MELODI & ICRP SESSION: Effects, Risks, and Detriment at Low Dose and Low Dose-Rate

Human Radiosensitivity and Prospects for Prediction

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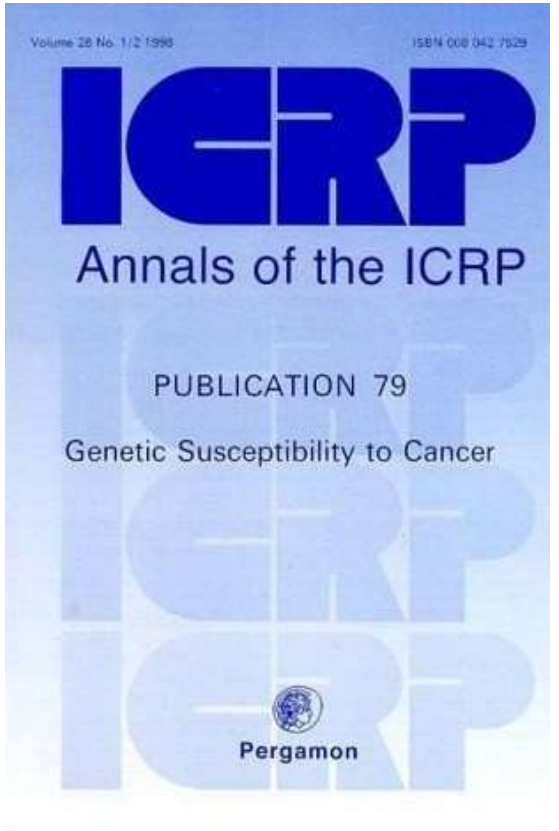
Members of the C1 WG on individual radiosensitivity:

Preetha Rajaraman
Michael Hauptman
Simon Bouffler (MC)

Major reports on human radiosensitivity and cancer susceptibility

2013

1999



Many definitions for the term „radiosensitivity“

- **Whole organism radiosensitivity**
  refers to radiation-related mortality due to deterministic effects

- **Normal tissue radiosensitivity or clinical radiosensitivity**
  refers to adverse reactions in non-target tissues as consequence of radiotherapy (deterministic effects)

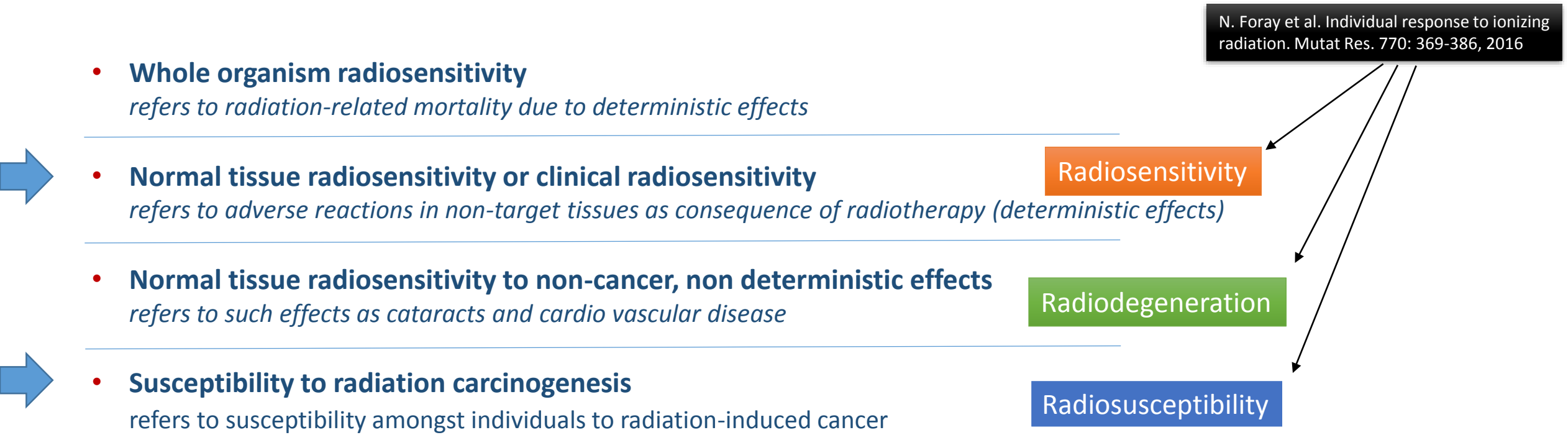
- **Normal tissue radiosensitivity to non-cancer, non deterministic effects**
  refers to such effects as cataracts and cardio vascular disease

- **Susceptibility to radiation carcinogenesis**
  refers to susceptibility amongst individuals to radiation-induced cancer

- **Tissue radiosensitivity for cancer**
  refers to in sensitivity of individual tissues to radiation-induced cancer

- **Cellular radiosensitivity**
  refers to endpoints measured at the cellular level such a DNA damage

N. Foray et al. Individual response to ionizing radiation. Mutat Res. 770: 369-386, 2016



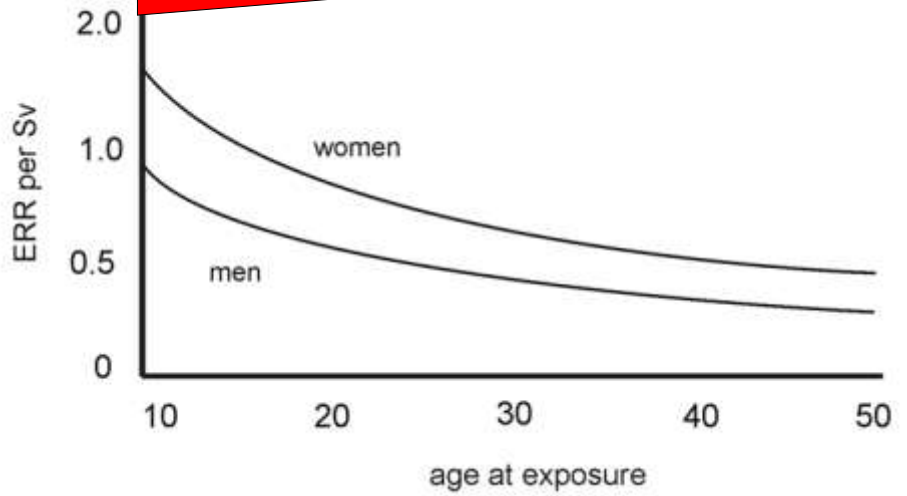
The importance of defining the endpoint when talking about individual radiosensitivity

Children are radiosensitive
with respect to stochastic effects

D.L. Preston et al. Radiat. Res. 168: 1- 64, 2007

ERR for cancer as a function of age and sex in the LSS chort

Radiosensitivity decreases with age



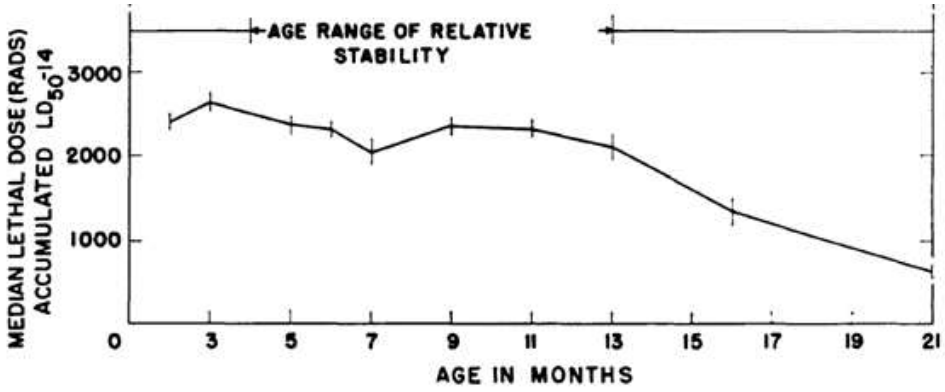
Biological explanation
- **age effect: long life expectancy, many cell divisions**
- **sex effect: mainly breast cancer**

Children are radioresistant
with respect to deterministic effects

J. Spalding and T.T. Trujillo Radiat. Res. 16:125-129, 1962

Median lethal dose as a function of age in mice

Radiosensitivity increases with age



Biological explanation
- **Decreasing regenerative capacity of tissues with age**

It is often assumed that the individual radiosensitivity and radiosusceptibility is genetically determined and is an „intrinsic“ trait

This is based on the established high radiosensitivity and/or radiosusceptibility of rare diseases associated with impaired DNA repair capacity

Source: N. Foray et al. Mutation Research 770:369–386, 2016.

