

Essential Radiobiology for Radiation Epidemiologists



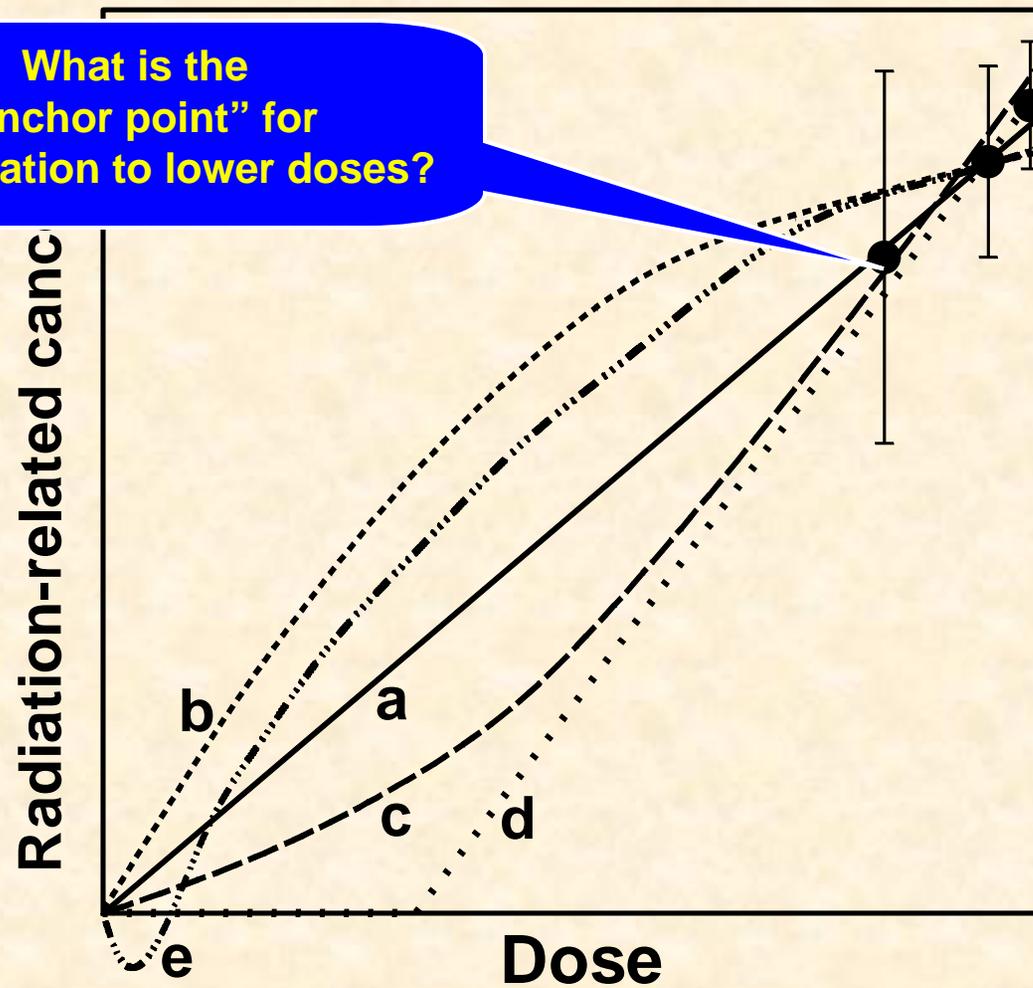
What every
epidemiologist needs
to know about
radiobiology,
but was too deep
in the bunker to ask

Do epidemiologists need radiobiology?

- The exposure situations that we are interested in are generally **not** those that are amenable to quantitative epidemiology
- Extrapolations:
 - ⇒ *Dose*
 - ⇒ *Dose rate*
 - ⇒ *Radiation quality*

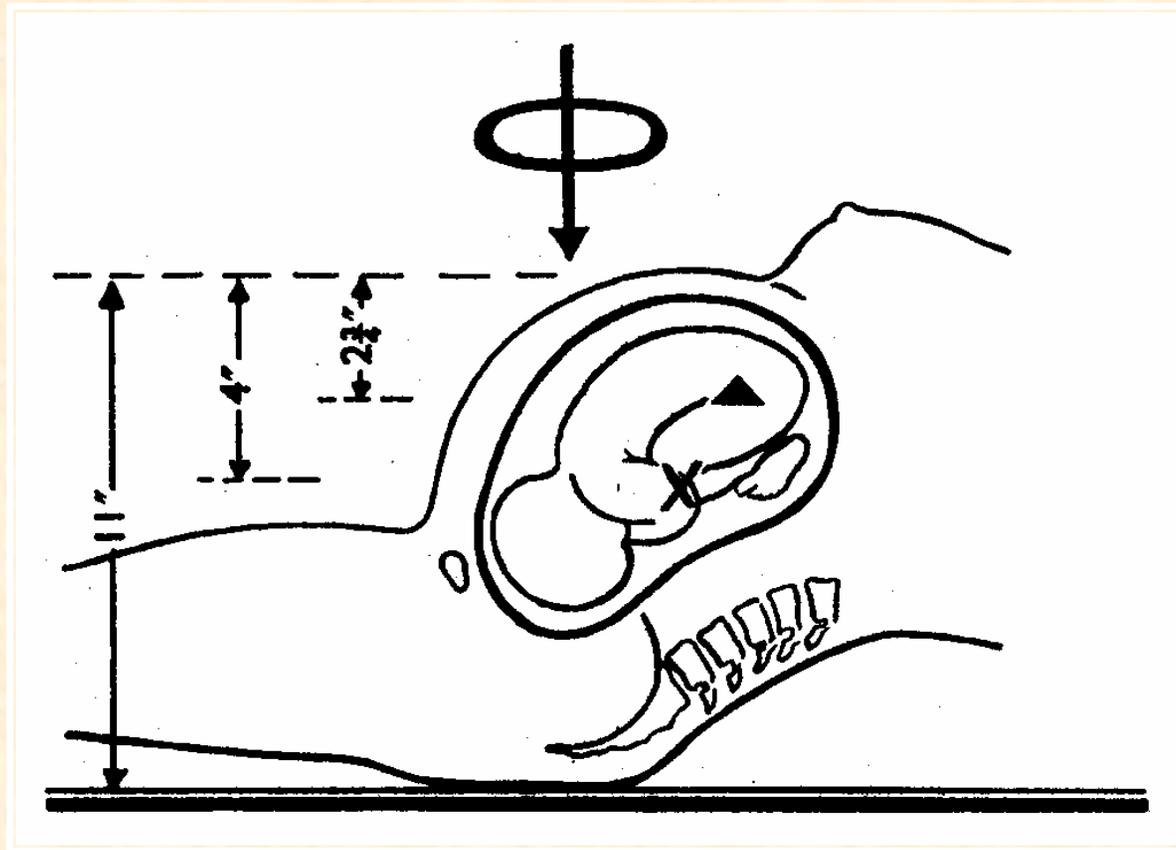
Different possible low-dose extrapolations

What is the
“anchor point” for
extrapolation to lower doses?



In-Utero x-ray exposure:

Pelvimetry, obstetric abdominal exam



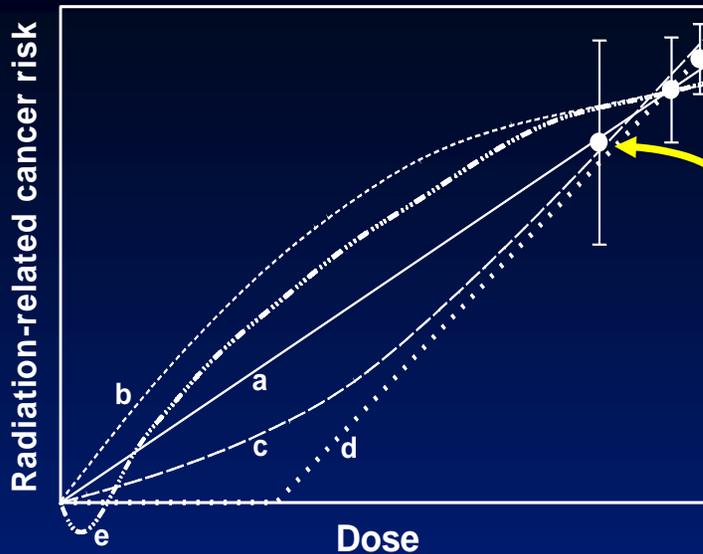
Mean dose 5-10 mGy, 80 kVp x rays

Mole (1990)

“The odds ratio for childhood cancer deaths after X-raying in birth years 1958-61 (1.23, 95% CI 1.04-1.48) and the mean fetal whole body dose from obstetric radiography in 1958 (6 mGy) can each be derived from nationwide surveys in Britain.....

This seems to be the only value for risk of cancer mortality after irradiation *in utero* based on independent determinations of dose and risk in nationwide samples of the same population of subjects. It is not based on extrapolation or on an unreliable dose response”

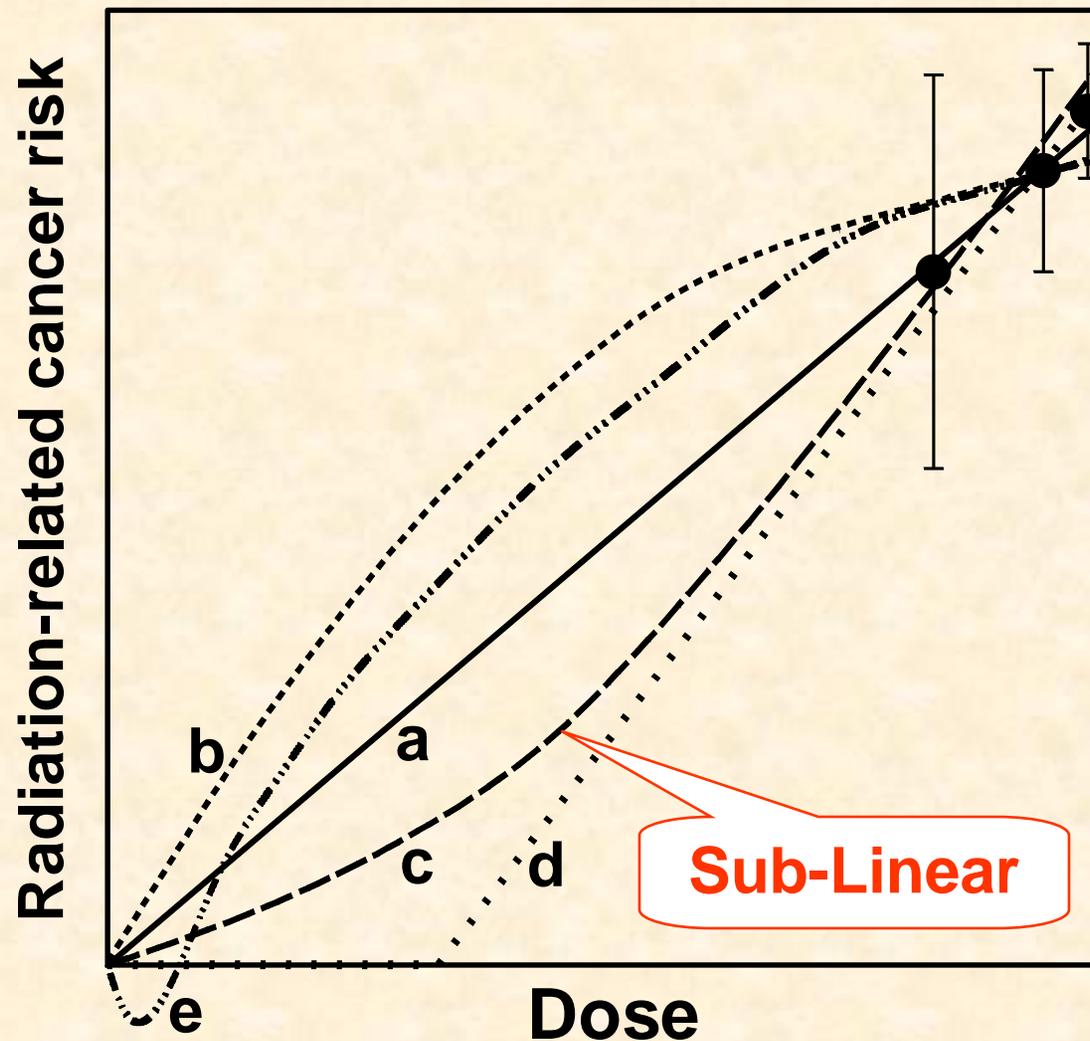
Brit. J. Cancer, 62, 152-68 (1990)



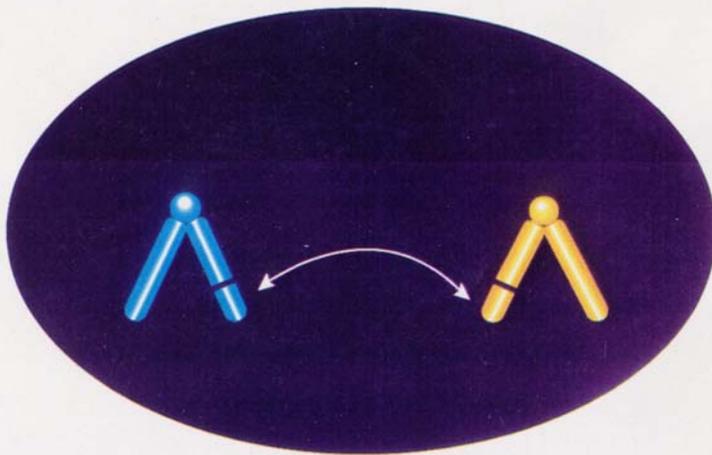
**So our “anchor point”
is about 5-10 mGy**

- **We know there are cancer risks at this dose**
- **It is unlikely that we will be able to directly estimate risks at much lower doses**
- **What can we do?**

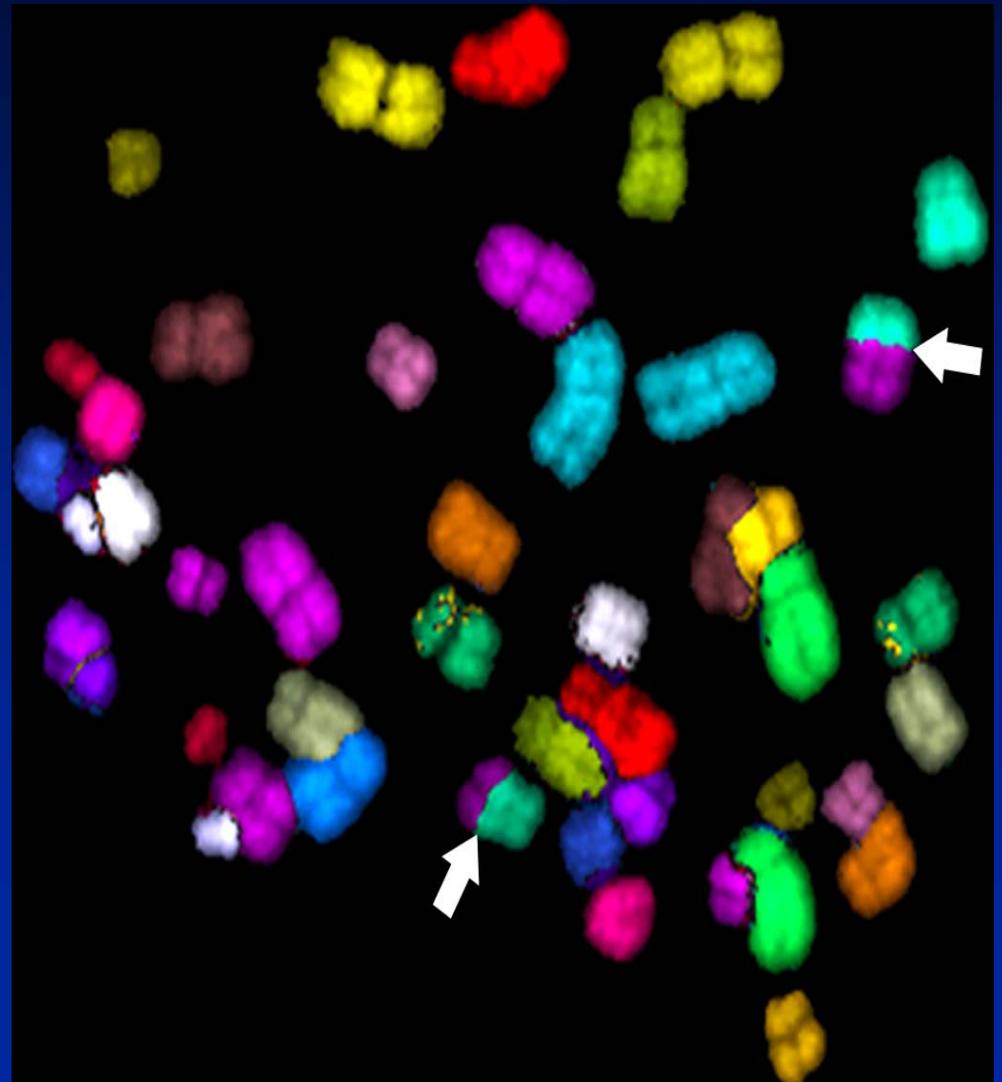
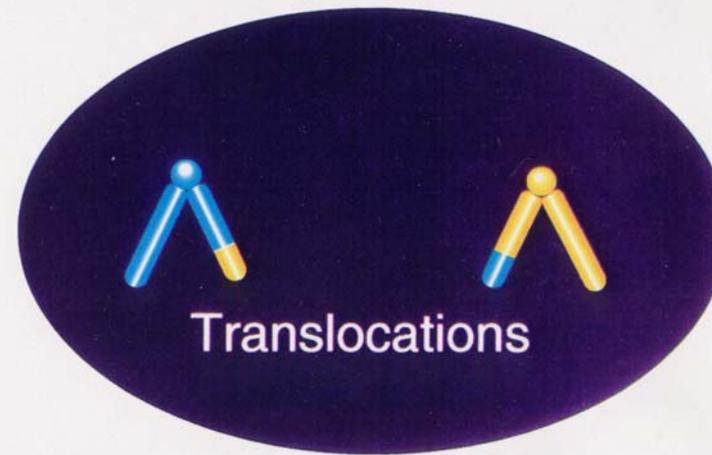
Different possible low-dose extrapolations



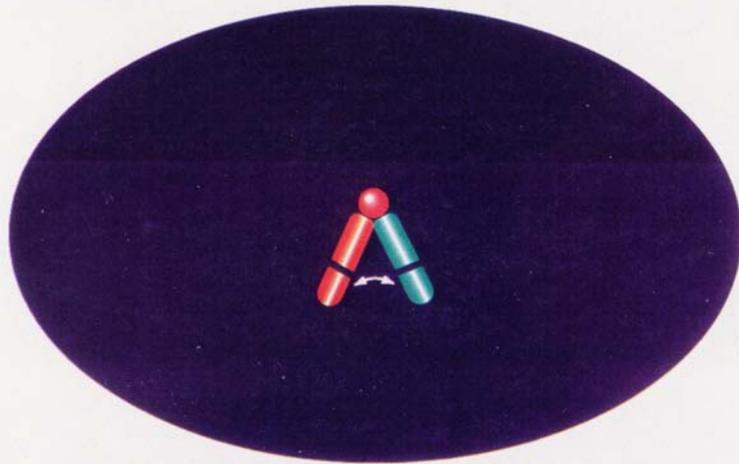
"Two break" stable aberrations: inter-arm (translocation)



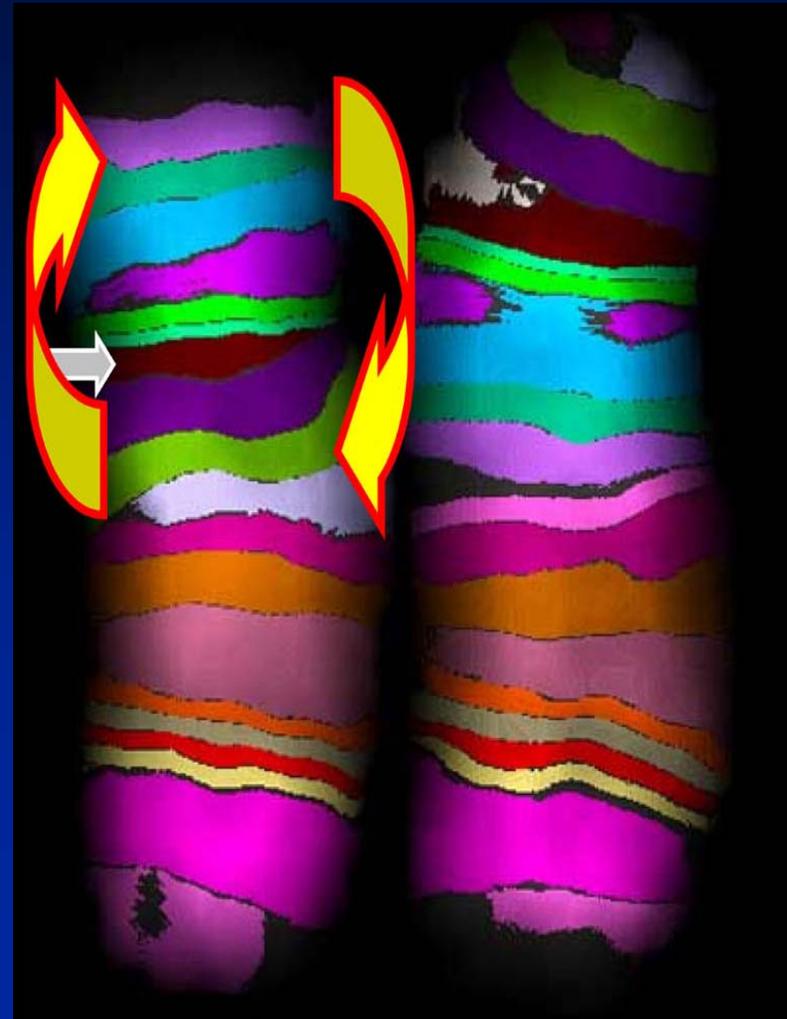
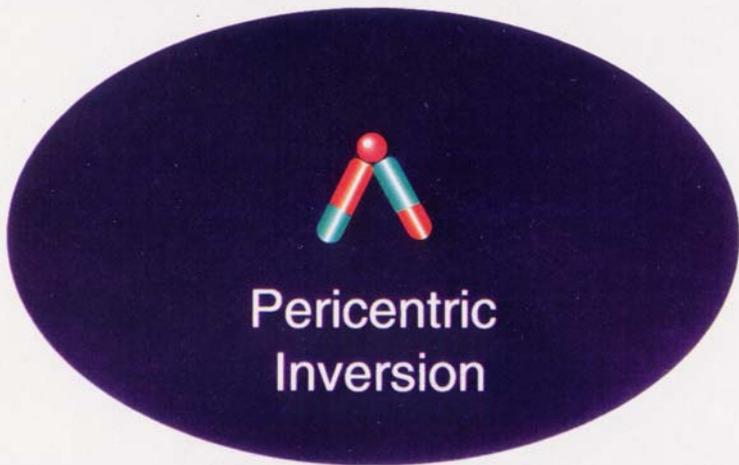
Incorrect ↓ Rejoining



