# DNA mismatch repair microsatellite instability IHC vs PCR vs NGS

Siska Dedeurwaerdere

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IPG (Gosselies)

# DNA mismatch repair microsatellite instability

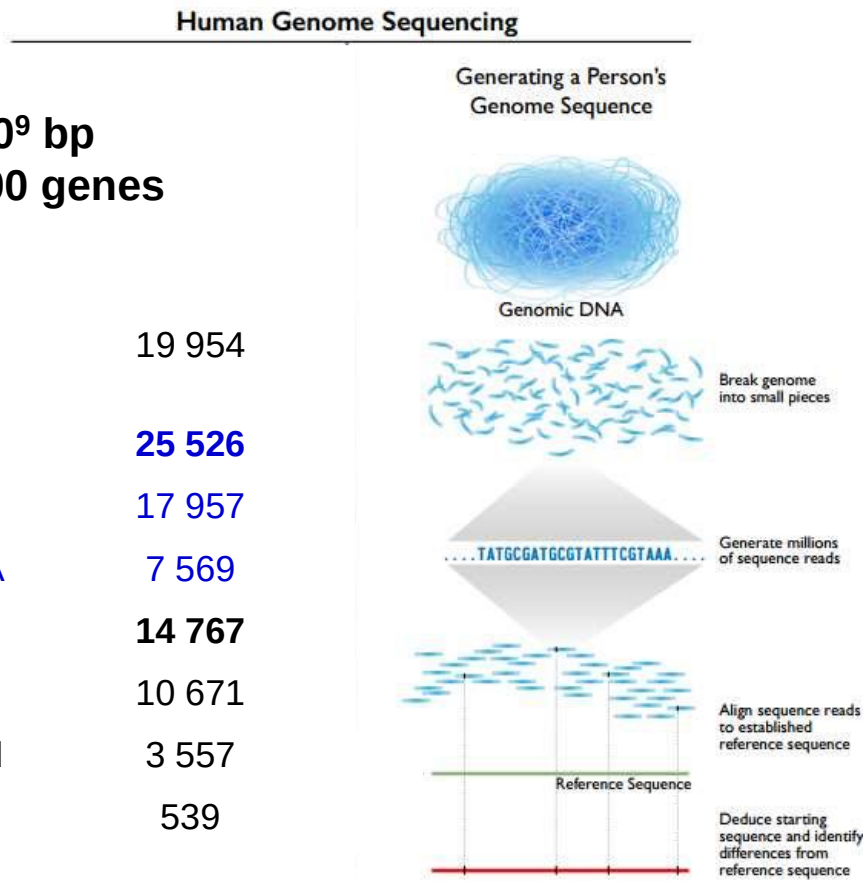
1) What?
2) Why?
3) How?

# HUMAN GENOME

**$6.10^9$ bp**
**# 60 000 genes**

| | |
|---|---|
| Protein-coding genes | 19 954 |
| **RNA genes** | **25 526** |
| Long ncRNA | 17 957 |
| Small ncRNA | 7 569 |
| **Pseudogenes** | **14 767** |
| Processed | 10 671 |
| Unprocessed | 3 557 |
| Other | 539 |

*GENCODE version 35 (GRCh38.p13)*




https://www.genome.gov/sequencingcosts/

- 50% REPEAT SEQUENCES
- 30% CODANT GENE (1,5% proteins)
- 20% INTER-GENIC REGIONS

# microsatellites

A DNA sequence block that consists of a succession of repeating units (5-50 times) of a nucleotide sequence.

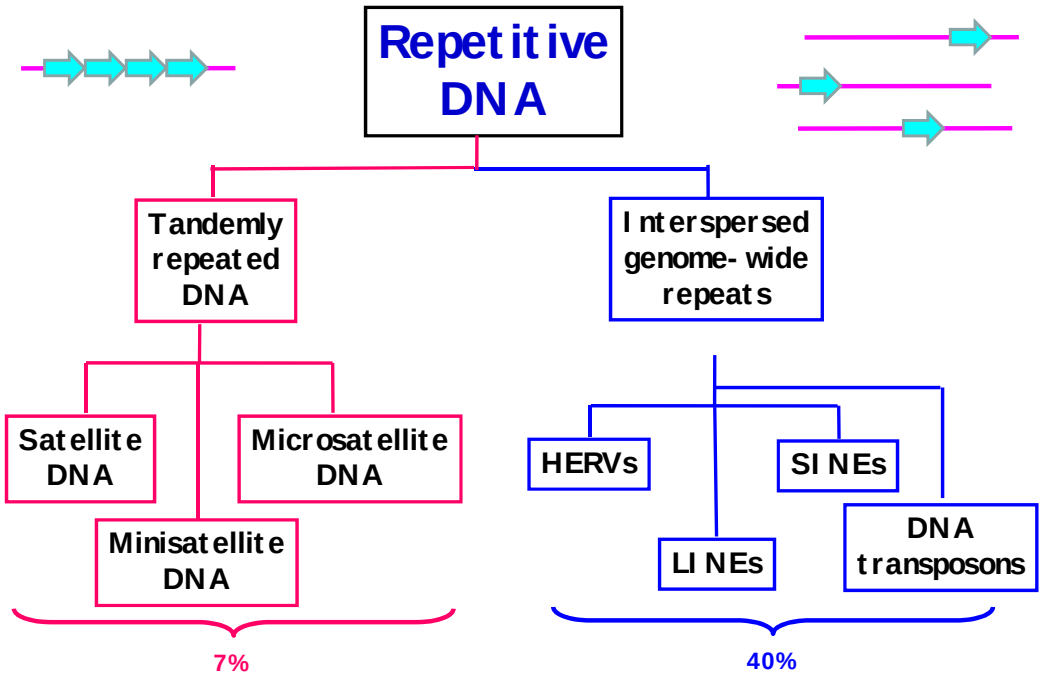
Synonym: short tandem repeat (STR)

Human genomes contains 50,000-100,000 dinucleotide microsatellites

Mono-repeats: AAAAA (A5)
Di-repeats: ATATATAT (AT4)
Tri-repeats: GTCGTCGTCGTCGTC (GTC5)
Tetra-repeats
Penta-repeats

| Repeat Type | Sequence | Name and Location |
|---|---|---|
| Mononucleotide | AGGTAAAAAAAAAAAAAAAAAAAAAAAAAGGGT<br>$(A)_{26}$ shown | *BAT26*, Intron 5 of *MSH2* gene<br>Chromosome 2 |
| Dinucleotide | TGTACACACACACACATCGA<br>$(CA)_6$ shown | D5S346<br>Chromosome 5 |
| Tetranucleotide | ATATTCTATCTATCTATCTATCTATCTG<br>$(TCTA)_5$ shown | D14S608<br>Intergenic region chromosome 14 |

# HUMAN GENOME




**Séquences répétées :** *S. cerevisiae* **(3.4%)**, *D. melanogaster* **(12%)**

Tandemly Repeated DNA

| TYPE | TOTAL LENGTH | REPEAT LENGTH | GENOME LOCALISATION |
|---|---|---|---|
| Satellite | 300Kb-10Mb | 5-171pb<br>Ex:&satellite 171 | Centromere (heterochromatine) |
| Minisatellite | 0,1-20Kb | 9-64pb*<br>(TTAGGG) | Telomere<br>Subtlomeric regions |
| Microsatellite | <100pb | 1-4pb*<br>Ex: CA (2pb) | Regular distribution |

* Allelic Polymorphism

# HUMAN GENOME

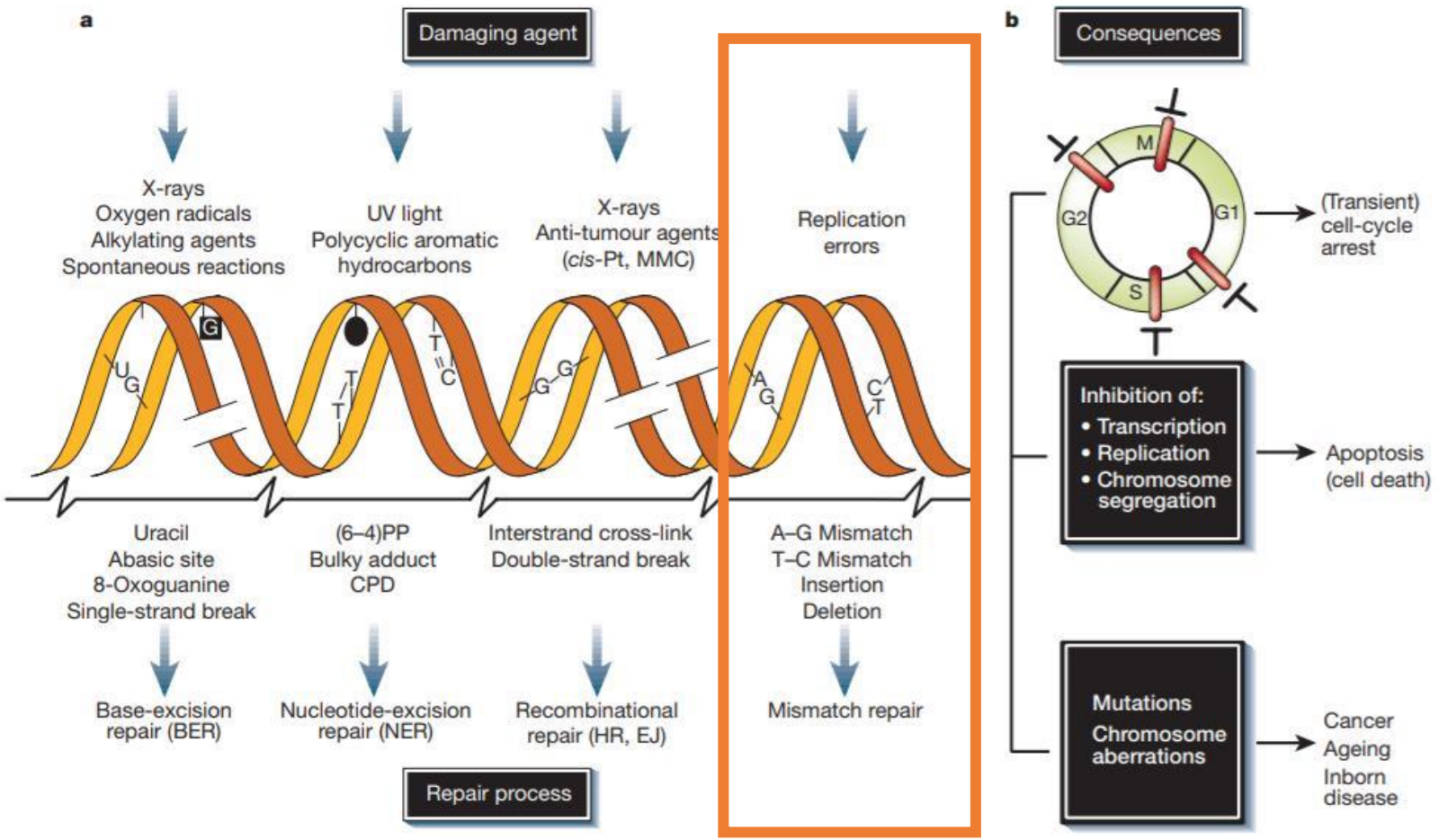
5′GCTATACATGACATGACAGTA
GCAGATGACATAGACATGAGTAC
ACCTTCATTCACTCACAGATCAG
ATTGTGCAC**CACACACACACACA**
**CACACACACACACACACACA**TGATG
ACAGATGAGATGGATGATCTGAT
TGGTGGTAGACAGCATTCATACA
GATGCAGATACA 3′

**Microsatellite $(CA)_{16}$**

5′GCTATACATGACATGACAGTA
GCAGATGACATAGACATGAGTAC
ACCTTCATTCACTCACAGATCAG
ATTGTGCAC**CACACACACACACA**
**CACACACACACACACACACACACAC**
**ACA**TGATGACAGATGAGATGGAT
GATCTGATTGGTGGTAGACAGCA
TTCATACAGATGCAGATACA 3′

**Microsatellite $(CA)_{20}$**

# DNA mismatch repair



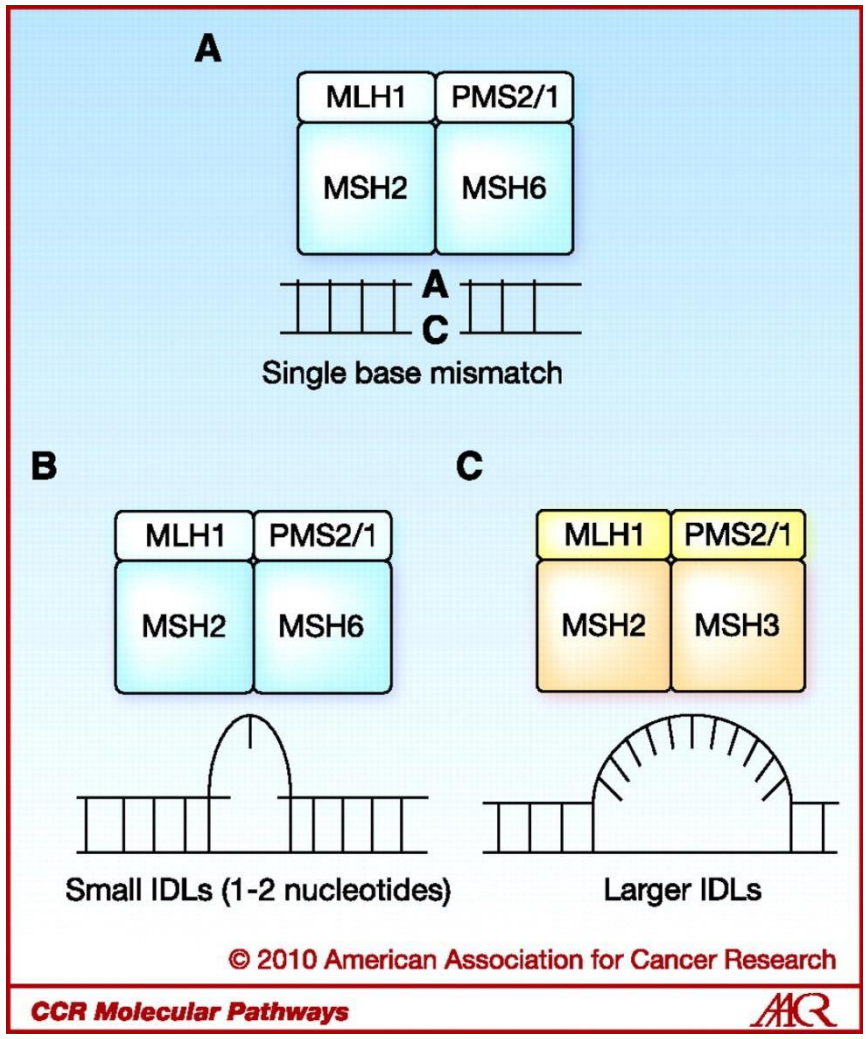

**Exogenous factors:**

- UV radiation
- Ionizing radiation
- Genotoxic chemicals

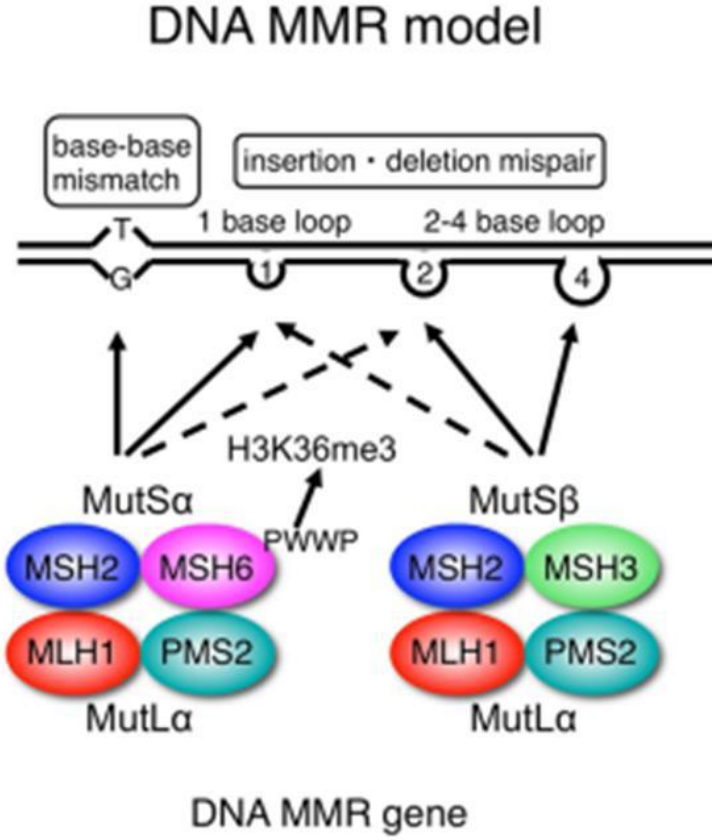
**Endogenous factors:**

- Spontaneous or enzymatic reactions
- Chemical modifications
- Replication errors
- Replication stress

Genome maintenance mechanisms for preventing cancer
Jan H. J. Hoeijmakers, NATURE, VOL 411; pp366-375 (2001)