Table 1
The major human syndromes associated with radiosensitivity and/or radiosusceptibility[a].

Syndromes	Mutated Genes	Major defective mechanism	Cancer predisposition	Clinical sensitivity to IR[b]
Ataxia telangiectasia	ATM homozygous mutations	DSB signaling and repair	Leukemia, Lymphoma	+++
Ligase IV	Lig IV homozygous mutations	NHEJ	Leukemia, Lymphoma	+++
Nijmegen	NBS1 homozygous mutations	DSB signaling and repair	Leukemia, Lymphoma	+++
Hutchinson-Gilford (progeria infantum)	Lamin A homozygous mutations	Nuclear membrane	No	+++
Bruton's disease (agammaglobulinemia)	BTK homozygous mutations	V(D)J recombination	No	+++
Hypo-gammaglobulinemia	Lig I	NER	No	+++
Glutathione synthetase deficiency	GSS	Glutathione cycle	No	+++
ICF syndrome	DNMT3B	DNA methylation DSB signallng and repair	No	+++
Huntington's disease	IT15	DNA methylation DSB signaling and repair	No	++
Neurofibromatosis type I (Von Recklinghausen)	NF1	DSB signaling and repair	Central and peripheral nervous system tumors	++
Tuberous sclerosis	TSC genes	DSB signaling and repair	Central and peripheral nervous system tumors	++

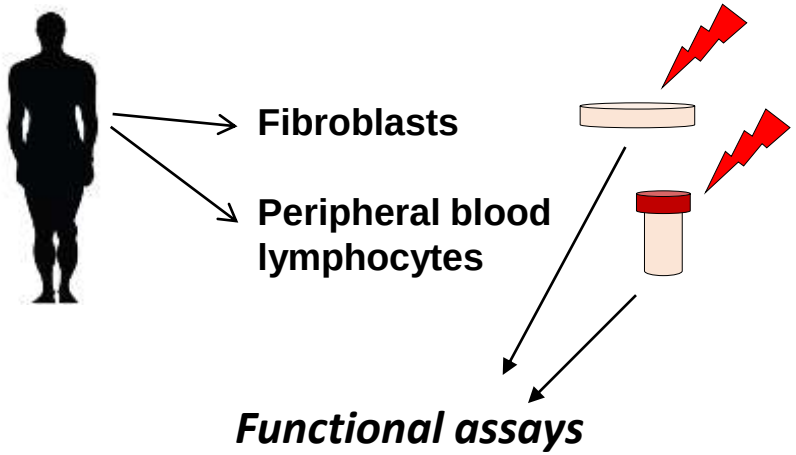
radiosusceptibility

Correlation?

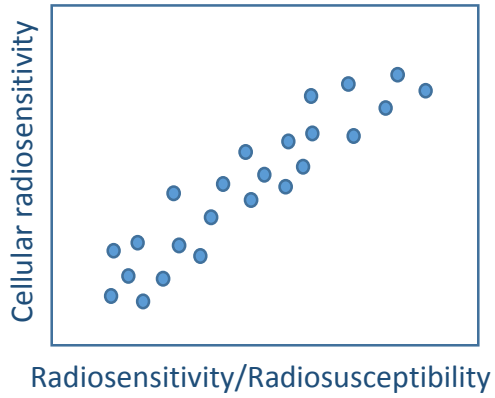
radiosensitivity

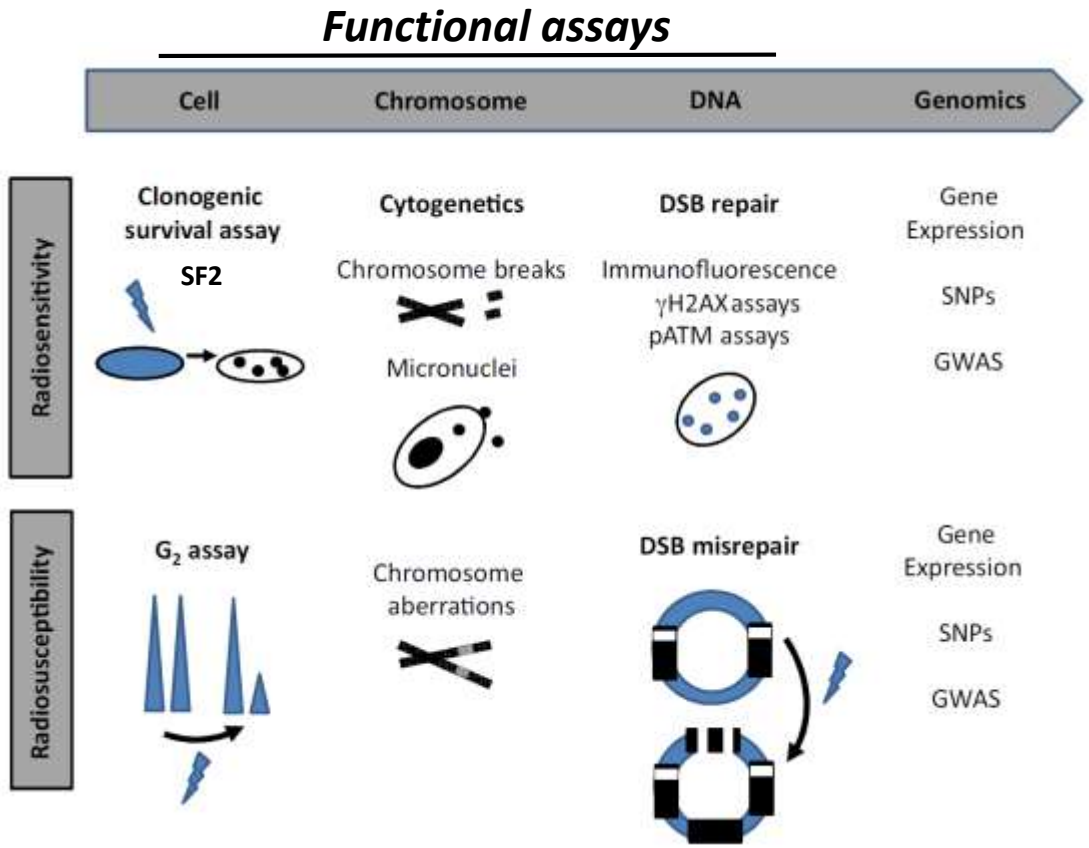
*All together, **ca 15** disorders are known showing increased cellular radiosensitivity They are generally the result of **low frequency, high penetrance** mutations that are **not often** seen in the general population*

If the individual radiosensitivity and radiosusceptibility is an „intrinsic“ trait then the radiosensitivity of cells isolated from an individual should correlate with his/her radiosensitivity and radiosusceptibility



The ideal outcome...

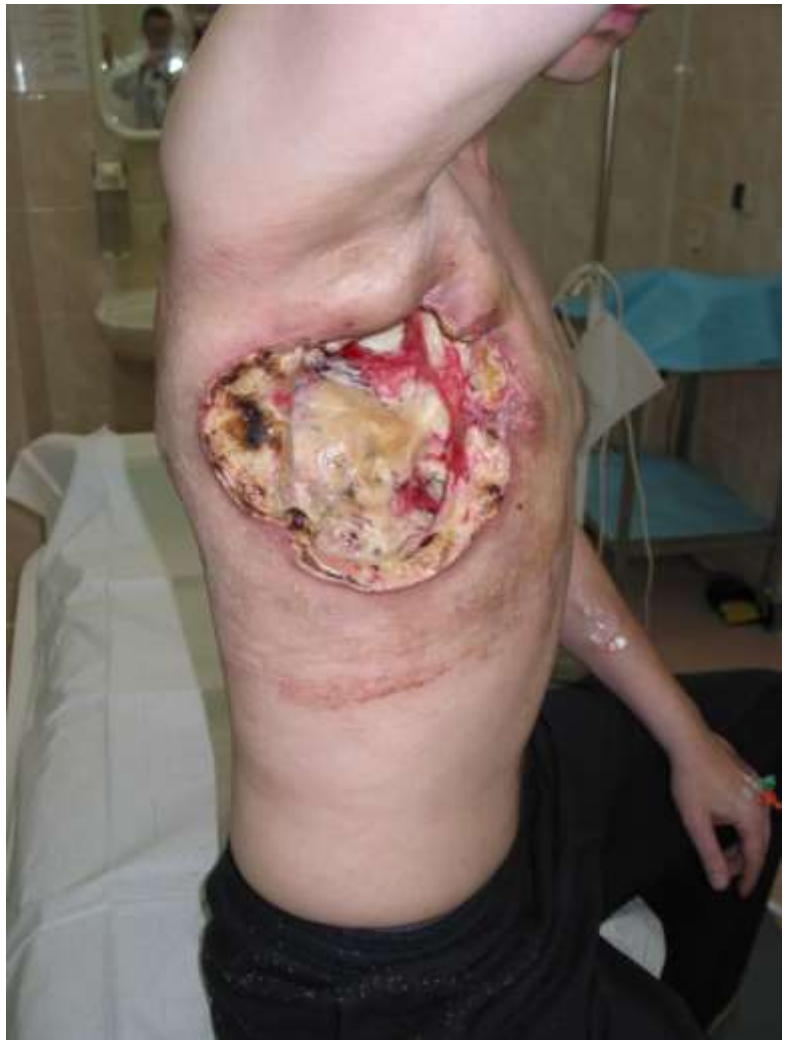




Source: N. Foray et al. Individual response to ionizing radiation. Mutat Res. 770: 369-386, 2016

