



MICROSATELLITE INSTABILITY AND LYNCH SYNDROME IN COLORECTAL CARCINOMA

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Colorectal Carcinoma

- **Why it is important to understand molecular pathways?**
- **Is it clinically important to detect MSI and Lynch syndrome?**
- **How do you identify MSI and LS patients?**
- **Clinical testing in practice**



Colorectal Cancer

15%

85%

MSI+
(Microsatellite Instability)

CIN
(Chromosome Instability)

2-3%

13%

<1%

85%

Lynch Sx

Sporadic MSI(+)

FAP

Sporadic

Germline Mutation
MMR genes
MLH1
MSH2
MSH6
PMS2

•Epigenetic silencing of
MLH1 by hypermethylation
of its promoter region

Germline
Mutation
APC

Acquired
APC, p53,
DCC, kras,
LOH,...

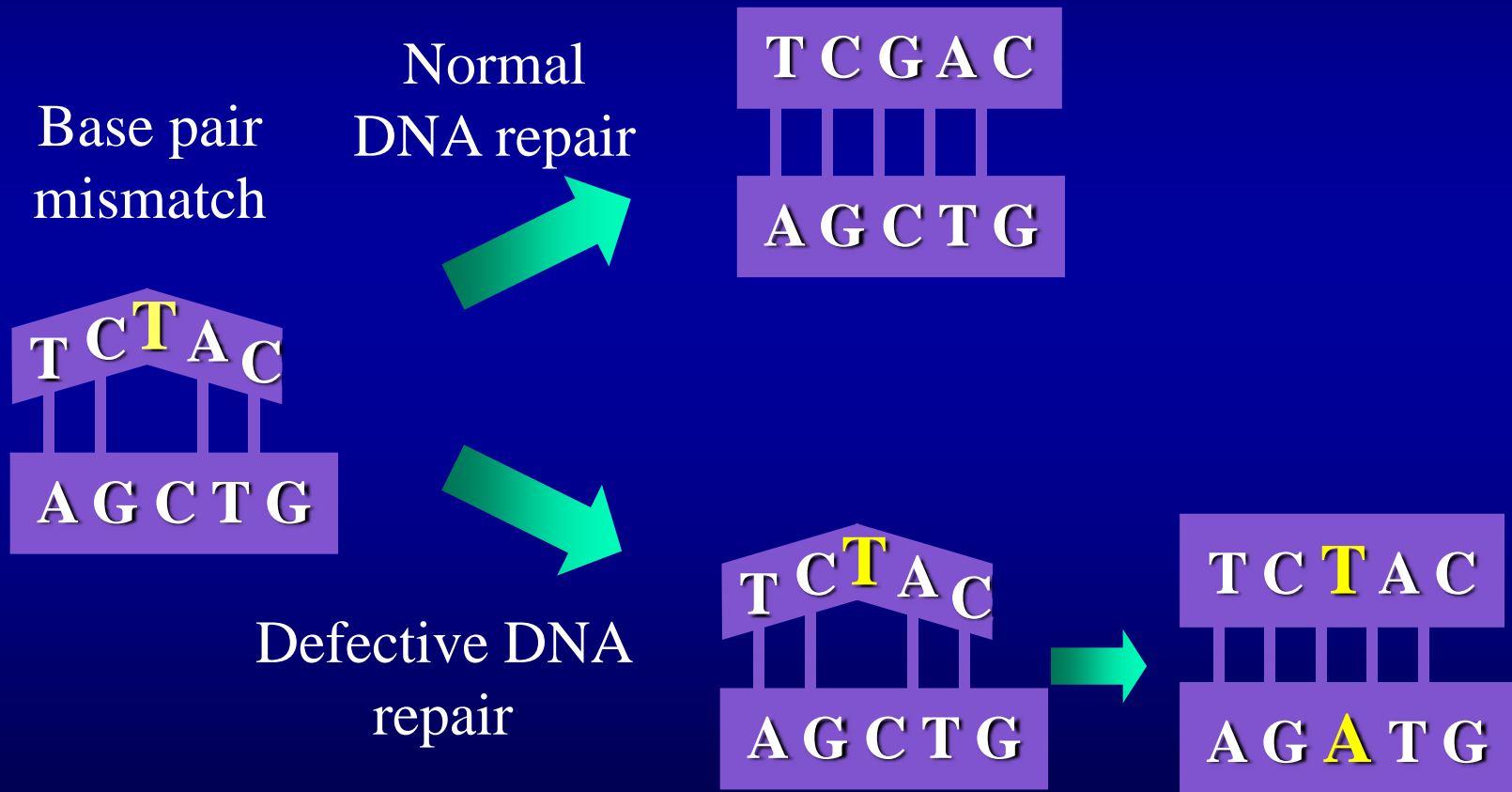


Why Determine Which Pathway?

- **LS patients at high risk for second primary cancers (CRC and others)**
- **LS patients have at-risk relatives who could benefit from genetic testing**
- **All MSI+ CRC patients have a better prognosis**
- **MSI+ CRC patients MAY need different treatment in future**



MSI is Caused by Failure of Mismatch Repair (MMR) Genes



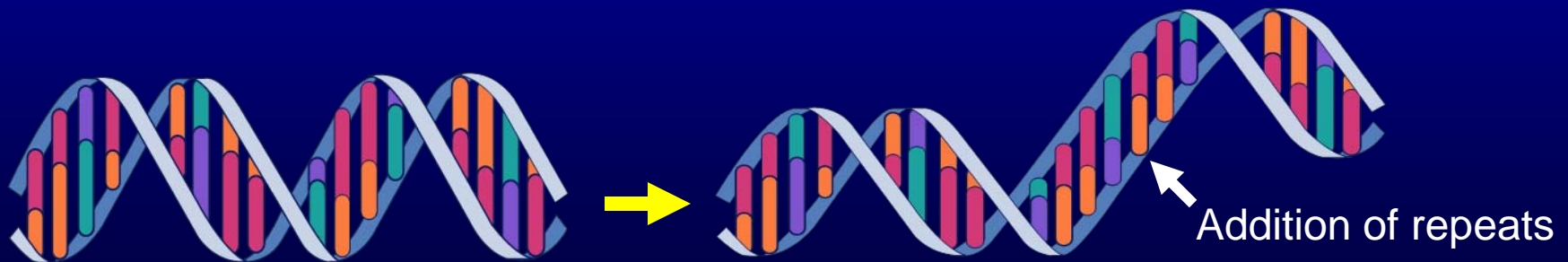


MSI Detects Mismatch Repair Failure

- Repetitive DNA sequences 1- 4 nucleotides (microsatellites) normally found genome
- With MMR failure, variability in repeats

