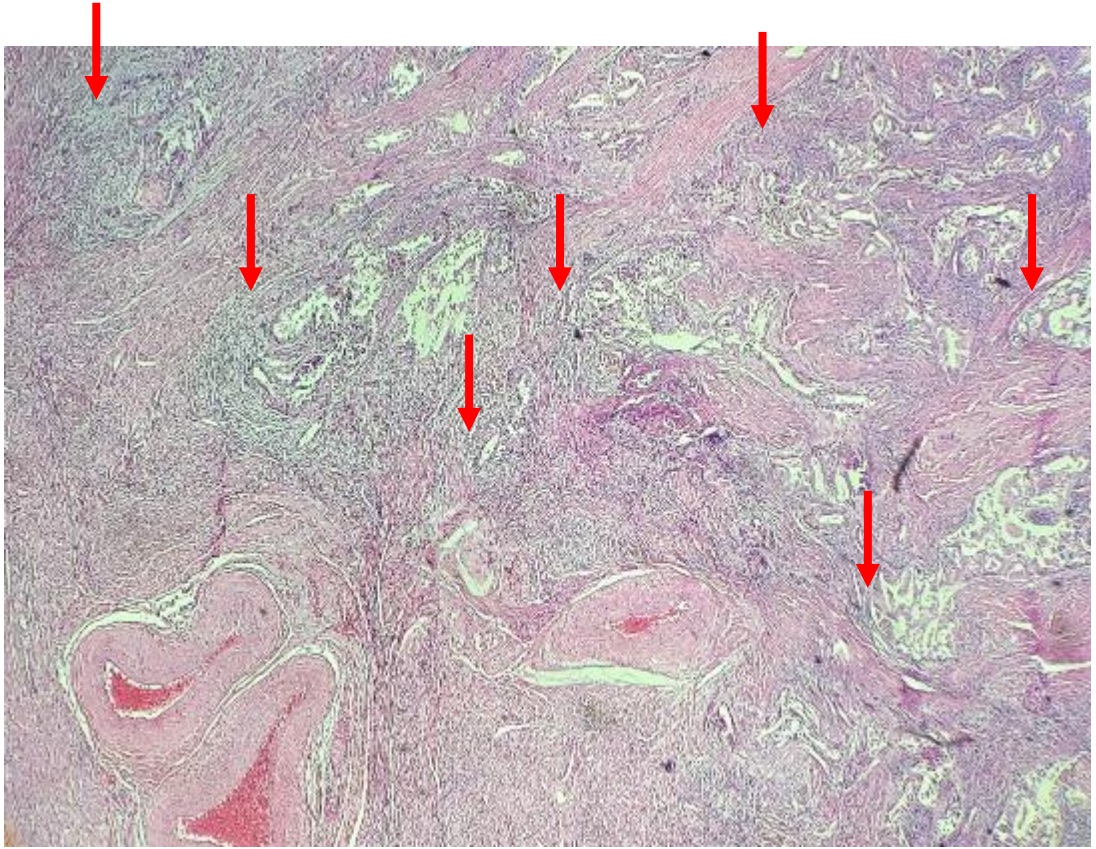
Staging of endometrial cancer

- Confined to the uterine corpus: FIGO stage I
  - Less than one half myometrial invasion: T1a, FIGO stage IA
  - More than one half myometrial invasion: T1b, FIGO stage IB
- Extension into cervix: FIGO stage II
- Extension into serosa/adnexa: FIGO stage IIIA
- Vaginal/parametrial involvement: FIGO stage IIIB
- Positive lymph nodes: FIGO stage IIIC
- Distant metastases: FIGO stage IVB

Source: College of American Pathologists Cancer Protocol - Endometrium (Aug 2018)

Problematic case

- Tumor diffusely involve the myometrium superficially and “deeply”
- Where does tumor invasion start?
- Where is adenomyosis?
- Which foci are invasive?
- Where does one begin with the depth of invasion assessment?



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Adenomyosis vs. invasive foci

Adenomyosis

- Glands are typically organized and round
- No architectural or cytological atypia
- Endometrial-type stroma surrounding glands
- No desmoplastic reaction

Invasive foci

- Glands are angulated
- Architectural and cytological atypia comparable to overlying surface tumor
- No endometrial-type stroma surrounding glands
- Desmoplastic reaction

Tumor involving adenomyosis: look for endometrial-type stroma surrounding tumor.

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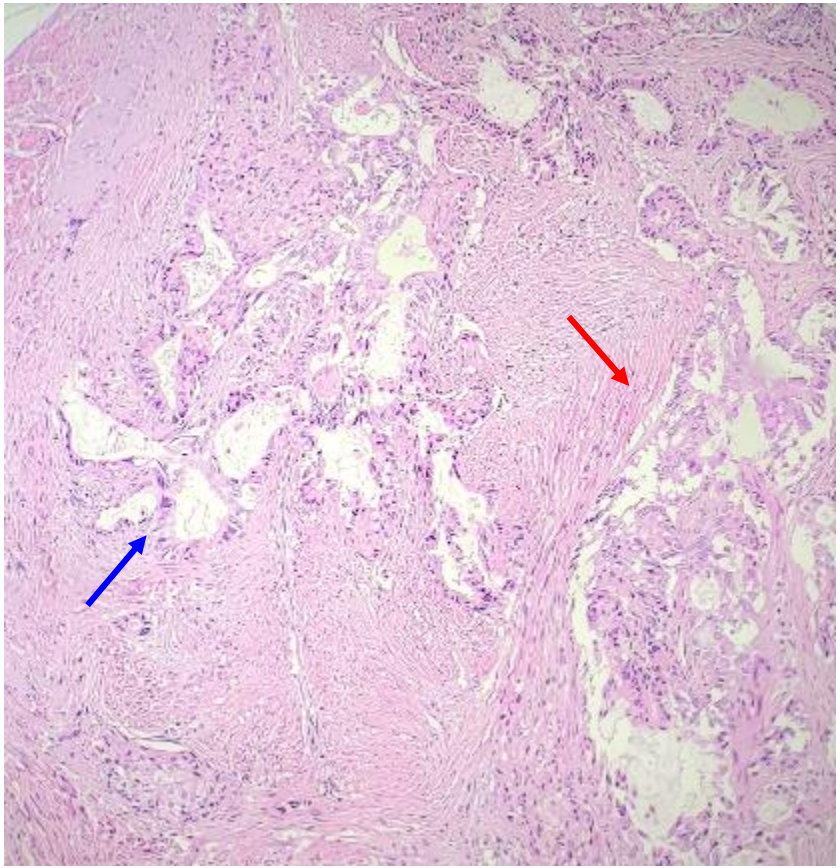
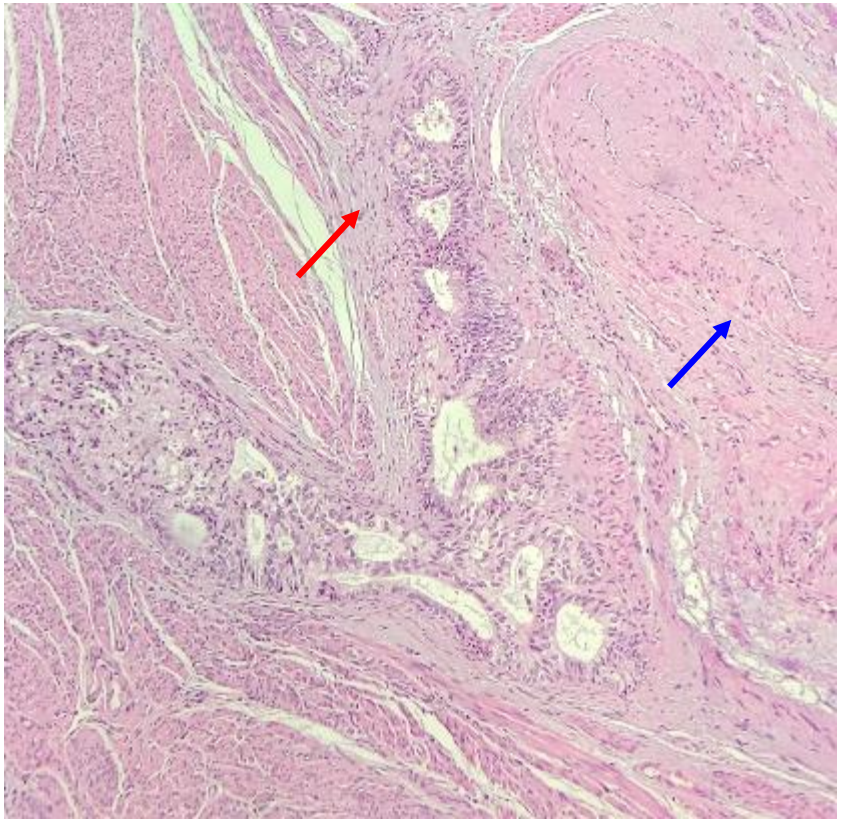
Invasive foci of tumor

