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OSPEDALE POLICLINICO SAN MARTINO
Sistema Sanitario Regione Liguria

Anatomo Patologo

DNA Mismatch Repair deficiency (dMMR)

Reflex testing and Lynch Alert

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Defective DNA mismatch repair complex (dMMR)/microsatellite instability (MSI)

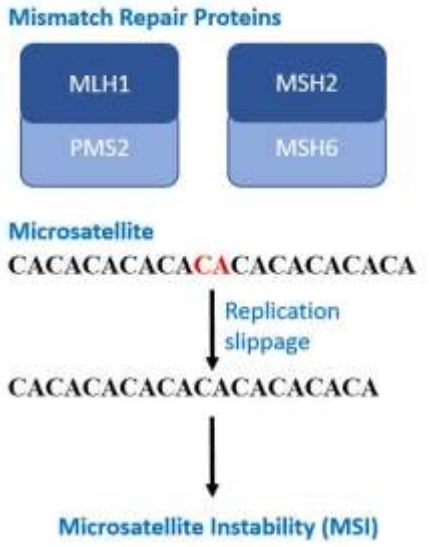
Highly conserved protein complex - recognizes and repairs erroneous short insertions, short deletions and single base mismatches that can arise during DNA replication and recombination.

MLH1
MSH2
MSH6
PMS2
EPCAM

Lynch Syndrome

monoallelic germline mutation

(2-3% of CRC)



Sporadic MSI-H/MMRd CRC (12% of CRC) – 60% BRAF mut and methylation of MLH1 promoter

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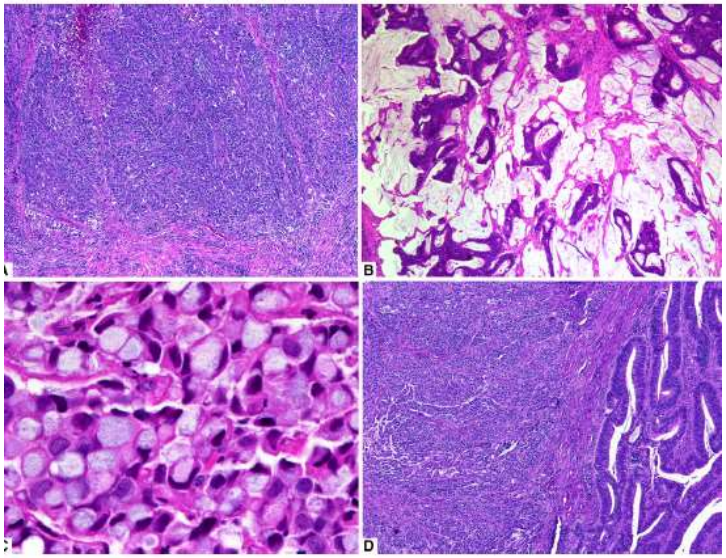
UNIVERSAL SCREENING

Most dMMR/MSI CRC – associated characteristics

(significant intra/peri-neoplastic lymphocytic infiltration, phenotypic heterogeneity, mucinous histology, medullary carcinoma and signet-ring cell adenocarcinoma)



6% – no detectable dMMR/MSI histologic characteristics

(Shia J, Holck S, Depetris G, et al. Lynch syndrome-associated neoplasms: a discussion on histopathology and immunohistochemistry. Fam Cancer 2013;12:241-60)

The use of immunohistochemistry (pMMR vs dMMR) or PCR (MSS/MSI-L vs MSH-H) is recommended in all the cancers belonging to the spectrum of cancers found in Lynch syndrome

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EGAPP RECOMMENDATION STATEMENT

Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives

*Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group**

1. **NOT ALL CENTRES PERFORM UNIVERSAL SCREENING**
2. **DIFFERENT DIAGNOSTIC TESTS**
3. **EXPERTISE REQUIRED**
4. **CONCLUSIONS NECESSARY**
5. **REFLEX TESTING AND LYNCH ALERT**

2) DIFFERENT DIAGNOSTIC TESTS: IHC or PCR

High concordance rate between IHC and PCR

Why prefer IHC ?

- costs less
- lower turnaround time
- all pathology labs have IHC
- allows to identify the altered protein/s
- requires limited amount of tissue

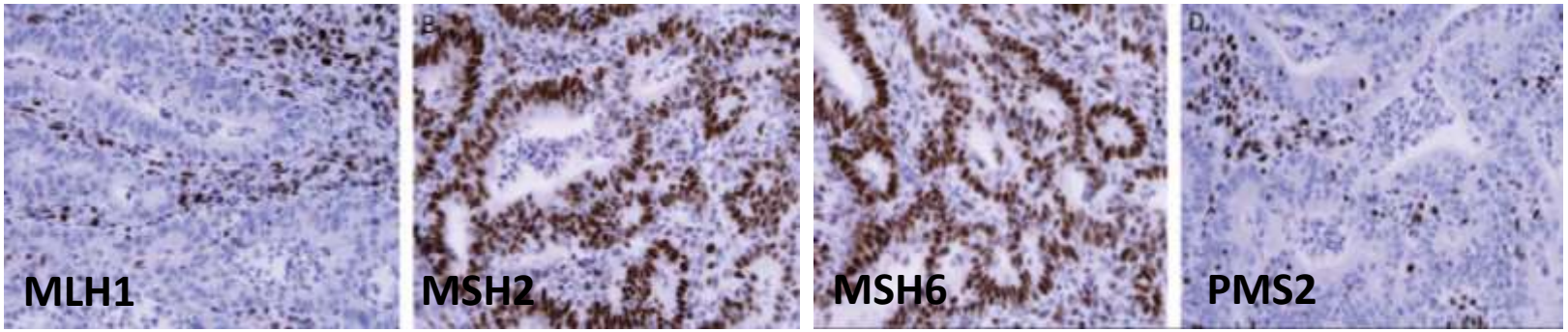
Histopathology

Histopathology 2020 DOI: 10.1111/his.14233

Identifying mismatch repair-deficient colon cancer: near-perfect concordance between immunohistochemistry and microsatellite instability testing in a large, population-based series