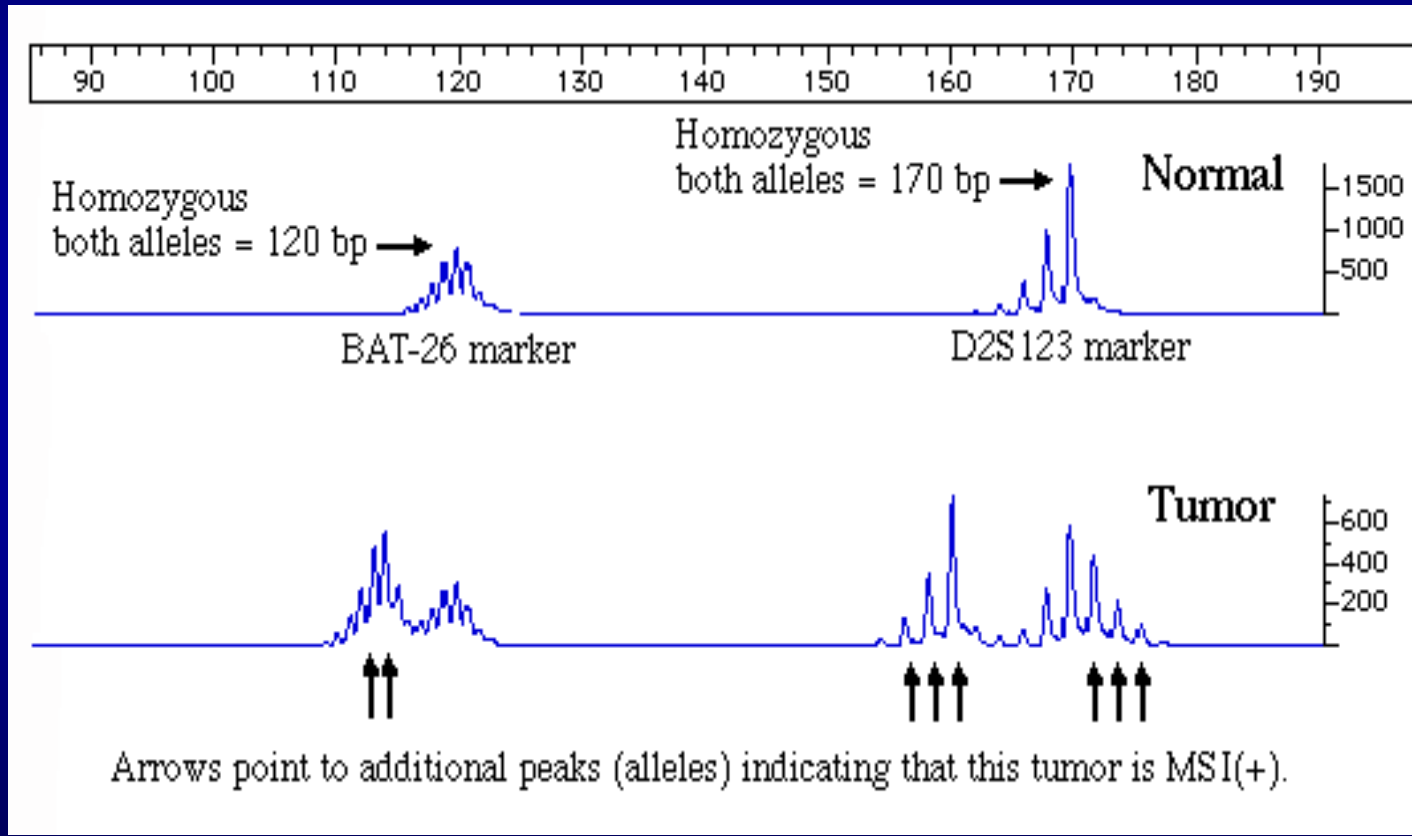
Mono... e.g. TCGAGG AAAAAAA GGAGCT

Di ... e.g. TCGAGG CACACACACACA GGAG





Microsatellite Instability BAT-26 and D2S123 - Genotype





Lynch Syndrome

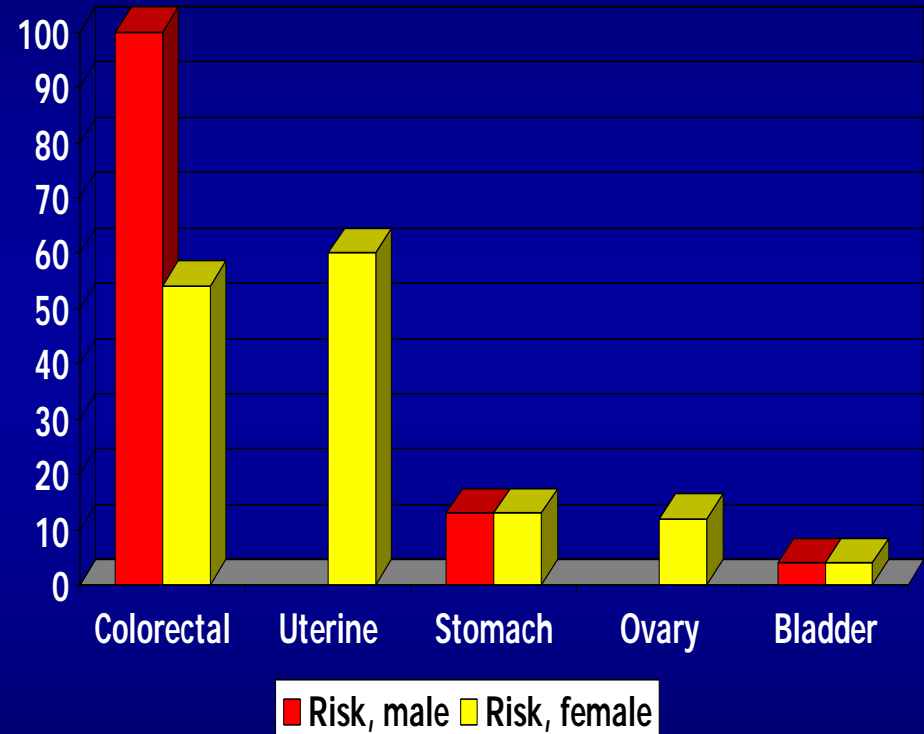
Hereditary Non-Polyposis Colorectal Carcinoma (HNPCC)

- Most common hereditary CRC syndrome
- 2-3% of CRCs
- Autosomal dominant, penetrance 80%
- Susceptibility to CRC & extracolonic cancers
- Germline mutation in genes belong to DNA MMR family- *MLH1, MSH2, MSH6, PMS2*
- Mutations in these genes lead to defective DNA repair and microsatellite instability



Clinical Features of LS

- Early but variable age at CRC diagnosis (~45 years)
- Tumor in proximal colon predominates
- Extracolonic cancers: endometrium, ovary, stomach, urinary tract, small bowel, bile ducts, sebaceous skin tumors





Clinical Utility of Identifying CRC with MSI+

- Strong evidence that individuals with MSI+ CRCs have a better prognosis than those with MSS tumors (32 studies pooled, >7000 patients, MSI+ >15% improvement in survival; Popat, JCO, 2005.)
- Less strong evidence that individuals with MSI+ CRCs may not respond as well to 5-FU based chemotherapy regimens (Ribic, NEJM, 2003; Carethers, Gastroenterol, 2004; Lynch, Eur J Hum Genet, 2006; Ward, 2005, J Pathol; Jover, Gut, 2006)



Detection of Patients with MSI+ and LS

1. Is this clinically useful?

YES for MSI and Lynch Syndrome

2. How identify patients?

History

- Less useful due to smaller families and endoscopic polypectomy**
- Amsterdam & Bethesda are <50% sensitive in unselected patients**

Histology

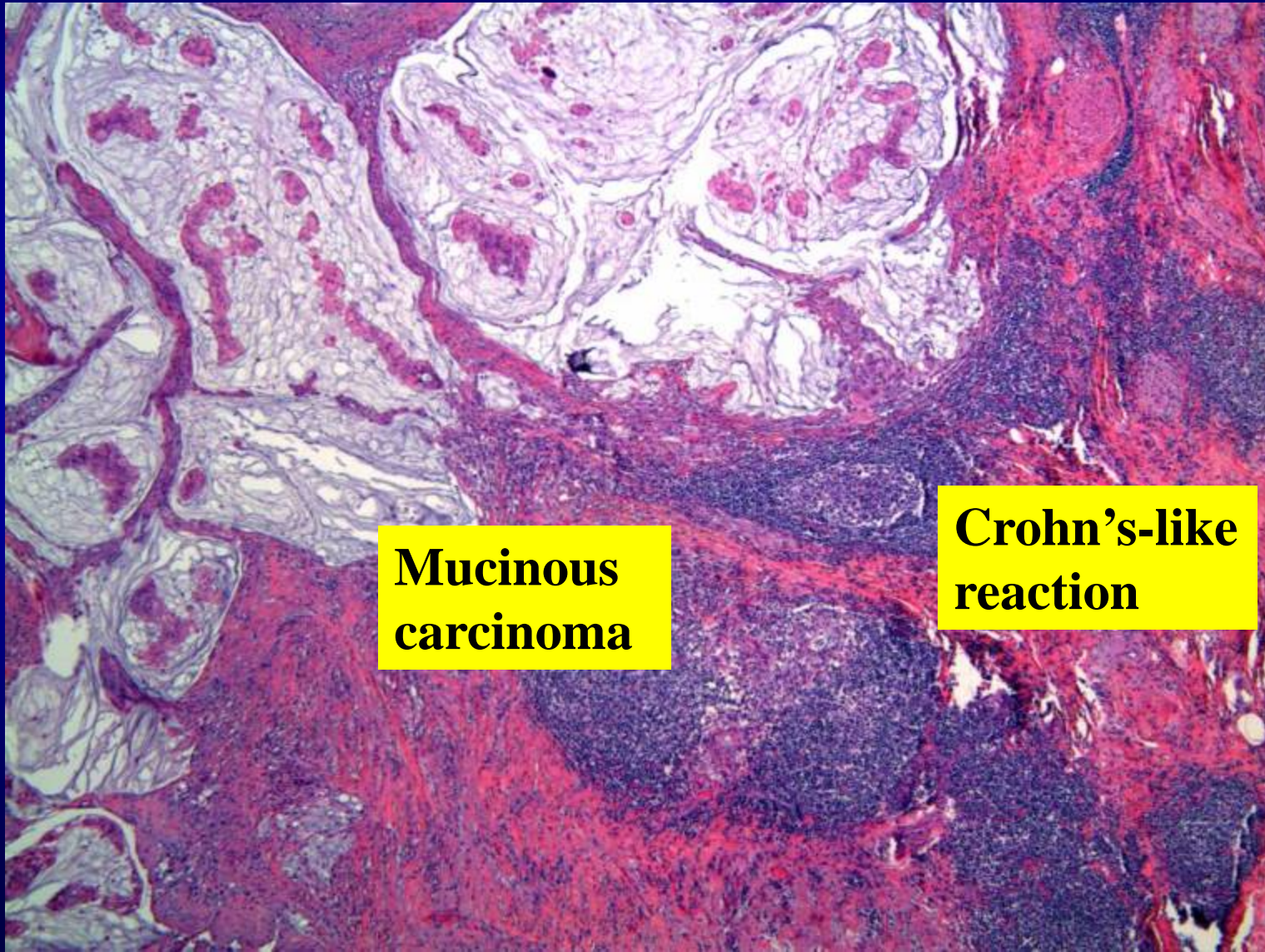


Histology MSI +

- Intraepithelial lymphocytes- most sensitive
- Mucinous and signet ring cell types
- Poorly differentiated, medullary carcinoma, tumor heterogeneity
- Low sensitivity (14 - 38%)
- Specificity (85 - 90%)
- Serration and sporadic methylation

