Individual Sensitivity

Mike Atkinson

Institute of Radiation Biology
National Centre for Radiation Sciences
Helmholtz Centre Munich

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“All animals are created equal,
...but some are more equal than othe

George Orwell, Animal Farm

What if ....

There are differences in the genetic composition between individuals and amongst populations.

Will this create individual differences in the risk of adverse effects after exposure to radiation?

Diversity of the global population:

The global human population is genetically diverse (and almost half of them are males!).

Anatomically modern humans (*Homo sapiens sapiens*) arose 150000 - 200000 years ago in Sub-Saharan Africa.

Genetic diversity amongst the populations migrating out of Africa.

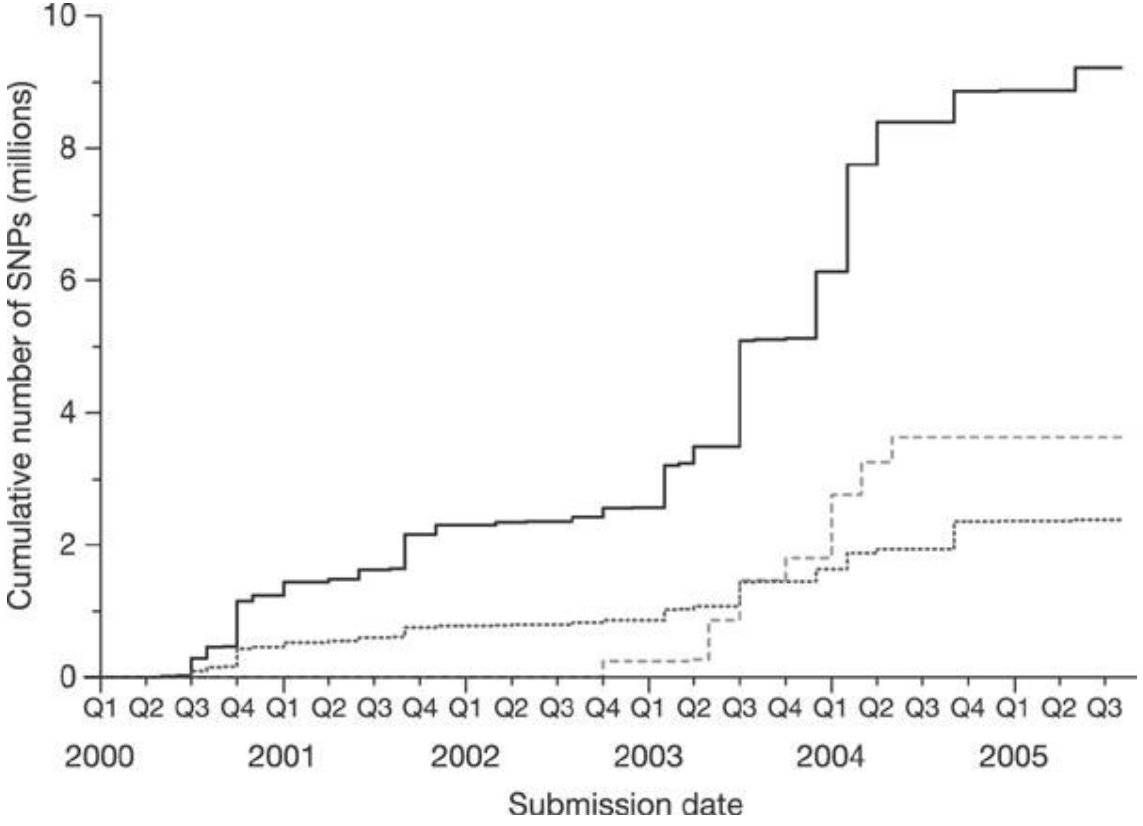
Populations of the different global regions show genetic diversity both within their own population and between populations.

“Population islands” remain, due to linguistic-cultural (e.g. Saami), religious (e.g. Mennonites) or physical separation (e.g. island populations).

A population island can be distributed globally (e.g. Ashkanazim).

The human genome is genetically diverse:

Analysis of genetic variations within man reveal over 9 million single nucleotide polymorphisms (SNPs).



Cumulative SNPs

1x validated

2x validated

A haplotype map of the human genome
The International HapMap Consortium
Nature 437, 1299-1320 2005

What do 9 Million SNPs mean for variability?

SNPs are common polymorphisms.

Assume 10% coding DNA, therefore potentially 300,000 coding SNPs per individual.

However, post-genomic era suggests a significant proportion of the genome is actually regulatory (microRNAs).

Here a single polymorphims may affect expression of 100-200 different genes.

What if ....

Disease occurrence is influenced by the various genetic variations in the human genome?

Genetics and disease

Family history is a major factor for developing common diseases (e.g. cardiovascular disease, diabetes, cancer), indicating a genetic component.

Breast cancer is 2x more frequent in first-degree relatives of women with breast cancer than in the rest of the population.

10% of MaCa show inheritance of one of two genes (*BRCA1* & *BRCA2*).

Other less frequent genes involved (e.g. *CHK2*, *ATM*). This still leaves 75% of familial risk unexplained.

