Microsatellite instability in colorectal cancers: how to deal with?

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Colorectal cancer can develop via two major molecular pathways:

- **CIN / MSS**: 85% of colorectal cancers
  - Chromosomal instability
  - K-RAS and P53 mutations

- **MSI**: 15% of colorectal cancers
  - Microsatellite instability
  - Lynch syndrome
  - Sporadic colorectal cancer
  - Widespread
  - Predominant
  - Poorly differentiated
  - Lynch

CIN: chromosomal instability
MSI: microsatellite instability
Microsatellites are:
- short DNA strengths composed of tandem repetitive sequence of 1-6 bases

examples: .....CATGCATGCATGCATG\textsubscript{n}......

.....CACACACACACA\textsubscript{n}......

...AAAAA\textsubscript{n}......

- scattered throughout the human genome, most commonly as dinucleotide (CA)\textsubscript{n}

50.000 to 100.000 per genome
Microsatellite instability

MSI and affected genes

LYNCH SYNDROME
- Median age at diagnosis: 42 years
- Germline mutation in one of the MMR gene: 90% MLH1 or MSH2, 10% MSH6 or PMS2

SPORADIC CASES
- Median age at diagnosis: >70 years
- > 90% MLH1 promoter hypermethylation
- Frequent somatic BRAF V600E mutation

COLORECTAL CANCERS
2-5%

12-15%

MSI PHENOTYPE
≈15% CRC

MAJOR MSI TARGET GENES

DNA REPAIR
- MRE11: 73%
- POLD3: 43%
- MSH3: 42%
- RAD50: 31%
- BRCA2: 27%
- MSH6: 26%
- MBD4: 26%
- PRKDC: 23%
- MLH3: 21%
- BLM: 17%
- LIGASE 3: 13%
- REV1L: 13%
- REV3L: 11%

DNA DAMAGE SIGNALLING & APOPTOSIS
- CASP 5: 49%
- BAX: 42%
- ATR: 25%
- CHEK1: 12%
- CDC25: 11%
- BCL10: 10%

SIGNAL TRANSDUCTION
- ACVR2: 86%
- TGFBR2: 75%
- EPHB2: 42%
- PTEN: 31%
- PIK3CA: 21%
- IGF2R: 20%
- WISP3: 19%

TRANSCRIPTION REGULATION
- TCF4: 83%
- TAF1B: 79%
- CREBBP: 29%
- HDAC2: 23%
- PRDM2: 18%

BAX
TGFBR2
IGF2R
The CRC related to high levels of microsatellite instability (MSI-H) can also be named **MMR-deficient (dMMR) CRC**

The CRC associated with CIN are described as **microsatellite stable (MSS)** or **MMR-proficient (pMMR) CRC**

Among MSI-H colorectal cancer:

- **Lynch syndrome:**
  - autosomal dominantly inherited predisposition to early onset multiple tumours
  - Germline mutations of MMR genes: ~30% for *MLH1*, ~40% for *MSH2*, ~15% for *MSH6* and ~15% for *PMS2*.
  - nonsense mutation (codon STOP) leading to truncated RNA with its subsequent degradation

- **Sporadic dMMR CRC**
  - Epigenetic *MLH1* inactivation through hypermethylation of its promoter
  - Activating *BRAFV600E* mutation in ~60% of sporadic dMMR CRC but **not** in Lynch syndrome cases!!
Identification of MSI+ CRC: clinical relevance

- diagnosis of Lynch CRC patients and germ-line mutation carriers
- prognostic impact
- predictive impact (for adjuvant chemotherapy)

MSI CRC may require different treatments
Identification of Lynch syndrome

- Proband: increased risk of developing secondary carcinomas in the colon and/or other extracolonic cancers (endometrial carcinoma!)

- First-degree relatives of the patient have a 50% chance of being MMR gene mutation carriers

- MMR gene mutation carriers: fivefold to sixfold increased risk of carcinoma
  
  Germline *MLH1* and *MSH2* mutation carriers: 30-80% of lifetime risk for CRC

  ➔ Benefit for early identification and regular surveillance of mutation carriers (increased clinical screening and early detection of disease in mutation carrier’s relatives)
Pronostic impact of MSI status

- MSI-H CRCs show better survival rates compared with MSS CRCs
  - Lower tumor stage at diagnosis
  - MSI-H are rare in metastatic CRCs
  - Longer OS and higher rate of DFS

- Why?
  - Aneuploidy in CIN/MSS CRCs vs diploidy in MSI-H CRCs?
    (aneuploidy = marker of poor prognosis – cf P53 deletion or KRAS activation)
  - Excess of tumor-infiltrating cytotoxic lymphocytes (TIL) in response to neopeptides generated by frameshift mutations in coding sequences
    ➔ eliciting a protective anti-tumour immune response?
MMR status may predict the response to adjuvant chemotherapy

- Stage II and III MSI-H CRCs: no benefit in OS and DFS from 5-FU adjuvant therapy in contrast to MSS(CIN) CRCs. MSI-H patients could be spared from unnecessary treatment-related toxic effects.

   ➤ Current clinical use of MMR status to guide adjuvant 5-FU therapy decisions in stage II and III CRC patients.

