

8^e ÉDITION

JOURNÉES DU GFCO 2022

Biomarqueurs et analyses moléculaires en oncologie

Avec la participation
scientifique du



BIOMARQUEURS EN ONCOLOGIE

Actualités et vision projective

Modérateurs : Lucie Karayan-Tapon, Poitiers & Pierre-Jean Lamy, Montpellier

**Approche agnostique : nouveautés sur les marqueurs
d'instabilité microsatellitaire et techniques de détection
en NGS**

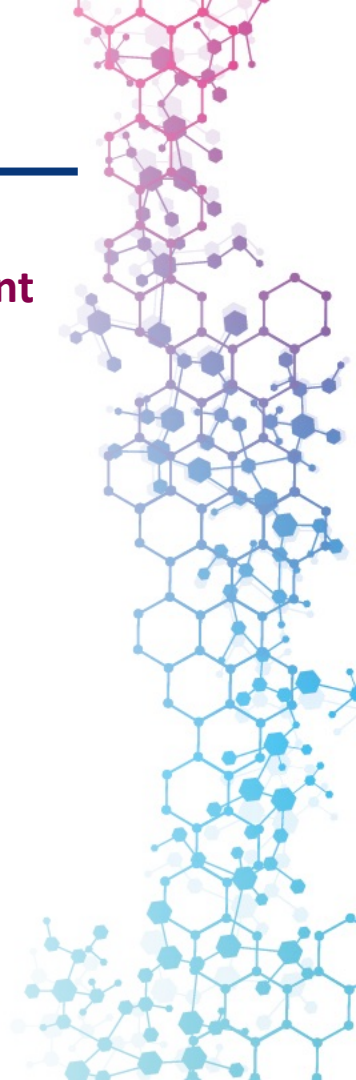
Alex Duval

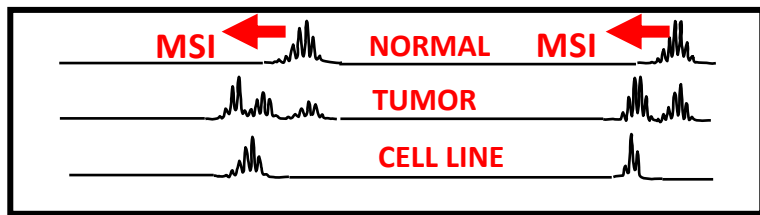
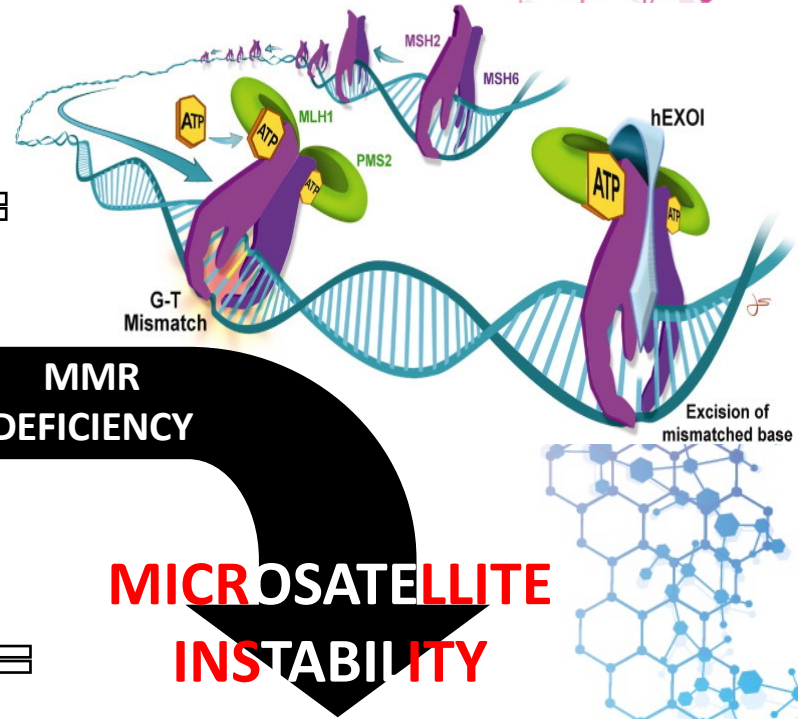
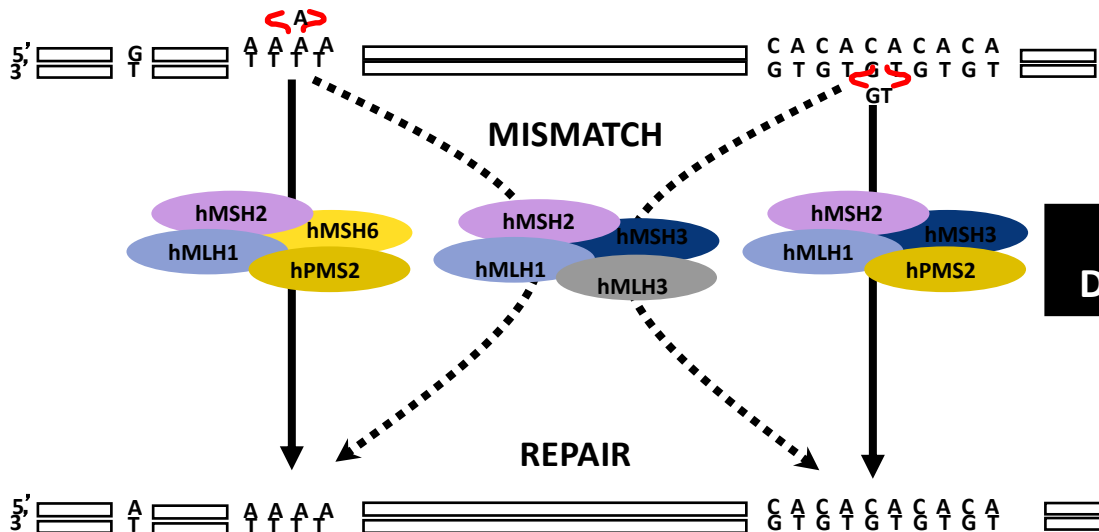
**Equipe Inserm Instabilité des Microsatellites et Cancer,
Hôpital Saint-Antoine, Paris**



LIENS D'INTÉRÊT

- **MSDAvenir: Lauréat de la Fondation MSDAvenir 2022 pour le Financement du projet visant à la valorisation de l'outil diagnostique MSICare (Financement 2022 - 2025)**
- **Start-Up MSInsight, fondateur et Directeur Scientifique**