"Two break" stable aberrations: inter-arm: pericentric inversion



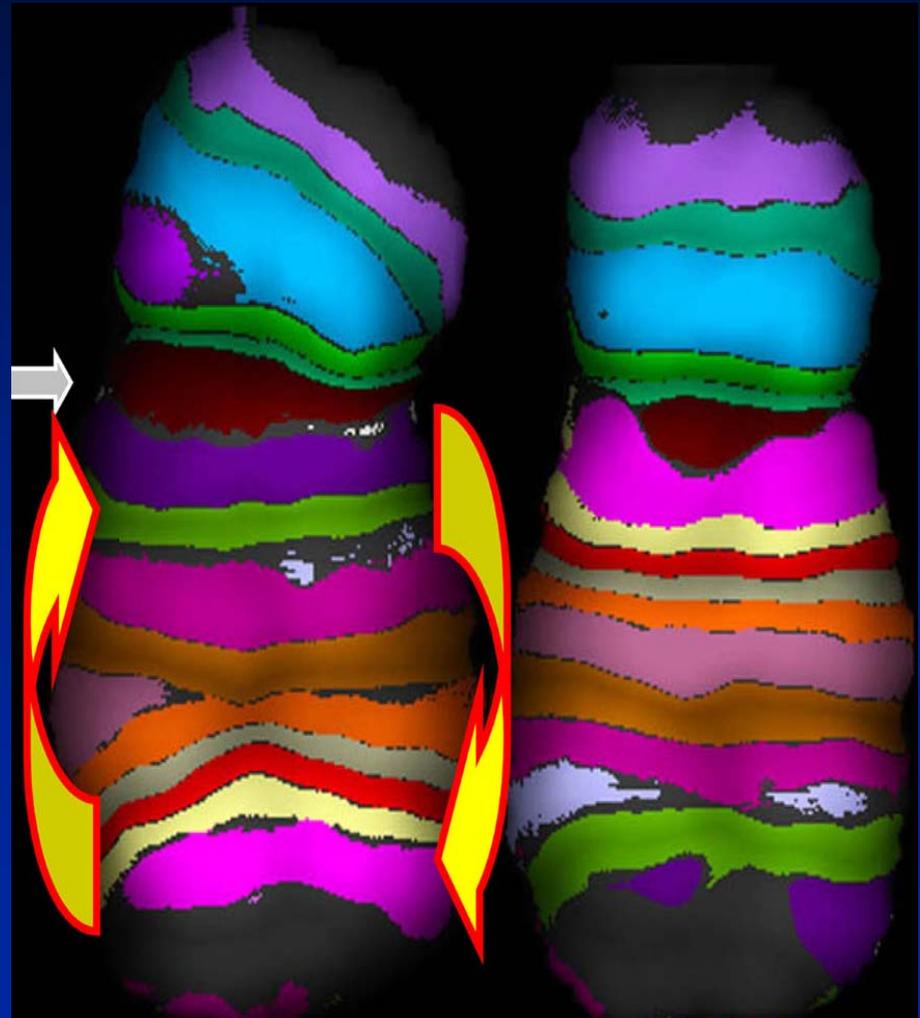
Incorrect ↓ Rejoining



“Two break” stable aberrations: intra-arm: Paracentric Inversion

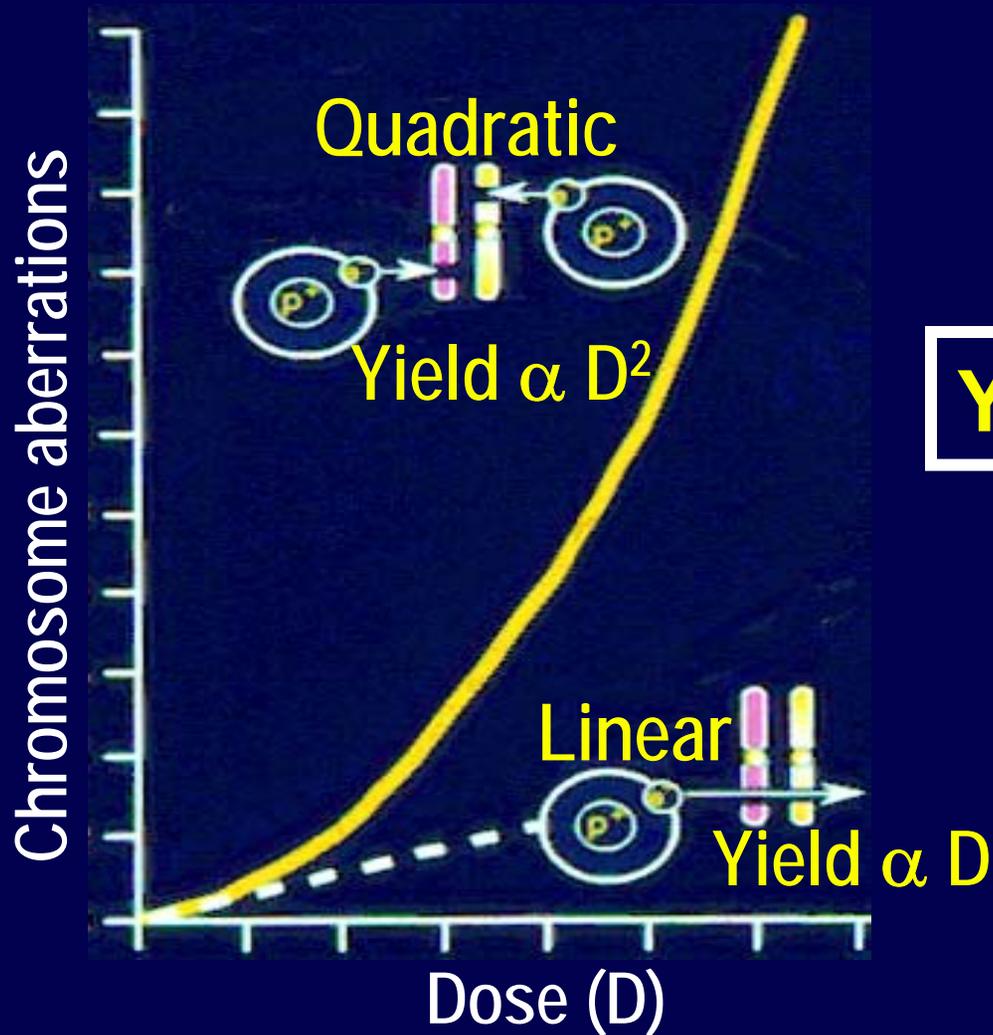


Incorrect Rejoining



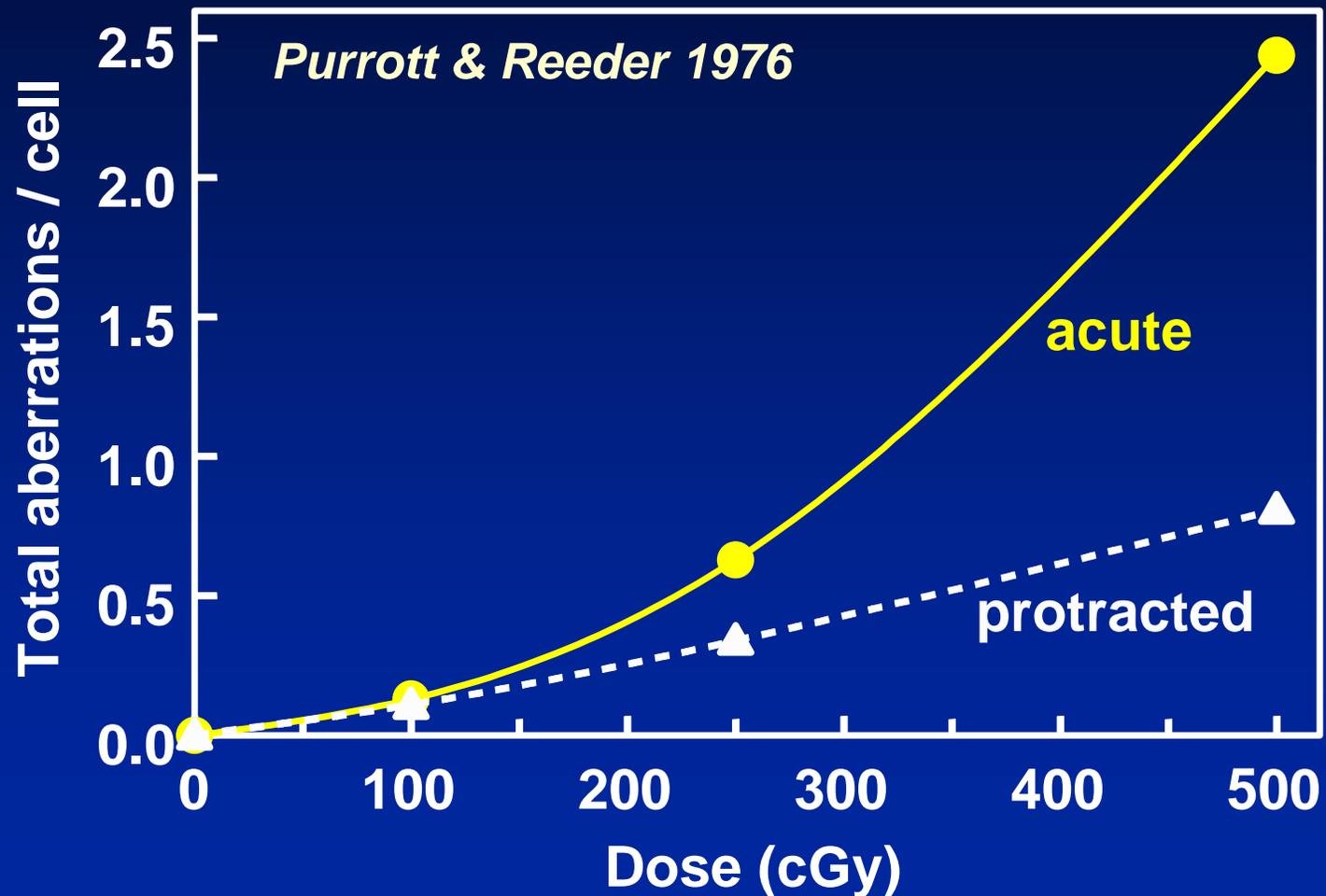
1 hit → linear

2 independent hits → quadratic

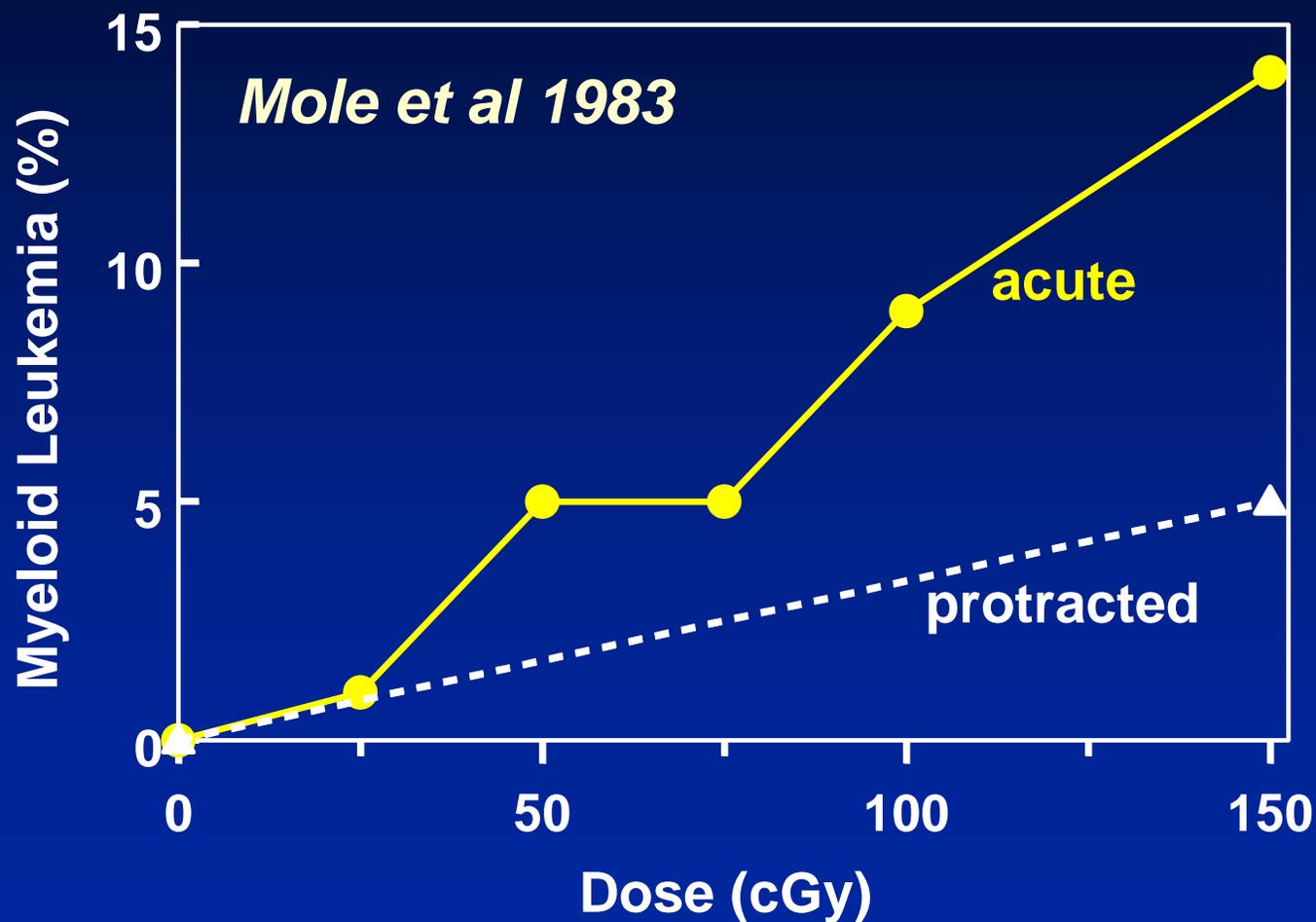


$$\text{Yield} = \alpha D + \beta D^2$$

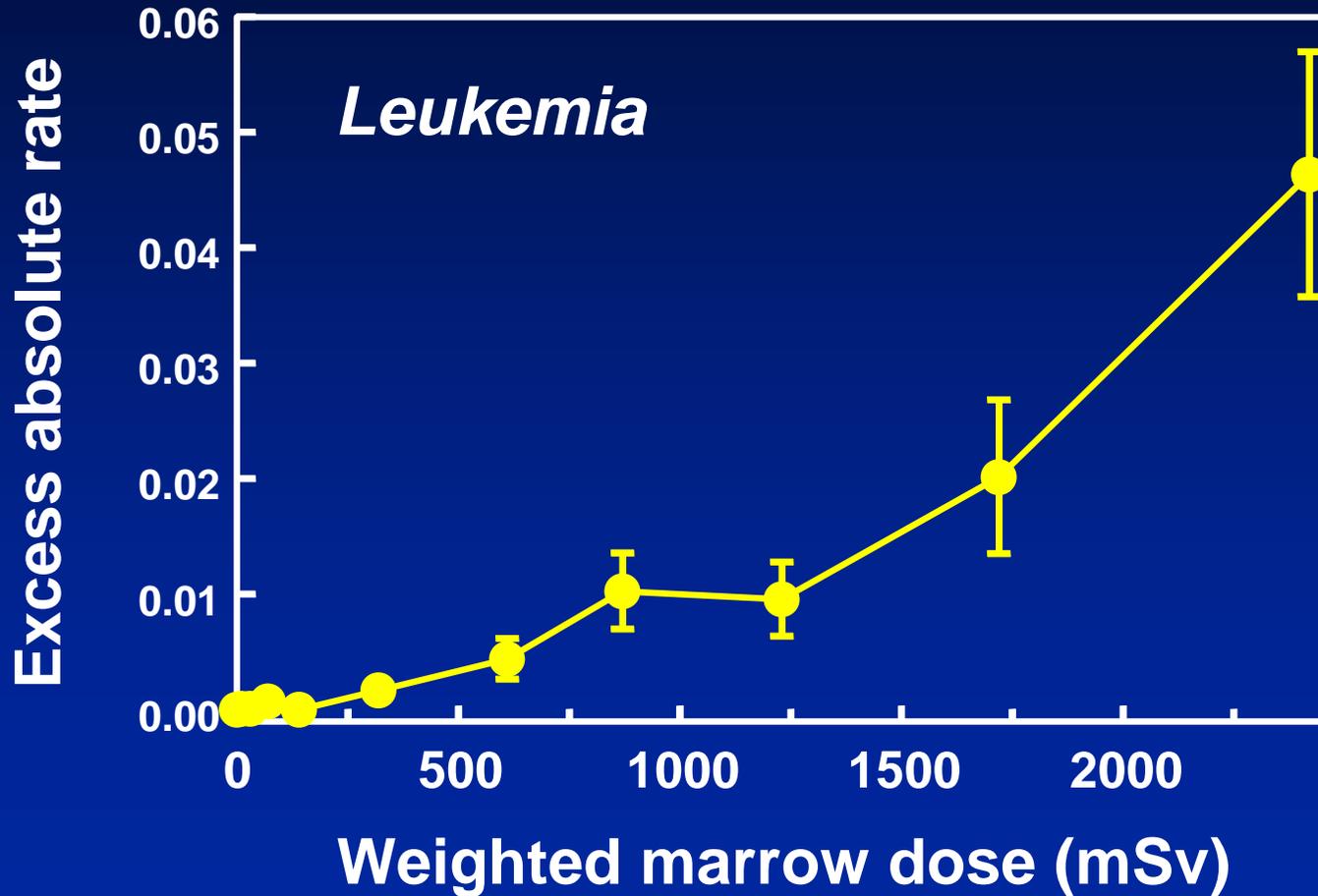
Aberration induction in human lymphocytes



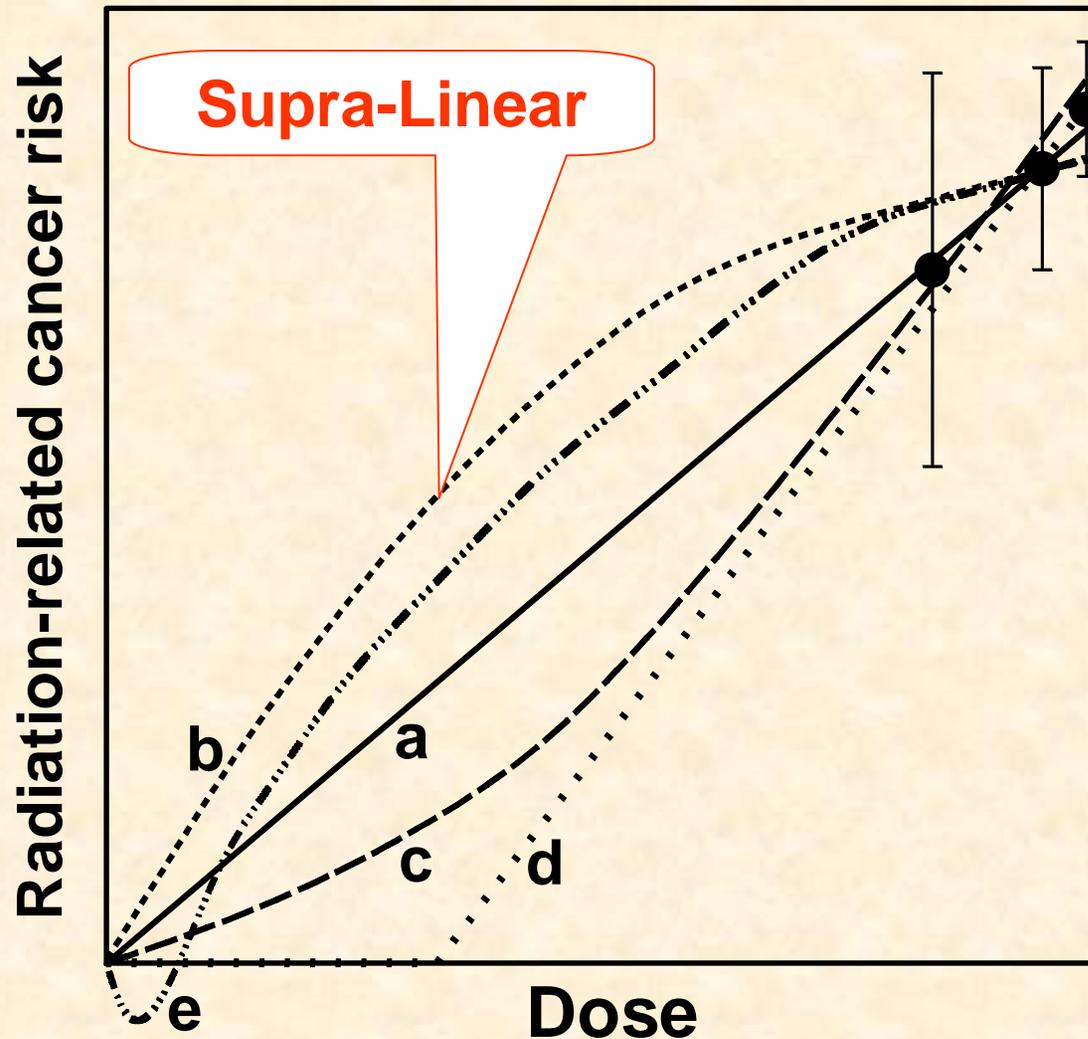
X-ray induction of myeloid leukemia in CBA/H mice



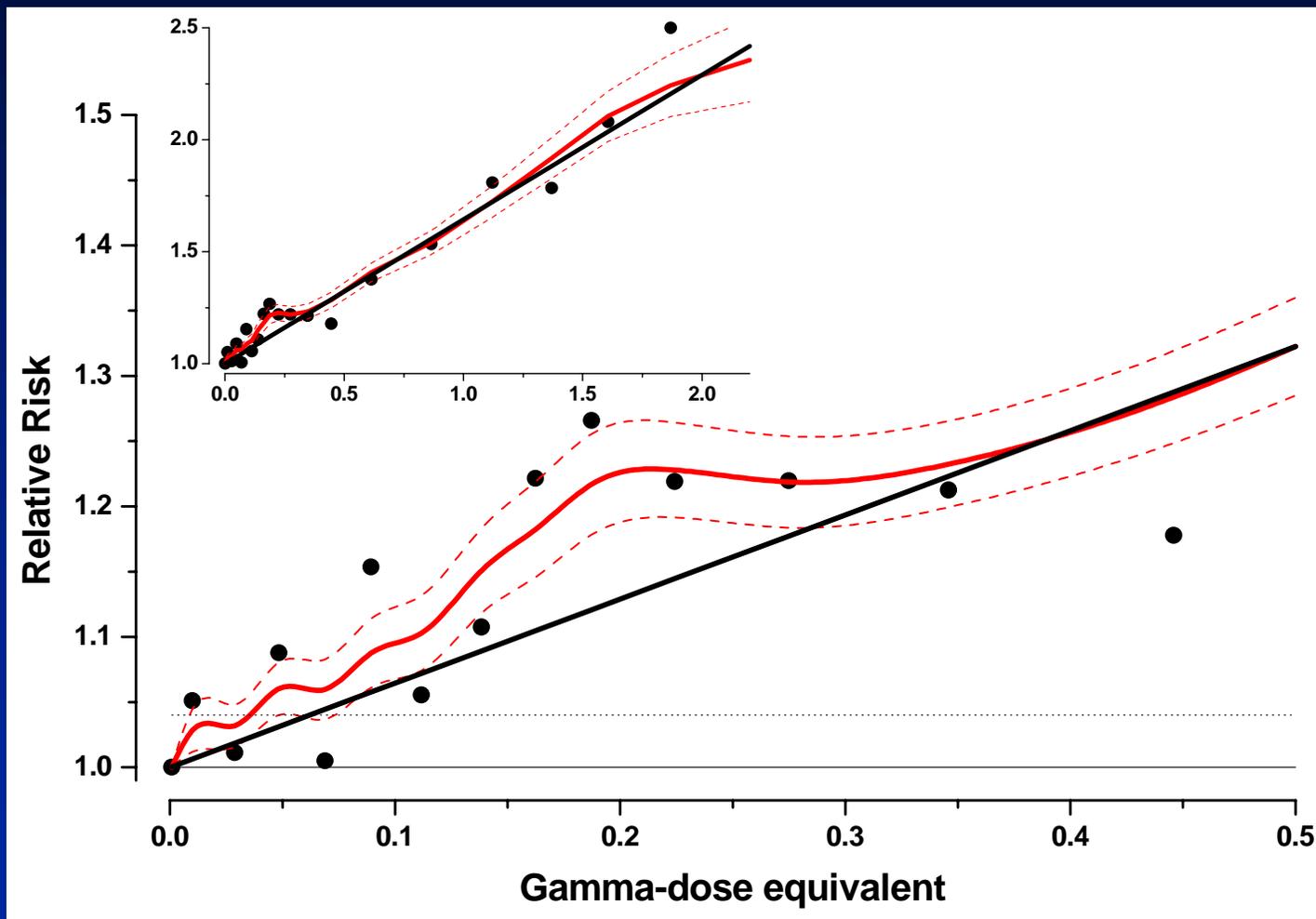
Excess leukemia in A-bomb survivors (Pierce et al 1996)



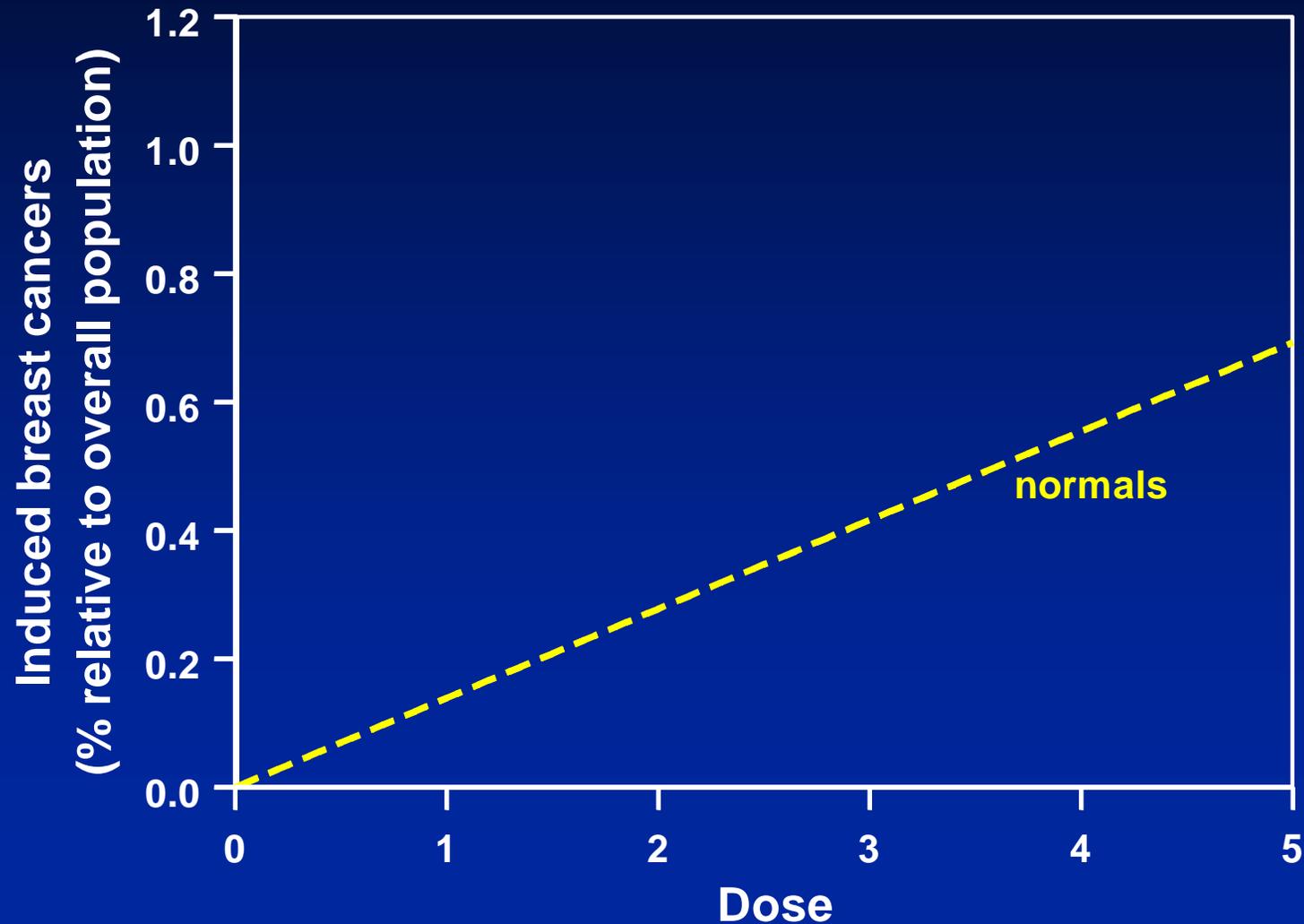
Different possible low-dose extrapolations



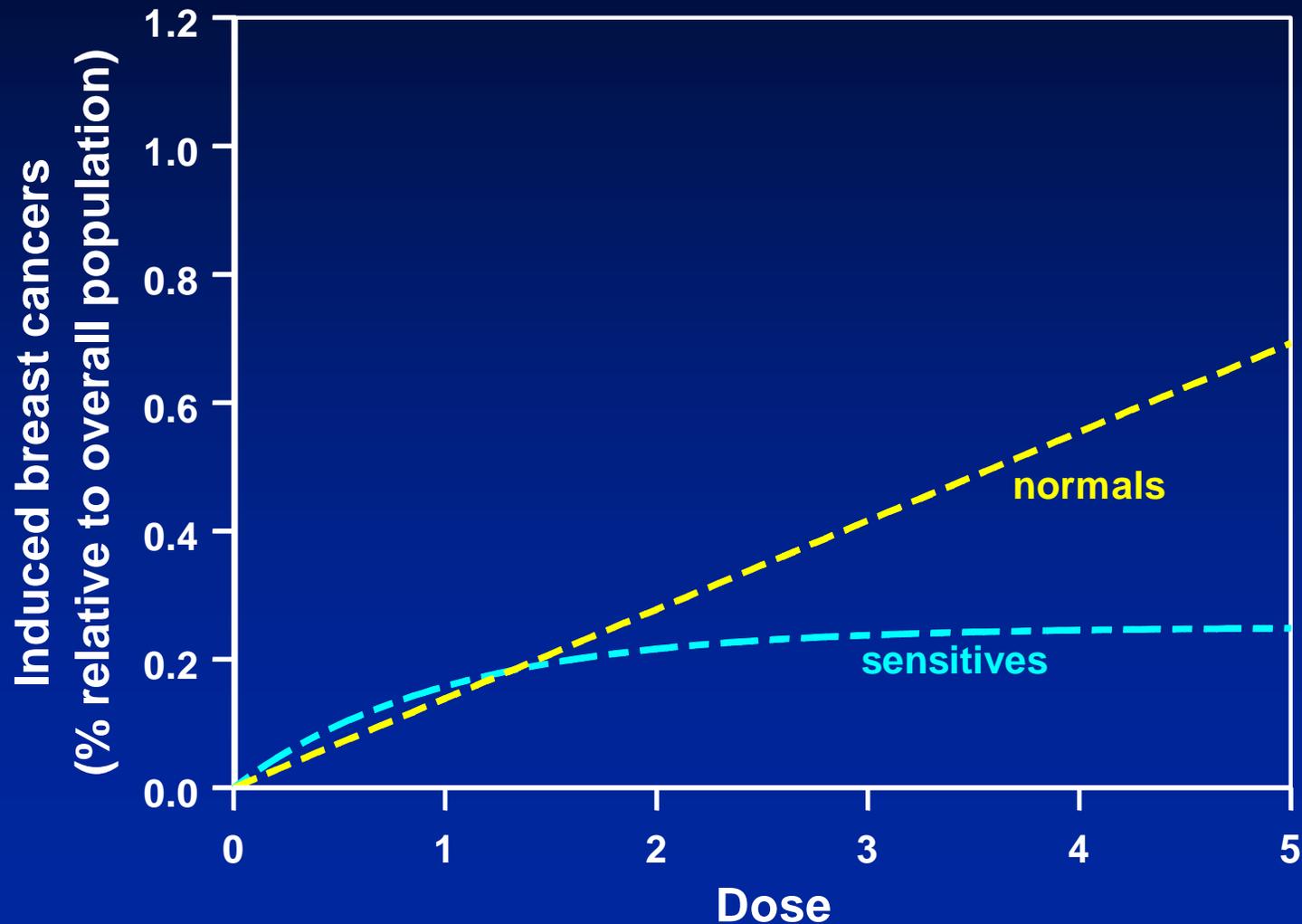
"Evidence" for downwardly-curving dose-effect relations – Solid cancer incidence at low doses in A-bomb survivors



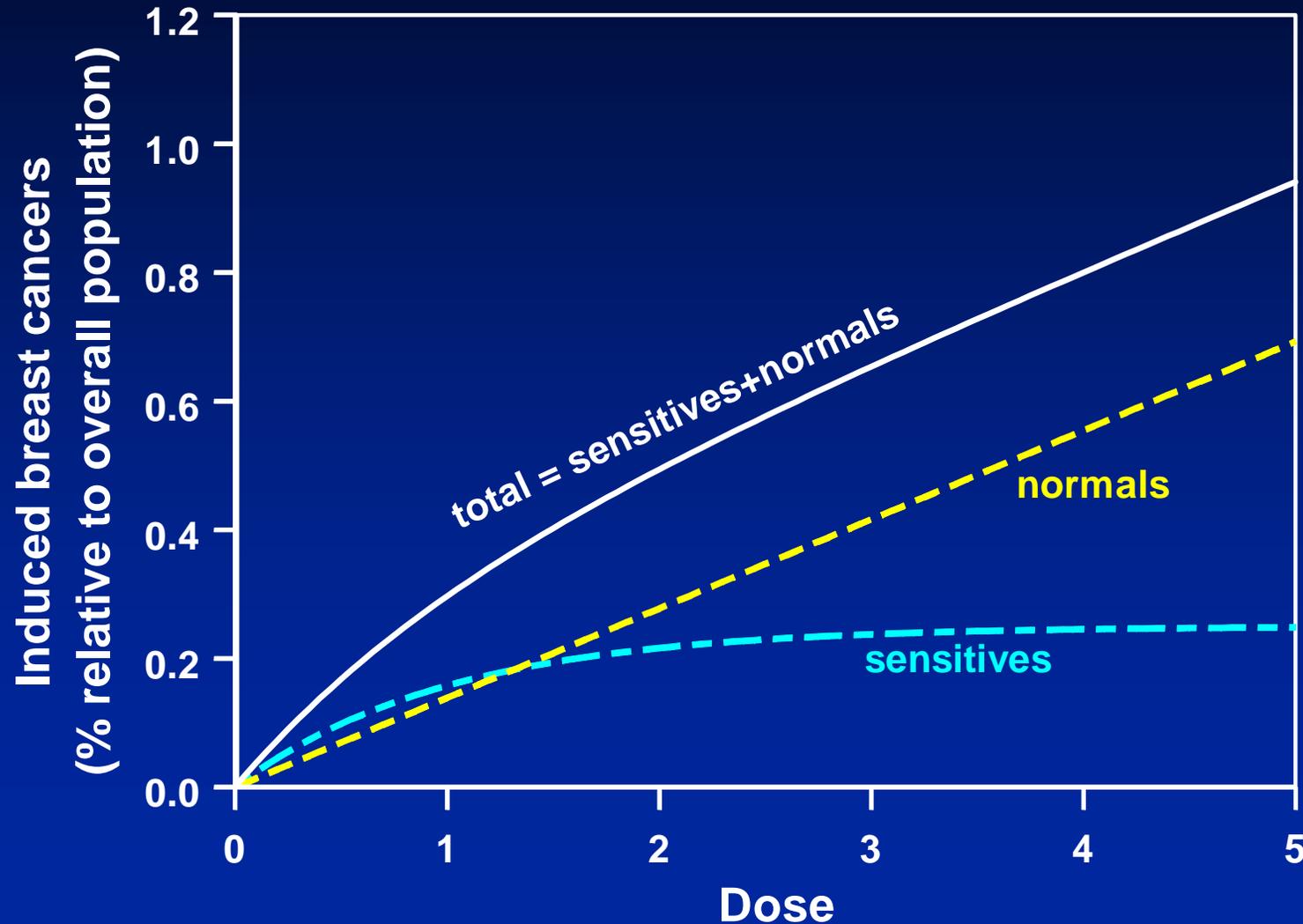
A scenario for downwardly curving dose responses – a highly radiosensitive subpopulation



