Webinar ItaLynch

21 ottobre 2020

FONDAZIONE GISCAD

GRUPPO ITALIANO PER LO STUDIO DEI CARCINOMI DELL'APPARATO DIGERENTE

Mutazioni in *BRAF* e ipermetilazione di *MLH1*

Matteo FASSAN, MD, PhD

Professor of Pathology

Department of Medicine (DIMED)

Surgical Pathology Unit

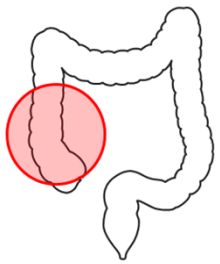
University of Padua - ITALY

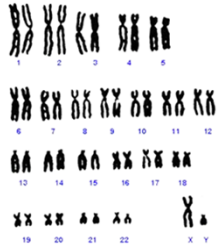
SIAPEC - IAP

1222·2022 800 ANNI

UNIVERSITÀ DEGLI STUDI DI PADOVA

15% CRC are defective in DNA mismatch repair (dMMR)





proximal

diploid

20%

"Double hit"

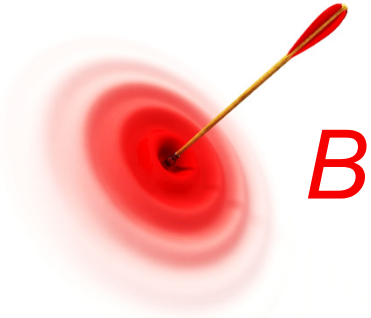
hereditary
mutations in MMR genes

HT Lynch

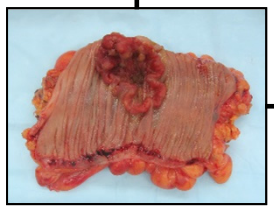
80%

Methylator
phenotype

sporadic
MLH1 methylation



BRAF



Specimen

MSI testing

Normal

MSI high

IHC

Loss of MLH1 & PMS2

Loss of other MMR proteins

BRAF or *MLH1* promoter status

Presence of *BRAF* mutation and/or *MLH1* promoter hypermethylation

No further testing

Absence of *BRAF* mutation and/or *MLH1* promoter hypermethylation

Refer to genetic counseling for consideration for germline testing (guided by IHC)

Universal screening Lynch syndrome – Targeted therapy

urrent prognostic and predictive biomarkers for gastrointestinal tumors in the linical practice

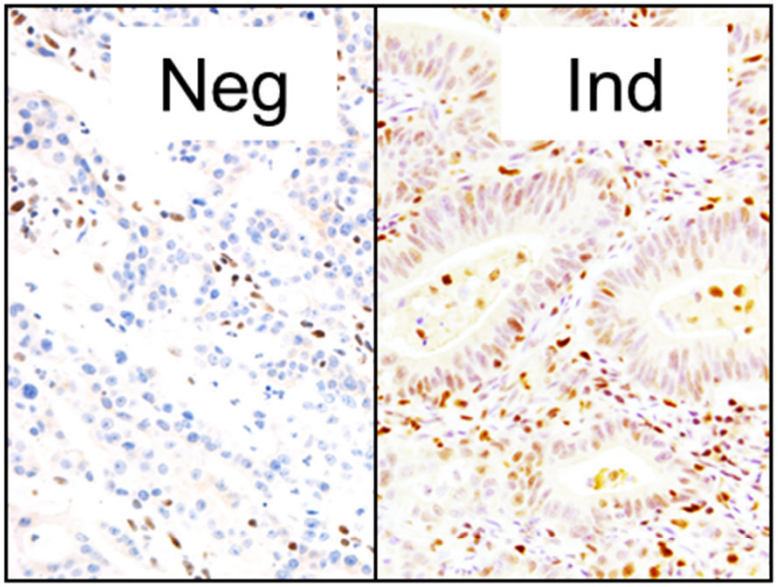
atteo Fassan[1,2], Aldo Scarpa[3,4], Andrea Remo[5], Giovanna De Maglio[6], Giancarlo Troncone[7], Antonio archetti[8], Claudio Doglioni[9,10], Giuseppe Ingravallo[11], Giuseppe Perrone[12], Paola Parente13, Claudio uchini[4], Luca Mastracci[14,15]

