Microsatellite Instability in Colorectal Cancer

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Topics

- Some facts and numbers
- Why talk about microsatellite instability?
- Microsatellites and microsatellite instability (MSI)
- Clinical background
- Pathology of MSI colorectal cancers
- Testing for MSI

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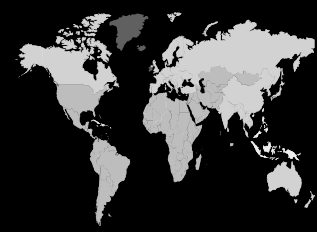
Statistics

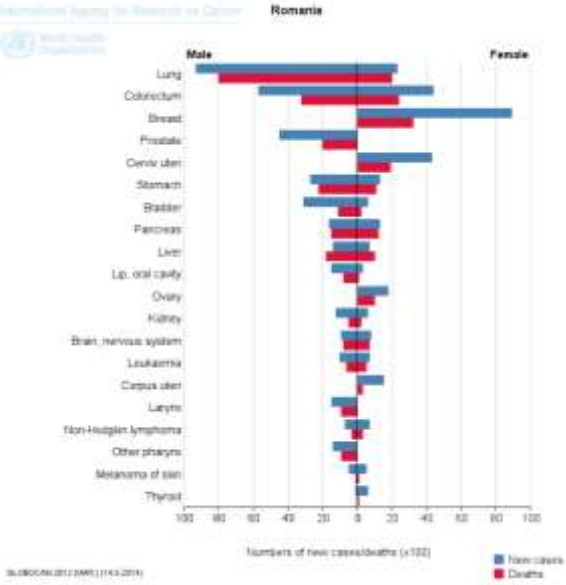
Statistics world

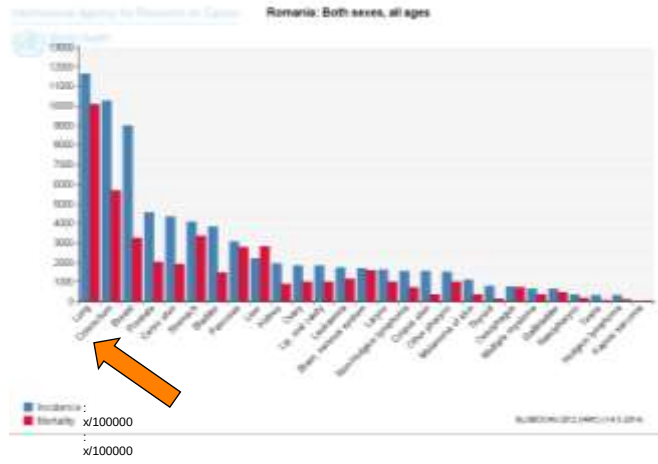
- About 1,24 milion new cases, 9% of all cancers.
- Male : 0,75 million, 10% , 3rd
- Female : 0,57 million, 9,4% , 2nd
- Incidence : 3rd
- Mortality : 4th (0,6 million)



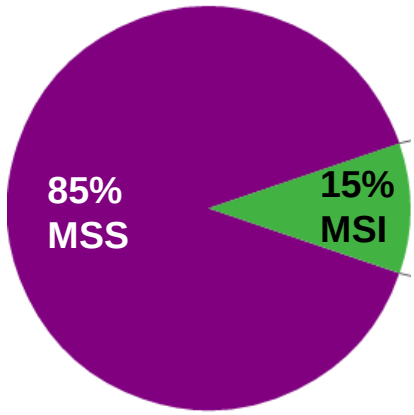
Statistics adapted from the American Cancer Society's publication, Cancer Facts & Figures 2008 and Globocan 2012.

CRC in Romania





Microsatellite Instability (MSI) and ColoRectal Cancer (CRC)



Most CRC are microsatellite stable (MSS).

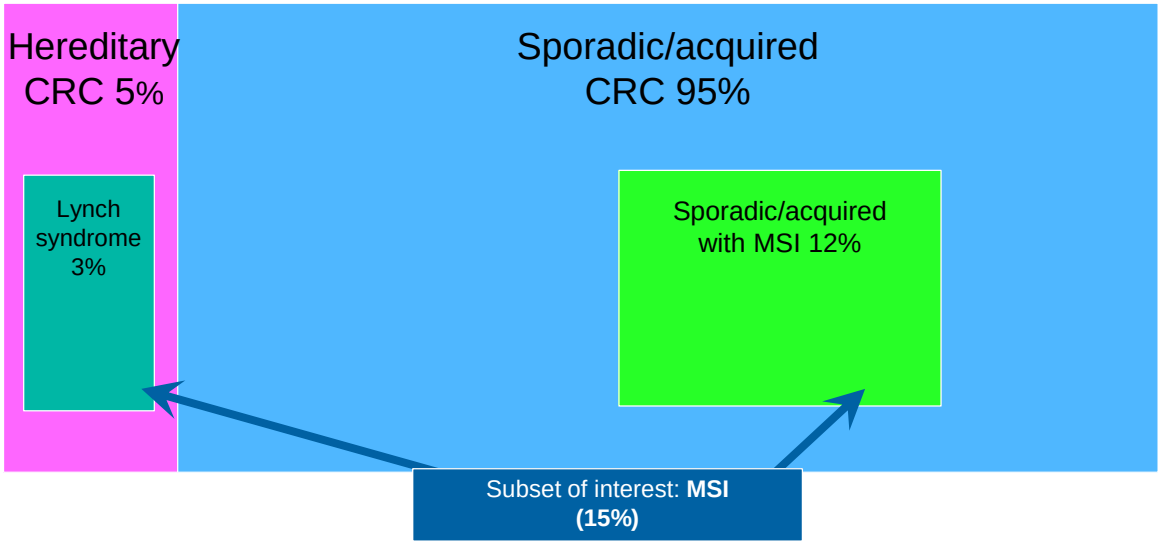
A subset demonstrate microsatellite instability-high (MSI-H).