MSI MSI

Mononucleotide Repeats (X)n

TCCAAAAAAAAAAAAAAAAAGTC
TCCAAAAAAAAAAAAAAAAAGTC
TCCAAAAAAAAAAAAAAAAAGTC
TCCAAAAAAAAAAAAAAAAAGTC

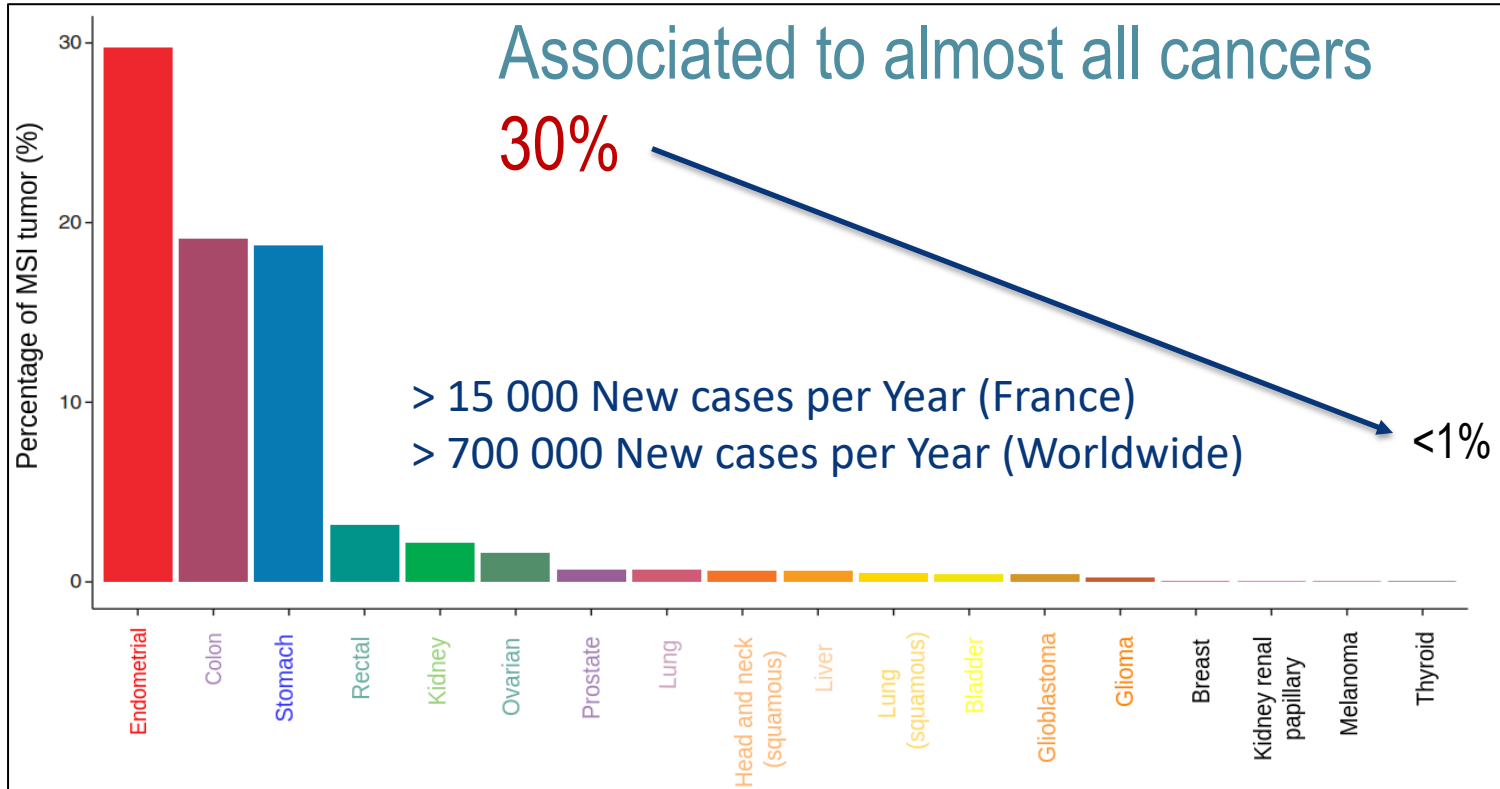
Dinucleotide Repeats (XY)n

GCTTACACACACACACACATCC
GCTTACACACACACACACATCC
GCTTACACACACACACATCC
GCTTACACACACACATCC

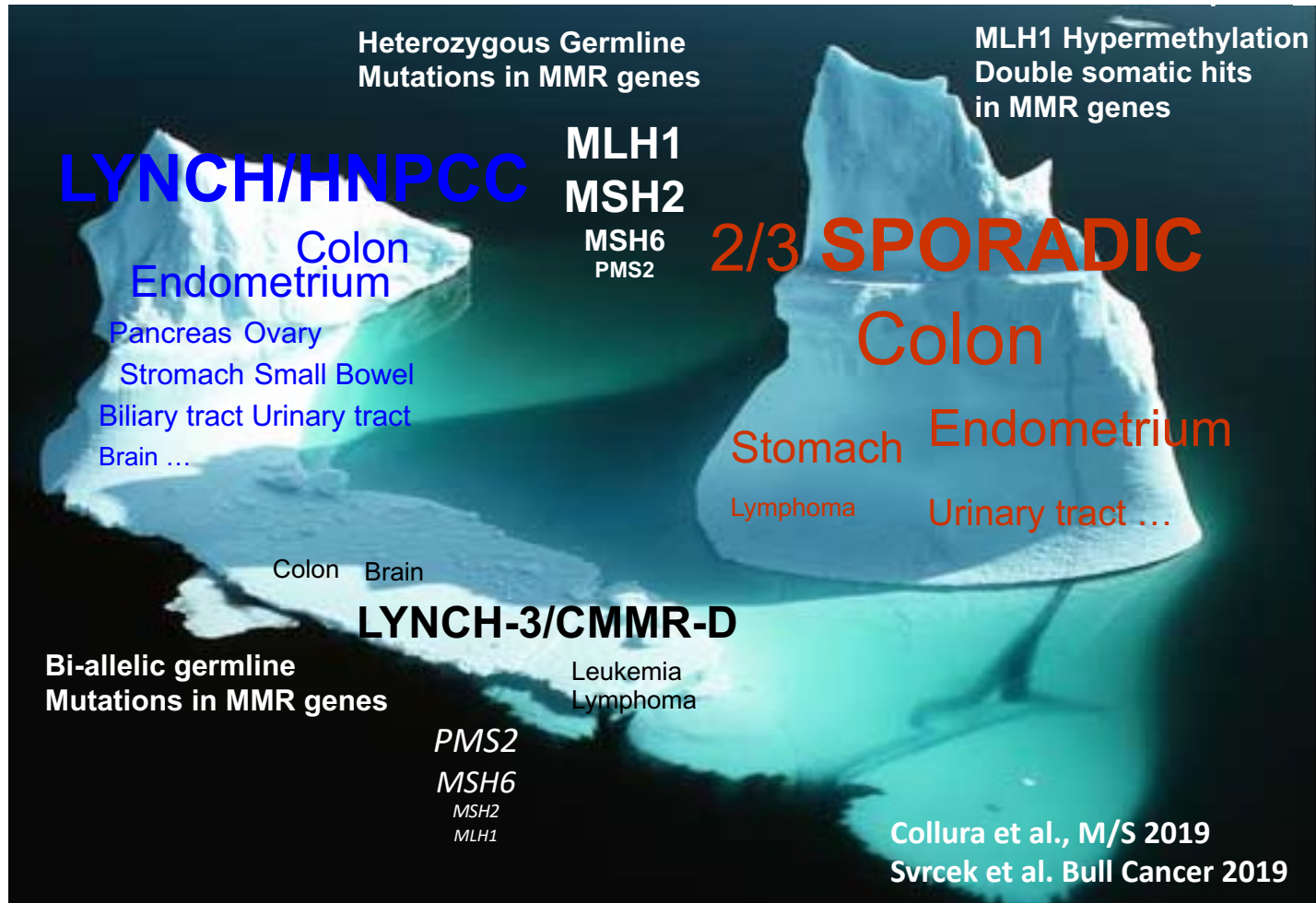
Leach Cell 1993
Thibodeau Science 1993

Fishel Cell 1993
Ionov Nature 1993

MSI TUMORS: CLINICAL SPECTRUM



MSI TUMORS: SPORADIC AND HEREDITARY SETTINGS

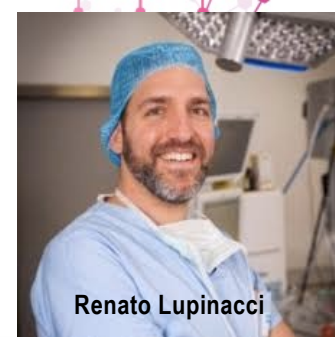


BASIC AND TRANSLATIONAL—PANCREAS

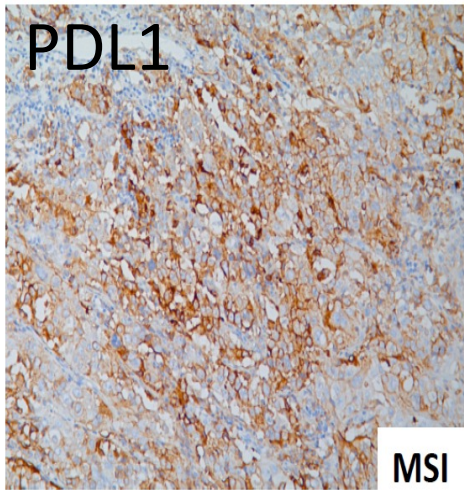
Prevalence of Microsatellite Instability in Intraductal Papillary Mucinous Neoplasms of the Pancreas



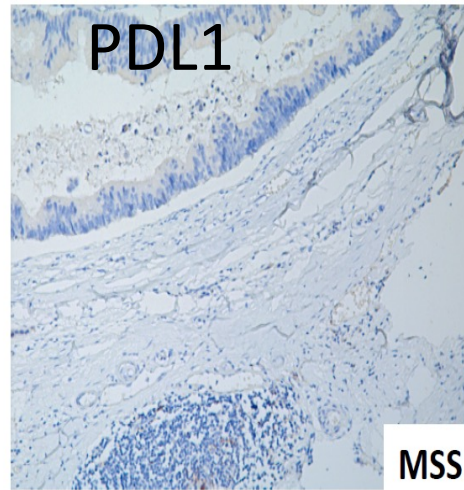
Renato M. Lupinacci,^{1,2,3,*} Anastasia Goloudina,^{1,4,*} Olivier Buhard,¹ Jean-Baptiste Bachet,^{2,5} Raphaël Maréchal,⁶ Pieter Demetter,⁷ Jérôme Cros,^{8,9} Armelle Bardier-Dupas,¹⁰ Ada Collura,^{1,2} Pascale Cervera,^{1,2,11} Aurélie Scriva,¹¹ Sylvie Dumont,² Pascal Hammel,^{9,12} Alain Sauvanet,^{9,13} Christophe Louvet,¹⁴ Jean-Robert Delpéro,¹⁵ François Paye,^{2,16} Jean-Christophe Vaillant,^{2,17} Thierry André,^{2,18} Jean Closset,⁶ Jean-François Emile,¹⁹ Jean-Luc Van Laethem,⁶ Vincent Jonchère,¹ Issam Abd Alsamad,²⁰ Martine Antoine,^{2,21} Anita Rodenas,^{2,21} Jean-François Fléjou,^{1,2,11} Nelson Dusetti,²² Juan Iovanna,²² Alex Duval,^{1,2,§} and Magali Svrcek^{1,2,11,§}



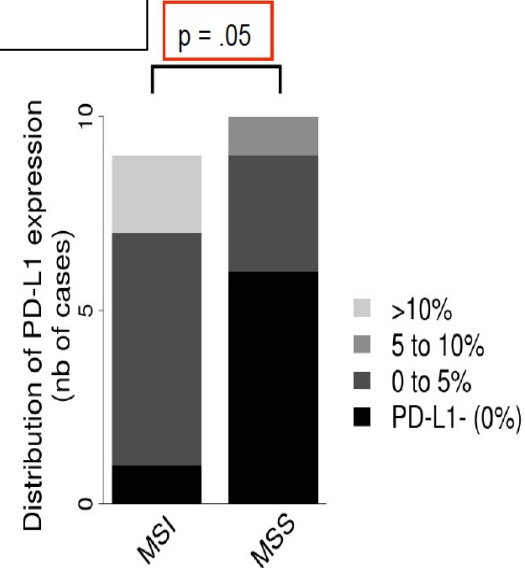
Renato Lupinacci



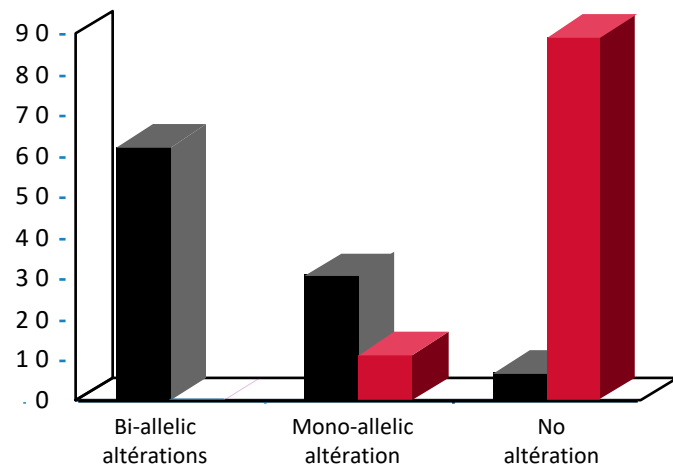
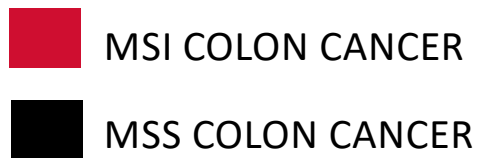
MSI



MSS

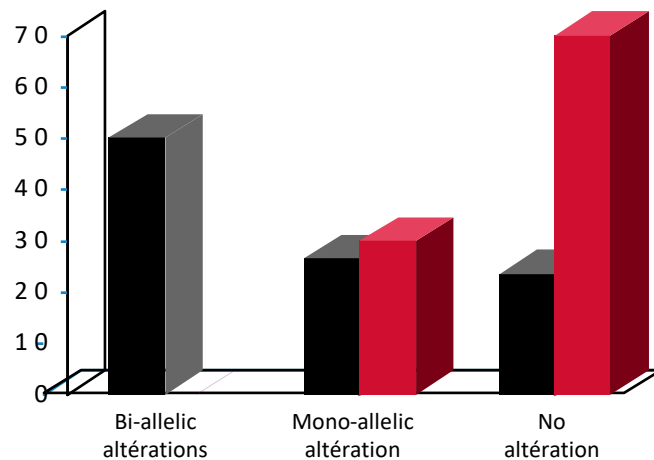


We still need to further define the clinical spectrum of MSI cancers in human ...