- Irregular appearing/angulated malignant glands (blue arrow)
- Desmoplastic reaction around glands (red arrow)
- This foci can be used to measure depth of invasion



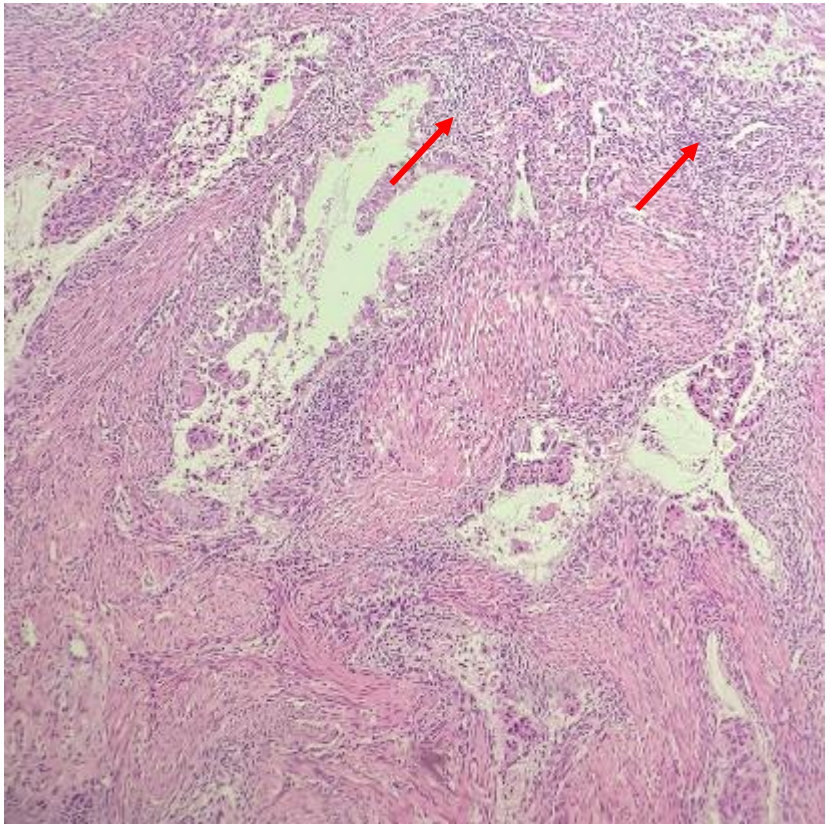
Invasive foci of tumor

- Irregular appearing/angulated malignant glands
- Desmoplastic reaction around glands (red arrow)
- Note proximity to uterine subserosal vessel (blue arrow)
- This foci can be used to measure depth of invasion

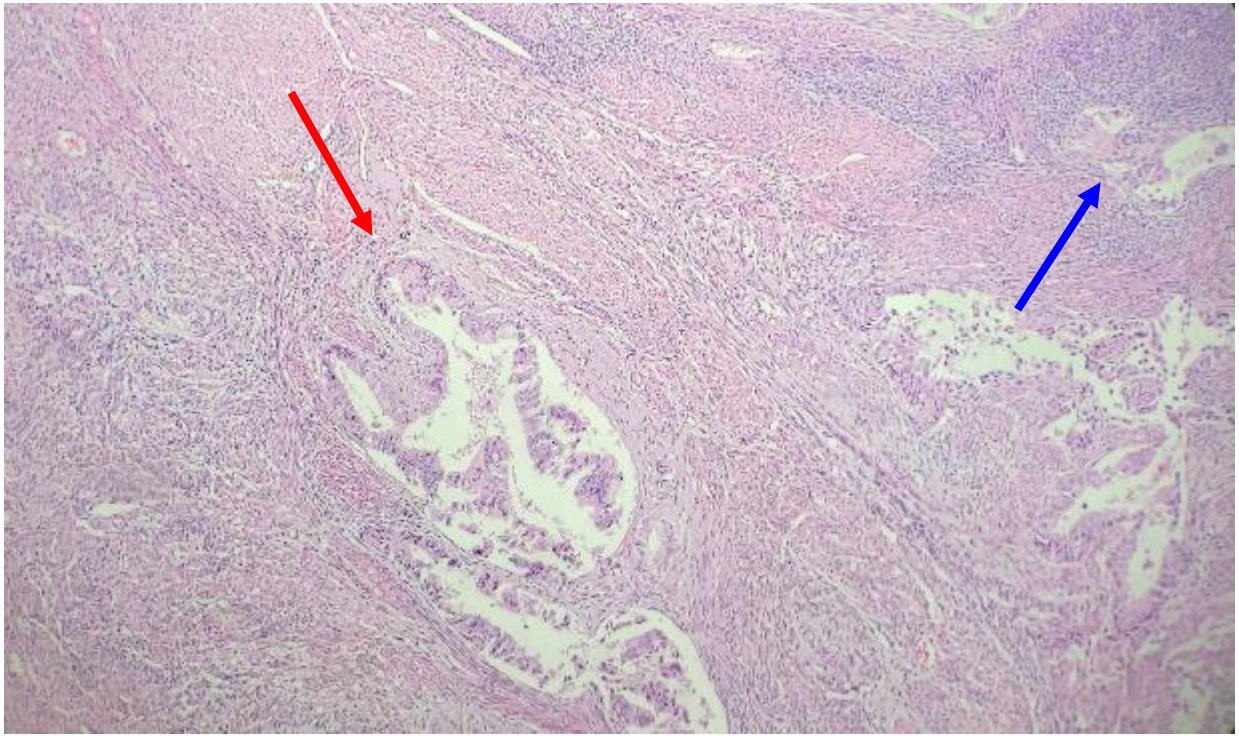


Tumor involving adenomyosis

- May be irregular shaped or more round/oval.
- Note the endometrial-type stroma surrounding the glands (red arrows)
- These foci should NOT be used to measure true depth of invasion.



