Molecular Pathology, Predictive markers and ‘omics’ in GI Tumours Present status and the future

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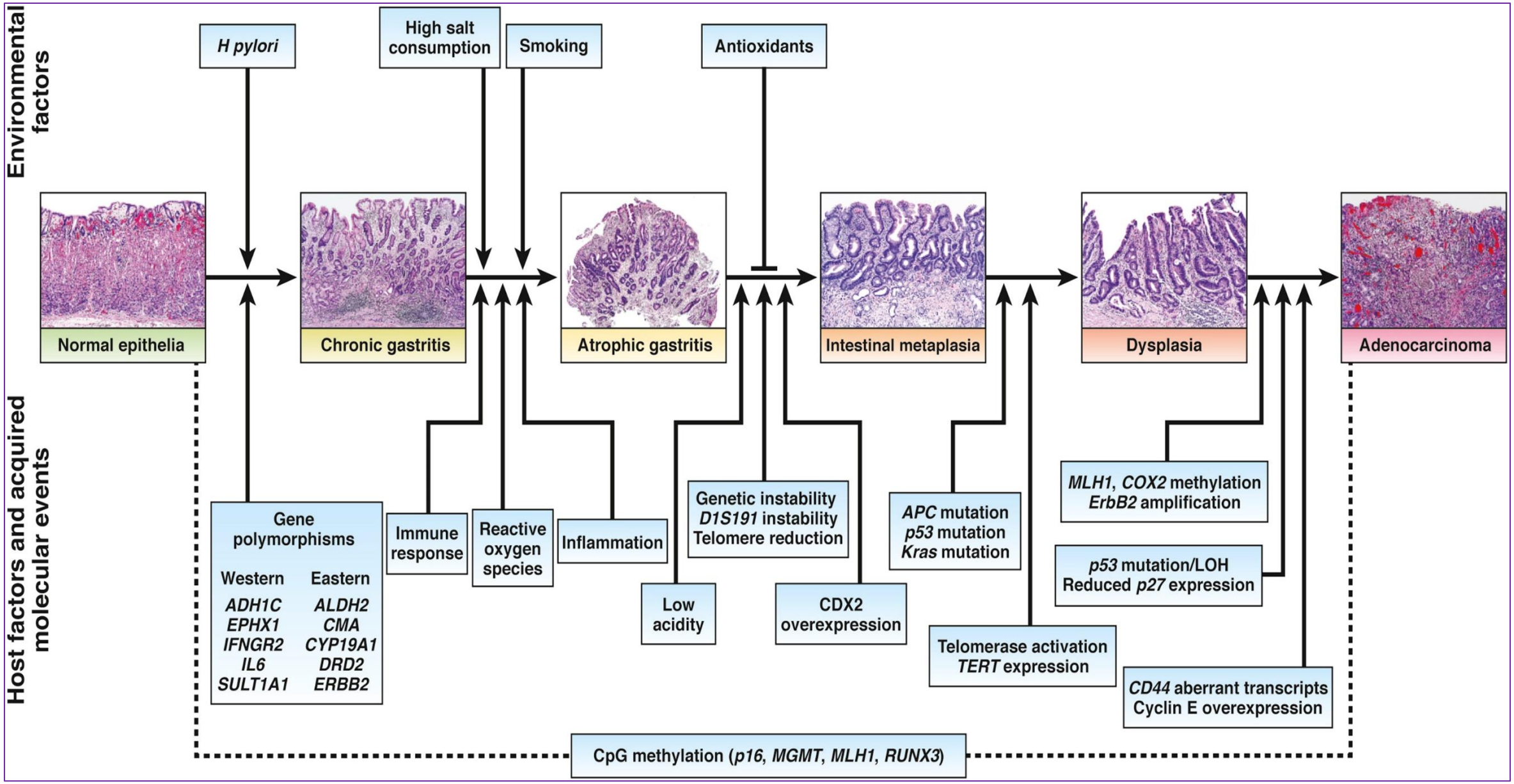
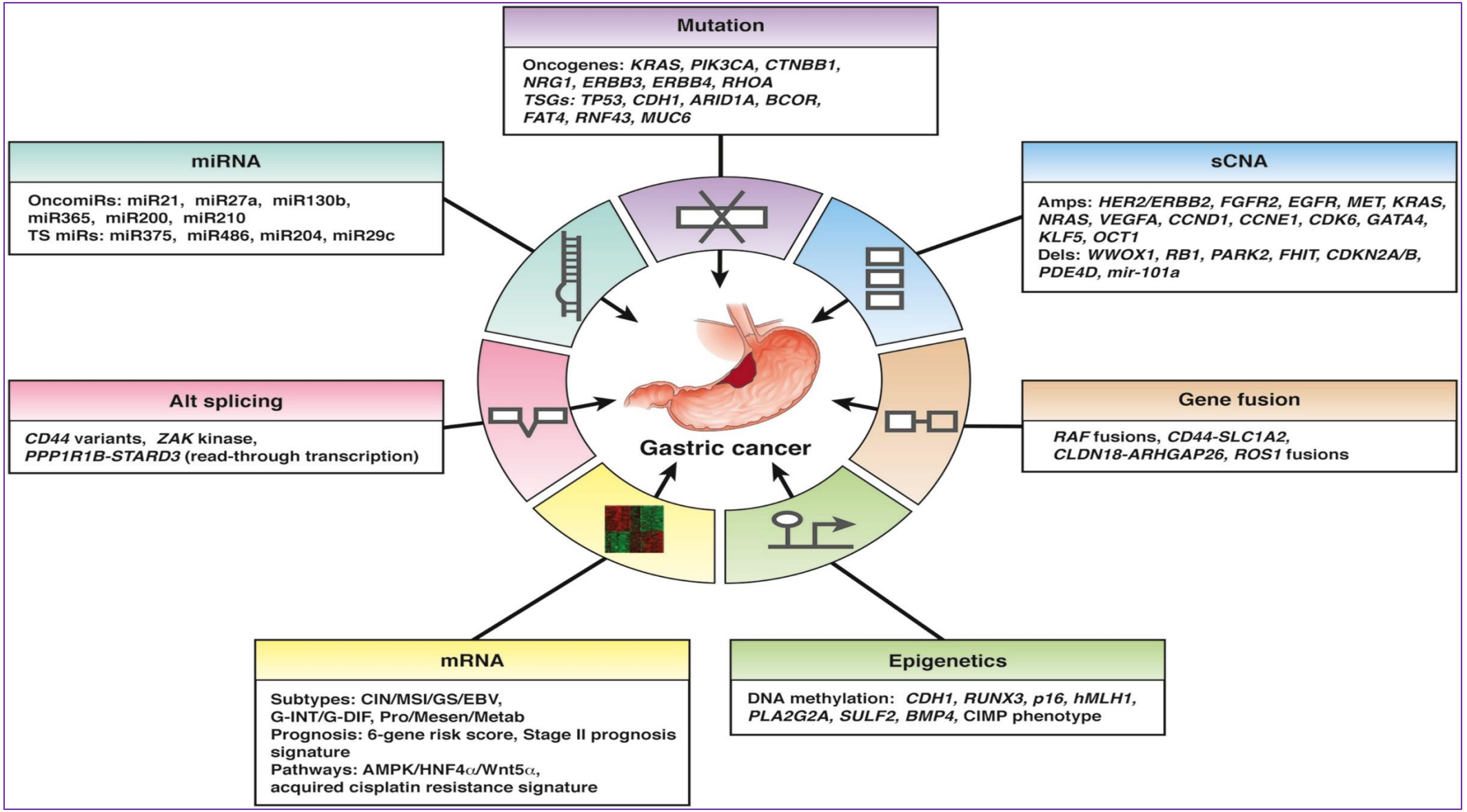
Cause and pathogenesis of intestinal-type GC



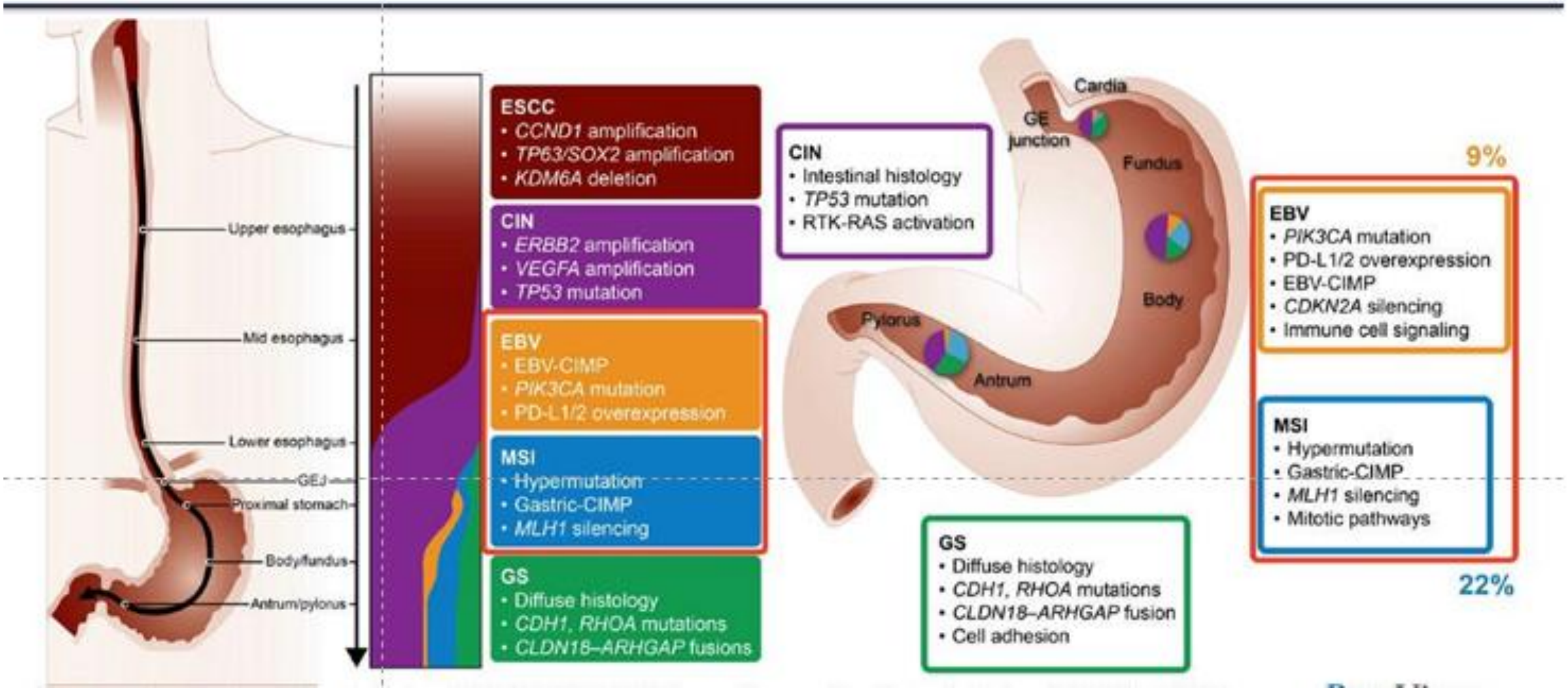
Gastroenterology 2015 149, 1153-1162.e3DOI: (10.1053/j.gastro.2015.05.059)

Molecular Genetic Landscape of Gastric Cancer



Gastroenterology 2015 149, 1153-1162.e3DOI: (10.1053/j.gastro.2015.05.059)

Esophageal & Gastric Cancer Subtypes



Cancer genome Atlas network. Nature 2014; 513: 202-209

Jass´s Molecular Classification of Colorectal Cancer

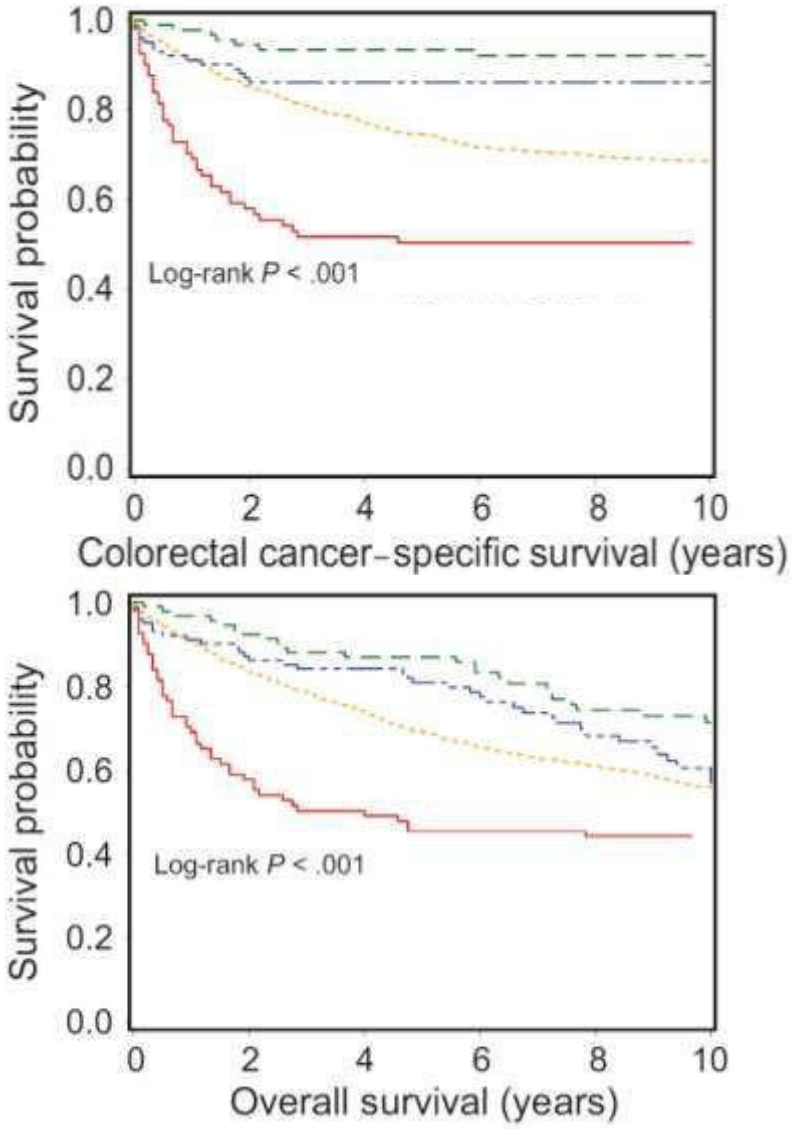
Jeremy R. Jass
1951 - 2008

Precursor lesions		
Serrated polyps/adenomas	**Group 1** CIMP-H, MSI-H, BRAFmut	12 %
	Group 2 CIMP-H, MSS/MSI-L, BRAFmut	8%
Adenomas with villi	**Group 3** CIMP-L, MSS/MSI-L, KRASmut	20 %
Tubular adenomas	**Group 4** CIMP negative, MSS	57 %
Lynch syndrome adenomas	**Group 5** CIMP negative, MSI-H	3 %