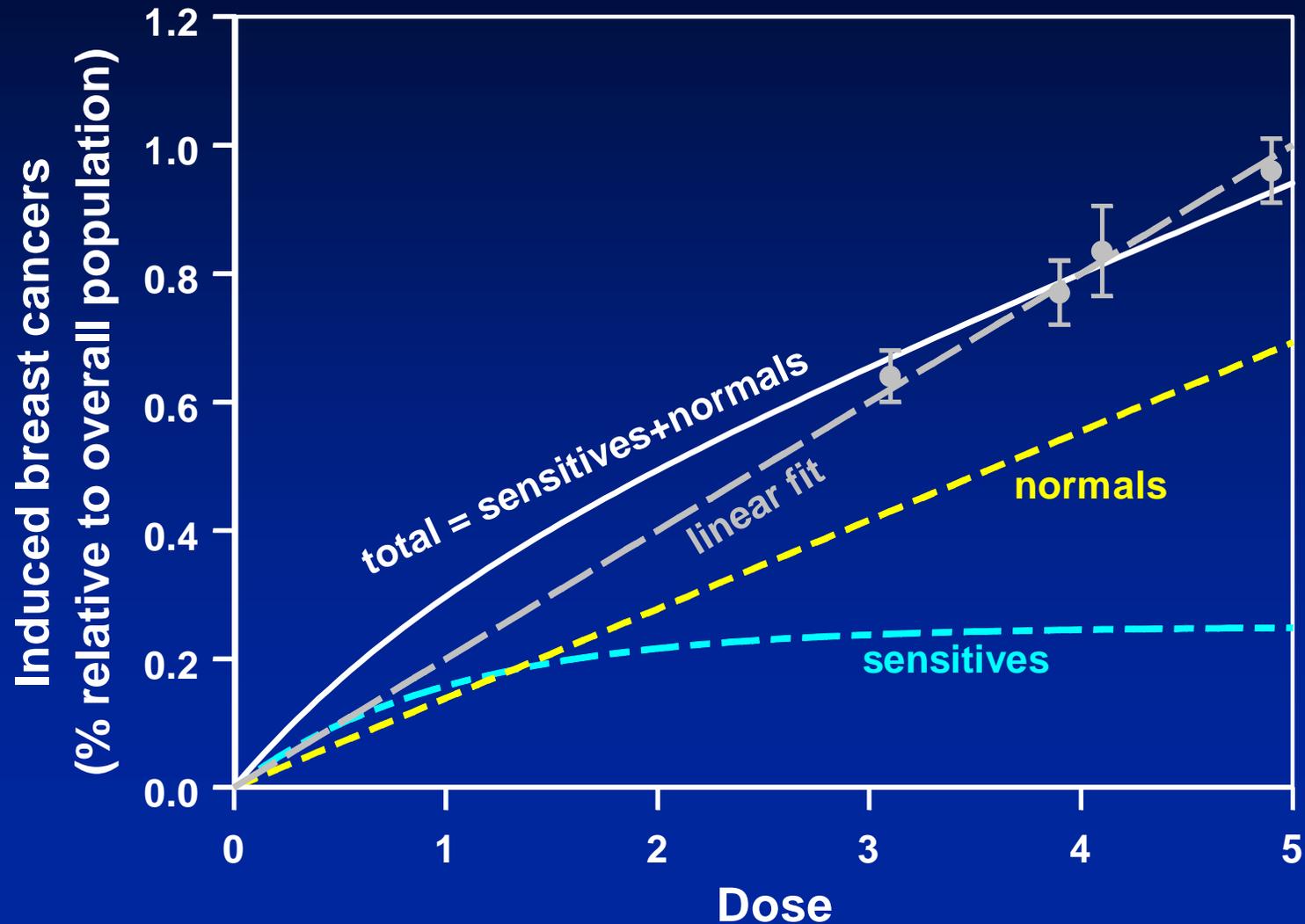
A scenario for downwardly-curving dose responses – a highly radiosensitive subpopulation



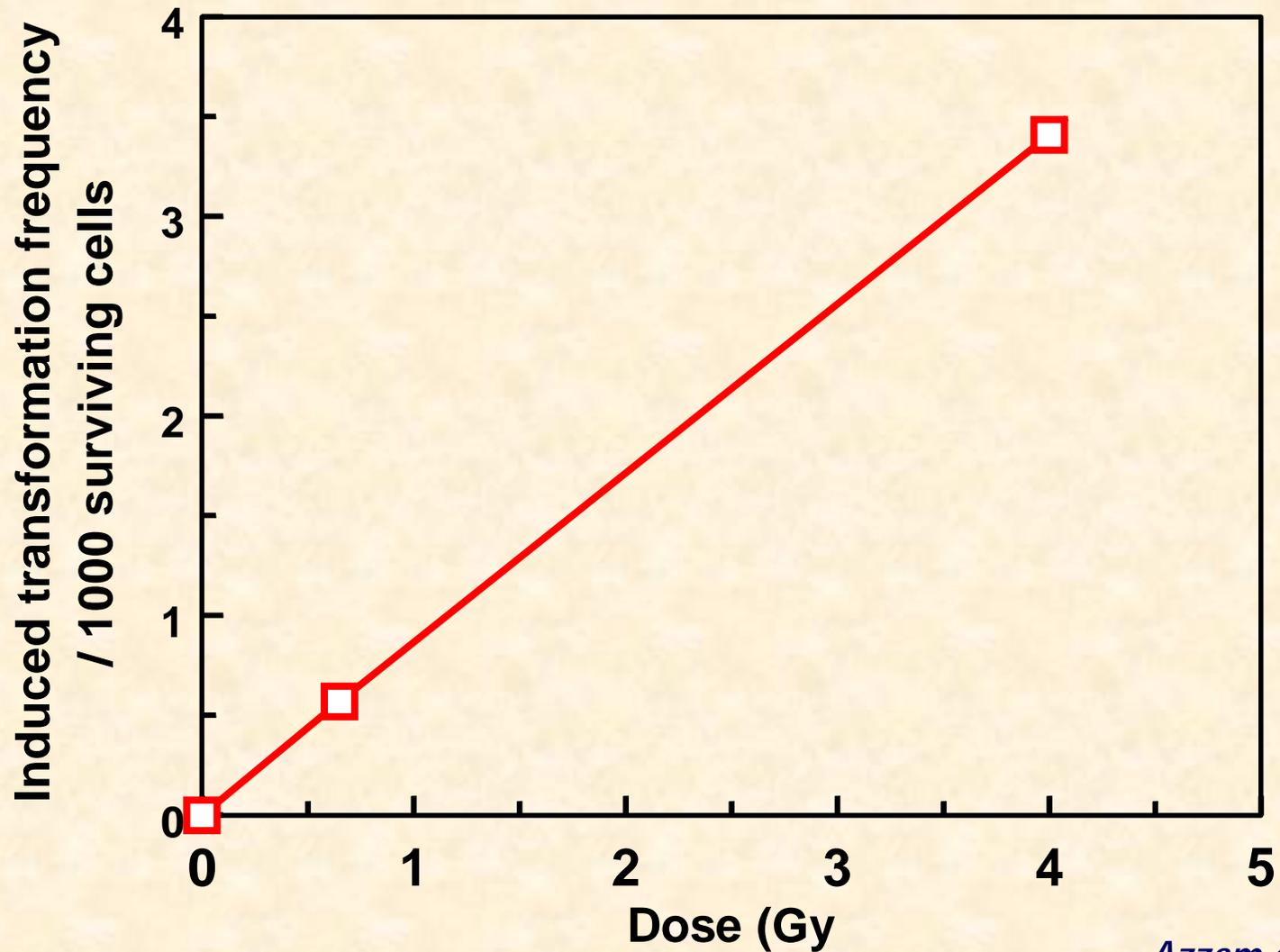
A scenario for downwardly-curving dose responses – a highly radiosensitive subpopulation



A scenario for downwardly-curving dose responses – a highly radiosensitive subpopulation

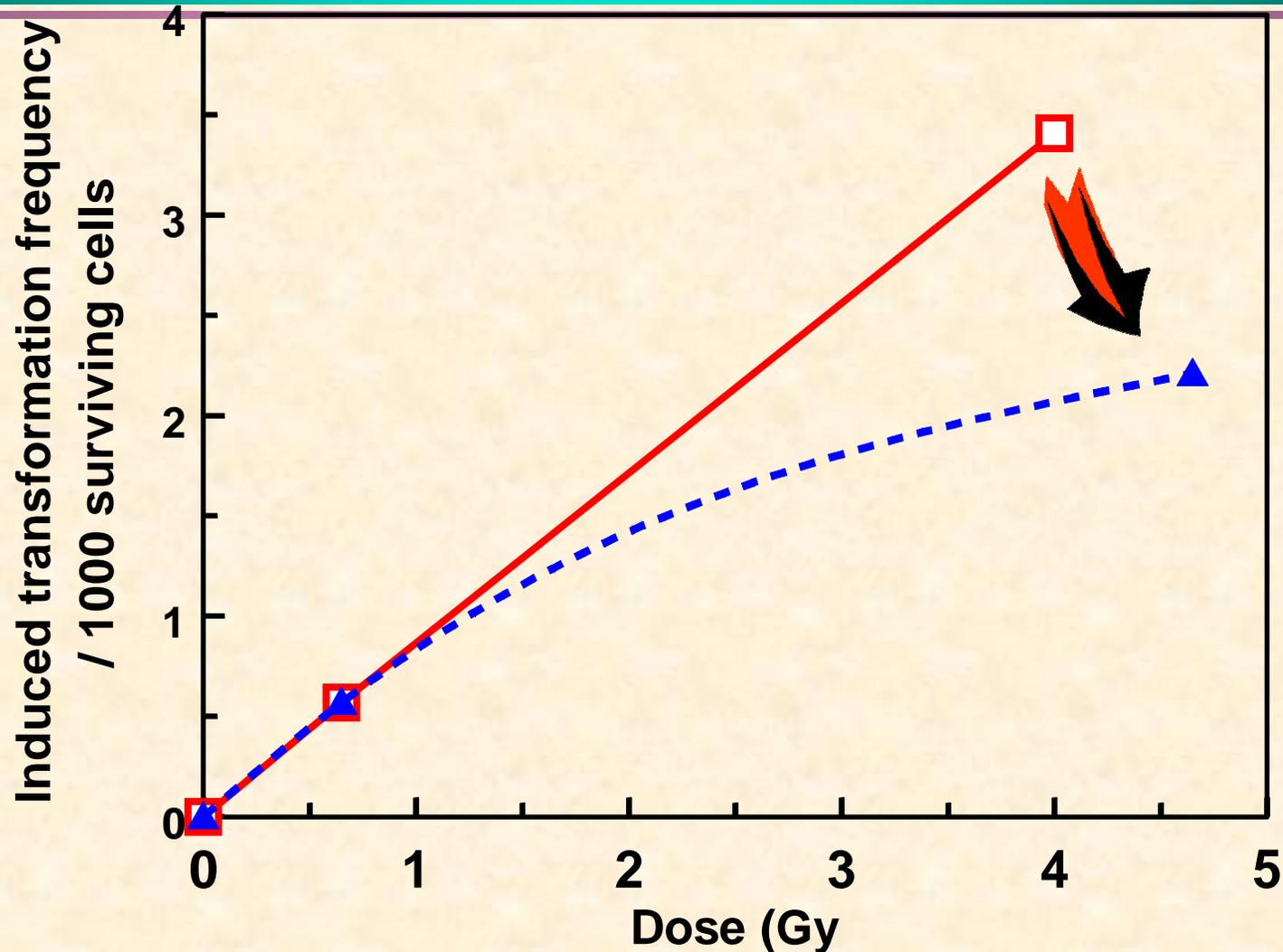


A scenario for downwardly-curving dose responses – An adaptive response



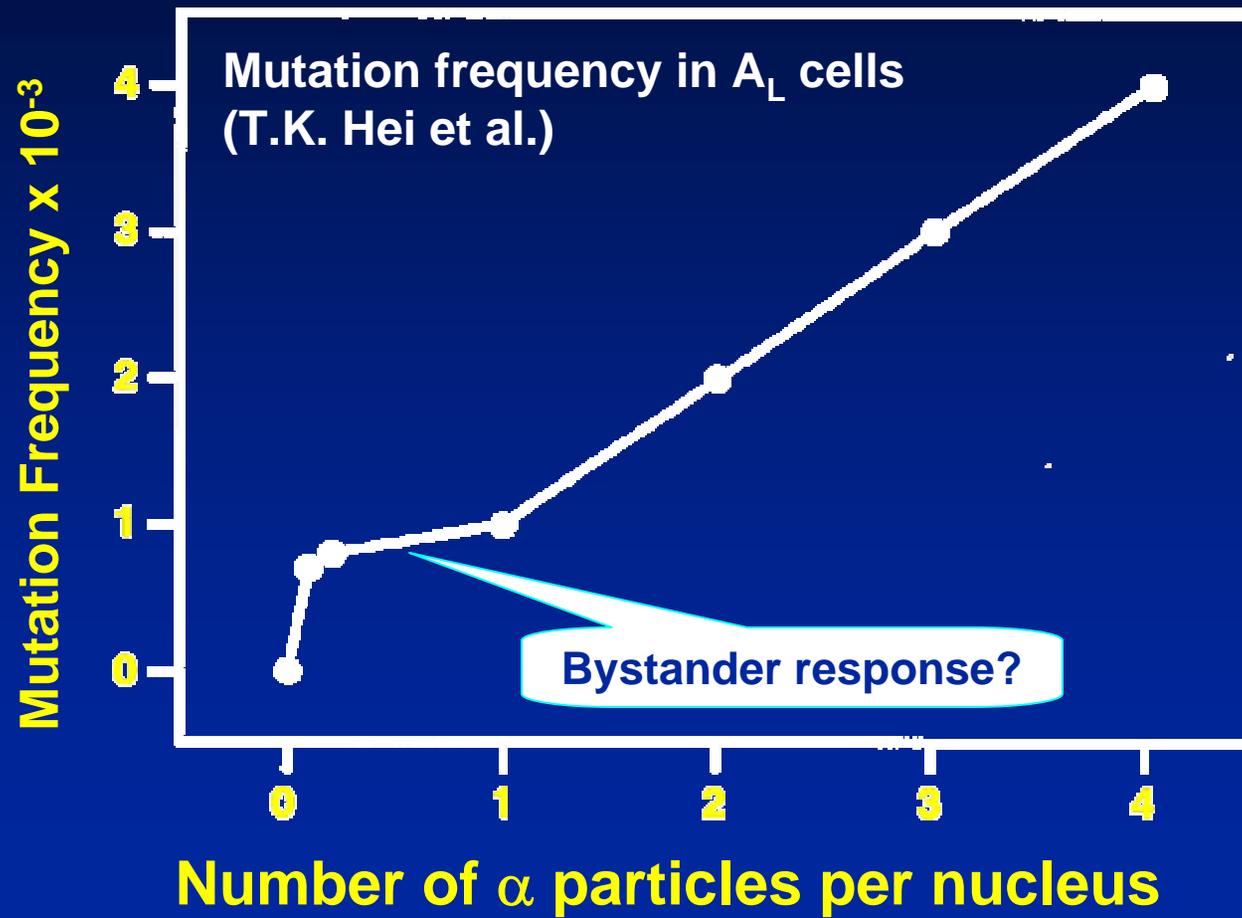
Azzam et al. 1994

A scenario for downwardly-curving dose responses – An adaptive response

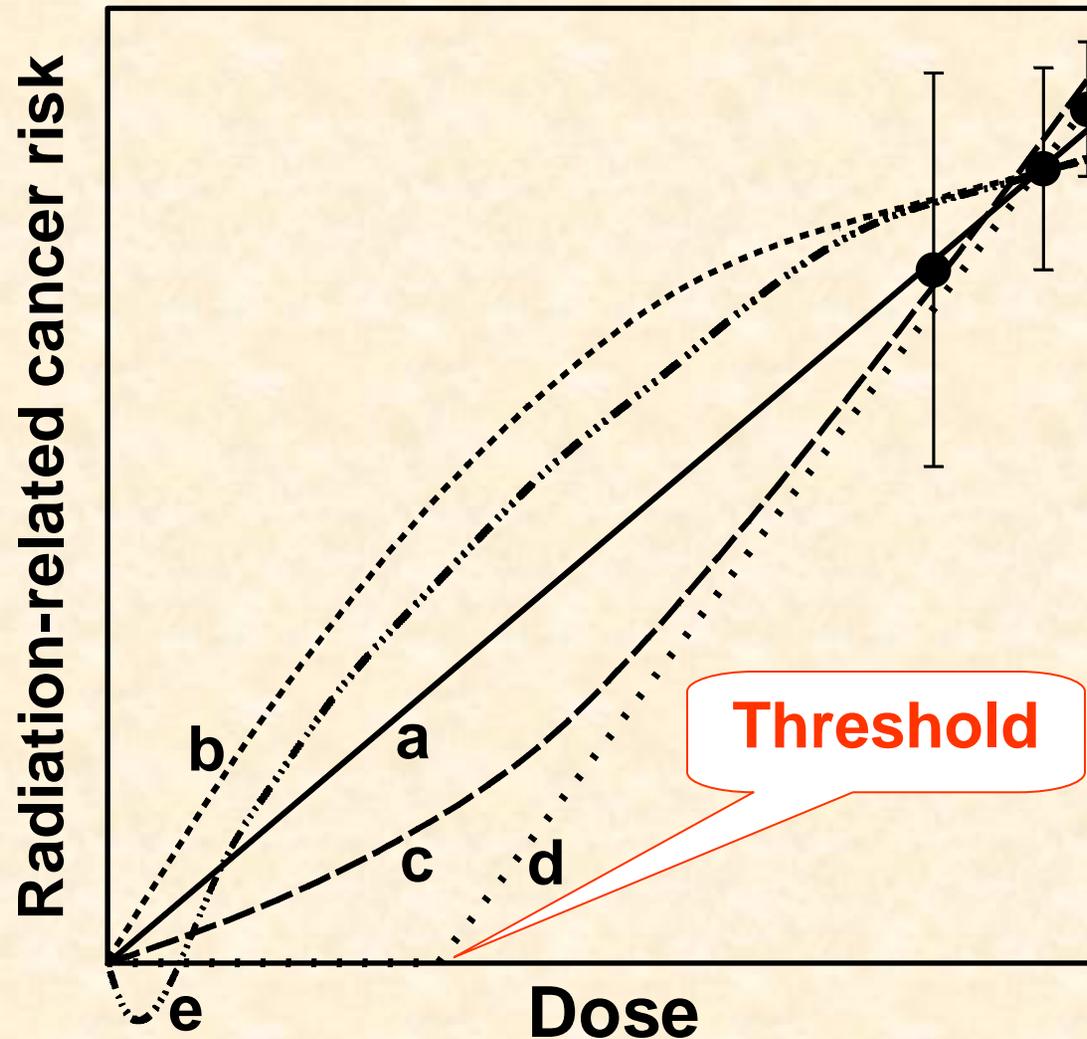


Azzam et al. 1994

A scenario for downwardly-curving dose responses – Bystander effects



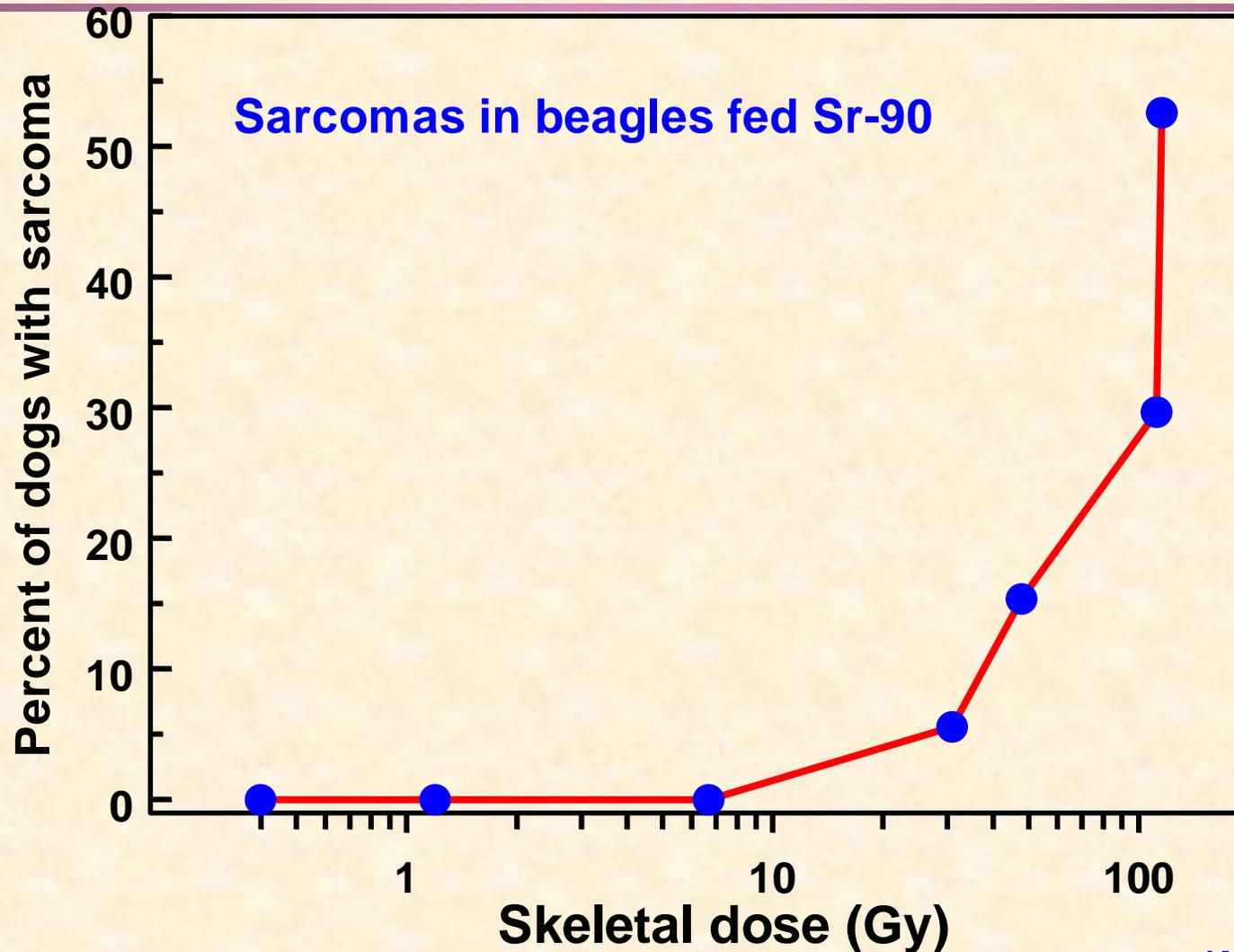
Different possible low-dose extrapolations



Thresholds for radiation-induced sarcomas

- **Non-cycling cells need a large dose to stimulate then to cycle**
- **Evidence in animal studies**

A threshold response - bone sarcomas in beagles

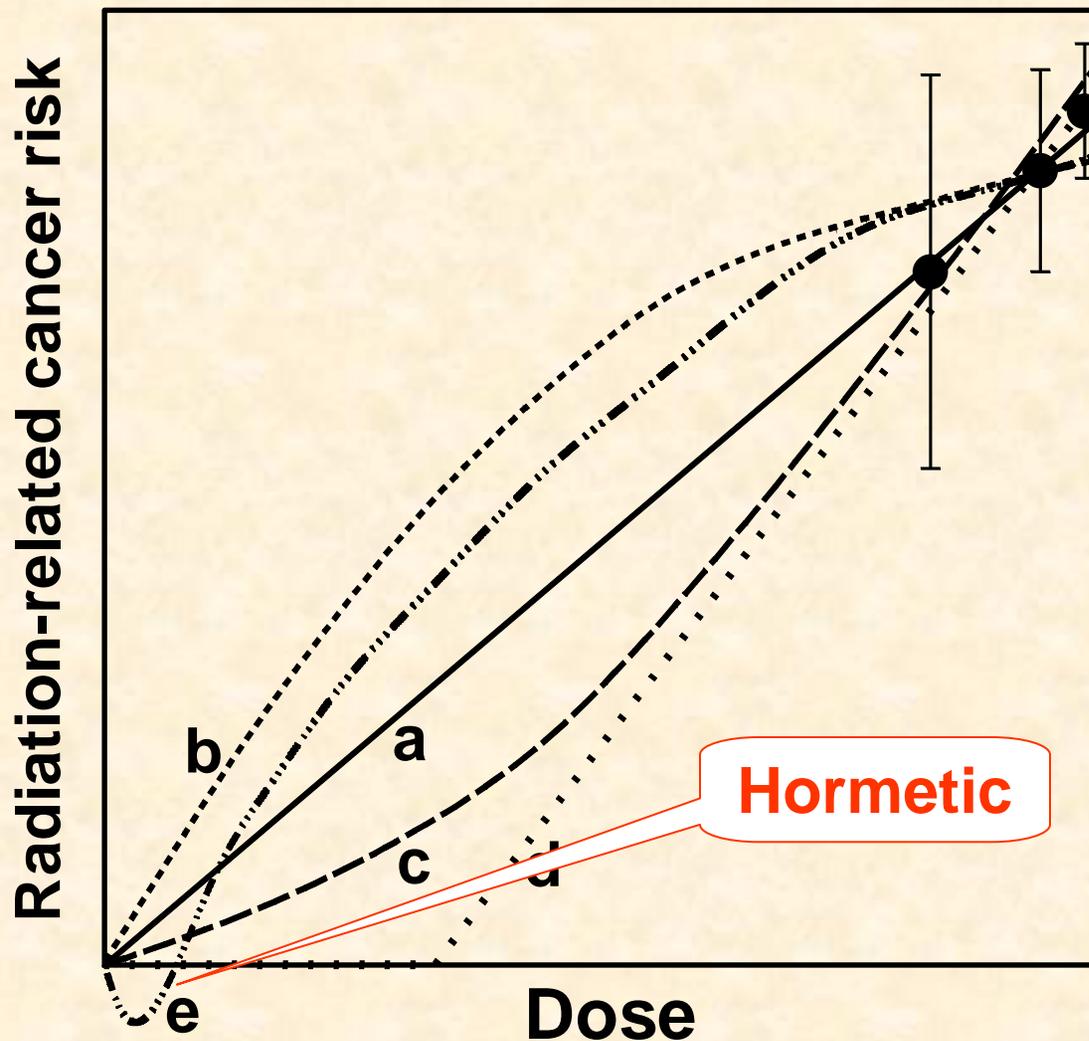


White *et al* 1993

Thresholds for radiation-induced sarcomas

- **Non-cycling cells need a large dose to stimulate them to cycle**
- Evidence in animal studies
- **Evidence for thresholds in induced sarcomas after RT**
- Evidence in A-bomb survivors
 - » Mean dose 200 mSv
 - » No significance increase in bone cancers
 - » Significant increase in **carcinomas**

Different possible low-dose extrapolations



Hormesis:

Can low doses of radiation increase longevity?

Mice: 500 mGy acute whole-body exposure

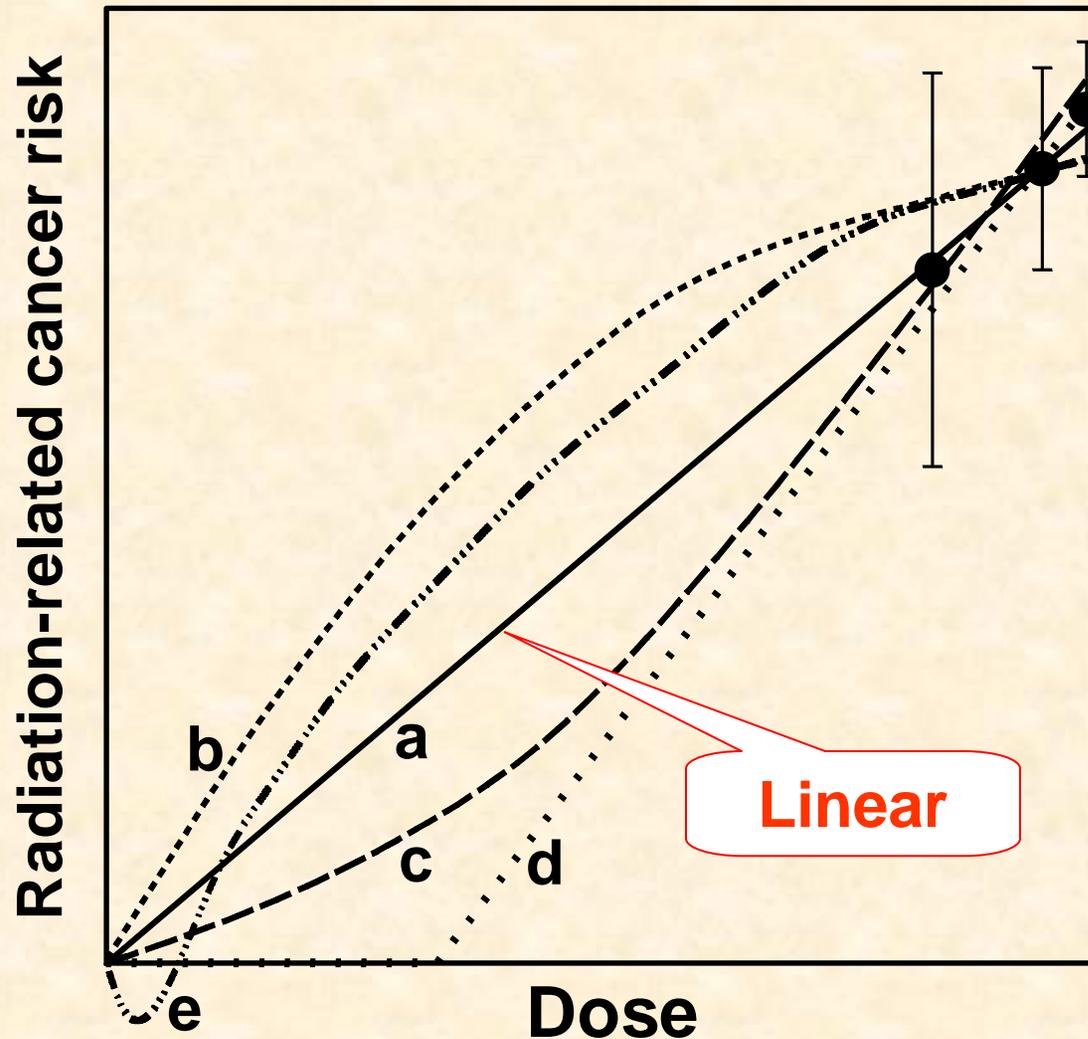
	Maisin <i>et al.</i> (1996)	Storer <i>et al.</i> (1979)
Number of mice	138	1,390
Life shortening (days)	-50	75

Hormesis:

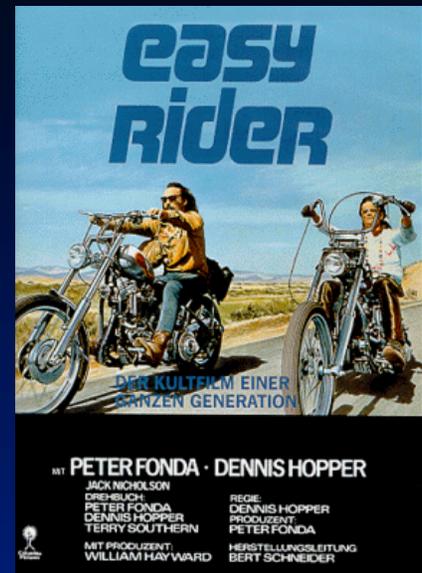
DNA repair *vs.* immune response

- In those animal experiments in which an increase in lifespan has been observed, the gain has generally **not reflected a reduction in malignant disease**, but rather an **early reduction in mortality from infections** and other non-malignant diseases.
- This suggests that a lifespan increase, if real, is less likely to be associated with a radiation-related stimulation of DNA repair mechanisms, and more likely to be associated with a **radiation-induced enhancement in the immune system**.