Article in press - 2020

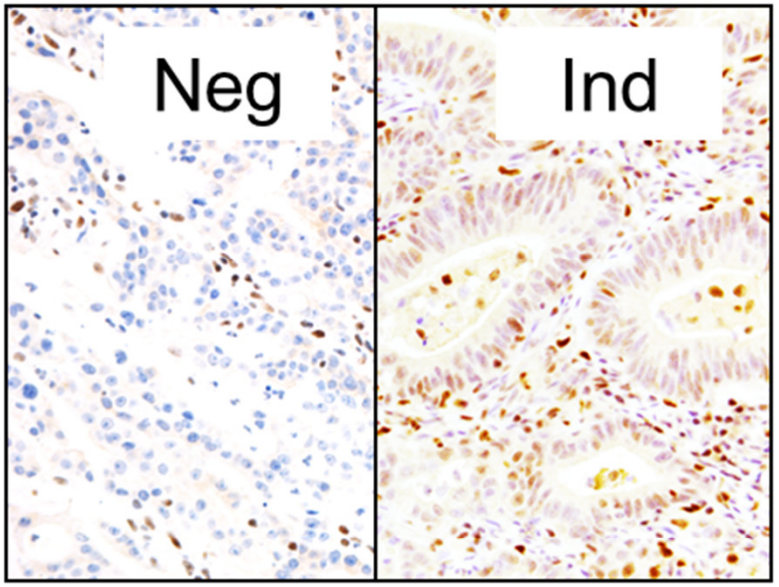
800 ANNI 1222·2022

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				

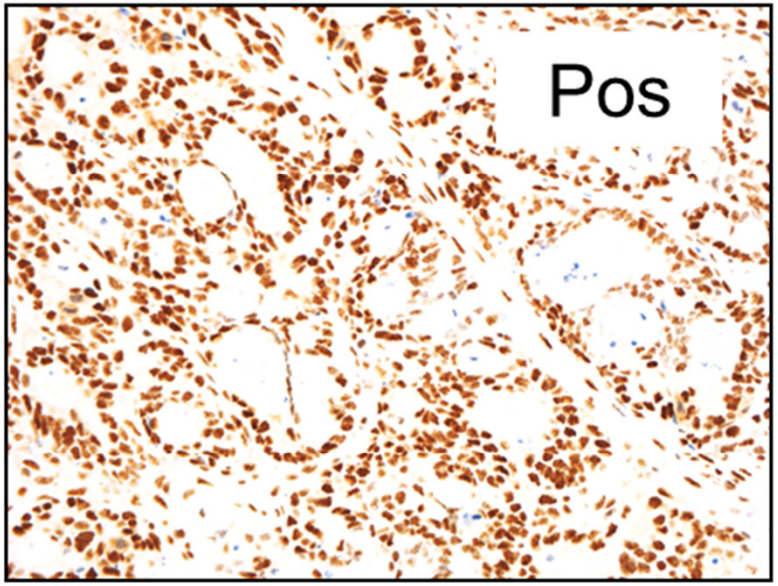
MLH1



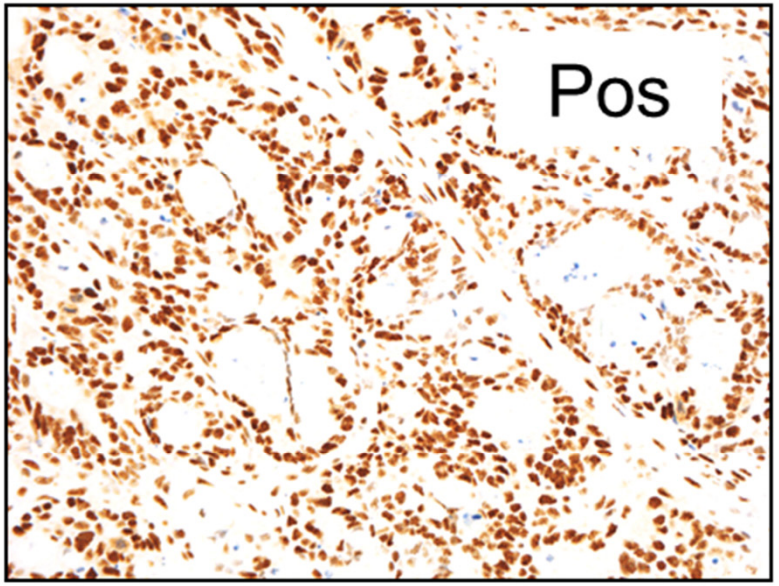
PMS2

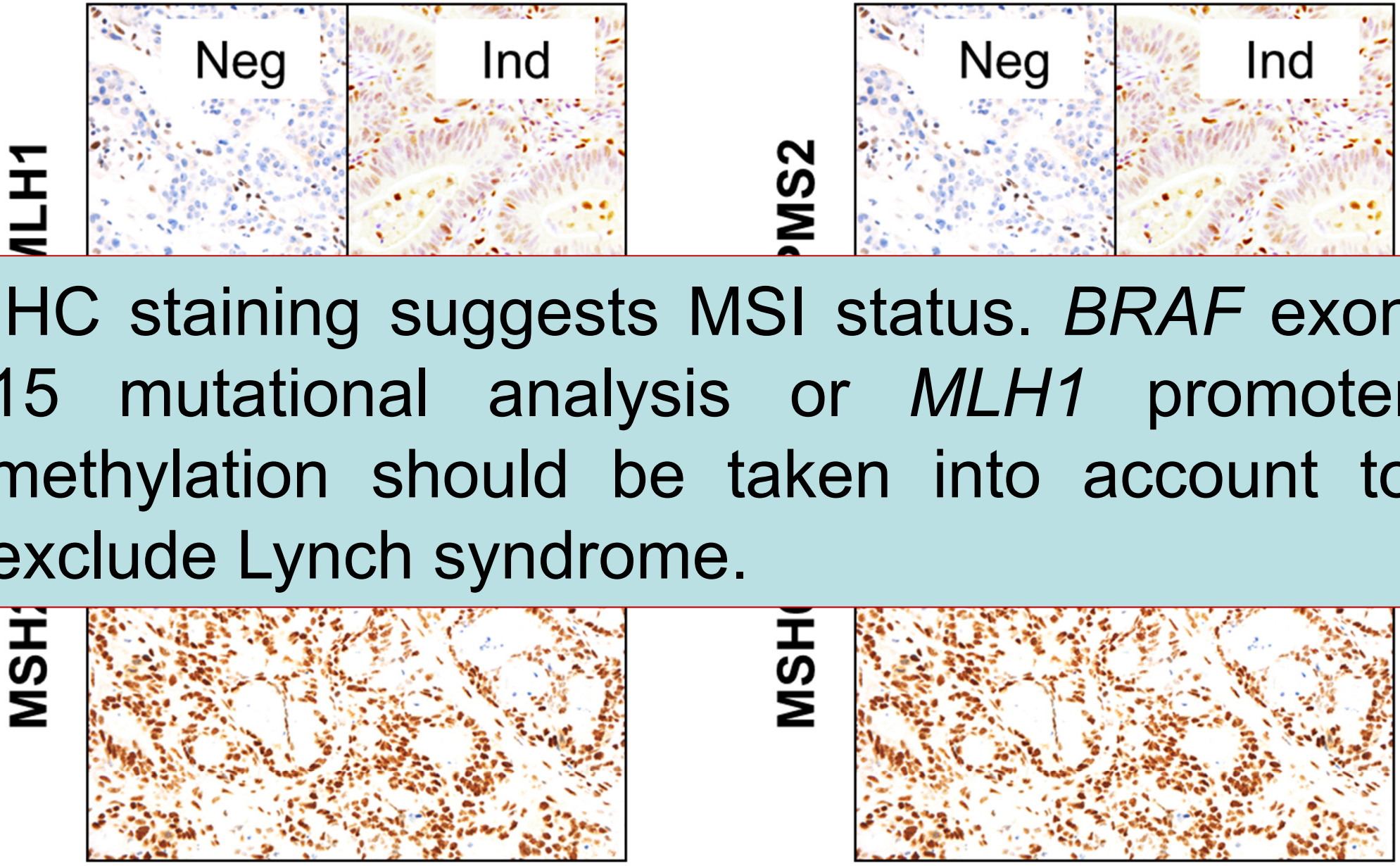


MSH2



MSH6





IHC staining suggests MSI status. *BRAF* exon 15 mutational analysis or *MLH1* promoter methylation should be taken into account to exclude Lynch syndrome.

EMQN Colon 2020

The European Molecular Genetics Quality Network

Methodology
Commercial kit/gene
Agena Bioscience
OncoFOCUS™ Panel
AmoyDx
AmoyDX® BRAF Mutations Detection Kit
AmoyDX® BRAF Mutations Detection Kit v2
AmoyDX® BRAF V600E Mutations Detection Kit
AmoyDX® EGFR Mutations Detection Kit
AmoyDX® KRAS Mutations Detection Kit
AmoyDX® KRAS Mutations Detection Kit v1
AmoyDX® KRAS Mutations Detection Kit v2
AmoyDX® KRAS/NRAS Mutations Detection Kit
AmoyDX® KRAS/NRAS/BRAF Mutations Detection Kit
AmoyDX® KRAS/NRAS/PIK3CA/BRAF Mutations Detection Kit
AmoyDX® NRAS Mutations Detection Kit v1
BioCartis
Idylla™
Idylla™ EGFR mutation test
Idylla™ KRAS mutation test
Idylla™ KRAS mutation Test V3.3
Idylla™ KRAS, NRAS-BRAF Mutation test
Idylla™ NRAS - BRAF Mutation Test
Idylla™ NRAS/BRAF mutation test
Idylla™ NRAS-BRAF Mutation Test
Idylla™ KRAS Mutation Test CEIVD
Idylla™ KRAS Mutation Test V3/ Idylla™ NRAS-BRAF Mutation Test V1
Idylla™ KRAS/NRAS/BRAF Mutation Test CEIVD
Idylla™ NRAS-BRAF Mutation Test CE-IVD
BioRad
Biorad ddPCR™ BRAF 600 Screening Kit
Diatech Pharmacogenetics
Anti-EGFR MoAb response® (BRAF STATUS)
Anti-EGFR MoAb response® (BRAF/KRAS/NRAS STATUS)
Anti-EGFR MoAb response® (KRAS STATUS)
Anti-EGFR MoAb response® (NRAS STATUS)
Anti-EGFR MoAb response®BRAF status /Easy® KRAS/NRAS/BRAF/EGFR
Easy® BRAF
Easy® KRAS
Easy® KRAS/NRAS/BRAF
Easy® KRAS/NRAS/BRAF/EGFR
Easy® NRAS
EasyPGX® ready BRAF
EasyPGX® ready KRAS
EasyPGX® ready KRAS/ BRAF,Easy® NRAS,
EasyPGX® ready KRAS/ BRAF/NRAS
EasyPGX® ready NRAS
Myriapod® Colon Status
Myriapod® Colon Status/lung status
PentaPanel® KRAS/NRAS/BRAF/EGFR/PIK3CA
EntroGen
BRAF Mutation Detection Kit
EGFR Mutation Detection Kit
KRAS Mutation Detection Kit
KRAS/BRAF Mutation Detection Kit
KRAS/BRAF/NRAS Mutation Detection Kits
KRAS/NRAS Mutation Detection Kit
KRAS/NRAS Mutation Detection Kit/BRAF Codon 600 mutation detection kit II/RAS c.59/117 Mutation Detection Kit
NRAS Mutation Detection Kit
RAS mutation screening panel V1.4
RAS mutation screening panel V1.6
Panagene
PNAClamp™ KRAS V4
Qiagen
therascreen® BRAF Pyro Kit
therascreen® KRAS Pyro Kit
therascreen® Ras Extension Pyro Kit
therascreen® BRAF RGQ PCR Kit
therascreen® BRAF RGQ PCR Kit v2
therascreen® KRAS Pyro® Kit v1, therascreen® RAS Extension Pyro Kit v1, therascreen® BRAF Pyro Kit v2
ViennaLab
KRAS XL/BRAF 600/601/NRAS XL StripAssay®
High Resolution Melting
Sequencing (NGS)
Sequencing (NGS - exome)
Sequencing (Sanger)
Other LDT
Grand Total

Raccomandazioni per la determinazione dello stato mutazionale di *BRAF* nel melanoma

A cura del Gruppo di Lavoro di AIOM e SIAPEC-IAP

AIOM: Referenti Programma Nazionale: Carmine Pinto (Bologna), Nicola Normanno (Napoli); Esperti: Paolo Ascierto (Napoli), Alessandro Testori (Milano), Michele Del Vecchio (Milano), Vanna Chiarion Sileni (Padova), Michele Maio (Siena), Paola Queirolo (Genova)