Jass J.R. Histopathology 2007, 50: 113-30

Prognostication of colorectal cancer

Jass´s Classification	Prognostic Groups	N = 1253 (100 %)
Group 5	MSI BRAFwt	92 (7,3 %)
Group 1	MSI BRAFmut	101 (8,1 %)
Group 4	MSS BRAFwt	979 (78%)
Group 2	MSS BRAFmut	81 (6,5 %)



Lochhead P et al. JNCI 2013; 105:1151

ARTICLE

doi:10.1038/nature11252

Comprehensive molecular characterization of human colon and rectal cancer

The Cancer Genome Atlas Network*

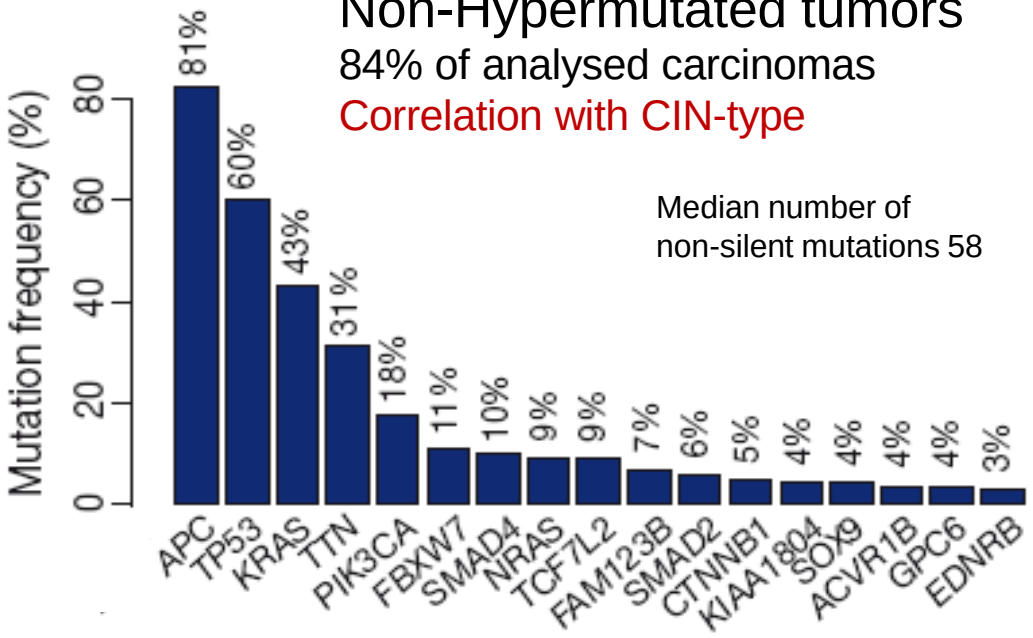
330 | NATURE | VOL 487 | 19 JULY 2012

Non-Hypermutated tumors

84% of analysed carcinomas

Correlation with CIN-type

Median number of non-silent mutations 58

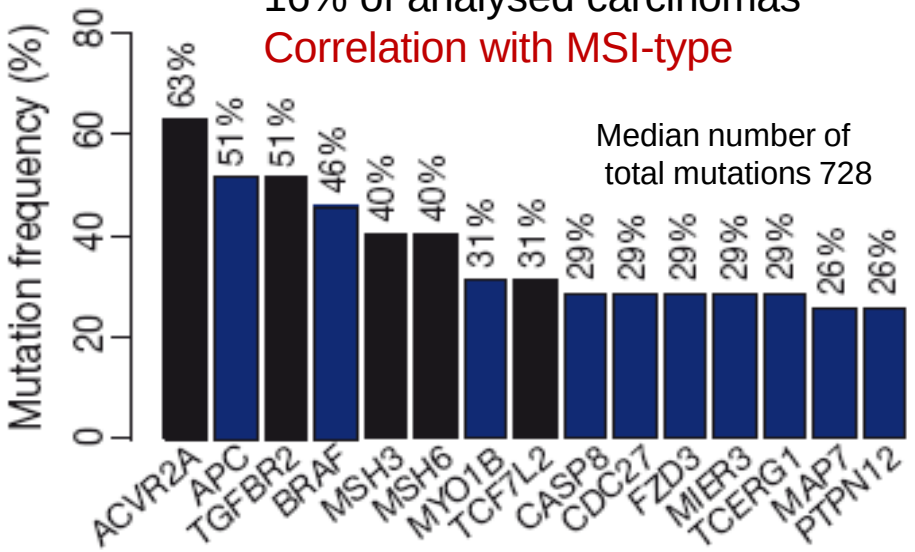


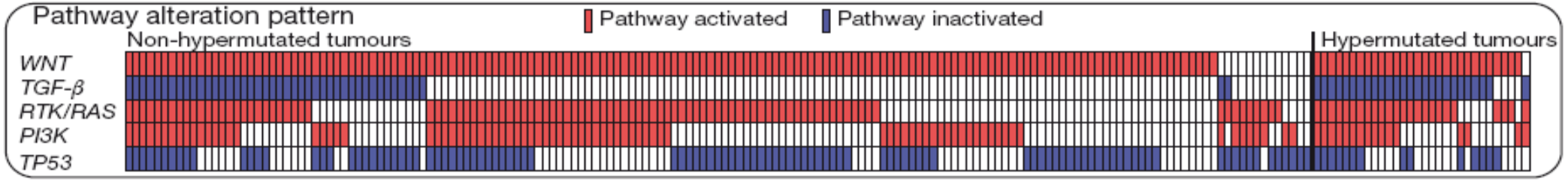
Hypermutated tumors

16% of analysed carcinomas

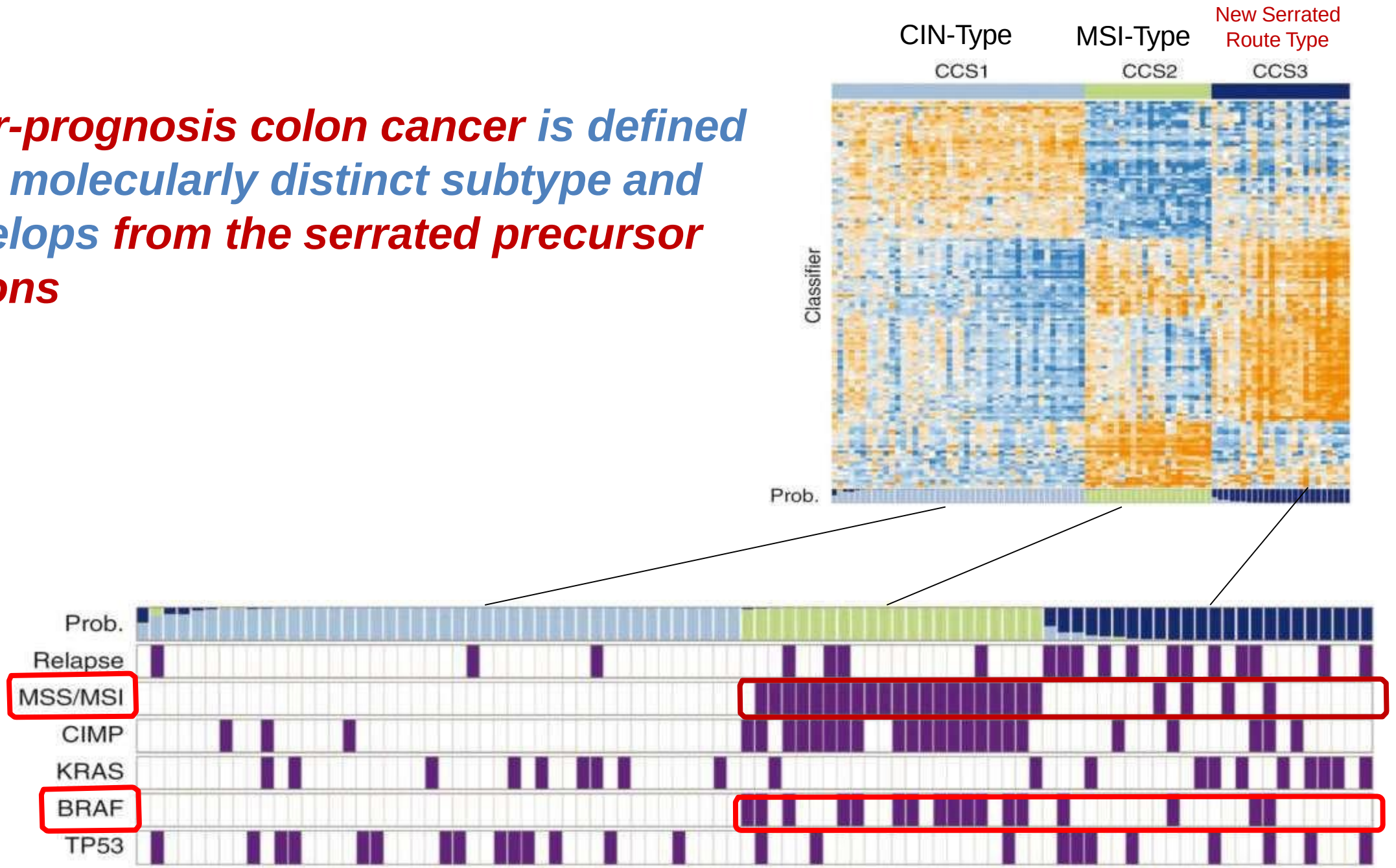
Correlation with MSI-type

Median number of total mutations 728





Poor-prognosis colon cancer** is defined by a molecularly distinct subtype and develops **from the serrated precursor lesions



De Sousa E Melo F, …Vermeulen L; Nature Med 19, 614-618 (2013)

Consensus molecular subtypes of colorectal cancer

based gene expression profiling in 18 CRC data sets (N= 4161 patients)

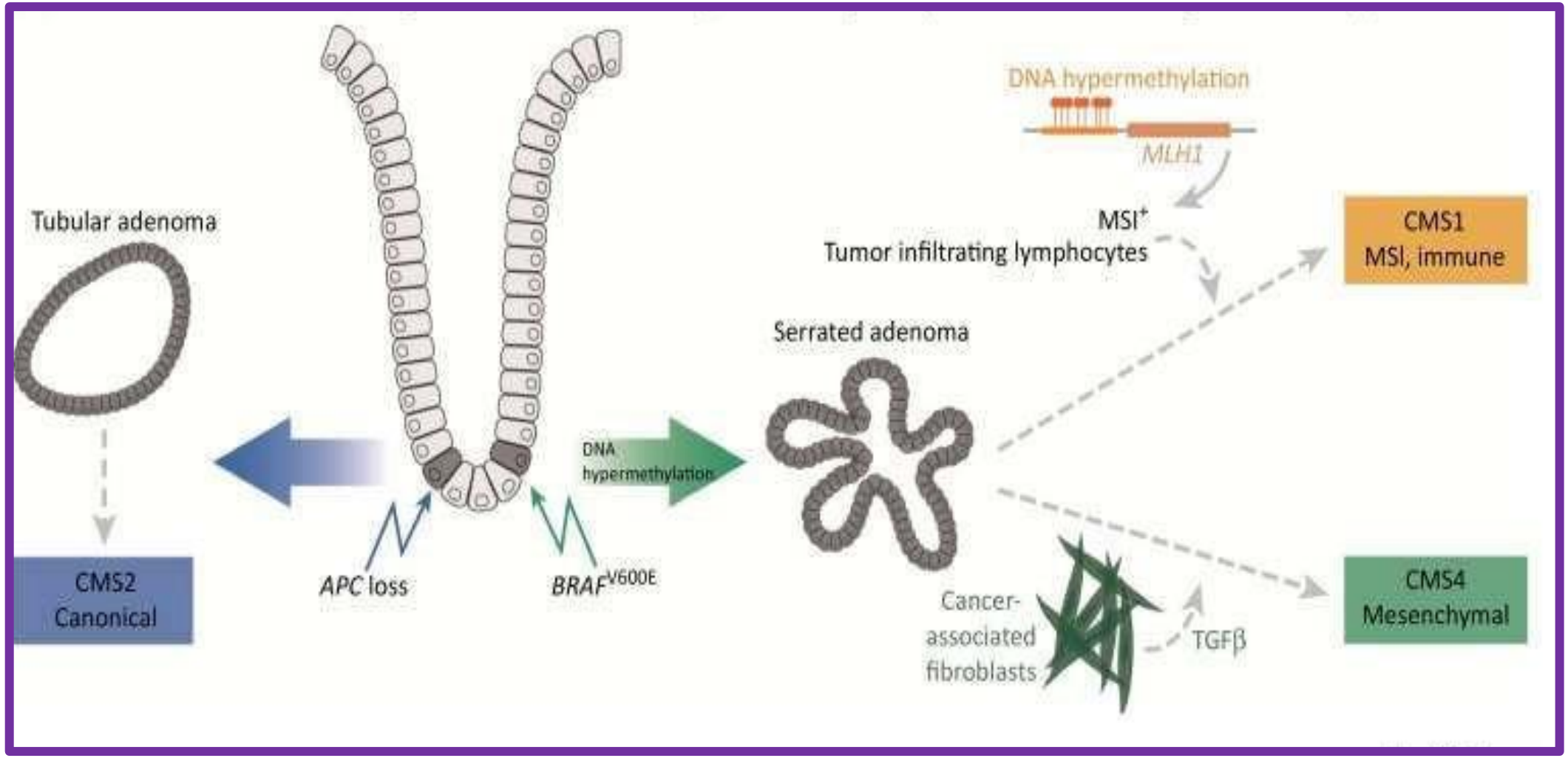
CMS1 MSI Immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGFβ activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