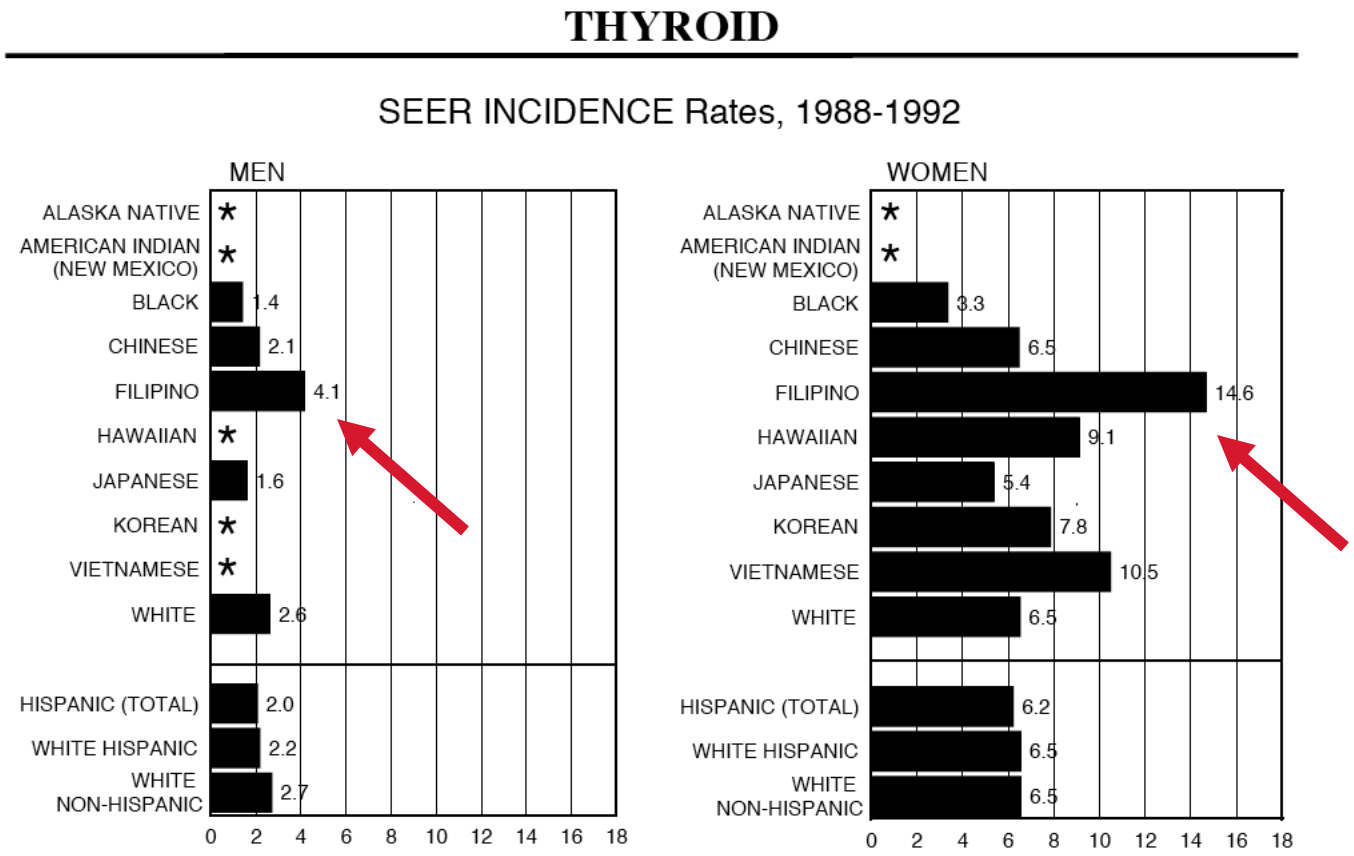
Recent genetic study (21,860 cases) revealed 5 new loci contributing to risk.

(Nature 447:1087 2007).

What if....

As a consequence of the differences in genetic composition (and gene-environment interactions) cancer risk varies globally?

Racial and gender differences in cancer rates



So how do genetic differences influence risk of cancer ?

Two genetic mechanisms responsible for the development of disease (including cancer).

- Monogenic gene mutations.

- Polygenic inheritance.

Cancer as the result of a single gene mutation

There are 1000+ rare and highly heritable “mendelian” traits.

Inheritance of a single allele is both necessary and sufficient to produce the disease phenotype (monogenic, dominant, high pentrance).

The mutations responsible are present by definition in less than 1% of the population, actually much less frequent.

Suggested to be responsible for between 1 and 15% of all cancers.

Example of a monogenic "familial cancer syndrome"

Multiple Endocrine Neoplasia (MEN1, 2 and X).

MEN1 germ-line inactivation of *MENIN* tumour suppressor gene.

MEN2 germ-line mutation of *RET* protooncogene.

MEN-X germ line mutation of *p27* tumour suppressor gene.

All three mutant genes lie in a single signal transduction pathway linking cell proliferation and differentiation.

Not all cancer syndromes are caused by classical ONC/TSGs
e.g. E-Cadherin in familial gastric cancer and DNA mismatch repair genes in hereditary non-polyposis colon cancer

What if....

The same genetic mechanisms also influence the radiation response and hence the risk of an adverse reaction?

Radiation hypersensitivity:

An unpredictable acute reaction to high doses of radiation.

Monogenic component indicated by individuals with SCID / DNA repair deficiency syndromes.

Polygenic component indicated by numerous association studies showing non-random distribution of alleles between normal and hypersensitives.

Uncertainties exist in the broader application of this phenomenon:

Acute tissue reaction may not have any relationship to the risk of cancer (no oncogenes / TSGs influence this pathway, no clear mechanism).

Association studies are statistically weak and have not been reproduced.

However, DNA repair defects frequently show hypersensitivity, and may be associated with an increased cancer risk.

The biological response to high doses causing acute effects may be quite different to the response at low and moderate doses (50-500mGy).

Monogenic disposition to radiation-induced cancer

Genetic predisposition to radiation-induced (secondary) cancers clearly established.

- Mechanisms and tissue involvement show a plausible causality.

- Monogenic traits associated with an increased risk of• "sporadic" cancer, show increased rate of secondary cancers.

- Heterozygous animal models reiterate phenotype.

Gorlin syndrome (PTCH1)

PTCH1 gene regulates differentiation of embryonic cells via TGF-beta and Wnt pathways.

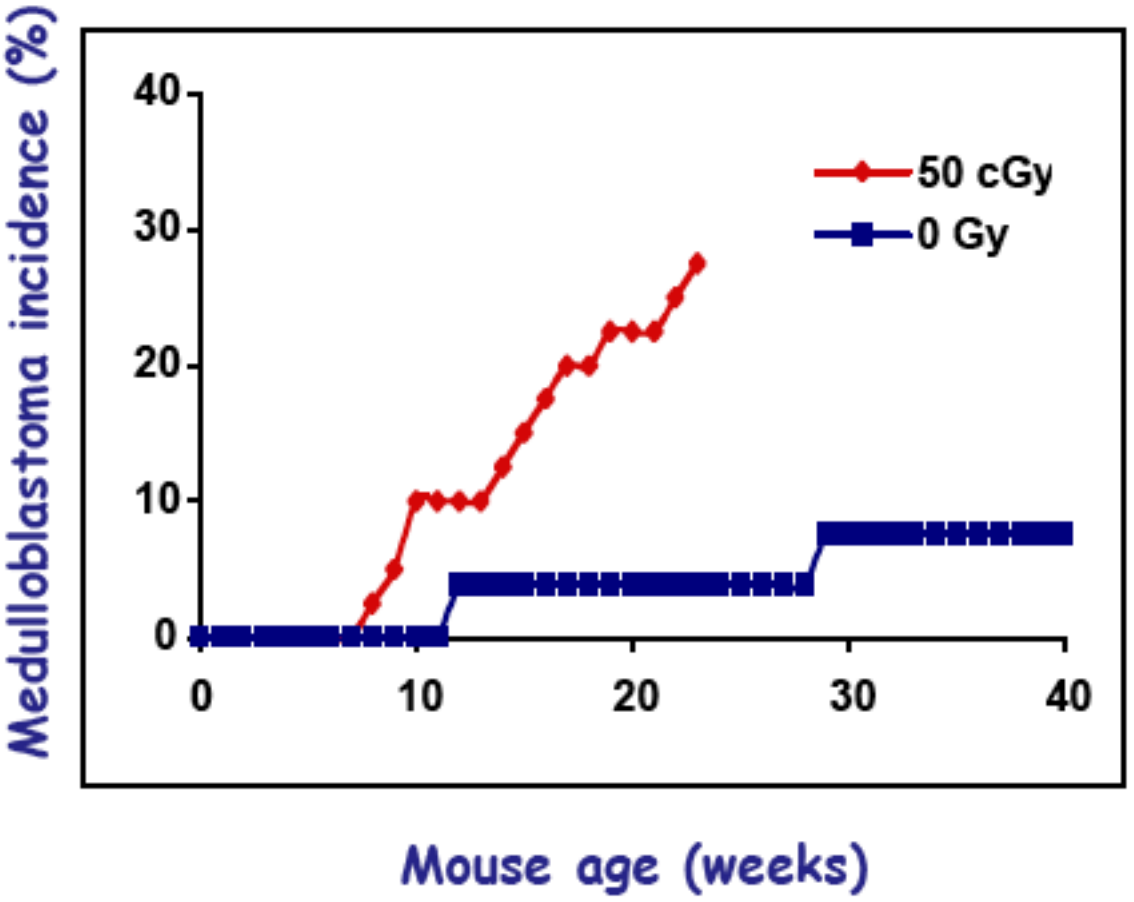
Affects 1:50 000 to 1:150 000 in population.

