Institute of Experimental Medicine, CAS

Genomic instability, microenvironment and telomere homeostasis in colorectal cancer

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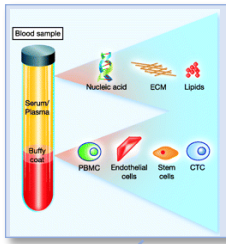
VISION

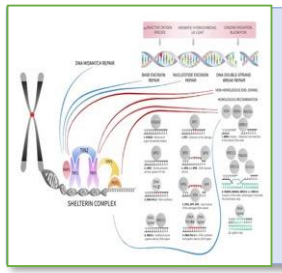
Research Focus of the Department of the Molecular Biology of Cancer

COLORECTAL CANCER BIOMARKERS
related to DNA damage and its repair



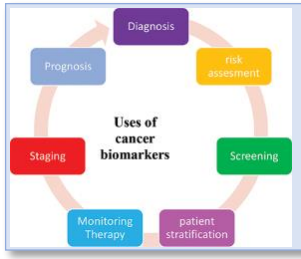
SEARCHING FOR BIOMARKERS
→ DNA, mRNA, microRNA, long non-coding RNA , circulating tumor cells, cell-free DNA

Genomic (in)stability



TELOMERE HOMEOSTASIS

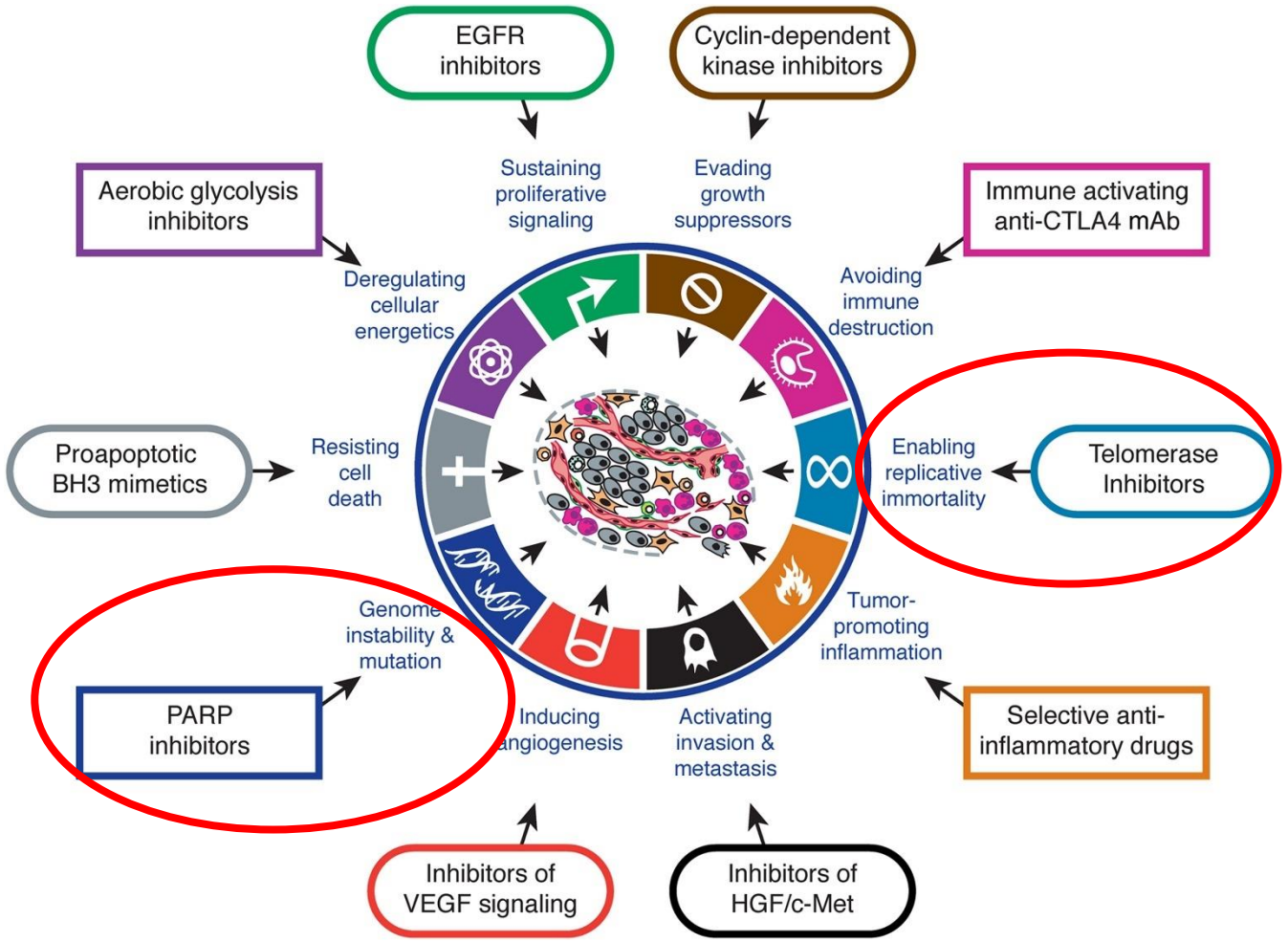
Department of the Molecular Biology of Cancer



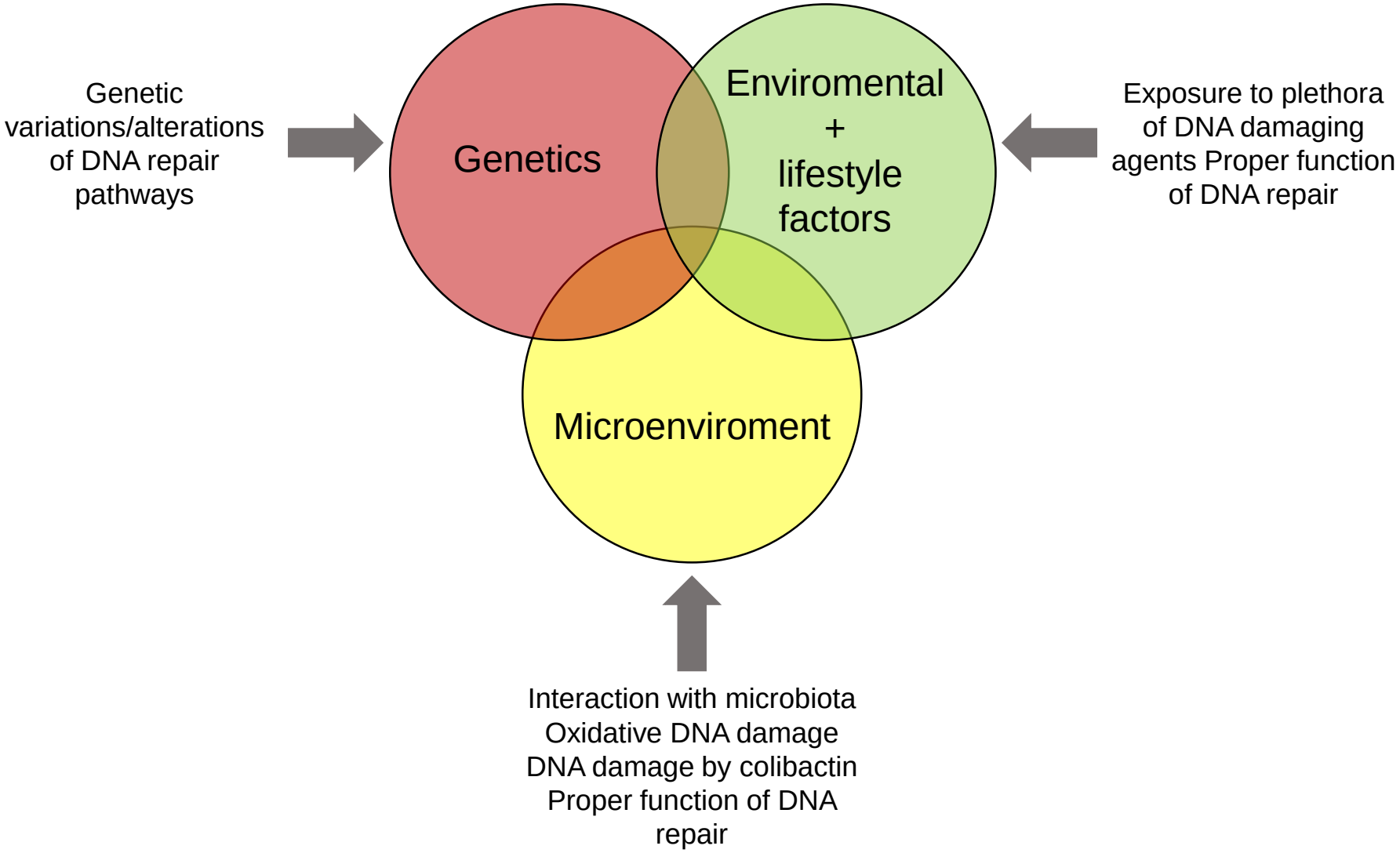
OUTCOMES

- Cancer risk assessment
- Options of cancer treatment and its prediction
- Cancer patients prognosis

Hallmarks of cancer



Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. CELL 144: 646-674, 2011


Genetic variations/alterations of DNA repair pathways
Genetics
Enviromental + lifestyle factors
Exposure to plethora of DNA damaging agents Proper function of DNA repair
Microenviroment
Interaction with microbiota
Oxidative DNA damage
DNA damage by colibactin
Proper function of DNA repair

Colorectal cancer (CRC)

Worldwide, colorectal cancer is the **third most common** form of cancer. In 2012, there were an estimated 1.36 million new cases of colorectal cancer and 694,000 deaths.[Ferlay et al. 2012]

In the United States

New cases of rectal cancer: 239,610.

New cases of colon cancer: 93,090.

Deaths: 49,700 (colon and rectal cancers combined).

In Europe

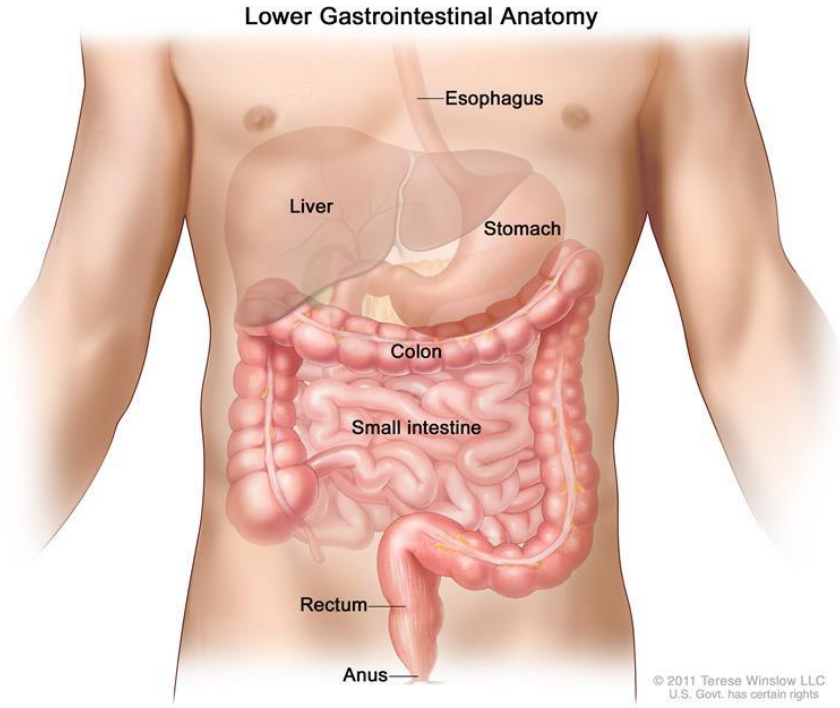
New CRC cases 447,136

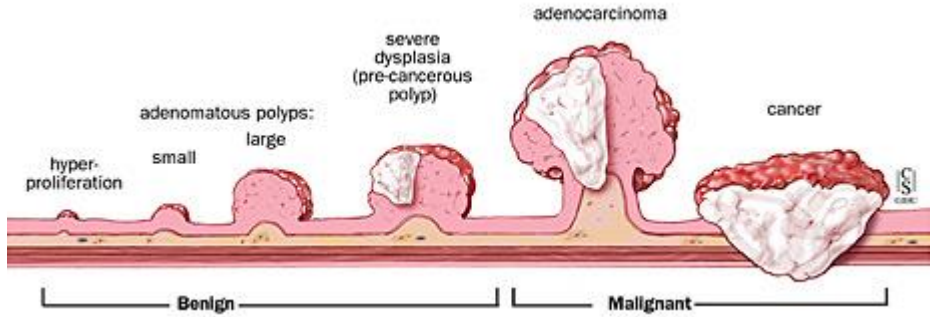
Deaths: 214,866 (colon and rectal cancers combined).

In Czech Republic

New CRC cases 8,336

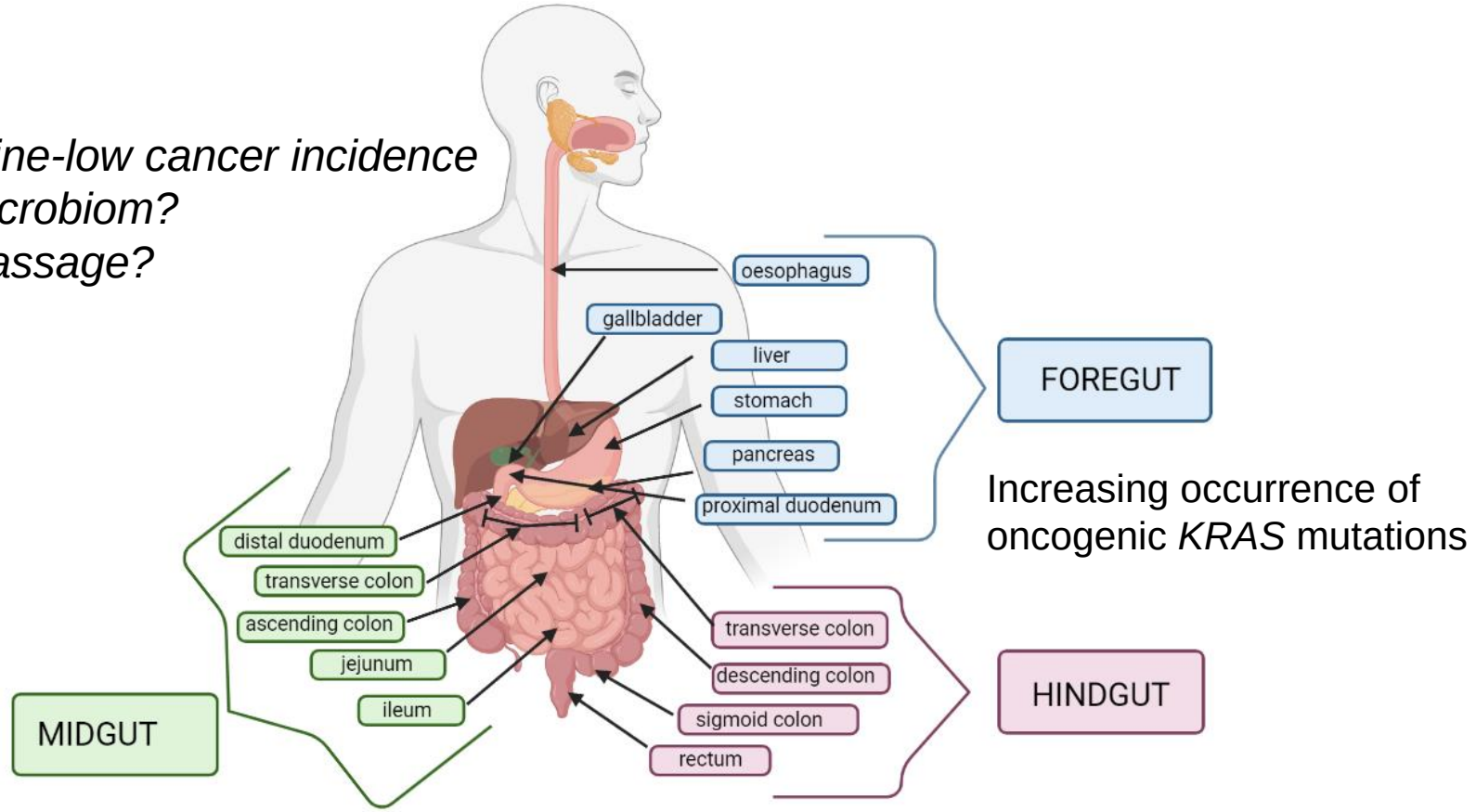
Deaths: 3628 (colon and rectal cancers combined).



