Understanding Your Pathology Report and What to Do Next: Challenging Topics and Their Clinical Relevance

# Challenges in Colorectal Cancer Lynch Syndrome Screening

***Wendy L. Frankel***
*Chair Pathology, Director GI/Liver Fellowship*
***Peter P. Stanich***
*Gastroenterology, Director GHN Section of Intestinal Neoplasia and Hereditary Polyposis*

The Ohio State University
Wexner Medical Center

# The Speakers have no Conflicts to Disclose

# Objectives

1. To review microsatellite instability in colorectal cancer.
2. To understand the importance of clear reporting in Lynch syndrome screening.
3. To know the importance of communication between pathologists and gastroenterologists.

# Outline

- **Case presentation**
- **CRC and Lynch syndrome (LS) screening**
  - Young patients
  - Causes other than LS or methylation for mismatch repair deficiency (Lynch-like)
    - Double somatic mutations
- **Clinical implications**

# Case- Clinical

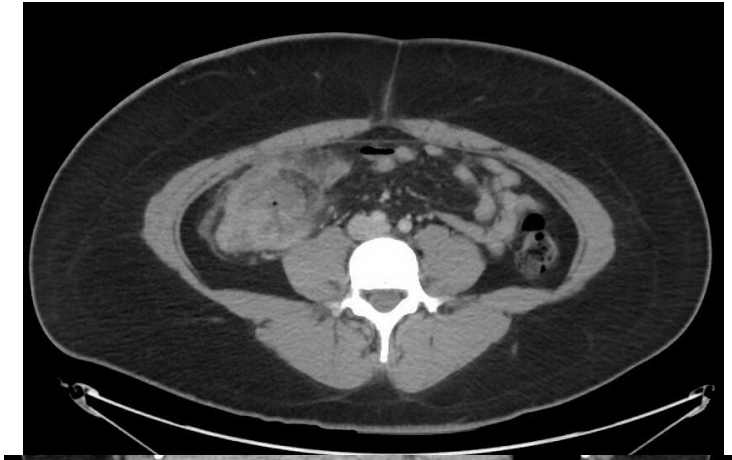
27 year old woman

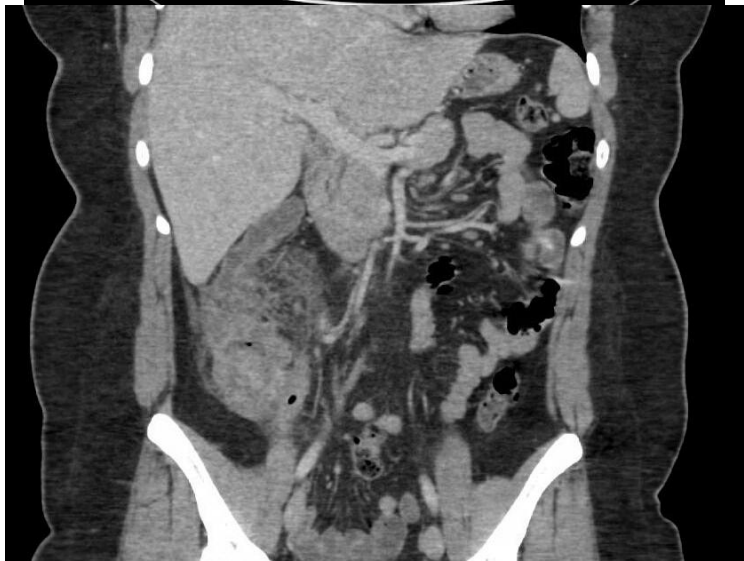
- Presented to ER with abdominal pain and fevers
- CT scan with ascending colon inflammation
- Discharged with antibiotics

- Returned after 4 weeks with similar complaints

# Repeat CT Scan

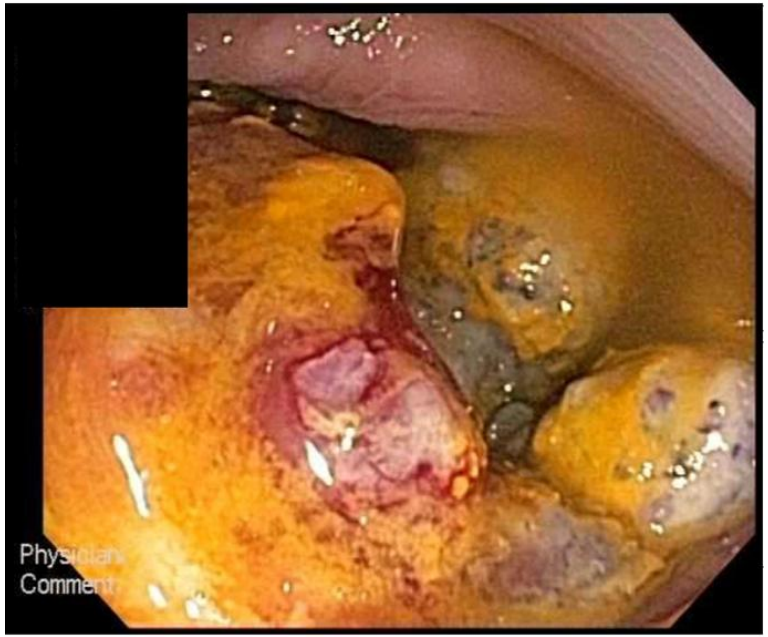
Findings compatible with nonspecific post-inflammatory changes and enterocolitis or typhilitis involving primarily the cecum which may be secondary to infectious or inflammatory etiology, including Crohn’s disease.  Neoplastic process less likely, but not completely excluded.  Ovarian cyst. Clinical correlation and follow-up suggested.