Maurice B Loughrey,[1,2,3] Jason McGrath,[4] Helen G Coleman,[2,3] Peter Bankhead,[5] Perry Maxwell,[4] Claire McGready,[4,6] Victoria Bingham,[4] Matthew P Humphries,[4] Stephanie G Craig,[2] Stephen McQuaid,[1,4,6] Manuel Salto-Tellez[1,2,4] & Jacqueline A James[1,2,4,6]

[1]Department of Cellular Pathology, Belfast Health and Social Care Trust, Belfast, [2]Patrick G. Johnston Centre for Cancer Research, Queen's University Belfast, Belfast, [3]Centre for Public Health, Queen's University Belfast, Belfast, [4]Precision Medicine Centre of Excellence, Queen's University Belfast, Belfast, [5]Edinburgh Pathology/Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, and [6]Northern Ireland Biobank, Health Sciences Building, Queen's University Belfast, Belfast, UK



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3) EXPERTISE REQUIRED in IHC interpretation

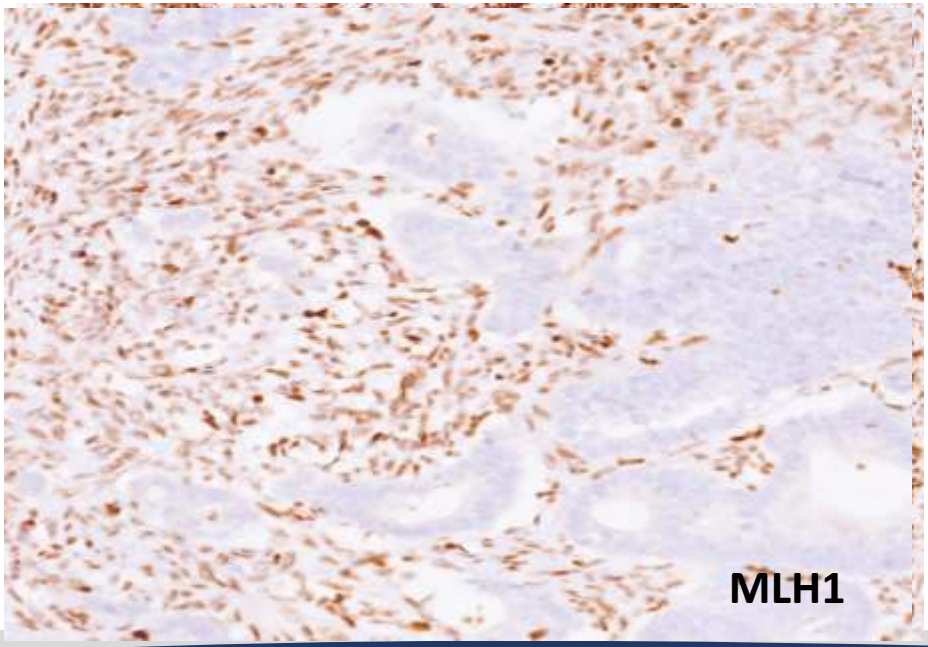
Immunohistochemical evaluation of mismatch repair proteins in colorectal carcinoma: the AIFEG/GIPAD proposal

A. REMO[1*], M. FASSAN[2*], G. LANZA[1,2] ON BEHALF OF AIFEG AND GIPAD
[1] Italian Association studying Familial and Hereditary Gastrointestinal Tumors (AIFEG): Chair: GB. Rossi. Directive members: D.Barana, B. Bonanni, M. Pedroni, A. Remo, E.D. Urso, M. Vitellaro; [2] Italian Group of Gastrointestinal Pathologists (GIPAD): Chairs: M. Guido & L. Saragoni. Scientific committee: F.P. D'Armiento, M. Fassan, L. Mastracci
[*] These authors contributed equally to this work.
PATHOLOGICA 2016;108:1-6

MMR protein expression is interpreted as:

(i) retained, when a moderate to strong expression (similar to what is observed in the stromal cells as internal control) is present in ≥ 10% tumor cells;

(ii) loss, in case of complete loss of nuclear expression in cancer cells;



MLH1

3) EXPERTISE REQUIRED in IHC interpretation

(iii) indeterminate, if IHC staining intensity in tumor cells is lower than the internal control or the tumor is positive in < 10%.

Possibly due to:

- **Variable epitope expression due to pre-analytical factors (**fixation, processing, storage, antigen specificity, staining**)**
- **Expression related to variable differentiation**
- **Second hit mutations or methylation in selected tumor clones**
- **Factors linked to the tumor microenvironment such as hypoxia and oxidative stress (eg neoadjuvant treatment and loss of MSH6)**

