Genomic Instability at low dose levels

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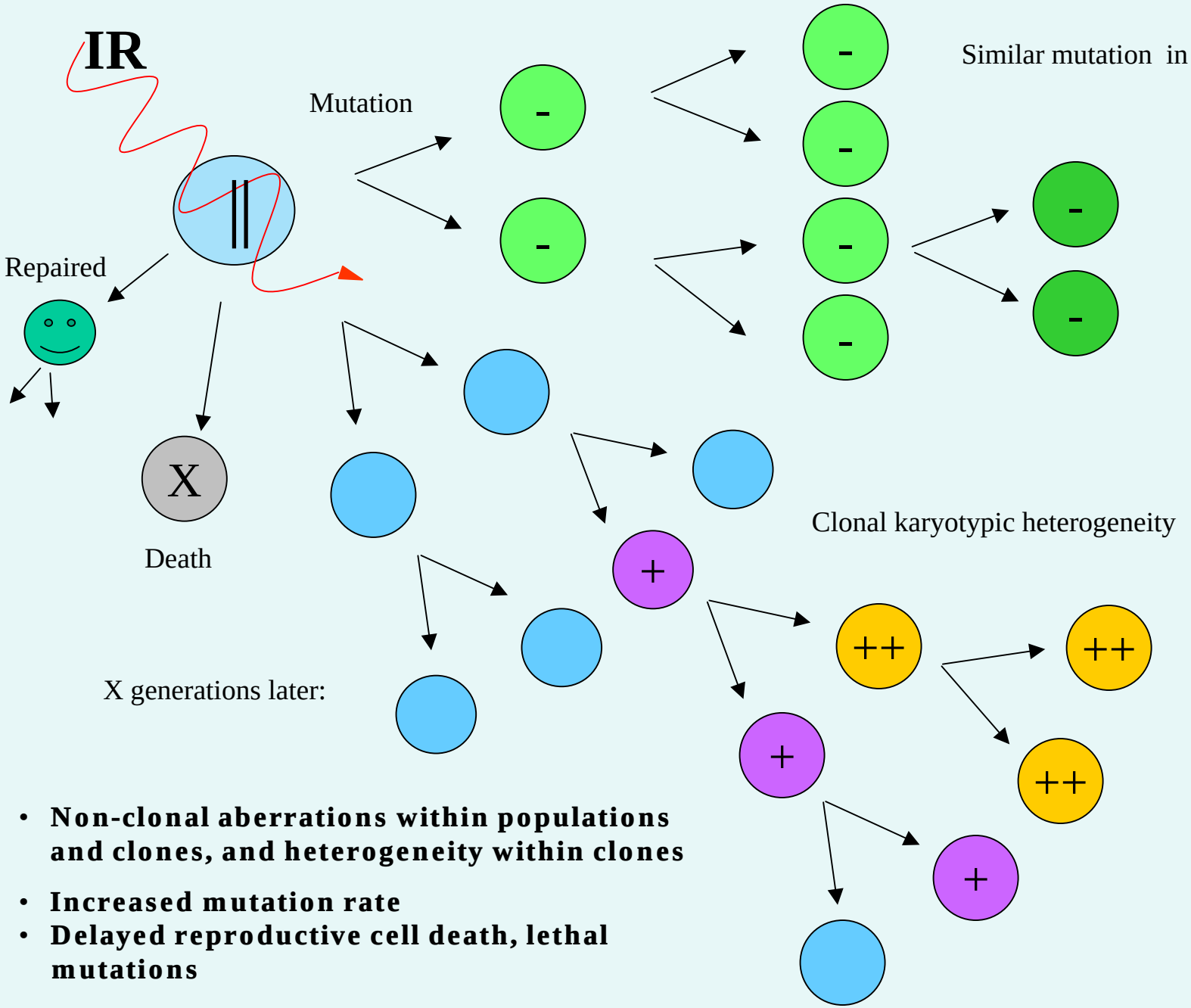
Genomic Instability (GI)

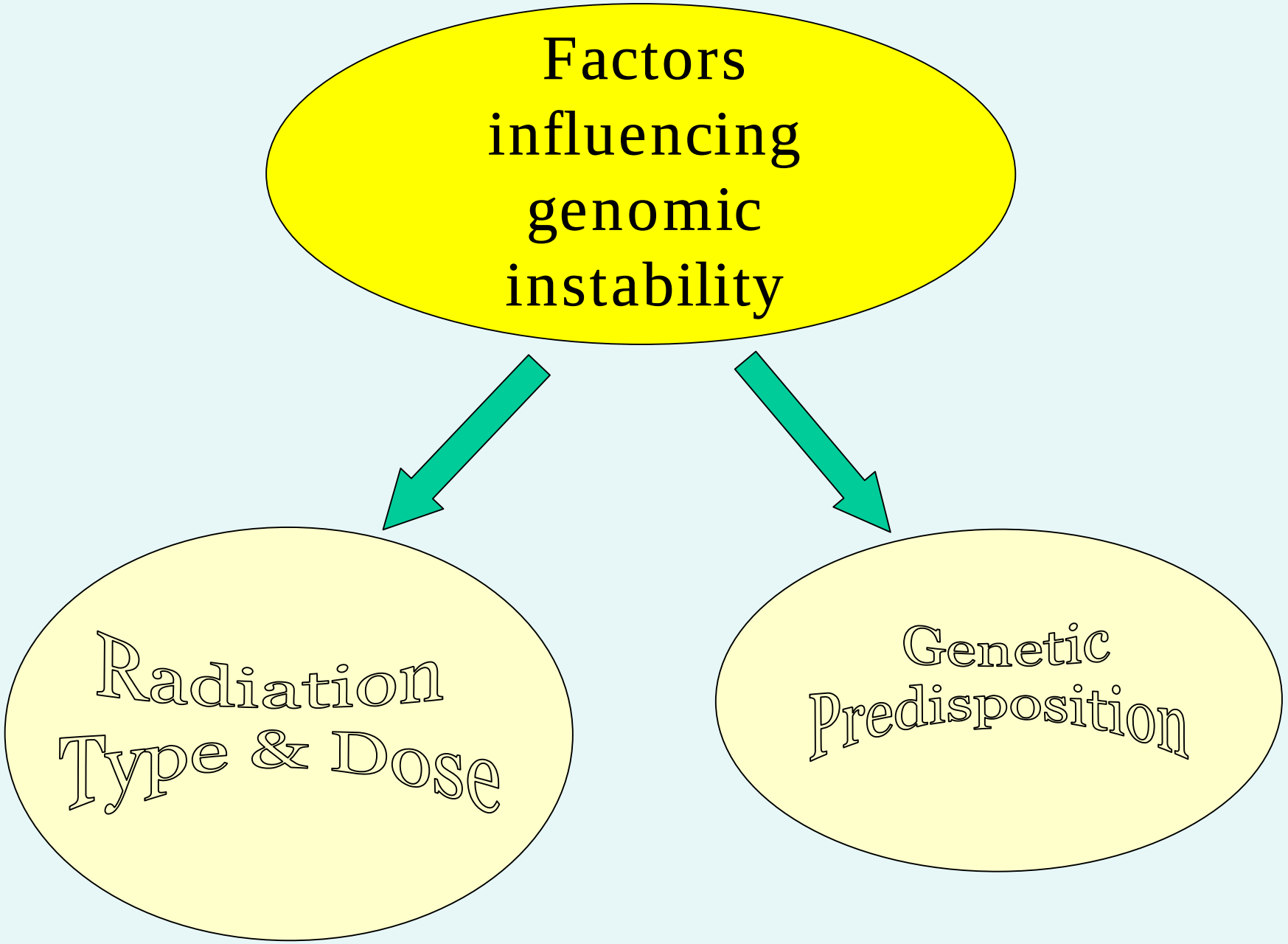
- The hallmark of tumorigenic progression
- Persistent elevated rate of accumulation of genetic alterations within a clonal population
- Its heterogeneous phenotypic manifestations:
  - delayed chromosomal aberrations
  - delayed reproductive cell death
  - increased mutation rate
- Exposure to Ionizing radiation induces a phenotype in the surviving progeny resembling that observed in tumors

Factors affecting expression of GI:

- Radiation dose, LET, and exposure conditions
- Genetic predisposition
  - Protein expression or activity differences



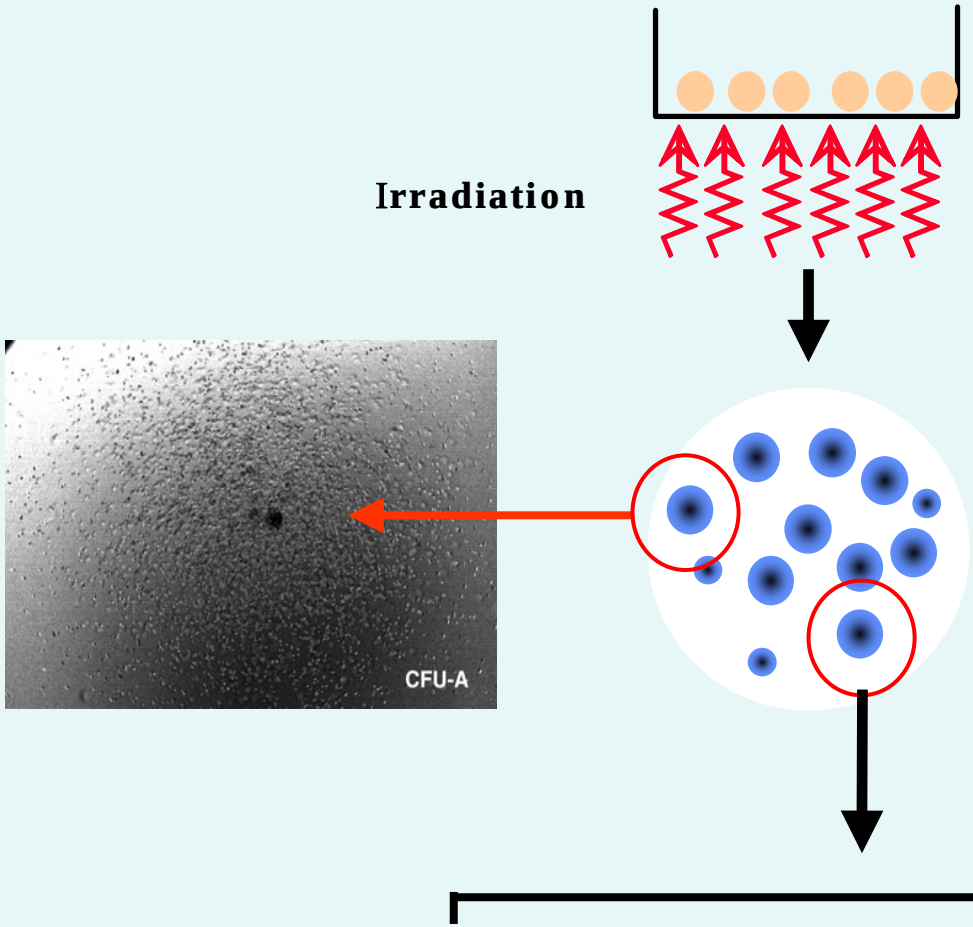
- **Non-clonal aberrations within populations and clones, and heterogeneity within clones**
- **Increased mutation rate**
- **Delayed reproductive cell death, lethal mutations**
- **Induced at higher frequency than expected**



Early studies in our lab

- Irradiated mouse haematopoietic stem cells:
  - Broadfield alpha particle irradiation (^{238}Pu) (0.25 through 1 Gy)
  - 250 KeV X-ray (3 Gy)
- Cytogenetically analysed individual surviving clones.