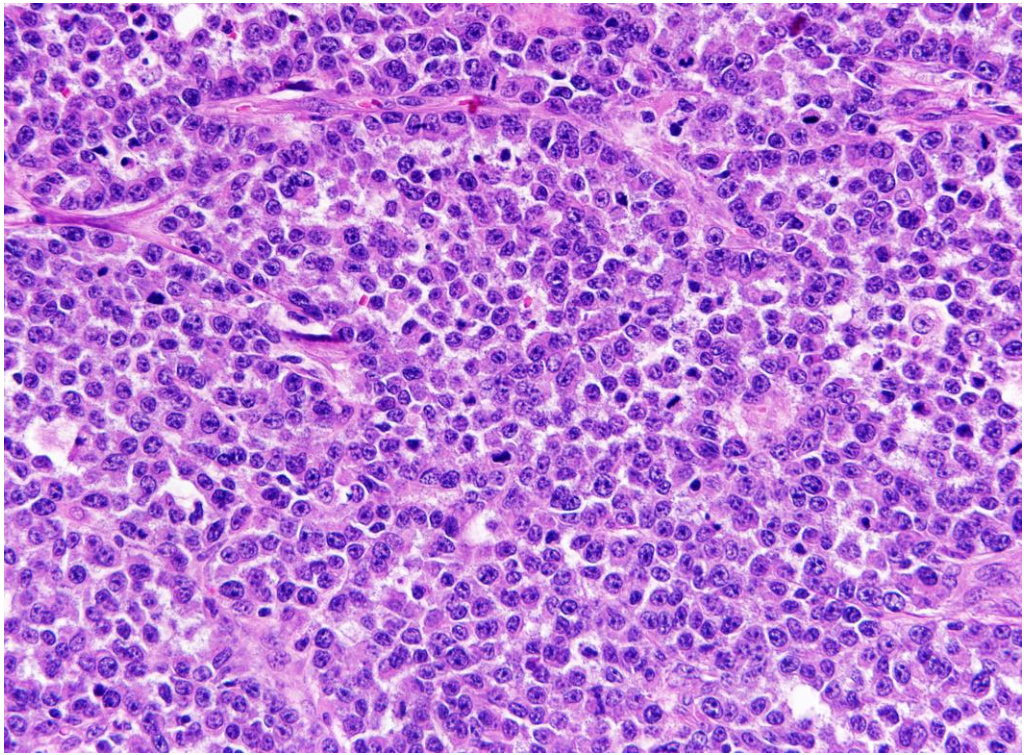


# Case

- Colonoscopy
  - Cecal mass
- Biopsy

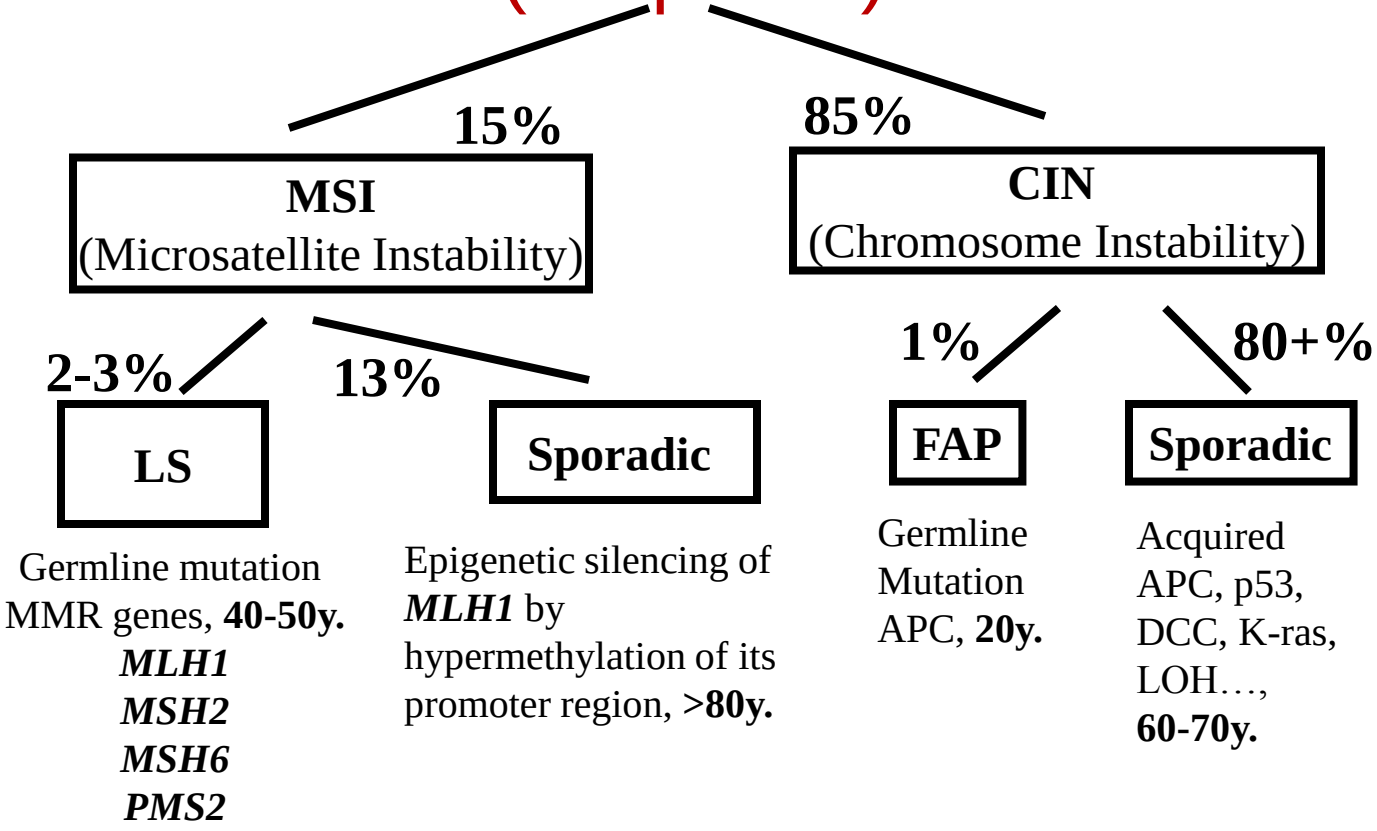


# Pathology- Poorly Differentiated Adenocarcinoma



(+) Cytokeratin AE1/3, Cytokeratin 20; (-) Cytokeratin 7, Chromogranin and Synaptophysin

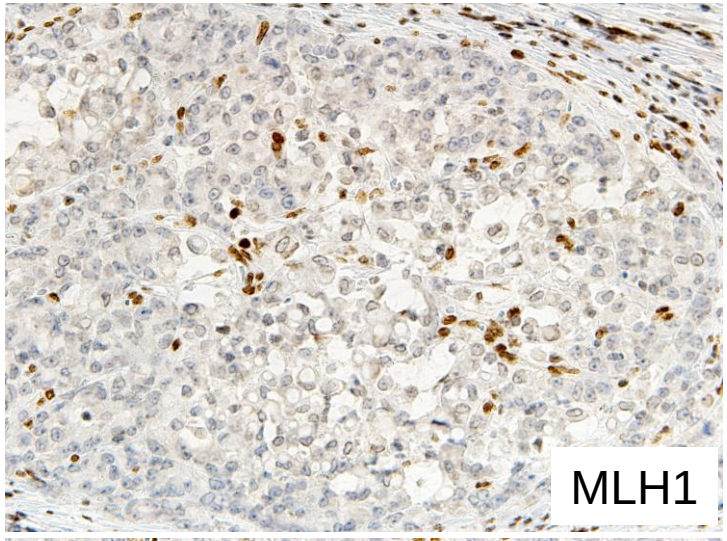
# Colorectal Cancer (simplified)