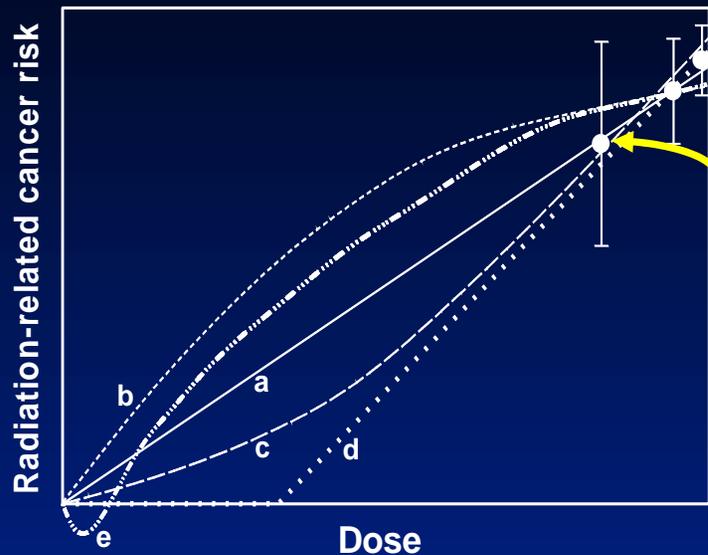
Different possible low-dose extrapolations



Once we are down to doses corresponding to about 1 electron track per cell, extrapolation to still lower doses becomes an easier task



All that happens at still lower doses is that fewer cells feel the same type of damage....



Our “anchor point” is about 6 mGy (from in utero data)

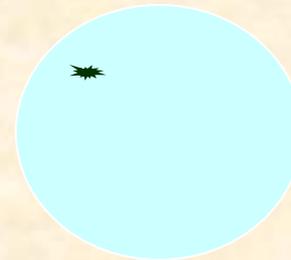
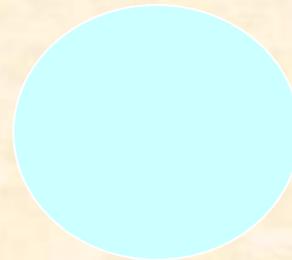
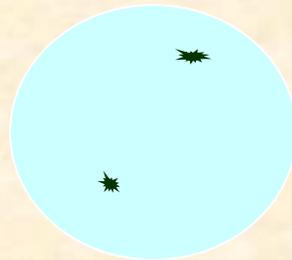
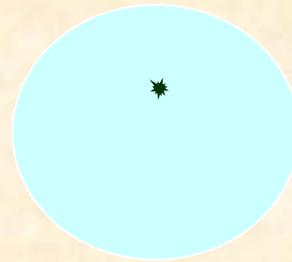
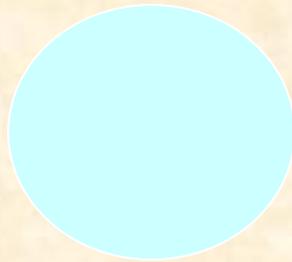
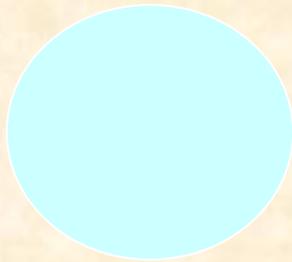
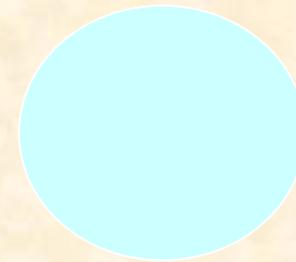
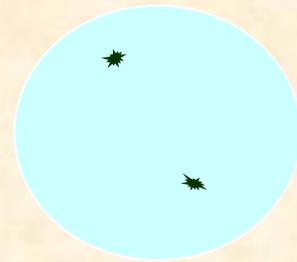
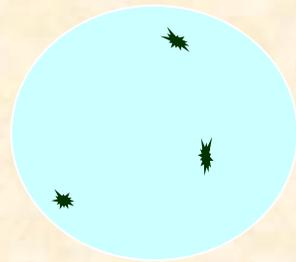
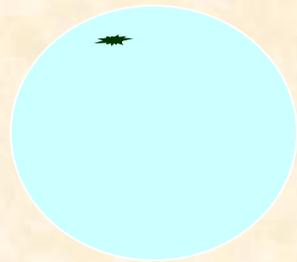
- **6 mGy of 80 kVp x rays corresponds to a mean of about one electron track per nucleus**

The Biophysical Argument for Linearity

- 1. There is direct epidemiological evidence that a dose of 6 mGy of diagnostic x rays is associated with an increase in cancer risk.**
- 2. At a dose of 6 mGy of diagnostic x rays, most irradiated cell nuclei will be traversed by 1 or at most a few physically-distant electron tracks. Being so physically distant, it is unlikely that these few electrons tracks could produce DNA damage in a joint, cooperative way; rather these electron tracks will act independently.**

**Twelve 6-micron cell nuclei exposed to
6 mGy of 80 kVp x rays**

Mean number of electron tracks / nucleus: 1



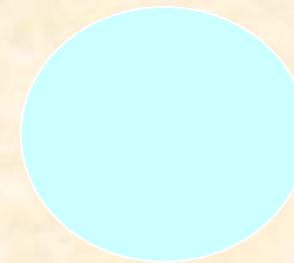
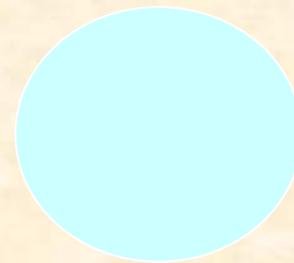
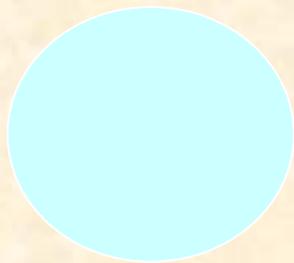
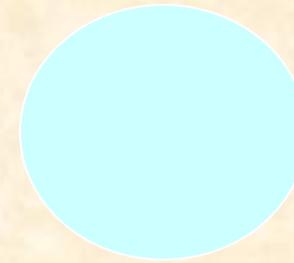
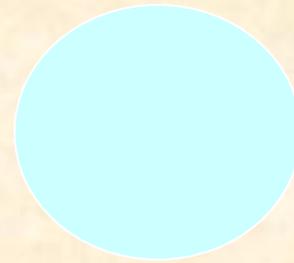
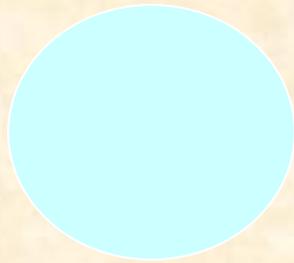
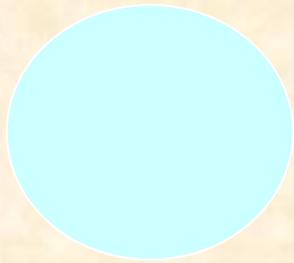
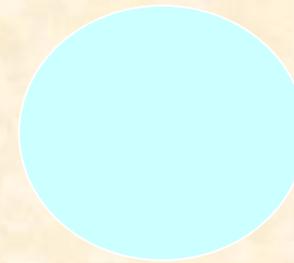
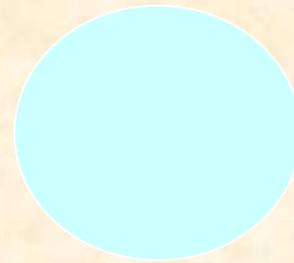
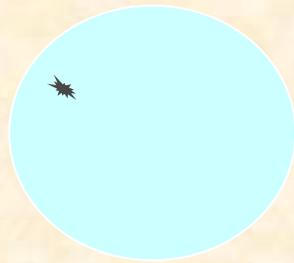
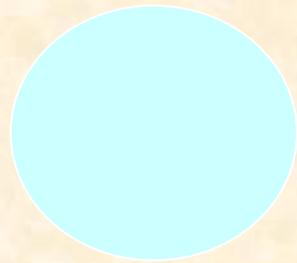
The Biophysical Argument

(continued)

3. If the dose is decreased, say by a factor of 10, this will simply result in proportionately fewer electron tracks and fewer hit cells. It follows that those fewer cells that are hit at the lower dose....
 - a) will be subject to the same types of electron damage
 - b) will be subject to the same radiobiological processes as would occur at 6 mGy.

**Twelve 6-micron cell nuclei exposed to
0.6 mGy of 80 kVp x rays**

Mean number of electron tracks / nucleus: 0.1



The Biophysical Argument

(continued)

4. Decreasing the number of damaged cells by a factor of 10 would be expected to decrease the biological response by the same factor of 10, i.e. response would decrease linearly with decreasing dose.

One could not expect qualitatively different biological processes to be active at 0.6 mGy that were not active at 6 mGy.

The argument suggests that the risk of most radiation-induced endpoints will decrease linearly, without threshold, from ~6 mGy down to arbitrarily low doses.

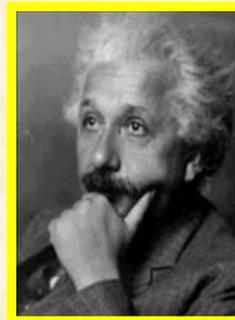
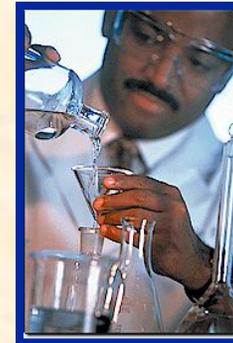
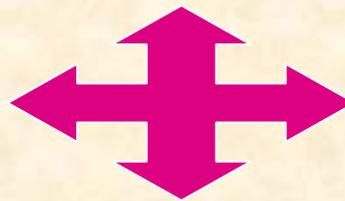
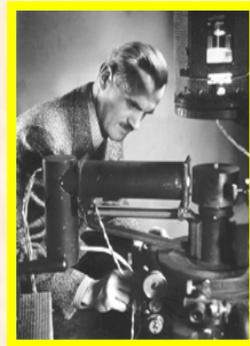
The Biophysical Argument

(continued)

- 5. This argument would potentially not hold if other irradiated cells could decrease the probability that any given initially radiation-damaged cell develops into, say, a cancer, in a way which is non-linear with dose.**

In fact those cooperative effects that have been observed, such as bystander and delayed-instability, have shown saturation at low doses, and thus would increase rather than decrease the probability that any initially radiation-damaged cell would ultimately result in a cancer.

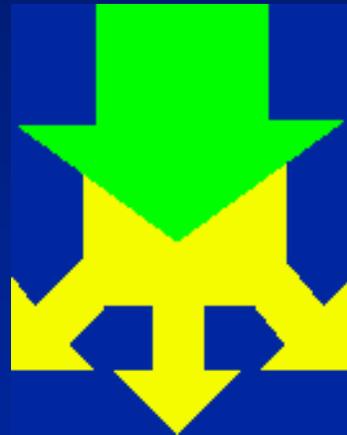
We do not know if the biophysical argument is correct, though it is probably the strongest we have at very low doses



We still need all four approaches to pushing risk estimates still lower

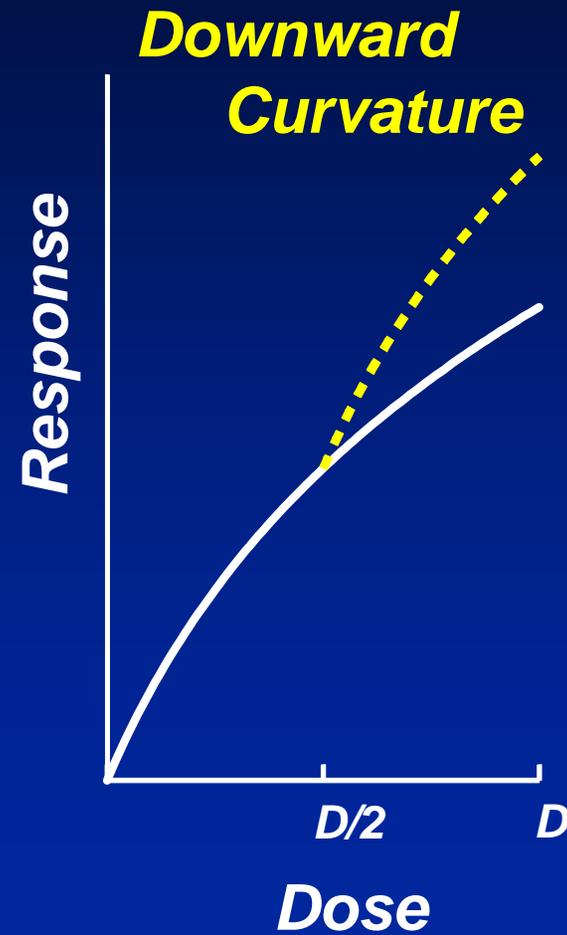
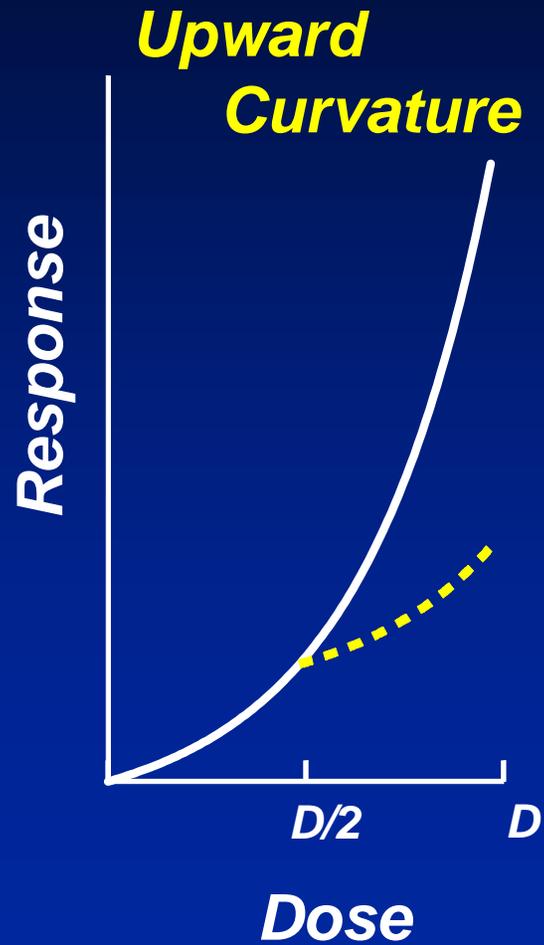
Dose Rate Effects

**Shape of the
acute dose-response curve
at low doses**



Dose rate effects

Splitting the Dose into Fractions

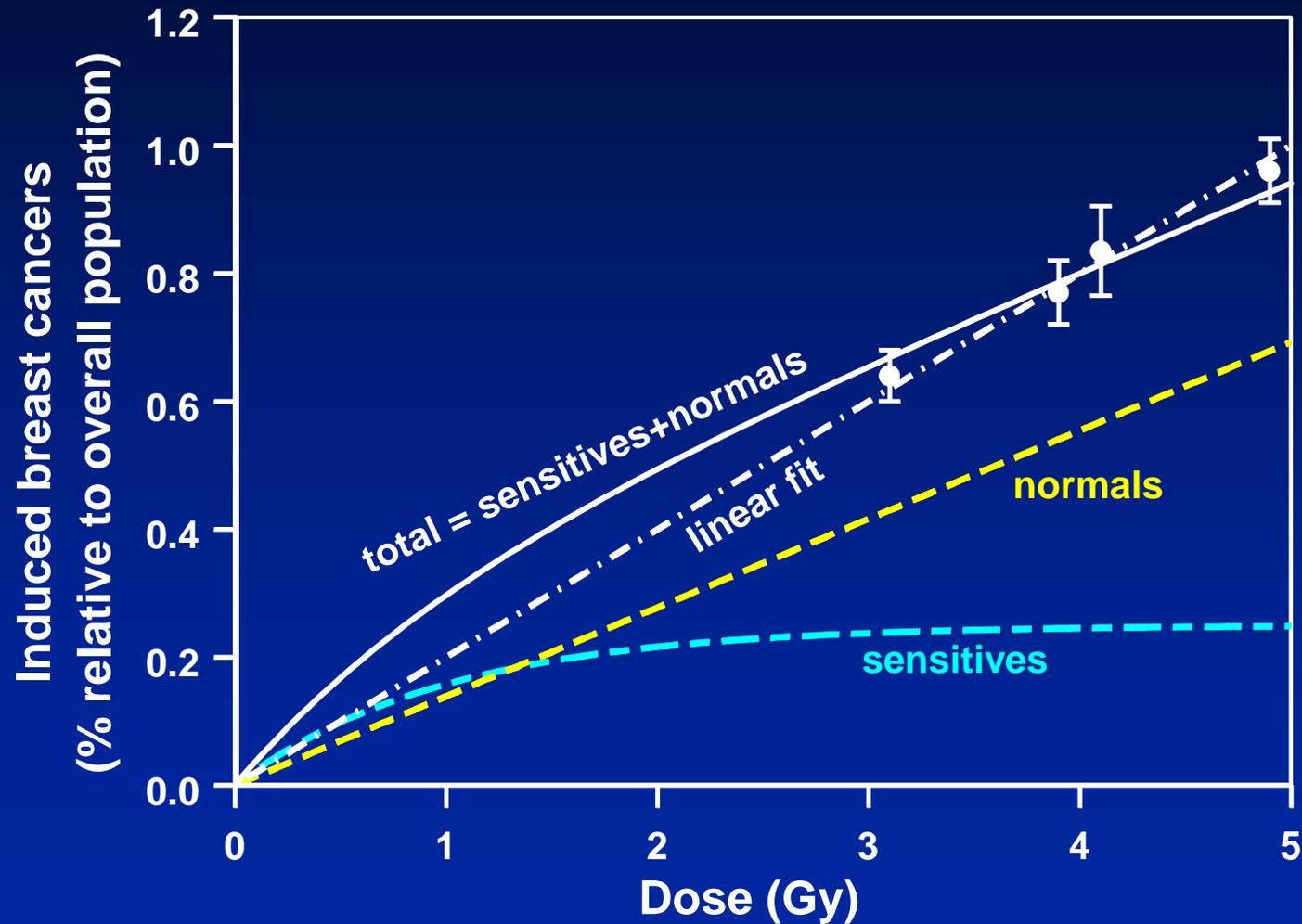


The “Repeater” Rule

The Repeater Rule for protraction only applies if the population radiosensitivity **restores** during the protracted exposure

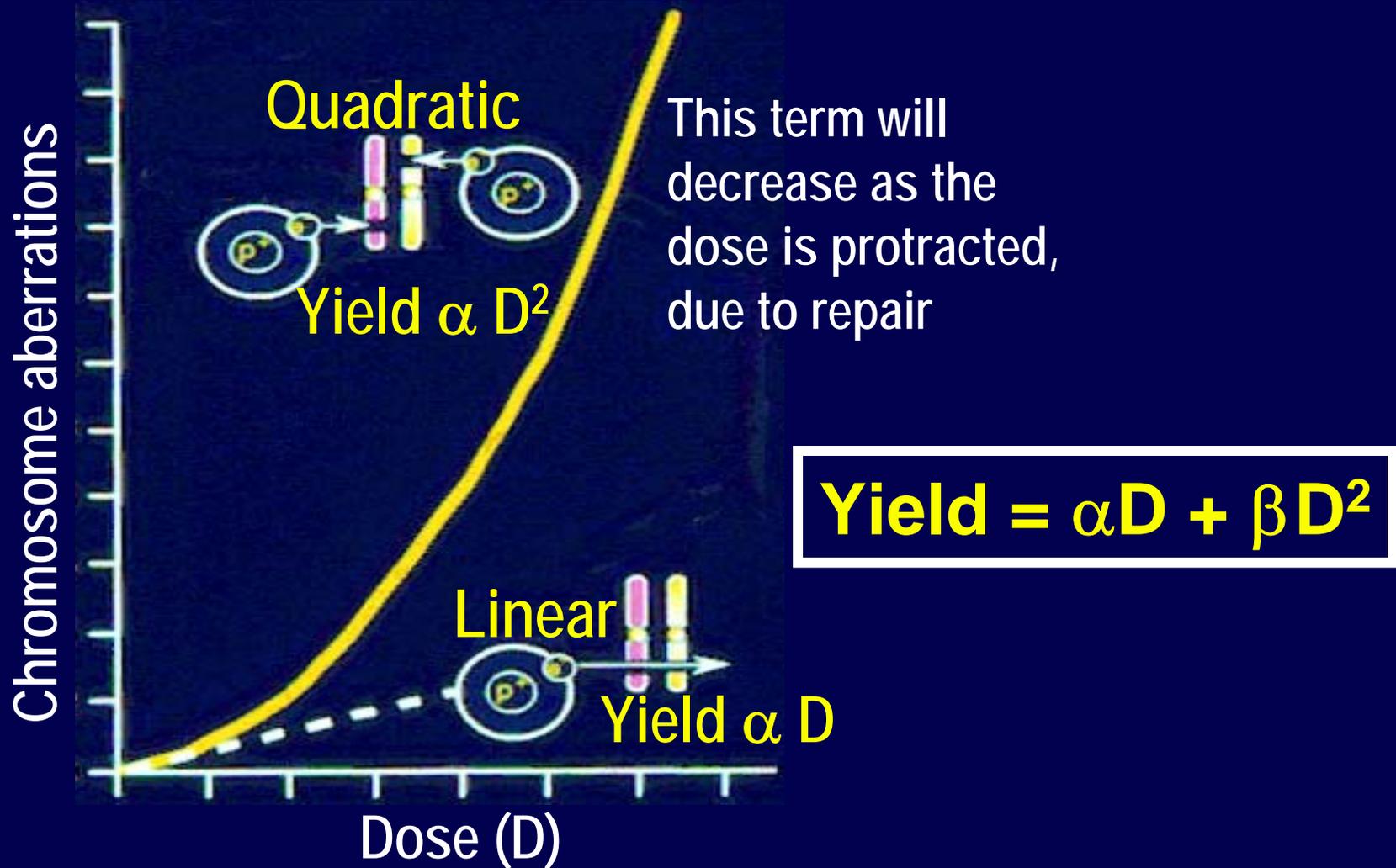
e.g., the distribution of radiosensitivity is roughly the same before the 2nd fraction as it was before the 1st fraction

The potential effect of a sensitive subpopulation



1 hit → linear

2 independent hits → quadratic



The standard linear-quadratic model (LQ)

$$\text{Yield} = \alpha D + G \beta D^2$$

for continuous exposure...

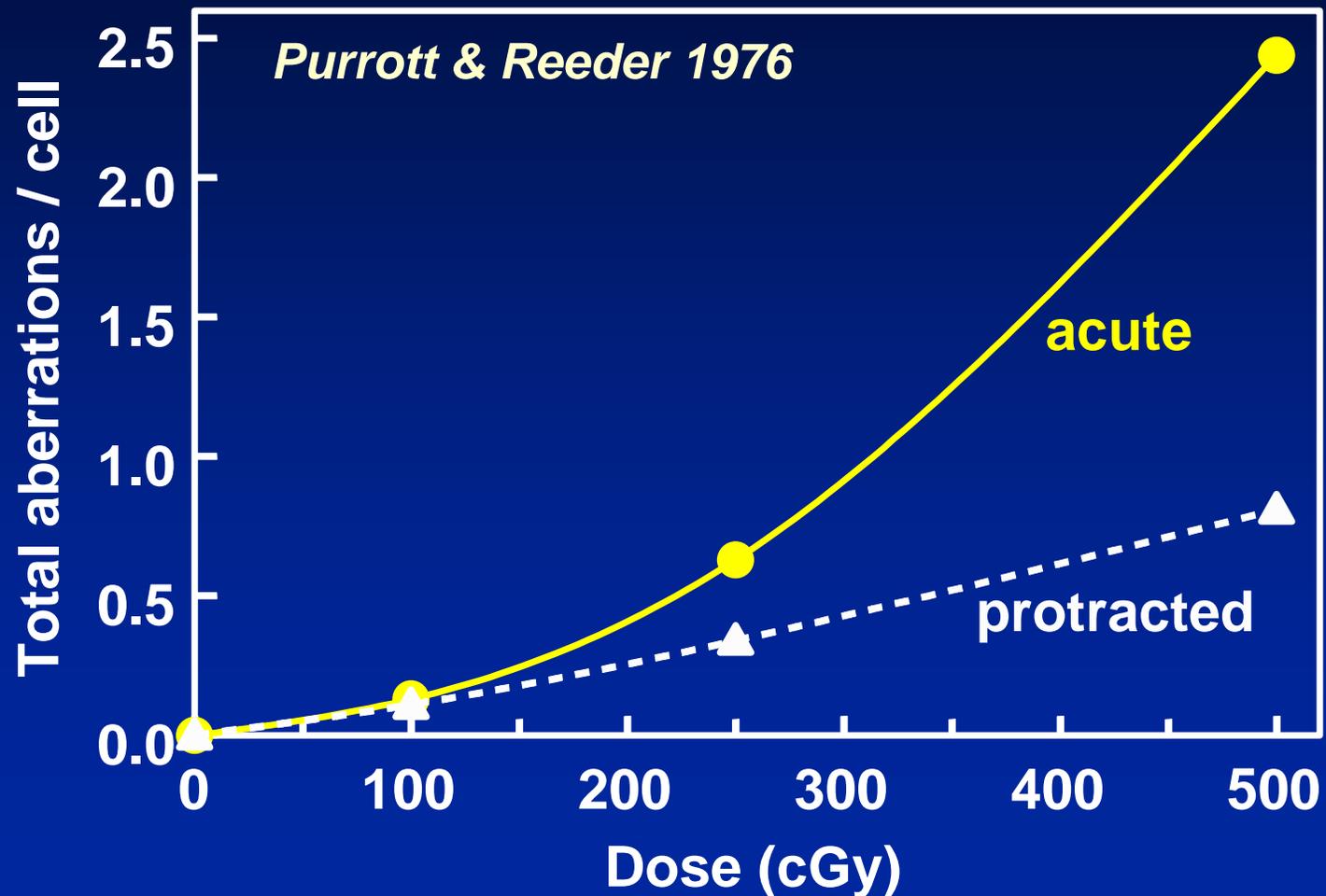
$$G = 2(T/\tau)^2 [(T/\tau) - 1 + \exp(-T/\tau)]$$