Sarah A. Martin et al. Clin Cancer Res 2010;16:5107-5113

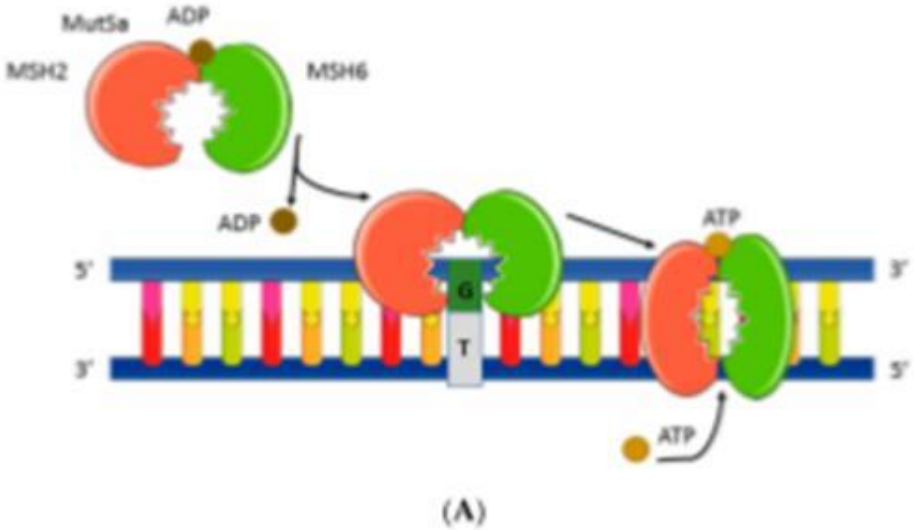



Arch Toxicol (2015) 89:899–921
DOI 10.1007/s00204-015-1474-0

REVIEW ARTICLE

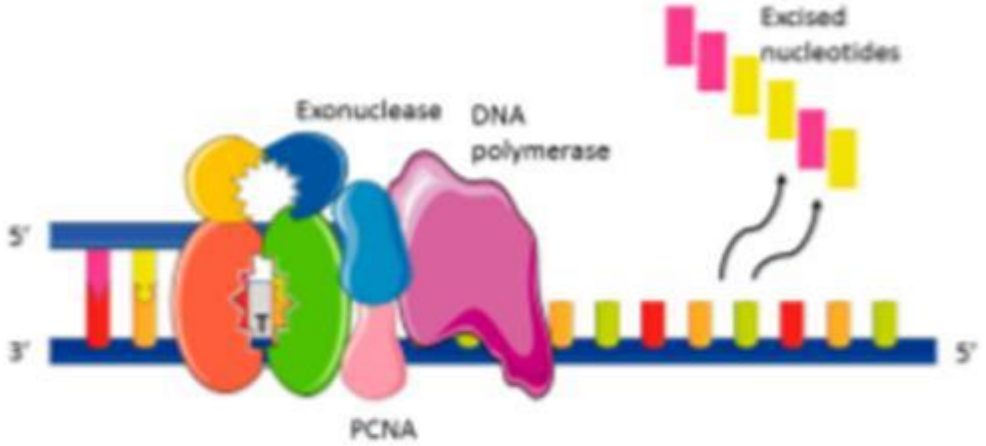
**Microsatellite instability: an update**

Hiroyuki Yamamoto · Kohzoh Imai




(A)

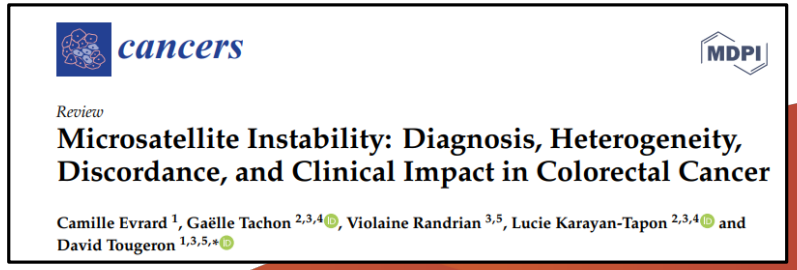

(B)

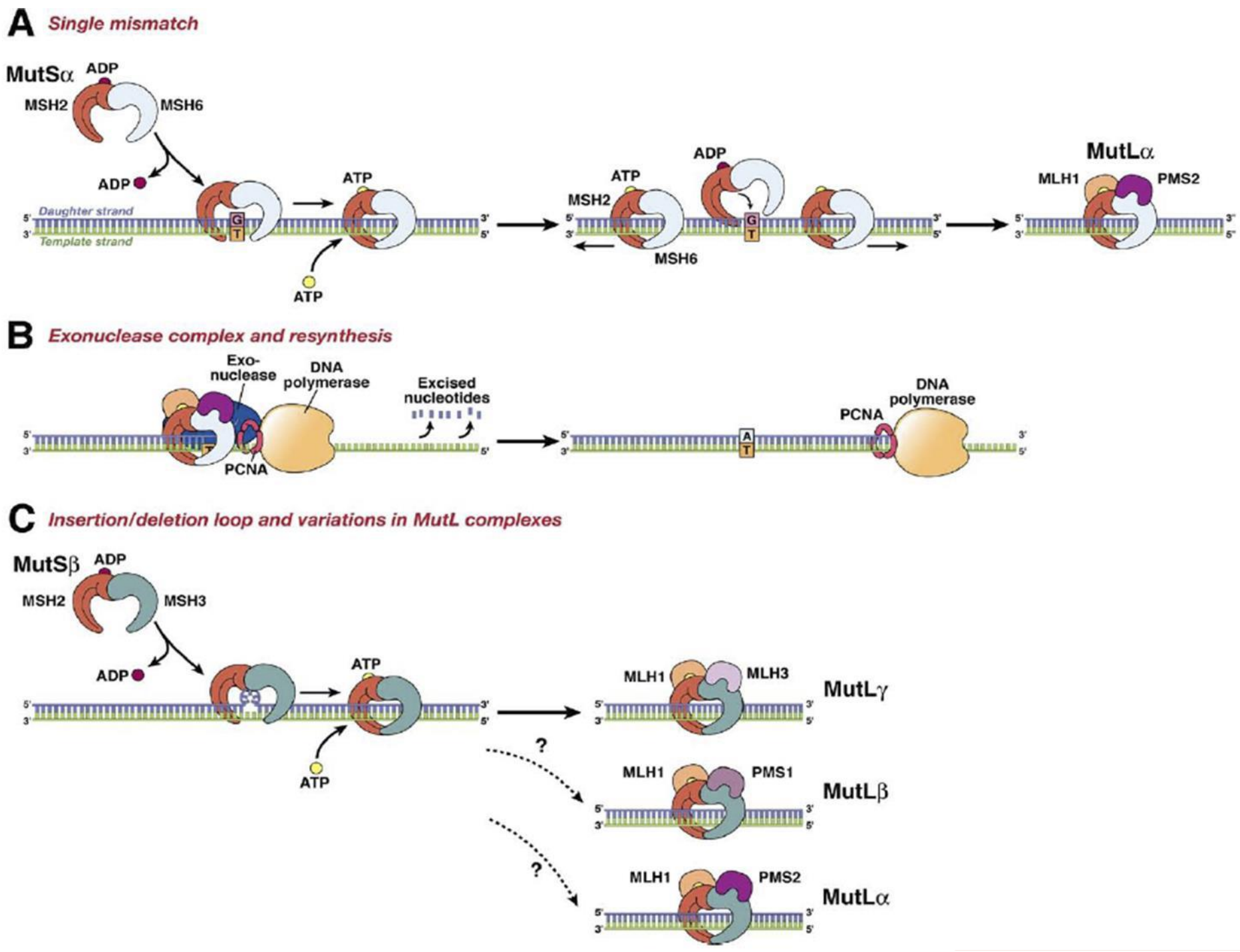



(C)


(D)

A) mismatch recognition bij MutSα complex (MSH2/MSH6)
B) Recruitment of additional MMR factors (MutLα complex, MLH1/PMS2)
C) Interaction with exonuclease to excise mismatch
D) Resynthesize DNA strand




A
Single mismatch
MutSα
ADP
MSH2
MSH6
ADP
Daughter strand
Template strand
G
T
ATP
ATP
ADP
ATP
MSH2
MSH6
MutLα
MLH1
PMS2
B
Exonuclease complex and resynthesis
Exo-nuclease
DNA polymerase
Excised nucleotides
PCNA
DNA polymerase
PCNA
A
T
C
Insertion/deletion loop and variations in MutL complexes
MutSβ
ADP
MSH2
MSH3
ADP
ATP
ATP
MLH1
MLH3
MutLγ
?
MLH1
PMS1
MutLβ
?
MLH1
PMS2
MutLα

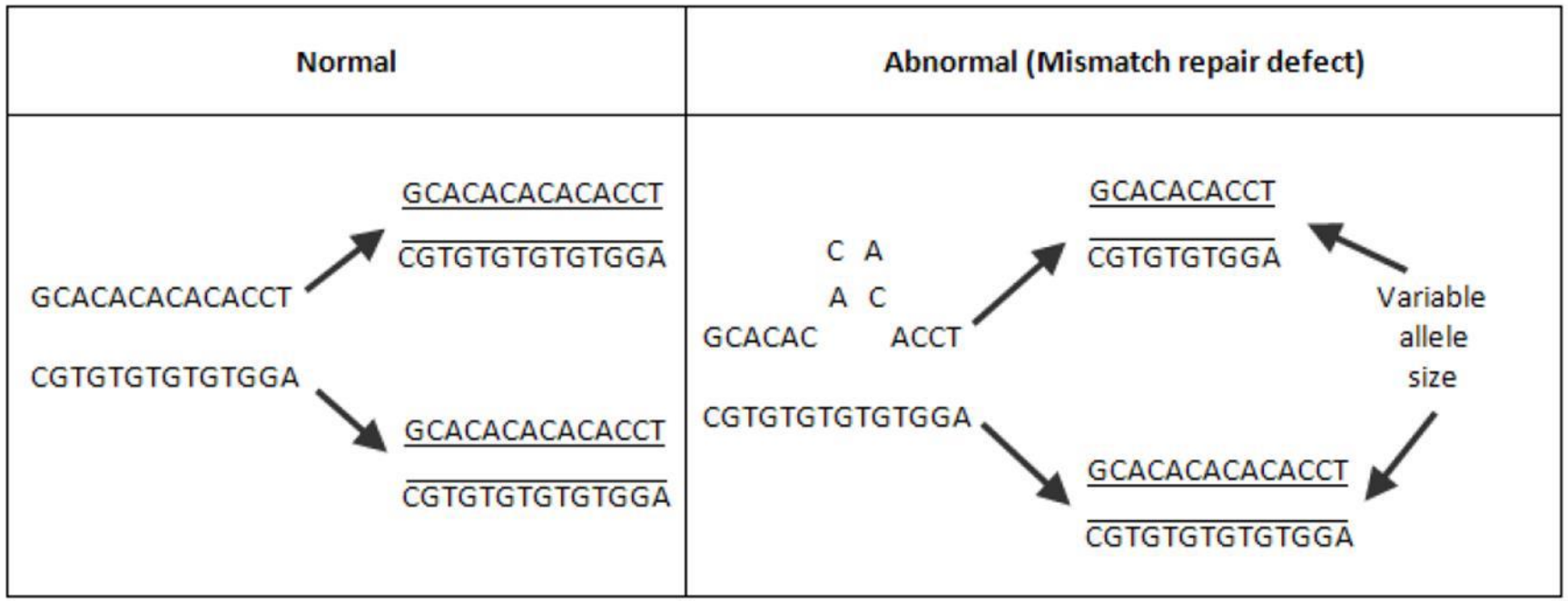
# Defective mismatch repair


Deficient mismatch repair = dMMR

Proficient mismatch repair = pMMR

Microsatellites: particularly prone to replication errors in the case of deficiency of the MMR system (dMMR)

## Microsatellite Replication




Title: Lynch Syndrome *GeneReview*: Microsatellite Instability (MSI) Testing
Authors: Kohlmann W, Gruber S
Date: February 2018

# Microsatellite instability

dMMR: replication errors are not restored

- accumulation of mutations throughout the genome: ‘hypermutated’.

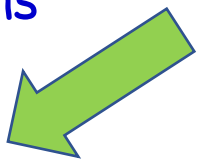
  -> increasing risk for development of neoplasia

- Microsatellites show increasing variation in length (usually shorter, sometimes longer) = microsatellite instability (MSI)

**MSI = a phenotypical feature of dMMR**

# Causes of dMMR/MSI

Mecanisms of alterations in human tumors



| Oncogenes | Tumor suppressor genes |
|---|---|
| • Activating mutations | • Inacativating mutations |
| • Gene amplifications | • Deletions (+/- larges) |
| • Translocations | • Epigenetic alterations |
| • Insertions (virus, ALU, HERV…) | • Insertions (virus, ALU, HERV…) |

## 1. Mutation in one of the MMR genes

a) Germline mutation = Lynch syndrome

b) Somatic/sporadic

## 2. Inactivation of an MMR gene

Usually by silencing of MLH1 by hypermethylation of the gene promoter

Usually somatic event, rarely constitutional

A defect in MMR is NOT manifested until BOTH alleles of an MMR gene are inactivated. **A cell develops a DNA repair defect only when its second copy of the gene also becomes non-functional** (Knudson's two-hit hypothesis) as a result of a random mutation (somatic mutation of the second allele of the same MMR gene).

# Lynch syndrome

(originally termed ‘hereditary non-polyposis colorectal cancer’ = HNPCC)

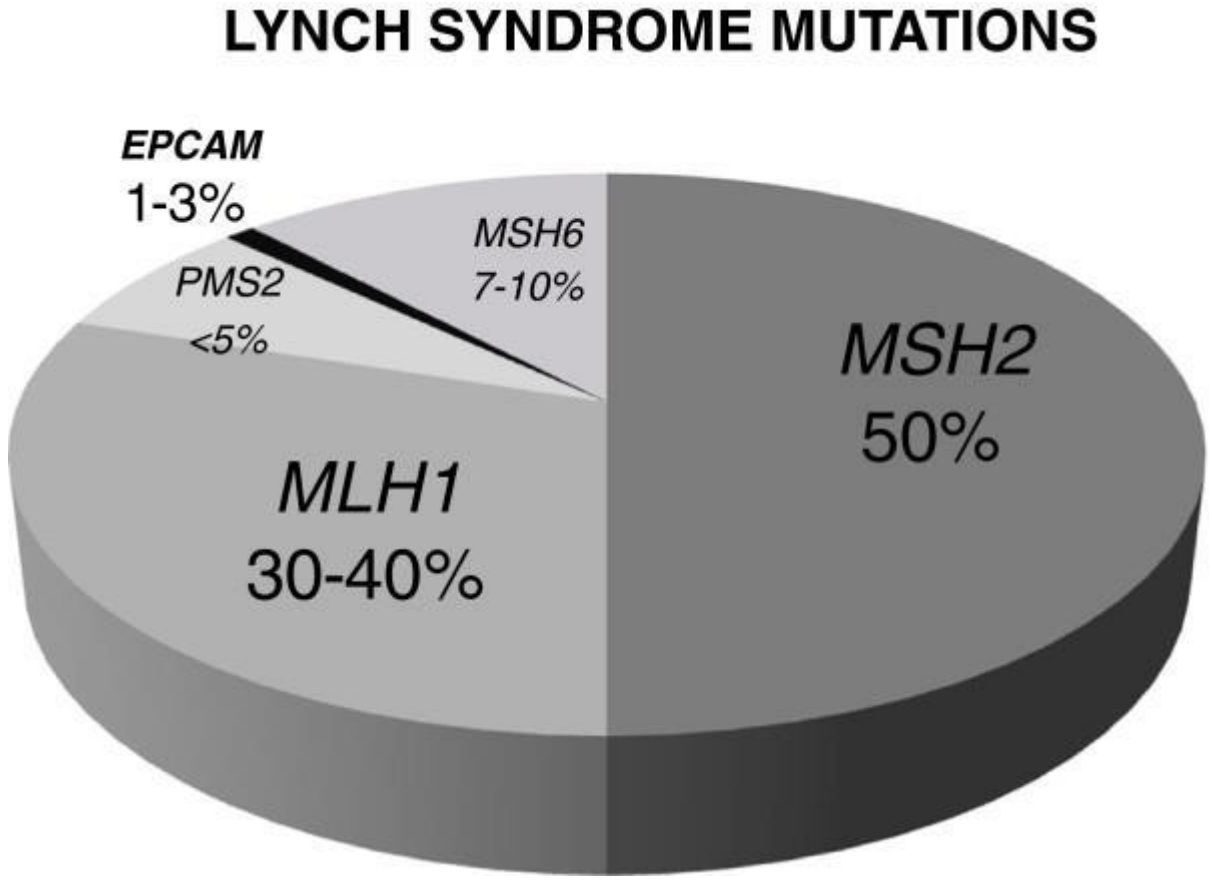
- Increased risk for developping colorectal cancer and endometrial cancer
- also tumors of stomach, duodenum, pancreas, biliary tract, ureter/pyelum, ovary, sebaceous glands and brain
- Autosomal dominant

**Table 1** Neoplasms associated with Lynch syndrome[4 13 30]

| Cancer type | Notes |
|---|---|
| Gastrointestinal | |
| Colorectal carcinoma (CRC)* | Accounts for 3%–5% of all CRC |
| Gastric adenocarcinoma | |
| Small intestinal adenocarcinoma | |
| Pancreatic adenocarcinoma | |
| Cholangiocarcinoma | |
| Gynaecological | |
| Endometrial carcinoma* | Accounts for 2%–3% of all endometrial cancers |
| Ovarian carcinoma | |
| Other sites | |
| Urinary tract carcinoma (transitional cell) | |
| Prostatic carcinoma | |
| Cutaneous sebaceous tumours† | Muir-Torre syndrome |
| Glioblastoma | |
| Adrenocortical carcinoma | |
| Germ cell tumours | |
| Mesothelioma | |
| Melanoma | |
| Sarcoma | |

*CRC and endometrial carcinoma are the two neoplasms most commonly associated with Lynch syndrome.
†There is also an increased risk of development of keratoacanthoma.