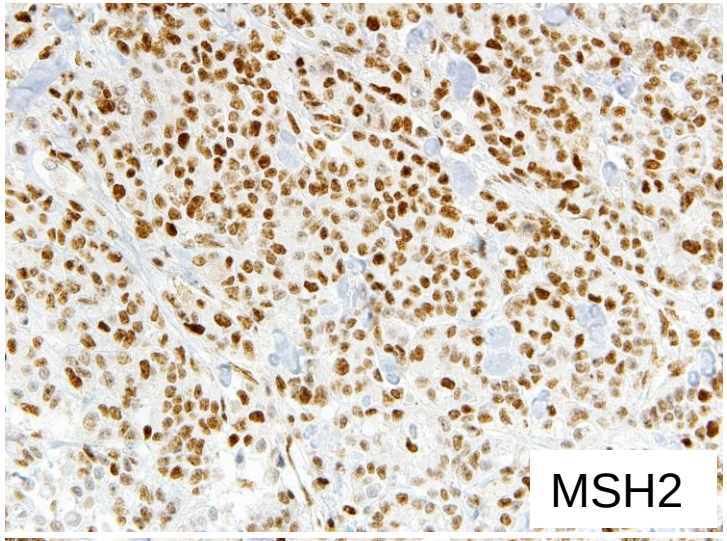


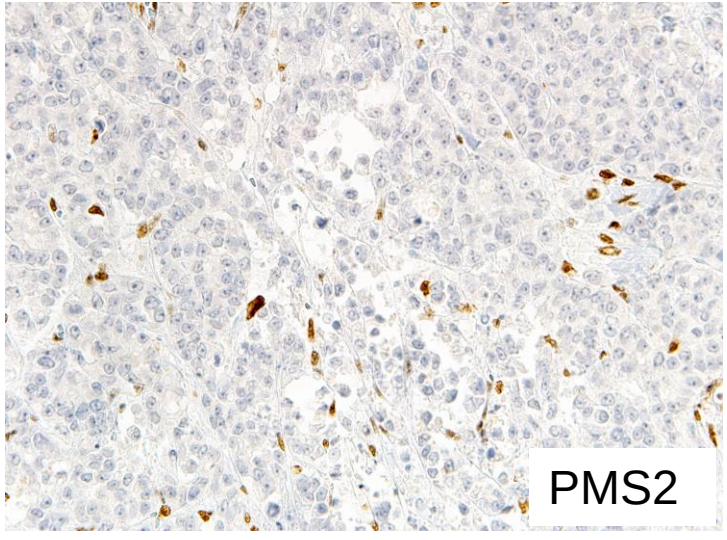
# Why is MSI Important?

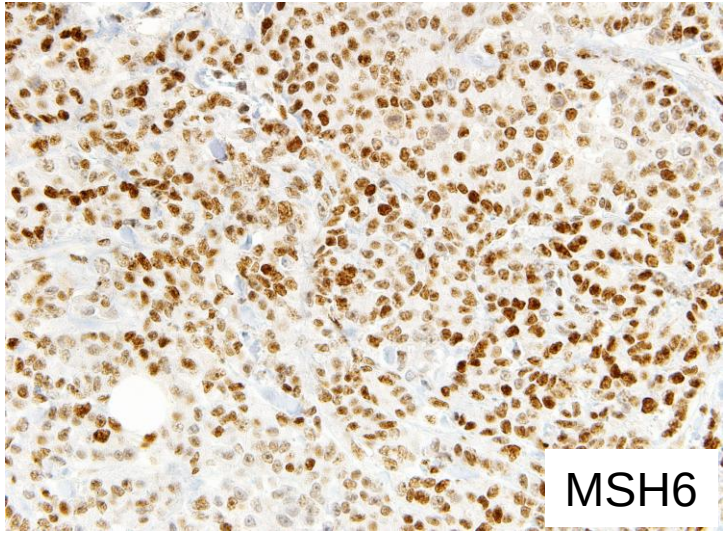
- MSI- Deficient DNA MMR, instability, ↑↓ repetitive nucleotide sequences
- All MSI CRC patients better prognosis (sporadic and germline/Lynch)
- Identification Lynch Syndrome (LS) helps patients/families
  - Colonoscopic screening ↓ CRC & death
  - LS patients risk 2nd primary (CRC & others)
  - LS patients' relatives benefit from testing
- Predictive/treatment
  - MSI CRC do not respond to 5FU-based chemotherapy
  - MSI predictive of response to PD-1/PD-L1 inhibitors (immune therapy with checkpoint blockade using pembrolizumab)

Ribic, NEJM 2003; Carethers, Gastroenterol 2004; Popat, J Clin Onc 2005; Lynch, Eur J Hum Genet 2006; Ward, J Pathol 2005; Jover, Gut 2006; Sargent, J Clin Onc 2010; Des Guetz, EJC 2009; Le, NEJM, 2015


MLH1


MSH2


PMS2

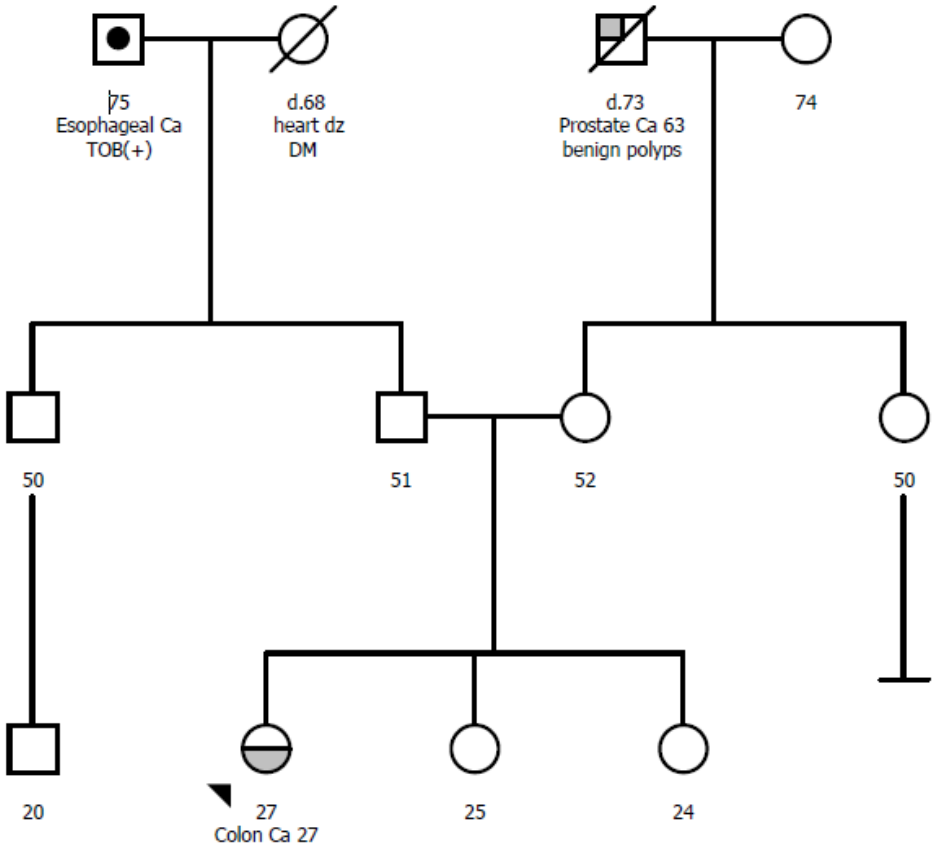

MSH6

# Case

- Patient referred to colorectal surgery
  - Concern for Lynch syndrome
  - Discussed total colectomy with possible hysterectomy and oophorectomy given ovarian cyst if Lynch syndrome

- Referred to Cancer Genetics prior to surgery
  - Personal and tumor testing concerning for Lynch syndrome
  - Family history not consistent with inherited cancer syndrome

# Pedigree


75
Esophageal Ca
TOB(+)
d.68
heart dz
DM
d.73
Prostate Ca 63
benign polyps
74
50
51
52
50
20
27
Colon Ca 27
25
24

# Lynch Syndrome

- Most common hereditary CRC syndrome
  - ~ 4% of CRCs
- Autosomal dominant
- 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 Reviews, March 2015; Pai, Am J Surg Pathol, 2016

# Cancer Risk in Lynch Syndrome

- **Cancer risks dependent on mutation**
- Colon and endometrial are highest risk, but multiple other cancers are associated