Patched acts as a tumor suppressor gene, loss of function derepresses signal pathways and prevents differentiation.

Irradiation leads to multiple basal cell carcinoma and medulloblastoma in radiation field.

Mouse model reiterates human radiosensitivity phenotype.

Medulloblastoma induced by 50cGy X-rays in Ptch1 +/- mice



Data from Saran and Pazzaglia RISCRAD

Li-Fraumeni syndrome (TP53)

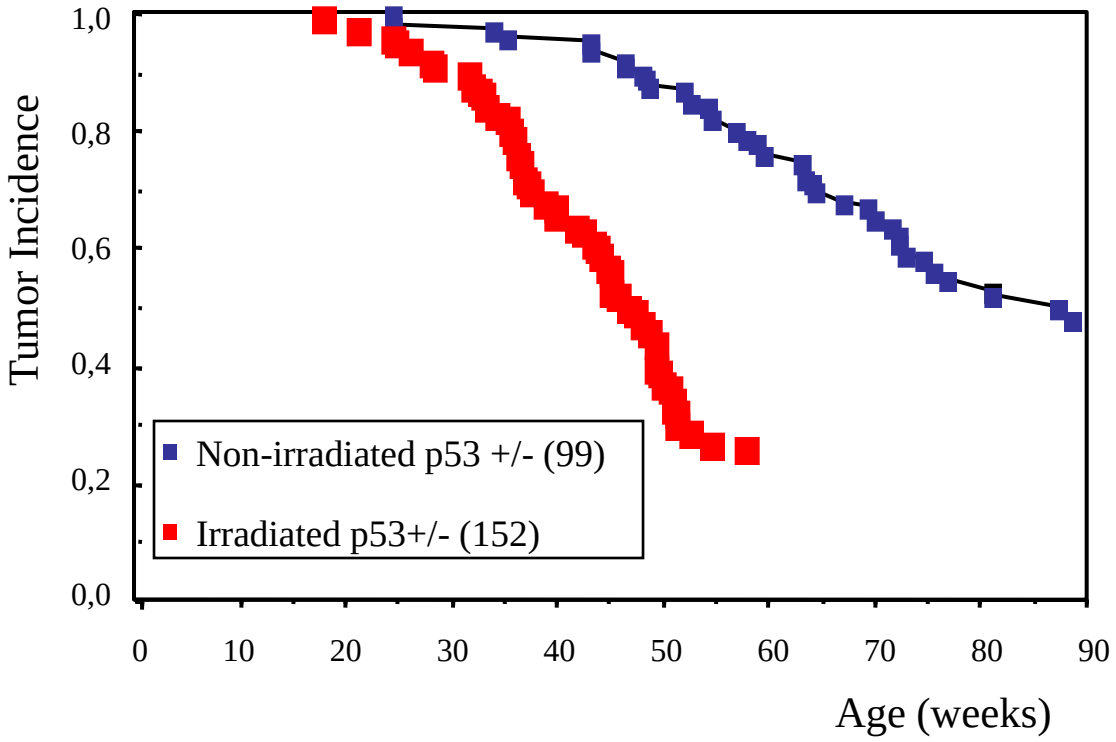
Tumor supprerssor gene regulating response to DNA damage, including cycle arrest and apoptosis.

Sporadic cancers of multiple tissues (soft tissue sarcoma, osteosarcoma, breast cancer, brain tumors, & leukemia).

Irradiation induces cancers in same organs, skeleton most sensitive.

Mouse model reiterates human syndrome.

Skin bone and lymphoma cancer incidence in p53 heterozygous mice



Data from Brown & Balmain GENRAD

Retinoblastoma (RB1)

RB1 tumor suppressor gene regulates cell cycle entry.