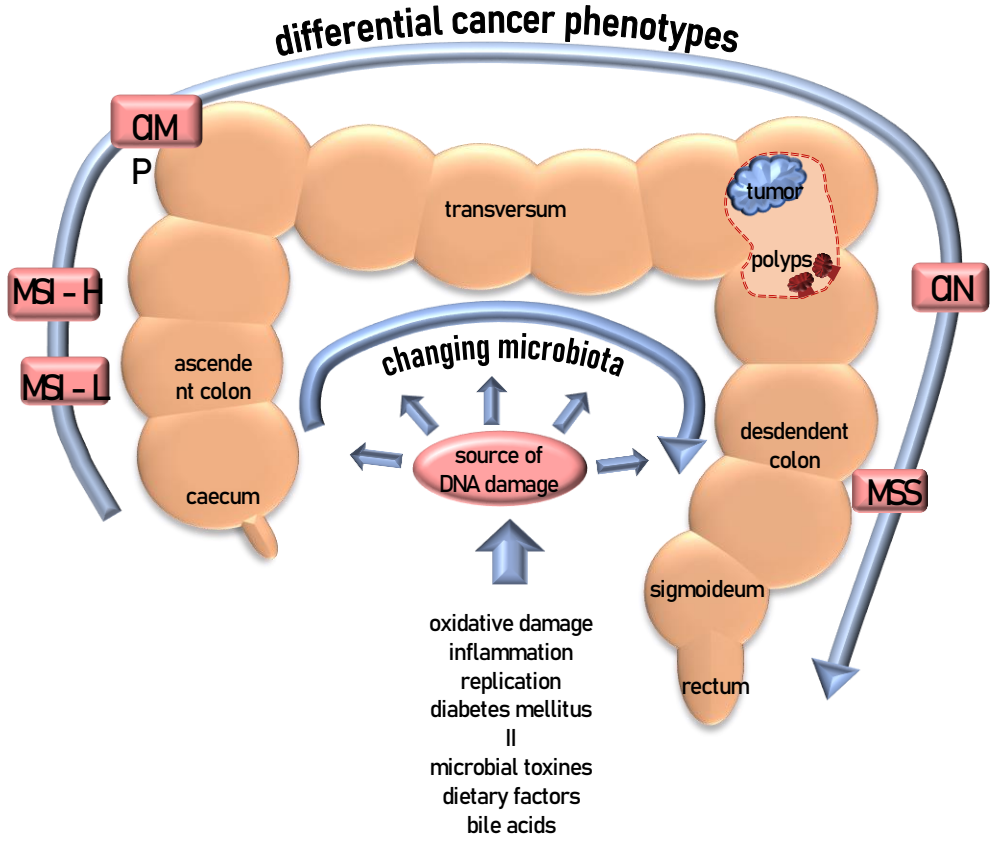


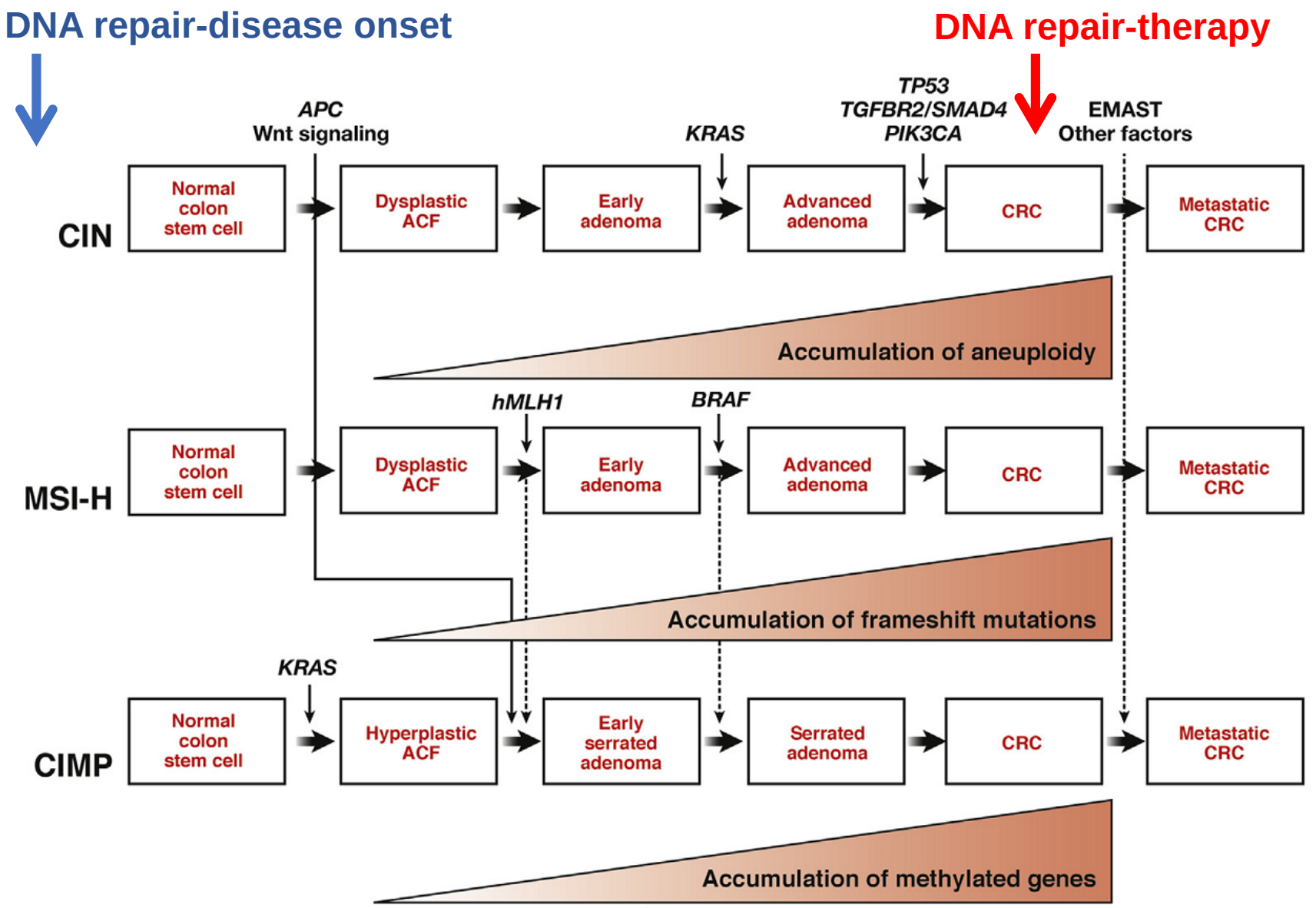
KRAS mutation, right colon with MSI exclusivelly BRAF

Note: small intestine-low cancer incidence vs colorectum. Microbiom? Genetics? Fast passage?



Increasing occurrence of oncogenic *KRAS* mutations


differential cancer phenotypes
CIMP
MSI - H
MSI - L
CIN
MSS
transversum
tumor
polyps
ascende nt colon
caecum
changing microbiota
source of DNA damage
desdendent colon
sigmoideum
rectum
oxidative damage
inflammation
replication
diabetes mellitus II
microbial toxines
dietary factors
bile acids



Carethers & Jung, Gastroenterology 2015

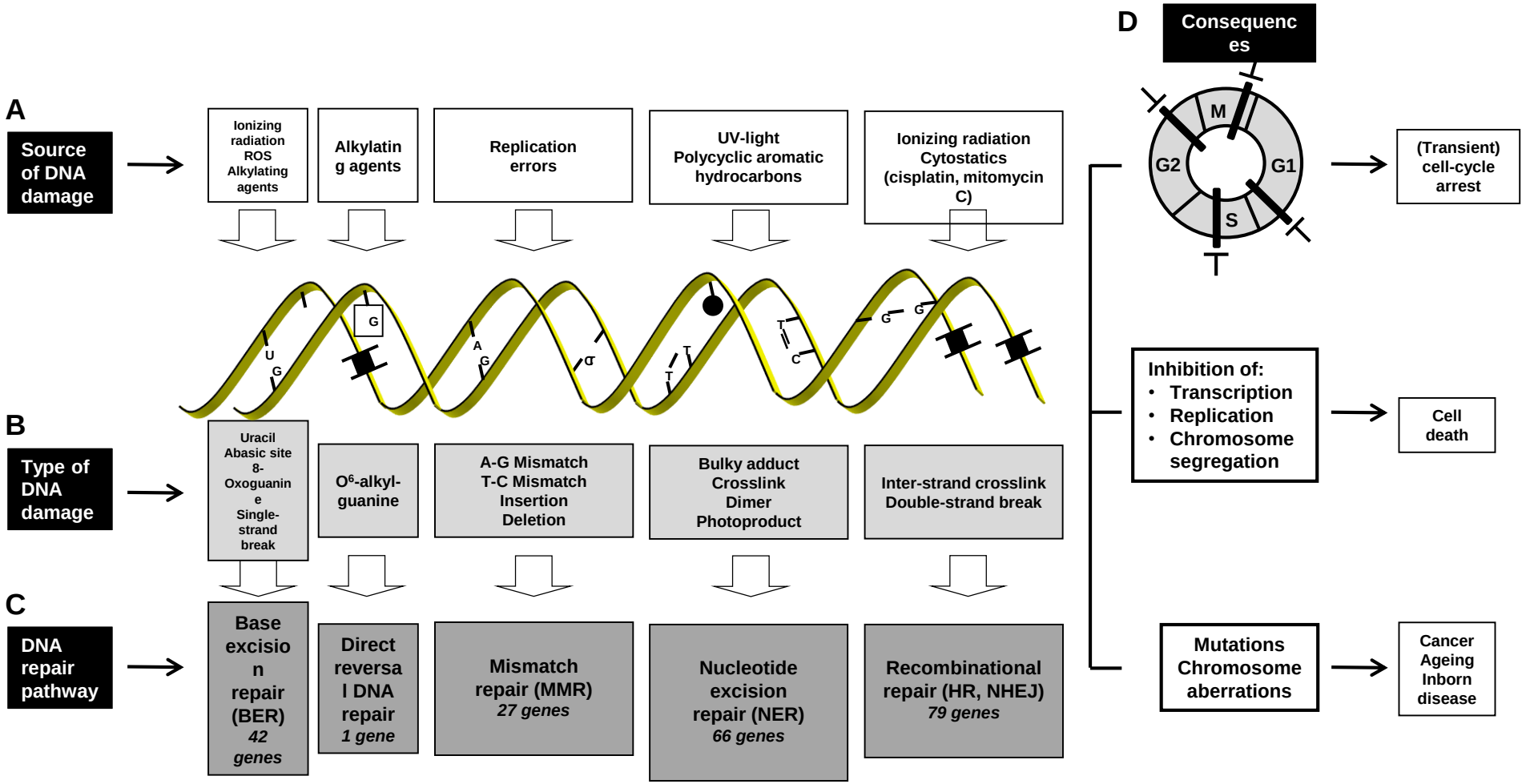
Mismatch repair deficient CRC is apparently resistant to 5-fluorouracil adjuvant chemotherapy while data suggest chemosensitivity to oxaliplatin. *Tougeron D, JNCI 2016*

Anatomical CRC sublocation and distinct germline genetics.

- Heterogeneity among colorectal cancer (CRC) tumours originating at different locations of the colorectum-observed in **somatic genomes**, **epigenomes** and **transcriptomes**, and in some established environmental risk factors for CRC
- Clinical and genome-wide genotype data of 112 373 CRC cases and controls searched for distinct genetic architecture of CRC subgroups defined by anatomical sublocation.
- We discovered **13 new loci at genome-wide significance** ($p<5\times10^{-8}$) that were specific to certain anatomical sublocations
- **Strong candidate target genes** at several of these loci, including PTGER3, LCT, MLH1, CDX1, KLF14, PYGL, BCL11B and BMP7 were found.
- **Distal colon and rectal cancer have very similar germline genetic aetiologies**.

Huyge et al., Genetic architectures of proximal and distal cancer are partly distinct. Gut 2020

DNA damage, their repair and possible cellular consequences



DNA repair genes and genetic susceptibility to (sporadic) CRC.

