Microsatellite instability in colorectal cancers: how to deal with?

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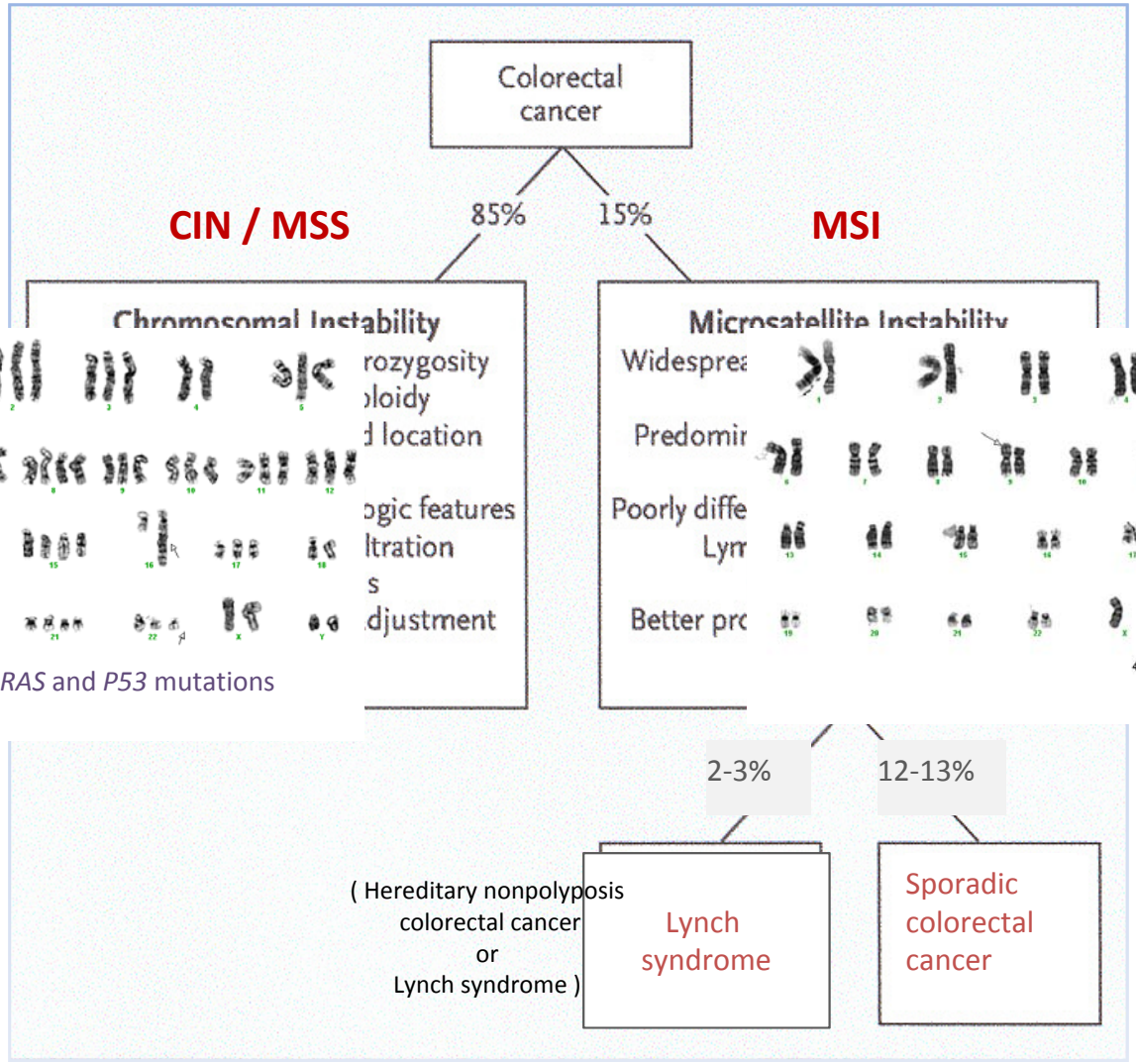
MOLECULAR MECHANISMS OF COLORECTAL CARCINOGENESIS