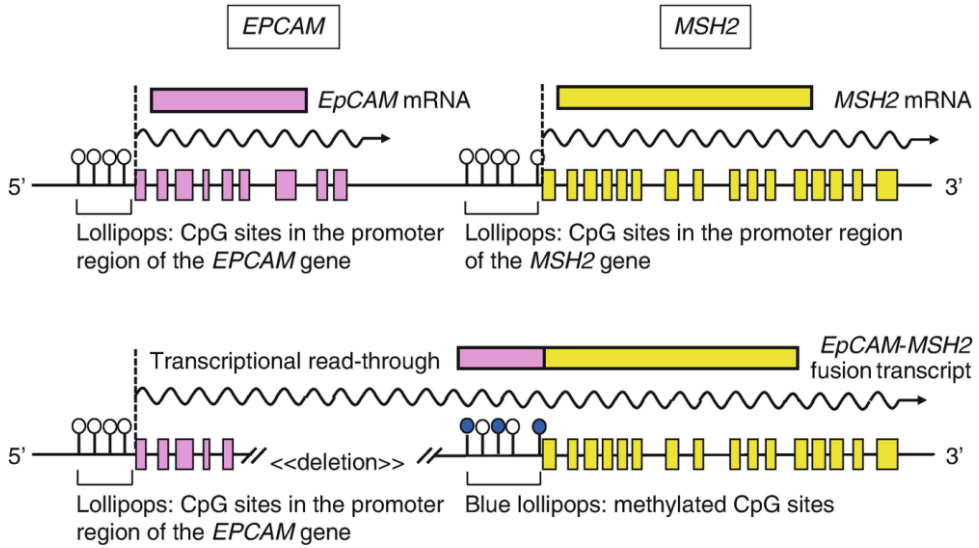
Bateman AC. J Clin Pathol 2021;74:264–268

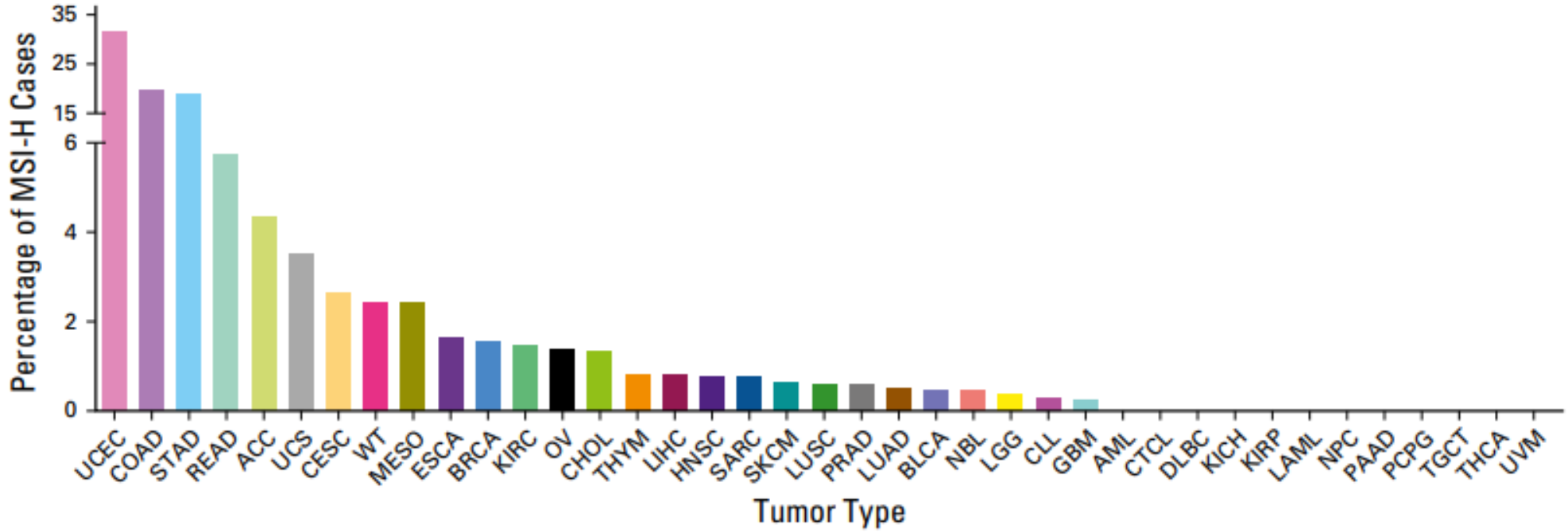



Tutlewska et al. Hereditary Cancer in Clinical Practice 2013, 11:9

## EPCAM MUTATIONS AND dMMR

IHC loss of MSH-2 expression but no germline mutation in MSH-2.
germline mutation at the 3’ end of the EPCAM gene, which results in hypermethylation of the MSH-2 promoter sequence and inactivation of MSH-2.






ACC, adrenocortical carcinoma; AML, pediatric acute myeloid leukemia ; BLCA, bladder carcinoma; BRCA, breast carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma;CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; CTCL, cutaneous T-cell lymphoma; DLBC, diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia (TCGA); LGG, lower-grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; NBL, pediatric neuroblastoma; NPC, nasopharyngeal carcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TCGT, testicular germ cell tumor; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; WT, Wilms tumor

## Landscape of Microsatellite Instability Across 39 Cancer Types

Crossref DOI link: https://doi.org/10.1200/PO.17.00073

| Tumor | %MSI-H |
|---|---|
| Uterine corpus endometrial carcinoma | 28.30%-29.75% |
| Stomach adenocarcinoma | 18.71%-21.92% |
| Colon adenocarcinoma | 16.61%-19.05% |
| Rectal adenocarcinoma | 3.13%-5.26% |
| Ovarian cancer | 1.59%-3.21% |
| Hepatocellular carcinoma | 0.59%-2.93% |
| Renal clear cell carcinoma | 1.06%-2.15% |
| Breast cancer | 0%-1.74% |
| Head and neck squamous cell carcinoma | 0.59%-1.19% |
| Glioblastoma multiforme | 0.38%-1.27% |
| Lung squamous cell carcinoma | 0.45%-1.23% |
| Prostate adenocarcinoma | 0.6%-0.65% |
| Urothelial bladder cancer | 0.4%-0.54% |
| Lung adenocarcinoma | 0.21%-0.63% |
| Papillary kidney carcinoma | 0%-0.7% |
| Low-grade glioma | 0.19%-0.58% |
| Cutaneous melanoma | 0% |
| Thyroid cancer | 0% |

**NOTE**: Prevalence of MSI-high (MSI-H) below 1% is highlighted in yellow, between 1% and 10% in blue, and more than 10% in violet.

DOI: 10.1158/2159-8290.

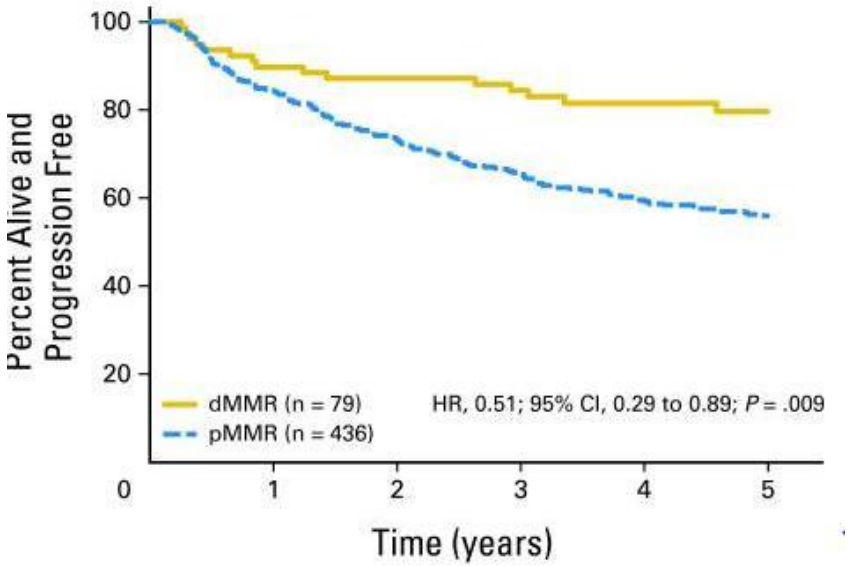
# QUESTION: BIOMARKER?

- PROGNOSTIC // PREDICTIF

- PROGNOSTIC BIOMARKER
  - Prediction of survival (PFS/OS)
  - Risk Factor but not prediction for a treatment
- PREDICTIVE BIOMARKER
  - Prediction of sensibility/resistance to a treatment

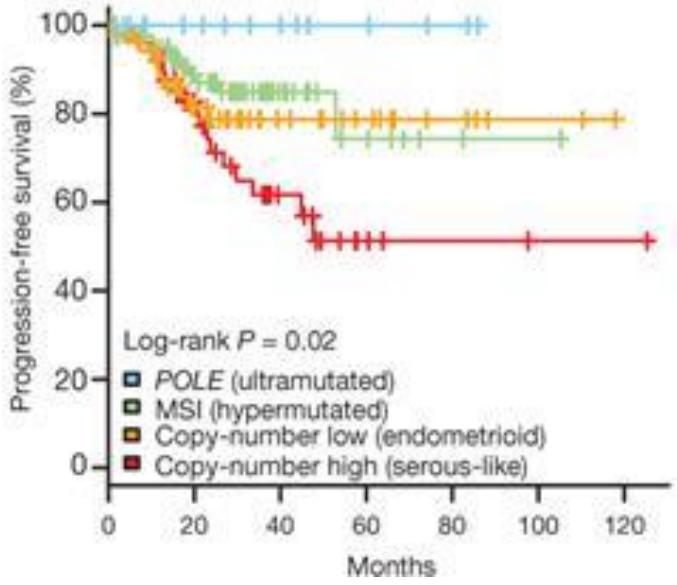
# Reasons to look for dMMR/MSI

- Identify patients with Lynch syndrome
  - (secundary) prevention of malignancy
  - Screen family members
- Prognostic marker:  CRC




J Clin Oncol. Jul 10, 2010; 28(20): 3219–3226.

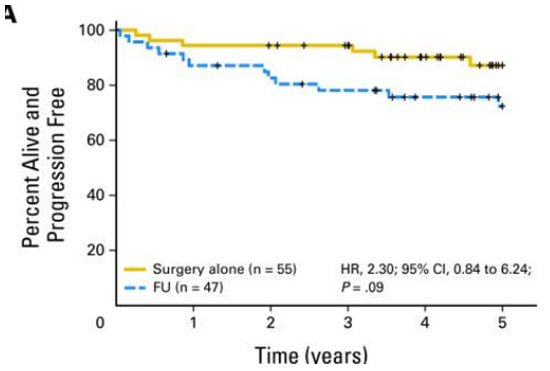
 endometrial cancer




G Getz et al. Nature **497**, 67-73 (2013)

# Reasons to look for dMMR/MSI

- predictive marker:
  - CRC: lack of benefit from 5-FU when MSI




J Clin Oncol. Jul 10, 2010; 28(20): 3219–3226.

  - endometrial cancer showing dMMR: may show an improved response to adjuvant radiotherapy
  - link between MSI and response to immunotherapy
  - Pembrolizumab (Keytruda ®): FDA approval for metastazised, non-resectable MSI-H and/or TMB-high solid tumors in adults and children with progression after previous line(s) of therapy without other therapeutic options, irrespective of primary origin.

    = first tumor-agnostic FDA approval

# Immunotherapy and MSI



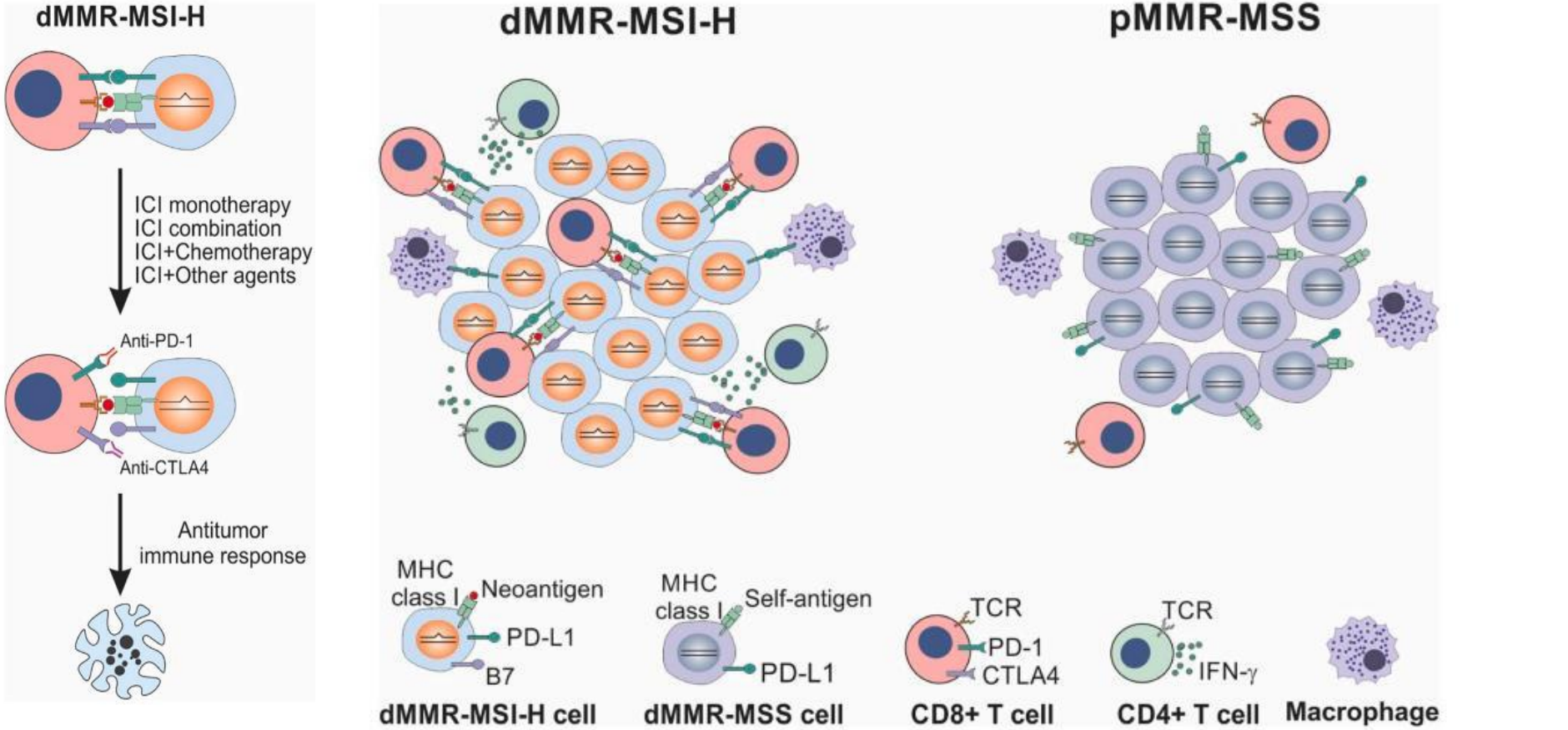



**Fig. 2.** Comparison between dMMR/MSI-H and pMMR/MSS colorectal cancers. dMMR/MSI-H CRCs have a high mutational burden and persistent renewal of neoantigens, which are favorable for immune surveillance. Neoantigens are presented by MHC class I molecules to attract CD8+ T cells to the tumor microenvironment via T cell receptor (TCR) engagement with MHC class I molecules. However, interactions between immune checkpoint proteins expressed on the surface of T cells and their ligands on antigen presenting cells, such as PD-1/PD-L1 and CTLA-4/B7 interactions, suppress antitumor immune response. In contrast, pMMR/MSS CRCs have a low mutational burden and lack immune surveillance.

Immunotherapy (anti PD1, anti PDL1, anti CTLA4) is based on boosting an antitumour immune response by patients' own immune systems, usually by blocking molecular mechanisms that tumours use to evade host attack.

dMMR leads to an increased mutational burden and the generation of novel peptide sequences by cancer cells, representing an enhanced range of epitopes that are potentially recognisable by the host immune system. Therefore, tumours with dMMR may respond more favourably to immunotherapy than those lacking this feature.