Lynch associated CRC constitutes about 1/3 of MSI-H CRCs

Capper et al. Int J Cancer 2013

Aaltonen et al. Clin Cancer Res. 2007: 356-361

Microsatellite Instability (MSI) and ColoRectal Cancer (CRC)



Fleming et al. Journal of gastrointestinal Oncology, Vol3 ,no 3 September 2012.

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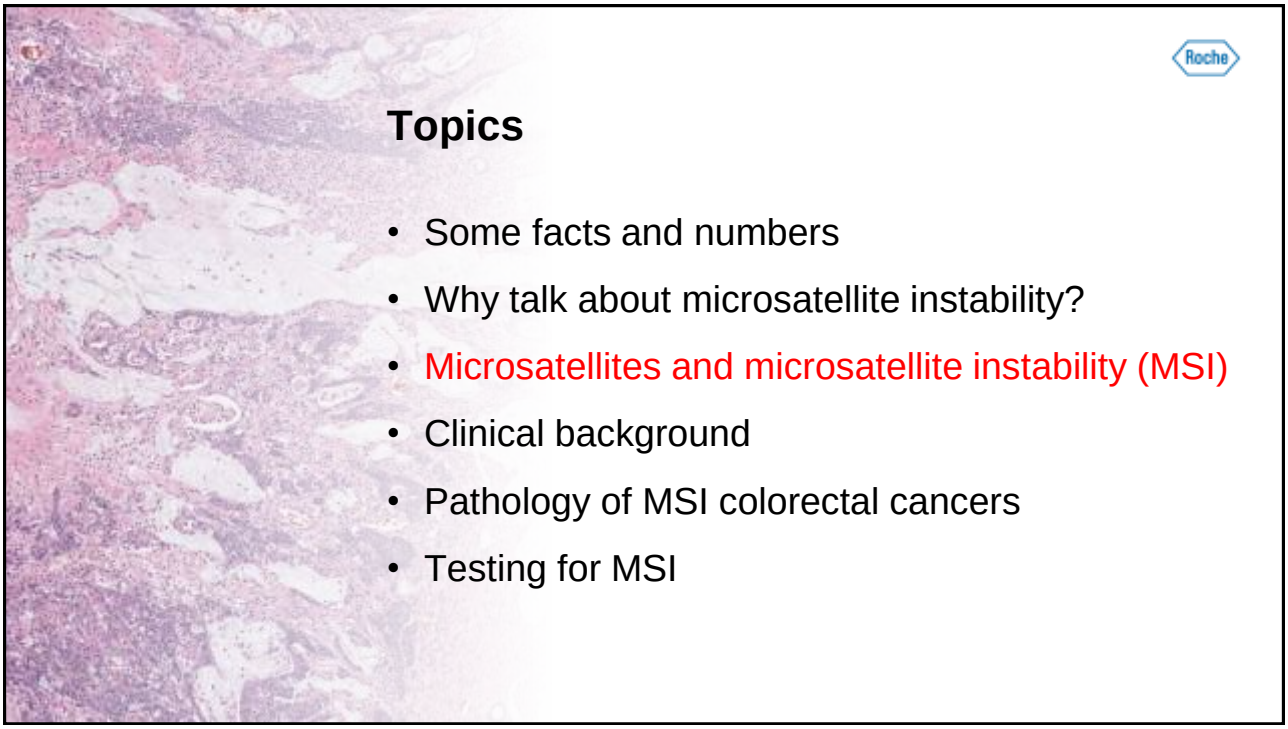
Why Distinguish MSI in CRC Tumors?

- Personalized healthcare
- MSI tumors in general have:
  - Better prognosis
    - Increased overall survival
    - Lower incidence of metastasis
  - Difference in treatment
    - Appear not to respond to traditional 5-FU chemotherapy
    - Irinotecan may be particularly effective for these tumors
- Additionally, Lynch syndrome identification important
  - Hereditary basis of transmission
  - Identify family members with syndrome
  - Increased and earlier cancer screening



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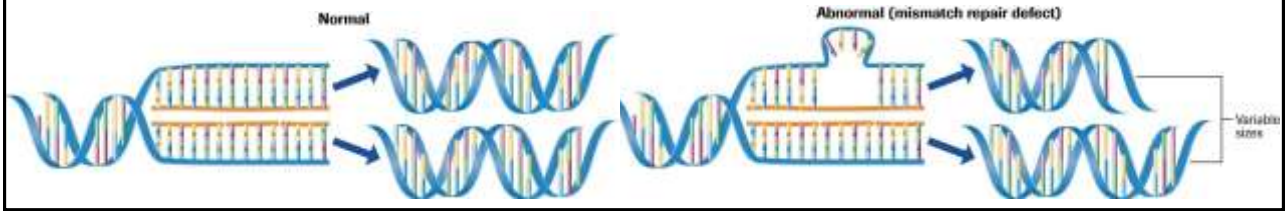
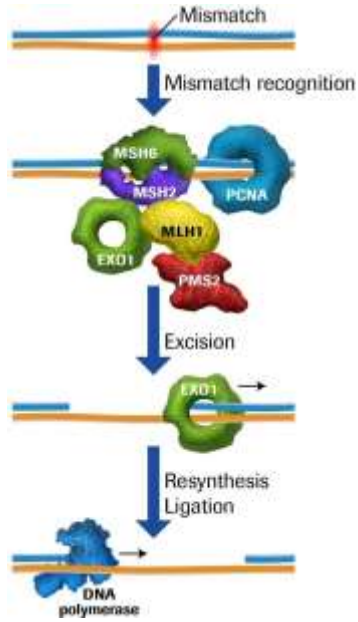
What is Microsatellite Instability (MSI)?

