Chromosomal aberrations as markers of genotoxic effects and carcinogenesis

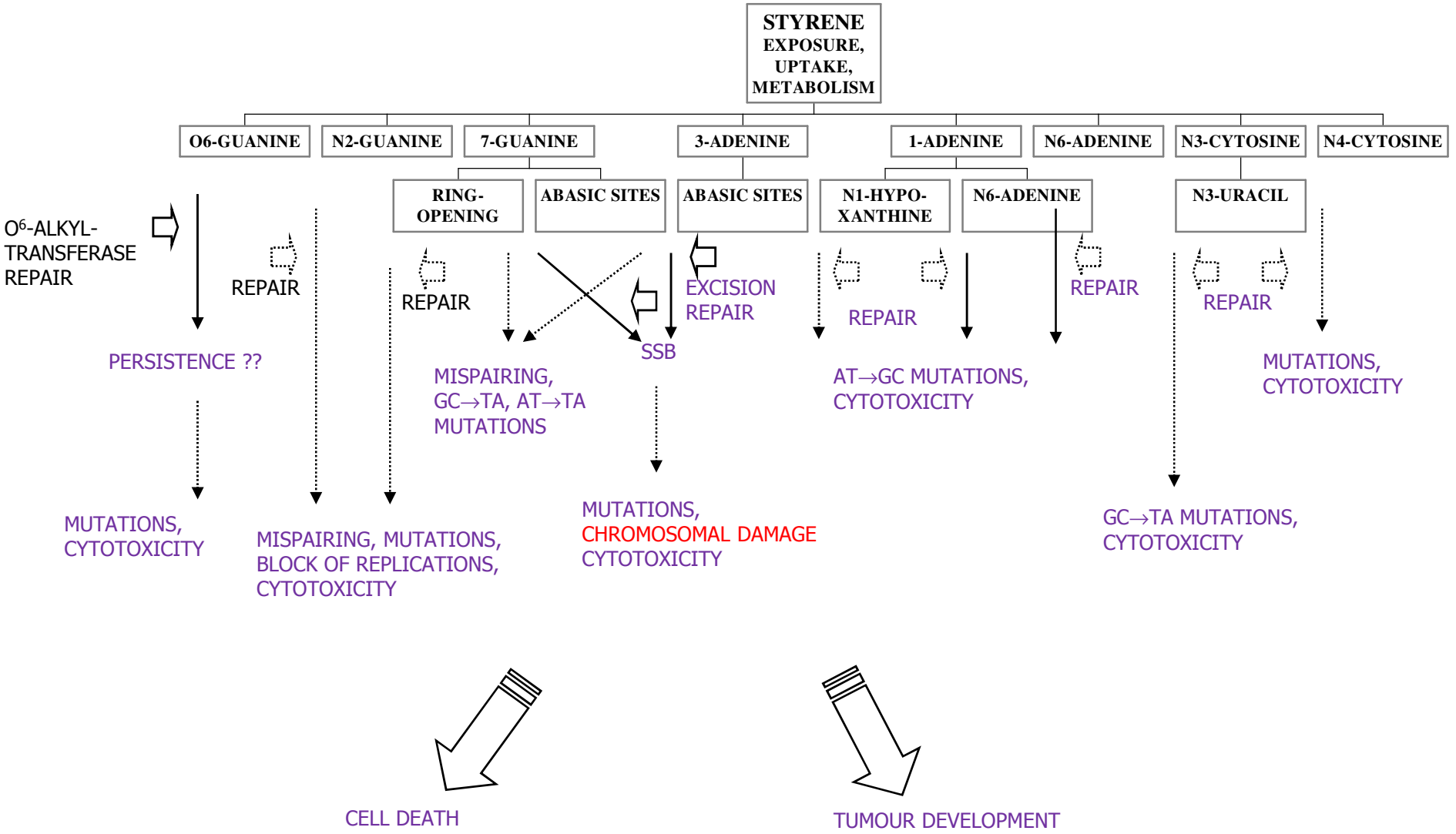
Chicago, IL, USA, October 20th-22nd, 2014

Pavel Vodicka a collaborators

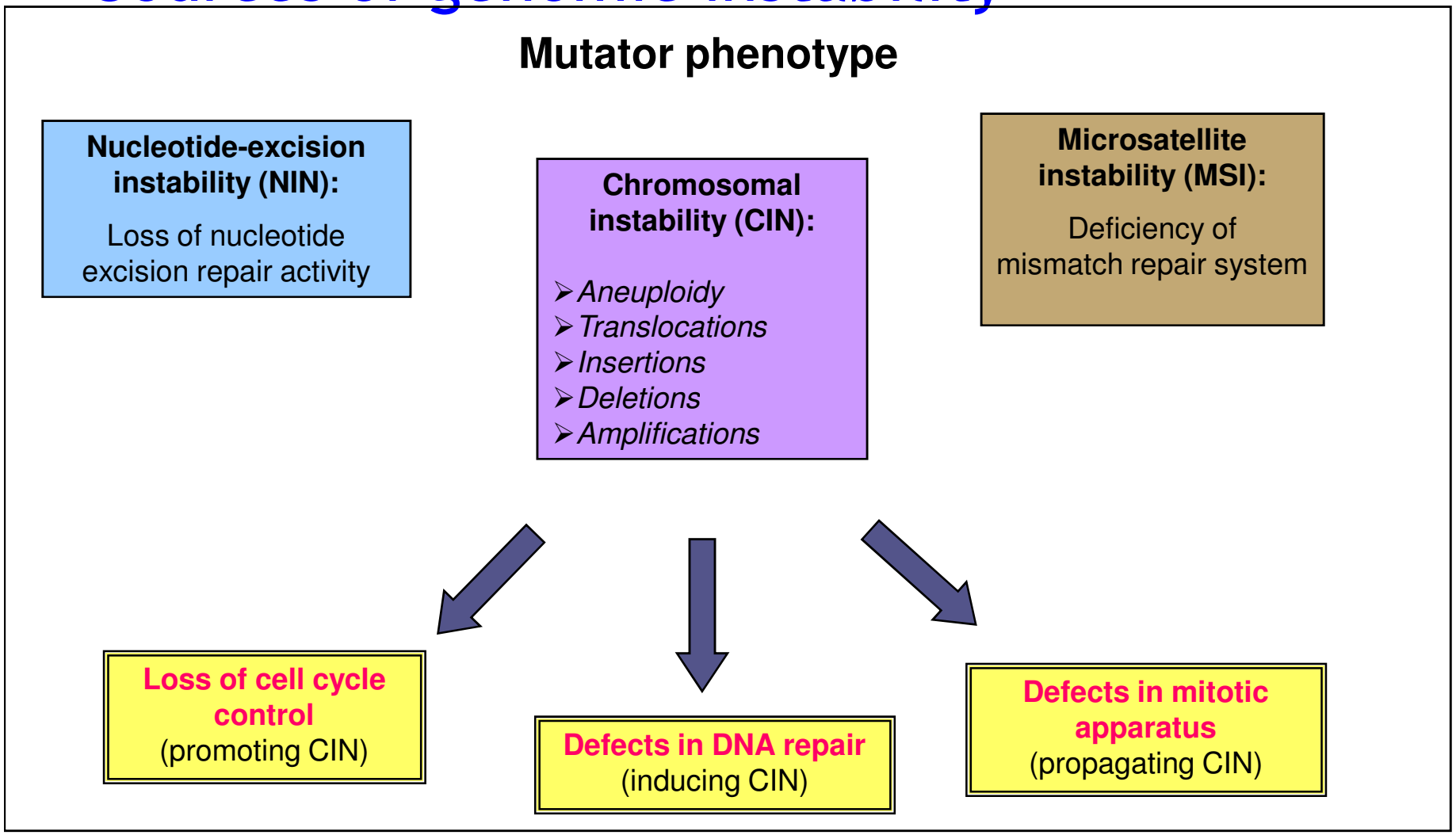
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EU Centre of Excellence

Department of the Molecular Biology of Cancer,
Institute of Experimental Medicine,
The Academy of Sciences of the Czech Republic

Tentative consequences of various DNA adducts



Sources of genomic instability



Chromosomal aberrations and cancer risk

• Majority of human cancer cases arise due to the inability of cells to maintain genomic stability

• **Chromosomal damage and susceptibility**
•chromosomal aberrations – a consequence of unrepaired or incorrectly repaired DNA damage (formation of double strand breaks, DSBs)

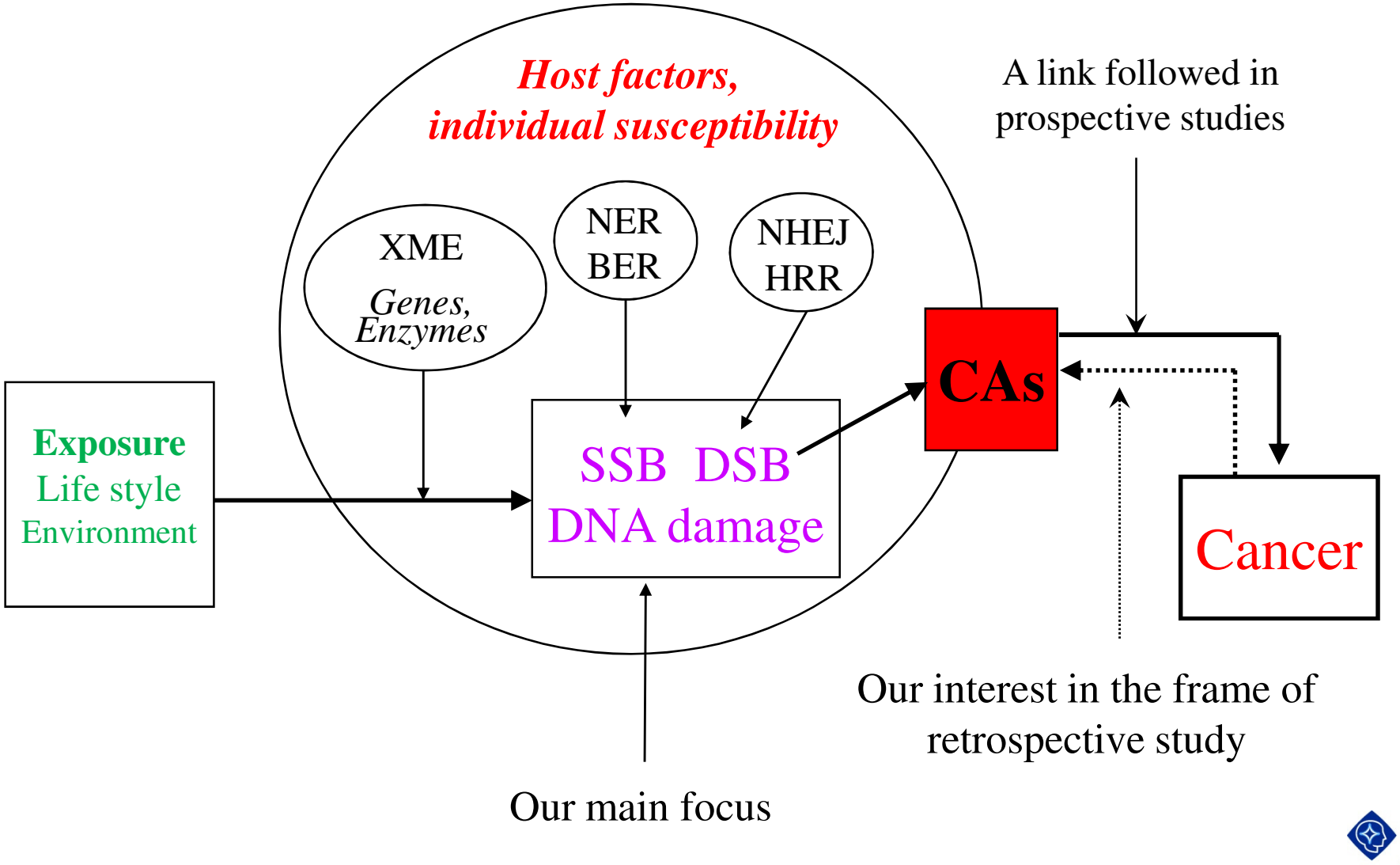
from peripheral blood lymphocytes we retrieve following information:
a) *individual exposure to genotoxic factors*
b) *individual susceptibility to these genotoxic factors* (genetically and environmentally influenced equilibrium between the DNA damage and DNA repair)