Tumor in adenomyosis (blue arrow) vs invasive foci (red arrow)



Depth of myometrial invasion

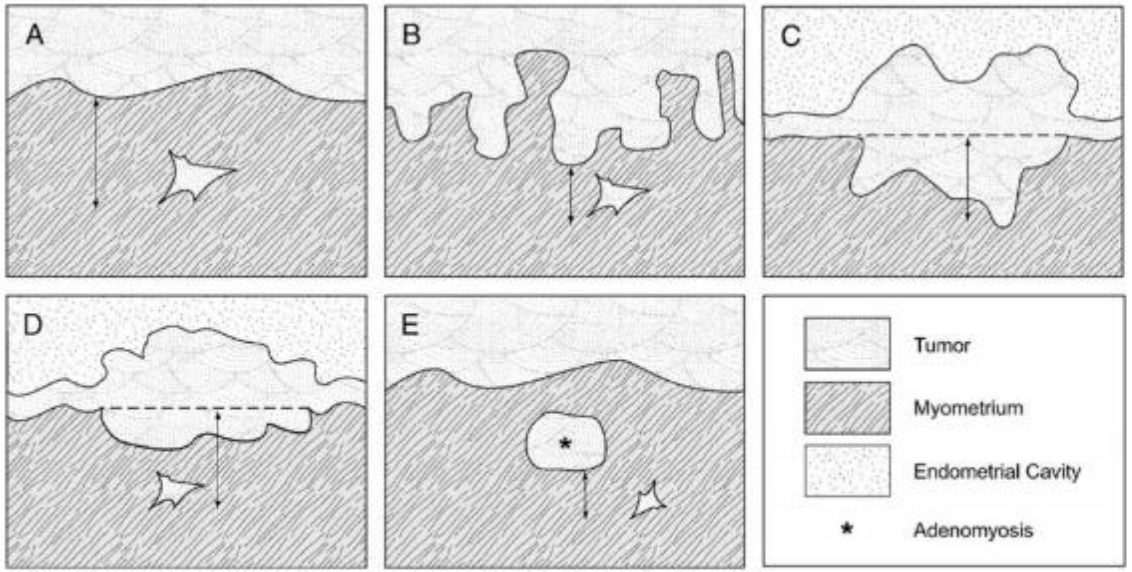


Figure 1. Schematic of measurement of depth of invasion in (A) tumor with a regular interface; (B) tumor with an irregular endomyometrial interface; (C) and (D) tumor with an exophytic growth; (E) tumor arising from adenomyosis. From Ali A, Black D, Soslow RA. Difficulties in assessing the depth of myometrial invasion in endometrial carcinoma. *Int J Gynecol Pathol.* 2007;26:115-123. Copyright © 2007, Wolters Kluwer Health. Reproduced with permission.

Source: College of American Pathologists Cancer Protocol - Endometrium (Aug 2018)

Depth of myometrial invasion

- Measured from the endomyometrial junction to the deepest point of invasion
  - Looking for normal residual endometrial glands
- Depth of invasion is divided by total myometrial thickness
- <50% vs >50%
- May be requested to evaluate by frozen section

Source: College of American Pathologists Cancer Protocol - Endometrium (Aug 2018)

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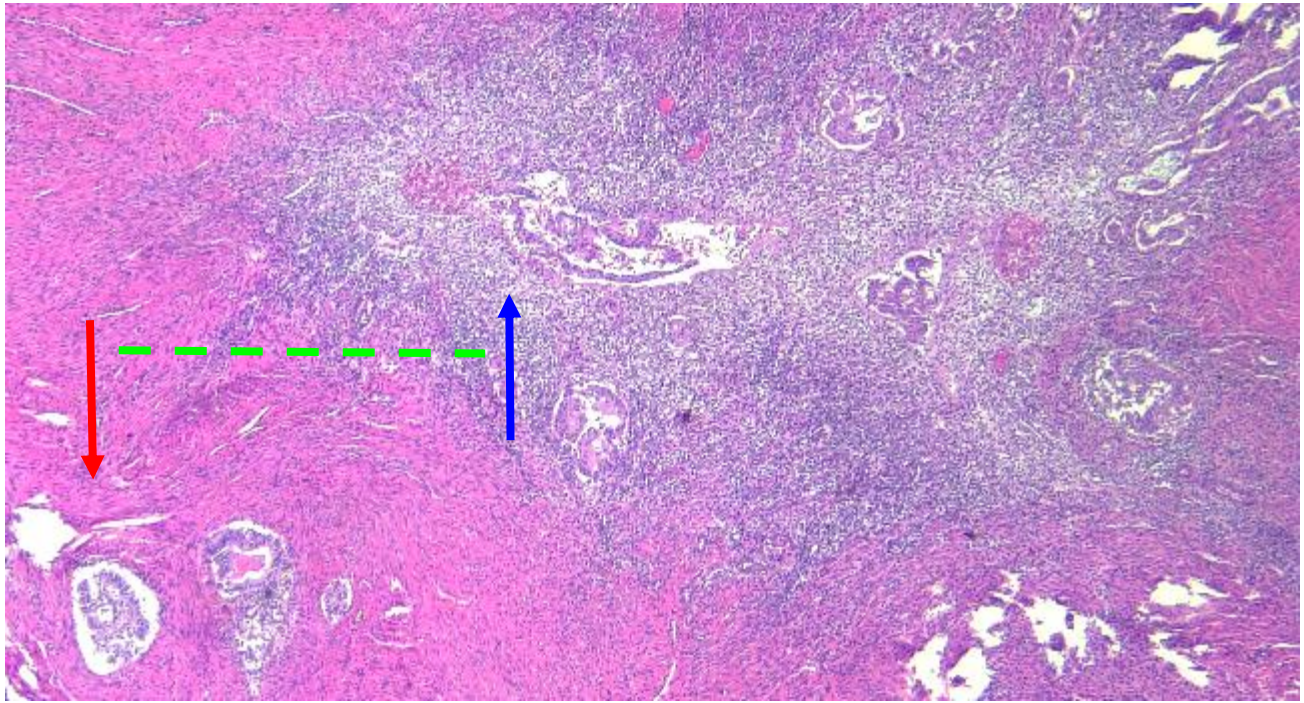
Depth of invasion (different case)



Begin with the deepest foci of adenomyosis (blue arrow), down to the deepest invasive gland. Measure the distance of the green dashed line.