Biomarkers of individual radiosensitivity

The horror scenario for a radiation oncologist: skin necrosis – severe late side effect to radiotherapy



1999: RT for Hodgkin's disease

picture taken in 2005

The total dose was 32 Gy in 20 fractions of 1.6 Gy given 5 days a week with 9 MeV photons.

Patient did not show an in vitro radiosensitive phenotype (chromosomal aberrations)

Small-scale studies using functional assay yield controversial results

Examples: Residual DNA damage (repair foci) and clinical radiosensitivity

O Nuta et al. *Correlation between the radiation responses of fibroblasts cultured from individual patients and the risk of late reaction after breast radiotherapy.* Cancer Lett. 374:324-330, 2016.	**Residual 53BP1 foci counts 24 h after in vitro irradiation were significantly higher in fibroblasts from RT-sensitive versus RT-resistant patients**
P. Lobachevsky et al. *Compromized DNA repair as a basis for identification of cancer radiotherapy patients with extreme radiosensitivity.* Cancer Lett. 383:212-219, 2016.	**The most powerful predictor of extreme toxicity was a combination of the fraction of the unrepairable component of γ-H2AX foci and repair rate in PBL**
M. Chua et al. *DNA double-strand break repair and induction of apoptosis in ex vivo irradiated blood lymphocytes in relation to late normal tissue reactions following breast radiotherapy*. Radiat Environ Biophys. 53:355-364, 2014.	**No association was observed between apoptosis and residual focus levels in breast cancer patient groups with various late toxicities**
K. Brzozowska et al. *In vivo versus in vitro individual radiosensitivity analysed in healthy donors and in prostate cancer patients with and without severe side effects after radiotherapy.* Int J Radiat Biol. 88: 405–413, 2012.	**There is no obvious correlation between clinical and cellular radiosensitivity in lymphocytes of prostate cancer patients**

Today, GWAS appears to be the best way forward

- Complex diseases or traits are often associated with a specific pattern of SNP variants. Available GWAS results suggest that the same may be true for radiosensitivity. A SNP fingerprint will be specific for each type of late toxicity.
- Currently, several large studies are in progress whose main goal is to discover new SNPs and validate previously identified genetic biomarkers of radiosensitivity.

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journal homepage: www.ebiomedicine.com

Research Paper

Meta-analysis of Genome Wide Association Studies Identifies Genetic Markers of Late Toxicity Following Radiotherapy for Prostate Cancer

Sarah L. Kerns [a,b,1], Leila Dorling [c,1], Laura Fachal [d,e,1], Søren Bentzen [f,g], Paul D
Antonio Gómez-Caamaño [h], Ana M. Carballo [h], David P. Dearnaley [i], Paula Pele
Emma Hall [j], Kyriaki Michailidou [c], Ángel Carracedo [e,k], Michael Sia [l], Richard
Matthew R. Sydes [m], Jonathan P. Tyrer [c], Shahana Ahmed [d], Matthew Parliam
Barry S. Rosenstein [b,q,r,2], Ana Vega [e,l,2], Neil G. Burnet [s,2], Alison M. Dunning [d,
Catharine M.L. West [u,*,2], on behalf of the, Radiogenomics Consortium:

NATURE GENETICS VOLUME 46 | NUMBER 8 | AUGUST 2014

A three-stage genome-wide association study identifies a susceptibility locus for late radiotherapy toxicity at 2q24.1

Laura Fachal[1,2], Antonio Gómez-Caamaño[3], Gillian C Barnett[4], Paula Peleteiro[3], Ana M Carballo[3], Patricia Calvo-Crespo[3], Sarah L Kerns[5], Manuel Sánchez-García[6], Ramón Lobato-Busto[6], Leila Dorling[4], Rebecca M Elliott[7], David P Dearnaley[8], Matthew R Sydes[9], Emma Hall[10], Neil G Burnet[11], Ángel Carracedo[1,2,12], Barry S Rosenstein[5], Catharine M L West[7], Alison M Dunning[4] & Ana Vega[1,2]

Seminars in RADIATION ONCOLOGY

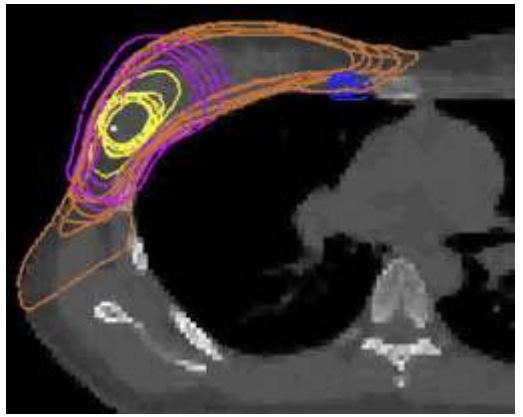
Radiogenomics: Identification of Genomic Predictors for Radiation Toxicity

Barry S. Rosenstein, PhD*,†

Semin Radiat Oncol 27:300-309 © 2017

BUT: there are major confounding factors in identifying markers of radiosensitivity

Treatment planning – Significant differences between hospitals/RT professionals in contouring of PTV and organs at risk.

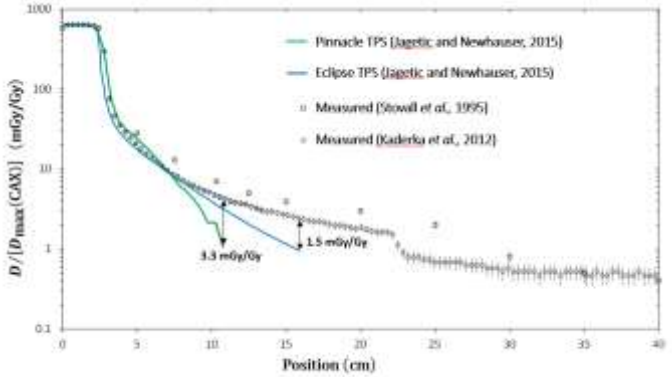


Contours of the internal mammary nodes, the lumpectomy cavity, boost PTV, and the breast volume in an axial plane.

Source: Li et al. Variability of target and normal structure delineation for breast cancer radiotherapy: an RTOG Multi-Institutional and Multiobserver Study. Int. J. Radiation Oncology Biol. Phys., 73: 944–951, 2009.

Dosimetry —detailed treatment and dosimetric data is essential (DVH) but often lacking. Moreover, some TPS poorly estimate doses to tissues distal to PTV.

Remember: we may be looking at side effects to a RT carried out many years ago



Source: W Newhauser. Physical Aspects of Radiation Therapy Exposures of Relevance to Second Cancers. Workshop on SMN, Stockholm 2016

Measures and scales used to assess adverse effects – different measures and scales are used accross hospitals.

Outcomes – multiple measures of toxicity for the same outcome are used.

Barnett GC, West CM, Coles CE: Standardized Total Average Toxicity score: A scale- and grade-independent measure of late radiotherapy toxicity to facilitate pooling of data from different studies. Int J Radiat Oncol Biol Phys 82:1065-1074, 2012

REQUITE

Biomarkers of individual radiosusceptibility

- A factor which contributes to intrinsic cancer susceptibility is the **genetic background** which is associated with **genomic instability** leading to an increased level of mutations and to sensitivity to environmental factors.

- Genomic instability can be identified as increased spontaneous or radiation-induced frequency of chromosomal aberrations. The latter is called the **Mutagen Sensitivity Assay**. Radiation can be substituted by bleomycin (BLM).

