Lynch Syndrome screening: What do we need to know in 2019?

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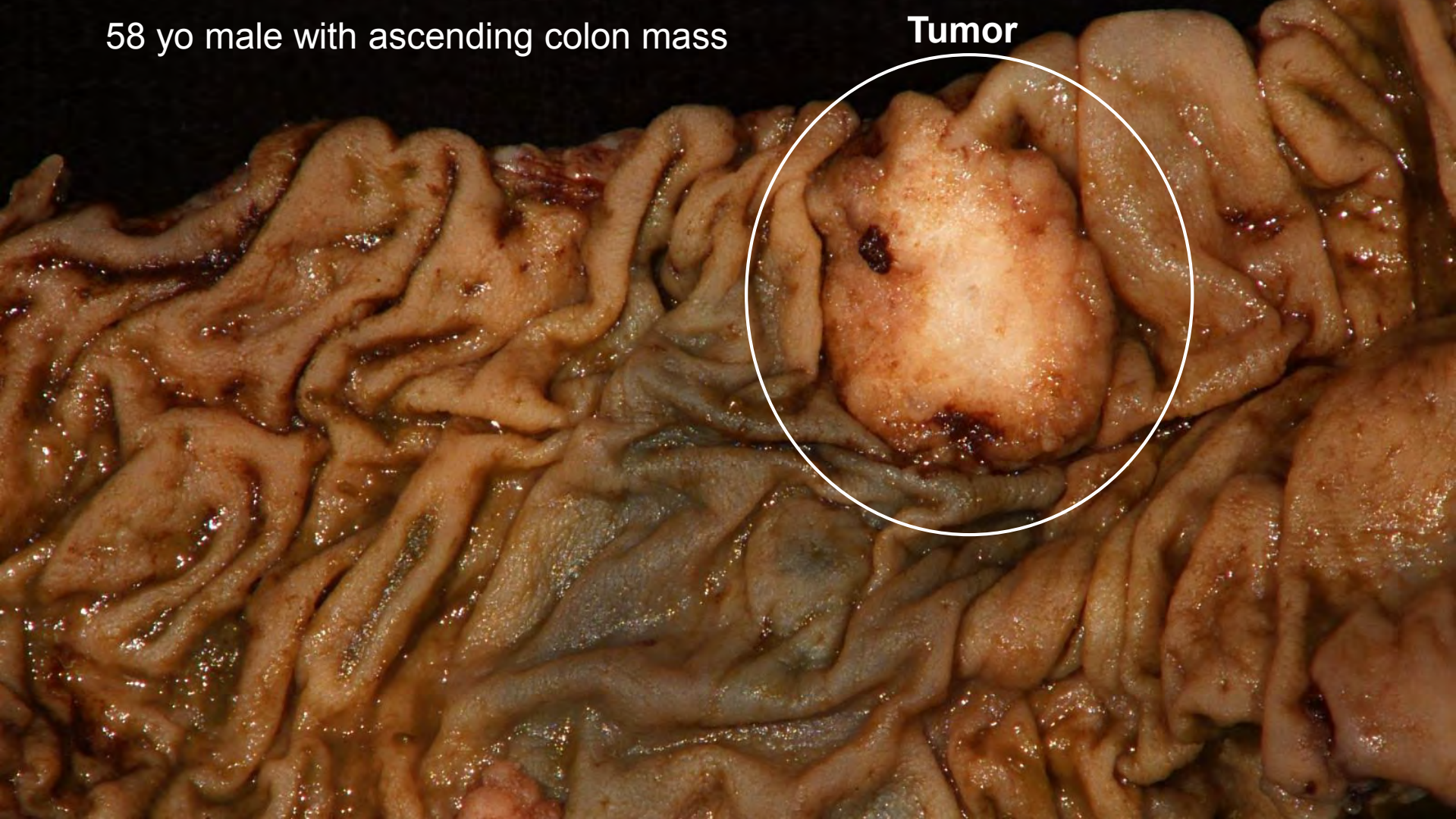
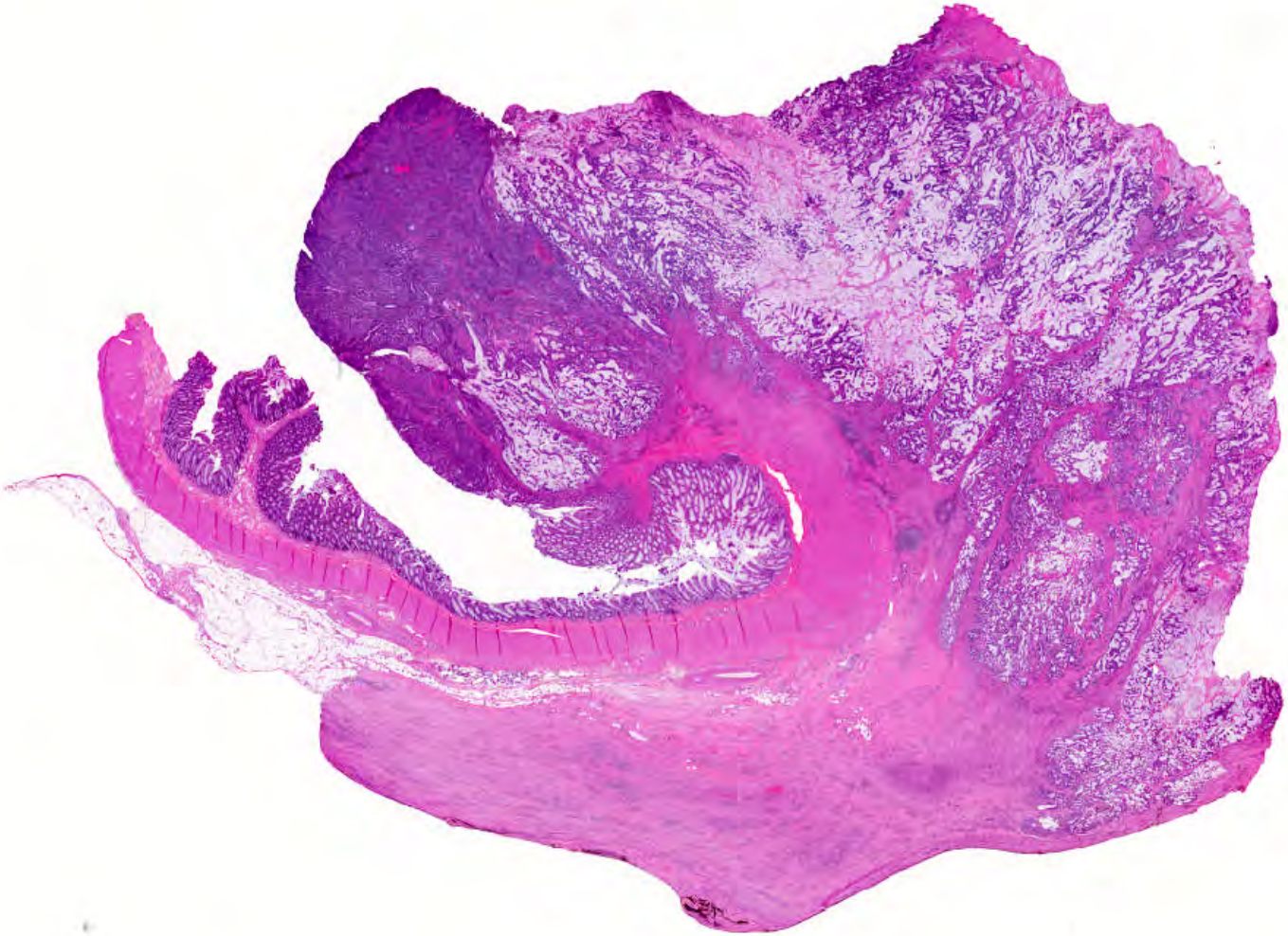
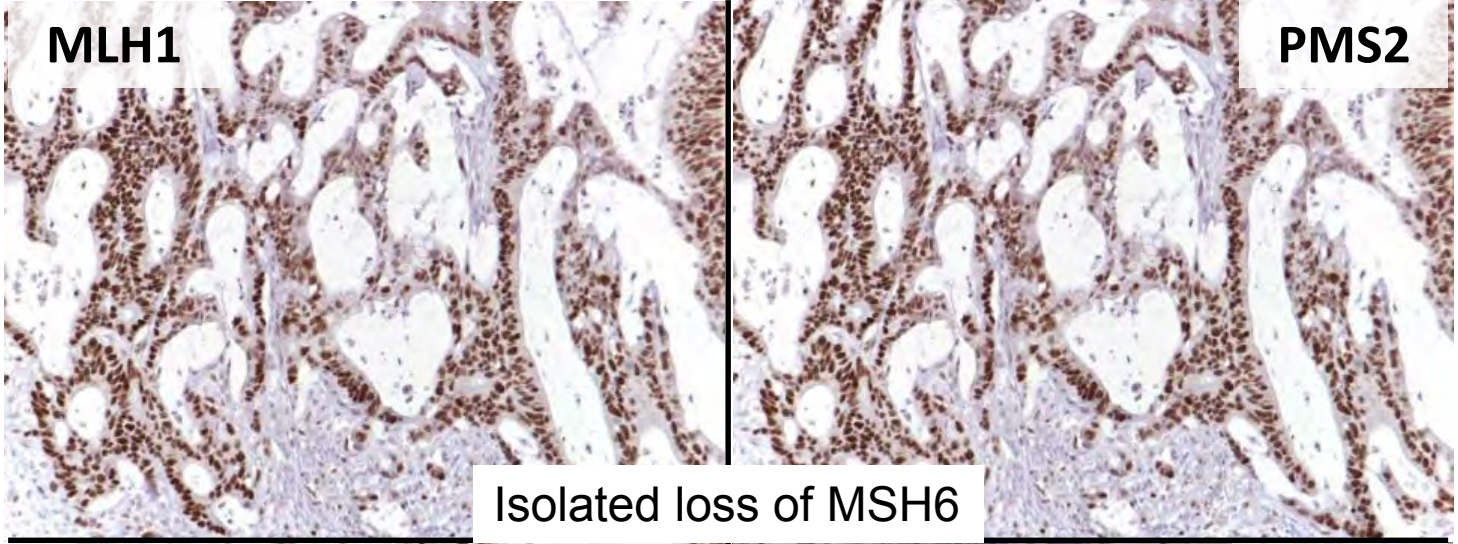
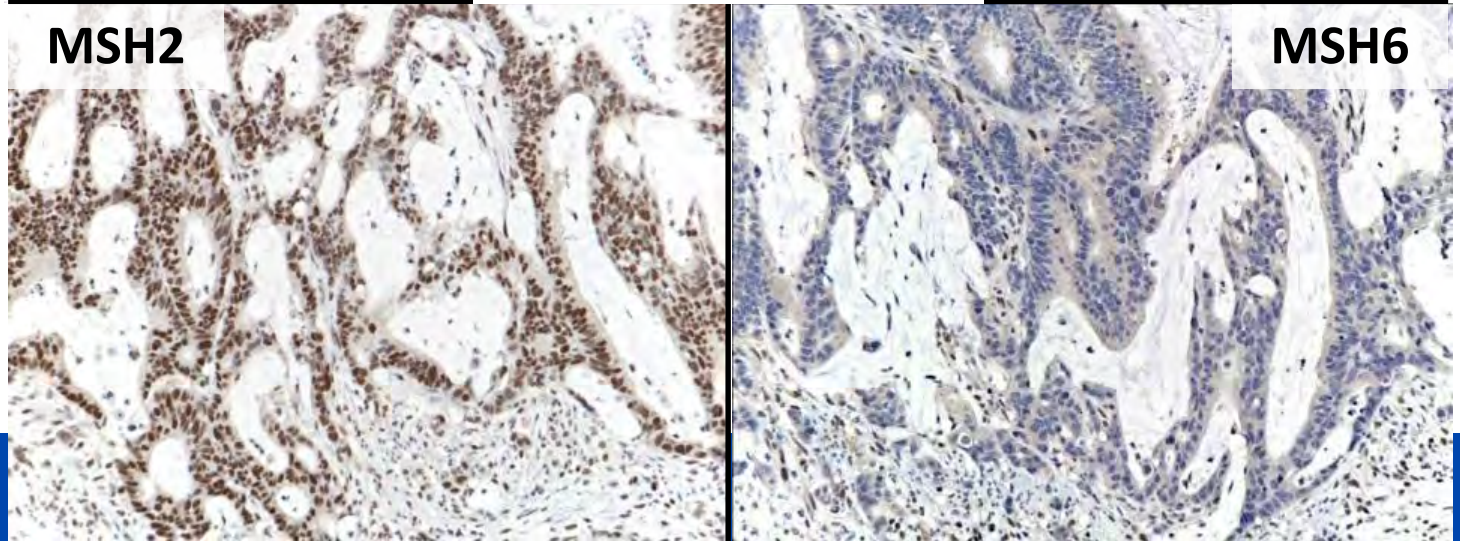

58 yo male with ascending colon mass
Tumor





Isolated loss of MSH6



Question 1:

Which of the following is true of the MMR IHC results?

A. This pattern is diagnostic of Lynch Syndrome
B. This pattern is diagnostic of sporadic MMRD carcinoma
C. Defects in *MSH6* can be either somatic or germline
D. This tumor likely arose from a sessile serrated polyp

Question 1:

Which of the following is true of the MMR IHC results?

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Reporting the result

RESULTS

- IHC: Isolated loss of MSH6, preserved expression of MSH2, MLH1 and PMS2

METHOD

Immunohistochemical staining (IHC) is used to determine the presence or absence of protein expression for MLH1, MSH2, MSH6, and PMS2. Lymphocytes and normal epithelium exhibit strong nuclear staining and serve as positive internal controls for staining of these proteins.

INTERPRETATION

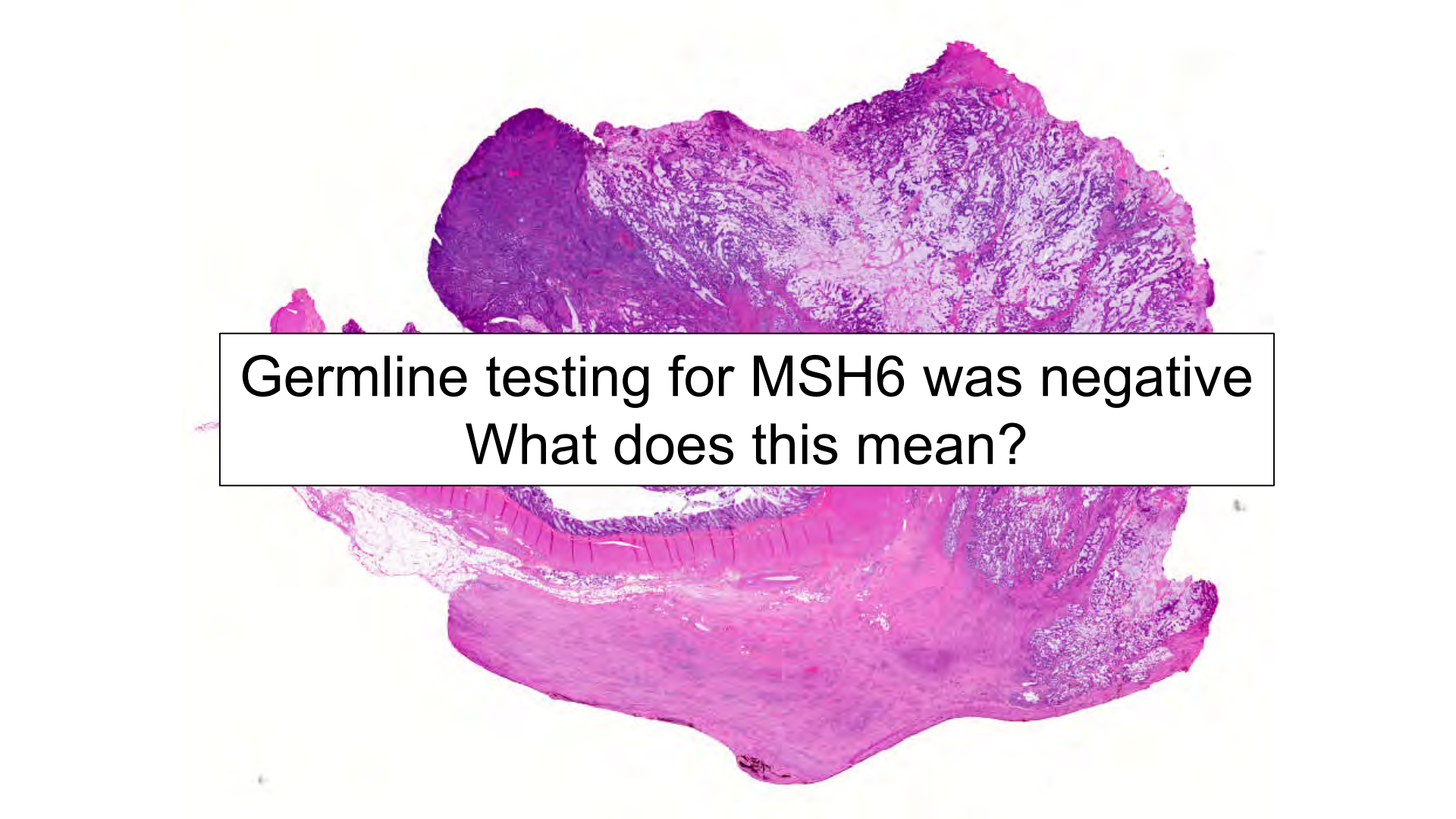
These results indicate loss of normal Deoxyribonucleic Acid (DNA) mismatch repair function within the tumor. Isolated loss of MSH6 expression is frequently associated with the presence of a germline (heritable) mutation in MSH6. Thus, this individual, and other family members, are at increased risk for having an inherited colon cancer syndrome due to defective DNA mismatch repair (Lynch syndrome).

It is important to note that these results do not distinguish between somatic and germline mutations. Germline testing of MSH6 on an additional blood sample may help distinguish between these two possibilities and provide the opportunity for predictive testing for at risk family members.

Additional information regarding this testing may be obtained by ordering a consultation through the inherited cancer clinic. (480-342-6263)

CAUTIONS

Test results should be interpreted in context of clinical findings, family history, and other laboratory data. If results obtained do not match other clinical or laboratory findings, please contact the laboratory for possible interpretation. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.


Germline testing for MSH6 was negative
What does this mean?