- Colorectal cancer can develop via two major molecular pathways



CIN: chromosomal instability
MSI: microsatellite instability

A FEW DEFINITIONS

Microsatellites are:

- short DNA strengths composed of tandem repetitive sequence of 1-6 bases

examples : …..CATGCATGCATGCATG$_n$……

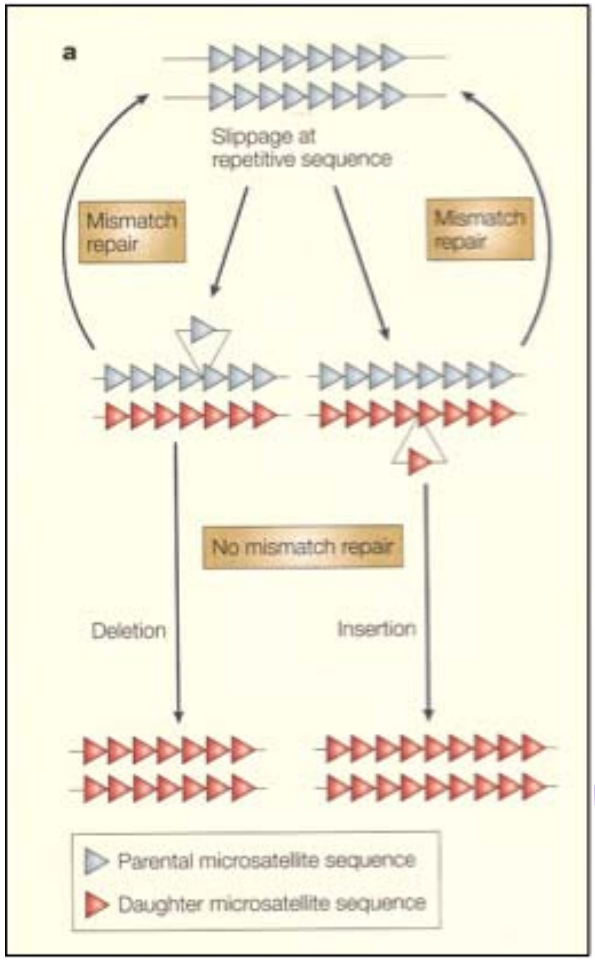
…..CACACACACA$_n$…..

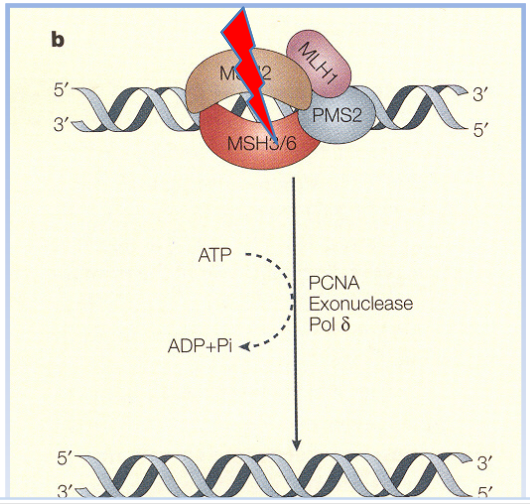
…AAAAAAAAA$_n$…..