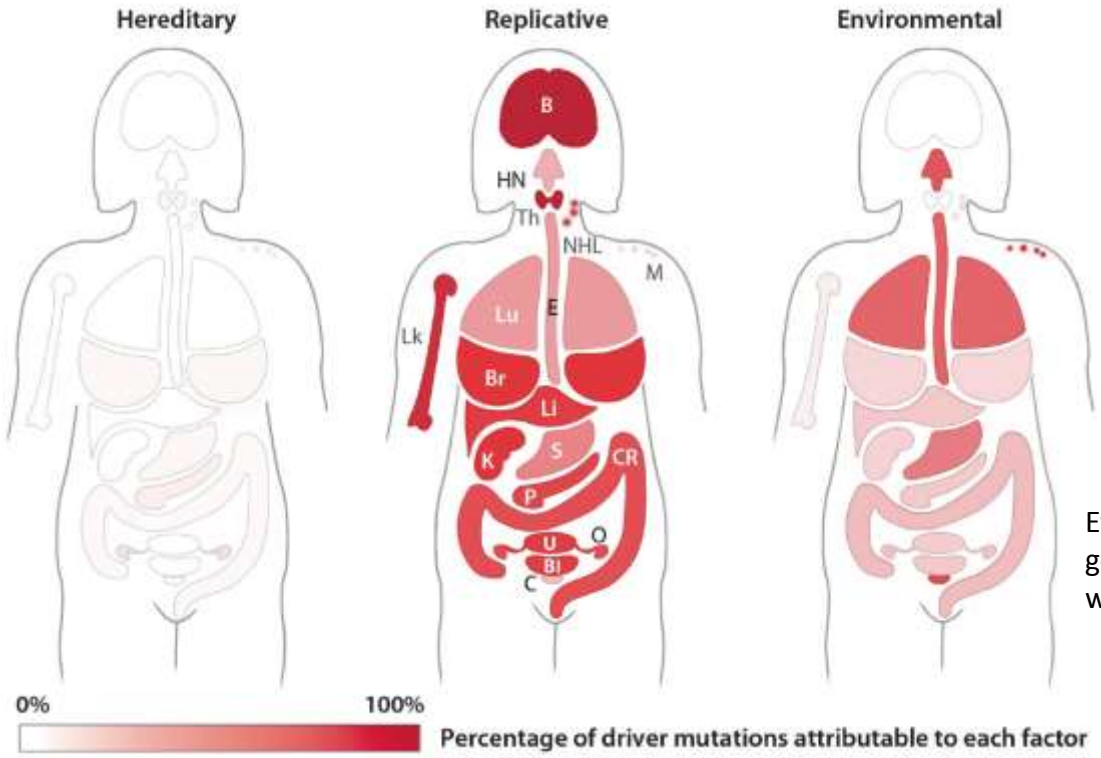
However, the fraction of cancers attributed to genetic background is low

Proportion of cancer susceptibility accounted for by genetic factors

Thyroid cancer	53%
Endocrine glands	28%
Testis	25%
Breast	25%
Cervix	22%
Melanoma	21%
Colon	13%
Nervous system	12%
Rectum	12%
Non-Hodgkin lymphoma	10%
Lung	8%
Kidney	8%
Urinary bladder	7%
Stomach	1%
Leukaemia	1%

Source: K. Czene et al. Environmental and heritable causes of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. Int J Cancer 99:260-266, 2002.

Replication errors in stem cells may be responsible for ca 70% of the mutations in human cancers



Etiology of driver gene mutations in women with cancer

Source: C. Tomasetti et al. Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. Science 355: 1330–1334, 2017

Nevertheless: a high chromosomal radiosensitivity of skin fibroblasts is a hallmark of cancer susceptibility

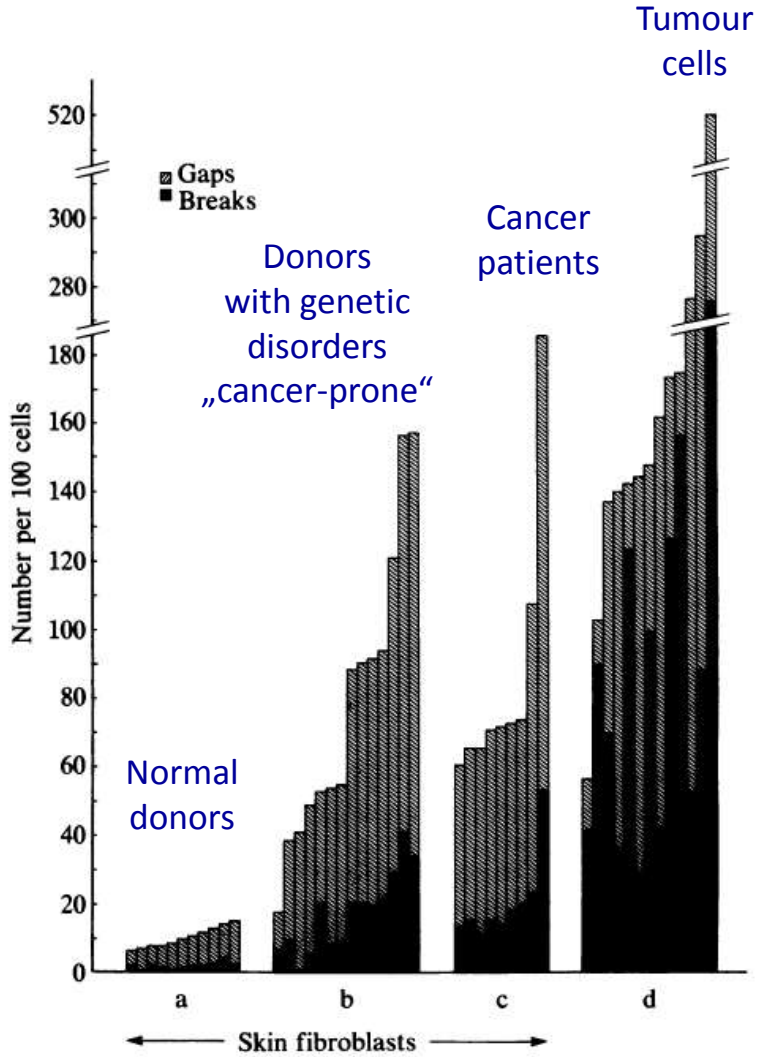
Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 5400–5403, August 1985
Cell Biology

Chromosomal radiosensitivity during the G_2 cell-cycle period of skin fibroblasts from individuals with familial cancer

(chromatid gaps and breaks/cell-cycle-related radiosensitivity)

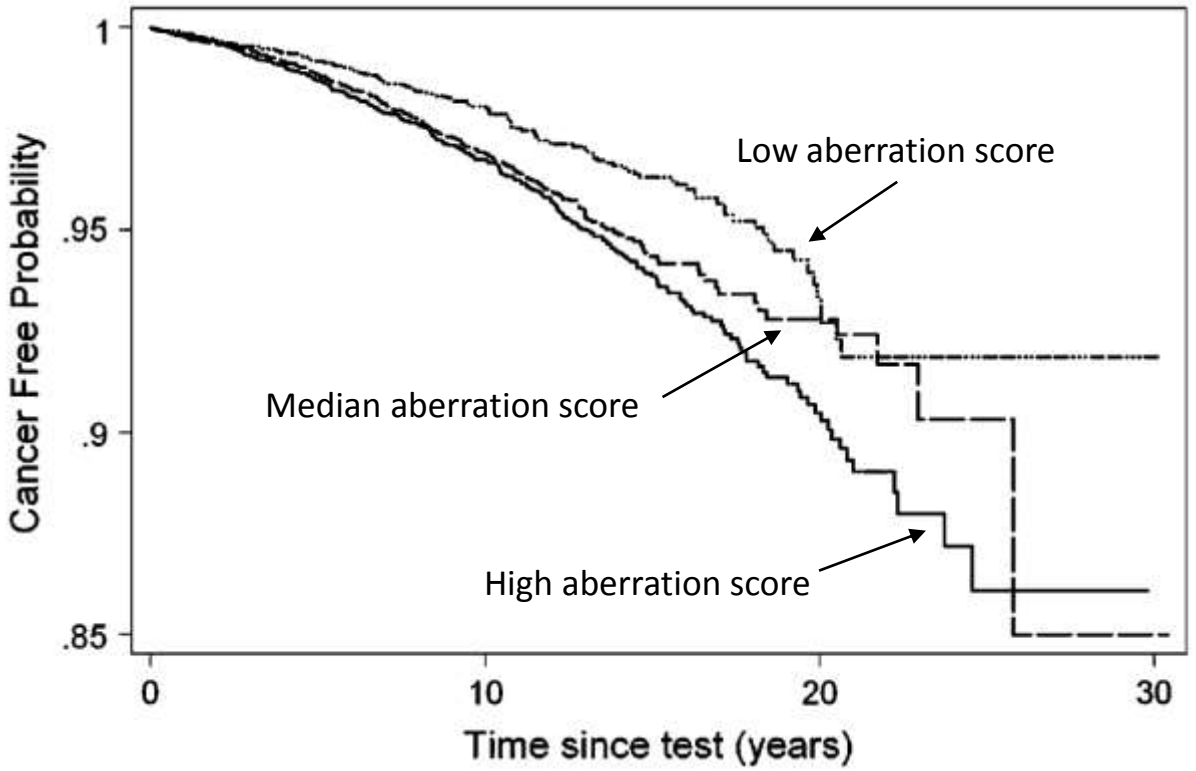
RAM PARSHAD*, KATHERINE K. SANFORD†‡, AND GARY M. JONES†