Outline:

- Pathways to colon cancer
- Definition of Lynch Syndrome and goals of screening
- Principles of MMR IHC as a screening tool
- Issues with MMR IHC interpretation
- “Lynch-like” syndrome

Subtypes of colorectal carcinoma

Comprehensive molecular characterization of human colon and rectal cancer

Nature 2012

The Cancer Genome Atlas Network*

Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions

Nat Med 2013

Felipe De Sousa E Melo[1,7], Xin Wang[2,7], Marnix Jansen[3], Evelyn Fessler[1], Anne Trinh[2], Laura P M H de Rooij[1], Joan H de Jong[1], Onno J de Boer[3], Ronald van Leersum[1], Maarten F Bijlsma[1], Hans Rodermond[1], Maartje van der Heijden[1,4], Carel J M van Noesel[3], Jurriaan B Tuynman[5], Evelien Dekker[6], Florian Markowetz[2], Jan Paul Medema[1,7] & Louis Vermeulen[1,4,7]

A colorectal cancer classification system that associates cellular phenotype and responses to therapy

Anguraj Sadanandam[1,2], Costas A Lyssiotis[3,4,14,15], Krisztian Homicsko[2,5,15], Eric A Collisson[6], William J Gibb[7], Stephan Wullschleger[2], Liliane C Gonzalez Ostos[2], William A Lannon[3,14], Carsten Grotzinger[8], Maguy Del Rio[9], Benoit Lhermitte[10], Adam B Olshen[11,12], Bertram Wiedenmann[8], Lewis C Cantley[3,4,14], Joe W Gray[13] & Douglas Hanahan[2]

Nat Med 2013

Proteogenomic characterization of human colon and rectal cancer

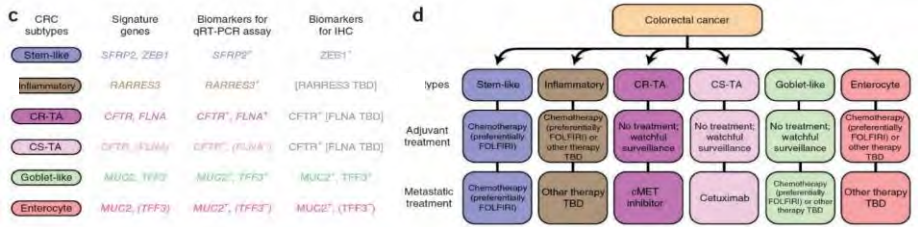
Nature 2014

Bing Zhang[1,2], Jing Wang[1], Xiaojing Wang[1], Jing Zhu[1], Qi Liu[1], Zhiao Shi[3,4], Matthew C. Chambers[1], Lisa J. Zimmerman[5,6], Kent F. Shaddox[6], Sangtae Kim[7], Sherri R. Davies[8], Sean Wang[9], Pei Wang[10], Christopher R. Kinsinger[11], Robert C. Rivers[11], Henry Rodriguez[11], R. Reid Townsend[8], Matthew J. C. Ellis[8], Steven A. Carr[12], David L. Tabb[1], Robert J. Coffey[13], Robbert J. C. Slebos[2,6], Daniel C. Liebler[5,6] & the NCI CPTAC*

Subtypes of colorectal carcinoma

CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

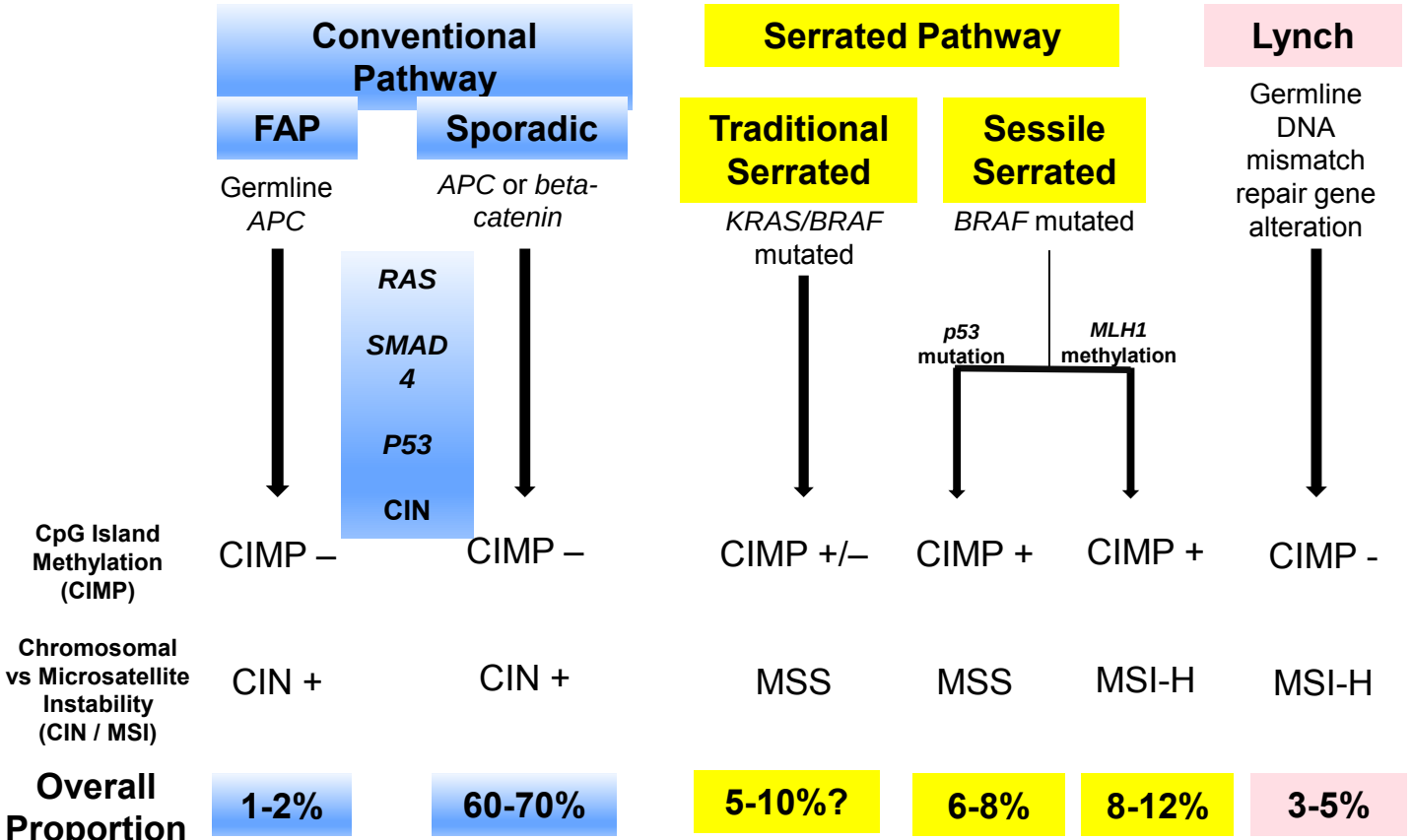
TCGA Molecular Subtypes (2012)



Subtypes based on Cell Type (2013)

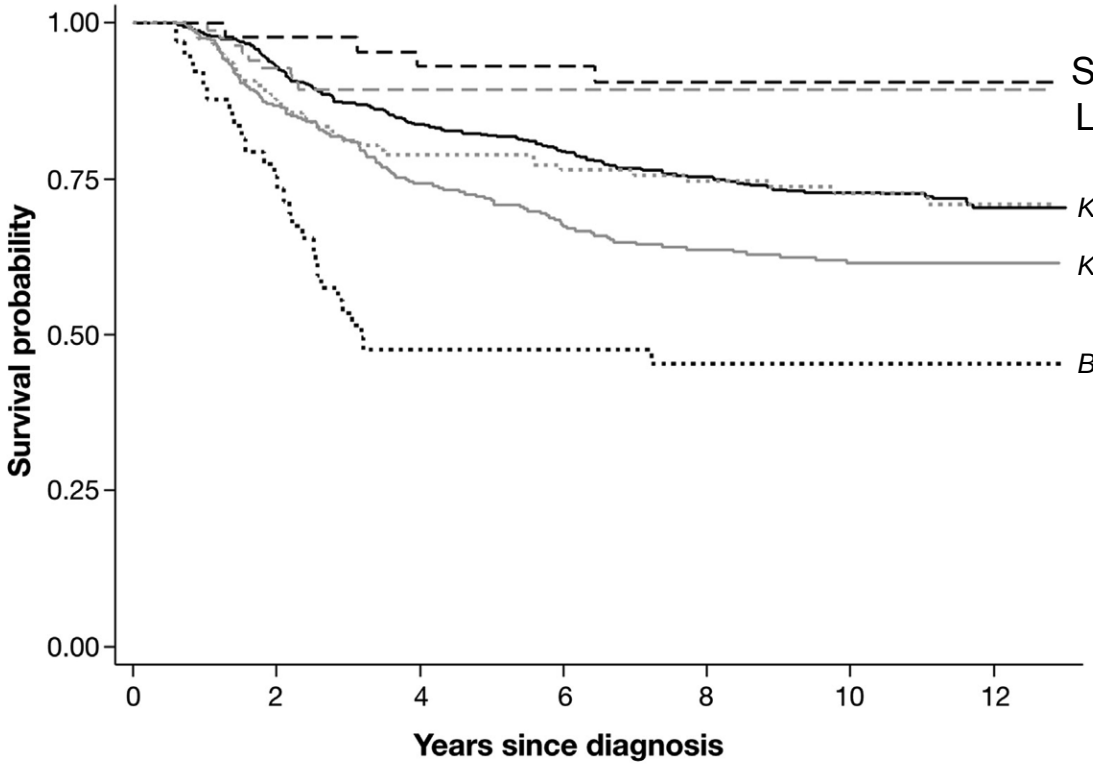
- These classification schemes are not very practical
- The Jass classification scheme is more useful to pathologists

Pathologists' view of Colon Cancer: Modified Jass classification



Why does molecular classification matter?

- Prognostic implications
- Predictive of response to certain treatments
- **Provides a framework for screening for Lynch syndrome**

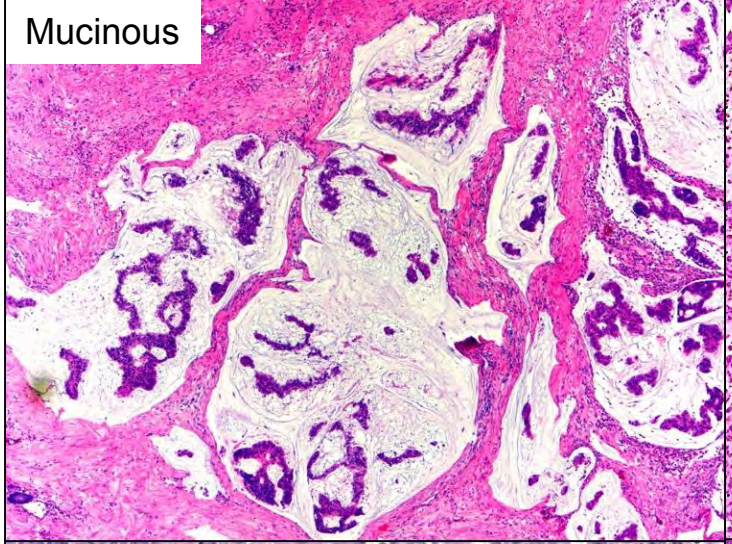


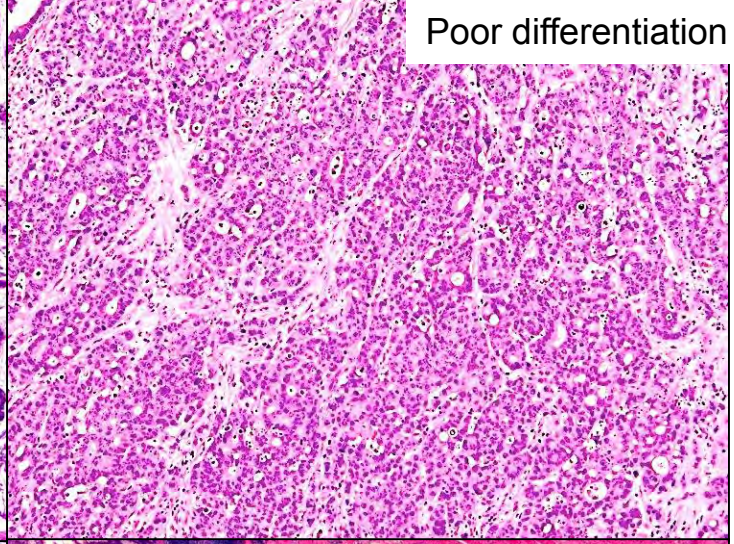
Number at risk

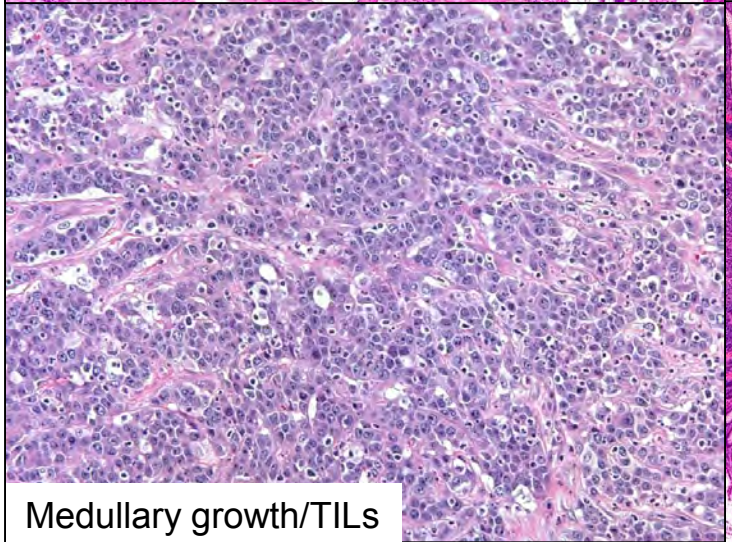
Type 1	100	78	71	66	56	43	14
Type 2	55	38	24	23	21	20	4
Type 3	353	268	223	191	160	130	37
Type 4	631	541	473	433	370	300	113
Type 5	50	43	39	37	32	26	10
Other	155	120	103	96	84	65	22

Figure 1. Kaplan-Meier survival curves for disease-specific survival.

Phipps AI, *Gastroenterology* 2015;148:77-87.


Mucinous


Poor differentiation


Medullary growth/TILs


Crohn’s-like reaction

Lynch Syndrome Screening: Goals

- Identify MSI-H tumors
- Separate out sporadic MSI-H tumors
- Identify those patients that need germline testing
- Identify deleterious mutations in MMR genes
- Identify affected family members
- Enroll affected individuals in lifelong screening program

Lynch Syndrome Screening: Goals

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Lynch Syndrome Definition

- Germline mutations in DNA mismatch repair (MMR) genes:
  - *MLH1* (50%)
  - *MSH2* (40%)
  - *MSH6* (7%-10%)
  - *PMS2* (<5%)
- Deletions in *EPCAM/TACSTD1* (1-3%)
  - Result epigenetic silencing of the *MSH2* gene by hypermethylation

Lynch Syndrome: Cancer Risk

Cancer Type	General Population Risk	Lynch Syndrome	
		Risk	Mean Age of Onset
Colon	**4.8%**	**52%-82%**	**44-61 years**
Endometrium	**2.7%**	**25%-60%**	**48-62 years**
Stomach	<1%	6%-13%	56 years
Ovary	1.4%	4%-12%	42.5 years
Hepatobiliary tract	<1%	1.4%-4%	Not reported
Urinary tract	<1%	1%-4%	~55 years
Small bowel	<1%	3%-6%	49 years
Brain/central nervous system	<1%	1%-3%	~50 years
Sebaceous neoplasms	<1%	1%-9%	Not reported

Who to screen for Lynch Syndrome

- **Universal screening of all patients with CRC**
  - Endorsed by the following organizations:
    - National Comprehensive Cancer Network (NCCN), EGAPP (working group sponsored by the CDC), American Society of Medical Oncology (ASCO), US Multi-Society Task Force on Colorectal Cancer, American College of Gastroenterology (AGA)
- **Selective Screening of all patients <70 years of age & in patients >70 years fulfilling revised Bethesda guidelines** (misses up to 5% of patients with Lynch syndrome)
  - Endorsed as an option by the following organizations:
    - National Comprehensive Cancer Network (NCCN) and the American Society of Medical Oncology (ASCO)

Molecular Biomarkers for the Evaluation of Colorectal Cancer

Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology

Antonia R. Sepulveda, MD, PhD,[1] Stanley R. Hamilton, MD, PhD,[2] Carmen J. Allegra, MD,[5] Wayne Grody, MD, PhD,[6] Allison M. Cushman-Vokoun, MD, PhD,[7] William K. Funkhouser, MD, PhD,[8] Scott E. Kopetz, MD, PhD,[3] Christopher Lieu, MD,[9] Noralane M. Lindor, MD,[10] Bruce D. Minsky, MD,[4] Federico A. Monzon, MD,[11] Daniel J. Sargent, PhD,[12] Veena M. Singh, MD,[13] Joseph Willis, MD,[14] Jennifer Clark, SCT, MB(ASCP)^{cm},[15] Carol Colasacco, MLIS,[16] R. Bryan Rumble, MSc,[17] Robyn Temple-Smolkin, PhD,[18] Christina B. Ventura, MT(ASCP),[16] and Jan A. Nowak, MD, PhD[19]

Recommendation

- Mismatch repair status testing in patients with colorectal cancers should be performed for the identification of patients at high-risk for Lynch syndrome and/or prognostic stratification.

- Testing can be performed by immunohistochemistry or by MSI DNA-based testing.

Flip the paradigm: Tumor sequencing

Validation of a targeted next-generation sequencing approach to detect mismatch repair deficiency in colorectal adenocarcinoma

Modern Pathology (2018) 31:1882–1890

David J. Papke Jr.[1] · Jonathan A. Nowak[1] · Matthew B. Yurgelun[2] · Alexander Frieden[1] · Amitabh Srivastava[1] · Neal I. Lindeman[1] · Lynette M. Sholl[1] · Laura E. MacConaill[3] · Fei Dong[1]

- 275 gene panel (training set of 243 CRC)
- 298 gene panel (validation set of 436 tumors)
- 13 indels per Mbp in MMRD vs. 0.45 indels/Mbp per tumor MMRP

- Training set: ≥ 3 indels/Mbp identified 22 of 23 MMRD and 218 of 218 MMRP tumors (96% sensitivity and 100% specificity)

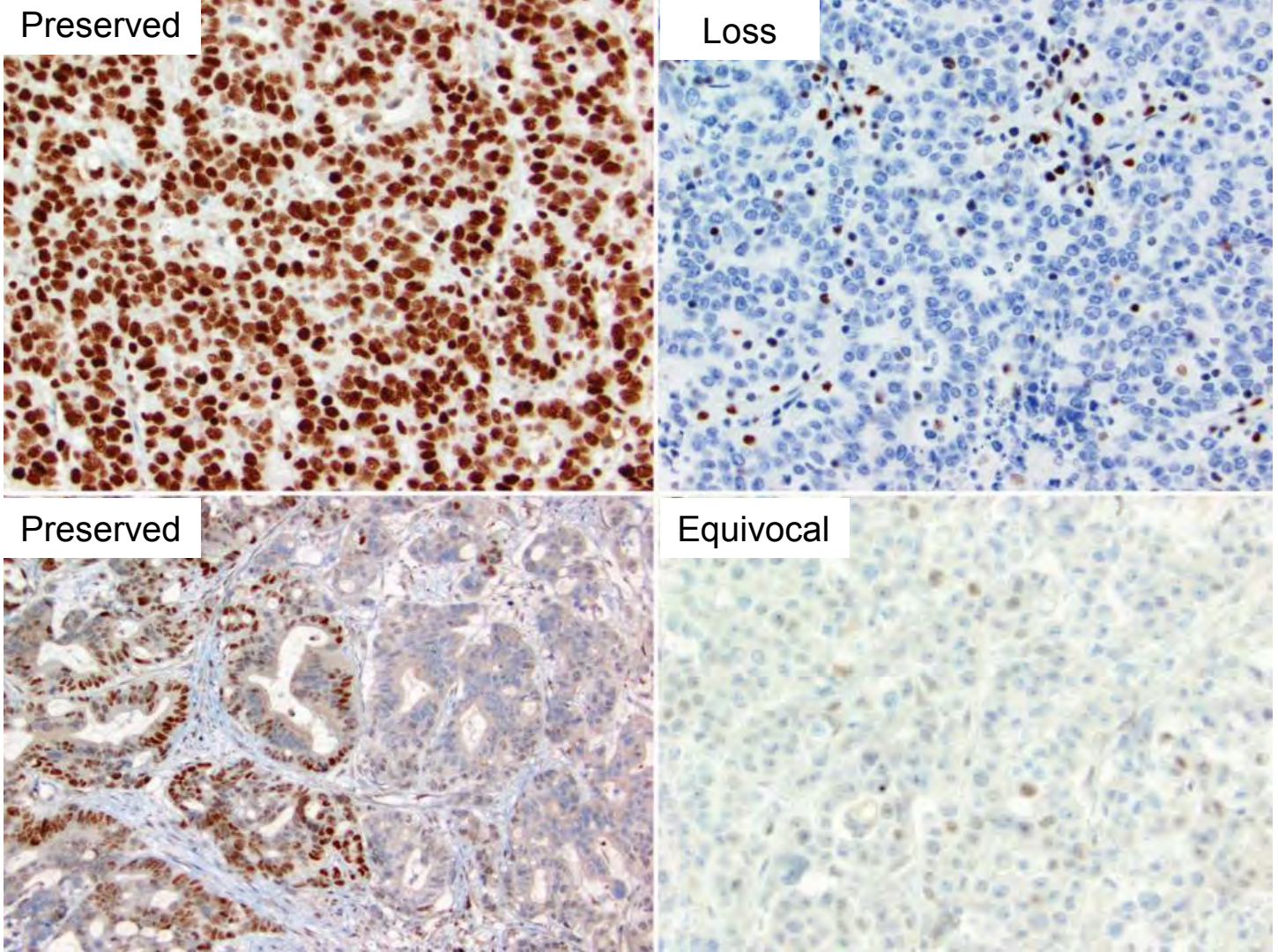
- Validation set: ≥ 3 indels/Mbp identified 44 of 46 MMRD and 388 of 290 MMRP tumors (96% sensitivity and 99% specificity)

Screening for Lynch Syndrome

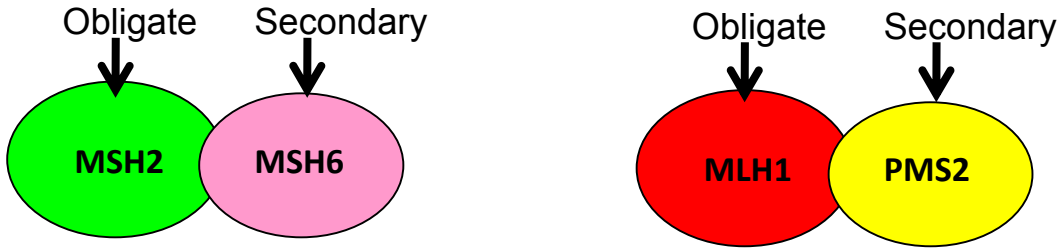
- Who to screen has been answered: **Universal Screening** is the best (for both CRC and Endometrial carcinoma)
- Many issues remain
  - Correct interpretation of MMR IHC
    - Unusual MMR IHC staining patterns
    - Pitfalls in interpretation
  - How do you set up a successful program?
  - Should we screen other GI tract carcinomas? Polyps?
  - MMR IHC and other tests suggest LS but germline testing is negative, now what? Does pathology play any role in this scenario?