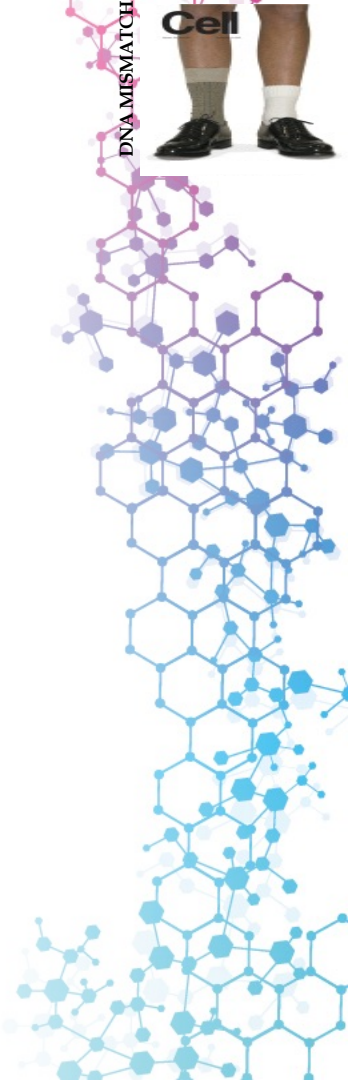
$P < 10^{-4}$

APC

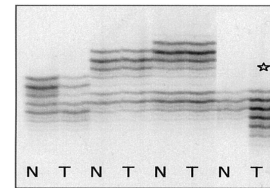
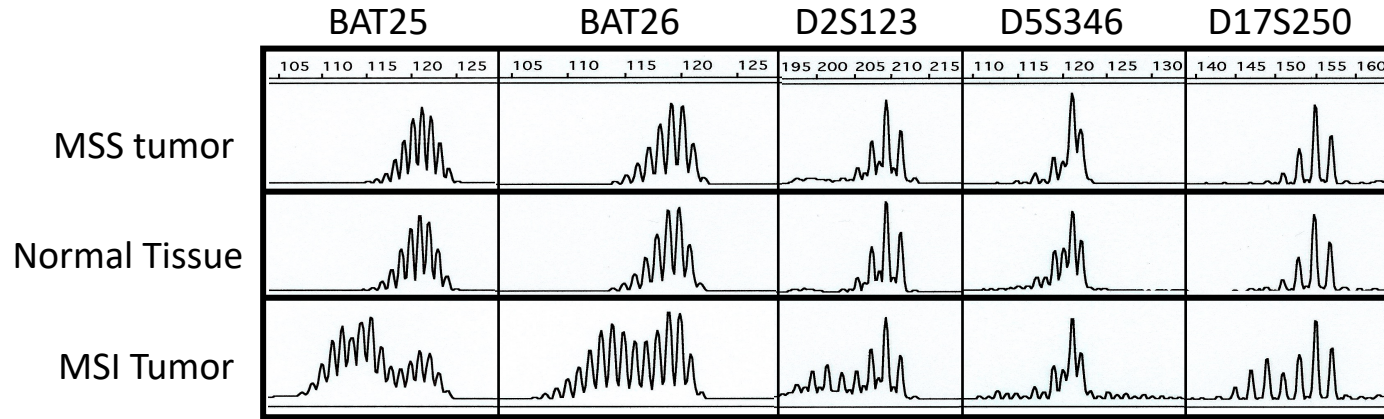


$P = 6.10^{-4}$

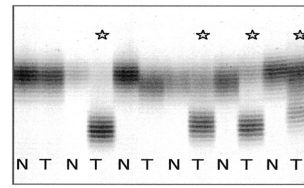
P53



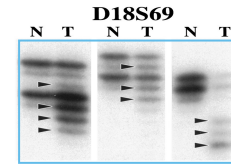
Identification of MSI in colon cancer



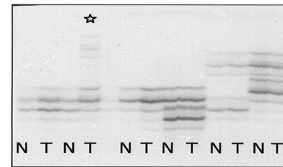
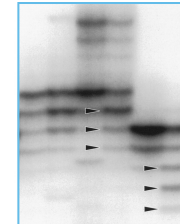
D17S250



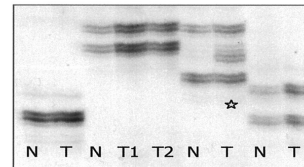
Bat 26



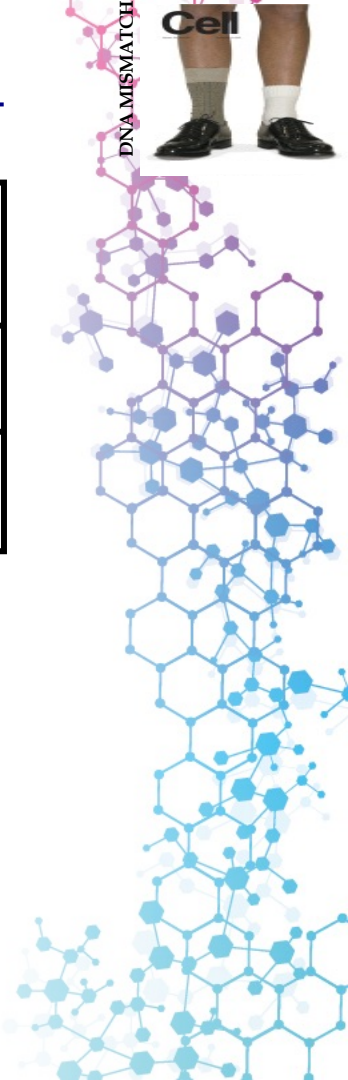
D18S64



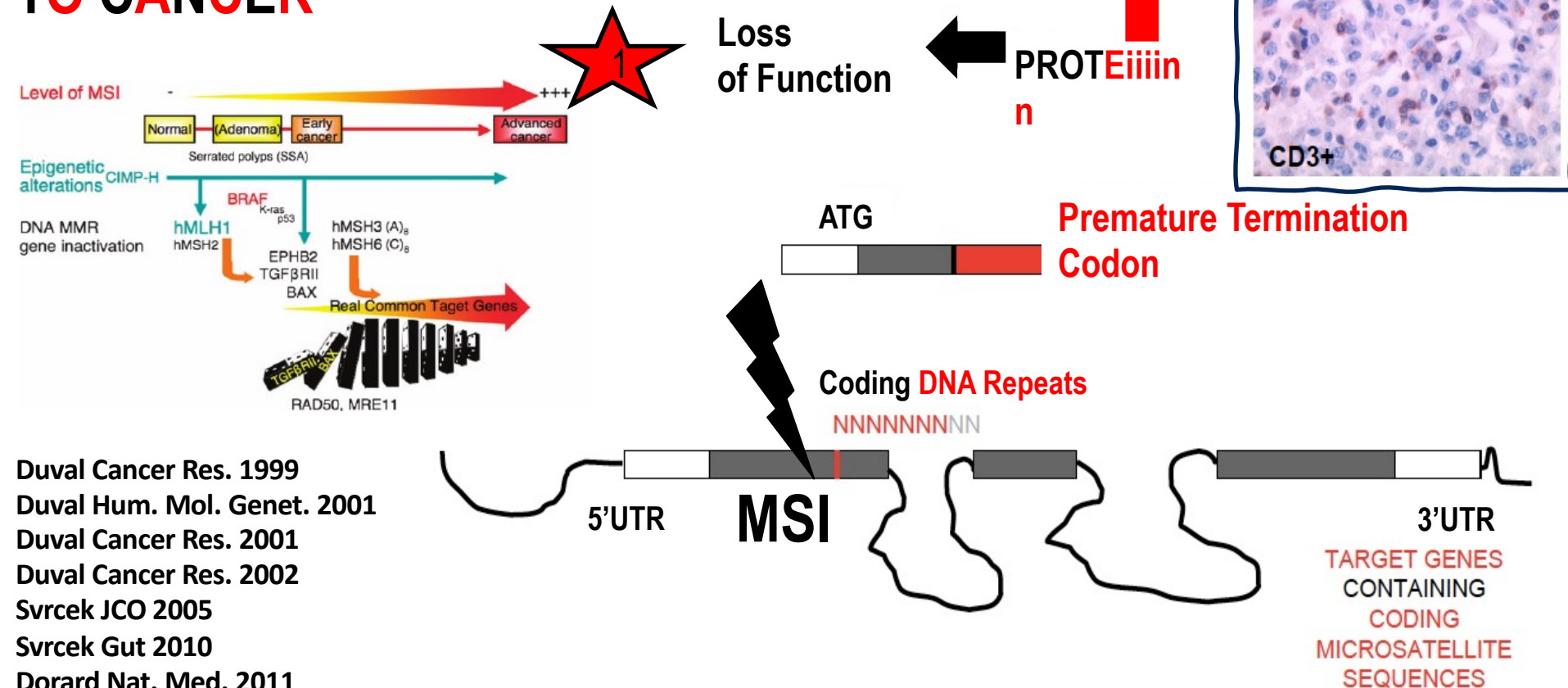
D5S346



MYCL



THE CODING MSI-DRIVEN PATHWAY TO CANCER



Duval Cancer Res. 1999
 Duval Hum. Mol. Genet. 2001
 Duval Cancer Res. 2001
 Duval Cancer Res. 2002
 Svrcek JCO 2005
 Svrcek Gut 2010
 Dorard Nat. Med. 2011
 Collura M/S 2019

THE CODING MSI-DRIVEN PATHWAY TO CANCER

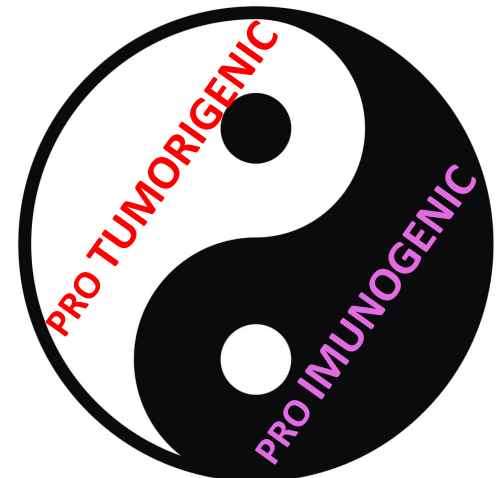


RECQL	41 aa	649aa
AXDND1	66 aa	1012aa
PRR11	30aa	360aa
SULF1	81	871aa
TMPO	109 aa	694aa
LNPEP	149aa	1025aa
FGF13	40	245aa
DHX36	179aa	1008aa
UGDH	88aa	494aa
DIAT	123 aa	647aa
TCF25	146aa	676aa
FXR1	136aa	621aa
EIF3I	64 aa	258aa
C11orf54	79 aa	315aa
BOP1	188	746aa
ARFGAP2	134 aa	521aa
AKAP7	90aa	348aa
RALGAPB	390aa	1494aa
UBA6	305aa	1052aa
BAX	58aa	192aa
EIF2AK1	198aa	630aa
TGFBR2	186aa	592aa
UGT1A3	172aa	534aa
LTN1	613aa	1812aa
C1orf112	301 aa	853aa
COIL	207aa	576aa
MSH3	413aa	1137aa
PSORS1C1	64aa	152aa

