Colorectal Cancer Screening for Lynch Syndrome and MSI: Past, Present, and Future

PRESENTED BY

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USCAP 108TH ANNUAL MEETING
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Wendy L Frankel reported no relevant financial relationships.

Outline

- MSI and Lynch syndrome
- Past- History
  - Histology
  - Ordered by request only
- Present- Universal tumor screening
  - Reflex ordering
  - MSI by PCR
  - IHC
- Future- Universal tumor screening for Lynch and others
  - NGS

Past

Why is MSI Important?

- All MSI CRC patients better prognosis (sporadic and germline/Lynch)
- MSI CRC do not respond to 5FU-based chemotherapy
- Identification Lynch Syndrome (LS) helps patients/families
  - Colonoscopic screening ↓ CRC & death
  - LS patients risk 2nd primary (CRC & others)
  - LS patients' relatives benefit from testing

Jarvinen, 1995 and 2000; Ribic, NEJM 2003; Carethers, Gastroenterol 2004; Popat, J Clin Onc 2005; Lynch, Eur J Hum Genet 2006; Ward, J Pathol 2005; Jover, Gut 2006; Jover, Eur J Cancer 2008; Sargent, J Clin Onc 2010, ASCO; Des Guetz, EJC 2009

Lynch Syndrome

- Most common hereditary CRC syndrome
- 2-4% of CRCs
- Autosomal dominant, penetrance up to 80%
- Early, variable age at CRC diagnosis, 45 y/o
- Susceptibility to CRC & extracolonic cancers
- Germline mutation in genes belonging to DNA MMR family- *MLH1, MSH2, MSH6, PMS2, EPCAM*
- Mutations lead to defective DNA repair & MSI

Hampel, NEJM 2005; Lynch, Nature Rev Cancer 2015; Pai, Am J Surg Pathol 2016

Terminology and Timeline

- 1895 Warthin studied Family G (Univ Michigan)-1913 published
- 1966 Lynch published Family N and M
- 1971 Cancer Family Syndrome (CFS) coined by Henry Lynch
- 1984 CFS renamed Lynch syndrome (LS) by Boland
- 1984 HNPCC term introduced (to differentiate from FAP)
- Mid 1990s mismatch repair genes identified
- Late 1990s Lynch syndrome = families with known mutation in mismatch repair gene

Warthin, Arch Intern Med 1913; Lynch Arch Intern Med 1966; Lynch, Surg Gynecol Obstet 1971; Boland, Ann Intern Med 1984; Lynch, Nature Rev Cancer 2015

Terminology and Timeline

- 1991 Amsterdam I Criteria- clinical criteria to aid diagnosis LS
  - 1999 amended to include extracolonic (Amsterdam II)
- 1997 Bethesda Guidelines to select CRC that warranted MSI
  - 2004 revised
    - Decision to call it Lynch syndrome and not HNPCC
- 2005 Familial colorectal cancer type X (FCC-X) described
  - Meet Amsterdam, lack MMR defects
- 2006 Jeremy Jass wrote paper explaining differences between HNPCC, Lynch syndrome and FCC-X but misuse of terms continued
- 2013 Lynch-like syndrome used to describe patients with defective mismatch repair but no identified germline mutation in MMR genes

Vasen, Dis Colon Rectum 1991; Vasen, Gastroenterol 1999; Rodriguez-Bigas, J Natl Cancer Inst 1997; Umar, J Natl Cancer Inst 2004; Lindor, JAMA 2005; Jass, World J Gastroenterol 2006; Rodriguez-Soler, Gastroenterol 2013

How was Lynch Syndrome Diagnosed Past?

- Family history
- Amsterdam II criteria (CRC and others)
  - 3 cases of LS-associated cancers
  - 2 generations affected at least
  - 1 affected individual is a first-degree relative of the other 2
  - 1 diagnosed <50
- Bethesda
  - CRC dx <50
  - 3 cases of LS-associated cancers at any age
  - CRC + 1 relative with a LS-associated cancer dx <50
- Do not work very well even if with a good family history