13 % of all CRC can not be assigned to a consensus subtype

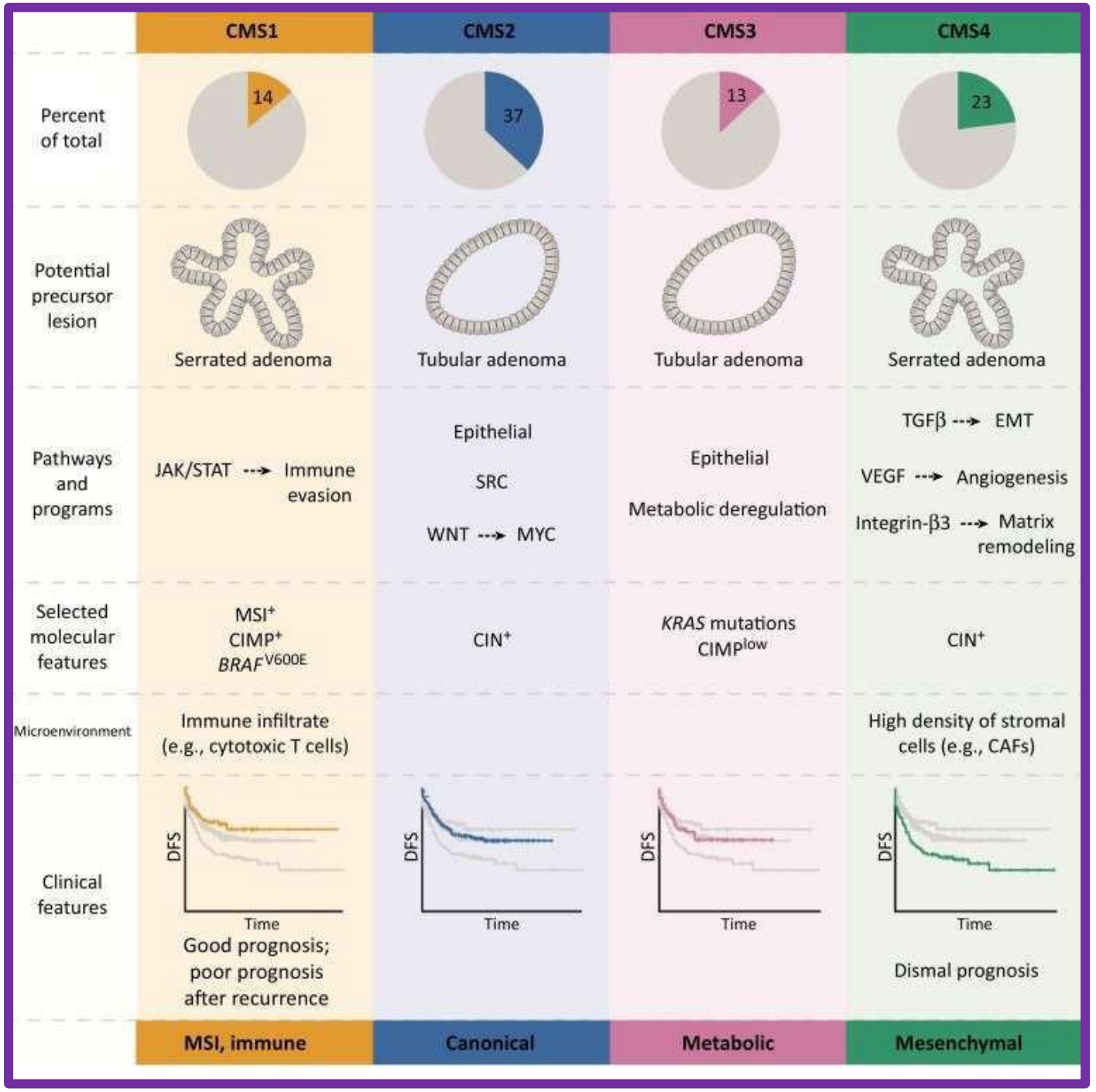
Cuinney J. et al. Nat Med **2015**; 21: 1350-6

The Consensus Molecular Subtypes of Colorectal Cancer

Pathways of carcinogeneis and precursor lesions

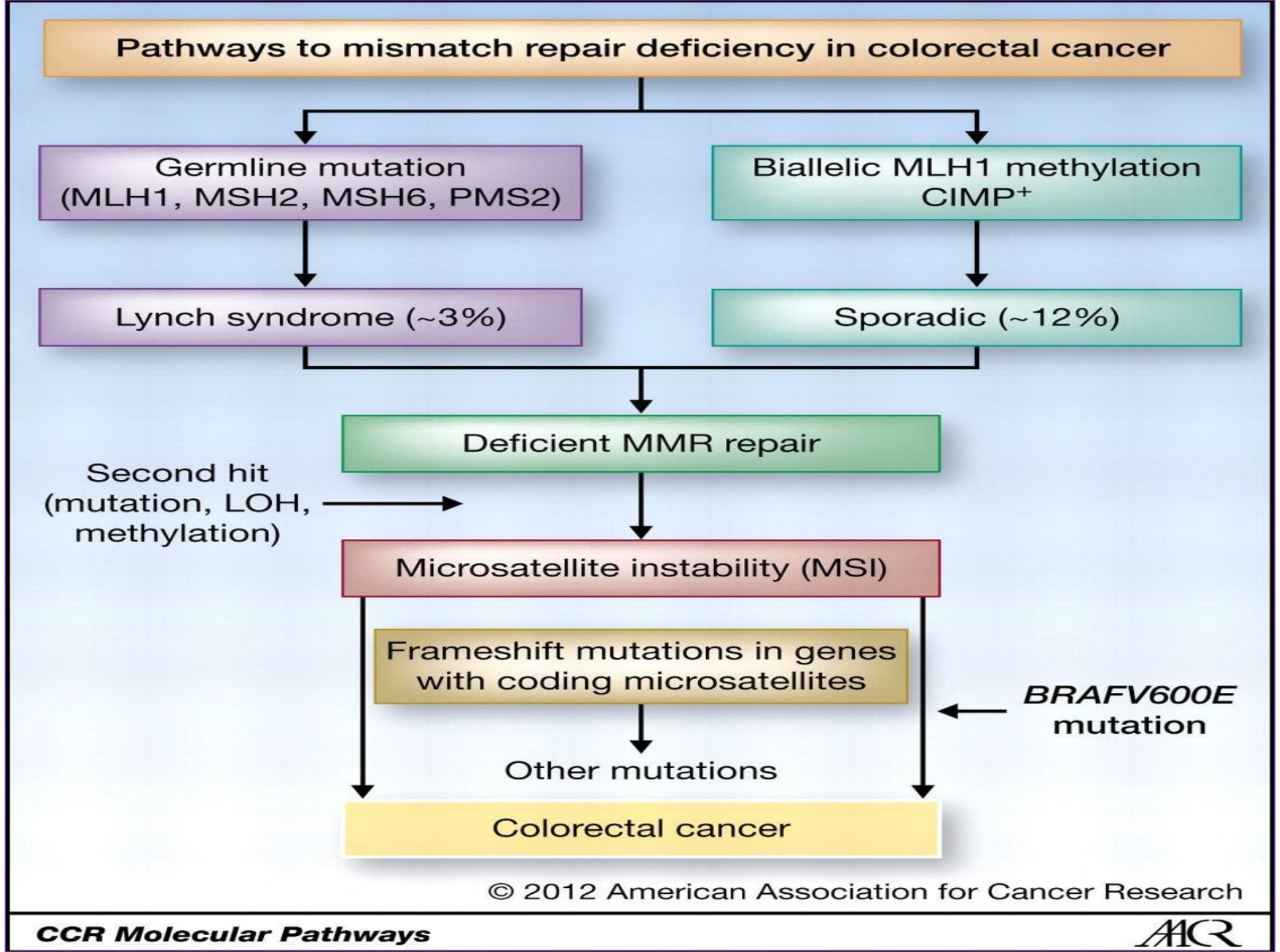


Fessler E, Medema JP, Trends in Cancer 2016; 2: 505-518



The Consensus Molecular Subtypes of Colorectal Cancer Represent Biologically and Clinically Distinct Subgroups (Entities)

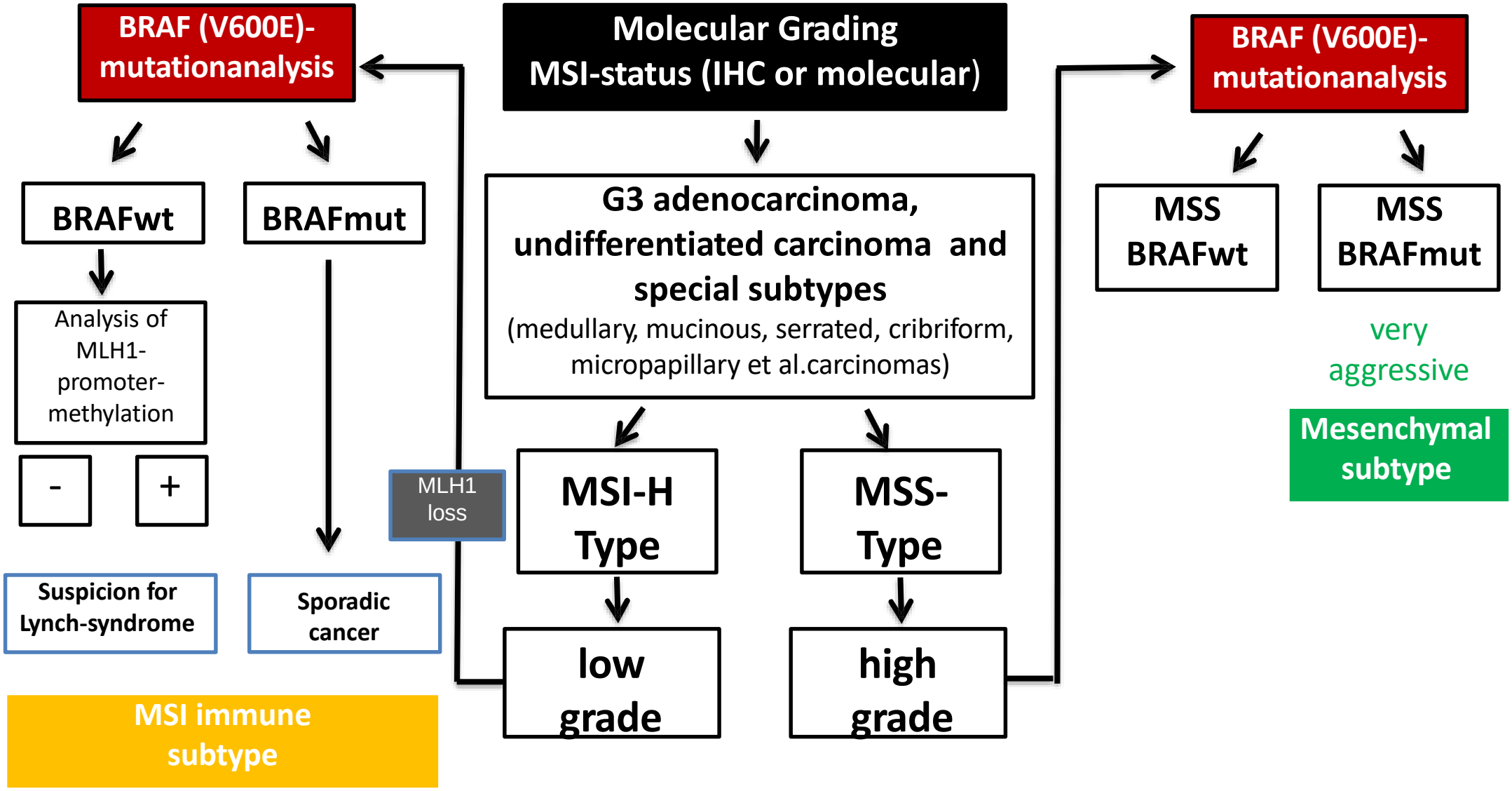
Fessler E, Medema JP, Trends in Cancer 2016; 2: 505-518


Pathways to mismatch repair deficiency in colorectal cancer
Germline mutation (MLH1, MSH2, MSH6, PMS2)
Biallelic MLH1 methylation CIMP+
Lynch syndrome (~3%)
Sporadic (~12%)
Deficient MMR repair
Second hit (mutation, LOH, methylation)
Microsatellite instability (MSI)
Frameshift mutations in genes with coding microsatellites
BRAFV600E mutation
Other mutations
Colorectal cancer
© 2012 American Association for Cancer Research
CCR Molecular Pathways
AACR

MSI-testing by immunohistochemistry

Markers	Prediction of MSI-H	
	Sensitivity	Specificity
MLH1 & MSH2	92.6 %	99.1 %
PMS2 & MSH6	100 %	98.2 %