MNS1	209 aa	495aa
ICA1	207aa	483aa
ACBD3	231 aa	528aa
CREBBP	1097aa	2442aa
SMAP1	218aa	467aa
ARAF	293	609aa
TRAPP8	695 aa	1435aa
CYB5R4	258aa	521aa
SSB	204 aa	408aa
SLC35F5	268 aa	523aa
PIGB	290 aa	554aa
TBCK	470 aa	893aa
G2E3	375 aa	706aa
CDC25C	257aa	473aa
PPP1R12A	561 aa	1030aa
TNKS2	673 aa	1190aa
NDUFA10	203 aa	355aa
CDS1	267 aa	461aa
CEP57	291 aa	500aa
PXDN	888 aa	1479aa
ARFGAP3	319 aa	516aa
SMC2	758 aa	1197aa
SENP1	412 aa	644aa
ARV1	179 aa	271aa
DDX60	1135 aa	1712aa
WISP3	248 aa	372aa

TEAD2	308aa	450aa
HSP110	588 aa	858aa
PANK1	421aa	598aa
CBX5	135 aa	191aa
ZNRF1	161aa	227aa
PNISR	581 aa	805aa
SRFBP1	309 aa	429aa
NAA15	634 aa	866aa
JAK1	874 aa	1154aa
CTSA	383 aa	498aa
RBM27	819 aa	1060aa
CYC1	252 aa	325aa
SCLT1	542 aa	688aa
DCAF17	432 aa	520aa
MXRA8	406 aa	450aa
PDSSB	1392	1447aa
SAMD9L	1524	1584aa

**CODING
DNA**



- Frameshift mutator mutation
- Neoantigenic aberrant C-terminal tail

- Tumor suppressor Gene, Genome Stability
- Oncogene
- Metabolism
- Cell Structure
- Other signalling pathways and Enzymes

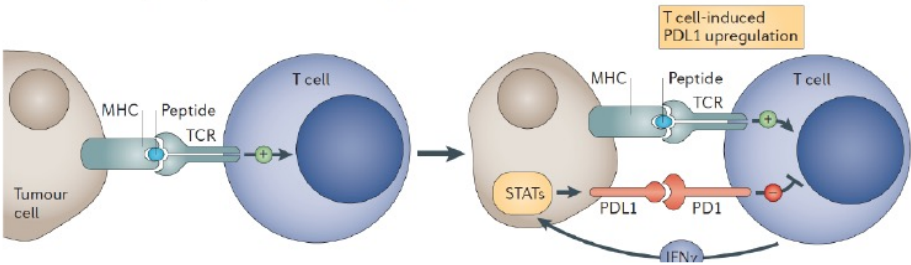
AntiTumor Immunity in MSI Cancers: The role of ICKs

Published in final edited form as:
Cancer Discov. 2015 January ; 5(1): 43–51. doi:10.1158/2159-8290.CD-14-0863.

Cancer Discovery 2015

ORIGINAL ARTICLE

The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints



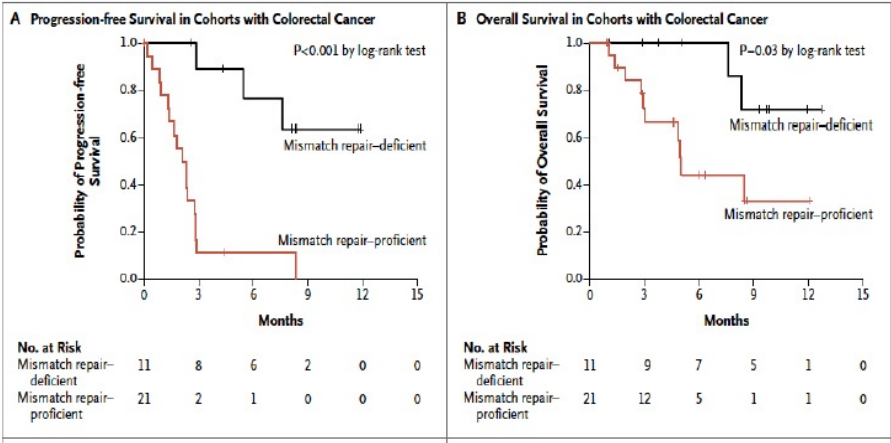
PD-1 Blockade in Tumors
with Mismatch-Repair Deficiency

N ENGL J MED 372;26 NEJM.ORG JUNE 25, 2015

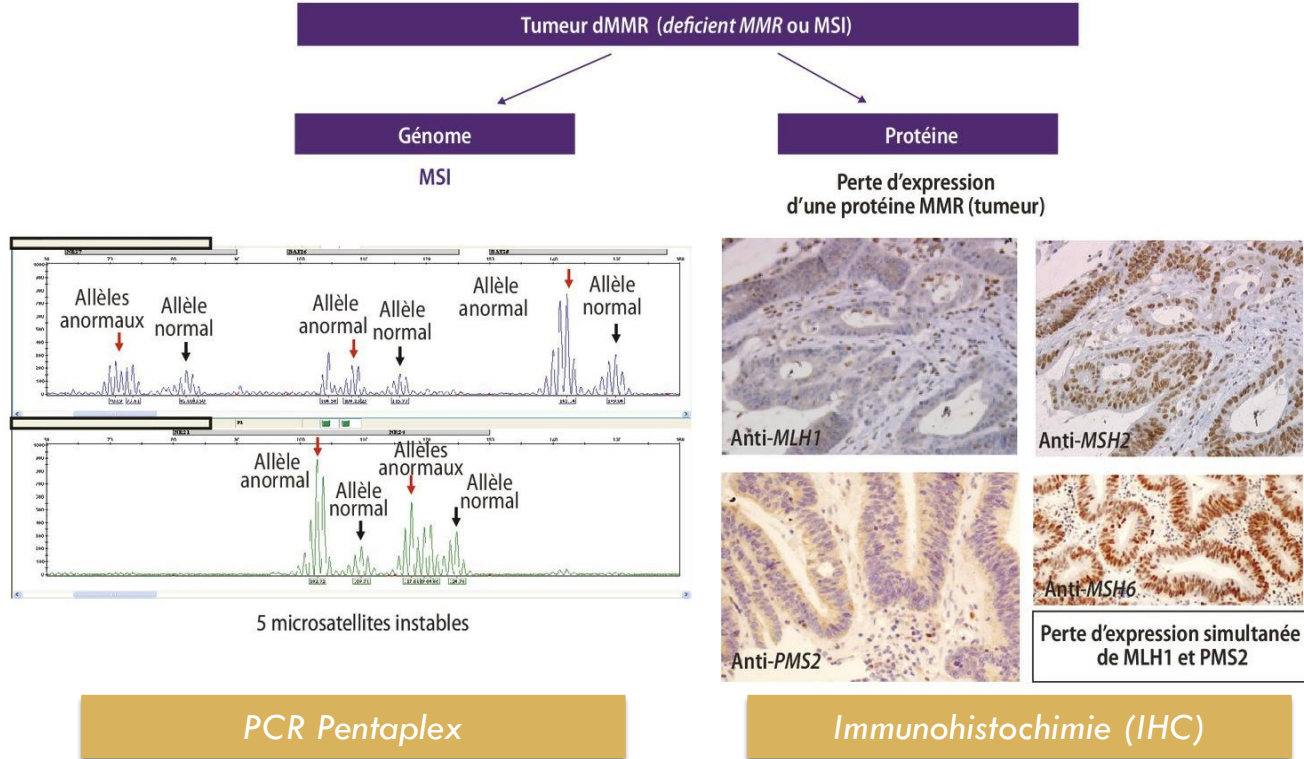
Nobel Prize in Physiology and Medicine
2018

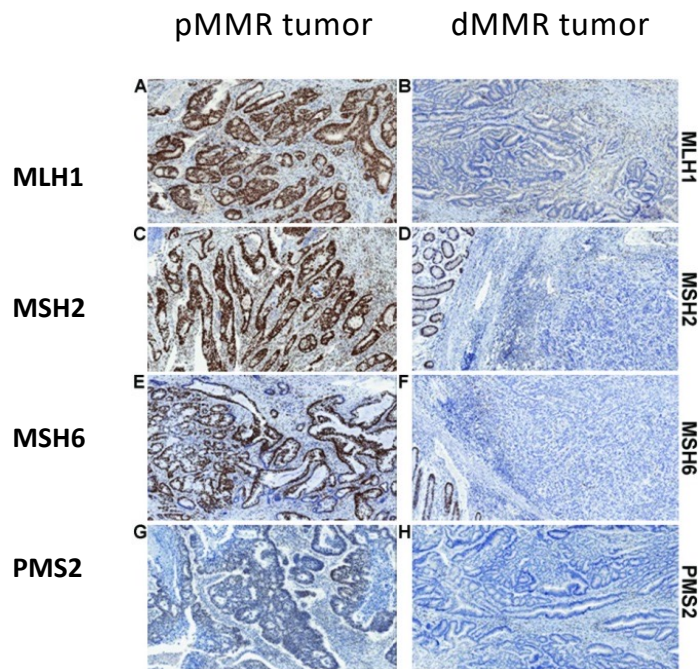


James P. Allison and Tasuku Honjo



Gold Standard Methods for MSI Diagnostics in Human Tumors





Diagnosing MSI in Cancer

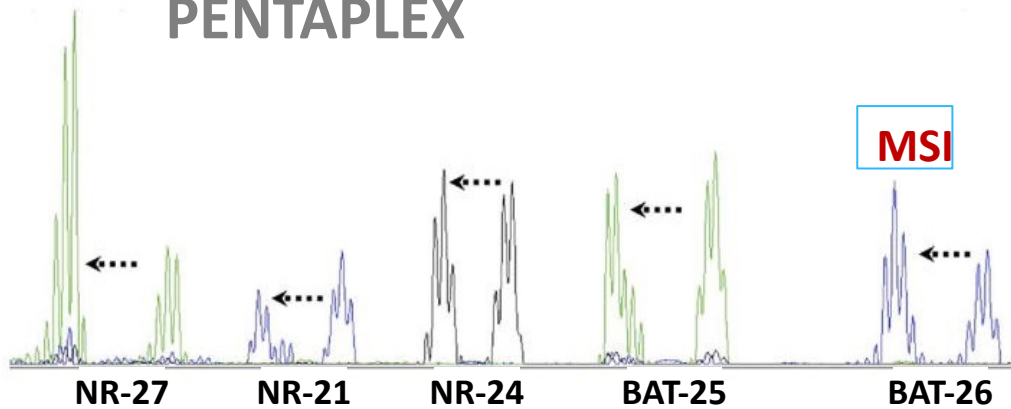
1/ ImmunoHistoChemistry

- Co analysis of MMR protein by IHC (MLH1, MSH2, MSH6, PMS2)
- Highly Sensitive and specific in pan-cancer. However, there are **some false negative results**
- **Need of tumor material, hard to standardize, impossible to use in liquid biopsy (Circulating Tumor DNA)**
- **Not suitable for high throughput diagnosis**