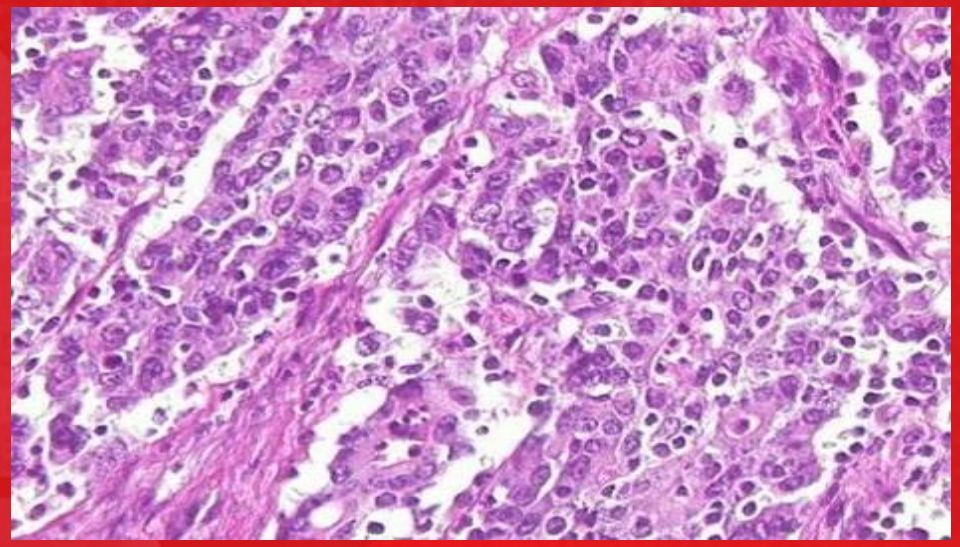
Detection of MSI and LS Patients

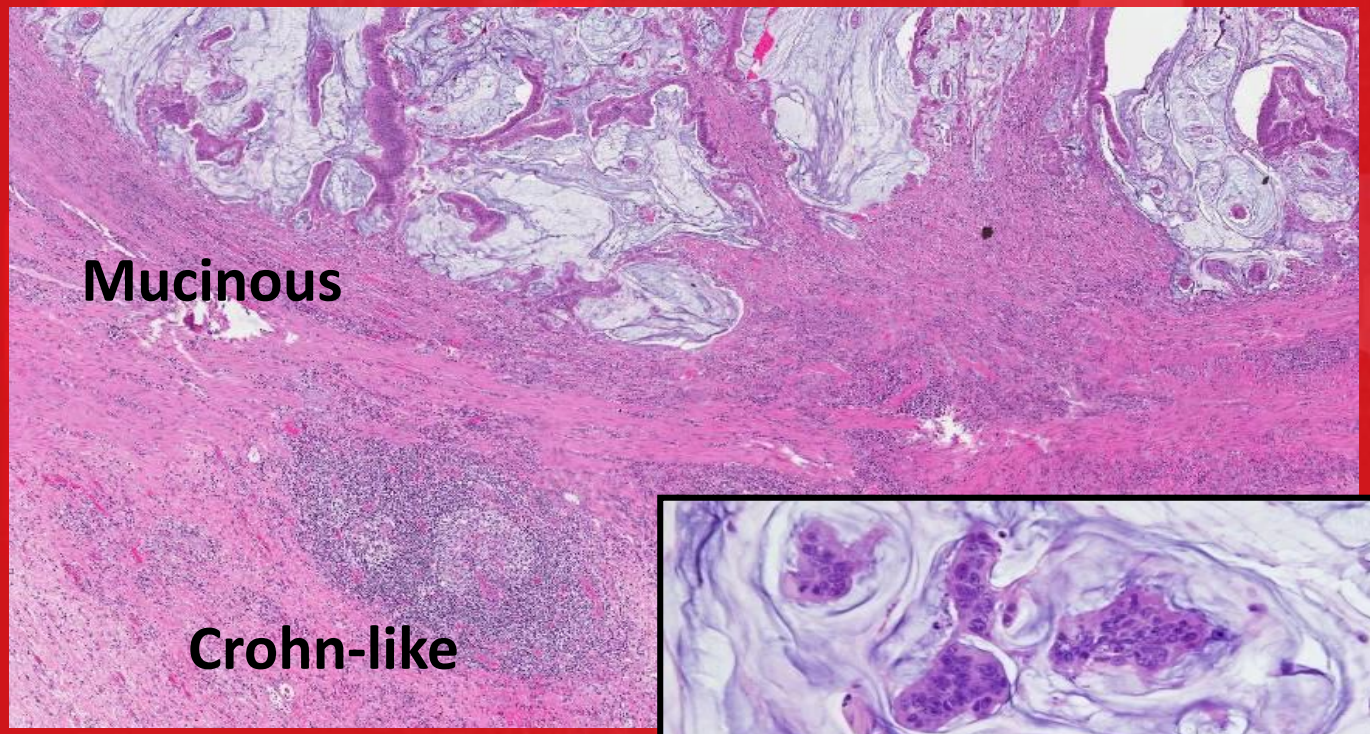
- History (LS)
  - Less useful; smaller families, polypectomy
  - Amsterdam & Bethesda < 50% sensitive
- Histology (MSI and Lynch)
  - Intratumoral lymphocytes (TIL)
  - Peritumoral lymphocytes (Crohn-like)
  - Mucinous & signet ring cells
  - Poorly differentiated, medullary

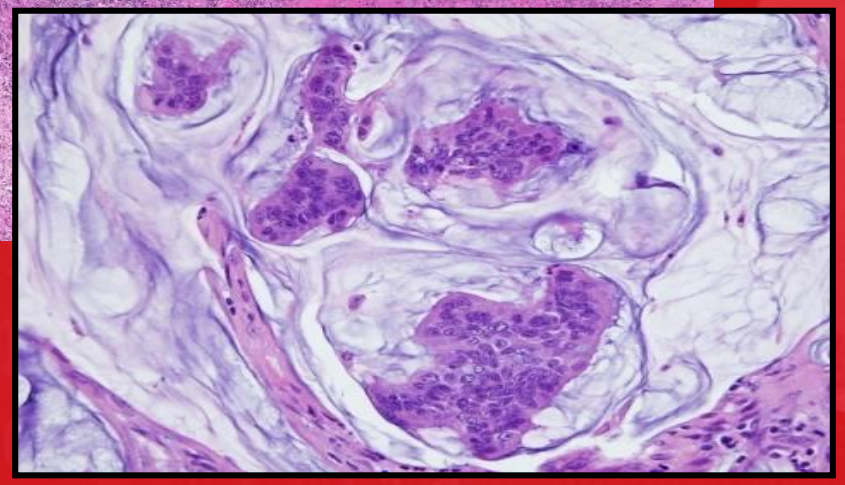
Jass , Gut 1998; Jass, Fam Cancer 2004; Alexander, Am J Pathol 2001; Yearsley, Hum Pathol 2006; Greenson, Am J Surg Pathol 2009