- ❖ subtle inter-individual differences in the DNA repair systems modulate the individual risk of developing CRC
- ❖ Meta-analysis conducted by Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) and the Colon Cancer Family Registry (CCFR)
- ❖ over 27,000 individuals
- ❖ 15,400 single nucleotide polymorphisms (SNPs) within 185 DNA repair genes analyzed analysed by GWAS

Pardini et al. Int J Cancer 2020

Results

	ht SNP ID	localization	OR (95% CI)	p-value for SNP effect	BSGoF-Adjusted p-value
MLH1 *(MMR*	rs1800734	colon	1.13 (1.07-1.18)	**$3.48X10^{-06}$**	0.019
RAD51B *(HR)*	rs2189517	rectum	1.15 (1.08-1.22)	**$5.73X10^{-06}$**	$1.24X10^{-05}$

Significant results after Bonferroni correction

CRC treatment

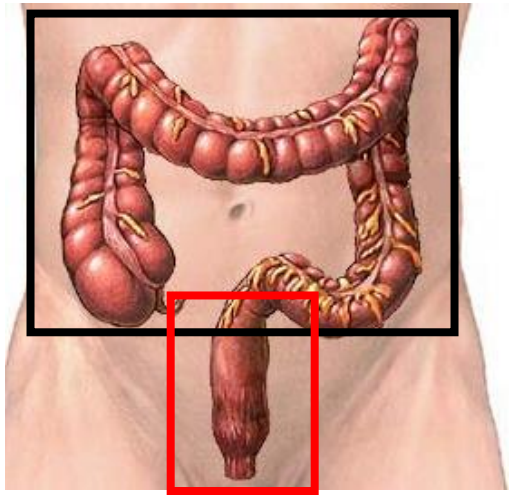
Depends on tumor stage and its localization

- **rectum** – neoadjuvant chemoradiotherapy followed by surgery
- **colon** – surgical resection of the tumor and/or adjuvant chemotherapy

Chemotherapy

- conventionally on the basis of **5-fluorouracil (5-FU)**

→ at present, 5-FU is the main compound in combination chemotherapy regimens (FUFA, FOLFIRI, FOLFOX)



Cytostatics

- cytotoxic effect on tumor cells (induction of DNA damage followed by apoptosis)
- the goal is to reduce distant metastases and to extend survival of patients with advanced stages of CRC
- **Main problems:** Acquired resistence and severe non-selective side effects and toxicity

DNA NER in relation to therapy

48 controls

~ 64 years

Peripheral blood

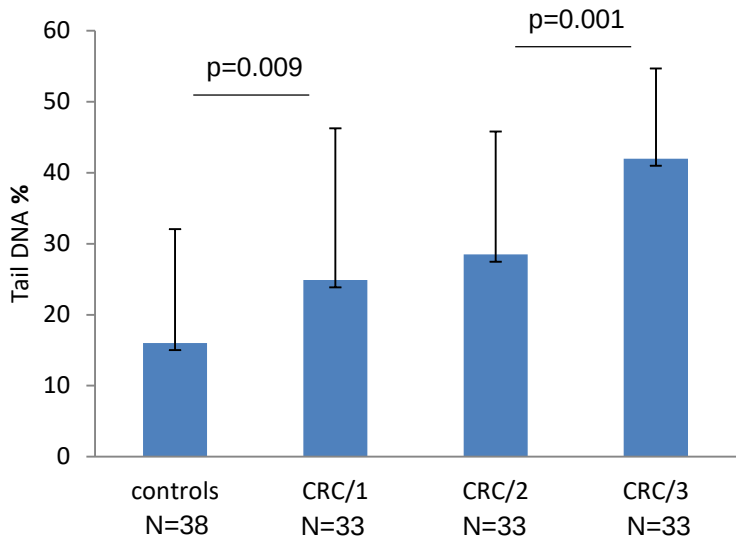
39 CRC patients (initially 100)

~ 64 years

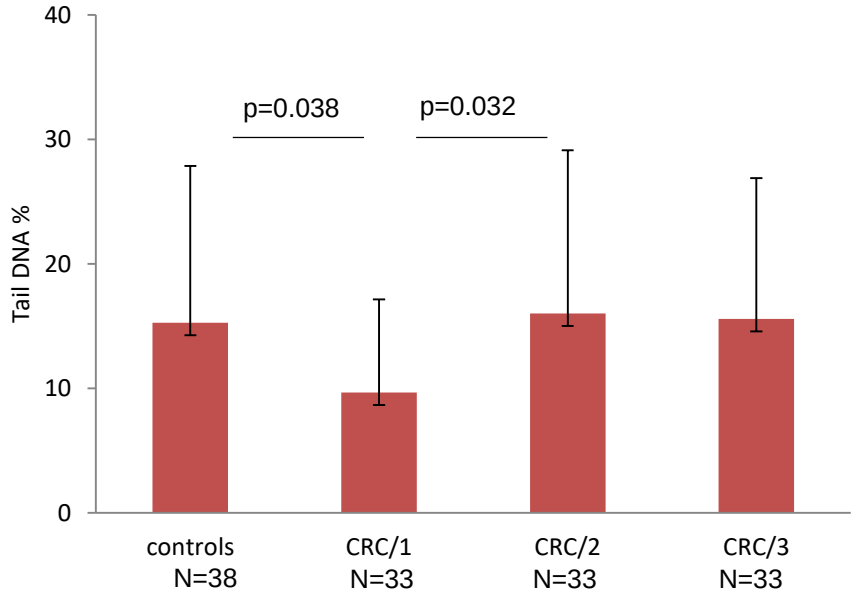
Slyskova J et al, *Molecular Carcinogenesis,* 2015

Slyskova J et al, *Molecular Carcinogenesis,* 2015

DNA damage

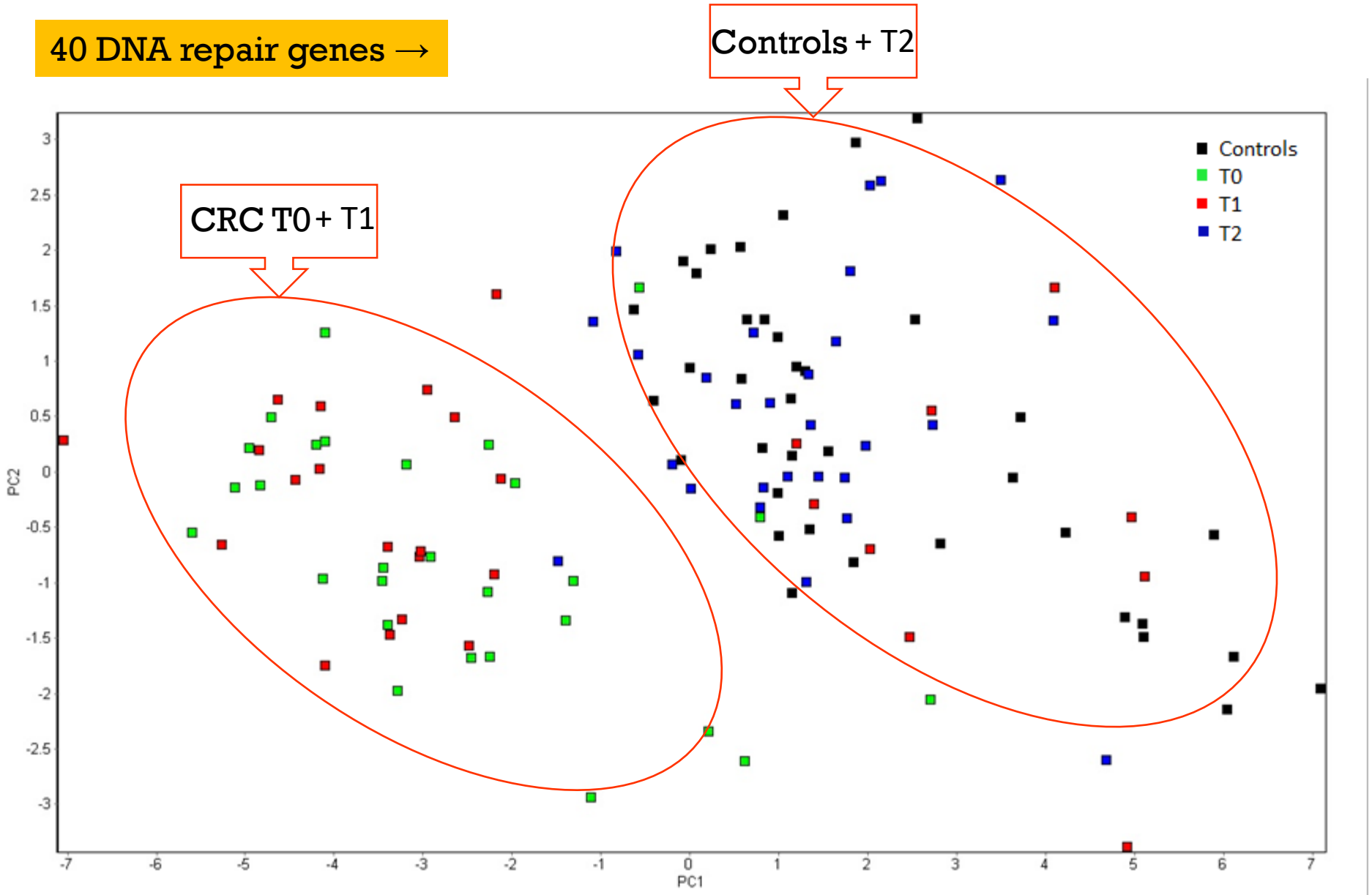


NER capacity



DNA repair in relation to therapy

40 DNA repair genes →



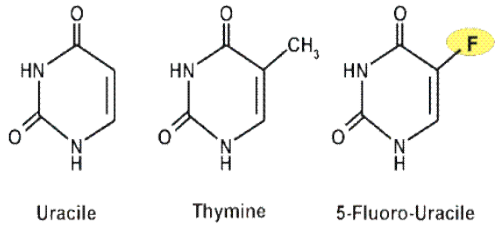
Slyskova J et al, *Molecular Carcinogenesis, 2015*

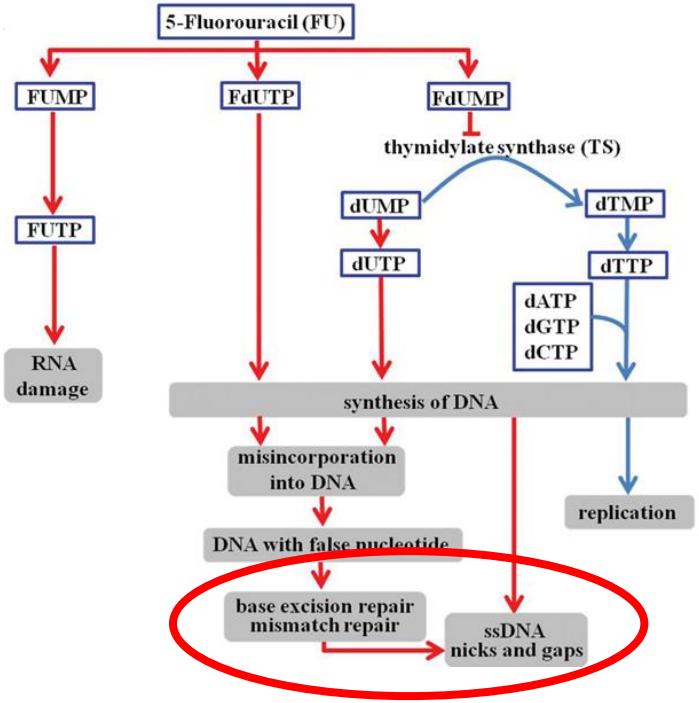
DNA damage and BER

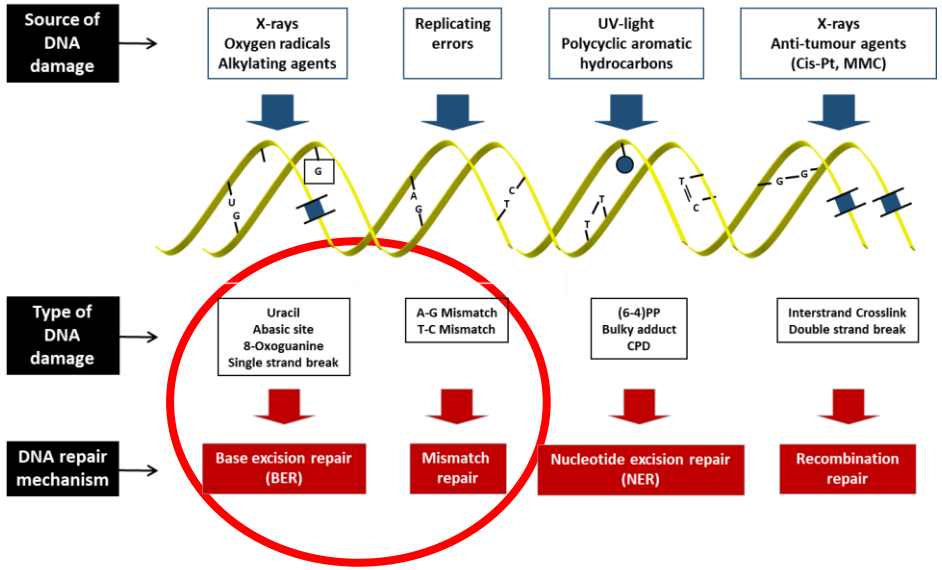
- chemotherapy - cytotoxic effect on rapidly dividing cells, such as cancer cells

5-fluorouracil

- base analog, halogenated pyrimidine

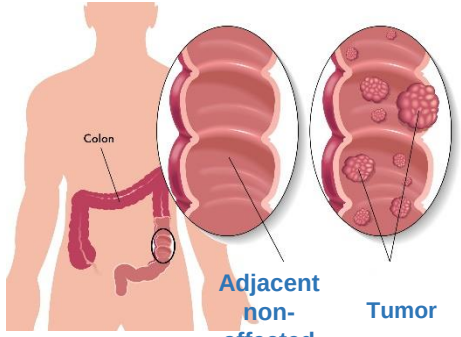






Workflow strategy

Surgical resection before any therapy



Adjacent non-affected mucosa

Tumor

Collection of samples (-80°C)

Selection of patients

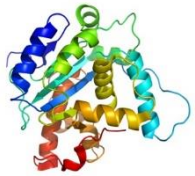
DIAGNOSIS
only colon cancer patients

FOLLOW-UP
for at least 30 months

THERAPY
adjuvant therapy with 5-FU

FINAL SET
pair samples from 94 patients

Experimental analyses



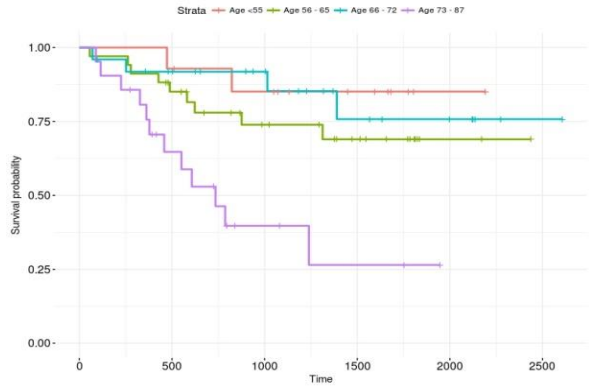
- Measurement of microsatelite instability
- Functional measurement of BER
- Protein expressions measurement