SIAPEC-IAP: Referenti Programma Nazionale: Claudio Clemente (Milano), Gian Luigi Taddei (Firenze); Esperti: Massimo Barberis (Milano), Gerardo Botti (Napoli), Guido Collina (Bologna), Gerardo Ferrara (Benevento), Antonio Marchetti (Chieti), Daniela Massi (Firenze), Maria Cristina Montesco (Padova), Stefania Staibano (Napoli)

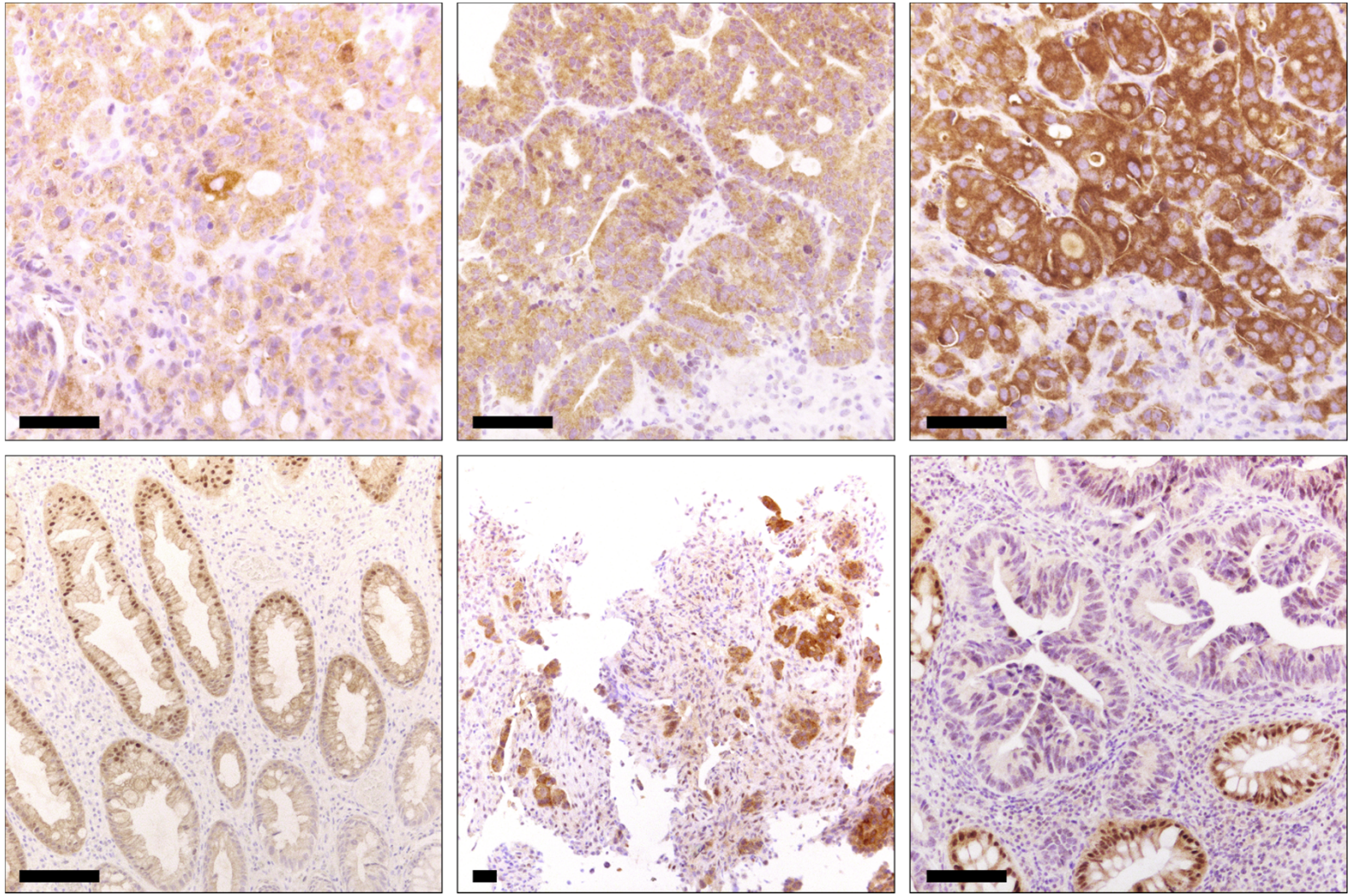
AIOM

SIAPEC – IAP

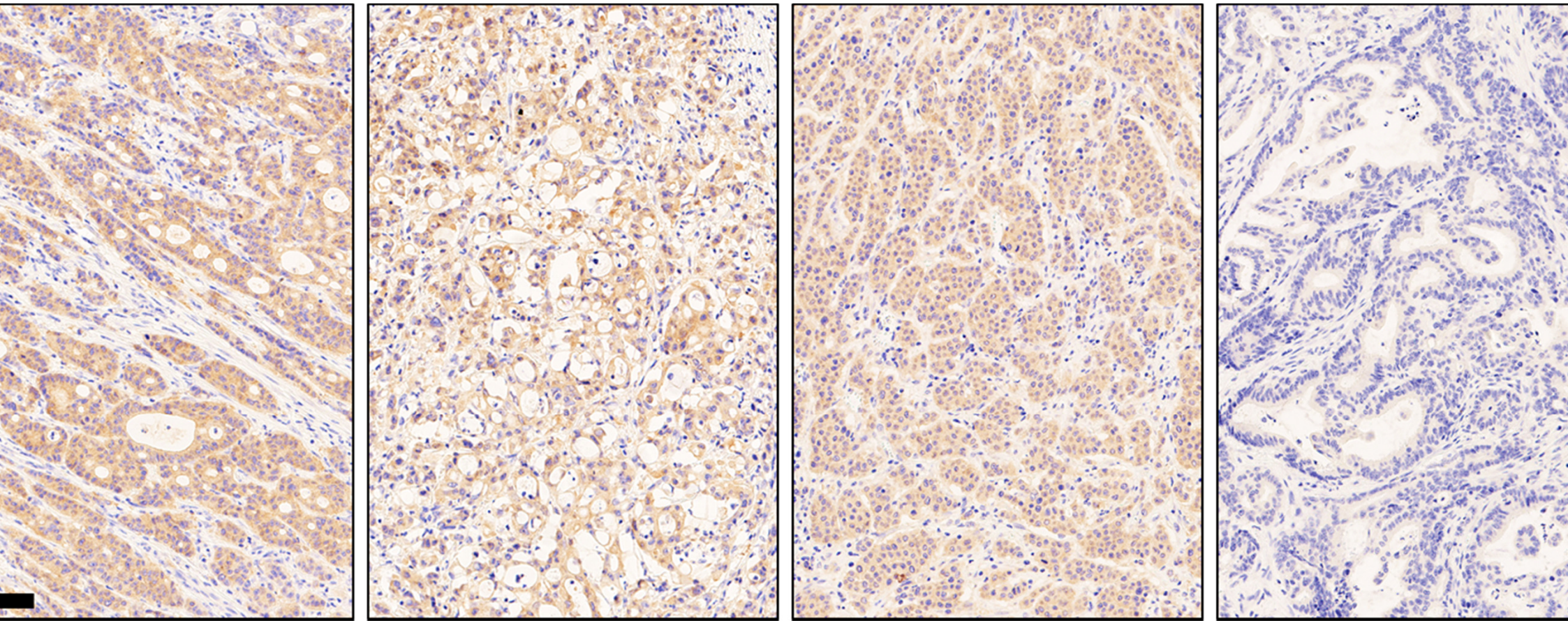
1222·2022 800 ANNI

BRAF p.V600E-specific immunohistochemical assessment in colorectal cancer endoscopy biopsies is consistent with the mutational profiling

Galuppini F, *et al*. Histopathology 2017



BRAF p.V600E-specific IHC assessment in old FFPE specimens



Luchini C, *et al*. – In preparation

JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology

STAGE IV

KRAS/NRAS/BRAF

ALL STAGES

MMR or MSI

Evaluation performed by the pathologist on metastatic samples

Loss of MLH1 in stages I-III

BRAF p.V600E

No *PI3KCA*, *PTEN*, CIMP

J Clin Oncol 2017

The NEW ENGLAND JOURNAL of MEDICINE

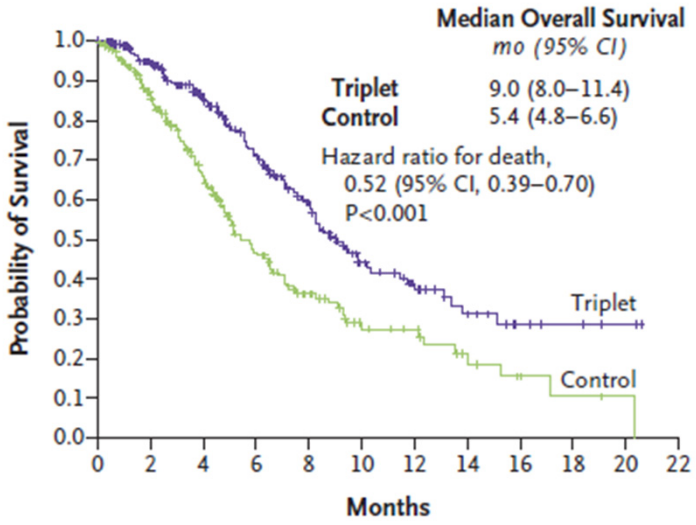
ORIGINAL ARTICLE

Encorafenib, Binimetinib, and Cetuximab in *BRAF* V600E–Mutated Colorectal Cancer