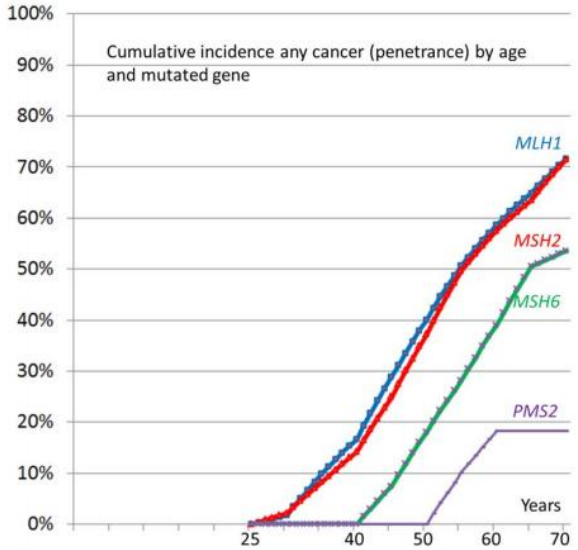



**Figure 1** Calculated cumulative incidences by age and mutated gene for any cancer.

| | MLH1 & MSH2 | MSH6 & PMS2 | Gen Pop |
|---|---|---|---|
| CRC | 52 - 82% | 10 - 20% | 4.5% |
| EC | 25 - 60% | 15 - 26% | 2.7% |

National Comprehensive Cancer Networks Clinical Practice Guidelines in Oncology, 2018 and Moller et al. Gut 2017.

# Ohio- City and Statewide Results

- Citywide (Columbus)
  - 44 LS out of 1566 (2.8%)
  - Average age 51(23-87), 50% >50
  - 25% not meet Amsterdam/Bethesda
  - 109/249 family members tested, LS
- Statewide (Ohio)
  - 191 LS out of 3309 (4.3%), average age 60 (17-96)
  - 1/14 (7%, 231) at least 1 hereditary cancer syndrome
- IHC and MSI by PCR both work well

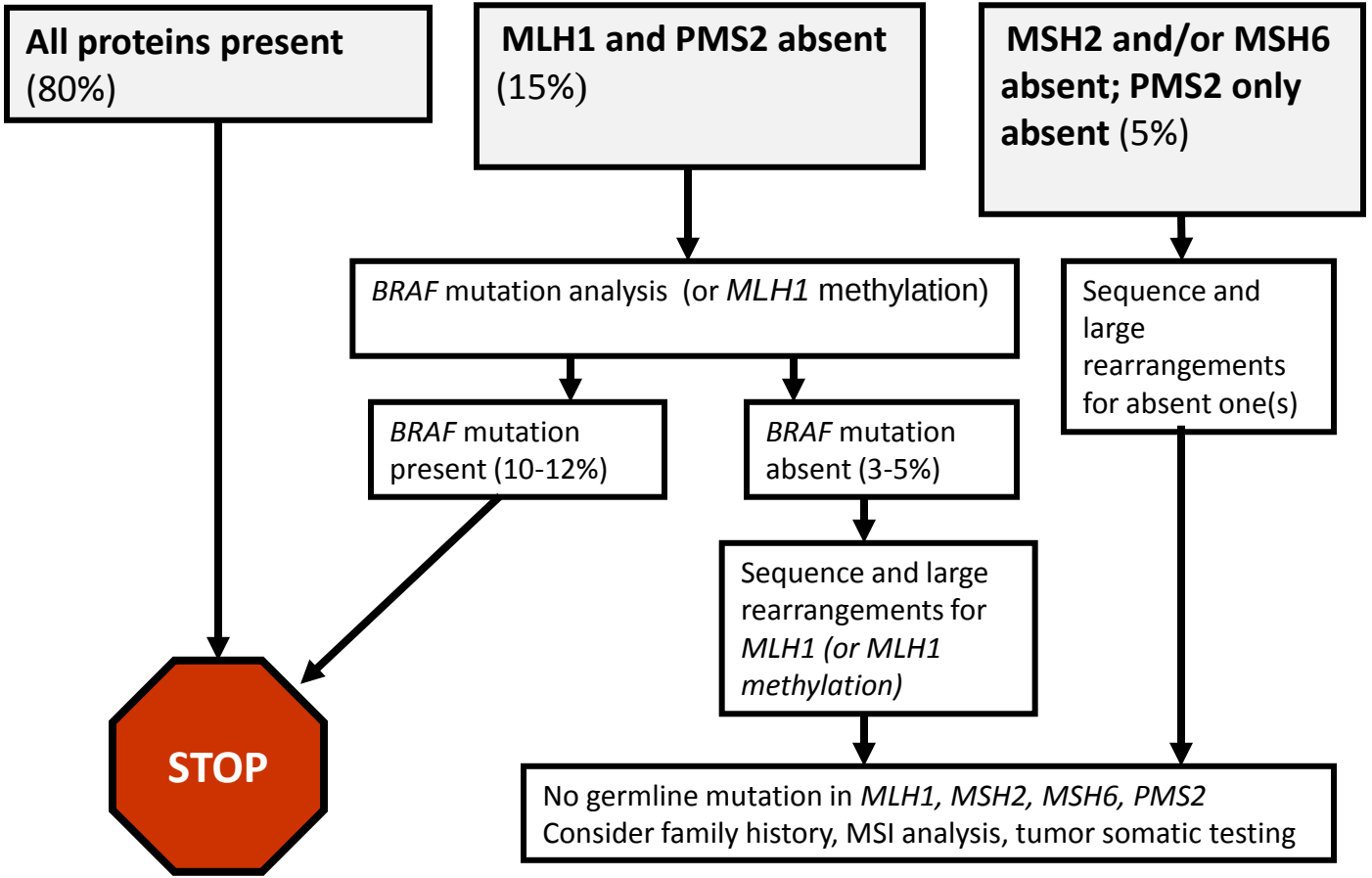
Pearlman, Nat Soc Gen Counselor ab, 2017; Hampel, NEJM 2005; Hampel, J Clin Oncol 2008

# Impact- Columbus 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

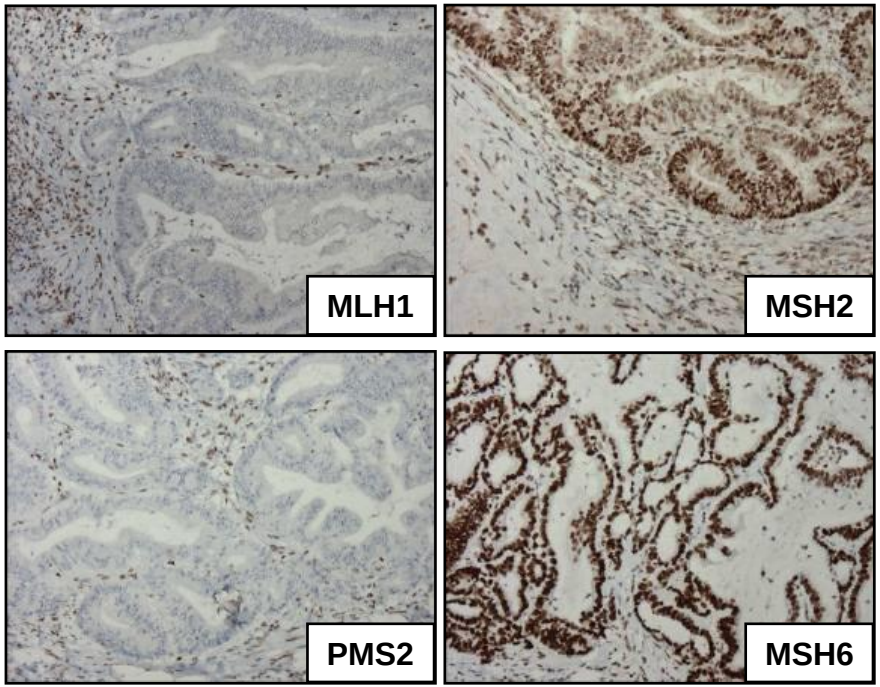
Mvundura, Genet Med 2010; Grosse, Genetic in Med 2015; EGAPP, Genet Med 2009; Giardiello, Am J Gastroenterol 2014; ACOG & SGO Practice Bulletin Number 147, 2014