T: time of exposure,

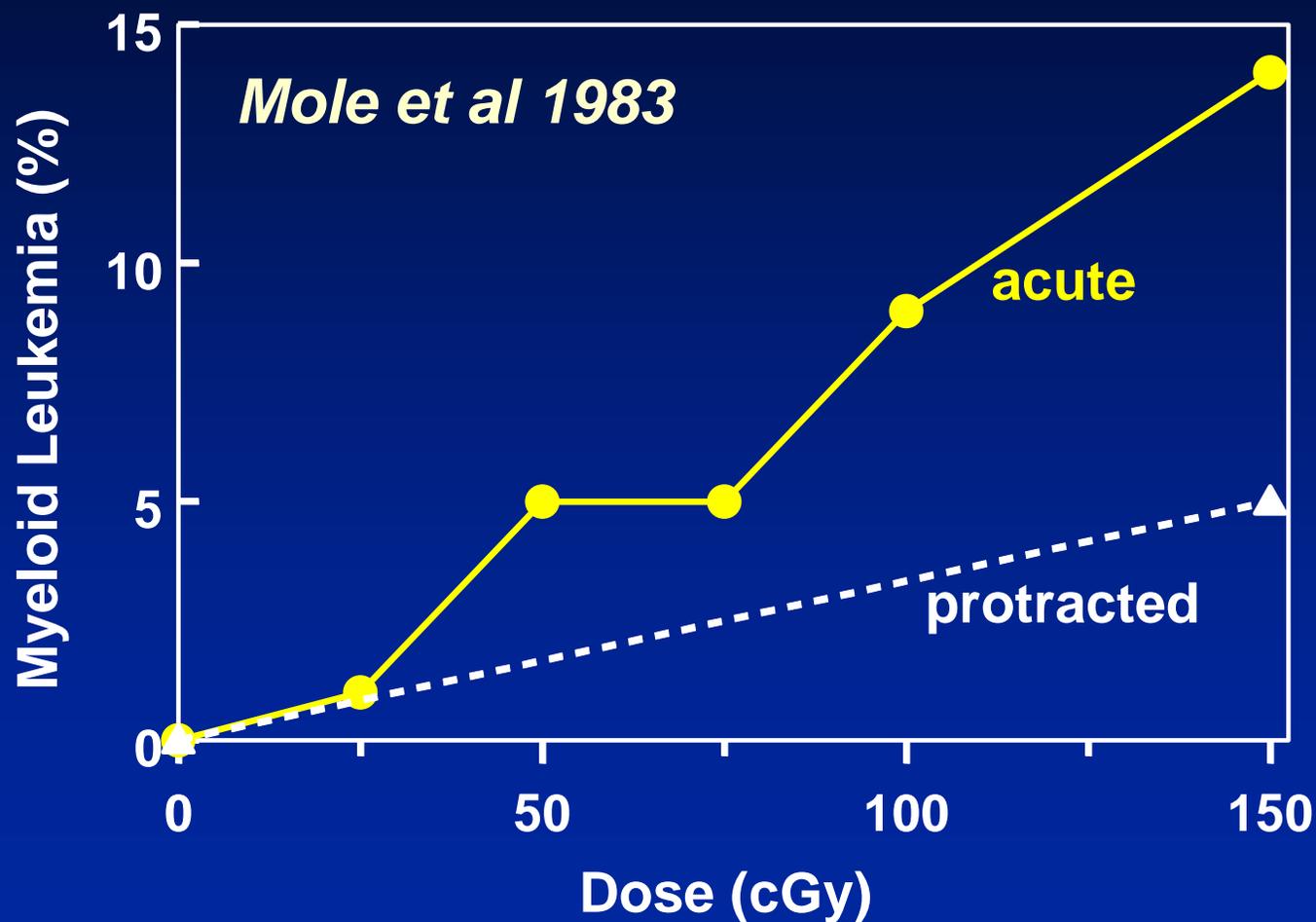
τ , characteristic repair time

- For very short exposures, $G=1$
- For very long exposures, $G=0$

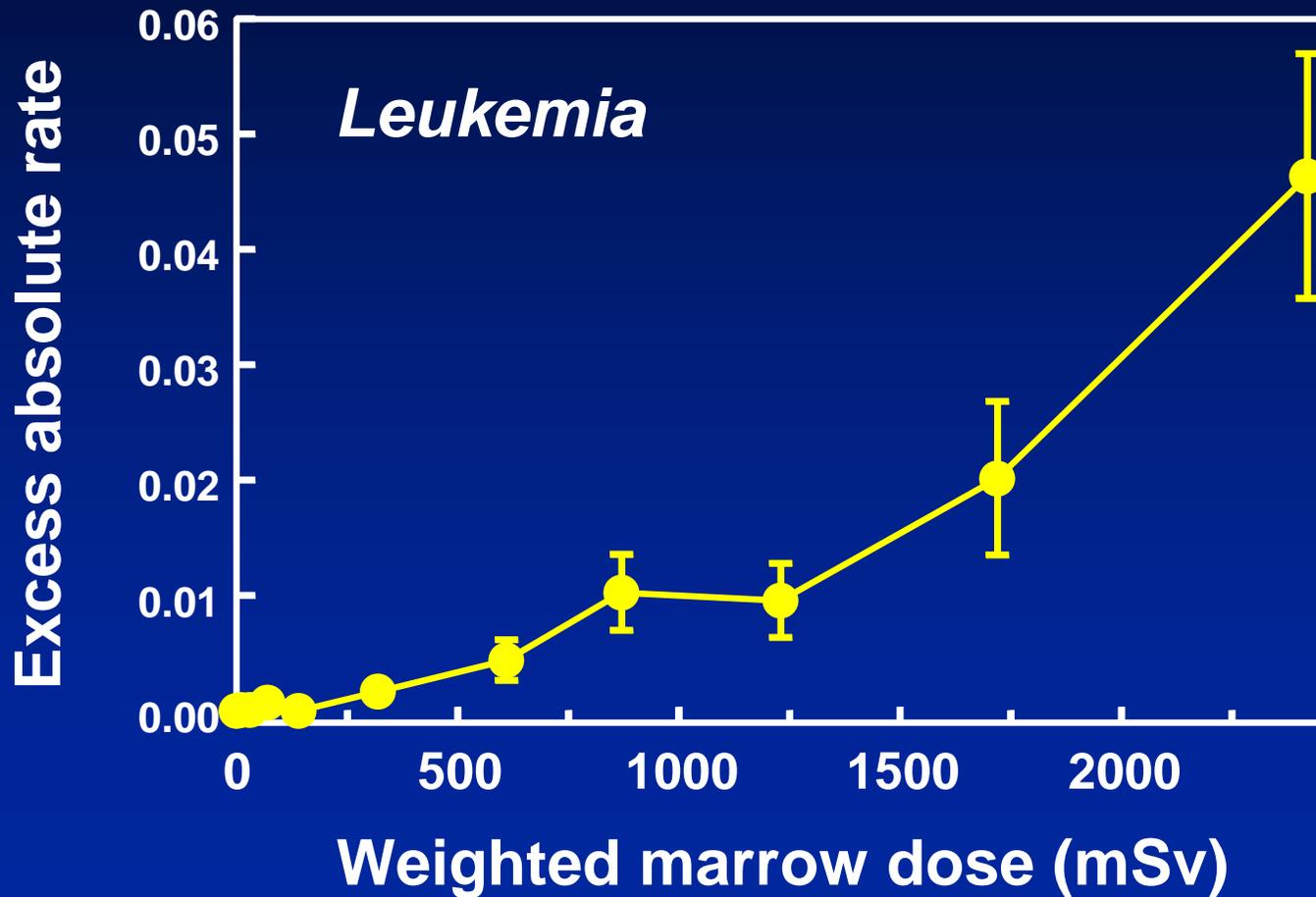
Aberration induction in human lymphocytes 10 cGy/h vs 400 cGy/h



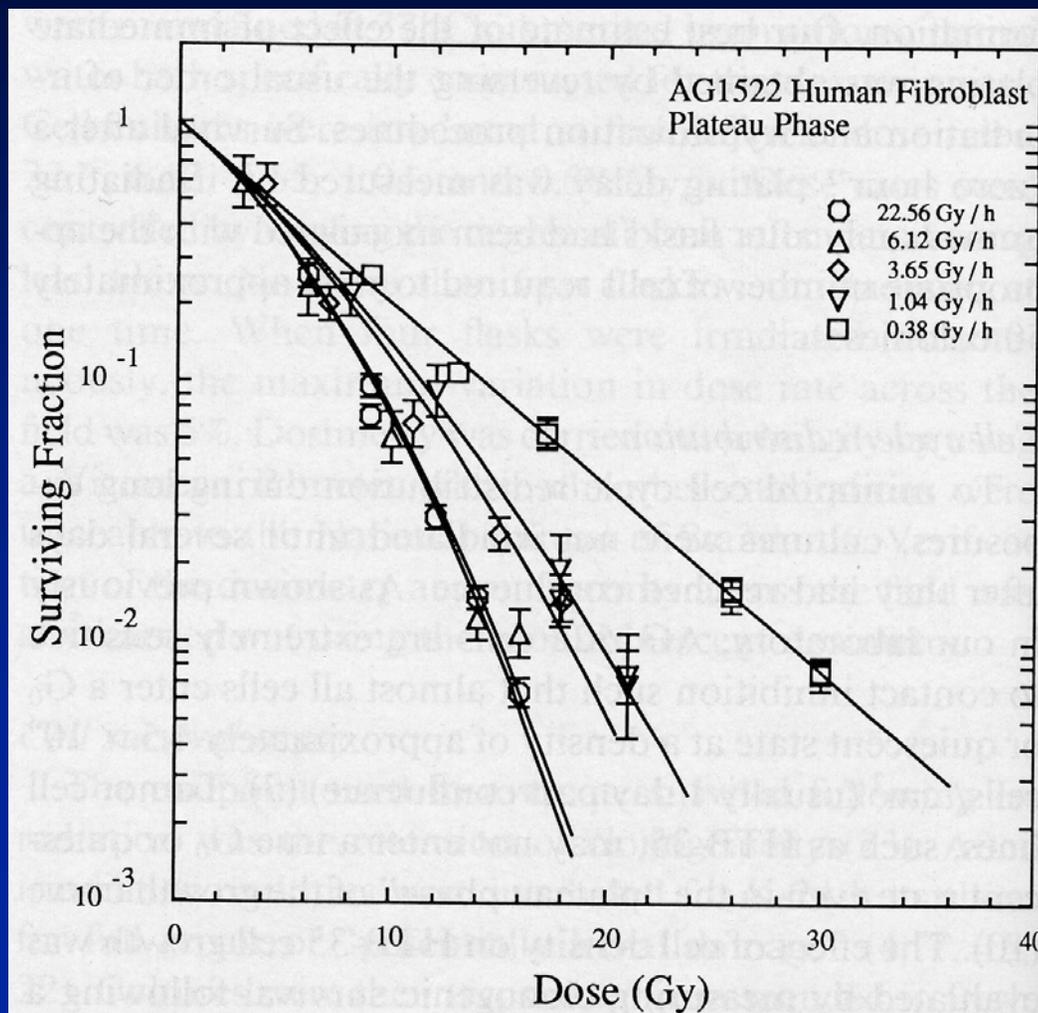
X-ray induction of myeloid leukemia in CBA/H mice



Excess leukemia in A-bomb survivors (Pierce et al 1996)



Dose rate effects for cell killing in normal human cells



**Amdur &
Bedford 1994**

The inverse dose-rate effect for radon

For a given dose of
densely-ionizing radiation,
lowering the dose rate
increases the cancer risk

The inverse dose-rate effect

IDR effect observed from
late 70's in animal
experiments **for high-LET**
carcinogenesis

The inverse dose-rate effect for radon

Independently, radiation (radon) epidemiologists were seeing the IDR...

- Kunz 1979
- Hornung 1981
- Howe 1987
- Darby 1990
- Xuan 1993
- Lubin 1994

The inverse dose-rate effect for radon

Laboratory experiments stimulated mechanistic studies (e.g. Barendsen 1985, Rossi 1986, Brenner 1990)

Clear biophysical conclusion:

IDR effect must decrease with decreasing dose

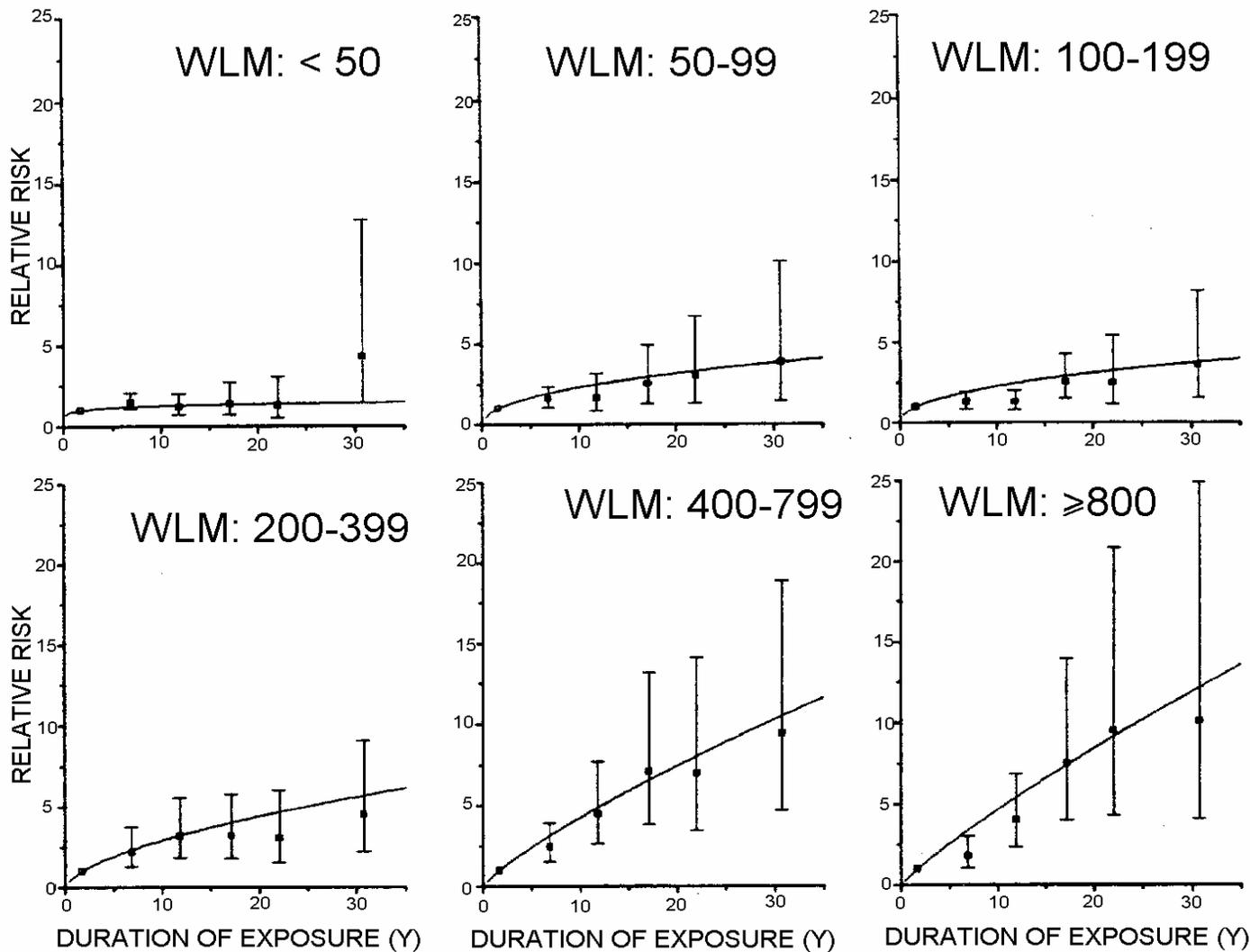
Inverse Dose Rate Effect

If target cell(s) are hit by one or zero alpha particles, there will not be any dose-rate effect of any kind

So the IDR

- **increases** as the **exposure rate decreases**
- **decreases** as the **exposure decreases**

Influence of radon exposure on the dose rate effect



Lubin *et al.* 1995

Relative Biological Effectiveness

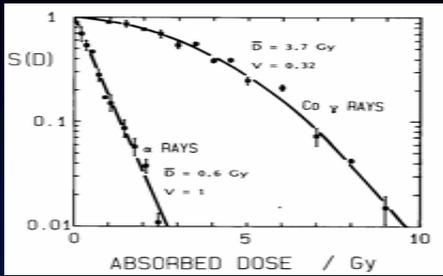
RBE =

**Dose for given probability of effect
by reference radiation**

**Dose for given probability of effect
by test radiation**

Relevance of RBE

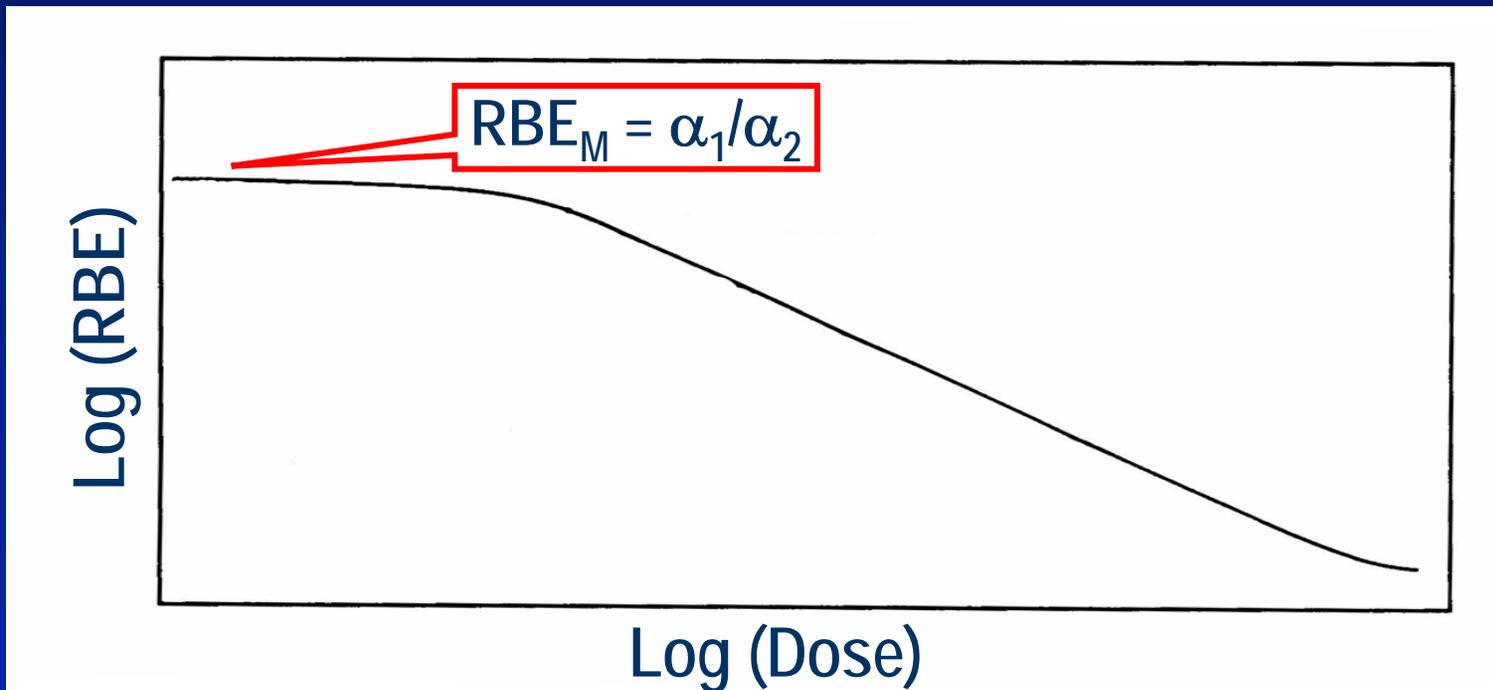
- Radon
- Mammography
- Neutrons
- I-131



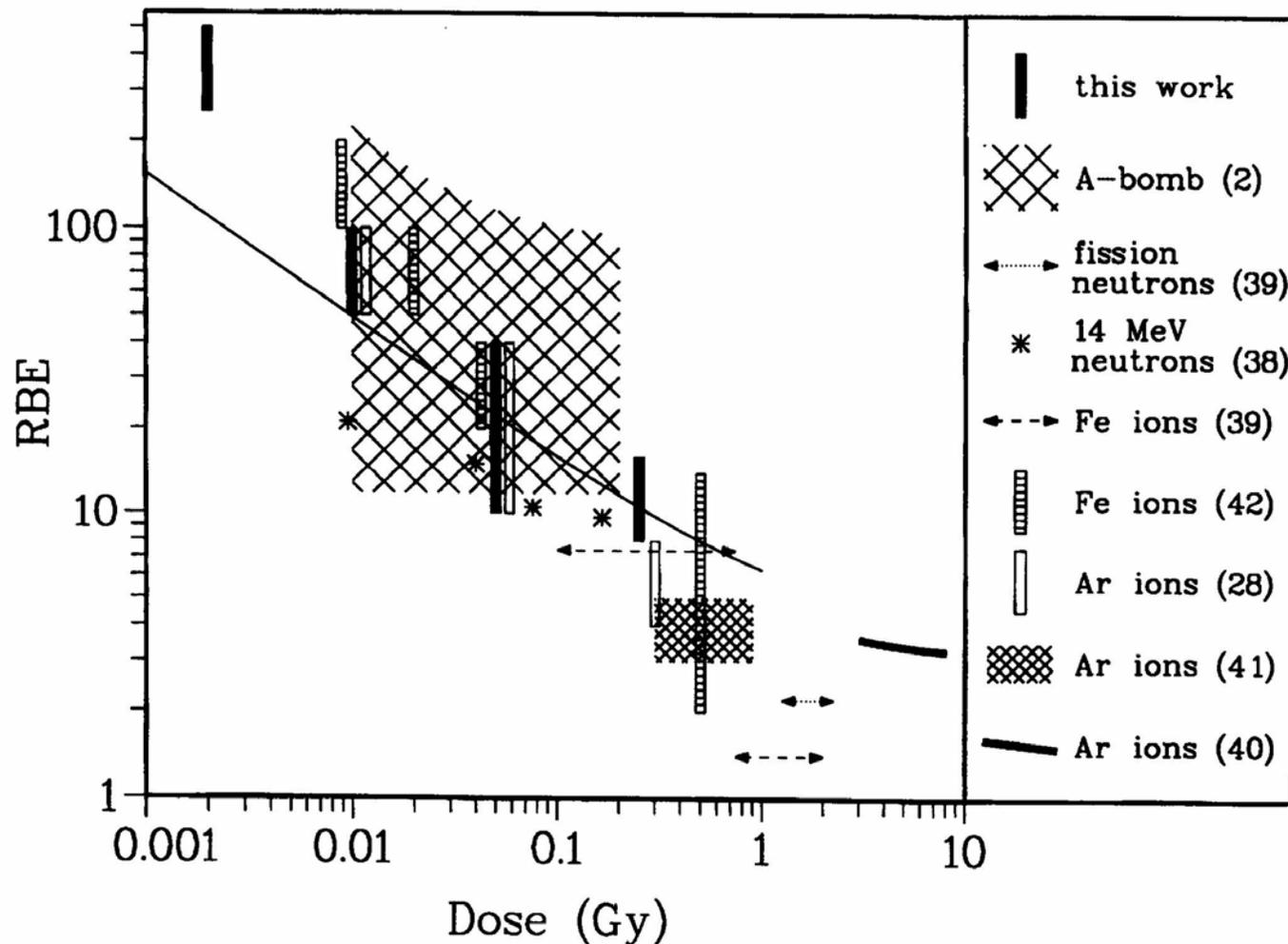
RBE is typically dose dependent

$$\alpha_1 D_1 + \beta_1 D_1^2 = \alpha_2 D_2 + \beta_2 D_2^2$$

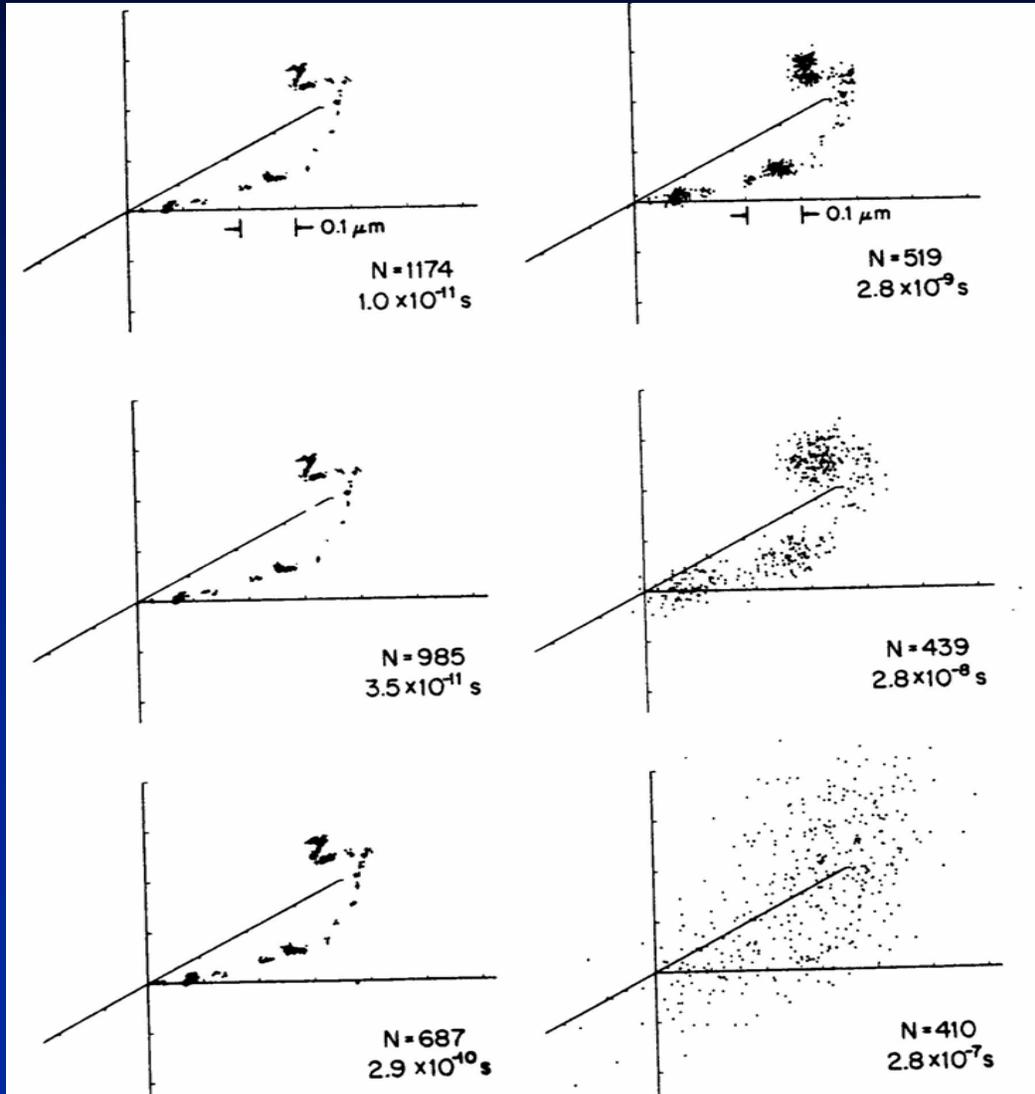
$$\text{RBE} = D_1 / D_2$$



RBE for cataract induction by high LET radiations



RBE must be due to the initial track structure

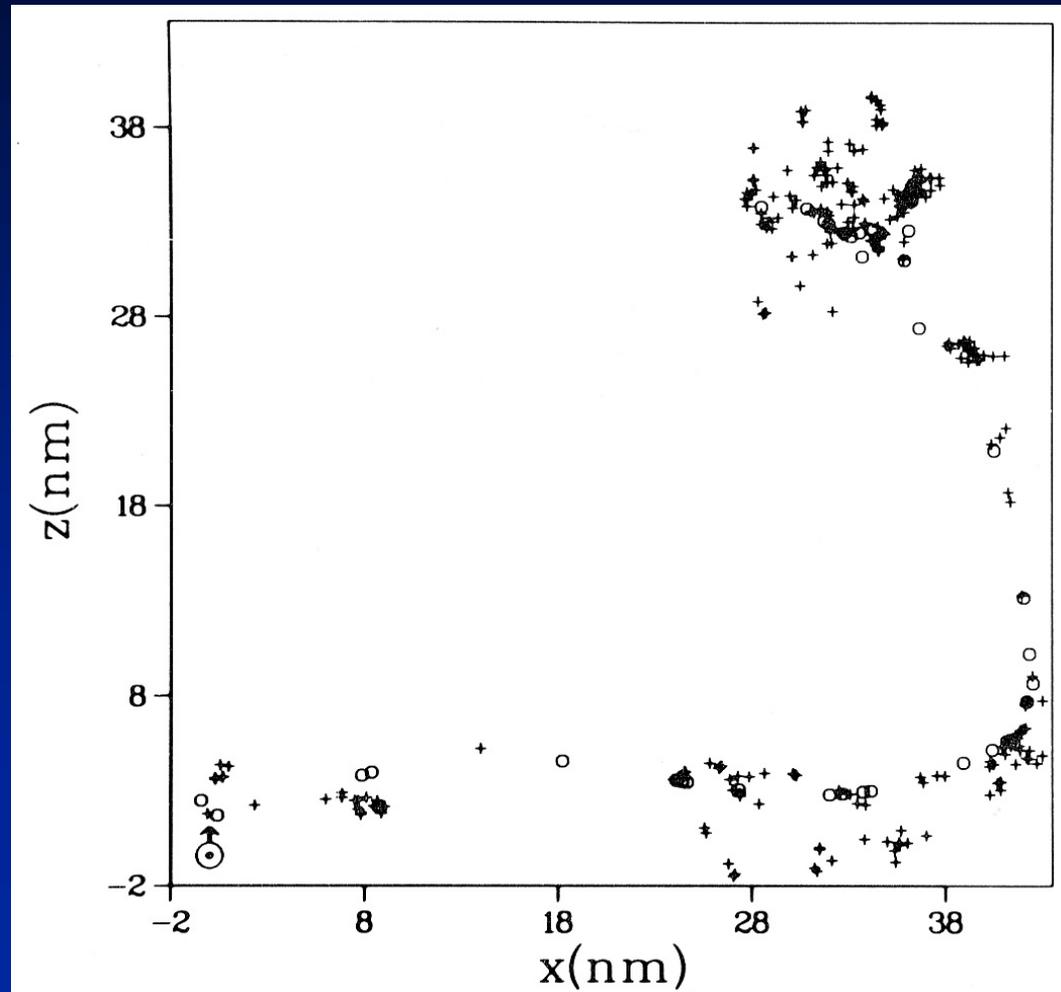


Wright et al
1982

Microdosimetry - The Study of Track Structure

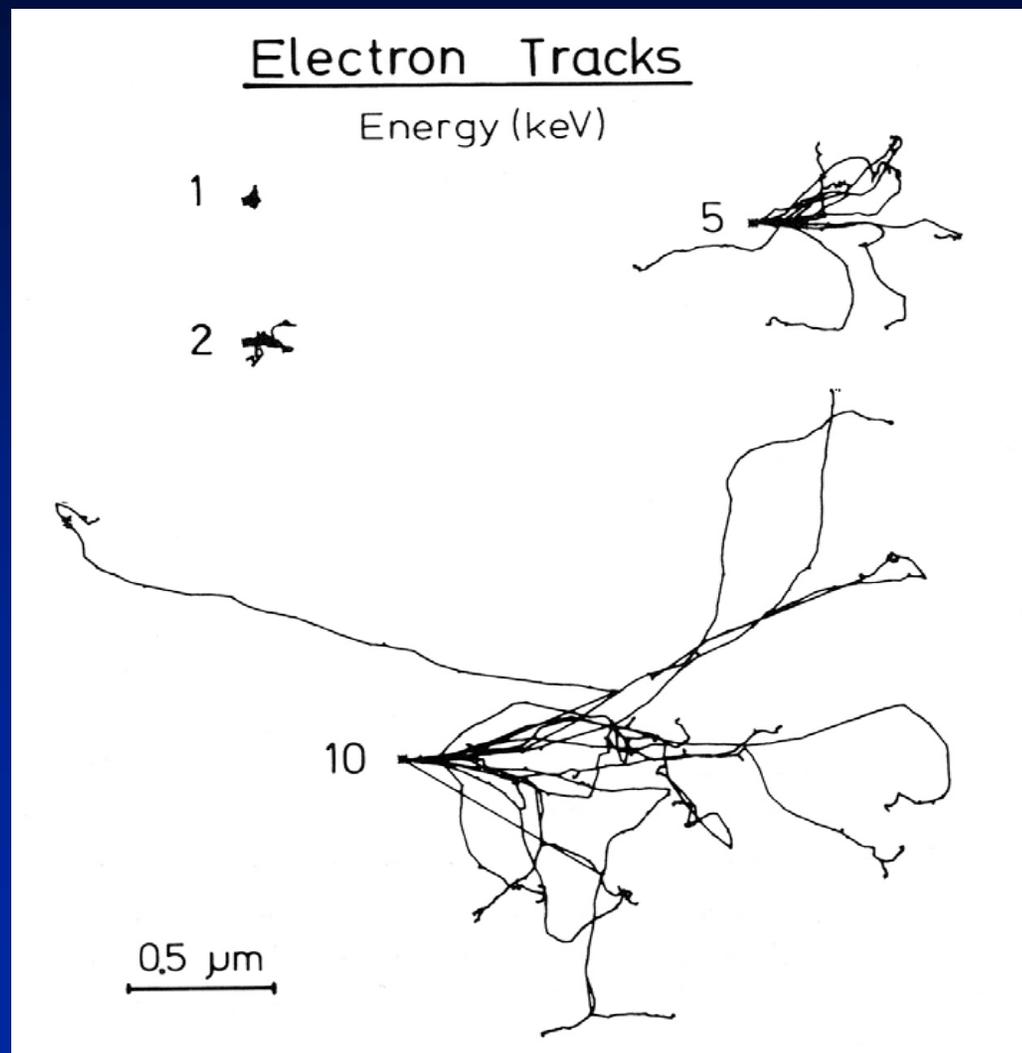
- Ionizing radiations deposit energy in a fundamentally way from that of other mutagens or carcinogens
- The energy imparted, and the subsequent radiation products are not distributed in simple uniform patterns.
- The radiation track is structured, with energy depositions occurring in clusters along the trajectories of charged particles.
- The characterization of energy depositions on micrometer (and smaller) scales is the field of *microdosimetry*