FIG. 1. Comparison of chromatid damage induced by x-irradiation (100 R) during G_2 phase of skin fibroblasts from normal donors (a), skin fibroblasts from individuals with genetic disorders associated with a high risk of cancer (b) (13), skin fibroblasts from cancer patients (c), and human tumor cells (d) (9). The genetic disorders represented, in order of increasing chromatid damage, were xeroderma pigmentosum variant, Gardner syndrome (GS), xeroderma pigmentosum, complementation group E (XP-E), GS, Bloom syndrome, XP-C, familial polyposis, ataxia telangiectasia heterozygotes (five individuals) and homozygotes (two individuals) (10, 13). Data on XP-A cells have not been included; for explanation, see ref. 13. The tumor cells were from malignancies of diverse tissues of origin and histopathology (9).



High spontaneous aberration frequency in lymphocytes is a hallmark of cancer susceptibility

Kaplan–Meier curves for total cancer incidence tertile of CA frequency based on pooled data from 11 European cohorts. Cancer-free probability refers to time from CA test to the first cancer diagnosis.



High CA frequency was associated with the risk of stomach cancer.

The presence of chromosome instability stomach cancers may be linked to the metabolisms of agents involved in stomach carcinogenesis, such as folic acid and vitamin B12.

However, *Helicobacter pylor*i infection is also known to increase the level of chromosomal damage in lymphocytes.

The idea of the study

Find a database with spontaneous aberration scores in lymphocytes of a cohort

⇩

Follow up the cohort for cancer incidence/mortality

⇩

Correlate the aberration score with RR (calculated as SIR, SMR or HR)

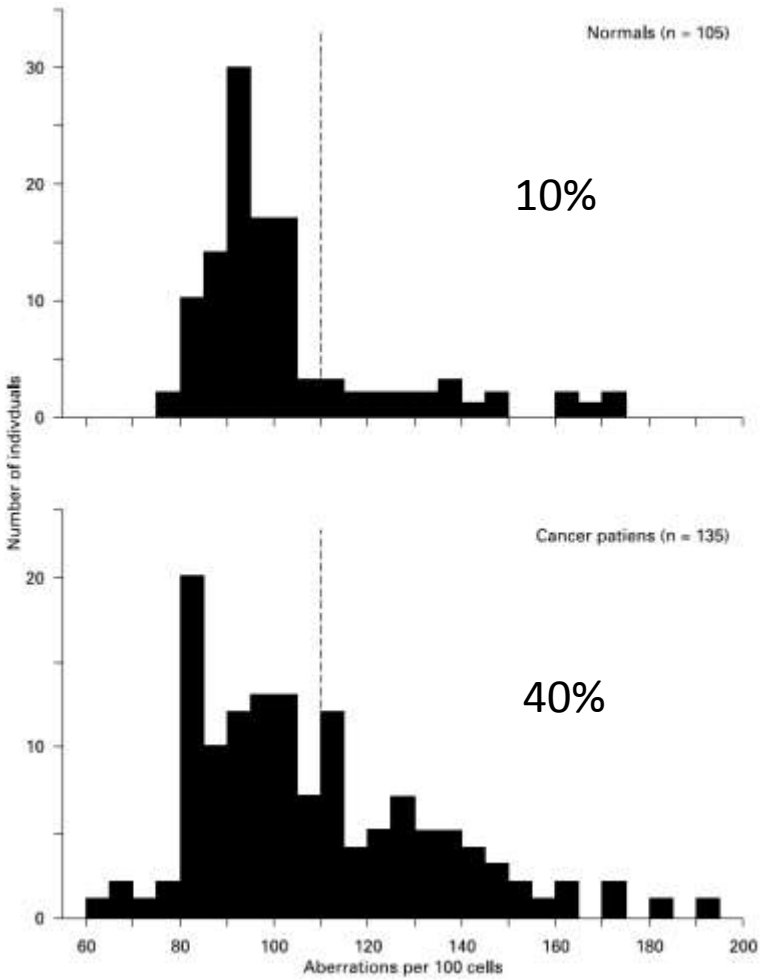
Source: S. Bonassi et al. Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22 358 subjects in 11 countries. Carcinogenesis 29: 1178–1183, 2008.

Lymphocytes of breast cancer patients show an enhanced radiation-induced aberration frequency (G_2 test)

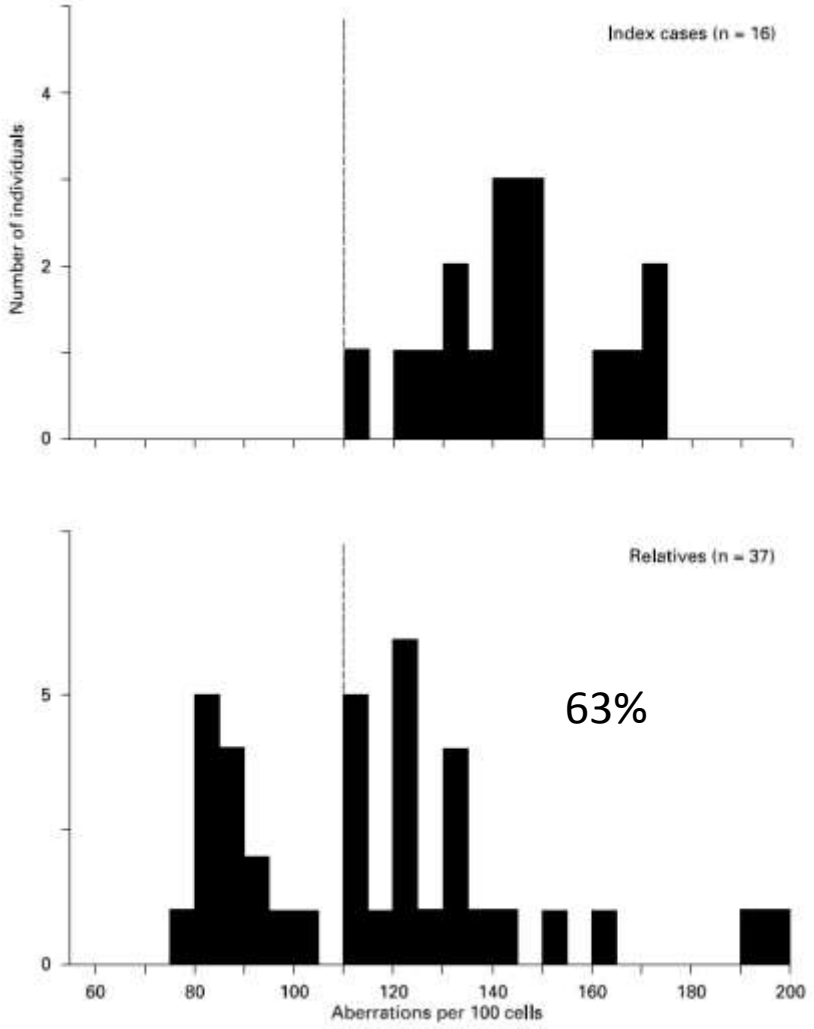
Chromosomal radiosensitivity and low penetrance predisposition to cancer

D. Scott

Cytogenet Genome Res 104:365–370 (2004)



G_2 chromosomal radiosensitivity of normal donors and breast cancer patients. The dashed vertical lines indicate the cut-off point between a normal and a sensitive response.