- Microsatellite
  - Normally present DNA sequence
  - Consists of nucleotide repeats (100 to 200 base pairs)
    - Mononucleotide: AAAAAAAAA
    - Dinucleotide: CACACACACA
  - **Number of repeats is stable** from cell to cell
- Micro**s**atellite instability
  - **Change in the number of repeats**: gain or loss due to *insertion-deletion loops*
  - Leads to **frameshift mutations**
  - Tumours have a **different number of repeats** than normal tissue



DNA Errors and the Mismatch Repair System

- Mismatch repair (MMR) system is one of the mechanisms available to fix DNA errors
  - Repairs errors that lead to MSI
- Defects in the MMR system result in tumors with MSI



MSI Leads to Frameshift Mutations in Adjacent Genes

TGFBR2	MBD4	Important biological functions such as
BAX	TCF4	
ACVR2	STK11	• signal transduction
IGF2R	RAD50,	• apoptosis regulation
BLM	CHK1	• cell cycle regulation
MSH3	AXIN2	• cell proliferation
MSH6	WISP3	• cell differentiation
E2F4	B2M	• DNA MMR
PTEN,	MYO1A	
AIM2	CDX2	
CASPASE5		

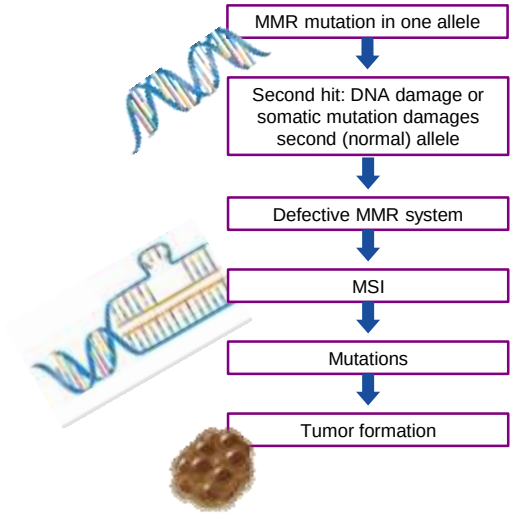
Jung Ho Kim, Gyeong Hoon Kang, *World J Gastroenterol 2014 April 21; 20(15): 4230-4243*

MSI in Lynch syndrome

- Inherited mutation of one of the following MMR gene alleles:

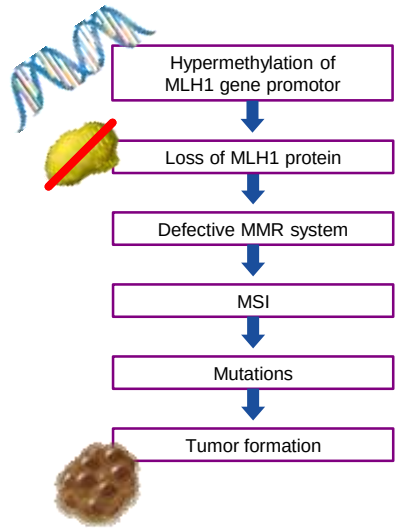
  MLH1 MSH6 MSH3 PMS1
  MSH2 PMS2 MLH3

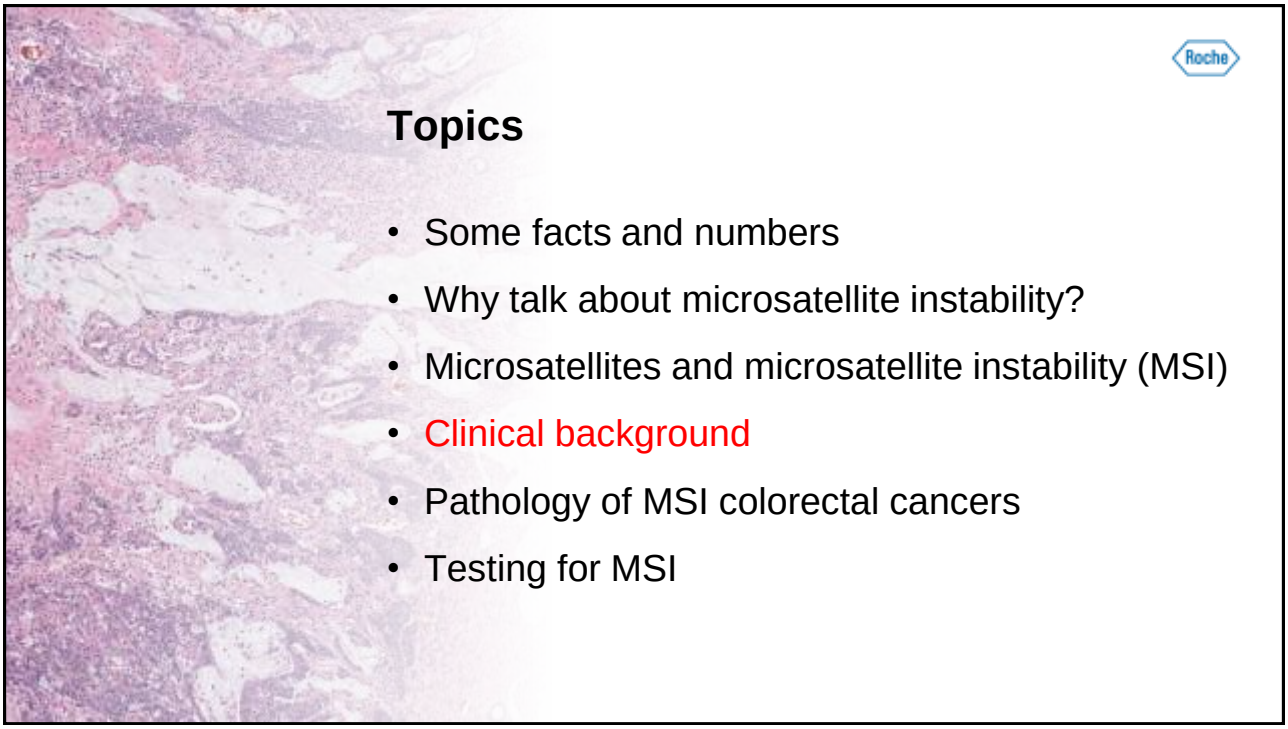
- Frequency of mutations
  - MLH1: 40% 3p21
  - MSH2: 40% 2p21
  - MSH6: 10% 2p16
  - PMS2: 5% 7p22
- Most mutations lead to prematurely truncated, unstable proteins
- Development of cancer