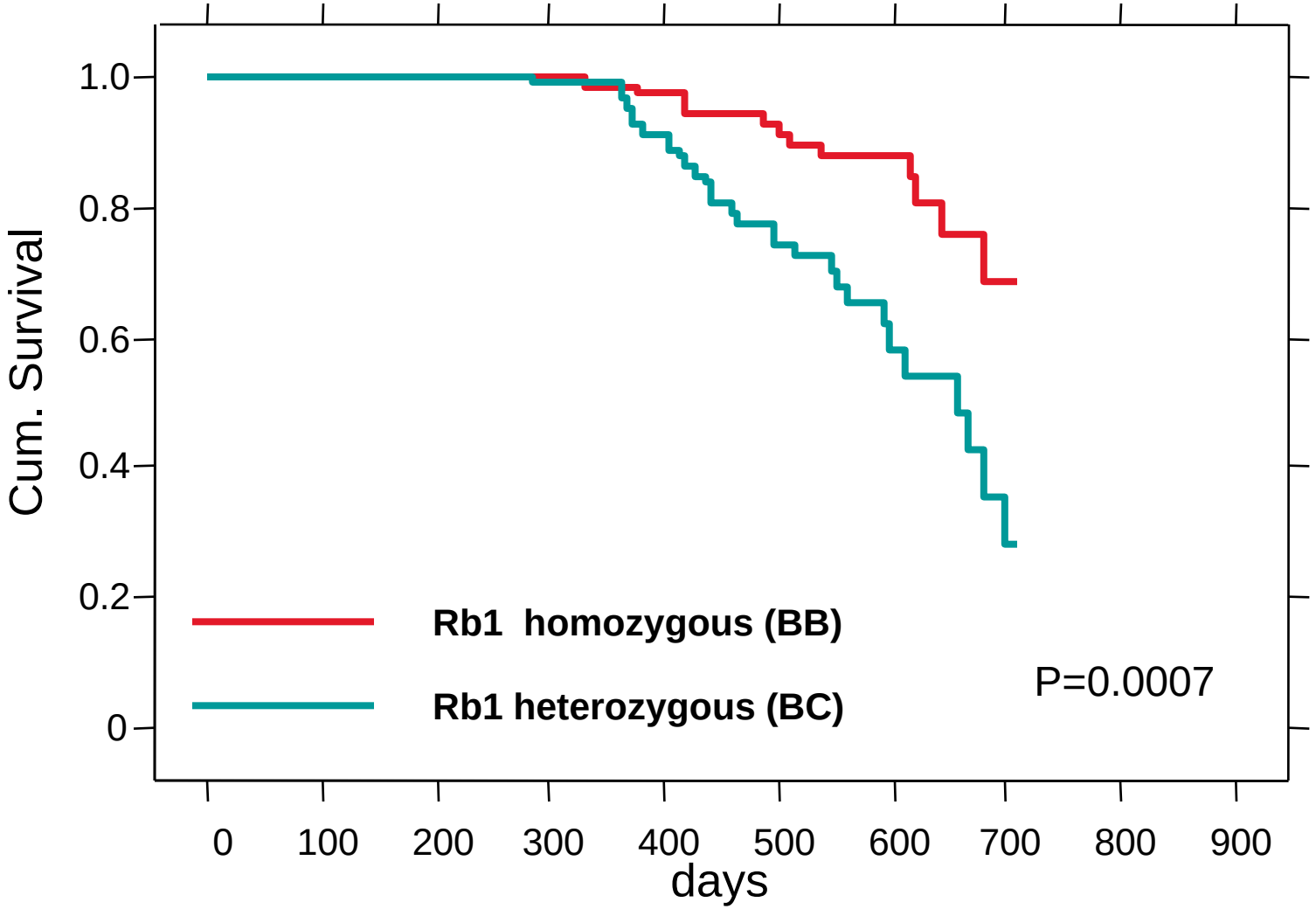
1:23 000 frequency.

Loss of function leads to sporadic retinoblastoma and osteosarcoma.

In radiation field the risk of osteosarcoma rises manyfold.

Mouse model does not develop retinoblastoma. Sensitivity to radiation-induced osteosarcoma (RISCRAD).

Mice bearing one hypomorphic Rb1 allele are more sensitive to radiation-induced osteosarcoma



Data from Rosemann & Atkinson RISCRAD

European Project
IP-FP6
RISC-RAD
2004-2008

CDKN2A (p16)

A CDKI protein, regulator of cell cycle entry.

Tumor suppressor gene causing familial melanoma.

A recent case report describes a patient exposed to atmospheric A Bomb testing. Developed multiple melanoma on exposed side of body.

Eliason et al Arch Dermatol 143:1409 2007

Mouse model under test using alpha, neutron & x-irradiation (RISCRAD)

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Inherited defects in DNA repair

Over 50 DNA repair genes. Loss of function mutation usually phenotypically recognisable (XP, AT, NBS etc).

Due to the lack of control of genomic integrity many affected individuals develop sporadic cancer at an early age.

Radiation exposure of such sensitive individuals is not normally performed.

Animal models frequently lethal or hypersensitive to radiation (e.g. *Rad51, Xrcc3, Rad50, Mbs1, Lig4, Brca1*).

Heterozygous effects under test (RISCRAD).

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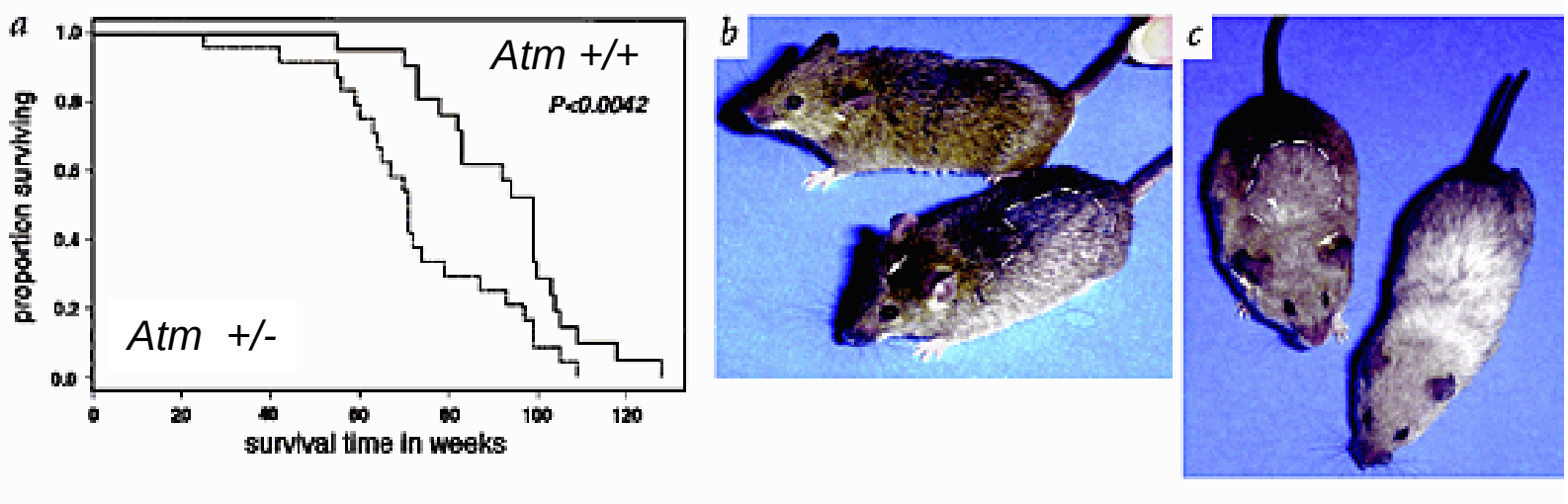
ATM: mediates DNA damage response.

Disruption of ATM leaves mouse with growth retardation, infertility and neurologicla dysfunction.

Cells are hypersensitive to radiation

Mice develop lymphoid cancer at early age (2-4 months)

Radiation cannot show much effect as all die anyway



Barlow *Nature Genetics* **21**, 359 1999

What if ....

Hypomorphic alleles of the known monogenetic genes influence susceptibility to cancer ?

What if ....

Polygenic inheritance influenced susceptibility ?