Clonal analysis

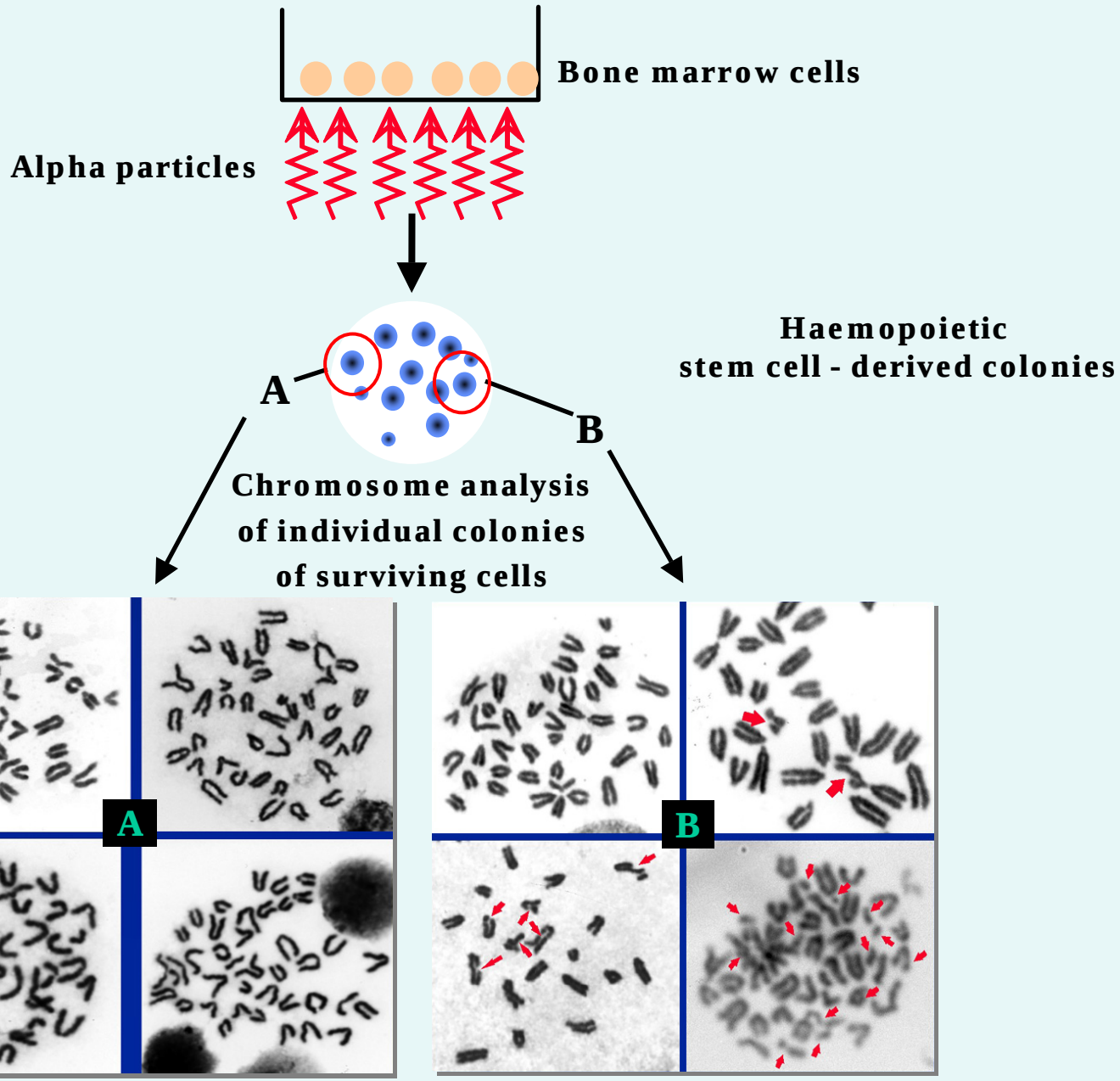


Haemopoietic
stem cell - derived colonies

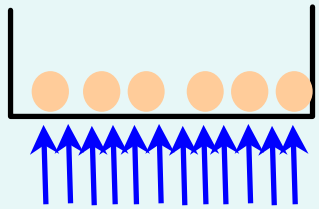
Determination of survival

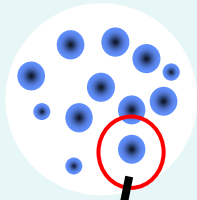
Chromosome preparation from individual colony for chromosome analysis

Analysis of γ-H2AX
Western Blot for p53, ATM
(and phoso-forms)



Unexpected and large heterogeneity in chromosomal damage within each aberrant colony

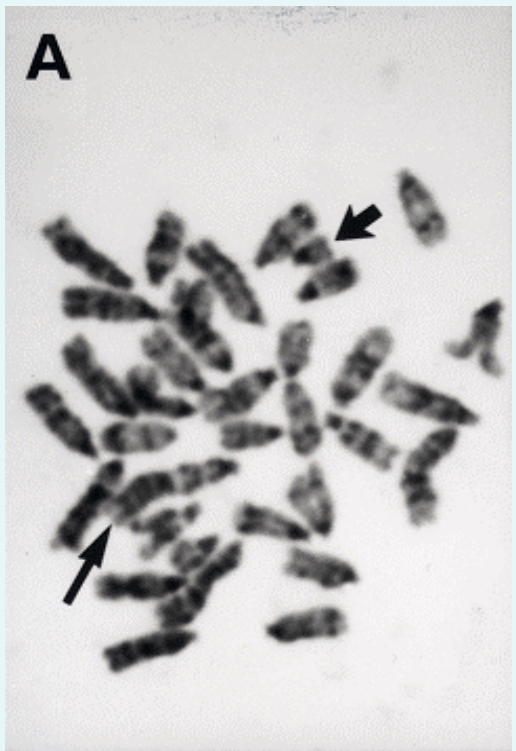




Haemopoietic
stem cell - derived colonies

Clonal descendants of an X-irradiated normal murine haemopoietic stem cell

Clonal cytogenetic analysis



Arrows:
t(2;17)(q;qter),
+frag(2)(cen)

Non-clonal cytogenetic aberrations (GI) in colonies derived from CFU-A in marrow exposed to alpha-particles, but not 3 Gy X-rays

Radiation Exposure (Gy)	Metaphases with aberrations (%)	Mean aberrations per cell
0	7/432 (1.6)	0.02
alpha-particles		
0.25	8/29 (27.6)	0.34
0.50	19/92 (20.7)	0.27
1.00	26/107 (24.3)	0.41
X-rays		
3.0	2/409 (0.5)	0.04

Kadhim *et al.* Nature 1992

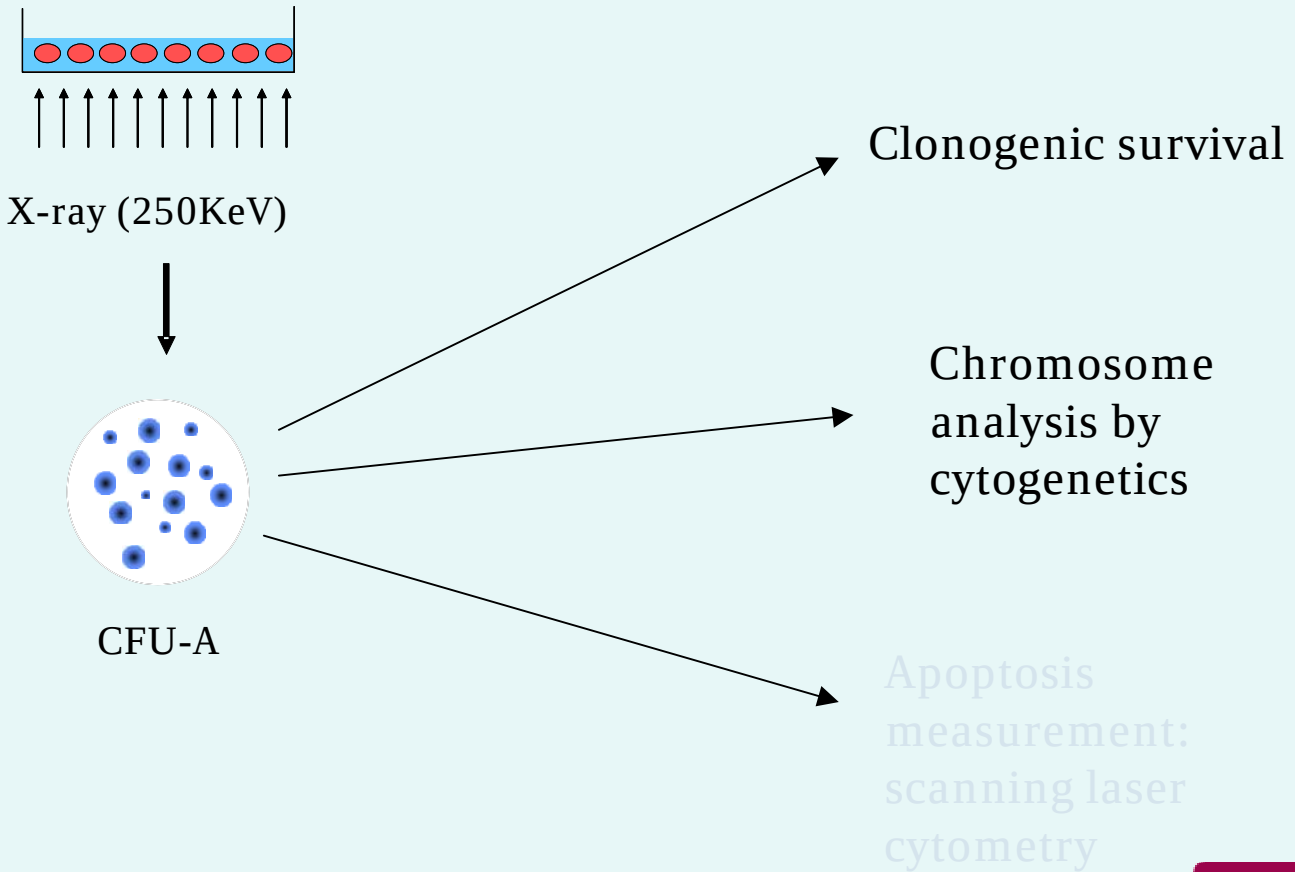
Discrepancy between high and low LET with respect to initiation of GI, could be due to several factors:

1. Track structure? No.
2. Interaction between hit and non-hit cells (BE)? Likely.
3. Dose?

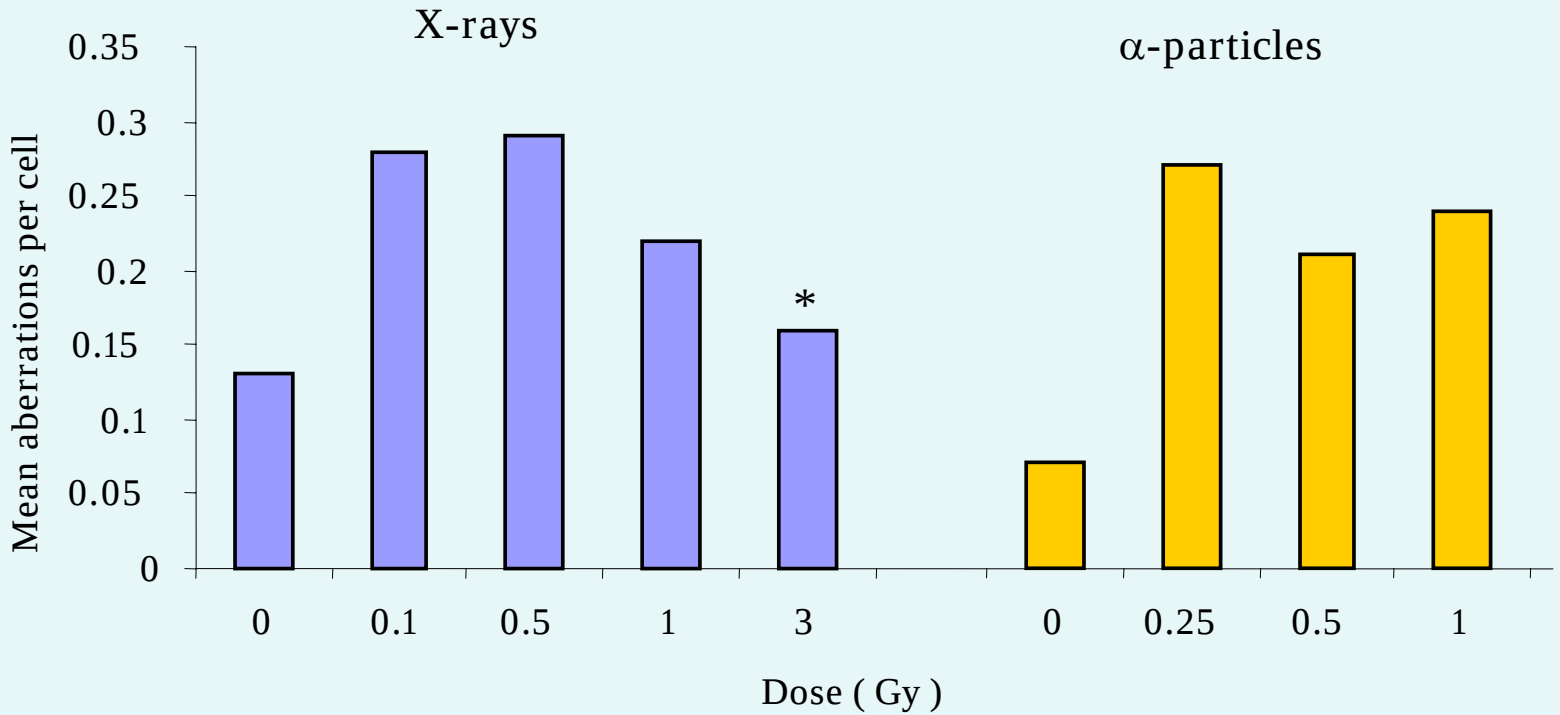
Methods to address X-ray dose issue:

Femoral bone marrow isolated from CBA/H mice

Exposed to several doses (0.1 – 3 Gy) 250 KeV X-rays



Low LET induced chromosomal instability at low doses



* Not significant vs. control

These results suggest that instability is a consequence of dose with low LET irradiations.

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Low LET radiation

Genetic differences in susceptibility to GI have been observed after low doses of high LET and high doses of low LET radiations...

Are these same genetic differences evident after low (environmentally-relevant) doses of low LET radiation?

Genetics and GI: Human genetic disorders

Important genetic disorders exhibit GI and are radiosensitive

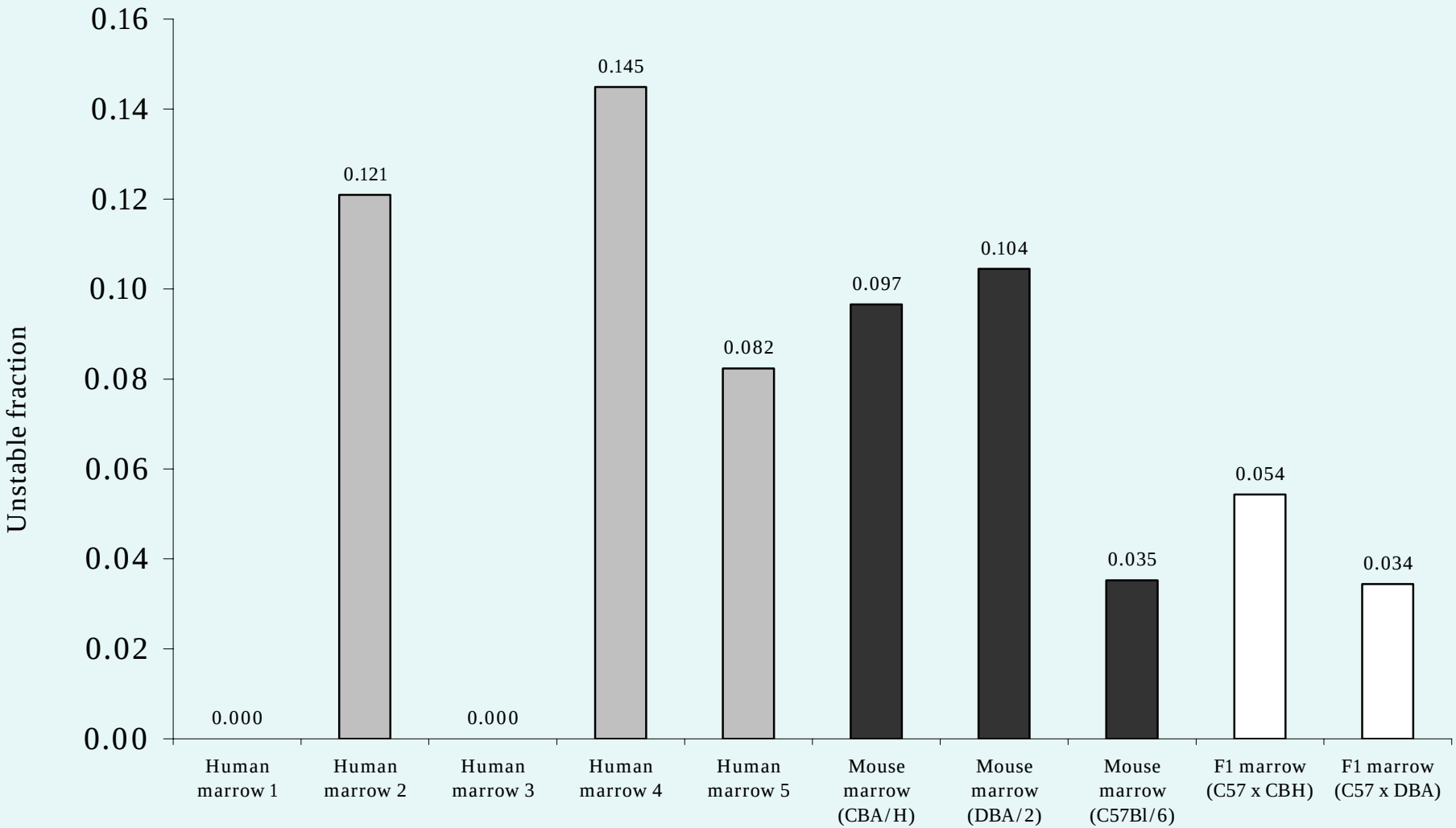
Fanconi Anemia
Li Fraumeni syndrome
Nijmegen Breakage Syndrome
Ataxia Telangiectasia
Cockayne's Syndrome

- Fanconi Anemia
  - Spontaneous CS breakage
  - Aberrant superoxide metabolism
  - Failure to thrive
  - Predisposition to develop leukemia

Genetics and GI: Induced by Radiation

- High dose (3 - 50 Gy) γ-irradiated mouse mammary epithelial cells (Ponnaiya *et al.* 1997).
  - Deficiency in DNA/PKcs (Okayasu *et al.* 2000).
- α-partical Irradiated human& mouse heamopoietic stem cells (Kadhim et al. 1994; Watson *et al.* 1997)
  - In mouse, GI is linked to difference in respiratory burst activity (Watson *et al.* 1997)
- Irradiated human & mouse urothelial cells (up to 5 Gy ^{60}Co) (Mothersill *et al.* 1999).
  - In mouse, GI phenotype is related to the apoptotic initiator, bcl2.
- Irradiated primary human fibroblasts (Kadhim *et al.* 1998).
  - Complex/unknown genetic component
- Variation in background and IR-induced cytokine levels from healthy human donors, may be related to differences observed in GI (Moore *et al.* in revision).

Differential induction of genomic instability in human and mouse bone marrow following alpha particle irradiation



Kadhim, Oncogene 2003 ; Kadhim *et al.*, 1994; Watson *et al.*, 1997

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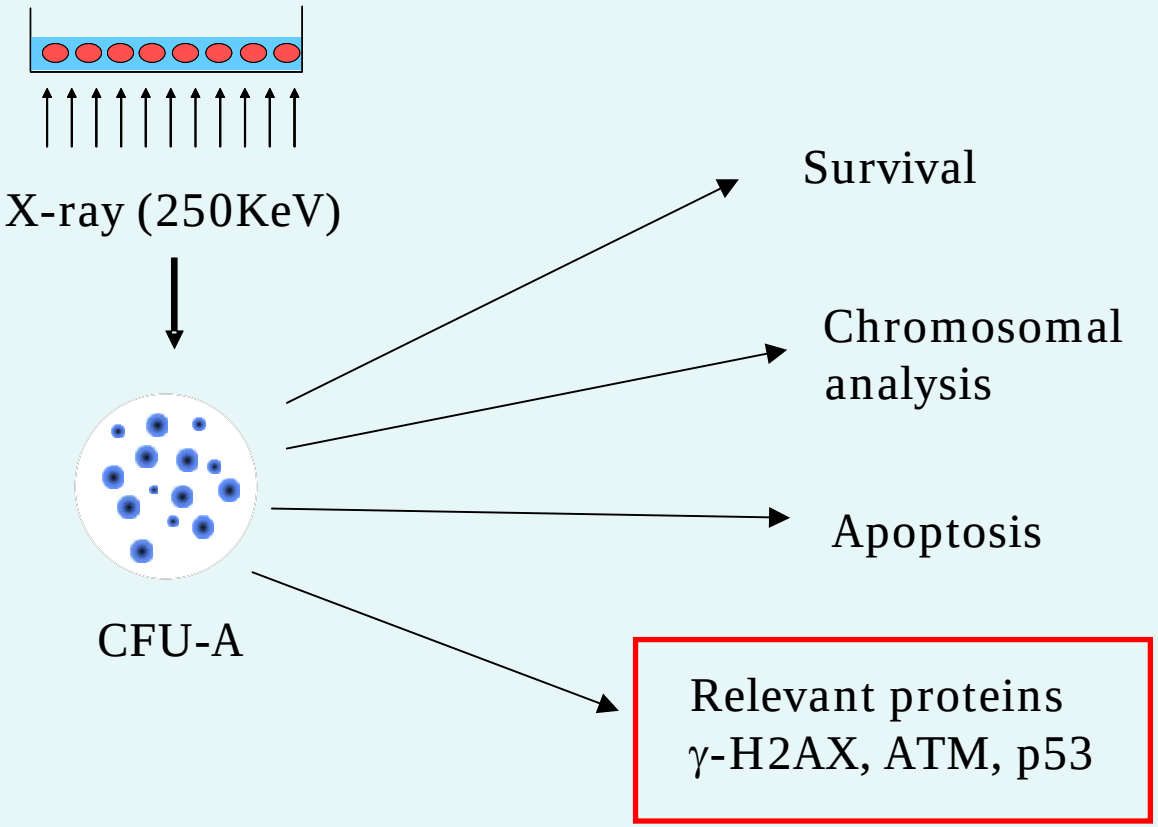
Low LET radiation

To Investigate in to the genetic differences after low doses of low LET radiation?