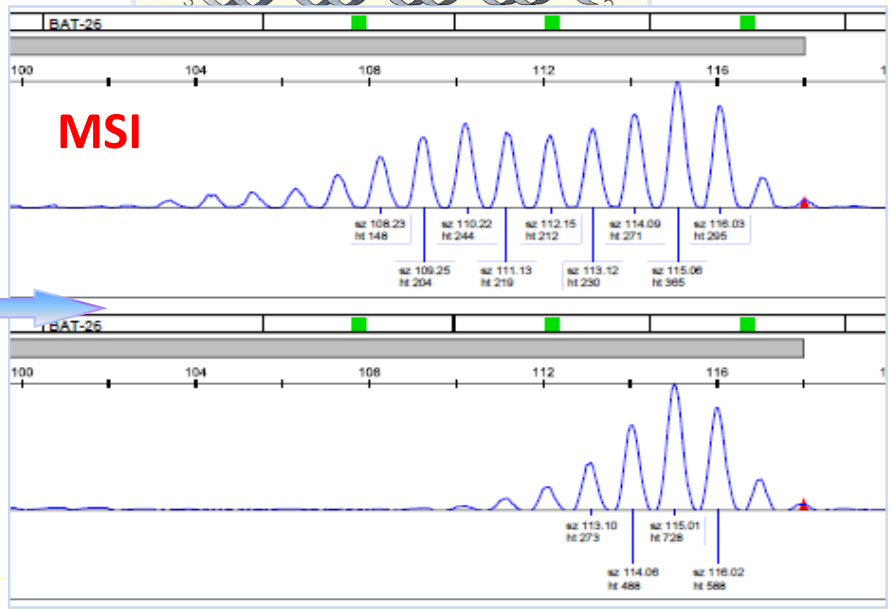
- scattered throughout the human genome, most commonly as dinucleotide $(CA)_n$