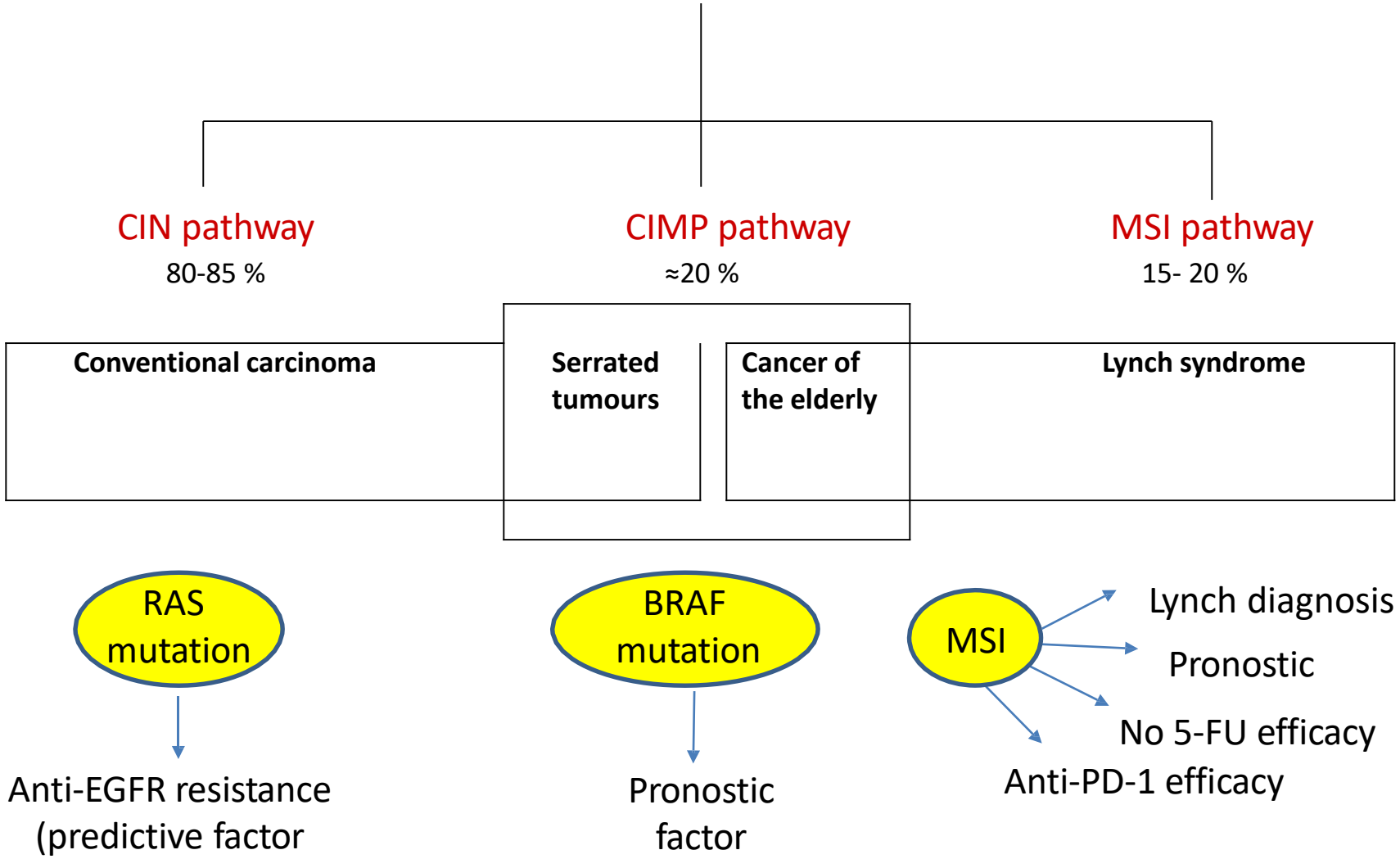
Yuan L et al. Int J Clin Exp Med. 2015



Neumann J, Kirchner T . Pathologe 2014; 35: 615

Biomarkers of GI Tumors

Molecular CRC classification- Useful biomarkers



CRC molecular biomarkers and targets

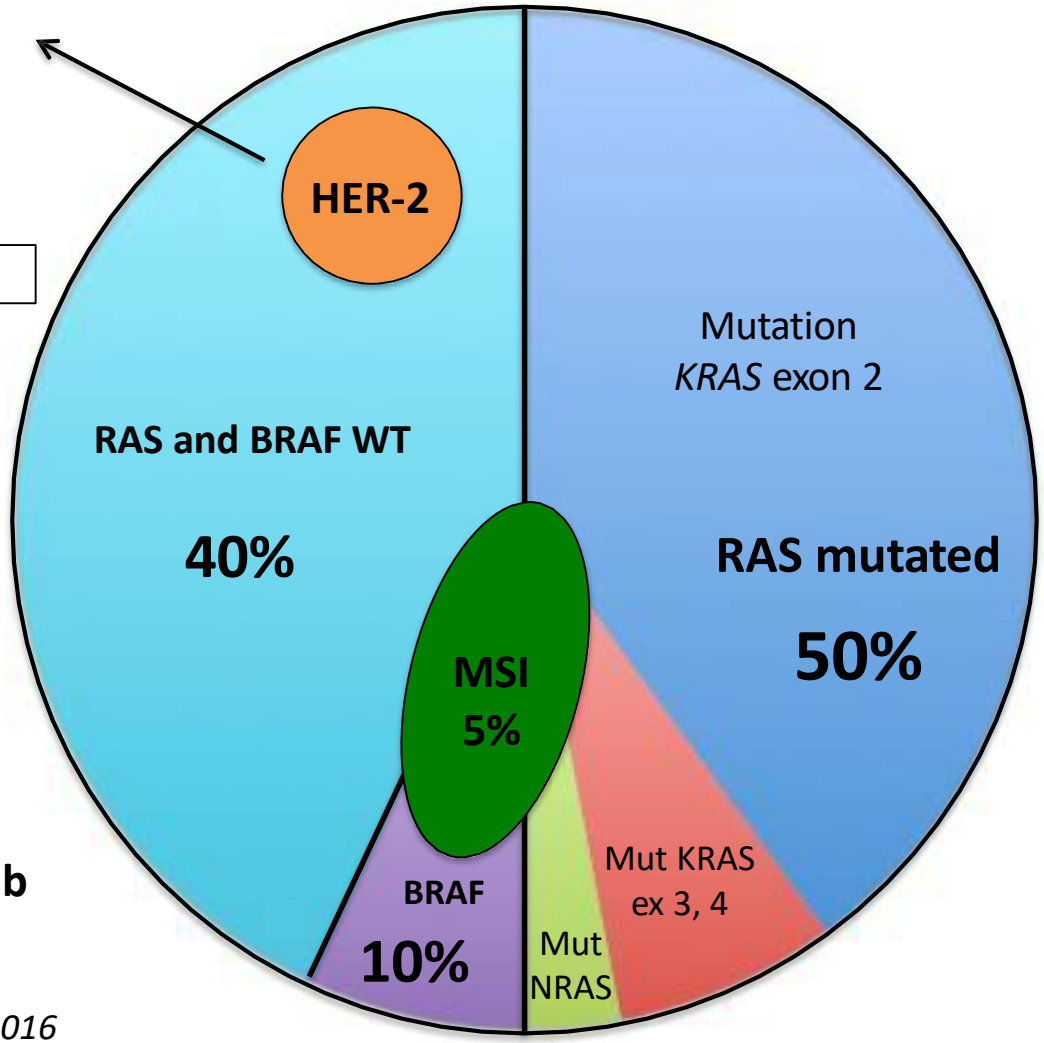
Amplifications: 2,5%
Mutations: 1,9%

Anti-EGFR resistance ?

Raghac ASCO 2016

Anti-HER2 Targeted therapies?

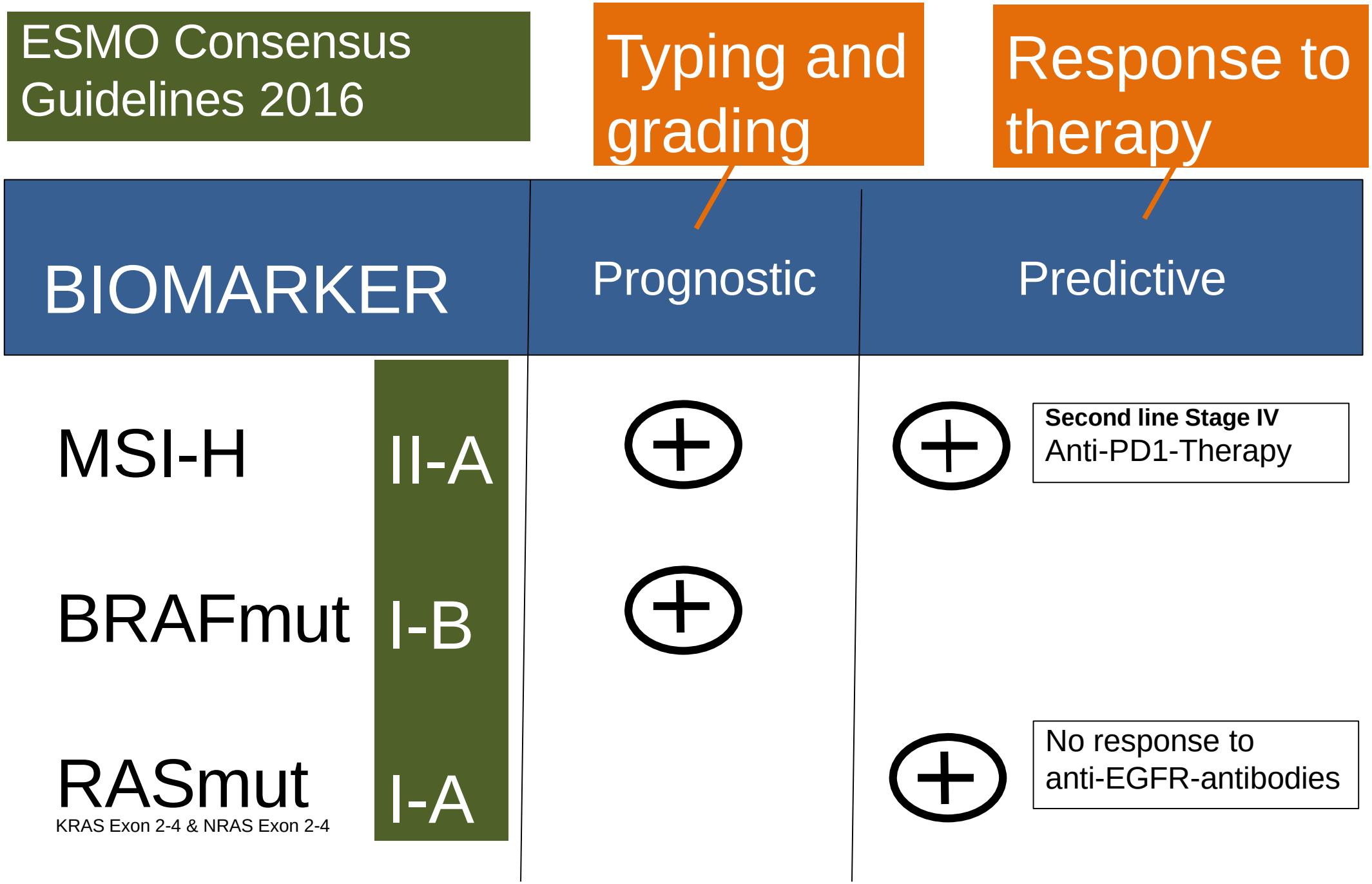
Trastuzumab + lapatinib
(HERACLES)
Trastuzumab + pertuzumab



Sartore-Bianchi Lancet Oncol 2016
Hurwitz ASCO GI 2016
Marsoni AACR 2017

SPECTAcolor: Folprecht ESMO 2016, abst 4580

ESMO Consensus Guidelines 2016



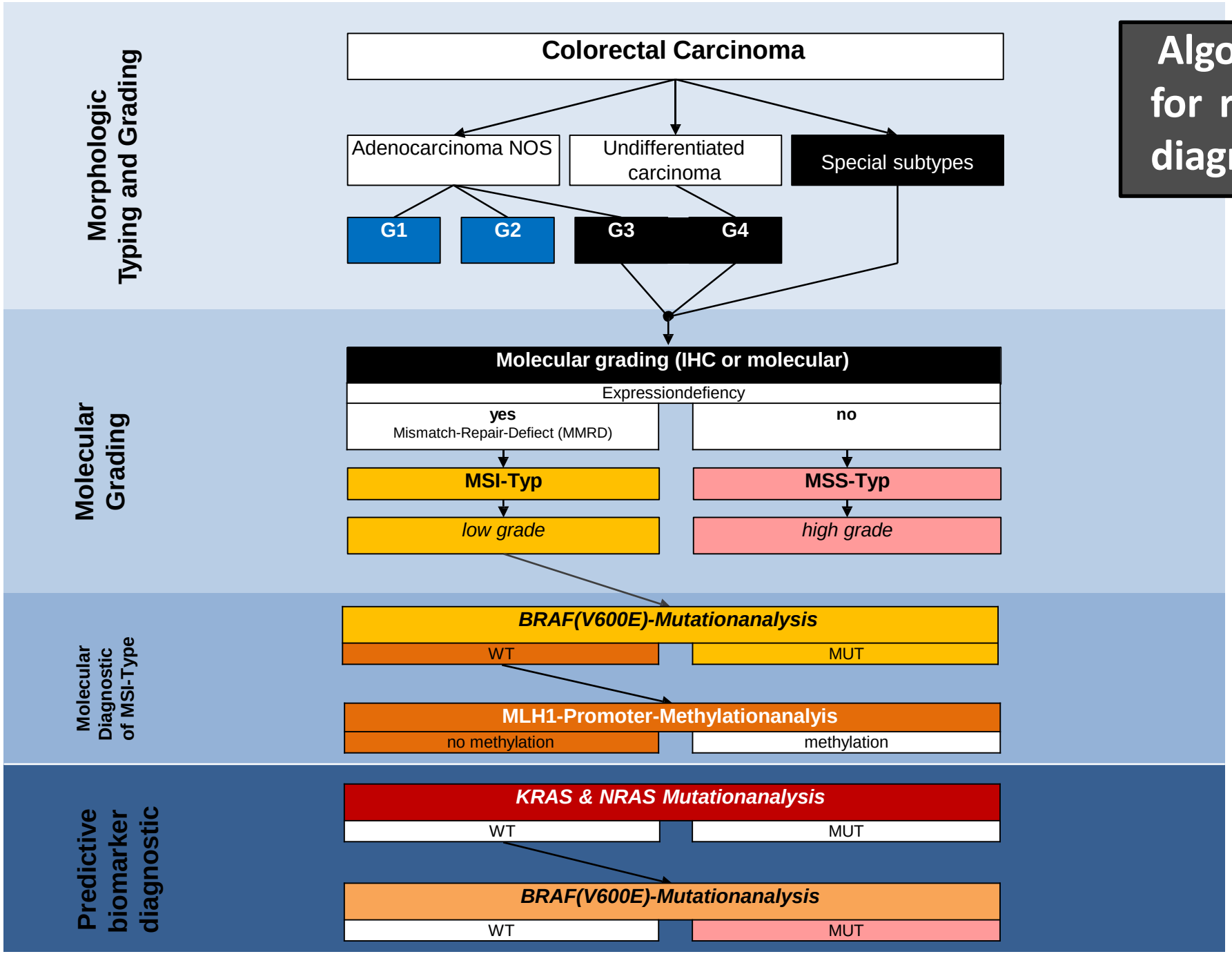
Van Cutsem E et al, Ann Oncol 2016; 0: 1-38