S. Kopetz, A. Grothey, R. Yaeger, E. Van Cutsem, J. Desai, T. Yoshino, H. Wasan, F. Ciardiello, F. Loupakis, Y.S. Hong, N. Steeghs, T.K. Guren, H.-T. Arkenau, P. Garcia-Alfonso, P. Pfeiffer, S. Orlov, S. Lonardi, E. Elez, T.-W. Kim, J.H.M. Schellens, C. Guo, A. Krishnan, J. Dekervel, V. Morris, A. Calvo Ferrandiz, L.S. Tarpgaard, M. Braun, A. Gollerkeri, C. Keir, K. Maharry, M. Pickard, J. Christy-Bittel, L. Anderson, V. Sandor, and J. Tabernero

A combination of encorafenib (anti BRAF), cetuximab, and binimetinib (anti MEK) resulted in significantly longer overall survival and a higher response rate than standard therapy in patients with metastatic colorectal cancer with the *BRAF* V600E mutation.

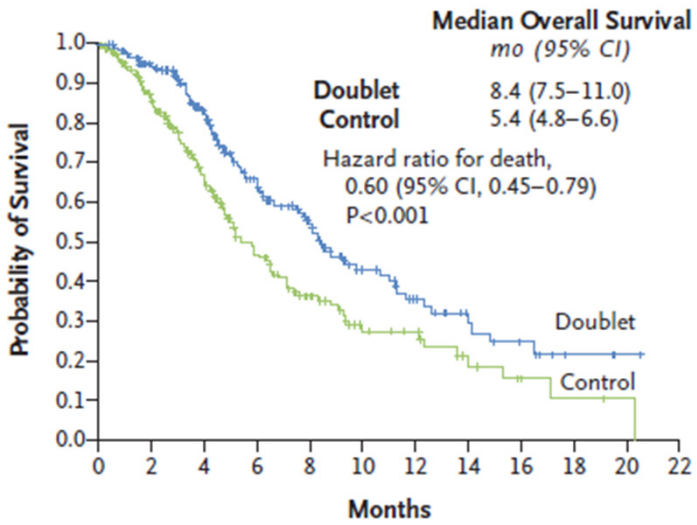
Overall Survival, Triplet Regimen vs. Control



No. at Risk

Triplet	224	186	141	103	69	37	24	14	6	4	2	0
Control	221	158	102	60	34	18	15	7	4	2	1	0

Overall Survival, Doublet Regimen vs. Control

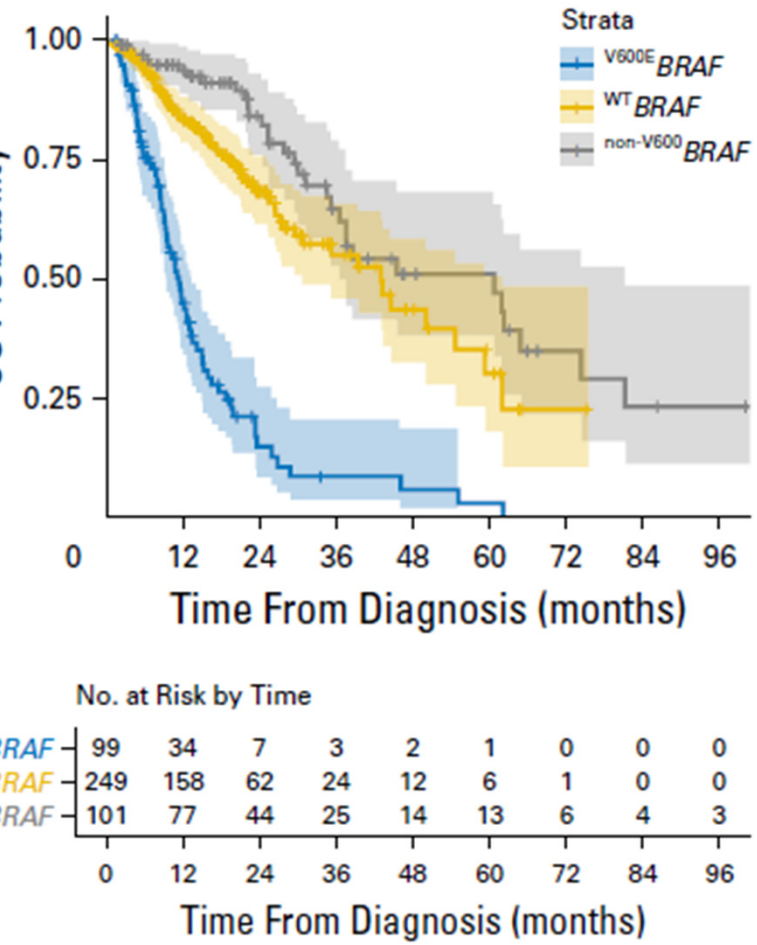


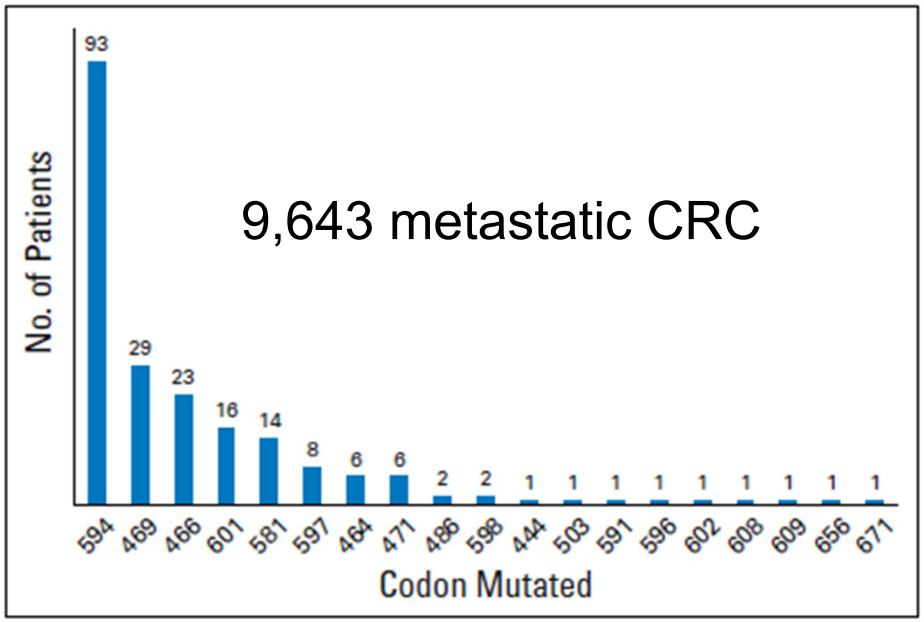
No. at Risk

Doublet	220	184	133	87	57	33	21	12	8	3	1	0
Control	221	158	102	60	34	18	15	7	4	2	1	0

The heterogeneous mutational landscape of the *BRAF* gene in CRC

Non-V600BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer





*non-V600*BRAF* mutations occurred for 22% of all *BRAF* mutations identified and were characterized by younger age, fewer female patients, fewer high-grade tumors or right-sided primary tumors.