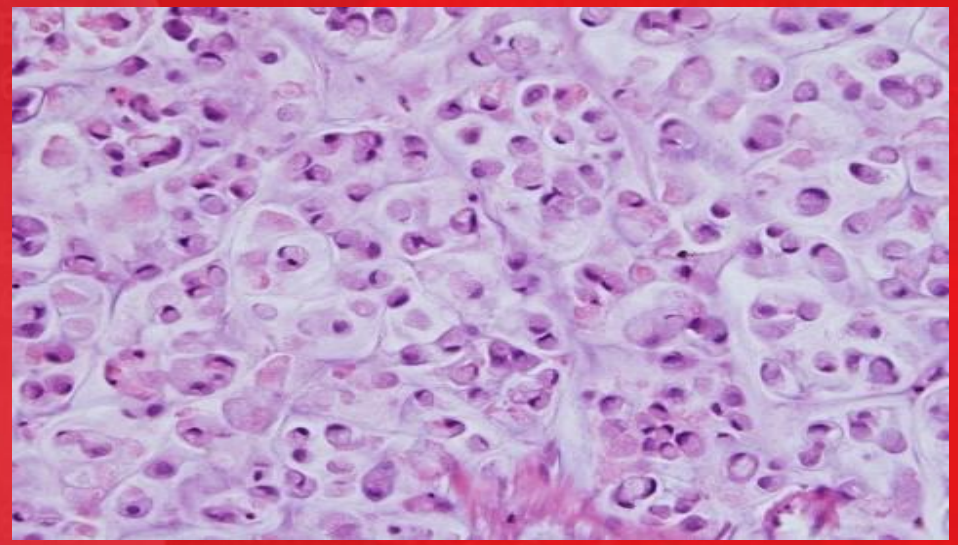
Histology MSI CRC

TILs



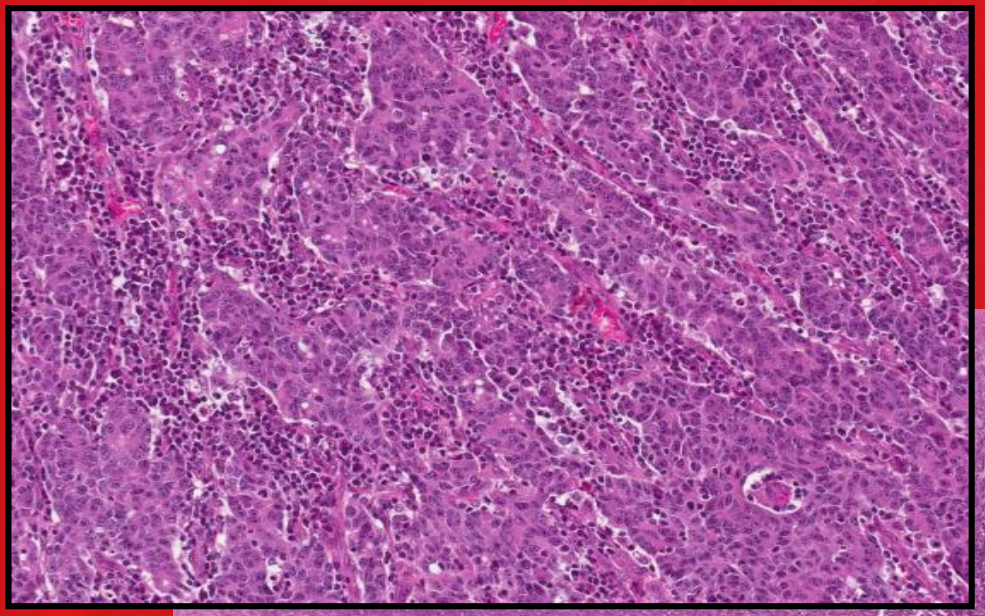


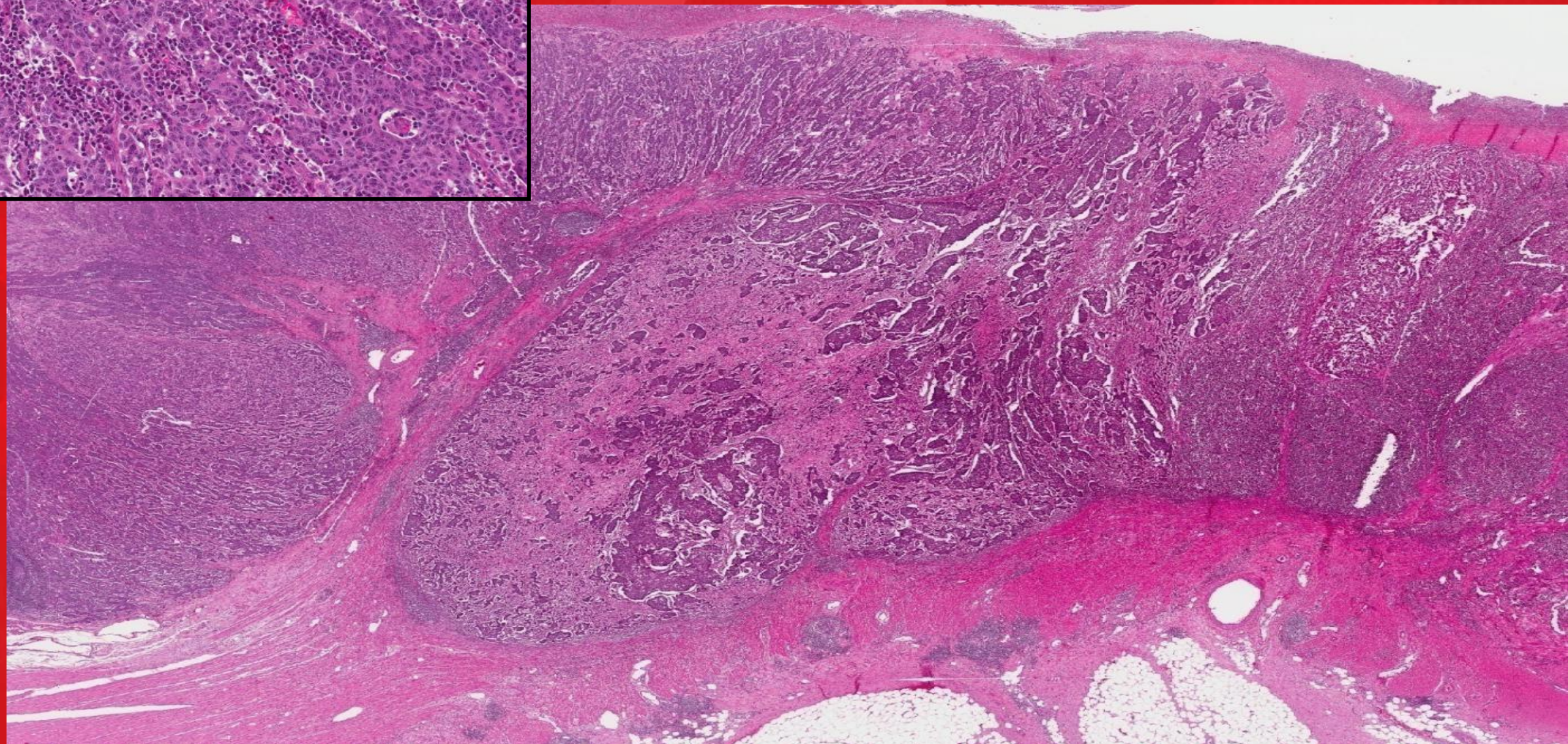




Signet ring cells

Histology and History not Enough to Identify MSI & LS





Past Concerns for Testing

- Is IHC genetic testing- do we need consent?
- Worry about follow-up if Pathologist reflex tests
- Worry whether biopsy is as good as resection
- Should primary or metastatic tumors be tested?
- How can we save money?
  - IHC for MLH1/MSH2; most common mutations
  - IHC for MSH6/PMS2; taking advantage heterodimer partners
- What is better IHC or MSI by PCR?

Live Content Slide

When playing as a slideshow, this slide will display live content

Poll: Which of the Following is True of Screening Colorectal Cancer for Lynch Syndrome:

What we Learned

- IHC is not genetic test (protein), many abnormals are somatic
- Reflex to *BRAF* or Methylation can save costs and anxiety
  - Methylation picks up more cases (68% methylated are *BRAF* mutated)
- Biopsies work as well as resections
  - Education essential for follow-up
- Metastases work as well as primaries
- If greater than 1 tumor, screen all if initial is MSS

Shia, Am J Surg Pathol 2011; Jin, Am J Clin Pathol 2013; Haroldsdottir Fam Cancer 2016; Roth, Am J Clin Pathol 2016

What we Learned

- 2 stain rather than 4 is not worthwhile
  - We will miss cases
- IHC and MSI by PCR similar rates of detection LS
- Double somatic is common cause of Lynch-like
  - Tumor testing essential

Haroldsdottir, Gastroenterol 2014; Pearlman, Mod Pathol 2018

Ohio- City and Statewide Results

- Citywide (Columbus) CRC
  - 44 LS out of 1566 (2.8%); 1 out of 35 unselected CRC
  - Average age 51(23-87); 50% >50
  - 25% not meet Amsterdam/Bethesda
  - 109/249 family members tested, LS
- Statewide (Ohio) CRC
  - 191 LS out of 3309 (4.3%); 1 out of 25; average age 60 (17-96)
  - 1 /14 (7%) at least 1 hereditary cancer syndrome
  - <50 years old (or suspicious) need genetics referral, panel testing
    - 1/6 (16%) <50 have genetic syndrome
- IHC and MSI by PCR both work well (similar results)

Hampel, NEJM 2005; Hampel, J Clin Oncol 2008; Sachak, Mod Path ab 2018; Pearlman, Nat Soc Gen Counselor ab 2017

Present

Impact- Ohio Study and Others

- Universal tumor screening is feasible
- Universal tumor screening is cost effective
- Universal tumor screening recommended by
  - Evaluation of Genetic Applications in Practice & Prevention (CDC) since 2009
  - NCCN since 2013
  - US Multi-society Task Force on CRC since 2014
  - Society for Gynecologic Oncology & ACOG since 2014
  - Healthy People 2020 goal: Increase # of newly diagnosed CRC patients screened for LS at dx
- Histologic features of MSI no longer in CAP CRC synoptic

Mvundura, Genet Med 2010; Grosse, Genetic in Med 2015; EGAPP, Genet Med 2009; Giardiello, Am J Gastroenterol 2014; ACOG & SGO Practice Bulletin Number 147, 2014

Universal Tumor Screening (UTS)

- 2012; Slow adoption- 80 institutions
  - 38% UTS, big gap
    - 71% NCI CC Centers
    - 36% COS accredited community hospital CC programs
    - 15% community hospitals
- 2018; Progress- 96 institutions surveyed (59 academic)
  - 86% academic and nonacademic UTS- no gap
  - 76% IHC, 20% PCR (most used with IHC; decreased PCR)
  - 9% UTS extra-colonic GI

Beamer, JCO 2012; Hissong, Mod Pathol 2018

Why is MSI Important?