Jass, Gut, 1998; Jass, Fam Cancer, 2004;
Alexander, Am J Pathol, 2001; Yearsley, Hum
Pathol, 2006

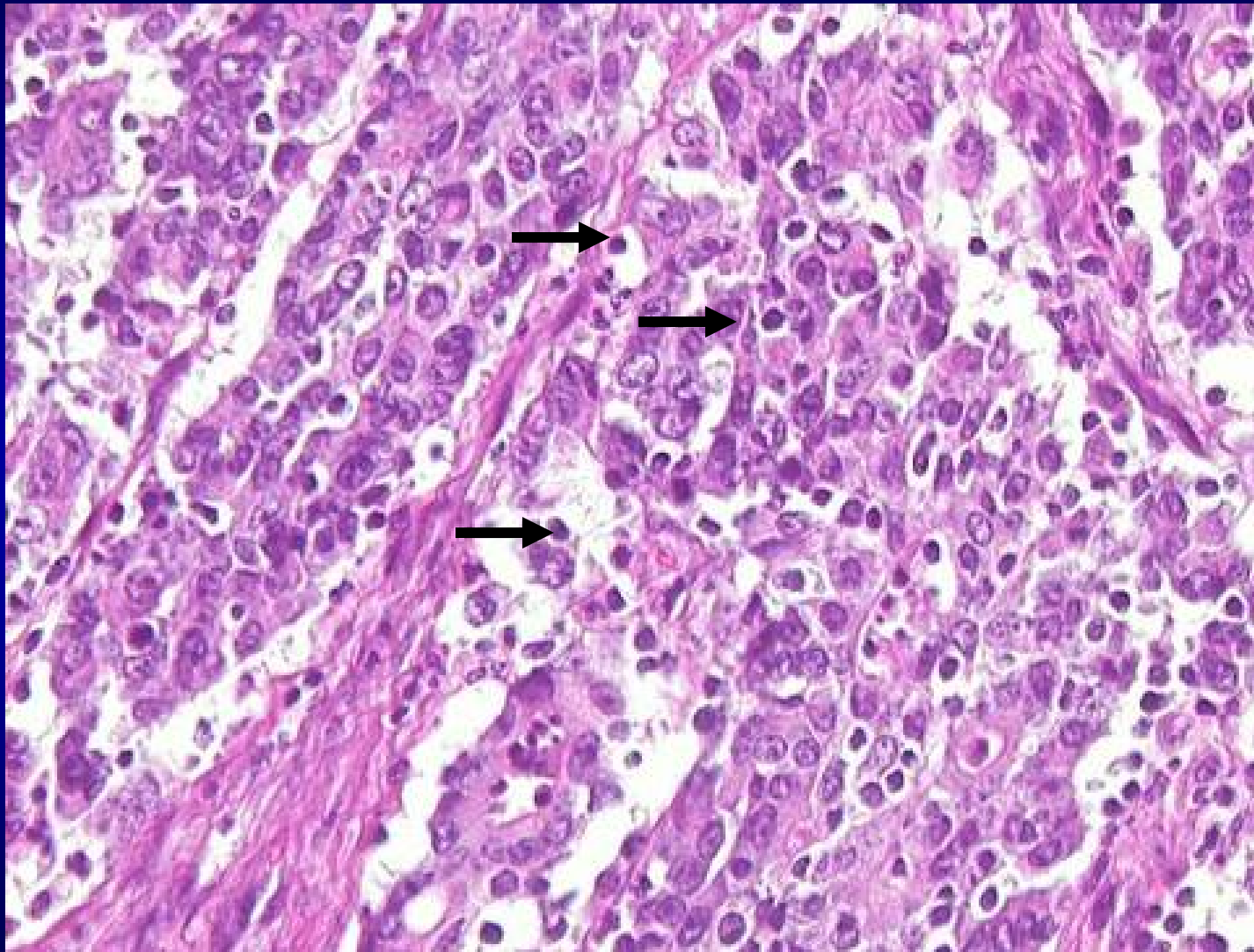
Histology MSI+ CRC



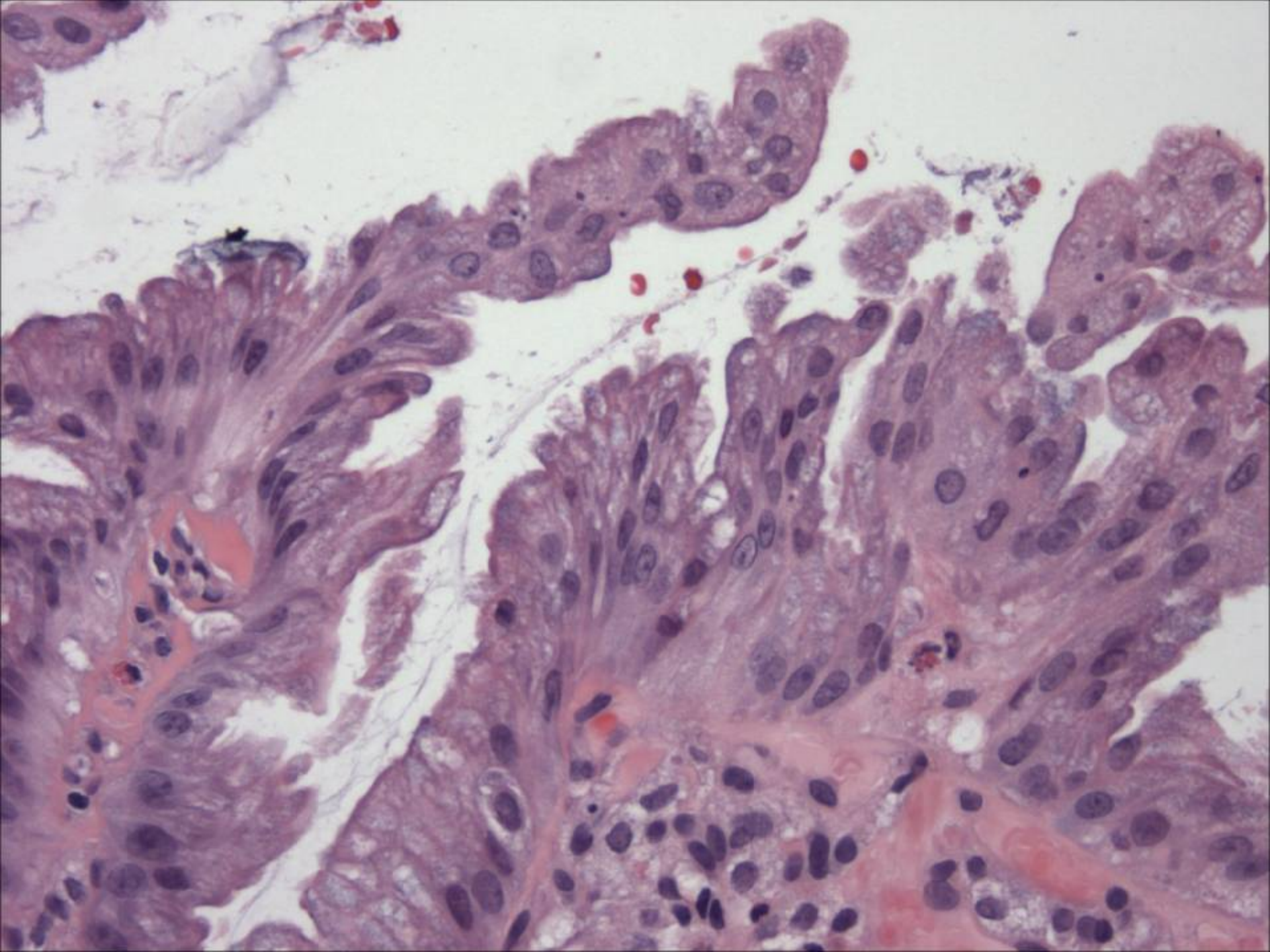
**Mucinous
carcinoma**

**Crohn's-like
reaction**

Histology of MSI+ CRC



Intraepithelial Lymphocytes





Detection of MSI + and LS Patients

1. Is this clinically useful?

YES

2. How identify patients?

History }
Histology } Not enough

Defective MMR system

MSI – Tumor DNA

IHC – Tumor protein



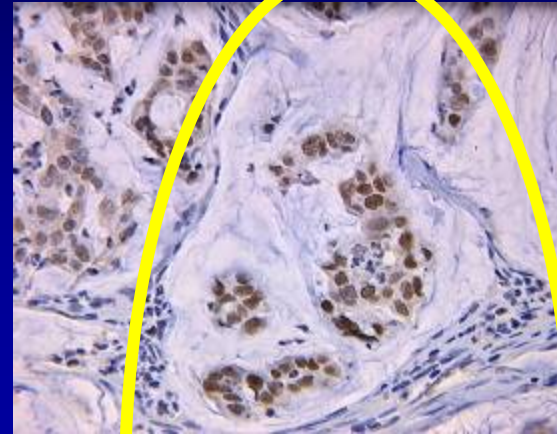
How can you identify CRC with MMR Deficiency