Typing and grading

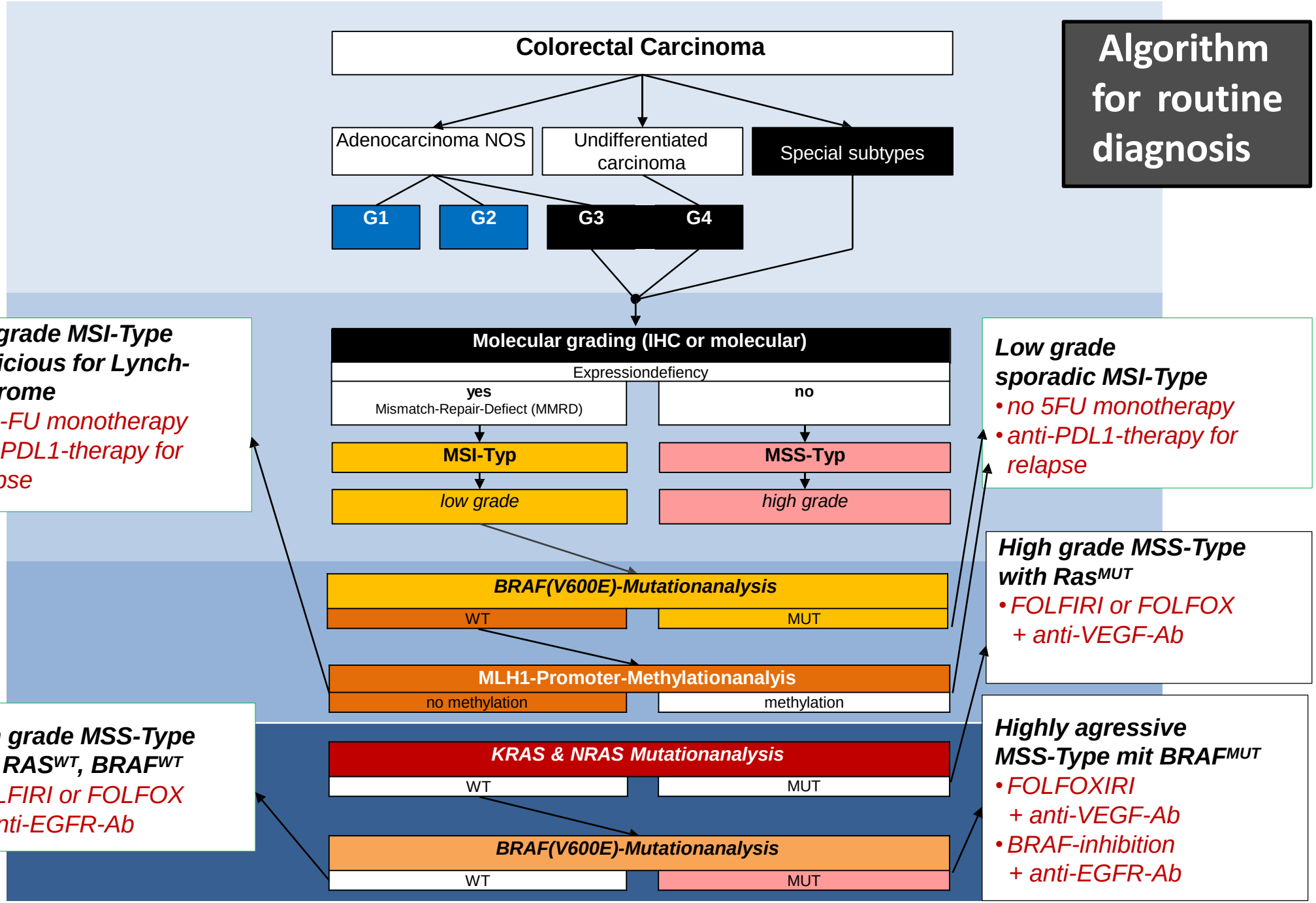
Response to therapy

BIOMARKER	Prognostic	Predictive	
MSI-H	⊕	⊕	**First line Stage III** 5-FU-monotherapy **Second line Stage IV** Anti-PDL1-Therapy
$BRAF_{(V600E)}$ mut	⊕	⊕	FOLFOXIRI + anti-VEGF-AK BRAF-inhibition + anti-EGFR-AK
RAS mut KRAS Exon 2-4 & NRAS Exon 2-4		⊕	No response to anti-EGFR-antibodies

Algorithm for routine diagnosis



Algorithm for routine diagnosis



Infrequent actionable mutations in colorectal cancer (CRC)

Care for the rare

Targeted therapy	Frequency *% in CRC*	Mutation	EMA – approval or successful trial
Ceritinib	*2,1%*	ALK-translocation	
Crizotinib	*1,6%*	ROS1-translocation	Lung cancer (NSCLC)
	1,1%	RET-translokation/-mutation	
Afatinib Erlotinib Gefitinib	*1,1%*	EGFR-activating mutation	Lung cancer (NSCLC)
Trastuzumab & Lapatinib	*2,7–10 %*	HER2-amplification	Gastric cancer Breast cancer

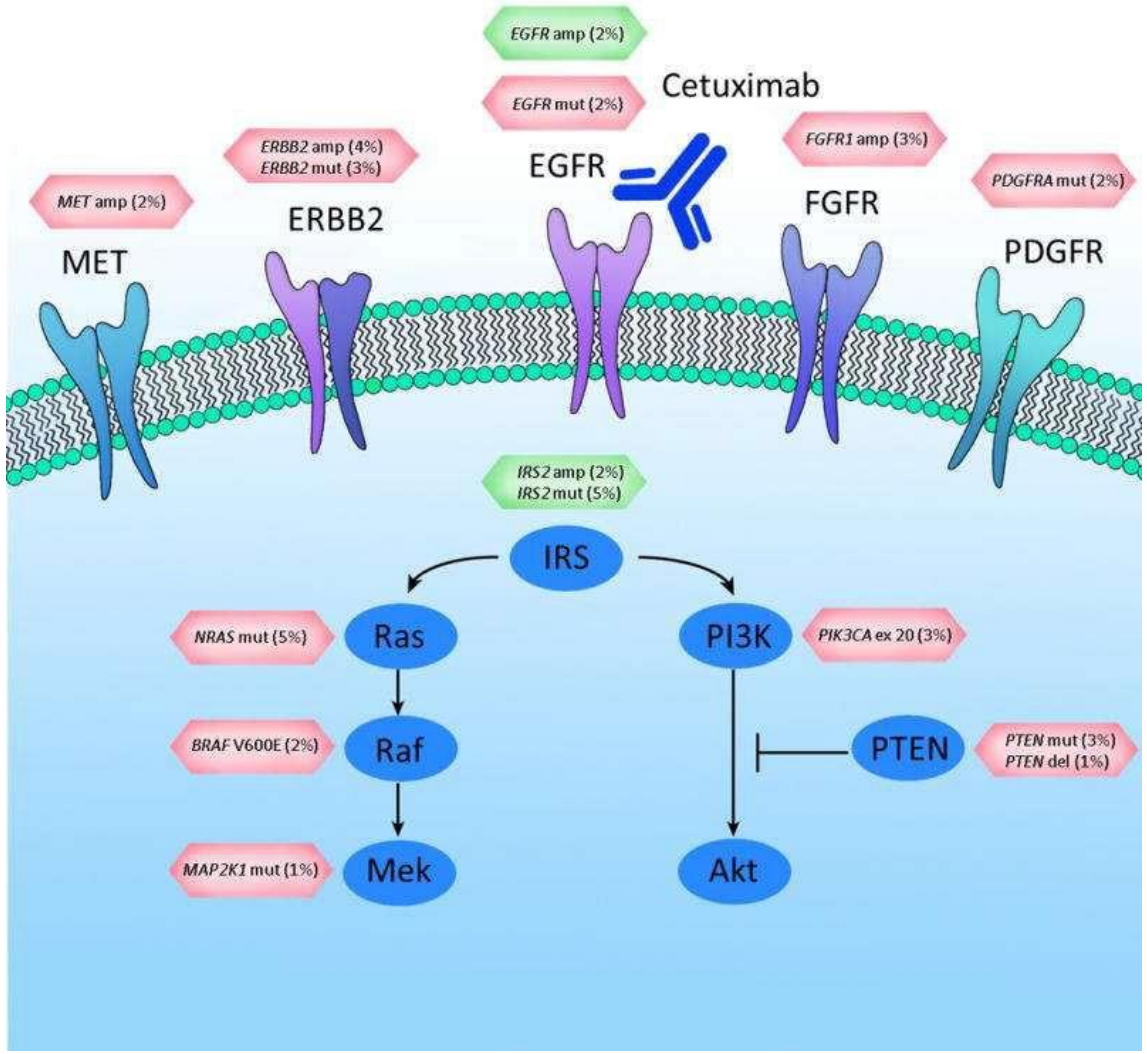
Update 2017
German Guideline for
Stage IV CRC

Typing and grading

Response to therapy

BIOMARKER	Prognostic	Predictive	
MSI (immunhistochemisch)	⊕	⊕	**First line Stage III** 5-FU-monotherapy **Second line Stage IV** Anti-PDL1-Therapy
BRAF(V600E) mut	⊕	⊕	FOLFOXIRI + Anti-VEGF-AK BRAF-Inhibition + anti-EGFR-A
RAS mut KRAS Exon 2-4 & NRAS Exon 2-4		⊕	No reponse to anti-EGFR-AK
HER2 Amplification		⊕	**Second line stage IV** Trastuzumab & Lapatinib

Genes involved in cetuximab resistance or sensitivity in CRCs with KRAS wildtype



Bertotti A. et al. Nature **2015**; 526: 263

Acquired resistance to EGFR blockade in mCRCs

Amplifications associated with acquired resistance

- HER2 (ERBB2)
- MET
- KRAS

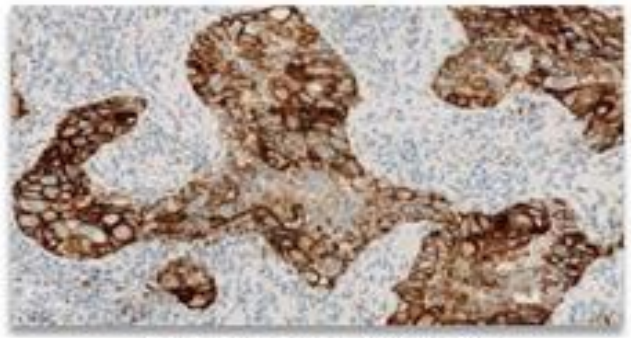
Mutations associated with acquired resistance

- KRAS
- NRAS
- BRAF
- EGFR

Misale S. et al. Cancer Discov **2014**; 4: 1269

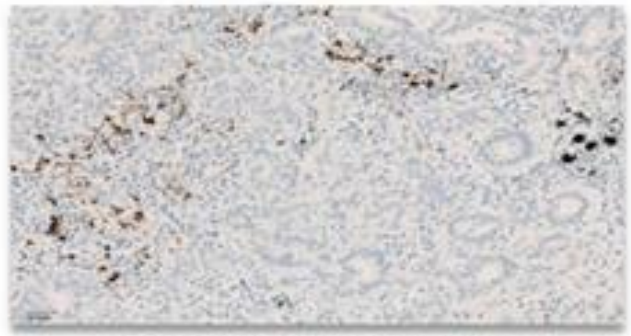
PD-L1 Expression Assessment With IHC

TC and IC Staining can be Reliably Distinguished With SP142



Staining in tumor cells

Staining in tumor cells and immune cells



Staining in immune cells

PD-L1 TC Staining Criteria	
TC Score[a]	% of PD-L1–Expressing TC
TC3	≥50%
TC2	≥5% and <50%
TC1	≥1% and <5%
TC0	<1%

PD-L1 IC Staining Criteria	
IC Score[a]	% of PD-L1–Expressing IC
IC3	≥10%
IC2	≥5% and <10%
IC1	≥1% and <5%
IC0	<1%

[a] Intensity of PD-L1 expression is not considered for scoring.