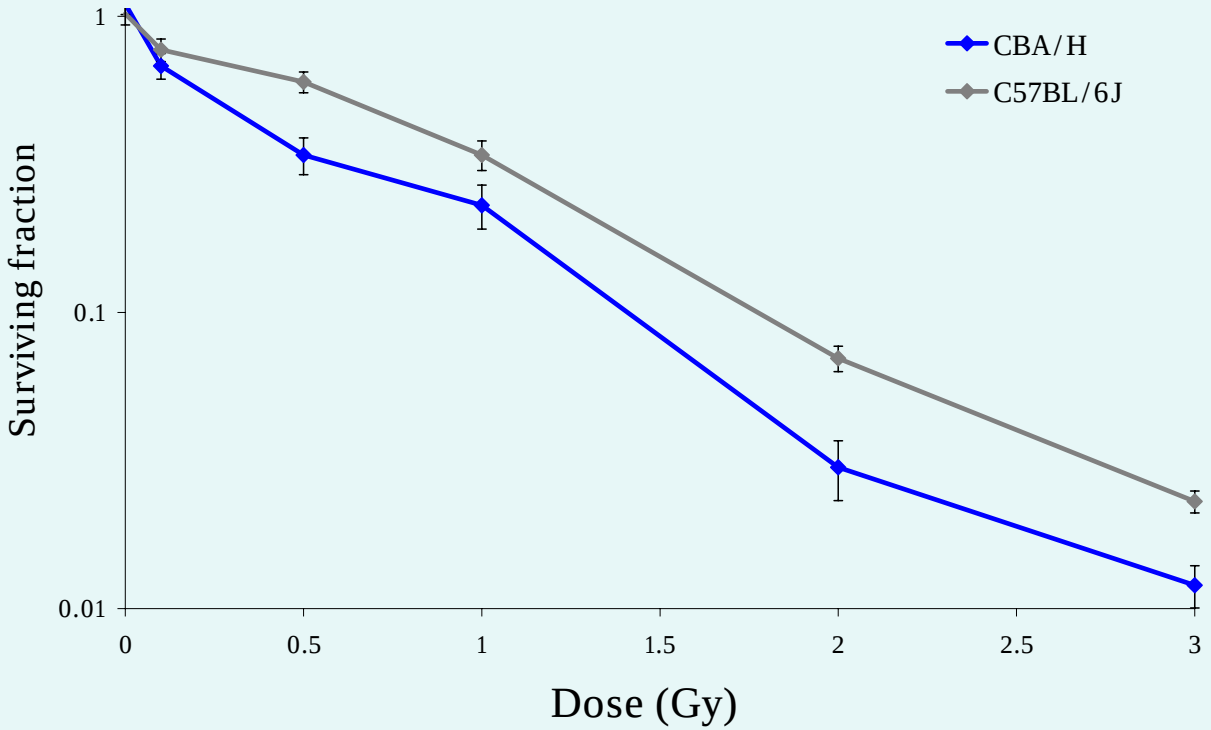
Low Dose low LET - Genetics Predisposition?

- Femoral bone marrow isolated from CBA/H and C57BL/6J mice
- Exposed to several doses (0.1 – 3 Gy) 250 KeV X-rays



Survival and cell target number

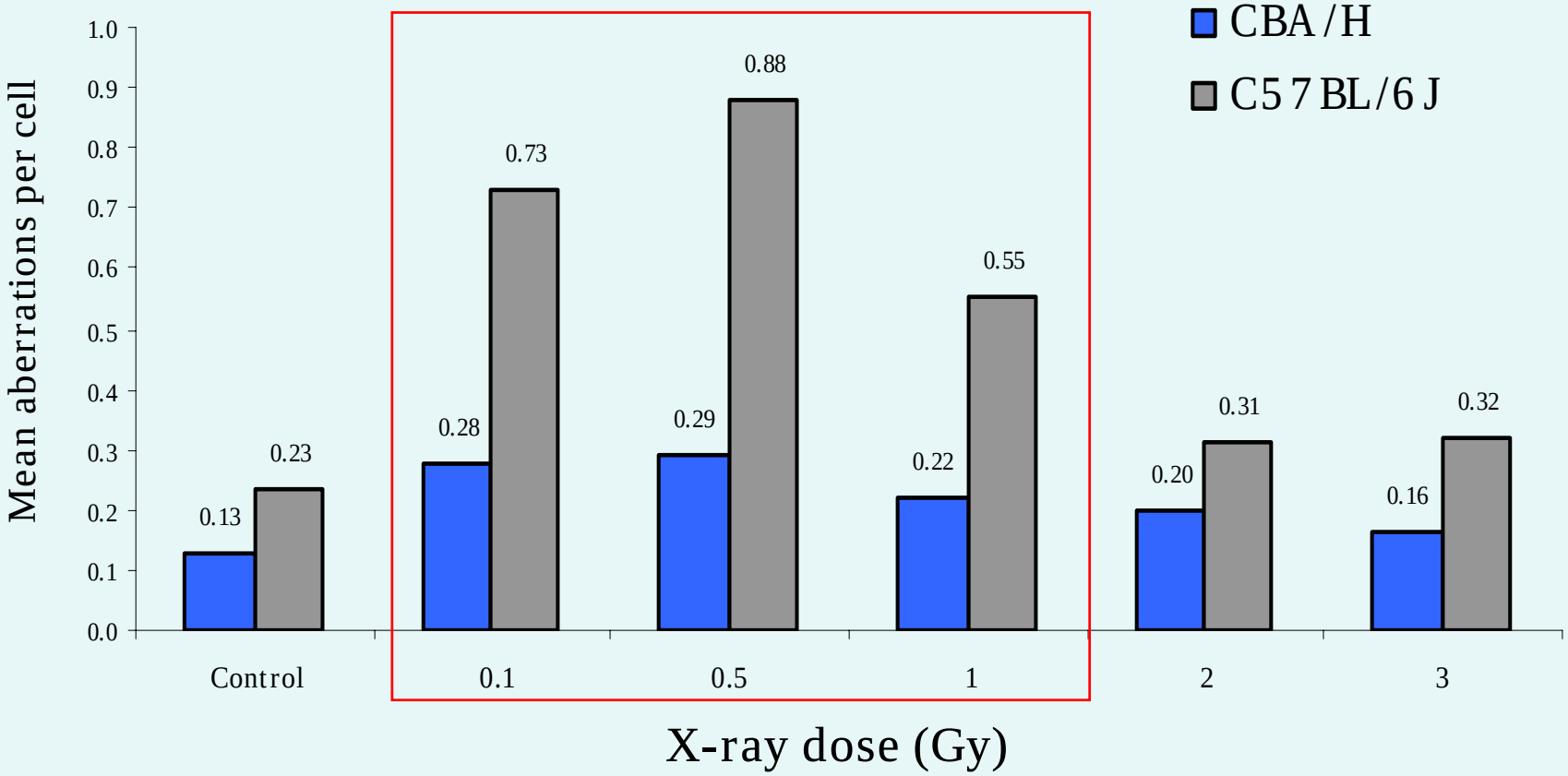
Overall, survival is significantly reduced in CBA/H compared to C57BL/6J after low LET X-rays (p=0.013).



Potential target cell number are 2 – 7 x greater in C57BL/6J

	Mouse Strain		
Stem cells	CBA/H	C57BL/6J	Fold difference
CD34+	0.27%	1.23%	4.6
LY6+	0.07%	0.50%	7.1
CD34+/LY6+	0.08%	0.15%	1.9

Magnitude of TGI is strain-dependent

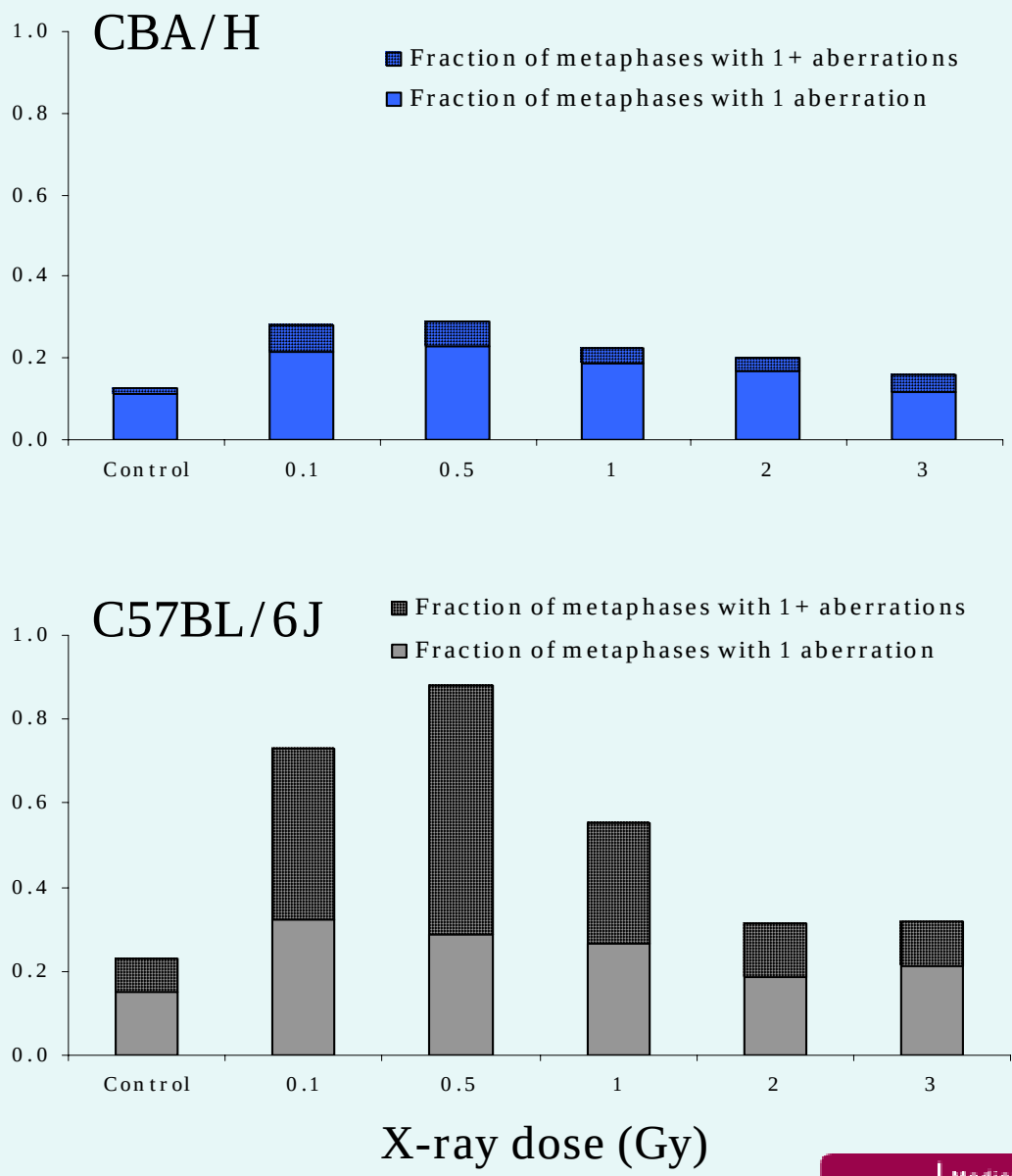


- Instability is induced in clones derived from both mouse strains
- No significant induction of GI at high doses (2 and 3 Gy)
- Magnitude of induction shows some strain specificity. May be due to the contribution of heavily damaged cells.