Simulated track of 1 keV electron



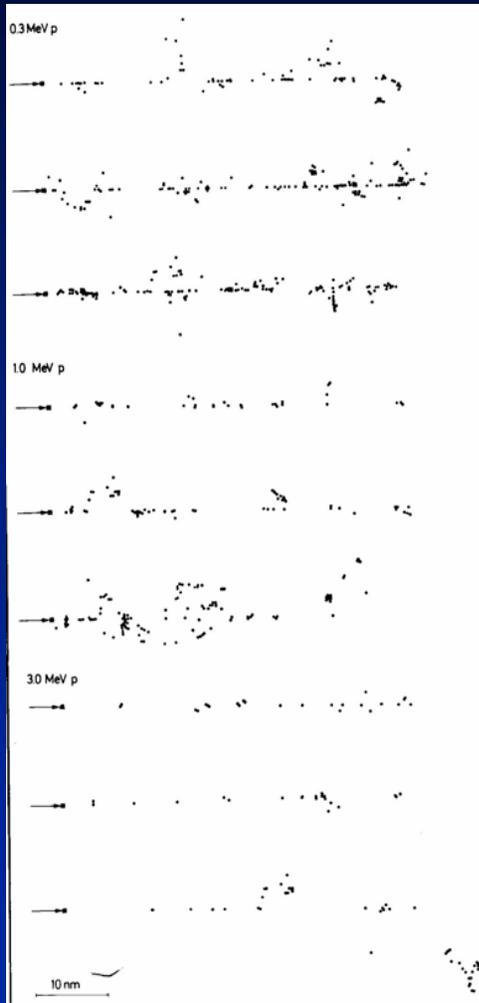
(Zaider & Brenner 1983)

Electron tracks of different energies

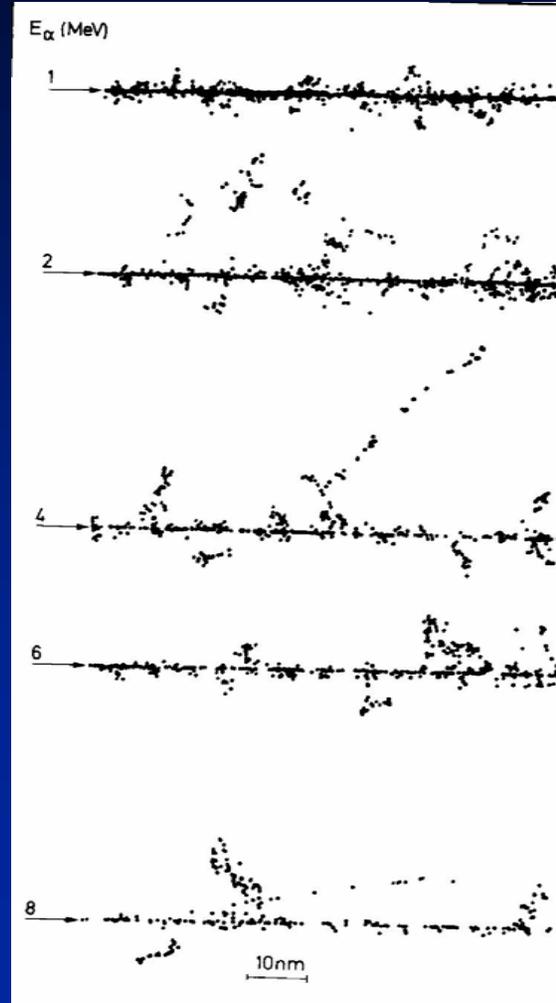


Paretzke 1987

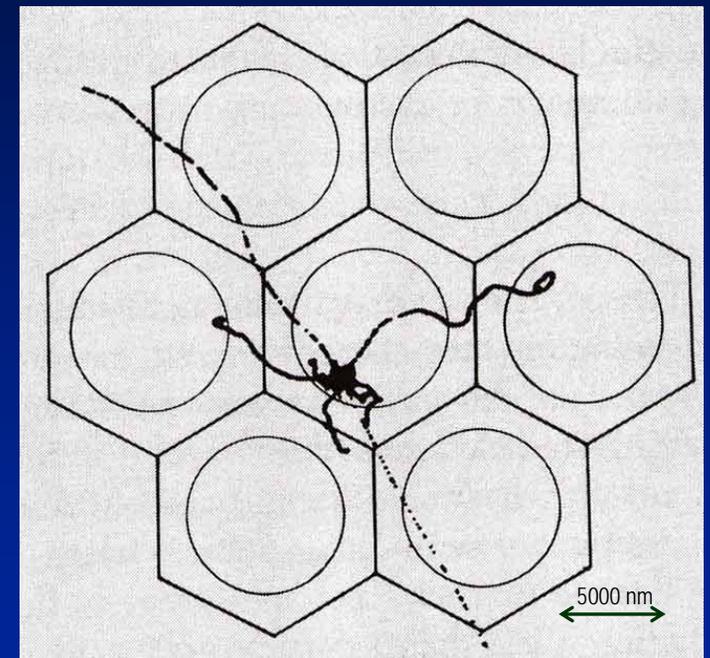
Simulated charged-particle tracks



protons



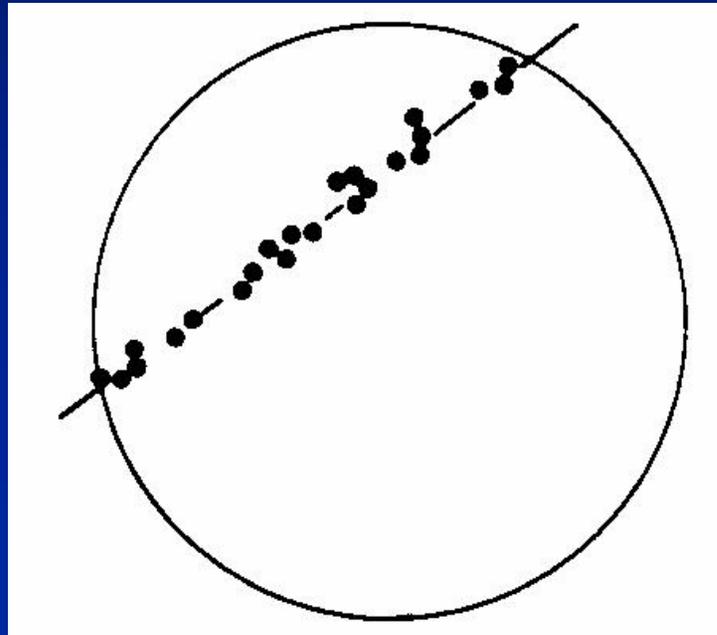
alpha particles



Cosmic-ray iron ion
passing through
lens of eye

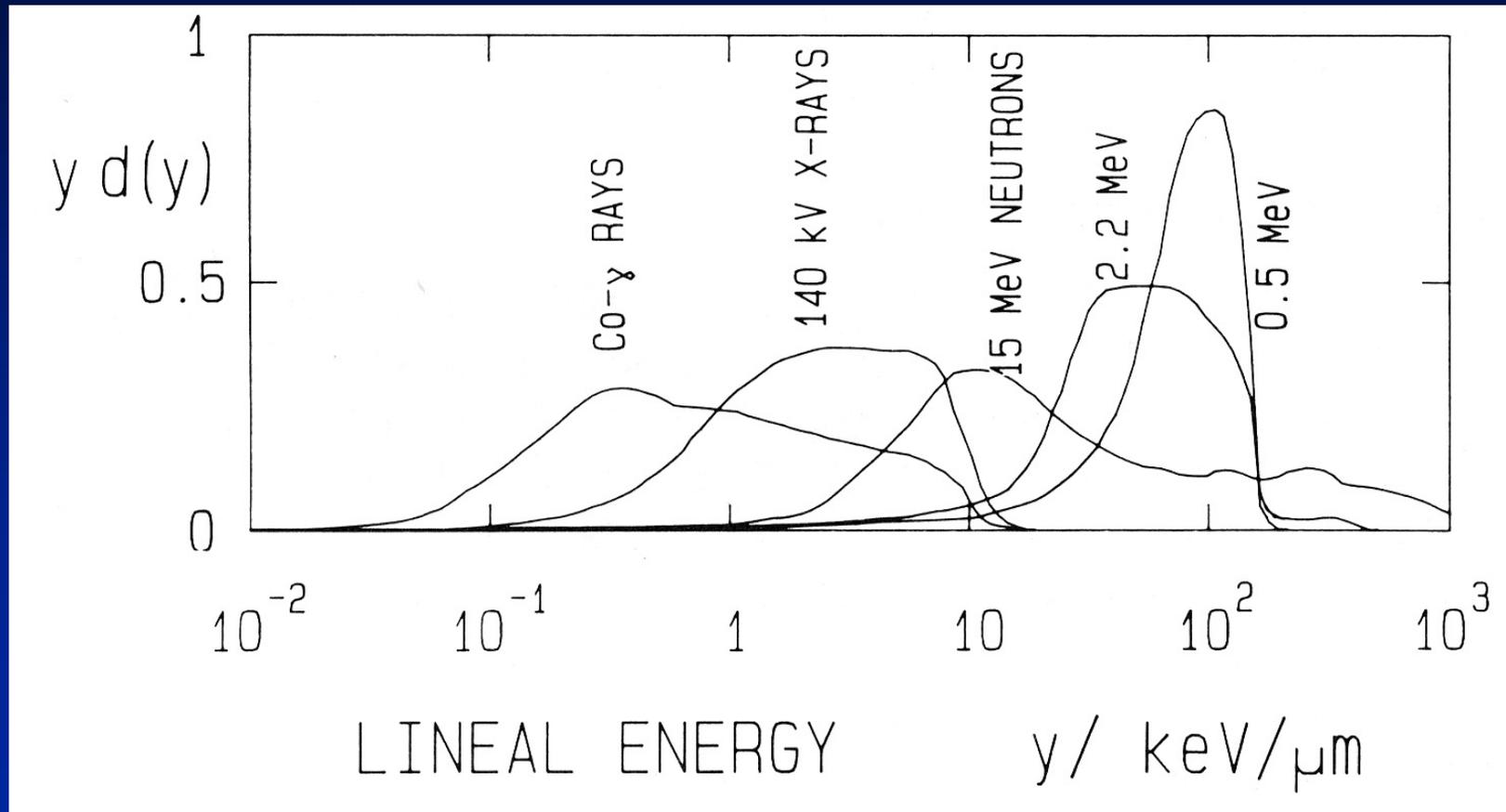
Microdosimetry: ***Lineal Energy (y)***

**Energy deposited in a target
by a single radiation track, divided by
the mean chord length of the target**

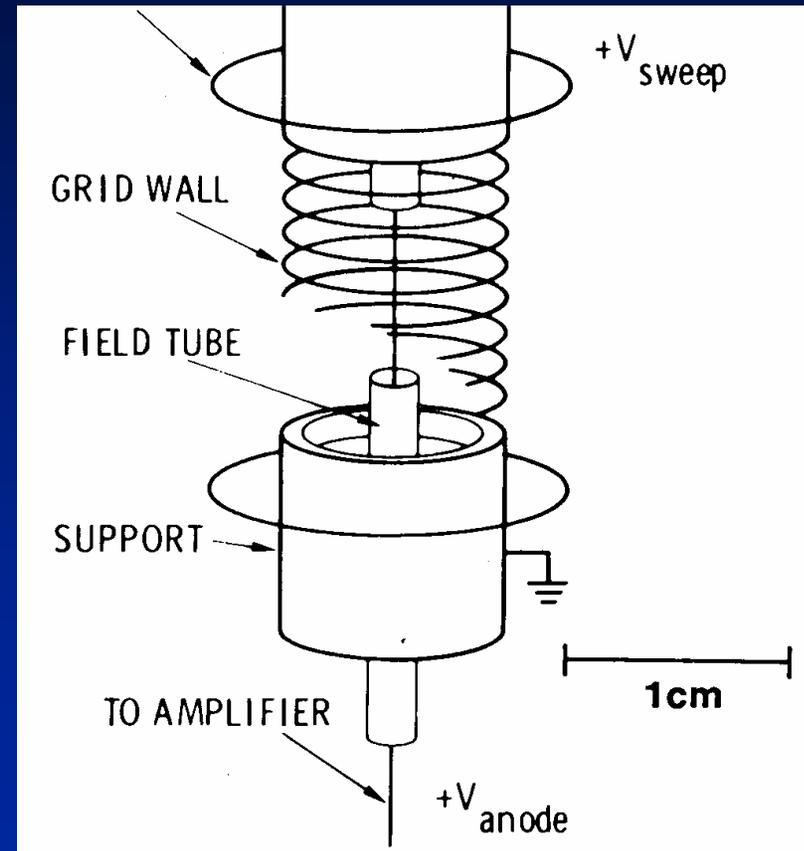


Microdosimetric Distributions:

Distributions of energy deposition in micron site sizes



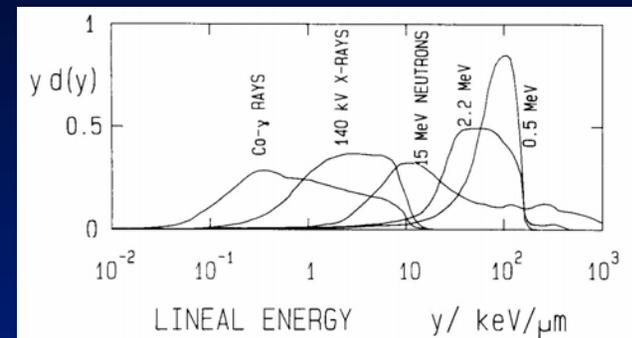
Microdosimetric spectra can be calculated or measured



From track structure to RBE_M

1. Site model (empirical)

$$\text{RBE}_M = \int d(y) r(y) dy$$

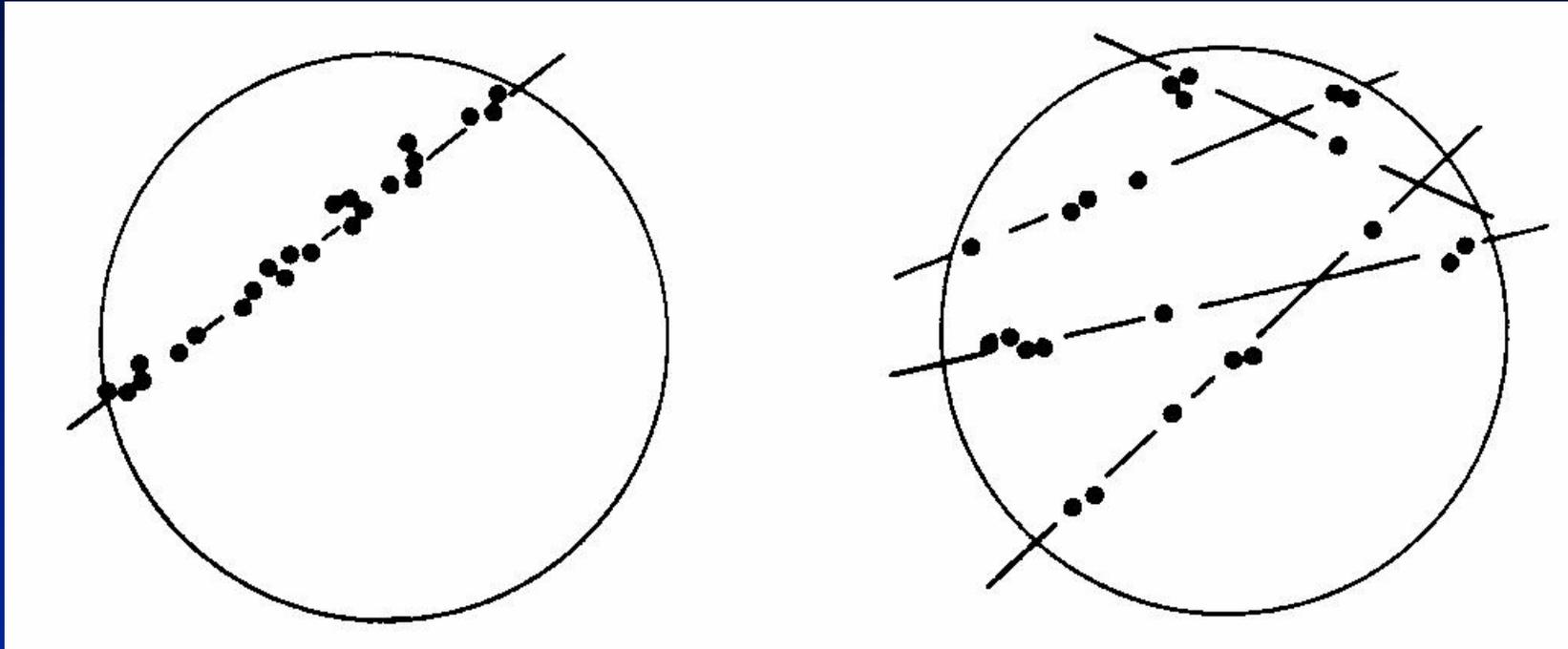


2. Distance model (mechanistic)

$$\text{RBE}_M = \int t(x) \gamma(x) dx$$



Low dose and high-dose track structures are different

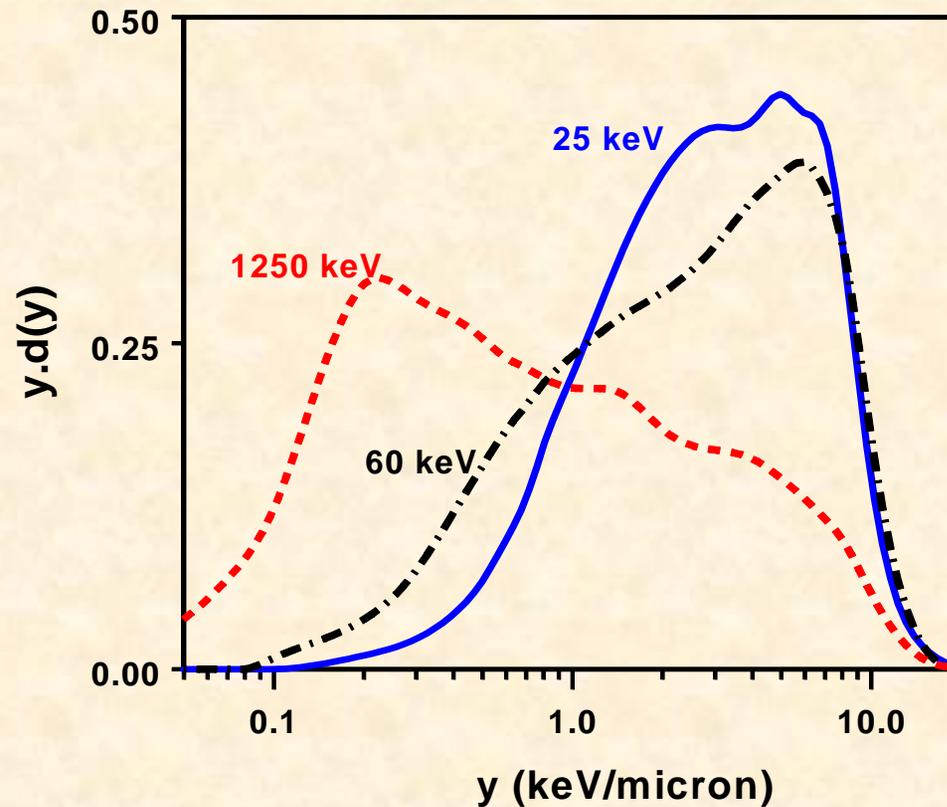


but you can calculate
high doses from low doses

What is a low dose?

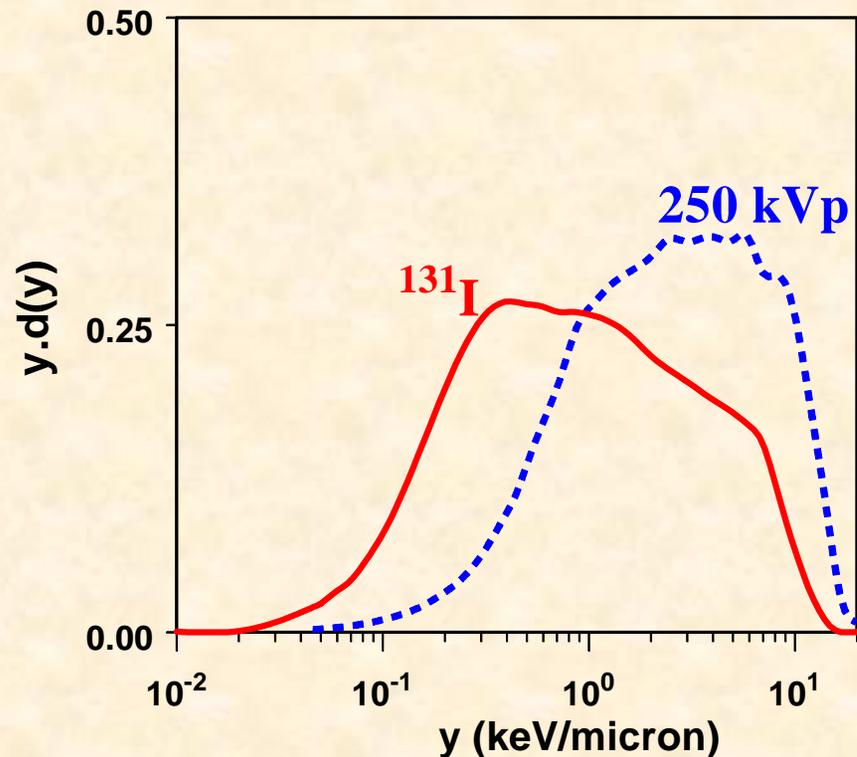
Target diameter → Radiation type ↓	1.9 μm <i>(nucleotides)</i>	7.7 μm <i>(nucleus)</i>	d=22 μm <i>(cluster of cells)</i>
1.25 MeV γ rays	1.5 cGy	0.09 cGy	0.01 cGy
25 kVp x rays	10	0.45	0.05
0.44 MeV neutrons	200	5	0.4
100 keV/ μm α particle	550	30	3

Different photon energies produce quite different microdosimetric spectra



So, for example, mammographic x rays have an RBE of 2-3, compared to high energy photons

Low dose RBE of ^{131}I vs. 250 kVp x rays

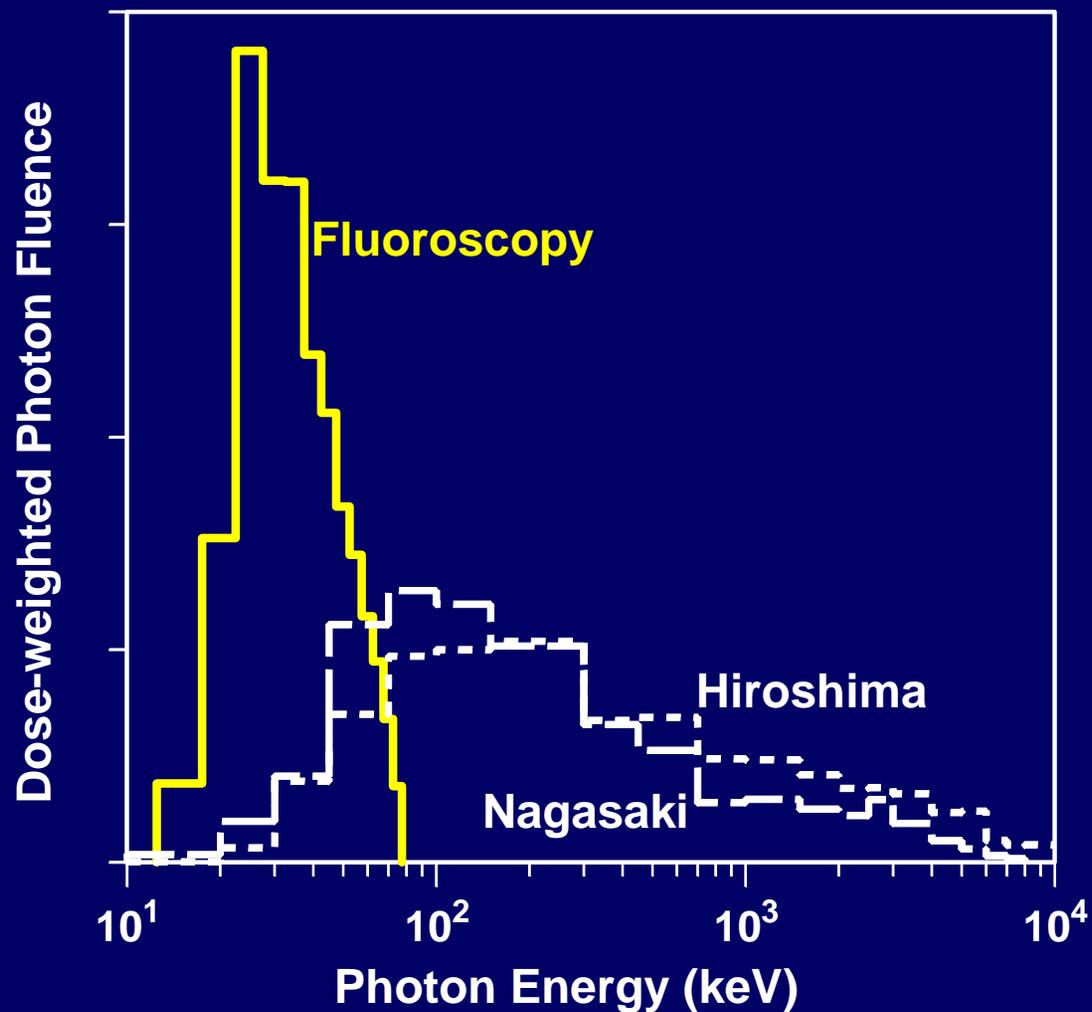


- Mean secondary electron energy from ^{131}I is ~ 200 keV
- Mean secondary electron energy from 250 kVp x rays is ~ 20 keV
- Based on microdosimetric spectra, $\text{RBE}_M \sim 0.6$

Fractionation and radiation-induced breast cancer

- **A-bomb survivors show about the same risk per unit dose for breast cancer as do the TB fluoroscopy cohorts**
- **So no fractionation effects?**

Photon energy spectra for fluoroscopy-energy x rays vs A-bomb γ rays



Calculated low-dose relative risks for fluoroscopy-energy x rays vs A-bomb γ rays

Endpoint ↓	Hiroshima bomb (1.5 km ground distance)	Nagasaki bomb (1.9 km ground distance)	80 kVp x rays
Exchange-type chromosome aberration formation	1	1.06	1.61
Mutation at HPRT locus	1	1.04	1.72
<i>In-vitro</i> oncogenic transformation	1	1.06	1.90

So the similarity between fluoroscopy risk and A-bomb survivor risk could well be due to the cancellation of an RBE effect and a fractionation effect

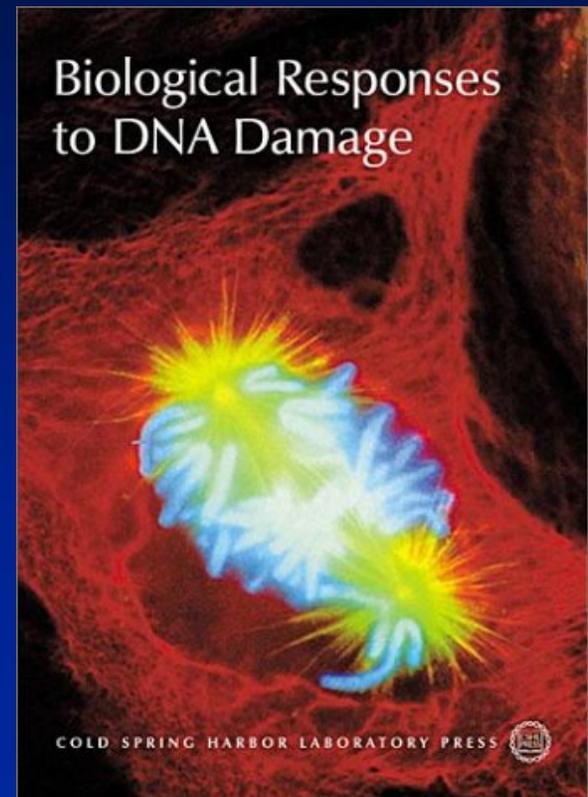
Bystander Effects

Unirradiated “bystander” cells respond to signals emitted by nearby irradiated cells



