Ciências ULisboa

REPÚBLICA PORTUGUESA
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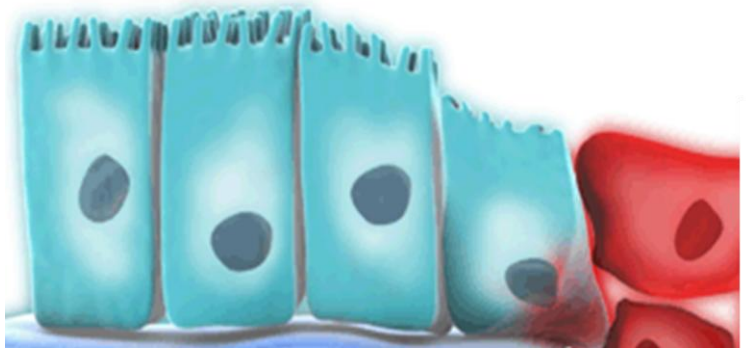
Instituto_Nacional de Saúde Doutor Ricardo Jorge



Oncobiology

Margarida Gama-Carvalho (DQB/FCUL) and Peter Jordan (INSA)

Molecular subtypes of sporadic colorectal cancer

International Agency for Research on Cancer
World Health Organization

GLOBAL CANCER OBSERVATORY

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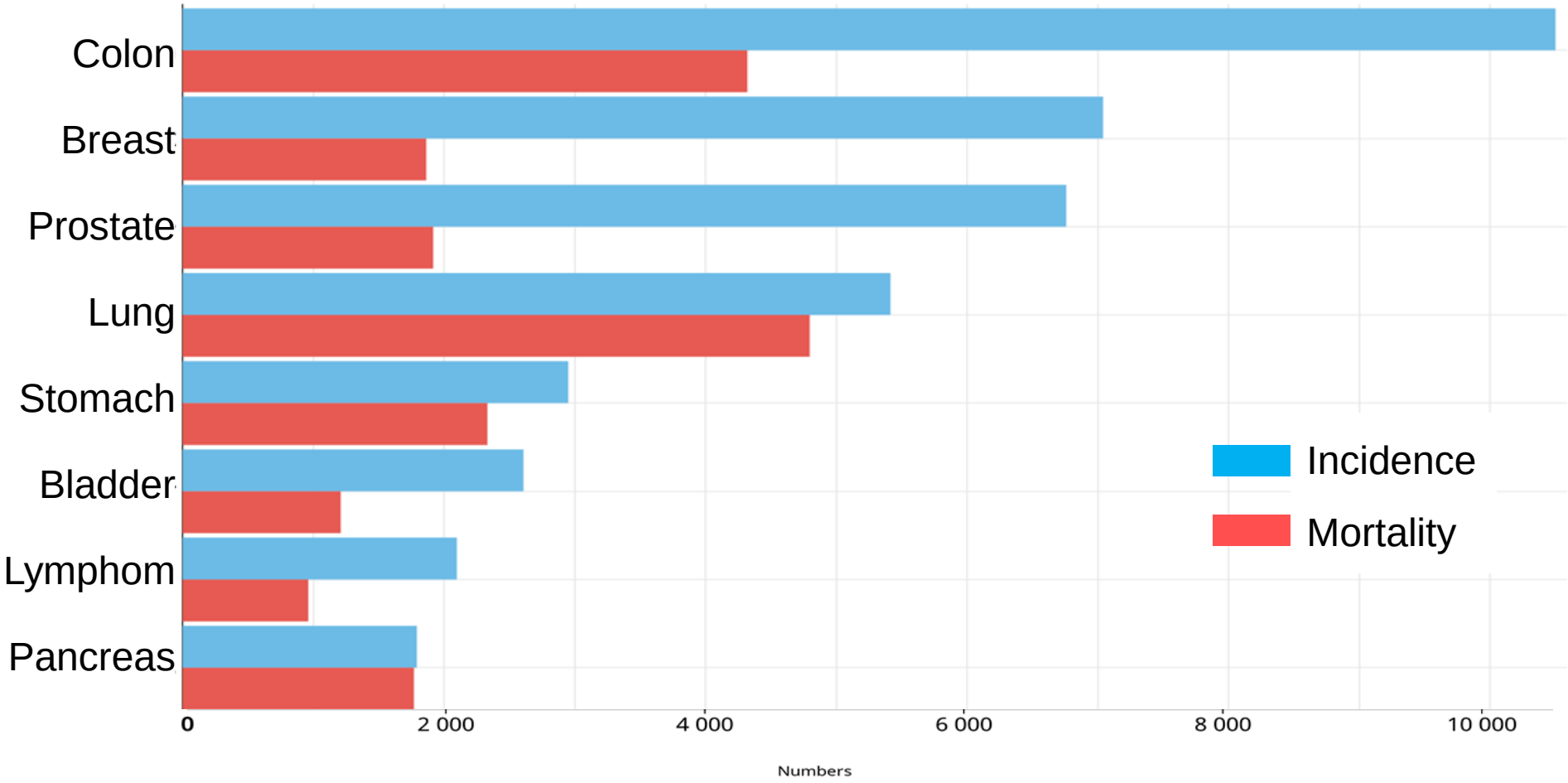
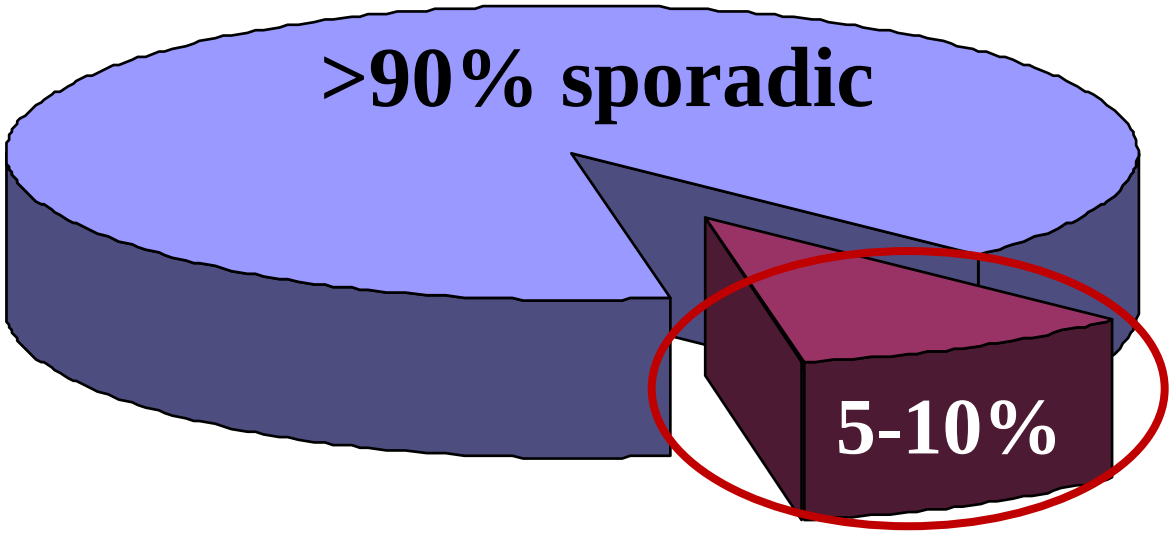
Estimated number of new cases and deaths from cancer in Portugal (2020)



Forms of colorectal cancer



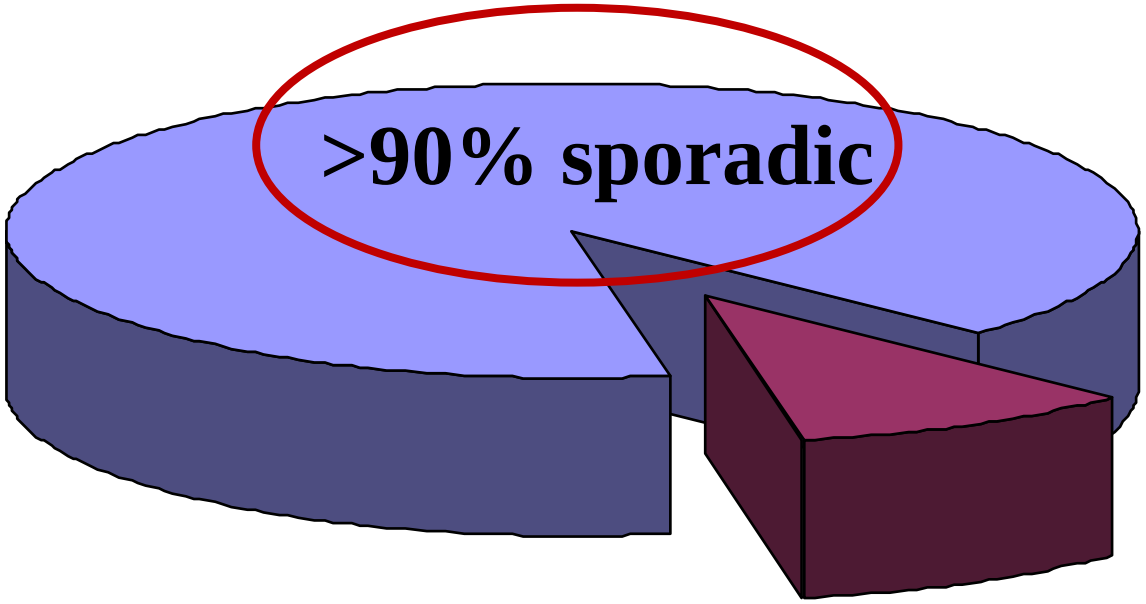
hereditary

Germline mutation in a *tumour suppressor gene*

Reminder:
major hereditary colorectal cancer syndromes

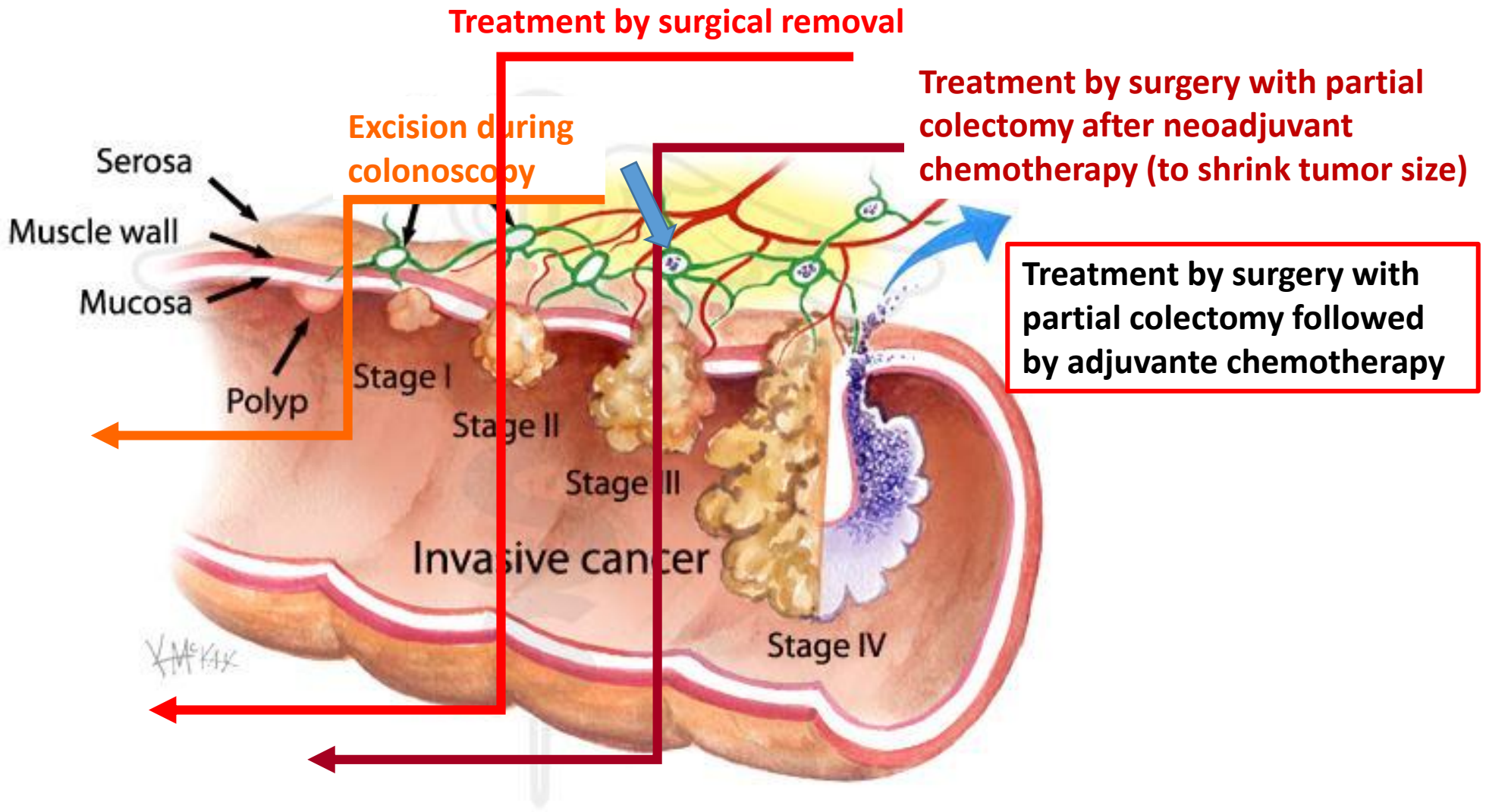
	Syndrome	Gene	Features
with polyps	Familial adenomatous polyposis (FAP)	APC	>100 polyps (adenomas) in the colon detectable in second decade of life; autosomal dominant inheritance
	Attenuated FAP	APC	20 -100 polyps in the colon,
	MYH polyposis	MYH	~10 polyps in the colon, recessive inheritance of mutated DNA glycosylase in Base Excision Repair
no polyps	HNPCC or Lynch syndrome	MSH2 or MLH1 or MSH6 or PMS2	- **no polyps** formed; - high frequency of DNA sequence mutations; - most common hereditary colon cancer syndrome, - can also cause endometrial or ovarian cancer;

Forms of colorectal cancer



A heterogeneous disease: challenge and opportunity

Anatomical stages of colorectal cancer



Stages of colorectal tumour development

Chemotherapy options for colorectal cancer

commonly used regimens:

FOLFOX (leucovorin, 5-FU, and oxaliplatin)

FOLFIRI (leucovorin, 5-FU, and irinotecan)

CAPEOX (capecitabine and oxaliplatin)

FOLFOXIRI (leucovorin, 5-FU, oxaliplatin, irinotecan)

Side effects

Targeted therapy or
Precision medicine or
Personalized medicine

5-FU: (5-fluorouracil- anti-metabolite)

decreased dTTP levels lead to FdUTP incorporation during DNA replication

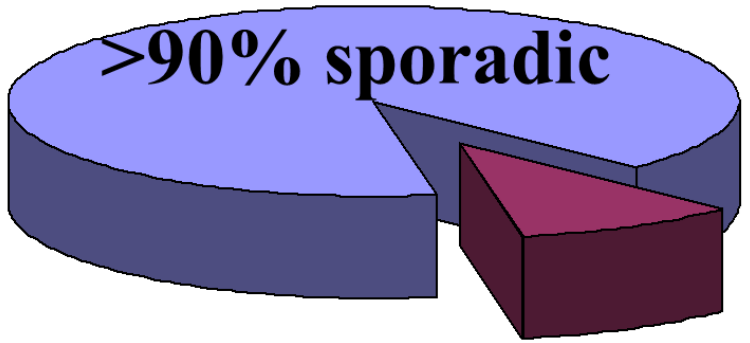
Leucovorin: Folinic acid, stabilizes 5-FU binding to thymidylate synthase

Capecitabine: 5-FU precursor

Oxaliplatin: DNA alkylating agent (DNA damage)

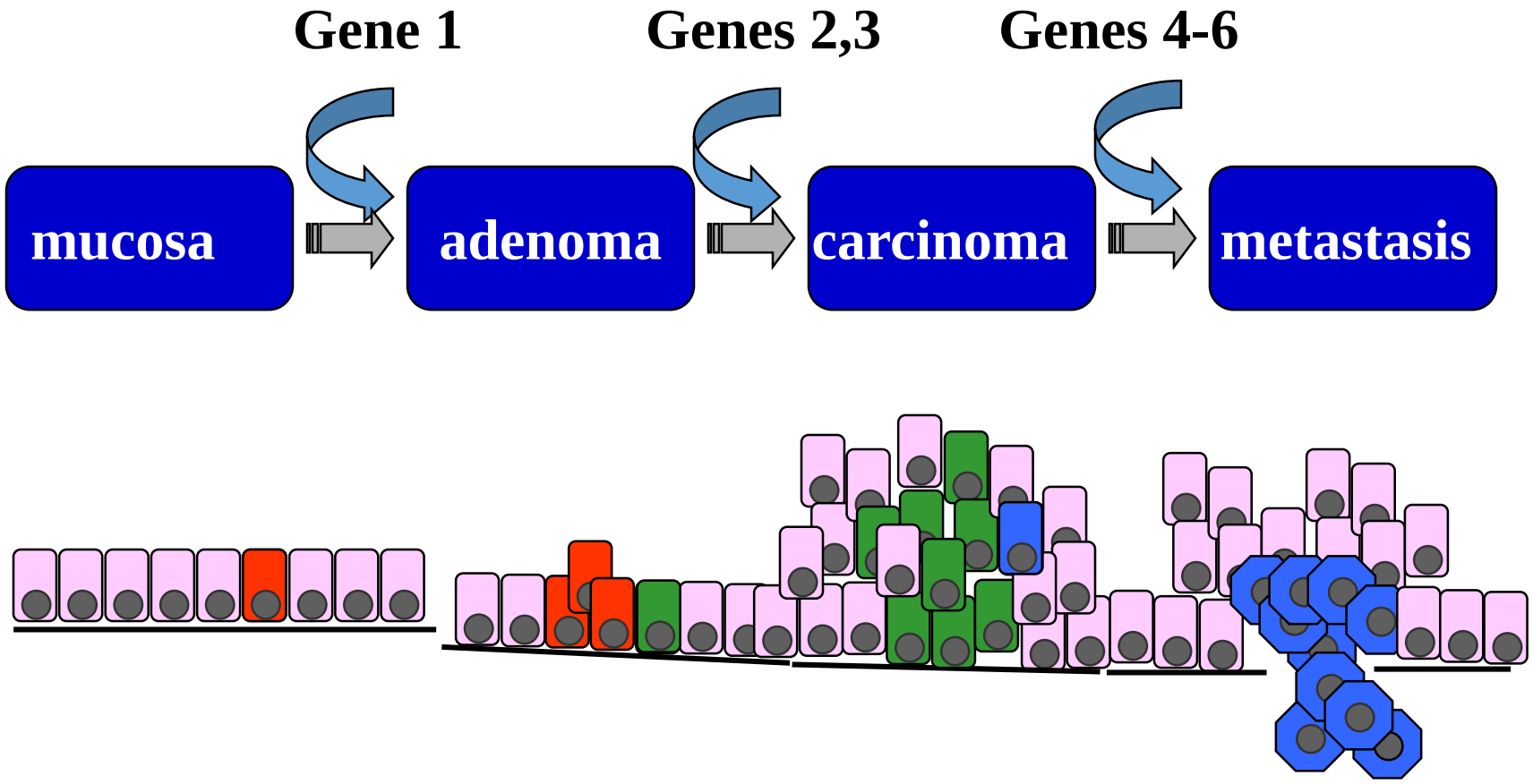
Irinotecan: Topoisomerase I inhibitor (DNA replication)