- All MSI CRC patients better prognosis (sporadic and germline/Lynch)
- MSI CRC do not respond to 5FU-based chemotherapy
- Identification Lynch Syndrome (LS) helps patients/families
  - Colonoscopic screening ↓ CRC & death
  - LS patients risk 2nd primary (CRC & others)
  - LS patients' relatives benefit from testing

- **MSI predictive of response to PD-1 inhibitors (immune therapy with checkpoint blockade using pembrolizumab)**

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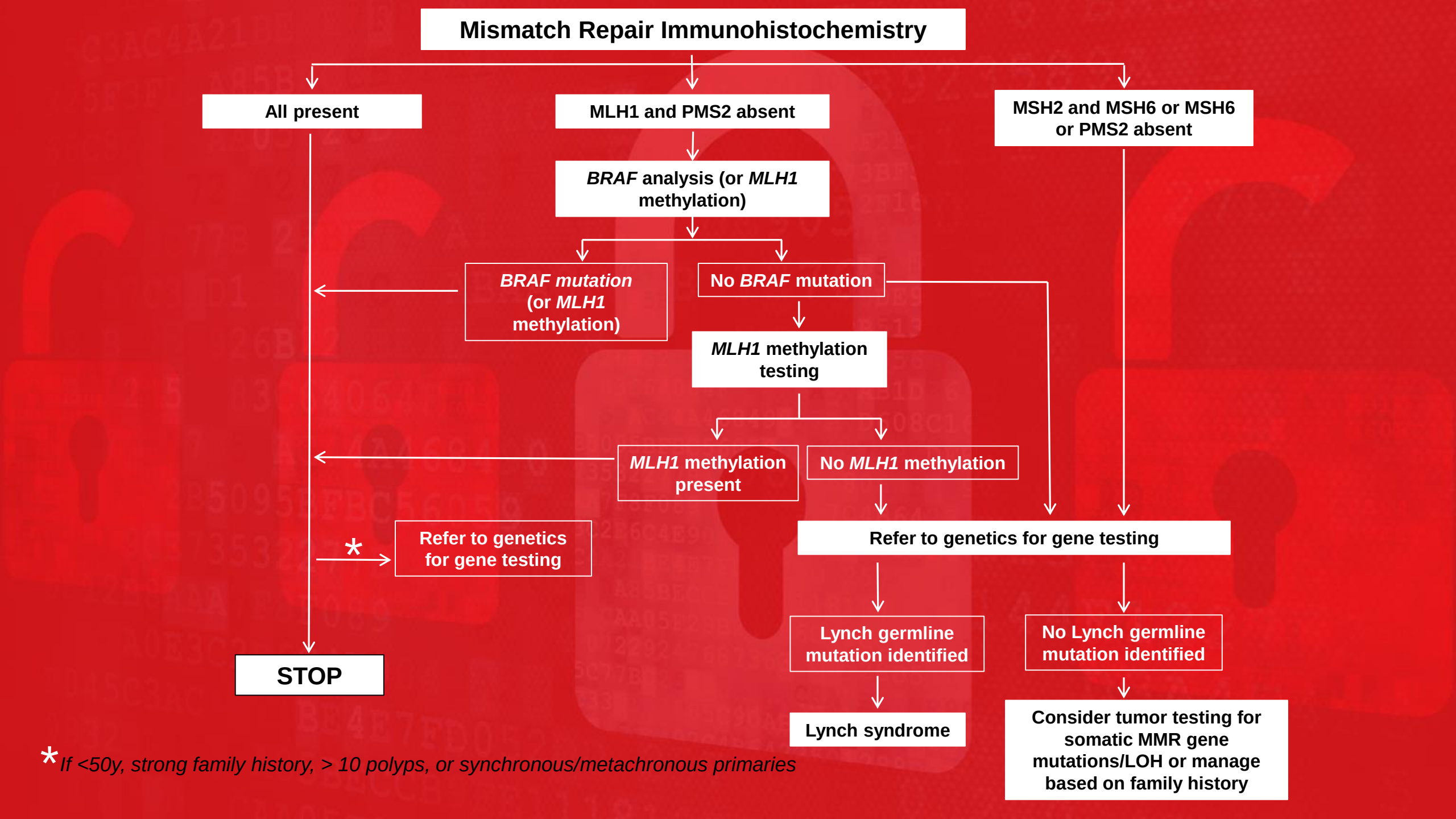
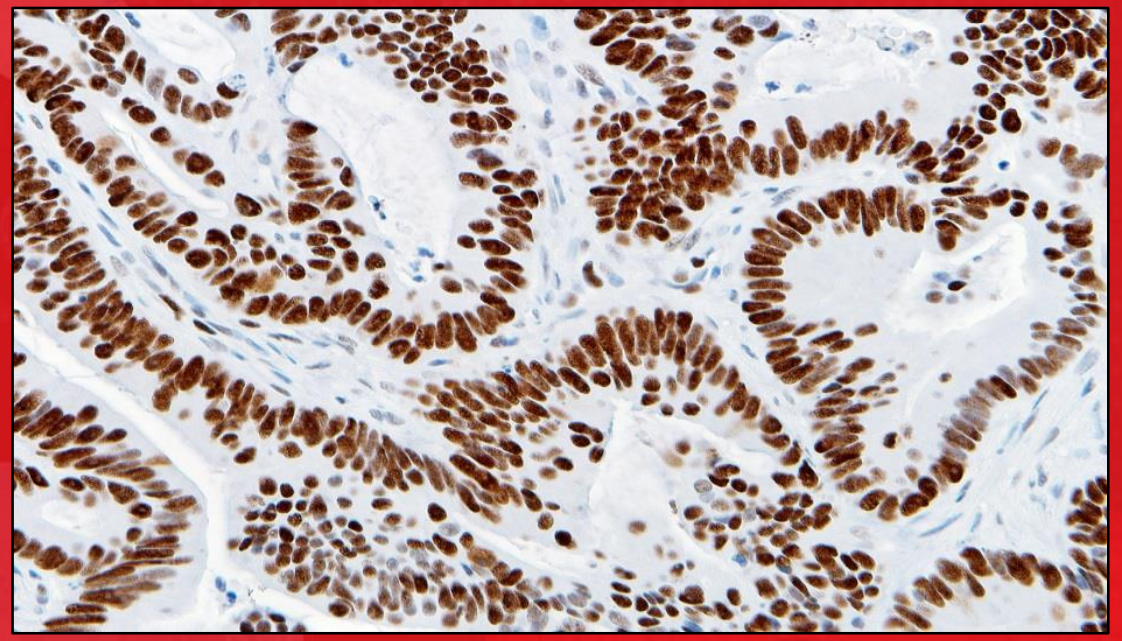
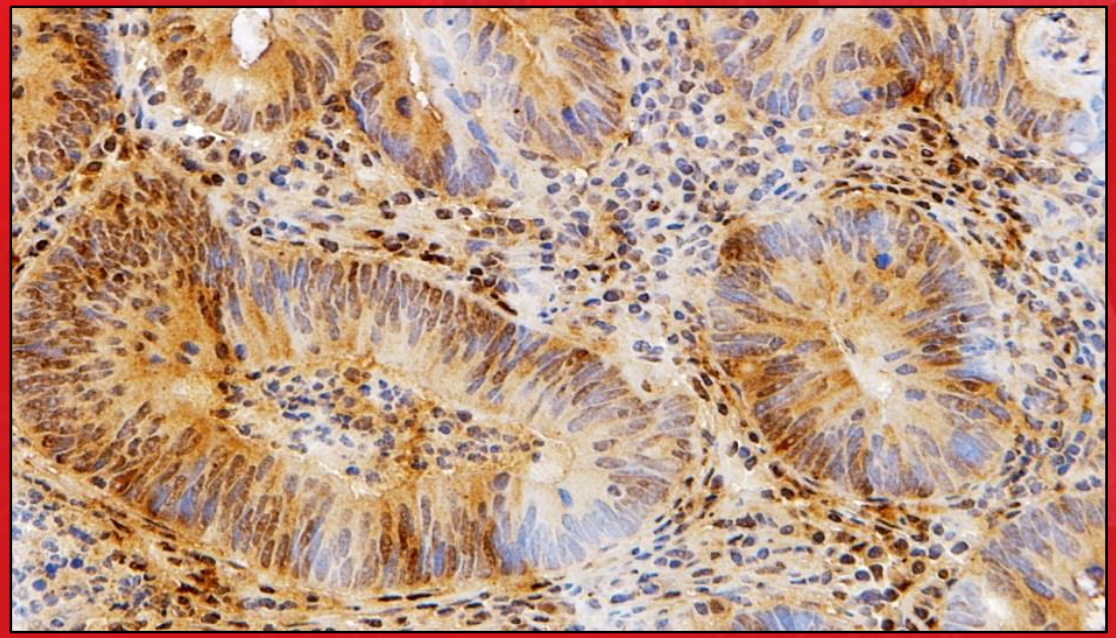
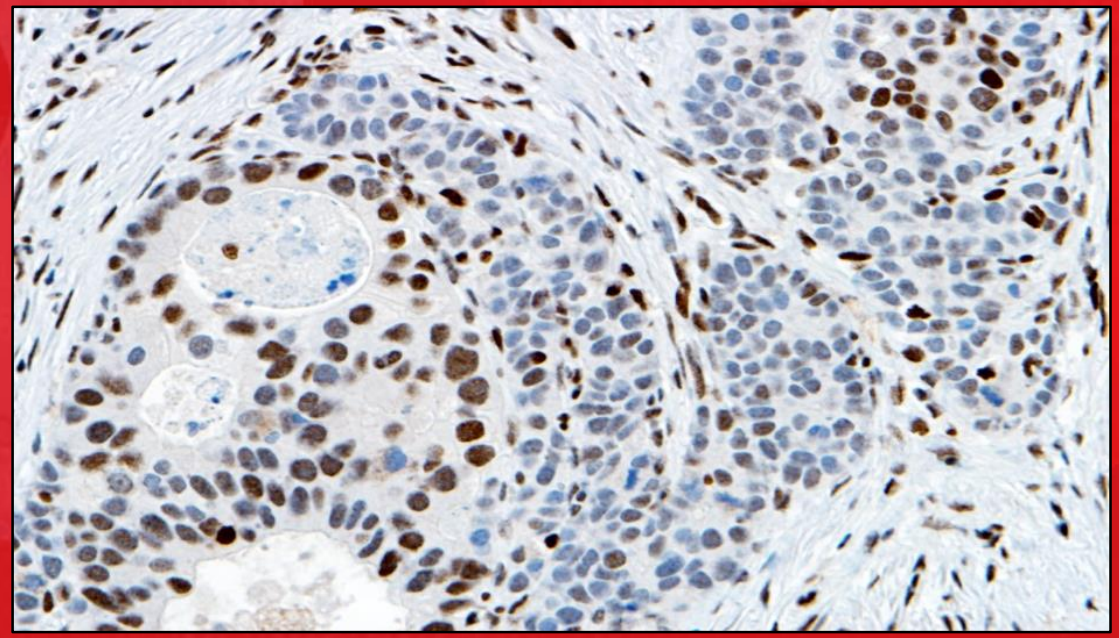