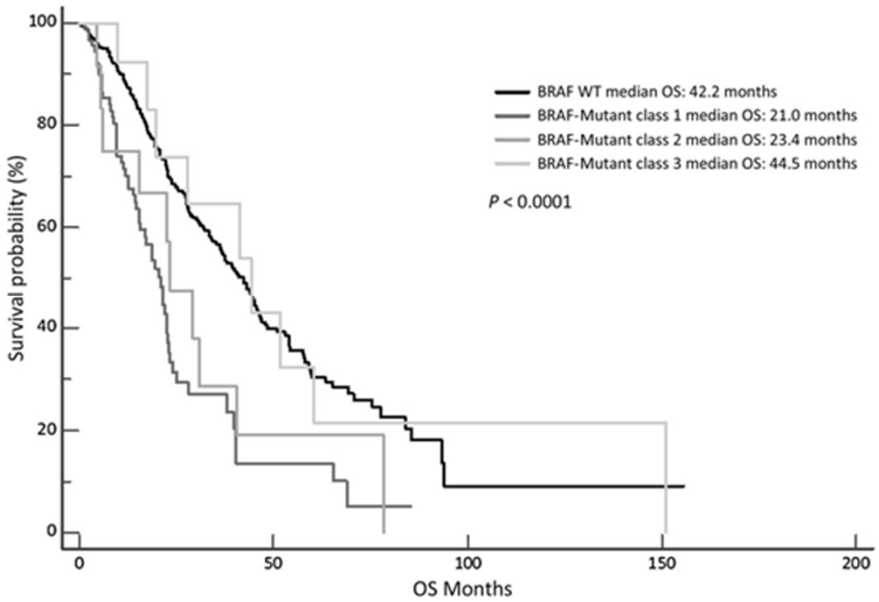
Median overall survival was significantly longer in patients with *non-V600*BRAF*-mutant metastatic CRC compared with those with both *V600E*BRAF*-mutant and wild-type *BRAF* (60.7 v 11.4 v 43.0 months, respectively; P =0.001).

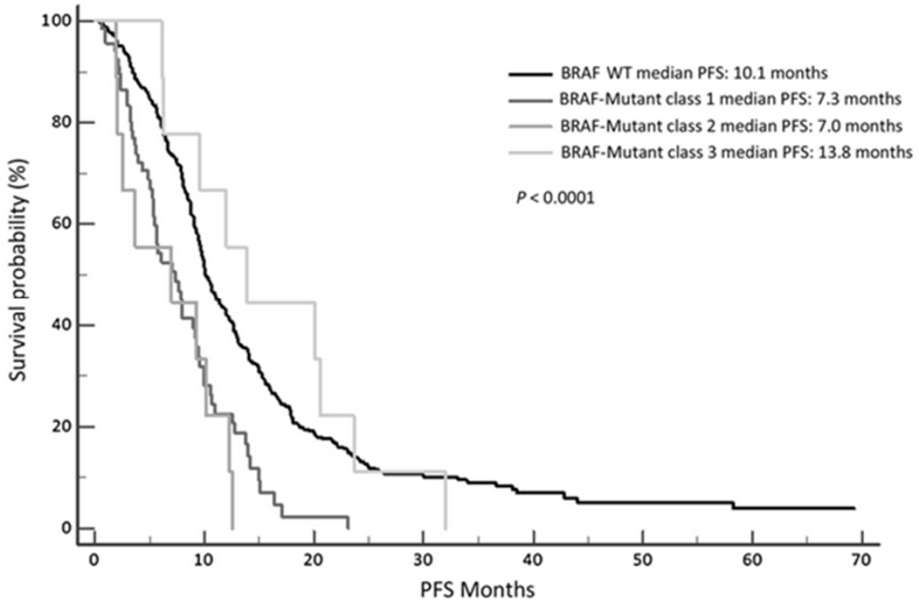
Jones JC, *et al.* – J Clin Oncol 2017

Translational Cancer Mechanisms and Therapy

Clinical Cancer Research

Class 1, 2, and 3 *BRAF*-Mutated Metastatic Colorectal Cancer: A Detailed Clinical, Pathologic, and Molecular Characterization