MSI in Sporadic CRC

- 12% of sporadic CRC
- Acquired inactivation of MMR genes by promoter hypermethylation
  - Methylation of gene causes loss of this MMR protein
  - Usually the MLH1 gene
  - Because of the now defective MMR system, MSI and subsequent frameshift mutations can occur





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CRC with MSI

- There are two types of CRC with associated MSI:
  - **Inherited**: Lynch syndrome (former HNPCC)
  - **Acquired**: sporadic CRC with MSI

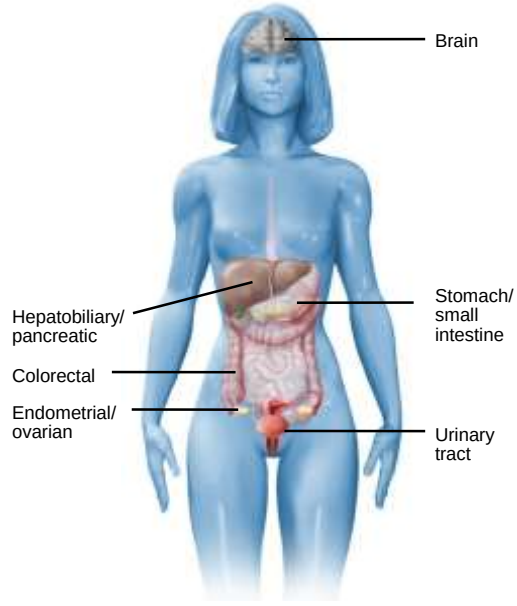
What is Lynch Syndrome?

- Hereditary Nonpolyposis Colorectal Carcinoma (HNPCC)
- Autosomal dominant condition
- Germline (inherited) mutation in a mismatch repair gene allele (MMR)
- 60% to 80% lifetime risk of CRC compared to 7% lifetime risk in the general population
- Average age of onset
  - Sporadic CRC 67 years vs. Lynch Syndrome 45 years
- Accelerated carcinogenesis
  - Adenoma → Carcinoma
    - 2-3 years in Lynch Syndrome
    - 8-10 years in general population
- Increased risk of additional CRC
  - 25-30% of pts with Lynch Syndrome related CRC will have second CRC primary within 10 years (if surgery was less than a subtotal colectomy)

Lynch Syndrome Tumor Sites

Tumor Site	Lifetime Risk With MMR Mutation (%)
Colonic	
• Men	28 to 75
• Women	24 to 52
Endometrial	27 to 71
Ovarian	3 to 13
Gastric	2 to 13
Small bowel	4 to 7
Urinary tract	1 to 12
Bile duct/gallbladder	2
Brain	1 to 4

- Variants: Turcot, Muir-Torre



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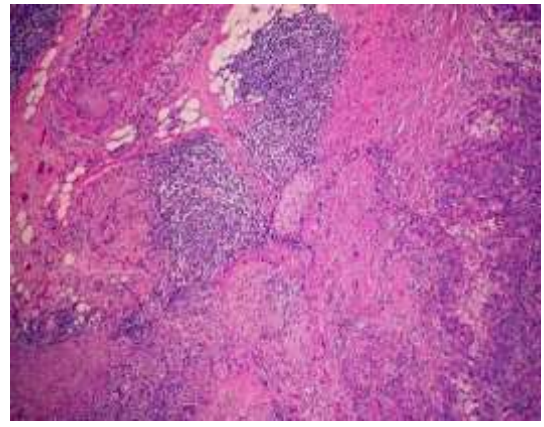
Common Features of MSI tumors