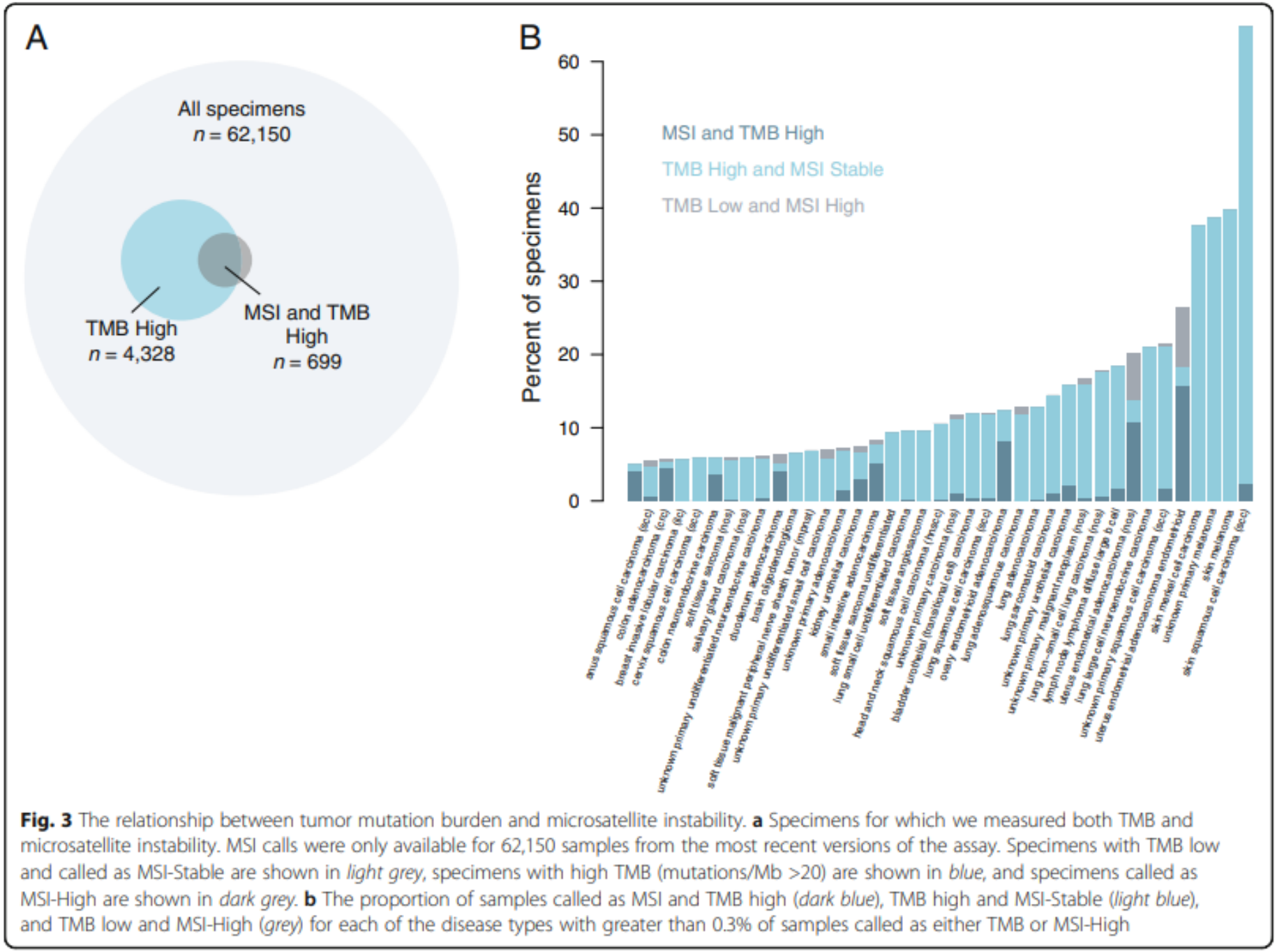



**Fig. 3** The relationship between tumor mutation burden and microsatellite instability. **a** Specimens for which we measured both TMB and microsatellite instability. MSI calls were only available for 62,150 samples from the most recent versions of the assay. Specimens with TMB low and called as MSI-Stable are shown in *light grey*, specimens with high TMB (mutations/Mb >20) are shown in *blue*, and specimens called as MSI-High are shown in *dark grey*. **b** The proportion of samples called as MSI and TMB high (*dark blue*), TMB high and MSI-Stable (*light blue*), and TMB low and MSI-High (*grey*) for each of the disease types with greater than 0.3% of samples called as either TMB or MSI-High

Chalmers et al. Genome Medicine (2017) 9:34

## MSI/dMMR and high tumoral mutational burden (TMB-high)

**overall:**
83-97% of MSI-H tumors are TMB-H
16% of TMB-H tumors are MSI-H

**in GI tract cancers**: MSI-H and TMB-H nearly always co-occur

**skin cancer** (SCC and melanoma) **and lung cancer**: high prevalence of TMB-H but MSI-H very uncommon

**causes of TMB-H**:
exogeneous agents: smoking, UV
dMMR
POLE mutation  ultramutated

# How to look for MSI/dMMR?

1) Immunohistochemistry
2) Molecular techniques:
    a) PCR
    b) Idylla
    c) NGS

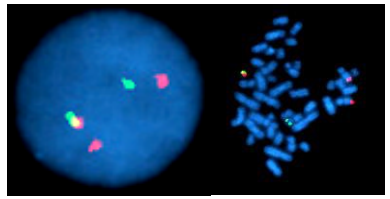
# MOLECULAR ANOMALIES DETECTIONS (Biomarkers)

| DNA | RNA | PROTEINE |
| --- | --- | --- |
| Amplifications | RNA quantity | Proteine Quantity |
| Translocations | Alternatif Transcrits | Proteine Activity |
| Mutations | | |
| CGH | RT-PCR | Wester-Blot |
| FISH | Transcriptional chip | Immunohistochemestry |
| DNA seq | RNA seq | Enzymatic Activity |

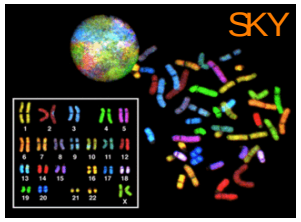
# Analytic Step

- Wide range of technics ever-evolving

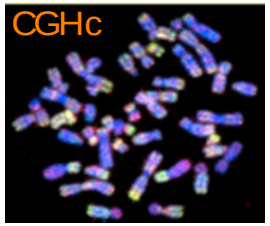
FISH



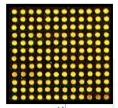
SKY

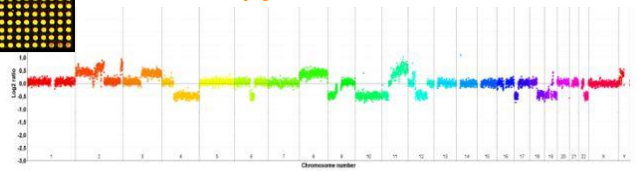


CGHc

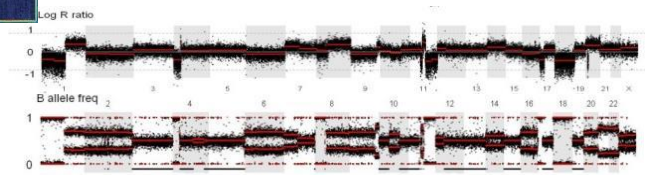


aCGH copy Number

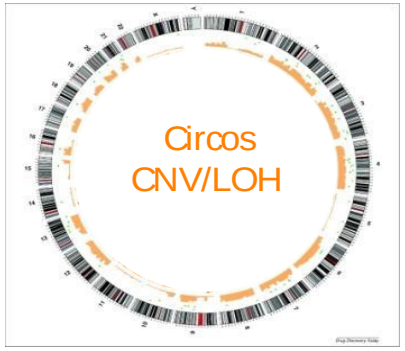




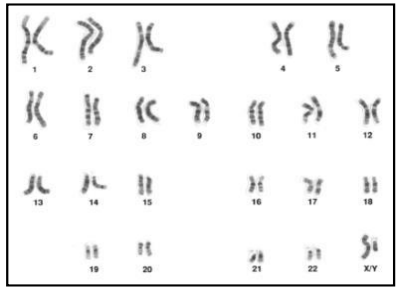
aCGH CNV LOH




Circos CNV/LOH




Banding



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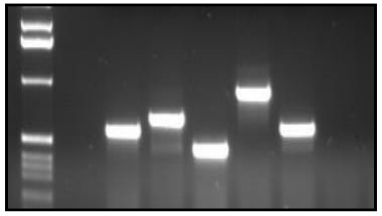
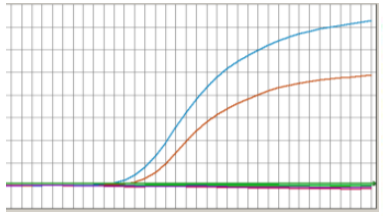
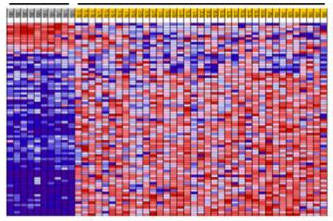
PCR



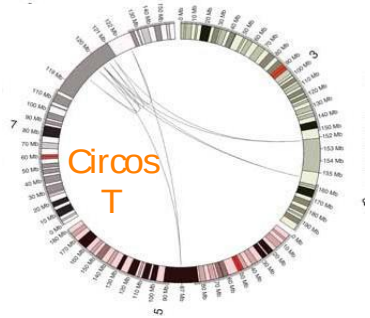
RT QPCR



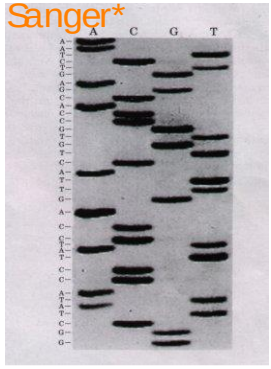
Expression Assays



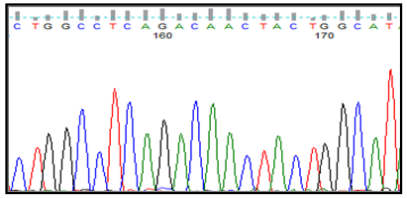
Circos T



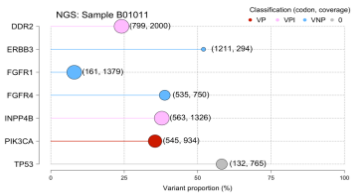

Sanger*



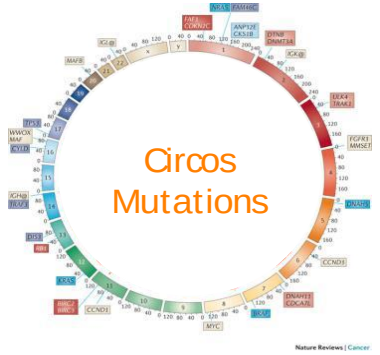
Sanger



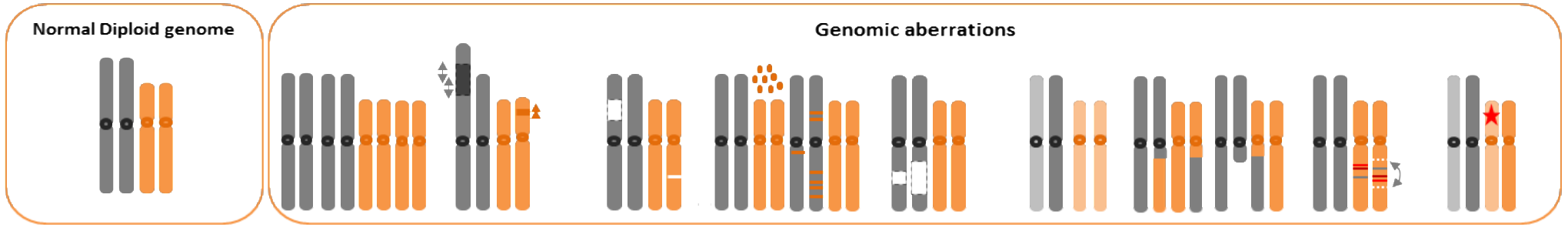
NGS Panel




Circos Mutations



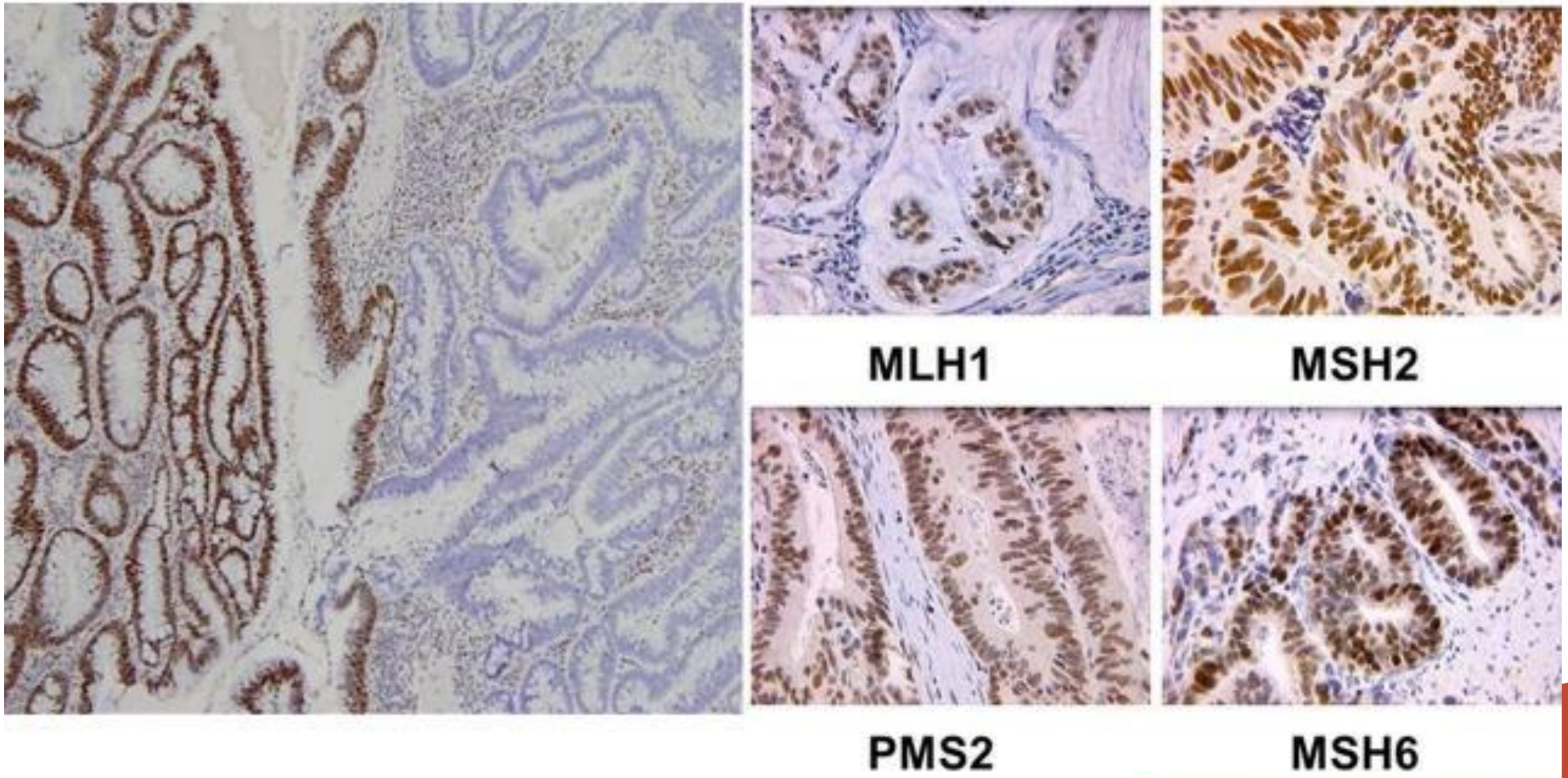
# MOLECULAR ANOMALIES DETECTIONS (Biomarkers)



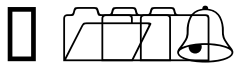

| Techniques | Nbre de marqueurs explorés | Polyploïdie | Gains | | Pertes | | Amplifications | Délétion Homozygotes | Isodisomies | Translocations | Inversions | Mutations Ponctuelles |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Larges | Focaux | Larges | Focales | | | | | | |
| Caryotype | N | + | + | | + | | + | + | | + | +/- | |
| FISH | 1 | + | + | | + | | + | + | | + | +/- | |
| PCR | 1 | | | + | | + | + | + | | + | + | |
| Séq. Sanger | 1 | | | +/- | | +/- | | + | + | + | + | + |
| QPCR | 1 / 3 | | | + | | + | + | + | | | | |
| RT-QPCR | 1 / 3 | | | + | | + | + | + | | + | + | |
| MLPA | N | | + | + | + | + | + | + | | | | |
| aCGH | N | | + | + | + | + | + | + | | * | | |
| aCGH/LOH | N | + | + | + | + | + | + | + | + | * | | |
| Panel NGS | 10 / 50 | | | + | | + | + | + | + | | + | + |
| RNAseq | N | | + | + | + | + | + | + | + | + | + | +/- |
| WES | N | + | + | + | + | + | + | + | + | +/- | +/- | + |

For each molecular marker a proper tool

# Immunohistochemistry for MSH2, MSH6, MLH1 and PMS2



# Pitfalls/remarks

- Sometimes retained expression of non-functional protein: sensitivity
  
- Heterogenous, weak expression (influence of pre-analytics/fixation)
- No consensus on cut-off (5-10% staining nuclei = preserved expression)
- Diminished expression after neo-adjuvant therapy, especially for MSH6
- Some advocate testing only for MSH6 and PMS2
- 2/3 adenomas in Lynch syndroom has disturbed IHC-profile
- Test is usually performed for predictive value, but sometimes a hereditary condition is found