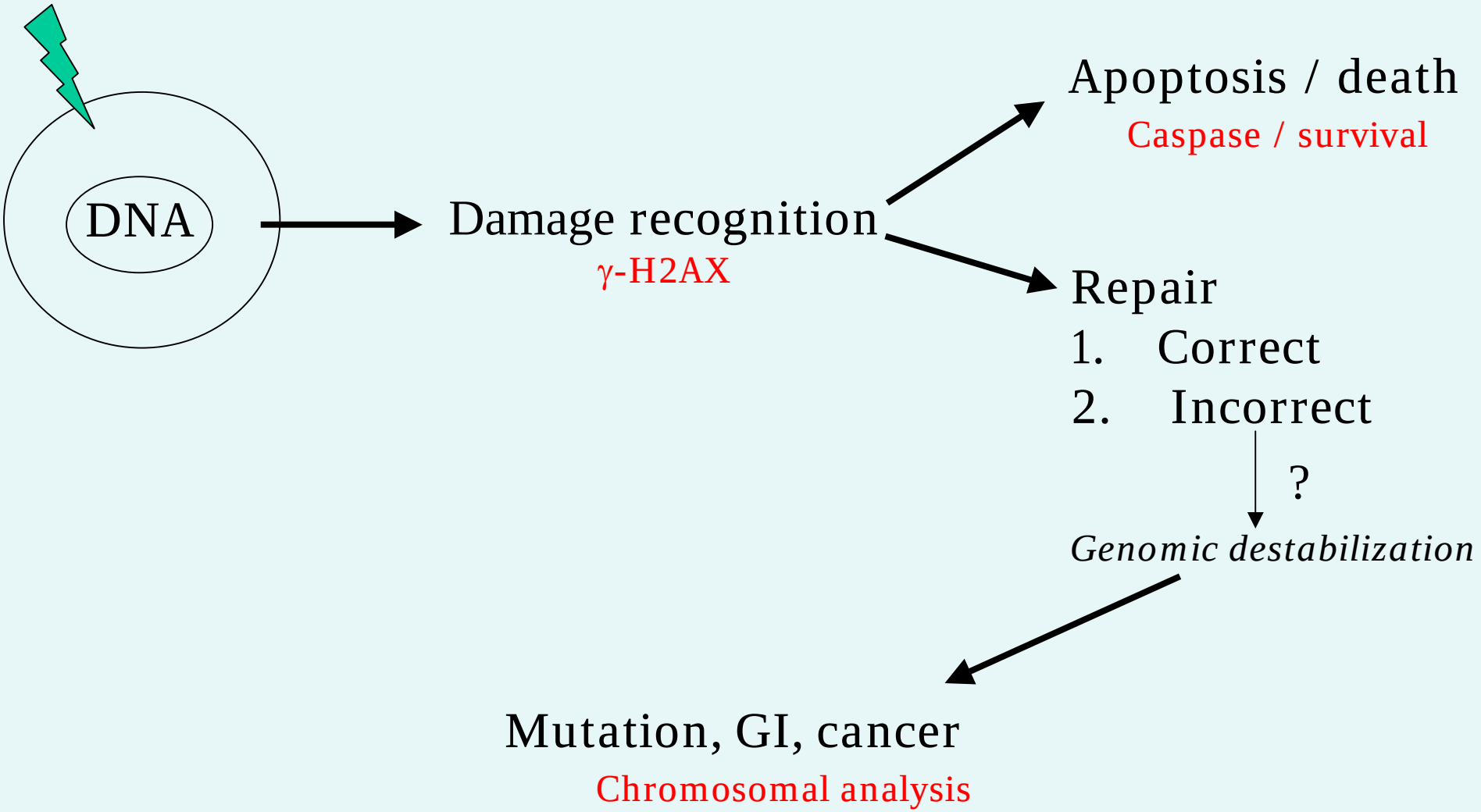
Qualitative GI differences between strains are evident

Heavily damaged cells (chequered):

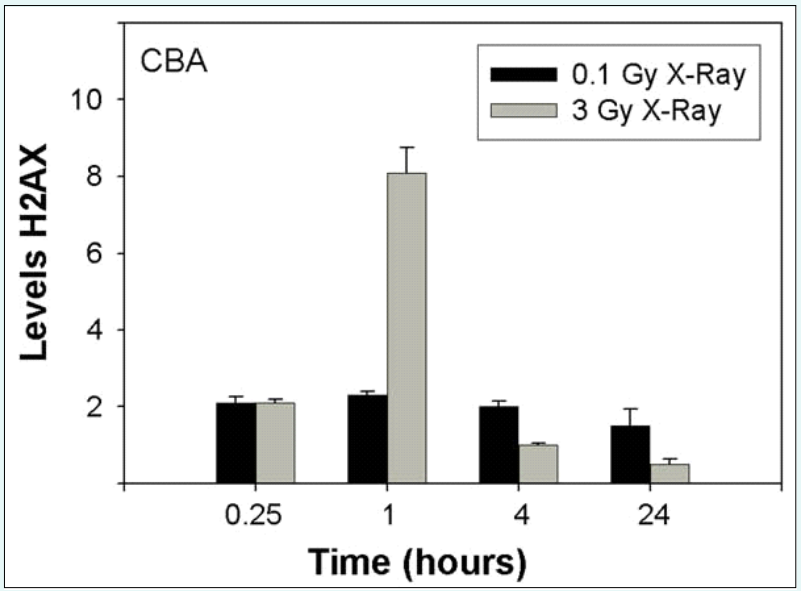
- Greater in C57 at all doses
- Inversely dose-dependent within the C57 strain
- The level of instability between the strains is similar if only moderately damaged cells are considered (solid).

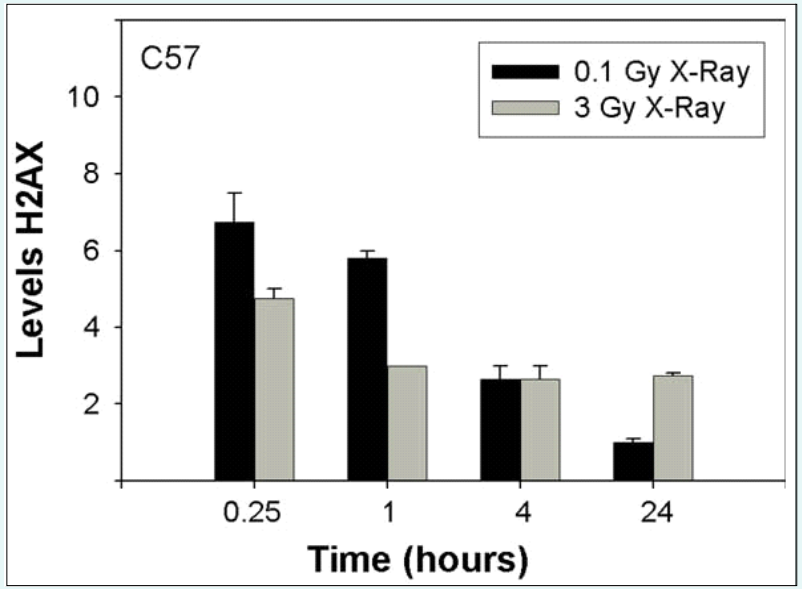


Genetic Predisposition - The How & Why questions.



Recognition of DNA damage

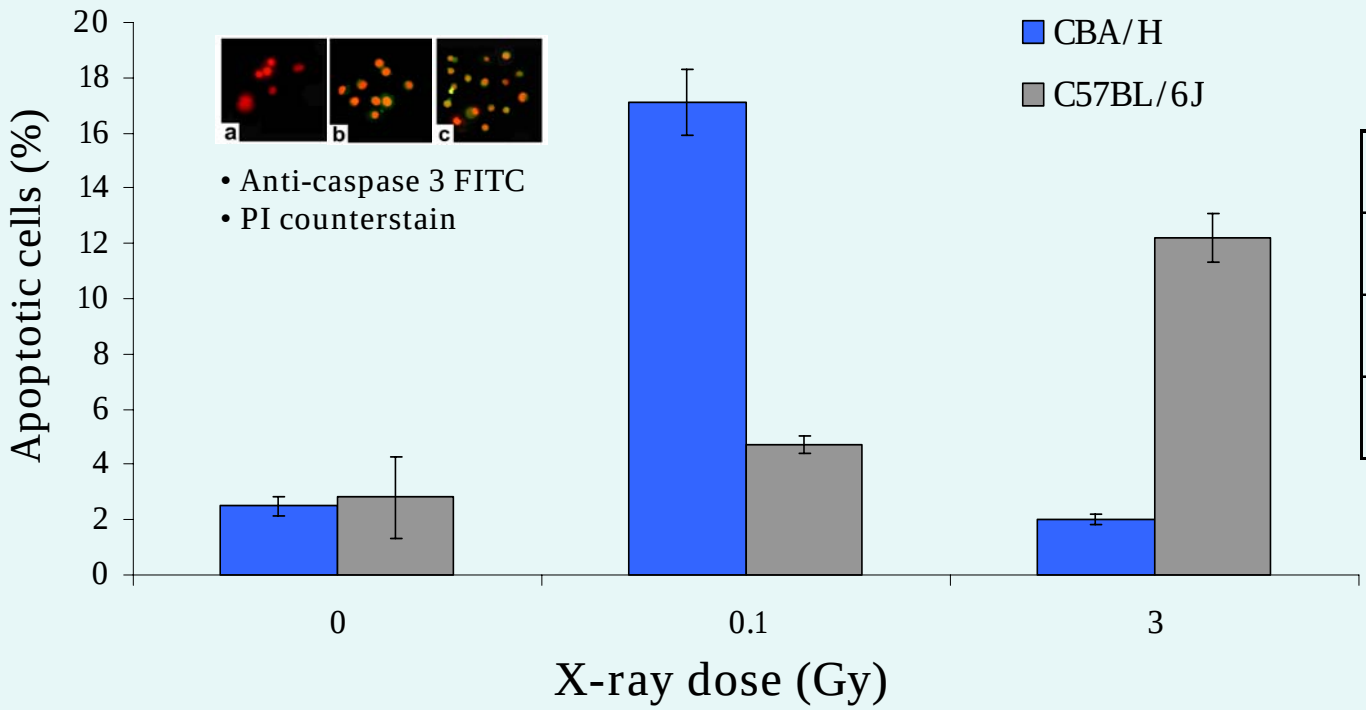




After low dose irradiation, DNA damage is not recognized as efficiently in the CBA strain as in the C57 strain.
DNA damage is recognized efficiently in both strains after high doses, but in the C57 strain residual damaged cells persisted up to 24hrs.

Although levels of damage (by γ-H2AX staining) drop in C57, the delayed chromosomal aberration indicate that the 'repaired' breaks are likely misrepaired, leading to cells with more than one aberration.

Clearance of damaged cells is strain-dependent



	Fraction of metaphases with aberrations	
Dose (Gy)	**CBA/H**	**C57BL/6J**
0	0.11	0.15
0.1	0.21	0.32
3	0.12	0.21

At the time when GI was measured, CBA was still efficiently removing via apoptosis that weren't originally recognized as damaged, and apoptosis was low in the 3 Gy group, where damage may have been efficiently recognized and repaired earlier.

Apoptosis was much lower in C57 at low doses, as damaged cells were recognized early. Apoptosis is higher in the 3 Gy group as the γ-H2AX data showed that damaged cells remain.