Set of patients

Established prognostic factors

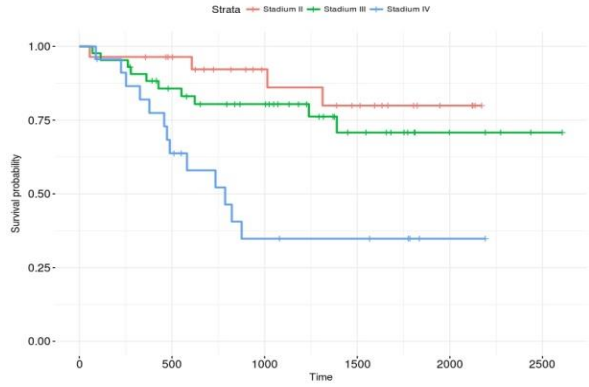
PERSONAL DATA		
Age at diagnosis (mean ± SD)		65.6 ± 9.8
Sex	Female	45%
	Male	55%
Smoking	Non-smokers	46%
	Current smokers	24%
	Ex-smokers	30%
BMI (mean ± SD)		27.3 ± 4.6
Diabetes	Yes	20%
	No	80%
Family history of cancer	Yes	64%
	No	36%
Family history of CRC	Yes	22%
	No	78%
DIAGNOSIS		
Stage (by TNM)	II	30%
	III	46%
	IV	24%
Grade	G0	3%
	G1	1%
	G2	3%
	G3	60%
	G4	31%
	No data	2%
THERAPY AND FOLLOW-UP		
Adjuvant	5-FU	39%
	5-FU + oxaliplatin	54%
	No specification	6%
Recidive	Yes	20%
	No	77%
	No data	3%
Living status	Alive	71%
	Dead	29%

Age



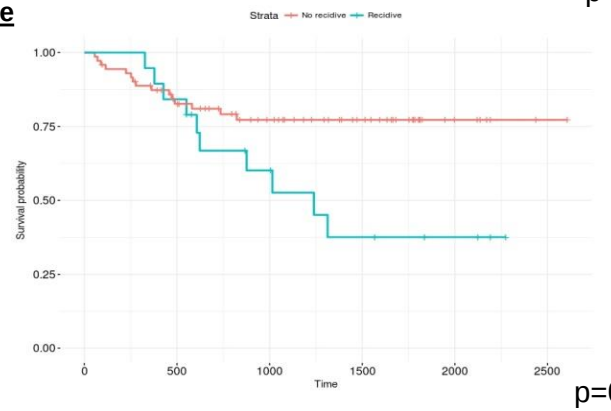
p=0.009**

Stage



p=0.003**

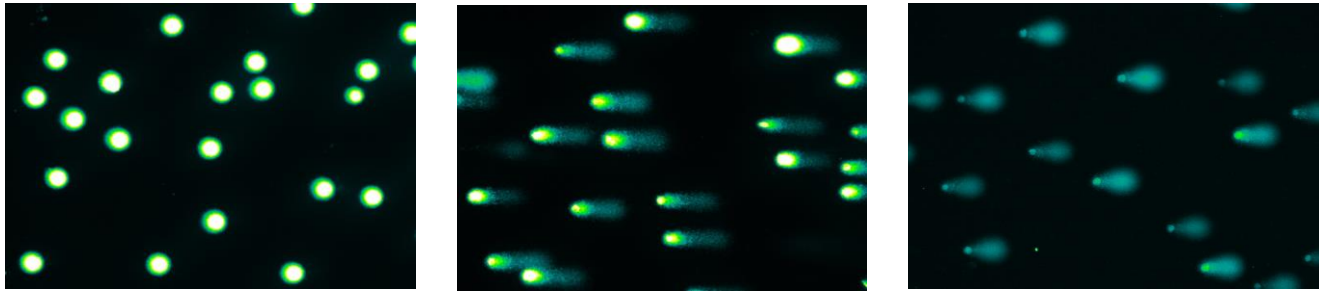
Recidive

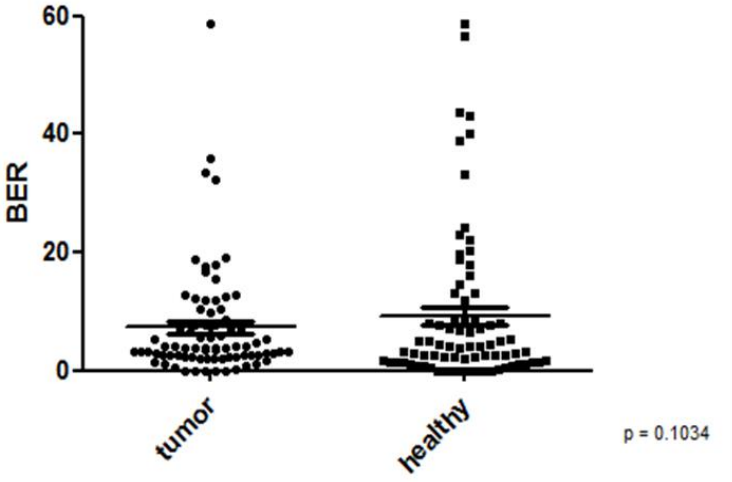


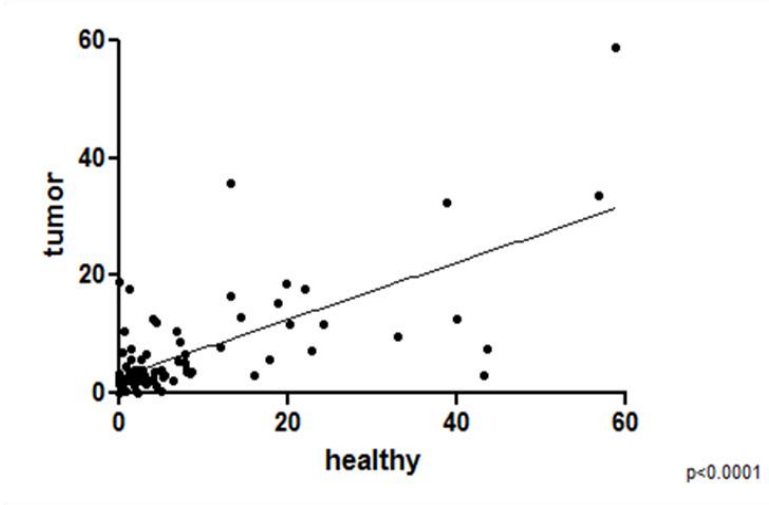
p=0.03*

BER capacity in CRC tumor and adjacent mucosa

(on 100 independent sporadic CRC patients)





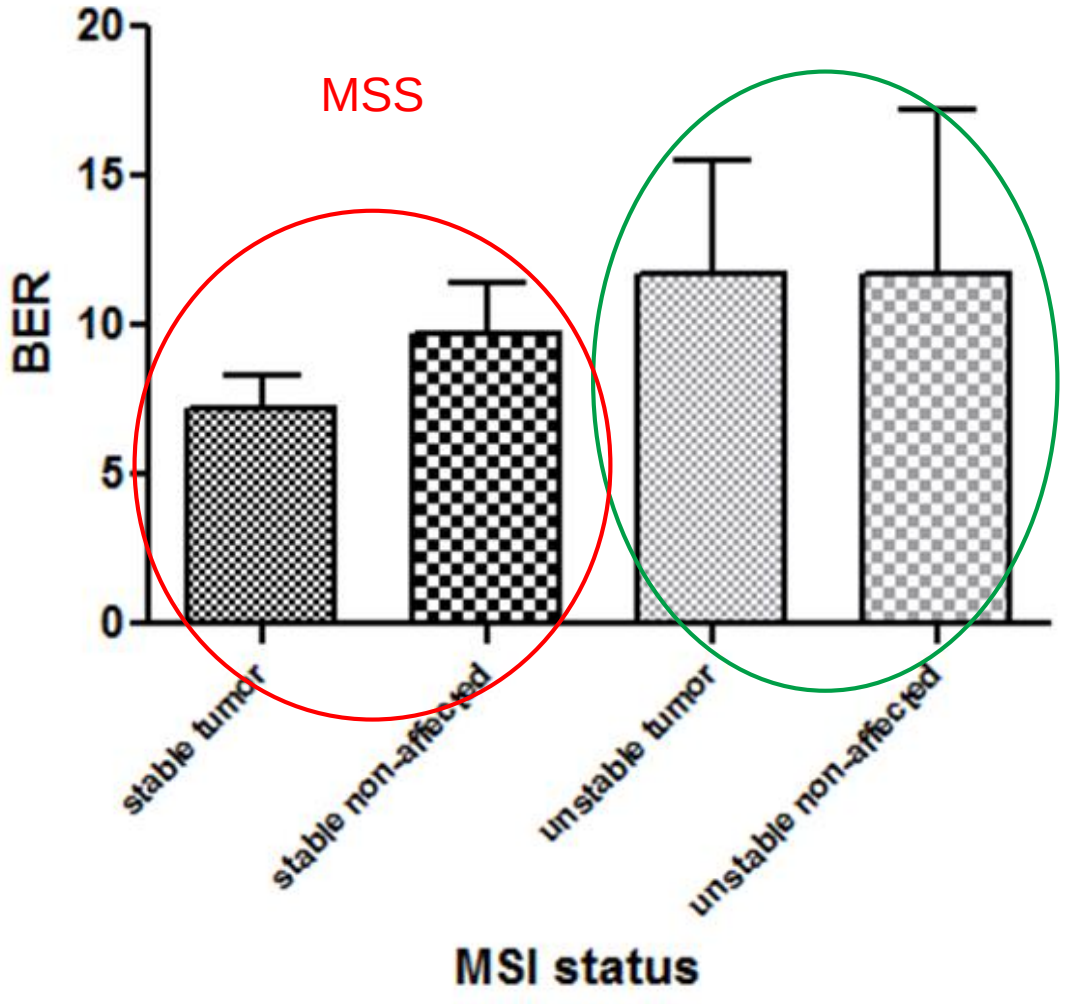


Incision rate of BER was not significantly different between tumor tissue and adjacent mucosa (p=0.09)

Incision rate of BER in tumor tissue significantly correlated with that in mucosa (p<0.0001)