- **Microsatellite instability testing**
 - Direct assessment on molecular level
 - Requires DNA from tumor and normal
 - Molecular laboratory
- **IHC for the MMR proteins**
 - Assesses presence/absence of protein
 - 100% tumors with abnormal IHC are MSI+
 - 95% MSI+ tumors have abnormal IHC
 - Any Pathology Department

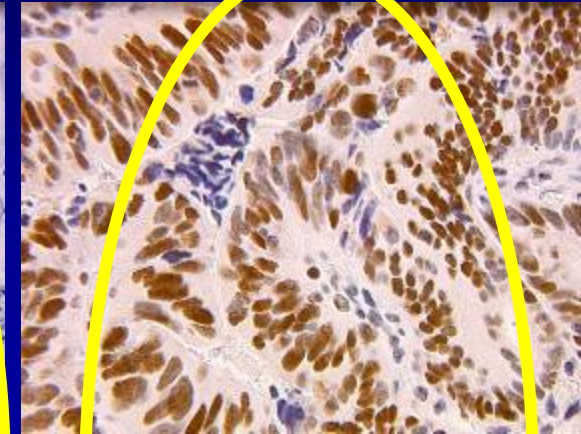


Immunohistochemistry

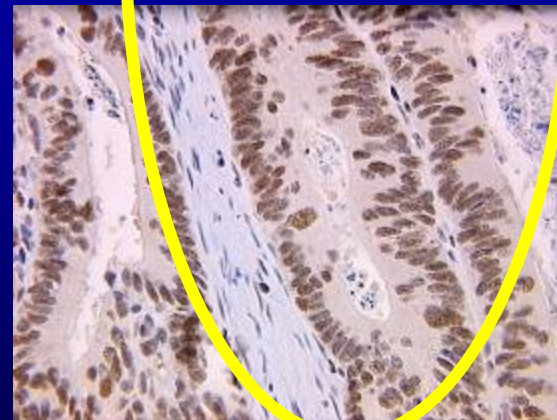
- Identify MMR proteins
- Normally present
- If protein is absent, gene is not being expressed (mutation or methylation)
- Helps direct gene testing by predicting likely involved gene
- If abnormal IHC (absent), MSI+



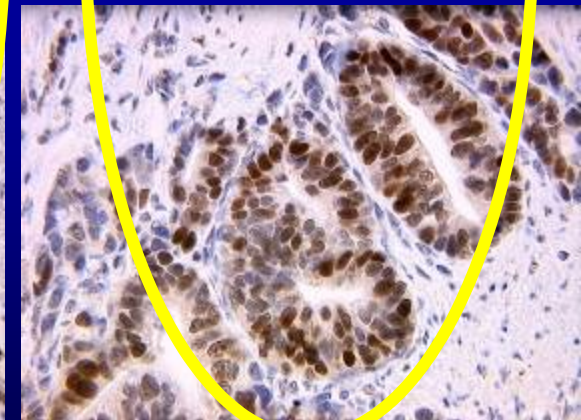
MLH1



MSH2



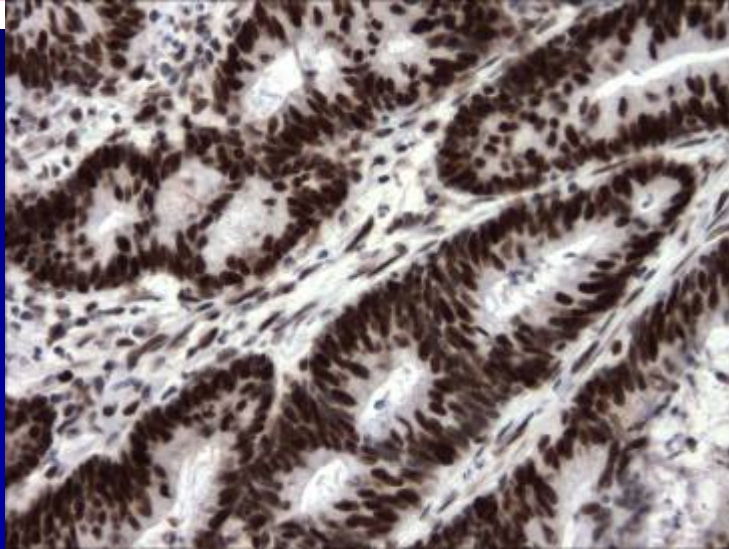
PMS2



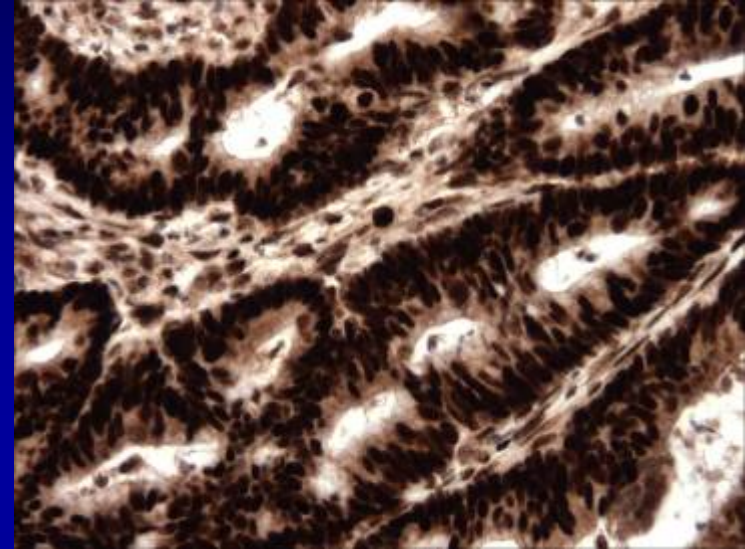
MSH6



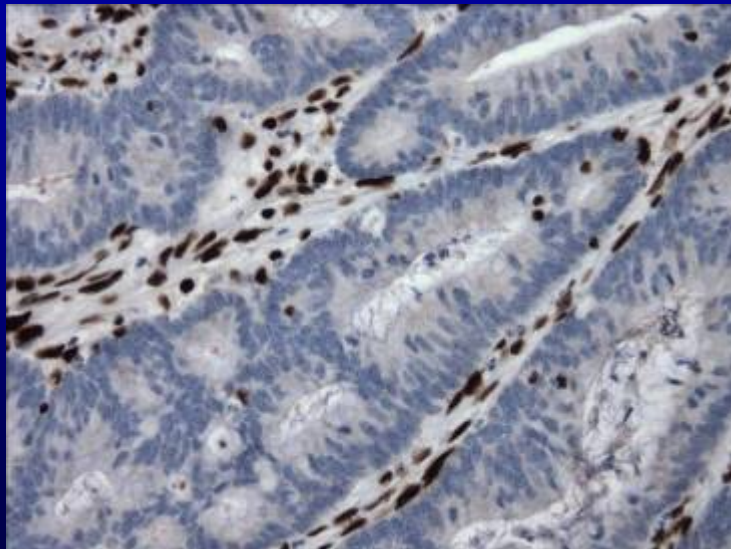
Methylation or Germline Mutation?



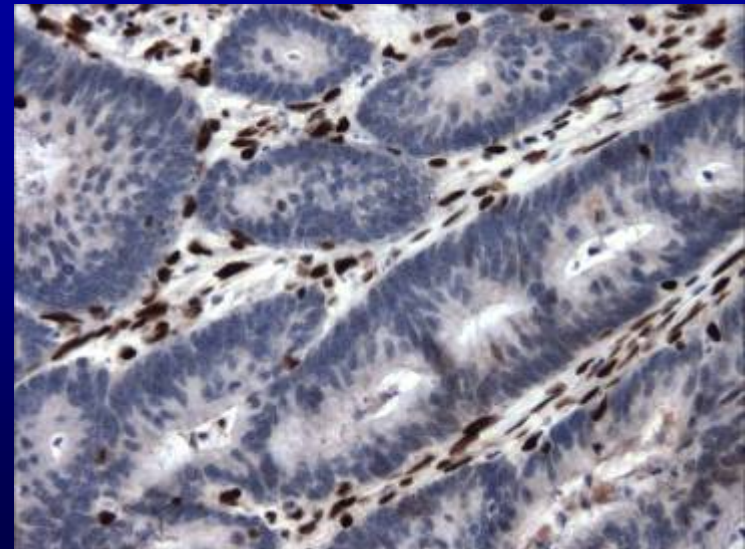
**MLH2
Present**



**MSH6
Present**



**MLH1
Absent**



**PMS2
Absent**



The Columbus-Area LS Study Aims

- Determine the frequency of LS among newly diagnosed CRC cases
- Determine the frequency of LS among newly diagnosed EC cases
- Determine the feasibility of screening unselected cases