BOTH sporadic and Lynch syndrome tumors

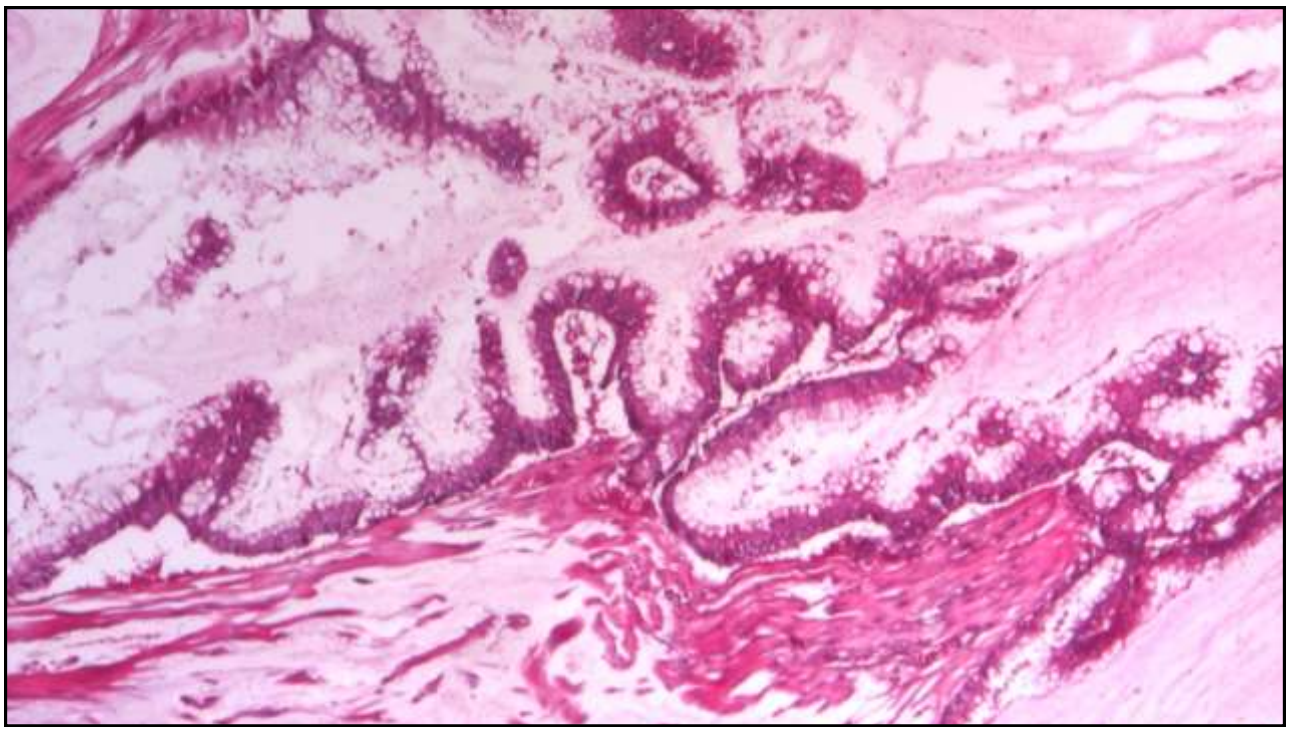
- Presentation
  - Located in the right colon
  - Large at presentation

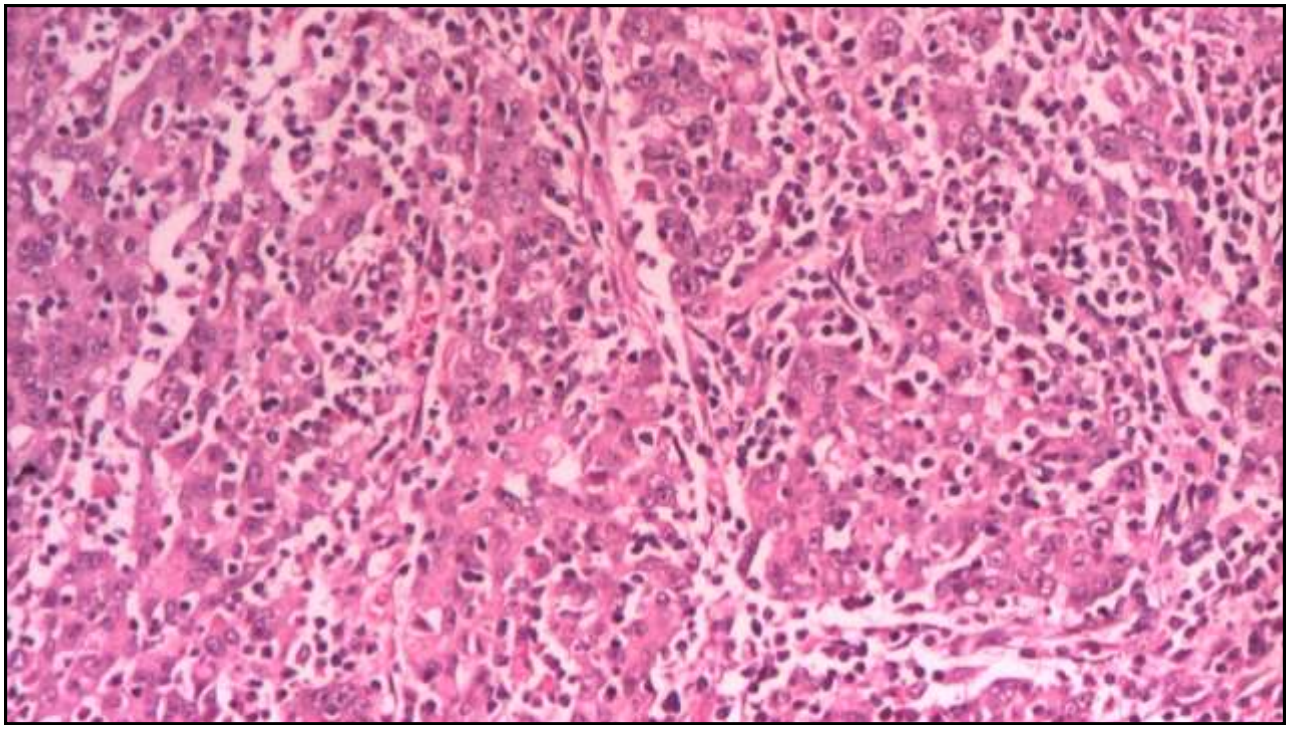
Common Features of MSI tumors

- BOTH sporadic and Lynch syndrome tumors
- Common histological features
  - Mucinous, signet ring or medullary pattern
  - Prominent peritumoral lymphocytes, nodular pattern: Crohn-like reaction
  - Prominent tumor infiltrating lymphocytes
  - Pushing border
  - Heterogeneous histological patterns
  - Well or poorly differentiated
  - Lack of “dirty necrosis”