•chromosomal aberrations as a biomarker:
prospective studies have shown that of CHAs are predictive of cancer risk

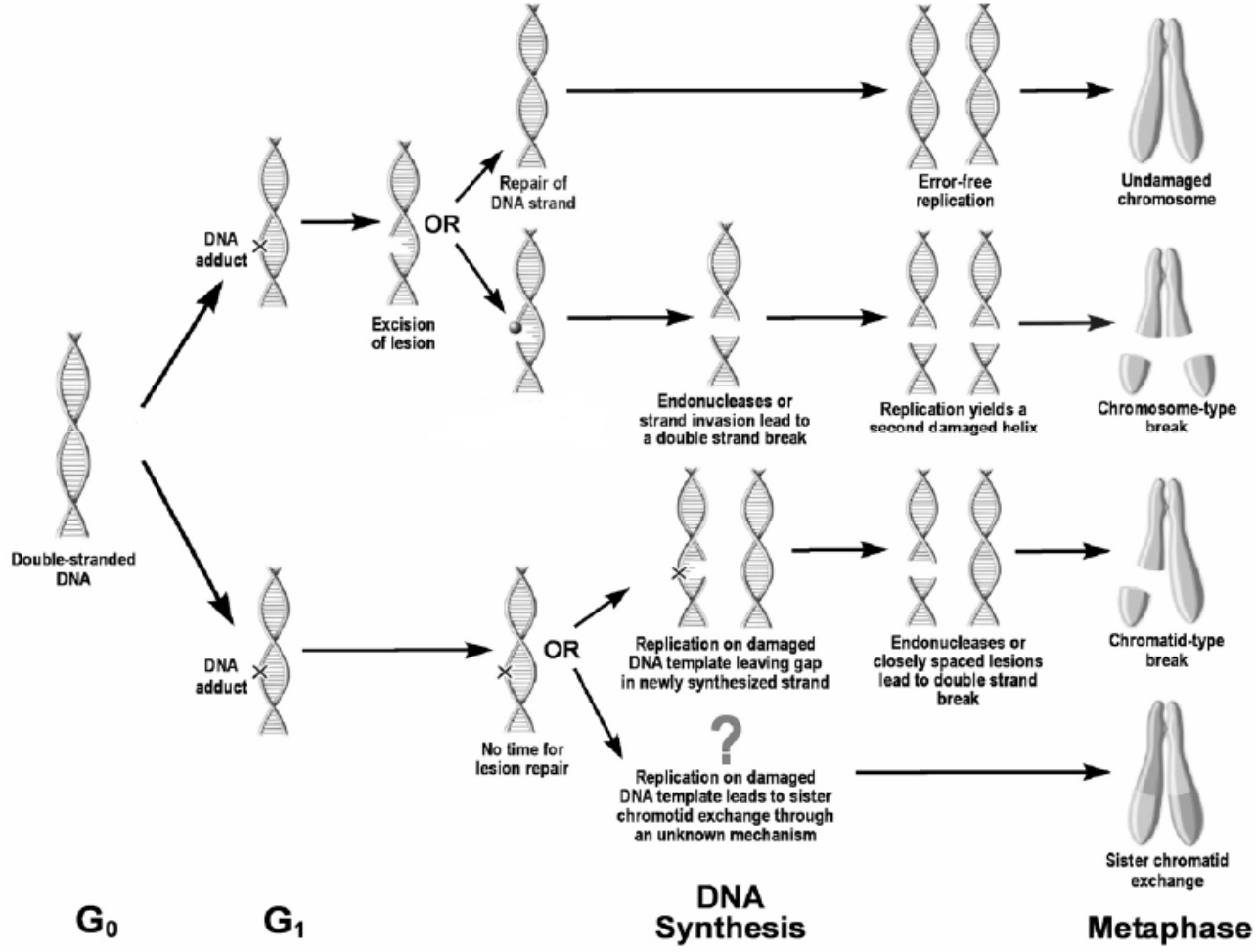
(*Bonassi et al. 2004, Hagmar et al. 2004, Norppa et al. 2006, Boffeta et al. 2007, Bonassi et al. 2008)*

Do CHAs represent transient biomarker of carcinogenesis?

A schema of factors involved in CAs formation and tentative link to cancer



Chromosomal aberrations and cancer risk - Mechanism



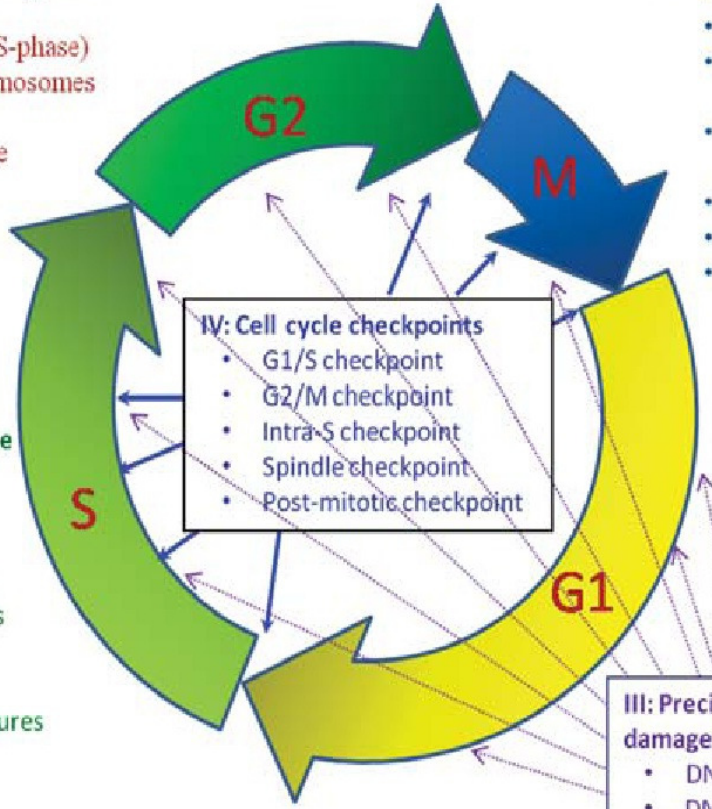
NHEJ acts throughout whole cell cycle, HR during G2/S phase

Modified from A.D. Kligerman, Y. Hu / Chemico-Biological Interactions 166 (2007) 132-139

Chromosomal aberrations and cancer risk - Preconditions

During the cell division cycle, four major mechanisms are involved in maintaining the genomic stability:

I. Fidelity of DNA replication (S-phase)
II. Accurate segregation of chromosomes (M-phase)
III. Precise repair of DNA damage (throughout the cell cycle)
IV. Cell cycle checkpoints



II: Segregation of chromosomes in mitosis
- Chromosome condensation
- Sister chromatid cohesion
- Kinetochore assembly and attachment
- Centrosome duplication and separation
- Spindle formation and checkpoint
- Chromatid segregation
- Cytokinesis

IV: Cell cycle checkpoints
- G1/S checkpoint
- G2/M checkpoint
- Intra-S checkpoint
- Spindle checkpoint
- Post-mitotic checkpoint

I: Fidelity of DNA replication in S-phase
- DNA polymerase
- Mismatch repair
- Replication licensing
- Maturation of Okazaki fragment
- Restart of stalled replication forks
- Re-chromatinization
- Telomere maintenance
- Preservation of epigenetic signatures

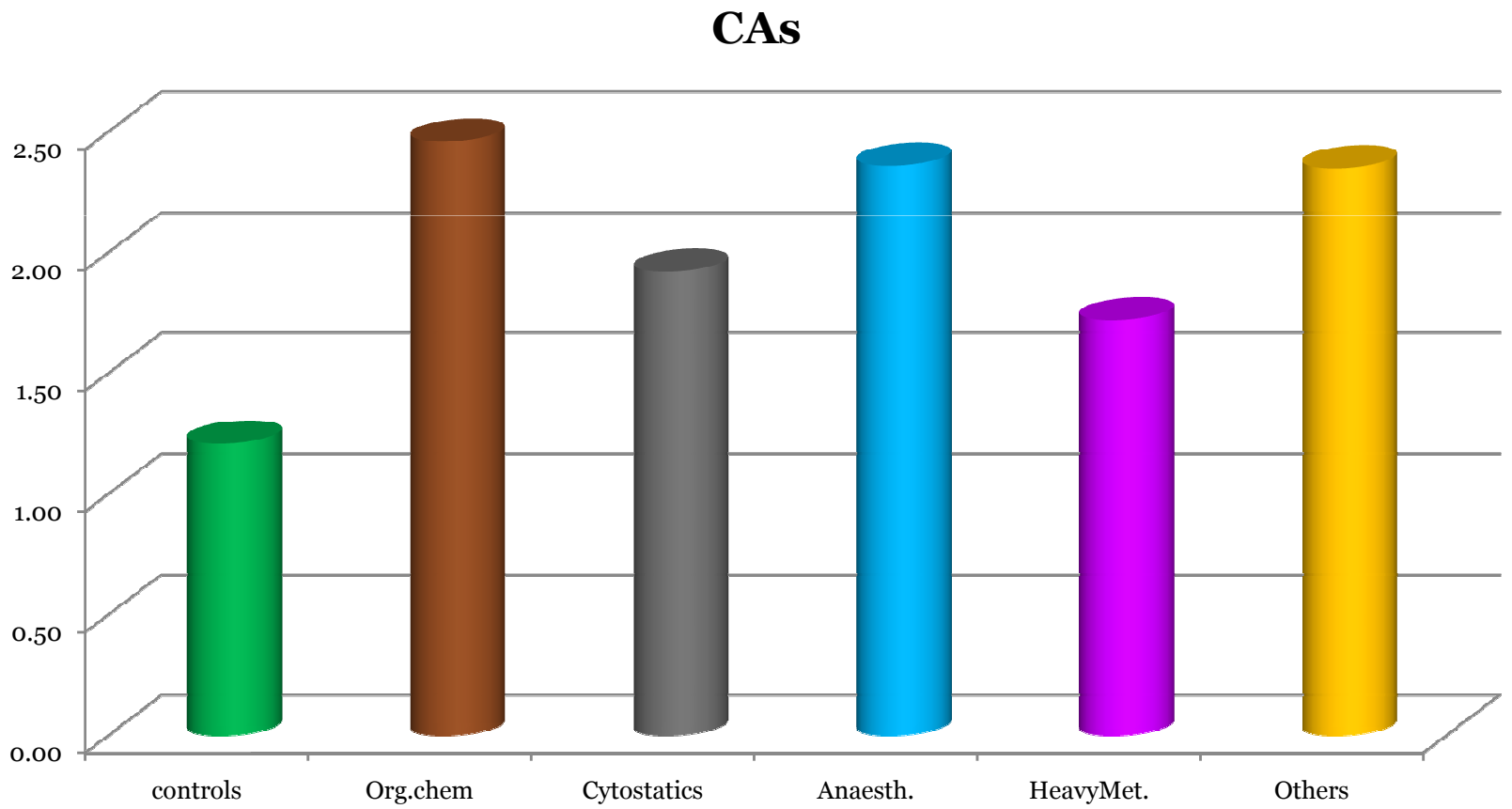
III: Precise repair of sporadic DNA damage *throughout the cell cycle*
- DNA repair pathways
- DNA damage signaling