MMR Immunohistochemistry

- Defective MMR genes results in ***loss of immunohistochemical expression***
- All 4 antibody testing (MLH1, PMS2, MSH2 and MSH6)
  - If >10% of tumor nuclei demonstrate expression, then protein expression is preserved.
  - If <10% of tumor nuclei demonstrate expression, then protein expression is equivocal. Repeat stain, or reflex to MSI PCR.
- **Must see <u>complete lack of staining</u> to call loss of expression.**

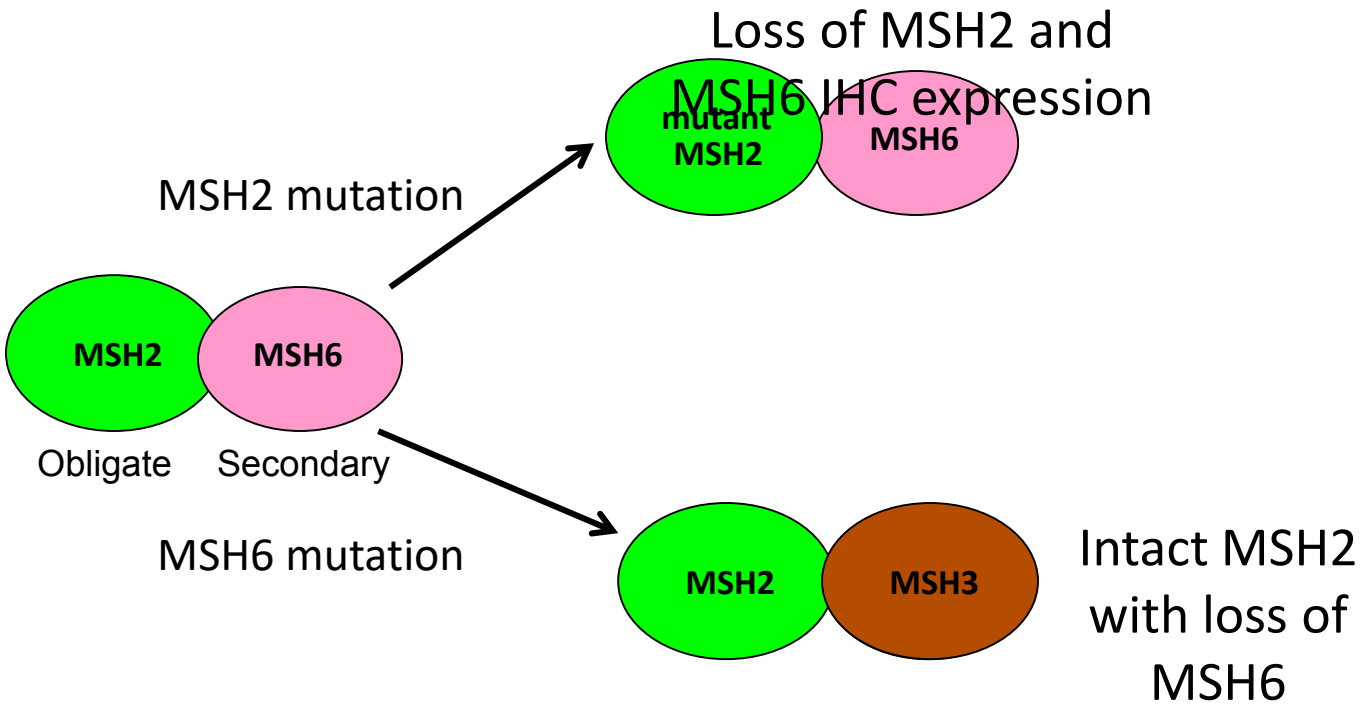

Preserved
Loss
Preserved
Equivocal

MMR Proteins: Basic Biology



- Dimers of obligate and secondary partner.
- Loss of obligate partner results in proteolytic degradation of the respective secondary partner.
- Loss of secondary partner still results in intact expression of obligate partner as it can bind to other partner proteins preventing degradation.

MMR Proteins: Basic Biology

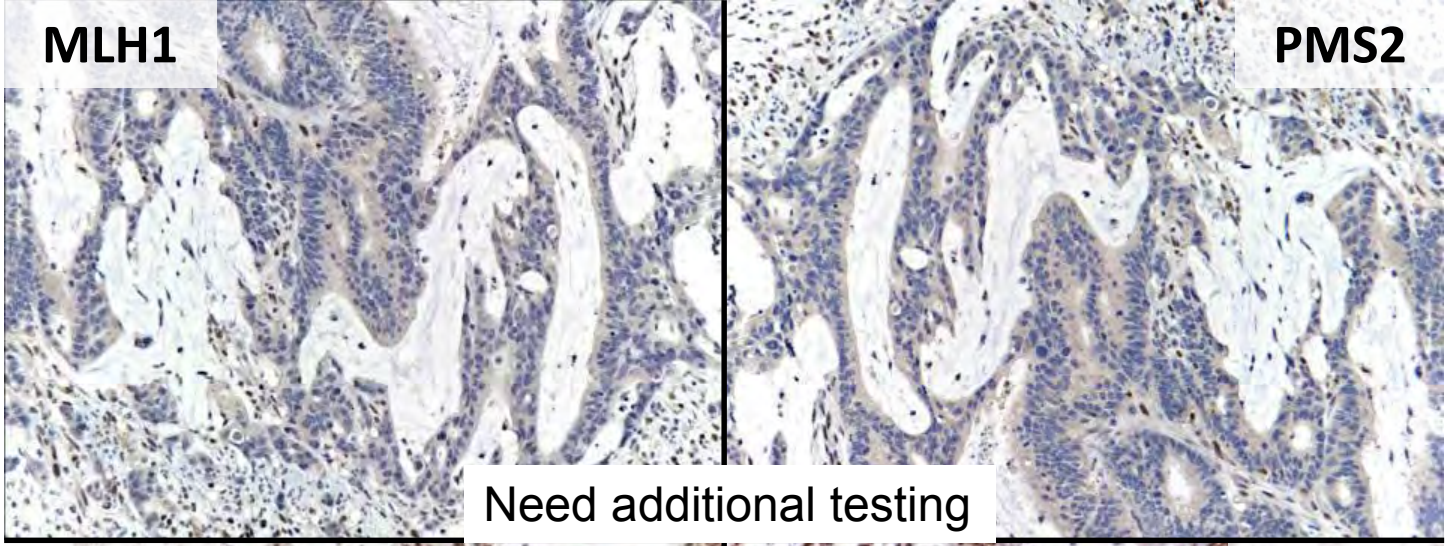


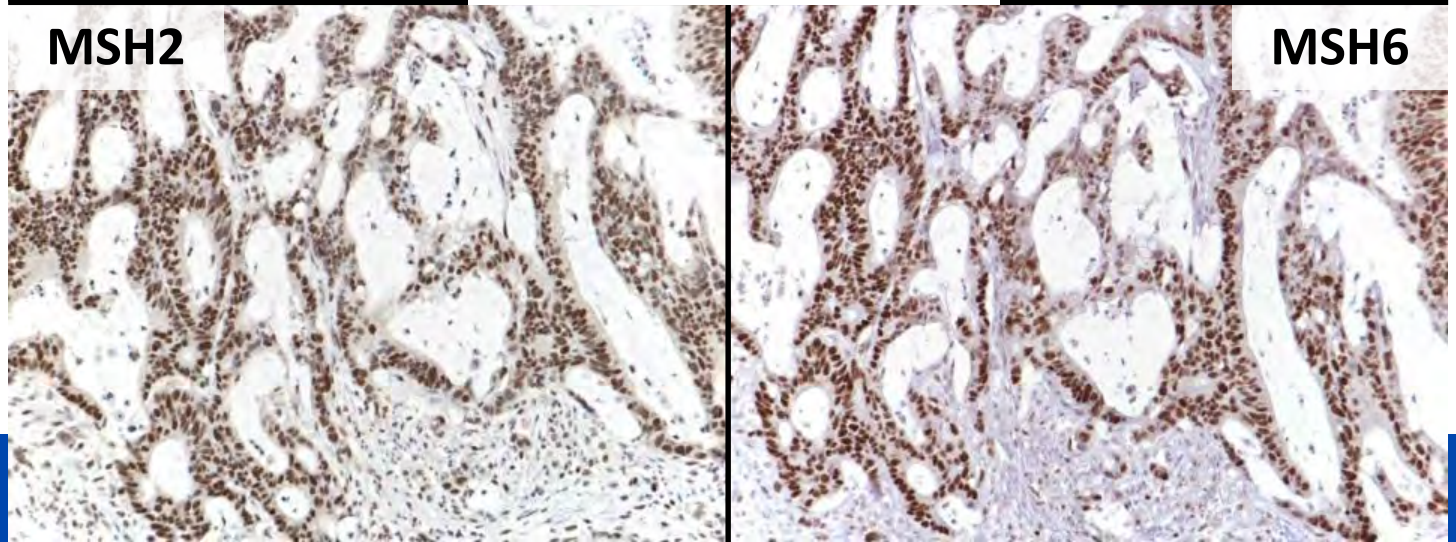
MMR IHC as a screening tool

IHC result	Most likely defective gene
Loss of MLH1 and PMS2	
Loss of MSH2 and MSH6	
Isolated loss of MSH6	
Isolated loss of PMS2	

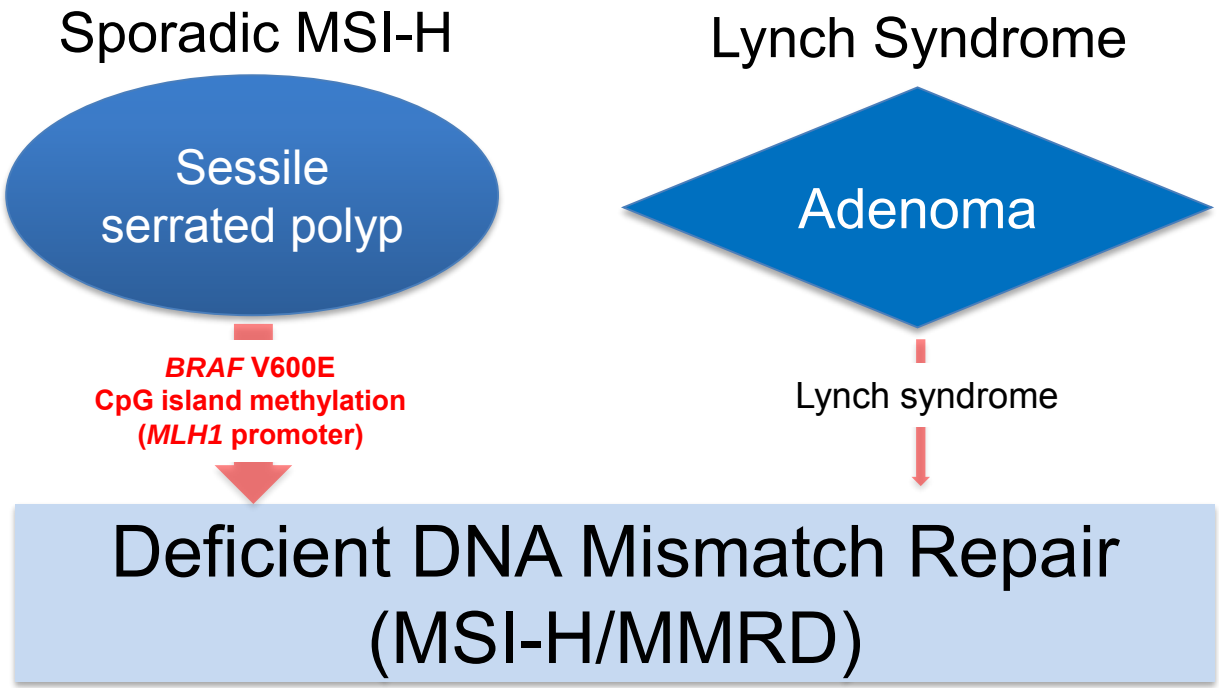
Seen in sporadic MSI-H and Lynch syndrome

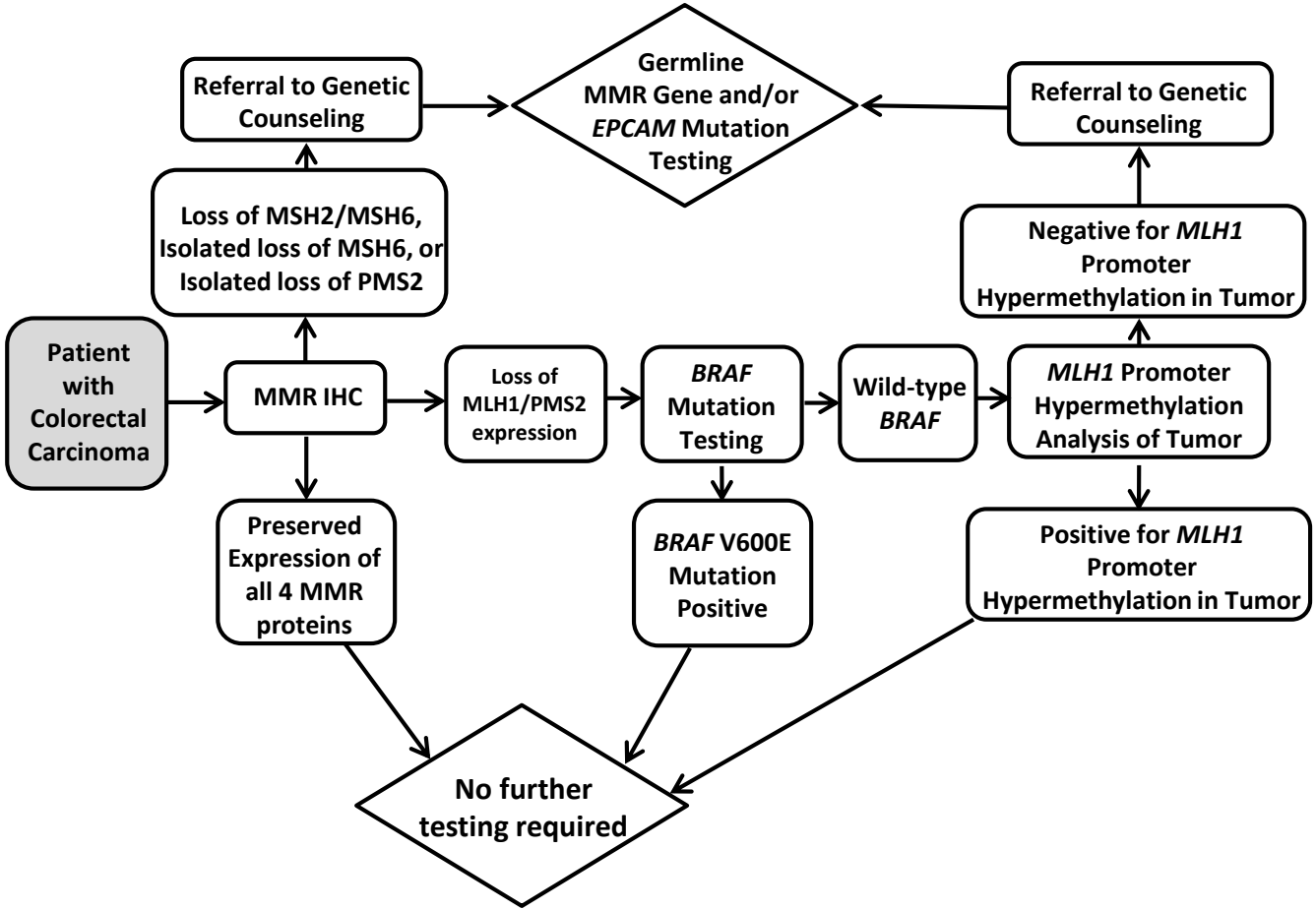
Concerning for Lynch syndrome ***but not diagnostic***





Pathways to MMR Deficiency





Pai RK and Pai RK. Am J Surg Pathol 2016;40:e17-34.

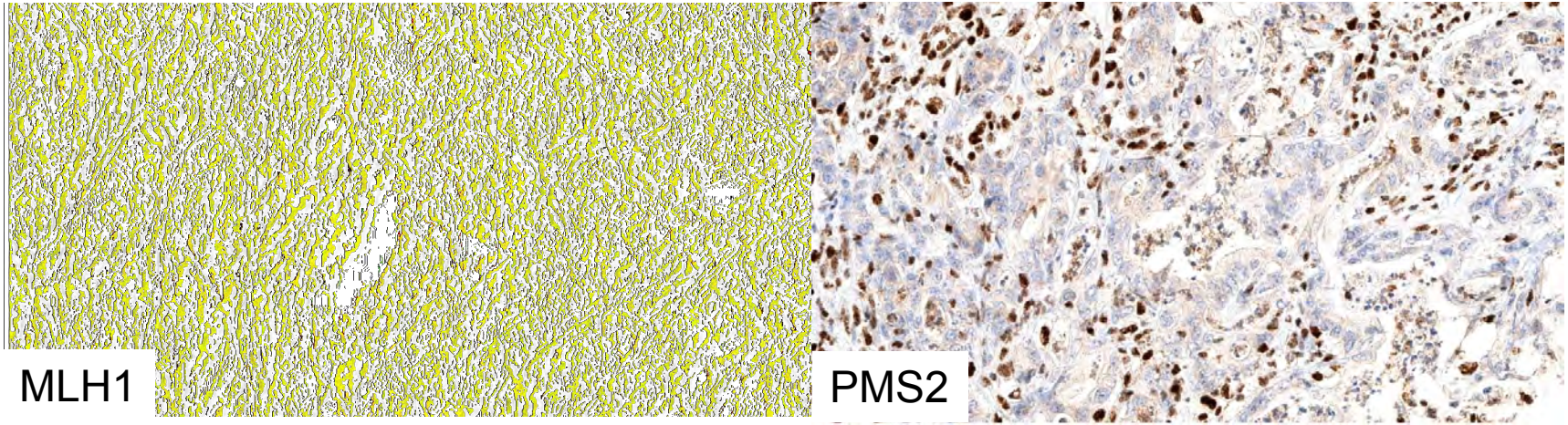
MMR IHC as a screening tool

IHC result	Most likely defective gene
Loss of MLH1 and PMS2	*MLH1*
Loss of MSH2 and MSH6	*MSH2* or *EPCAM*
Isolated loss of MSH6	*MSH6*
Isolated loss of PMS2	*PMS2* or *MLH1*

Rarely, other patterns can be seen

Unusual MMR IHC Patterns

- **Punctate/speckled nuclear MLH1**
  - Typically seen with concurrent PMS2 loss and *BRAF* V600E mutation/*MLH1* promoter hypermethylation.
  - Likely a technical issue with staining protocol.