Diagnosing MSI in Cancer

2/ PCR and AFLP

PENTAPLEX



Normal (contaminating DNA)

Suraweera Gastroenterology 2002

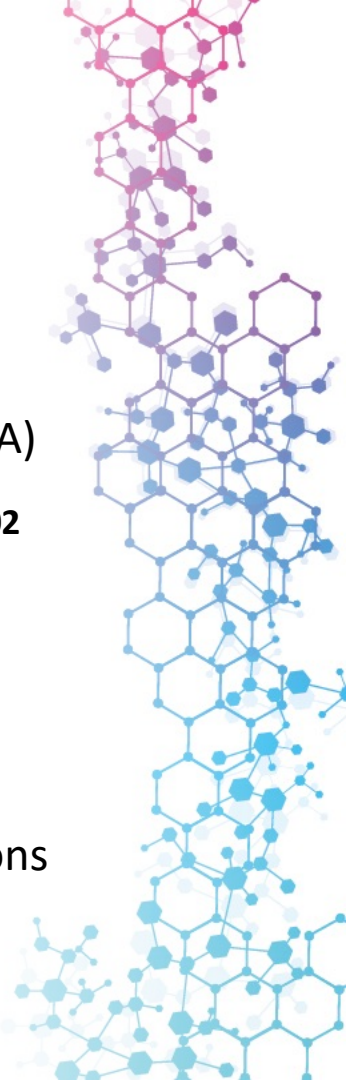
Buhard JCO 2006

Patented, Licenced Promega

- Co amplification of 5 monomorphic markers (mononucleotide repeats)
- No need of matching normal DNA (Colorectal cancer)
- Matching normal DNA is required when testing other primary tumor locations for MSI (Endometrial tumors, others)
- PCR is poorly sensitive for diagnosing MSI outside CRC

Olivier Buhard

Richard Hamelin



Romain Cohen



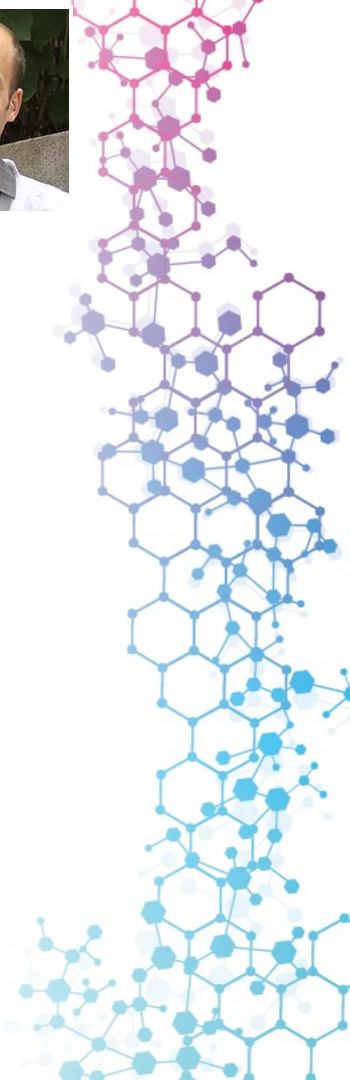
Research

JAMA Oncology | **Brief Report**

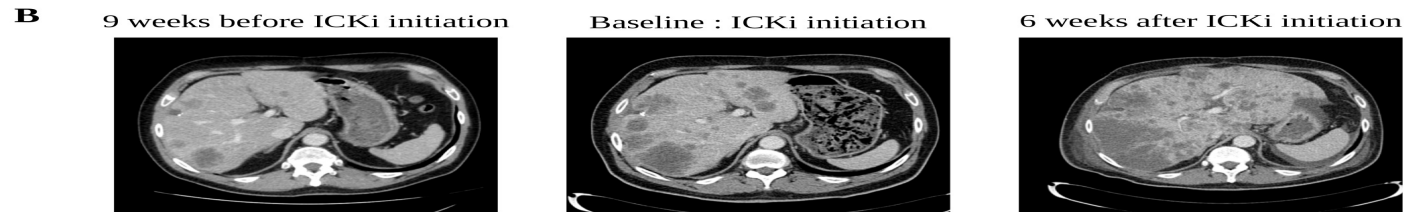
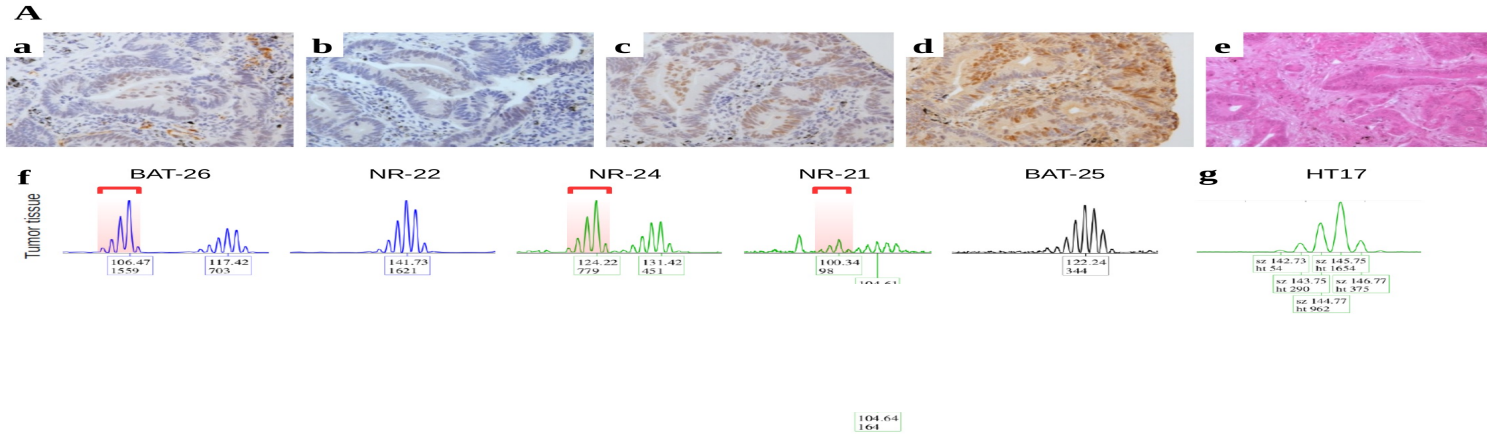
Association of Primary Resistance to Immune Checkpoint Inhibitors of Metastatic Colorectal Cancer With Misdiagnosis of Microsatellite Instability or Mismatch Repair Deficiency Status

Romain Cohen, MD; Elisabeth Hain, MD; Olivier Buhard, MSc; Agathe Guilloux, PhD; Armelle Bardier, MD; Rachid Kaci, MD; Philippe Bertheau, MD, PhD; Florence Renaud, MD, PhD; Frédéric Bibeau, MD, PhD; Jean-François Fléjou, MD, PhD; Thierry André, MD; Magali Svrcek, MD, PhD; Alex Duval, MD, PhD

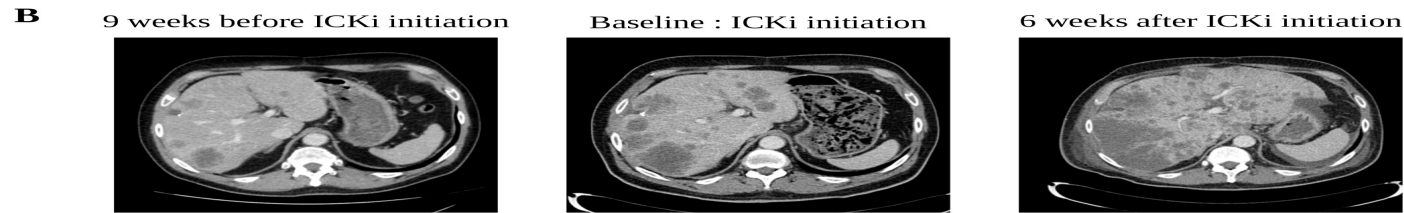
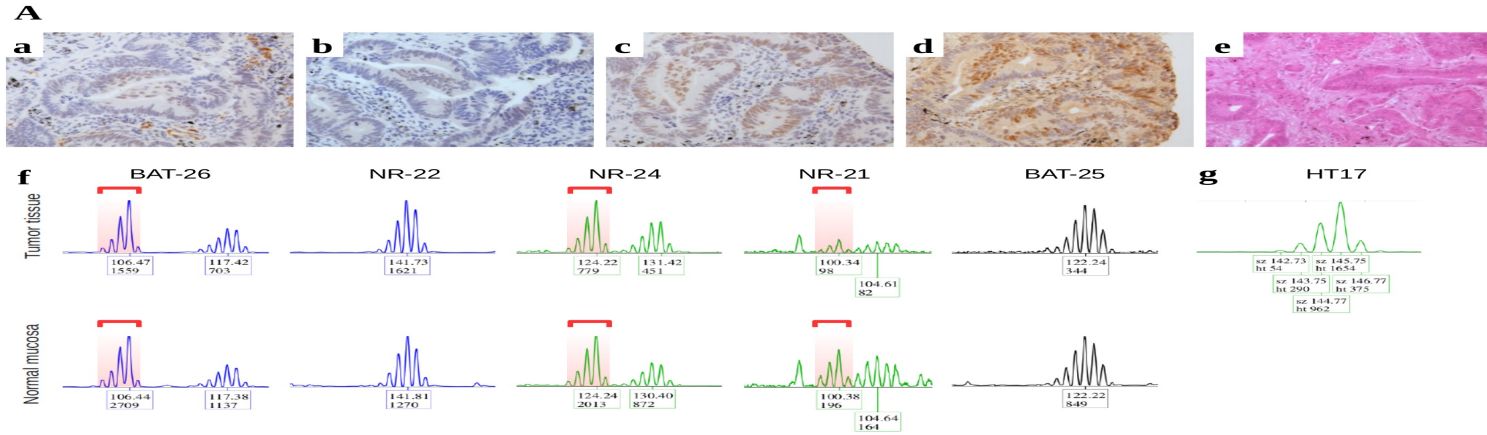
Cohen Jama Oncol. 2019



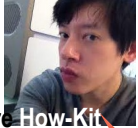
Be Careful MSI Diagnosis using PCR Before to Use ICKi !



Be Careful MSI Diagnosis using PCR Before to Use ICKi !