50.000 to 100.000 per genome

Microsatellite instability

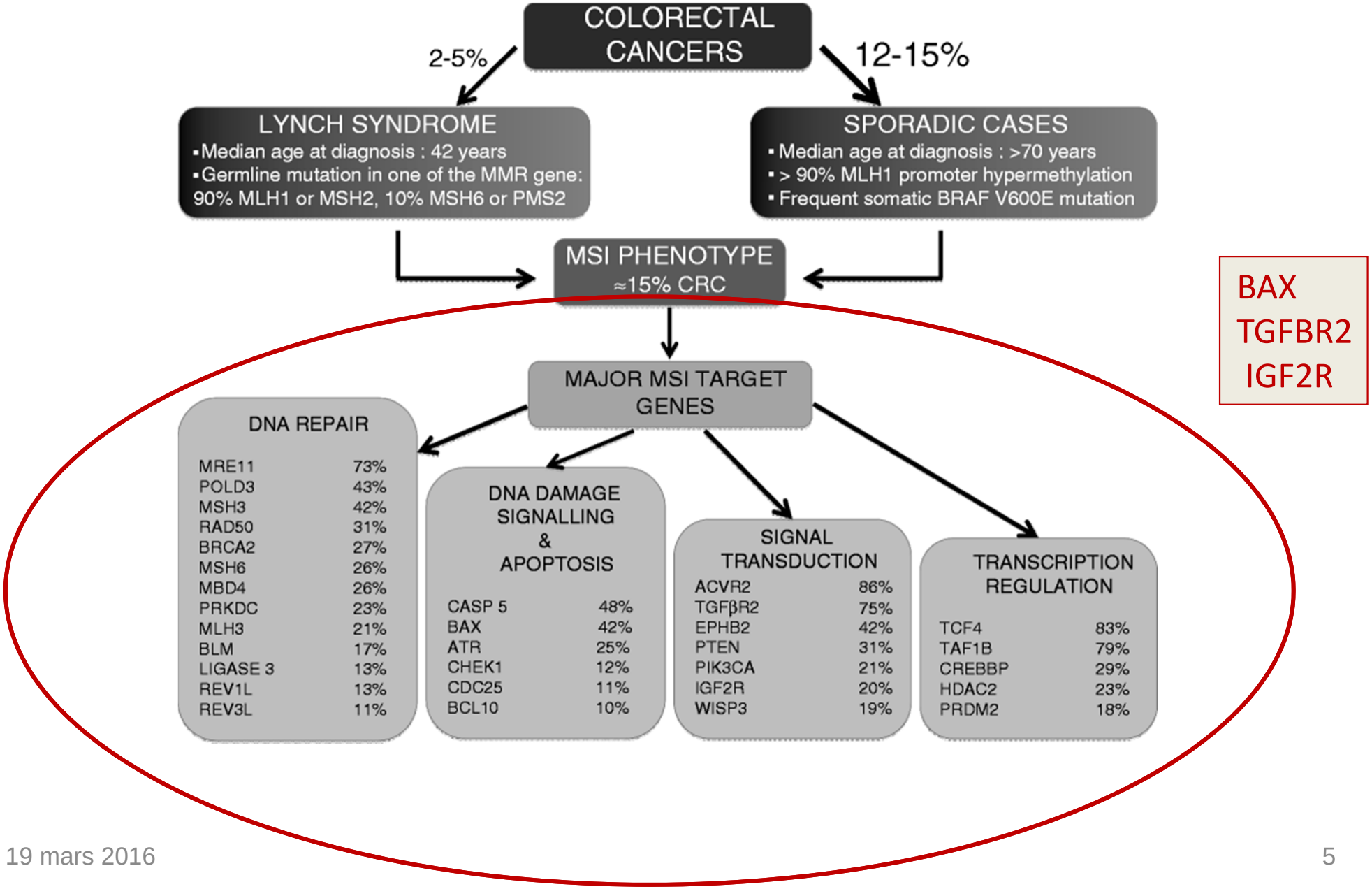






Umar A et al. *Nat Rev Cancer*(2004)

MSI and affected genes



MSI COLORECTAL CANCERS

- 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 mutiple 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?

Predictive impact of MSI status

- 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

CLINICAL GUIDELINES USED TO SELECT FAMILIES WITH HIGH-RISK OF LYNCH SYNDROME

Hôpital Erasme ULB

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

Hôpital Erasme | ULB

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

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)*

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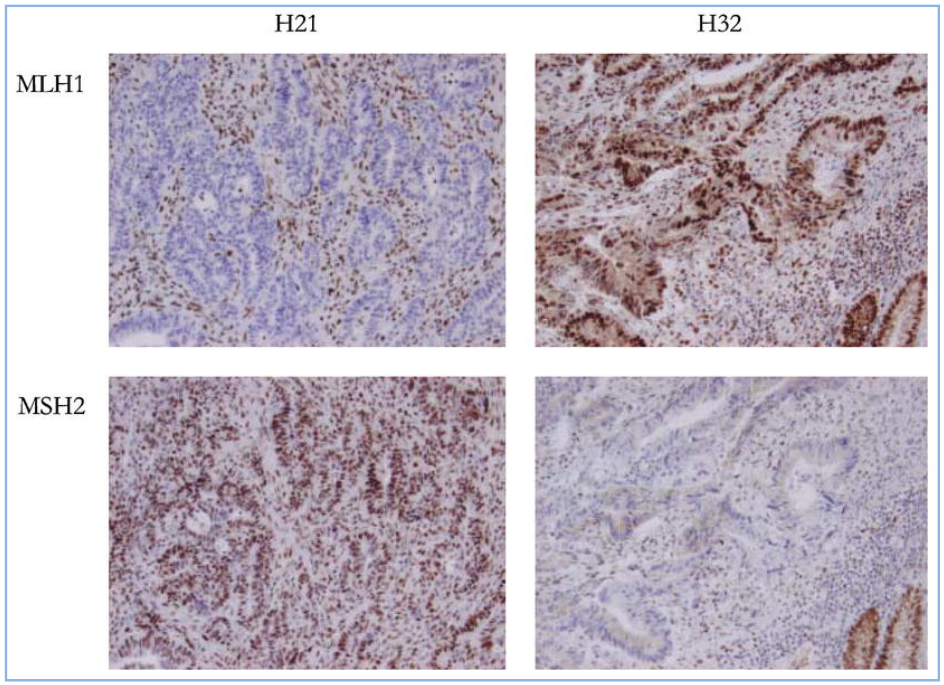