Greenson JK, et al. Am J Surg Pathol 27:563-570, 2003.
Bellizzi, Surgical Pathology Clinics, 2013





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How to recognize Lynch Syndrome

- **Amsterdam Criteria (1999)**
  - Clinical guidelines for when to suspect Lynch Syndrome
- **Bethesda Guidelines (2004)**
  - Guidelines for when to do MSI testing

- **American Gastroenterology Association (AGA) Guidelines (2014)**
  - Screen all newly diagnosed colon cancers in patients 70yrs old or younger
  - Pts older than 70yrs with family history concerning for LS

Testing for MSI in CRC tumors

- Screening tests
  - Direct detection of MSI – **PCR**
    - MSI-H
    - MSI-L – similar clinically and pathologically to MSS
    - MSS
  - Indirect indication of MSI by detecting MMR defects – **IHC staining** for MMR proteins
- Further testing to differentiate and confirm hereditary vs sporadic mechanism
  - Germline mutation testing
  - Methylation of MLH1 promoter
  - *BRAF* mutation testing

Testing for MSI in CRC tumors

Immunohistochemistry – Detects a MMR defect

- Readily available to pathology labs
- Effective screening tool to identify MSI-associated CRC
- 92.3% sensitive and 100% specific for MSI
- Nuclear expression of MMR proteins: MLH1, MSH2, MSH6 and PMS2
- Testing is performed on neoplastic and non-neoplastic tissue (internal control)

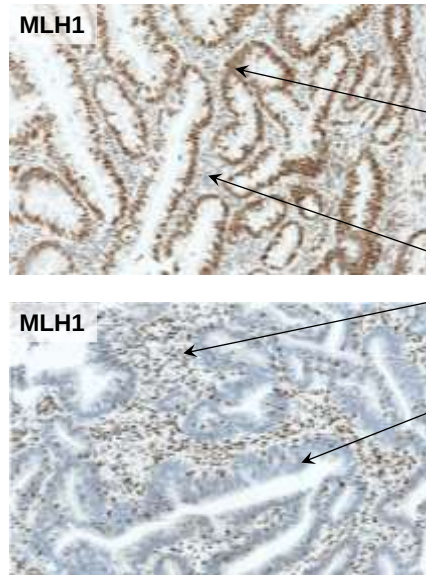
Interpretation of MMR IHC

- **Protocol with OptiView +/- AMP**
- Positive internal control – **crucial**
  - Fibroblasts, lymphocytes, normal mucosa

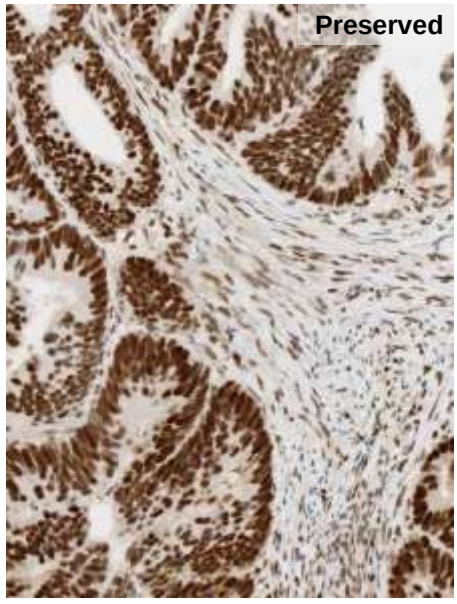
Evaluation of staining

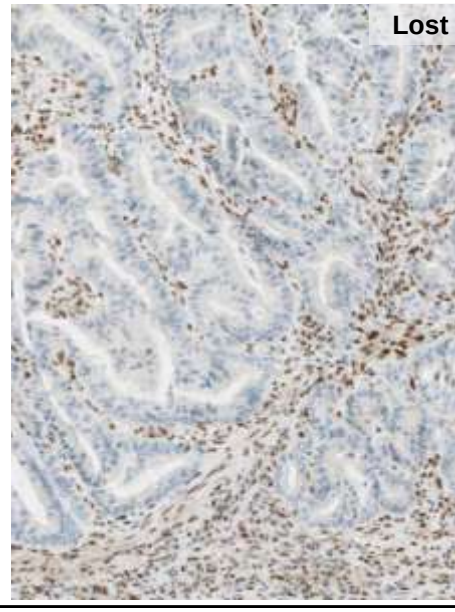
- *MMR intact (preserved)* - **any** positive staining of tumor nuclei
- *MMR deficient (lost)* - no tumor nuclei staining
- *Not evaluable* - negative tumor with absent internal control
  - Retest or choose another block
  - Test with other method: PCR