Mismatch Repair Immunohistochemistry
All present
MLH1 and PMS2 absent
MSH2 and MSH6 or MSH6 or PMS2 absent
BRAF analysis (or MLH1 methylation)
BRAF mutation (or MLH1 methylation)
No BRAF mutation
MLH1 methylation testing
MLH1 methylation present
No MLH1 methylation
*
Refer to genetics for gene testing
Refer to genetics for gene testing
STOP
Lynch germline mutation identified
No Lynch germline mutation identified
Lynch syndrome
Consider tumor testing for somatic MMR gene mutations/LOH or manage based on family history
* If <50y, strong family history, > 10 polyps, or synchronous/metachronous primaries

IHC MMRP- Interpretation

- Strong, diffuse nuclear
- Variability
- Cytoplasmic staining
- Tissue and fixation
  - Controls important

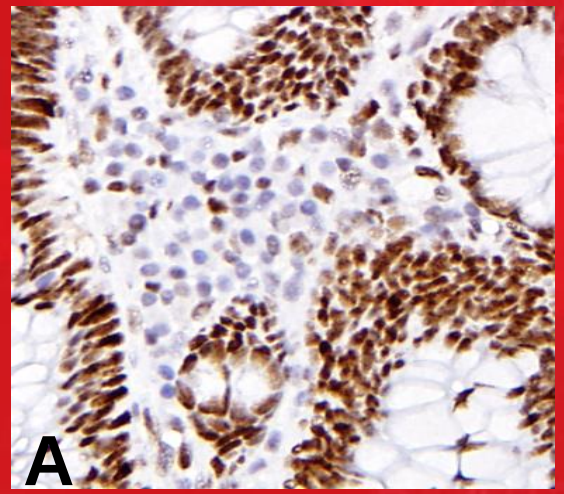


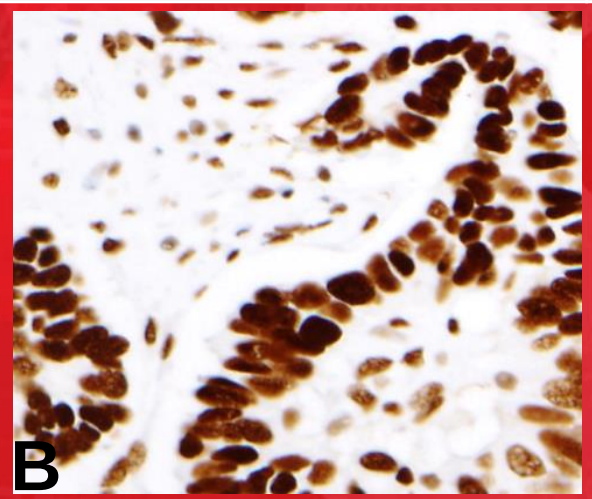


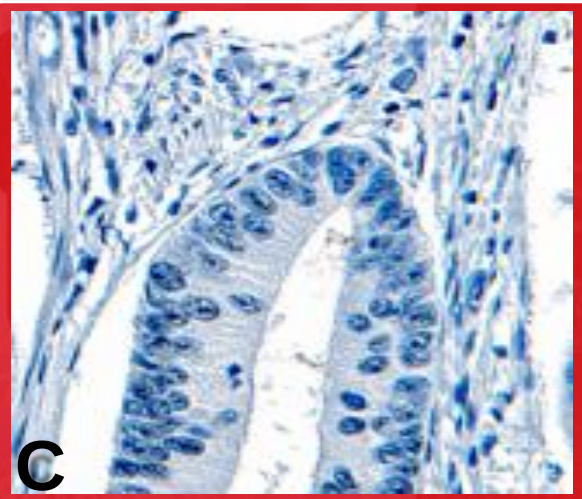


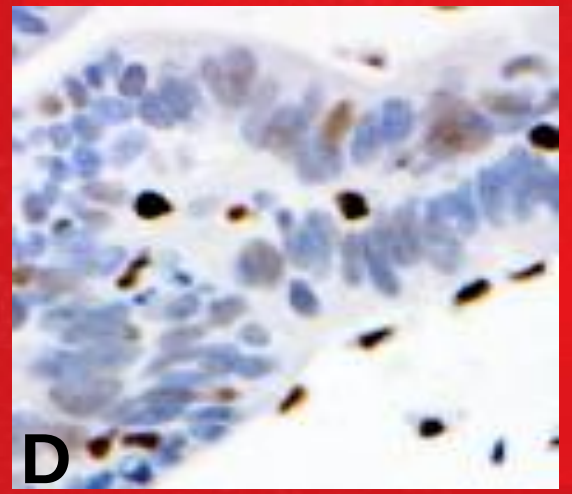
Control Is the Key

Do's	Don'ts
Check Internal Control!!! (Nuclear reactivity in normal crypts, lymphocytes, and stromal cells)[A]	Call absent/lost staining, if internal control cells are not staining[C]
Ok to call present/intact staining, if tumor stains stronger than control[B]	Call present/intact staining, if tumor stains weaker than control[D]









IHC MMRP- Interpretation and Reporting

- Report as present or absent not + or -
- Resection or biopsies?
  - Stains work well on both
  - Advantage biopsies- may do different surgery if LS
  - Disadvantage biopsies- risk lost to follow-up
- Cutoff- any convincing nuclear staining
  - 1%, 5% (we use this)
  - 10%
  - Must be as strong as control

Shia, Am J Surg Pathol 2011; Bartley, CAP Colon Rectum Biomarker Protocol, Arch Pathol Lab Med 2014; Pai, Am J Surg Pathol 2016; Chen, Mod Pathol In press

MMR IHC Interpretation Challenges

Unusual IHC	Action
Cytoplasmic staining	Repeat if nucleus obscured; Call absent
Tumor weaker than control	Check control; Repeat; Call equivocal/absent
Post-neoadjuvant therapy abnormal staining	Repeat; Test pre-treatment Bx; Call present/intact
Heterogeneous tumor staining	Check control; Check for edge artifact; Call present/intact

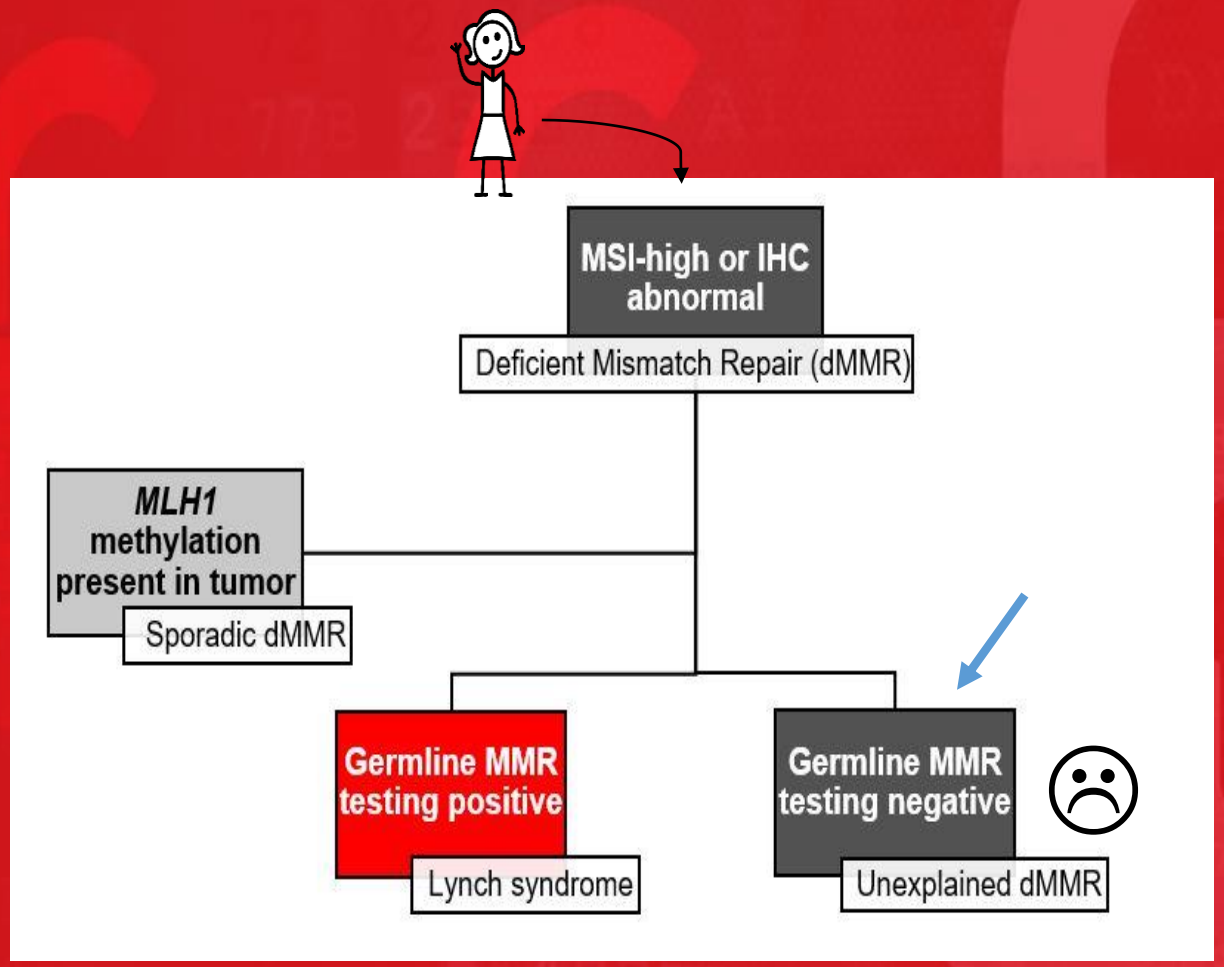
Bao, Am J Surg Pathol 2010; Radu, Hum Pathol 2011; Shia, Mod Pathol 2013; Graham, Am J Surg Pathol 2015; Pai, Am J Surg Pathol 2016; Kuan, Hum Pathol 2017; Chen, Diagn Pathol 2017; Markow, Surg Pathol Clinics 2017; Pearlman, Mod Pathol 2018