# Universal Screening Algorithm



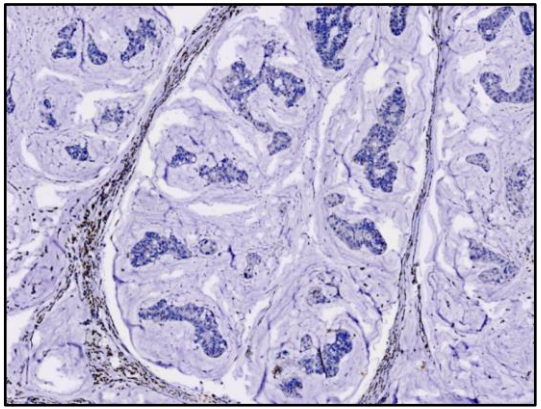
# MLH1 & PMS2 Lost

- 15% of the time
- CRC is MSI
- Better prognosis
- 80% sporadic, acquired methylation *MLH1*
- Could be LS
- Test *BRAF* or methylation *MLH1 promoter*

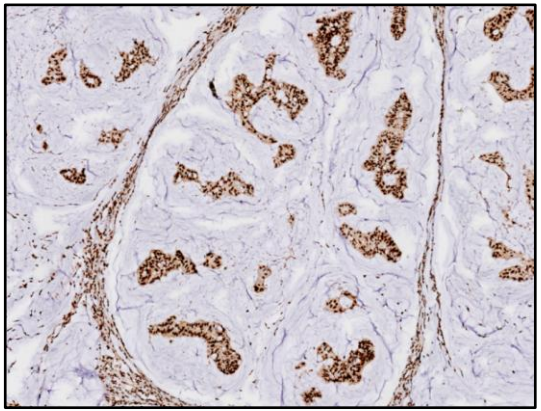


# Germline or Sporadic Methylation? BRAF Testing

- Kinase encoding gene *ras/raf/mapk*
- Present in 5 to 22% CRC
- DNA test on tumor
  - Exon 15 amplification with PCR
  - Sequencing point mutation V600E
- If mutated, not Lynch syndrome
  - Presumed sporadic methylation
  - No need workup
    - Cost savings by *BRAF* (48% OSU)
  - Some use *MLH1* methylation (pyrosequencing) instead of *BRAF*
    - 68% methylated cases- *BRAF* mutation



MLH1, PMS2

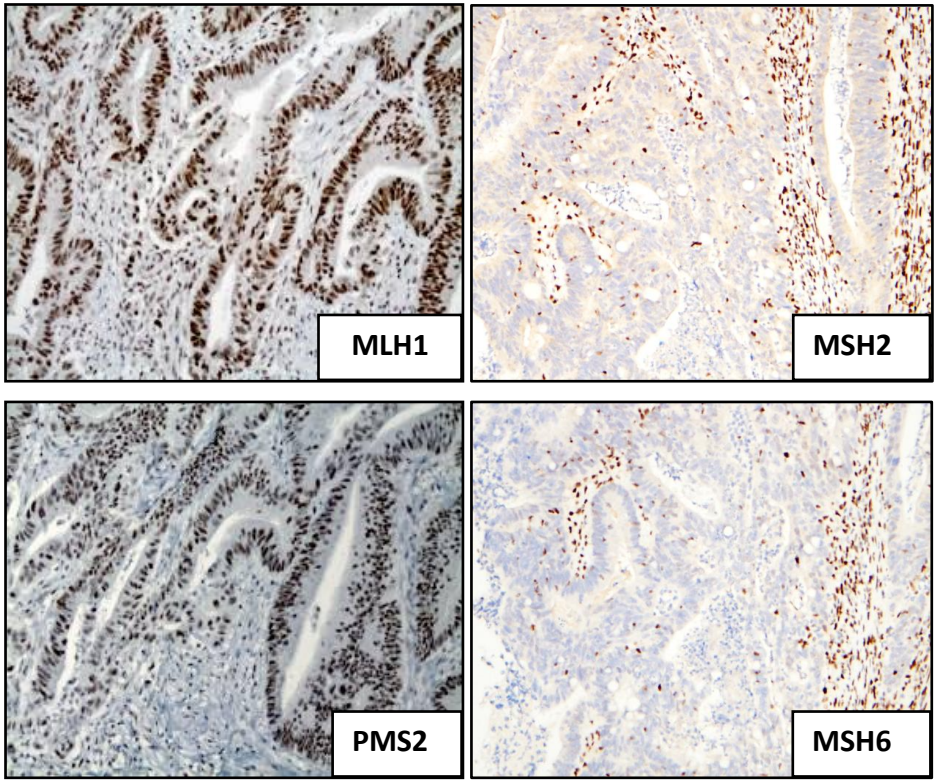


MSH2, MSH6

Jin, Am J Clin Pathol, 2013

# MSH2 & MSH6 Lost

- 3% of the time
- CRC is MSI
- Better prognosis
- Could be LS due to *MSH2* (*MSH6* less likely) mutation
- Refer to Genetics
- MSH6 and PMS2 only similar



# Mismatch Repair Protein (MMR) Nuclear Expression by IHC

MLH1: Absent
PMS2: Absent
MSH2: Present
MSH6: Present

____No loss of nuclear expression of MMR proteins: low probability of microsatellite instability-high (MSI-H)

__x_ Loss of nuclear expression of MLH1 and PMS2: testing for methylation of the *MLH1* promoter and/or mutation of *BRAF* is indicated (the presence of a *BRAF* V600E mutation and/or *MLH1* methylation suggests that the tumor is sporadic and germline evaluation is probably not indicated; absence of both *MLH1* methylation and of *BRAF* V600E mutation suggests the possibility of Lynch syndrome, and sequencing and/or large deletion/duplication testing of germline *MLH1* may be indicated)#

# MLH1 Promoter Hypermethylation

**Loci Tested:**
CpG1: Not hypermethylated
CpG2: Not hypermethylated
CpG3: Not hypermethylated
CpG4: Not hypermethylated

**Interpretation:**
Results from this analysis demonstrate the absence of MLH1 promoter hypermethylation within the tumor.
Loss of MLH1 protein expression could result from germline MLH1 mutation(s) or somatic/epigenetic inactivation of MLH1 transcription. **Absence of MLH1 promoter hypermethylation in this tumor sample suggests a germline MLH1 mutation might be present and genetic consultation may be beneficial for the next step of clinical care.**

# Could our Patient have Lynch Syndrome?

- Prevalence of **Lynch syndrome** among early onset CRC patients
  - **4%** (38/870) dx <55 (no *PMS2*) – Barnetson 2006
  - **8.4%** (22/263) dx <50 – Hampel 2005, Hampel 2008
  - **12%** (23/193) dx ≤35 – Mork 2015
  - **13.6%** (59/434) dx <50 – Yurgelun 2015