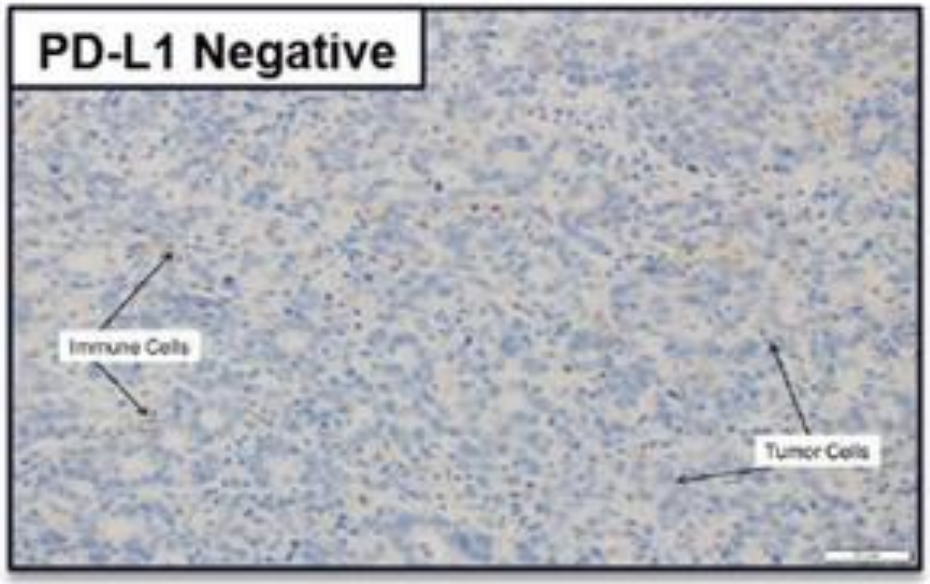
PeerView.com

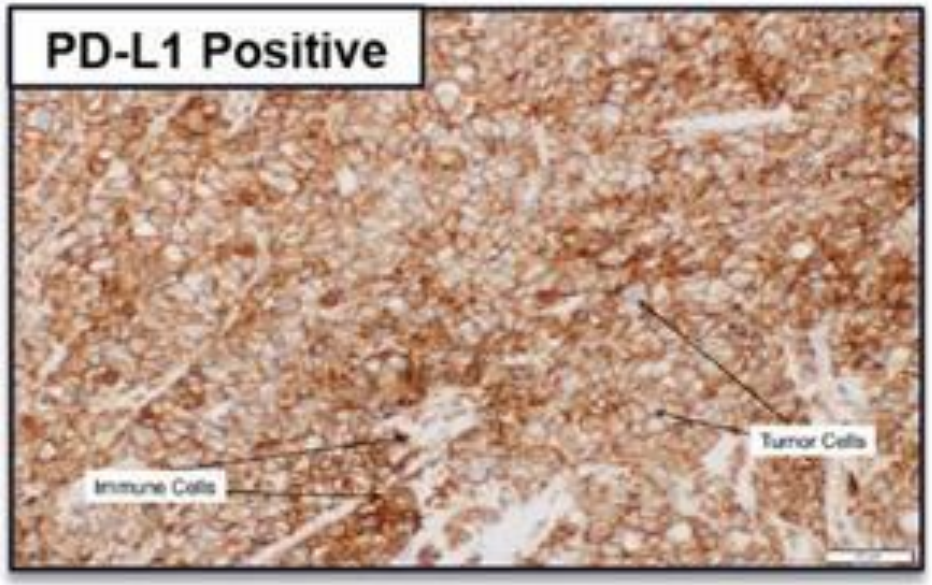
PD-L1 Expression IHC[a]

- PD-L1 expression in gastric cancer is determined by **c**ombined **p**ositive **s**core (**CPS**)

$$\textbf{CPS} = \frac{\text{No. of PD-L1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total no. of viable tumor cells}} \times 100$$

- A specimen is considered to have positive PD-L1 expression if CPS ≥1





[a] 22C3 pharmDx kit, Agilent Technologies, Carpinteria, CA.

PeerView.com

Revised Scoring and Interpretation for Gastric or Gastroesophageal Cancer[a]

CPS Numerator Inclusion/Exclusion Criteria

Tissue Elements	Included in the Numerator	Excluded From the Numerator
Tumor cells	Convincing partial or complete linear membrane staining (at any intensity) of viable invasive gastric or GEJ adenocarcinoma tumor cells	• Non-staining tumor cells • Tumor cells with only cytoplasmic staining • Adenocarcinoma, dysplasia, and carcinoma in situ
Immune cells	Membrane and/or cytoplasmic staining (at any intensity) of MICs within tumor nests and adjacent supporting stroma: • Lymphocytes (including lymphocyte aggregates) • Macrophages Only MICs directly associated with the response to the tumor are scored	• Non-staining MICs • MICs associated with adenoma, dysplasia, and carcinoma in situ • MICs (including lymphoid aggregates) associated with ulcers, chronic gastritis, and other processes not associated with the tumor • MICs associated with normal structures • Neutrophils, eosinophils, and plasma cells
Other cells	Not included	• Normal cells (including ganglion cells) • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

[a] The revised label from the Dako 22C3 IVD.

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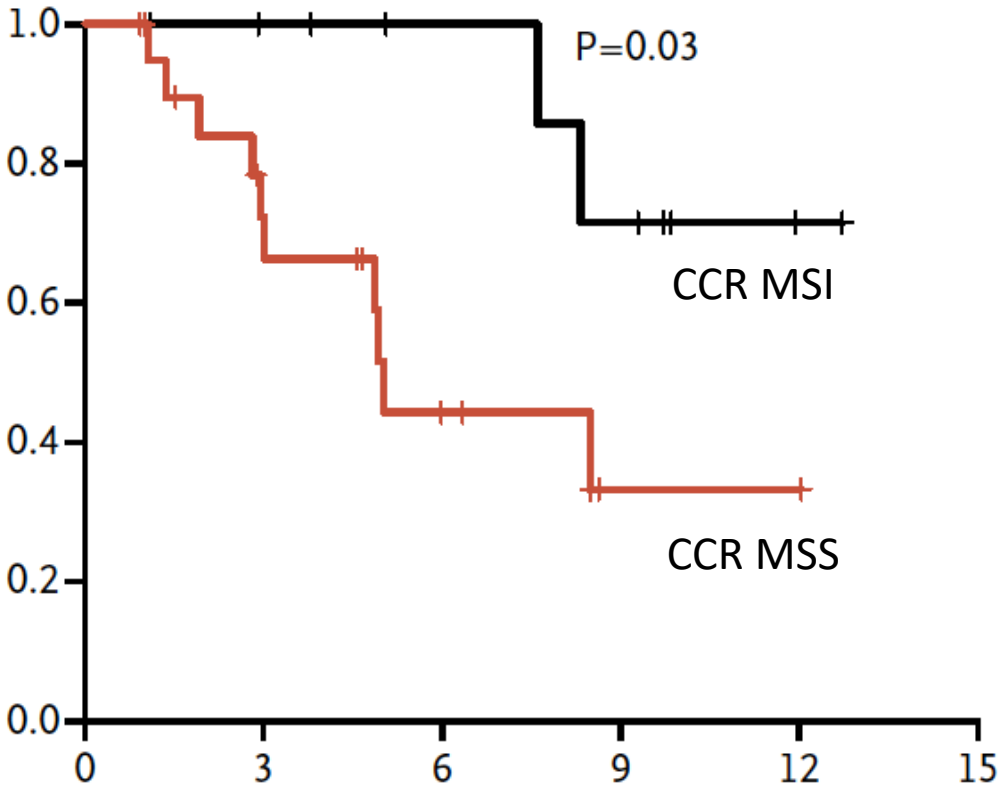
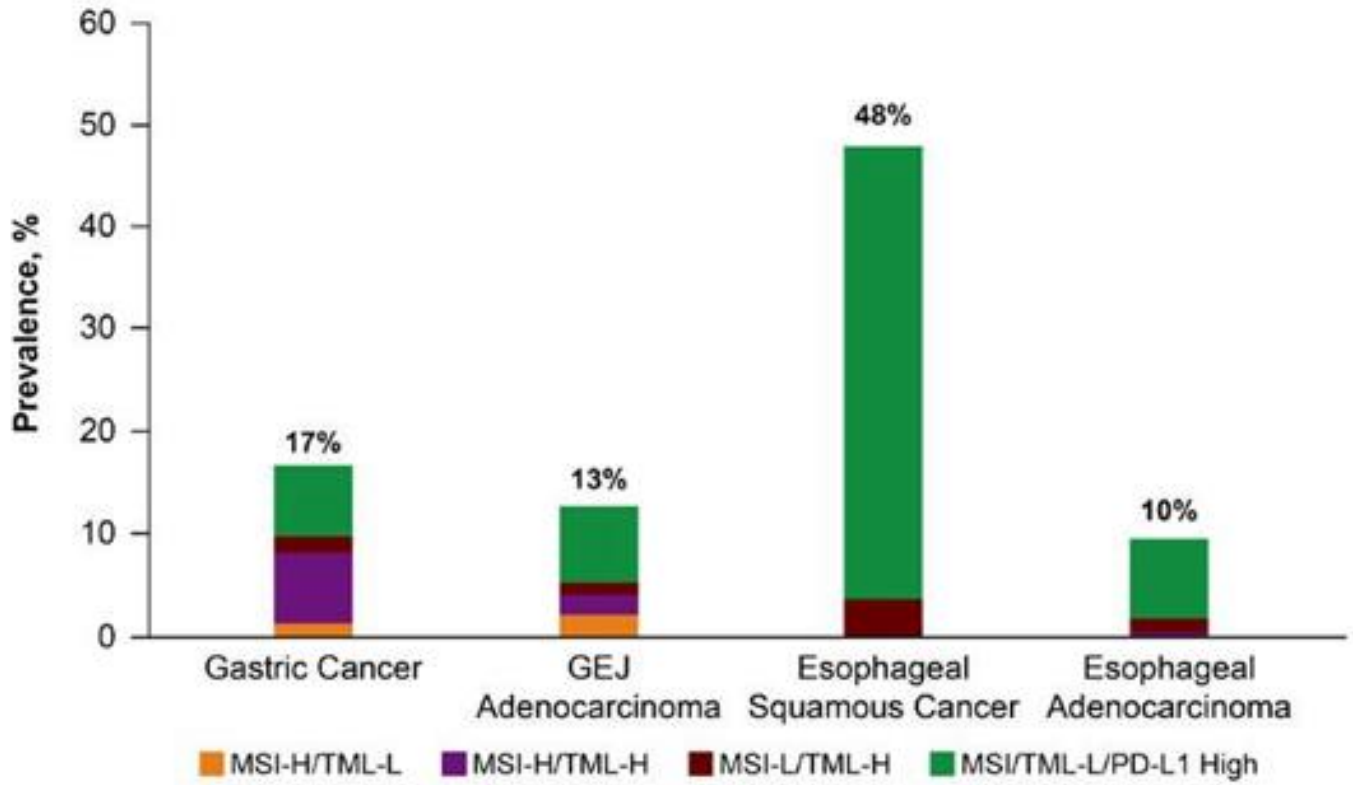
Immunotherapy

Anti-PD-1 treatment: overall survival



⟶ Selection of patients based on MSI status

PD-L1 Expression, MSI Status, and Mutational Load in Gastric and Esophageal Cancers[1]



1. Salem ME et al. *Mol Cancer Res*. 2018;16:805-812.

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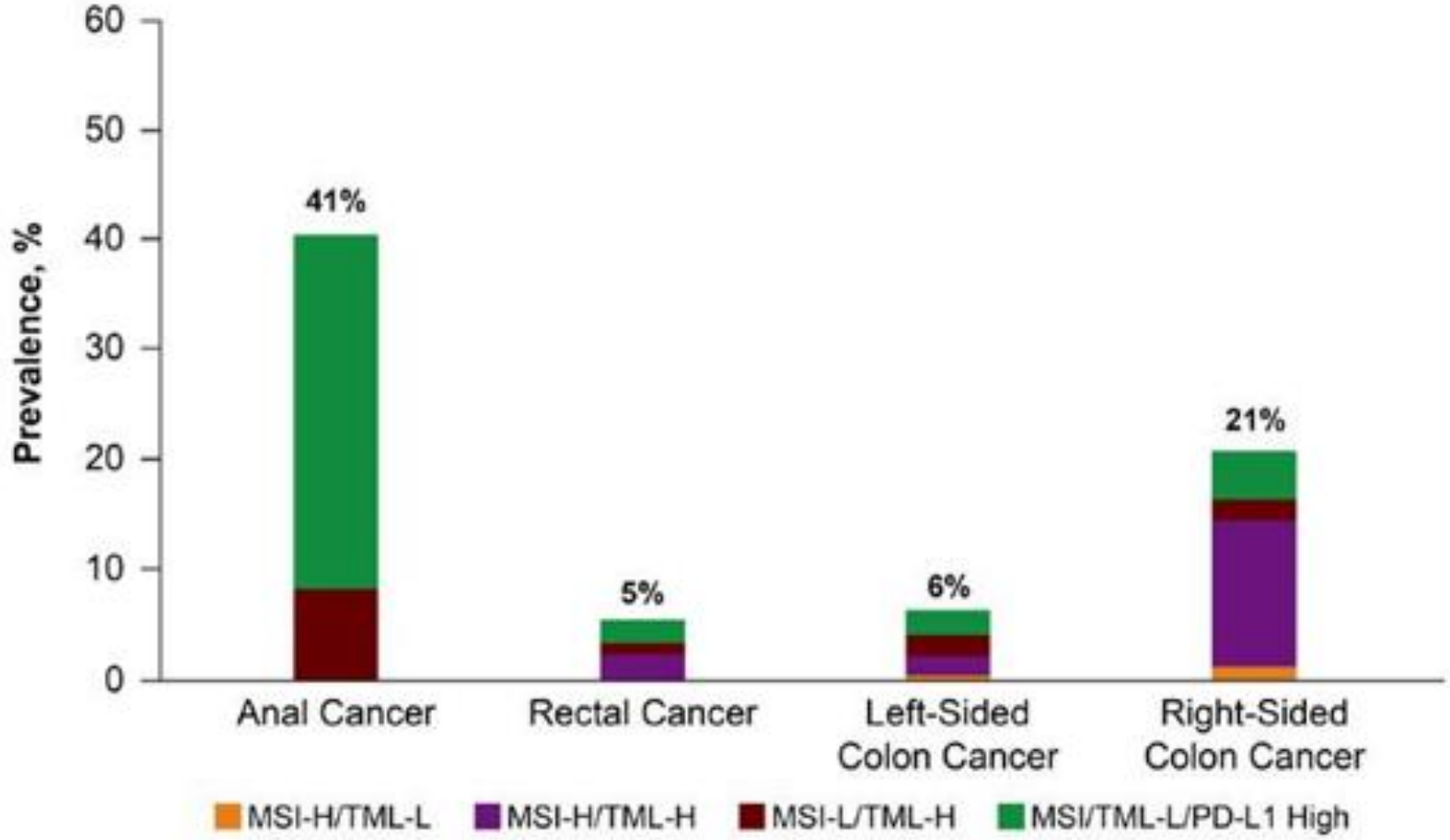
PD-L1 Expression, MSI Status, and Mutational Load in Colorectal Cancers[1]