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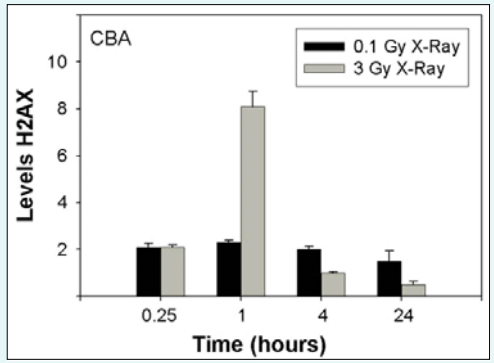
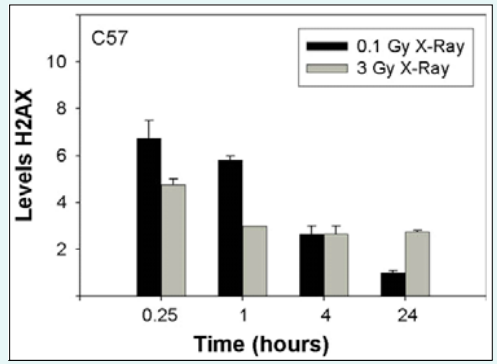
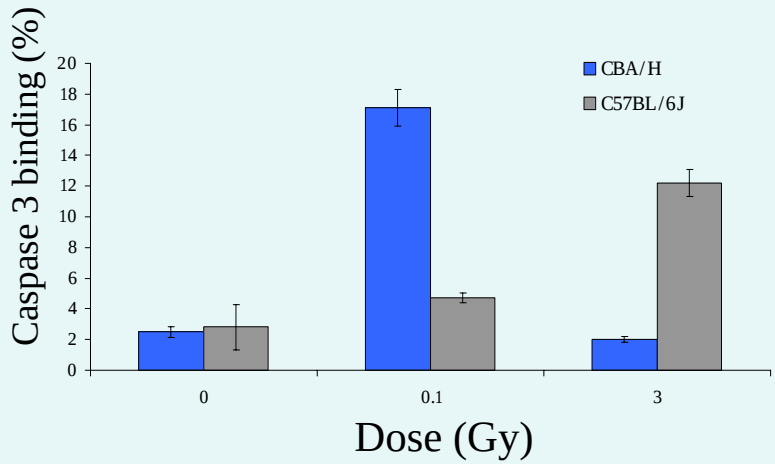
After irradiation, DNA is not recognized as efficiently in the CBA strain (except after high doses, where the damaged cells are removed or repaired rapidly) as in the C57 strain where damaged is recognized efficiently at both doses, but residual damaged cells remain with time.



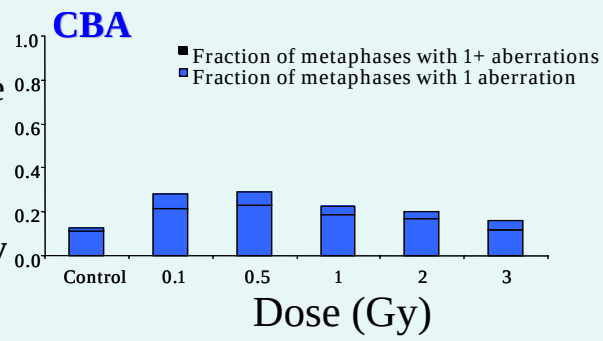


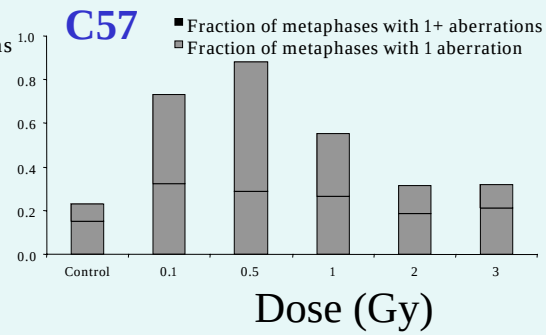
At 7 days post-irradiation, CBA is removing cells via apoptosis that weren't originally recognized as damaged, and apoptosis is low in the 3 Gy group, where damage was efficiently recognized.
Apoptosis is much lower in C57 at low doses, as damaged cells were recognized early.
Apoptosis is higher in the 3Gy group as the γH2AX data showed that damaged cells remain.



Although levels of damage (γH2AX) drop in C57, the delayed chromosomal aberration data indicate that the 'repaired' breaks are likely misrepaired, leading to cells with more than one aberration, cf. CBA.

These results indicate that subtle genetic differences between the strains (CBA is likely deficient in ATM and C57 is likely deficient in a protein that is integral to cell fate decisions, such as p53).





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Conclusions

- Significant induction of GI was observed in both strains with low doses of low LET.
- Genetic differences between the strains could be attributed to a contribution from heavily damaged cells, likely removed efficiently by apoptosis in CBA.
- With high levels of background γ-H2AX (and likely DNA damage), it is possible that cellular programs are up-regulated to deal with exogenous damaging agents via efficient apoptosis or repair.
- The results indicate the chromosomal instability may be a non-threshold response, but apoptosis appears to exhibit a genetically-dependent threshold.
- The genetic differences reported here may have implications for risk assessment in heterogeneous populations.

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Funding

Medical Research Council

Commission of the European Communities

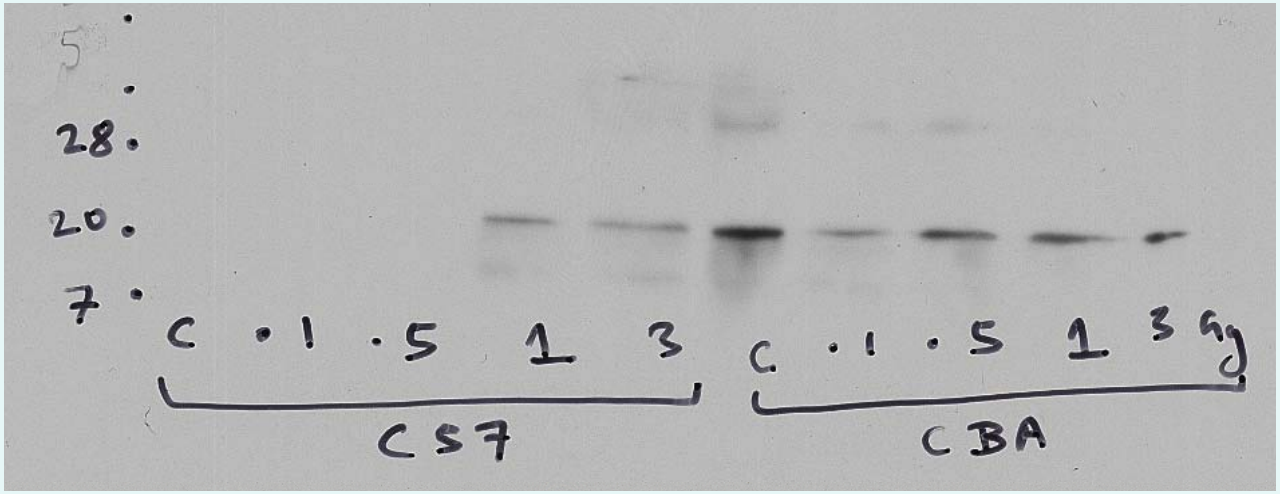
US Department of Energy

MRC Medical Research Council

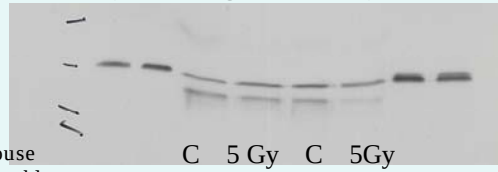
Thank You

Any Questions?

γ-H2AX Western Blot, t=0hr



Actin (loading control)



Cells were collected immediately post-IR and proteins were evaluated using Western blot.

In C57: As dose increased, so did protein levels

In CBA: High levels of background protein were observed (background damage?) which decreased after IR

	CBA		C57	
	C	IR	C	IR
γ-H2AX	+	+++	-	+
S P53	-	-	-	-

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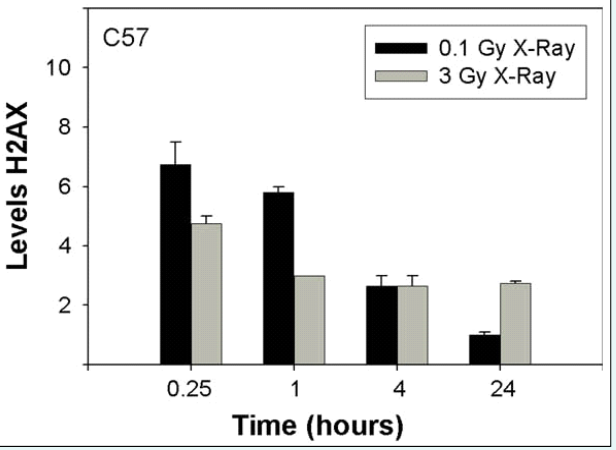
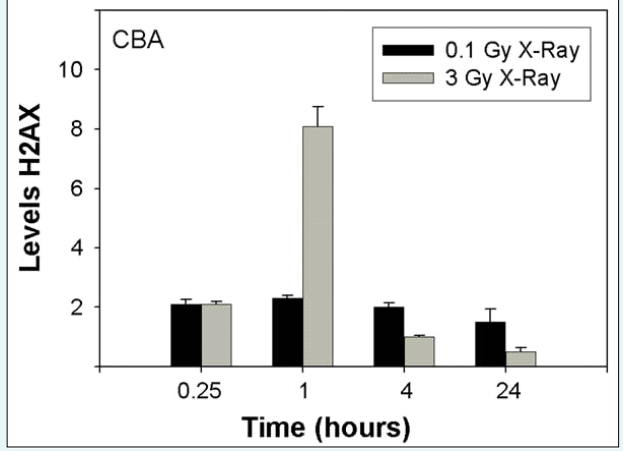
Temporal Vs. Spatial Aspects

In collaboration with the Biophysics group we have set up a study to determine whether quality dependence is primarily due to spatial or temporal properties of radiation. High-LET radiations are characterized by dense ionizations along each track, but also by an instantaneous (~10^{-12} s) higher dose (=0.2Gy) to the traversed cell. It is currently unknown, as to which of these properties is primarily responsible for instability induction, as a high frequency effect. Primary cells were irradiated with ultrasoft x-rays (usx) from a high intensity pulsed laser-plasma source previously developed in collaboration with the Rutherford Appleton Laboratory. The full dose was delivered in two different modes:

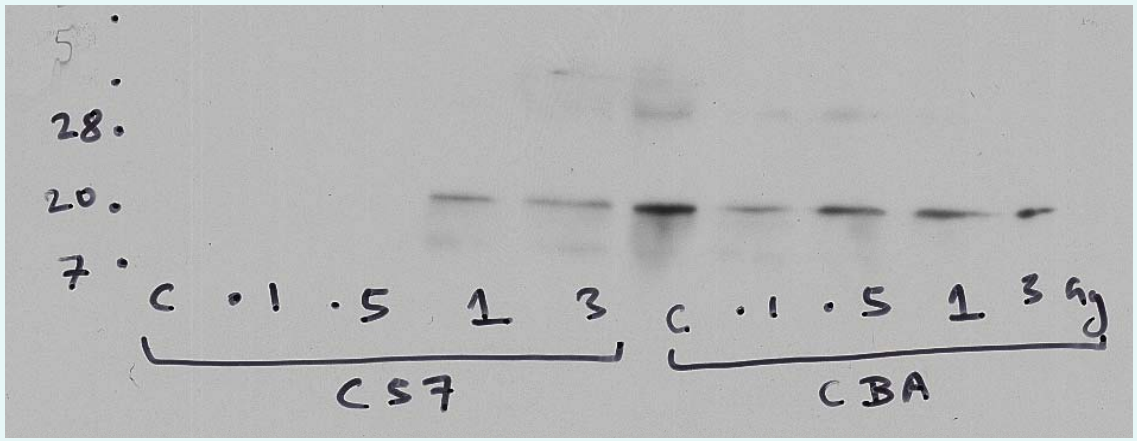
1. **High Intensity Pulsed X-ray (HIP) given in a few picosecond pulses, each pulse delivering more energy (dose) to each cell than a single α-particle track.**
2. **Low Intensity Pulsed X-ray (LIP), given in many low intensity pulses delivered in seconds to minutes similar to conventional hard X-ray irradiations.**

In both cases, we detected a consistent expression of chromosomal instability in the progeny of irradiated primary human fibroblasts. Both radiation modes are effective in inducing instability with no obvious dose dependency at the doses used. The data do not seem to be significantly different from those previously obtained with high LET radiation. It is clear that HIP and LIP modes do not differ with regard to cellular inactivation but also to the induction of transmissible chromosomal instability





The levels of γ-H2AX here are corrected to control values, ie. increase over controls. The levels of γ-H2AX here are at taken at least 15 min. post-IR



The levels of γ-H2AX here represent total protein, not in relation to control values as above. The levels of γ-H2AX here are taken immediately post-IR.