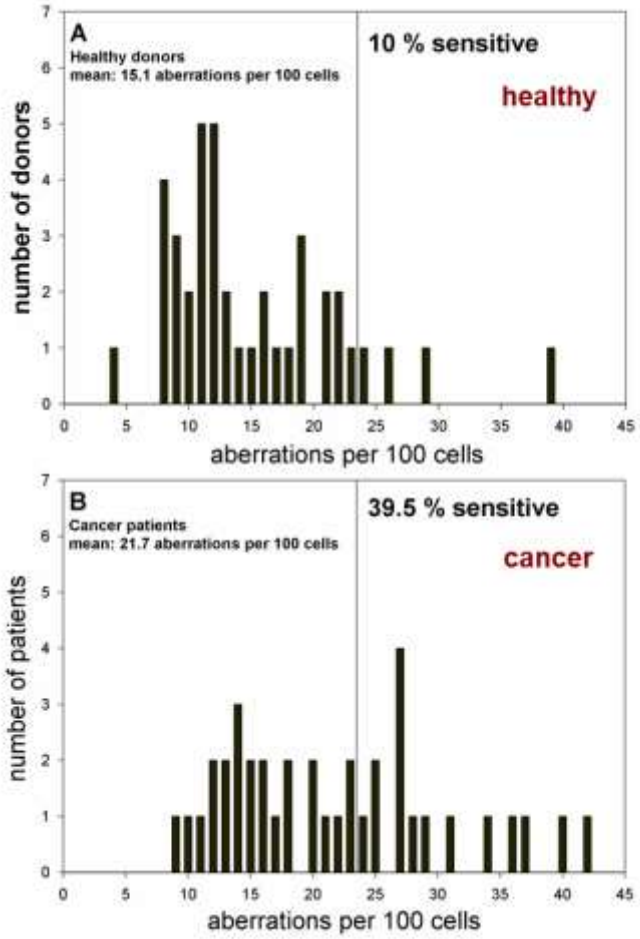


A heritable trait?

G_2 chromosomal radiosensitivity of patients with breast cancer selected as being sensitive in the assay and first degree relatives. The dashed vertical lines indicate the cut-off point between a normal and a sensitive response, from historic control data (see Fig. to the left).

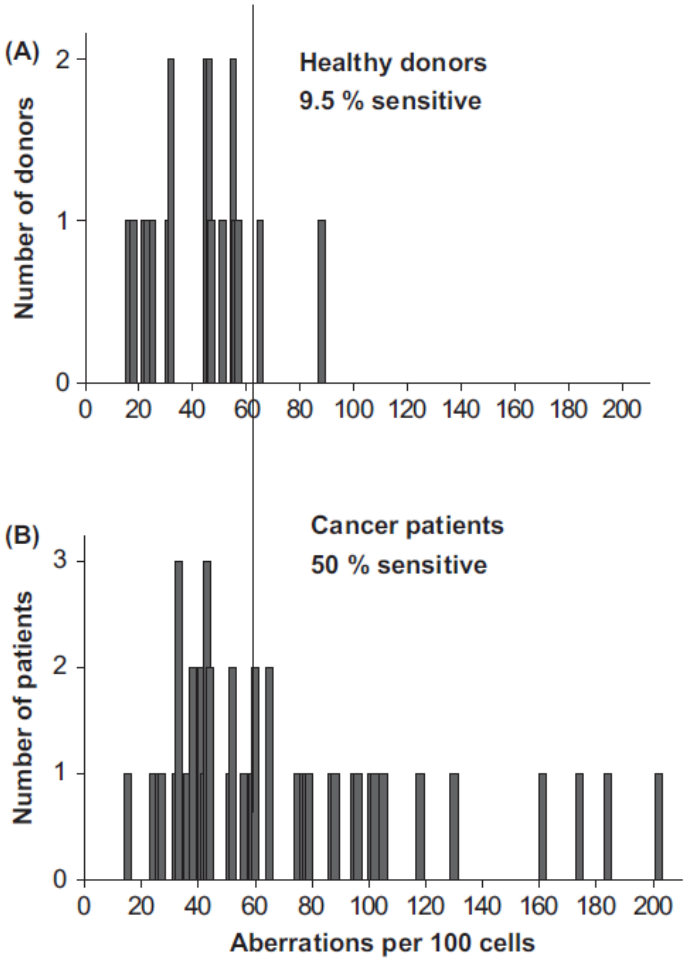
Lymphocytes of patients with some other cancers may also show enhanced radiation-induced aberration frequencies

H&N cancer



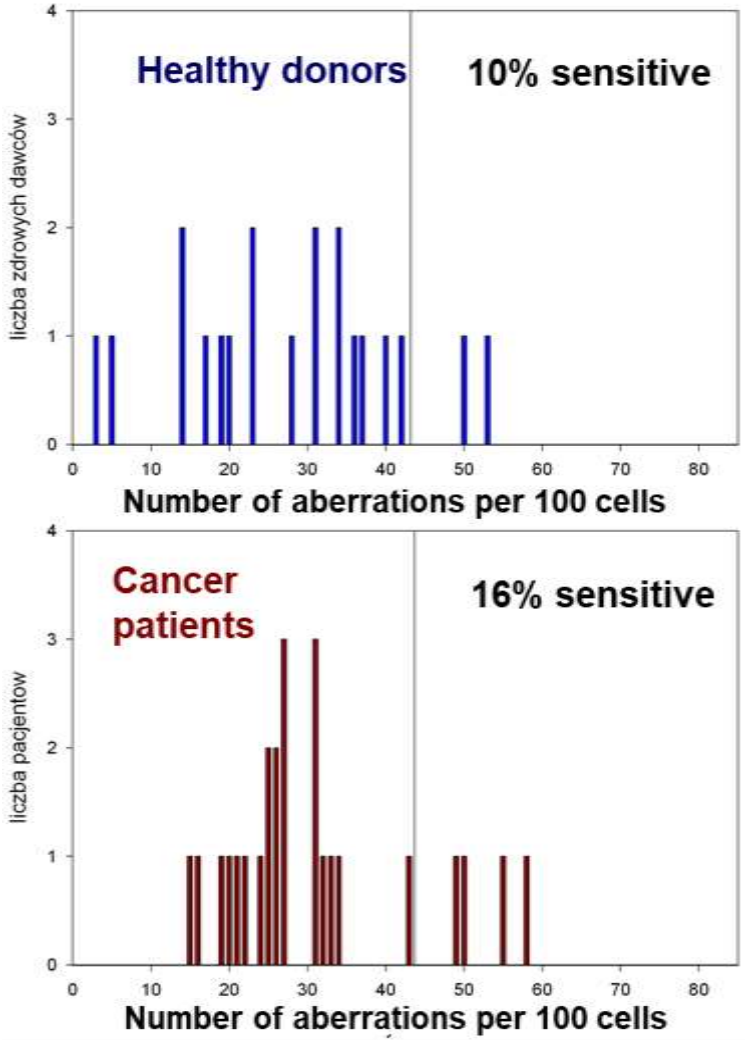
Source: Lisowska et al. Int. J. Radiation Oncology Biol. Phys., 66: 1245–1252, 2006

Prostate cancer



Source: Brzozowska et al. Int. J. Radiat. Biol. 88: 405–413, 2012

Gynecological cancers



Source: Padjas, PhD thesis, Kielce, unpublished

Mutagen sensitivity studies suggest a much higher genetic component in cancer susceptibility than epidemiological genetic linkage studies

X. Wu et al. Mutagen Sensitivity: A Genetic Predisposition Factor for Cancer. Cancer Res 67: 3493-3495, 2007.

Table 1. Contribution of genetic and environmental variance components to mutagen sensitivity

Variance components	Mutagen			
	Bleomycin	BPDE	γ-Radiaton	4NQO
Genetic contribution (%)	40.7	48.0	62.5	58.8
Nonshared environment (%)	33.3	31.8	37.5	39.7
Shared environment (%)	26.0	20.2	0	1.5

Abbreviations: BPDE, benzo[*a*]pyrene diol epoxide; 4NQO, 4-nitroquinoline 1-oxide.

Is a high mutagen sensitivity really a marker of genetically-determined cancer susceptibility?

G. Szekely et al. Does the bleomycin sensitivity assay express cancer phenotype? Mutagenesis 18: 59–63, 2003

Comparison of PBL sensitivity to BLM in H&N cancer patients, healthy normal people and healthy alcoholics.

No difference between cancer patients and alcoholics.

BLM assay seems to be a tool for characterization of genotoxic exposure to heavy tobacco and alcohol use rather than for individual susceptibility to cancer.