Class 1: codon 600

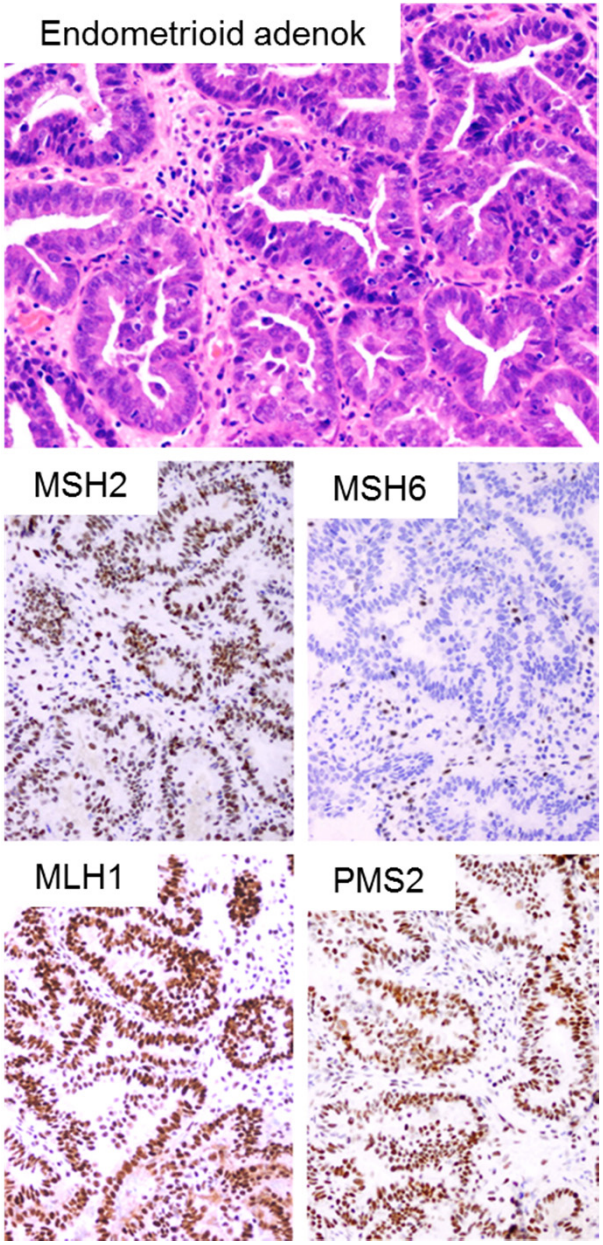
Class 2: codons 601 and 597

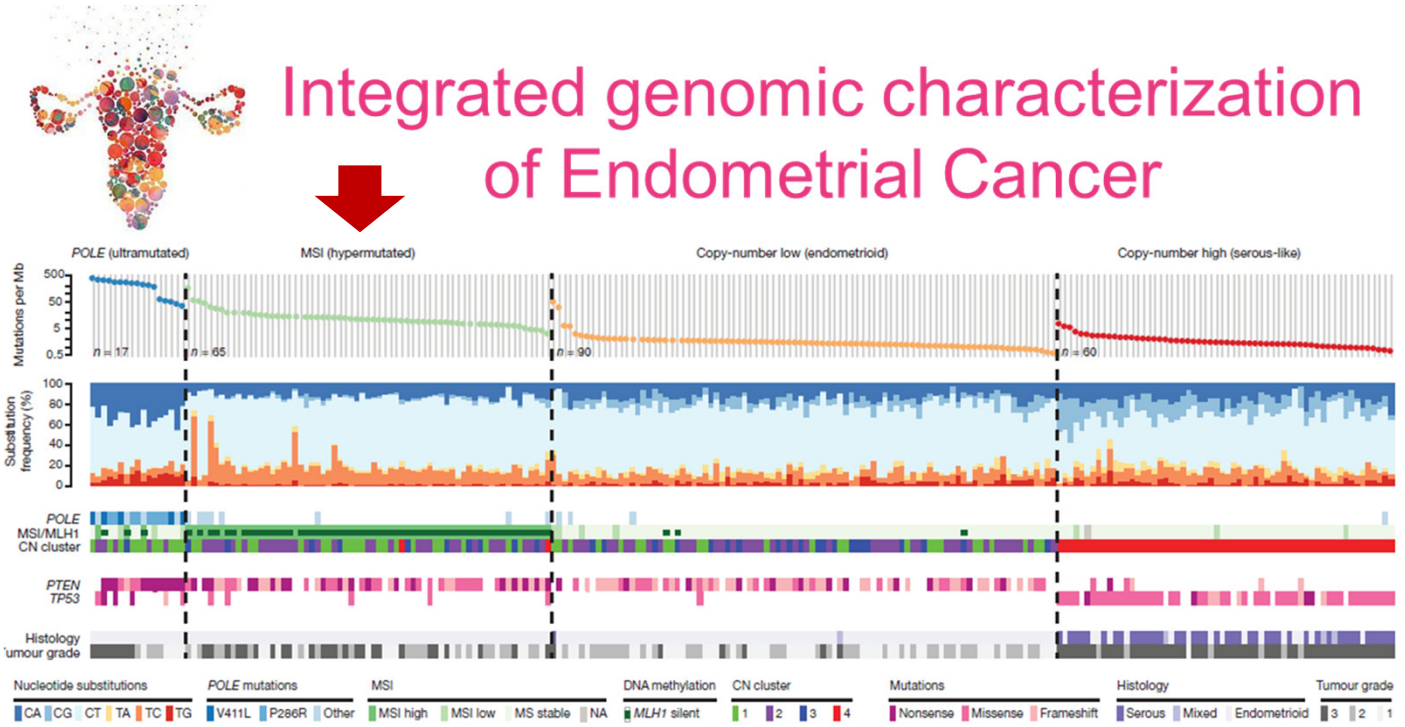
Poor prognosis

Class 3: codons 594 and 596

Similar to *BRAF* wt

Schirripa M, *et al.* – Clin Cancer Res 2019

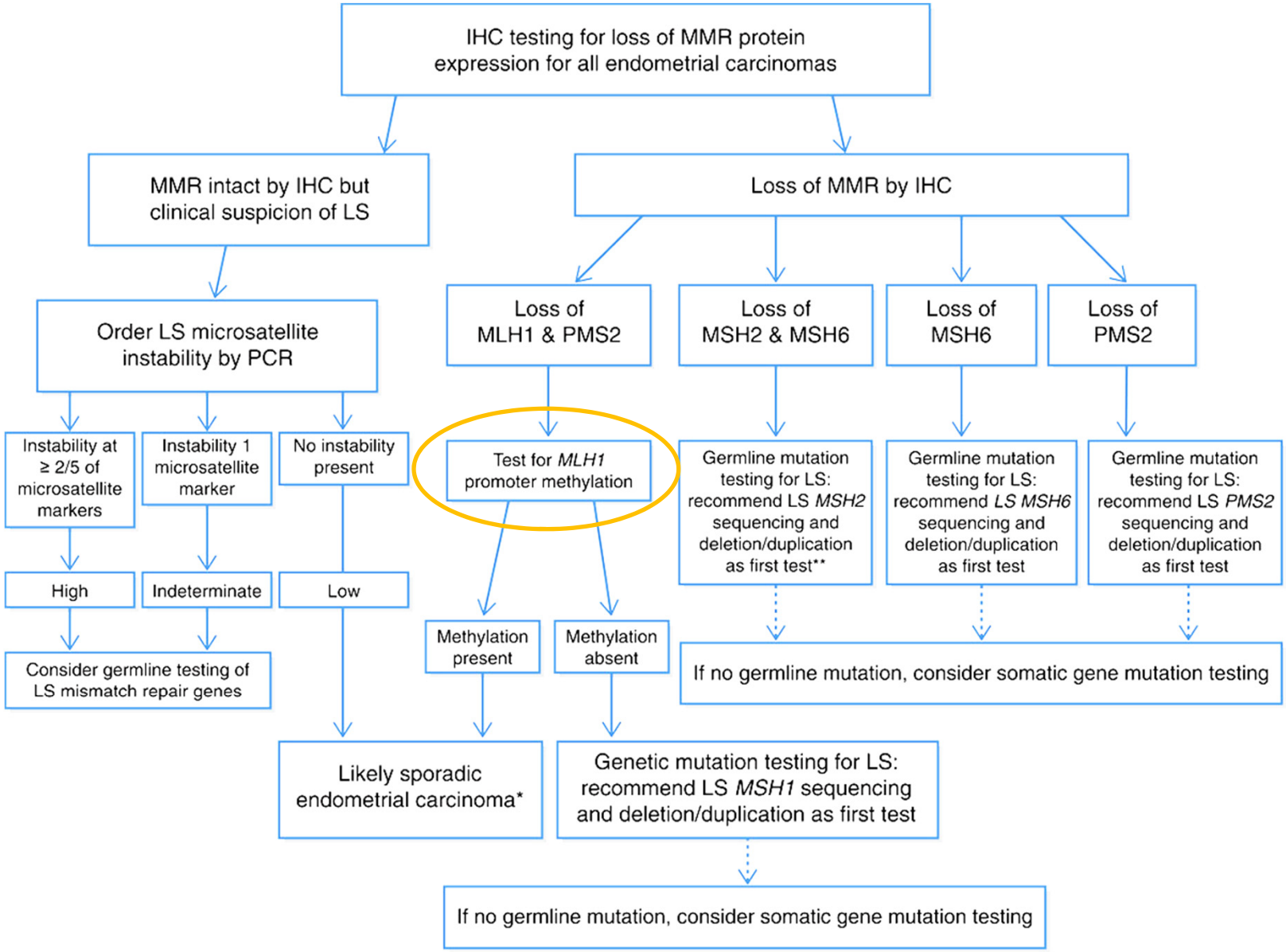




Lynch syndrome in endometrial cancer

- **5%** of endometrial cancers and ~1% of ovarian cancers.
- **No *BRAF*** mutation testing! Only PCR-based methylation testing.
- ***MSH6* mutations** are relatively more common (often MSI-low or MSS). Rethinking of MSI testing.

Mills AM & Longacre TA – Am J Surg Pathol 2016 / The Cancer Genome Atlas Research Network. – Nature 2013

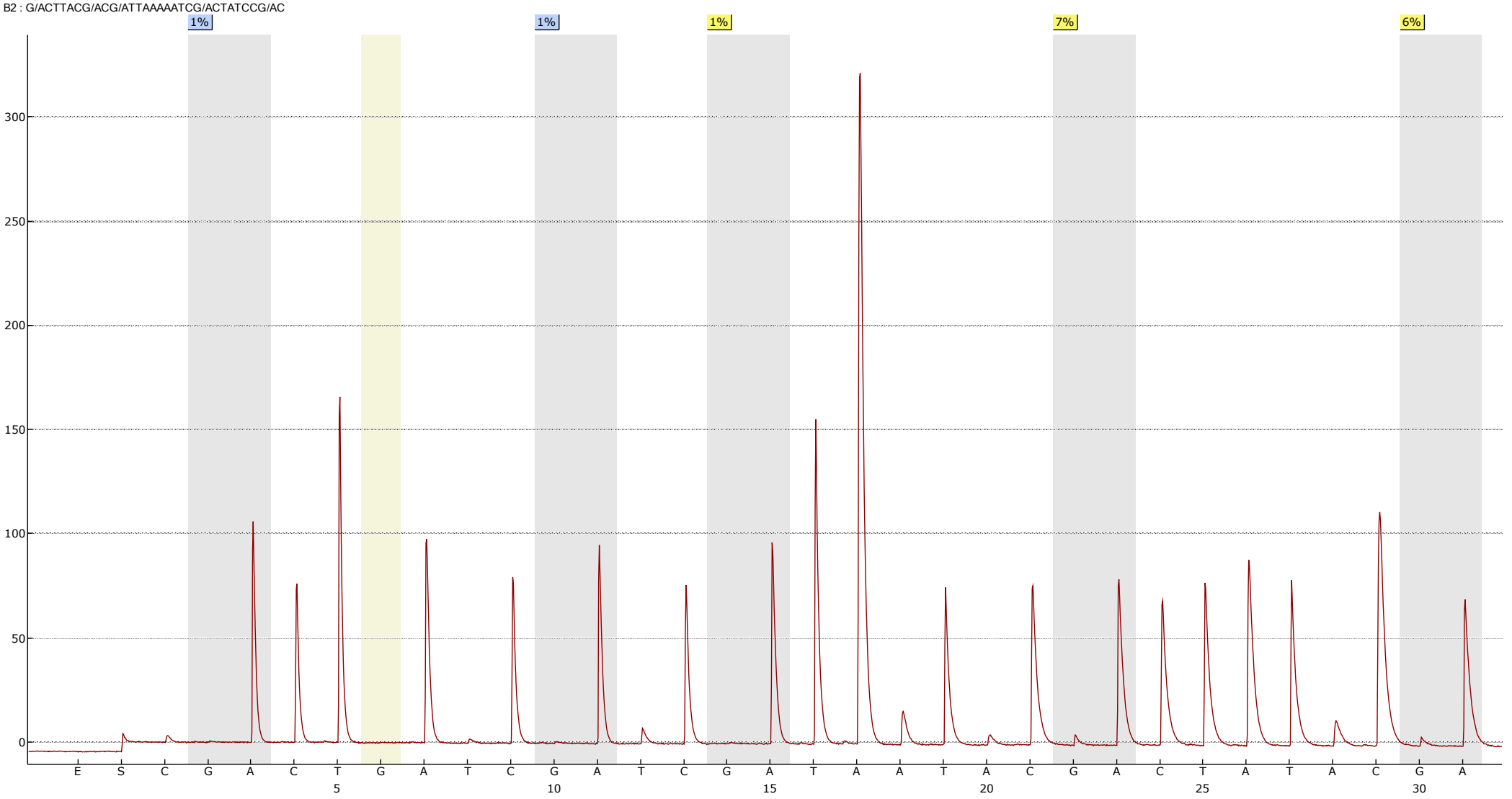


*If strong clinical suspicion for LS, consider *MLH1* promoter methylation analysis of non-neoplastic tissue/peripheral blood to evaluate for germline epigenetic *MLH1* promoter methylation.