New PCR and AFLP Methods for Improving MSI detection In solid and liquid biopsies containing tumor DNAs



Alexandre How-Kit

Detection of MSI using PCR and
AFLP can be improved using
HSP110 T17
by modifying Technical protocols ...

DOI: 10.1002/humu.23379

METHODS

WILEY



**Major improvement in the detection of microsatellite
instability in colorectal cancer using HSP110 T17
E-ice-COLD-PCR**

Alexandre How-Kit¹ | Antoine Daunay¹ | Olivier Buhard² | Clément Meiller³ |
Mourad Sahbatou⁴ | Ada Collura² | Alex Duval² | Jean-François Deleuze^{1,5}

How-kit Hum Mut 2018

Clinical Chemistry 64:8
1252-1259 (2018)

**Improved Microsatellite
Instability Detection
and Identification by
Nuclease-Assisted
Microsatellite Instability
Enrichment Using
HSP110 T17**

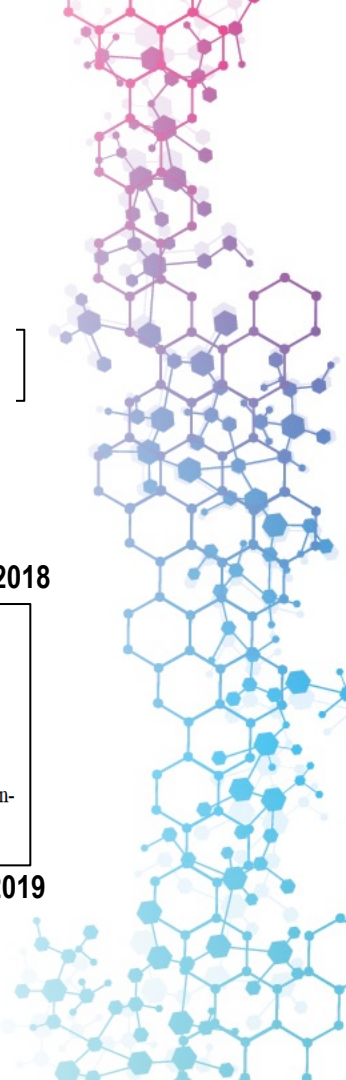
Laura G. Baudrin^{2,3}
Alex Duval⁴
Antoine Daunay²
Olivier Buhard⁴
Hung Bui²
Jean-François Deleuze^{2,5}
Alexandre How-Kit^{2*}

Baudrin Clin Chem 2018

Low temperature isothermal amplification of microsatellites drastically
reduces stutter artifact formation and improves microsatellite instability
detection in cancer

Antoine Daunay¹, Alex Duval², Laura G. Baudrin^{1,3}, Olivier Buhard², Victor Renault⁴, Jean-
François Deleuze^{1,5} & Alexandre How-Kit^{1*}

Daunay Nucl Acid Res 2019

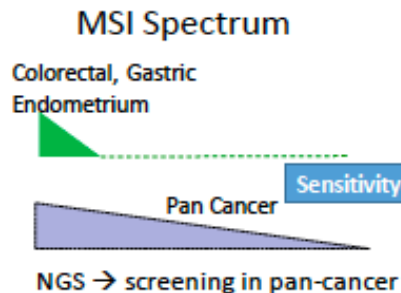


Diagnosing MSI in Cancer

3/ Next Generation Sequencing

Emerging methods:
Next Generation Sequencing

- ❖ Multiple markers
- ❖ MSI → Increasing depth

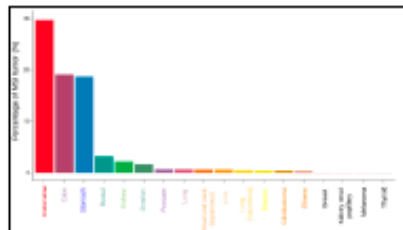
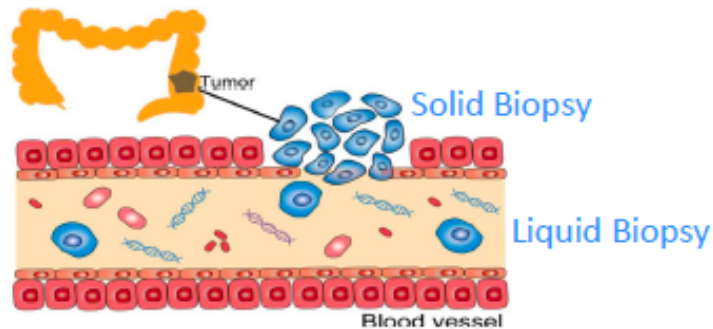


MSI Progression

Adenoma Adeno Carcinoma Metastasis

MSI PCR

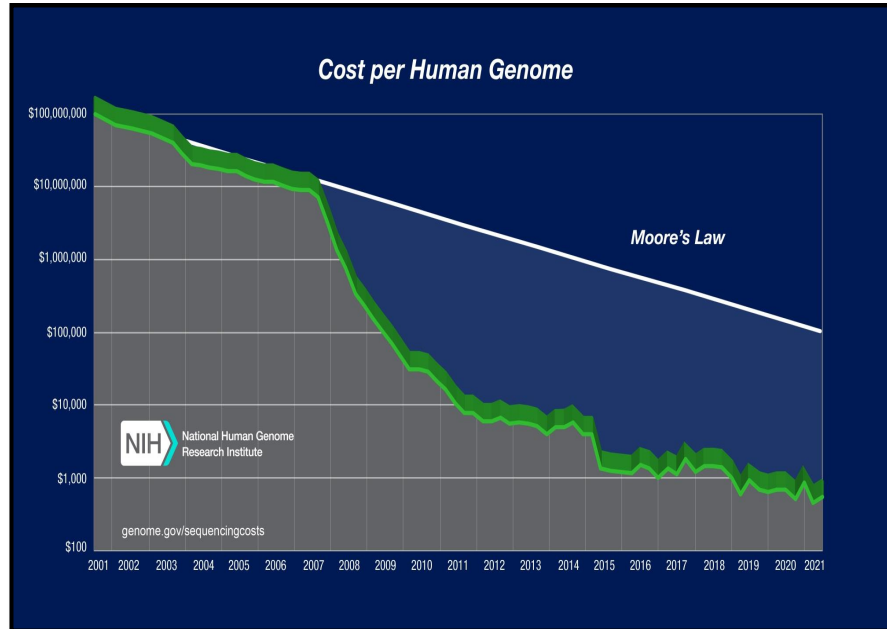
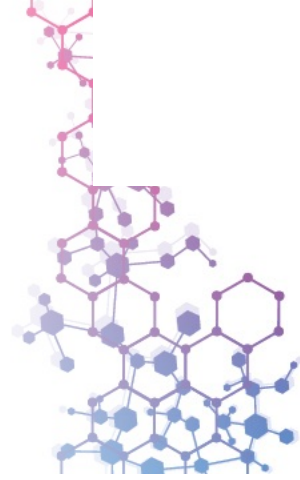
MSI NGS



Diagnosing MSI in Cancer

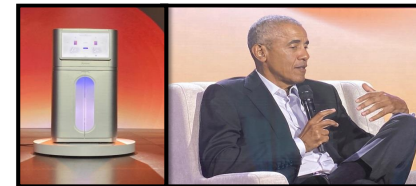
3/ Next Generation Sequencing

Moving towards the genome's democratization



□ Séquençage du génome humain → changement d'échelle en termes de prix

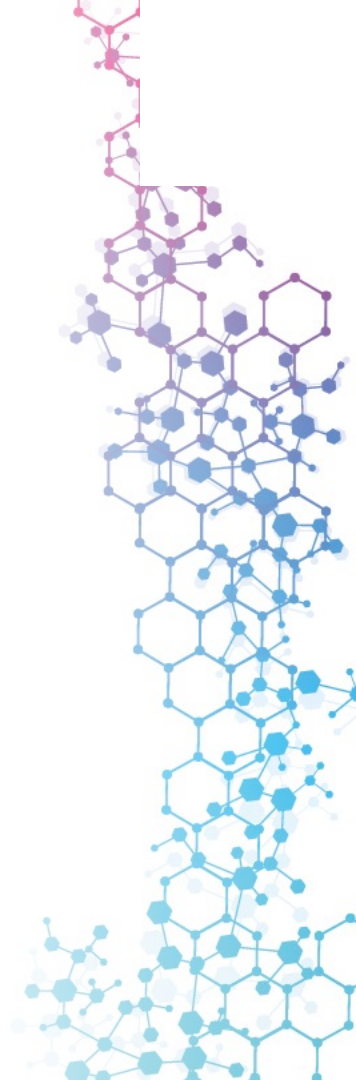
- 1990-2004 : Projet génome humain → 3Mds \$
- 2007 : James Watson (Roche 454®) → 1M \$
- 2010 : HiSeq2000 (Illumina®) → 10 000 \$
- 2013 : Ion Proton (Life Technologies®) → 1000 \$
- 10/2022 : NovaSeq X séries (Illumina®) < 200 \$
- 10/2022 : PacBio® Revio (single molecule real time sequencing) → Génome Long-reads < 1 000 \$



Diagnosing MSI in Cancer

3/ Next Generation Sequencing

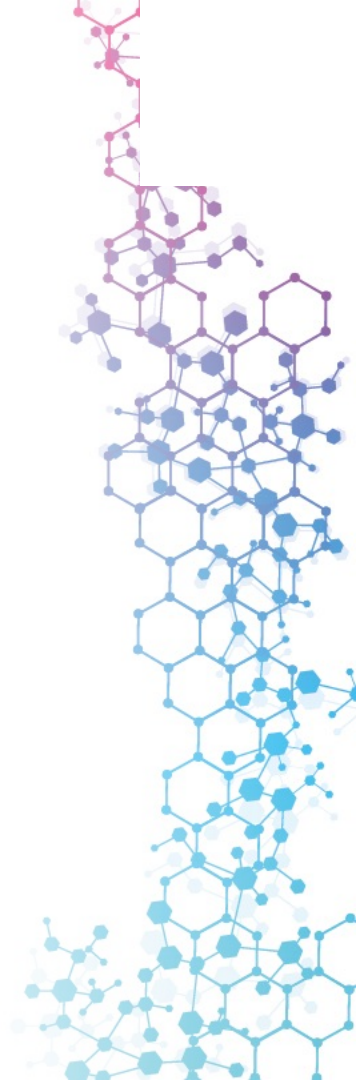
Méthode	Type d'échantillons	Méthode de diagnostic
MSIsensor	Tumeur vs tissu normal apparié (v1) ou tissu tumoral seul (v2)	Classificateur binaire (MSS/MSI) Seuil MSI-H > 3,5% (FDA > 10%) v1 ; seuil >20% pour v2
MSIsensor-pro	Tumeur vs base de référence	Classificateur binaire (MSS/MSI) Seuil complexe
MSIsensor-ct	ADNtc vs base de référence	Classificateur binaire (MSS/MSI) Seuil MSI > 20%
MANTIS	Tumeur vs tissu normal apparié	Classificateur binaire (MSS/MSI) Agrégat des scores MSI > 0,4
mSINGS	Tumeur vs base de référence	Classificateur binaire (MSS/MSI) Seuil MSI-H > 20% des loci instables