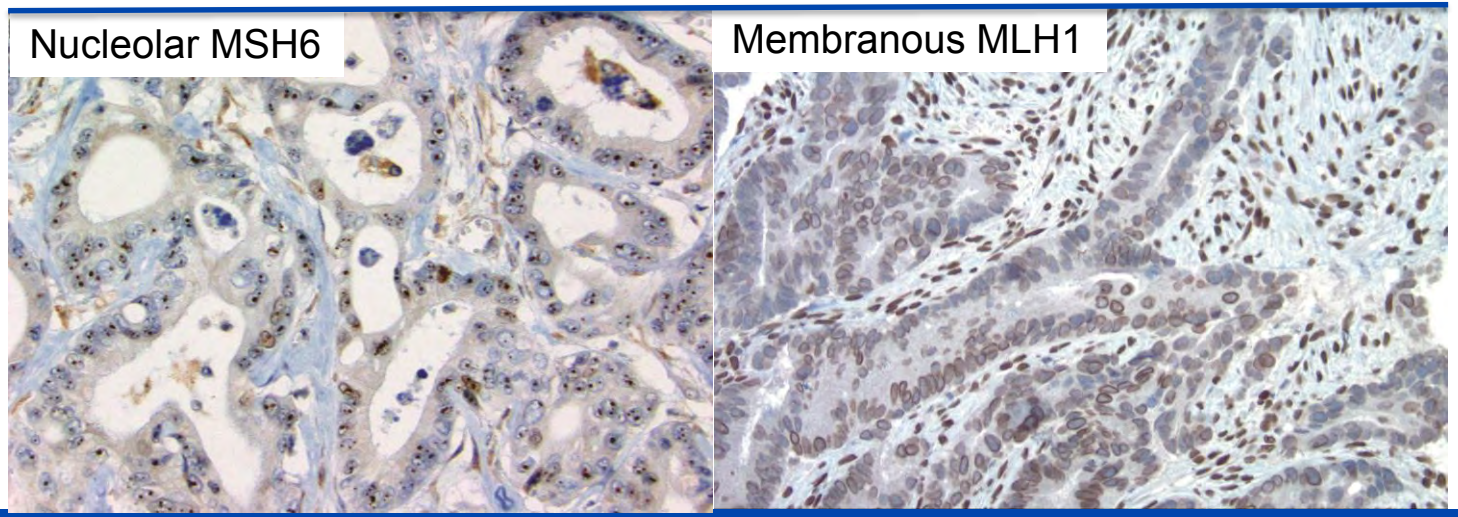


Don’t interpret as isolated loss of PMS2

Loughrey, M., et al. Histopathology. 2018. epub ahead of print

Unusual MMR IHC Patterns

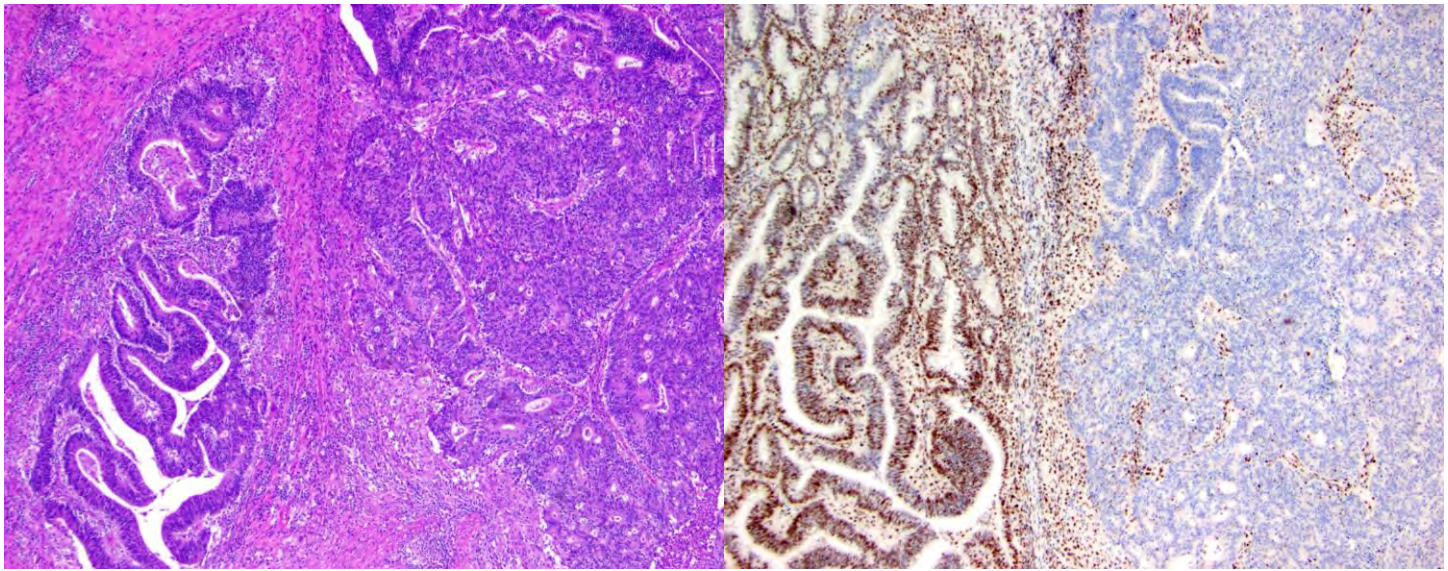
- **Nucleolar MSH6 *or* Membranous MLH1**
  - Should not be taken as evidence of intact expression. MSI PCR should be performed.
  - Likely a technical issue with staining protocol.



Unusual MMR IHC Patterns

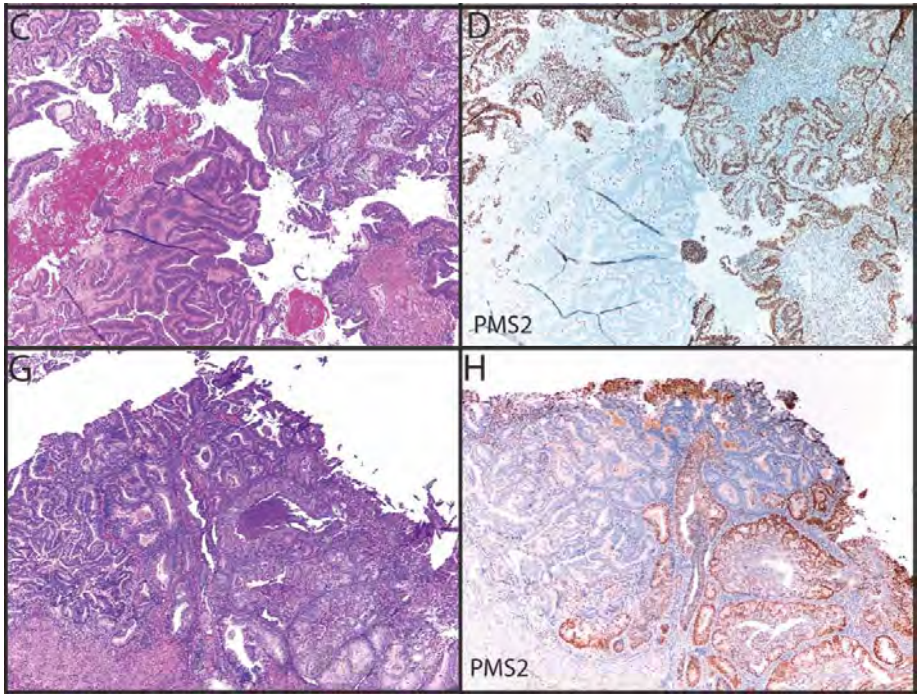
Clonal/Subclonal Loss of MLH1 and PMS2

- Large areas of tumor show abrupt loss of expression



Unusual MMR IHC Patterns

- **Clonal/Subclonal Loss of MLH1 and PMS2**
  - Large areas of tumor show abrupt loss of expression
  - Result of differential *MLH1* hypermethylation within these different areas



Pai, RK et al. Am J Surg Path. 2015. 39(7):993-999

Unusual MMR IHC Patterns

- **Decreased MMR expression after neoadjuvant therapy**

Neoadjuvant Therapy Induces Loss of MSH6 Expression in Colorectal Carcinoma

Fei Bao, MD, Nicole C. Panarelli, MD,† Hanna Rennert, PhD,† David L. Sherr, MD,‡ and Rhonda K. Yantiss, MD† (Am J Surg Pathol 2010;34:1798–1804)*

Decreased MSH6 in 20% of treated tumors

How reliable is immunohistochemical staining for DNA mismatch repair proteins performed after neoadjuvant chemoradiation?☆,☆☆,★

Human Pathology (2014) 45, 2029–2036

Alex Vilkin MD[a], Marisa Halpern MD[b], Sara Morgenstern MD[c], Eli Brazovski MD[d], Rachel Gingold-Belfer MD[a], Doron Boltin MD[a], Ofer Purim MD[d], Yulia Kundel MD[d], Sara Welinsky MD[a], Baruch Brenner MD[d,e], Yaron Niv MD[a,e], Zohar Levi MD[a,e,*]

Decreased expression of all MMR proteins after treatment

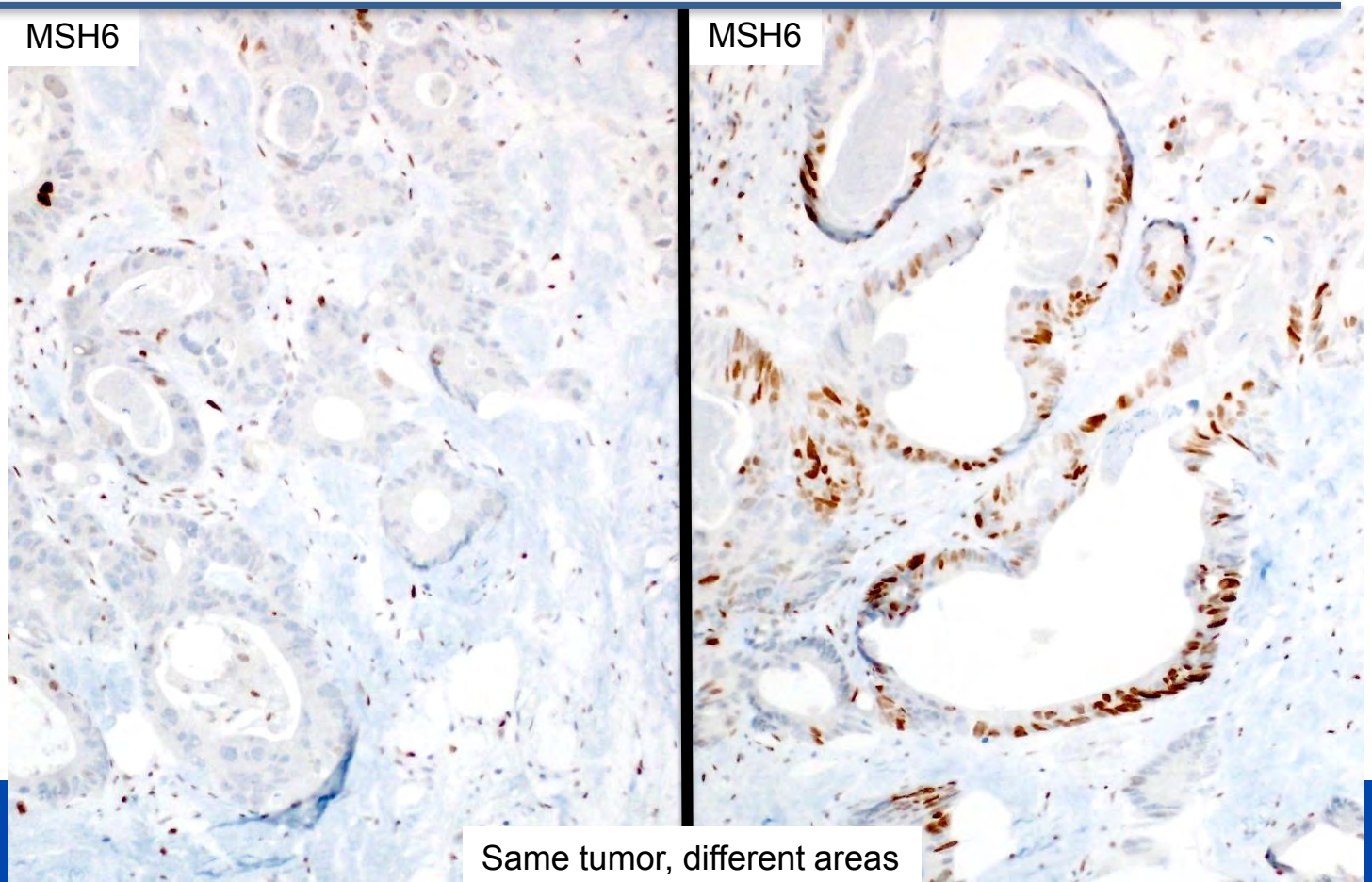
Neoadjuvant therapy in microsatellite-stable colorectal carcinoma induces concomitant loss of MSH6 and Ki-67 expression☆

Shih-Fan Kuan MD, PhD[a,*], Bing Ren MD, PhD[a], Randall Brand MD[b], Beth Dudley MS[b], Reetesh K. Pai MD[a]

Human Pathology (2017) 63, 33–39

Decreased expression correlated with proliferation

Unusual MMR IHC Patterns



Same tumor, different areas

Unusual MMR IHC Patterns

- **Concurrent Loss of MLH1, PMS2, and decreased MSH6**
  - Decreased MSH6 (<5% expression) is most often due to secondary **somatic** mutation of a coding microsatellite within the *MSH6* gene.

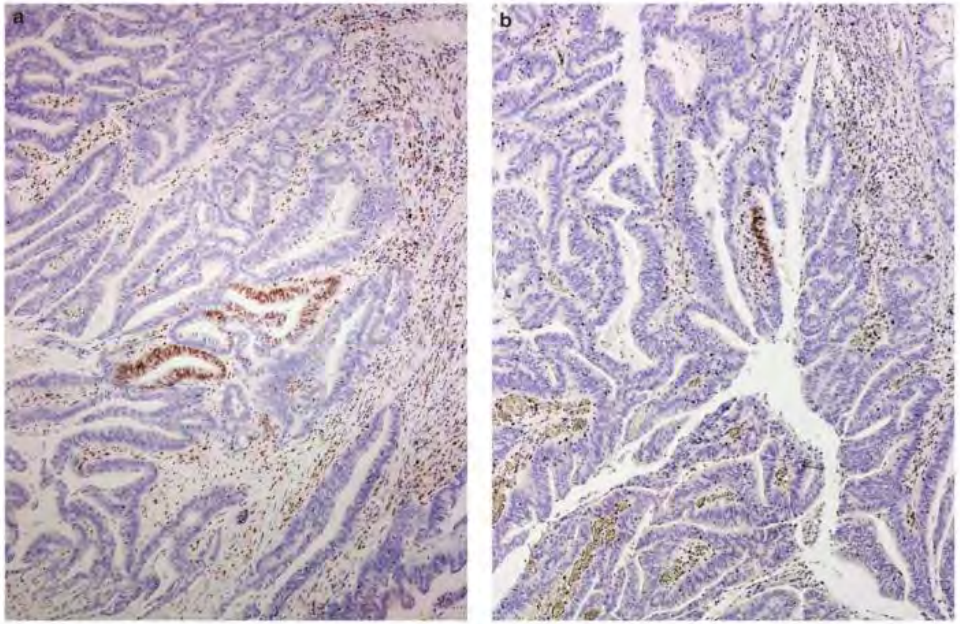
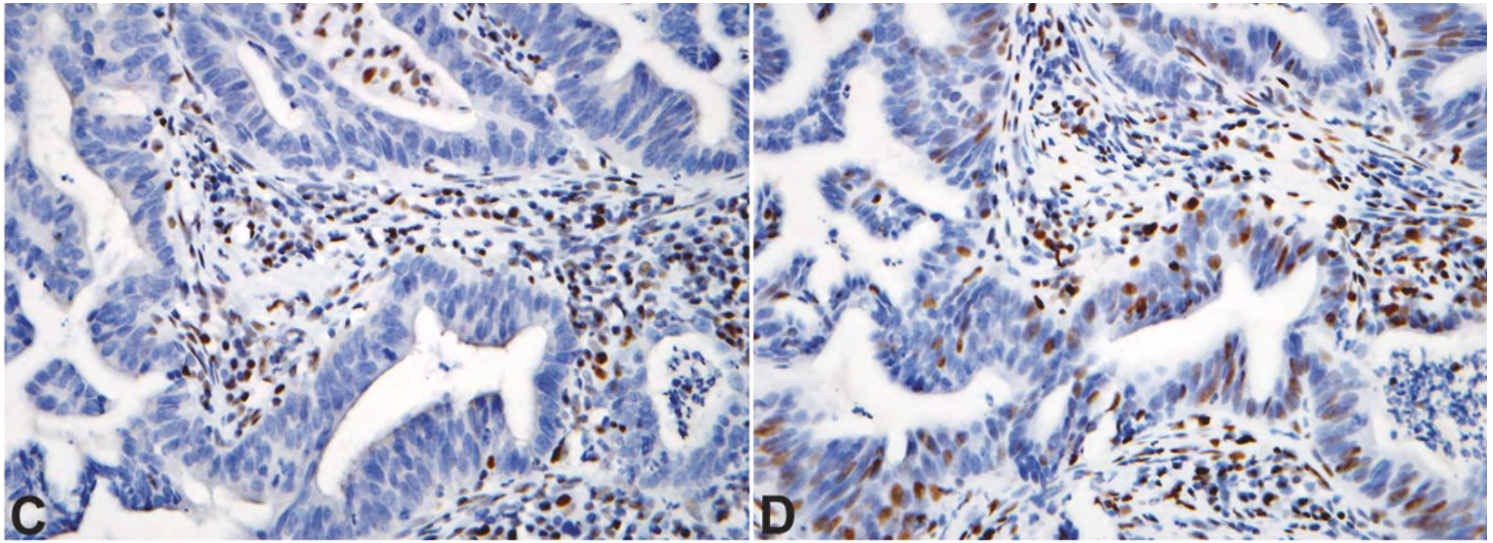


Figure 1 Immunohistochemical staining showing scanty MSH6 staining in a colonic adenocarcinoma; **a** and **b** represent two areas from one tumor where there is distinct nuclear staining for MSH6, but the staining is present only in a limited number of tumor cells. Note the presence of tumor-infiltrating lymphocytes that stain positively for MSH6. This tumor has intact expression of MSH2 and complete loss of MLH1 and PMS2 (staining not shown).

Shia, J, et al. Mod Pathol. 2013 Jan;26(1):131-8.