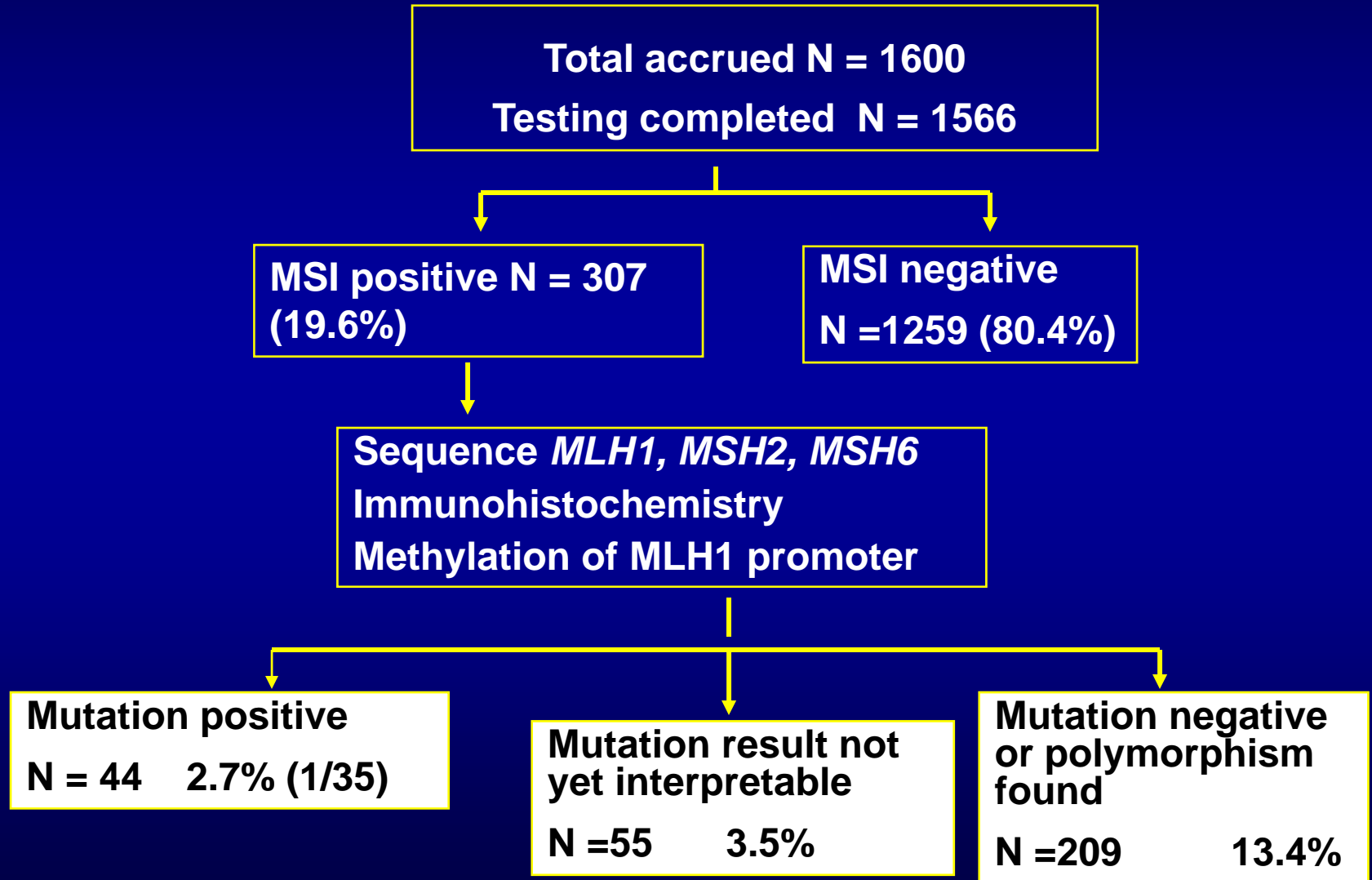


Design

- **Newly dx CRC or EC regardless age/family history- 1999**
- **MSI by modified Bethesda panel (BAT-25, BAT-26, D18S69, D2S123, D5S346)**
- **MSI+; IHC and mutation analysis of *MLH1*, *MSH2*, *MSH6* (and *PMS2*) genes by full sequencing of genomic DNA**
 - **Methylation status *MLH1* promoter evaluated by methylation-specific PCR and bisulfite-PCR followed by restriction digestion of tumor DNA**



Columbus LS Study CRC





CRC Proband Characteristics

- Mean age at diagnosis – 51.4
– Range, 23 to 87
- 22/44 (50%) diagnosed \geq 50 yo
- 11/41 (27%) did not meet Amsterdam or Bethesda criteria
- 23 *MSH2* mutations, 9 *MLH1* mutations, 6 *MSH6* mutations, 6 *PMS2* mutations



Columbus LS Study - EC

Endometrial cancer
Presently accrued N = 565
Presently analyzed N = 562

MSI positive
N = 131 (23.4%)

MSI negative
N = 430* (76.6%)

Sequence *MLH1*, *MSH2*, *MSH6*
Immunohistochemistry
Methylation of *MLH1* promoter

Mutation positive
N = 12* 2.1%

Mutation result not yet interpretable
N = 23 4.1%

Mutation negative or polymorphism found
N = 96 17.1%



EC Proband Characteristics

- Mean age at diagnosis – 54.1
 - Range, 39 to 69
- 8/12 EC probands were dx \geq 50
- 8/12 EC probands did not meet Amsterdam or Bethesda criteria
- 7 MSH6 mutations, 3 MLH1 mutations, 2 MSH2 mutations



CRC and EC Summary Results

- **1600 CRC; 19.6% MSI, 2.7% LS**
- **565 EC; 23.4% MSI, 2.1% LS**
- **For CRC (44 LS):**
 - 35 proband families evaluated so far
 - 196 family members tested
 - 89 positives
- **For EC (12 LS):**
 - 11 proband families evaluated so far
 - 33 family members tested
 - 14 positives



Conclusions

- **Morbidity and mortality likely reduced by identifying probands and family members at risk and counseling**
- **For CRC and EC in the Columbus area, the rate LS is 2.7 and 2.1%**
- **Half patients would have been missed by history and age under 50**
- **Large scale screening is feasible**



Choosing the Screening Test: MSI vs. IHC

- IHC is available in virtually all hospitals
- MSI requires molecular diagnostics and normal for comparison
- IHC with 4 antibodies is similar in cost to MSI with 5 markers
- IHC directs gene testing saving money
- Ethical issues surrounding IHC
- IHC and MSI have limitations



Comparison of MSI to IHC in 500 CRC MSI as the Screening Test

		Lynch		
		Yes	No	
MSI	High	18	47	65
	Low	0	34	34
	Stable	0	401	401
		18	482	500

$$\text{DR} = 18/18 = 100\%$$

$$\text{FPR} = 47/482 = 10\% \text{ (including MSI-low with MSI-stable)}$$



Comparison of MSI to IHC in 500 CRC

IHC as the Screening Test

Lynch

		Lynch		
		Yes	No	
IHC Abnormal (1 or more protein)	Yes	17	56	73
	No	1	426	427
		18	482	500

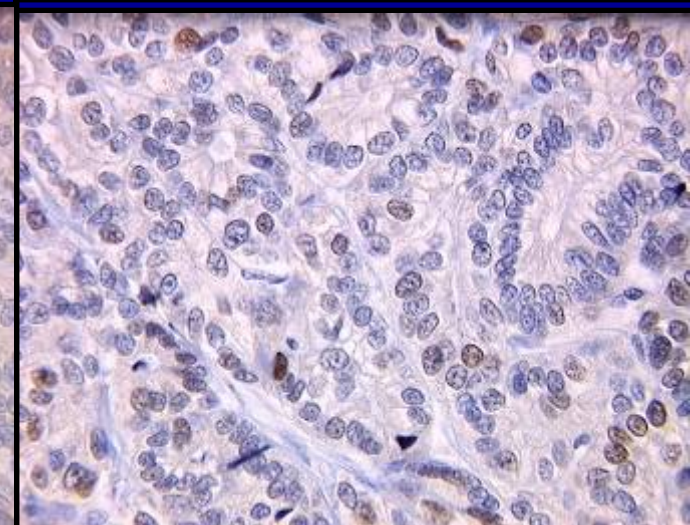
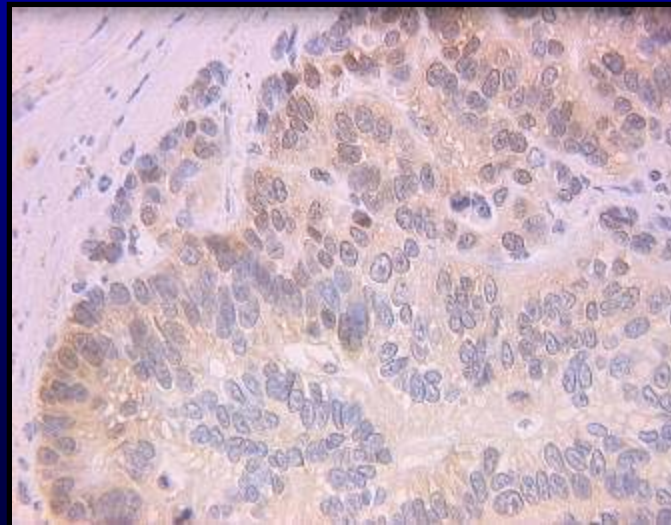
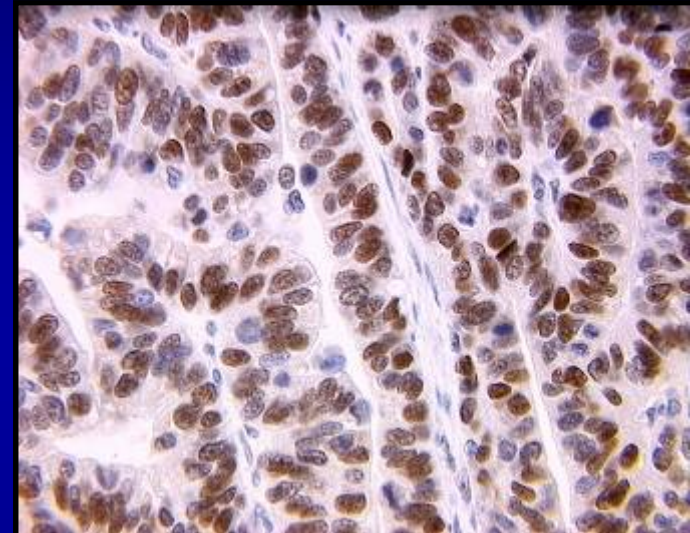
$$DR = 17/18 = 95\%$$