- Prevalence of **other cancer syndromes** among early onset CRC patients largely unknown until recently

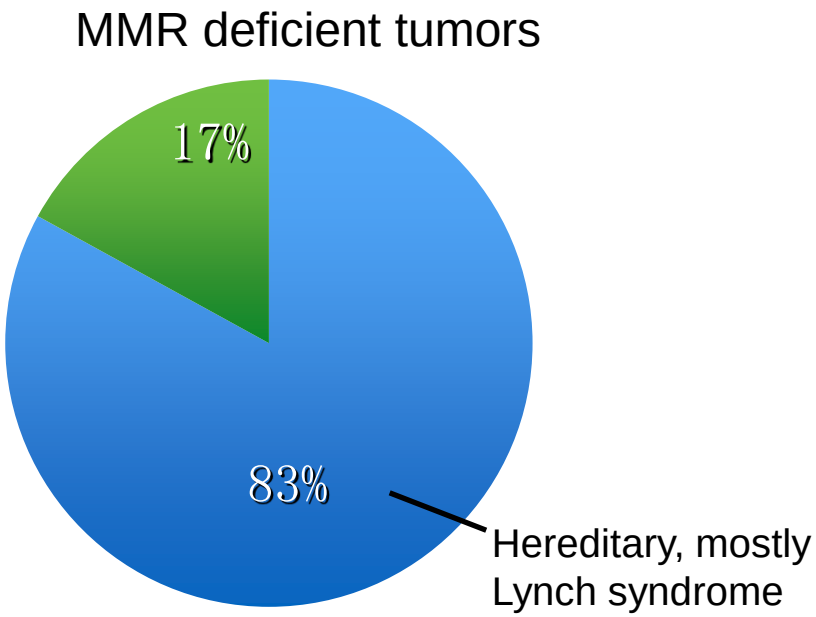
Barnetson, NEJM, 2006; Hampel, NEJM, 2005; Hampel, J Clin Oncol, 2008; Mork, J Clin Oncol, 2015; Yurgelun, Gastroenterology, 2015

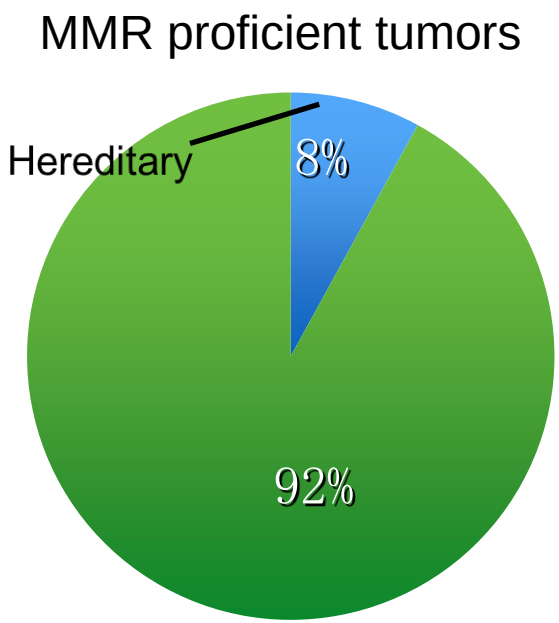
# Ohio Colorectal Cancer Prevention Initiative (OCCPI) Under 50 Study

- 450 Ohioans under 50 with CRC
- 72 (16%) had at least 1 clinically actionable mutation
  - 8% Lynch syndrome
  - 7.6% other syndrome (including 6 with BRCA1/2!)
  - 0.4% 2 syndromes (PMS2 and APC mutation)
- If only targeted-testing had been performed, 17 (31%) would have been missed
- All early-onset CRC patients should be referred for genetic testing with a comprehensive hereditary cancer gene panel

Pearlman, JAMA Oncol 2016

# Tumor Screening and Likelihood of Positive Genetic Testing



# Case Workup

- Germline testing
  - University of Washington BROCA panel on serum
  - 66 genes that increase risk hereditary cancers including MLH1, PMS2, MSH2, MSH6, EPCAM

# Genetic Testing Results

## BROCA Cancer Risk Panel

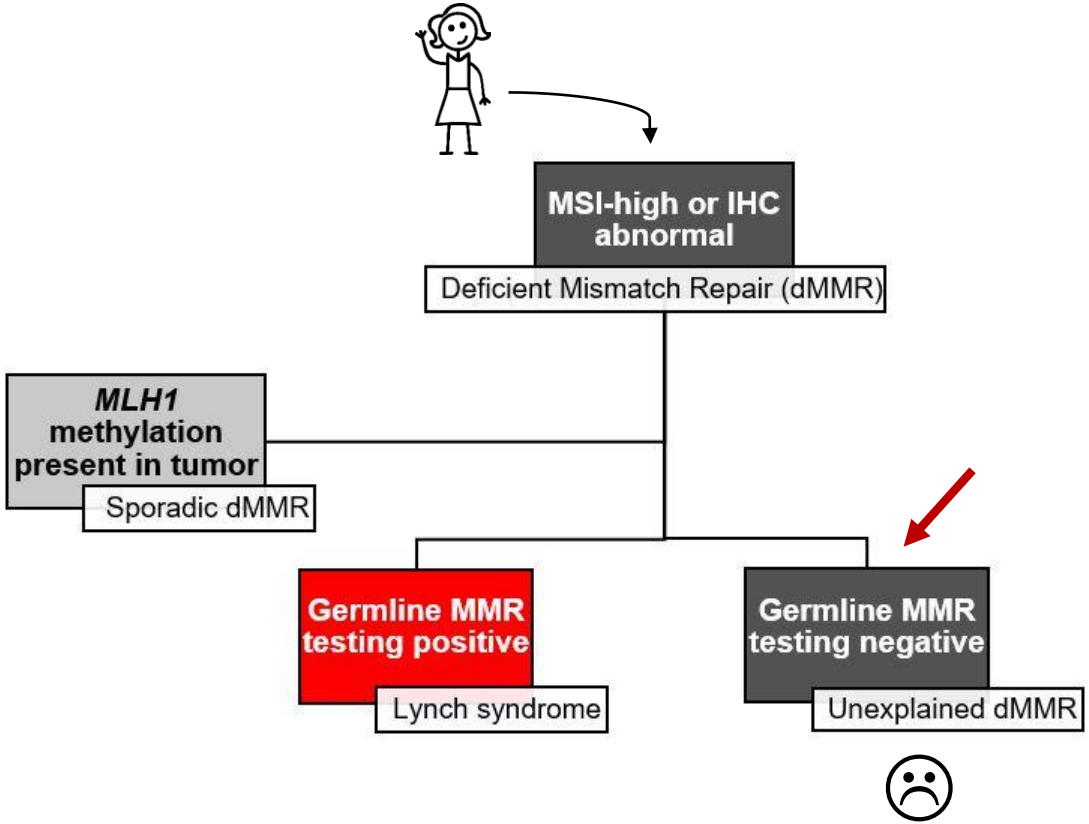
### BROCA Result

NEGATIVE for mutations (see interpretation).