CHA: Studied healthy subjects

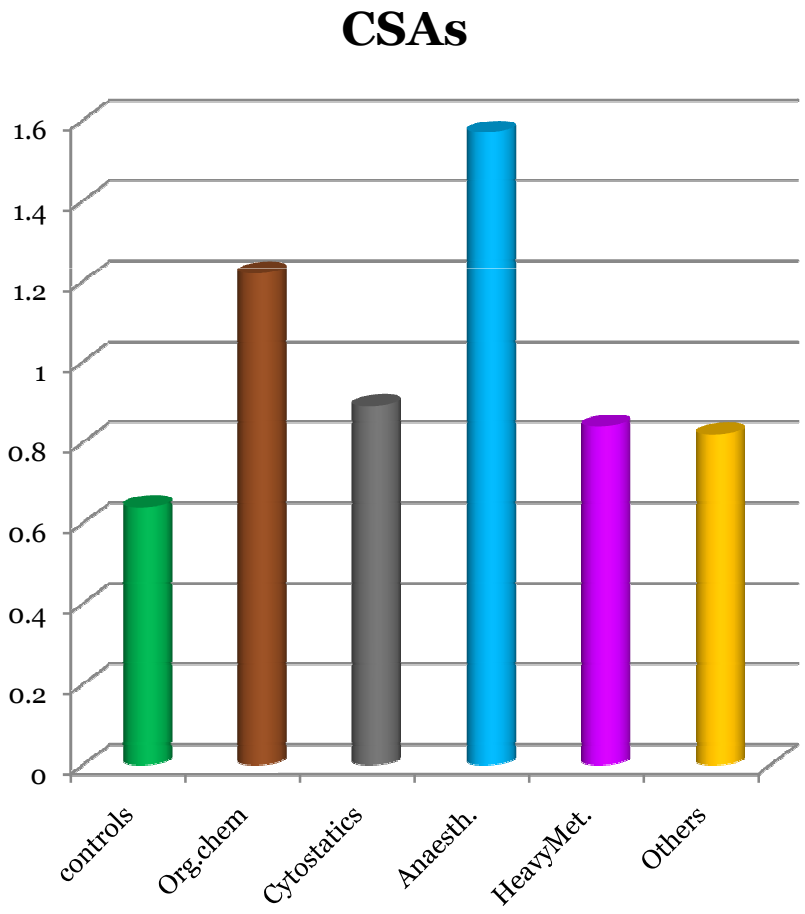
All subjects	Healthy controls	751
	Healthy exposed controls	1028
Age (years)		42.7 ± 14.9 (median 41.0; min 18; max 88)
Sex (in %)	Females	53.3
	Males	46.7
Smoking (in %)	Non-smokers	71.3
	Current smokers	28.6
Occupational exposure (in %)	No	42.2
	Yes	57.8
Agent (in %)	Non-exposed controls	42.2
	Mixture of organic monomers	21.1
	Cytostatics	4.6
	Anaesthetics	4.2
	Heavy metals	5.5
	Mineral fibers	21.4
	Other occup. exp.	1.0

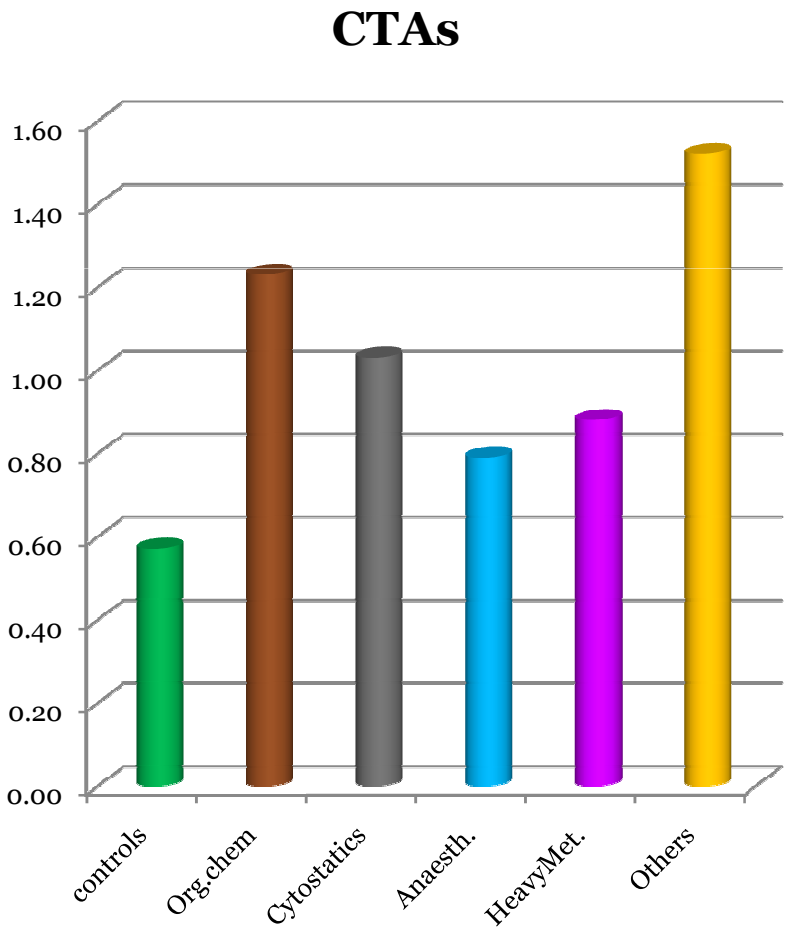
Numbers may not add up to 100% of available subjects because of missing data.