Unusual MMR IHC Patterns

- “Isolated loss of MSH2” with patchy but convincing staining for MSH6



Most patients will have *MSH2* mutations similar to those with complete loss of MSH2 and MSH6

Pearlman R, et al. Modern Pathology (2018) 31:1891–1900

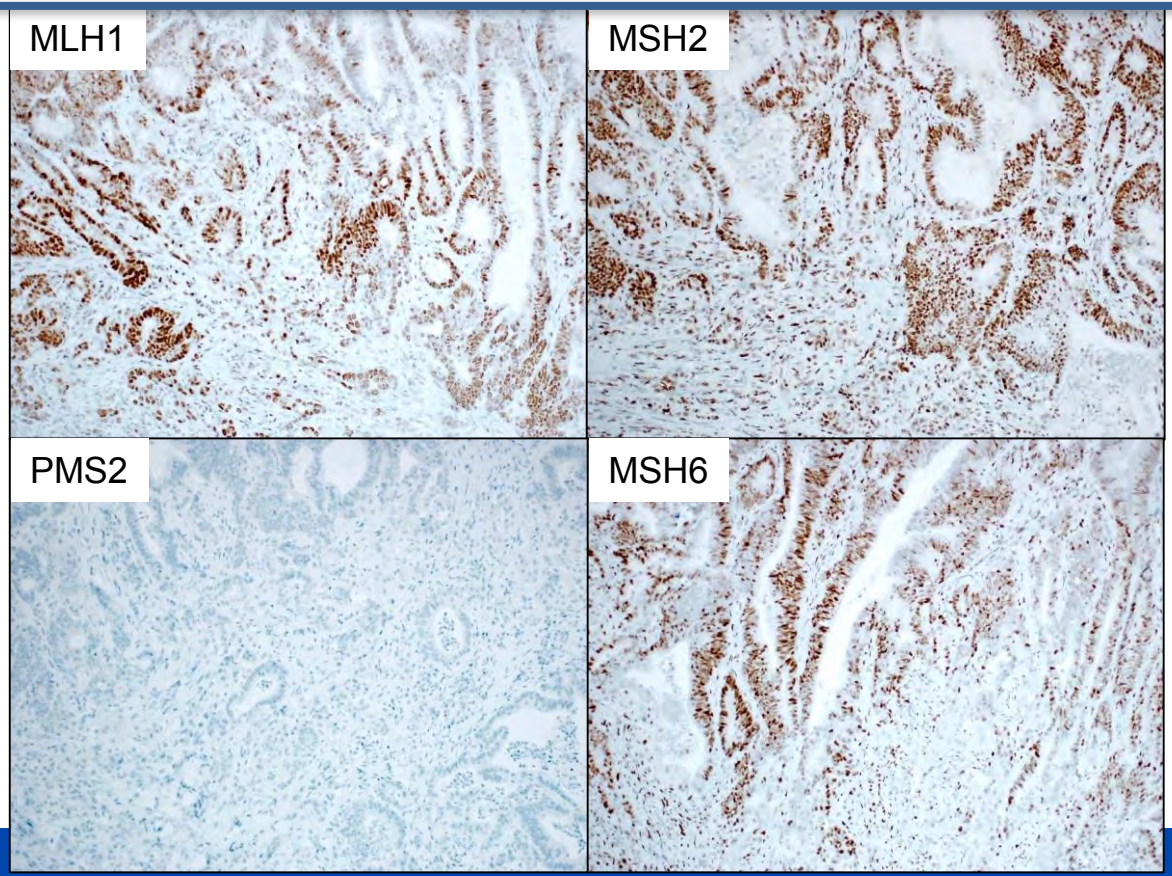
MMR IHC as a screening tool

Most common patterns

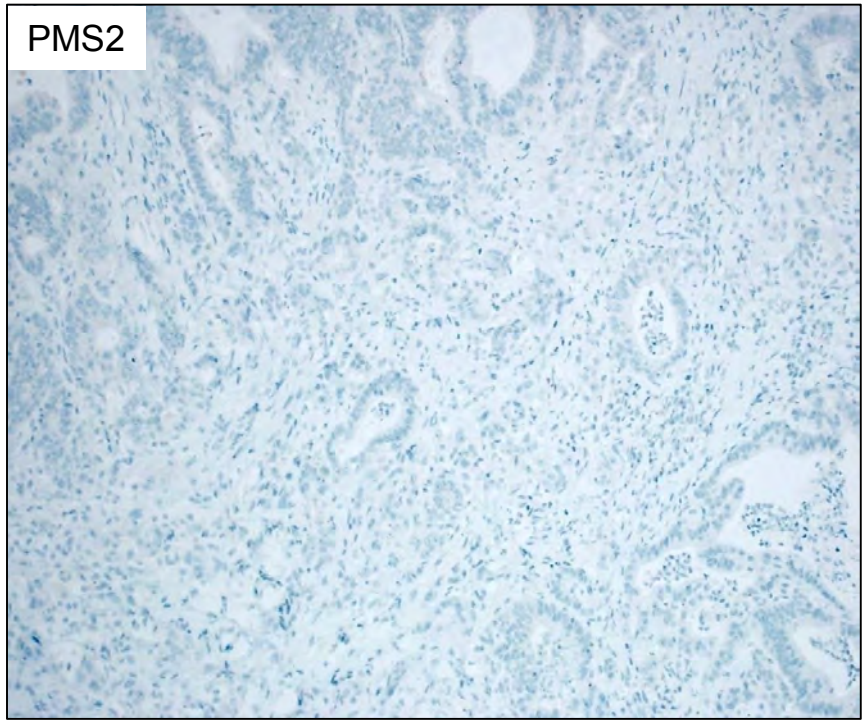
IHC result	Most likely defective gene
Loss of MLH1 and PMS2	*MLH1*
Loss of MSH2 and MSH6	*MSH2* or *EPCAM*
Isolated loss of MSH6	*MSH6*
Isolated loss of PMS2	*PMS2* or *MLH1*

- Punctate MLH1, PMS2 loss
- Nucleolar MSH6
- Membranous MLH1
- Clonal loss of MLH1/PMS2
- Punctate MLH1, PMS2 loss
- Reduced MSH6
- Concurrent loss of MLH1, PMS2 and focal MSH6

40 yo with ascending colon tumor: MMR IHC



40 yo with ascending colon tumor: MMR IHC



What are your next steps?

- Repeated PMS2 x3:
  - Same result
- Performed MSI testing by PCR
  - Only 3 of 5 loci were evaluable
  - 2 of 3 were unstable (MSI-H)

40 yo with ascending colon tumor: MMR IHC

CONSENSUS GUIDELINES

Recommendations on Surveillance and Management of Biallelic Mismatch Repair Deficiency (BMMRD) Syndrome: A Consensus Statement by the US Multi-Soci

Gastroenterology 2017;152:1605–1614

Colorectal Cancer

Carol Durno,[1] C. Richard Boland,[2] Shlomi Cohen,[3] Jason A. Dominitz,[4,5] Frank M. Giardiello,[6] David A. Johnson,[7] Tonya Kaltenbach,[8] T. R. Levin,[9] David Lieberman,[10] Douglas J. Robertson,[11,12] and Douglas K. Rex[13]

- LS-associated cancers
- Polyposis
- Café-au-lait macules
- Loss of affected MMR protein expression in **tumor** and **normal**
- PMS2 and MSH6 are the most affected

Table 1. Estimated Penetrance and Age of Onset Neoplasms in BMMRD

Organ	Estimated penetrance, %	Age at diagnosis, median (range), *y*
Small-bowel adenomas[a]	50	12 (10–20)
Colorectal adenomas[a]	>90	9 (6–15)
Small-bowel cancer	10	28 (11–42)
Colorectal cancer[b]	70	16 (8–48)
Low-grade brain tumors	Unknown	Unknown
High-grade brain tumors[c]	70	9 (2–40)
Lymphoma	20–40	5 (0.4–30)
Leukemia	10–40	8 (2–21)
Endometrial cancer	<10	(19–44)
Urinary tract cancer	<10	(10–22)

Screening for Lynch Syndrome: Goals

- Identify MSI-H tumors
- Separate out sporadic MSI-H tumors
- Identify those patients that need germline testing
- Identify deleterious mutations in MMR genes
- Identify affected family members
- Enroll affected individuals in lifelong screening program

Setting up a screening program

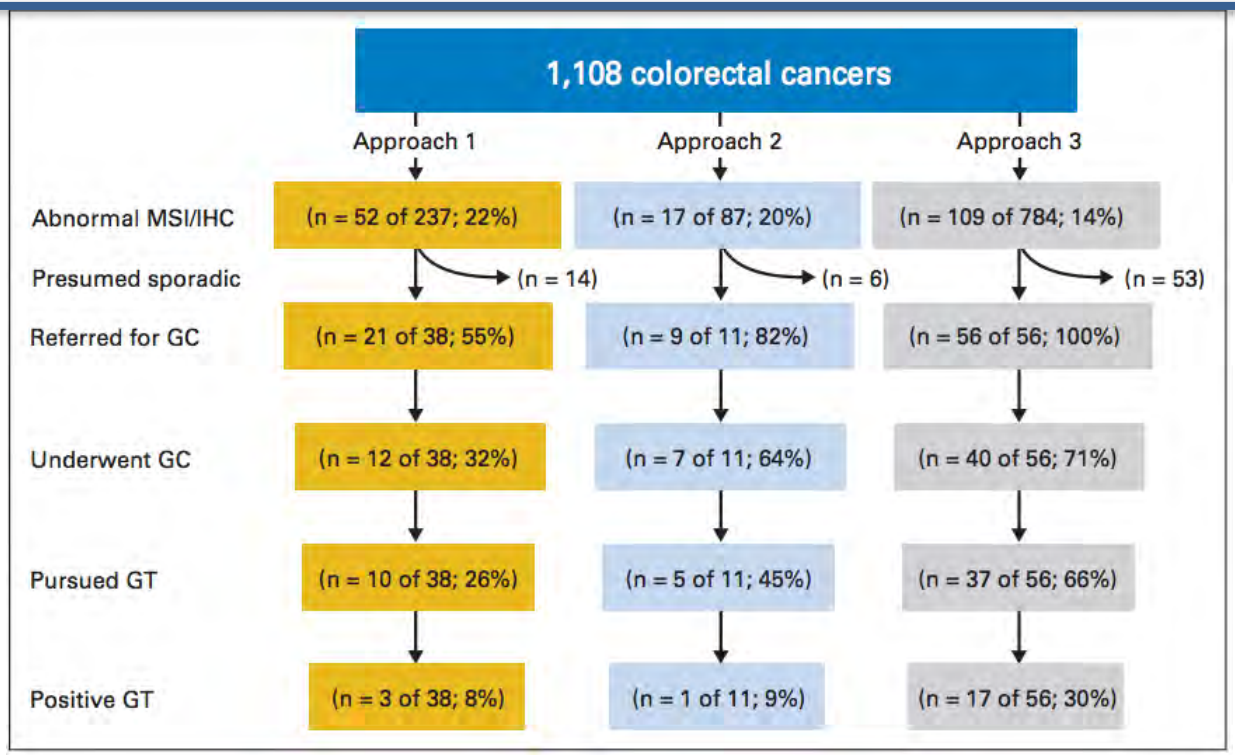
Implementation of Universal Microsatellite Instability and Immunohistochemistry Screening for Diagnosing Lynch Syndrome in a Large Academic Medical Center

Brandie Heald, Thomas Plesec, Xiuli Liu, Rish Pai, Deepa Patil, Jessica Moline, Richard R. Sharp, Carol A. Burke, Matthew F. Kalady, James Church, and Charis Eng

J. Clin Oncol. 2013 31(10):1336-40

- Approach 1: Provide results to the surgeon who would decide who to refer to genetic counseling.
- Approach 2: Provide results to surgeon and GC. GC would contact surgeon and not patient.
- Approach 3: Provide results to surgeon and GC. GC would contact patient directly

Setting up a screening program



Approach 3 is superior

Screening for Lynch syndrome

- Who to screen has been answered: **Universal Screening** is the best (for both CRC and Endometrial carcinoma)
- Many issues remain
  - Correct interpretation of MMR IHC
    - Unusual MMR IHC staining patterns
    - Pitfalls in interpretation
  - How do you set up a successful program?
  - Should we screen other GI tract carcinomas? Polyps?
  - MMR IHC and other tests suggest LS but germline testing is negative, now what?

Question 2:

Screening which of the following has the highest yield for detection of Lynch Syndrome?

A. Gastric adenocarcinoma
B. Cholangiocarcinoma
C. Colonic adenomas in patients < 40 yrs
D. Small bowel adenocarcinoma

Question 2:

Screening which of the following has the highest yield for detection of Lynch Syndrome?

A. Gastric adenocarcinoma

B. Cholangiocarcinoma

C. Colonic adenomas in patients < 40 yrs

D. Small bowel adenocarcinoma

Small bowel adenocarcinomas

Table 3. Standardized incidence ratios (SIRs) and corresponding 95% confidence intervals (CIs) of primary extracolonic cancers following colorectal cancer for carriers of mismatch repair gene mutation*

Site of cancer	O	E	Median age at diagnosis, y(min–max)	Median no. of years from colorectal cancer to following cancer diagnosis (min–max)	SIR	(95% CI)
Both sexes						
Kidney etc.†	25	1.99	60 (35–78)	14 (1–40)	12.54	(7.97 to 17.94)
Urinary bladder	18	2.49	65 (54–84)	11 (2–34)	7.22	(4.08 to 10.99)
Small intestine	17	0.23	55 (31–67)	13 (1–28)	72.68	(39.95 to 111.29)
Stomach	9	1.59	69 (55–79)	19 (1–38)	5.65	(2.32 to 9.69)
Hepatobiliary tract‡	7	1.18	62 (39–73)	6 (2–13)	5.94	(1.81 to 10.94)
Brain	5	1.15	68 (62–80)	16 (10–33)	4.36	(0.79 to 9.55)
Hematopoietic tissue	5	1.61	57 (41–75)	12 (2–18)	3.11	(0.63 to 6.10)
Lung	4	9.48	57 (48–65)	13 (1–18)	0.42	(0.10 to 0.91)
Pancreas	3	1.62	65 (46–67)	13 (9–23)	1.86	(0.00 to 4.31)
Bone	2	0.11	68 (64–71)	3.5 (3–4)	17.99	(0.00 to 45.41)
Men						
Prostate	19	9.25	64 (55–77)	14 (4–33)	2.05	(1.23 to 3.01)
Women						
Endometrium	45	1.12	50 (35–69)	8 (1–34)	40.23	(27.91 to 56.06)
Breast	20	11.34	60 (43–79)	16 (1–23)	1.76	(1.07 to 2.59)
Ovary	6	1.43	52 (48–61)	10 (1–26)	4.19	(1.28 to 7.97)

J Natl Cancer Inst. 2012 Sep 19;104(18):1363–72

Small bowel adenocarcinomas

Small bowel adenocarcinoma phenotyping, a clinicobiological prognostic study

T Aparicio[*,1], M Svrcek[2], A Zaanan[3,4], E Beohou[5], A Laforest[4], P Afchain[6], Emmanuel Mitry[7], J Taieb[3], F Di Fiore[8], J-M Gornet[9], A Thirot-Bidault[10], I Sobhani[11], D Malka[12], T Lecomte[13], C Locher[14], F Bonnetain[5] and P Laurent-Puig[4]

- MMRD only in duodenum and jejunum
- 9/14 MMRD were associated with Lynch syndrome (14% of all SB adenoCA in this series)

Table 3. Tumour characteristics according to the primary site

Tumour characteristic	Duodenum, $n=32$ (51%)	Jejunum, $n=18$ (29%)	Ileum, $n=13$ (20%)	*P*-value
Stages I–II ($n=19$)	8 (42%)	7 (37%)	4 (21%)	
Stage III ($n=22$)	13 (59%)	5 (23%)	4 (18%)	0.81
Stage IV ($n=20$)	9 (45%)	6 (30%)	5 (25%)	
Poorly differentiated ($n=13$)	5 (38%)	3 (24%)	5 (38%)	0.23
P53 overexpression ($n=26$)	13 (50%)	8 (31%)	5 (19%)	0.89
Abnormal β-catenin ($n=12$)	4 (33%)	3 (25%)	5 (42%)	0.16
dMMR phenotype ($n=14$)	9 (64%)	5 (36%)	0 (0%)	0.07
Mutated *KRAS* ($n=21$)	12 (57%)	6 (29%)	3 (14%)	0.73
HER2 expression 2+ ($n=2$)	0 (0%)	0 (0%)	2 (100%)	—

Br J Cancer. 2013 Dec 10;109(12):3057–66

Colonic polyps in Lynch Syndrome

Immunohistochemical testing of conventional adenomas for loss of expression of mismatch repair proteins in Lynch syndrome mutation carriers: a case series from the Australasian site of the colon cancer family registry

MODERN PATHOLOGY (2012) 25, 722–730

Michael D Walsh[1,2], Daniel D Buchanan[1], Sally-Ann Pearson[1], Mark Clendenning[1], Mark A Jenkins[3], Aung Ko Win[3], Rhiannon J Walters[1], Kevin J Spring[1], Belinda Nagler[1], Erika Pavluk[1], Sven T Arnold[1], Jack Goldblatt[4,5], Jill George[5], Graeme K Suthers[6,7], Kerry Phillips[7], John L Hopper[3], Jeremy R Jass[8], John A Baron[9], Dennis J Ahnen[10], Stephen N Thibodeau[11], Noralane Lindor[12], Susan Parry[13], Neal I Walker[14], Christophe Rosty[1,2,15] and Joanne P Young[1,2]

- dMMR by IHC in 79% of LS-adenomas
- 27/29 (93%) with villous component
- 47/65 (73%) w/o villous component
- 12/12 (100%) with HGD
- No diff between <10mm and >10 mm

Microsatellite Instability and DNA Mismatch Repair Protein Deficiency in Lynch Syndrome Colorectal Polyps

Cancer Prev Res; 5(4) April 2012

Matthew B. Yurgelun[1], Ajay Goel[4], Jason L. Hornick[2], Ananda Sen[5], Danielle Kim Turgeon[5,6], Mack T. Ruffin IV[5,6], Norman E. Marcon[6,8], John A. Baron[6,9], Robert S. Bresalier[6,10], Sapna Syngal[2,3,6], Dean E. Brenner[5,6,7], C. Richard Boland[4], and Elena M. Stoffel[5]

- 18/36 (50%) of adenomas, 0/21 HP, 0/2 SSPs
- >8mm were more likely to demonstrate dMMR

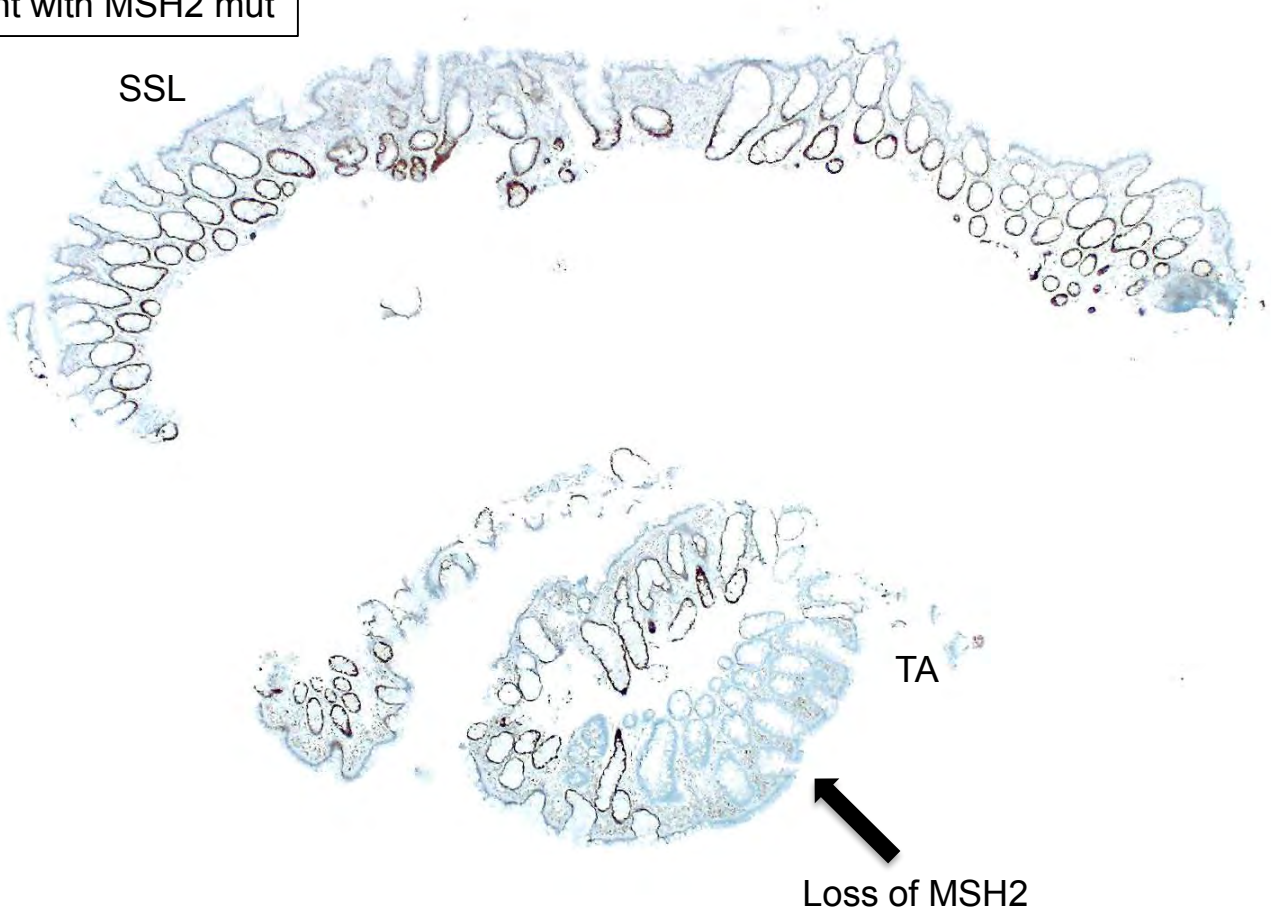
Mismatch repair deficiency commonly precedes adenoma formation in Lynch Syndrome-Associated colorectal tumorigenesis

MODERN PATHOLOGY (2017) 30, 1144–1151

Shigeki Sekine[1,2,3], Taisuke Mori[1,2,3], Reiko Ogawa[2], Masahiro Tanaka[1], Hiroshi Yoshida[1], Hirokazu Taniguchi[1], Takeshi Nakajima[3,4], Kokichi Sugano[3,5], Teruhiko Yoshida[3,6], Mamoru Kato[7], Eisaku Furukawa[7], Atsushi Ochiai[8] and Nobuyoshi Hiraoka[1,2,3]

- dMMR by IHC in 79% of LS-adenomas

LS patient with MSH2 mut



Should we screen adenomas?