Indeterminate IHC results should undergo to MSI testing

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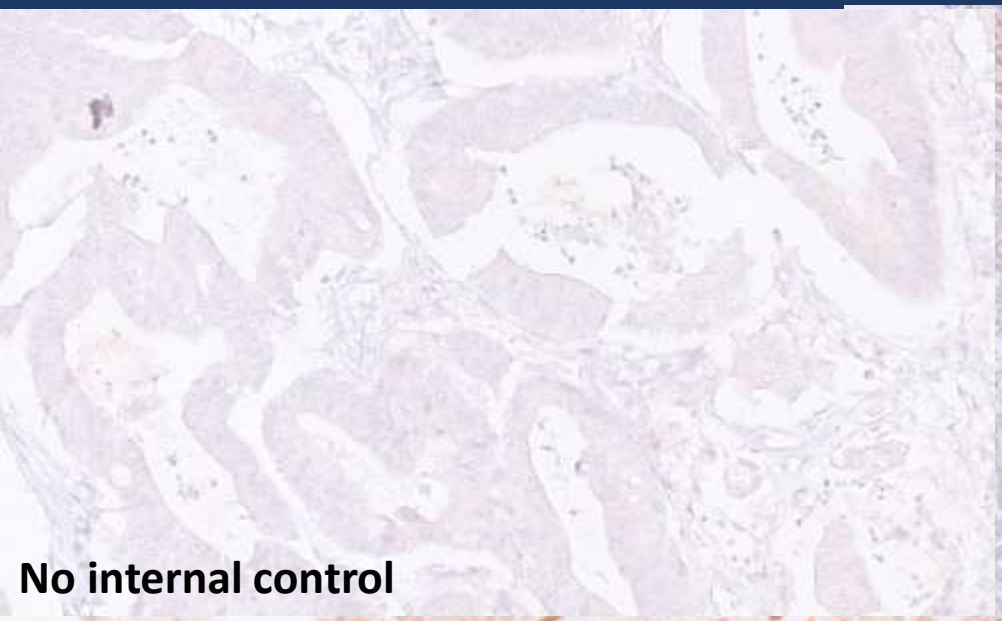
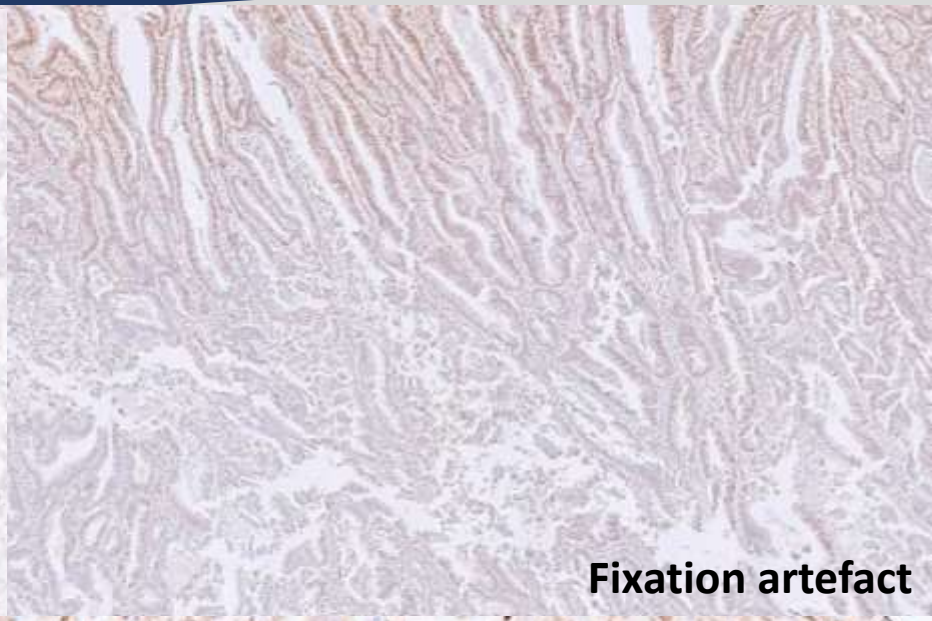
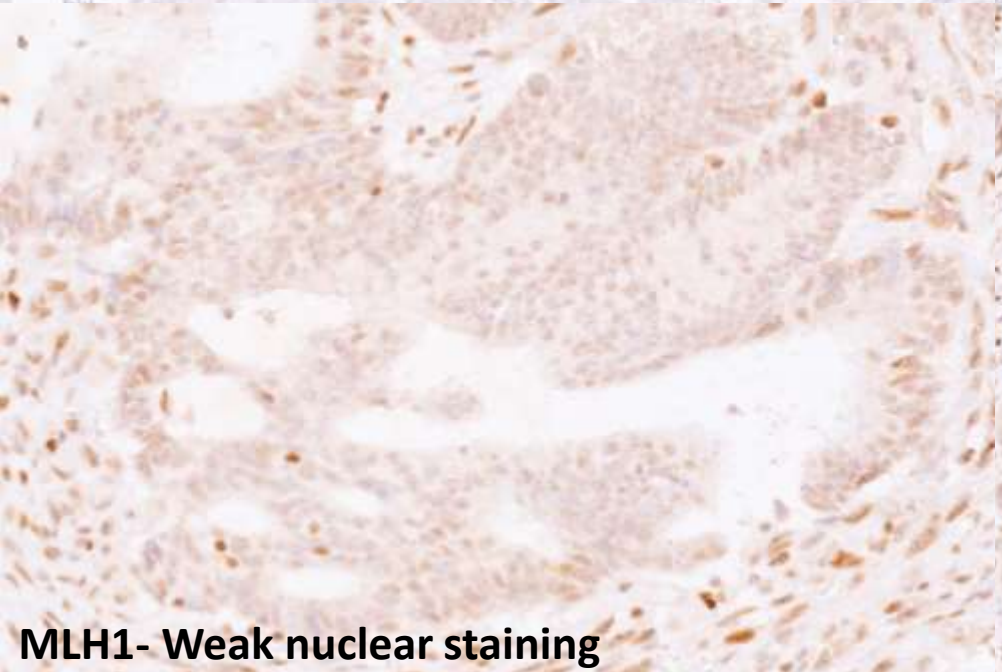
3) EXPERTISE REQUIRED in IHC interpretation