(Check 14 min-video animation- DNA damage exploited by chemotherapy agentes from lecture 7-DNA repair)



targeted therapy design: based on understanding the molecular subtypes of sporadic colorectal cancer

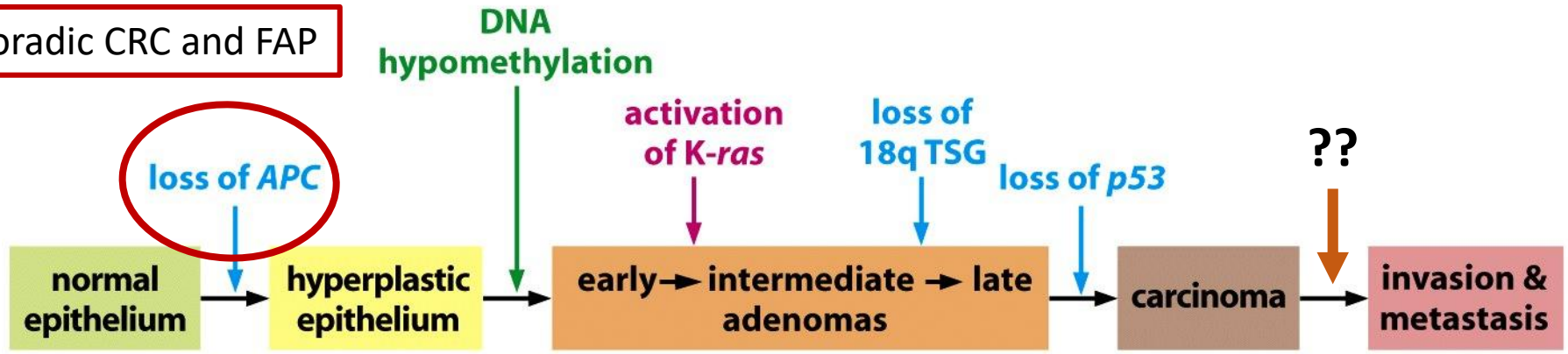
The classical model of *clonal selection* in colorectal tumorigenesis



Accumulation of genetic changes in a cell

In the early days…

in sporadic CRC and FAP



Fearon and Vogelstein, A Genetic Model for Colorectal Tumorigenesis, Cell 61, 759-767 (1990)

"…Thus, most colorectal carcinomas probably arise from a minimum of five genetic alterations….

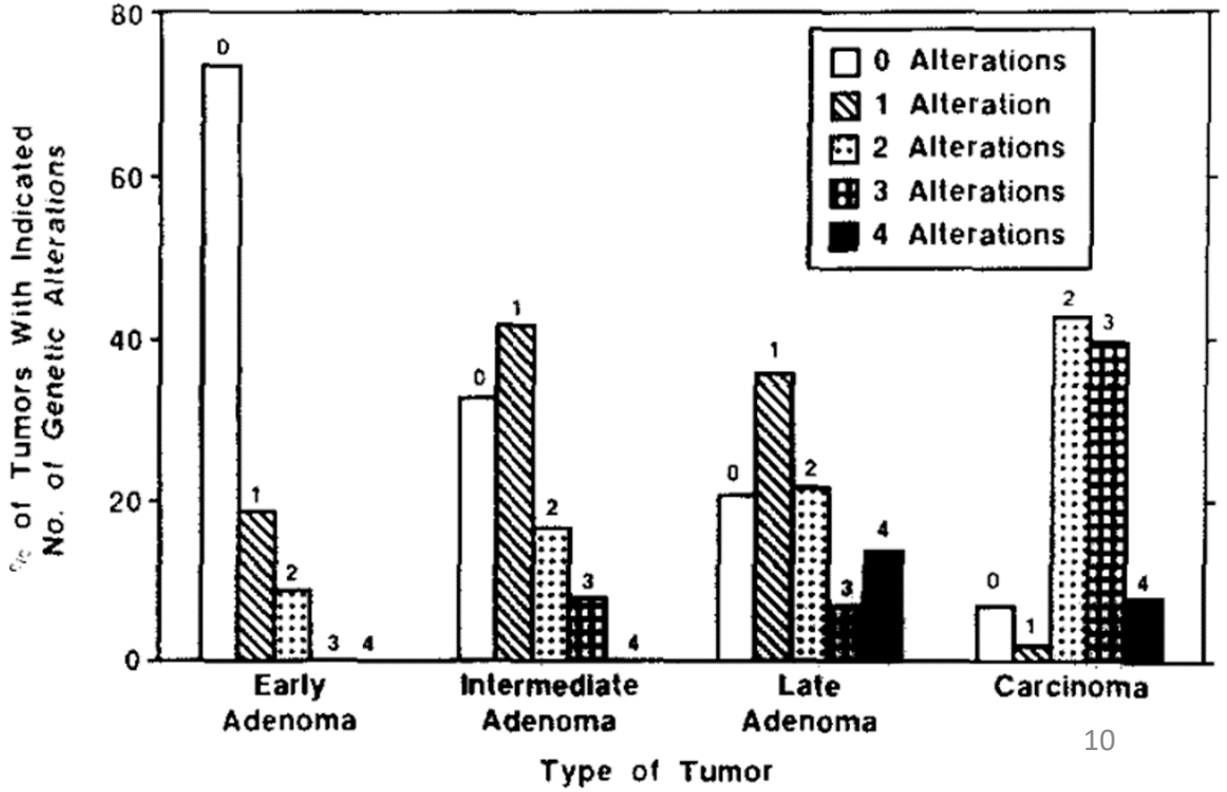
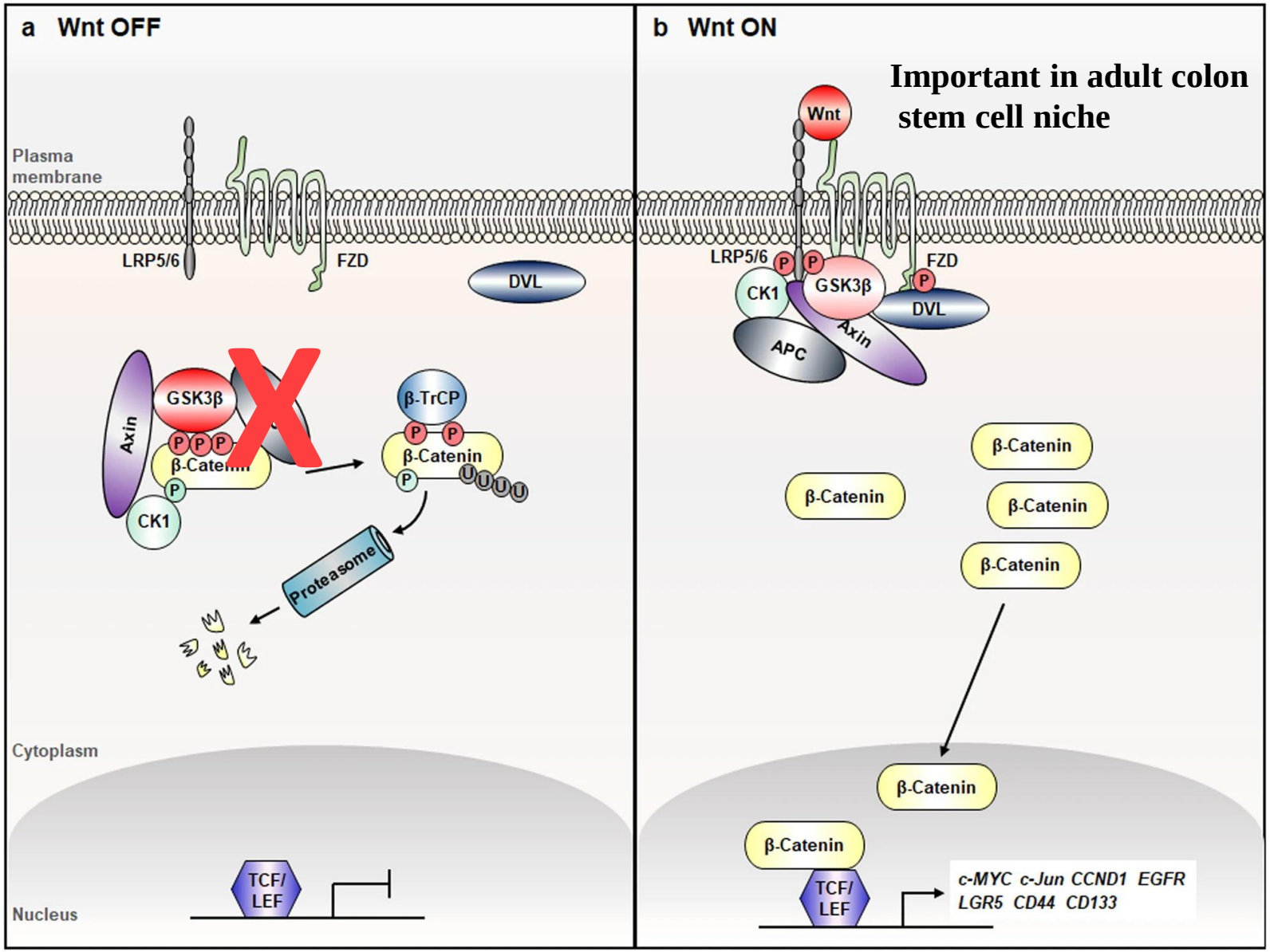


Figure 11.10 *The Biology of Cancer* (© Garland Science 2007)

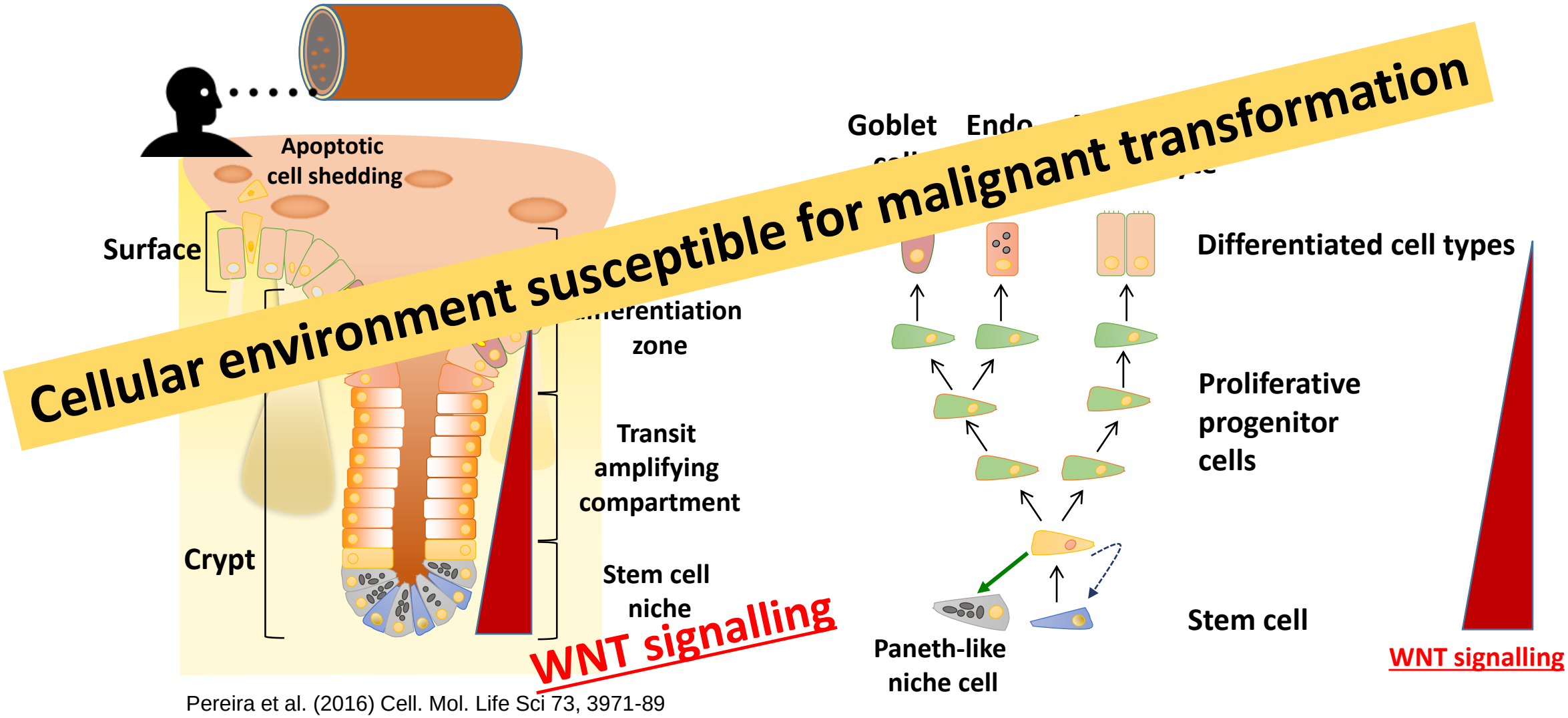
APC tumor supressor gene controls the transcriptional cofactor β-catenin



Wnt signaling

Wnt/β-catenin pathway

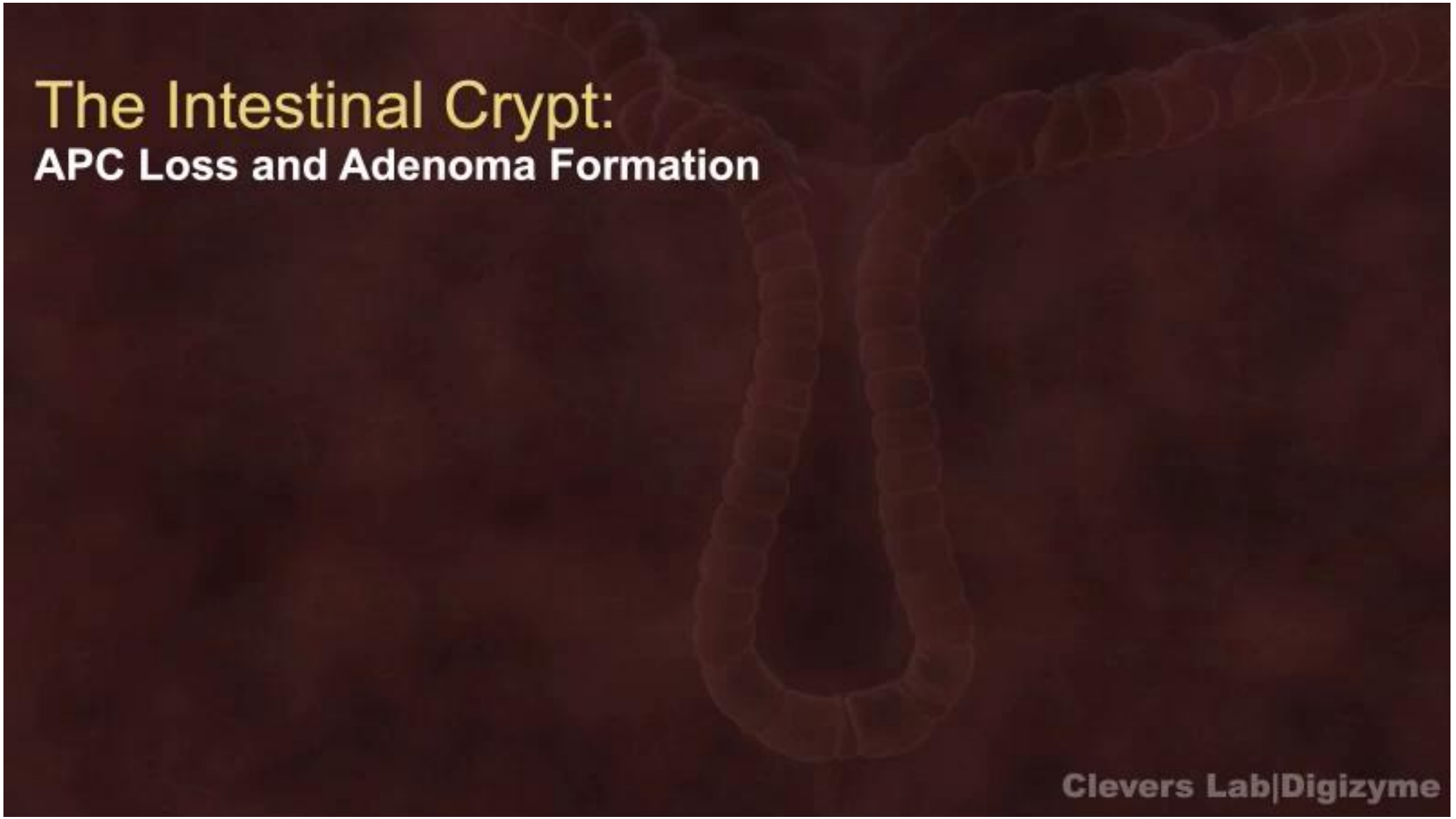
β-catenin/Wnt signalling and colon crypt organization with stem cells:

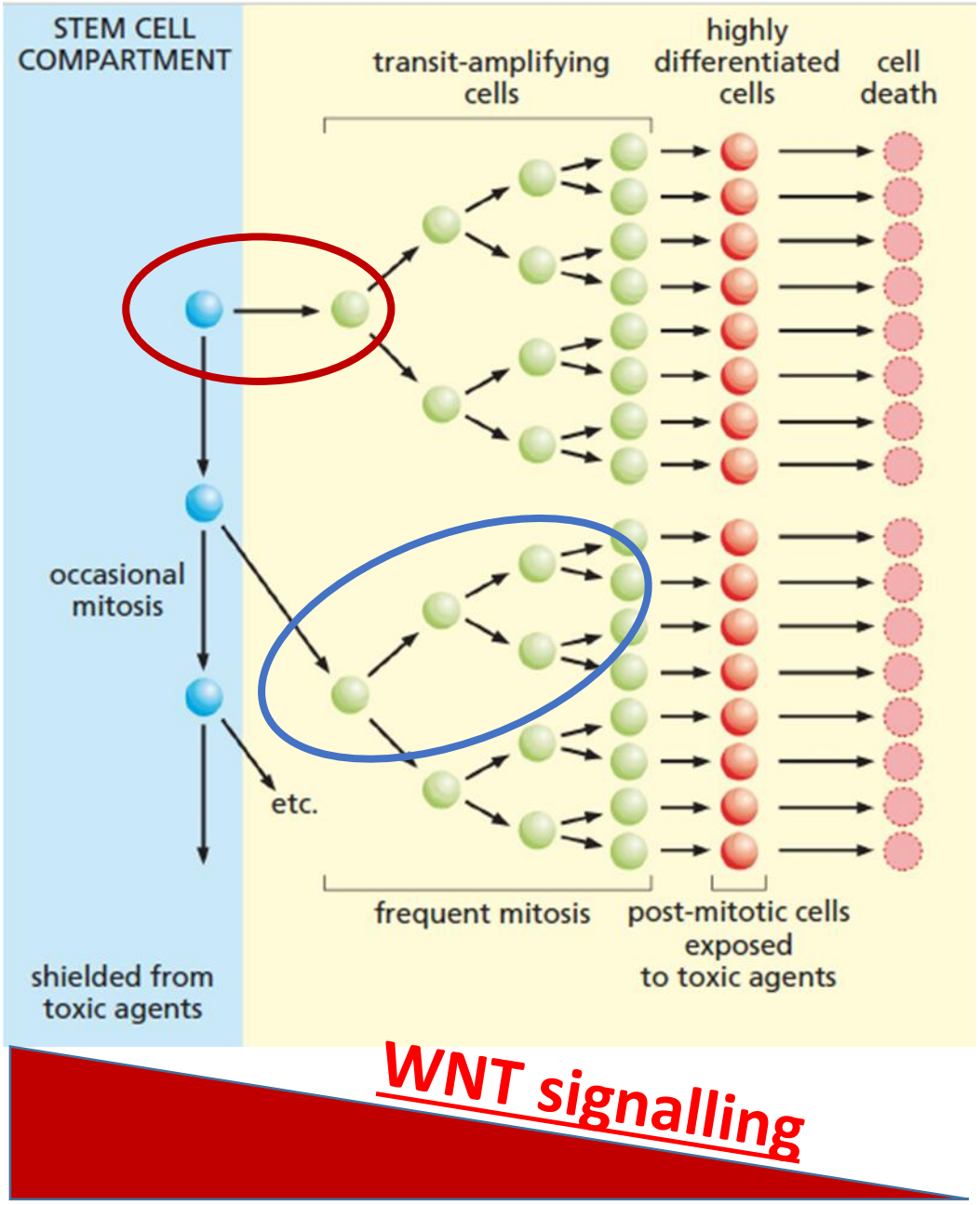


Cellular environment susceptible for malignant transformation

Pereira et al. (2016) Cell. Mol. Life Sci 73, 3971-89

Hans Clevers lab- Utrecht





Stem cell biology and cancer stem cells

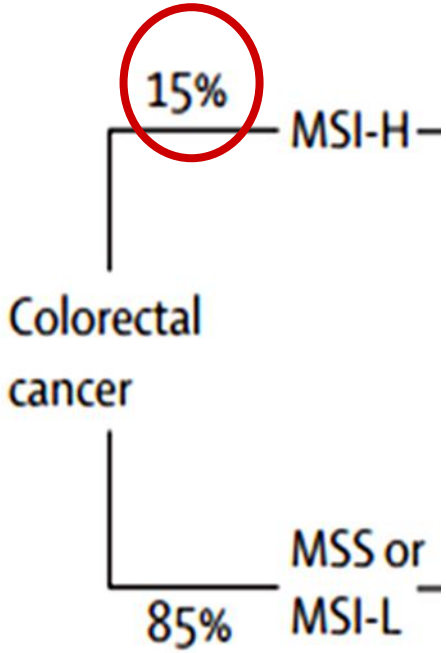
stem cell (blue) divides only occasionally in an asymmetric fashion to generate a new stem cell daughter and a transit-amplifying daughter.

The transit-amplifying cells (*green*) undergo repeated rounds of growth and division, expanding the cell population before starting to differentiate

beyond APC…

molecular subtypes of sporadic colorectal cancer-

Genetic heterogeneity in sporadic CRC



MicroSatellite Instability

Microsatellites are

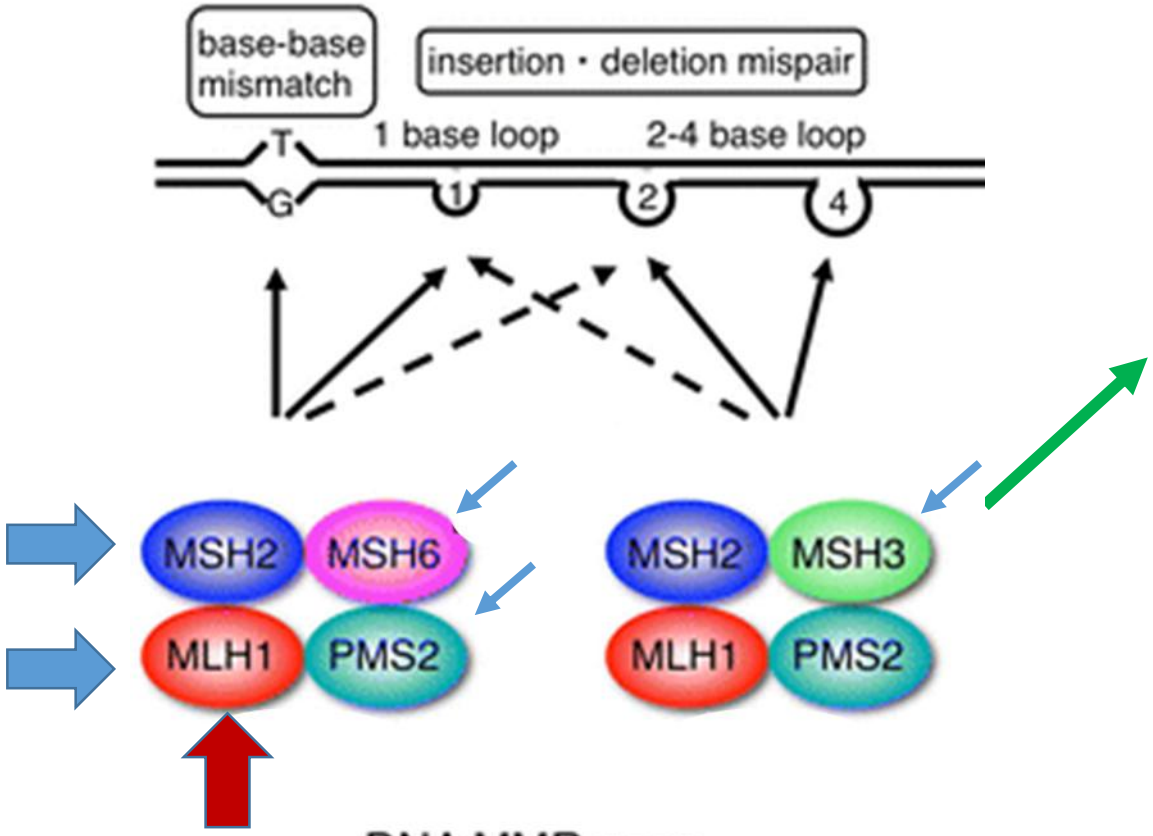
non-coding genome regions enriched in mono- or di-nucleotide repeats

=

prone to slippage errors during replication

Adapted from Brenner et al Lancet 2014

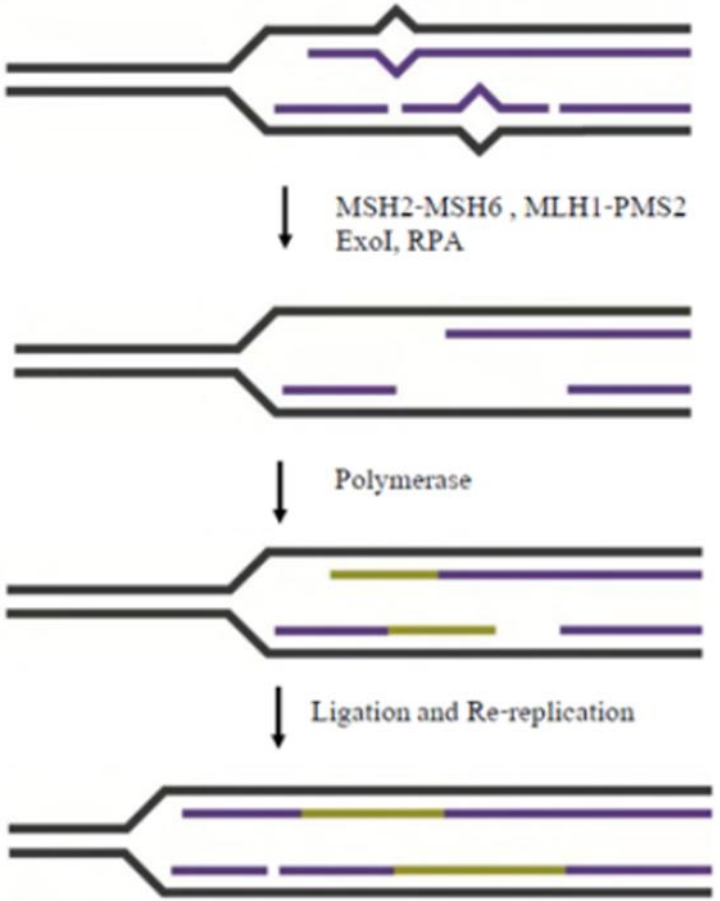
Mismatch error recognition



Mutated in hereditary colon cancer

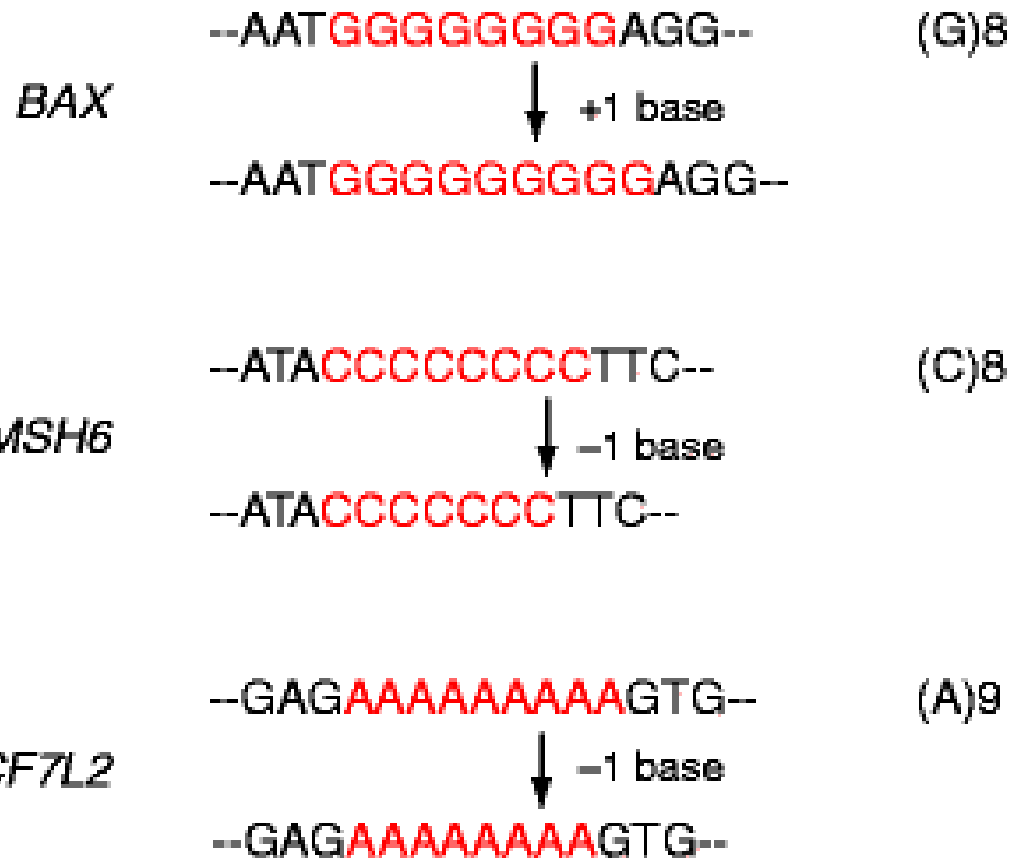
Gene silenced in sporadic MSI-CRC

Error repair



..what happens in microsatellites also happens genome-wide,
therefore also in coding sequences of some tumor supressor genes