MSIsensor v1

- ❑ Approuvé par la *FDA* comme test compagnon en NGS (seuil > 10% de locis instables)
- ❑ Utilisable en théorie en pan-cancer
- ❑ Marqueurs microsatellites multiples

$$\text{MSISensor Score} = \frac{\text{number of Mutated Microsatellites}}{\text{number of investigated Microsatellites}}$$



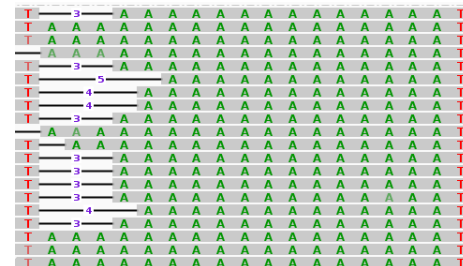
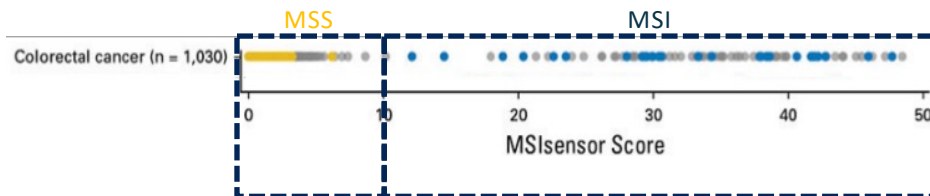
Diagnosing MSI in Cancer

3/ Next Generation Sequencing

$$\text{MSISensor Score} = \frac{\text{number of Mutated Microsatellites}}{\text{number of investigated Microsatellites}}$$

Published in final edited form as:
JCO Precis Oncol. 2017 ; 2017: .

Reliable Pan-Cancer Microsatellite Instability Assessment by Using Targeted Next-Generation Sequencing Data

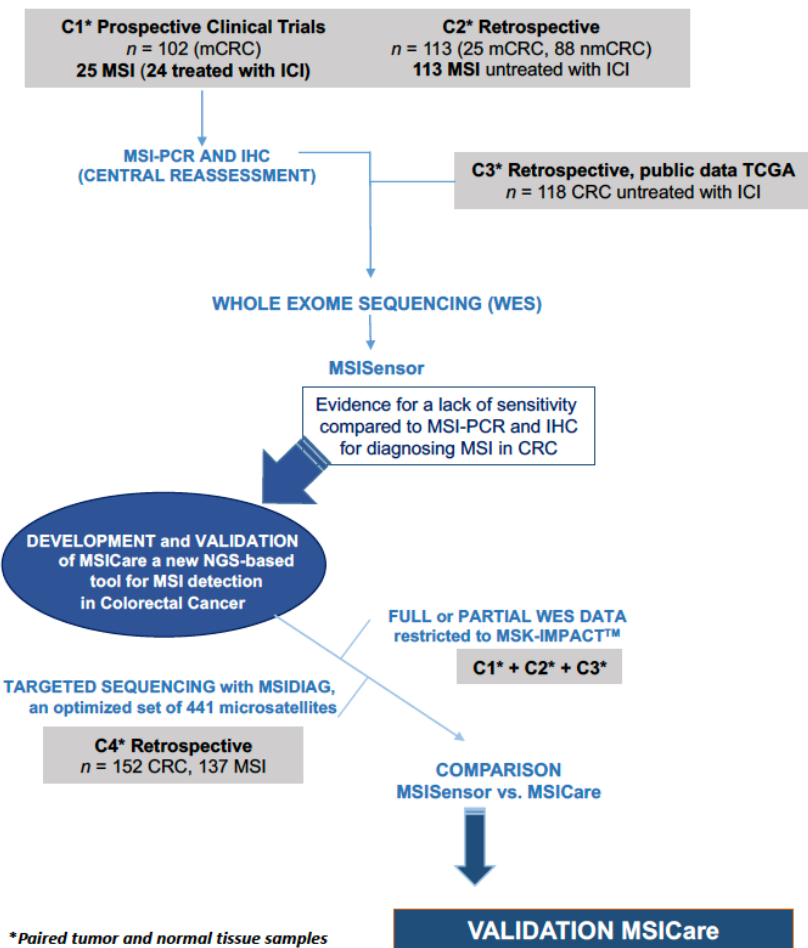


MSISensor Score > 10% → MSI

Validation status

- Validated MSI-H/MMR-D
- Validated MSS/MMR-P
- Not validated

STUDY DESIGN

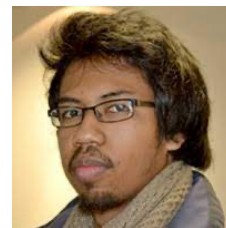


CLINICAL—ALIMENTARY TRACT

Gastroenterology 2021;161:814–826

Performance of Next-Generation Sequencing for the Detection of Microsatellite Instability in Colorectal Cancer With Deficient DNA Mismatch Repair

Toky Ratovomanana,¹ Romain Cohen,^{1,2} Magali Svrcek,^{1,3} Florence Renaud,^{1,4} Pascale Cervera,^{1,5} Aurélie Siret,¹ Quentin Letourneur,¹ Olivier Buhard,¹ Pierre Bourgoin,¹ Erell Guillemin,^{1,5} Coralie Dorard,¹ Remy Nicolle,⁶ Mira Ayadi,⁶ Mehdi Touat,^{1,7} Franck Bielle,^{7,8} Marc Sanson,^{7,8} Philippe Le Rouzic,^{1,5} Marie-Pierre Buisine,^{4,9} Guillaume Piessen,^{4,10} Ada Collura,¹ Jean-François Fléjou,^{1,3} Aurélien de Reyniès,⁶ Florence Coulet,^{1,5,9} François Ghiringhelli,^{11,12,9} Thierry André,^{1,2,9} Vincent Jonchère,^{1,5,9} and Alex Duval^{1,5,9}

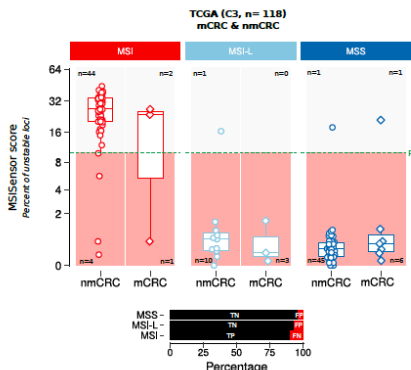
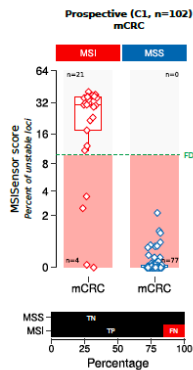


Toky Ratovomanana

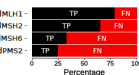
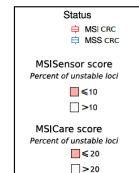
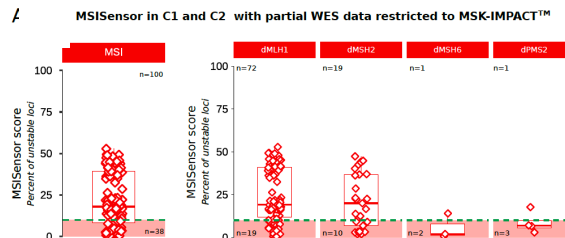
Ratovomanana Gastroenterology 2021

NGS-based Diagnosis of MSI using MSISensor (FDA-Approved)

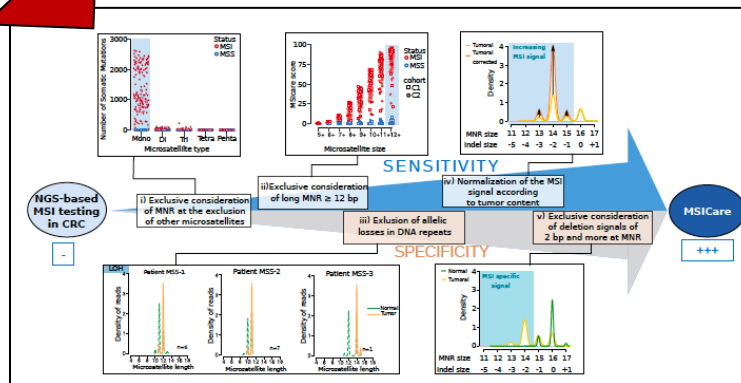
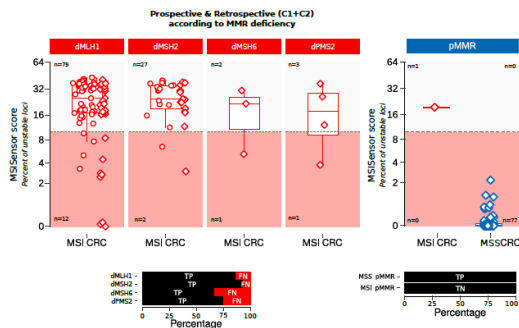
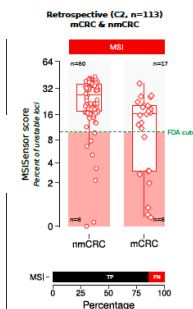
mCRC, Treated with ICI, whole Exome