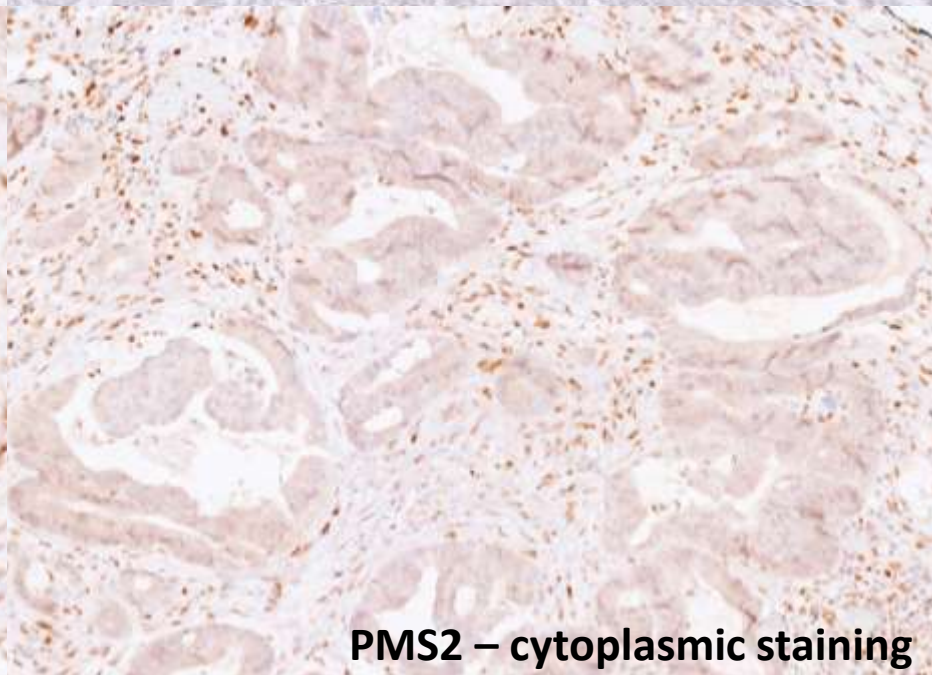
No internal control



Fixation artefact



MLH1- Weak nuclear staining



PMS2 – cytoplasmic staining

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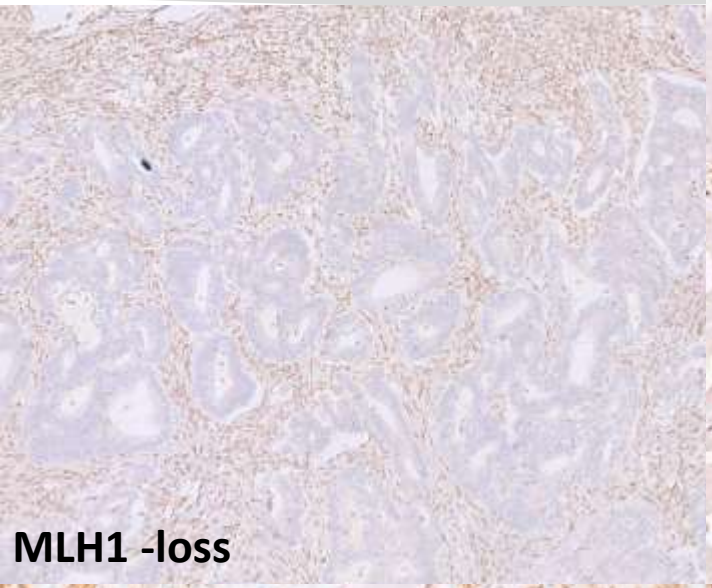
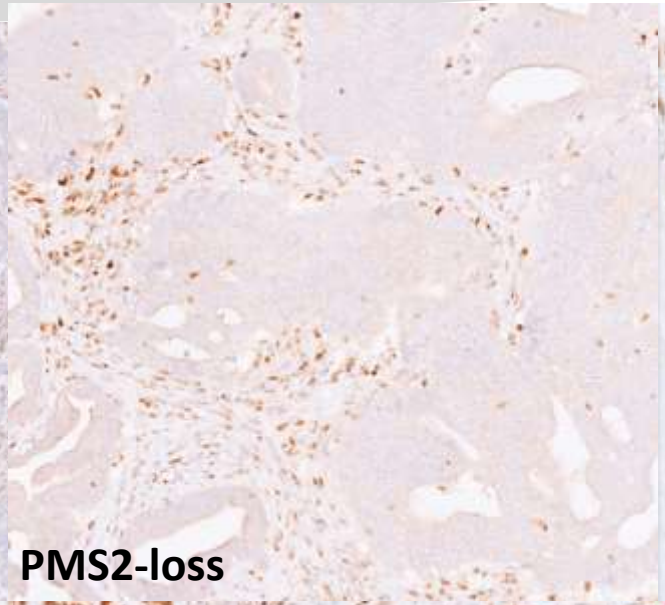
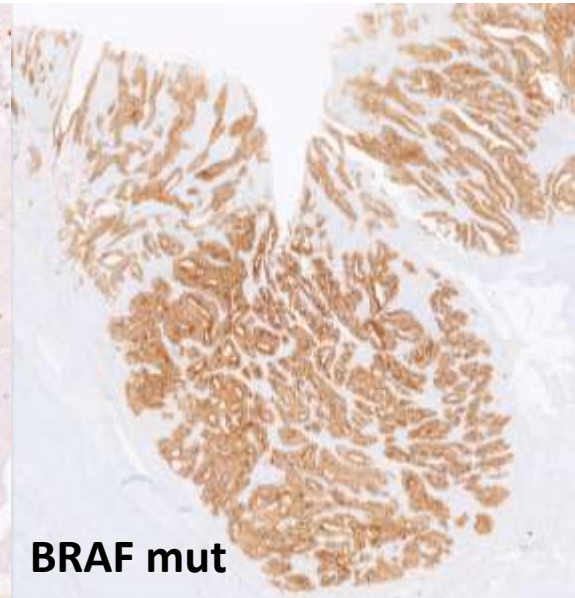
3) EXPERTISE REQUIRED in IHC interpretation