1. Salem ME et al. *Mol Cancer Res*. 2018;16:805-812.

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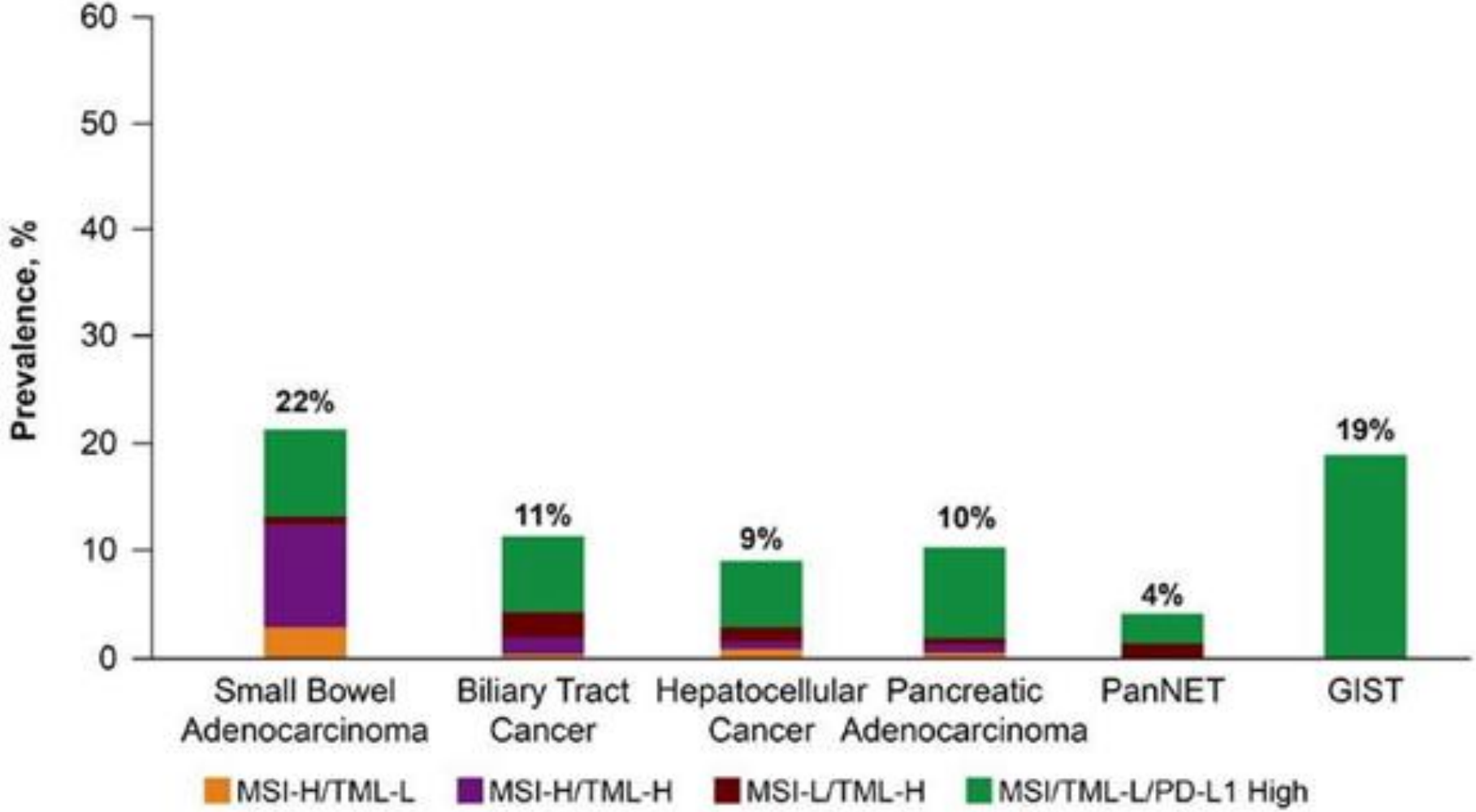
PD-L1 Expression, MSI Status, and Mutational Load in Other GI Cancers[1]



1. Salem ME et al. *Mol Cancer Res*. 2018;16:805-812.

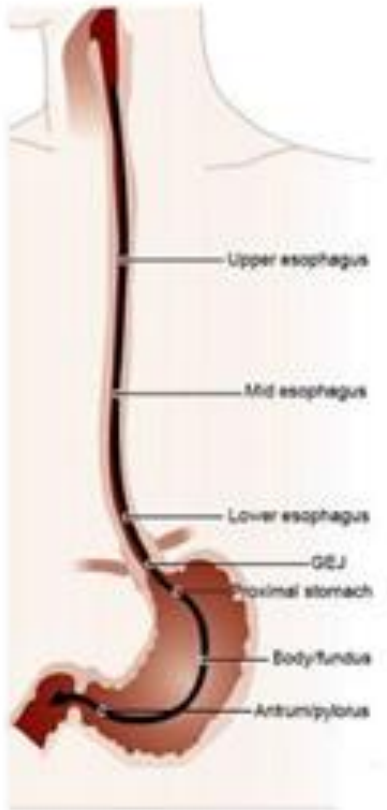
PeerView.com

‘New’ Targets in Gastric Cancer

Cytotoxics: Modest impact—median survival of doublets/triplets usually <12 mo

New Targets

- **HER2: Trastuzumab[a]**
- **Angiogenesis: Ramucirumab[a]**
- EGFR
- mTOR
- cMET
- **FGFR**
- **PD-1[b]/PD-L1**
- **CTLA-4**
- **Claudin**
- **Stem cell: STAT3**
- **MMP9**
- **PARP**
- …



[a] Globally approved. [b]Approved outside the EU.

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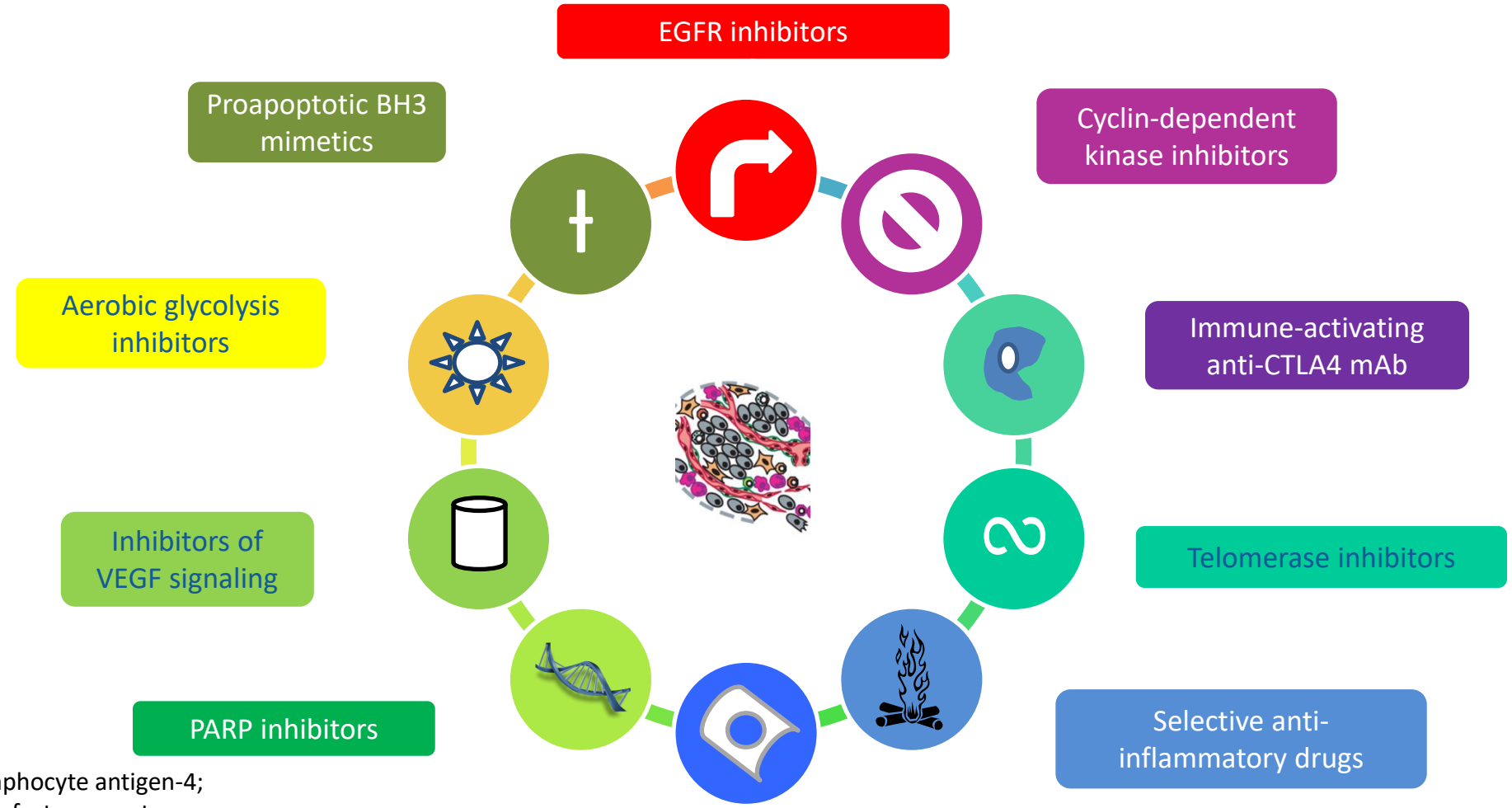
Therapeutic targeting of biomarkers in CRC

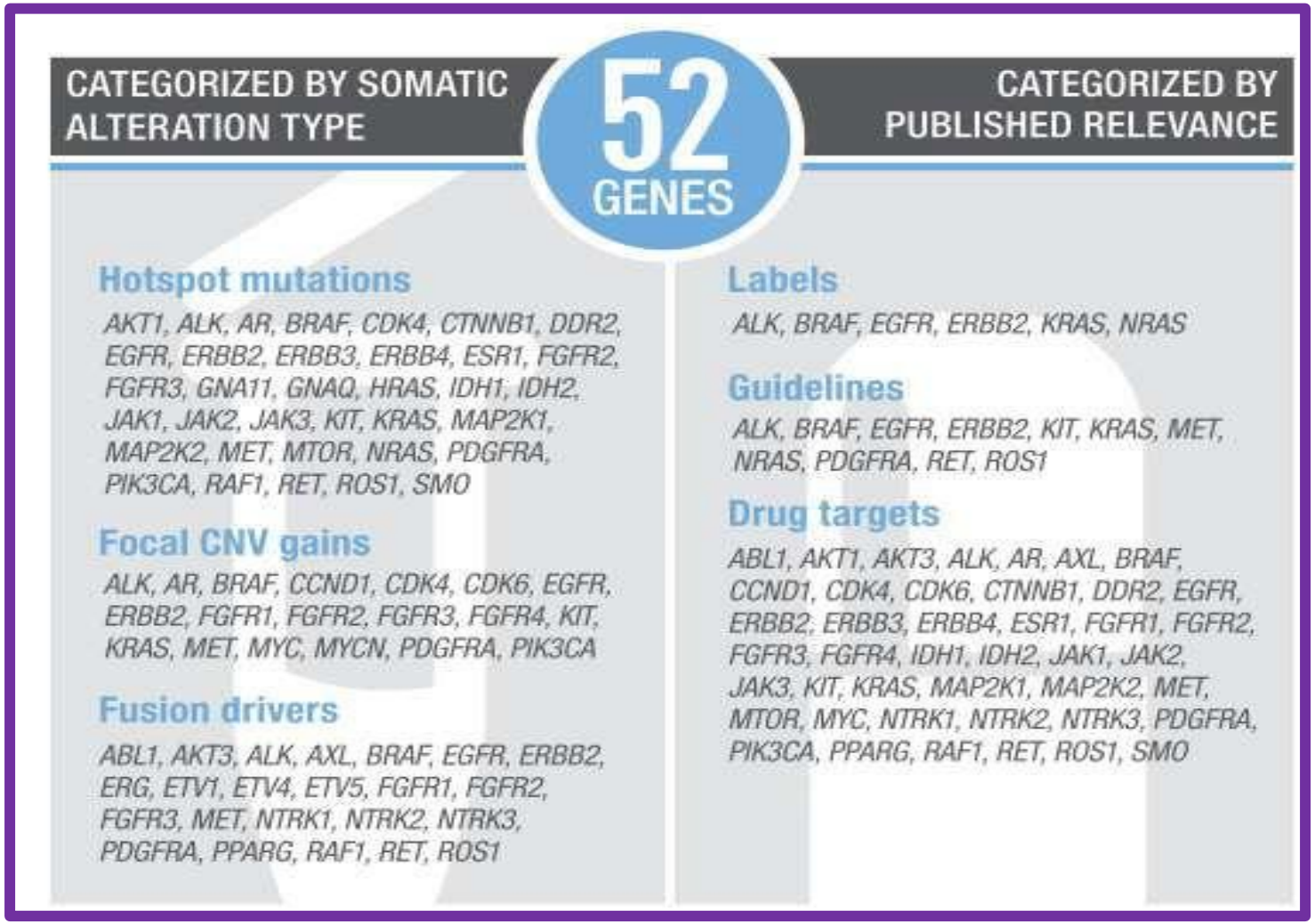


CTLA-4, cytotoxic T-lymphocyte antigen-4;
EGFR, epidermal growth factor receptor;
HGF, hepatocyte growth factor;
PARP, poly ADP ribose polymerase;
VEGF, vascular endothelial growth factor

Adapted from: Hanahan D, Weinberg RA. Cell 2011; 144: 646–674

Search for targetable mutations by NGS in recurrent cancer after the application of standard treatment

Oncomine Focus Assay



Conclusion for colorectal carcinomas

- Molecular subtyping/grading and predictice analysis for treatment is now well established in routine pathology.
- The ESMO Consensus Guideline 2016 for CRC and the German Guideline 2017 for Stage IV CRC recommend as biomarkers: MSI, BRAFmut, RASmut and ERBB2 (HER2).
- The morphological and molecular analysis should be integrated by a routine algorithm.
- Multigene panel analysis by NGS can be used for recurrent cancer after standard treatment.