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SLN processing

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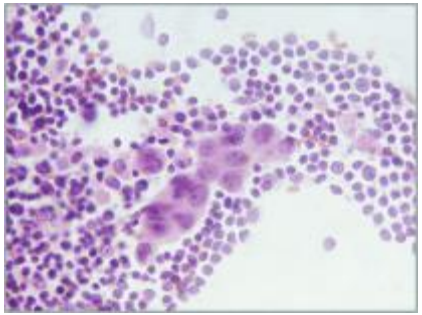
Sentinel Lymph Node processing

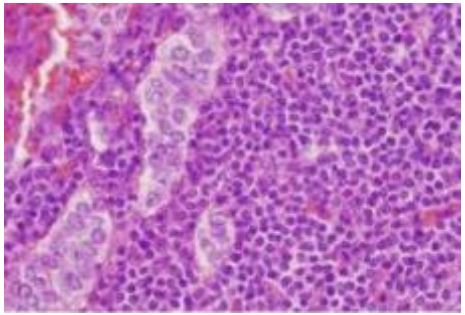
Touch Preparation

- Rapid assessment for the presence or absence of tumor cells
- No tissue freezing needed (morphology on permanents preserved)
- Easy to miss isolated tumor cells
- Fibrotic lesions difficult to obtain



Frozen Section

- Rapid assessment for morphology and architecture
- Requires tissue freezing which may affect morphology.
- Isolated tumor cells may be lost in between levels



Sentinel Lymph Node processing

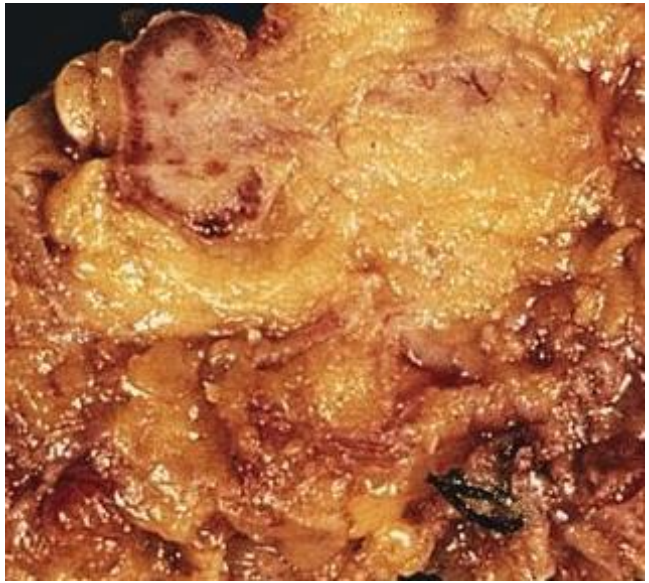
Lymph node versus fatty tissue?

Adipose tissue

- Soft, shiny, yellow in color
- May be lobulated

Lymph nodes:

- Soft, shiny, yellow-tan in color
- Well demarcated
- May resemble adipose tissue grossly.



The above is a different type of specimen
- for demonstration purposes only

Photo adapted from:
http://www.pathologyoutlines.com/topic/lymphnodesangiomyomatoushamartoma.html

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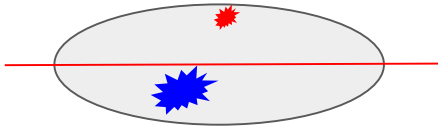
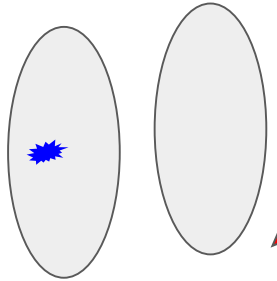
Tissue Processing of Sentinel LNs

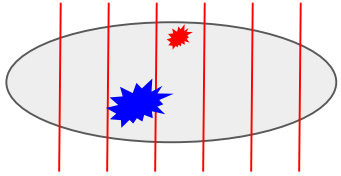
- Lymph node is sectioned at 1mm intervals perpendicular to the long axis
- Additional H&E levels and/or IHC obtained
- Goal: to increase the chances of seeing isolated tumor cells
- Frozen section: rapid intraoperative diagnosis if lymph node is overtly positive; may miss isolated tumor cells due to artifact and block re-embedding during permanent processing

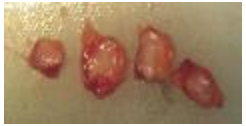
INCORRECT

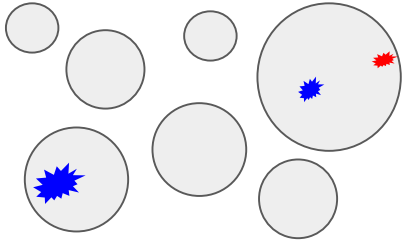




CORRECT







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MMR testing

Lynch syndrome (HNPCC)

- Affects 3-5% of population
- Inheritance: autosomal dominant
- Genetic mutations affecting the following mismatch repair proteins:
  - MLH1, PMS2, MSH2, MSH6
- ***Predisposition*** to colorectal, GI, pancreatobiliary, ovarian and endometrial malignancies
- Earlier onset of neoplasia / malignancy than the average population

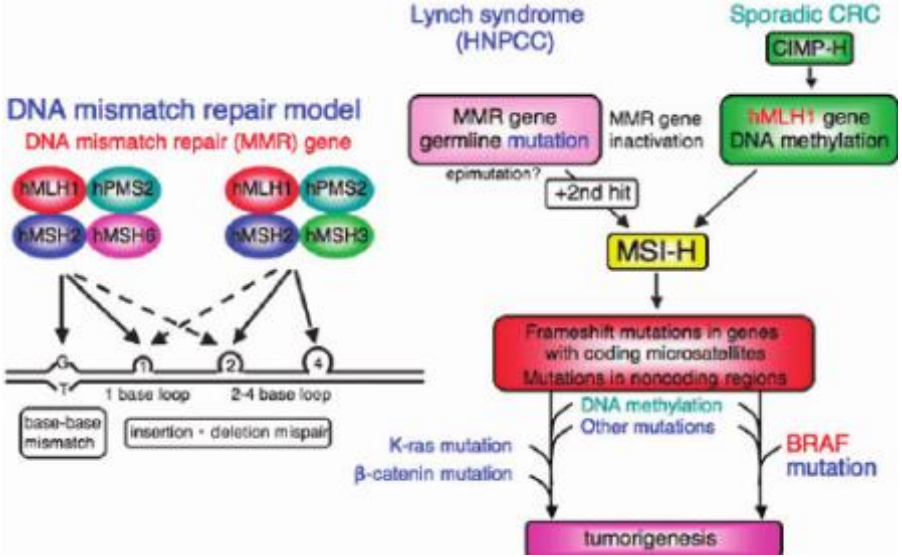

Figure adapted from:
https://www.researchgate.net/figure/A-model-of-DNA-MMR-and-molecular-pathways-for-CRCs-with-MSI-H_fig1_5904069