A Paradigm Shift in Interpreting Radiation Effects

Generations of students were taught that heritable biological effects require direct damage to DNA



Radiation-Induced Bystander Effects

- **First quantified by Nagasawa & Little (1992)**
- **Exposed cells to low doses of α particles, about 1% of cells were hit**
- **30% of cells showed increased in SCE**

Bystander effects have been reported for a variety of endpoints using single-cell systems

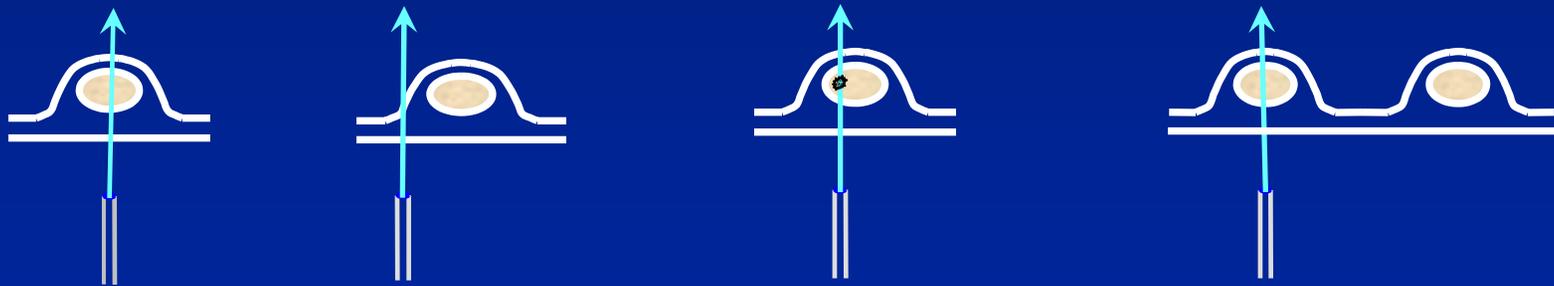
- ✓ **Sister-chromatid exchanges**
- ✓ **Cell killing (mitotic and apoptotic)**
- ✓ **Micronucleus induction**
- ✓ **Mutation induction**
- ✓ ***In-vitro* oncogenic transformation**
- ✓ **Changes in gene expression**
- ✓ **Altered cell growth**

Various experimental approaches to bystander studies

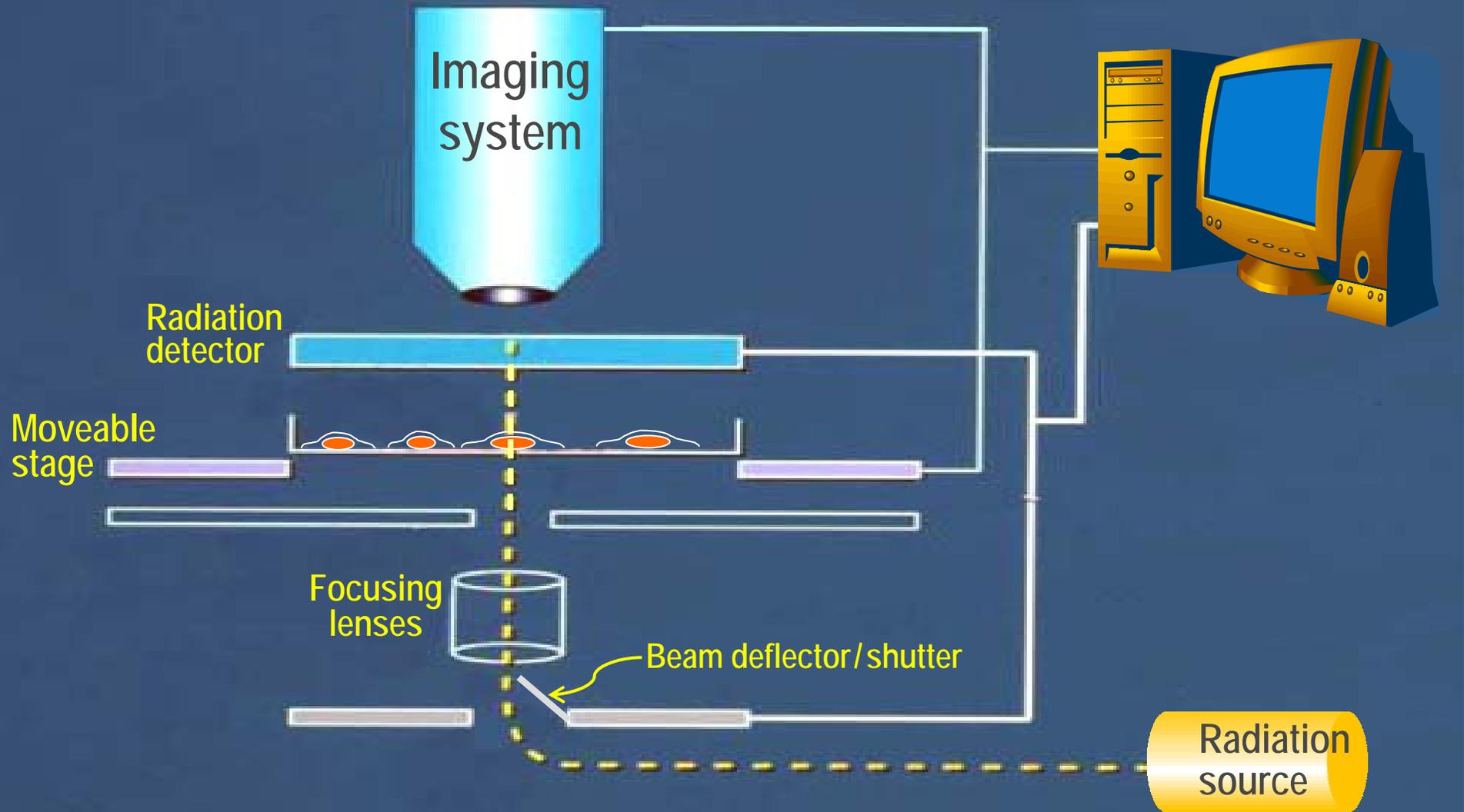
- Irradiate with a broad beam of high-LET radiation at a very low dose, such that most cells not hit
- Intra-media signal transfer
 - » Irradiate cells/medium, then transfer irradiated medium/cells onto fresh cells
 - » Co-culturing dishes
- ***Microbeam studies***
 - > Hit only specified cells in the field

Why Microbeams?

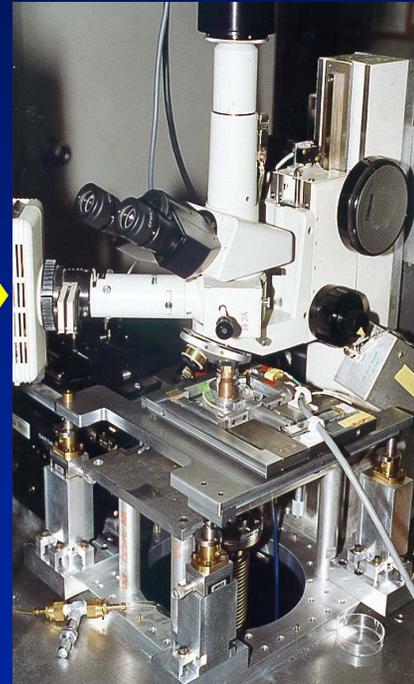
- The microbeam can deposit ionizing radiation damage in **microscopic** or **sub-microscopic** regions of cells
- Allows investigation of **intra-** and **inter-cellular** mechanisms of stress response



Single-Cell / Single-Particle Microbeams



The Columbia University Single-Cell / Single-Particle Microbeam



Microbeam Bystander Studies

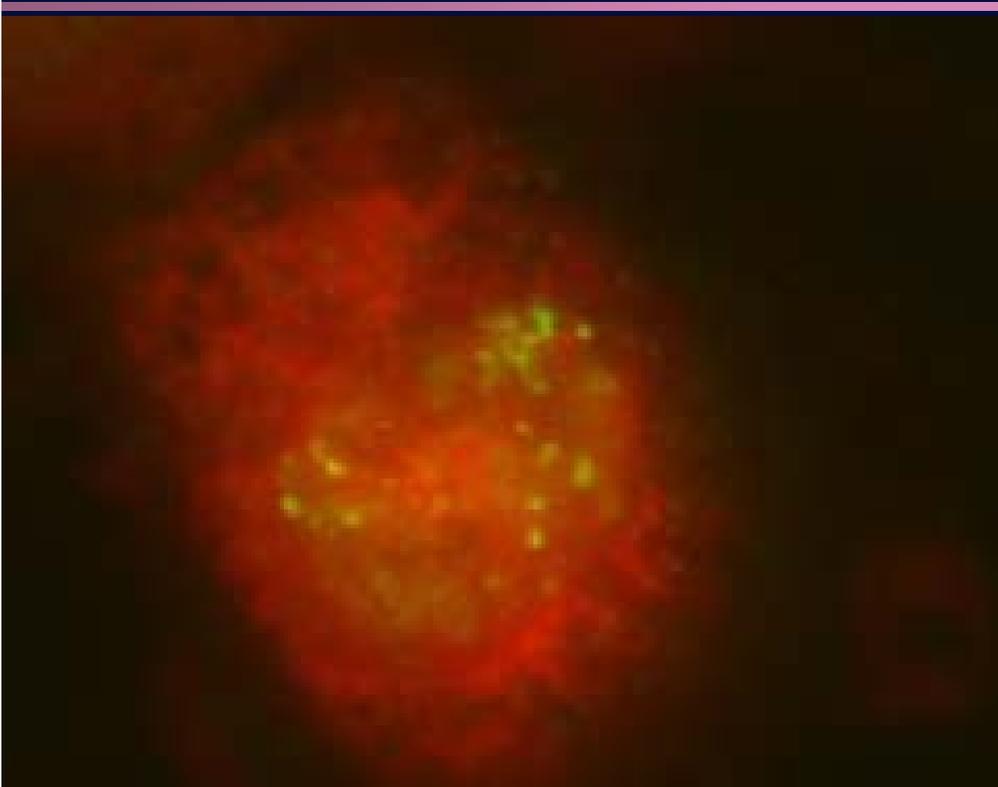


**Blue-stained
nuclei:
HIT cells**

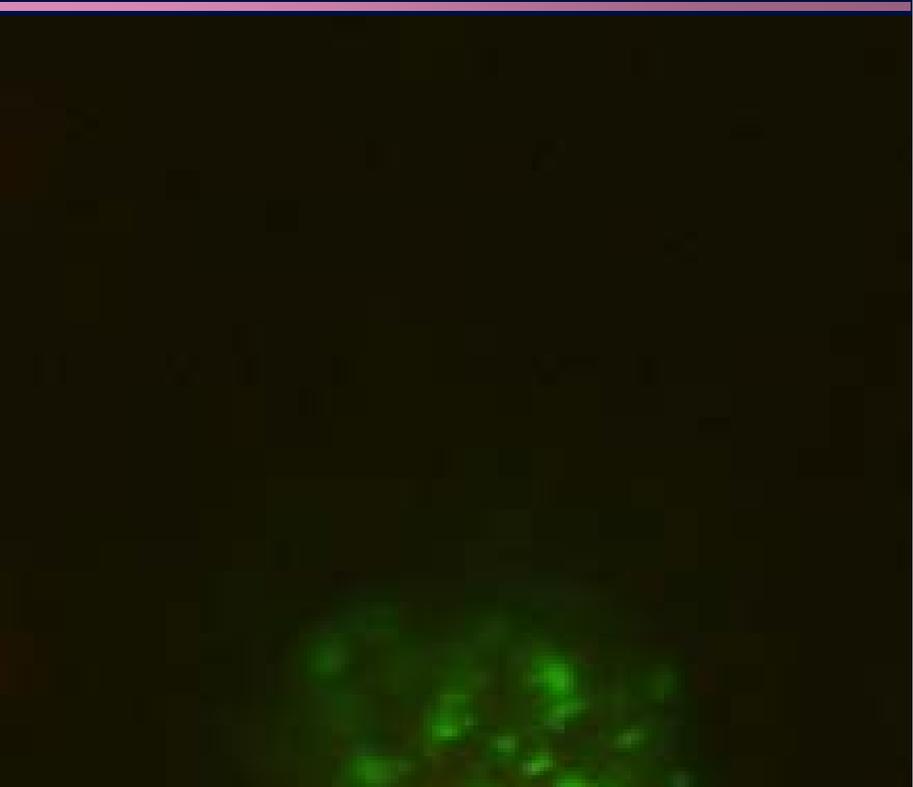
**Red-stained
cytoplasm:
NON-HIT cells**

Geard et al 2004

γ -H2AX Foci (Green)



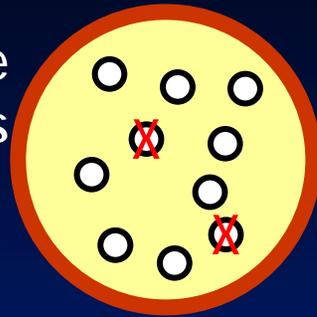
non-hit nucleus



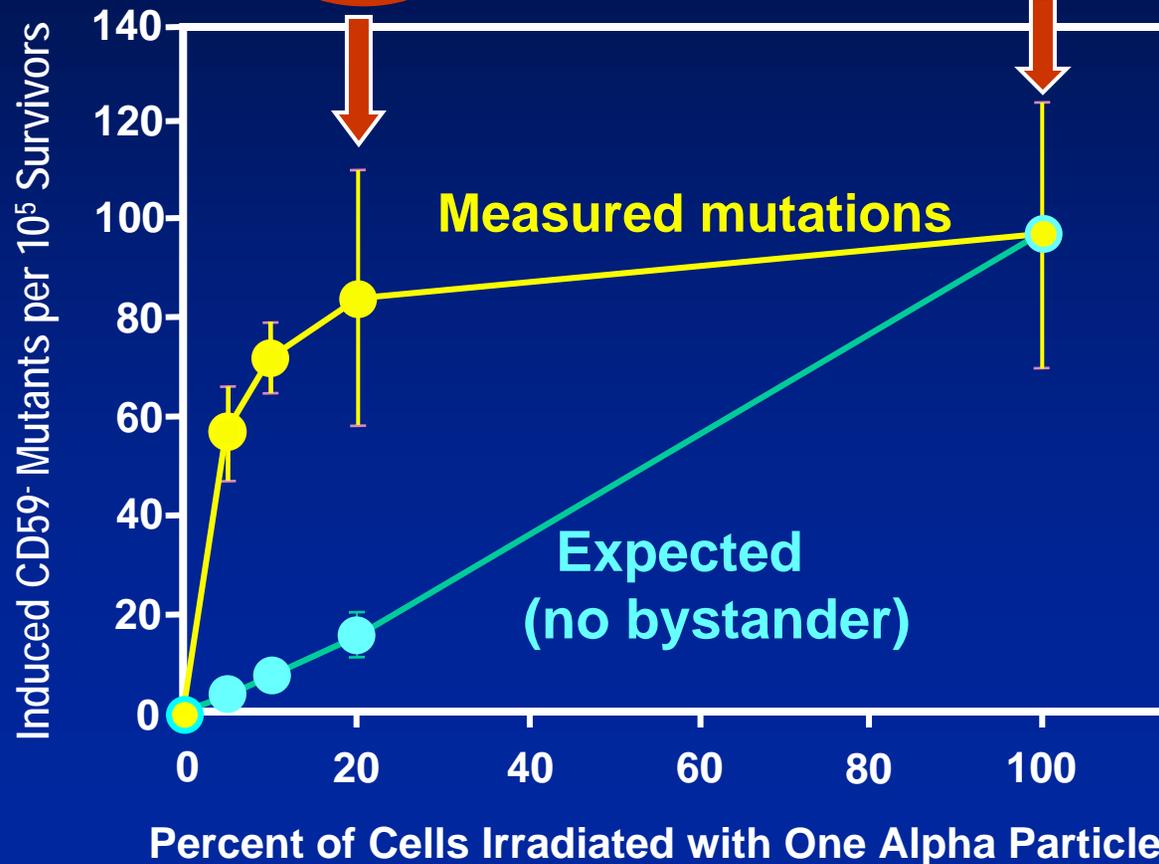
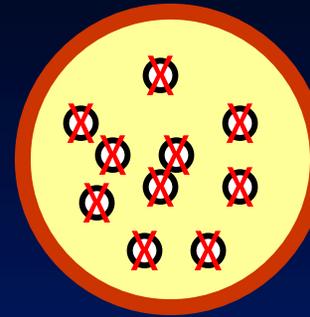
hit nucleus

A predetermined fraction of cells can be hit

One α particle hitting 20% of cells



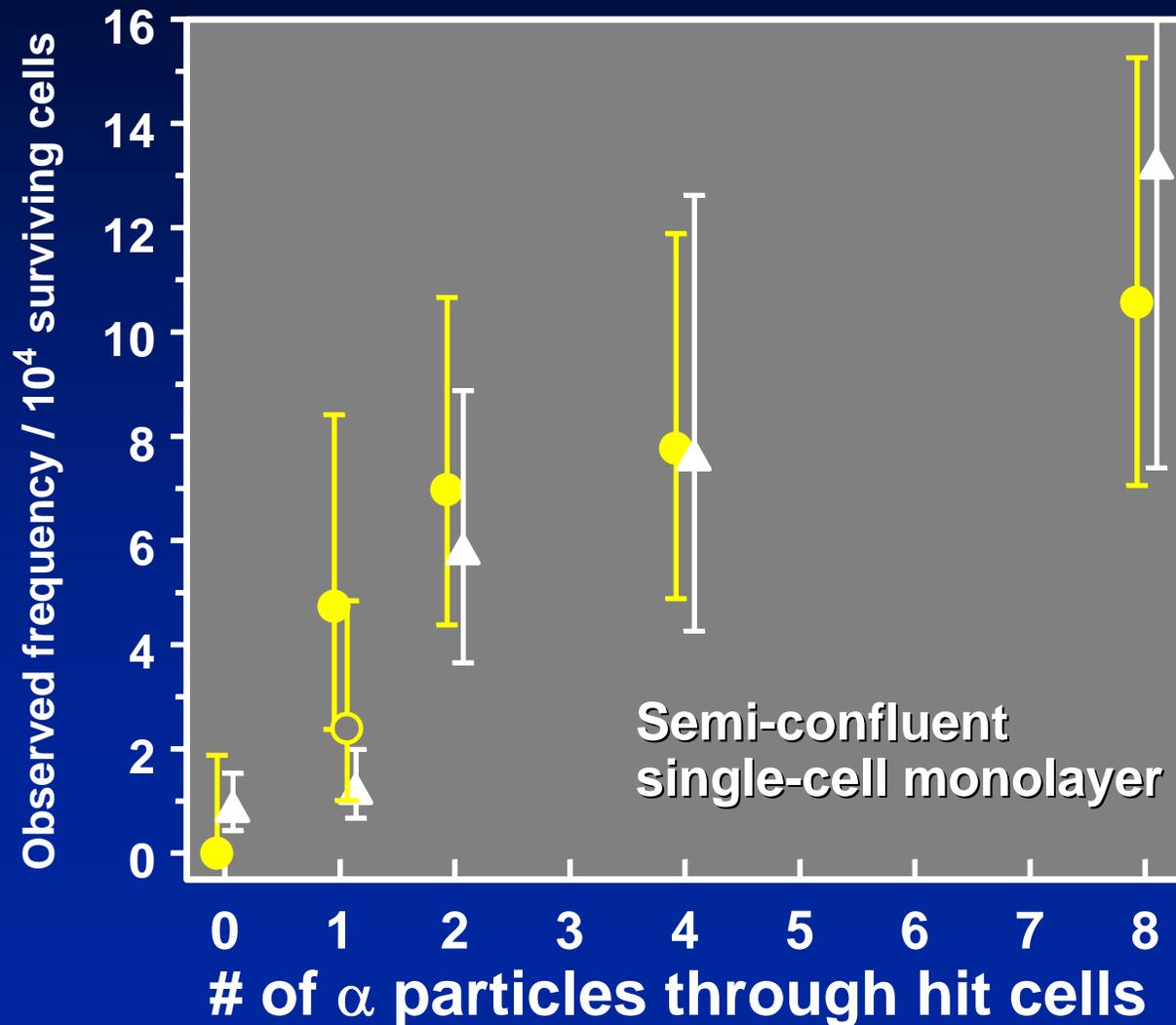
One α particle hitting every cell



T. K. Hei et al.

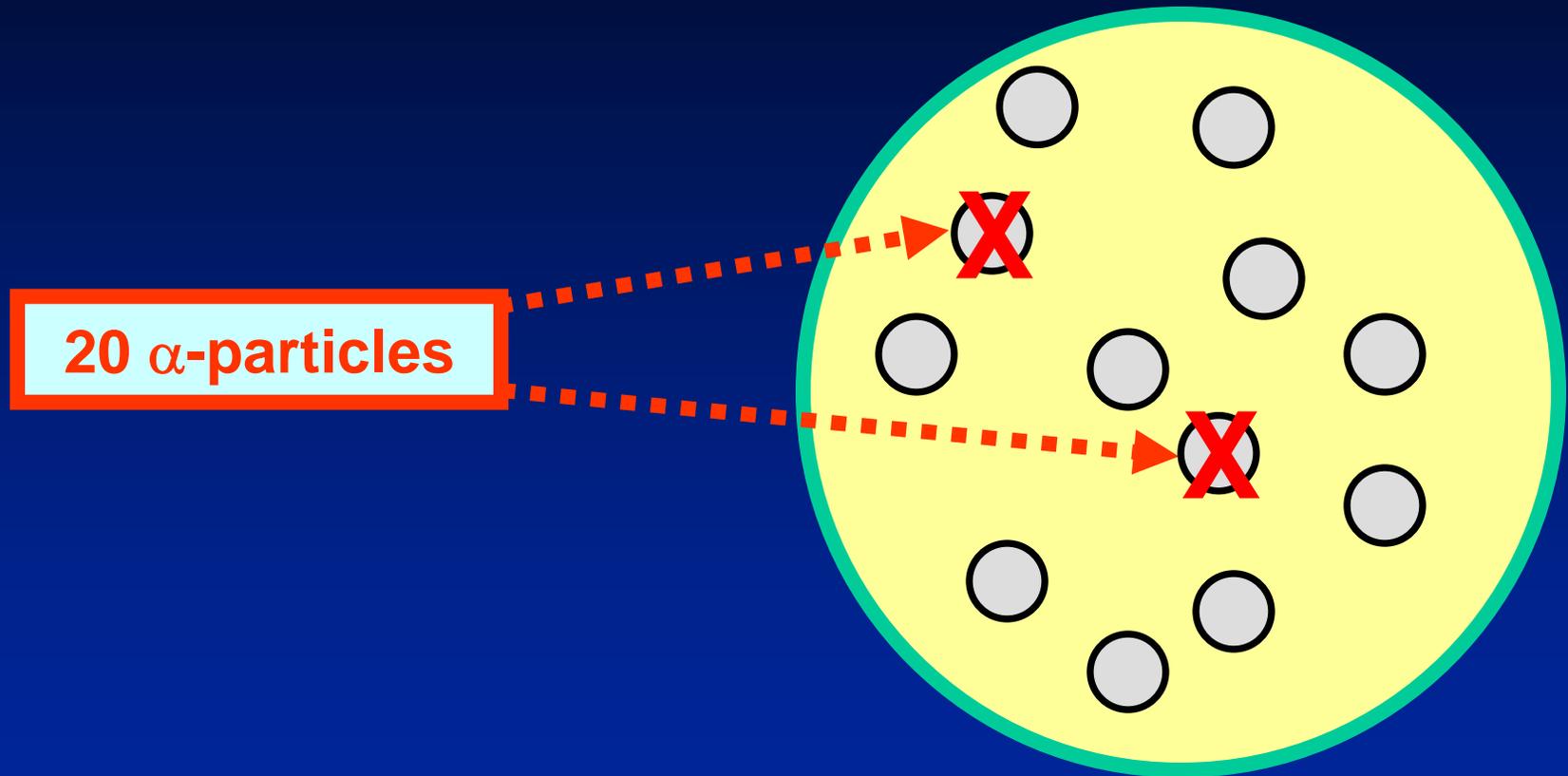
In-vitro oncogenic transformation with microbeam

White: All cells hit by α particles;
Yellow: Only 1 in 10 cells hit



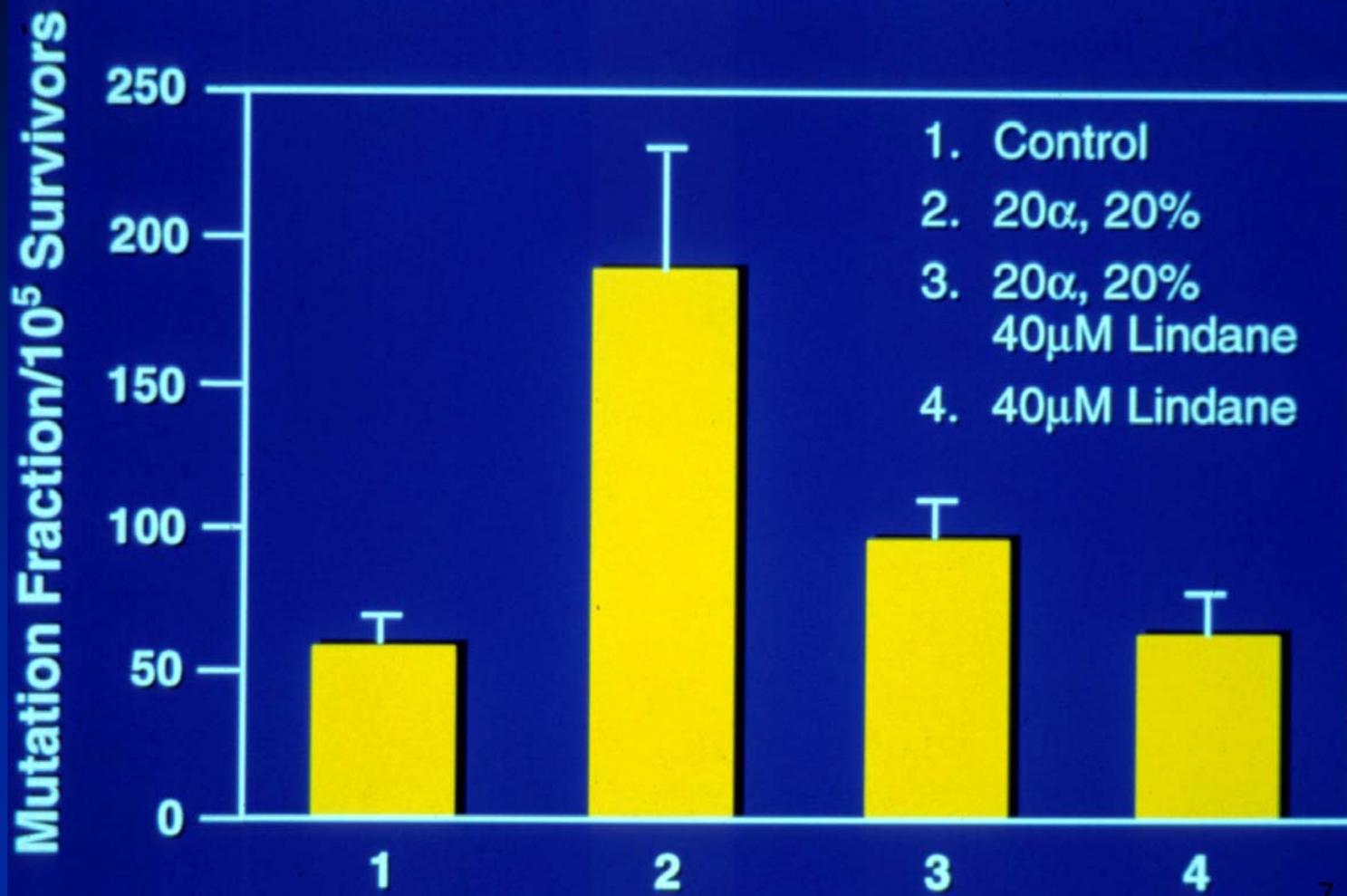
R. C. Miller et al.

A defined fraction of cells on a dish can be killed,
the remainder being not hit

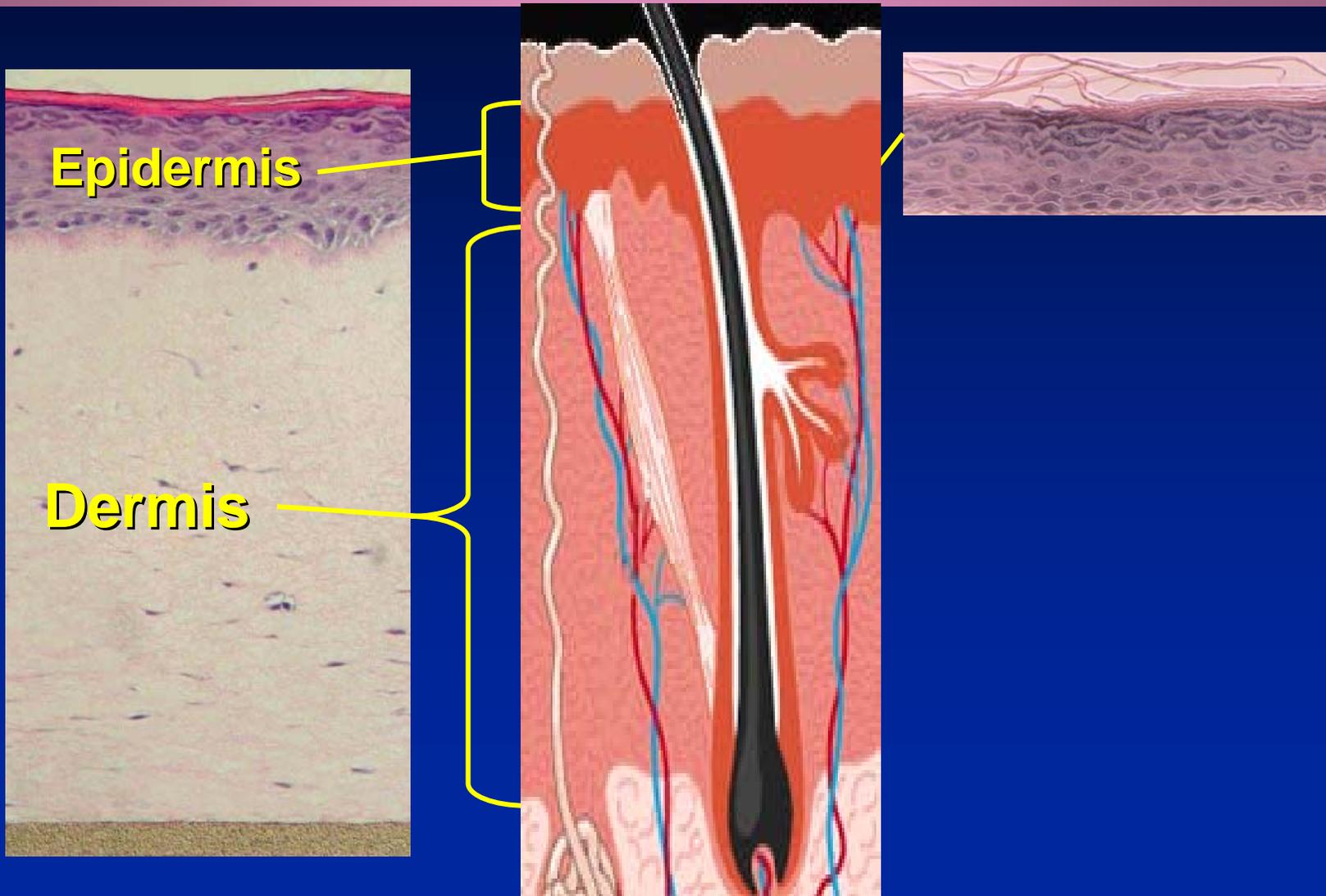


- 20 α -particles through the nucleus kills the cells that are hit ($SF \ll 1\%$)
- Mutations observed therefore come from unhit "bystander" cells.