Nucleotide repeats:
GGG- Gly
CCC- Pro
AAA- Lys

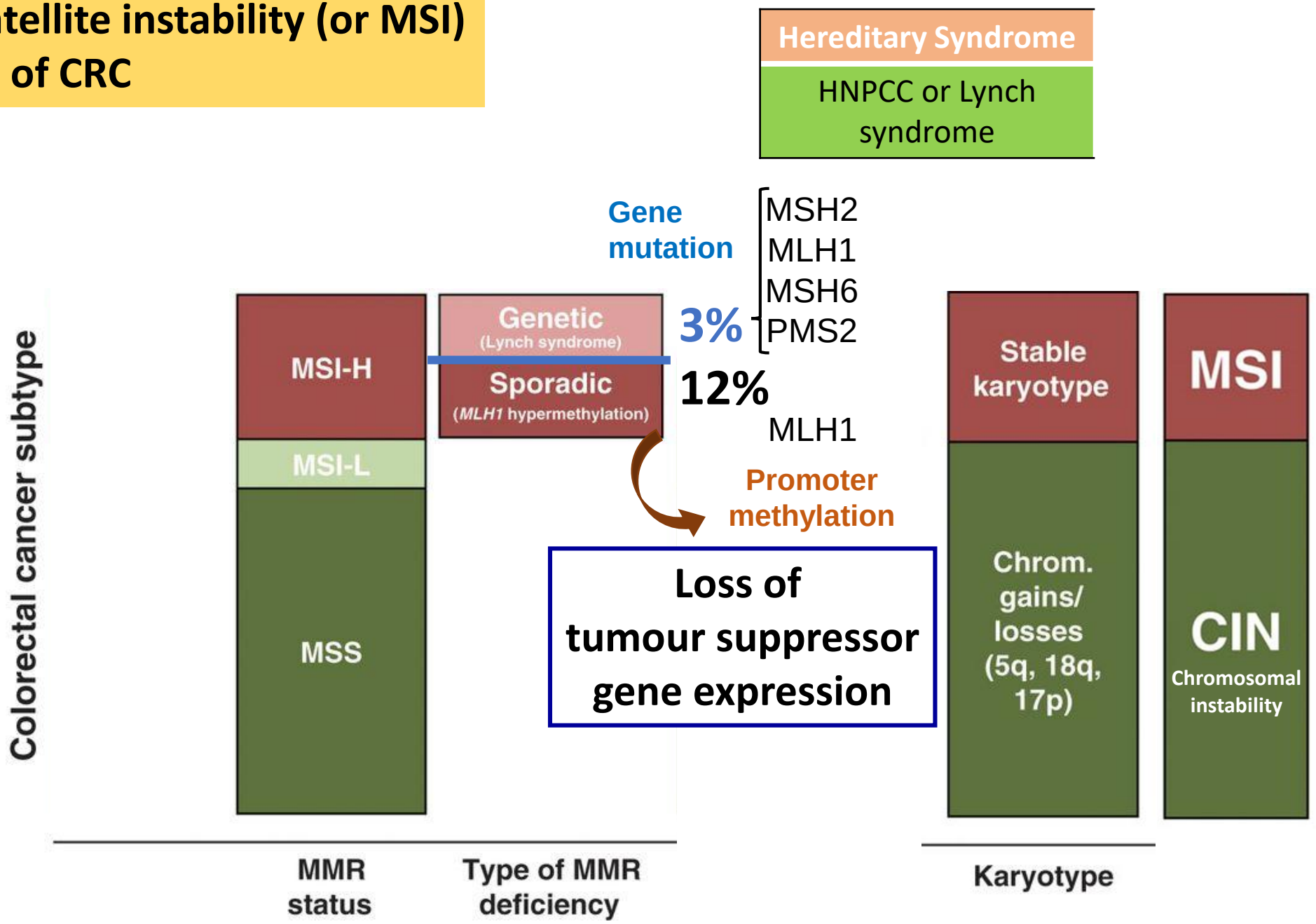


TGF-β is a growth-inhibitory signal for normal epithelial cells

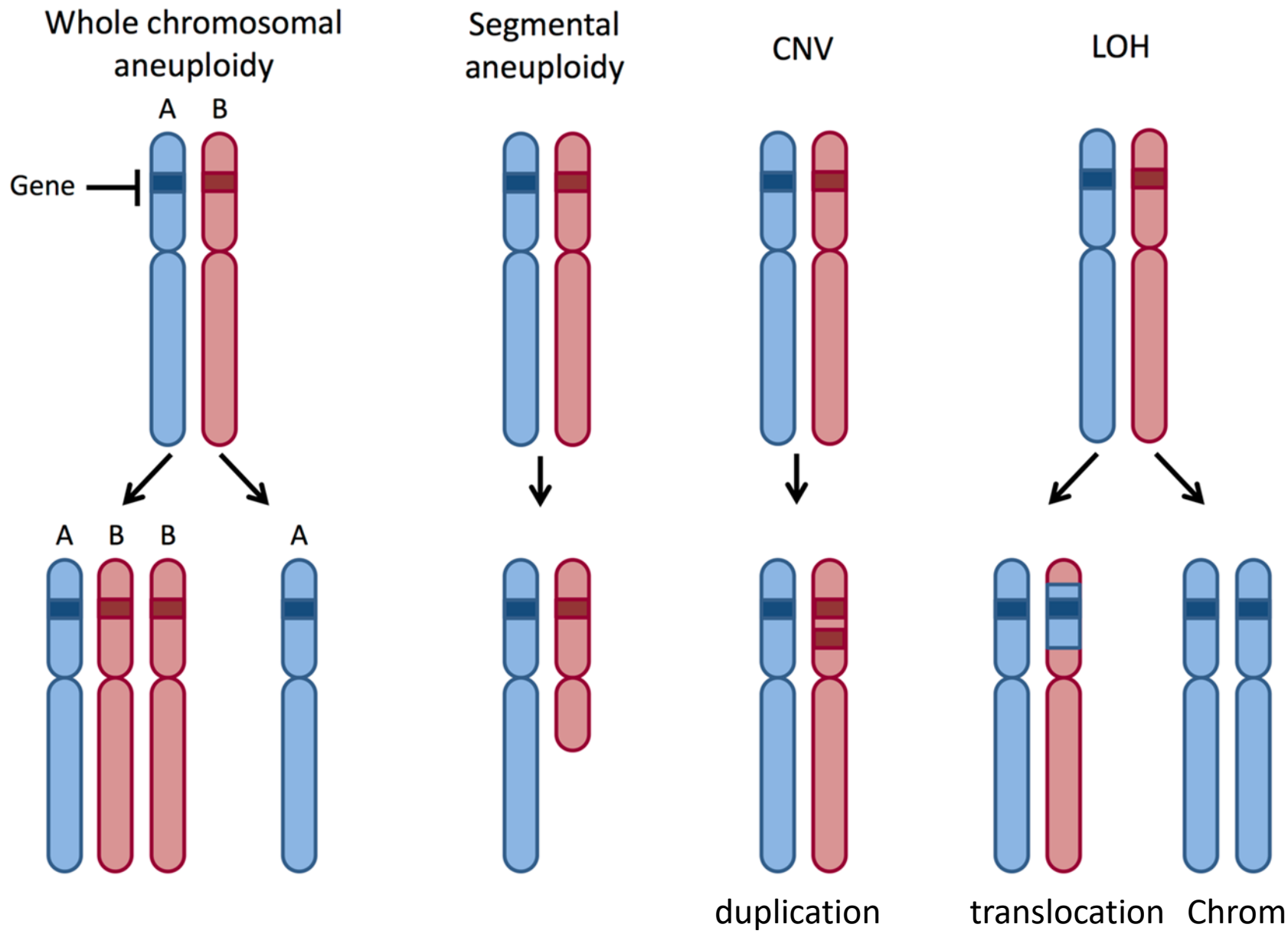
Designations

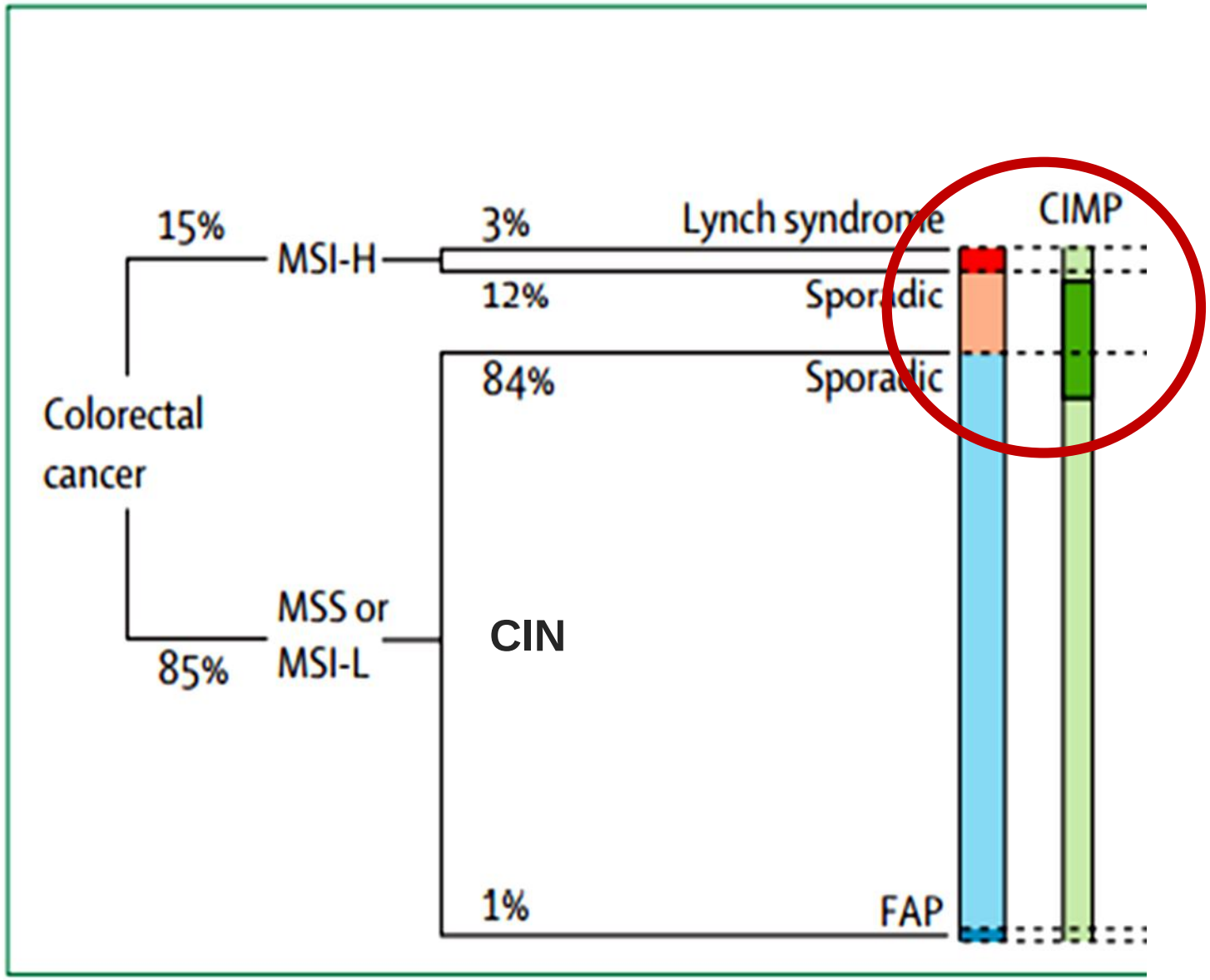
	Normal counterpart:
Microsatellite instability (or MSI),	**Microsatellite stability (or MSS),**
Mismatch repair deficiency (dMMR),	**Mismatch repair proficiency (pMMR),**
DNA sequence instability,	**DNA sequence stable**
Mutator phenotype,	
High tumour mutation burden ≥ 10 mut/Mb	**Low tumour mutation burden** ~1 mut/Gb

Microsatellite instability (or MSI) in ~15% of CRC



CIN and chromosomal alterations affecting gene dosage



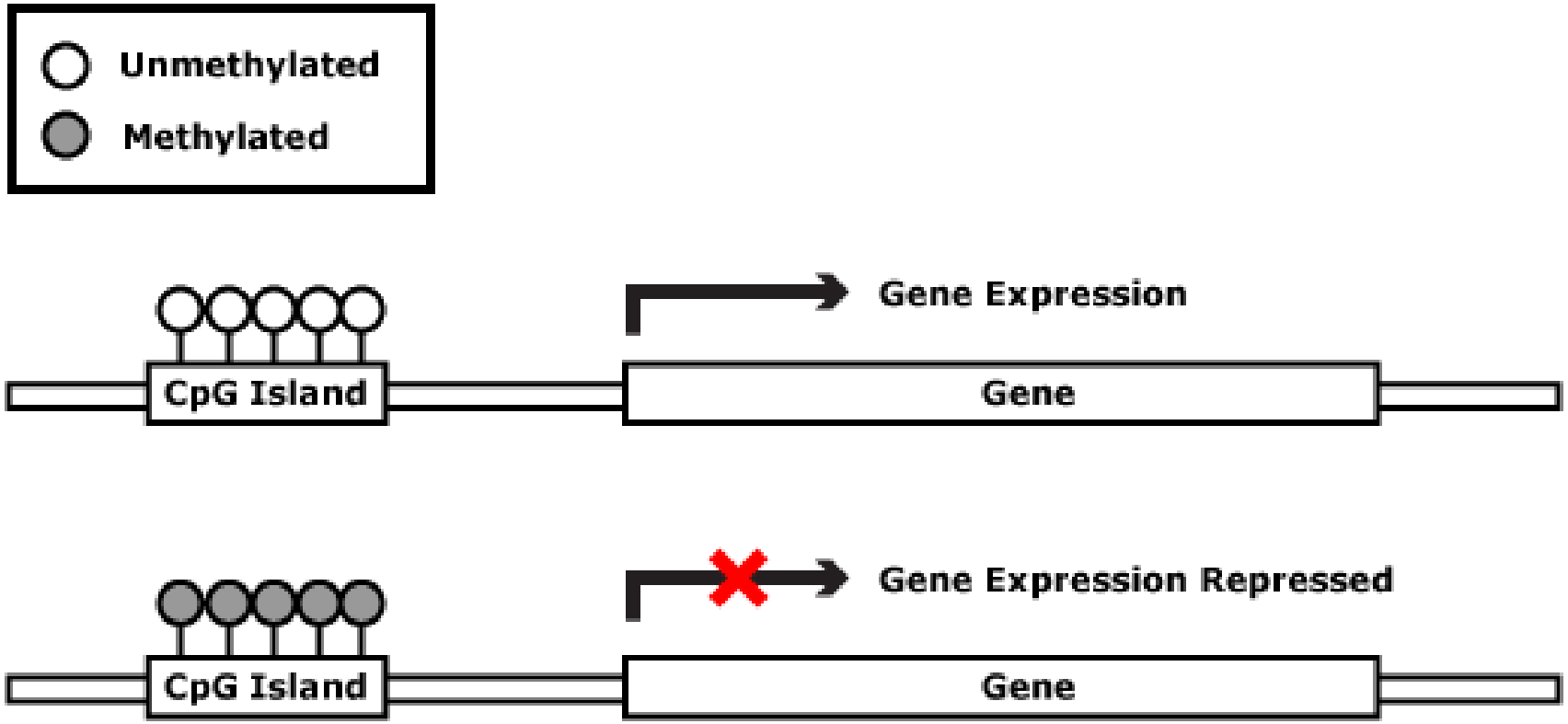


Partial overlap of MSI and CIMP

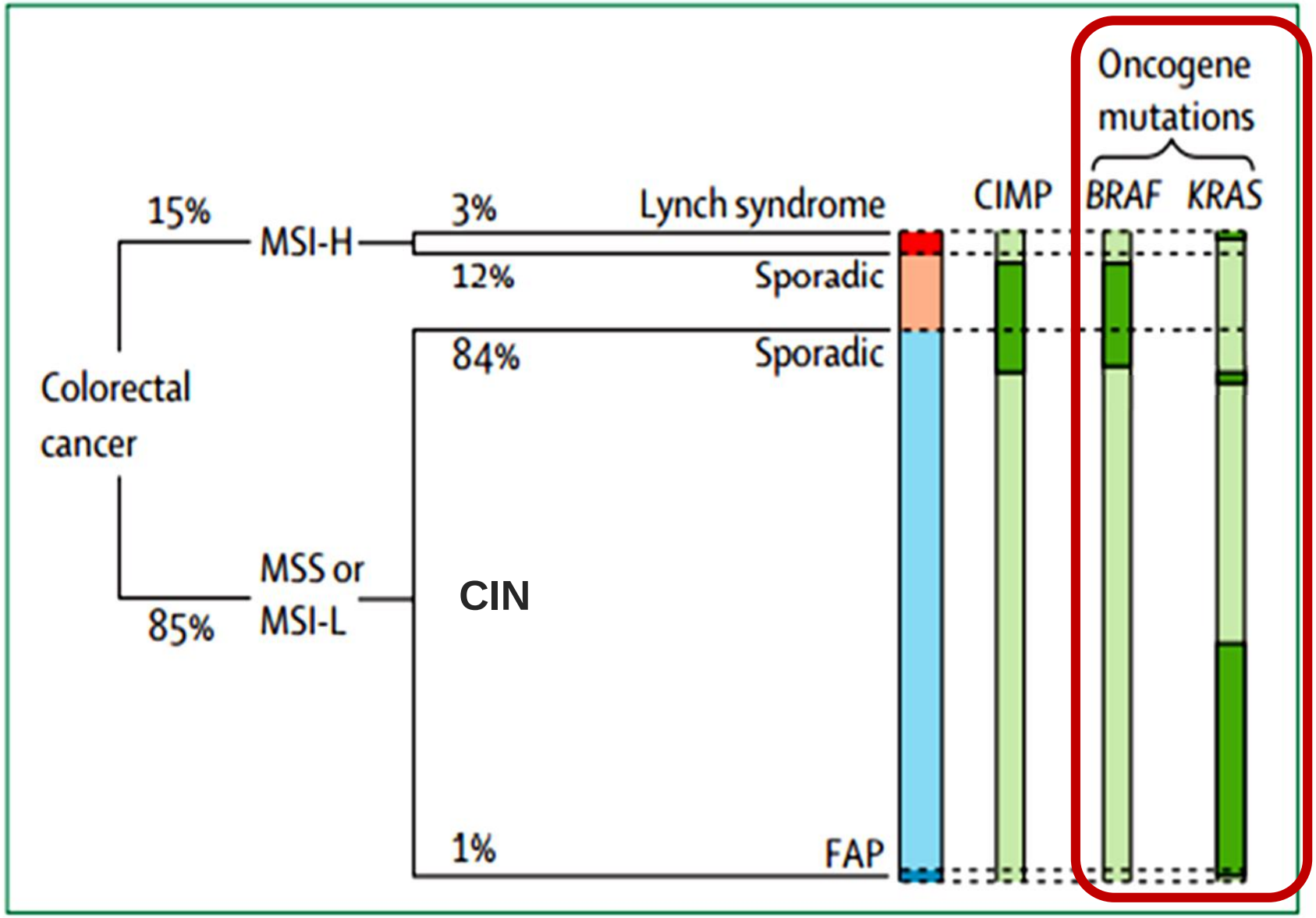
Molecular genetics of colorectal cancer subtypes

adapted from : Brenner et al Lancet 2014

CIMP- **C**pG **i**sland **m**ethylator **p**henotype



CIMP= Genome-wide epigenetic imbalance;
Eventually affects promoters of tumour supressor genes, such as p16**INK4a**, hMLH1, …