Mismatch Repair (MMR) Protein Testing

- Performing MSI & germline mutation sequencing on every case is costly and burdensome
- “Cheap”, fast **screening** tool by IHC for microsatellite instability and cases of HNPCC (Lynch syndrome)
- Caveats: screening for ***phenotypic*** abnormalities only; not genetic
- Detects the phenotypic presence or absence of the 4 **closely linked** MMR proteins (sporadic or germline mutations leading to microsatellite instability):
  - MLH1, PMS2
  - MSH2, MSH6
- What this means for patient care:
  - Earlier screening for breast, GI, urological, and gynecological malignancies
  - Potential candidates for additional immunotherapy

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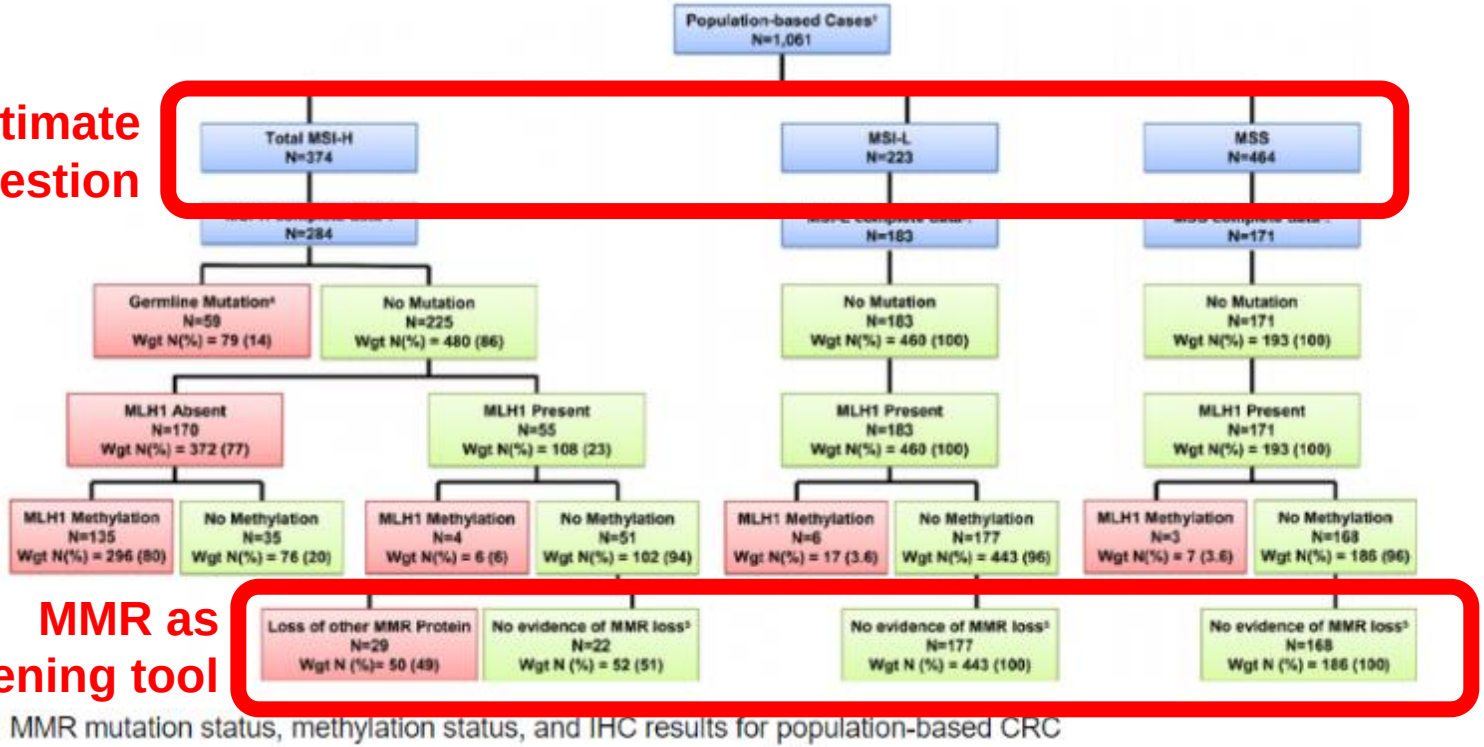
Treatment is dependent on MSI status

The ultimate question

MMR as screening tool



MMR mutation status, methylation status, and IHC results for population-based CRC

Figure adapted from: https://www.researchgate.net/figure/MMR-mutation-status-methylation-status-and-IHC-results-for-population-based-CRC_fig1_23457968

IHC testing schematic

With BRAF and hypermethylation

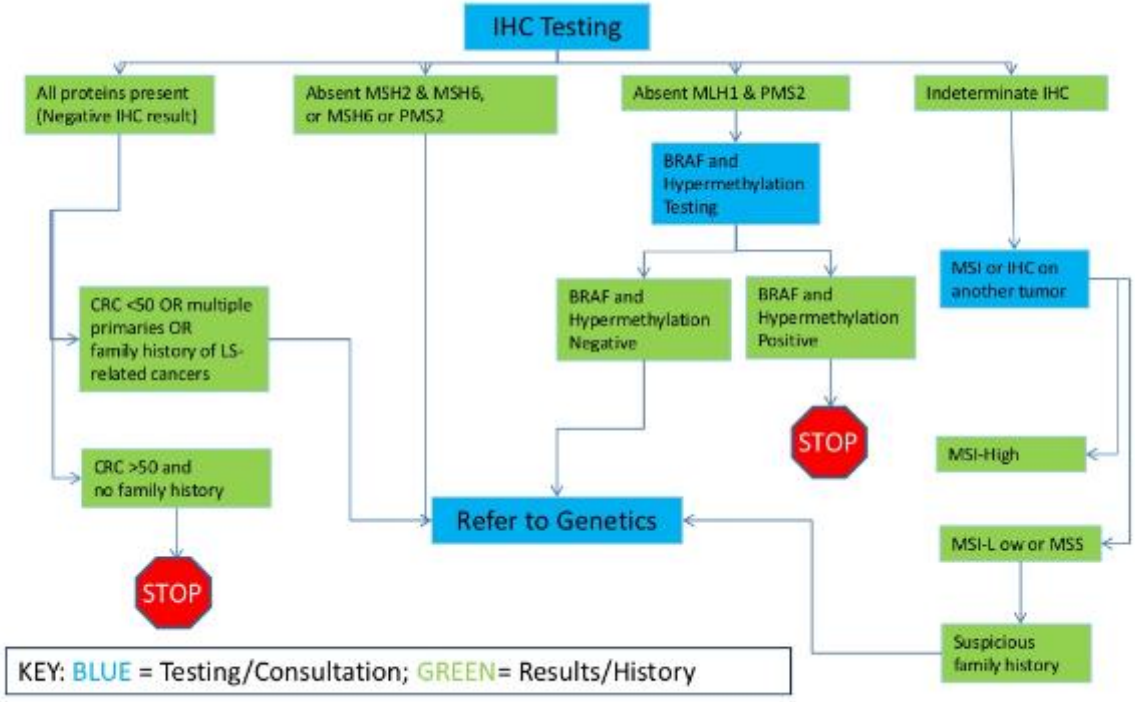


Figure adapted from Lynchscreening.net - https://www.lynchscreening.net/wp-content/uploads/2012/04/IHC-+methylBRAF.jpg