- Stage III MSI-H CRC patients could benefit from Irinotecan + LV adjuvant chemotherapy (improved 5-years DFS) in contrast to MSS(CIN) CRCs. But this predictive impact still awaits further evaluation.
Strategy to detect MSI+ colorectal cancers

- Evidence accumulates showing that it is time to diagnose MSI tumours in all patients with newly diagnosed CRC (+ patients with endometrial carcinoma before the age of 60 years)

- Guidelines to detect Lynch syndrome or sporadic MSI+ tumours keep changing as our knowledge improves and should not be seen as definitely established
Strategy to detect MSI+ colorectal cancers

Good practical test algorithm taking into account all the following criteria:

- Clinical criteria
- Morphological criteria
- Immunohistochemistry testing
- Molecular testing
  - MSI testing
  - DNA sequencing testing

All those criteria and tests are not 100% sensitive
Revised Bethesda Criteria

Just one of these criteria need to be met

- diagnosed with colorectal cancer before the age of 50 years or endometrial cancer before the age of 60 years;

- synchronous or metachronous CRC or other HNPCC-related tumours (which include stomach, bladder, ureter, renal pelvis, brain, biliary tract, sebaceous gland adenomas, keratoacanthomas and carcinoma of the small bowel), regardless of age;

- colorectal cancer with a high-microsatellite-instability morphology that was diagnosed before the age of 60 years;

- colorectal cancer with one or more first degree relatives with colorectal cancer or other HNPCC-related tumours. One of the cancers must have been diagnosed before the age of 50 years (including adenoma, which must have been diagnosed before the age of 40 years);

- colorectal cancer with two or more relatives with colorectal cancer or other HNPCC-related tumours, regardless of age.

Those revised criteria allow to predict Lynch syndrome with a sensitivity of 95% and a specificity of 39% - would miss 5% of Lynch syndrome cases - do not take the sporadic MSI+ CRC cases into account.
Pathological criteria for prediction of MSI+ CRC

MSI+ CRC can be suspected according to various pathologic features:

(From the PREDICT model: “Pathologic Role in Determination of Instability in Colorectal tumors”)

- Right-sided tumor location
- Mucinous component, signet ring or medullary histology
- Increase number of tumor-infiltrating lymphocytes
- Peritumoral lymphocytic reaction
- Increased stromal plasma cells, granulomatous reaction (Crohn-like)
- Absence of intraglandular neutrophil-rich “dirty” necrosis
- Sessile serrated adenoma/polyps* (as precursor lesions)

* Sessile serrated adenoma are precursors of sporadic MSI+ CRC while Lynch CRC arise in conventional adenomatous polyps

These morphological criteria have a sensitivity of 92% and would miss 8% of Lynch syndrome cases

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Hyde et al Am J Surg Pathol 2010
Principles

- the MMR gene nonsense mutations lead to the production of a truncated RNA and its subsequent degradation.
- IHC will thus have the advantage of identifying the affected gene by detecting loss of its specific protein product.

- MMR proteins function as heterodimers: 
  - MLH1-PMS2 and MSH2-MSH6

- Loss of MLH1 or MSH2 results in concomitant loss of their respective partner, while the reverse is not true.
**IMMUNOCHEMISTRY TESTING (2)**

### Advantages:
- Directing gene mutation screening
- Less expensive and faster than molecular methods
- Available in numerous pathology departments
- Great sensitivity

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Loss of MSH2/MSH6 expression

- MSH6 alone
- PMS2 alone

- Likely to be Lynch syndrome

Loss of MLH1

- ? Sporadic dMMR CRC
- ? Lynch syndrome CRC

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IHC tends to replace molecular MSI as a screening method for MMR-deficient tumors but…

- interpretation sometimes difficult
- ~ 11% of Lynch syndrome and ~ 4% sporadic dMMR CRC show MSI testing positivity without MMR protein loss:
  - retained MMR protein immunoreactivity in case of missense mutations
  - interobserver variabilities among pathologists

IHC criteria alone would fail to detect ~11% of new Lynch syndrome patients and 4% of sporadic cases
Principle

- MSI testing is performed on paraffin-embedded tumor tissue
- using a PCR-based assay for detection of instability at selected microsatellite loci
- panel of 5 quasi-monomorphic mononucleotide markers
- If available, comparison with normal DNA of each patient would facilitate the interpretation of the profile
- A minimum of 30% of tumoral cells in the sample is required
**MSI TESTING (2)**

- CRC can be classified as:
  - High-frequency MSI (**MSI-H**) if at least 2/5 microsatellite markers show instability (3/5 if no normal DNA sample available).
  - Low-frequency MSI (**MSI-L**) if only 1/5 microsatellite markers
  - Microsatellite stable (**MSS**) if none of the markers show instability

MSI-L and MSS cases are grouped together as they have similar clinical features and outcomes.

![Images showing normal colon and colon tumor](images)
MSI TESTING (3)

- Disadvantages:
  - more expensive and time-consuming than IHC staining
  - does not identify the affected MMR gene
  
  but...

  ~ 1% of false negative cases (Lynch syndrome and sporadic dMMR CRC respectively showing MMR protein loss without MSI-H)

MSI criteria alone would fail to detect only ~1% of new Lynch and sporadic dMMR CRCs

IHC staining and MSI testing are complementary methods as 100% of cases will be detected by one of the two methods
Proposal

MMR screening algorithm includes testing for

1. IHC (MLH1, MSH2, MSH6 and PMS2).

2. MSI (preferably with 5 mononucleotide markers)

3. \textit{BRAFV600E} mutation testing

at the time of any new diagnosis of CRC.
Use of this algorithm should allow MMR subgroup assignment for most cases.
THAT’S IT !!