Cancer as the result of a complex genetic inheritance

Transmission of the cancer phenotype due to inheritance of multiple common alleles (polymorphisms present in >1% of the population).

Examples of common alleles associated with disease:-

- HLA (autoimmunity and infection)
- APOE4 (Alzheimer's disease)
- Factor V Leiden (deep vein thrombosis)
- NOD2 (inflammatory bowel disease)
- Complement factor H (age-related macular degeneration)

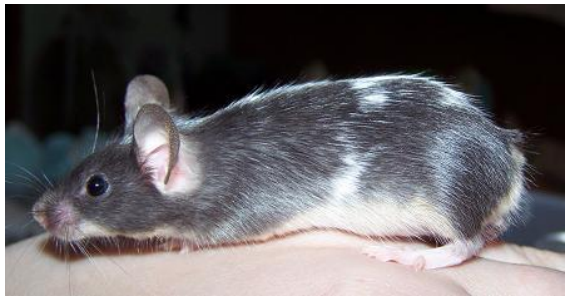
Each disease phenotype is the result of the interaction of multiple weak alleles, each of which makes only a small individual contribution.

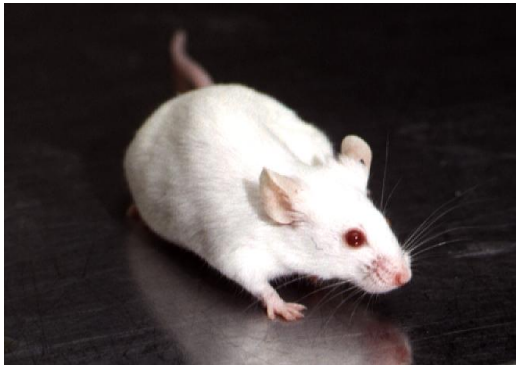
Human studies on complex genetic inheritance do not have the power to identify multiple weak alleles.

Note the breast cancer study mentioned earlier has a total of over 40 000 individuals.

So, what is the alternative ?

“All *mice* are created equal,
...but some *mice* are more equal than others”





Quantifying the genetic component of risk using mouse models

Discovery:

- Identify candidate genes modifying carcinogenic effects of radiation.

Validation:

- Test effects of the genetic component, in particular at low doses.

Application:

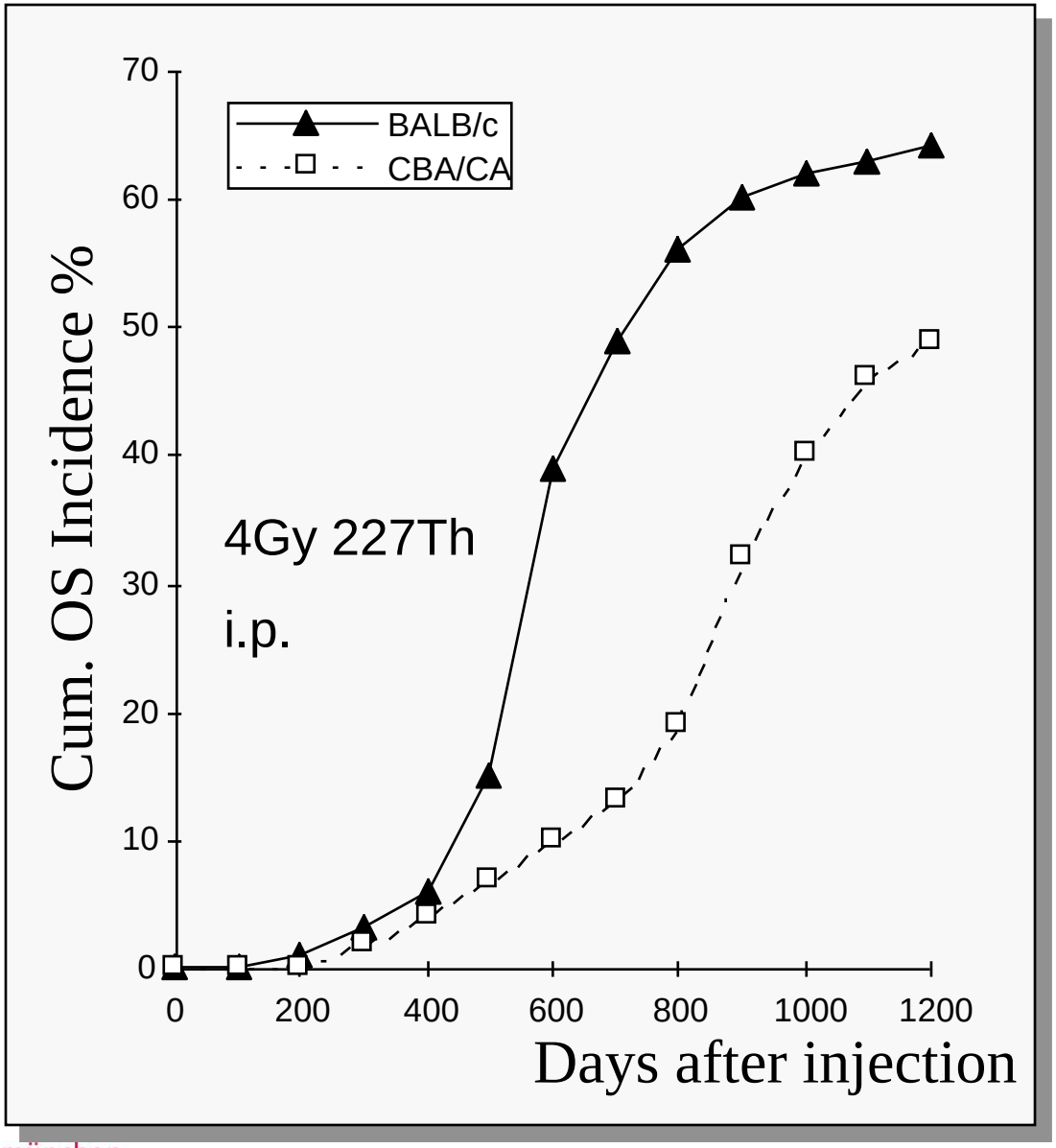
- Functional studies on mechanism of genetic susceptibility
- Create animal models to study biological responses at low doses
- Improve risk assessment models

Radiation-induced mouse osteosarcoma

- Radiation-induced osteosarcoma in man
  (Dial painters, Pu-workers, Ra & Th therapy).

Low sporadic incidence (high causality).
Strain differences in susceptibility.