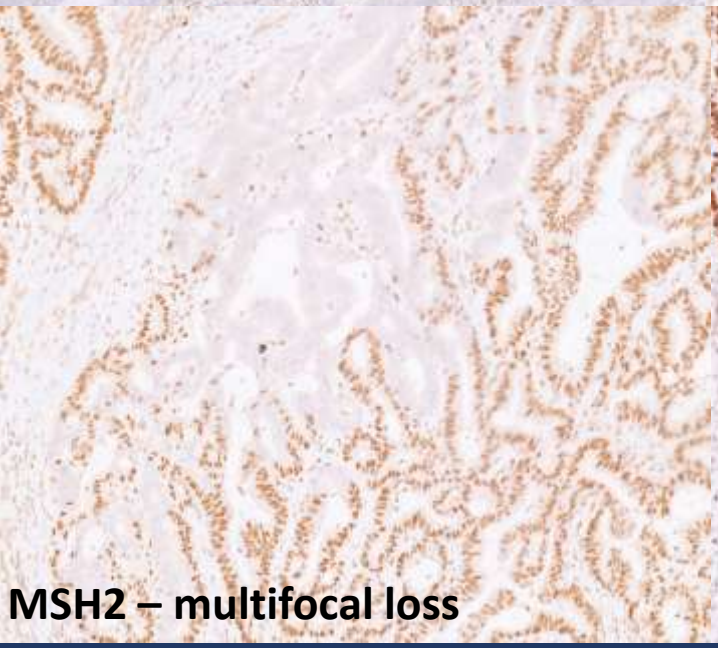
MLH1 -loss



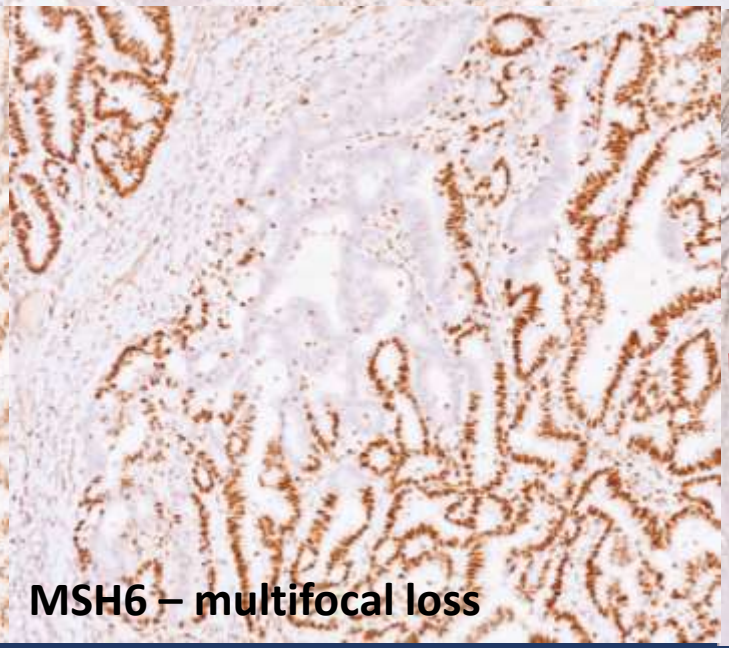
PMS2-loss



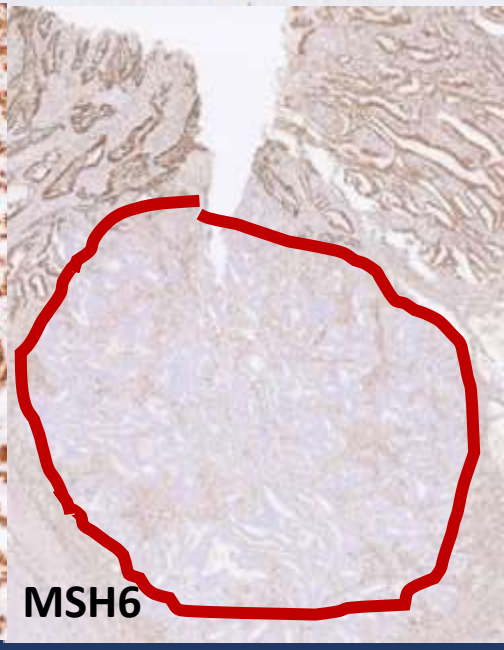
BRAF mut



MSH2 – multifocal loss



MSH6 – multifocal loss



MSH6

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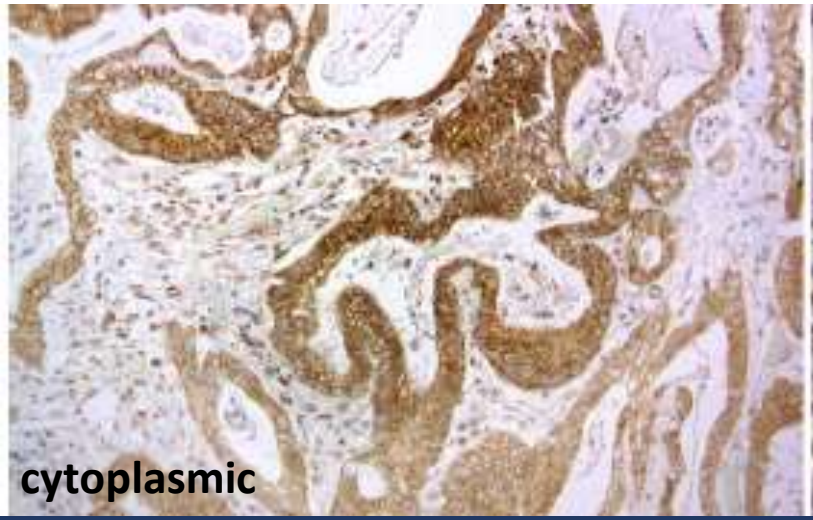
3) EXPERTISE REQUIRED in IHC interpretation

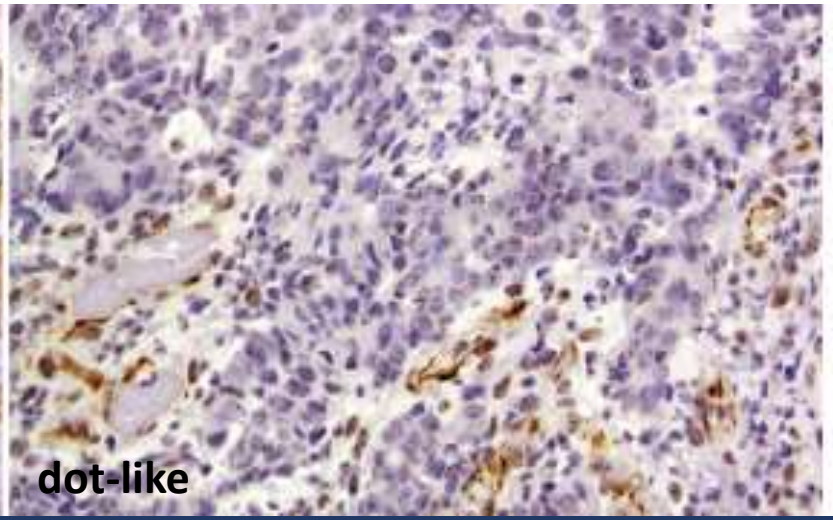
False negative MMR (dMMR but MSS)

- **pre-analytical issues** (tissue fixation) - recognized by the absence of signal in the internal positive controls (stromal cells or normal mucosa) – repeat on other tissue (eg. diagnostic biopsy) or send to MSI

False positive MMR (pMMR but MSI)

- **Aberrant staining patterns** (cytoplasmic, dot-like or perinuclear staining) - send to MSI
- **Catalytically inactive mutated MMR proteins, which retain their antigenic integrity**





4) IHC CONCLUSIONS NECESSARY

MLH1 – espressione nucleare conservata
PMS2 - espressione nucleare conservata
MSH2 - espressione nucleare conservata
MSH6 - espressione nucleare conservata

Tali aspetti suggeriscono un sistema MMR conservato (pMMR)

MLH1 – perdita completa di espressione nucleare
PMS2 – perdita completa di espressione nucleare
MSH2 - espressione nucleare conservata
MSH6 - espressione nucleare conservata

Tali aspetti suggeriscono un difetto del sistema del MMR (dMMR)

MLH1 – espressione nucleare conservata
PMS2 - espressione mal valutabile, per più probabili difetti preanalitici; si procede alla valutazione del campione bioptico/valutazione molecolare
MSH2 - espressione nucleare conservata