Halvarsson B et al. *Virchows Arch*(2004)

- Loss of MSH2/MSH6 expression
  MSH6 alone
  PMS2 alone

  ➔ likely to be Lynch syndrome

- Loss of MLH1

  ➔ ? Sporadic dMMR CRC
  ? Lynch syndrome CRC

- **Advantages:**
  - directing gene mutation screening
  - less expensive and faster than molecular methods
  - available in numerous pathology departments
  - great sensitivity

IMMUNOCHEMISTRY TESTING *(3)*

- 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

MSI TESTING *(1)*

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

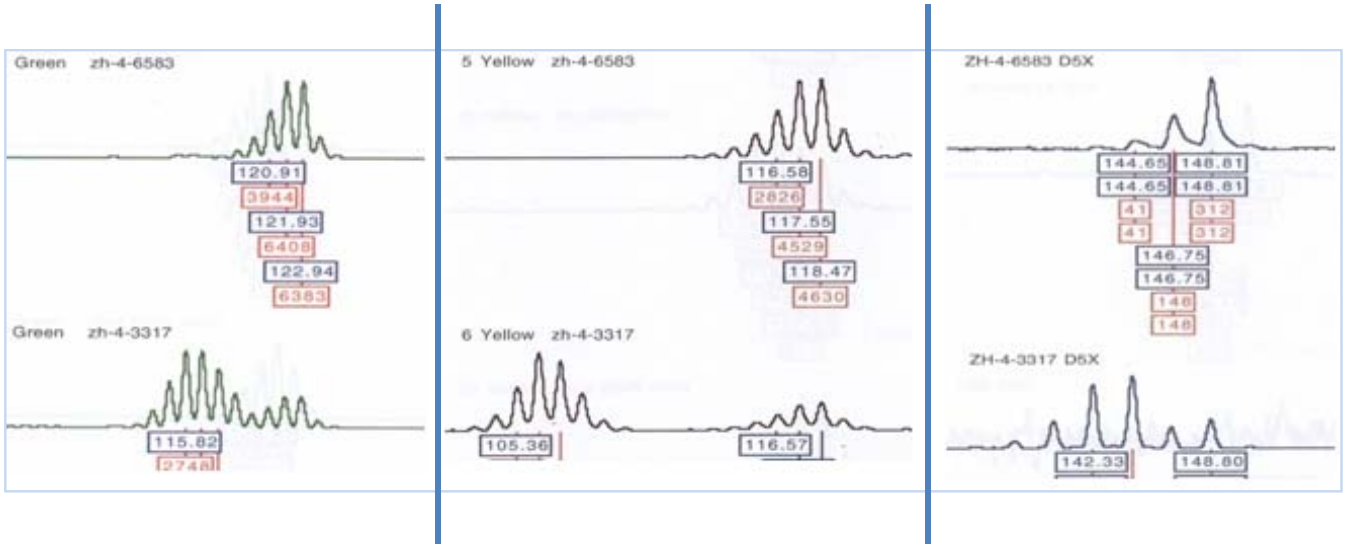
MSI TESTING *(2)*

- 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

Normal colon

Colon tumour



MSI TESTING *(3)*

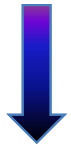
- Disadvantages:

  - 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 as100% of cases will be detected by one of the two methods