Osteosarcoma analysis in CBA and BALB/c mice



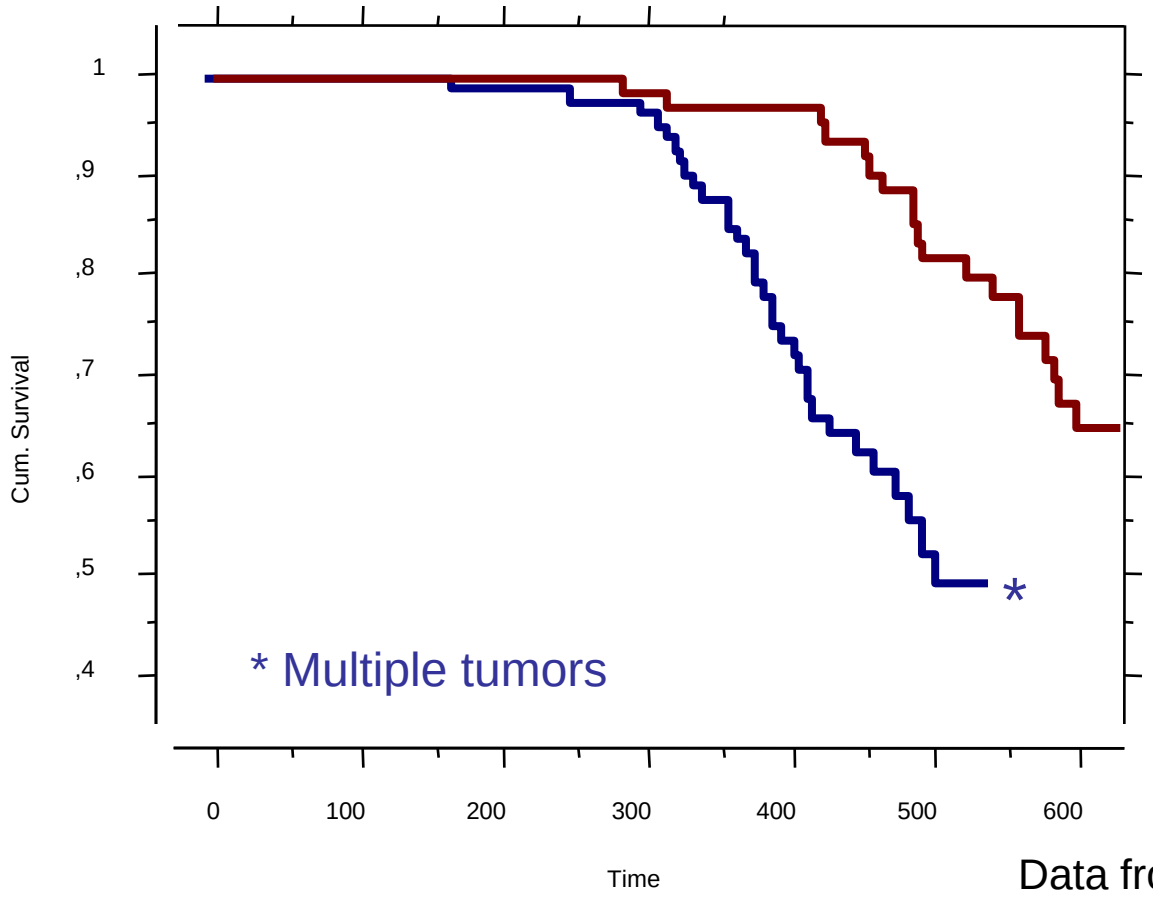
Data from Luz & Atkinson GENRAD

QTL mapping of low penetrance modifiers

In a backcross between resistant and sensitive strains the genes responsible for sensitivity will be distributed amongst the offspring.

Sensitivity (QTL latency) to induced ostesoarcoma can be correlated with genotype.