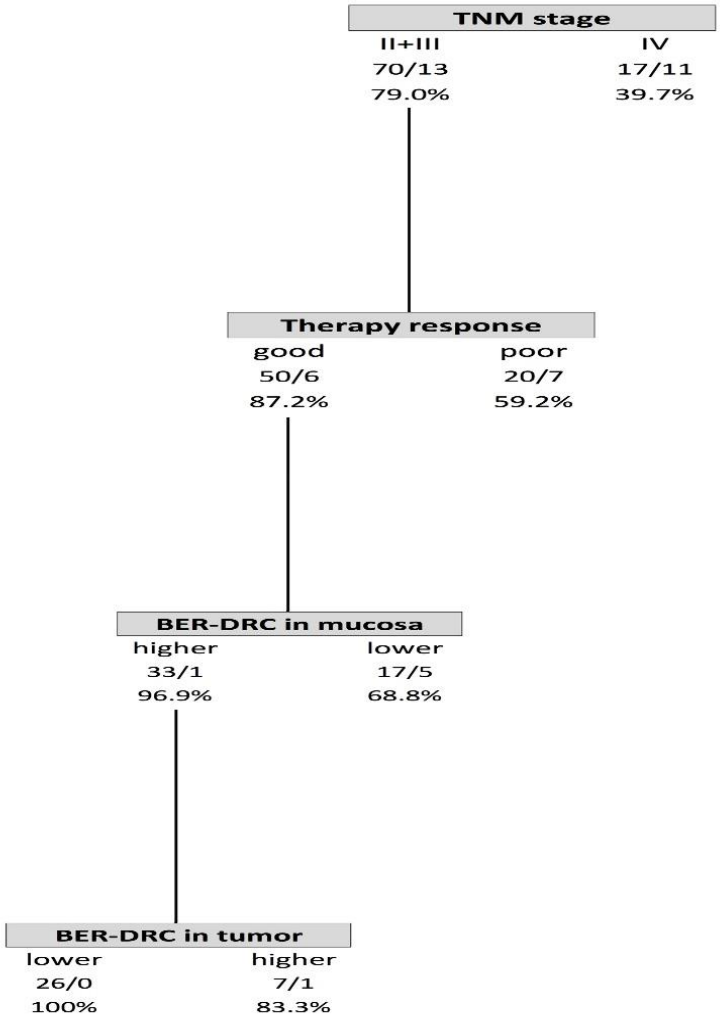
BER capacity in relation to MSI

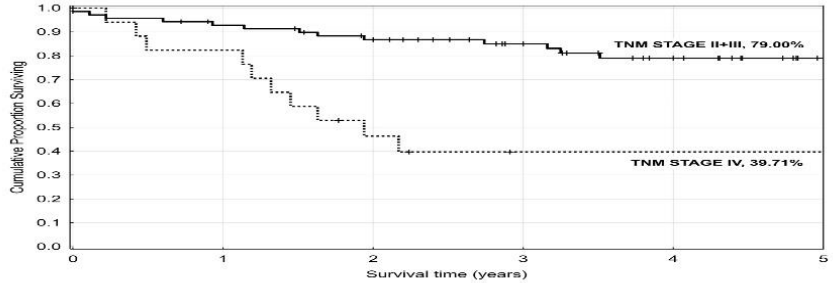
MSI or MSH

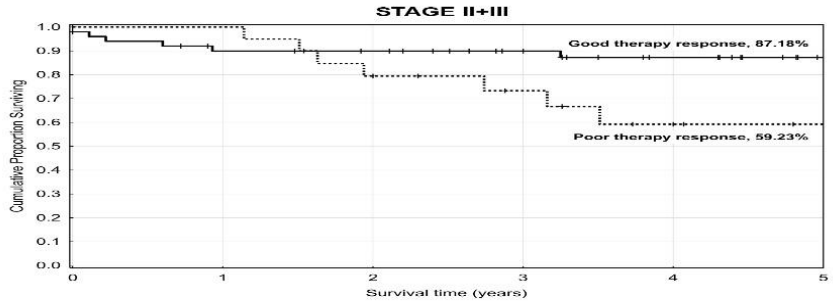


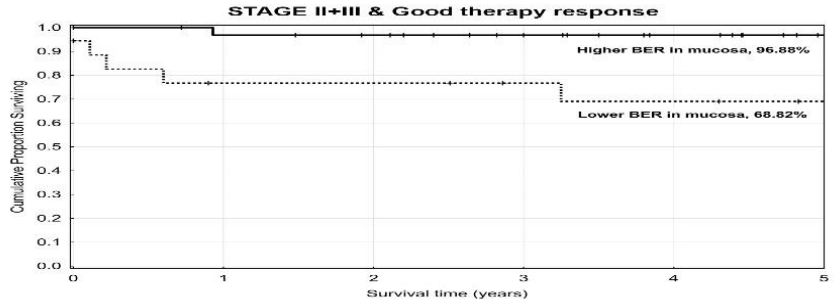
Perhaps BER compensates MMR deficiency

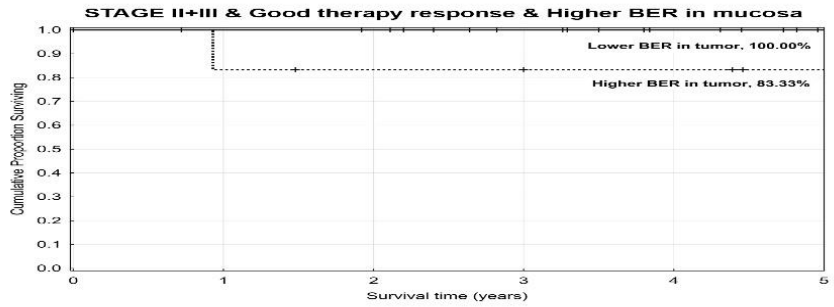
Multivariate analysis CART

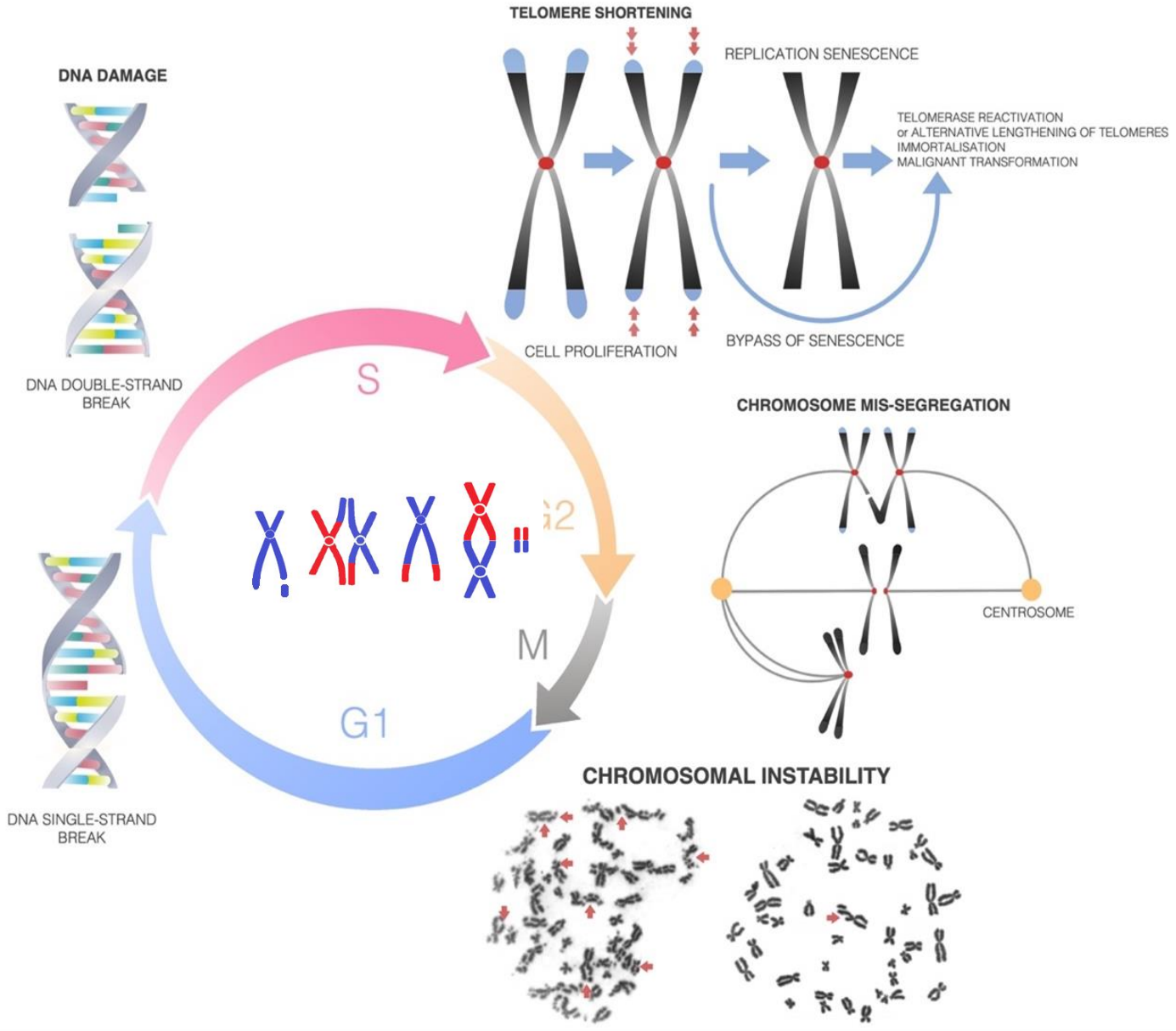




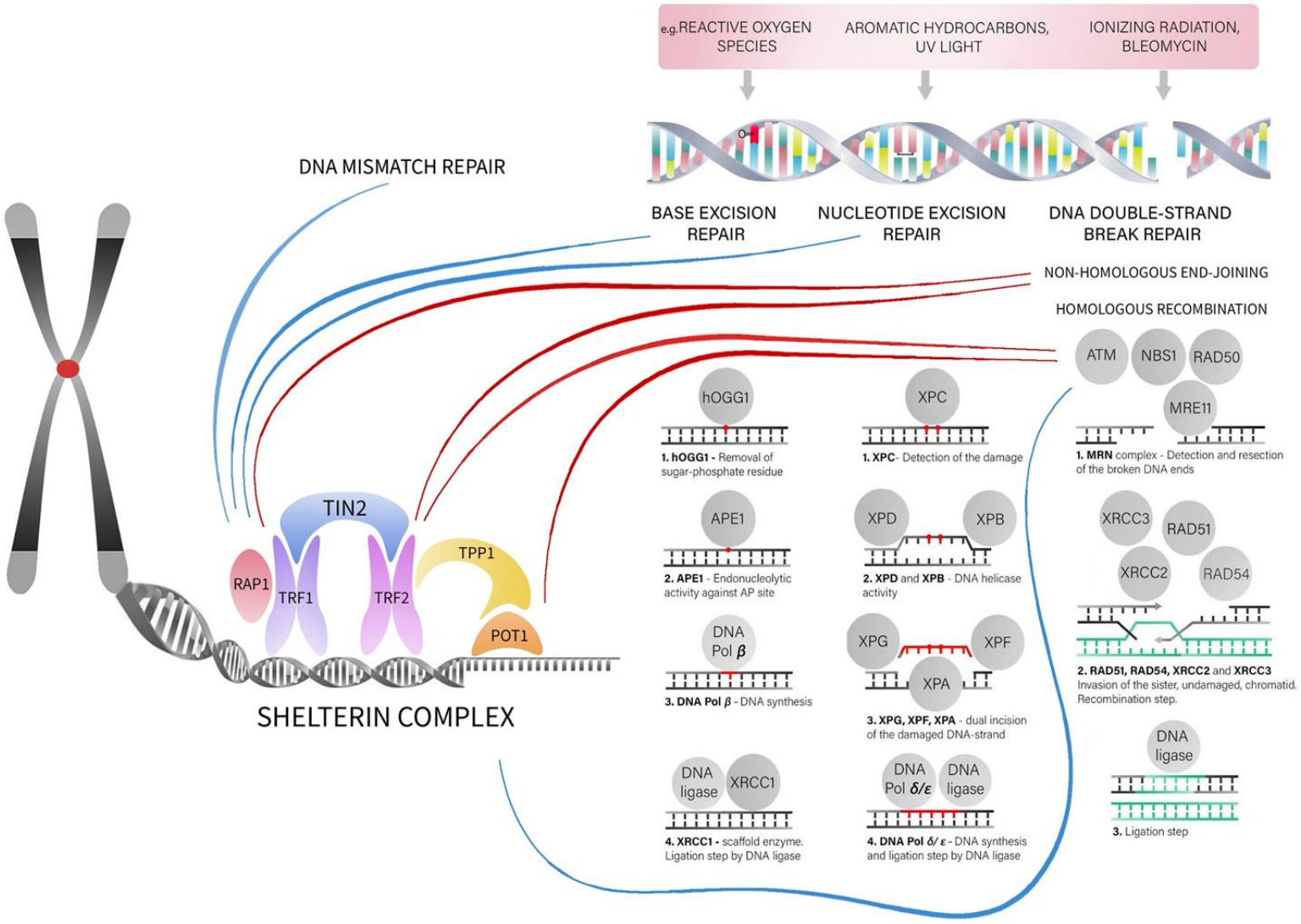




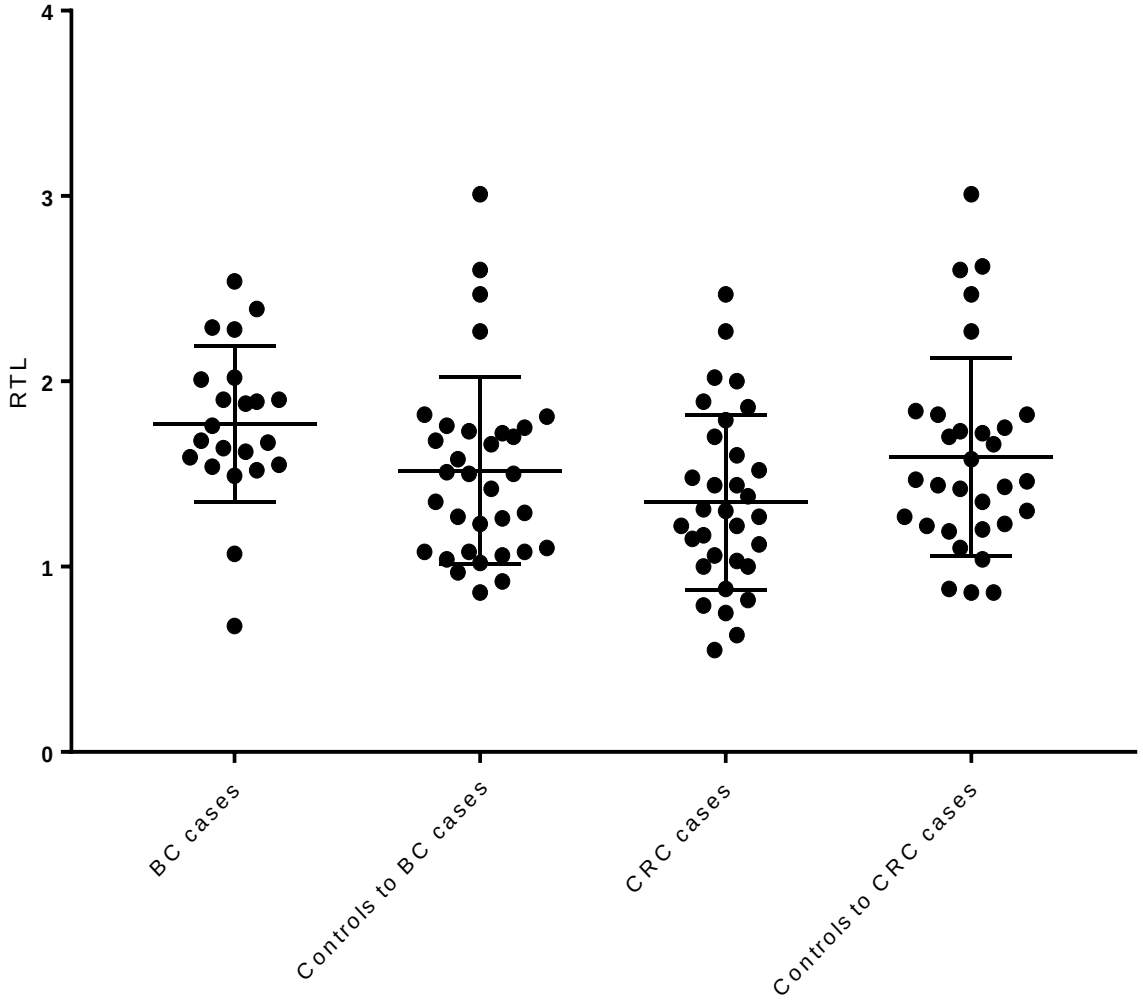



TELOMERE SHORTENING
REPLICATION SENESCENCE
TELOMERASE REACTIVATION
or ALTERNATIVE LENGTHENING OF TELOMERES
IMMORTALISATION
MALIGNANT TRANSFORMATION
DNA DAMAGE
CELL PROLIFERATION
BYPASS OF SENESCENCE
DNA DOUBLE-STRAND BREAK
S
G2
M
G1
CHROMOSOME MIS-SEGREGATION
CENTROSOME
DNA SINGLE-STRAND BREAK
CHROMOSOMAL INSTABILITY

ASSOCIATIONS BETWEEN DNA REPAIR AND TELOMERE MAINTAINANCE

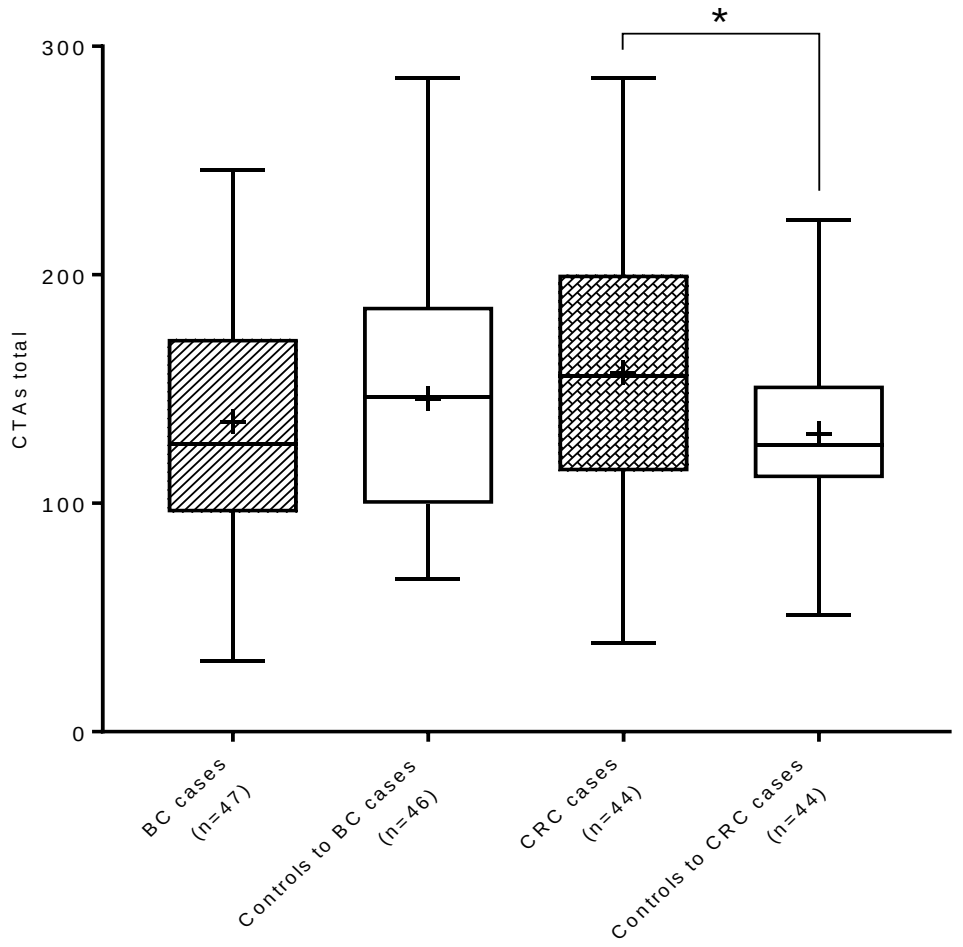


RTL measurement in all investigated groups



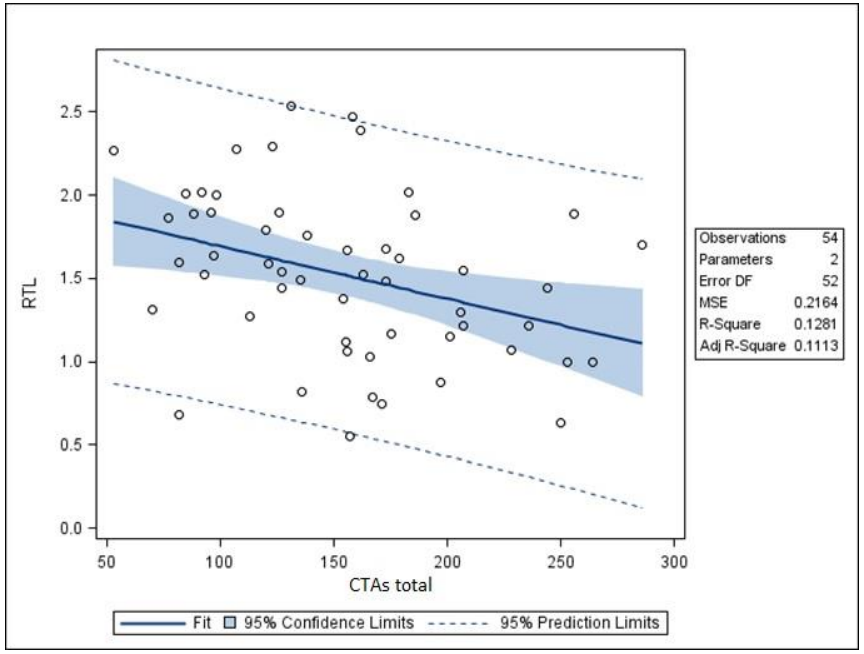
Chromatid-type aberrations in all investigated groups

*p=0.03.