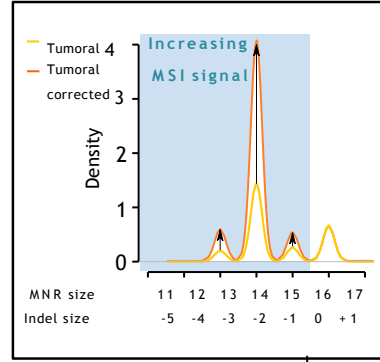
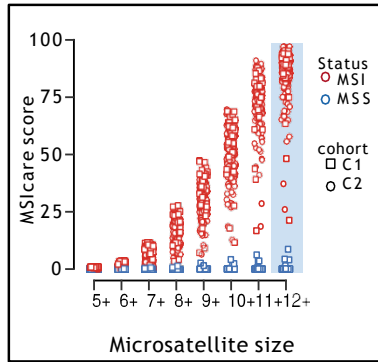
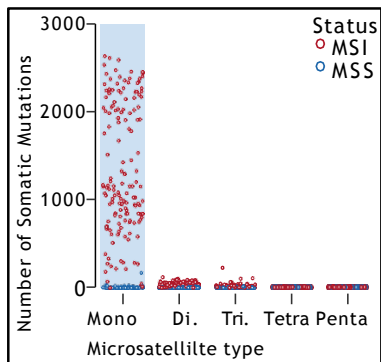


mCRC and nmCRC, MSK-Impact gene panel



nmCRC, whole Exome





NGS-based
MSI testing
in cancer

i) Exclusive
consideration of MNR

ii) Selection of long
MNR ≥ 12 bp

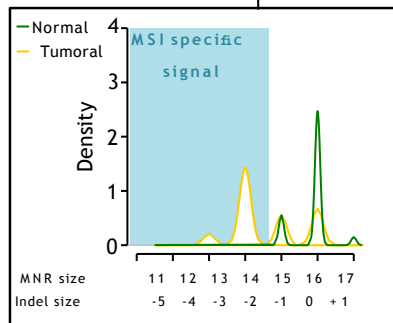
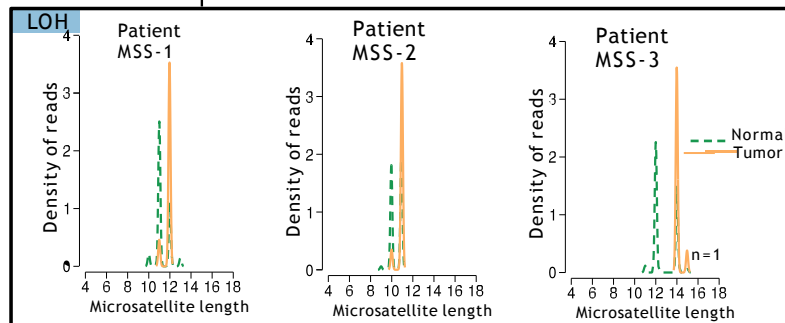
iii) Exclusion of
LOH at DNA repeats

iv) Normalization of the
MSI signal

v) Consideration of
deletion ≥ 2 bp

SENSITIVITY

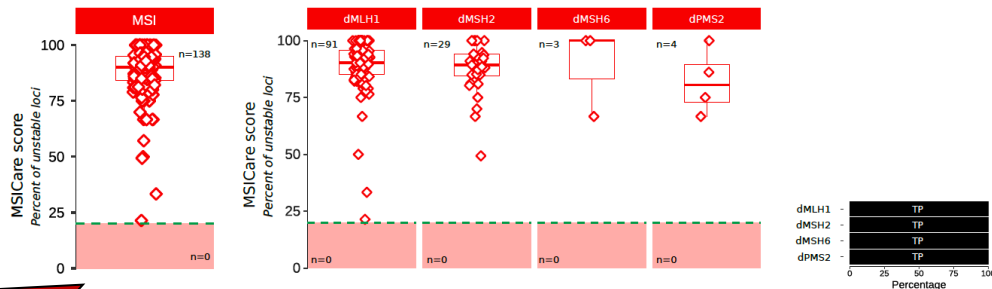
SPECIFICITY



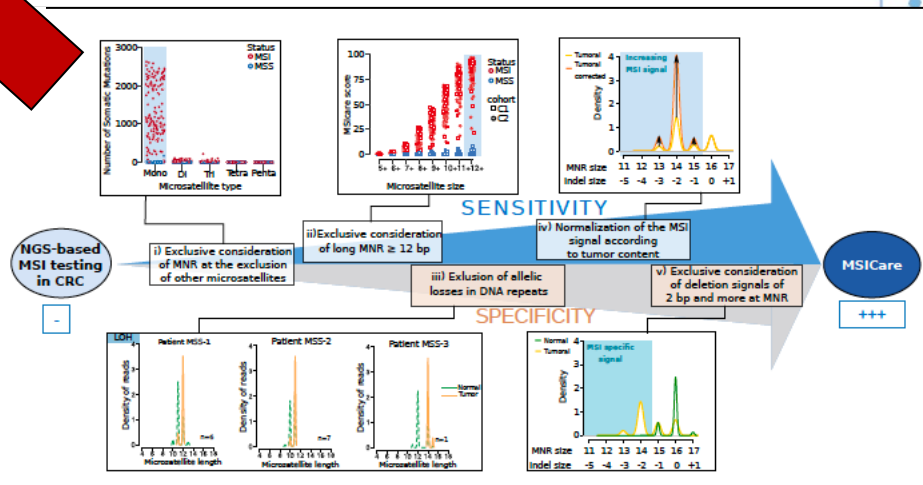
NGS
MSICare

+++

MSICare in C1 and C2 with partial WES data restricted to MSK-IMPACT™

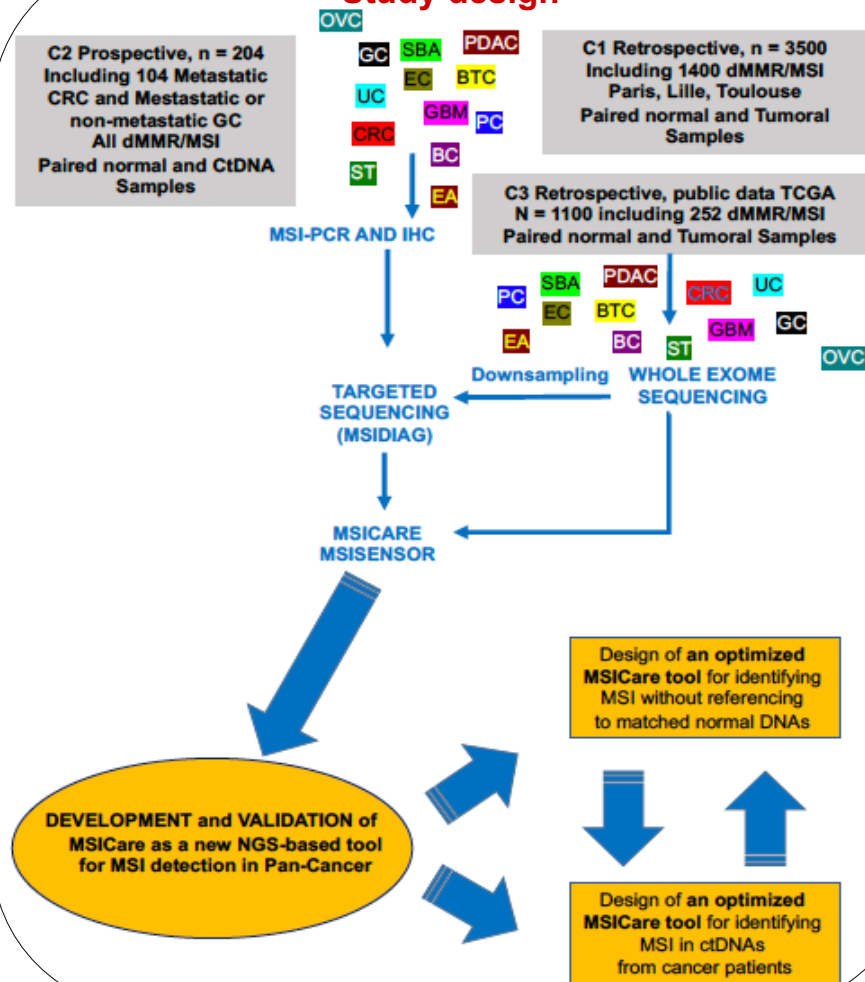


NGS-based MSI testing in CRC



Ratovomanana, in revision

Study design



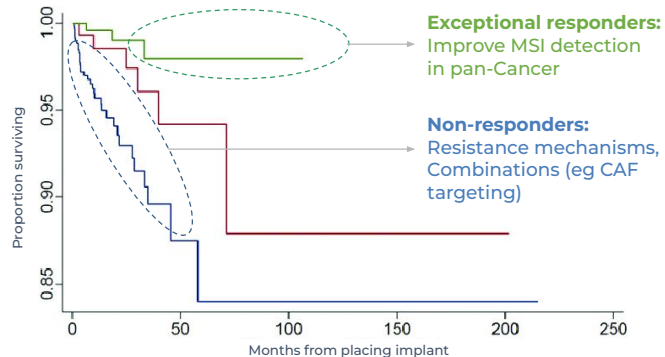
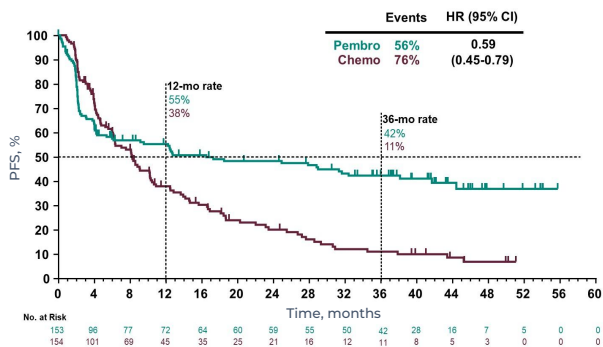
MSD  **VENIR**
améliorer la Vie ENsemble
par l'Innovation et la Recherche

Towards precision immunotherapy for MSI cancers

MSI cancers are among the cancer types that derive the highest benefit from treatment with immune checkpoint blockade

- ➔ To improve the methods for the **diagnosis** MSI using **MSICare** in pan-Cancer
- ➔ To identify **biomarkers of resistance** to refine therapeutic strategies

Pembro vs chemo in MSI/MMRd CRC



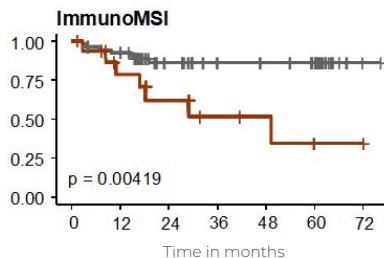
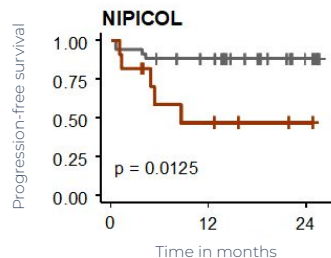
Refine strategies for MSI cancers not responding to single agent ICB



IRP3

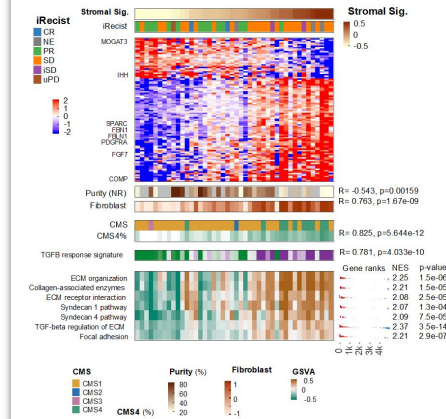
Identification of a stromal signature predicting resistance to ICB in patients with metastatic MMRd CRC

Revalidation in ongoing clinical trials



Stromal signature

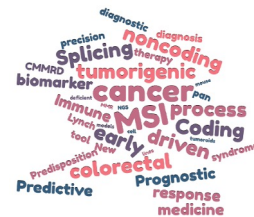
— low — high



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Founded: January 2006

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THANK YOU