CAs - individual exposures

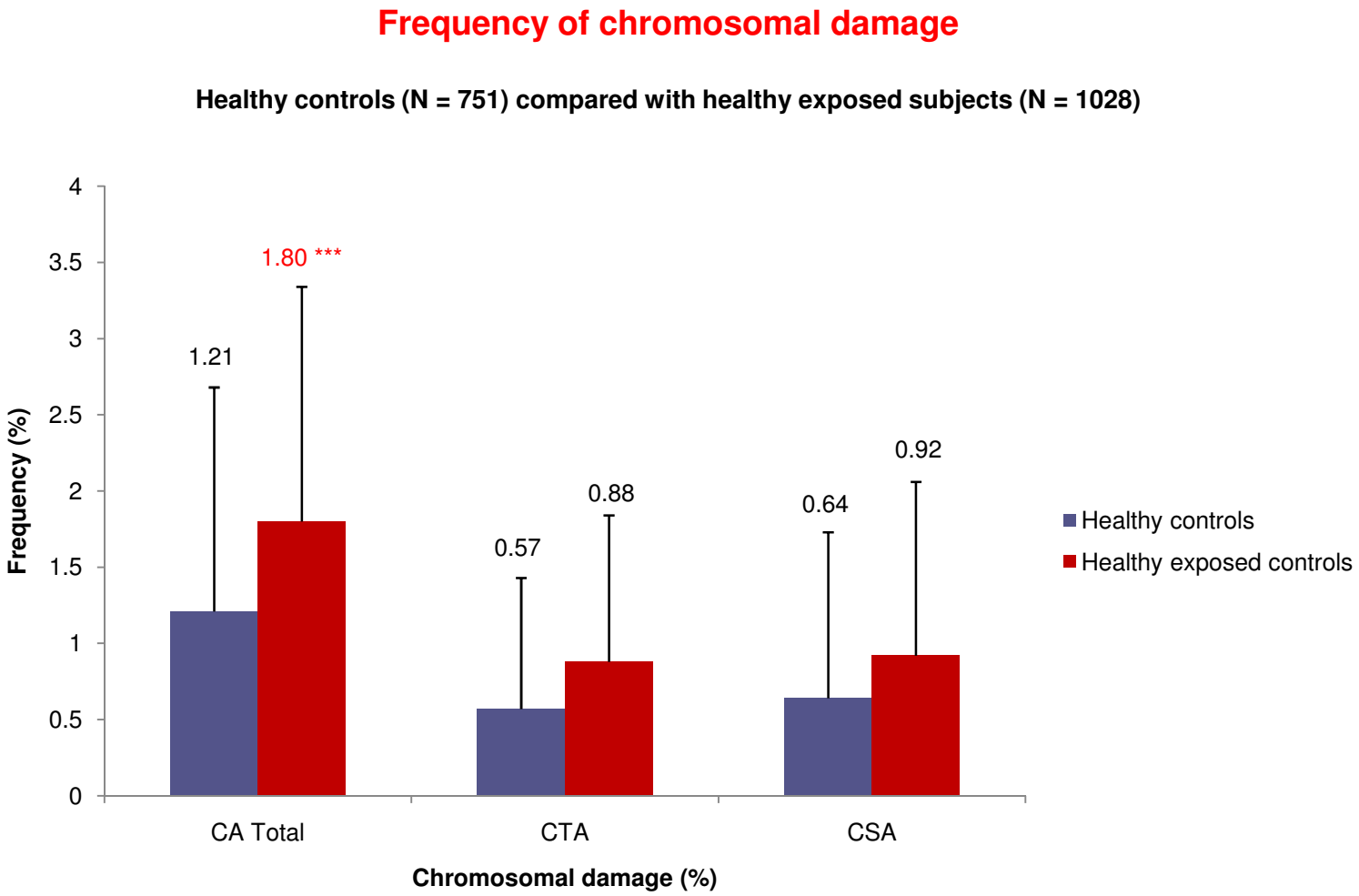


CSAs and CTAs - - individual exposures



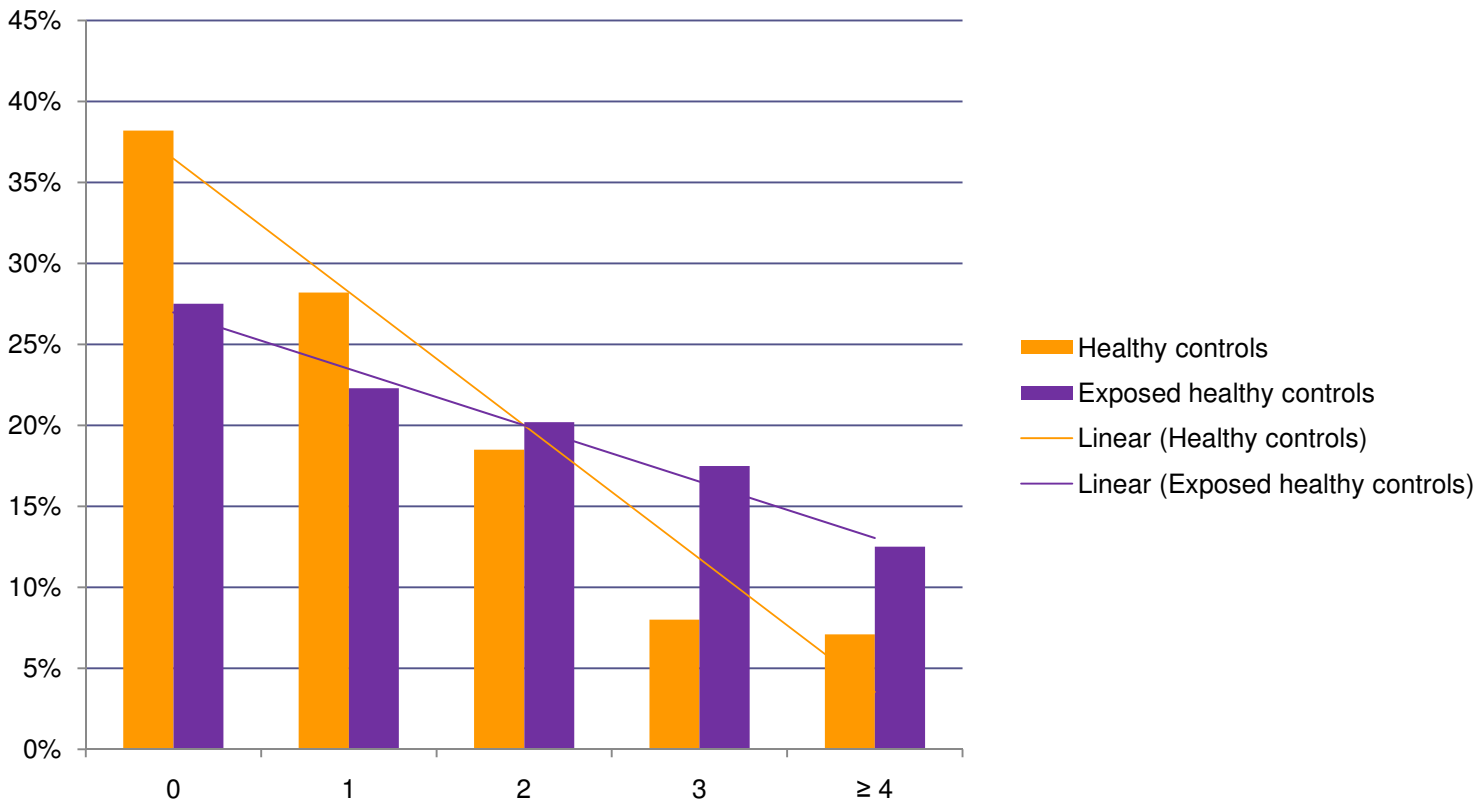


CHA: control and exposed populations

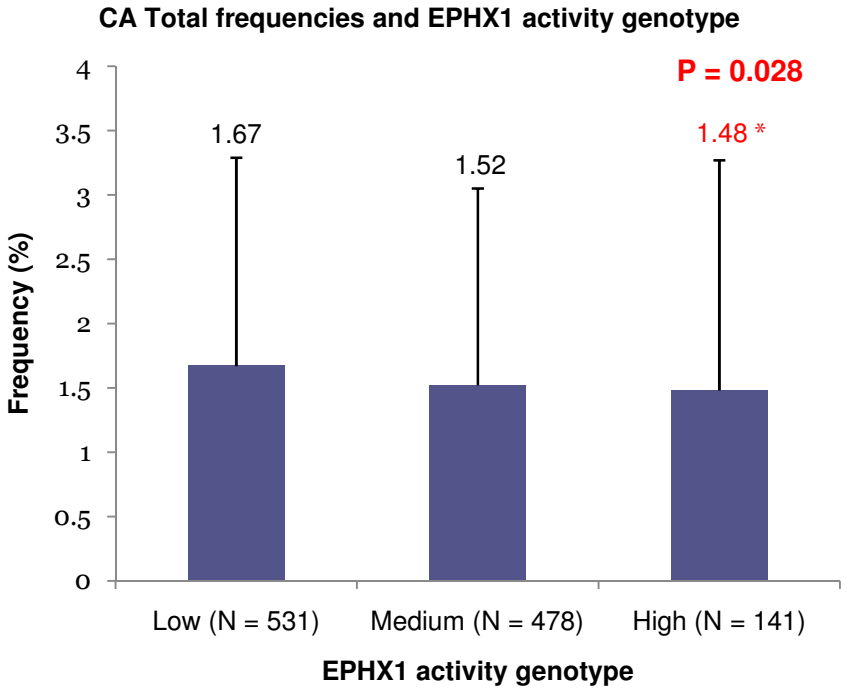


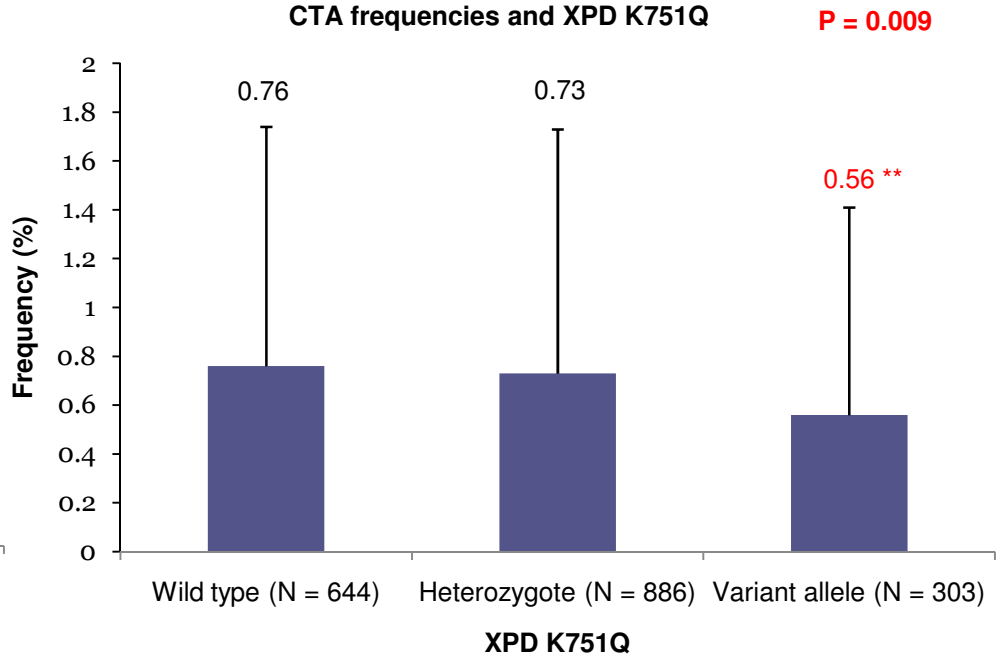
CHA: control and exposed population - distribution

Distribution of frequencies of CAs (N = 1779)



CHA: control and exposed population - genotypes





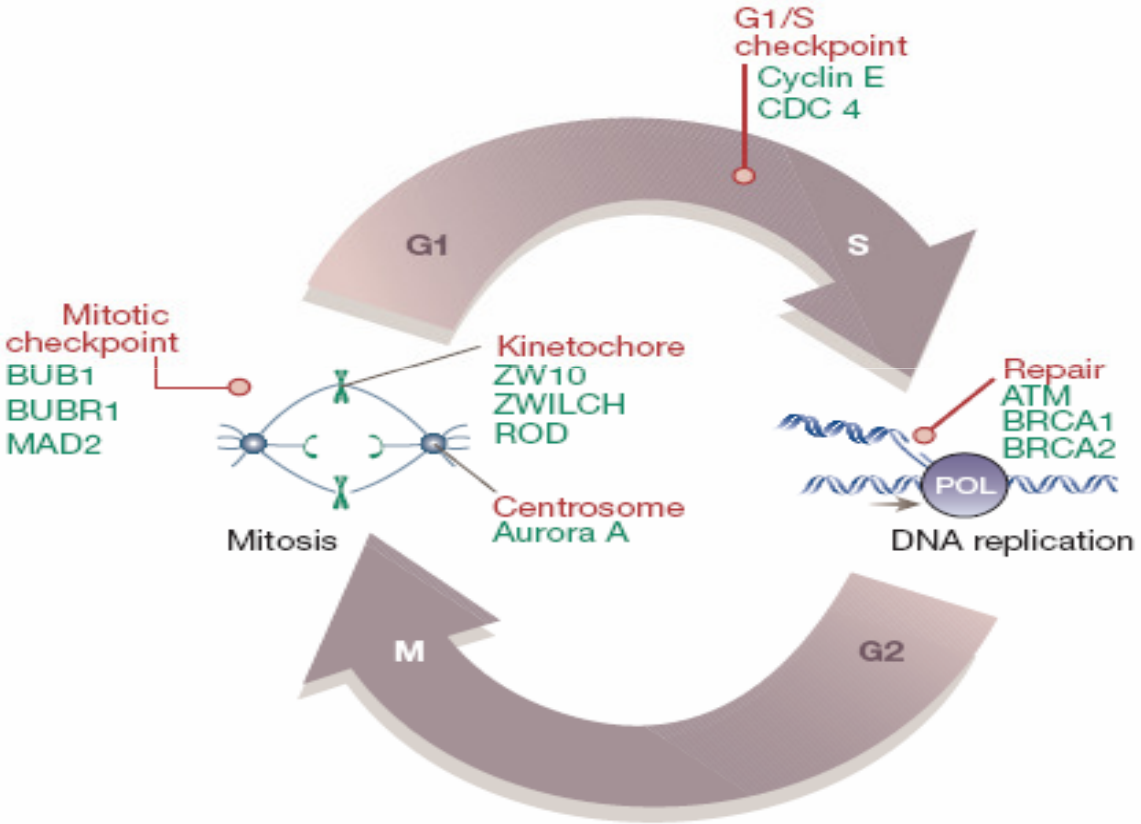
CHA: control and exposed population - confounders

Binary logistic regression models – chromosomal damage and genotype variants (adjusted odds ratio)

CAs	aOR	95% C.I. For OR	P-value
Smoking	1.36	1.05 – 1.76	0.018
Occupational exposure	1.42	1.08 – 1.87	0.013
EPHX1 high activity genotype	0.67	0.45 – 0.98	0.040

CTA	aOR	95% C.I. For OR	P-value
Occupational exposure	1.34	1.10 – 1.64	0.004
XPD23 k751Q (T/G)	0.65	0.49 – 0.86	0.003

Some processes implicated in the process of aneuploidy



From H. Rajagopalan, C. Lengauer / Nature 432 (2004) 338-341

CHA: control and exposed population – genes in cell division

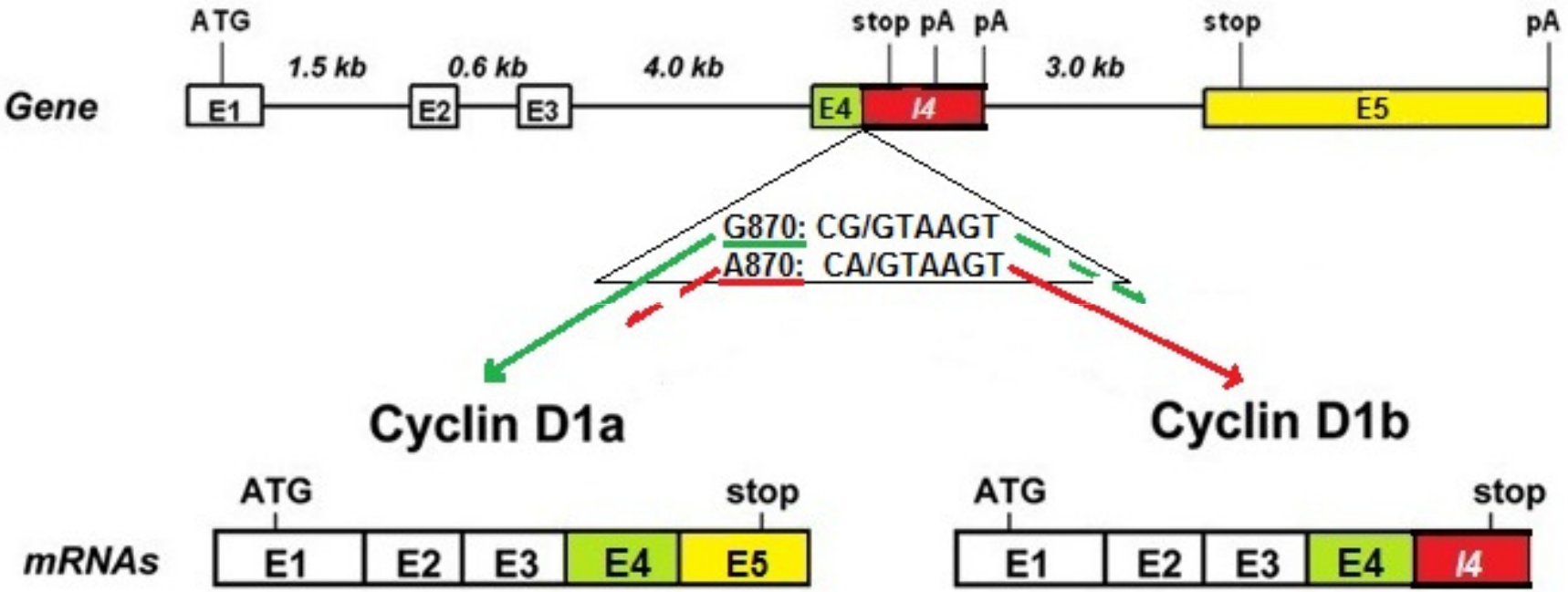
Binary logistic regression models – CTA (crude odds ratio)

		Categorized CTA					
		Low	High	P-value	cOR	95% C.I. For OR	P-value
XPD23	W/W	327	301	0.010	1.00	-	
	W/M	455	399		0.95	0.78 – 1.17	0.645
	M/M	185	112		0.66	0.50 – 0.87	0.004
BUB1B	W/W	69	92	0.031	1.00	-	
	W/M	76	72		0.71	0.45 – 1.11	0.135
	M/M	15	6		0.30	0.11 – 0.81	0.018
PTTG	W/W	278	111	0.032	1.00	-	
	W/M	184	86		1.17	0.84 – 1.64	0.361
	M/M	39	31		1.99	1.18 – 3.35	0.010
ZWINT	W/W	146	74	0.054	1.00	-	
	W/M	229	88		0.76	0.52 – 1.10	0.145
	M/M	98	27		0.54	0.33 – 0.91	0.019

Does cyclin D1 splice variants influence % of CAs?