$$FPR = 56/482 = 12\%$$



Accuracy of MSI and IHC

- 2 of 44 mutations (5%) missed by MSI in 1500 cases
 - Mucin and scant tumor cells
- 3 of 44 mutations (7%) missed by IHC in 1500 cases
 - Variability in staining
 - Weak nuclear staining
 - Cytoplasmic staining





Combined Conclusions

- **IHC is as sensitive as MSI to detect MMR protein deficiency**
- **IHC analysis leads to less complex and expensive molecular analysis than MSI**
- **If there is high suspicion for LS, and one screening test is negative, consider doing the other**



Clinical Testing for Lynch Syndrome and MSI - Choices

- **MSI and/or IHC ordered by clinician only**
 - Based upon clinical and family history
 - If abnormal results or results not consistent with history- genetic consult
- **MSI and/or IHC ordered by pathologist too?**
 - Histology
 - Call clinician to request test or pathologist directed?
- **Routine testing on all CRC**
 - IHC and/or MSI



OSU Plan

- **Due to clinical importance of detecting LS and MSI status (prognosis/predictive)**
- **IHC on all primary CRC at OSU 3/1/06**
- **Presented at OSU Surgical Oncology Tumor Board and GI Specific Committee and brochure created for all CRC patients admitted for surgery**
- **Surgery, Oncology, Cancer Genetics and Pathology involved in plan**



Immunohistochemistry (IHC) TESTING

What is IHC testing in Colorectal Cancer?

When you have surgery, the tumor from your colon or rectum will be closely studied. A tumor is a growth, which may or may not be cancer. The results will be given to your doctor in a pathology report about one week after your surgery. This pathology report helps the doctor to know:

- *How well you might do after surgery*
- *The size of the tumor*
- *If the tumor was cancer*
- *If the cancer has spread*

Your doctor may discuss this report with you in a visit after surgery.

The Ohio State University (OSU) has added a new test to this report for all colon and rectal cancers. This test is called IHC. IHC tells your doctor more about how well you might do and if you may have a hereditary form of cancer known as Lynch syndrome. Hereditary cancers are those that run in families. People who have Lynch syndrome have a high chance of having more than one cancer in their lifetime. Their close relatives may have Lynch syndrome too and may have an increased risk for cancer.

What does IHC test for?

IHC tests for four proteins in the tumor. A protein is a substance that helps your body work the right way. These proteins are present in normal colon cells. They may be absent in colon cancer cells. The IHC test will look for these proteins:

- *MLH1*
- *PMS2*
- *MSH2*
- *MSH6*

What do the results of IHC mean?

- *All four proteins are present in your tumor.* This result occurs about 80% of the time (8 out of every 10 tests). This result means that you have the most common type of colon or rectal cancer. Your doctors will decide how well you will do based on the stage of your cancer at diagnosis. This also means that you are at a low risk to have Lynch syndrome.
- *One or more of the proteins is absent in your tumor.* This result occurs about 20% of the time (2 out of every 10 tests). This result means that you have the less common type of colon or rectal cancer. Your chance of having a good outcome is better than someone with all four proteins present. This result also means that you may have Lynch syndrome. Your doctor may ask you to see a staff member in the OSU Clinical Cancer Genetics Program. The staff member will tell you more about your results. They will take your family history and may discuss the need for more testing.

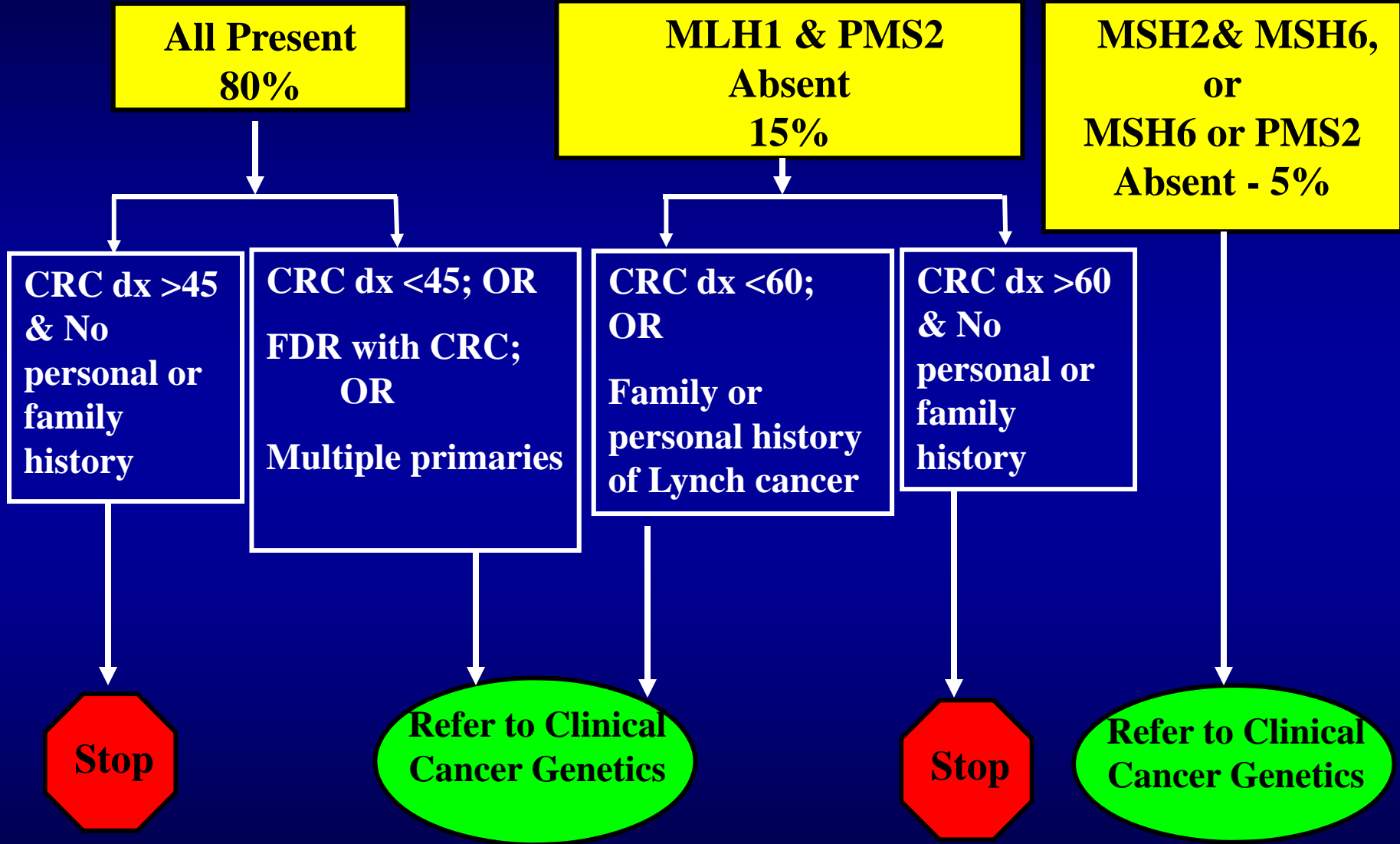
What if my tumor does not have some of the proteins?