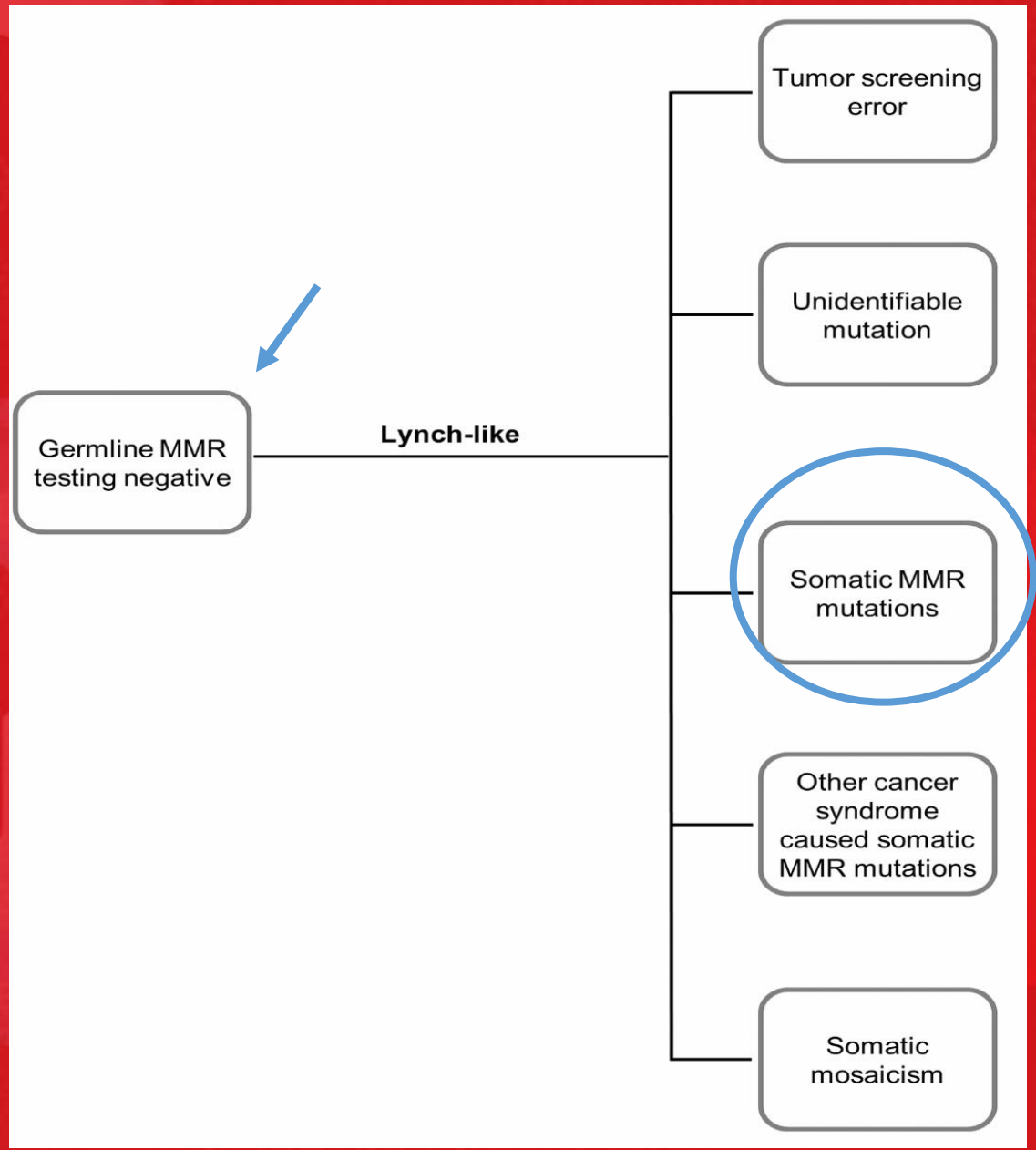
Problems in Interpretation

- MMRP present but 40yo, family hx, suspicious features
  - If MSI+ and MMR mutation found
    - Possibly protein present but not functional (missense)
- MMRP lost, gene mutation or methylation not found
  - “Lynch-like”- waste basket term
    - Double somatic mutation in tumor **(no need intensive family screening)**
    - Others possibly LS, limited by technology (inversions,..)
    - Other germline defects
    - Errors

Haroldsdottir, Gastroenterol 2014; Chen , USCAP 2019, Poster Session VI, GI, #103

Causes of Mismatch Repair Deficiency (dMMR)





Haroldsdottir, Gastroenterol 2014; Hemminger, Hum Pathol 2018

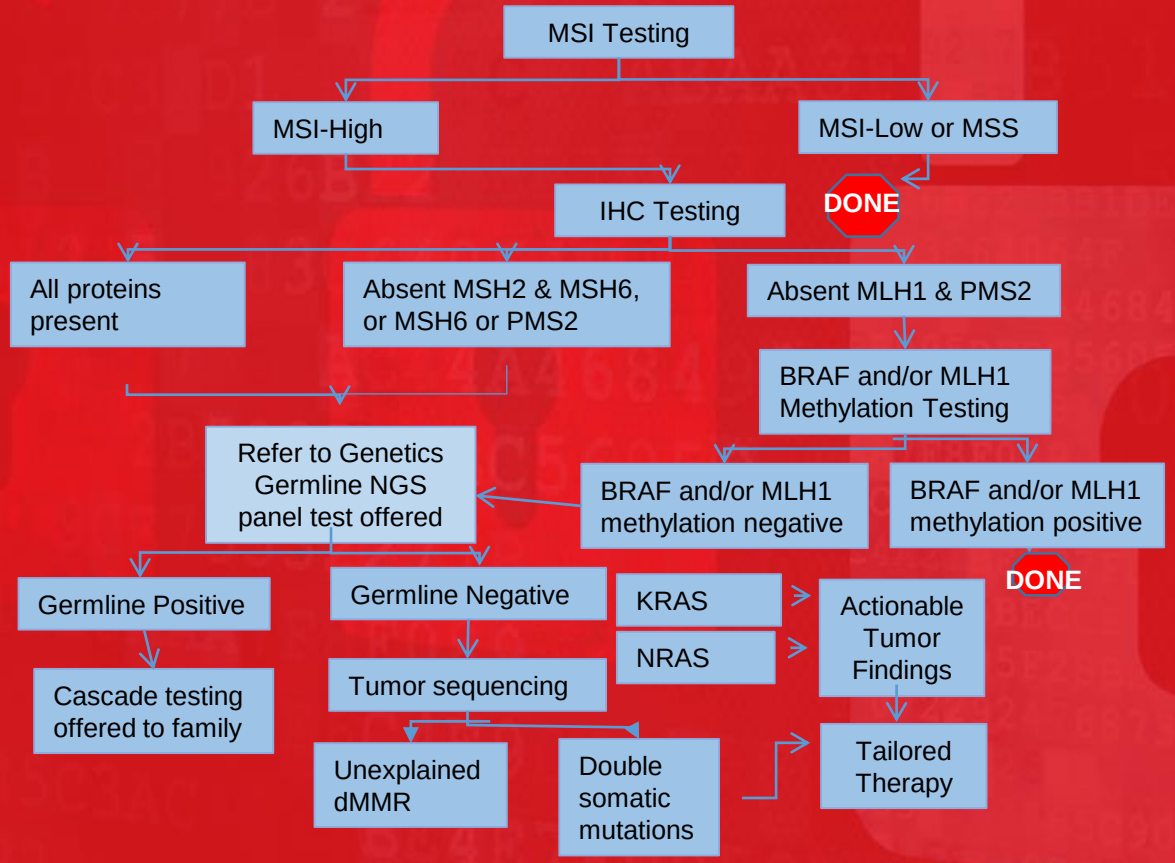
Future: It is Here

What if we Flip the Whole Paradigm?