Is a high mutagen sensitivity really a marker of genetically-determined cancer susceptibility?

M. Khosravifarsani et al. The study of radiosensitivity in left handed compared to right handed healthy women. BMC Medical Physics 12:3, 2012

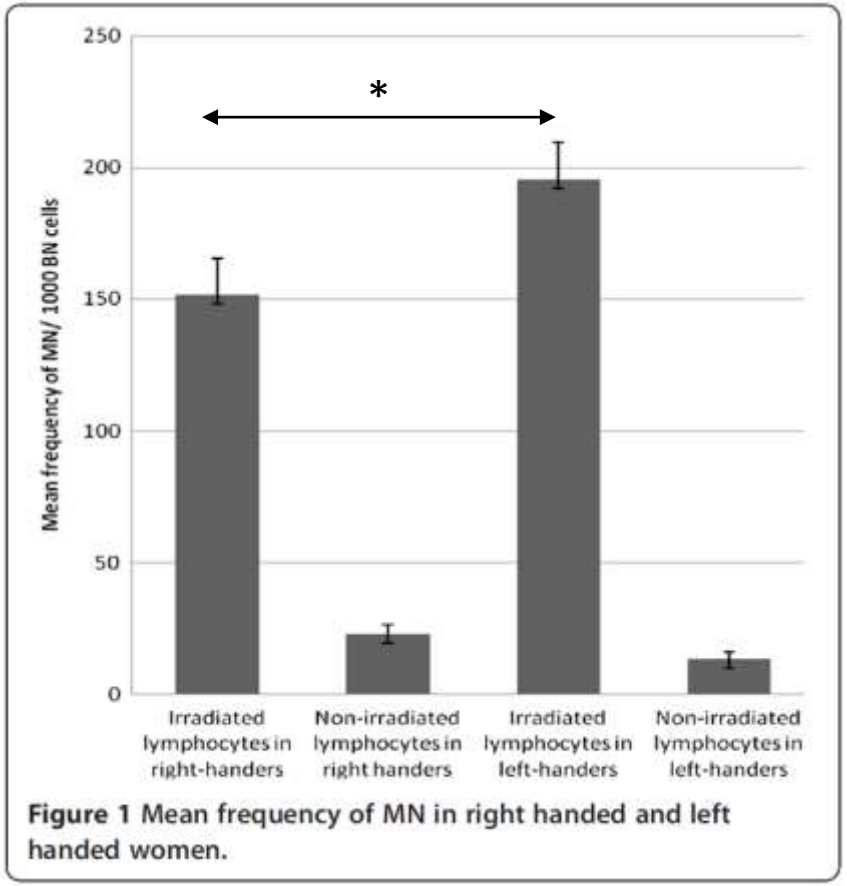


Figure 1 Mean frequency of MN in right handed and left handed women.

No evidence of genomic instability in survivors of childhood cancers

ELSEVIER

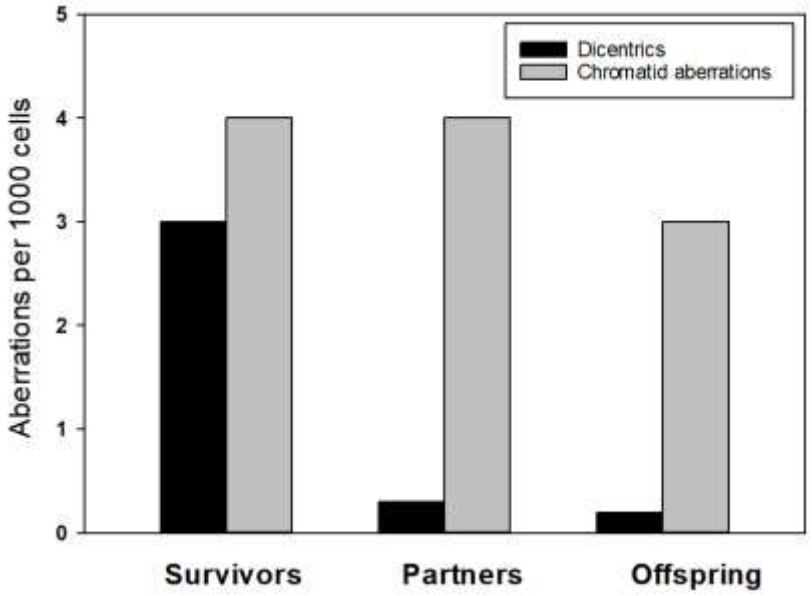
Mutation Research 465 (2000) 45–51

Genetic Toxicology and Environmental Mutagenesis

www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Sequential chromosome aberration analysis following radiotherapy — no evidence for enhanced genomic instability

E. Janet Tawn *, Caroline A. Whitehouse, Fiona A. Martin [1]



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Mutation Research 583 (2005) 198–206

Genetic Toxicology and Environmental Mutagenesis

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Chromosome analysis in childhood cancer survivors and their offspring—No evidence for radiotherapy-induced persistent genomic instability

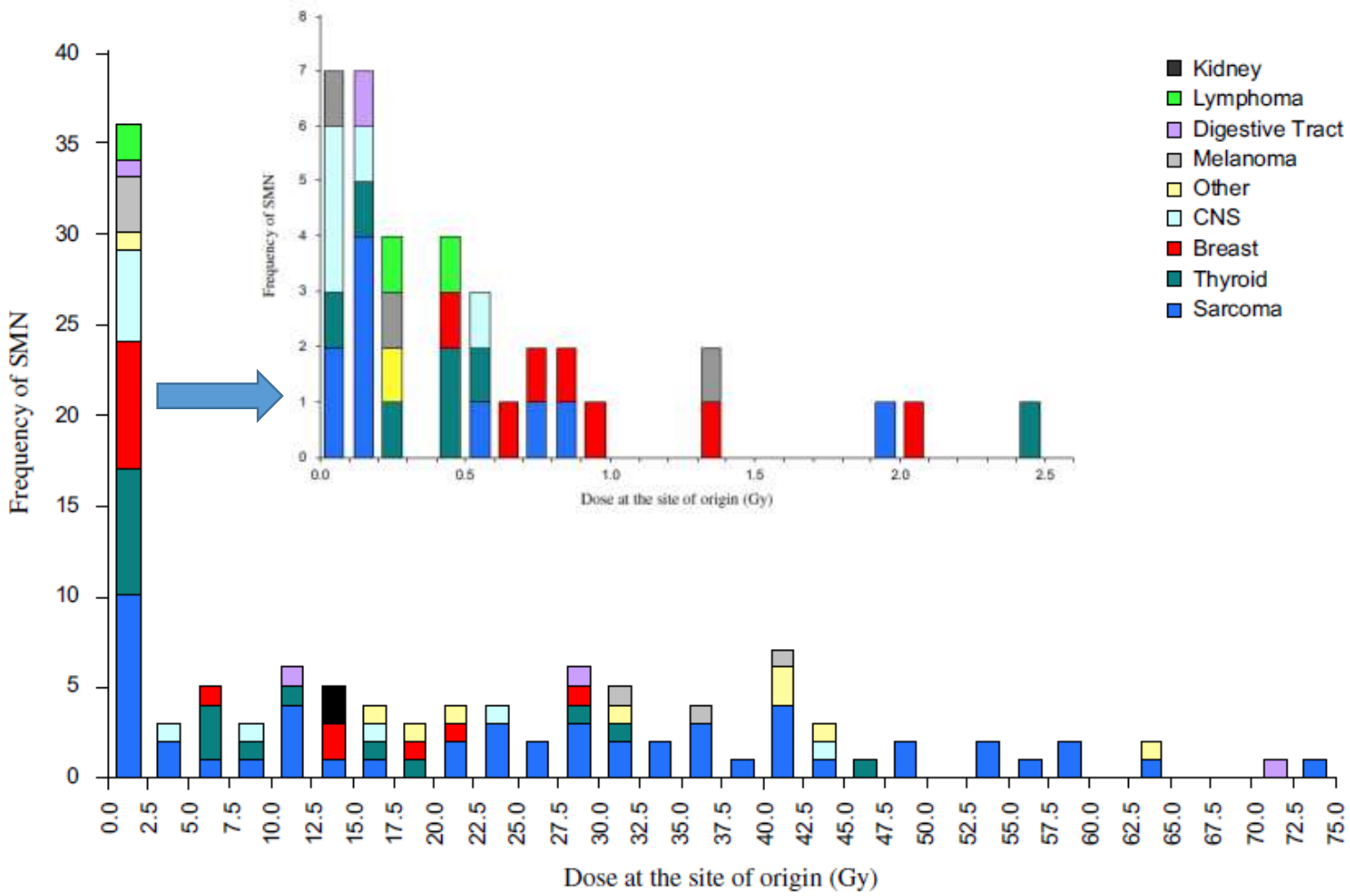
E. Janet Tawn [a,*], Caroline A. Whitehouse [a], Jeanette F. Winther [b], Gillian B. Curwen [a], Gwen S. Rees [a], Marilyn Stovall [c], Jørgen H. Olsen [b], Per Guldberg [d], Catherine Rechnitzer [e], Henrik Schrøder [f], John D. Boice Jr. [g,h]

Patients with SMN (second malignant neoplasms) appear to be an attractive cohort for studies of biomarkers of cancer susceptibility, BUT:

Problem 1: the dose-response relationship for SMN is not well known

Where do the SMN occur? Dose at the site of origin.