- Antigens are fixation dependent



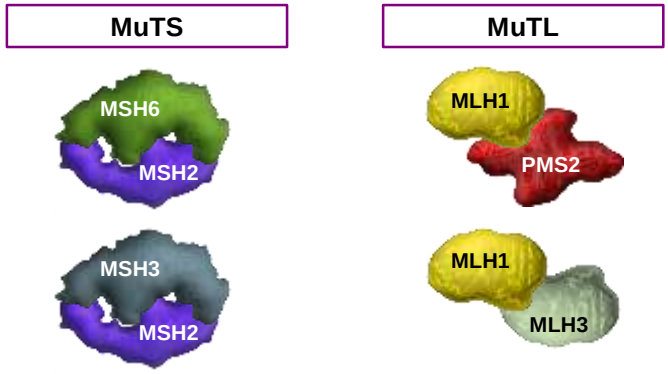
Colon Cancer Samples: MLH1 Staining



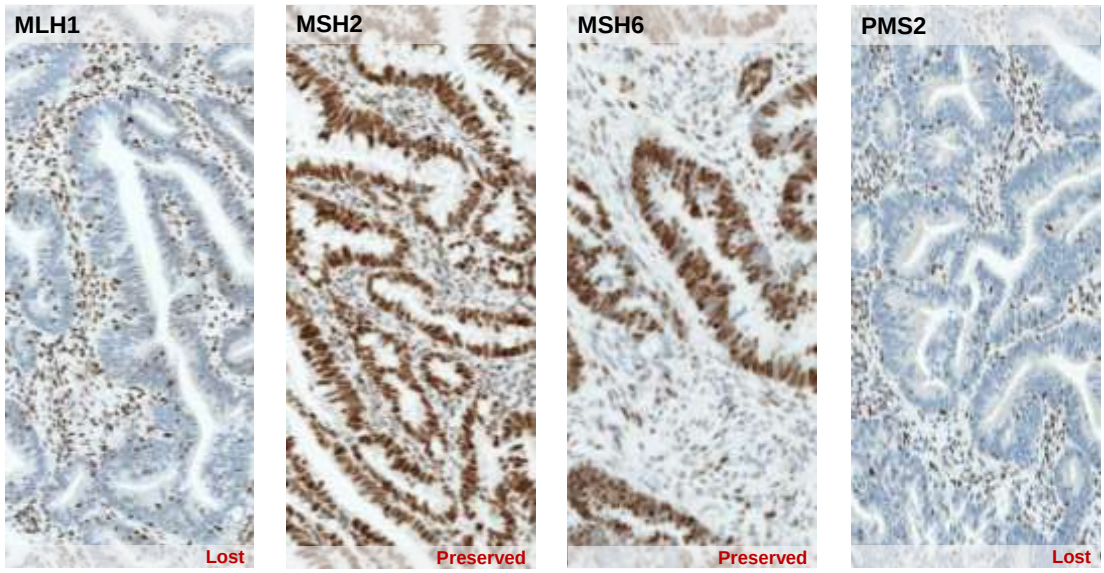


MMRs, Protein Stability, & IHC Staining Patterns

- MMR proteins work as heterodimers
  - This pairing affects IHC results and can act as a control



Interpretation of MMR IHC



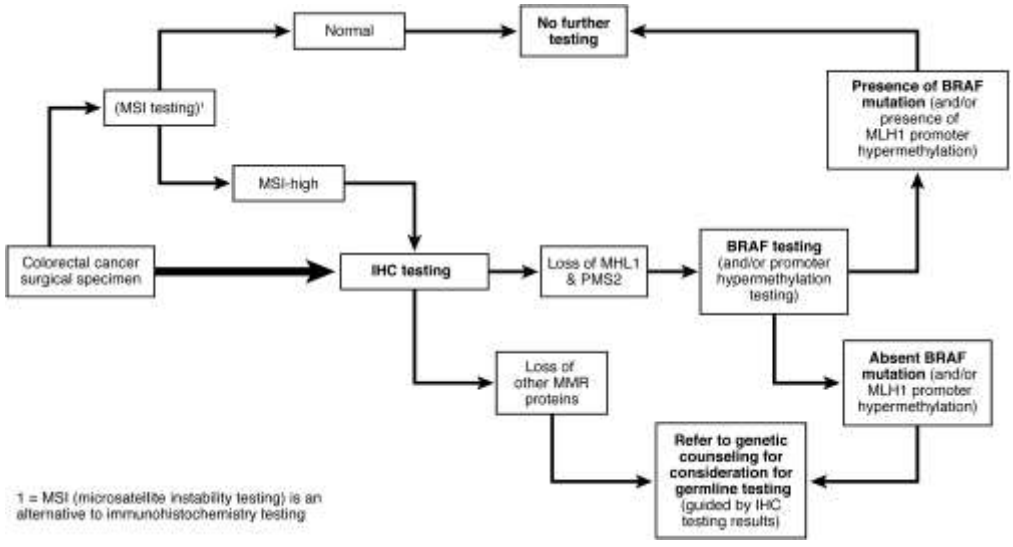
Adapted from Bellizzi, *Immunohistochemistry in Gastroenterohepatopancreatobiliary Epithelial Neoplasia*, September 2013

IHC result	Frequency	Interpretation	Action(s)
All 4 proteins intact	80-85%	Normal MMR function Unlikely LS	Consider follow-up MSI testing to confirm normal result Refer to Cancer Genetics if clinically appropriate
MLH1/PMS2 lost MSH2/MSH6 intact	15%	• Likely *sporadic* dMMR due to MLH1 promoter methylation • Less likely *LS* due to MLH1 (usually) or PMS2 (rarely) mutation	**BRAF V600E** and/or **MLH1 promoter methylation** testing If above are normal: MLH1 mutation testing (followed by PMS2 if needed)
MSH2/MSH6 lost MLH1/PMS2 intact	1-2%	Likely *LS* due to MSH2 (usually) or EPCAM deletion or MSH6 mutation (rarely)	**MSH2 mutation** testing (followed by EPCAM and MSH6 if needed)
MSH6 lost MLH1/PMS2/MSH2 intact	Up to 0.5%	Likely *LS* due to MSH6 (usually) or MSH2 (rarely) mutation	**MSH6 mutation** testing (followed by MSH2 if needed)
PMS2 lost MLH1/MSH2/MSH6 intact	Up to 0.5%	Likely *LS* due to PMS2 (usually) or MLH1 (rarely) mutation	**PMS2 mutation** testing (followed by MLH1 if needed)