- *MLH1 and PMS2 are absent.* This result will occur 15% of the time. Most people (4 out of 5) with absent MLH1 and PMS2 do not have Lynch syndrome. People with Lynch syndrome may have cancer at a younger age (under 60) and/or have a family history of certain cancers (colon, rectal, uterine, stomach, ovarian, ureter). If MLH1 and PMS2 are absent, it may be hard to tell if you may have Lynch syndrome so your doctor may ask you to see Clinical Cancer Genetics.
- *PMS2 alone, MSH2, or MSH6 are absent.* These results will occur only 5% of the time. Most people with these results have Lynch syndrome. It is very important that anyone who receives one of these results goes to Clinical Cancer Genetics.

If you have questions about your IHC result or to make an appointment with the OSU Clinical Cancer Genetics Program, please call 614-293-6694 (or toll free 1-888-329-1654).



How to Follow-up on IHC Results





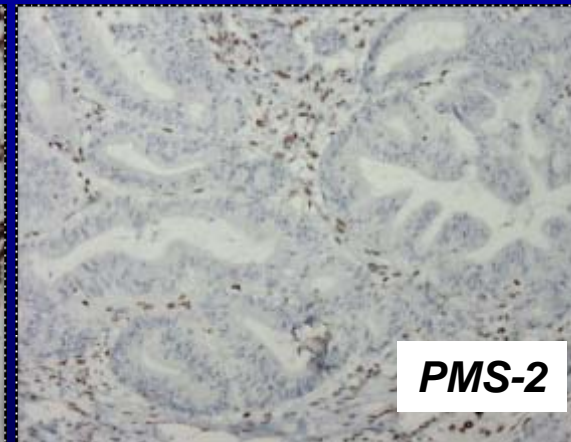
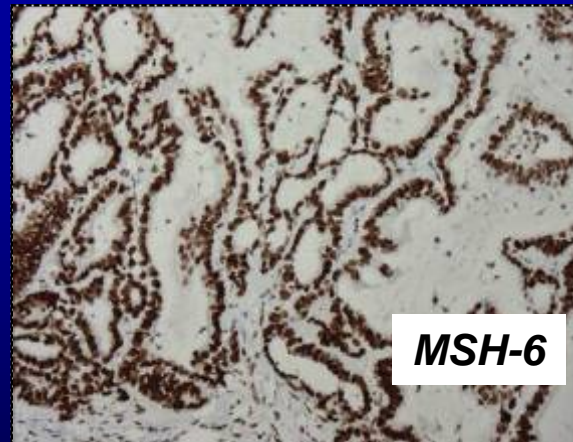
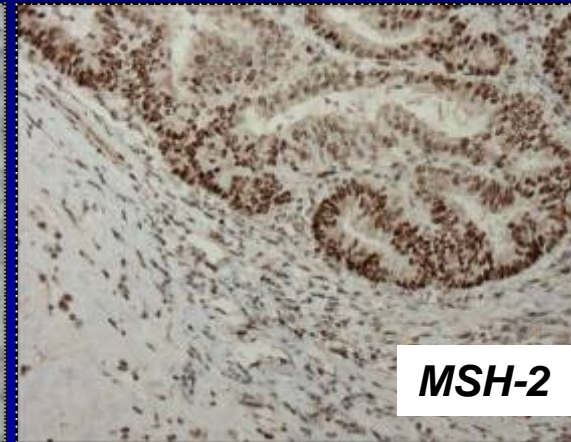
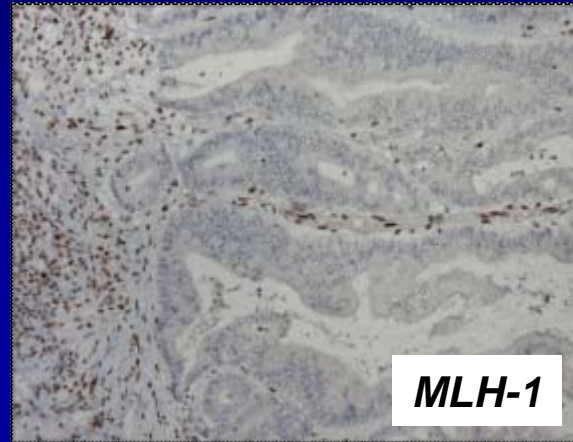
1. Normal – All 4 Stains Present

- 80% of the time you will get this result
- CRC is probably not MSI+
- Prognosis worse than if MSI+
- Refer to Genetics ONLY if you suspect polyposis, patient dx <45, patient has had multiple CRC primaries, or the patient has an FDR with CRC at any age



2. Abnormal – MLH1 & PMS2 Absent

- 15% of the time
- CRC is MSI+
- Better prognosis
- 80% acquired methylation of *MLH1*
- 20% will be LS





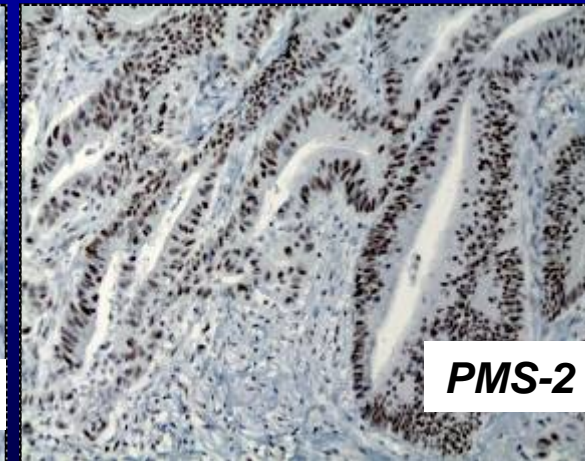
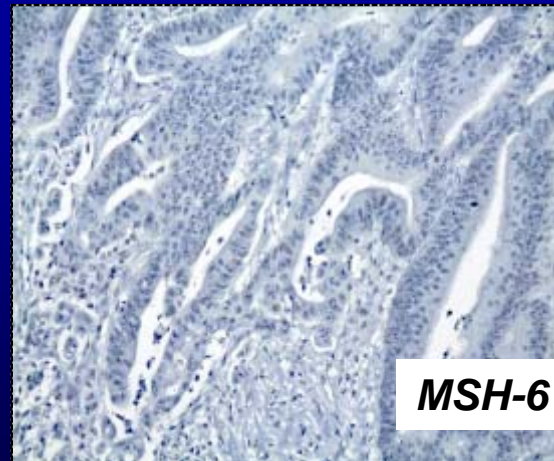
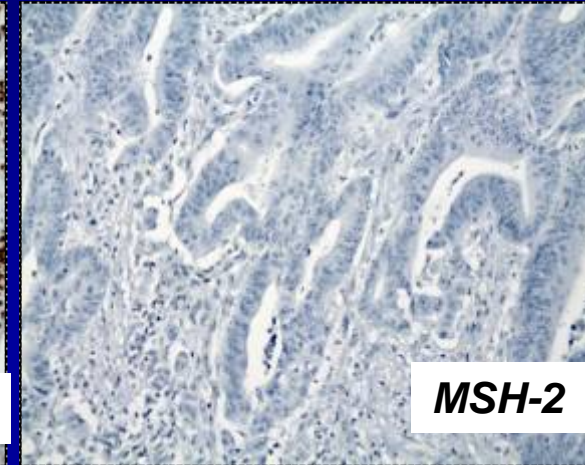
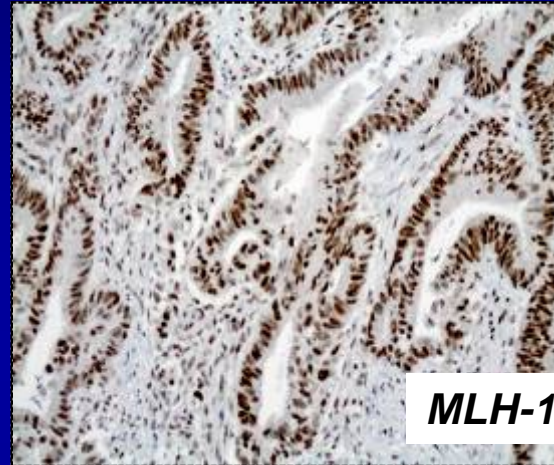
2. Abnormal – MLH1 & PMS2 Absent

- **Either refer all cases to Genetics
OR**
- **Refer those diagnosed under age 60,
those with multiple primary LS cancers,
and those with an FDR or SDR with a
LS cancer at any age**
- **No need to refer those diagnosed over
age 60 who have no personal or family
history**