C3H:102 congenic line shows increased sensitivity to alpha-induced osteosarcoma



Kong7RR *Ch7 C3HR:Ch14 102R*

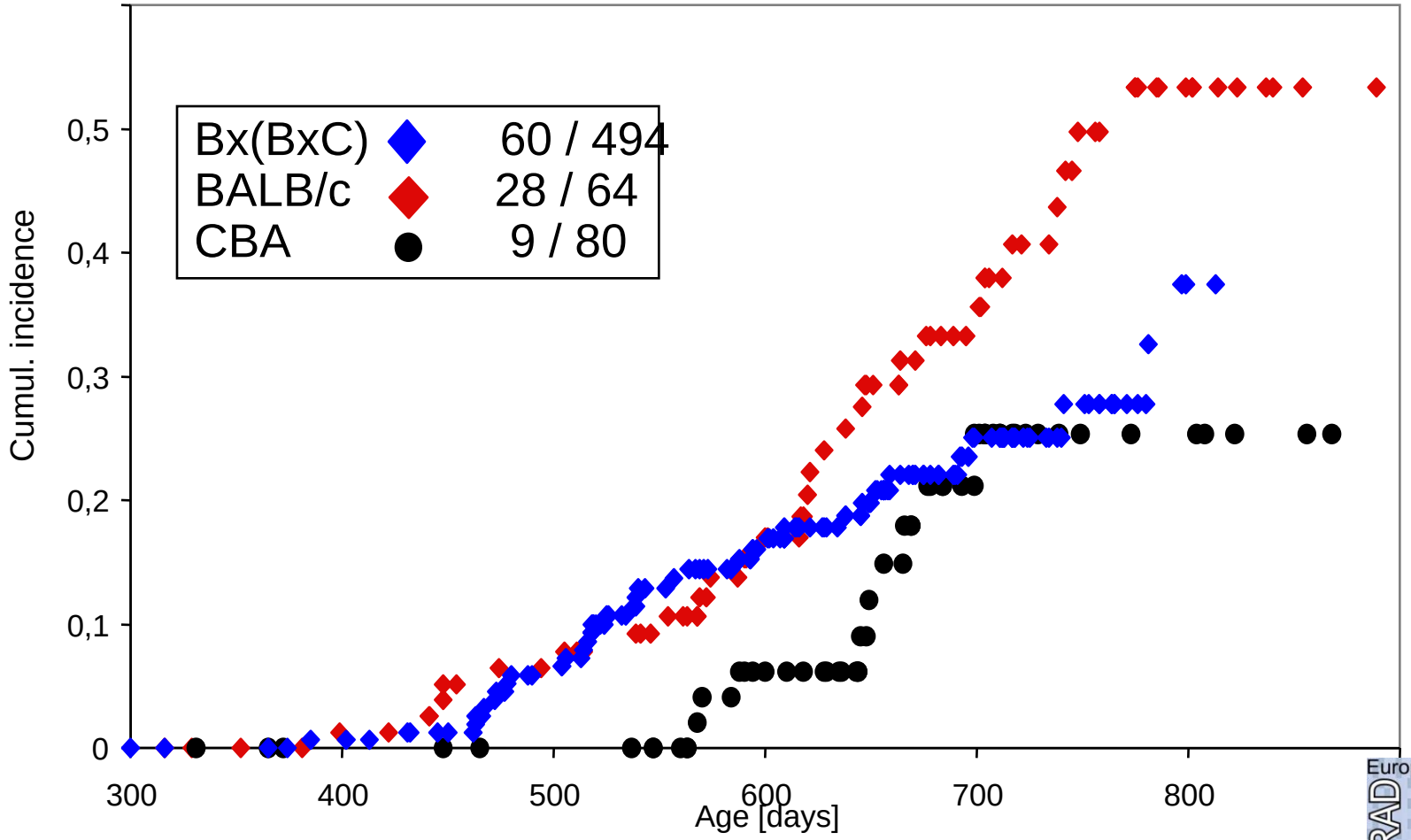
Kong14SS *Ch7 102S: Ch14 C3HS*

Data from Rosemann & Atkinson RISCRAD

HelmholtzZentrum münchen
German Research Center for Environmental Health

European Project IP-FP6 RISC-RAD 2004-2008

OS incidence in a backcross of BALB/c x (BALB/c x CBA)



Data from Rosemann & Atkinson RISCRAD

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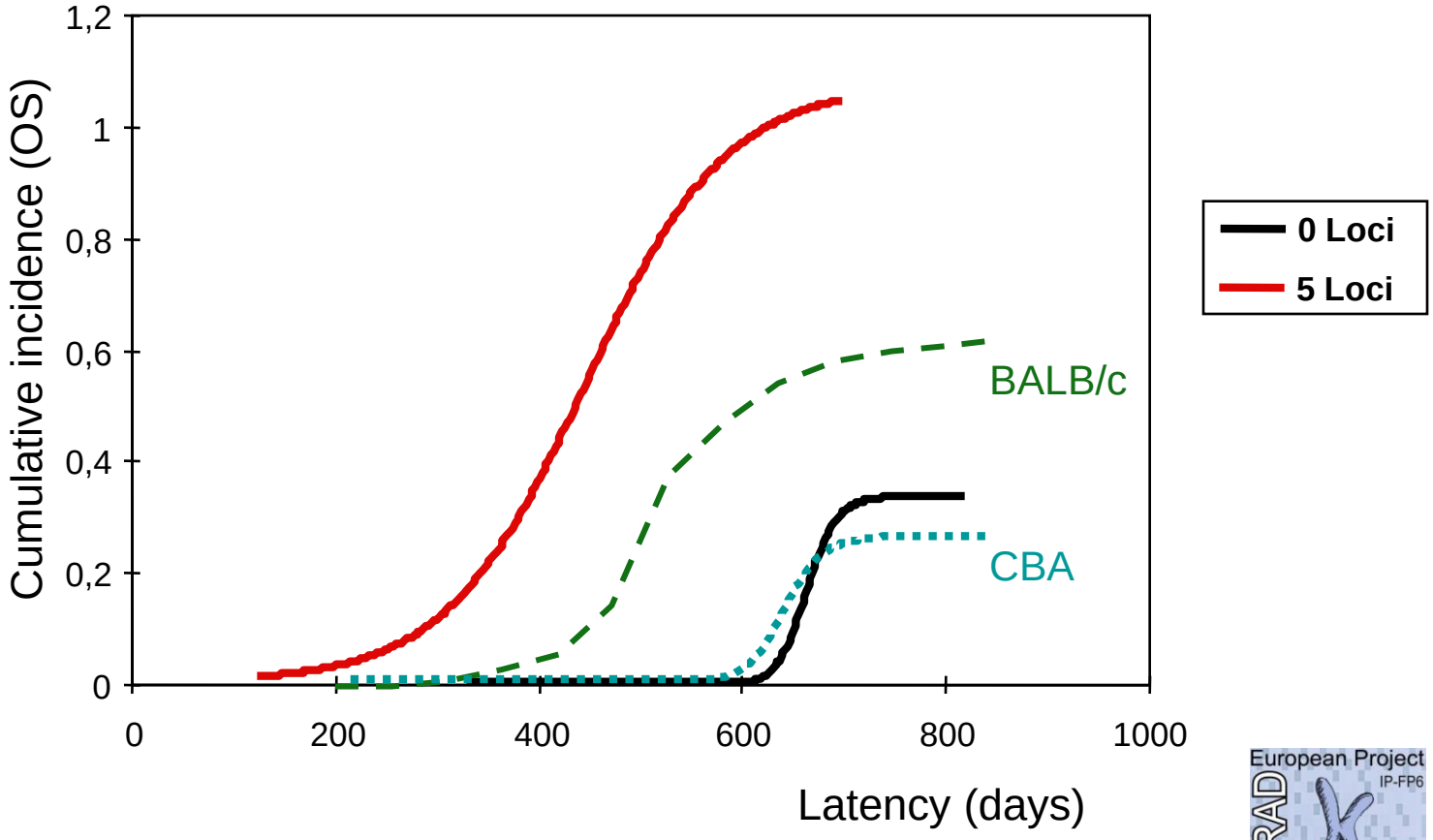
Multiple loci interact to determine sensitivity to Th227-induced osteosarcoma



Data from Rosemann & Atkinson RISCRAD

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RISCRAD has established that epistatic genes influence the sensitivity to a range of tumor types induced by different radiation qualities.

GENRISK-T has extended these observations to include thyroid cancer.

Strain-dependent distribution of proliferative thyroid lesions after treatment with Iodine 131

Genotype	Irradiated	Control
(C3H x C57BL6) F1 Hybrids	31% normal **69% lesion**	94% normal **6% lesion**
	Irradiated (29)	Non-irradiated (23)
(JF1 x C3H) F1 Hybrids:	76% normal **24% lesion**	100% normal **0% lesion**
	Irradiated (24)	Non-irradiated (9)
(BALB/c x C3H) F1 Hybrids:	38% normal **62% lesion**	89% normal **11% lesion**

Data from Rosemann & Atkinson GENRISK-T

What if ....

Mouse models of human radiation-induced cancer syndromes were used to address the problem of low dose sensitivity?