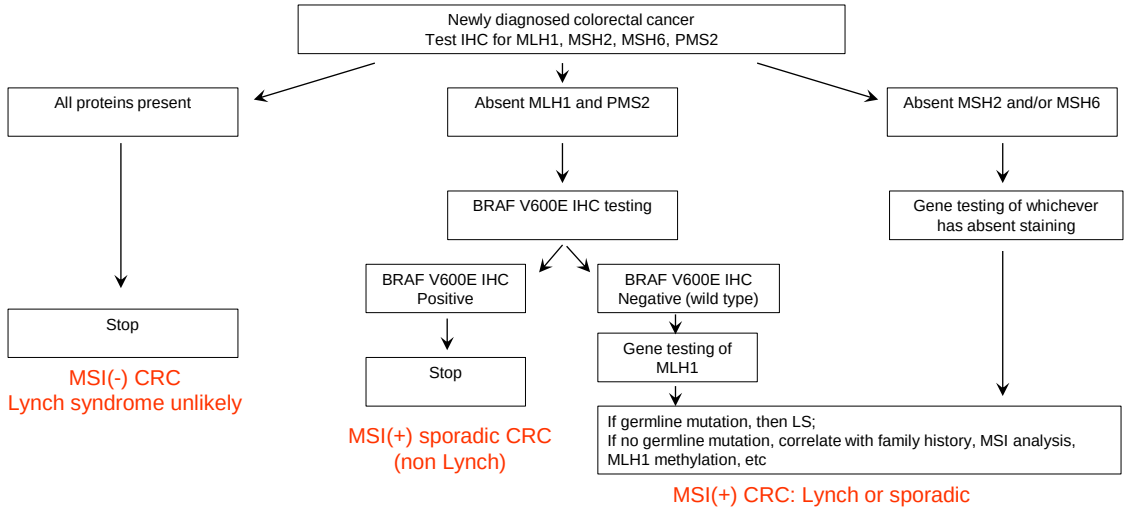
Detecting MSI and/or Changes in MMR Proteins

- **Immunohistochemistry** helps to
  - identify microsatellite unstable tumors
  - distinguish between Lynch syndrome and sporadic CRC with MSI
    - *BRAF V600E* mutation (virtually never seen in Lynch syndrome): sporadic CRC
- **Further testing**
  - Germline mutation testing: confirmatory for Lynch syndrome
    - IHC points to which gene to test
  - Methylation of MLH1 gene promoter testing: sporadic CRC
  - *BRAF* mutation testing: sporadic CRC

Guidelines on Genetic Evaluation and Management of Lynch Syndrome: A Consensus Statement by the US Multi-Society Task Force on Colorectal Cancer
Gastroenterology 2014 147, 502-526DOI: (10.1053/j.gastro.2014.04.001)



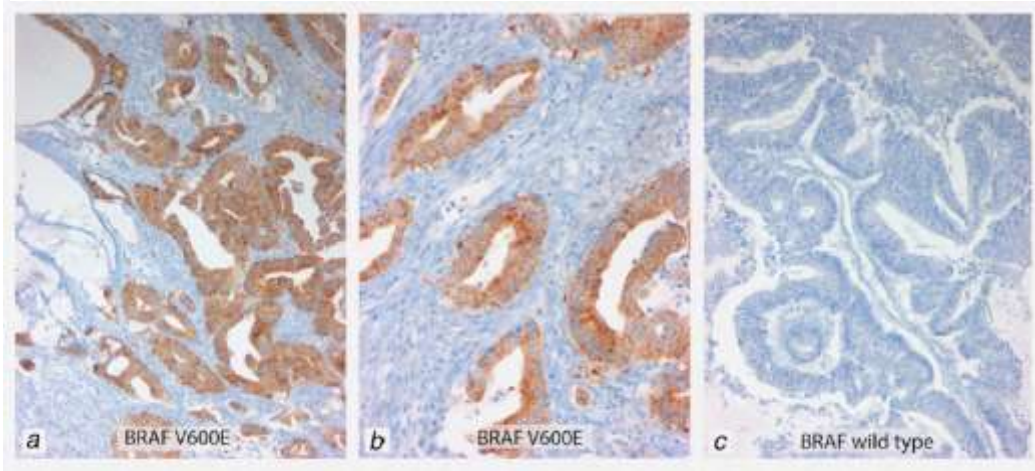
An algorithm for MMR and BRAF V600E testing of colorectal cancers



M, Hampel et al.. BRAF V600E mutation analysis simplifies the testing algorithm for Lynch syndrome. Am J Clin Pathol 2013.

BRAF V600E-specific immunohistochemistry for the exclusion of Lynch syndrome in MSI-H colorectal cancer

David Capper[1], Anita Voigt[2,3], Gergana Bozukova[2,3], Aysel Ahadova[2,3], Philipp Kickingereder[1], Andreas von Deimling[1], Magnus von Knebel Doeberitz[2,3] and Matthias Kloor[2,3]



Int. J. Cancer: 133, 1624–1631 (2013)

Conclusions

- MSI-H tumors account for about 15% of all colon cancers
- Lynch Syndrome associated CRC accounts for about one third of MSI-H colon cancers, and up to 5% of all colon cancers
- MSI-H colon cancers are biologically distinctive in their behavior
- MSI testing should be performed on all new colon cancer cases according to AGA
- MSI testing can be performed on fixed tissue
- Patients with MSI-H tumors are candidates for genetic counseling and further genetic testing

Thank you