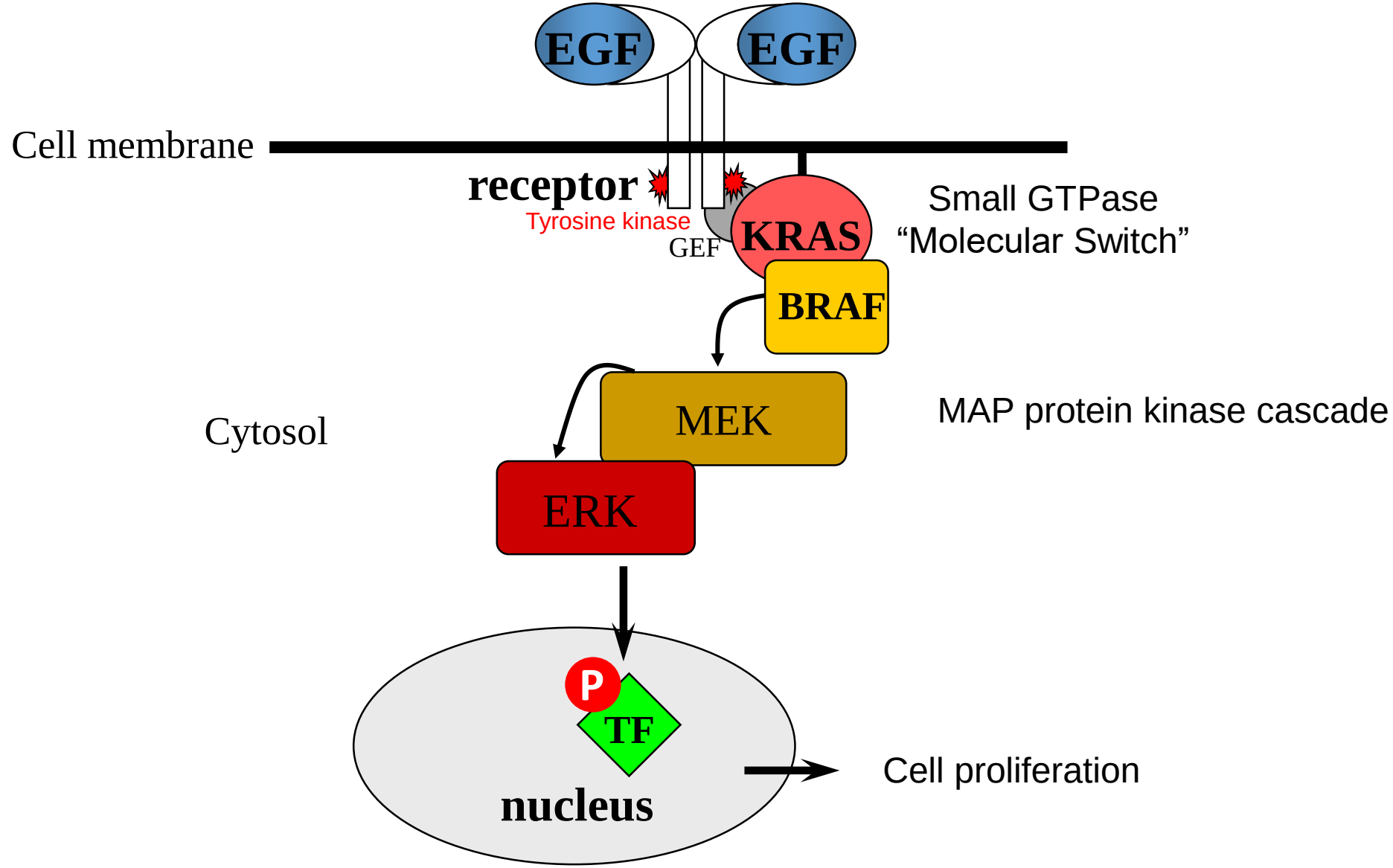
Correlation with mutated oncogenes

Molecular genetics of colorectal cancer subtypes

adapted from : Brenner et al Lancet 2014

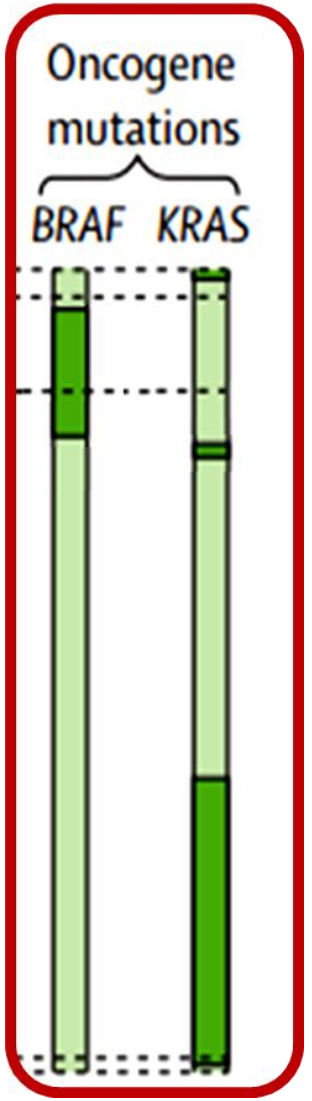
activation of
KRAS and *BRAF* oncogenes
in colorectal cancer

KRAS and *BRAF* oncogenes encode proteins of the MAP kinase pathway

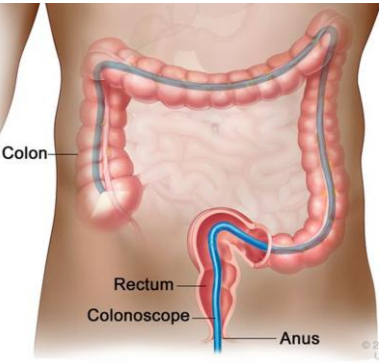


Major oncogenic mutations affecting MAPK signalling in CRC

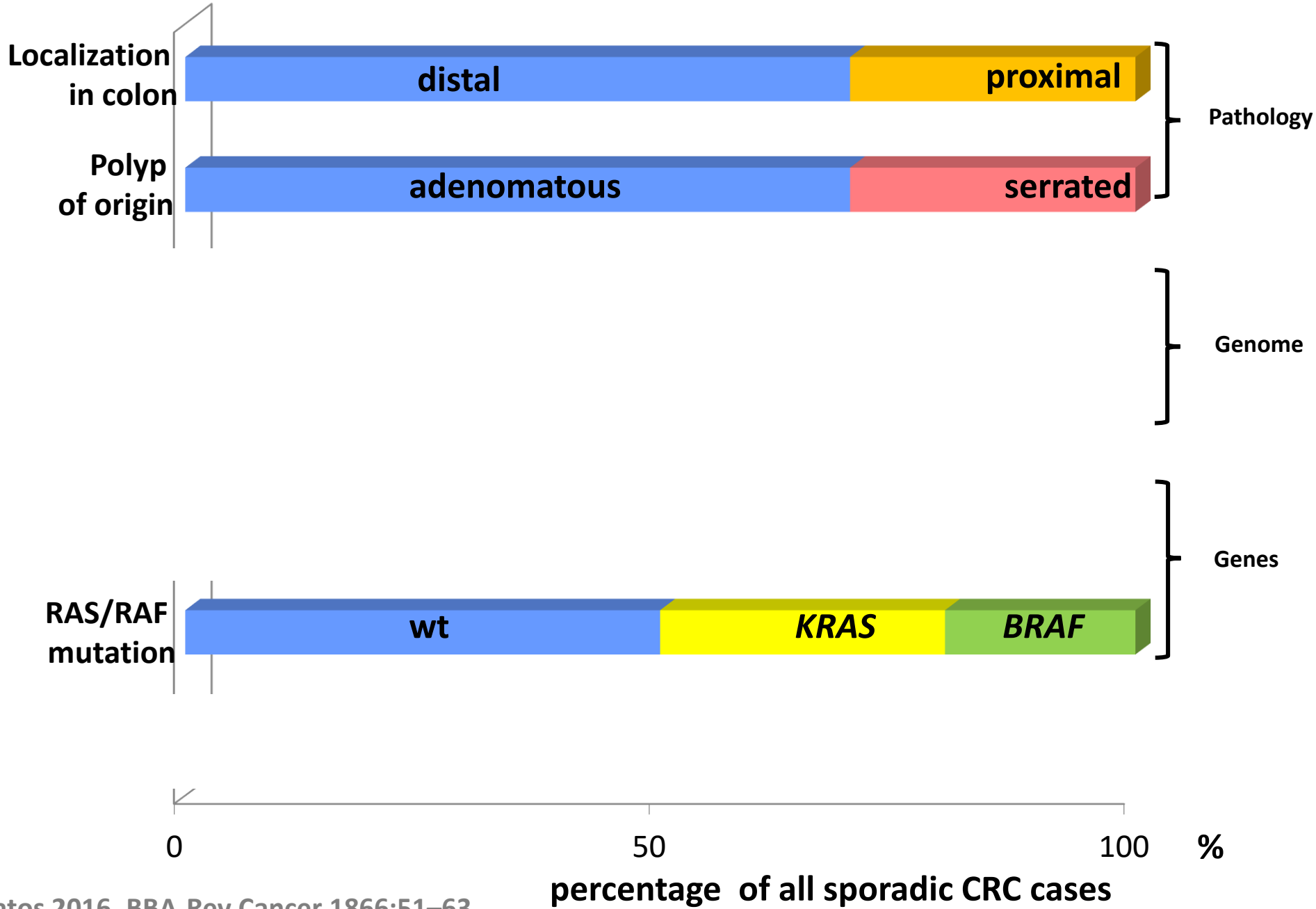
Gene	Mutated in % CRC	effect
KRAS	32–40%	Activating KRAS mutation in codons 12, 13 or 61 that activate the MAPK and PI3K pathways.
BRAF	15%	Activating BRAF mutations (V600E in 95%) activate MAPK but not PI3K pathway
KRAS mutations are mutually exclusive with BRAF mutation!		



Lancet Oncol 2011; DOI:10.1016/S1470- 2045(10)70209-6

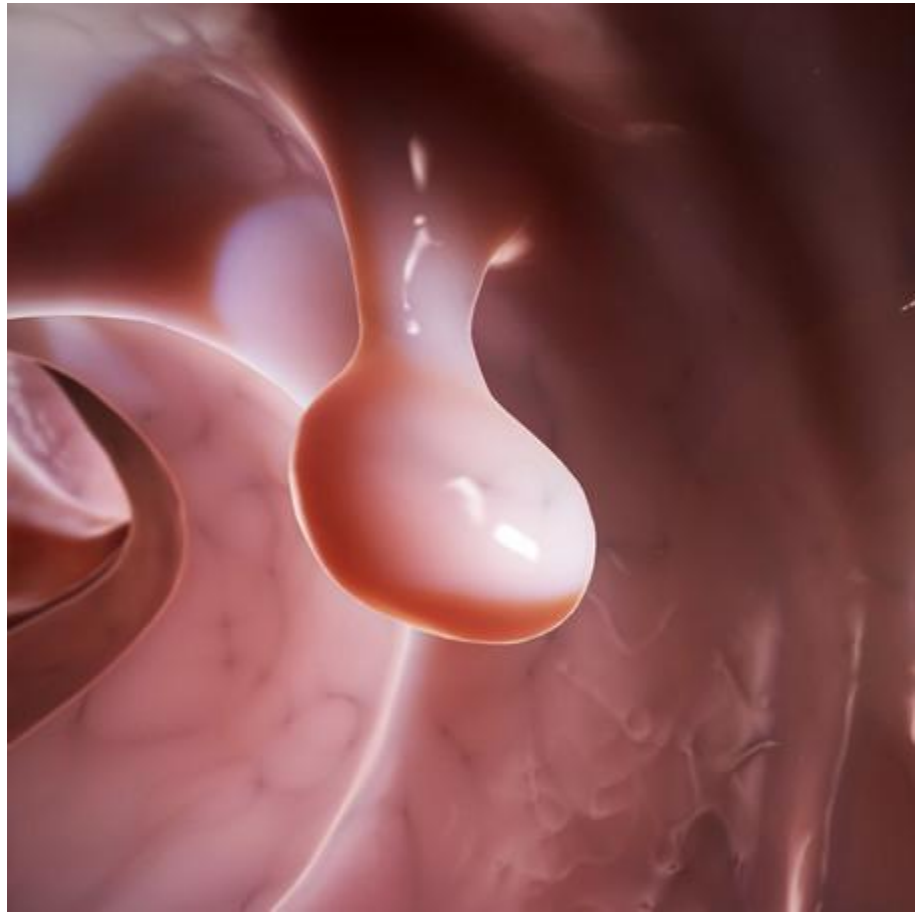


CRC classification:

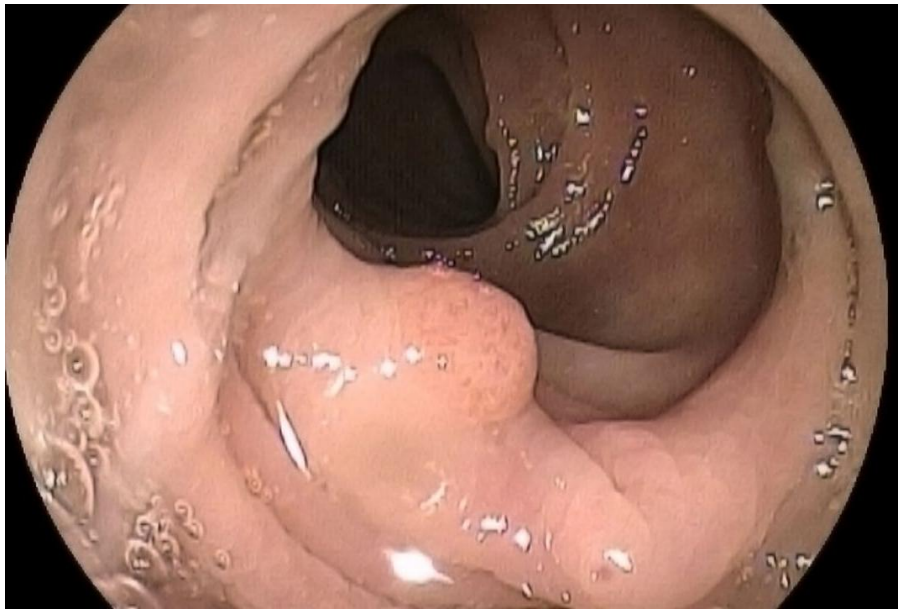


Matos 2016, BBA-Rev Cancer 1866:51–63

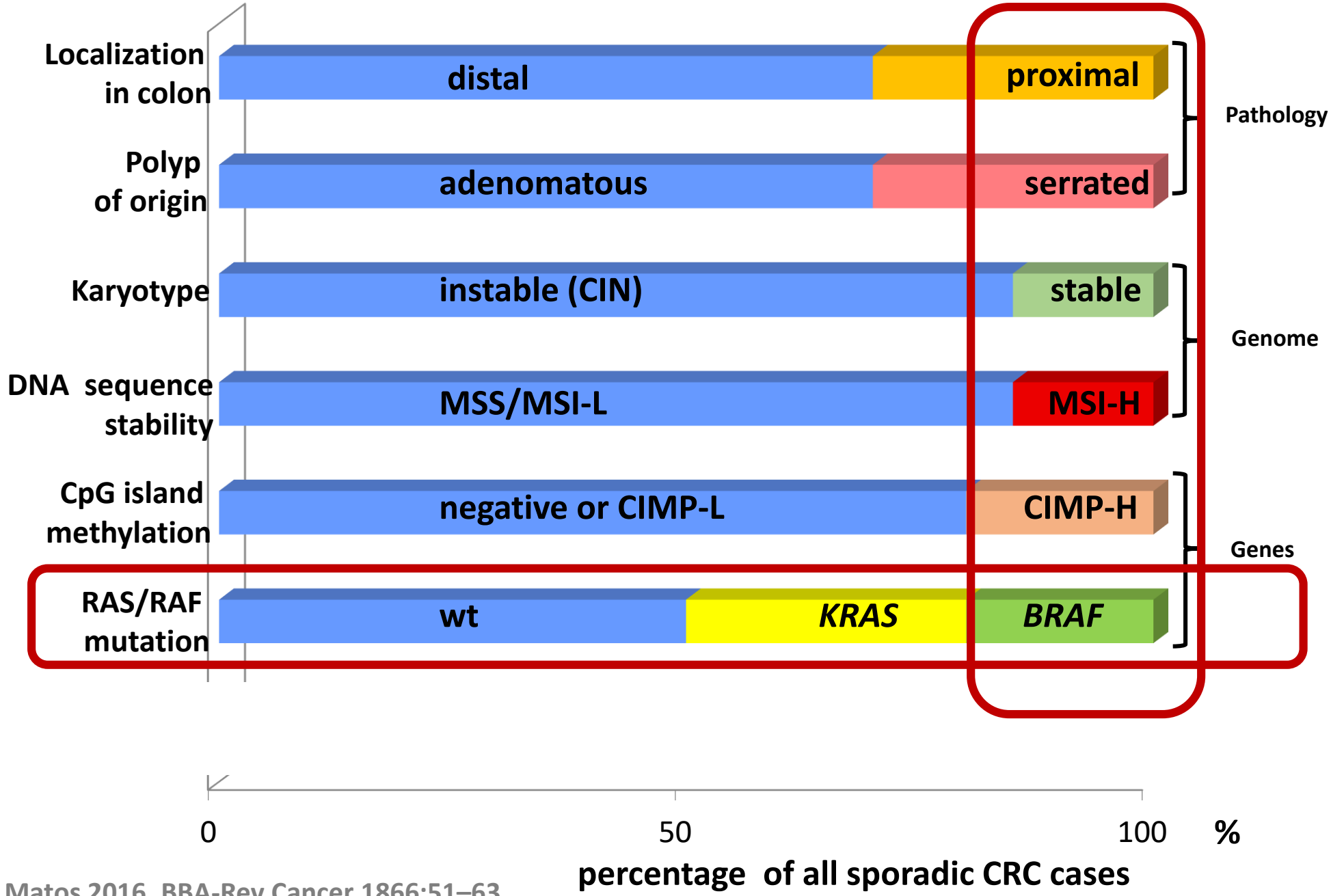
Adenomatous polyp



Sessil serrated polyp



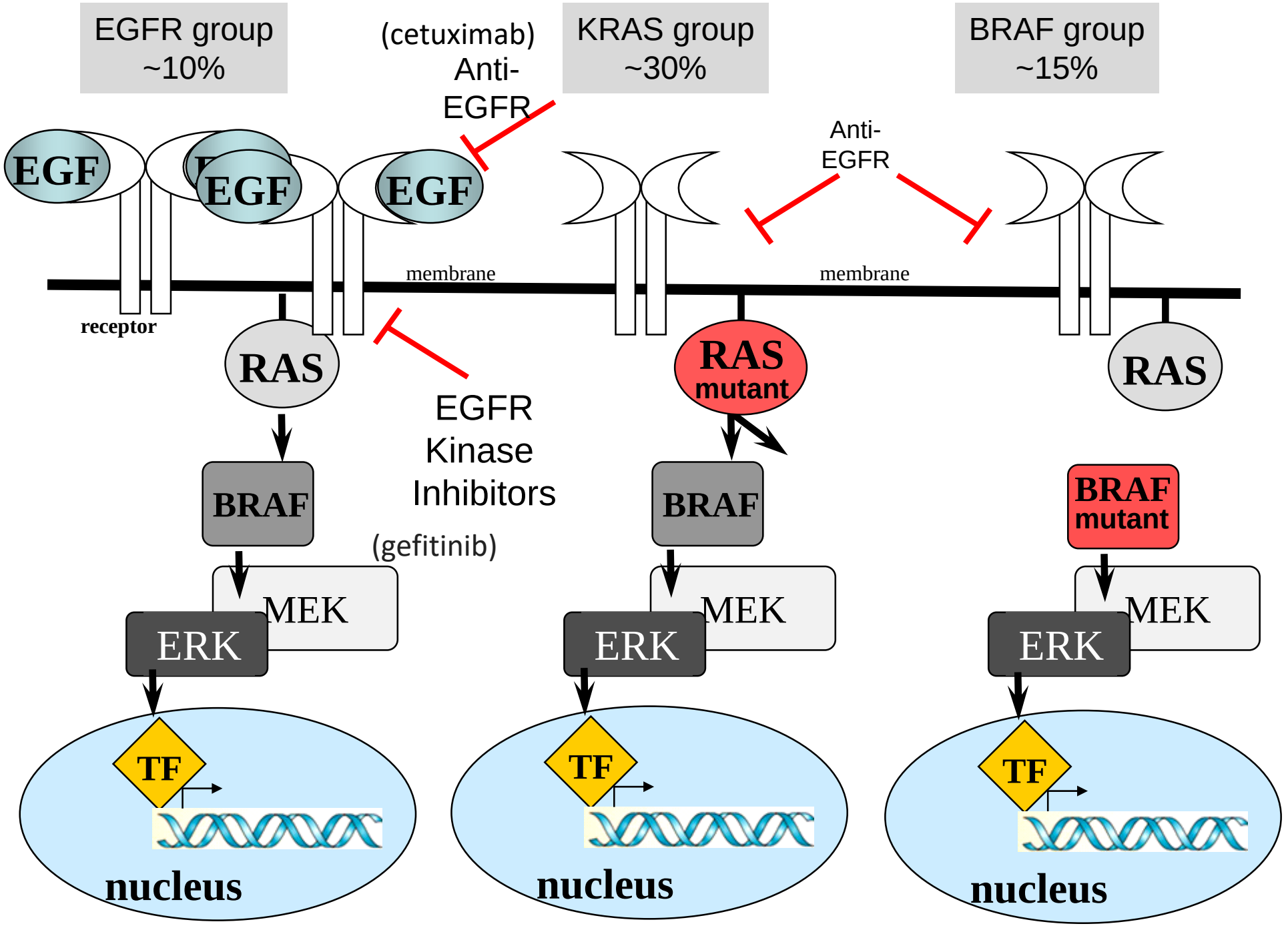
CRC classification:



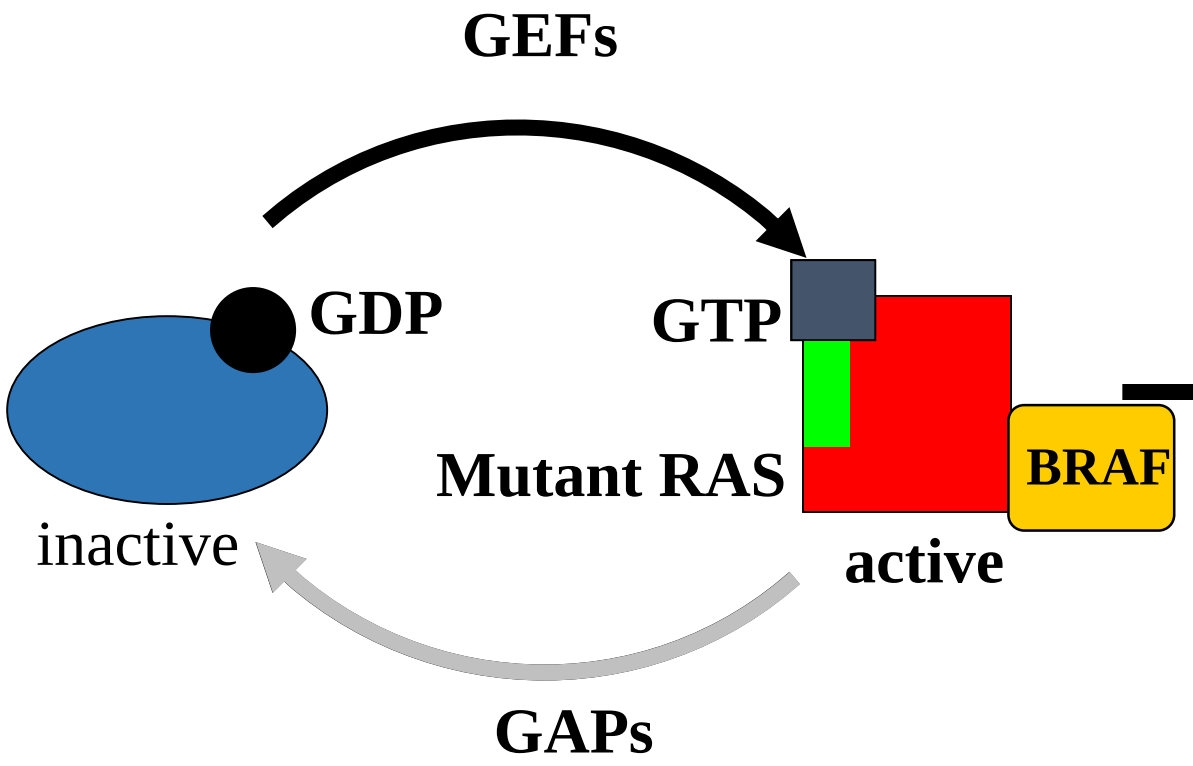
Matos 2016, BBA-Rev Cancer 1866:51–63

Personalized therapy in sporadic CRC:

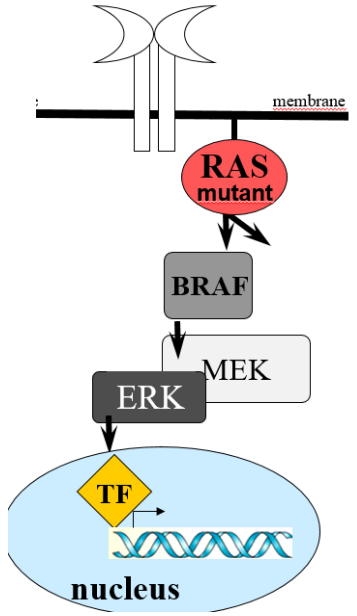
Routine genetic testing of tumours for
KRAS* and *BRAF mutations
to indicate primary resistance against EGFR therapy


EGFR group
~10%
(cetuximab)
Anti-
EGFR
KRAS group
~30%
BRAF group
~15%
Anti-
EGFR
EGF
EGF
EGF
membrane
membrane
receptor
RAS
RAS
mutant
RAS
EGFR
Kinase
Inhibitors
(gefitinib)
BRAF
BRAF
BRAF
mutant
MEK
MEK
MEK
ERK
ERK
ERK
TF
TF
TF
nucleus
nucleus
nucleus

The RAS GTPase cycle



signalling to MAPK



Most frequent mutations:
Gly12 → Val, Asp → impair GAP-binding and subsequent GTP hydrolysis

Cell proliferation

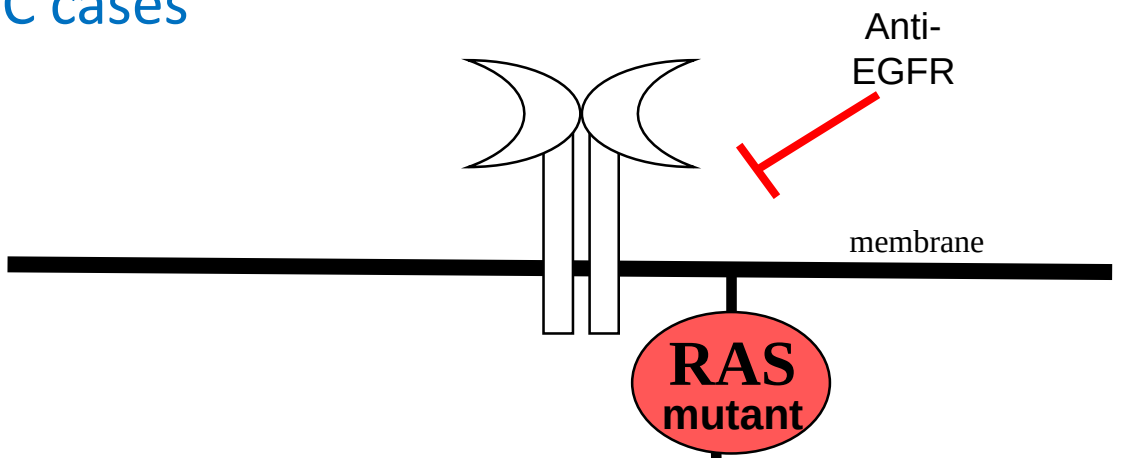
KRAS mutations in CRC

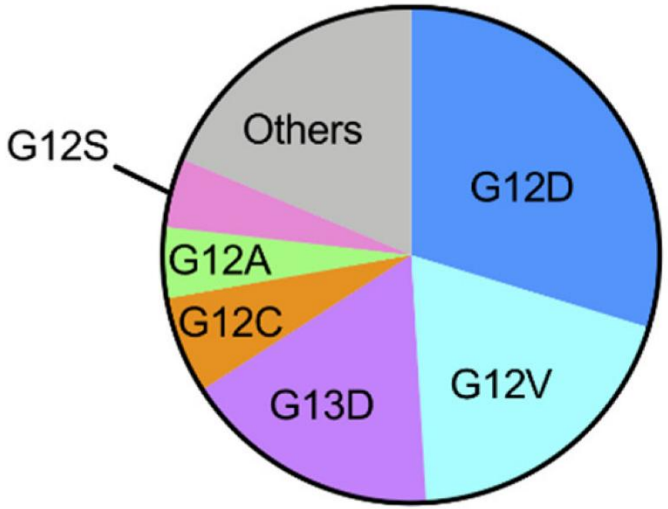
KRAS mutations in **~30%** of sporadic CRC cases

per Codon:
G12: 70%, G13 (25%) and Q61 (1%)

In Codon 12:
G12D (36%), G12V (28%) , **G12C (5%)**

KRAS group
~30%





Colorectal cancer

KRAS inhibition:

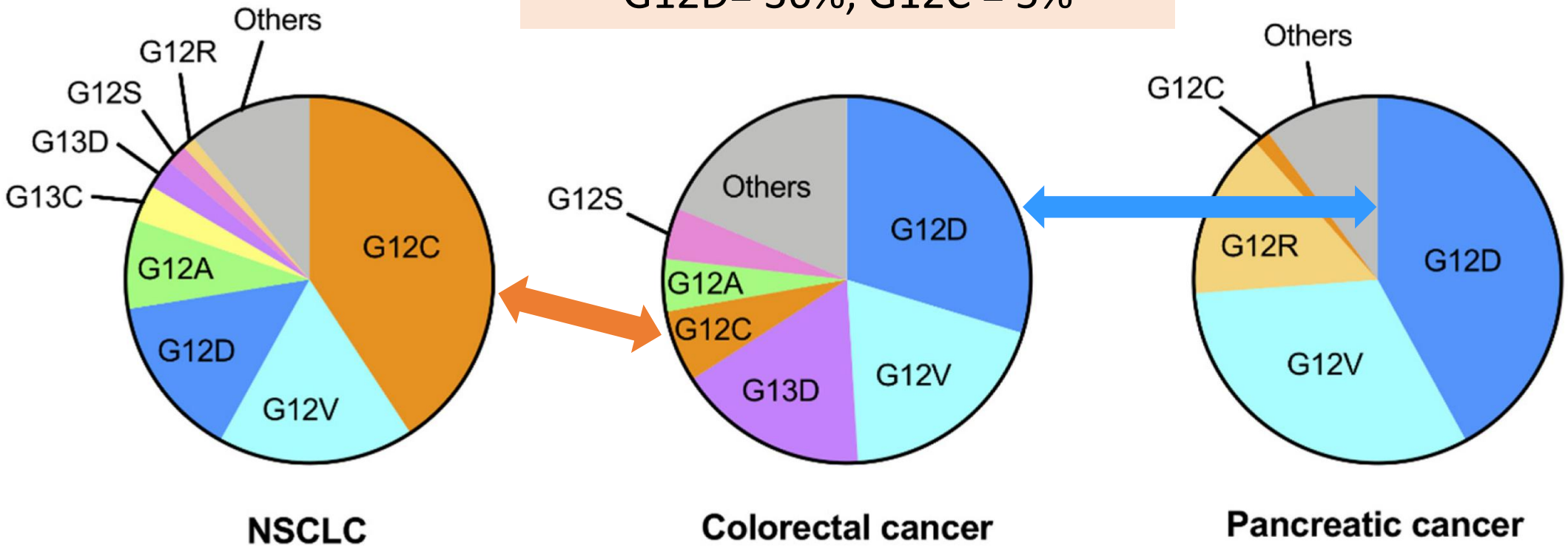
-No general inhibitors found or approved;
-Recently mutation-specific drugs were identified

https://www.mdpi.com/2072-6694/13/23/5956

- Inhibitors that form a **covalent bond with cysteine**, locking KRAS in the inactive state; approved in lung cancer with **G12C** mutant;
- In clinical trials: **G12D** inhibitor MRTX1133 in pancreatic cancer

AMG-510= Sotorasib,
MRTX849= Adagrasib

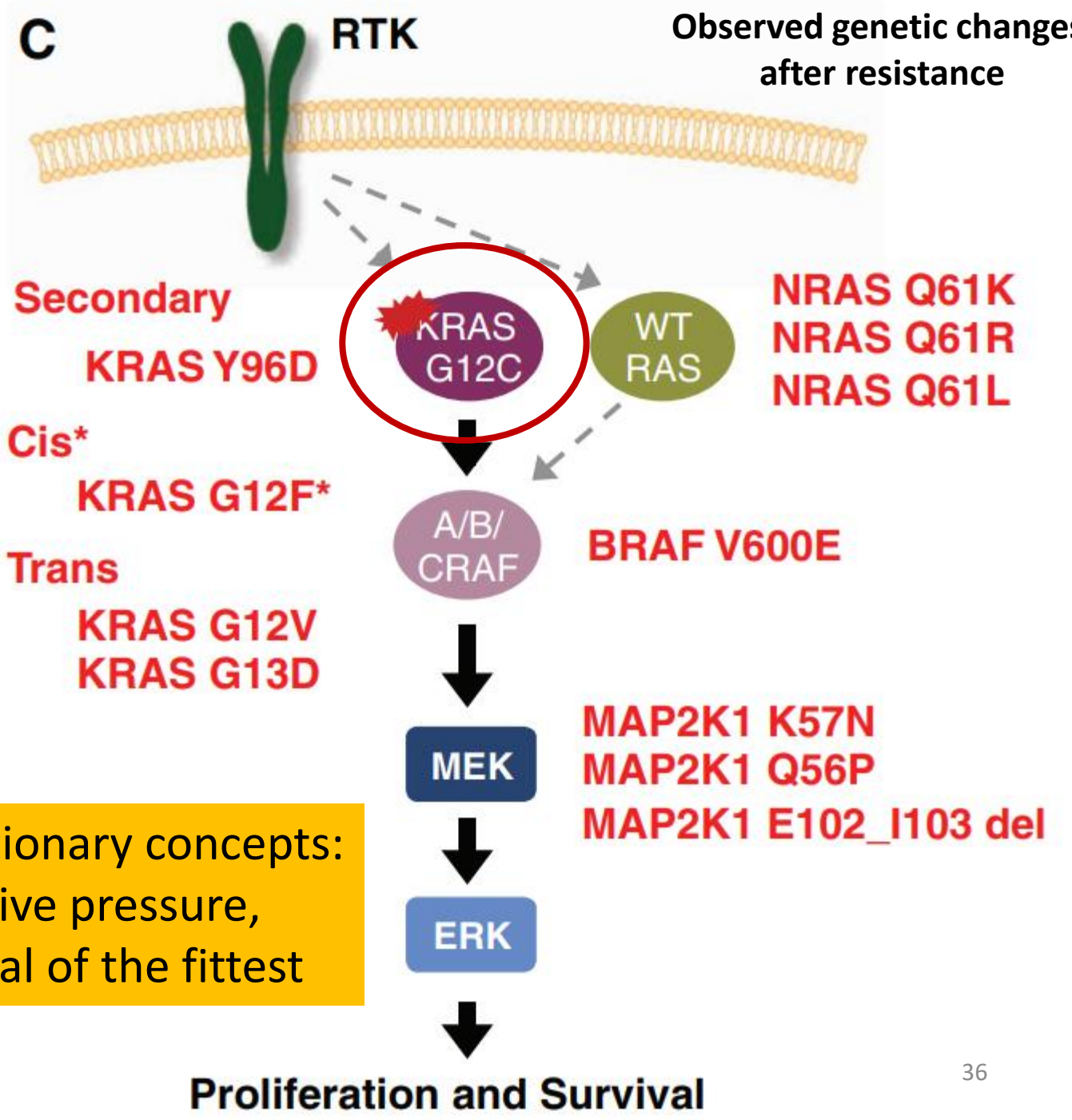
in codon 12 mutated CRC cases:
G12D= 36%, G12C = 5%



https://www.mdpi.com/2072-6694/13/23/5956

Response rate to **monotherapy**
with KRAS G12C-inhibitors
in patients with lung cancer
=
30–40%
with approximately 6-month
progression-free survival

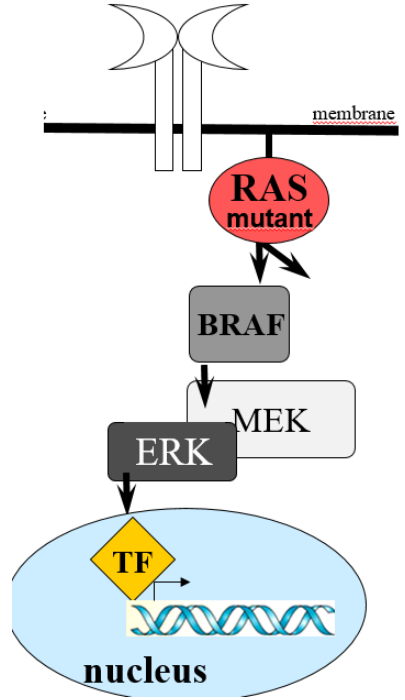
then:
Resistance developed



Evolutionary concepts:
Selective pressure,
Survival of the fittest

Cancer Discov 2021;11:1913–22
doi: 10.1158/2159-8290.CD-21-0365

Resistance to anticancer drugs



- Primary resistance:

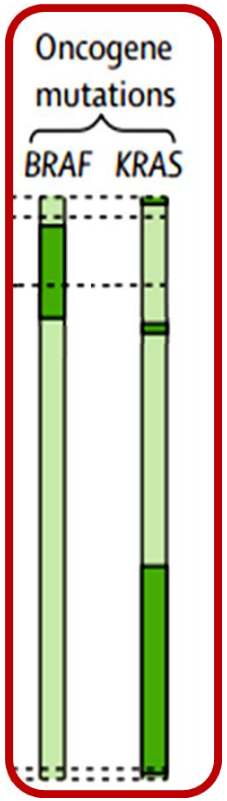
  tumour-intrinsic, i.e. resistance factor already present prior to treatment:

  - inherent genetic mutations
  - tumor heterogeneity
  - presence of cancer stem cells

- Acquired resistance:

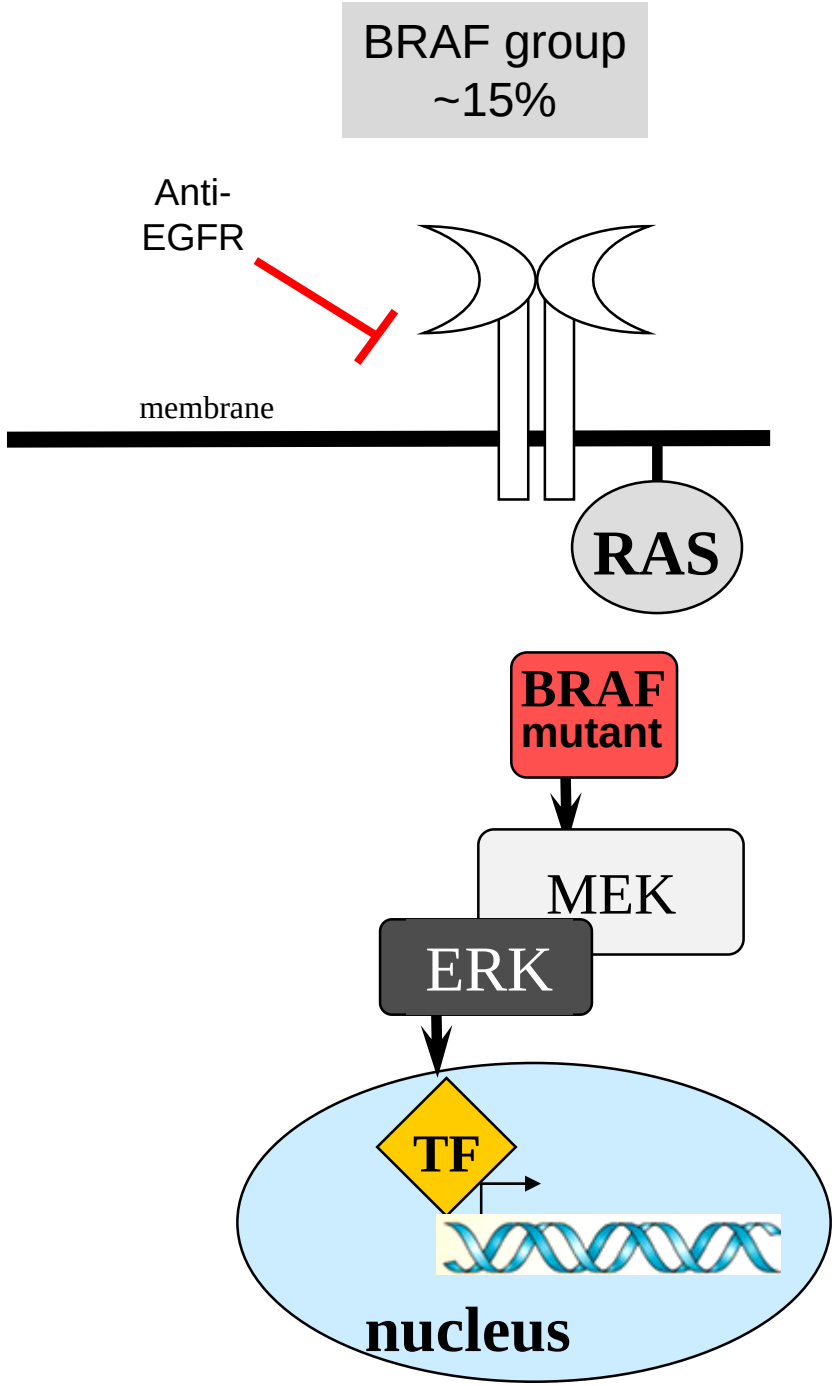
  adaptive response induced during the treatment of tumours that were initially sensitive

  - Activation of alternative genes or cellular pathways to overcome inhibitor action;
  - Expression of drug-eliminating transport channels (multi-drug resistance ABC transporter proteins) or drug-oxidizing enzymes (cytochrome P450)



BRAF mutations

- 90% are BRAF-V600E mutation;
- present in ~15% of CRC (frequent in melanoma and thyroid);
- activates catalytic kinase domain independent from KRAS



<u>BRAF kinase inhibitors</u>

- Promising as monotherapy in melanoma

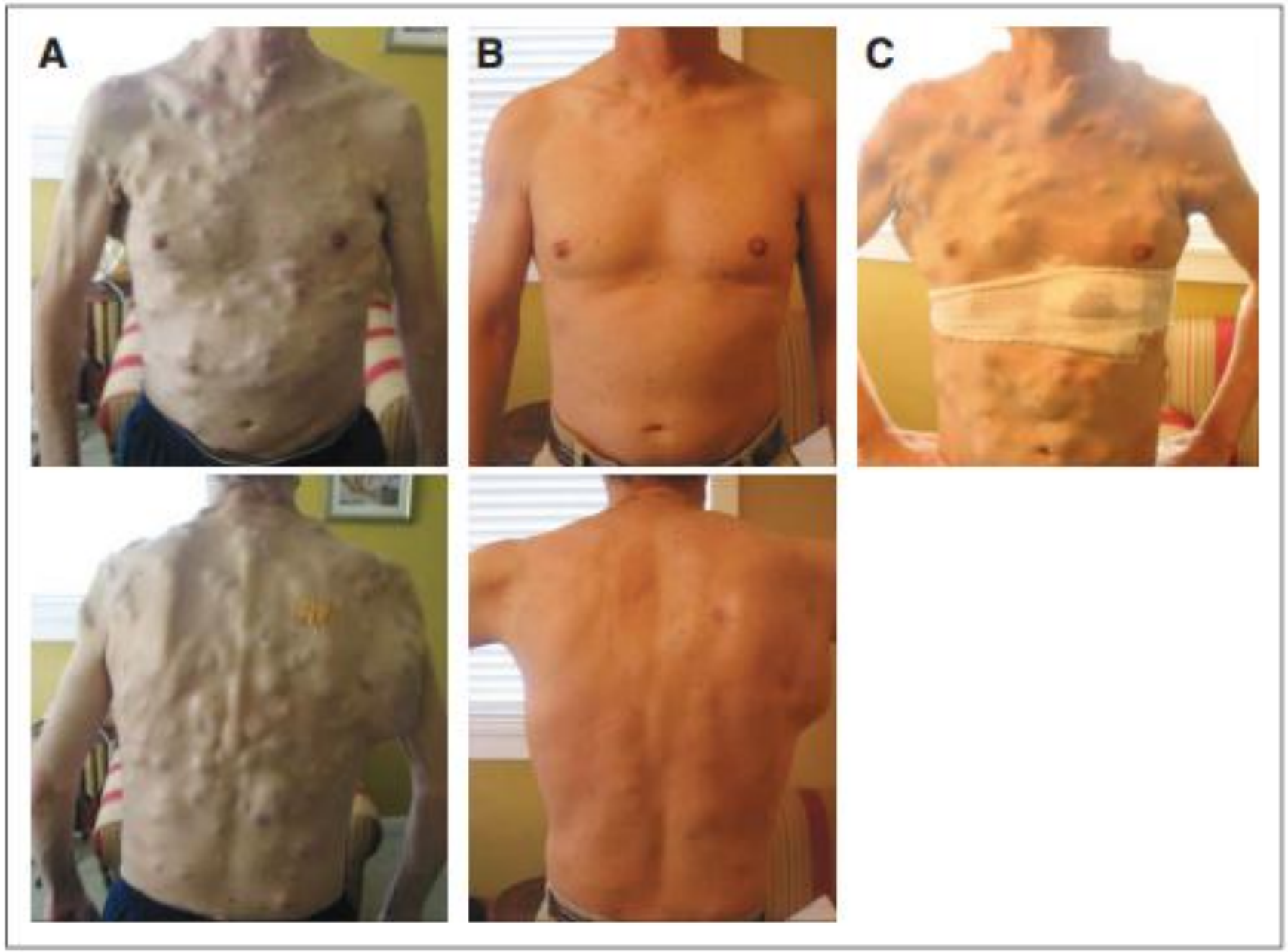
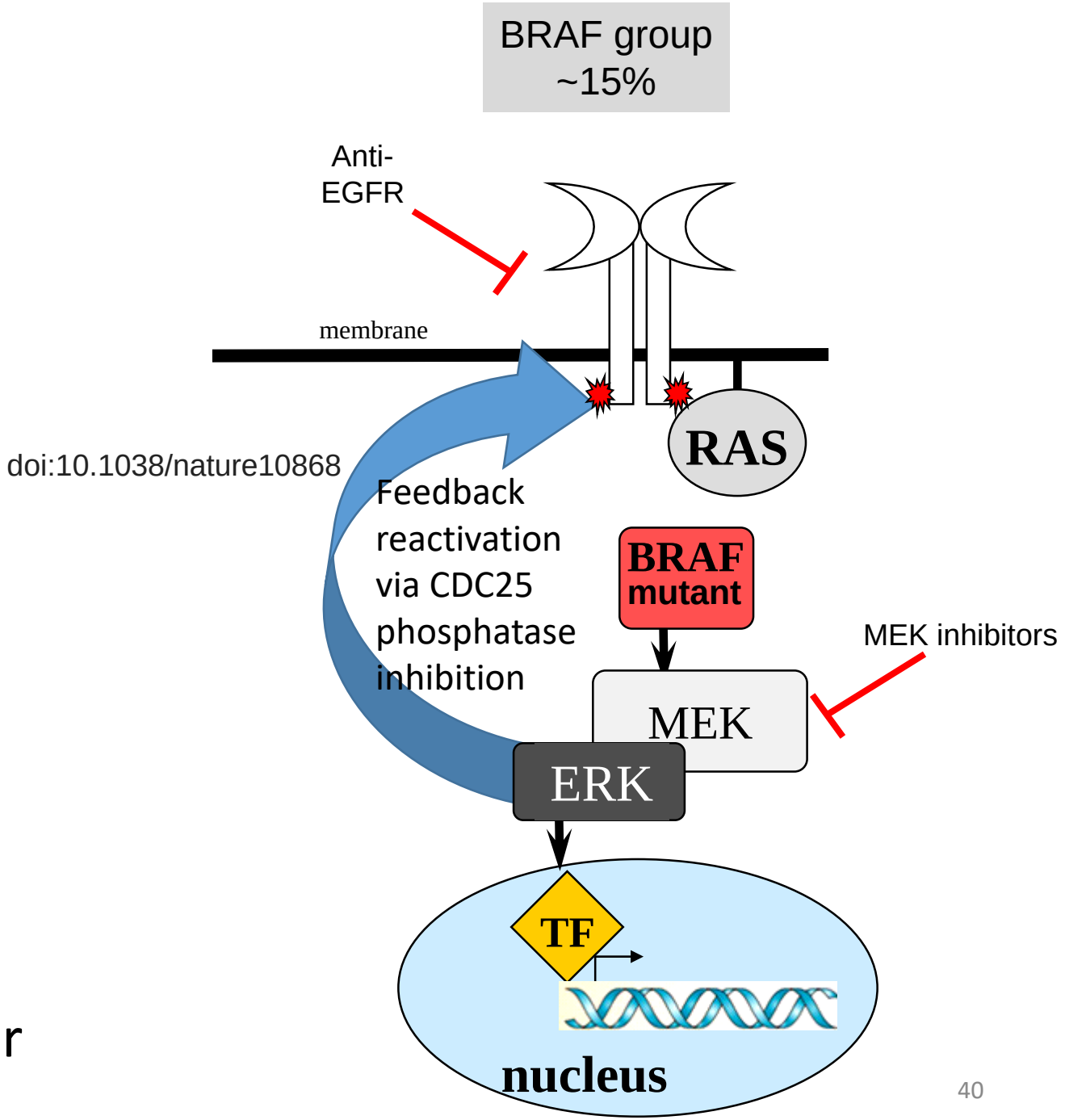


Fig 2. A 38-year-old man with *BRAF*-mutant melanoma and miliary, subcutaneous metastatic deposits. Photographs were taken (A) before initiation of PLX4032, (B) after 15 weeks of therapy with PLX4032, and (C) after relapse, after 23 weeks of therapy.

before treament week 15 weak 23

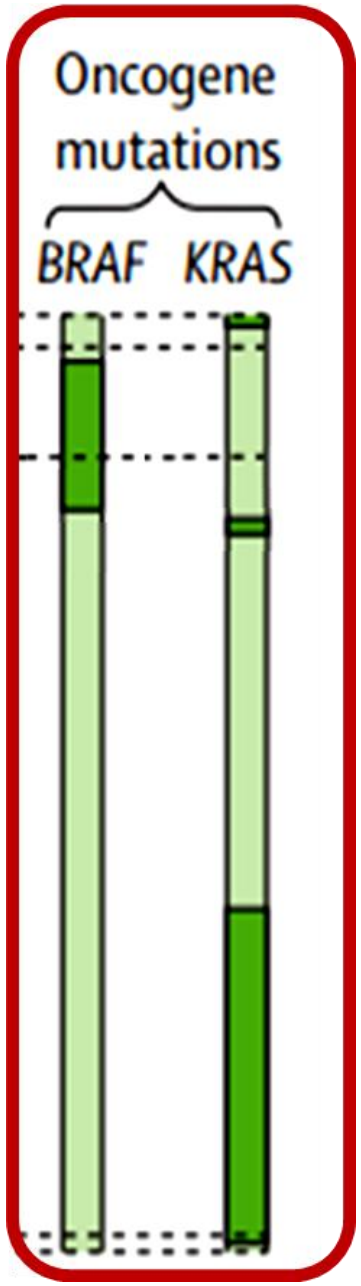
BRAF mutations



BRAF kinase inhibitors

- Promising as monotherapy in melanoma, but not in CRC;
- Feedback reactivation of EGFR occurs in CRC;

- Currently combination therapy with anti-EGFR and MEK inhibitor



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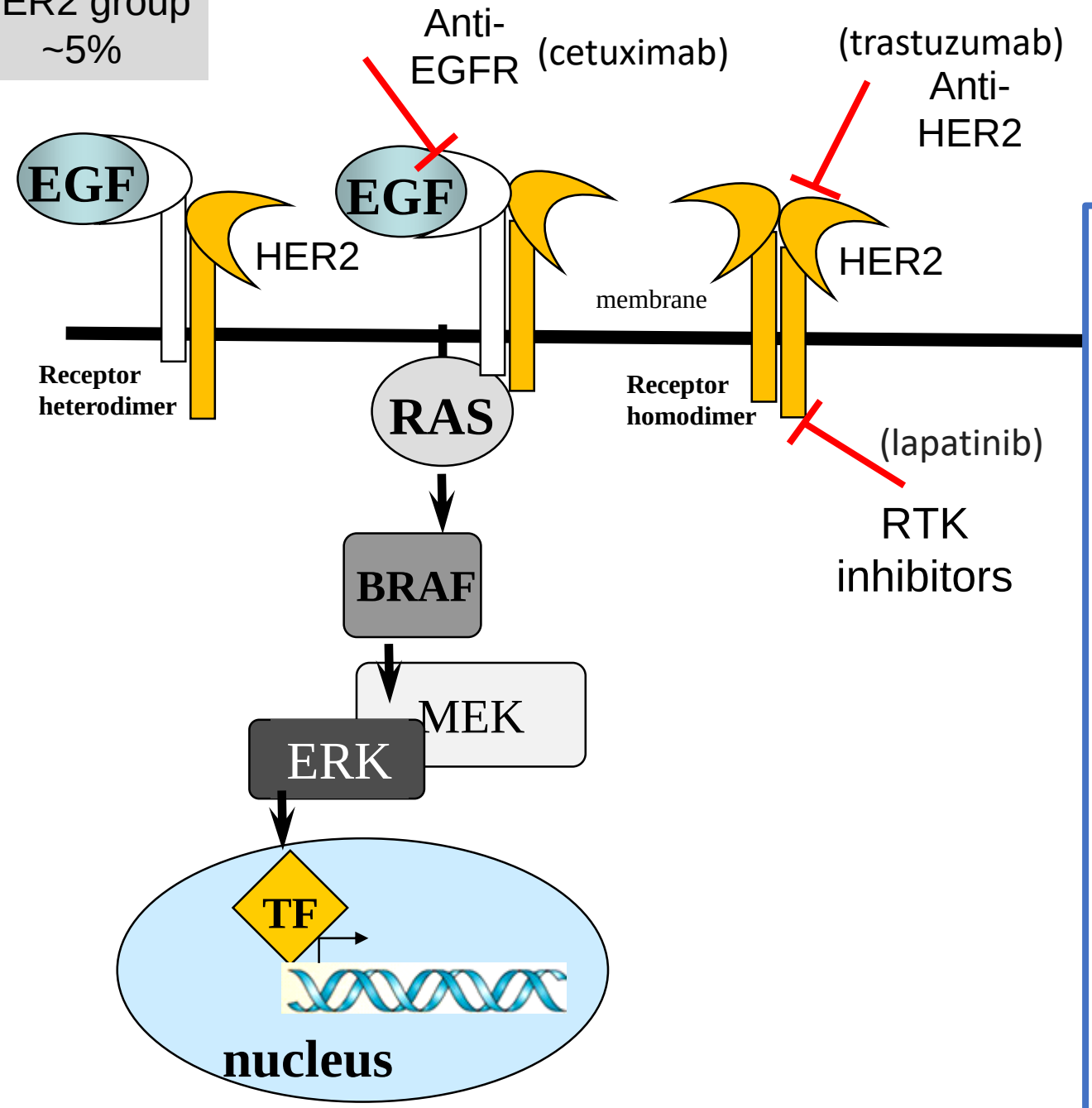
No mutations in KRAS or BRAF