Advanced Colorectal Adenomas in Patients Under 45 Years of Age Are Mostly Sporadic

Vladimir M. Kushnir · ILKe Nalbantoglu · Rao Watson · Jonathan Goodwin · Elyas Safar · Reena V. Chokshi · Riad R. Azar · Nicholas O. Davidson

Dig Dis Sci (2014) 59:2757–2764

- Identified 76 patients with adenomas < 45 y
- 64 patients had tissue available and only 1/64 probable LS patient was identified

Routine Molecular Analysis for Lynch Syndrome Among Adenomas or Colorectal Cancer Within a National Screening Program

Gastroenterology 2018;155:1410–1415

Anne Goverde,[1,2] Anja Wagner,[2] Marco J. Bruno,[1] Robert M. W. Hofstra,[2] Michael Doukas,[3] Marcel M. van der Weiden,[3] Hendrikus J. Dubbink,[3] Winand N. M. Dinjens,[3] and Manon C. W. Spaander[1]

Table 2. Results of Molecular Diagnostics

	n	Age, y (IQR)	Male gender, n (%)	MMR deficiency	MHL1 promoter methylation	Germline MMR mutation	Somatic MMR mutations
Patients included for IHC	456	67 (63–71)	296 (65)	8	5	0	3
Colorectal cancer	56	69 (63–72)	36 (64)	7	5	0	2
Advanced adenoma	370	66 (62–71)	237 (64)				
Villous component	186	65 (61–69)	124 (67)	1	0	0	1
High-grade dysplasia	42	67 (63–74)	30 (73)	0	0	0	0
4–10 nonadvanced adenomas	30	67 (63–74)	23 (77)	0	0	0	0

Screening for Lynch syndrome

- Who to screen has been answered: **Universal Screening** is the best (for both CRC and Endometrial carcinoma)
- Many issues remain
  - Correct interpretation of MMR IHC
    - Unusual MMR IHC staining patterns
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  - How do you set up a successful program?
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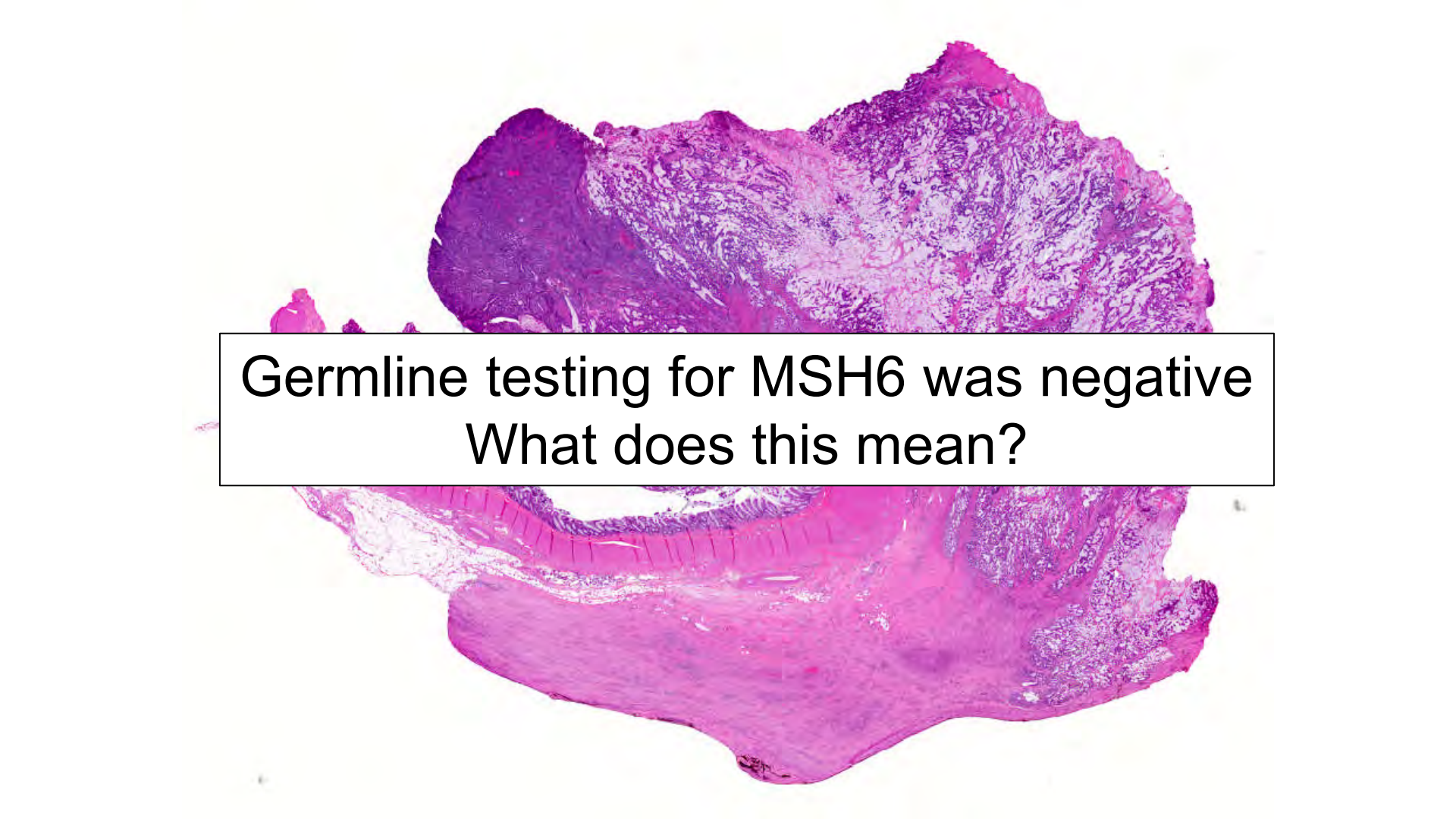
  - MMR IHC and other tests suggest LS but germline testing is negative, now what?


Germline testing for MSH6 was negative
What does this mean?

“Lynch-Like” Syndrome

- Deficient DNA mismatch repair protein expression with ***no deleterious germline mutation*** in mismatch repair genes or *EPCAM* and, if MLH1-deficient, no evidence of *BRAF* mutation or *MLH1* promoter hypermethylation.
- Also called “Suspected Lynch Syndrome” by Win and colleagues (*Gut* 2015;64:101-10)
- Accounts for between 2.5% and 3.9% of patients with colorectal carcinoma.
- **~30%** of patients with abnormal MMR protein expression within their tumor concerning for Lynch syndrome will have ***negative germline mutation studies***.

Issues with “Lynch-Like Syndrome”

1. Anxiety for patients as they are uncertain if they have a genetic disease with ramifications for their health and the health of their family.

2. Intensive lifelong screening protocols for patients with Lynch syndrome.
   a) Should it be applied to patients with “Lynch-like syndrome”?
   b) Most patients with “Lynch-like syndrome” have opt to follow a screening protocol as if they have confirmed Lynch syndrome.

Somatic MMR Gene Mutation

Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors

Isabelle Sourrouille · Florence Coulet · Jeremie H. Lefevre · Chrystelle Colas · Mélanie Eyries · Magali Svrcek · Armelle Bardier-Dupas · Yann Parc · Florent Soubrier

BRIEF REPORTS

Somatic Mutations in *MLH1* and *MSH2* Are a Frequent Cause of Mismatch-Repair Deficiency in Lynch Syndrome-Like Tumors

Arjen R. Mensenkamp,[1] Ingrid P. Vogelaar,[1] Wendy A. G. van Zelst-Stams,[1] Monique Goossens,[2] Hicham Ouchene,[1] Sandra J. B. Hendriks-Cornelissen,[2] Michael P. Kwint,[2] Nicoline Hoogerbrugge,[1] Iris D. Nagtegaal,[2] and Marjolijn J. L. Ligtenberg[1]

[1]Department of Human Genetics, [2]Department of Pathology, Radboud university medical center, Nijmegen, The Netherlands

Journal of Pathology
J Pathol 2014; 234: 548–559
Published online 30 September 2014 in Wiley Online Library (wileyonlinelibrary.com)

ORIGINAL PAPER

Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers

Willemina RR Geurts-Giele,[1]* Celine HM Leenen,[1] Hendrikus J Dubbink,[1] Isabelle C Meijssen,[1] Edward Post,[1] Hein FBM Sleddens,[1] Ernst J Kuipers,[2,3] Anne Goverde,[2,4] Ans MW van den Ouweland,[4] Margot GF van Lier,[5] Ewout W Steyerberg,[5] Monique E van Leerdam,[6]* Anja Wagner[1] and Winand NM Dinjens[1]

Colon and Endometrial Cancers With Mismatch Repair Deficiency Can Arise From Somatic, Rather Than Germline, Mutations

Sigurdis Haraldsdottir,[1] Heather Hampel,[2] Jerneja Tomsic,[3] Wendy L. Frankel,[4] Rachel Pearlman,[2] Albert de la Chapelle,[3] and Colin C. Pritchard[5]

[1]Department of Internal Medicine, Division of Medical Oncology, [2]Department of Internal Medicine, Division of Human Genetics, [3]Department of Microbiology, Virology, Immunology, and Medical Genetics, and [4]Department of Pathology, The Ohio State University Comprehensive Cancer Center, Columbus, Ohio, and [5]Department of Laboratory Medicine, University of Washington, Seattle, Washington

***Fam Cancer* 2013;12:27-33.**
(Sanger sequencing)

***Gastroenterology* 2014;146:643-646**
(Next-generation sequencing)

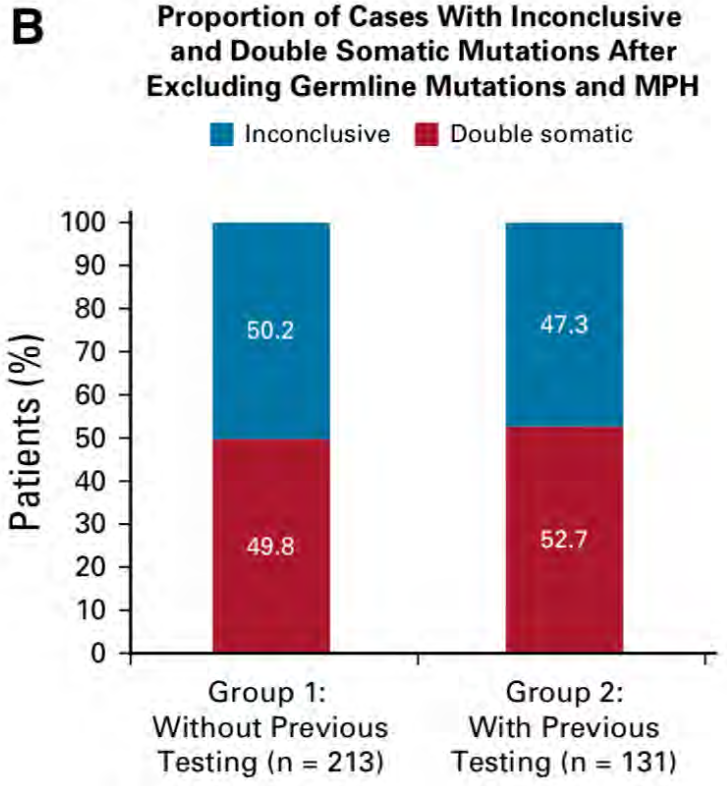
***J Pathol* 2014;234:548-559**
(Next-generation sequencing)

***Gastroenterology* 2014;147:1308-1316.**
(Next-generation sequencing)

Somatic MMR Gene Mutation in Colorectal Carcinoma in Patients with “Lynch-like Syndrome”

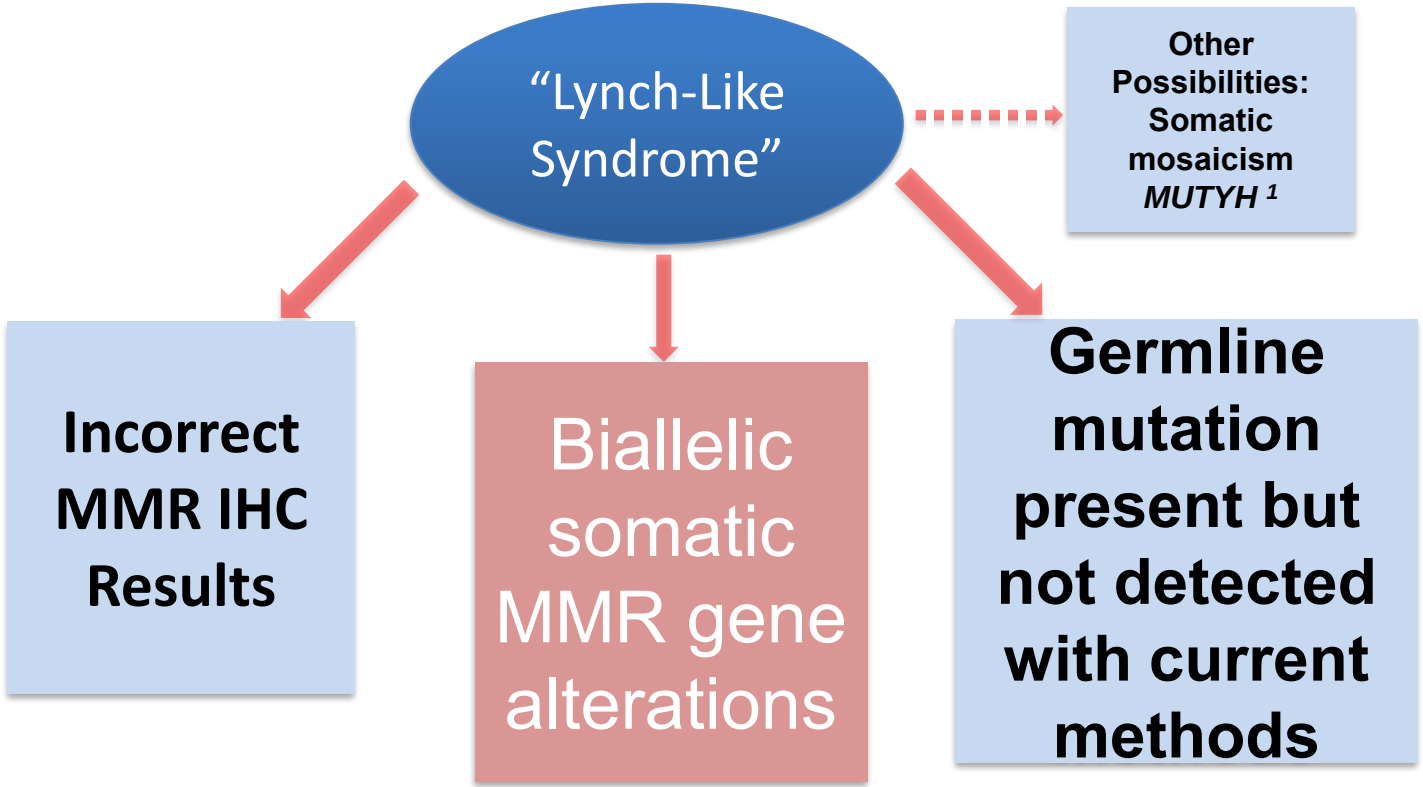
Somatic Alteration #1	Somatic Alteration #2	Loss of MLH1 & PMS2 (N=45)	Loss of MSH2 & MSH6 (N=22)
MMR gene deletion/frameshift/ insertion/duplication	MMR gene deletion/frameshift/ insertion/duplication	13%	41%
MMR gene deletion/frameshift/ insertion/duplication	MMR gene loss of heterozygosity (LOH)	58%	27%
% of “Lynch-like” carcinomas with biallelic somatic mutations explaining the abnormal MMR IHC results		71%	68%

Solving Lynch-Like cases: Current state



Ambry Genetics: J Clin Oncol. 2019 Mar 10;37(8):647-657.

Solving cases of “Lynch-like Syndrome”



Castillejo A, et al. Prevalence of *MUTYH* mutations among Lynch-like patients. *Eur J Cancer* 2014;50:2241.

MMR Deficient Colonic Crypts: A Novel Indicator of Lynch Syndrome

Prevalence of mismatch repair-deficient crypt foci in Lynch syndrome: a pathological study *Lancet Oncol* 2012; 13: 598–606

Matthias Kloor, Cathrin Huth*, Anita Y Voigt, Axel Benner, Peter Schirmacher, Magnus von Knebel Doeberitz, Hendrik Bläker*

Mismatch Repair-Deficient Crypt Foci in Lynch Syndrome – Molecular Alterations and Association with Clinical Parameters

PLoS One. 2015 10(3):e0121980

Laura Staffa[1], Fabian Echterdiek[1], Nina Nelius[1], Axel Benner[2], Wiebke Werft[2], Bernd Lahrmann[3], Niels Grabe[3], Martin Schneider[4], Mirjam Tariverdian[4], Magnus von Knebel Doeberitz[1], Hendrik Bläker[5☺], Matthias Kloor[1☺*]

- First reported by the same research group in Heidelberg, Germany (Kloor et al. and Staffa et al.)
  - Between 25% to 32% of patients with Lynch syndrome had MMR-deficient normal appearing crypts. Correlated with length of mucosa
- Can the identification of MMR deficient crypts help identify patients with Lynch syndrome?