BACK UP SLIDES

Biological Role	Biomarker	Abnormality	Mechanism of Action	Therapy If applicable
Screening	DNA panel	genetic mutations		
Screening	stool DNA profile	genetic mutations		
Screening	septin 9	genetic mutation		
Predictive	anti-EGF tyrosine kinase inhibitors (antibodies)	genetic mutation (15-20%)	Blocks EGFR signaling. KRAS mutations are associated with a lack of benefit from anti-EGFR antibodies. Wild-type KRAS + anti-EGFR antibodies+ chemotherapy enhance patient outcomes Testing of mutational status of KRAS is now standard practice in patient identification in metastatic CRC.	Cetuximab Panitumumab Imatinib
Predictive	KRAS	Genetic mutation exon 2 (4-15%).Used for patient selection with wild-type KRAS for treatment with anti-EGFR antibodies	A proto-oncogene involved in cellular response to extracellular stimuli. KRAS mutation involves A structural activation of downstream signaling pathways i.e. MAPK and P13K/AKT.	
Predictive	BRAF	Genetic V600E mutation in 10-20% of CRC	A signature of BRAF/KRAS a possible predictive factor for the response to EGFR inhibitors.	
Predictive	NRAS (neuroblastoma RAS viral (v-ras) oncgene)	Genetic mutation	The encoded protein, which has intrinsic GTPase activity, is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein.	
Predictive	PTEN (Phosphatase and tensin) homolog protein	Genetic mutation. Encoded by the PTEN gene	*PTEN* acts as a tumor suppressor gene through the action of its phosphatase protein product. This phosphatase is involved in the regulation of the cell cycle, preventing cells from growing and dividing too rapidly.	

1. *Kalia M (2015) Biomarkers of Colorectal Cancer. J Cancer Biol Res 3(1): 1058. .*

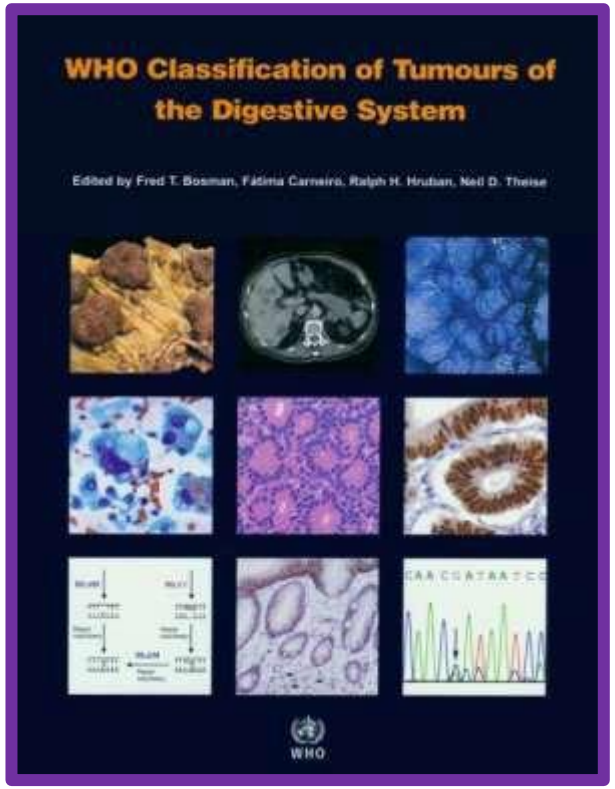
Biological Role	Biomarker	Abnormality	Mechanism of Action	Therapy If applicable
Predictive	PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha).	Like other kinases, PI3K adds a cluster of oxygen and phosphorus atoms (a phosphate group) to other proteins through a process called phosphorylation.	PI3K signaling is important for many cell activities, including cell growth and division (proliferation), movement (migration) of cells, production of new proteins, transport of materials within cells, and cell survival.	
Prognostic	BRAF	Genetic V600 mutation in 10-20% of CRC	Associated with sporadic MSI positive tumors through its relationship with CIMP-high. Mutated BRAF is one of the most powerful prognostic markers in CRC.	
Prognostic	VEGF	proangiogenic factor	Involved in cell proliferation, migration, and vascular permeability. Increase in VEGF expression is associated with poor prognosis, low response to preoperative radiotherapy and greater likelihood of Recurrence.	
Prognostic	TIMP metallopeptidase inhibitor	protein coding	involved in the degradation of the extracellular matrix	
Predictive of response to chemotherapy	Dihydropyrimidine dehydrogenase (DPD)	gene expression of DPD	enzyme involved in pyrimidine degradation. Involved in the degradation of chemotherapeutic drugs:5-fluorouracil and Tegafururacil.	5-fluorouracil and Tegafururacil
Response to chemotherapy	AP-2 epsilon (*TFAP2E*)	Genomic and epigenetic alterations gene encoding transcription factor	The gene encoding homolog 4 protein (*DKK4*) is a potential downstream target of *TFAP2E* and has been implicated in chemotherapy resistance.	
Predictive of toxicity of irinotecan	UGT 1A1	Genetic mutation	Responsible for irinotecan glucuronidation	Irinotecan
Risk assessment	MLH1/MSH2	Genetic mutation	Member of the MMR (mismatch repair) gene family. Increases the risk of tumor formation. example Lynch Syndrome	

1. *Kalia M (2015) Biomarkers of Colorectal Cancer. J Cancer Biol Res 3(1): 1058. .*

Typing and grading of colorectal carcinoma (WHO)

Adenocarcinoma, NOS (not otherwise specified)
Special types

- mucinous adenocarcinoma
- signet ring cell carcinoma
- medullary carcinoma
- serrated carcinoma
- cribriform comedo-like carcinoma
- micropapillary carcinoma
- adenosquamous carcinoma
- spindle cell carcinoma
- neuroendocrine carcinoma



WHO Classification of Tumours of the Digestive System, 4th Edition 2010

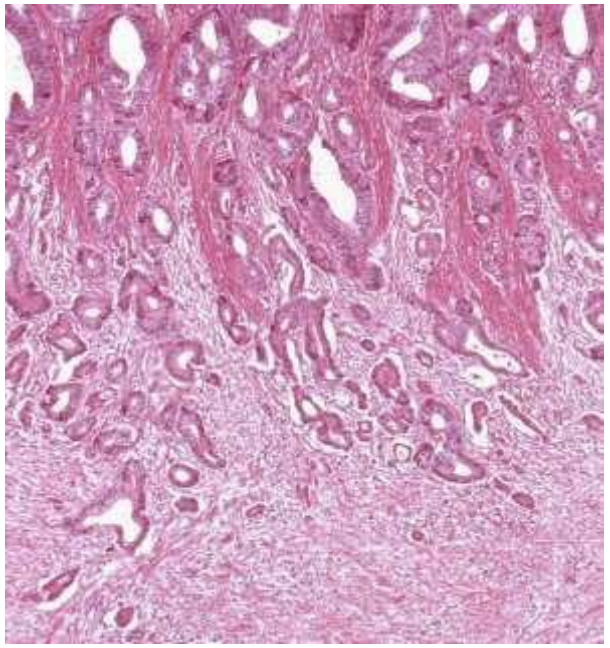
Adenocarcinoma NOS & undifferentiated carcinoma

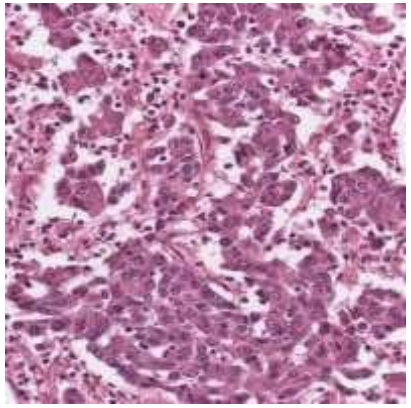
Morphological Grading

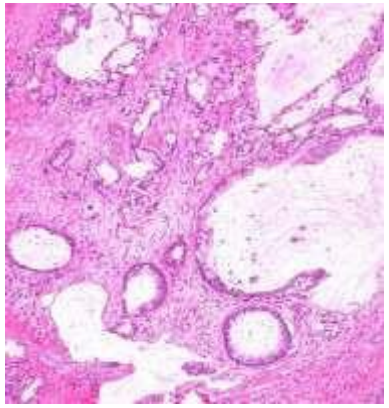
G1	G2	G3	G4
> 95 % glands	50–90 % glands	> 0–49 % glands	0 % glands

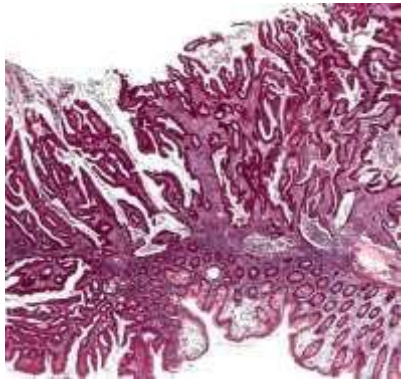
Special Subtypes

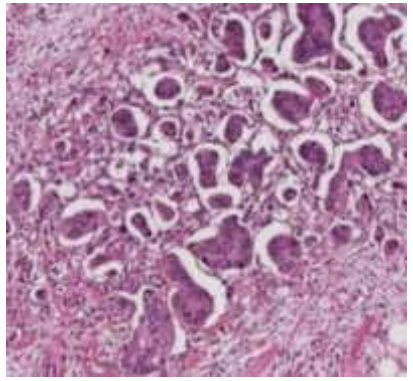
medullary, mucinous,
serrated, cribriform,
micropapillary
et al.
carcinomas











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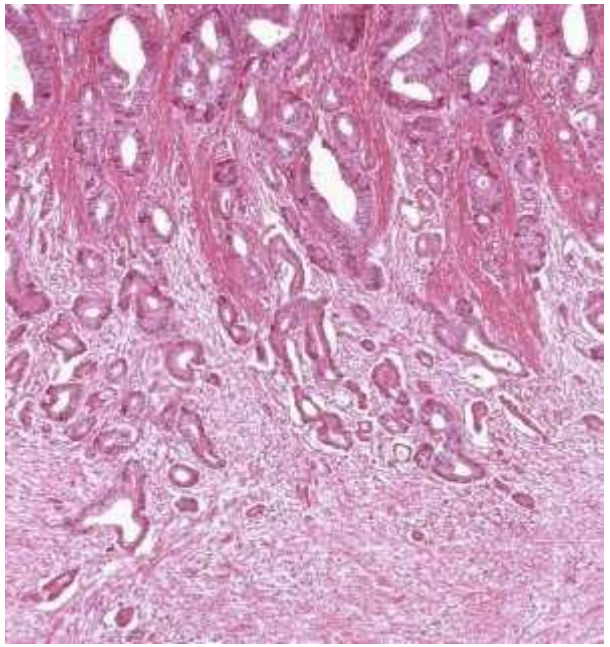
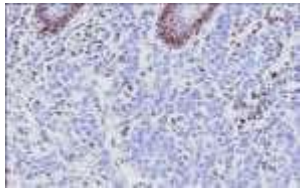
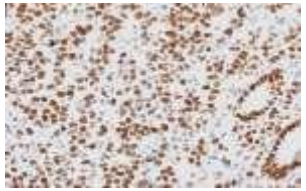
serrated, cribriform,
micropapillary
et al.
carcinomas

Molecular Grading
MSI-status (IHC)







MSI-H → **low grade**

MSS → **high grade**

WHO Classification Tumours of the Digestive System. 4th Edition 2010

Adenocarcinoma NOS & undifferentiated carcinoma

Morphological Grading

G1
> 95 % glands

G2
50–90 % glands

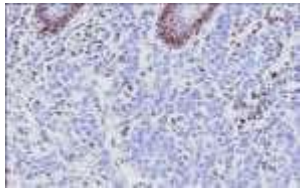
G3
> 0–49 % glands

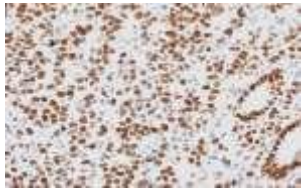
G4
0 % glands

Canonical/conventional subtype

G3 adenocarcinoma, undifferentiated carcinoma and special subtypes
(medullary, mucinous, serrated, cribriform, micropapillary et al. carcinomas)

Molecular Grading
MSI-status (IHC)





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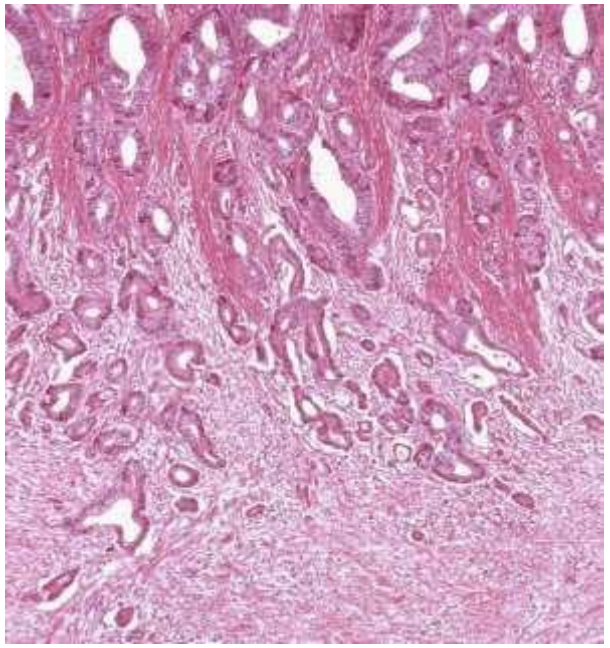

German S3-Guideline for Colorectal Cancer, 2014

Thank You