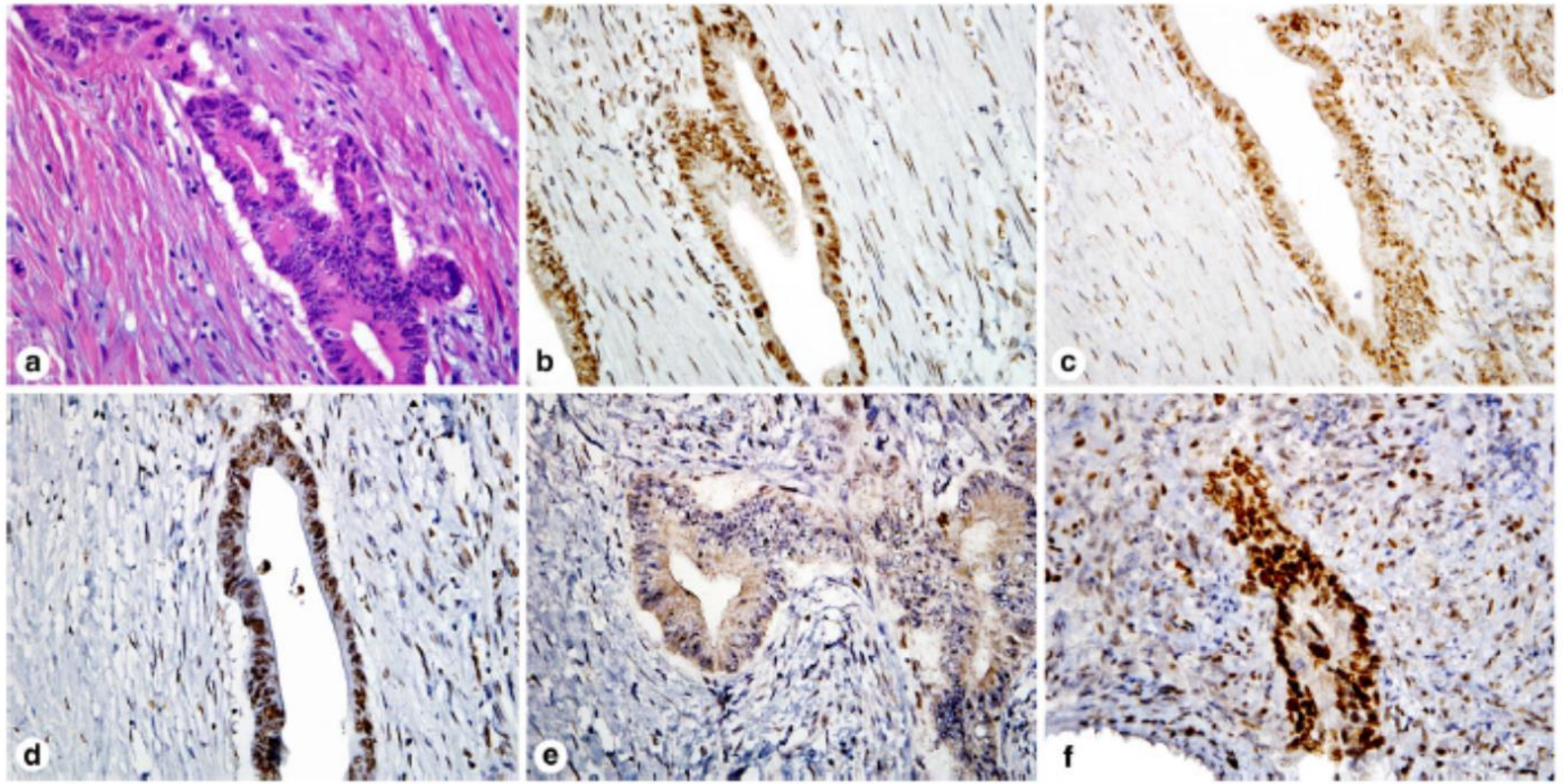
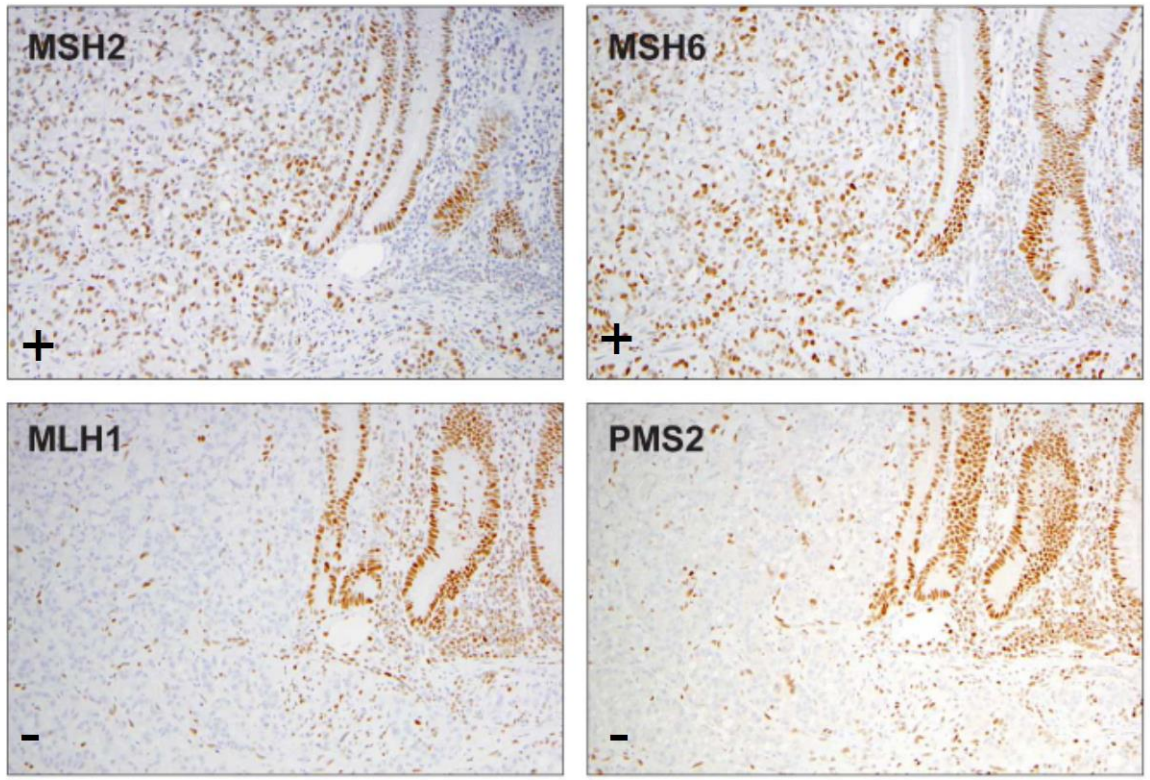



**Fig. 4** Immunohistochemistry for mismatch repair proteins in a patient that received neoadjuvant chemotherapy for rectal adenocarcinoma. H&E stain of the tumor in the resection specimen (**a**). The resection specimen showed intact MLH1 (**b**), PMS2 (**c**), and MSH2 (**d**) staining. MSH6 staining of the resection specimen showed focal nucleolar staining (**e**) that was originally interpreted as absent, but subsequent molecular sequencing did not reveal a mutation. The pretreatment tumor biopsy was stained for MSH6 and showed intact staining (**f**)

# Evaluation of MMR status by IHC for MMR protein expression




| MLH1 | PMS2 | MSH2 | MSH6 | In CRC, pattern suggests… |
|---|---|---|---|---|
| + | + | + | + | Intact MMR pathway, rare germline point mutations or other gene mutations |
| - | - | + | + | Somatic *MLH1* promoter methylation or, rarely, *MLH1* germline mutation |
| + | + | - | - | *MSH2* germline mutation |
| + | - | + | + | *PMS2* germline mutation |
| + | + | + | - | *MSH6* germline mutation |

Lack of expression of one or more MMR proteins is a very good surrogate test for MSI

Nowak JA, Hornick JL. Surg Path Clin 2016;9(3):427-439.

# Reflextesting by immunohistochemistry

standard of care for:

- All new diagnoses of colorectal adenocarcinoma
- All new diagnoses of endometrial adenocarcinoma

Recommended in:

- Sebaceous lesions (Muir-Torre)
- Gastric adenocarcinoma (classification)

## Table 2. Patterns of Mismatch Repair (MMR) Deficiency by Immunohistochemistry

| Protein | | | | | | |
|---|---|---|---|---|---|---|
| MLH1 | MSH2 | MSH6 | PMS2 | Interpretation | Inactivated Gene | Microsatellite Status |
| + | + | + | + | Intact MMR | None | MSS |
| − | + | + | − | Deficient MMR | *MLH1*[a] | MSI-H |
| + | − | − | + | Deficient MMR | *MSH2*[b] | MSI-H |
| + | + | − | + | Deficient MMR | *MSH6* | MSI-H |
| + | + | + | − | Deficient MMR | *PMS2* | MSI-H |

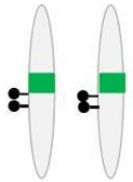
Abbreviations: +, intact/preserved nuclear staining; −, loss of nuclear staining; MSI-H, microsatellite instability–high; MSS, microsatellite stable.

[a] MLH1 can be inactivated because of sporadic, MLH1-promoter methylation (usually associated with *BRAF* V600E mutation) or germline mutation.

[b] Lack of expression of MSH2 and MSH6 is usually due to a germline mutation in *MSH2*, although it can also be caused by transcriptional read through of the neighboring *EPCAM* gene, which inactivates MSH2.

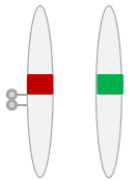
# Loss of MLH1/PMS2 on IHC

**Somatic** defect

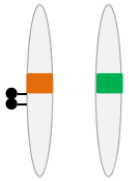


CpG island methylator phenotype
(CIMP)

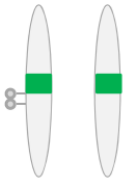
**Germline** defect



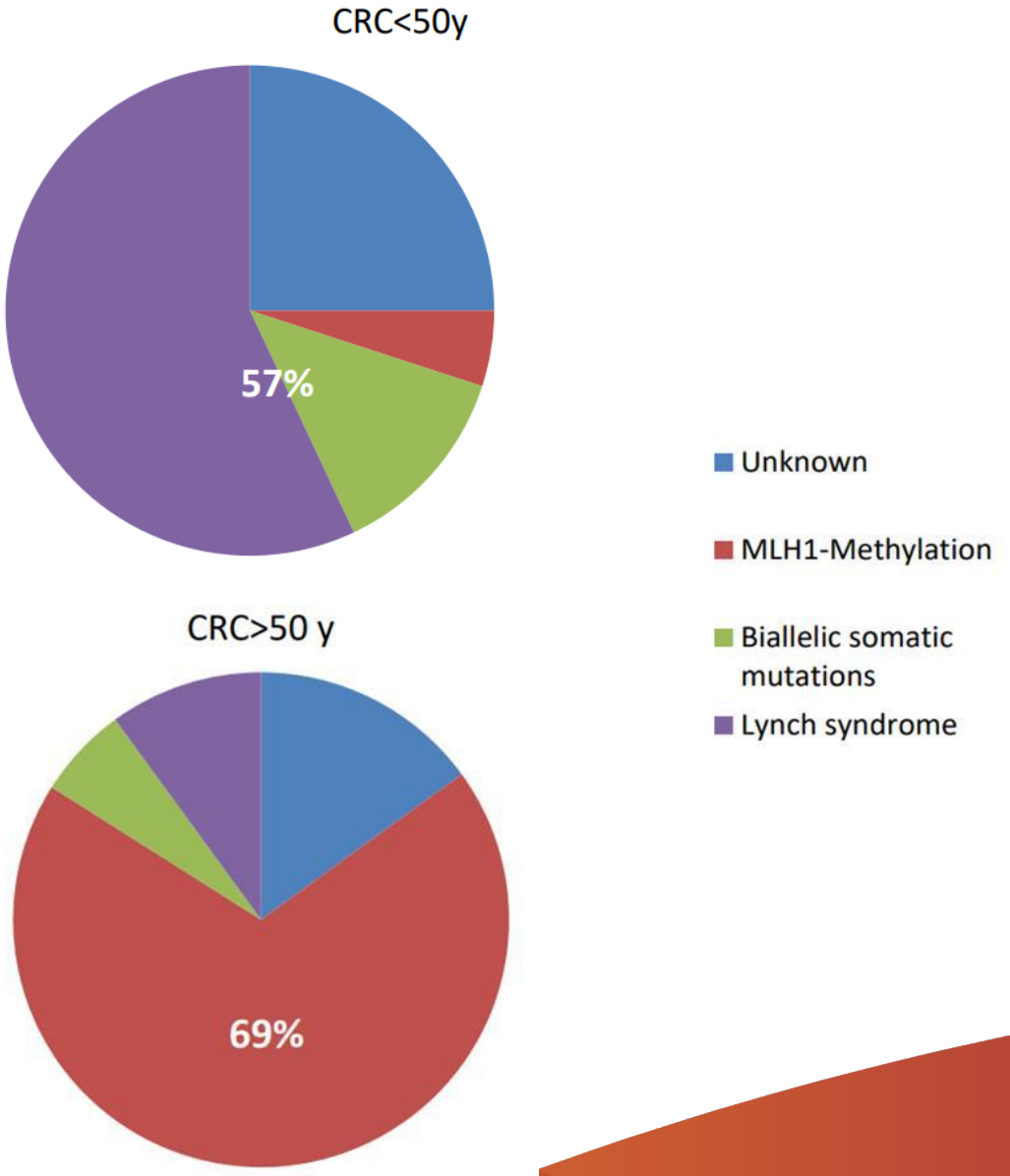
Lynch pathogenic MLH1 variant/ Class 5 variant



Constitutional MLH1 epimutation
+ presence of cis-acting MLH1 variants
**The haplotype is inherited in a methylated state**



Lynch suspected MLH1 deficient tumor unsolved



# MLH1 promoter hypermethylation

1. By MLPA-PCR

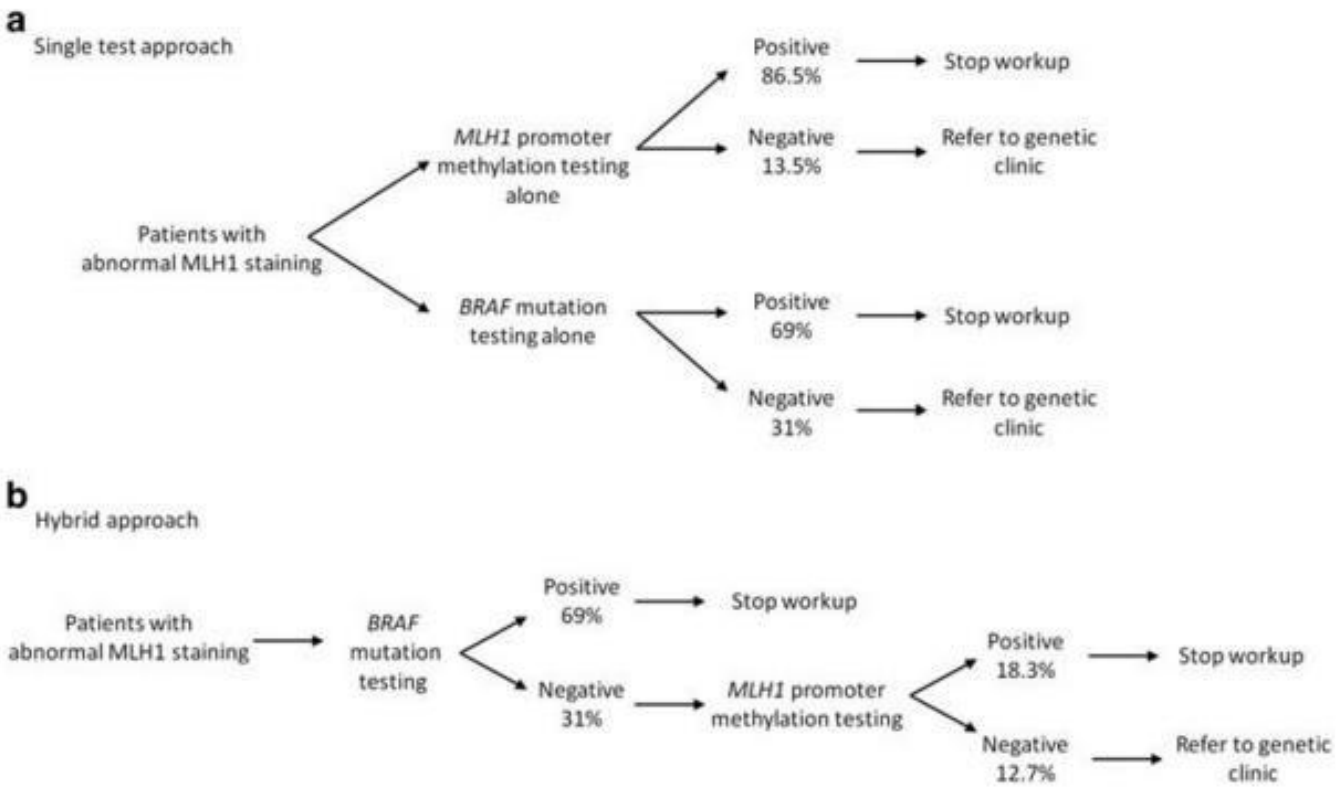
2. BRAF V600E = surrogate marker

MSI-high tumors with absent MLH1 immunostaining:

- positive predictive value of a *BRAF* mutation in predicting *MLH1* promoter methylation = 99%
- negative predictive value of a *BRAF* mutation in predicting *MLH1* promoter methylation = 41%

Remarks:

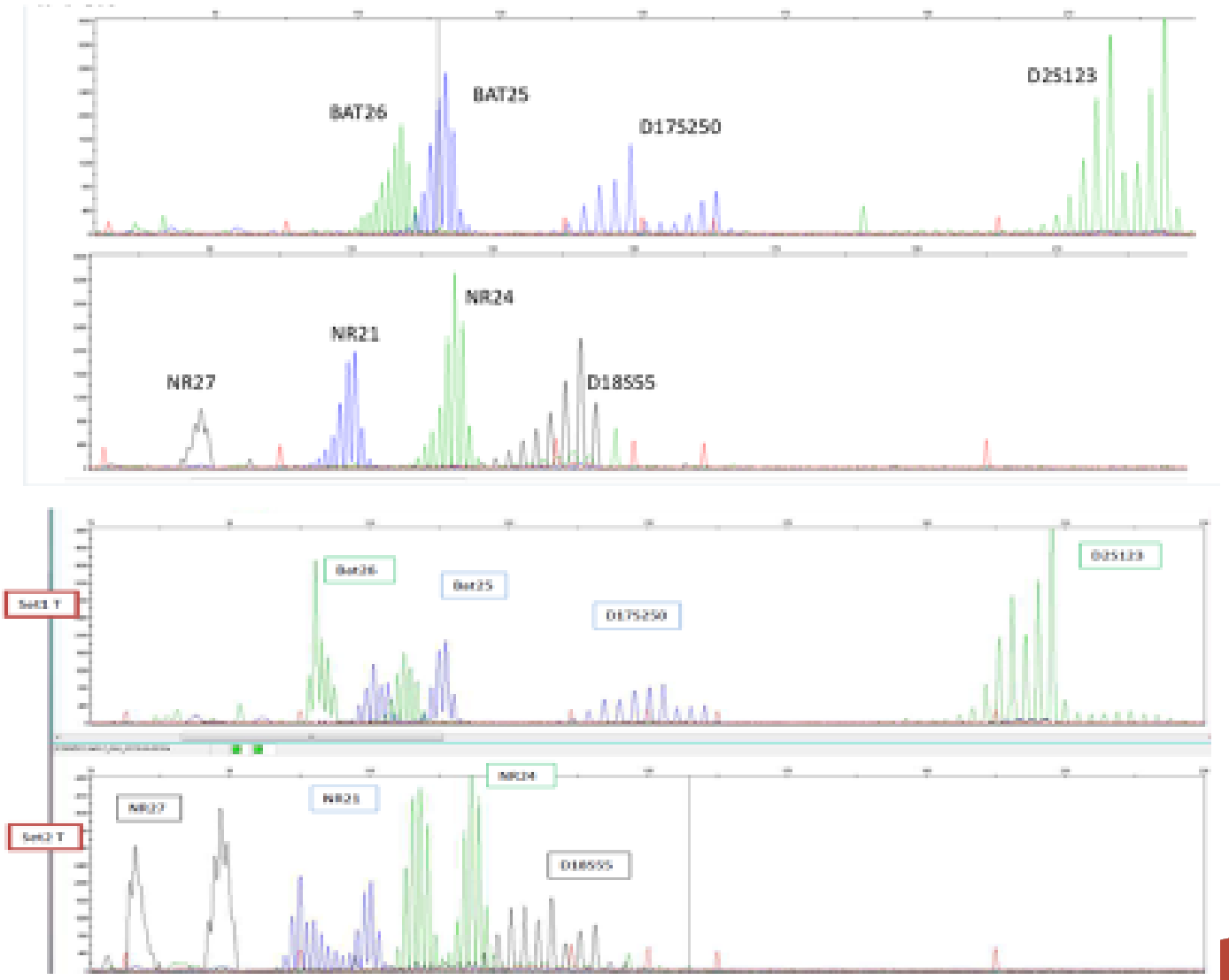
- 1-2% of Lynch cases (dMMR/MSI CRC with germline mutation) carry BRAF mutation
- Constitutional epimutation of MLH1 gene does exist
- BRAF as surrogate for MLH1 promoter methylation status is useless in MSI-H endometrial cancer as they only rarely have BRAF mutations.




Modern Pathology (2017) 30, 440–447

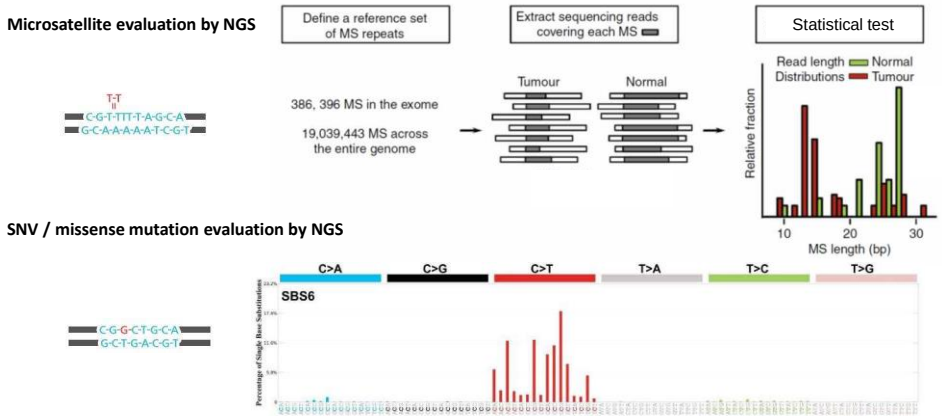
# PCR

- PCR with panel of 5 mononucleotide (BAT-25, BAT-26, NR-21, NR-24, NR-27) and 3 dinucleotide markers (D2S123, D17S250, D18S55)
- MSI-H if 2 or more loci are instable
- Healthy tissue sample is useful
- 3-10% discordance of MSI testing by ihc versus pcr:
  - -Most frequent discordance = loss MSH6 on ihc with MSS result on PCR
  - -wrong interpretation of ihc!

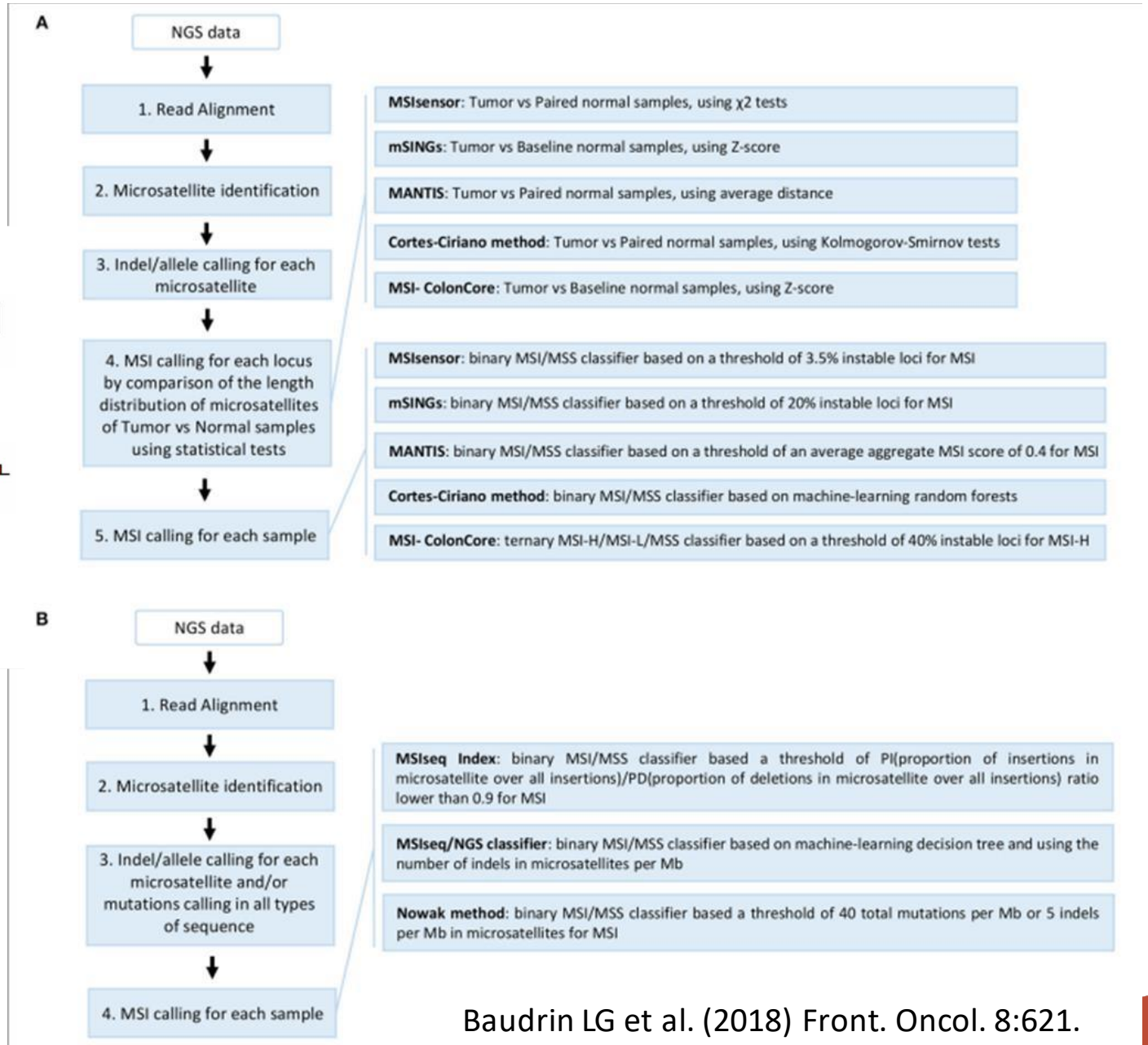


# MSI by NGS

## NGS approaches to detecting MMR deficiency




Cortes-Ciriano I, et al. Nat Comm. 2017;8:15180.
COSMIC Mutational Signatures v3.2 (March 2021)




Baudrin LG et al. (2018) Front. Oncol. 8:621.

**FIGURE 3 |** Overview of the different NGS-based computational methods developed for MSI detection in cancer. **(A)** Methods based on comparison of repeat length distribution of microsatellites including MSIsensor, mSINGs, MANTIS, Cortes-Ciriano method, and MSI-ColonCore. **(B)** Methods based on the total mutation burden in all sequences and/or the indel burden in microsatellites including MSIseq Index, MSIseq/NGS classifier, and Nowak methods. The steps 1–3 can be performed in

# Detection of MSI by NGS

Clinical Chemistry 60:9
1192–1199 (2014)

Molecular Diagnostics and Genetics

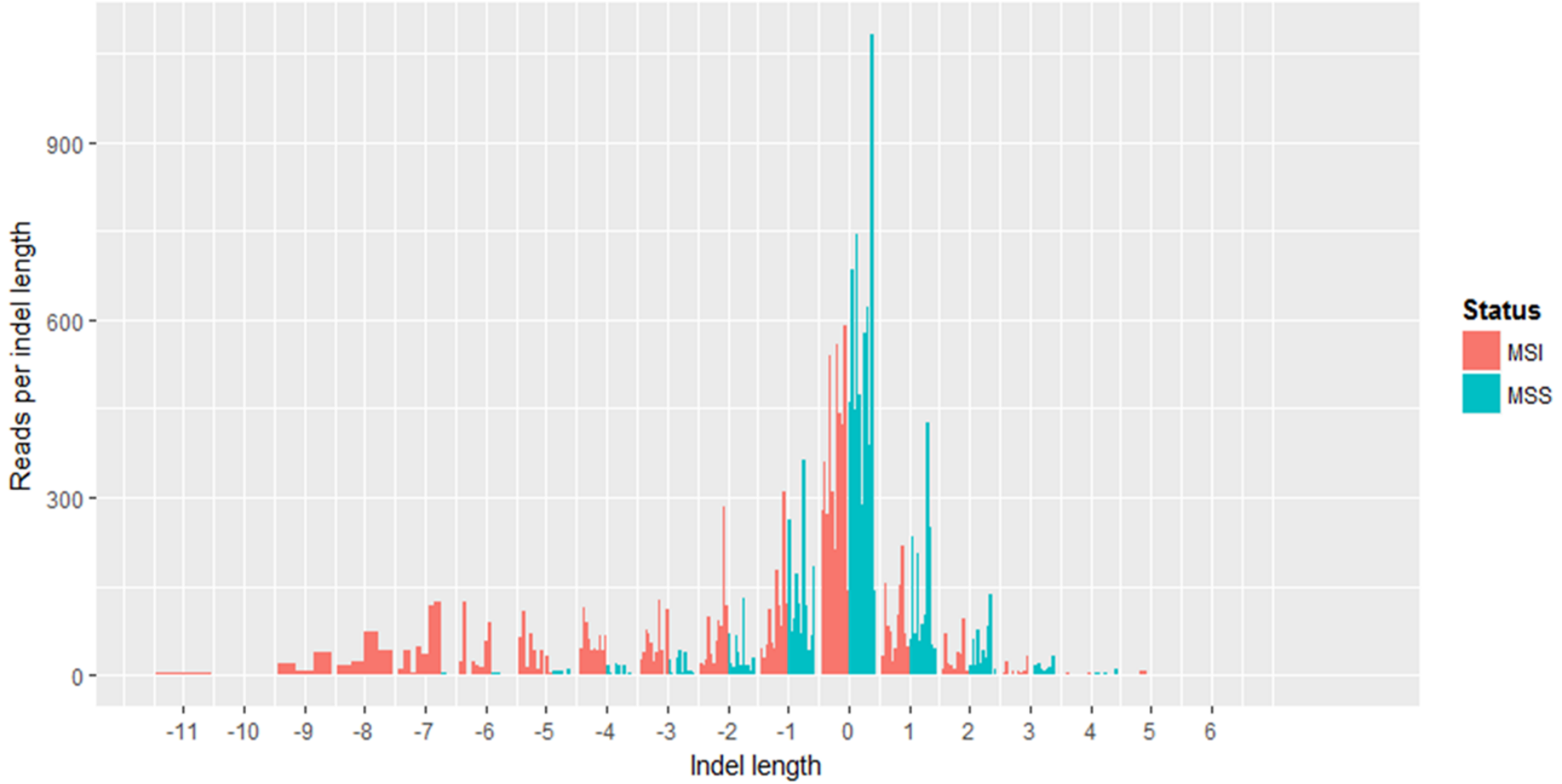
## Microsatellite Instability Detection by Next Generation Sequencing

Stephen J. Salipante,[1] Sheena M. Scroggins,[1] Heather L. Hampel,[2] Emily H. Turner,[1] and Colin C. Pritchard[1*]

- detection of MSI op based on 12 loci (KIF5B, ATM, KMT2A, CDK4, FLT1, GRIN2A, NF1, EML4, MSH6, BCL2L11, SMARCB1, TGFBR2, PBRM1, PTPRD en KDM6A)
- Analysis with mSINGS script (Salipante et al., Clinical Chemistry 2014;60:9,1192-1199).
- Script analyses per locus the number and distribution of indel length peaks in the sample (treshold for peak> 5% reads) and compares with the number of peaks in a reference set (10 pMMR CRC).
- Locus is MSI if more peaks than in the reference.
- sample is MSI if > 20% or > 2/12 unstable loci

# Variation in repeat length of microsatellites

Result of 3 MSI and 3 MSS experimental samples in 11 marker regions



# MSI detection by Idylla (Biocartis)

- Idylla ™ MSI test: full automated PCR on Biocartis Idylla device
- Detection of MSI based on 7 loci (ACVR2A, BTBD7, DID01, MRE11, RYR3, SEC31A en SULF2)
- fast, blackbox
- MSI if 2 or more loci are called unstable.

SAMPLE MSI STATUS: MSI-H

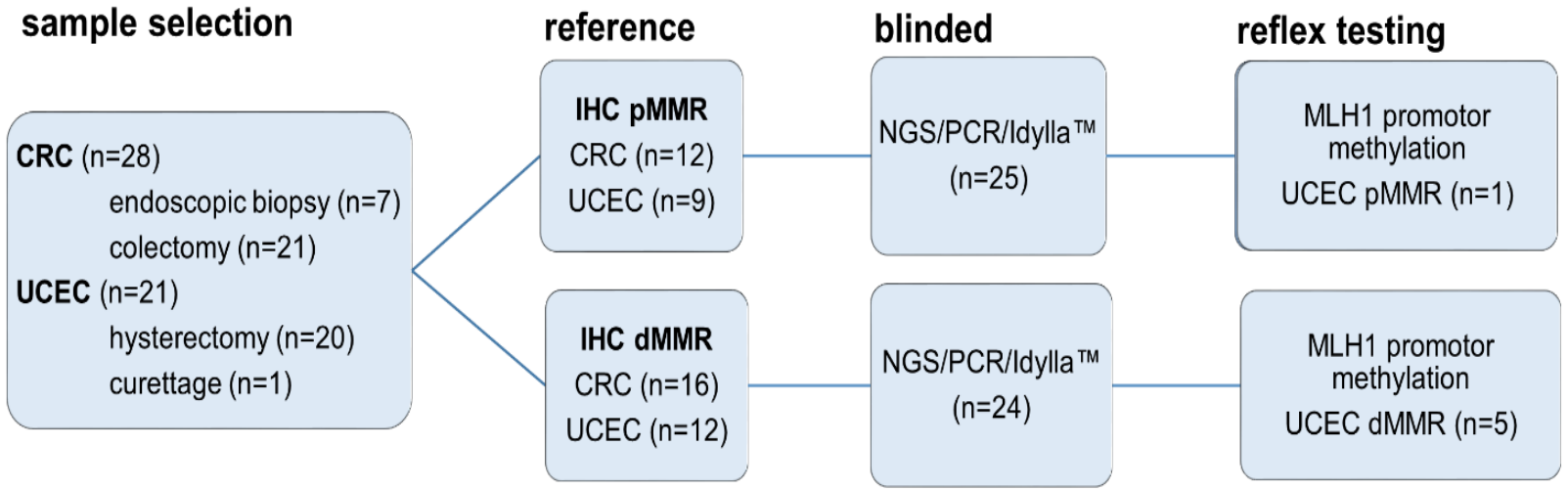
| TARGET | MSI SCORE |
|---|---|
| ACVR2A | 1.00 |
| BTBD7 | 0.19 |
| DIDO1 | 0.96 |
| MRE11 | 0.47 |
| RYR3 | 0.98 |
| SEC31A | 0.57 |
| SULF2 | 0.98 |

# Comparison of microsatellite instability detection by immunohistochemistry and molecular techniques in colorectal and endometrial cancer

Franceska Dedeurwaerdere [1,*,+], Kathleen BM Claes [2,5,7,+], Jo Van Dorpe [3], Isabelle Rottiers, Joni Van der Meulen [2,7], Joke Breyne [4], Koen Swaerts, Geert Martens [4,5,6]