MSH6 - espressione nucleare conservata

Valutazione se pMMR or dMMR in seguito

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Universal Screening Genova

MLH1	PMS2	MSH2	MSH6	Comment suggested to report in diagnosis
pos	pos	pos	pos	IHC staining suggests MSS status
neg	neg	pos	pos	IHC staining suggests MSI status. *BRAF* exon 15 mutational analysis or *MLH1* promoter methylation should be taken into account to exclude Lynch syndrome.
neg	neg/ind	pos	pos	
neg/ind	neg	pos	pos	
pos	pos	neg	neg	IHC staining suggests MSI status and patient should be referred to genetic counseling for Lynch syndrome.
pos	pos	neg	neg/ind	
pos	pos	neg/ind	neg	
pos	pos	pos	neg	
pos	neg	pos	pos	
ind	ind	pos	pos	IHC staining supports MSI, but MSI molecular testing is required.
pos	pos	ind	ind	
ind	ind	ind	ind	IHC staing should be repeated on a second block of the lesion for excluding technical artifacts. If obtained the same result, MSI molecular testing should be performed.
neg	pos	neg	pos	Biologically unlikely. For excluding technical artifacts IHC should be repeated and/or the sample should be analyzed for MSI molecular testing.
neg	pos	pos	neg	
pos	neg	neg	pos	
pos	neg	pos	neg	

pos= retained staining; neg= complete loss of staining; ind= indeterminate

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REFLEX TESTING and LYNCH ALERT

- Since April 2011
- Initial 2 antibody testing untill 2012– reduction in costs but disadvantages

- From mid 2012 – 2019 – 4 antibody testing
- Problems in fixation led to problems in antigenicity
- IHC interpretation – dMMR/MSI or pMMR/MSS or indeterminate (reason and action)