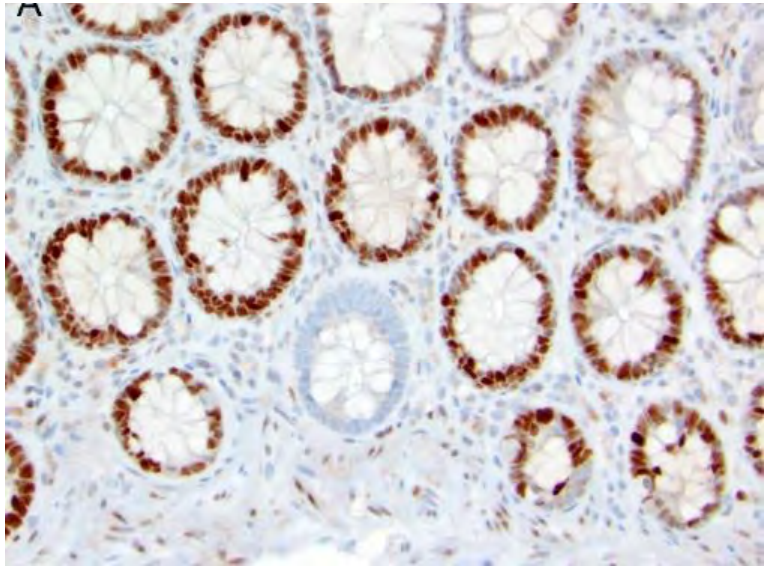
DNA mismatch repair protein deficient non-neoplastic colonic crypts: a novel indicator of Lynch syndrome

Rish K. Pai[1] · Beth Dudley[2] · Eve Karloski[2] · Randall E. Brand[2] · Neil O'Callaghan[3,4] · Christophe Rosty[3,4,5,6] · Daniel D. Buchanan[3,4,7] · Mark A. Jenkins[8] · Stephen N. Thibodeau[9] · Amy J. French[9] · Noralane M. Lindor[10] · Reetesh K. Pai[11]

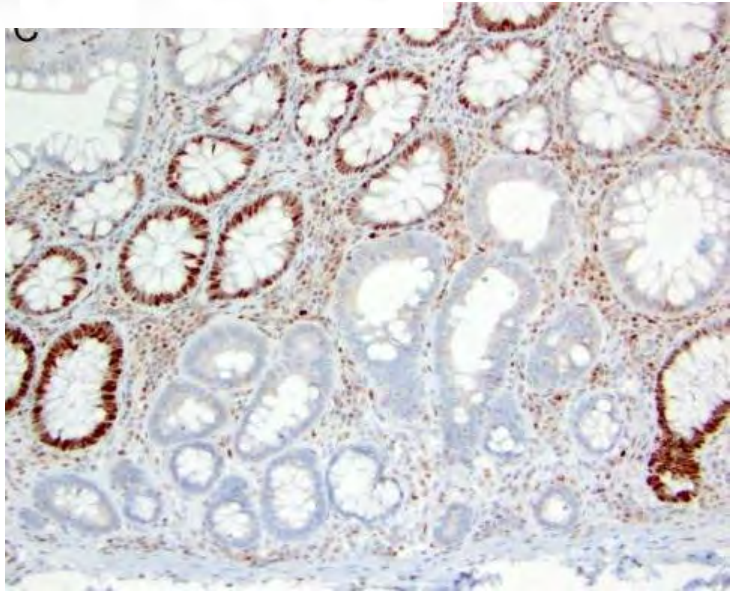
- Analyzed the following:
  - Normal mucosa from 52 patients with Lynch syndrome (LS) with known germline pathogenic variants and colorectal carcinoma
  - Normal mucosa from 30 MSS cancers and 30 sporadic MLH1 deficient colorectal cancers
- LS: IHC for known affected MMR gene
- MSS: IHC for all 4 MMR proteins
- Sporadic MLH1 deficiency: IHC for MLH1

DNA mismatch repair protein deficient non-neoplastic colonic crypts: a novel indicator of Lynch syndrome

Rish K. Pai[1] · Beth Dudley[2] · Eve Karloski[2] · Randall E. Brand[2] · Neil O'Callaghan[3,4] · Christophe Rosty[3,4,5,6] · Daniel D. Buchanan[3,4,7] · Mark A. Jenkins[8] · Stephen N. Thibodeau[9] · Amy J. French[9] · Noralane M. Lindor[10] · Reetesh K. Pai[11]

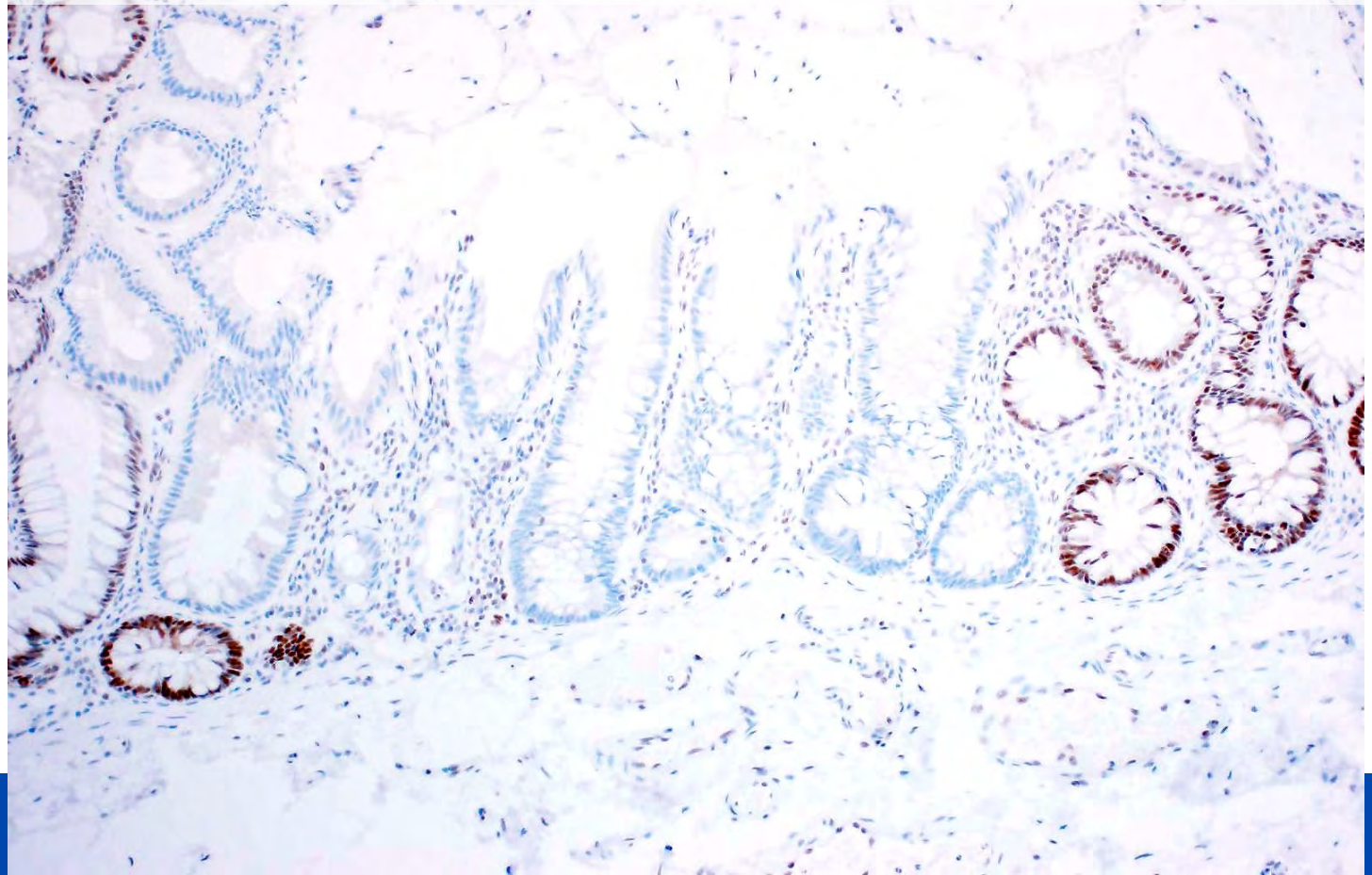


Solitary MSH6 deficient crypt in patient with germline pathogenic *MSH6* variant



Group of MSH6 deficient crypts in patient with germline pathogenic *MSH6* variant

MSH2 deficient crypts in patient with germline *MSH2* pathogenic variant



MMR Deficient Colonic Crypts in normal mucosa

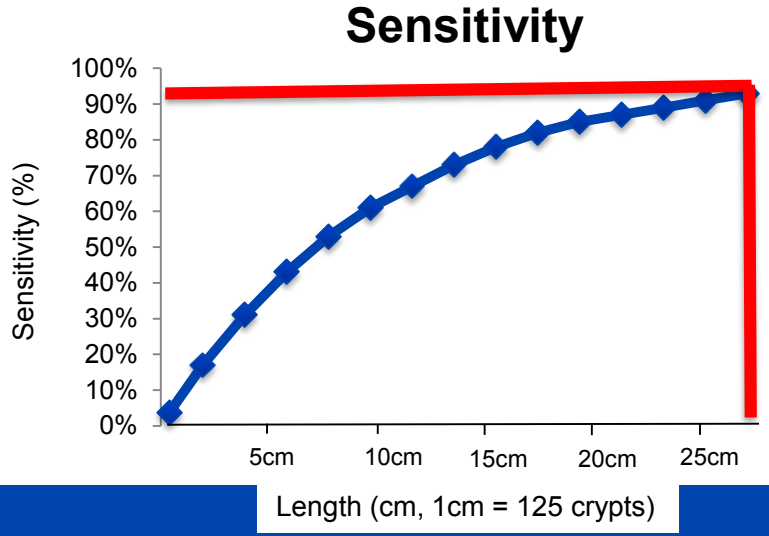
Table 3 Patients with Lynch syndrome stratified by the presence of DNA mismatch repair (MMR) protein-deficient colonic crypts

Clinicopathologic features	Lynch syndrome with MMR-deficient non-neoplastic colonic crypt *N* (%)	Lynch syndrome without MMR-deficient non-neoplastic colonic crypt *N* (%)	*p* value
No. of Cases	18	34	NA
Mean Age in years (range)	57 (27–79)	51 (28–80)	0.1
Gender, Male/Female	10 (56) / 8 (44)	17 (50) / 17 (50)	0.9
Location			
Right Colon	14 (78)	18 (53)	0.08
Left Colon / Rectum	4 (22)	16 (47)	
MMR IHC pattern in carcinoma			
Intact expression of all 4 proteins	0 (0)	1 (3)	0.7
MLH1 and PMS2 Loss	4 (22)	9 (26)	
MSH2 and MSH6 Loss	7 (39)	17 (50)	
Isolated MSH6 Loss	4 (22)	4 (12)	
Isolated PMS2 Loss	3 (17)	3 (9)	
Germline Mutation Analysis			
MLH1 pathogenic variant present	4 (22)	9 (27)	0.6
MSH2 pathogenic variant present	7 (39)	18 (53)	
MSH6 pathogenic variant present	4 (22)	4 (12)	
PMS2 pathogenic variant present	3 (17)	3 (17)	
Mean length of colonic mucosa evaluated by IHC in millimeters (range)	136 (21–336)	88 (5–244)	0.03
Mean estimated number of colonic crypts evaluated by IHC (range)	1508 (233–3733)	981 (56–2711)	0.03

MMR mismatch repair, *IHC* immunohistochemistry

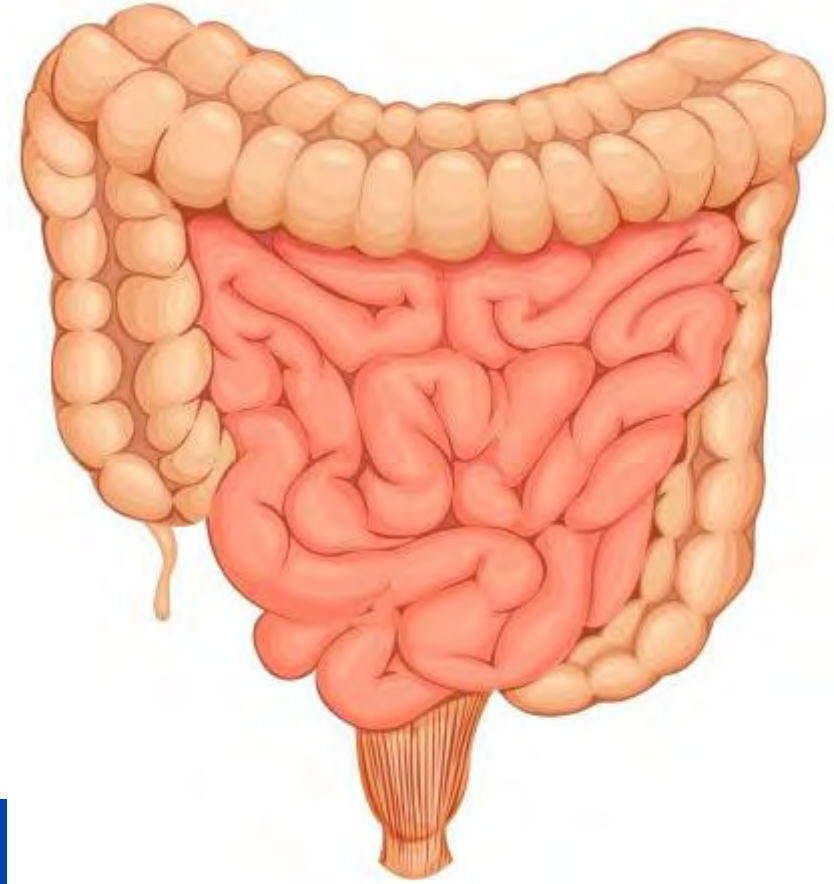
MMR Deficient Colonic Crypts in normal mucosa

- How to increase sensitivity?
- Estimated frequency of MMR deficient crypts:
  - Based on our initial data: 1 MMR-deficient crypt per ~1000 colonic crypts
  - **Evaluation of 3250 crypts would yield a 95% probability of detecting at least one MMR protein-deficient crypt.**

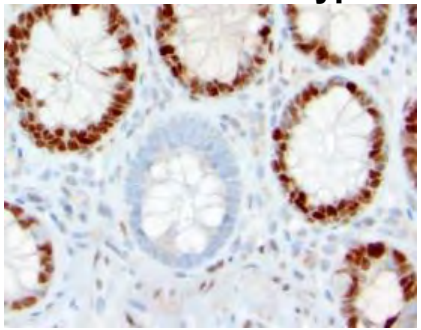


Based on Bernoulli trial using expected frequency of crypts with loss

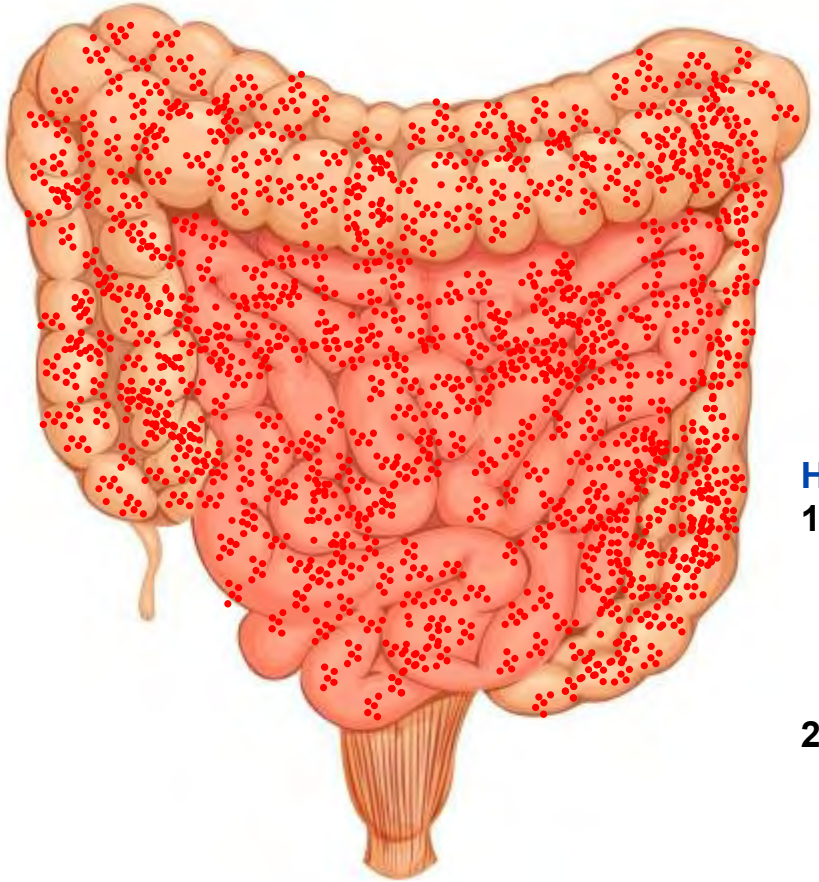
MMR Deficient Colonic Crypts in normal mucosa



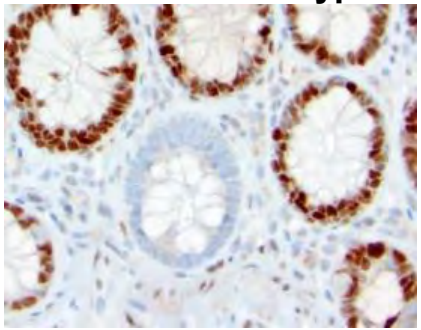
1 MMR-deficient crypt per ~1000 colonic crypts



MMR Deficient Colonic Crypts in normal mucosa



1 MMR-deficient crypt per ~1000 colonic crypts



Hypotheses:

1. **MMR deficient non-neoplastic crypts can be detected from biopsies of normal colorectal mucosa obtained during colonoscopy.**

2. **Detection of MMR deficient crypts can help identify patients with Lynch syndrome.**

MMR Deficient Colonic Crypts in normal mucosa

- **50 patients** undergoing screening colonoscopy
  - **33 patients** with Lynch syndrome: 22 with a cancer history, 11 with no cancer history
  - **13 patients** without Lynch syndrome (10 MSS CRC, 2 with biallelic *MLH1* somatic mutations, and 1 with *MLH1* hypermethylation).
  - **4 patients** with germline variants of uncertain significance (2 *MSH2* and 2 *MSH6*)
- **8 jumbo forcep biopsies** procured from each patient
  - 4 biopsies from right colon and 4 biopsies from left colon
  - The biopsies were sectioned at **100 µm** intervals to include 8 total sections per biopsy in order to evaluate >3250 crypts.

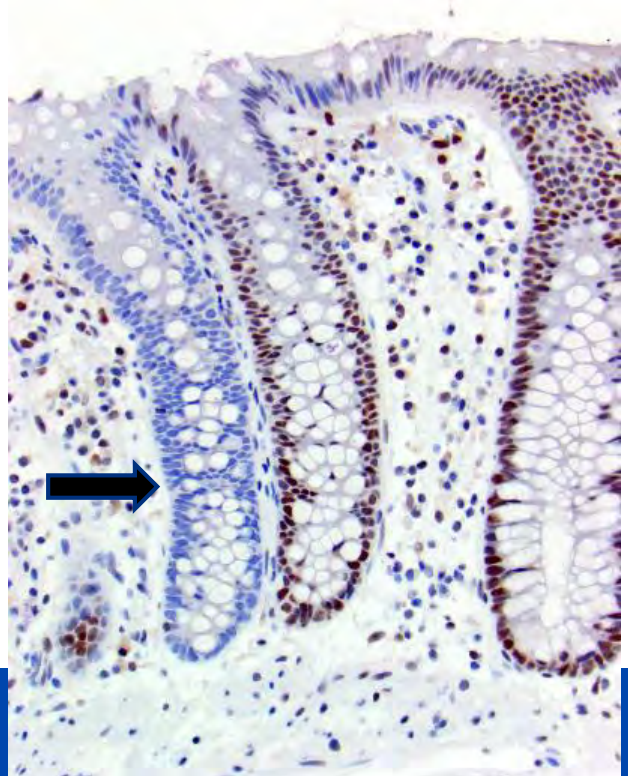
MMR Deficient Colonic Crypts in normal mucosa

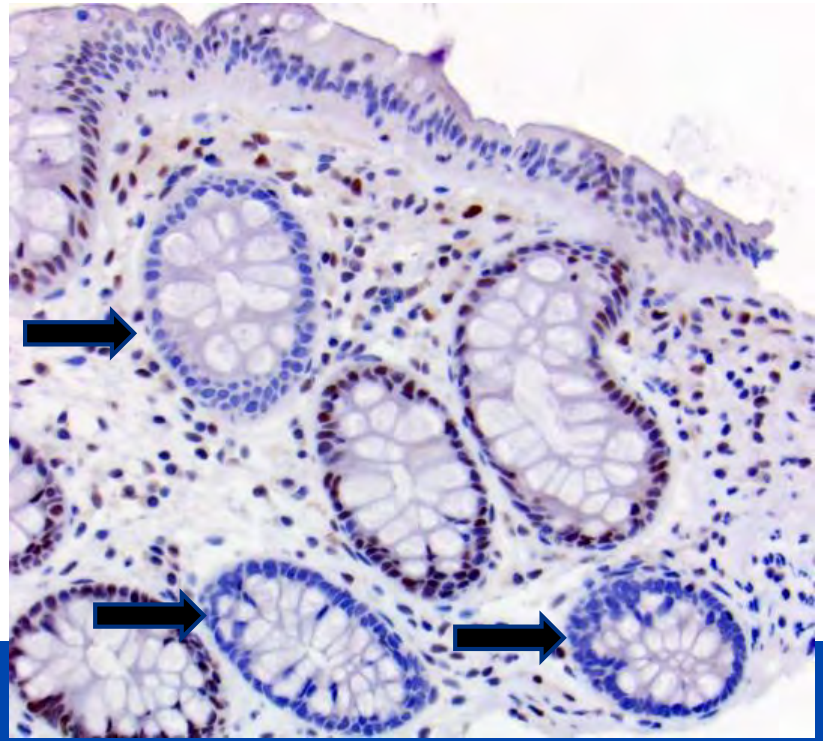
Clinicopathologic Features	Lynch Syndrome with MMR Deficient Crypts	Lynch Syndrome without MMR Deficient Crypts	*p*-value
No. of Cases	23	10	NA
Median Age in years (IQR)	**56 (19)**	**46 (29)**	**0.07**
Gender, Male/Female (%)	3 (13) / 20 (87)	4 (40) / 6 (60)	0.08
Lynch syndrome Type (%)			
Affected	15 (71)	6 (29)	0.8
Unaffected	8 (67)	4 (33)	
Germline Mutation Analysis (%)			
***MLH1* pathogenic variant present**	5 (71)	2 (29)	0.9
***MSH2* pathogenic variant present**	10 (63)	6 (38)	
***MSH6* pathogenic variant present**	4 (80)	1 (20)	
***PMS2* pathogenic variant present**	3 (75)	1 (25)	
***EPCAM* pathogenic variant present**	1 (100)	0	