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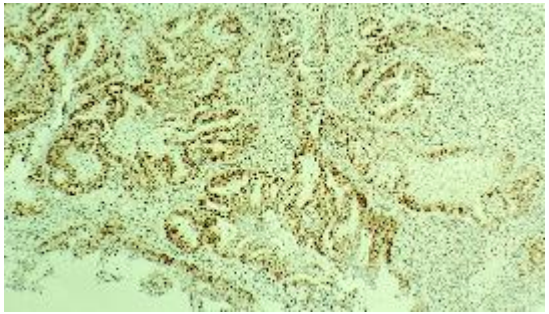
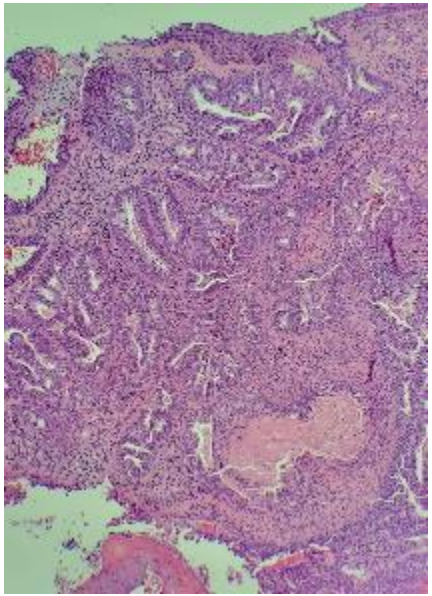
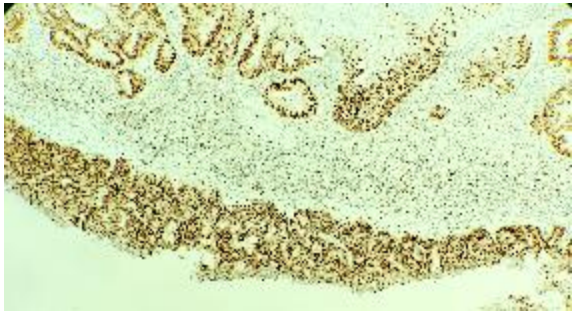
Normal - MMR by IHC

Same case as previous - all proteins present by IHC (nuclear positivity in tumor)

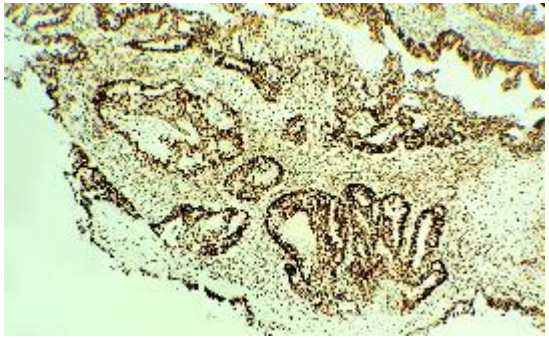


MLH1

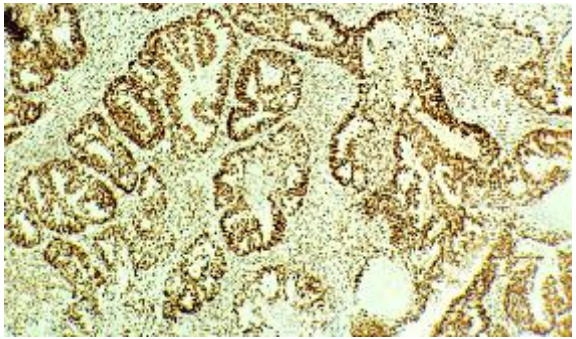




MSH2



PMS2



MSH6

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Suggestive of germline MSH2 mutation

Loss of MSH2 leading to loss of MSH6

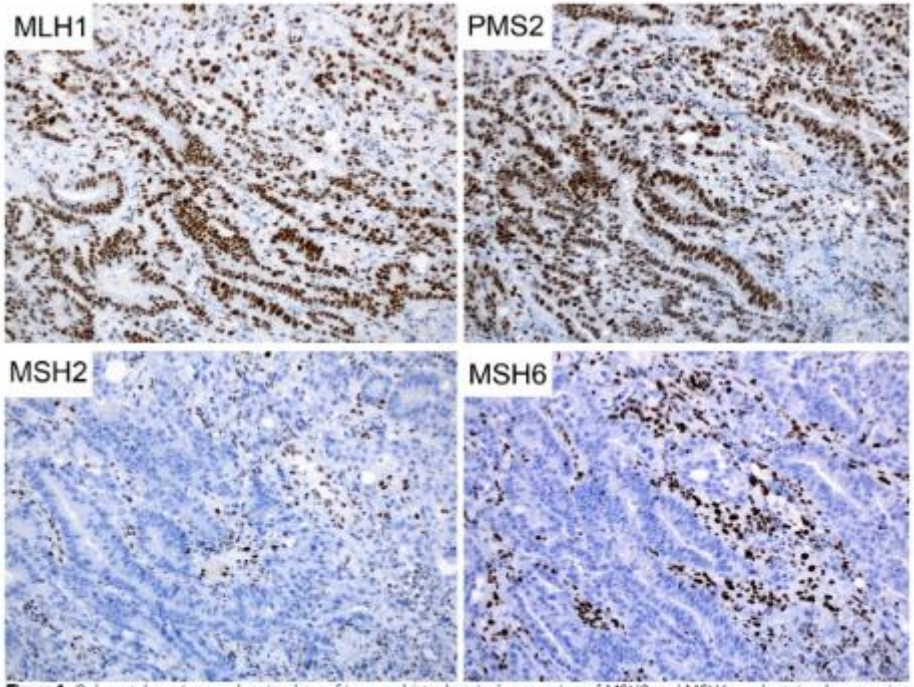

Figure 1. Colorectal carcinoma showing loss of immunohistochemical expression of MSH2 and MSH6, and retained expression of MLH1 and PMS2.

Figure adapted from: https://www.biorxiv.org/content/biorxiv/early/2018/02/23/270322.full.pdf

Requires additional testing

Sporadic loss of MLH1 can lead to loss of PMS2. Does NOT equal germline mutation.