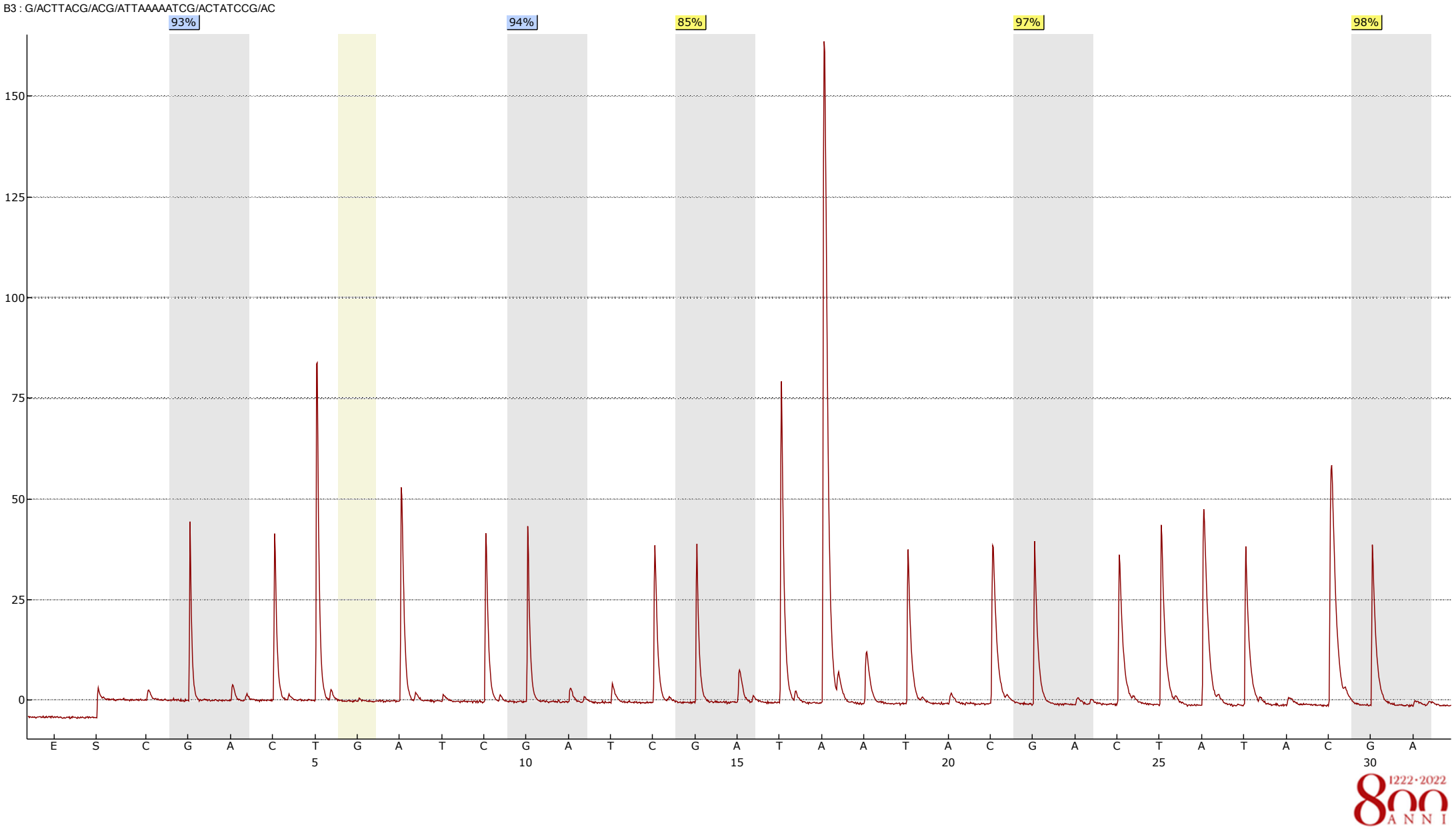
**If *MSH2* and *MSH6* unmutated, consider LS *EPCAM* sequencing and deletion/duplication.