MMDd crypts were not seen in non-LS patients

58 F with germline *PMS2* c.943C>T pathogenic variant

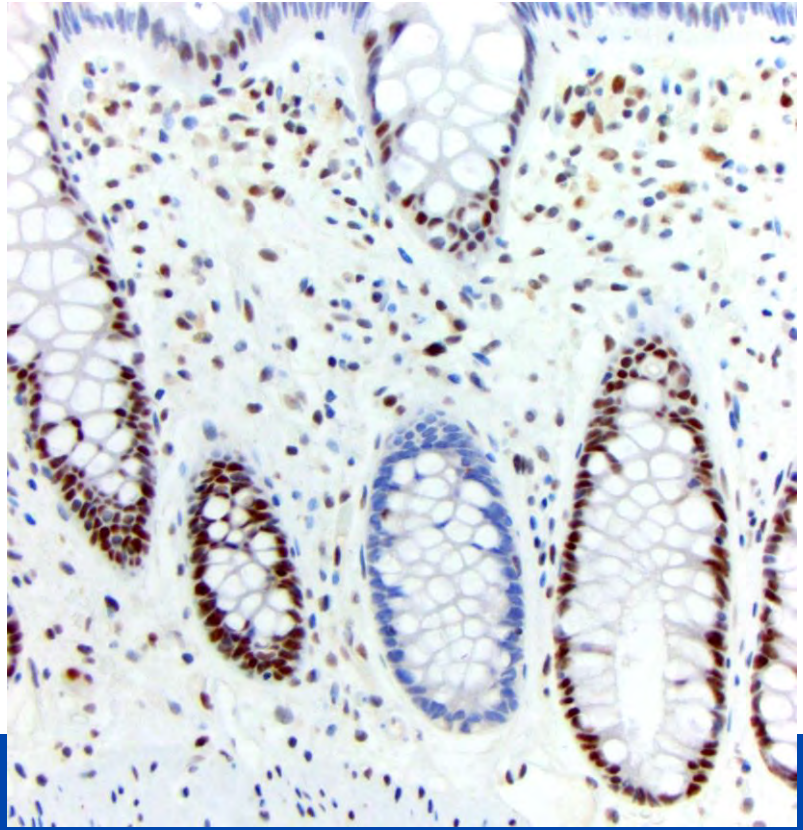
Multiple PMS2 deficient non-neoplastic colonic crypts in both the right and left colon





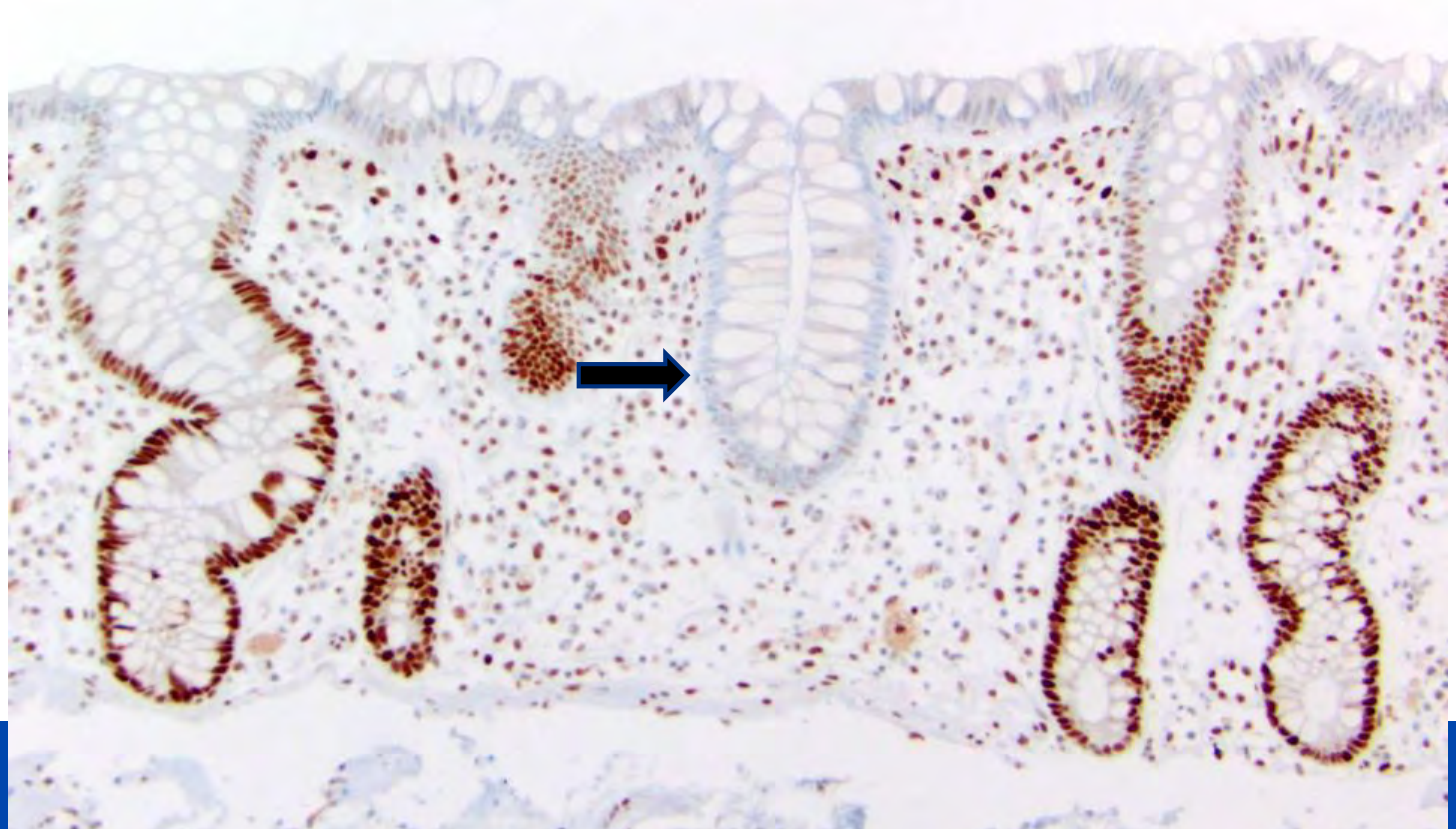
56 F with germline *MLH1* splice site c.208-3C>G likely pathogenic variant

Solitary MLH1 deficient non-neoplastic colonic crypt in the right colon



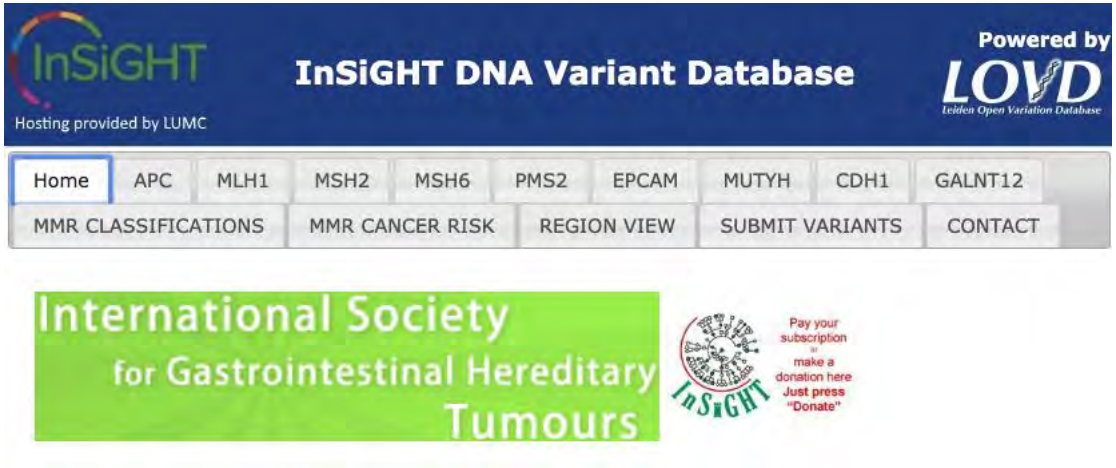
41 M with germline *MSH6* c.3226 C>T pathogenic variant

Solitary MSH6 deficient non-neoplastic colonic crypt in the right colon



Variants of uncertain significance (VUS)

- More than 700 VUS have been reported in the InSIGHT database
- More VUS will be identified as LS screening



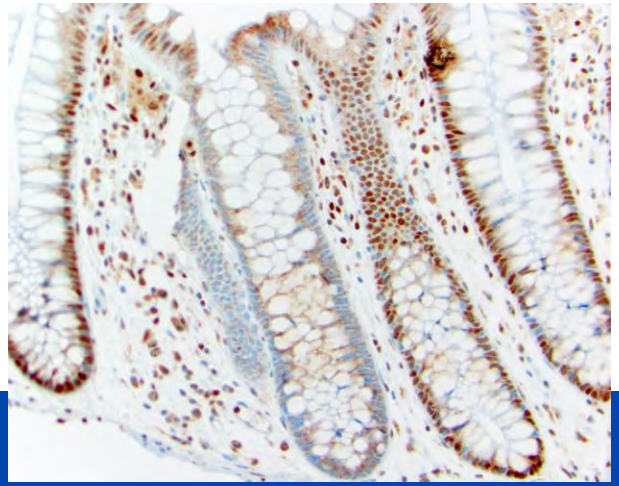
If MMR-deficient normal crypts = Lynch syndrome, then this simple test can help classify VUS

Variants of uncertain significance (VUS)

Case	Cancer History	Germline MMR Gene Variant of Uncertain Significance (VUS)	Age/ Sex	Number of MMR Deficient Crypts	Location of MMR Deficient Crypts
29	Uterine	MSH6 c.3385T>C	63/F	0	NA
30	**Colon**	**MSH6 c.3227G>A**	**67/F**	**1**	**R colon**
31	Colon	MSH2 c.166G>A	59/F	0	NA
32	**Colon**	**MSH2 c.1865C>A**	**59/M**	**1**	**L colon**

MSH2
c.1865C>A
VUS

Gene	Variant	Protein	Concensus InSiGHT Classification	Classification Date
MSH2	c.1865C>A View_InSiGHT_Database	p. (Pro622Glu)	Class 3: uncertain	2016/04/20 v1.9

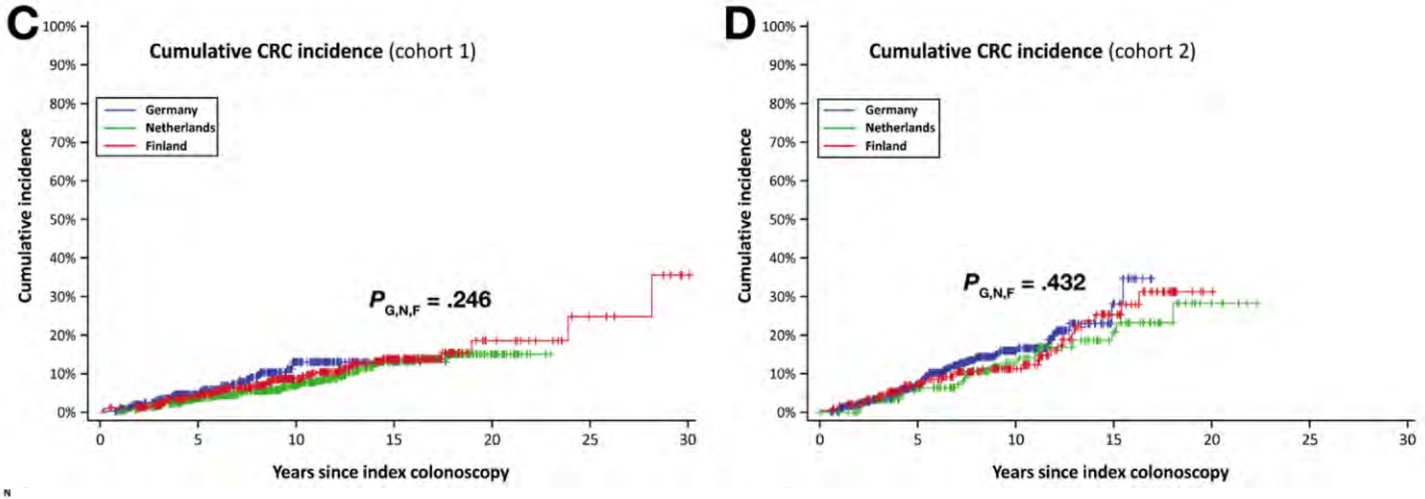


No Difference in Colorectal Cancer Incidence or Stage at Detection by Colonoscopy Among 3 Countries With Different Lynch Syndrome Surveillance Policies

Gastroenterology, 2018 Nov;155(5):1400-1409

No history of CRC

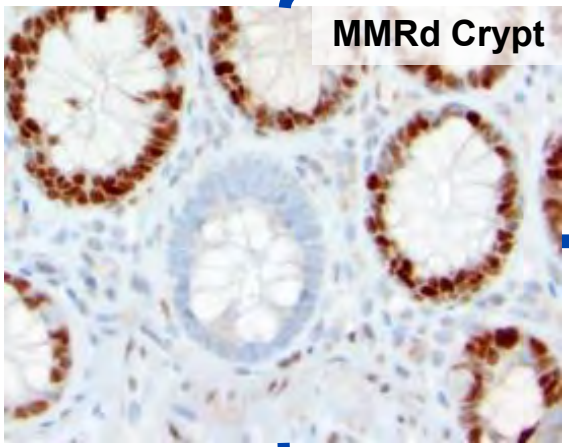
History of CRC

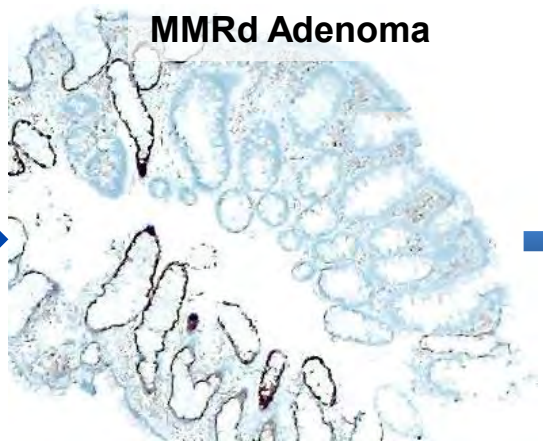


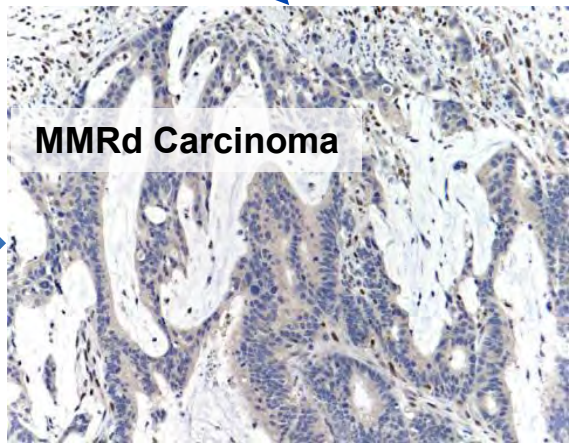
- Different colonoscopy intervals between 1 and 3 years. No difference in CRC detection
- Suggests screening does not improve CRC detection?
- Precursor lesions are not endoscopically visible?
- MMR-deficient crypts may contribute to CRC in Lynch without progressing through a visible adenoma? Flat adenoma or directly to CRC?

Lynch Syndrome Carcinogenesis

Bypass the visible adenoma to develop carcinoma?



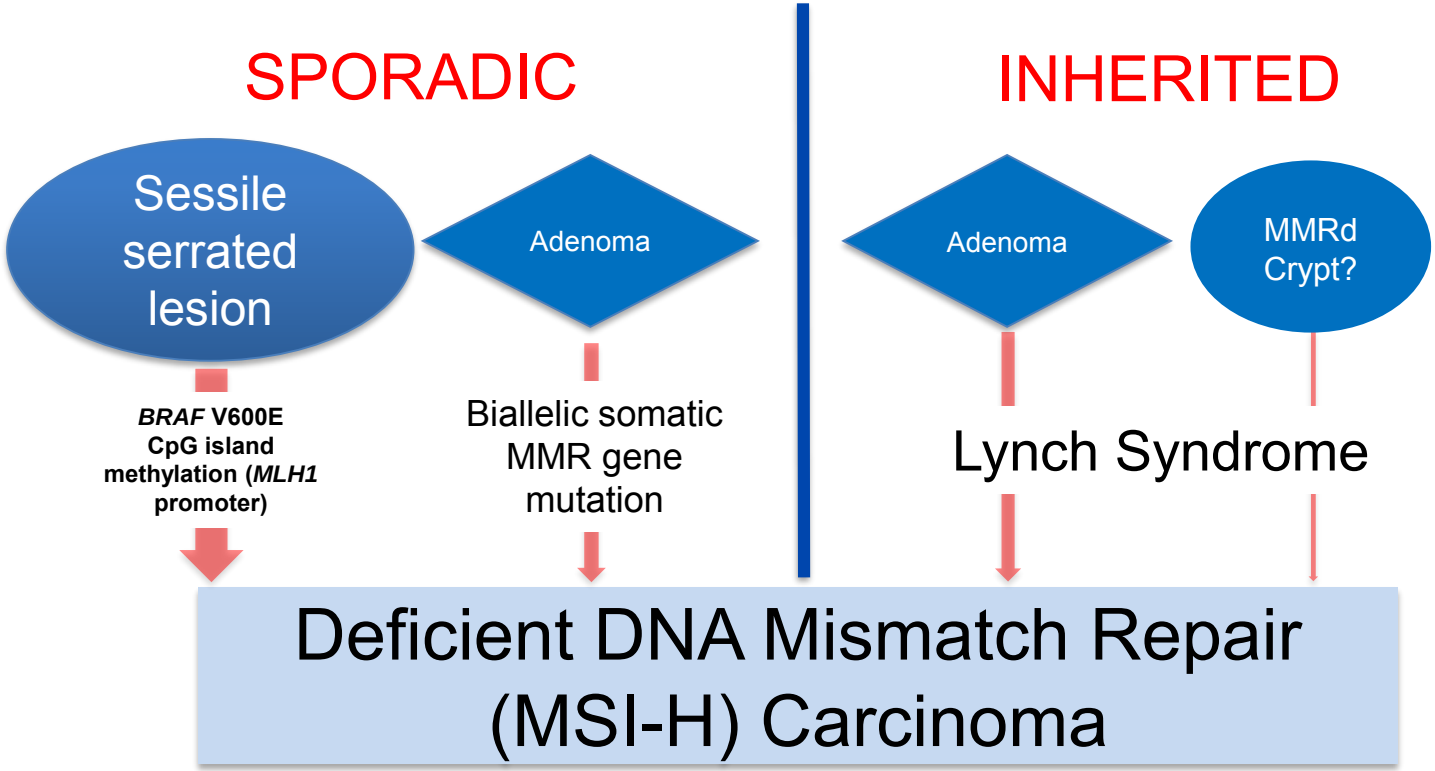




Eradication by normal cell turnover or possibly an immune mechanism

Neoantigens produced by MMRd crypts may induce an inflammatory response and subsequent crypt elimination.

Pathways to MSI-H



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Outline:

- Pathways to colon cancer
- Definition of Lynch Syndrome and goals of screening
- Principles of MMR IHC as a screening tool
- Issues with MMR IHC interpretation
- “Lynch-like” syndrome