From start of 2019 – now – 4 antibody testing, BRAF IHC and molecular testing +/- MLH1 methylation for MLH1/PMS2 negative - LS screening algorithm

Lynch Alert

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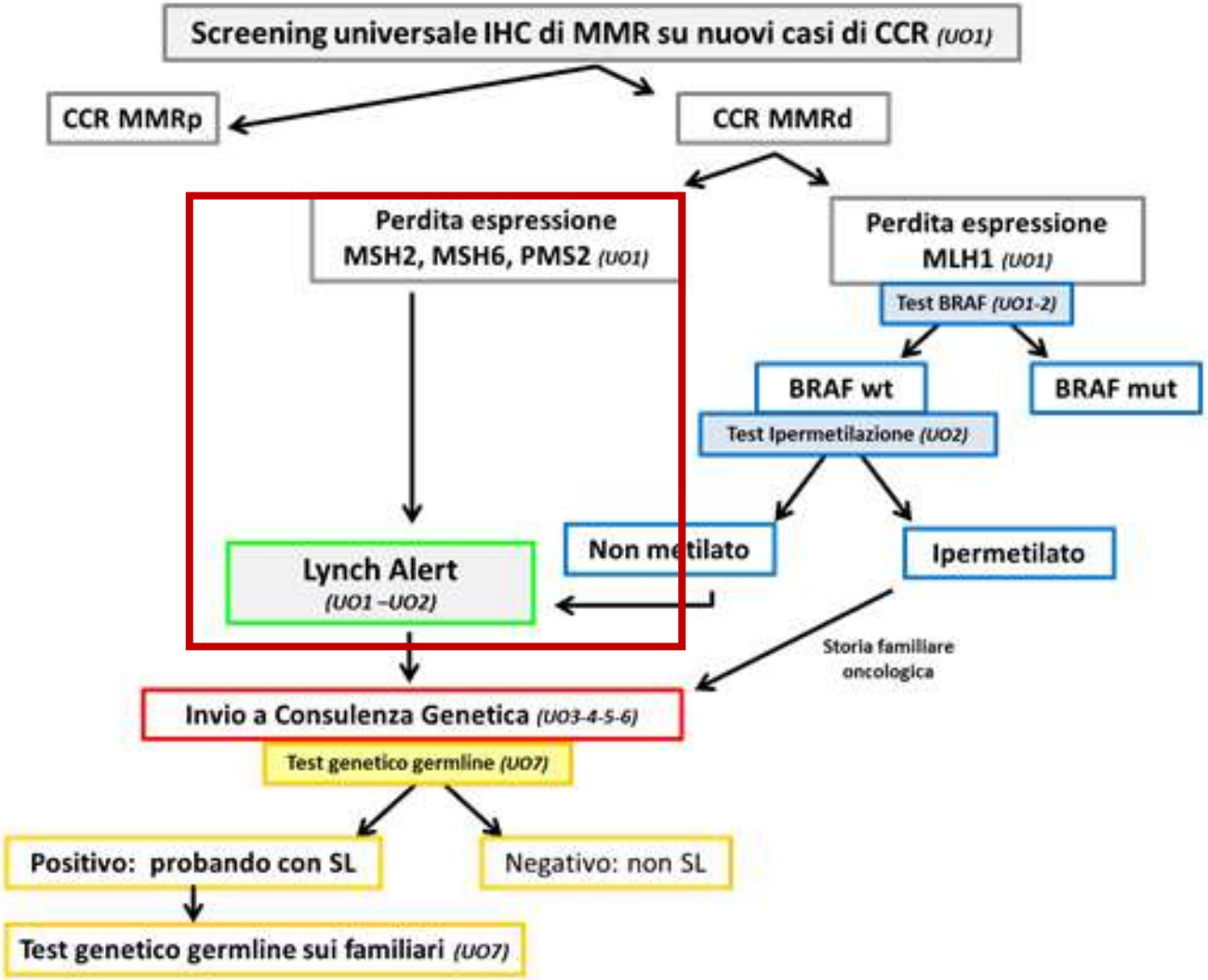
LS screening Genova



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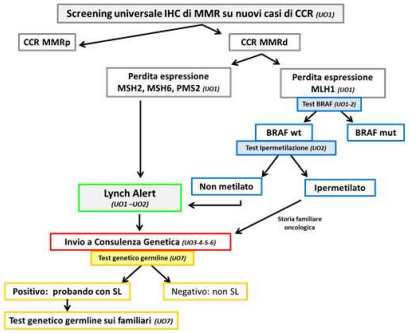
LYNCH ALERT - Genova



MLH1 – espressione nucleare conservata
PMS2 – espressione nucleare conservata
MSH2 - perdita completa di nucleare
MSH6 - **perdita completa di nucleare**

Tali aspetti suggeriscono un difetto del sistema MMR (dMMR)

LYNCH ALERT

Addendum in data XXXX: in considerazione del **difetto del sistema MMR (dMMR)** ed in particolare della **assenza di espressione nucleare immunoistochimica per MSH2/MSH6 o solo per MSH6 o solo per PMS2** è suggerito un:

Difetto del sistema MMR (mismatch repair) possibilmente legato a varianti costitutive.

Si consiglia visita di consulenza genetica per valutazione di sospetta S. di Lynch (Centro Tumori Ereditari, Ospedale Policlinico San Martino IRCCS).