[1]Department of Pathology, AZ Delta General Hospital, Roeselare, Belgium; [2]Center for Medical Genetics, Ghent University Hospital, Gent, Belgium; [3]Department of Pathology, Ghent University, Gent, Belgium; [4]Department of Laboratory Medicine, AZ Delta General Hospital, Roeselare, Belgium,[5]Department of Biomolecular Medicine, Ghent University, Gent, Belgium, [6]VUB Metabolomics Group, Brussels Free University, Brussels, Belgium; [7]Cancer Research Institute Ghent (CRIG), Gent, Belgium

Scientifc Reports | (2021) 11:12880



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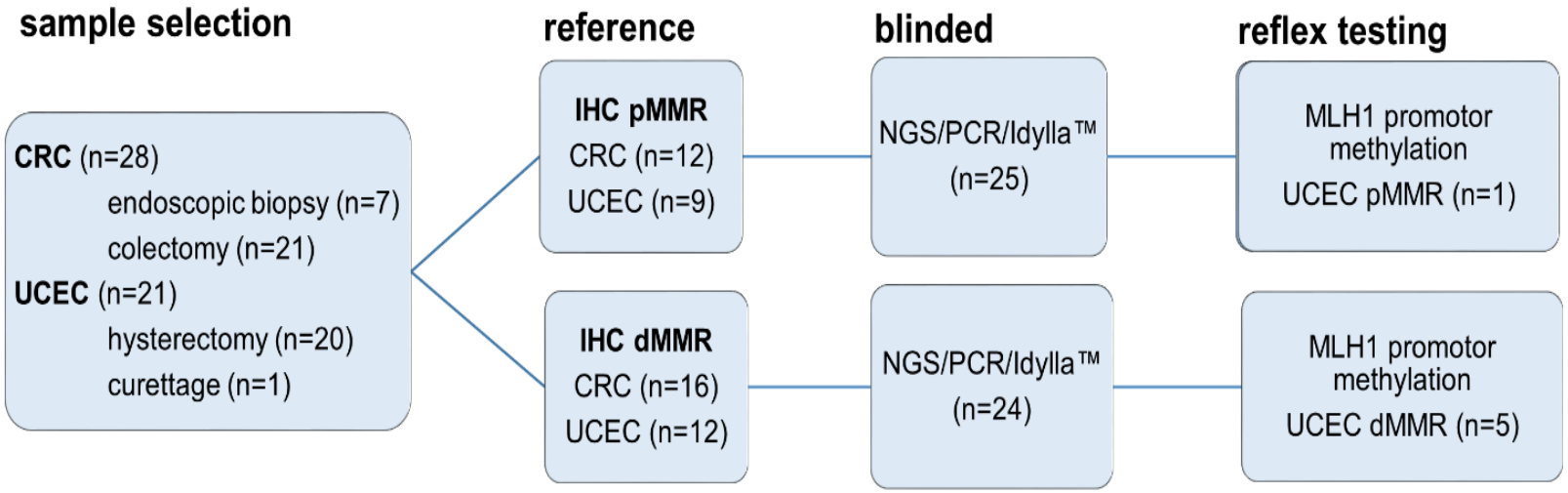
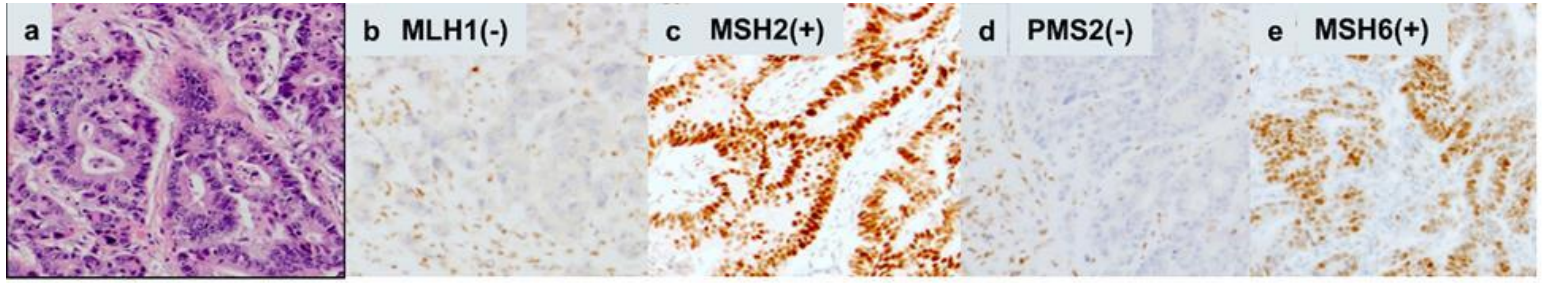
Scientifc Reports | (2021) 11:12880

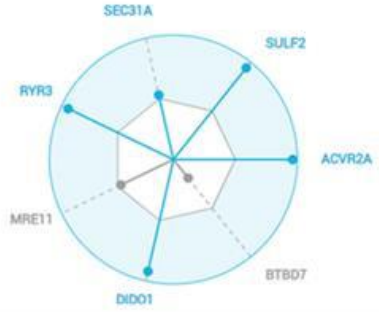




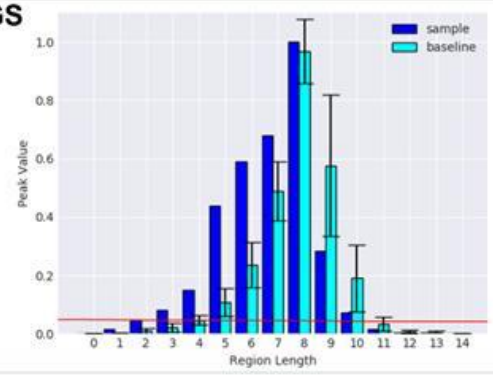

**f Idylla™ MSI assay**

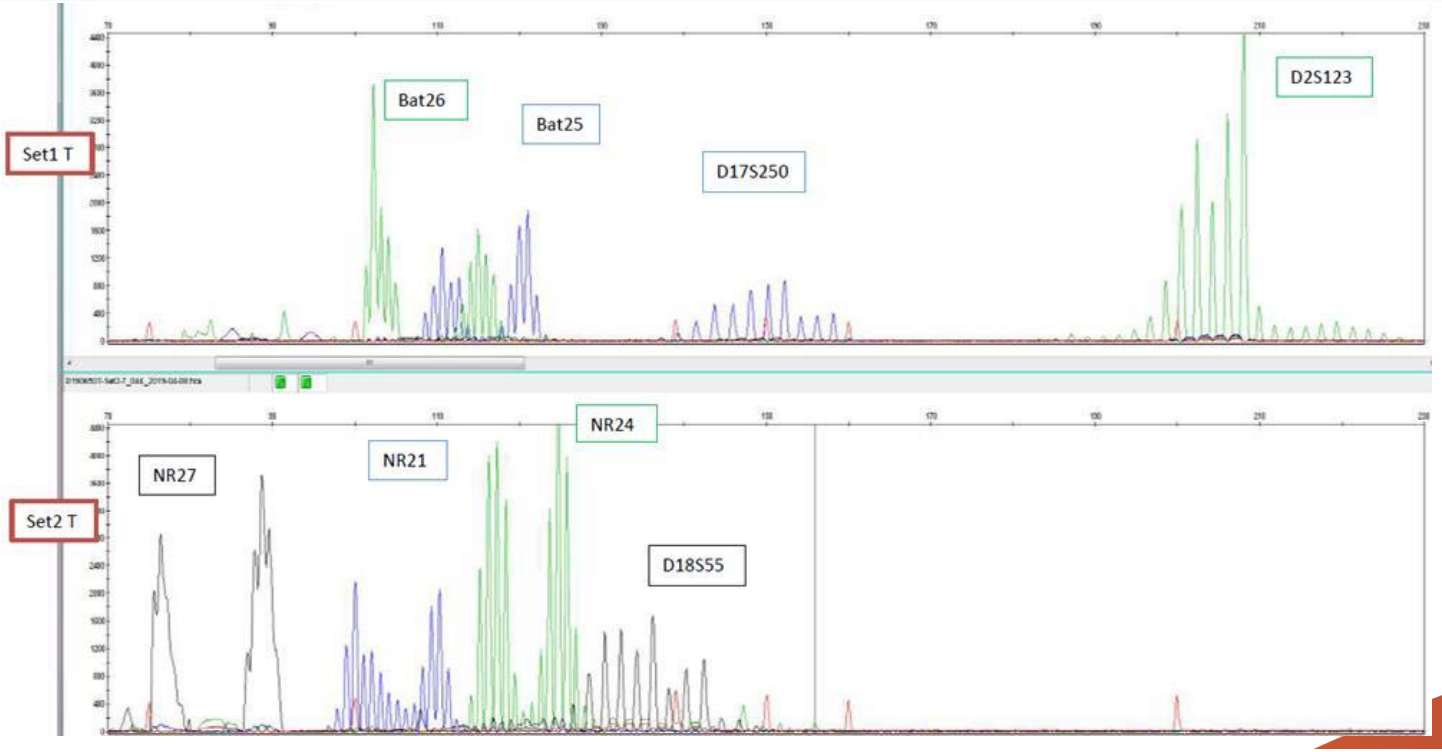
SAMPLE MSI STATUS: MSI-H

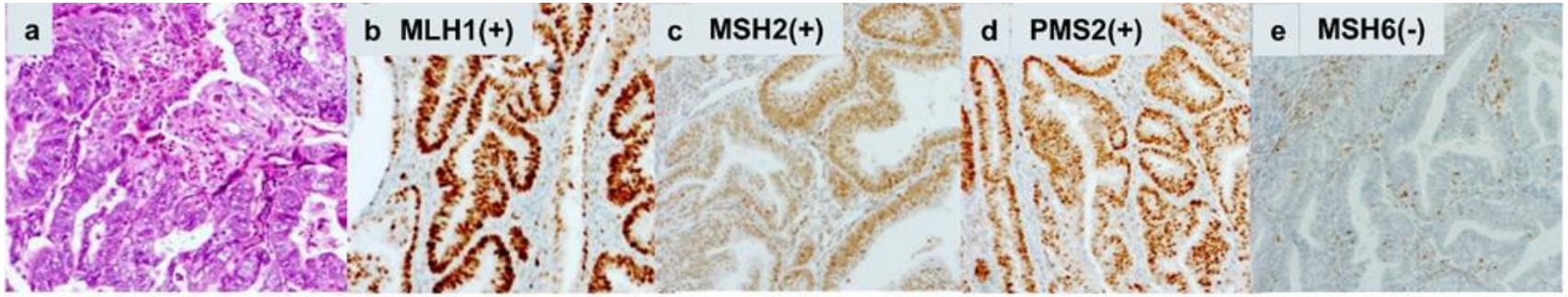
| TARGET | MSI SCORE |
|---|---|
| ACVR2A | 1.00 |
| BTBD7 | 0.19 |
| DIDO1 | 0.96 |
| MRE11 | 0.47 |
| RYR3 | 0.98 |
| SEC31A | 0.57 |
| SULF2 | 0.98 |




**g NGS**



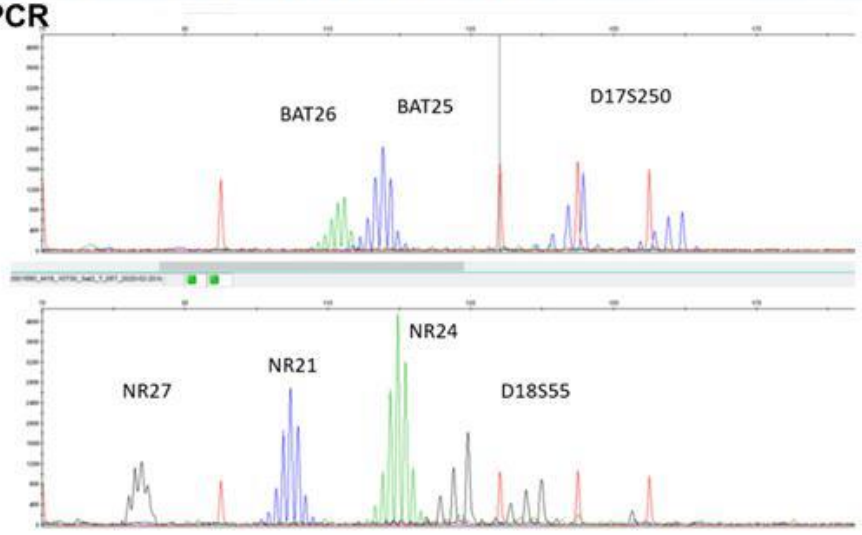



**f Idylla™ MSI assay**

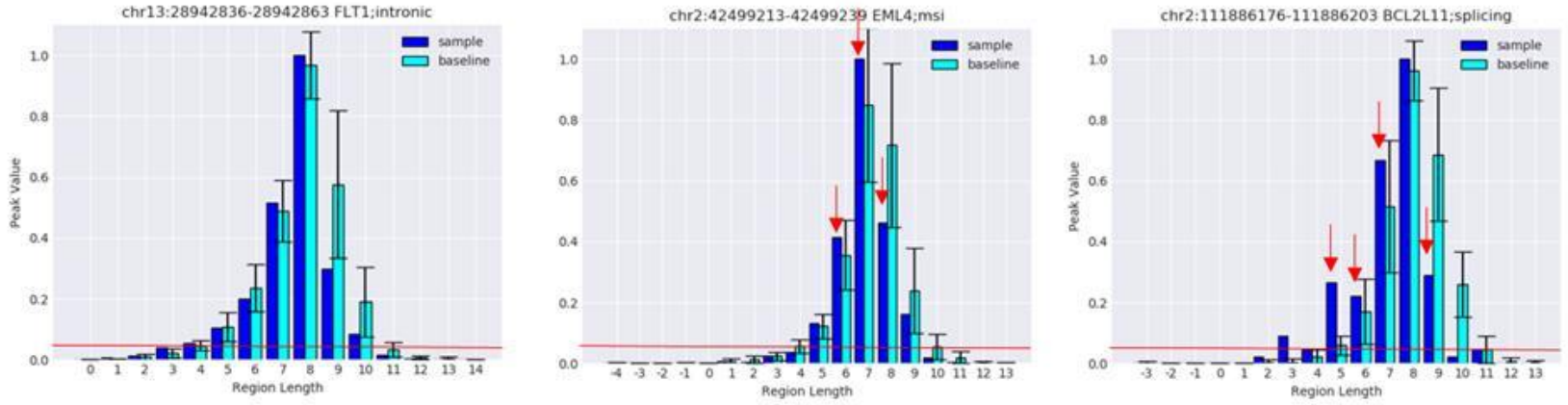
SAMPLE MSI STATUS: MSS

| TARGET | MSI SCORE |
|---|---|
| ACVR2A | 0.00 |
| BTBD7 | 0.00 |
| DIDO1 | 0.00 |
| MRE11 | 0.00 |
| RYR3 | 0.00 |
| SEC31A | 0.00 |
| SULF2 | 0.00 |


**g PCR**




**h NGS**



# Comparison of microsatellite instability detection by immunohistochemistry and molecular techniques in colorectal and endometrial cancer

- CRC: IHC and molecular techniques are equivalent. No difference in performance between PCR/NGS/Idylla. The molecular methods are very sensitive and specific.
- UCEC: molecular techniques are equivalent, but less sensitive than IHC.
  - IHC remains golden standard for UCEC
  - if dMMR on IHC: hypermethylation MLH1 promoter testing (in case of MLH1/PMS2 loss) and/or germline testing, irrespective of MSI-results PCR/NGS/Idylla
- Influence of tumor cell percentage, coverage and age FFPE bloc!