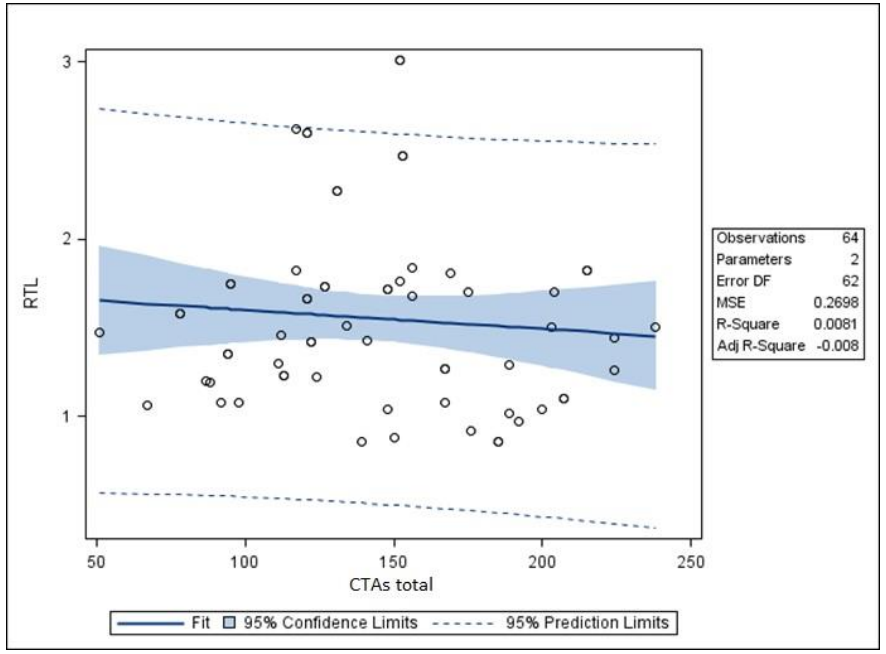


Spearman correlation of RTL with CTAs in a pooled group of cases and controls

DSB repair and RTL

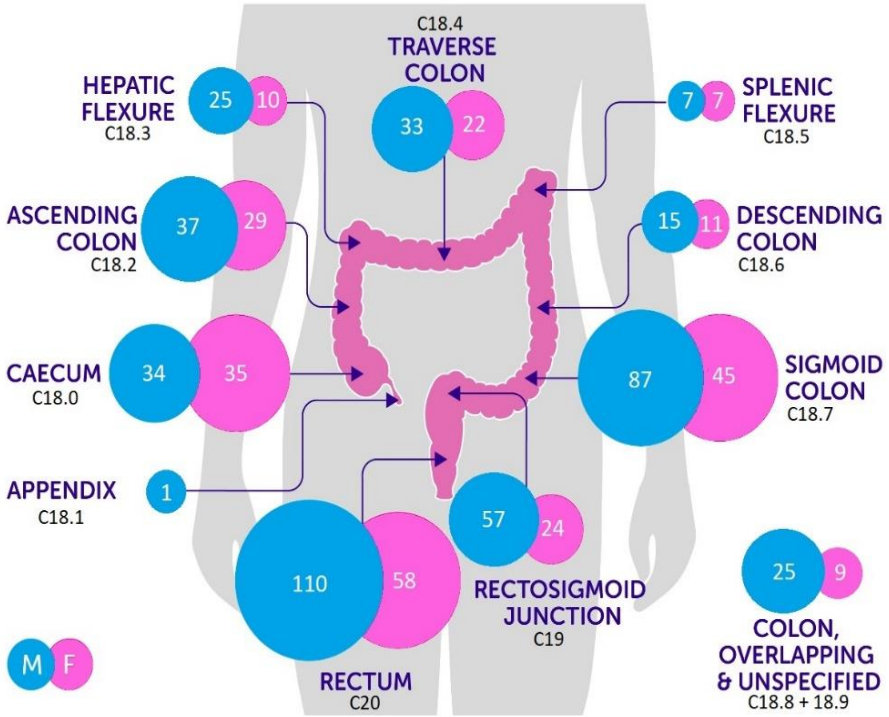


(R=-0.36; *p=0.02).

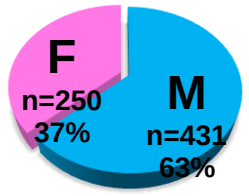


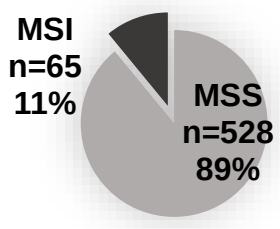
(R=-0.09)

POPULATION STUDY



edited from: www.cancerresearchuk.org

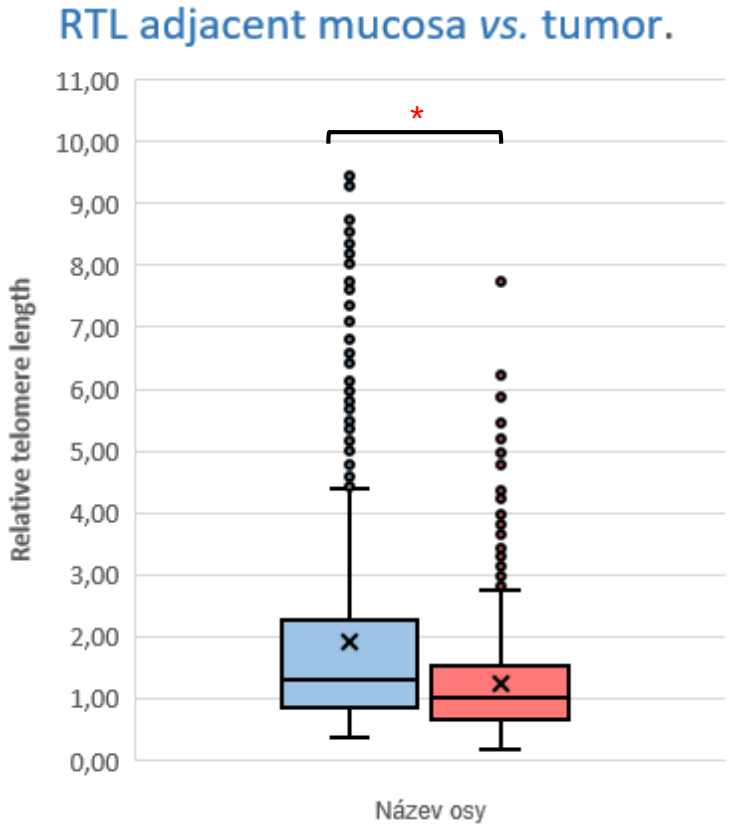


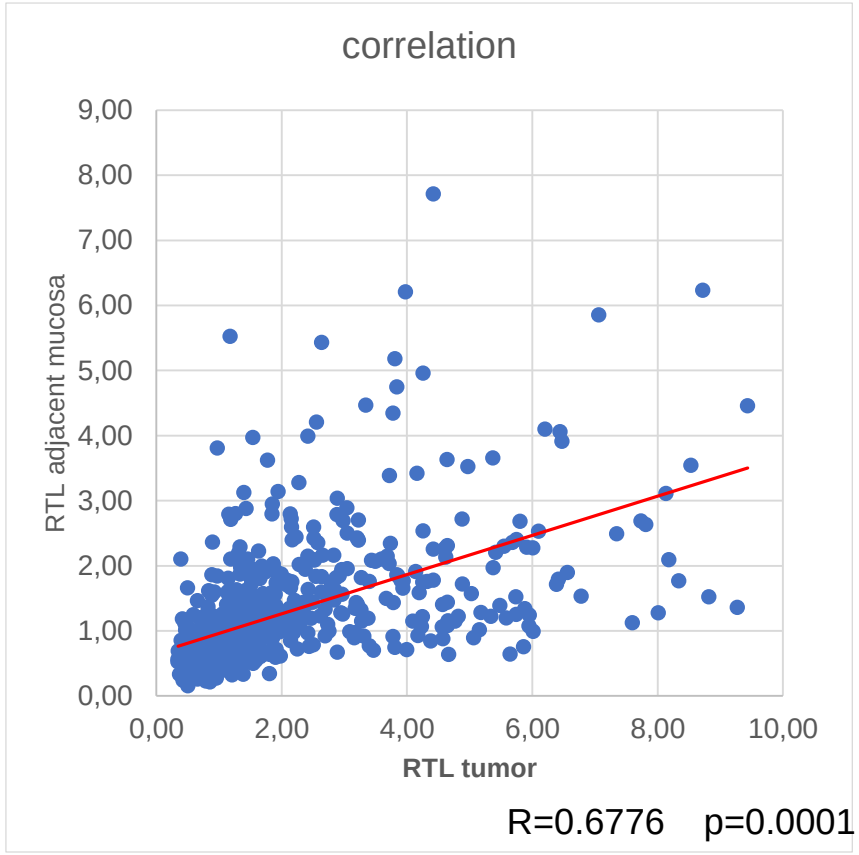


Characteristics of experimental population		n	%
CRC paired samples		681	
Blood		72	10.6
Gender	Male	431	63.1
	Female	250	36.9
Clinical Features			
Diagnosis	Proximal (C18.0-C18.4)	226	33.2
	Distal (C18.5-C19)	287	42.1
	Rectum (C20)	168	24.7
Stage (TNM)	I	100	15.1
	II	232	35.1
	III	204	30.9
	IV	125	18.9
MSI status	Stable	528	89.0
	Unstable	65	11.0
K-RAS	wild-type	53	48.6
	mutation	56	51.4
Grade	1	93	14.0
	2	453	68.3
	3	113	17.0
	4	4	0.6

Kroupa et al. Br J Cancer 2019

Telomere length in tumor tissue, adjacent mucosa and metastatic liver tissues

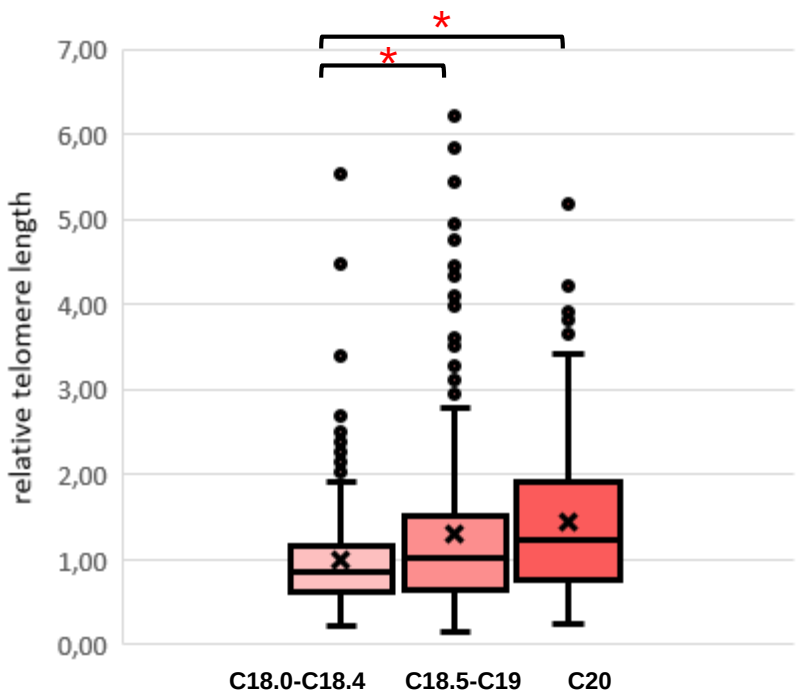




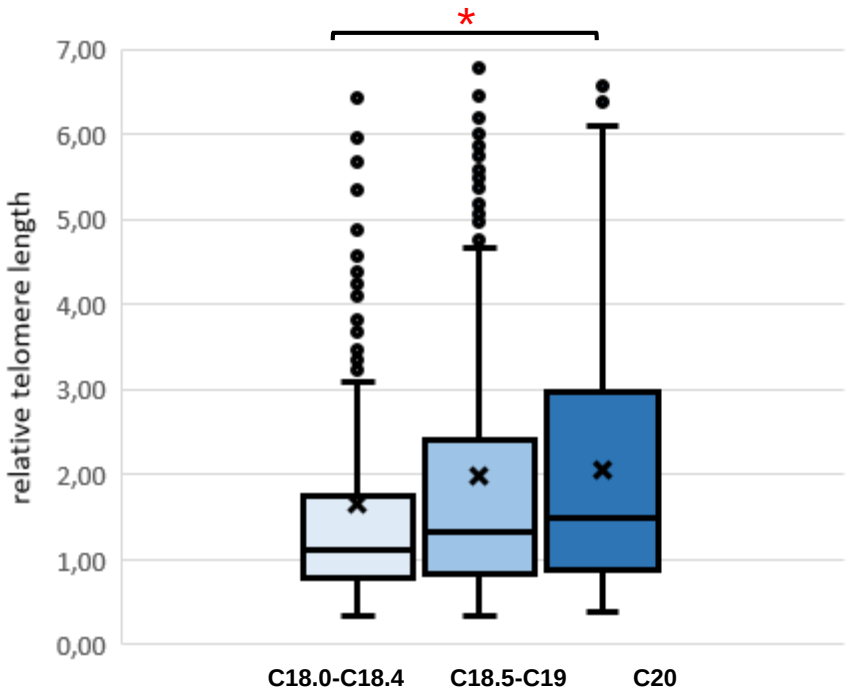
TL in patients with liver tissue: 0.97±0.42
and liver meta: 0.83±0.35
Ratio: 0.92±0.39