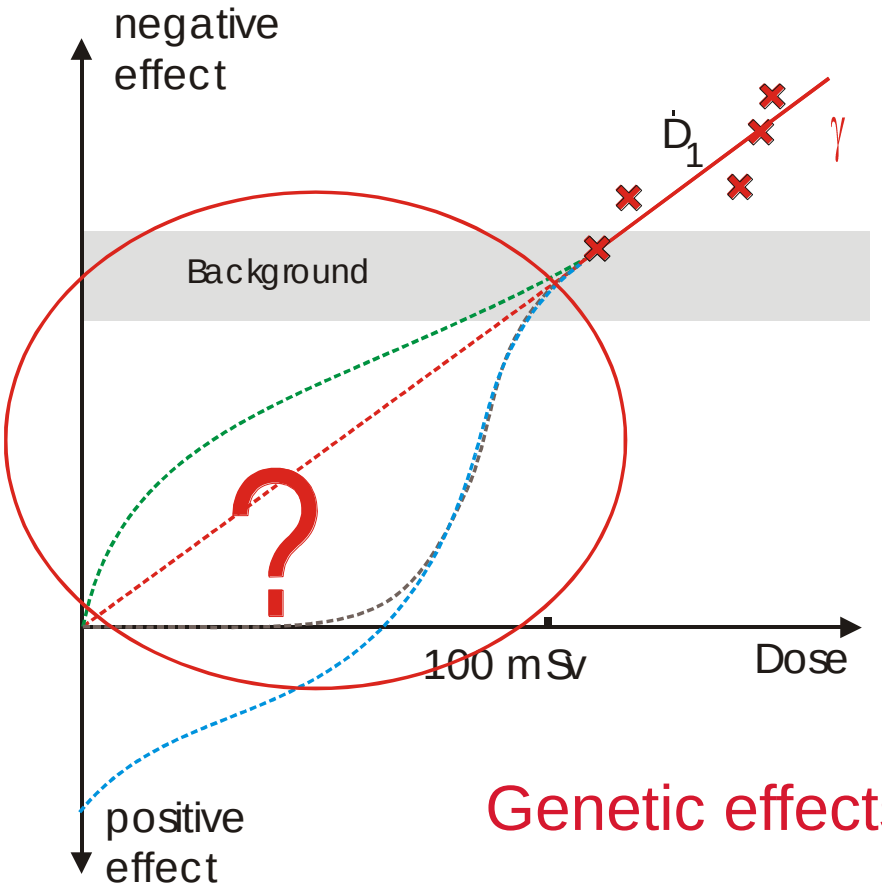
Genetic sensitivity and low dose responses

Assessment of risk,.....in the dose region from fractions of mGy to a few tens of mGy, would be greatly facilitated by knowledge of the ***shapes of the dose-response relationships*** for radiation induced cancers in humans.

.....not available and not likely to be obtained by direct observations.

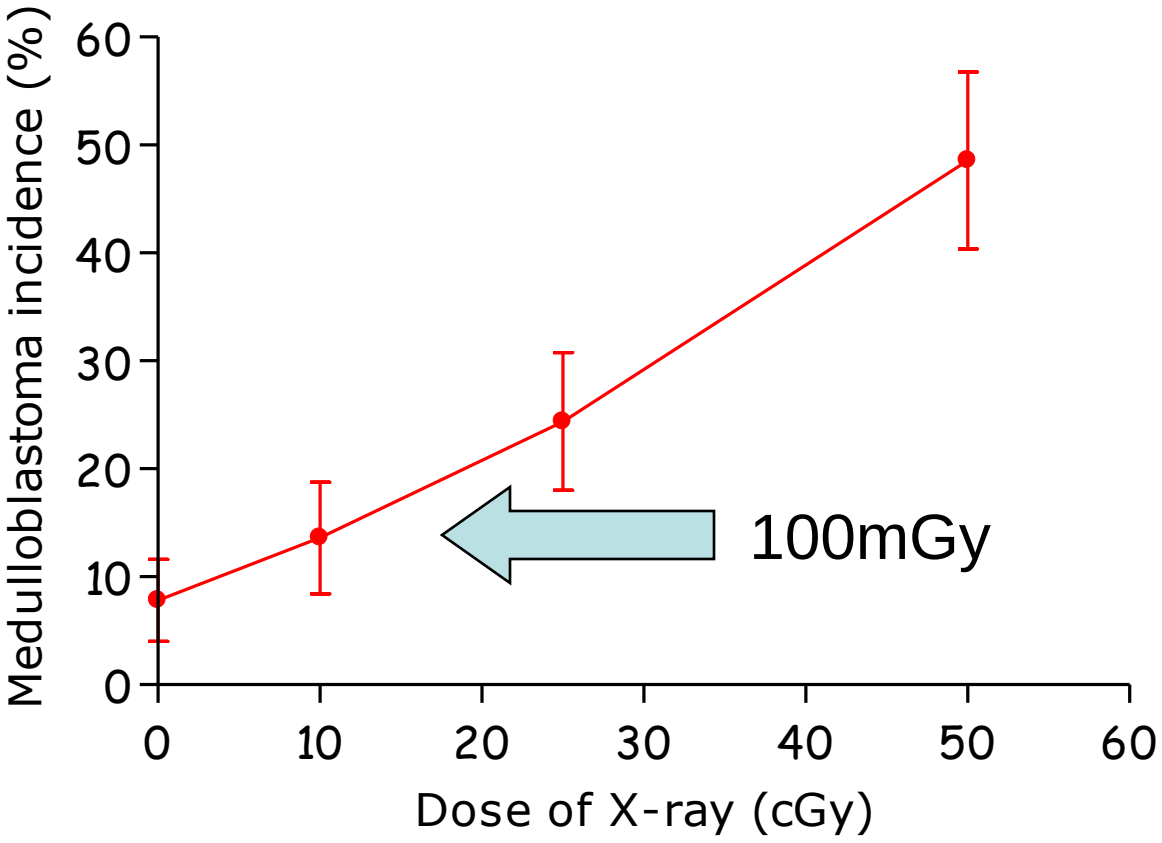
UNSCEAR 1986

Uncertainties in risk extrapolation to low doses



Genetic effects ?

Survival and tumorigenesis in Ptc1+/- mice irradiated with 250kV X-rays at postnatal day 1



Data from Saran & Pazzaglia, RISCRAD

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What if ....

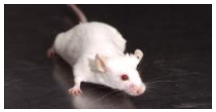
Are there differences between man and mouse?

Are the genetic mechanisms studied at high doses (secondary cancers) relevant to low dose risk?

If there is a genetic influence, how do we evaluate contribution to risk. Is it the same for all tissues and radiation qualities?

Does dose rate influence susceptibility?

Ethical concerns about prediction of phenotype from genotype?



Helmholtz München

Radiation Biology	Michael Rosemann Jan Smida Soile Tapio
Pathology:	Leticia Fend
Genetics:	Jochen Graw Kenji Imai Jack Favor
Radiation Protection	Wolgang Heidenreich Herwig Paretzke
Molecular Radiobiology	Horst Zitzelsberger

EU project partners

Osteosarcoma

CEA	Sylvie Chevillard
NRG	Rene Huiskamm

Patched mouse model

ENEA Rome	Anna Saran Simonetta Pagazzlia

Thyroid cancer

Imperial College	Gerry Thomas
Napoli	Massimo Santorino

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