- G870A gene polymorphism has been implicated as a risk factor for a number of cancers.
- We tested the relationship between structural CAs and CCND1 genotype by assaying for 751 healthy subjects.

Cyclin D1 isoforms



Cyclin D1 participates in DSB repair by binding to RAD51 (a main recombinase involved in HR). The induction of the DNA damage response is mediated by the cyclin D1a, whereas cyclin D1b lacks this activity.

Cyclin D1 (CCND1) G870A genotype, main confounders and frequency of CAs.

Variable	Persons	Significance	OR	95% C.I. for OR	
				Lower	Upper
Age (continuous)	730	.10	1.01	.99	1.03
Sex (M/F)	370/361	.08	1.33	.97	1.82
Smoking (S/NS)	250/481	.34	1.18	.84	1.64
Exposed/Unexposed	553/172	**.01**	**1.68**	**1.16**	**2.45**
CCND1_GG	200	-	1.00	-	-
CCND1_GA	379	.36	1.18	.83	1.69
CCND1_AA	151	**.01**	**1.85**	**1.17**	**2.93**

Hemminki et al. Leukemia 2013

Chromosomal aberrations and cancer risk –incident cancer patients

		CRC cases	LC cases	BC cases	General controls	Female controls
	All subjects	101	87	158	300	158
Diagnosis	Colon cancer	27	-	-	-	-
	Rectal + Anus cancer	74	-	-	-	-
	Bronchogenic cancer	-	27	-	-	-
	Pulmonary cancer	-	28	-	-	-
	Mediastinum cancer	-	2	-	-	-
	Ductal cancer	-	-	134	-	-
	Lobular cancer	-	-	16	-	-
	Ductolobuar cancer	-	-	4	-	-
Gender	Females	38	23	158	110	158
	Males	63	64	0	190	0
Age (years)		63.2 ±10.2	65.7 ± 10.3	60.0 ± 10.0	57.1 ± 13.6	62.9 ± 17.7
Smoking (in %)	Non-smokers	49.5%	20.7%	51.0%	62.3%	75.3%
	Exsmokers ≥ 5 years	15.8%	19.5%	15.0%	11.0%	6.3%
	Exsmokers < 5 years	5.0%	8.0%	3.9%	2.3%	1.9%
	Current smokers	29.7%	51.7%	30.1%	24.3%	16.5%
TNM stage	TNM 0, I, II	41	3	128	-	-
	TNM III, IV	53	31	30	-	-
Histopathological grading	G1, G2	60	1	99	-	-
	G3, G4	29	11	54	-	-
Receptors	Estrogen+/Progesterone+	-	-	108	-	-
	Estrogen+/Progesterone-	-	-	18	-	-
	Estrogen-/Progesterone+	-	-	7	-	-
	Estrogen-/Progesterone-	-	-	21	-	-

Numbers may not add up to 100% of available subjects because of missing data.

Chromosomal aberrations and cancer risk - Breast cancer

Female controls vs. BC cases

	Female controls (N=158)			BC cases (N=158)			
Number of evaluated cells	15800			15800			
Number of aberrant cells	288			414			
Chromosomal damage (%)	**Mean ± SD**	Median	Range	**Mean ± SD**	Median	Range	**P-value**
ACs	1.82 ± 1.38	2	0 - 6	2.62 ± 1.57	3	0 -7	**≤0.001**
CAs	1.93 ± 1.48	2	0 - 6	2.73 ± 1.64	3	0 - 7	**≤0.001**
CTA	1.11 ± 1.09	1	0 - 4	1.65 ± 1.35	1	0 - 6	**≤0.001**
CSA	0.80 ± 0.97	1	0 - 5	1.08 ± 1.02	1	0 - 4	**0.007**

The frequencies of chromosomal damage were tested with the non-parametric Mann-Whitney U-test.
The significance level is 0,05. Significant values are in bold.

Chromosomal aberrations and cancer risk - CRC

General controls vs. CRC cases

	General controls (N=300)			CRC cases (N=101)			
Number of evaluated cells	**30000**			**10100**			
Number of aberrant cells	**547**			**216**			
Chromosomal damage (%)	**Mean ± SD**	Median	Range	**Mean ± SD**	Median	Range	**P-value**
ACs	1.82 ± 1.32	2	0 - 6	2.14 ± 1.43	2	0 - 6	**0.057***
CAs	1.95 ± 1.47	2	0 - 7	2.27 ± 1.64	2	0 - 8	0.089
CTA	1.11 ± 0.99	1	0 - 4	1.45 ± 1.28	1	0 - 7	**0.031**
CSA	0.84 ± 1.13	0	0 - 6	0.82 ± 1.00	1	0 - 4	0.818

The frequencies of chromosomal damage were tested with the non-parametric Mann-Whitney U-test.
The significance level is 0,05. Significant values are in bold.
* on the borderline of significance.

Chromosomal aberrations and cancer risk – Lung cancer

General controls vs. LC cases

	General controls (N=300)			LC cases (N=87)			
Number of evaluated cells	**30000**			**8700**			
Number of aberrant cells	**547**			**249**			
Chromosomal damage (%)	**Mean ± SD**	Median	Range	**Mean ± SD**	Median	Range	**P-value**
ACs	1.82 ± 1.32	2	0 - 6	2.86 ± 1.45	3	0 - 6	**≤0.001**
CAs	1.95 ± 1.47	2	0 - 7	2.90 ± 1.49	3	0 - 7	**≤0.001**
CTA	1.11 ± 0.99	1	0 - 4	1.86 ± 1.30	2	0 - 6	**≤0.001**
CSA	0.84 ± 1.13	0	0 - 6	1.05 ± 0.98	1	0 - 4	**0.01**

The frequencies of chromosomal damage were tested with the non-parametric Mann-Whitney U-test.
The significance level is 0,05. Significant values are in bold.

Chromosomal aberrations and cancer risk – comparison between malignancies

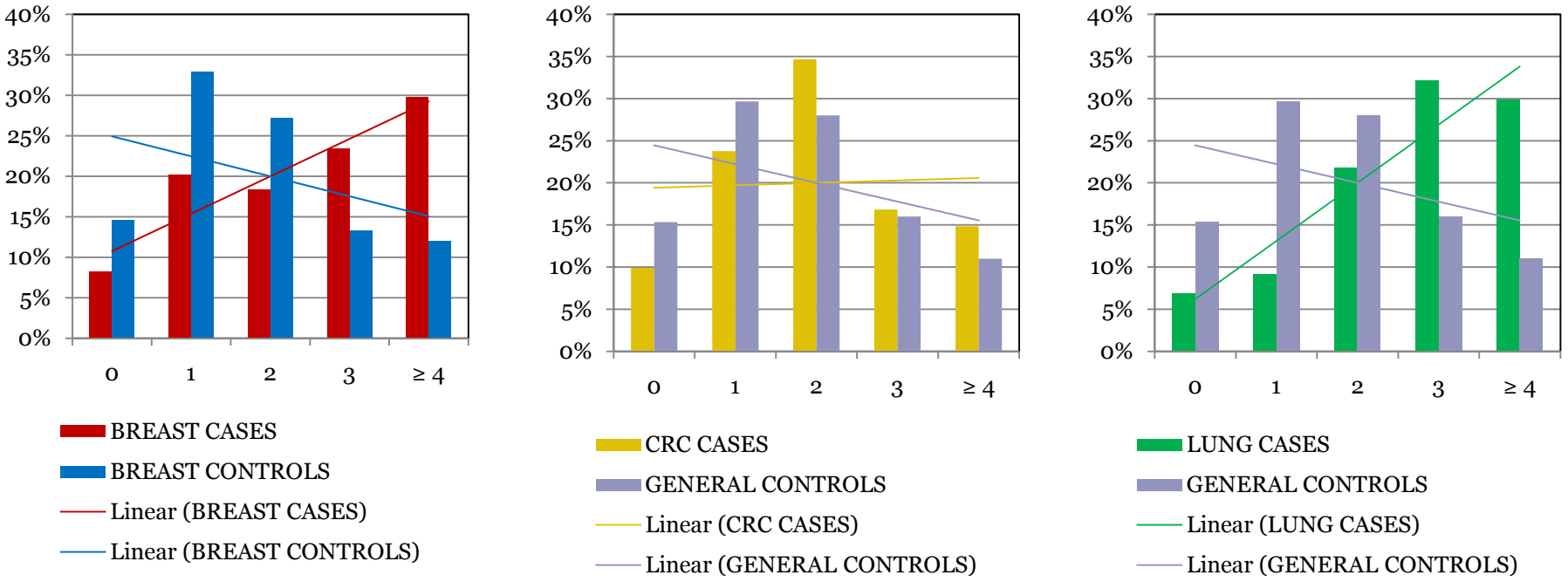
CRC cases vs. LC cases vs. BC cases

	CRC cases (N=101)			LC cases (N=87)			BC cases (N=158)			
Number of evaluated cells	10100			8700			15800			
Number of aberrant cells	216			249			414			
Chromosomal damage (%)	**Mean ± SD**	Median	Range	**Mean ± SD**	Median	Range	**Mean ± SD**	Median	Range	**P-value**
ACs	2.14 ± 1.43	2	0 - 6	2.86 ± 1.45	3	0 - 6	2.62 ± 1.57	3	0 -7	**0.001**
CAs	2.27 ± 1.64	2	0 - 8	2.90 ± 1.49	3	0 - 7	2.73 ± 1.64	3	0 - 7	**0.002**
CTA	1.45 ± 1.28	1	0 - 7	1.86 ± 1.30	2	0 - 6	1.65 ± 1.35	1	0 - 6	**0.04**
CSA	0.82 ± 1.00	1	0 - 4	1.05 ± 0.98	1	0 - 4	1.08 ± 1.02	1	0 - 4	**0.049**

Frequencies of chromosomal damage were tested with the non-parametric Kruskal-Wallis test.
The significance level is 0,05. Significant values are in bold.