Relative telomere length *vs.* tumor localisation

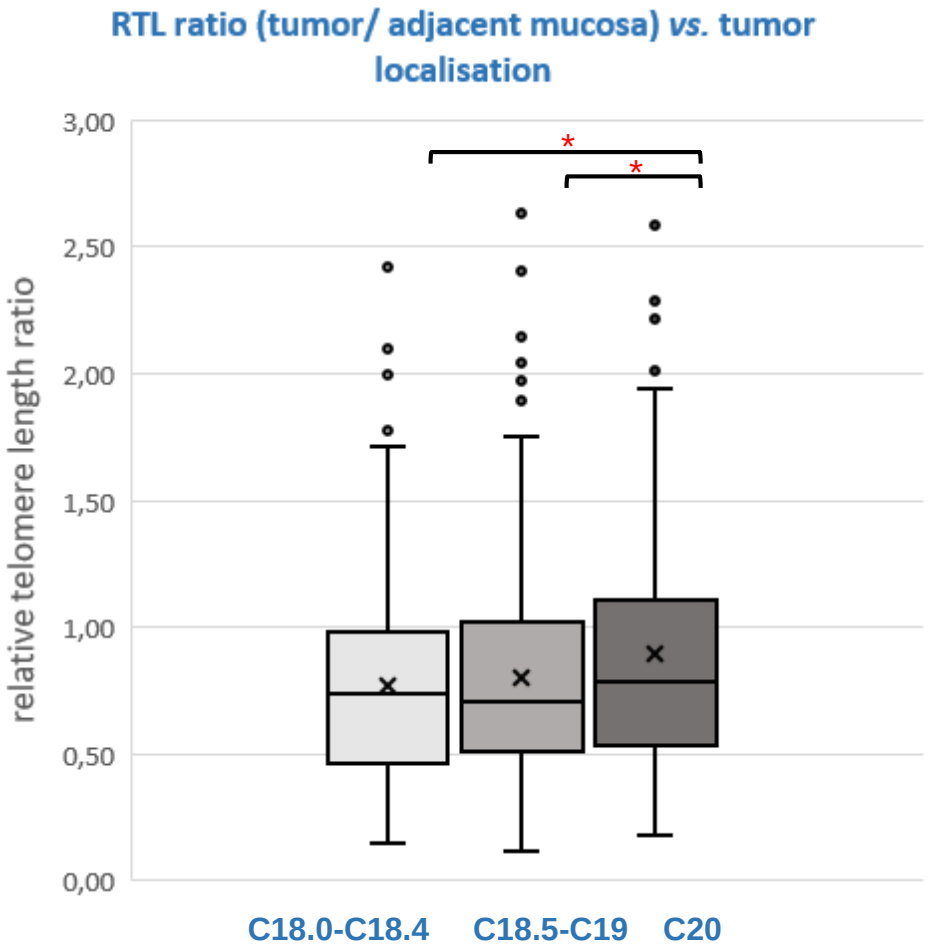
RTL *vs.* diagnosis: tumor



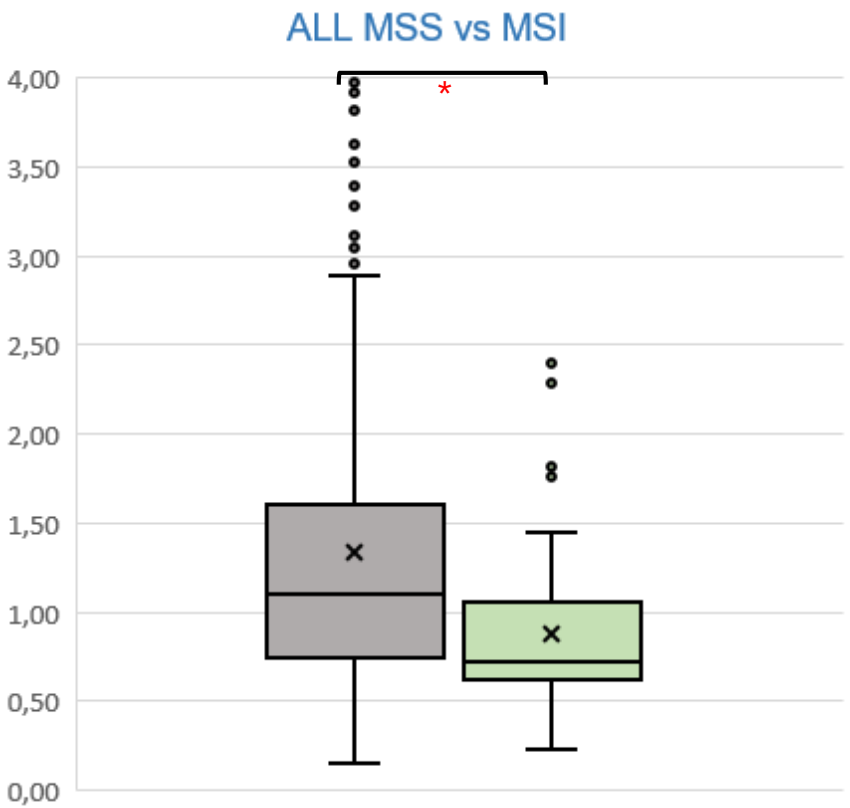
RTL *vs.* diagnosis: mucosa

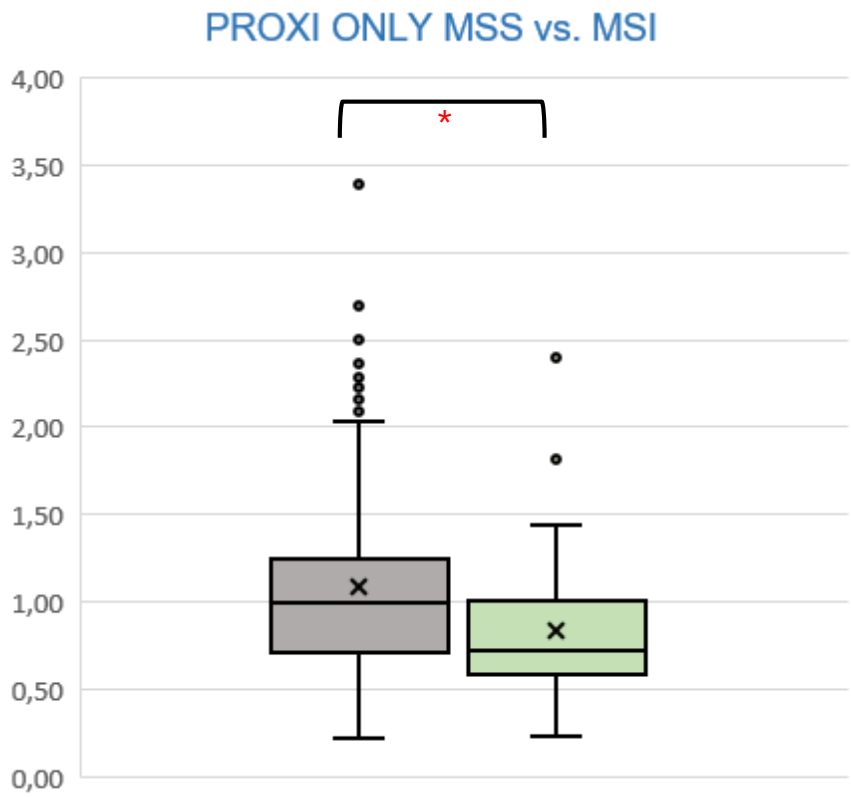


Relative telomere length ratio *vs.* tumor localisation

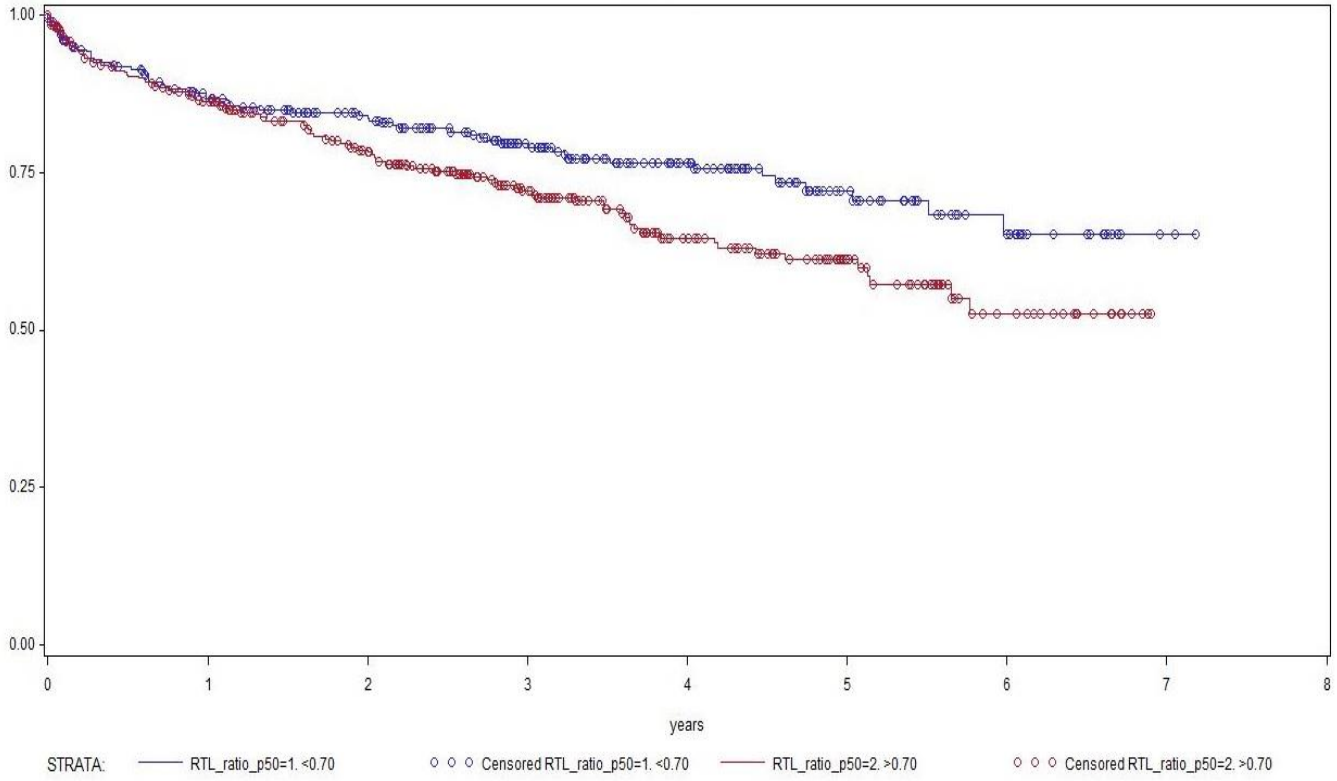


MSS *vs.* MSI (RTL)





Overall survival *vs.* RTL ratio (tumor/mucosa)



RTL ratio (tumor/ mucosa)

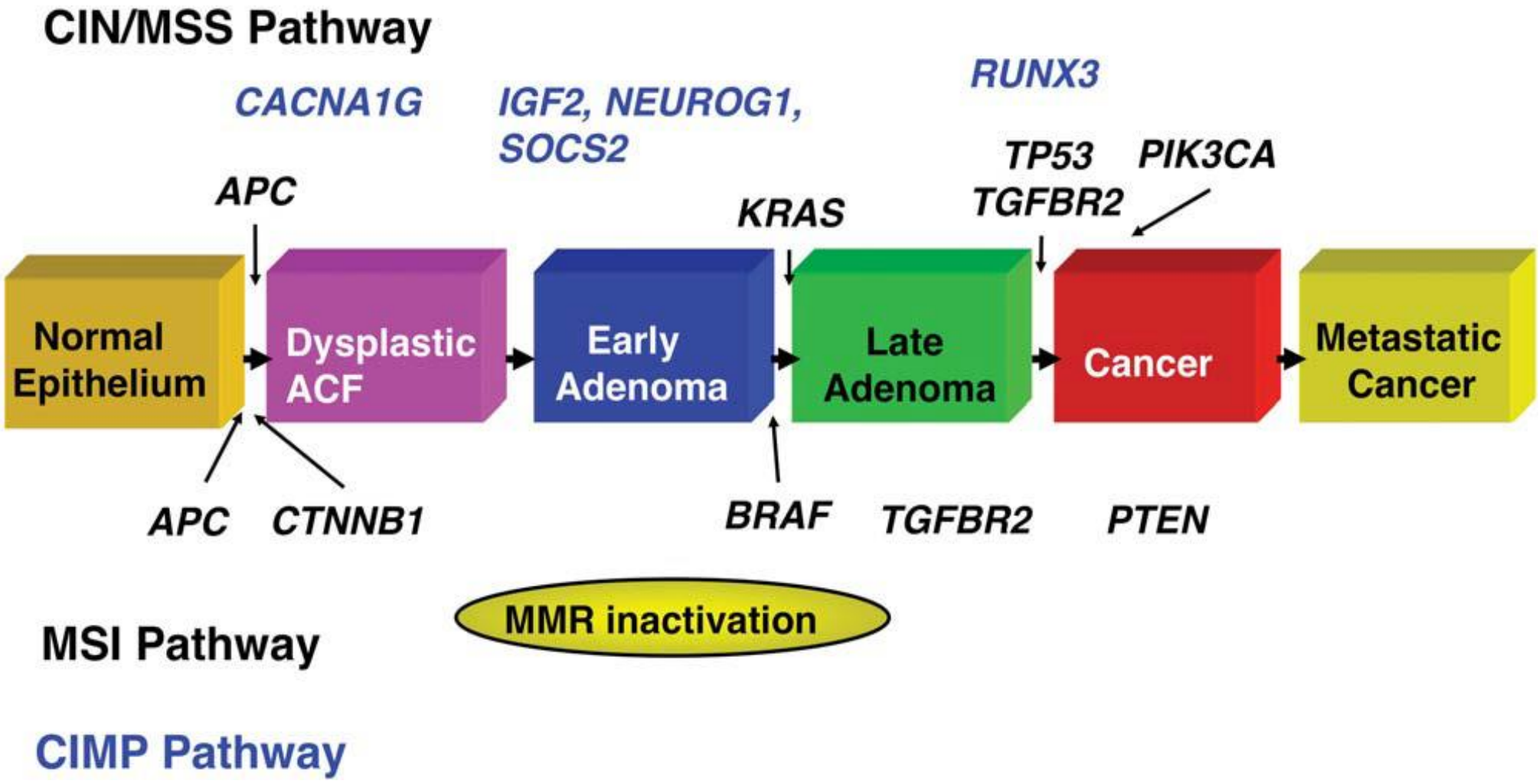
- <0.7
- >0.7

Overall Survival, RTL ratio (tumor/ adjacent mucosa); cut-off= 0.7, **p=0.022**.