Mills AM & Longacre TA – Am J Surg Pathol 2016

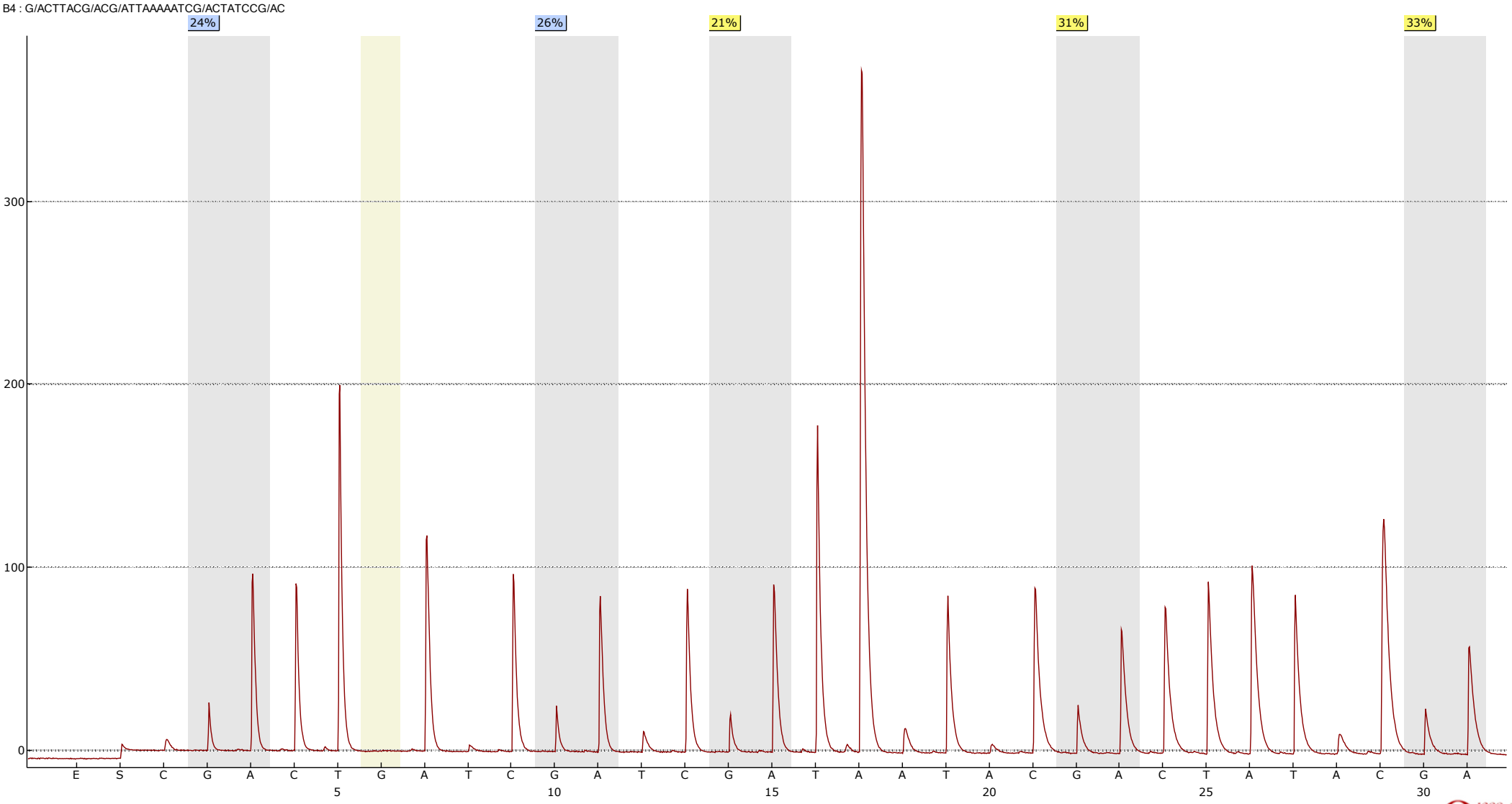
nmethylated *MLH1* promoter



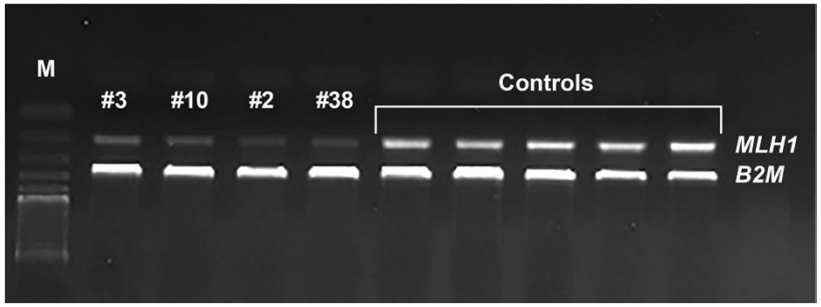
ethylated *MLH1* promoter

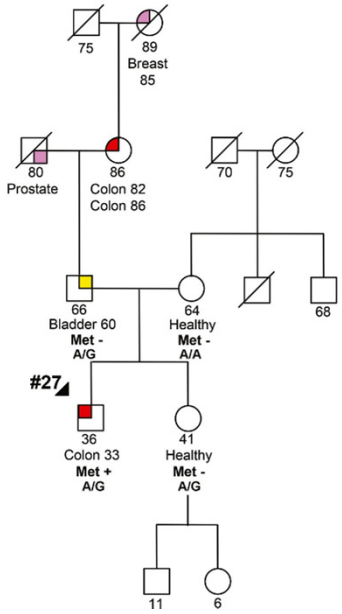


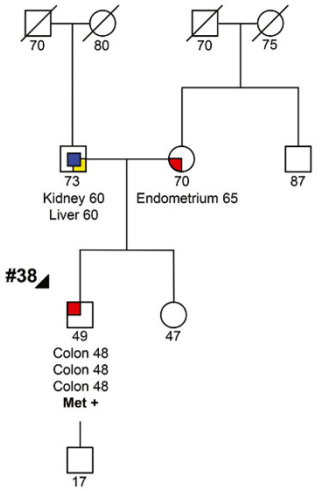
artially methylated *MLH1* promoter



ontribution of *MLH1* constitutional methylation for Lynch yndrome diagnosis in patients with tumor MLH1 downregulation







- Constitutional epimutation of *MLH1* and *MSH2* has been identified as an alternative mechanism that predisposes to the development of LS.
- *MLH1* constitutional hypermethylation is the molecular mechanism behind about 3% of LS
- Patients with early onset or multiple primary tumors without significant family history.

Pinto D, *et al* – Cancer Med 2018

1222·2022 800 ANNI

Rete Oncologica Veneta

Ricerca, innovazione, assistenza

REGIONE DEL VENETO

REGIONE DEL VENETO

ALLEGATO A DGR nr. 655 del 15 maggio 2018 pag. 1 di 5

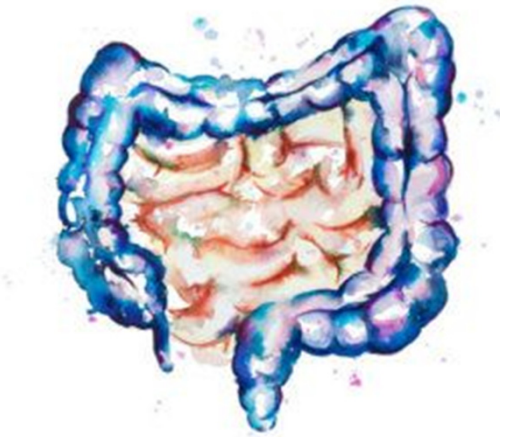
DOCUMENTO REDATTO DAL GRUPPO TECNICO DI LAVORO COSTITUITO DAL DIRETTORE GENERALE DELL'AREA SANITÀ E SOCIALE DELLA REGIONE VENETO

Coordinamento: dr. Tiziano Martello

(PROTOCOLLO REGIONE VENETO N 128975 DEL 30 MARZO 2017)

5 regional hubs for surgical pathology (~900.000 people)

3 regional hubs for molecular pathology (~1.500.000 people)



AZIENDA OSPEDALE UNIVERSITÀ PADOVA

Azienda Ospedale Università Padova

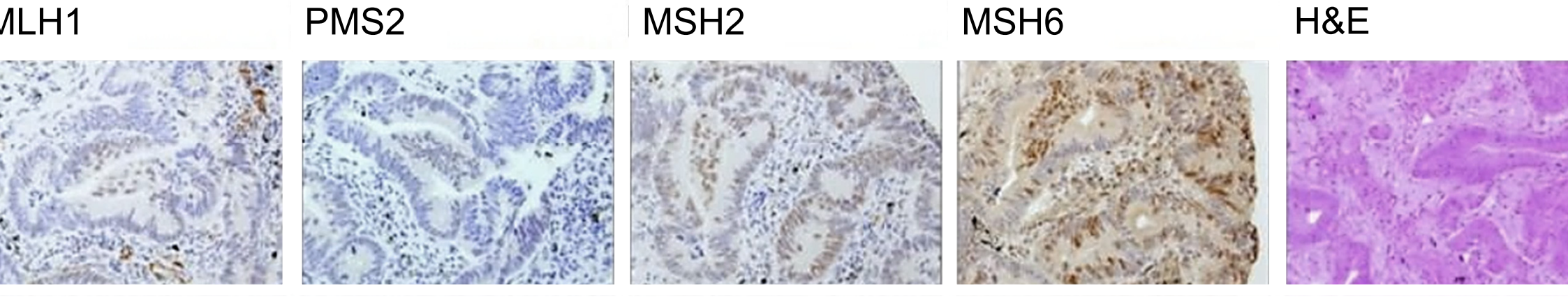
2019

590 CRC (*RAS/BRAF*)

1222·2022 800 ANNI

JAMA Oncology

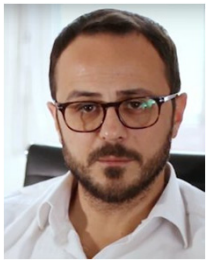
Association of Primary Resistance to Immune Checkpoint Inhibitors in Metastatic Colorectal Cancer With Misdiagnosis of Microsatellite Instability or Mismatch Repair Deficiency Status

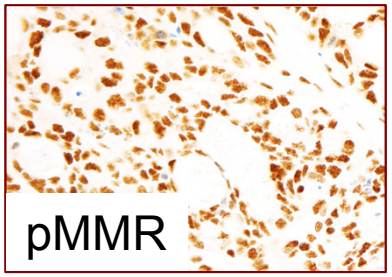


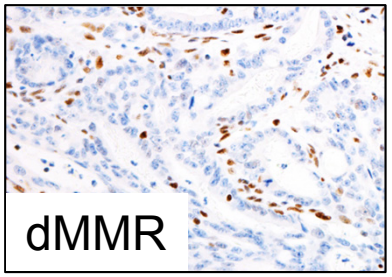
…"misdiagnosis of the MSI or dMMR status by local assessment was 10% (n = 9), with a positive predictive value of 90.3% (95% CI, 82.4%-95.0%)."

Cohen R, *et al* - JAMA Oncol 2019

DNA qualification may impact MSI testing results in mucinous colorectal adenocarcinoma







98.0%

81.8%

96.0%

45.4%

…"we demonstrated that preanalytical parameters as neoplastic cellularity and DIN may influence analytical performance for MSI testing. In particular, a minimum input of **50% of neoplastic cells** is fundamental to correct perform molecular analysis by using Idylla™ system. **DIN < 4** significantly affected TapeStation 4200 results."

Malapelle U, Parente P, *et al* - Cells 2020

Take home messages

- The clinical impact of the histology report
- MMR/MSI testing pre-analytical requirements
- The heterogeneous world of *BRAF*-mutated metastatic CRC
- The central role of the multidisciplinary team

Veneto Institute of Oncology – IOV
Fotios Loupakis
Sara Lonardi

The Institute of Cancer Research, Sutton, UK
Nicola Valeri
Chiara Braconi
Andrea Sottoriva

UniPD – Department of Molecular Medicine
Stefano Piccolo

UniPD – Department of Biology
Marina de Bernard

Padua University Hospital
Marco Scarpa

Fondazione Città della Speranza
Marco Agostini

The Ohio State University
Carlo M. Croce

University of Verona
Aldo Scarpa
Claudio Luchini

Institute of Oncology Research (IOR) - Bellinzon
Luciano Cascione

Cancer Research UK – Manchester Institute
Michela Garofalo

Semmelweis University Budapest
Andras Kiss

Ministero della Salute

AIRC

DIP. DI MEDICINA - DIMED
UNIVERSITÀ DEGLI STUDI DI PADOVA

I.O.
ISTIT
ONCOL
VEN
I.R.C.