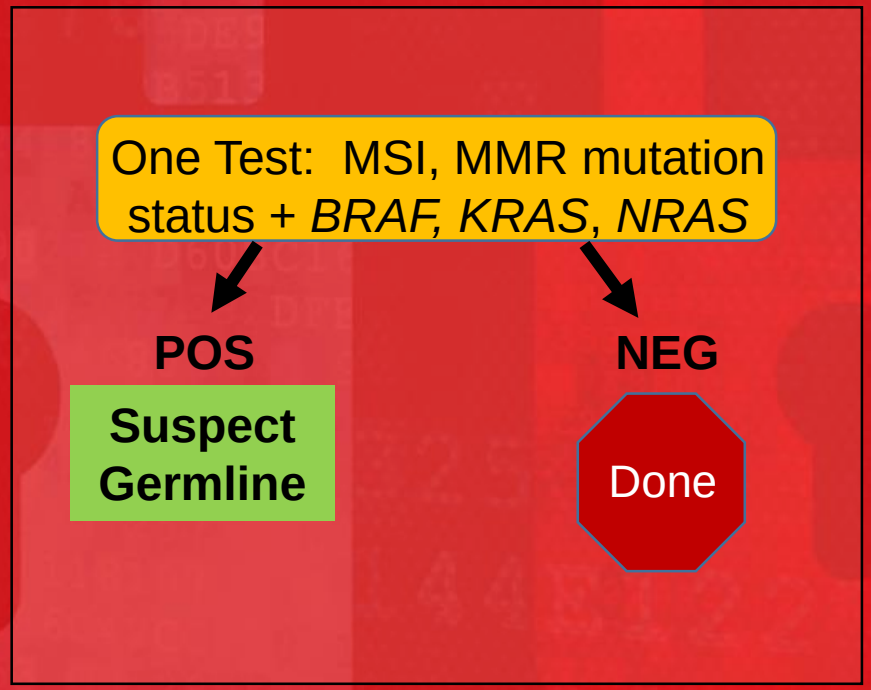
- Targeted tumor sequencing first
  - Detect *KRAS*, *NRAS*, *BRAF* mutations used in treatment stage IV CRC
  - Can detect MSI status by NGS profiling of multiple microsatellites
  - Test 4 MMR genes
- Allele fraction of mutations can tell if likely germline or somatic
  - Germline mutations have an allele fraction of ~50%
  - Somatic mutations may have allele fraction significantly less than 50% (depending on tumor %)
- Would only need to order single mutation analysis to rule out or suspect a germline mutation

Tumor Sequencing as First-Line Lynch Screening Simplifies Testing

- TRADITIONAL SCREENING



- TUMOR NGS SCREENING



Hampel, JAMA Oncol 2018

Tumor Sequencing Project

- 419 OCCPI patients underwent tumor sequencing in parallel to all standard universal tumor screening for LS
- 46 additional LS patients included to ensure adequate detection of germline MMR gene mutations
- Tumor sequencing performed for all 465 cases at UW
  - Determined *KRAS*, *NRAS*, and *BRAF* mutation status along with other potential therapeutic targets
  - Determined MSI status
  - Used *BRAF* mutation status for *MLH1* methylation
  - Assessed MMR genes for potential germline mutations

Hampel, JAMA Oncol 2018

A Brave New World: Tumor Sequencing Upfront?

Table 2. Analytic Validity of Tumor Sequencing for the Detection of Microsatellite Instability (MSI) and Lynch Syndrome (LS) Compared With MSI and Immunohistochemical (IHC) Staining Followed by *BRAF* Testing

Parameter	Tumor Sequencing Including *BRAF*	MSI + *BRAF*	IHC + *BRAF*
MSI-high detection, % (95% CI)			
Sensitivity	100 (95.3-100)	100 [Reference]	98.3 (94.0-99.8)
Specificity	99.7 (98.4-100)	100 [Reference]	99.7 (98.4-100)
PPV[a]	98.7 (91.5-99.8)	100 [Reference]	99.2 (94.3-99.9)
NPV[a]	100	100 [Reference]	99.4 (97.8-99.9)
LS mutation detection, % (95% CI)			
Sensitivity	100 (93.8-100)	91.4 (81.0-97.1)	89.7 (78.8-96.1)
Specificity	95.3 (92.6-97.2)	94.8 (92.2-96.8)	94.6 (91.9-96.6)
PPV[a]	40 (29.8-51.1)	34.4 (25.0-45.1)	33.3 (24.3-43.7)
NPV[a]	100 (99.1-100)	99.7 (98.3-100)	99.7 (98.3-100)
LS cases missed, No.	0	5	6

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

[a] Calculated using only the prospective cohort because disease prevalence can affect these measures.

Analytic Validity of Tumor Sequencing for the Detection of Microsatellite Instability (MSI) and Lynch Syndrome (LS) Compared With MSI and Immunohistochemical (IHC) Staining Followed by *BRAF* Testing

Hampel, JAMA Oncol 2018

Additional Benefits

- *DPYD* mutation detection
  - 8 patients found to have pathogenic variants in this gene that can cause toxic reactions to 5-FU based chemotherapy
- *KRAS*, *NRAS*, and *BRAF* mutation status along with other potential therapeutic targets
- Ability to identify germline mutations in other cancer susceptibility genes
  - 8 patients identified from tumor sequencing that turned out to have germline mutations in non-MMR genes
- Uses less tumor tissue – more left for other testing if necessary

Hampel, JAMA Oncol 2018

Number of Cases Needing Genetic Counseling Follow-up

- More difficult to be confident about double somatic calls vs. possible germline mutations with second hits
- If only screening for Lynch syndrome, similar numbers of patients would need to be seen by genetics
  - Tumor NGS = 30; TuNGS + *MLH1* methylation = 21
  - MSI + *BRAF* = 32; MSI + *MLH1* methylation = 26
  - IHC + *BRAF* = 33; IHC + *MLH1* methylation = 26
- If include other cancer genes with putative germline mutations, this number doubles
  - 30 for LS + 32 for other genes = 62 requiring genetic counseling

Cons of Upfront Tumor Sequencing

- Most commercial tumor testing laboratories NOT looking for germline genetic mutations
  - Detection of large rearrangements may be poor
  - Detection of *PMS2* mutation in exons 12-15 complicated by pseudogene interference
- Having *BRAF* instead of *MLH1* methylation incorporated leaves a lot of cases for genetic counseling
- Cost of tumor sequencing still high
  - Could be cost-effective because it reduces total number tests done in subset patients
- Turn-around time longer than for IHC alone
- Small tumors may fail tumor sequencing
  - IHC could still be done as back-up test

Future is Now

- Other cancer syndromes can be diagnosed
- Other targets may be found
- Approximately 16.3% of MSI tumors (all sites) are Lynch syndrome (Latham, 2018)
  - Should all tumors be screened?
- Vaccines for treatment and prevention LS associated tumors?
  - Mutations in microsatellite sequences may cause shifts of translational reading frames
  - Lead to generation of potentially immunogenic frameshift peptides
  - Host immune system may recognize these targets

Von Knebel Doeberitz, Familial Cancer 2013; Latham, J Clin Oncol 2018

Conclusions

- Screening CRC for LS is essential
- Paired tumor/normal tumor testing would be better test but…
  - No way to provide pre-test genetic counseling to every patient
  - Consider pre-test counseling video; post-test counseling for positives
- Upfront tumor sequencing alone solves consent problem
- This could be done now for Stage IV colorectal cancer patients
- MSI or IHC are sufficient for now but will likely be replaced by targeted tumor sequencing

Cancer Genetics OSUWMC/James/CCC

- Heather Hampel, MS, LGC
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- Richard Goldberg, MD
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- Dan Jones, MD, PhD, Weiqiang Zhao, MD, Tom Prior, PhD
- Debbie Knight, MS, Ahmet Yilmaz, Jason Bacher, Kristin Miller
- Ilene Lattimer, RN, OCN, CCRC
- Colin Pritchard, MD, PhD

THANK YOU

Questions?