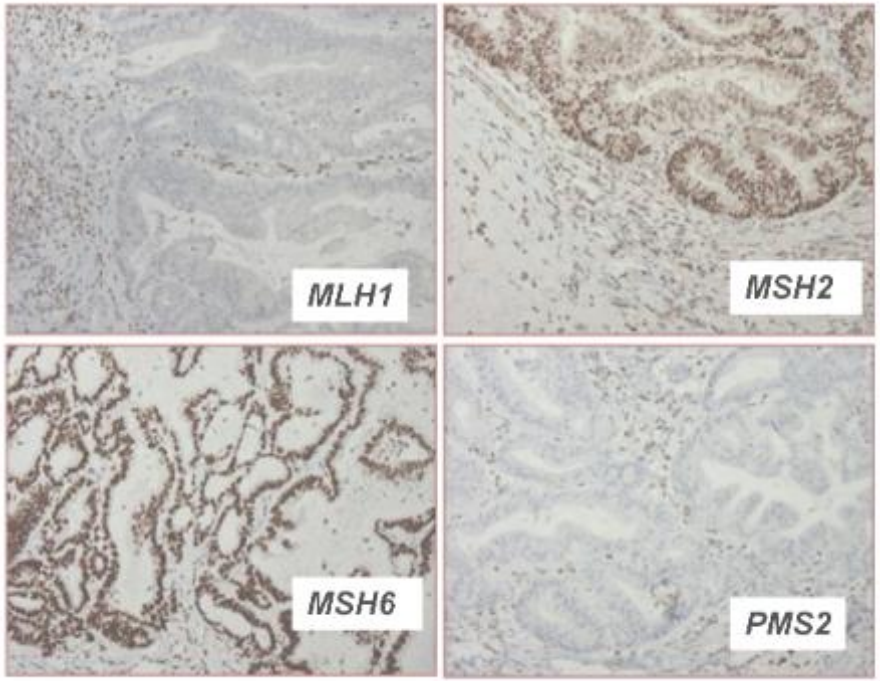

Figure adapted from: https://www.lynchscreening.net/implementation/immunohistochemistry-ihc-only-2/

Loss of PMS2?

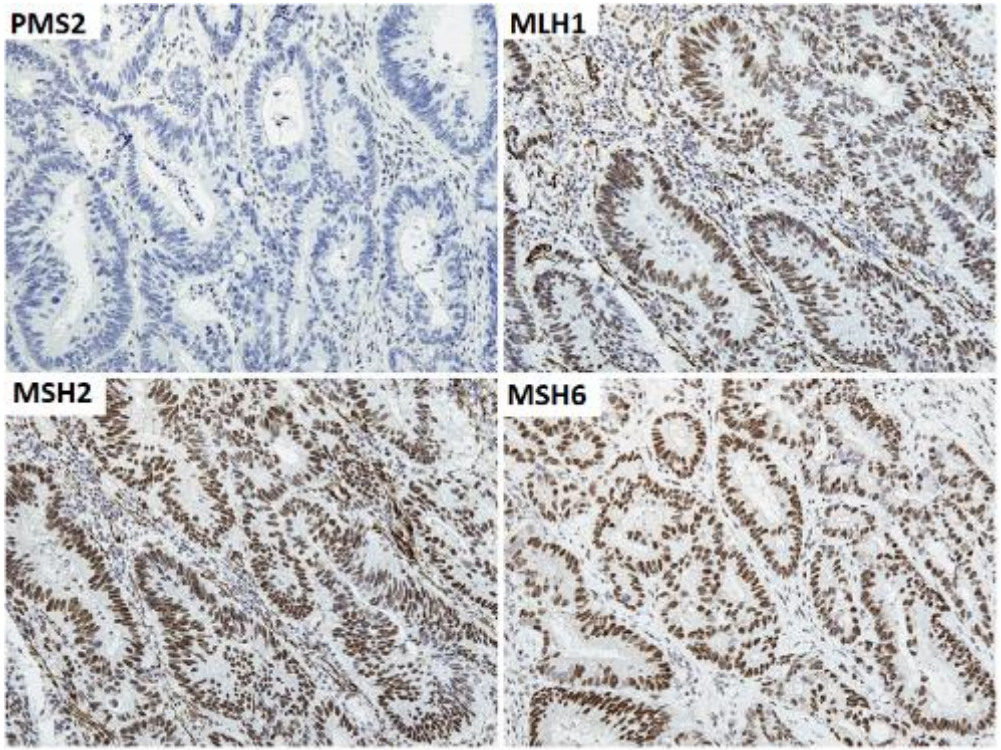

Figure adapted from: https://www.researchgate.net/publication/51057083_A_two-antibody_mismatch_repair_protein_immunohistochemistry_screening_approach_for_colorectal_carcinomas_skin_sebaceous_tumors_and_gynecologic_tract_carcinomas

Isolated Loss of PMS2 Immunohistochemical Expression is Frequently Caused by Heterogenous *MLH1* Promoter Hypermethylation in Lynch Syndrome Screening for Endometrial Cancer Patients

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Abstract: Lynch syndrome (LS) is an autosomal-dominant inherited disorder mainly caused by a germline mutation in the DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) and is associated with increased risk for various cancers, particularly colorectal cancer and endometrial cancer (EC). Women with LS account for 2% to 6% of EC patients; it is clinically important to identify LS in such individuals for predicting and/or preventing additional LS-associated cancers. *PMS2* germline mutation (PMS2-LS) is the rarest contribution to LS etiology among the 4 LS-associated MMR germline mutations, and its detection is complicated. Therefore, prudent screening for PMS2-LS is important as it leads to an efficient LS identification strategy. Immunohistochemistry is recommended as a screening method for LS in EC. Isolated loss of PMS2 (IL-PMS2) expression is caused not only by PMS2-LS but also by *MLH1* germline mutation or *MLH1* promoter hypermethylation (MLH-PHM). This study aimed to determine the association between MLH1-PHM and IL-PMS2 to avoid inappropriate genetic analysis. We performed *MLH1* methylation analysis and *MLH1*/*PMS2* germline mutation testing on the IL-PMS2 cases. By performing MMR-immunohistochemistry on 360 unselected ECs, we could select 8 (2.2%) cases as IL-PMS2. Heterogenous MLH1 staining and MLH1-PHM were detected in 4 of 8 (50%) IL-PMS2 tumors. Of the 5 IL-PMS2 patients who underwent genetic analysis, 1 had *PMS2* germline mutation with normal MLH1 expression (without MLH1-PHM), and no *MLH1* germline mutation was detected. We suggest that *MLH1* promoter methylation analysis for IL-PMS2 EC should be performed to exclude sporadic cases before further *PMS2* genetic testing.

Key Words: Lynch syndrome, endometrial cancer, PMS2, *MLH1* promoter hypermethylation, heterogenous

(*Am J Surg Pathol* 2016;40:770–776)

From the *Department of Obstetrics and Gynecology, Akita University Graduate School of Medicine; and †Department of Obstetrics and Gynecology, Noshiro Kosei Medical Center, Akita, Japan.