HER2 group
~5%



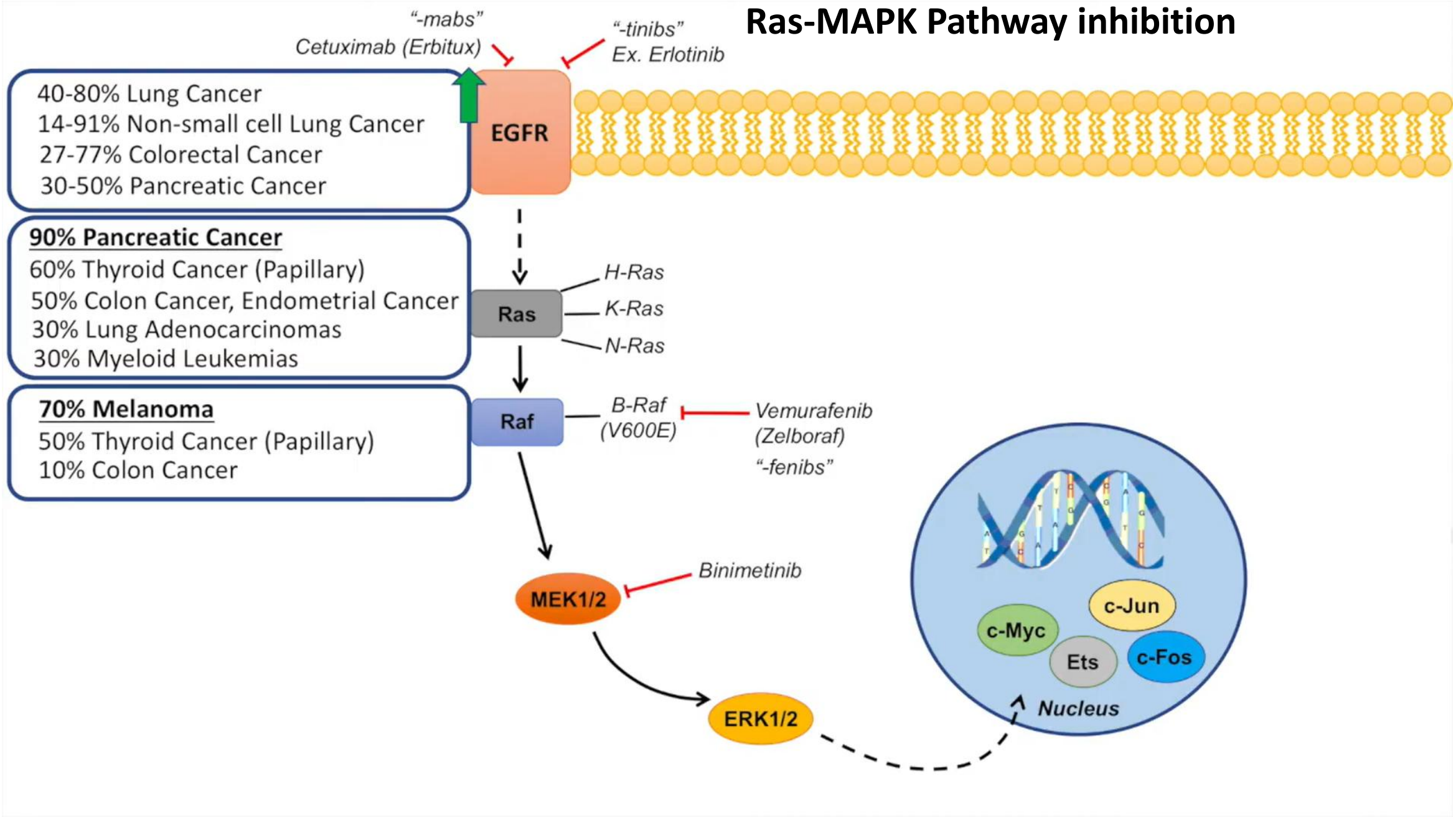
ErbB-1= epidermal growth factor receptor (EGFR)
ErbB-2= HER2 (*neu* in rodents)
ErbB-3= HER3
ErbB-4= HER4

HER2 overexpression/gene amplification in CRC:

- 3-5% of sporadic MSS distal CRC;
- Increased affinity to EGF by forming EGFR-HER2 heterodimers;
- Escape from standard anti-EGFR therapy

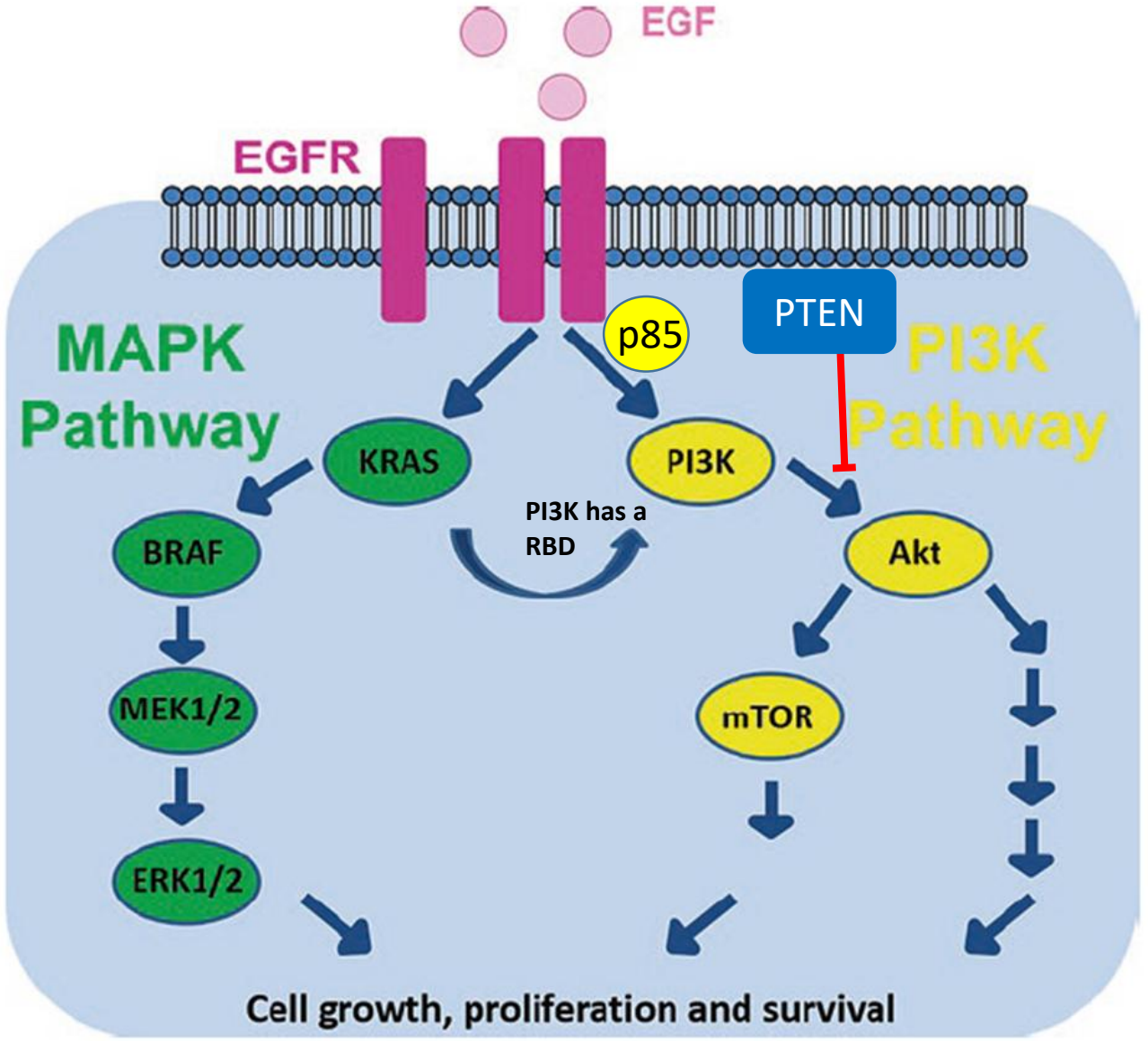
Doi: 10/1016/j.currproblcancer.2023/100960

Ras-MAPK Pathway inhibition



8-min vídeo animation MAPK Pathway and Cancer https://www.youtube.com/watch?v=NlFDea3ig7A

Understanding signalling to develop targeted therapy for colorectal cancer

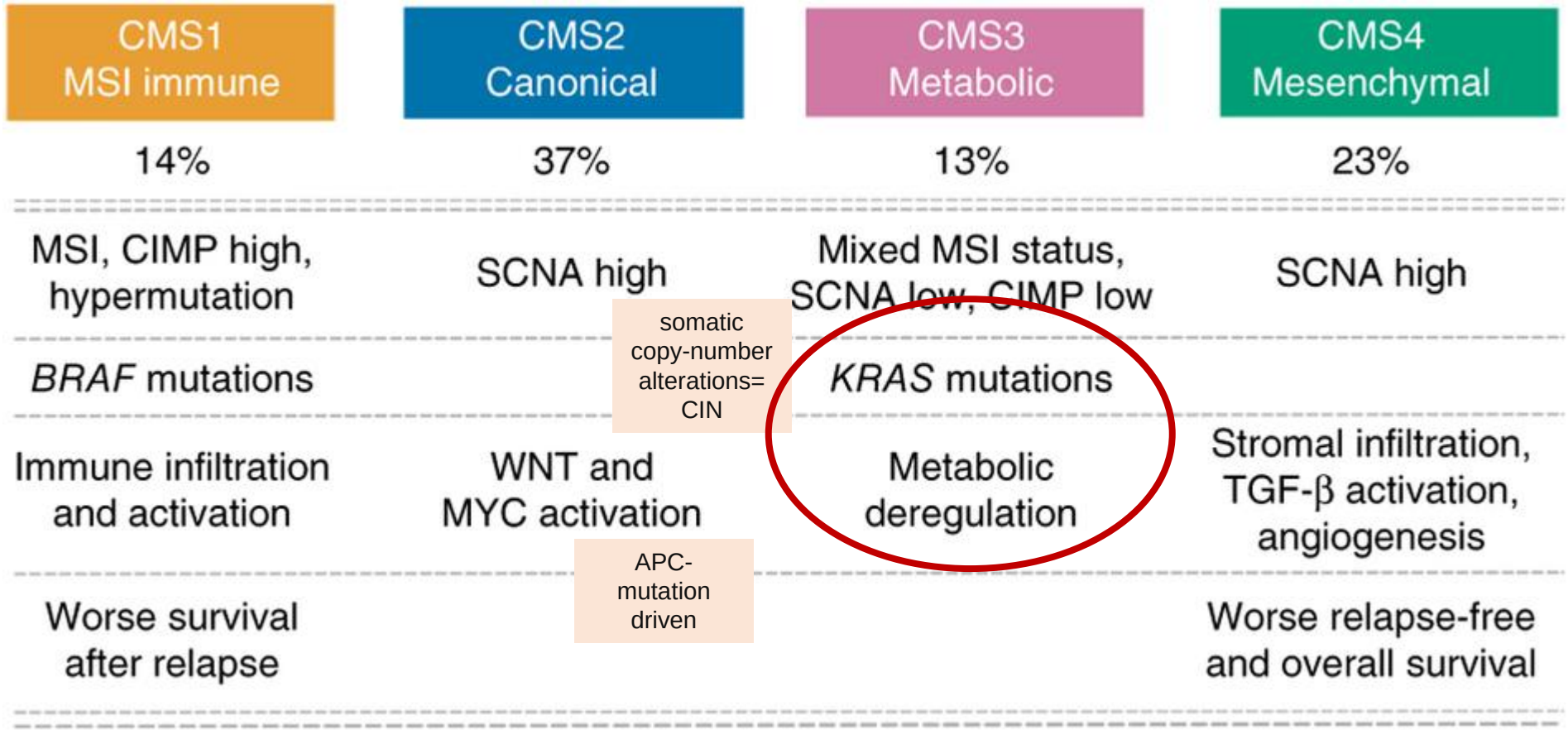


Oncogenic mutations affecting PI3K signalling in CRC

Gene	Mutated in % CRC	effect
PIK3CA	15-18%	*PIK3CA* =p110α isoform; Gain-of-function mutations - either **exon 9** (60–65%) (E542K, E545K), affect helical domain, require activation by direct interaction with RAS-GTP, but no longer by the p85 regulatory subunit; - or **exon 20** (20–25%) (H1047R); activates kinase domain, require activation the p85 regulatory subunit, but do not respond to RAS-GTP
PTEN	19-36%	PTEN tumour suppressor lost through: - mutations in *PTEN* (5%), especially in tumours with MSI high), - Deletions of chromosome 10q23 (23%), - hypermethylation of the *PTEN* promoter (9% in CRC with MSI high *vs*·2% in MSI low). Loss of PTEN has been found to co-occur with KRAS, BRAF and PIK3CA mutations
KRAS	32–40%	Activating KRAS mutation in codons 12 or 13 can directly activate PI3Kα.

Lancet Oncol 2011; DOI:10.1016/S1470- 2045(10)70209-6

Using gene expression profiles to classify colorectal cancer four consensus molecular subtypes (CMSs)



+ Mixed features 13%

doi:10.1038/nm.3967

Using gene expression profiles to classify colorectal cancer
Four consensus molecular subtypes (CMSs)

CMS1
MSI immune

14%

MSI, CIMP high, hypermutation

BRAF mutations

Immune infiltration and activation

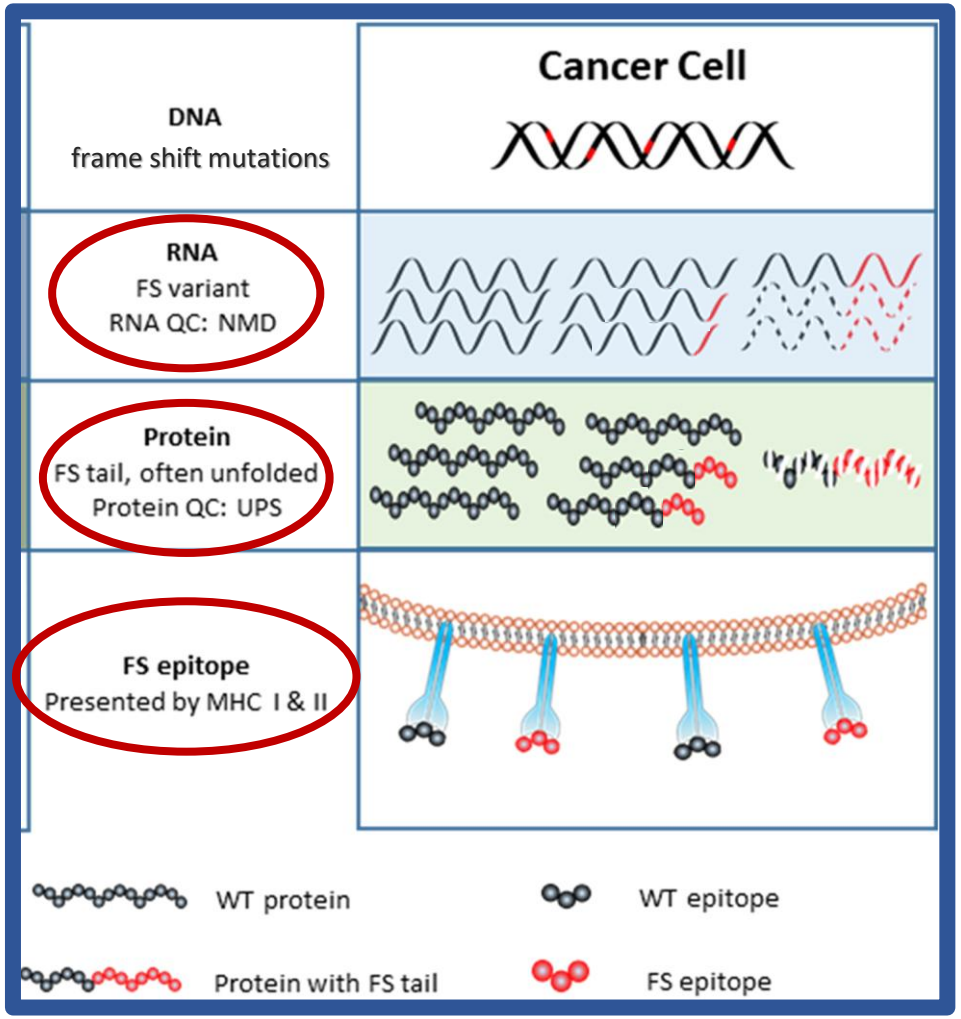
Good prognosis except after relapse

High tumour mutation burden

Neoantigen formation

Neoantigens are tumour-specific antigens derived from mutated proteins when frame-shift mutations occur in the DNA of tumor cells

→ immunotherapy



Molecular/genetic subtypes of sporadic colorectal cancer exist and offer an opportunity for patient-tailored targeted therapy

Compare to breast cancer:
ER/PR-positive, HER2 positive, Triple negative BC