3. Abnormal – MSH2 & MSH6 Absent

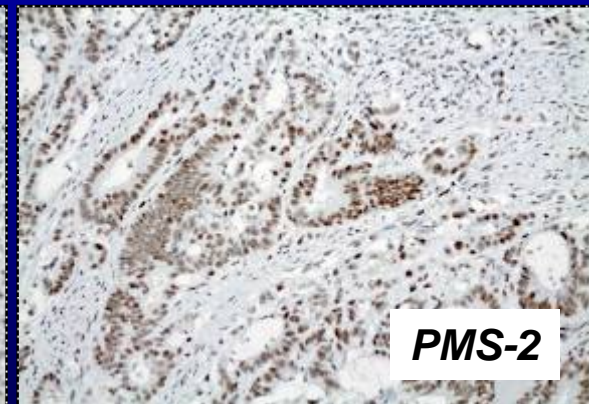
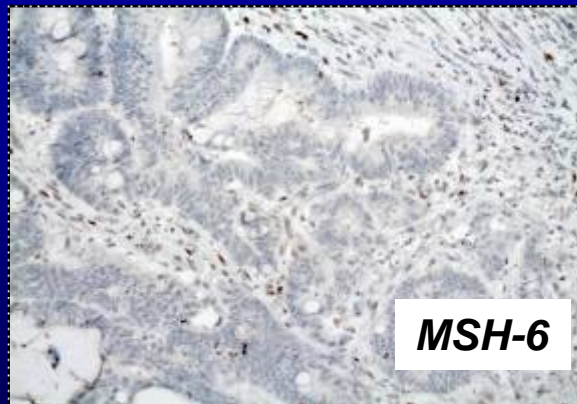
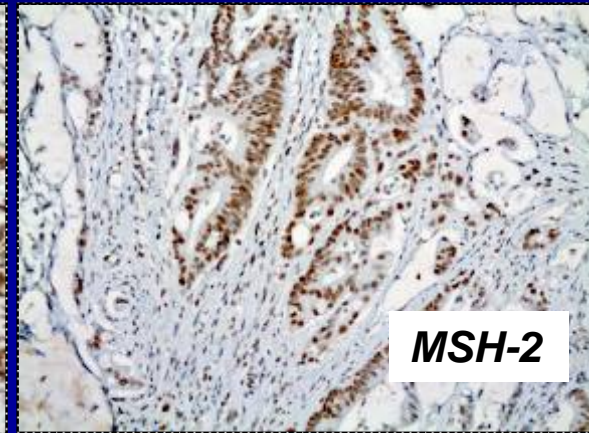
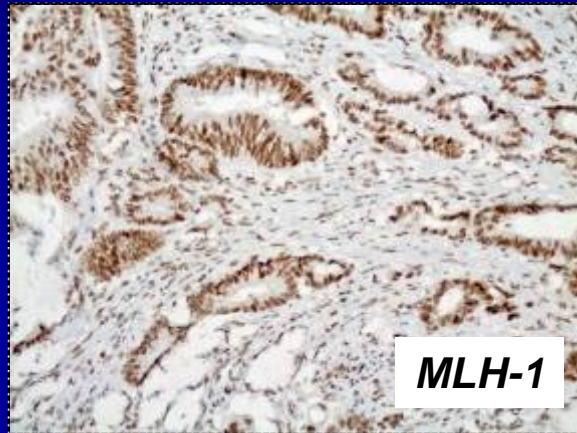
- 3% of the time
- CRC is MSI+
- Better prognosis
- Most likely LS due to either *MSH2* or *MSH6* gene mutation
- Always refer to Genetics





4. Abnormal – MSH6 Absent

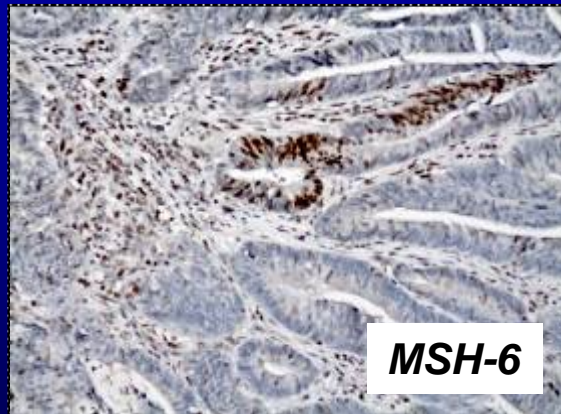
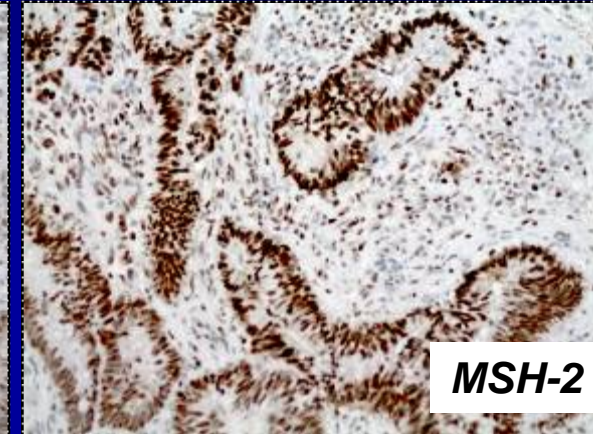
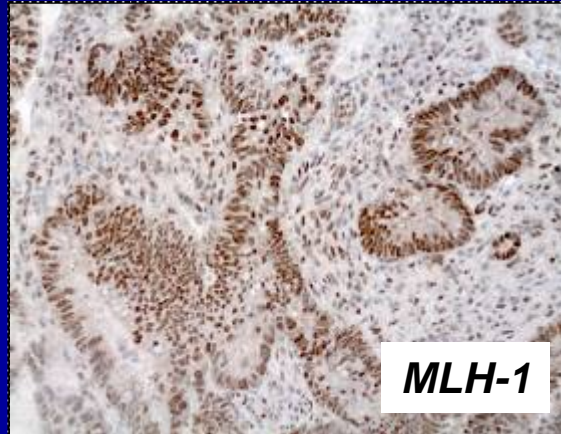
- 1% of the time
- CRC is MSI+
- Better prognosis
- Most likely LS due to an *MSH6* gene mutation
- Always refer to Genetics





5. Abnormal – PMS2 Absent

- 1% of the time
- CRC is MSI+
- Better prognosis
- Most likely LS due to an *PMS2* gene mutation
- Always refer to Genetics





OSU Experience since 3/1/06

- Reimbursement levels are ~50% which is average for Pathology stains
- 138 CRC tumors had IHC
- 24 patients have had abnormal IHC
- $24/138 = 17\%$ have abnormal IHC (within range expected), MSI+
- $114/138 = 83\%$ normal IHC



24 Abnormal IHC Cases

- 15 were absent MLH1 & PMS2
 - Mean age 72, 12/15 > 65
 - 3 or 4 suspicious for Lynch by age and FH

Probable Lynch

- 4 were absent MSH2 & MSH6
 - Mean age 58
 - 2 +FH, 1 +PH
- 4 were absent MSH6 alone
 - Mean age 53
- 1 was absent PMS2 alone



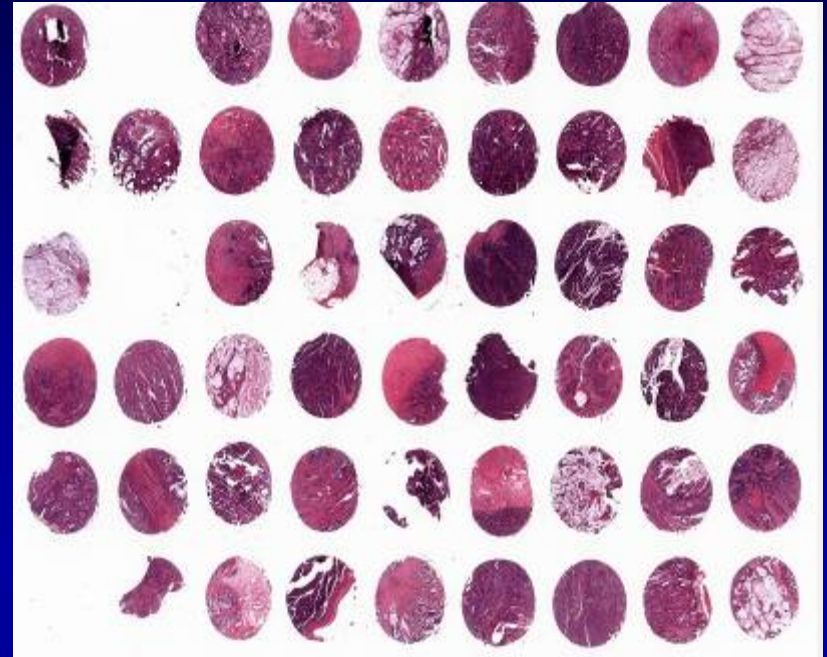
Self Evaluation

- Literature confirmed
- 24 MSI+ cases detected, improved prognosis
- 9 to 13 are suspicious for Lynch
- No patients initially seen by Cancer Genetics
 - Combination of reasons- patient and physician
 - Solution- Cancer Genetics to help contact all abnormalities with surgeons permission (Pathology notifies CG)
- Ethical dilemmas- who contacts (HIPPA, self referral), who is responsible if Lynch patient falls through the cracks, genetic testing????



Screening and Economics

- TMA approach for screening
- 168 unselected CRC
- 40 cases per TMA
- 4 MMR stains
- >99% correlation with whole sections
- Heterogeneous staining in 1 case; MSH2/MSH6





Summary CRC MSI / Lynch Syndrome

- The detection of MSI is important to identify LS and prognosis
- Identification is based upon history and laboratory testing
 - MSI and/or IHC
- OSU: MMR stains all primary CRC resections; Pathology, Surgery, Cancer Genetic and Oncology involved; MMR for EC, Gyn Onc request
- Economics and ethical issues
- Communication and team approach

Acknowledgements



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QUESTIONS