Among endometrial cancer (EC) patients, Lynch syndrome (LS) accounts for approximately 2% to 6% of cases.[1–5] LS is an autosomal-dominant inherited syndrome mainly caused by germline mutations in the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*.[6] Mutation carriers have an increased lifetime risk of developing colorectal cancer (CRC, 40% to 80%), EC (33% to 61%), ovarian cancer (9% to 12%), and other LS-associated cancers.[7] Thus, it is clinically relevant to identify LS women among EC patients to predict and prevent the development of other LS-associated cancers. It would also provide blood relatives an opportunity for genetic analysis and surveillance for LS-associated cancers. Each of the 4 MMR germline mutations lead to distinct molecular pathologies,[8] and thus individuals carrying different mutations should not be regarded as suffering from the same disease. *PMS2* germline mutation is associated with later onset, weaker family history, and a

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- Kato, et. al., AJSP 2016
- Hypermethaylation of MLH1 associated with *some* cases of loss of PMS2 by IHC *without* germline mutation of PMS2

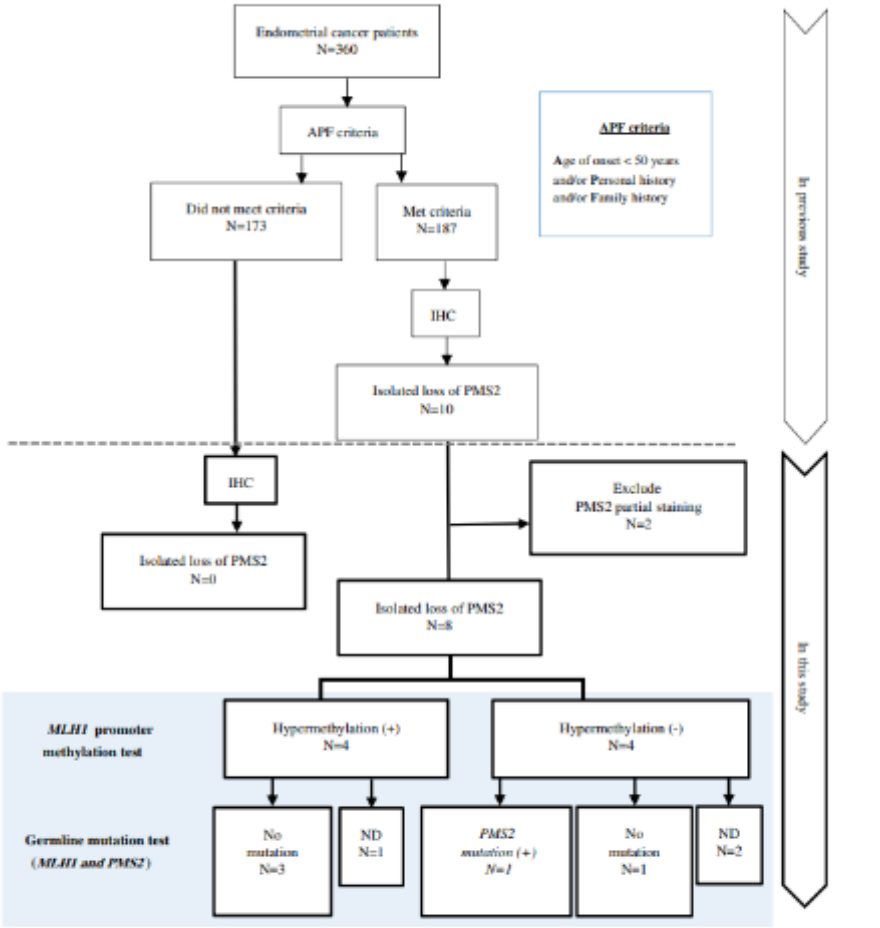

FIGURE 1. Summary of this study. The *MLH1* promoter methylation test and germline mutation test for *MLH1* and *PMS2* were performed for IL-PMS2 cases. APF criteria, our original criteria for selection according to Age of onset below 50 years and/or Personal/Family history of Lynch-associated cancer. IHC analysis for MLH1, MSH2, MSH6, and PMS2. ND indicates not done germline mutation test.

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IHC testing schematic

With BRAF and hypermethylation

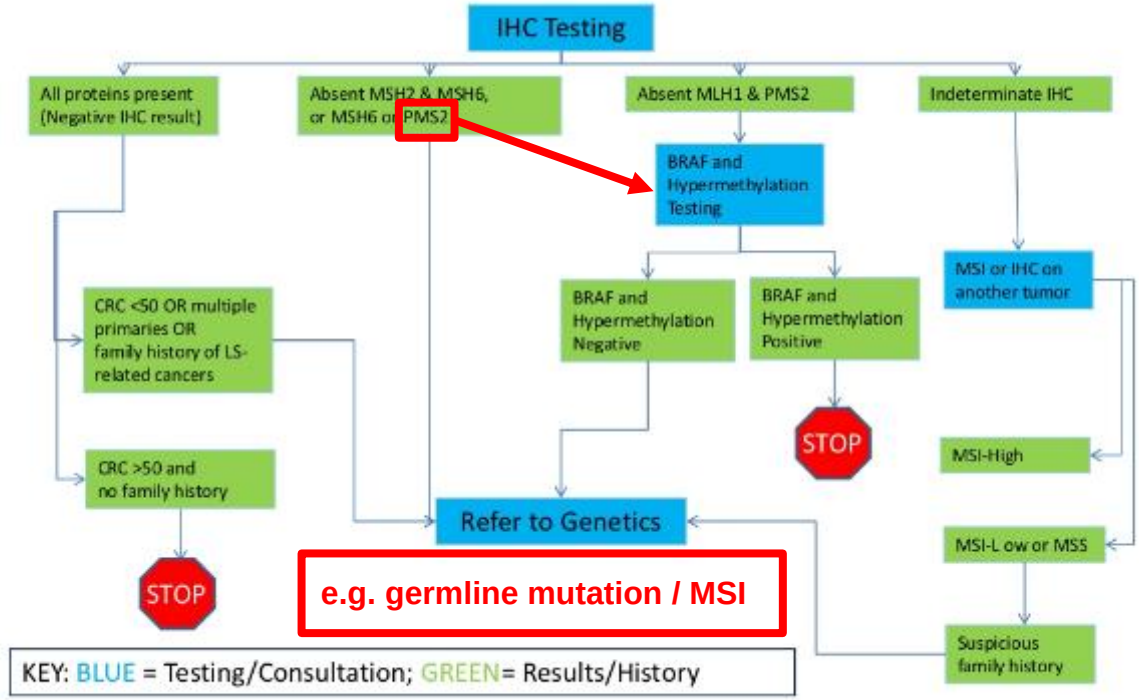

Figure adapted from Lynchscreening.net - https://www.lynchscreening.net/wp-content/uploads/2012/04/IHC-+methylBRAF.jpg

Questions?