C57BL/6J requires at least some time post-IR before γ-H2AX is observed. But it follows a normal response curve after the response is mounted – either repair or removal of damaged sites / cells.

CBA/H seems to have high background damage and subsequent activation of H2AX, so that levels of γ-H2AX don't substantially rise (except in 3 Gy at 1 hr) even after IR.

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Genomic Instability Studies in Murine Haemopoietic Stem Cells Following Exposure to Ionizing Radiation

Summary

Through analysis of immediate and delayed responses of murine haemopoeitic stem cells to varying doses of low LET irradiation, we observed distinct differences in response between two common inbred mouse strains, CBA/H and C57BL/6J.

These differences relate to recognition of DNA damage, clearance of damaged cells via apoptosis, and ultimately, the appearance of delayed chromosomal aberrations (genomic instability). Notably, genomic instability in one mouse strain was characterized by heavily damaged cells.

Experiments are currently being performed to determine the factors that might contribute to the phenotypes reported here, particularly with regard to ATM and p53.

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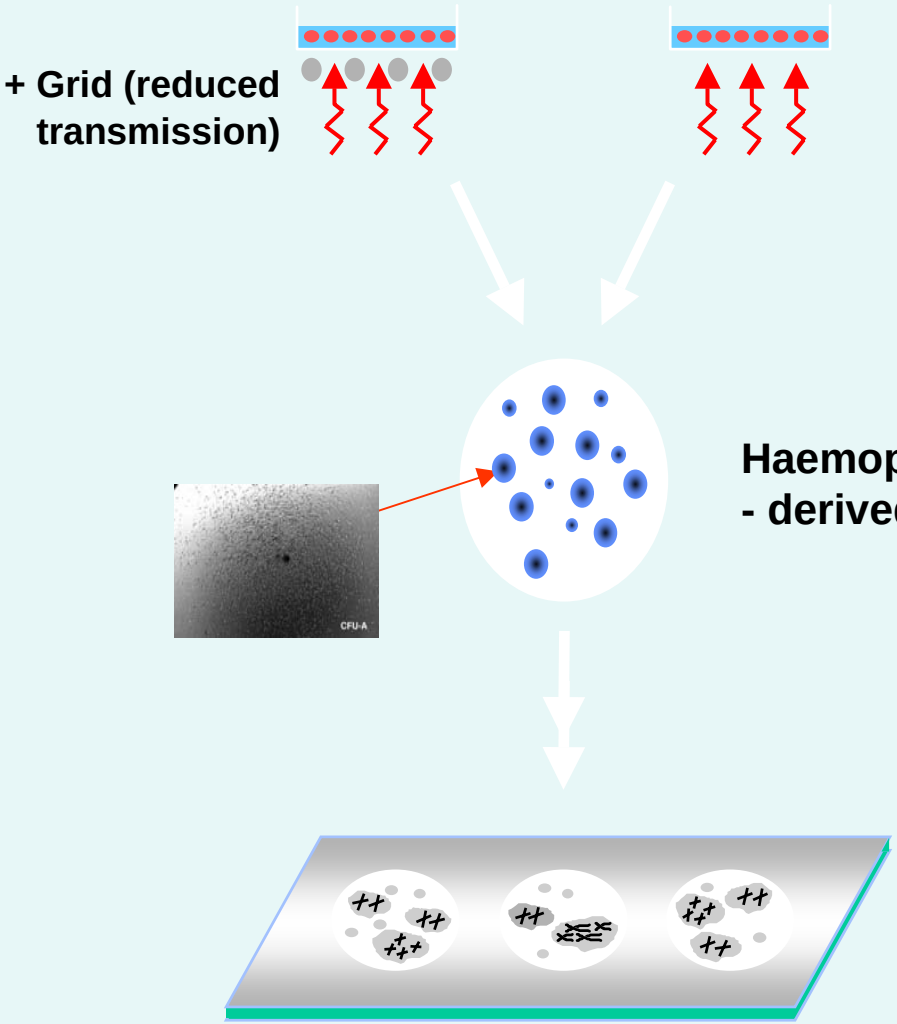
Methods - Radiation and Culture



- Femoral bone marrow cells irradiated
- Cells cultured

Haemopoietic stem cells - derived colonies

- Chromosome preparation from individual colony
- Chromosome analysis

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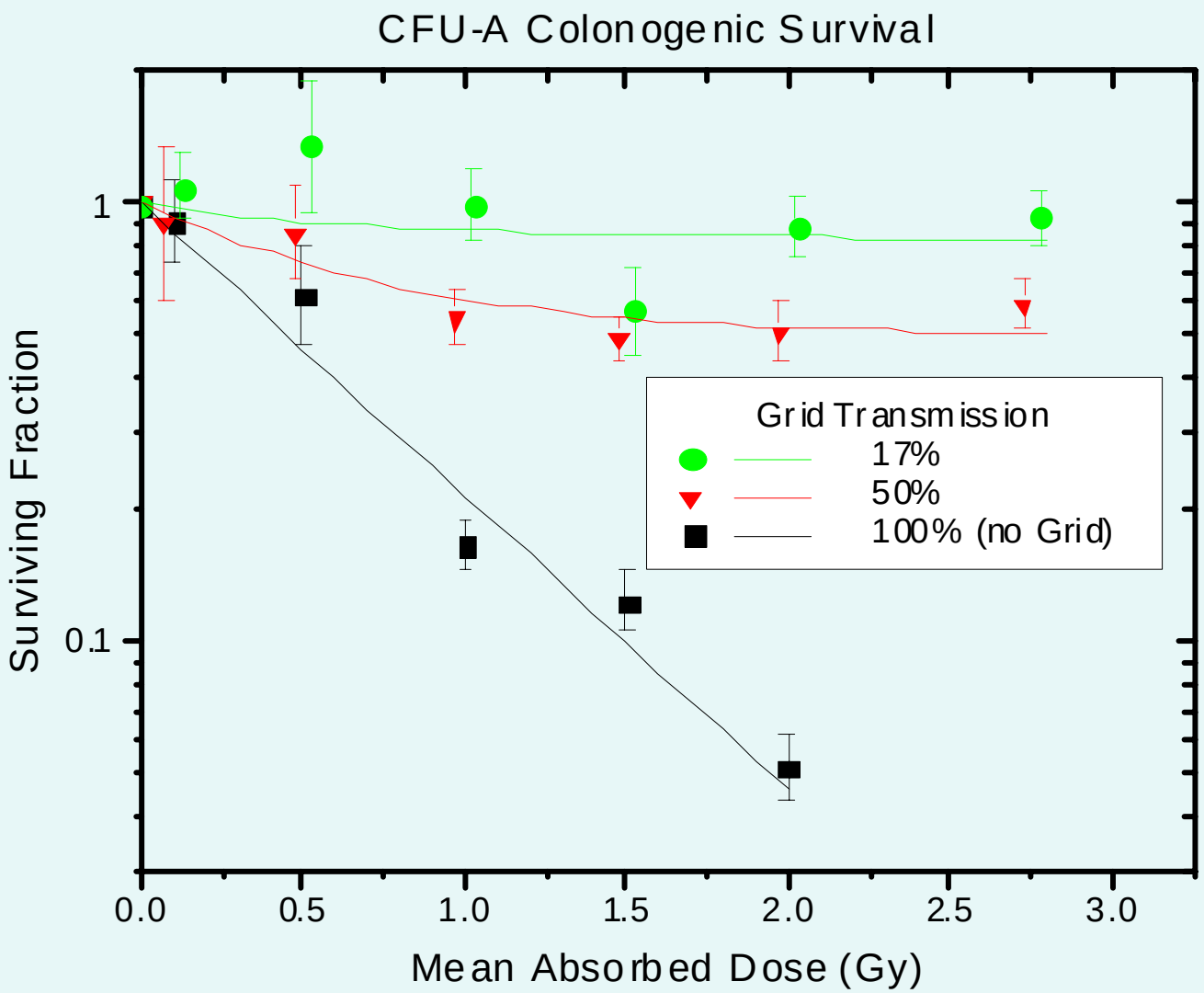
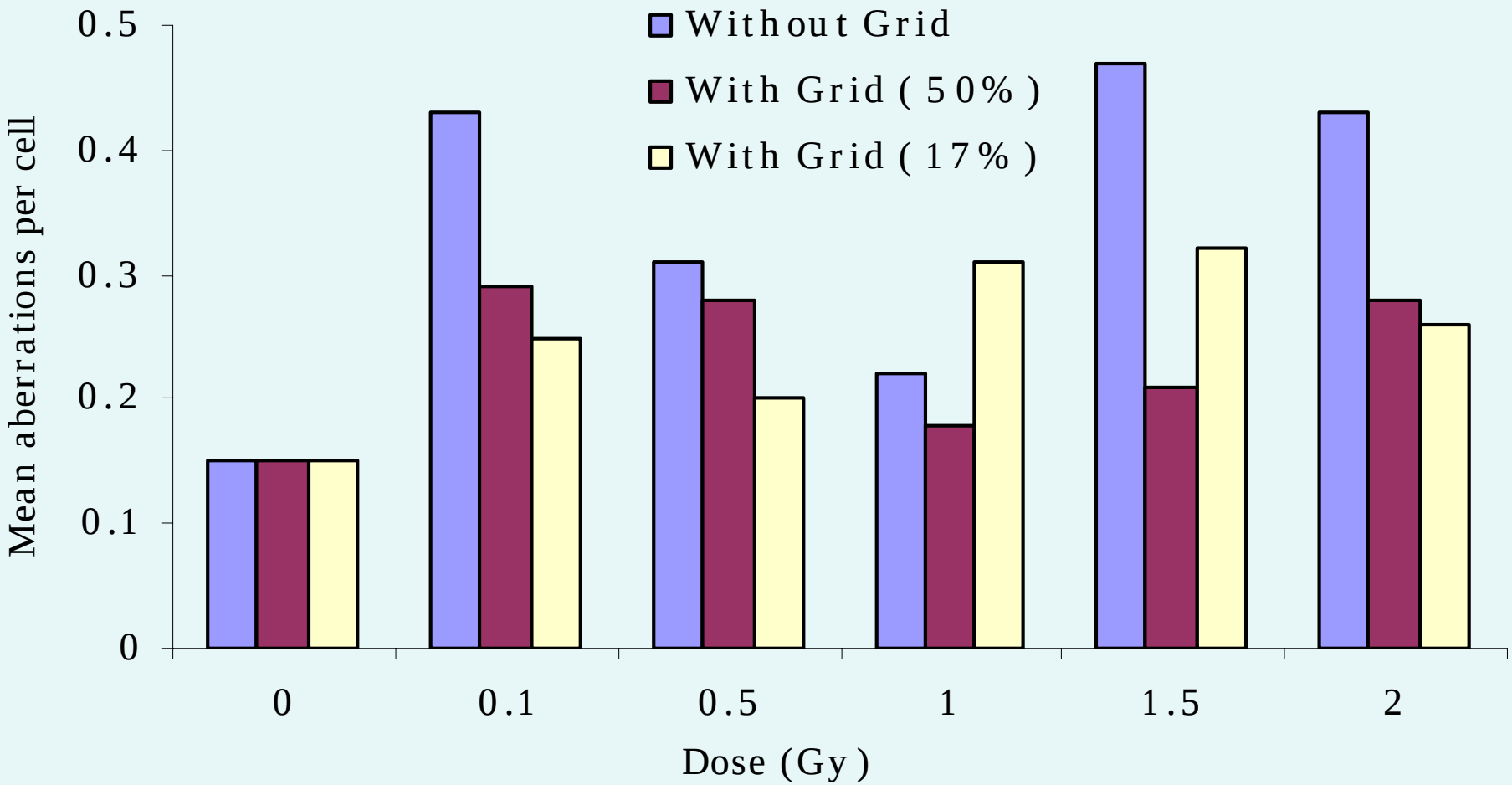