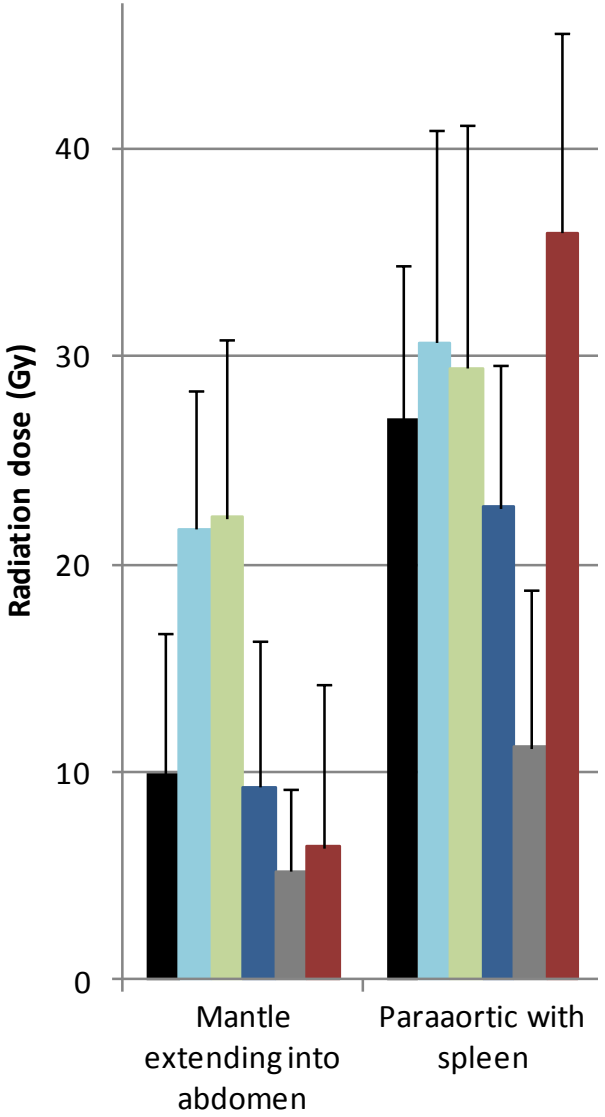
How precise is the dose estimate at the site of SMN? A problem is the long time span between RT and manifestation of SMN



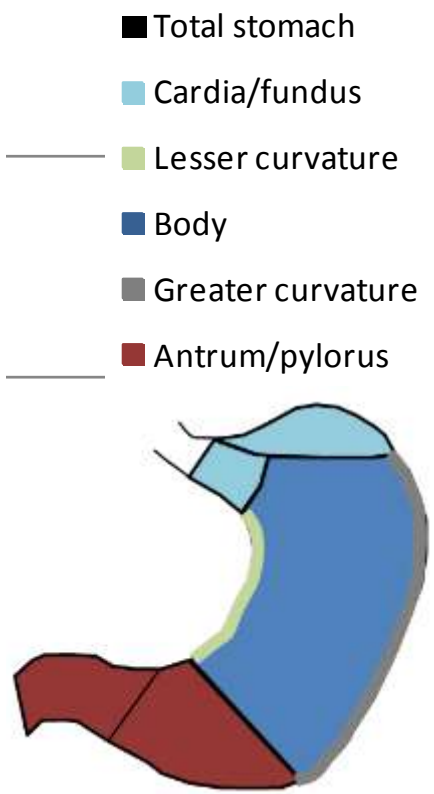
Source: Diallo et al Int. J. Radiation Oncology Biol. Phys. 74: 876–883, 2009

Patients with SMN (second malignant neoplasms) appear to be an attractive cohort for studies of biomarkers of cancer susceptibility, BUT:

Problem 2: the doses received by organs and tissues at risk are poorly defined



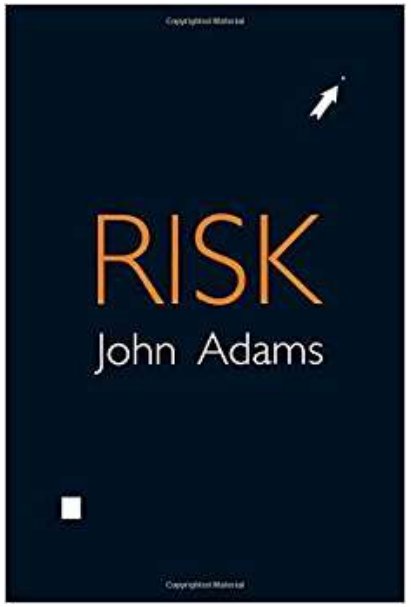
Doses to stomach after treatment for Hodgkin lymphoma



Which dose is considered in epidemiological studies?

Remember that we are analysing SMN induced by RT many years ago, when the TPS were different than today

Source: LM. Morton et al. Stomach cancer risk after treatment for Hodgkin lymphoma. J Clin Oncol 31:3369-3377, 2013



Can you reduce your individual radiosusceptibility?
Remember: all risks are conditional

- Cancer risk models recommended for use by the ICRP depend to a large extent on excess relative as opposed to excess absolute risk.
- This suggests that the risk of radiation-induced cancer is to a great extent determined by the same factors that determine cancer risk in the general population.
- **Therefore, measures that reduce population cancer risk incidence and mortality should help reduce the incidence of radiation-associated cancer in populations.**

- Can the risk of radiation-induced cancer be reduced **after** a radiation exposure has taken place?
- If this is the case then people who have been exposed to radiation (e.g. due to Chernobyl or Fukushima Daiichi accidents) can - to some extent - **control their risk**.
- This can have an enormous implication for their well being and, eventually, for their health.

Leisure-Time Physical Activity reduces the Risk of 26 Types of Cancer in 1.44 Million Adults

SC. Moore et al. JAMA Intern Med. 176:816-825, 2016.

1.44 million participants (59 [19-98] years), 57% females and 43% males, 186 932 cancers
High vs low levels of leisure-time physical activity were associated with lower risks of 13 cancers:

	Hazard ratio	95% CI
Esophageal adenocarcinoma	0.58;	0.37-0.89
Liver	0.73;	0.55-0.98
Lung	0.74;	0.71-0.77
Kidney	0.77;	0.70-0.85
Gastric cardia	0.78;	0.64-0.95
Endometrial	0.79;	0.68-0.92
Myeloid leukaemia	0.80;	0.70-0.92
Myeloma	0.83;	0.72-0.95
Colon	0.84;	0.77-0.91
Head and neck	0.85;	0.78-0.93
Rectal	0.87;	0.80-0.95
Bladder	0.87;	0.82-0.92
Breast	0.90;	0.87-0.93

Possible mechanism: stimulation of immune surveillance

Leisure-time physical activity was associated with higher risks of:

Malignant melanoma	1.27; 1.16-1.40
Prostate cancer	1.05; 1.03-1.08.

Smoking status modified the association for lung cancer but not other smoking-related cancers.

Conclusions

Functionality assays to detect individual radiosensitivity yield very conflicting results so their value is doubtful.

Radiosensitivity is a complex trait so SNP analysis by GWAS appears promising for identyfing radiosensitive patients – several large studies are ongoing.

Confounders such as variability in contouring the organs at risk and defining the adverse effects need to be reduced in order to better identify radiosensitive patients.

The value of testing for radiosusceptibility in the context of radiological protection of low-dose occupationally exposed individuals is doubtful because of the low contribution of genetic background.

The effect of lifestyle and other factors on risk following radiation exposure (“effect modifiers”) needs to be better understood so that a “cancer reducing” life style is promoted among exposed people. This will allow them to control the risk leading to increased well being and, eventually, improved health.