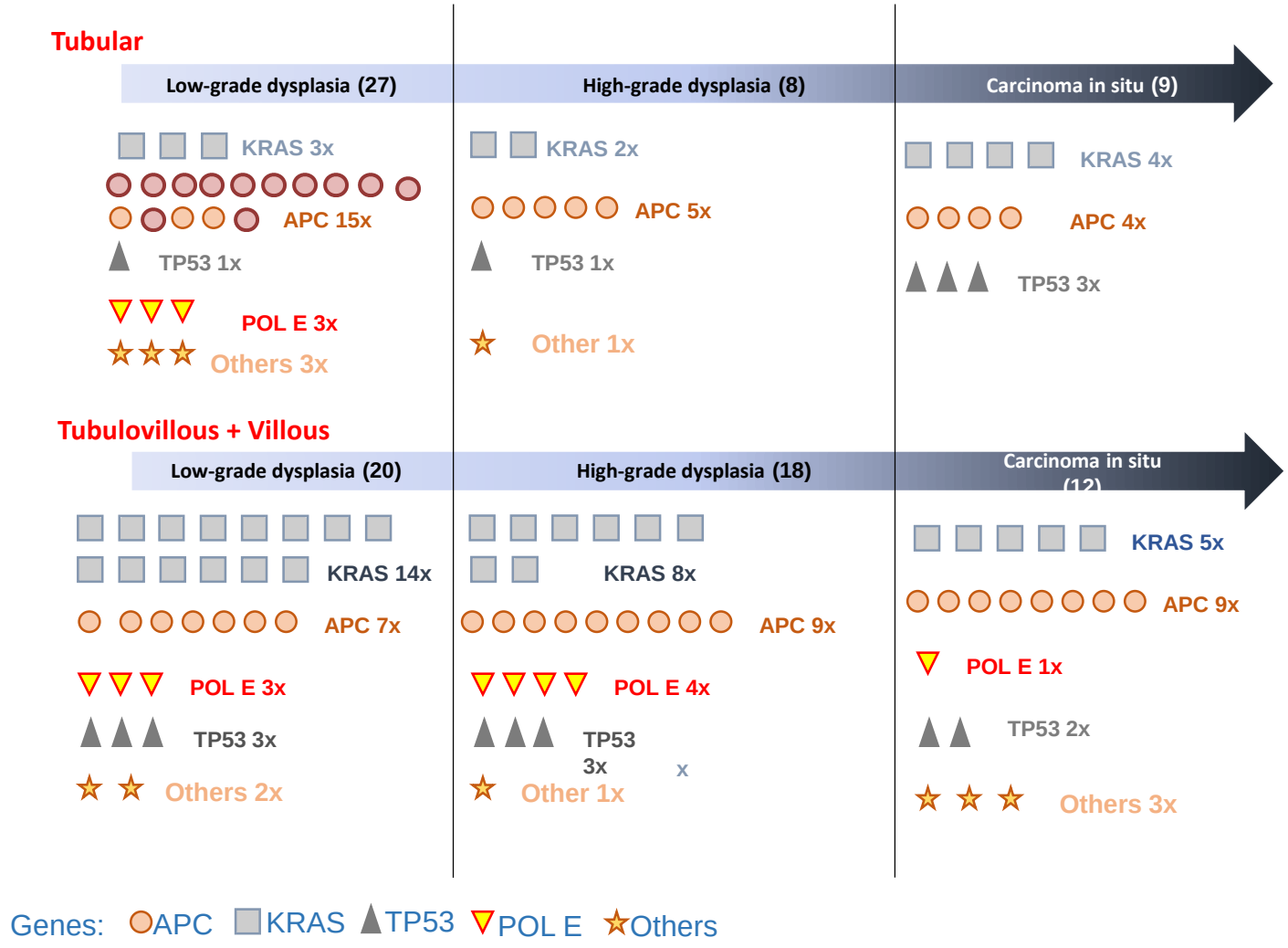
Prospective study of LTL in sporadic CRC patients

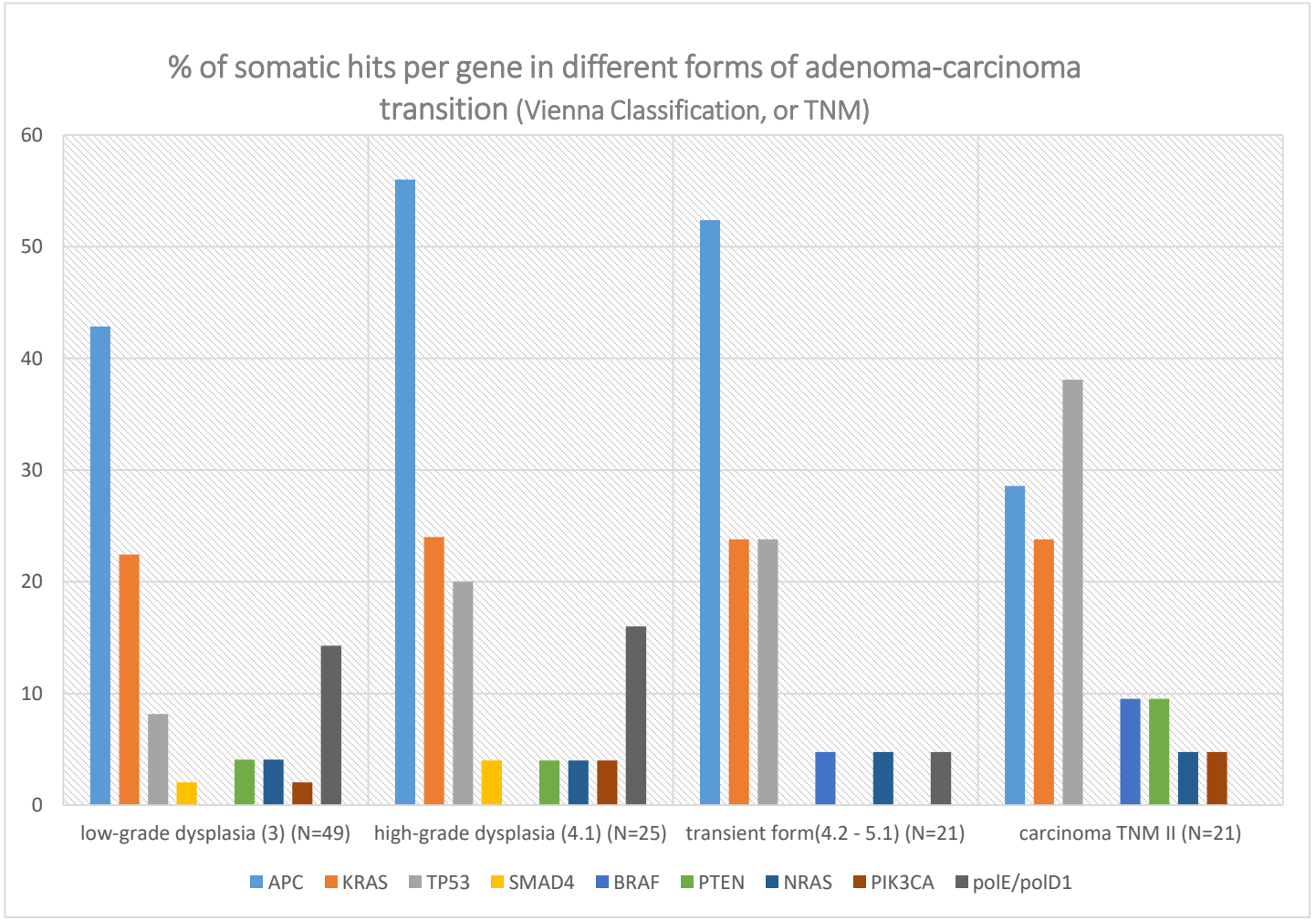
Studied cohort of patients	median age (years) [range]	66 [32-88]	
		n = 198	**%**
Gender	males	127	64.1
	female	71	35.9
Tumor site location[a]	proximal colon	35	18.0
	distal colon	86	44.3
	rectum	73	37.6
UICC TNM stage[b]	I + II	118	63.8
	III + IV	67	36.2
Microsatellite status	stable	138	85.2
	instable	24	14.8
Therapy response	good	64	70.3
	poor	27	29.7
Neoadjuvant therapy	yes	66	33.3
	no	132	66.6
Adjuvant therapy	yes	89	46.6
	no	102	53.4

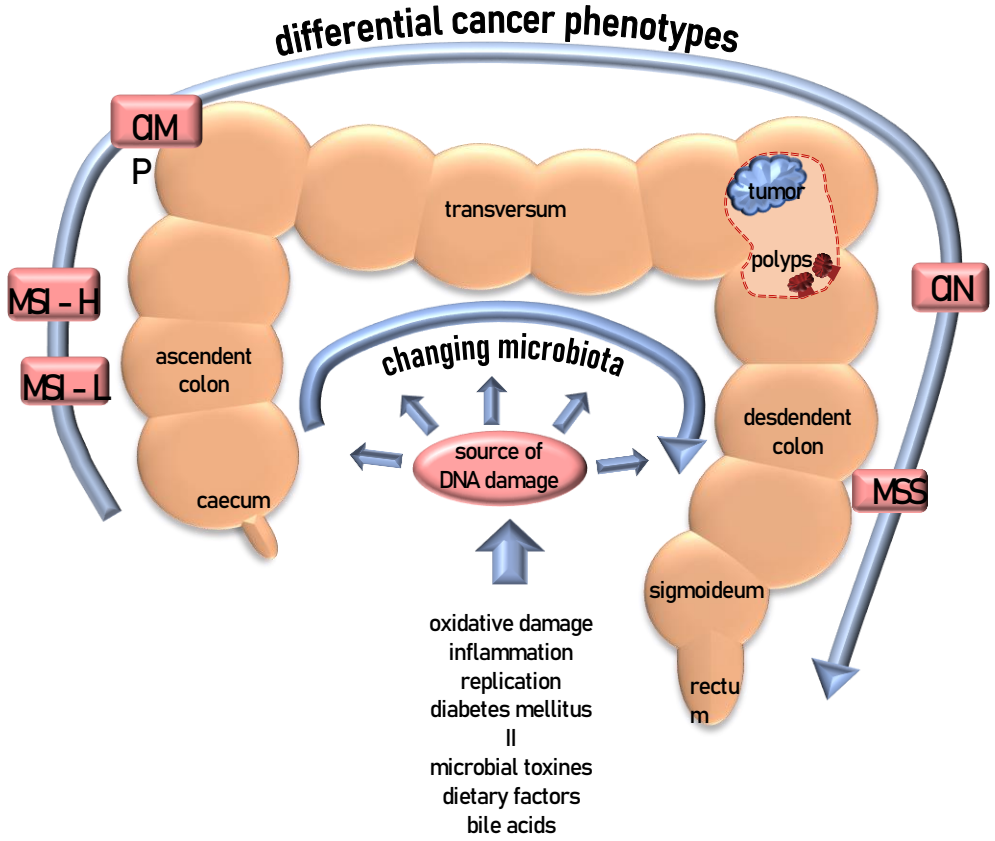
The polyp-to-carcinoma progression sequence



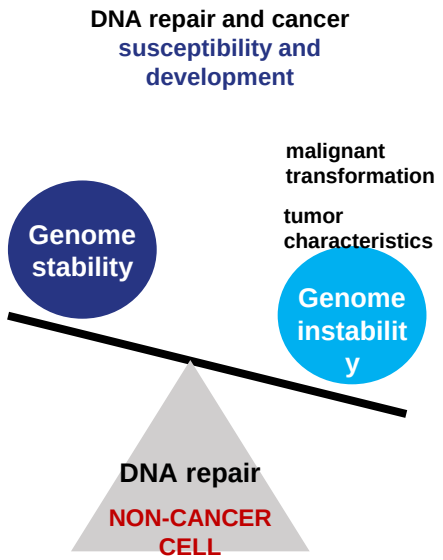
according to Vogelstein

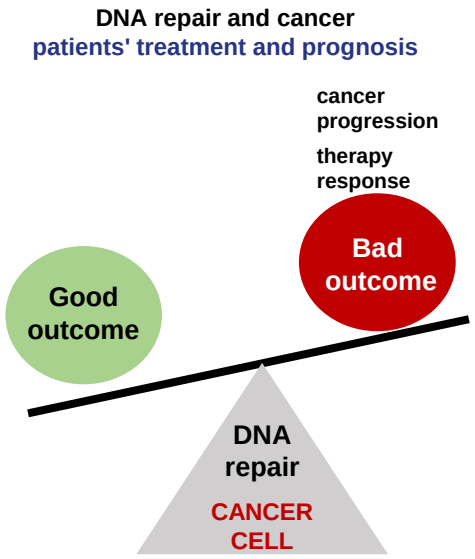

Tubular
Low-grade dysplasia (27)
High-grade dysplasia (8)
Carcinoma in situ (9)
KRAS 3x
APC 15x
TP53 1x
POL E 3x
Others 3x
KRAS 2x
APC 5x
TP53 1x
Other 1x
KRAS 4x
APC 4x
TP53 3x
Tubulovillous + Villous
Low-grade dysplasia (20)
High-grade dysplasia (18)
Carcinoma in situ (12)
KRAS 14x
APC 7x
POL E 3x
TP53 3x
Others 2x
KRAS 8x
APC 9x
POL E 4x
TP53 3x
Other 1x
KRAS 5x
APC 9x
POL E 1x
TP53 2x
Others 3x
Genes: APC KRAS TP53 POL E Others


% of somatic hits per gene in different forms of adenoma-carcinoma transition (Vienna Classification, or TNM)
60
50
40
30
20
10
0
low-grade dysplasia (3) (N=49)
high-grade dysplasia (4.1) (N=25)
transient form(4.2 - 5.1) (N=21)
carcinoma TNM II (N=21)
APC
KRAS
TP53
SMAD4
BRAF
PTEN
NRAS
PIK3CA
polE/polD1


differential cancer phenotypes
CIMP
MSI - H
MSI - L
CIN
MSS
transversum
tumor
polyps
ascendent colon
caecum
desdendent colon
sigmoideum
rectum
changing microbiota
source of DNA damage
oxidative damage
inflammation
replication
diabetes mellitus II
microbial toxines
dietary factors
bile acids

DNA DAMAGE AND DNA REPAIR IN TUMORIGENESIS





how to conclude?

There is a long way to go in understanding biology of human diseases-at least for few generations…

Thank you for your attention.

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Thank you for your attention

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