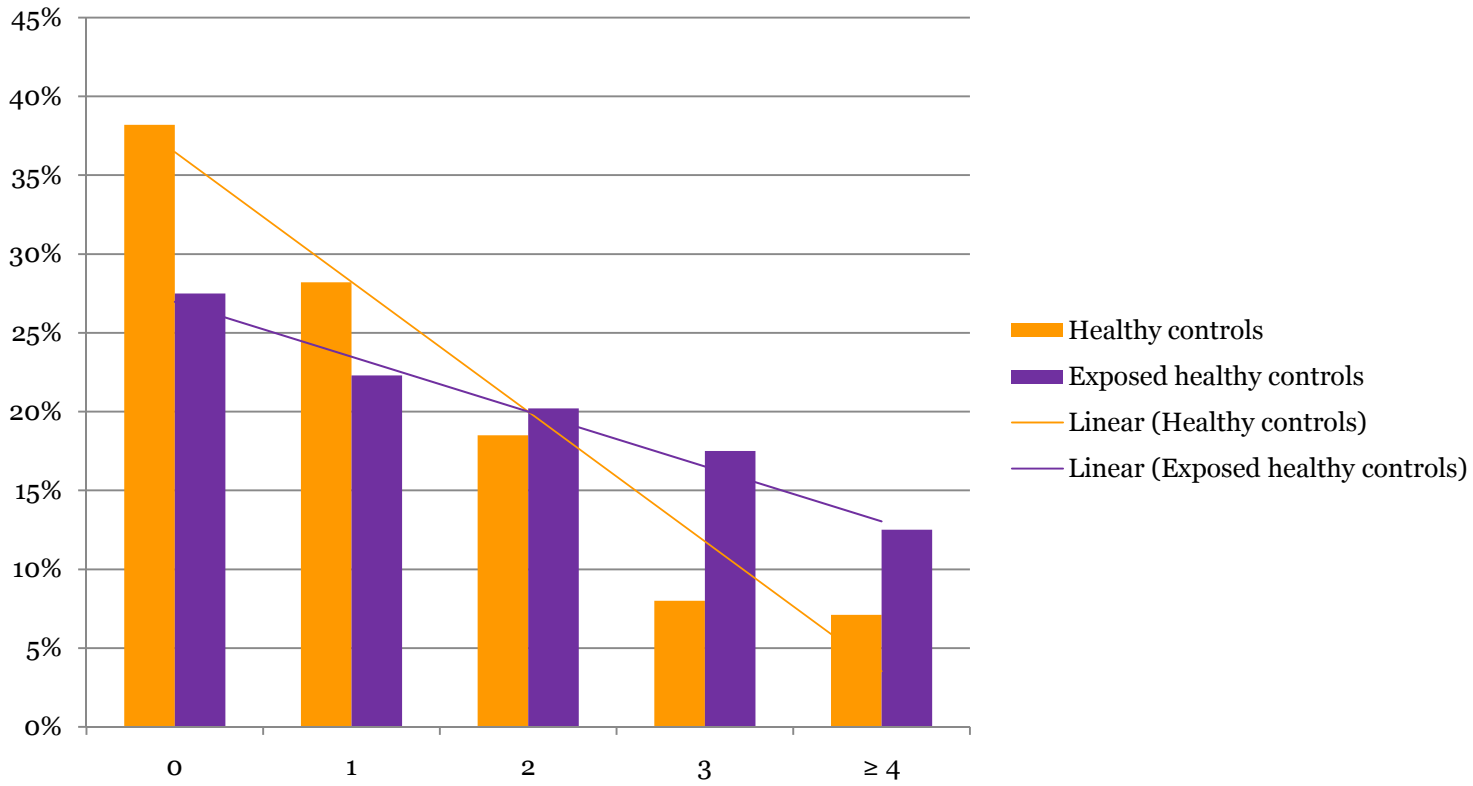
Chromosomal aberrations and cancer risk - distributions

Distribution of frequencies of ACs



CHA: control and exposed population - distributions

Distribution of frequencies of CAs (N = 1780)



Chromosomal aberrations and breast cancer risk - confounders

Binary logistic regression models to discern the modulation of incident cancer by chromosomal damage end points and major confounders (such as age, sex and smoking)

BC cases (N=158) compared with controls (N=158)

BC cases vs. Controls	aOR	CI	P-value
ACs (%)	1.41	1.19 – 1.66	**0.000**
Age (years)	0.99	0.97 – 1.01	0.189
Smoking (0 for non-smokers)	0.39	0.24 – 0.64	**0.000**
CAs (%)	1.36	1.16 – 1.60	**0.000**
Age (years)	0.99	0.97 – 1.01	0.153
Smoking (0 for non-smokers)	0.39	0.24 – 0.65	**0.000**
CTAs (%)	1.33	1.09 – 1.62	**0.005**
Age (years)	0.99	0.98 – 1.01	0.407
Smoking (0 for non-smokers)	0.39	0.24 – 0.64	**0.000**
CSAs (%)	1.45	1.13 – 1.86	**0.004**
Age (years)	0.99	0.97 – 1.00	0.141
Smoking (0 for non-smokers)	0.33	0.20 – 0.54	**0.000**

Chromosomal aberrations and CRC risk - confounders

Binary logistic regression models to discern the modulation of incident cancer by chromosomal damage end points and major confounders (such as age, sex and smoking)

CRC cases (N=101) compared with controls (N=300)

CRC cases vs. Controls	aOR	CI	P-value
ACs (%)	1.11	0.93 – 1.31	0.256
Age (years)	1.04	1.02 – 1.06	**0.000**
Smoking (0 for non-smokers)	0.55	0.34 – 0.89	**0.015**
CAs (%)	1.07	0.92 – 1.25	0.369
Age (years)	1.04	1.02 – 1.06	**0.000**
Smoking (0 for non-smokers)	0.54	0.33 – 0.88	**0.013**
CTAs (%)	1.25	1.01 – 1.55	**0.037**
Age (years)	1.04	1.02 – 1.06	**0.000**
Smoking (0 for non-smokers)	0.57	0.35 – 0.92	**0.021**
CSAs (%)	0.91	0.73 – 1.13	0.402
Age (years)	1.04	1.02 – 1.06	**0.000**
Smoking (0 for non-smokers)	0.50	0.31 – 0.81	**0.005**

Chromosomal aberrations and lung cancer risk - confounders

Binary logistic regression models to discern the modulation of incident cancer by chromosomal damage end points and major confounders (such as age, sex and smoking)

LC cases (N=87) compared with controls (N=300)

LC cases vs. Controls	aOR	CI	P-value
ACs (%)	1.48	1.21 – 1.81	**0.000**
Age (years)	1.07	1.05 – 1.10	**0.000**
Smoking (0 for non-smokers)	0.11	0.05 – 0.22	**0.000**
CAs (%)	1.33	1.11 – 1.59	**0.002**
Age (years)	1.07	1.05 – 1.10	**0.000**
Smoking (0 for non-smokers)	0.10	0.05 – 0.21	**0.000**
CTAs (%)	1.70	1.33 – 2.18	**0.000**
Age (years)	1.08	1.05 – 1.10	**0.000**
Smoking (0 for non-smokers)	0.10	0.05 – 0.20	**0.000**
CSAs (%)	0.97	0.76 – 1.24	0.815
Age (years)	1.07	1.05 – 1.10	**0.000**
Smoking (0 for non-smokers)	0.09	0.05 – 0.18	**0.000**

Clinical characteristics of Breast cancer

Breast cancer		Estrogen receptor	Progesterone receptor
Categories	Test	P-value	P-value
The distribution of **ACs** percentage in relation to the receptor	Kuskal-Wallis test	0.59	0.23
The distribution of **CAs** percentage in relation to the receptor	Kuskal-Wallis test	0.63	0.22
The distribution of **CTA** percentage in relation to the receptor	Kuskal-Wallis test	0.78	0.32
The distribution of **CSA** percentagein relation to the receptor	Kuskal-Wallis test	0.79	0.99
the significance level at 0.05			

TNM and Grading characteristics in solid tumors

TNM I +II vs. TMN III + IV		CRC	Lung cancer	Breast cancer
	Test	P-value	P-value	P-value
Percentage of ACs in relation to the TNM categories	Mann-Whitney U test	0.649	0.172	0.773
Percentage of CAs in relation to the TNM categories	Mann-Whitney U test	0.689	0.214	0.966
Percentage of CTAin relation to the TNM categories	Mann-Whitney U test	0.759	0.348	0.817
Percentage of CSA in relation to the TNM categoriesis	Mann-Whitney U test	0.428	0.645	0.444
The significance level at 0.05				

Grading G+G2 vs. G3+G4		CRC	Lung cancer	Breast cancer
Null Hypothesis	Test	P-value	P-value	P-value
Percentage of ACs in relation to the categories of grading	Mann-Whitney U test	0.701	0.500	0.219
Percentage of CAs in relation to the categories of grading	Mann-Whitney U test	0.684	0.500	0.249
Percentage of CTA in relation to the categories of grading	Mann-Whitney U test	0.978	0.833	0.174
Percentage of CSA in relation to the categories of grading	Mann-Whitney U test	0.940	0.333	0.426
The significance level at 0.05				

CRC-the effect of the localization on CAs

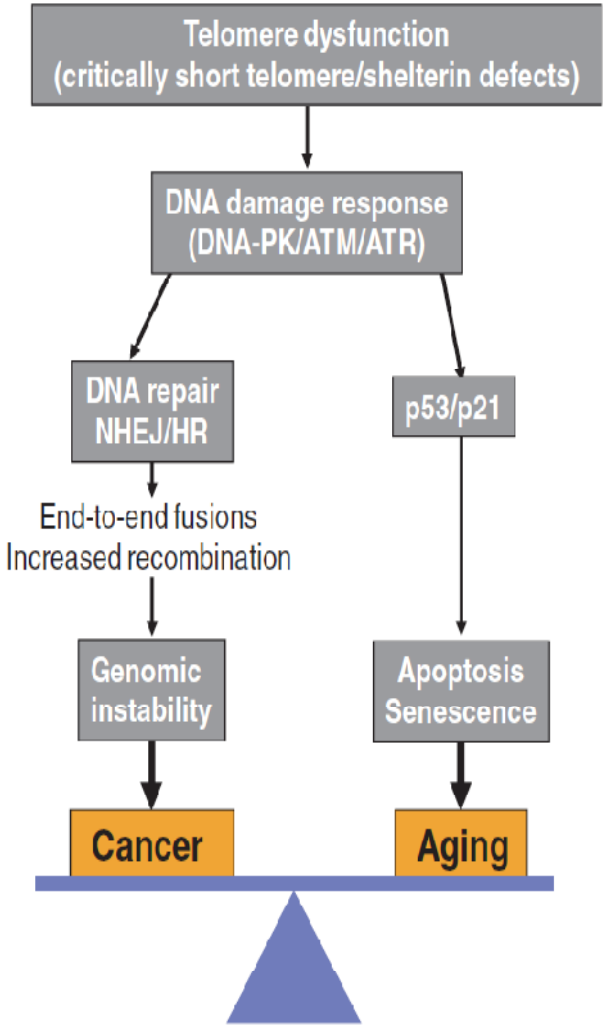
CRC laterality		
	Test	P-value
Percentage of **ACs** in relation to the CRC localization	Kuskal-Wallis test	0.426
Percentage of **CAs** in relation to the CRC localization	Kuskal-Wallis test	0.342
Percentage of **CTA** in relation to the CRC localization	Kuskal-Wallis test	0.815
Percentage of **CSA** in relation to the CRC localization	Kuskal-Wallis test	0.669