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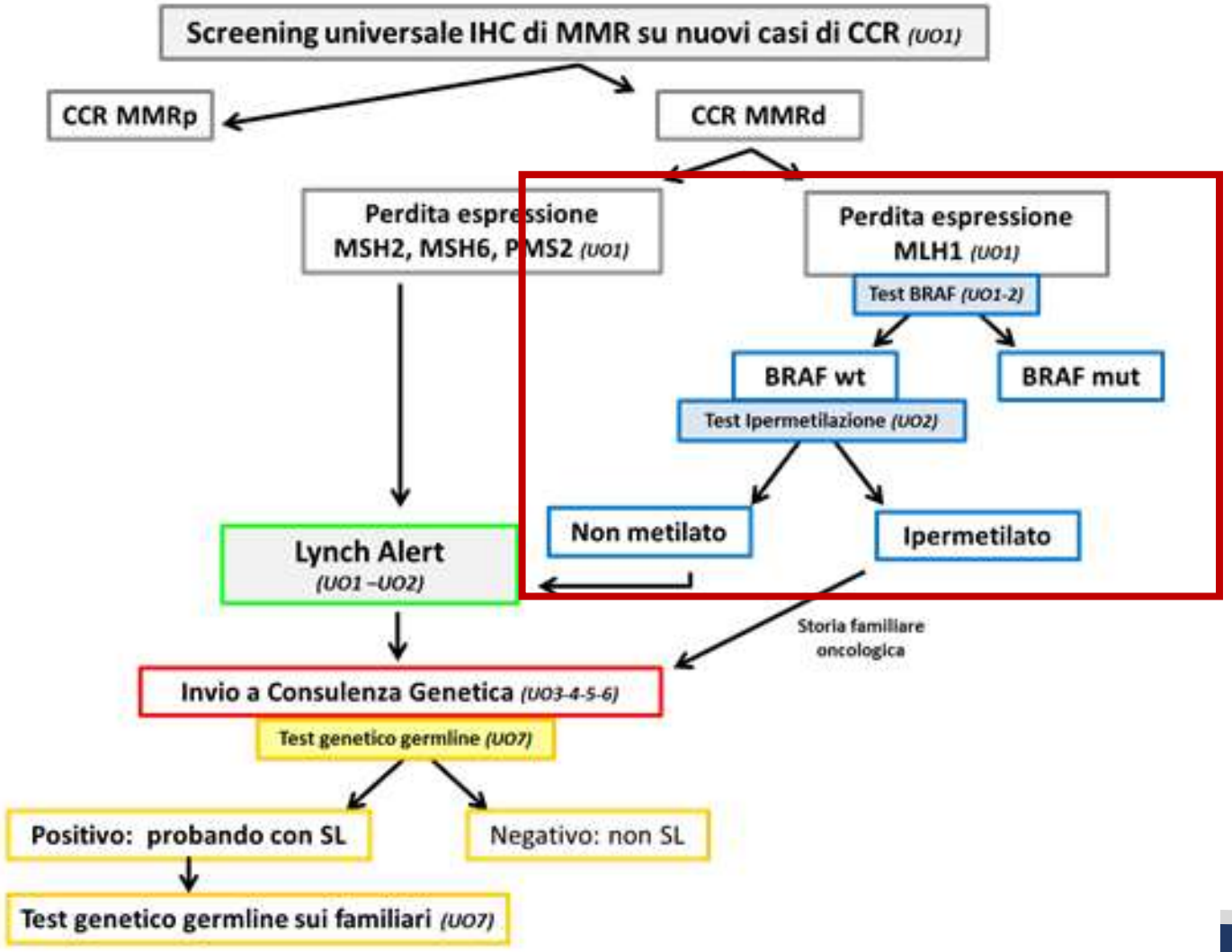
LS screening Genova



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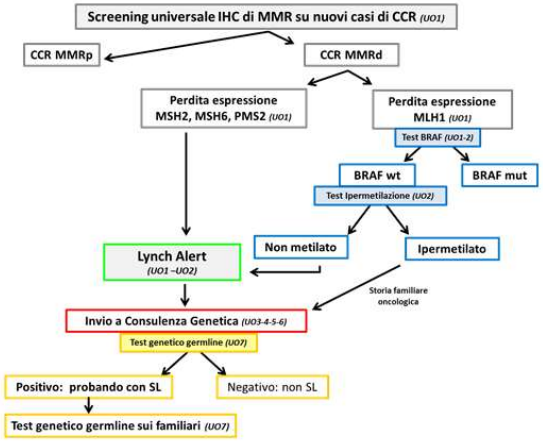
LYNCH ALERT - Genova



MLH1 – perdita completa di espressione nucleare
PMS2 – perdita completa di espressione nucleare
MSH2 - espressione nucleare conservata
MSH6 - espressione nucleare conservata

Tali aspetti suggeriscono un difetto del sistema MMR (dMMR)

BRAF IIC – score 0/1/2/3
Valutazione BRAF in molecolare a seguire

Addendum in data XXXX: in considerazione della **positività IHC e della conferma PCR di mutazione BRAF V600E** (vedi referto molecolare XXX/2020BM)**/della presenza di ipermetilazione del promotore di MLH1, seppur in assenza di mutazione in BRAF** (vedi referto molecolare XXX/2020BM) si conclude :

Difetto del sistema MMR (mismatch repair) presumibilmente variante somatica.

Addendum in data XXXX: in considerazione della **negatività immunoistochimica di BRAF e della assenza di mutazione in BRAF e assenza di evidente ipermetilazione del promotore di MLH1** (vedi referto molecolare XX/2020 BM) si conclude per:

Difetto del sistema MMR (mismatch repair) possibilmente legato a varianti costitutive.

Si consiglia visita di consulenza genetica per valutazione di sospetta S. di Lynch (Centro Tumori Ereditari, Ospedale Policlinico San Martino IRCCS).

Saluti da Genova