### BROCA Interpretation

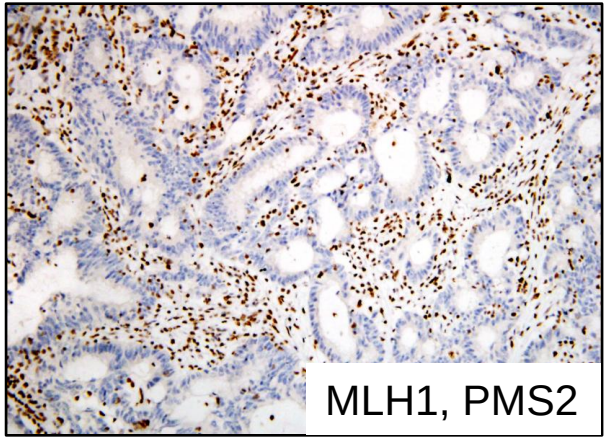
No mutations were found in AKT1, APC, ATM, ATR, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK1, CHEK2, CTNNA1, EPCAM, FAM175A, FANCM, FH, FLCN, GALNT12, GEN1, GREM1, HOXB13, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH6, MUTYH, NF1, NTHL1, PALB2, PALLD, PDGFRA, PIK3CA, PMS2, POLD1, POLE, POT1, PRKAR1A, PRSS1, PTCH1, PTEN, RAD51B, RAD51C, RAD51D, RB1, RECQL, RET, RINT1, RPS 20, SDHB, SDHC, SDHD, SLX4, SMAD4, SMARCA4, NBN, TP53, VHL, and XRCC2 genes by complete sequencing and deletion duplication testing. This result reduces the likelihood of a genetic predisposition to cancer. However, some mutations in this gene panel may not be detected by this test method, and other genes not included in this panel may also contribute to cancer risk. Genetic counseling is recommended. Note: variants from the reference sequences are not reported if they are considered neutral.

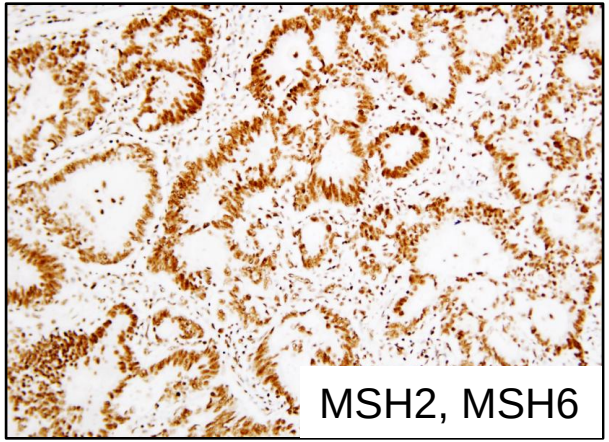
# Causes of Mismatch Repair Deficiency (dMMR)



# Lynch-Like

- IHC suggests MSI
- No *BRAF*, *MLH1* methylation or germline mutation
- “Lynch-like”- waste basket
- Must test tumor



# “Lynch-Like”- Additional Testing

- DS MMR gene mutations explain many unexplained cases
  - Up to 69% of cases contain DS MMR mutation or mutation and LOH
- Other rare causes
  - Missed germline mutation: 5.5% (1/18), 5% (2/40)
  - Somatic mosaicism: 5.5% (1/18)
  - Tumor screening errors: 18.8% (6/32)
- Clinical Characteristics of DS vs. LS
  - Age of DS cases similar to LS, younger than methylated cases
- Tumor histology no difference DS vs. LS

Sourrouille et al. *Fam Cancer* 2013; Mesenkamp et al. *Gastroenterology* 2014; Geurts-Giele et al. *J Pathol* 2014; Haraldsdottir et al. *Gastroenterology* 2014; Hemminger, Hum Pathol, In press

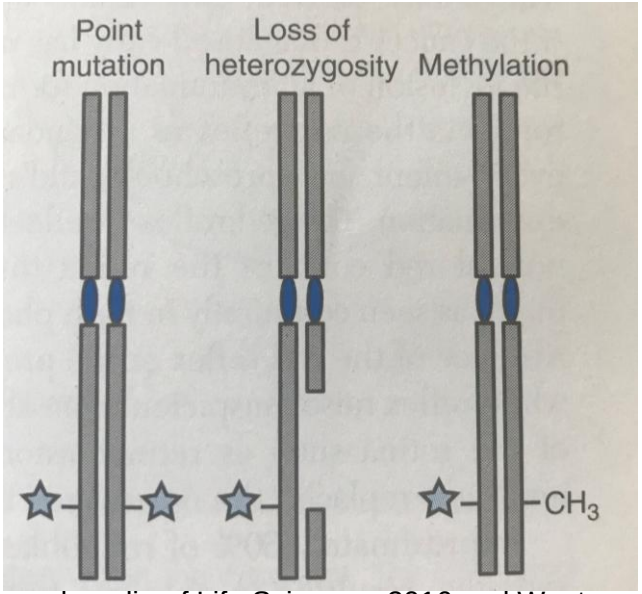
# Double Somatic Mutations Explain Many with Unexplained dMMR

| Publication | Somatic Mutations | Somatic Mutation + LOH | Somatic Mosaicism | Other |
|---|---|---|---|---|
| Sourrouille, *Fam Cancer* 2013 | 3/18 (16.7%) | LOH not studied but 5/18 patients had 1 somatic mutation (27.8%) | 1 /18 (5.5%) | 1/18 missed germline mutation |
| Mesenkamp, Vogelaar, *Gastroenterol* 2014 | 5/25 (20%) | 8/25 (32%) | Not assessed (Sanger sequencing) | |
| Geurts-Giele, *J Pathol* 2014 | 5/40 (13%) | 16/40 (40%) | 0/40 (0%) Not mentioned but probably assessed (Next-gen) | 2 probable germline mutations seen in T&N |
| Haraldsdottir, *Gastroenterol* 2014 | 12/32 (37.5%) | 9/32 (28.1%) | 0/32 (0%) | 6/32 had errors in tumor screening |
| **Total** | **45-69%** | | **0-5%** | **5-19%** |

8-50% of cases remain unexplained and are probable LS cases with an unidentified germline MMR gene mutation

# What is Loss of Heterozygosity (LOH)?

- Common genetic event in cancers
- Basically, **one allele is lost** resulting in a mutated allele that is no longer opposed by a normally functioning allele




Gorringe, Loss of Heterozygosity, Encyclopedia of Life Sciences, 2016 and Westman, Medical Genetics for the Modern Clinician, 2006

# What is Loss of Heterozygosity (LOH)?

- Can be caused by mitotic errors, chromothripsis (massive genomic rearrangement), inappropriate repair of DNA breaks
- This is a common second “hit” on tumor suppressor genes that allows for unchecked cell growth
- In somatic tumor testing, this is picked up by a single mutation seen in many more reads than expected