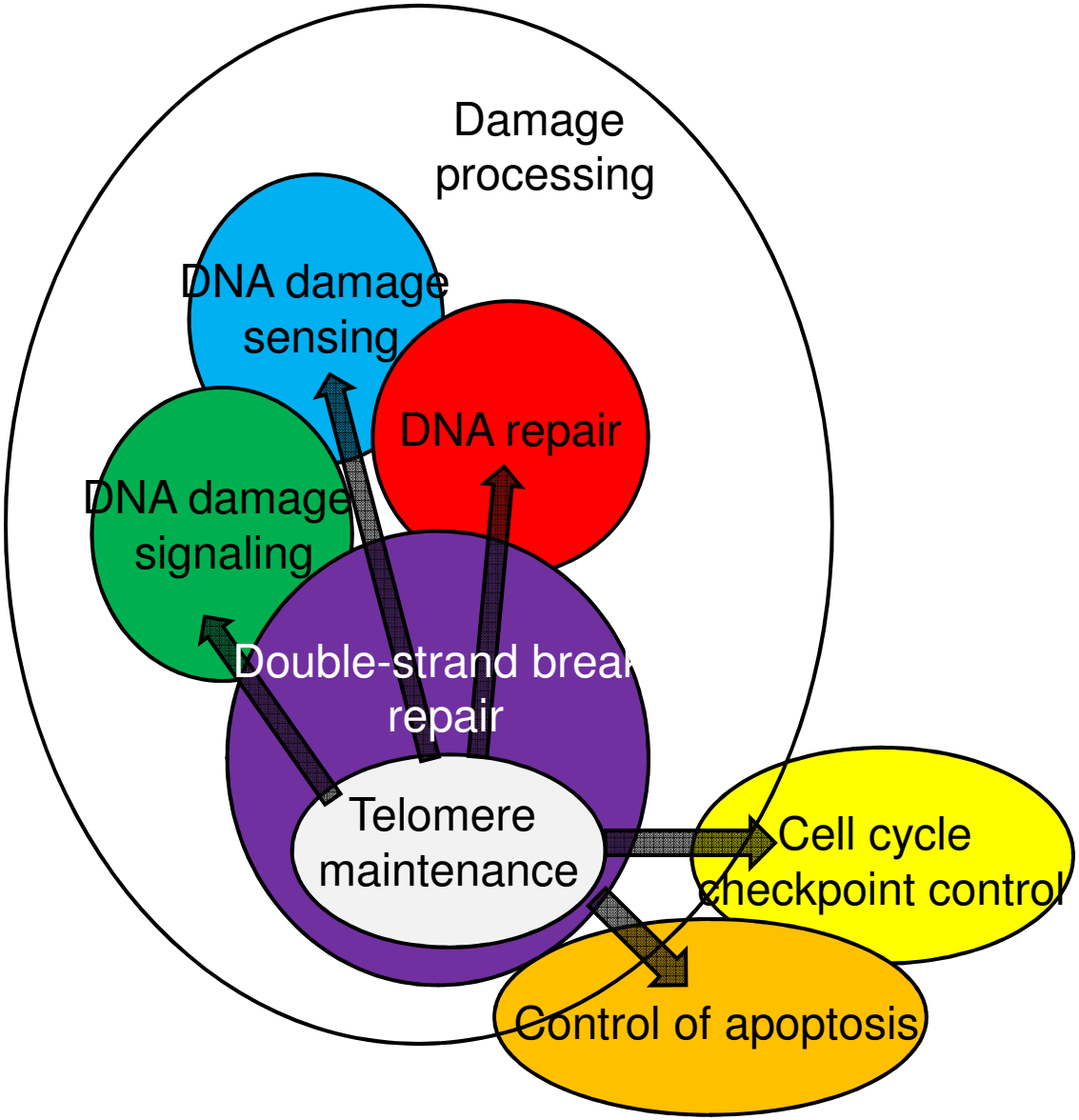
The significance level at 0.05 Note: prevalent rectal cancer

Chromosomal aberrations-Cox regression analysis

BRCA (EFS)		
	HR, 95%CI	**P**
ACs	0.93 (0.08-10.31)	0.960
CAs	0.86 (0.08-9.46)	0.899
CTAs	1.10 (0.15-8.59)	0.857
CSAs	1.18 (0.11-13.00)	0.890
Lung cancer (OS)		
ACs	1.64 (0.74-3.76)	0.222
CAs	1.66 (0.77-3.78)	0.220
CTAs	1.40 (0.77-2.53)	0.270
CSAs	1.35 (0.67-2.71)	0.390
CRC (OS)		
ACs	0.94 (0.31-2.82)	0.911
Cas	0.81 (0.27-2.41)	0.710
CTAs	*0.26 (0.06-1.16)*	*0.080*
CSAs	1.06 (0.30-3.81)	0.929

Telomere dysfunction/maintenance in DNA damage response mechanisms





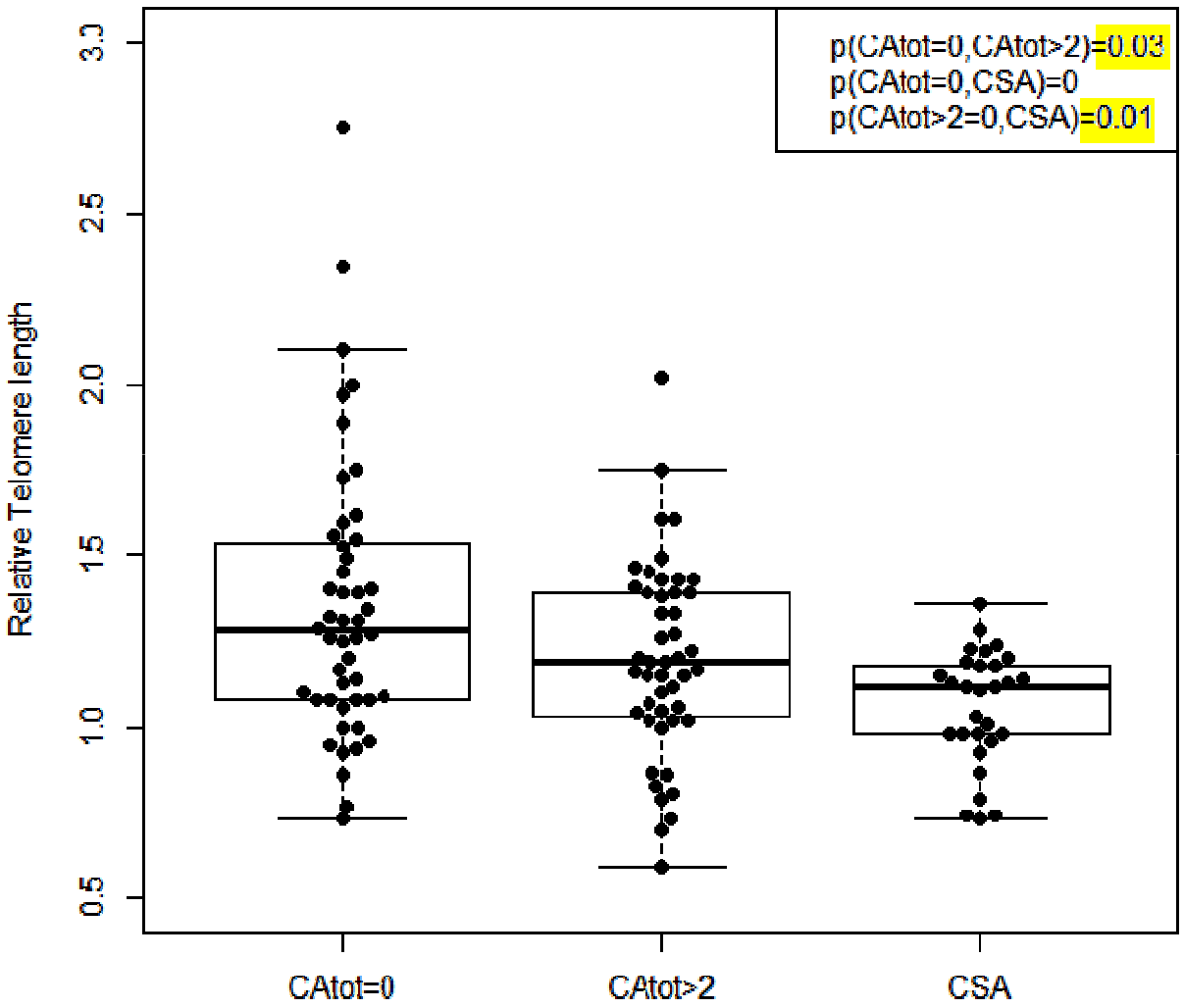
Martínez and Blasco, *Aging Cell.* 2010 Oct;9(5):653-66.

Telomeres and cancer

- Human telomeres: tandem repeats TTAGGG span 10-15 kb on average
- 150-200 nucleotide 3´-overhang of the G-rich strand
- Telomere DNA sequence bound to six-protein complex ***SHELTERIN***
- Abnormally shortened or prolonged telomeres are hallmark of cancer
- Telomere length assayed for in a) PBL DNA from healthy subjects with various levels of chromosomal damage
- b) In tumor tissue and adjacent mucosa of sporadic CRC patients

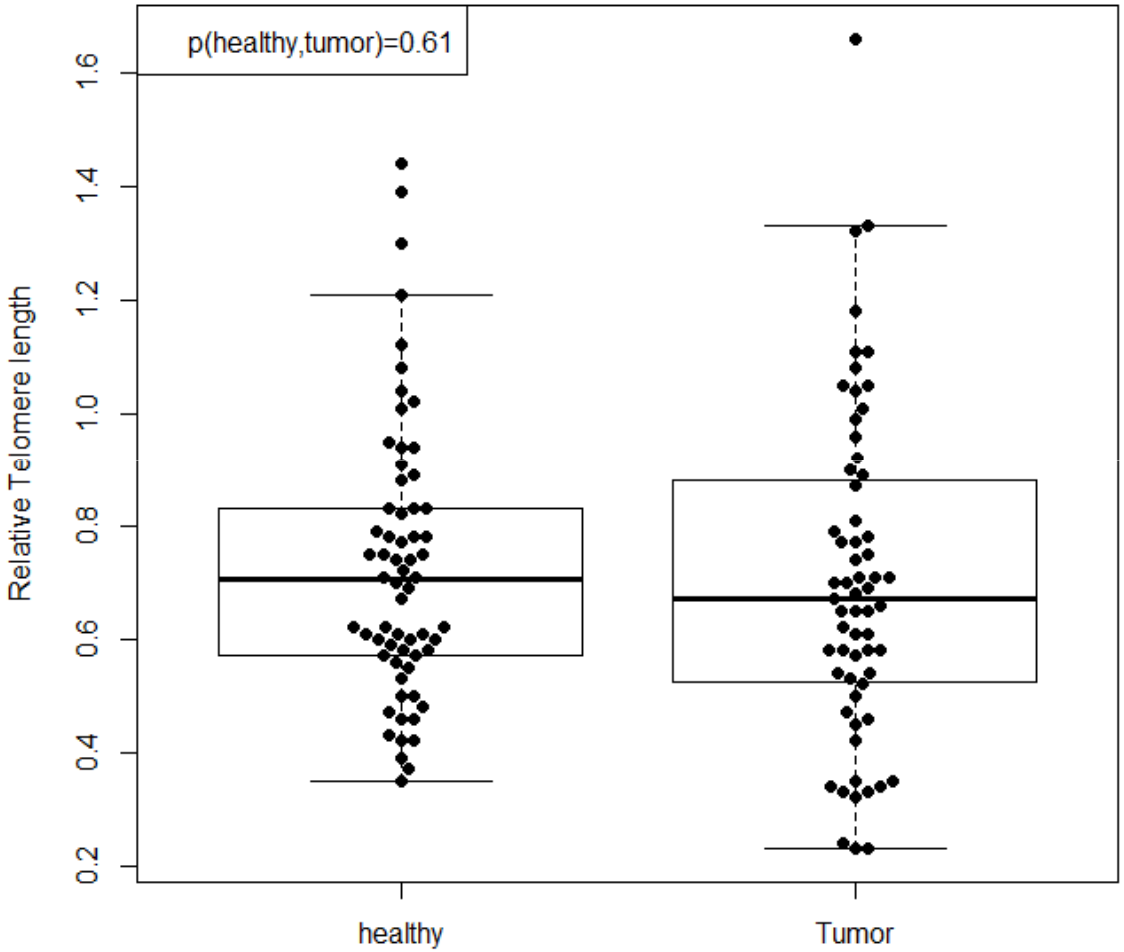
Heidenreich et al. Curr Opin Genet Develop 2014