The Journal of Molecular Diagnostics, Vol. 19, No. 1, January 2017

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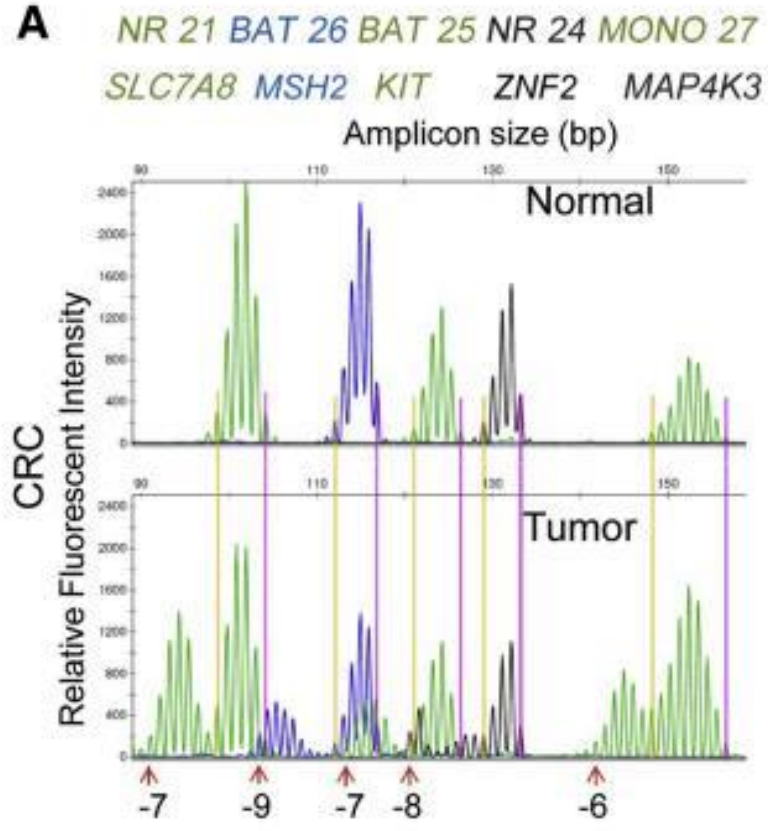
2017 JMD CME Program

# Differences in Microsatellite Instability Profiles between Endometrioid and Colorectal Cancers

## *A Potential Cause for False-Negative Results?*

Yang Wang, Chanjuan Shi, Rosana Eisenberg, and Cindy L. Vnencak-Jones

*From the Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee*



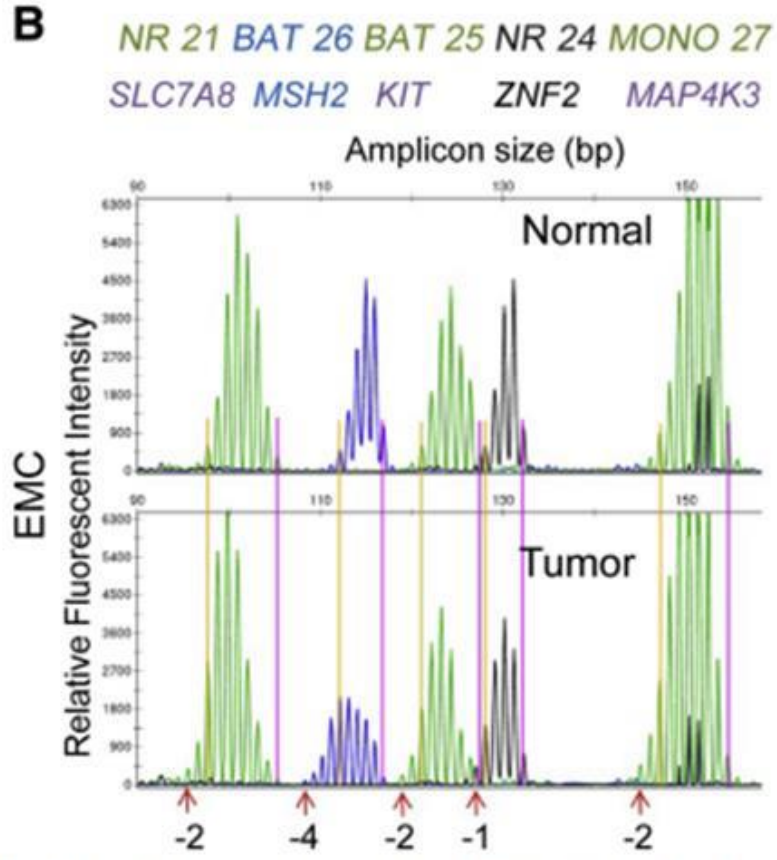




**Figure 1** Differences in microsatellite instability (MSI) profiles between colorectal cancer (CRC) and endometrioid cancer (EMC). **A:** MSI profile of a representative MSI—high (MSI-H) CRC with its paired normal control. Shifts in microsatellite repeat lengths are labeled at the bottom (eg, gene NR21/*SLC7A8*, −7 nt). **B:** MSI profile of a representative MSI-H EMC compared with its paired normal control. Shifts in microsatellite repeat lengths are labeled at the bottom (eg, gene NR21/*SLC7A8*, −2 nt). Common names and Human Genome Organisation nomenclature of genes containing microsatellite markers are listed.

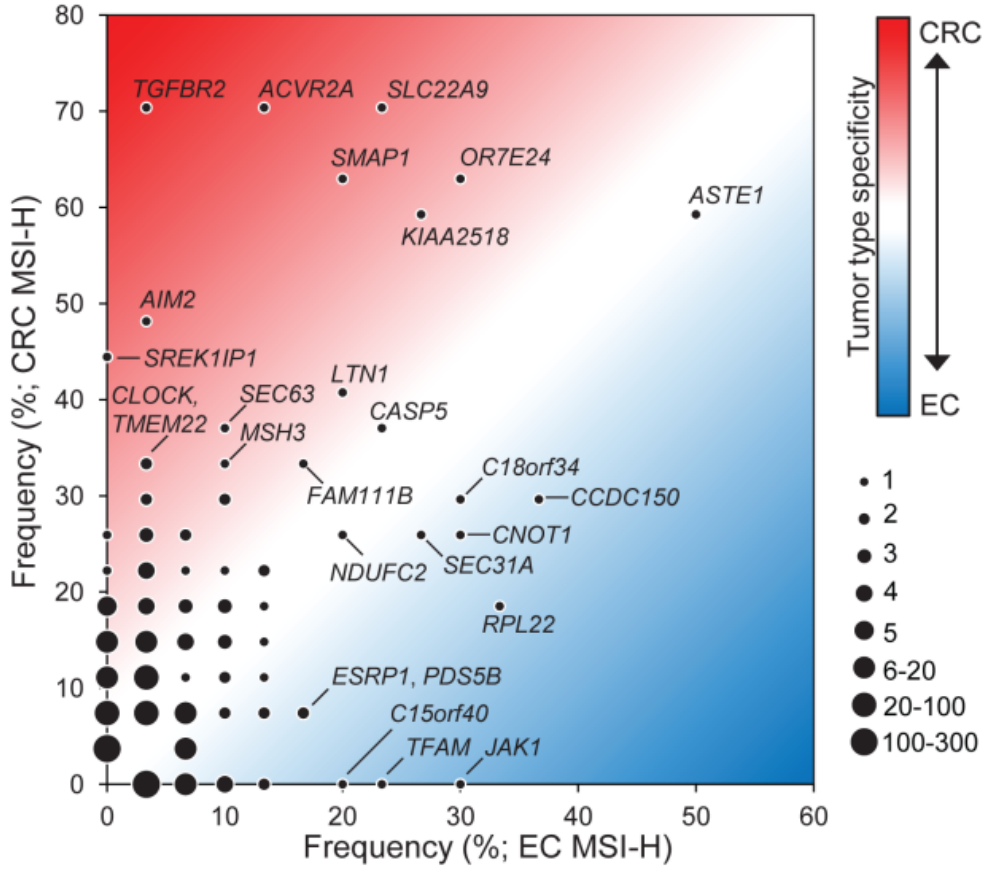
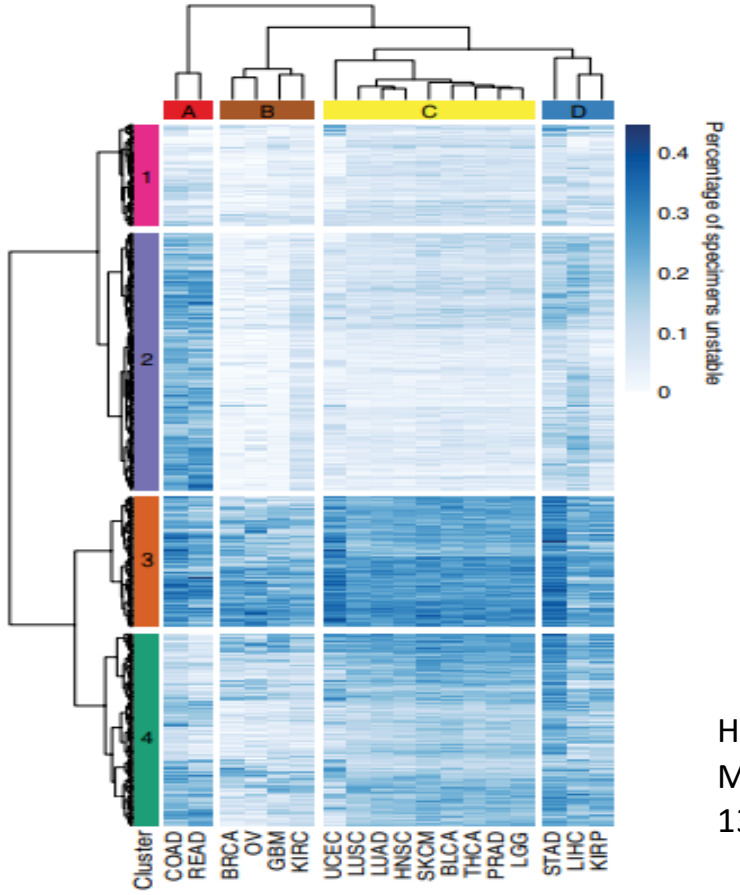



**Figure 3. The genes harboring frameshift MSI in CRC and EC genomes and tumor type specificity**

A scatter plot shows the distribution of genes with respect to their frequency of frameshift MSI in CRC and EC genomes. The 27 genes with frameshift MSI in >30% of CRC or in >15% of EC MSI-H genomes are noted. The color gradient indicates the extent of tumor type-specificity (red and blue for CRC- and EC-specificity, respectively). The size of the circles indicates the number of genes with the corresponding MSI frequencies. See also Figure S3 and Table S4.

Kim et al. Cell. 2013 Nov 7;155(4):858-68.




Hause et al. Nat Med **22,** 1342–1350 (2016).

**Figure 3** Cancer-specific signatures of MSI. Heatmap indicating the proportions of specimens within cancer types (columns) that were unstable at individual loci microsatellites (rows). Loci significant for differences among cancer types at FDR < 0.05 are shown. Colored microsatellite clusters (1–4, at left) denote groups of loci with similar instability trends based on Bayesian information criterion of the most likely model and number of clusters. Cancer types were also organized by hierarchical clustering into groups with similar patterns of MSI (A–D, top). UCEC, uterine corpus endometrial carcinoma; COAD, colon adenocarcinoma; STAD, stomach adenocarcinoma; READ, rectal adenocarcinoma; KIRC, kidney renal clear cell carcinoma; OV, ovarian serous cystadenocarcinoma; PRAD, prostate adenocarcinoma; LUAD, lung adenocarcinoma; HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; BLCA, bladder urothelial carcinoma; GBM, glioblastoma multiforme; LGG, brain lower grade glioma; BRCA, breast invasive carcinoma; KIRP, kidney renal papillary cell carcinoma; SKCM, skin cutaneous melanoma; THCA, thyroid carcinoma.

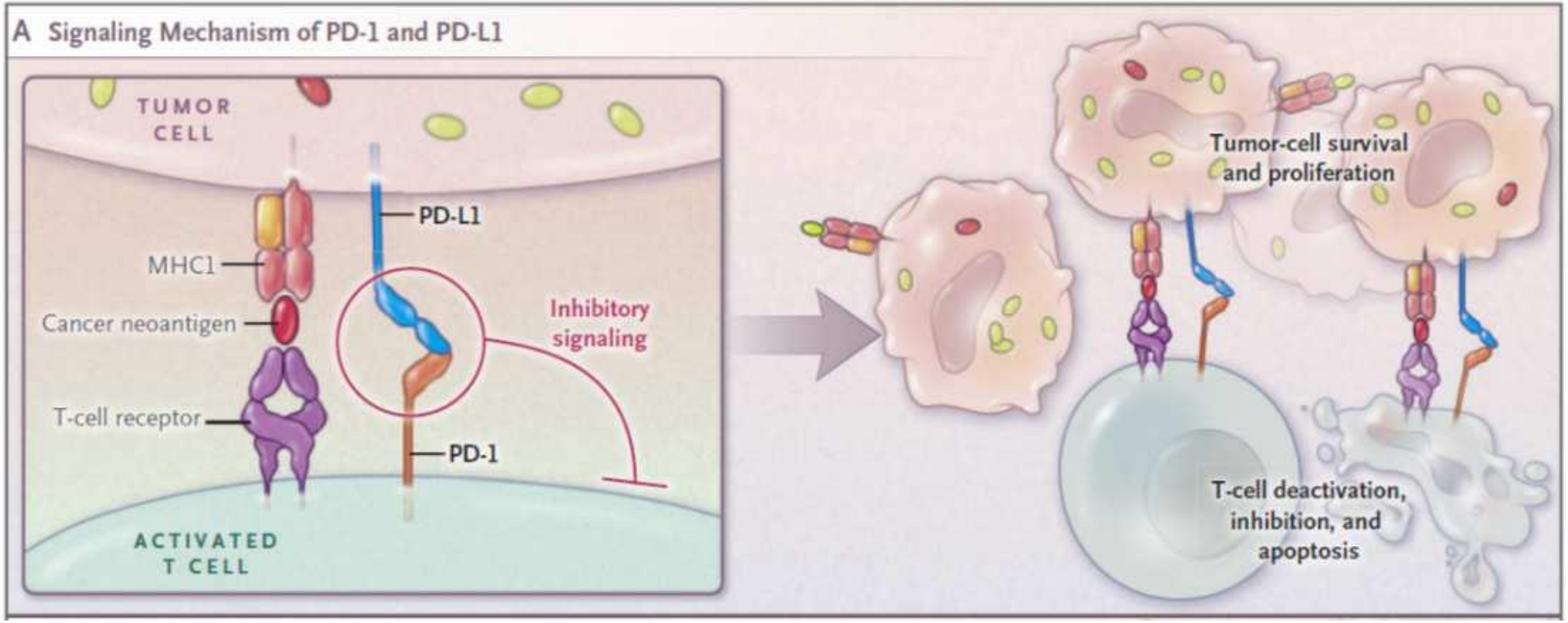
# In conclusion

| Characteristic | IHC | PCR | NGS | Idylla |
|---|---|---|---|---|
| **Cost/sample** | | | | |
| (1) NGS required by guidelines | low | low | low | high |
| (2) Stand-alone MSI testing | low | low | very high | high |
| Turnaround time (days) | 1-2 | 1-2 | 5-10 | 0.2 |
| Information on MMR driver gene | yes | no | no | no |
| Accessibility | high | intermediate | low | intermediate |
| Minimally required tumor cell percentage | 1% | 30% | 30% | 20% |
| Operator dependence | intermediate | intermediate | low | low |
| Normal tissue as internal control | no | difficult cases | no | no |
| Integration in standard workflow | standard | standalone test | possible | standalone test |
| MSI locus panel flexibility | low | high | high | low |
| CE-IVD/FDA | yes | yes | variable | yes |
| Other | - | - | - | dedicated instrument |

Comparison of microsatellite instability detection by immunohistochemistry and molecular techniques in colorectal and endometrial cancer

Franceska Dedeurwaerdere [1,*,+], Kathleen BM Claes [2,5,7,+], Jo Van Dorpe [3], Isabelle Rottiers, Joni Van der Meulen [2,7], Joke Breyne [4], Koen Swaerts, Geert Martens [4,5,6]

# A much broader indication for MMR testing…



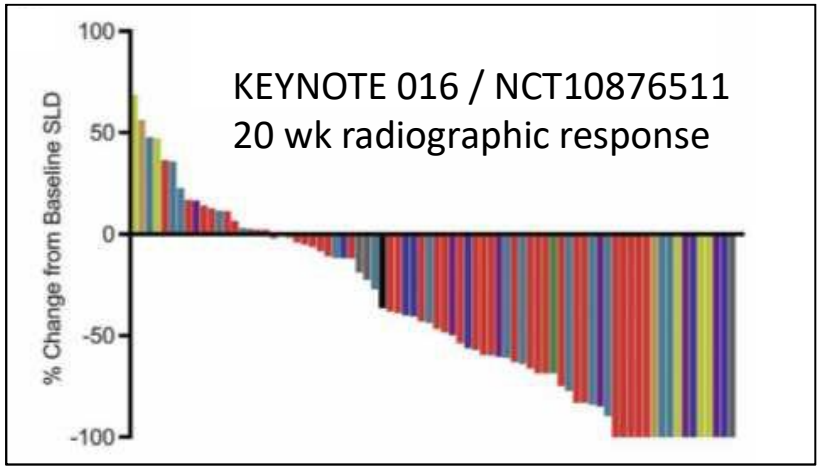

Lemery S, et al. NEJM. 2017;377(15)-1409-1412

# FDA approval for pembrolizumab in MMR-deficient solid tumors

- Data from 149 patients with MSI-H or MMR-D cancer across 5 clinical trials
- 90 patients had CRC, remainder had one of 14 other tumor types
- Patients identified using MMR IHC (n=47), MSI PCR (n=60), or both tests (n=42)
- Most patients had received two or more therapies for metastatic or unresectable disease
- Overall response rate 39.6% (CI 31.7-47.9%)
- Responses lasted ≥ 6 mos in 78% of patients that had a response
- 11 CRs and PRs

**Pembrolizumab Response Rate by Tumor Type.***

| Tumor Type | No. of Tumors | Patients with a Response | Range of Response Duration |
|---|---|---|---|
| | | *no. (%)* | *mo* |
| Colorectal cancer | 90 | 32 (36) | 1.6+ to 22.7+ |
| Endometrial cancer | 14 | 5 (36) | 4.2+ to 17.3+ |
| Biliary cancer | 11 | 3 (27) | 11.6+ to 19.6+ |
| Gastric or gastroesophageal junction | 9 | 5 (56) | 5.8+ to 22.1+ |
| Pancreatic cancer | 6 | 5 (83) | 2.6+ to 9.2+ |
| Small-intestine cancer | 8 | 3 (38) | 1.9+ to 9.1+ |
| Breast cancer | 2 | 2 (100) | 7.6 to 15.9 |
| Prostate cancer | 2 | 1 (50) | 9.8+ |
| Other cancers | 7 | 3 (43) | 7.5+ to 18.2+ |




Lemery S, et al. NEJM. 2017;377(15)-1409-1412., Le Dt, et al. Science. 2017;357:409-413.

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SPECIAL ARTICLE

# ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach

C. Luchini[1], F. Bibeau[2], M. J. L. Ligtenberg[3,4], N. Singh[5], A. Nottegar[6], T. Bosse[7], R. Miller[8], N. Riaz[9], J.-Y. Douillard[10], F. Andre[11*] & A. Scarpa[12]

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## DNA mismatch repair in cancer☆

Marina Baretti, Dung T. Le *

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Li *et al. Cancer Cell Int* (2020) 20:16
https://doi.org/10.1186/s12935-019-1091-8

Cancer Cell International

REVIEW Open Access

# Microsatellite instability: a review of what the oncologist should know

Kai Li[1,2,3†], Haiqing Luo[3†], Lianfang Huang[1,2], Hui Luo[2*] and Xiao Zhu[1,2*]