# Case- Tumor Testing

## UW-OncoPlex™ — Cancer Gene Panel

### OncoPlex Result

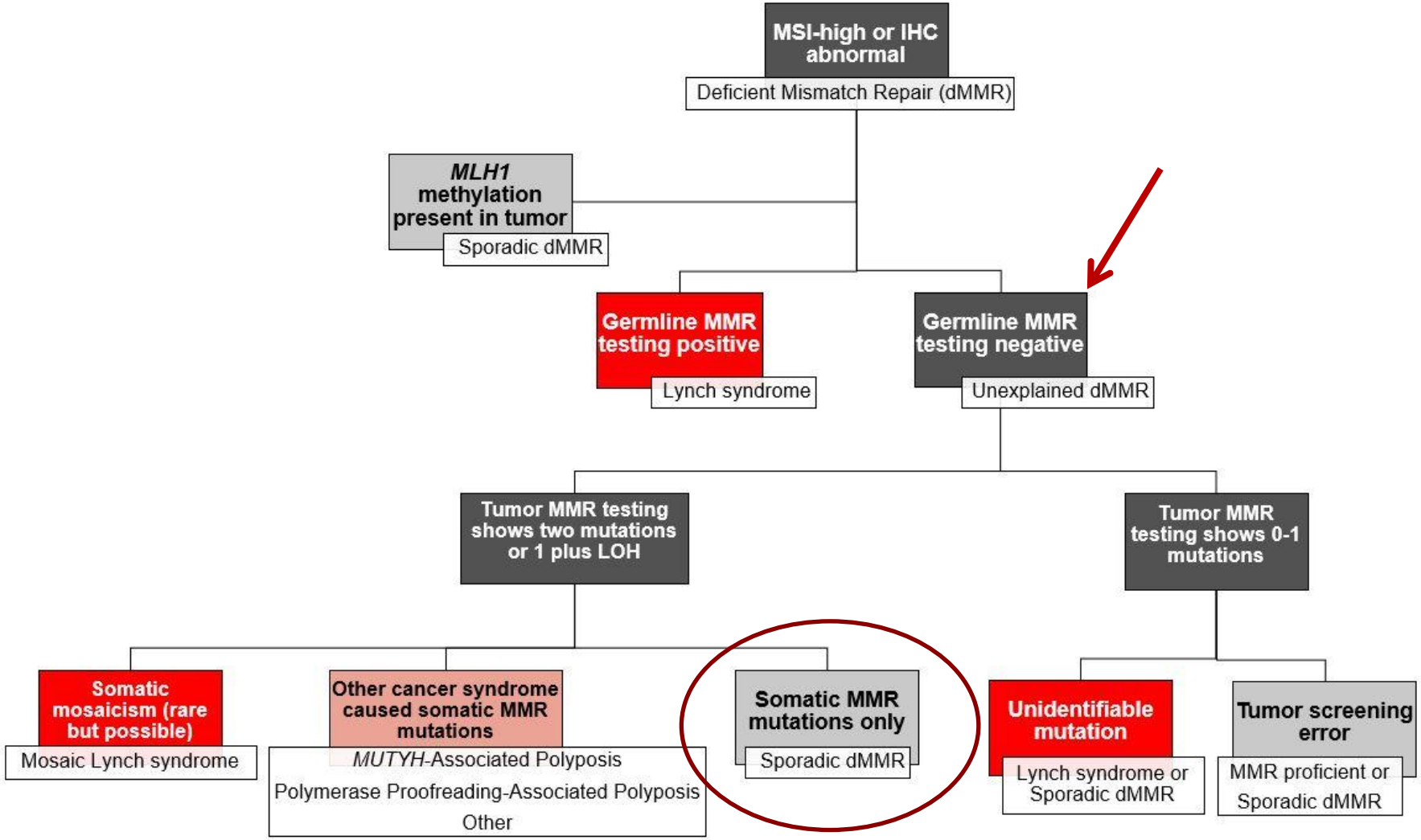
POSITIVE for double somatic mutation in MLH1 in tumor tissue (see interpretation).

### OncoPlex Interpretation

A pathogenic mutation was detected in MLH1 in the tumor sample tested (p.R100X, NM_000249.3:c.298 C to T). This mutation was detected in about twice as many sequencing reads compared to other somatic mutations in this tumor, supporting loss of heterozygosity (LOH) in the tumor cells. The result is most consistent with a functional double somatic mutation in the tumor. Double somatic mismatch repair gene mutations have been described in some tumor samples, and this finding may explain the results of screening tests on tumor tissue (Sourrouille 2013, Mensenkamp 2014, Geurts Giele 2014, Haraldsdottir 2014). No mutations were detected in non tumor tissue. In conjunction with the negative testing result in non tumor tissue, this reduces the probability that the patient has Lynch syndrome. Constitutional somatic mosaicism of mismatch repair gene mutations has been described in rare cases and cannot be excluded (Sourrouille 2013). Genetic counseling is recommended.

Microsatellite instability was confirmed using the mSINGS method (Salipante 2014).

No mutations were found in BRAF codon 600, MSH2, MSH6, PMS2, or EPCAM by complete sequencing and deletion duplication testing of each gene. There are mutations in these genes that this test will not detect. Note: variants from the reference sequence are not reported if they are considered neutral.


MSI-high or IHC abnormal
Deficient Mismatch Repair (dMMR)
MLH1 methylation present in tumor
Sporadic dMMR
Germline MMR testing positive
Lynch syndrome
Germline MMR testing negative
Unexplained dMMR
Tumor MMR testing shows two mutations or 1 plus LOH
Tumor MMR testing shows 0-1 mutations
Somatic mosaicism (rare but possible)
Mosaic Lynch syndrome
Other cancer syndrome caused somatic MMR mutations
MUTYH-Associated Polyposis
Polymerase Proofreading-Associated Polyposis
Other
Somatic MMR mutations only
Sporadic dMMR
Unidentifiable mutation
Lynch syndrome or Sporadic dMMR
Tumor screening error
MMR proficient or Sporadic dMMR

# Genetics Risk Assessment and Recommendations

- The cancer is considered sporadic rather than hereditary
- Likely not Lynch and not at increased risk for additional cancers
- Recommend standard treatment and follow-up (colonoscopy at 1 year, then if unremarkable 3 years, then every 5 years indefinitely)
- Risk for family members to develop colon cancer estimated based on the early-age of diagnosis
  - First-degree relatives 3.3x risk of colon cancer or 16.5% lifetime risk for developing colon cancer.
  - Colonoscopy every 5 years beginning at age 17 (10 years before earliest diagnosis in immediate family).

# Clinical Implications of Tumor Testing

- These were previously considered unexplained MMR deficiency
  - Patients treated like Lynch syndrome (colonoscopy every 1-2 years, TAHBSO)
  - First degree relatives also treated like Lynch!
  - High patient and family anxiety

- Now a majority of cases are able to be explained and treated like others with history of colon cancer

# Case Summary and Management

- 27 year old woman without suggestive family history
- Tumor MMR deficient by IHC
- MLH1/PMS2 absent, No methylation
- Germline testing negative
- Tumor testing consistent with sporadic cancer
- Hemicolectomy (rather than subtotal colectomy +/- TAH BSO)
  - pT4aN0 CRC (stage IIB)

# Summary

- Screening for MSI and Lynch syndrome essential
- Causes for MMR deficiency
  - Lynch syndrome
  - Methylation of MLH1 promoter (sporadic)
  - Double somatic MMR mutations (sporadic)
  - Other cancer syndrome causing somatic MMR
  - Unidentifiable, other
- Tumor sequencing necessary if no germline etiology
- Clear reporting and communication vital
  - Patient and family follow-up and testing impacted

# 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 et al. Assessment of Tumor Sequencing as a Replacement for Lynch Syndrome Screening and Current Molecular Tests for Patients With Colorectal Cancer. JAMA Oncol 3.2018 (epub ahead of print)

# Thanks!  Questions?