« Take Home Message » *(1)*

Proposal

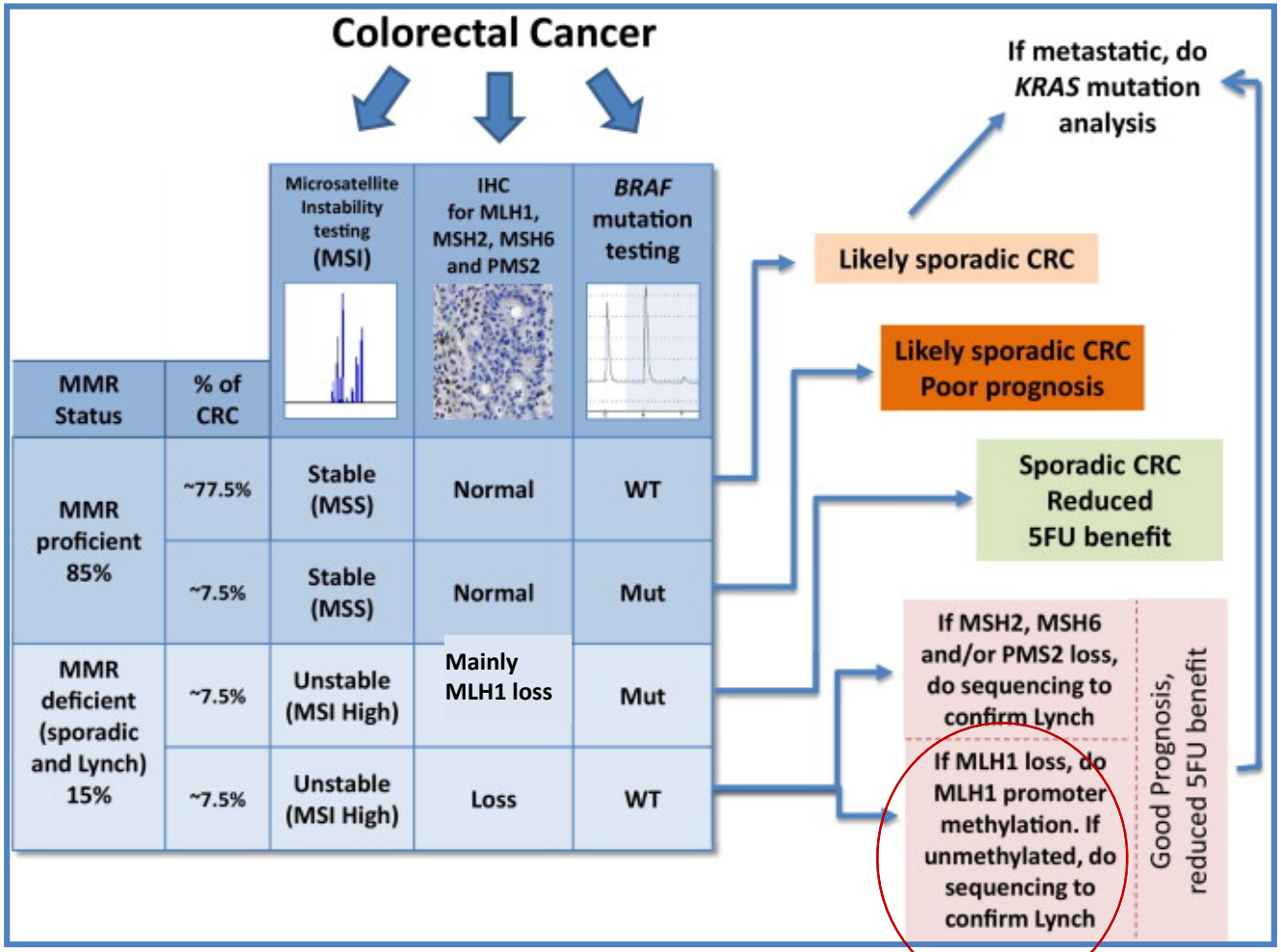
MMR screening algorithm includes testing for

1. IHC (MLH1, MSH2, MSH6 and PMS2).
2. MSI (preferably with 5 mononucleotide markers)
3. *BRAFV600E* mutation testing

at the time of any new diagnosis of CRC.

« Take Home Message » *(2)*

Use of this algorithm should allow MMR subgroup assignment for most cases



THAT'S IT !!