CFU-A Colonogenic Survival
Surviving Fraction
1
0.1
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Mean Absorbed Dose (Gy)
Grid Transmission
17%
50%
100% (no Grid)

Chromosomal instability in clonal descendants of Al X-ray-irradiated mouse stem cells



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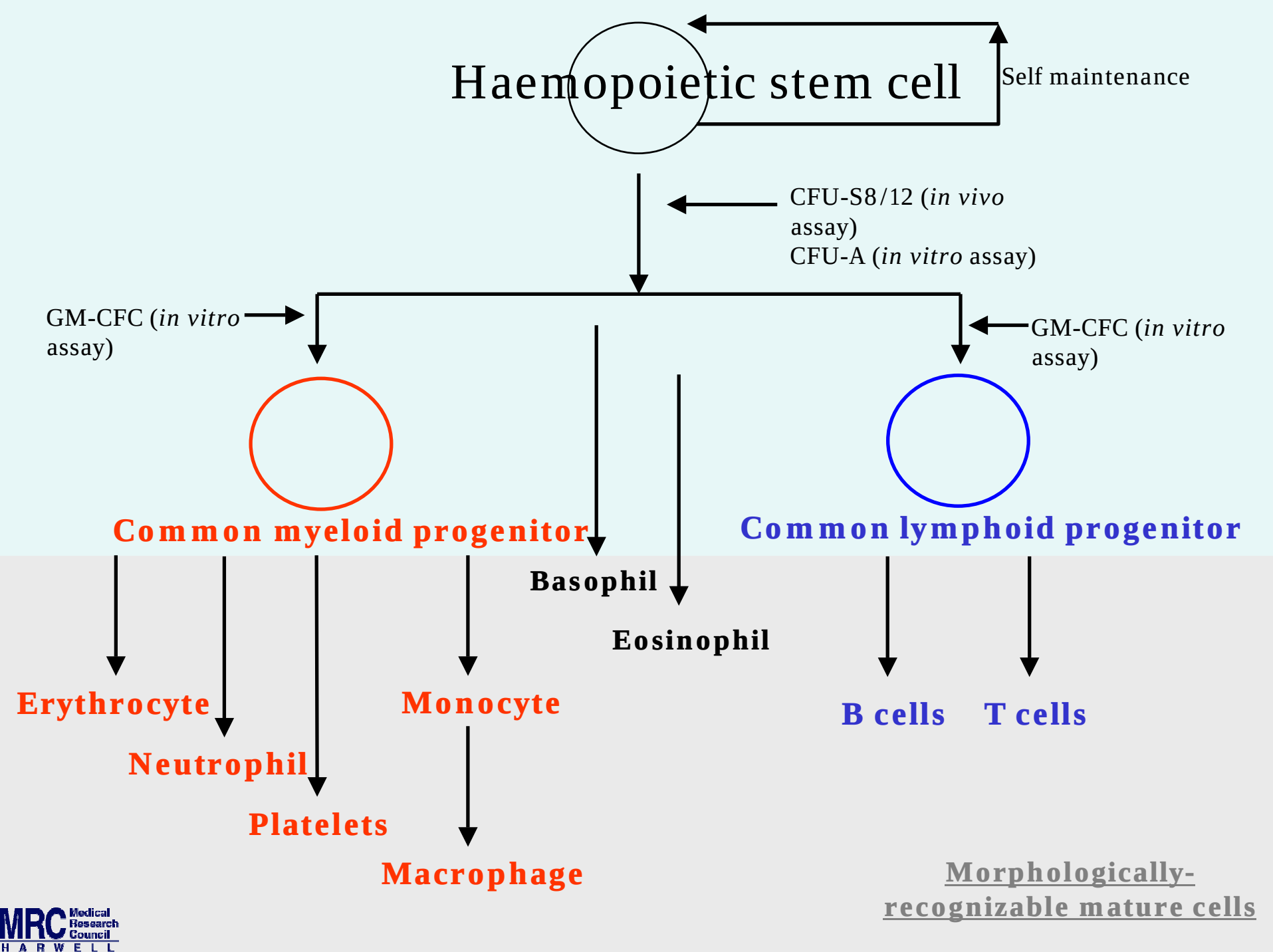

Genomic Instability Studies in Murine Haemopoietic Stem Cells Following Exposure to Ionizing Radiation

MA Kadhim[1], DA Macdonald[1], DA Bowler[1], SR Moore[1], D Papworth[1], BM Bianski[2],DS Gridley[2] GA Nelson[2], LM Green[2]
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2- Radiobiology Program, Department of Radiation Medicine, Loma Linda University, CA, USA

•The contribution of genetic factors to the radiation-induced genomic instability phenotype remains poorly understood.
• The aim of the current study was to interrogate this relationship after low LET X-irradiation in haemopoeitic stem cells derived from two widely-used inbred mouse strains known to differ in radiosensitivity. Several phenotypic endpoints associated with response to irradiation were measured in bone marrow stem cells isolated from CBA/H and C57BL/6J mice.
•The cells were exposed to X-ray doses from 0.1 to 3 Gy and γ-H2AX foci formation was measured immediately and at several times up to 24 hours post-irradiation. Apoptosis and chromosomal aberrations were assessed in clonal stem cell populations 15 population doublings post-irradiation.

•Formation of γ-H2AX foci was rapid and substantial in C57Bl/6J irrespective of dose, however, in bone marrow stem cells from the CBA/H mice the only significant increases were after the 3 Gy dose. The frequency of delayed chromosomal aberrations was significantly elevated in both strains after low doses, but not at the higher doses. In the C57BL/6J derived cells, the aberrant cells had multiple aberrations and thereby contributed more heavily to the sum of total aberrations scored. In contrast the CBA/H derived samples had very few such cells present. An inverse relationship between apoptosis and instability was evident in both strains; however it was more prominent in C57BL/6J.

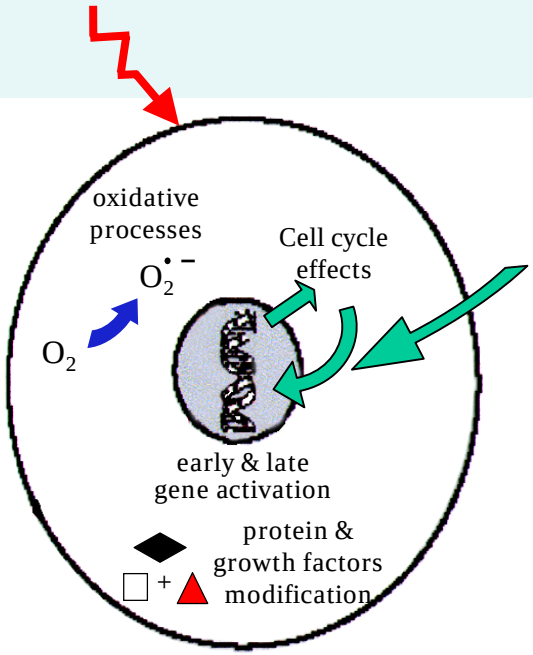
•Collectively, these results suggest that the differences in radiation-induced genomic instability between these strains may be related to genetic differences, especially with regard to DNA damage recognition, repair and apoptosis.

Chromosomal instability is thought to be maintained at low levels due to the removal of heavily damaged cells via activation of apoptosis following high LET or high doses of low LET irradiation. However, at low doses of low LET radiation, the results presented here suggest that the relationship between genetic predisposition and radiation-induced instability is rather complex, especially with regard to the balance between apoptosis and chromosomal instability. This implies that under low dose, low LET conditions, more of the population may be at risk than previously recognized, which may have important implications for human health and radiation risk assessment.

Genomic Instability, Bystander Effects & Adaptive Response: Mechanisms and Link

- *Radical paradigm shift in Radiation Biology* - Deterministic "hit-effect" relationship, to a complex model involving ongoing "cellular responses":
  - Non-targeted effects of radiation, viz. genomic instability, bystander effects and adaptive responses
  - These can occur at very low doses, or at time points far removed from the initial exposure.

    (Relevant to cancer risk associated with occupational and environmental exposure to radiation).
- *It is essential to understand*:
  - mechanisms underlying low-dose cellular responses,
  - interrelationships amongst these responses,
  - implications for radiotherapy - e.g. setting acceptable radiation exposure limits especially to low LET.



ATM/ATR

<u>Cell cycle checkpoints:</u>

CHK1, CHK2,

p53, p21, etc.

Rad9, Rad24

<u>DNA repair enzymes:</u>

DNA-PK/Ku70, Ku86

GADD45

hMLH1, hMSH2

....

....

Repair

Cell death

Genome stability

Loss of stability

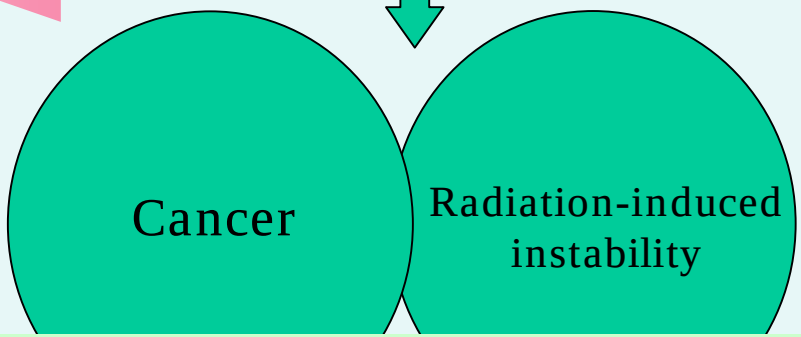
Evidence of correlation between radiation-induced genetic instability and:

Mammary tumours in BALB/c mice (Ulrich et al., 1997; Ponnaiya et al., 1997)

Leukaemia (AML) in CBA/H mice (Macdonald et al., 2001)

Pediatric thyroid carcinomas/post-Chernobyl (Nikiforov et al., 1998)

Human (AML)/A-bomb survivors (Nakanishi et al., 1999, 2001)



Delayed chromosomal abnormalities;
Increased mutation rate; Gene amplification...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for
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incidence
causal mechanisms
prevention