Healthy subjects



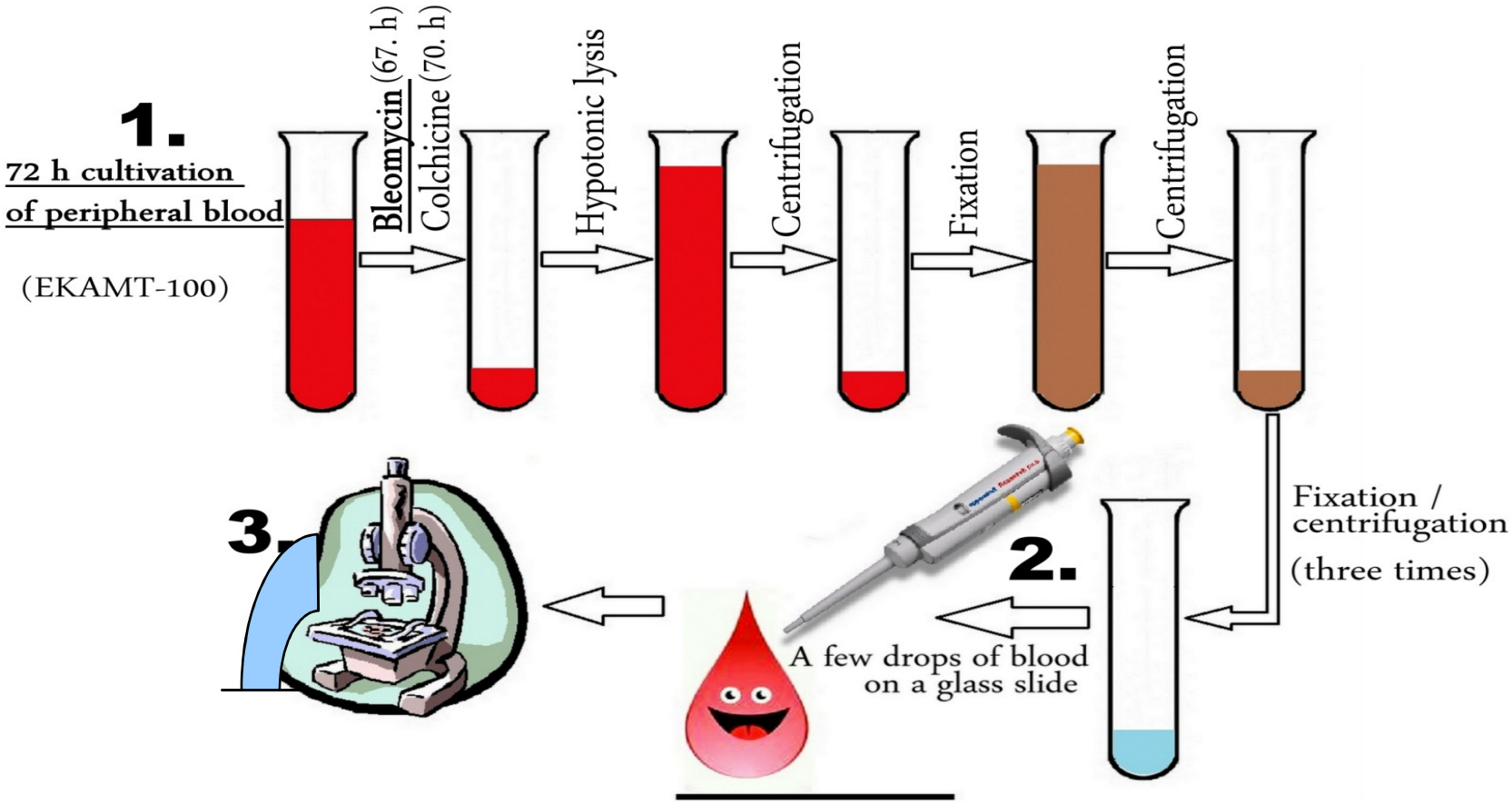
Type	number
CAtot	48
CATot>2	47
CSA	29

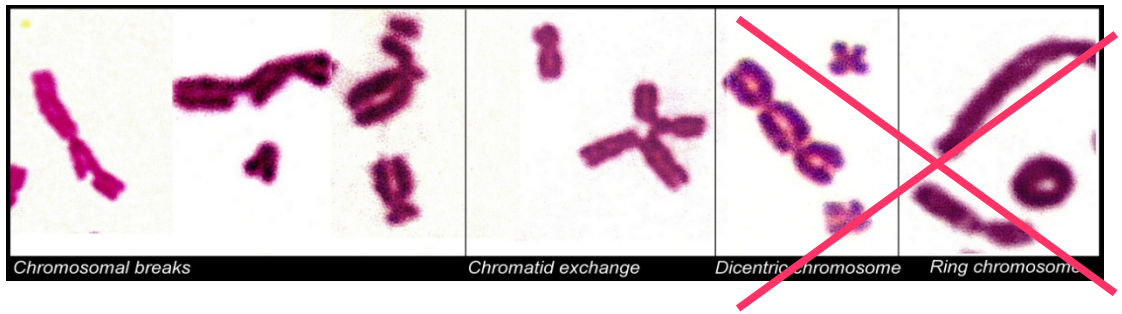
Colorectal tumors: Telomere analysis



Tissue	Number
healthy	64
Tumor	63

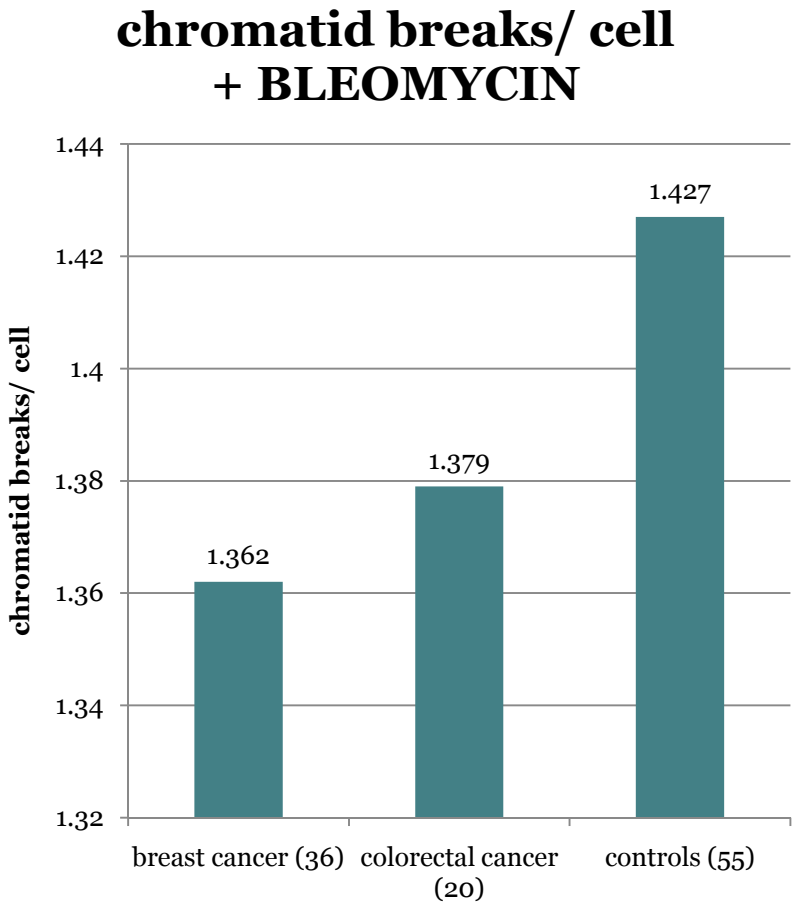
BLEOMYCIN CHALLENG ASSAY-DSB REPAIR

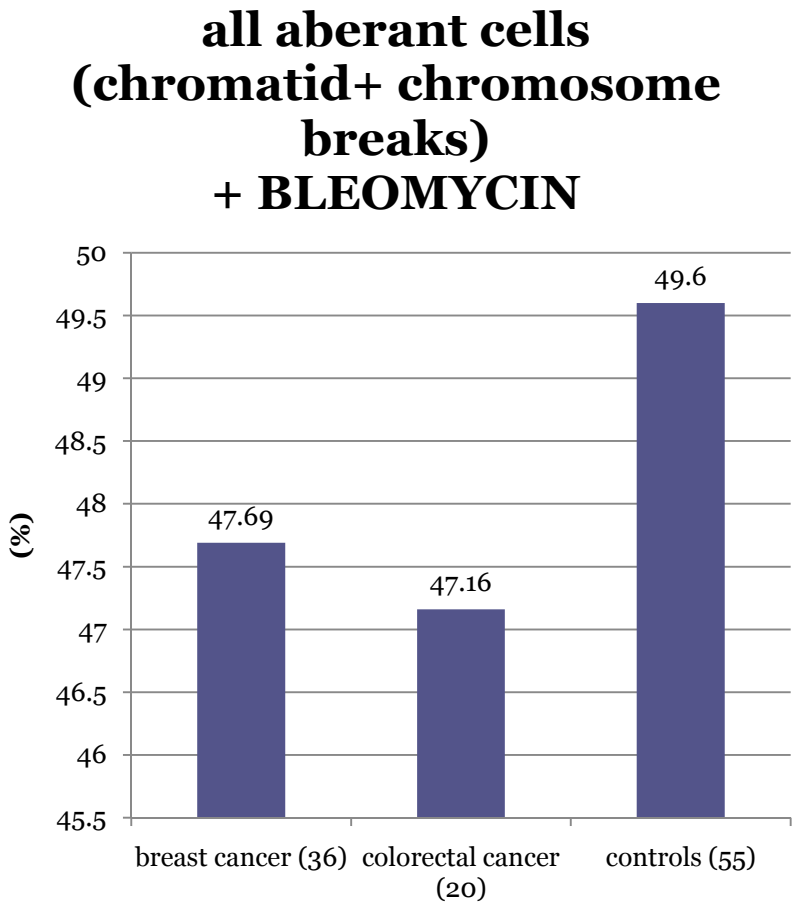




breaks/cell	classification
> 1	hypersensitivity
0.81-1.00	borderline sensitivity
< 0.81	low sensitivity

Bleomycin functional test

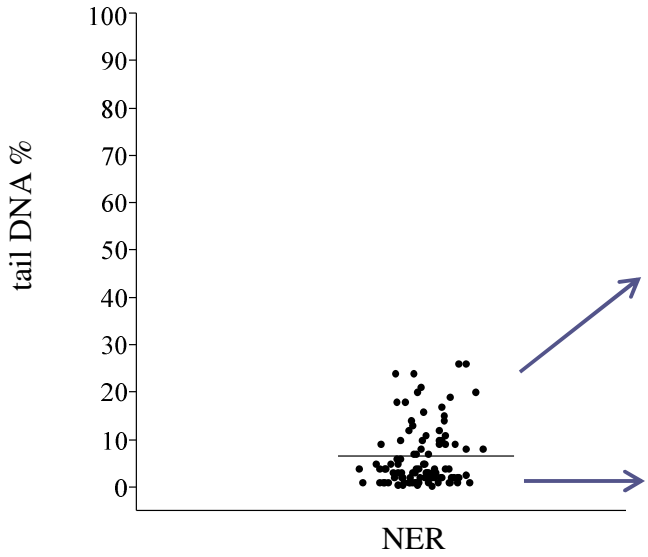




Questions to be addressed

Mechanism?

Cancer → temporary suppression of DNA repair WHERE? Target, surrogate? Interactions with other biological pathways?



Higher repair = Poor responders
Worse prognosis
Milder side effects

?

Lower repair = Good responders
Better prognosis
Advanced side effects

Preliminary conclusions

- The data suggest moderate, but significant, increase in structural chromosomal aberrations in cancer patients
- Chromosomal breaks, rearrangements and exchanges are associated with cancer risk
- Structural chromosomal aberrations, however, lack a correlation with the stage, grade and histology of the investigated solid tumour
- Association of structural chromosomal aberrations with PFS, OS and efficacy of the therapy
- The role of BUB and MAD genes in aneuploidy, cancer pathogenesis and therapy is very likely, so is the telomere length
- The relationship between DNA damage, DNA repair characteristics and CIN in tumorigenesis warrants further attention.

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Thank you for your attention!