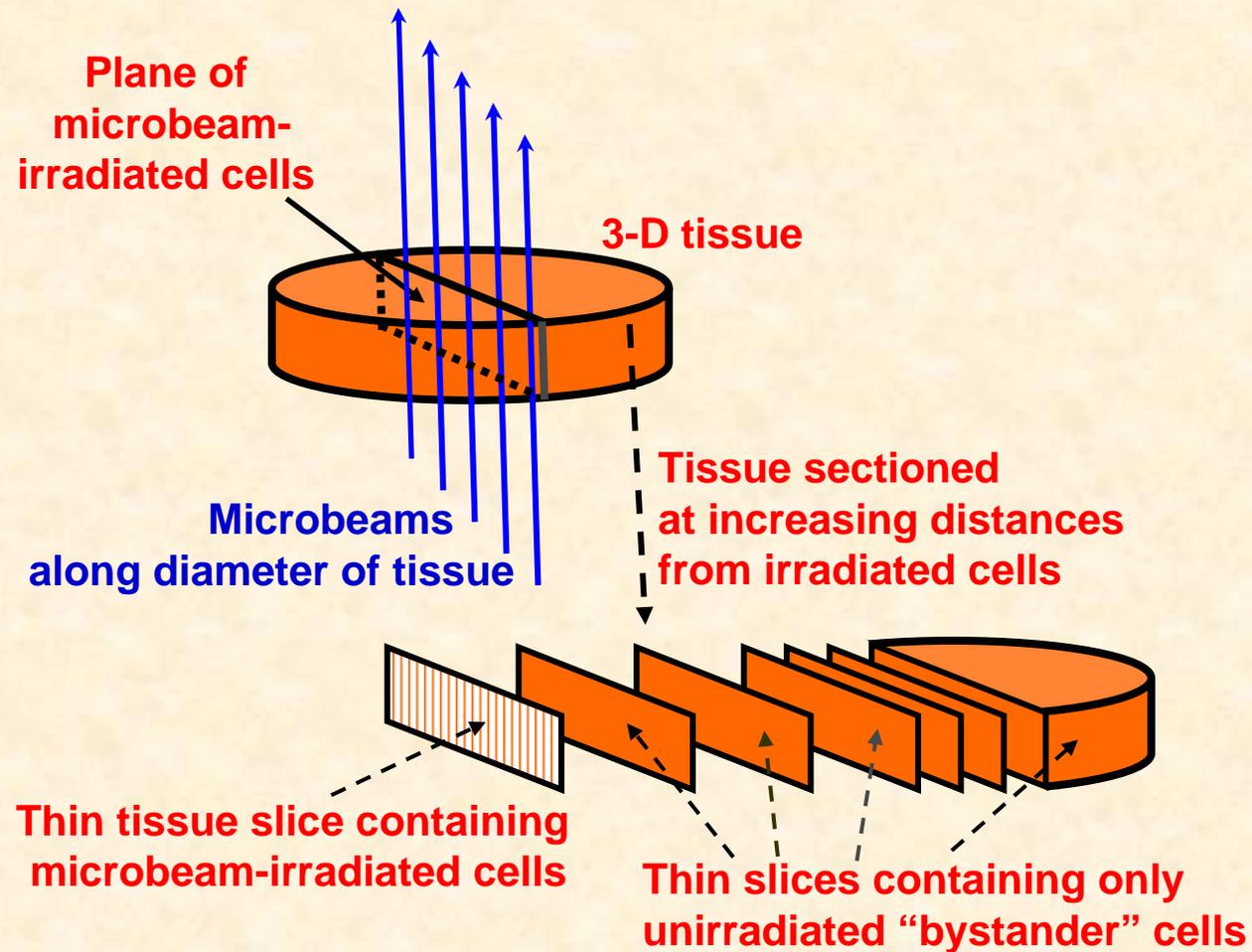
A defined fraction of cells on a dish can be killed,
the remainder being not hit



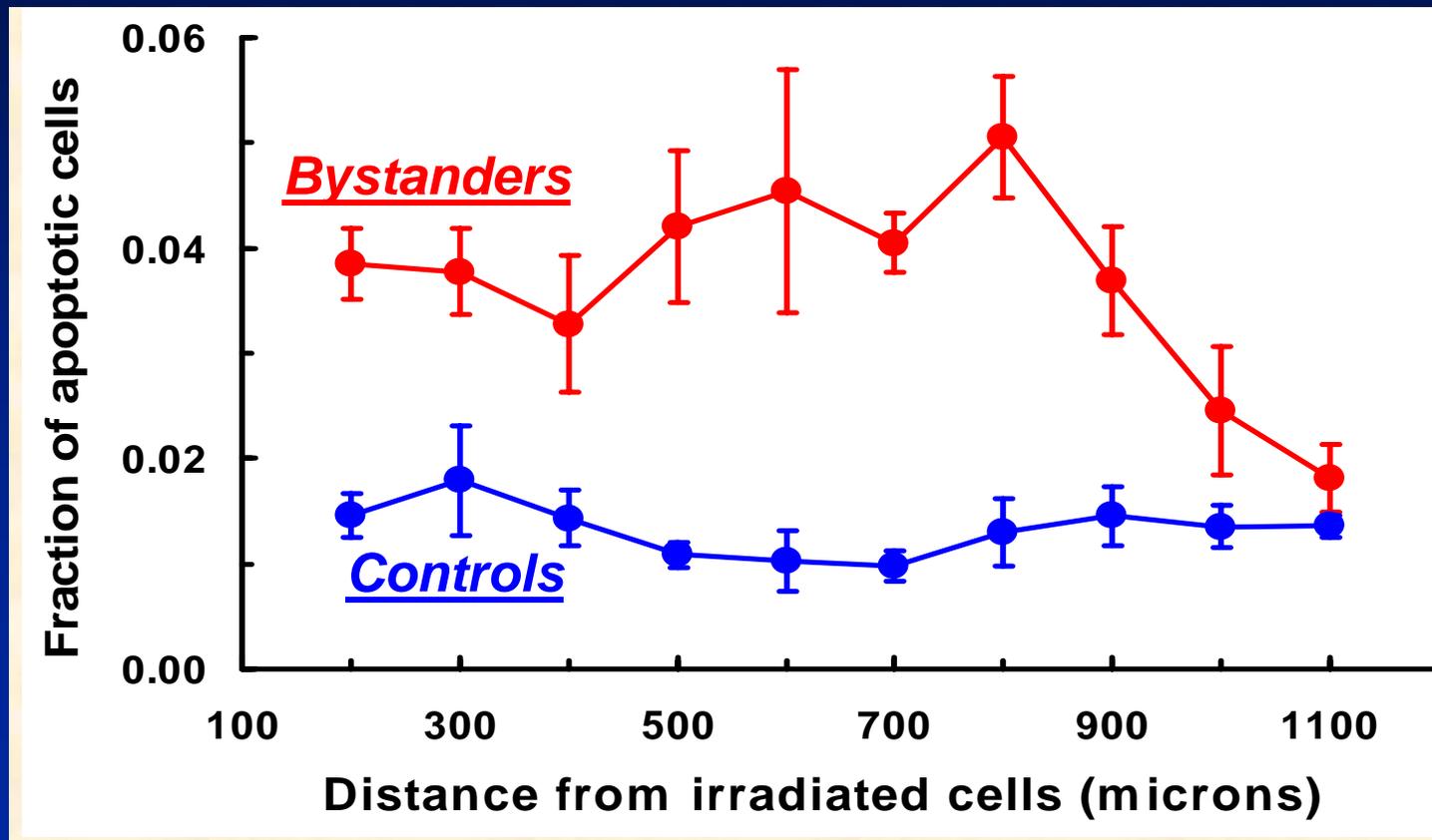
Microbeam-based bystander studies in human artificial 3-D skin



Microbeam-based bystander experiments in human 3-D tissue systems



Bystander Effects in 3-D Artificial Human Skin



Where might bystander effects be important?

- **RADON!**
- **Neutrons**
- **A Mars mission**

- **Low doses of photons??**

Large uncertainties in risk estimates from domestic radon case-control studies

So we are forced to extrapolate risks from uranium miners to domestic radon exposure

→ Uranium miners

- * High dose (few non-hit bystanders)
- * High dose rate

→ Domestic Exposure

- * Low dose (many non-hit bystanders)
- * Low dose rate

Why might bystander effects be relevant for domestic vs miner exposure?

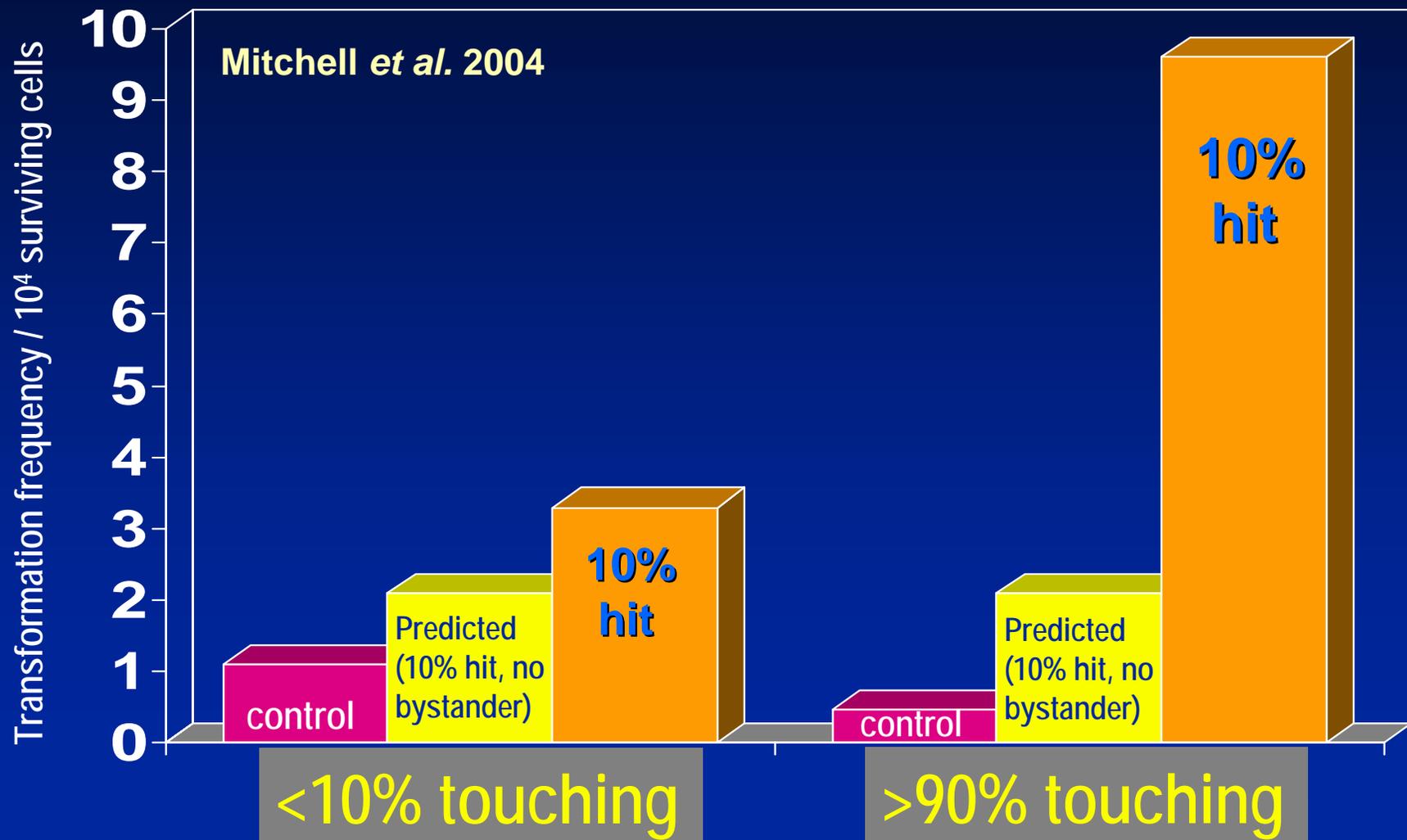
- **Cells are directly hit less frequently at low doses compared to high doses**
- **So the proportion of the overall risk due to bystander effects may be larger at lower doses**
- **Variations in the proportion of the response due to bystander effects can lead to non-linear dose-effect relations**

What do we know about bystander effects?

- **There's more than one bystander effect**
- **Only a small subpopulation of cells are sensitive to bystander signals (cell cycle??)**
- **At high doses, “classical” direct effects dominate**

The Two Bystander Effects:

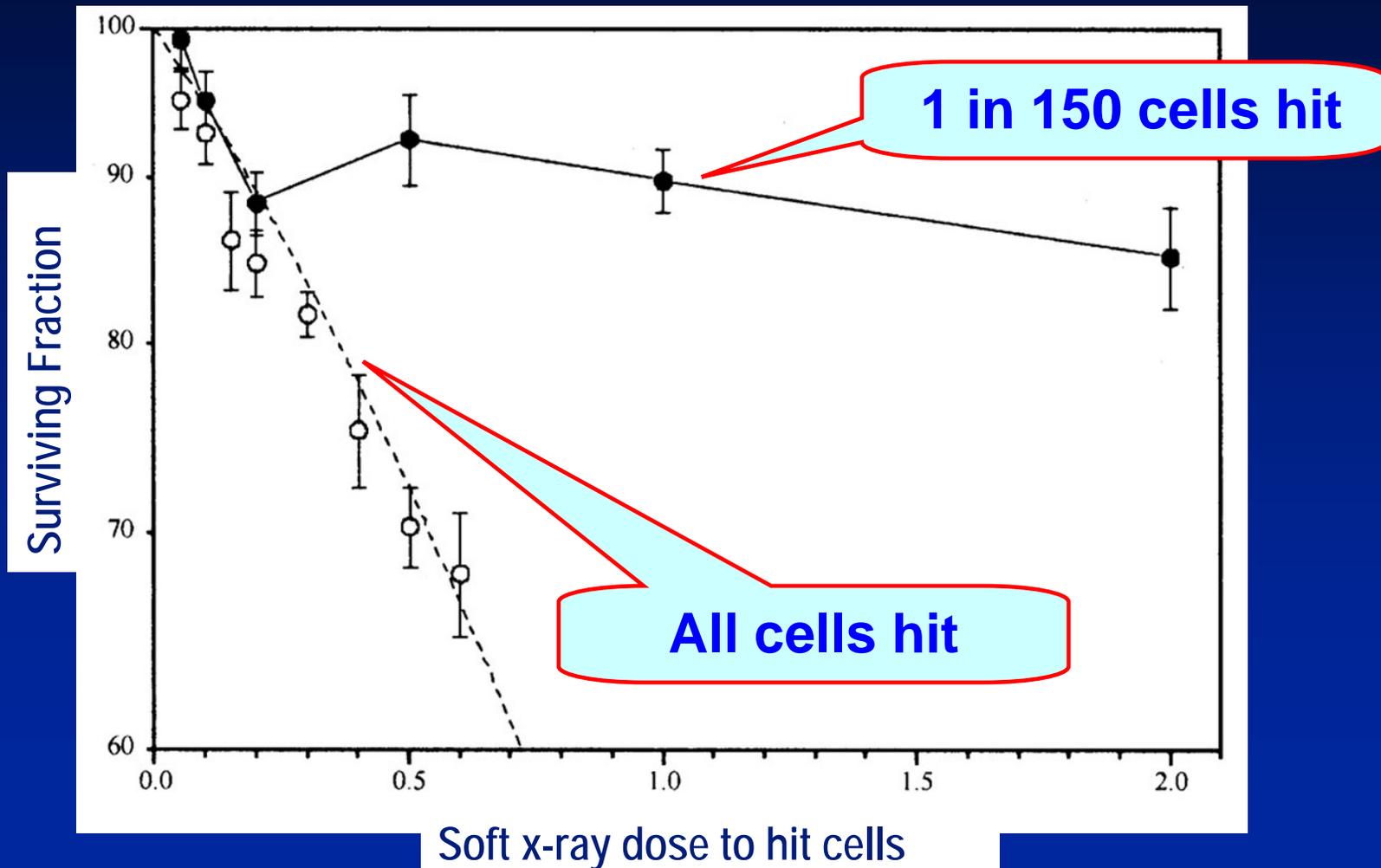
The effect of cell-to-cell contact on oncogenic transformation



What do we know about bystander effects?

- **There's more than one bystander effect**
- **Only a small subpopulation of cells are sensitive to bystander signals (cell cycle??)**
- **At high doses, “classical” direct effects dominate**

Bystander effects plateau at high doses...



What do we know about bystander effects?

- **There's more than one bystander effect**
- **Only a small subpopulation of cells are sensitive to bystander signals (cell cycle??)**
- **At high doses, “classical” direct effects dominate**

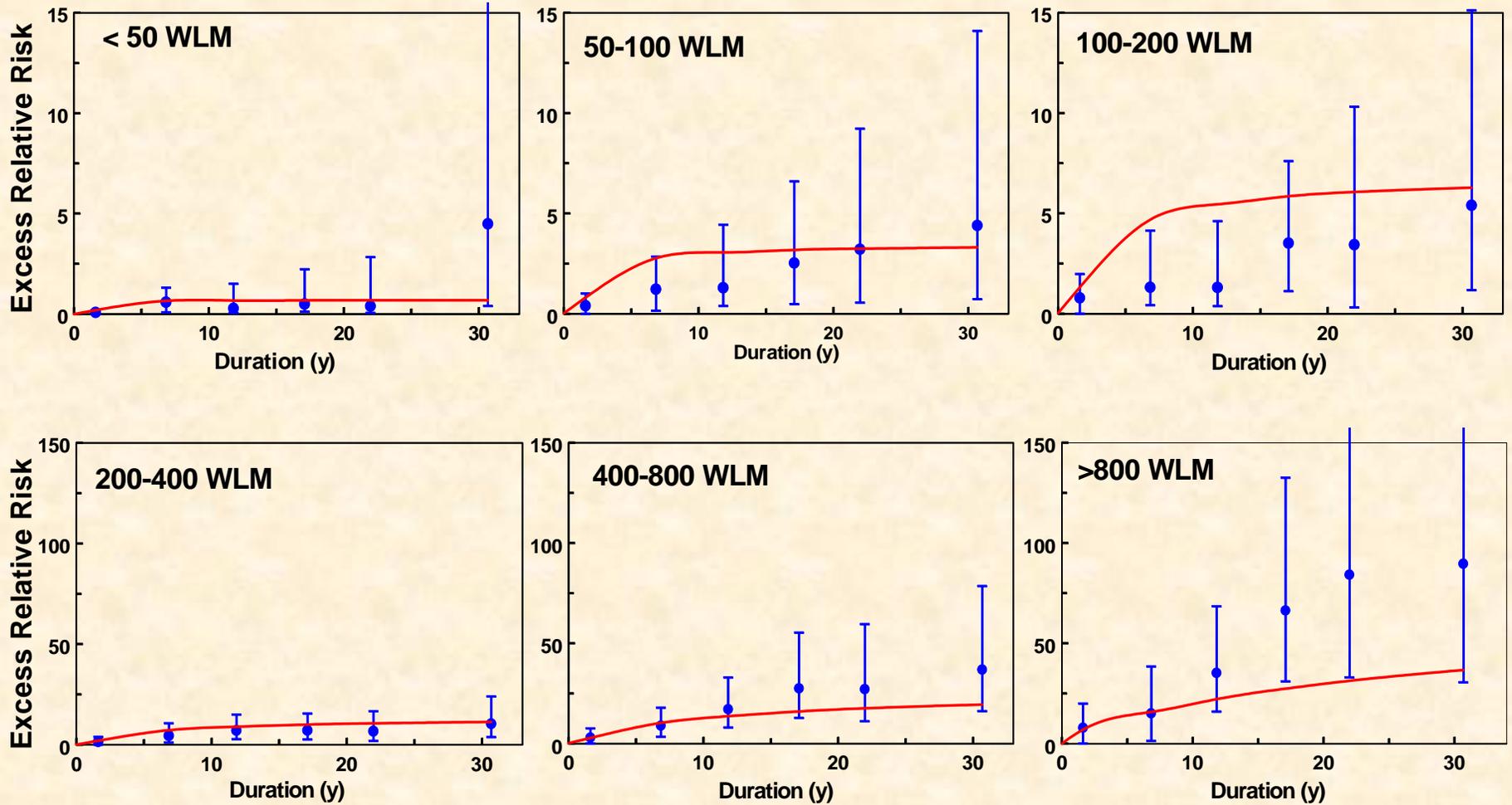
Quantitative BaD Modeling of Bystander and Direct Effects

The body of in-vitro experimental data on the bystander effect suggest that...

- Overall risk is a sum of direct and bystander effects
- A directly-hit cell sends out a signal to k neighbor cells
- Only a small subpopulation is sensitive to bystander signals
- During prolonged exposure, the bystander-signal-sensitive subpopulation will be replenished through endogenous processes

Excess relative risk in uranium miners as a function of *exposure time* and *exposure*.

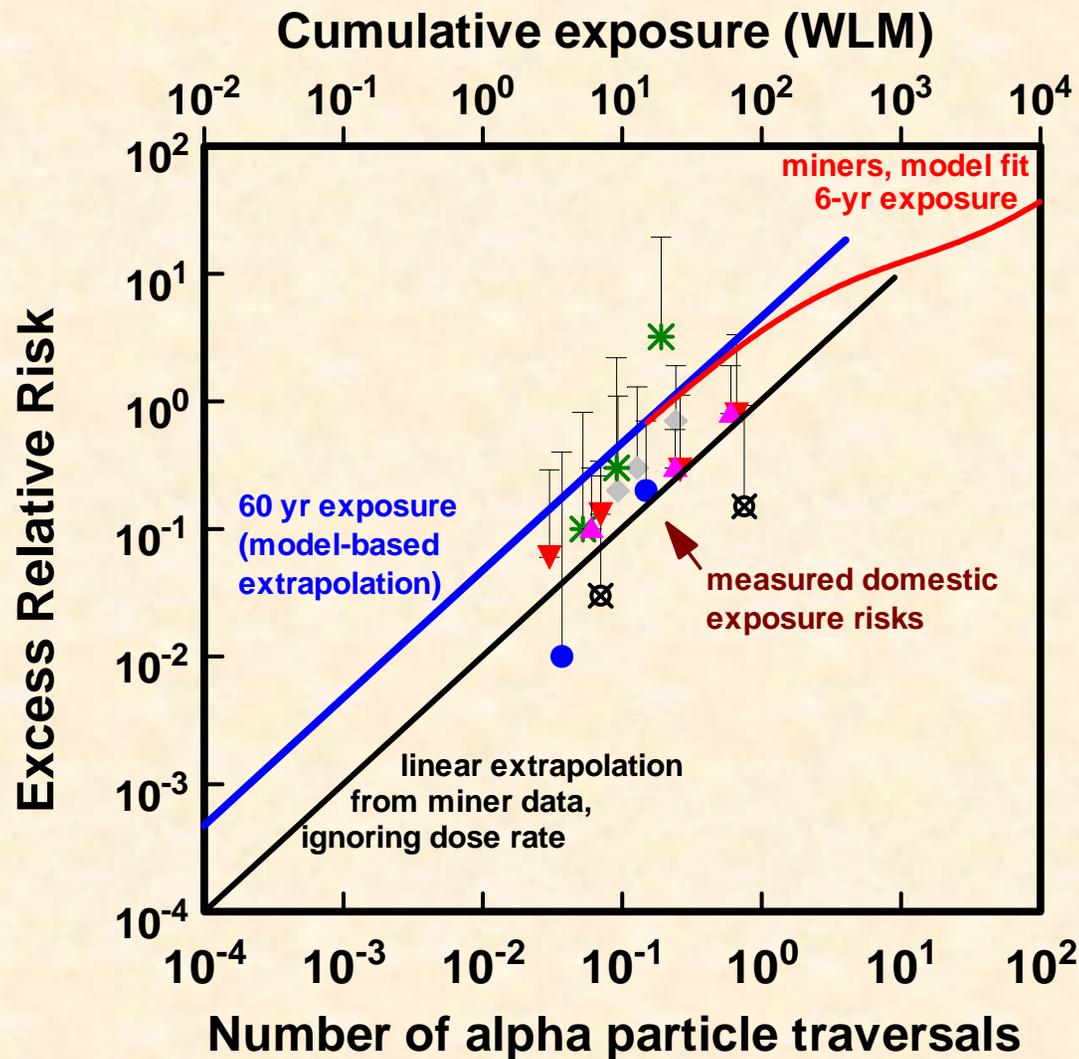
Red lines: Fit with extended 4 parameter BaD model



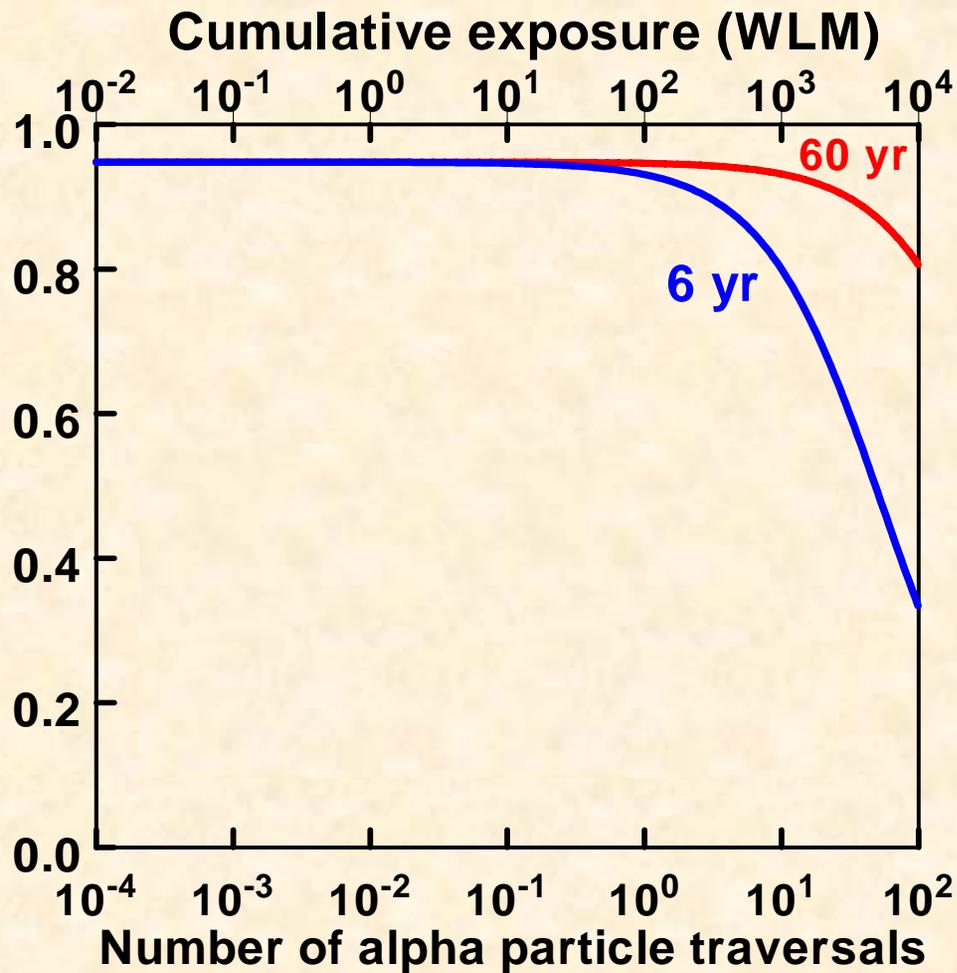
Relevant parameters as determined by fit to uranium miner data

- *Number of target cells potentially affected by bystander signal emitted by hit cell ≈ 50*
- *Replenishment rate constant of damaged cells $\approx 2/\text{month}$*
 - **The parameter values are not unreasonable**
 - **They are of the same order of, but certainly different from, those obtained in vitro**

Risk extrapolation from **miners (shorter, higher exposures)** to **domestic radon (protracted low exposure)**



Proportion of risk due to bystander response...



Model parameters
set by fit to uranium
miner data...

Are bystander effects important for radon risk estimation?

- ☛ The patterns of radon risks as a function of dose and time are highly suggestive that bystander effects are important at low doses
- ☛ Significant bystander effects would lead to **non-linear** dose-response relations
- ☛ In such situations, naïve linear extrapolation of risk from high to low doses could produce misleading results - typically under-predicting the true risk

So bystander effects are probably relevant to domestic radon exposure

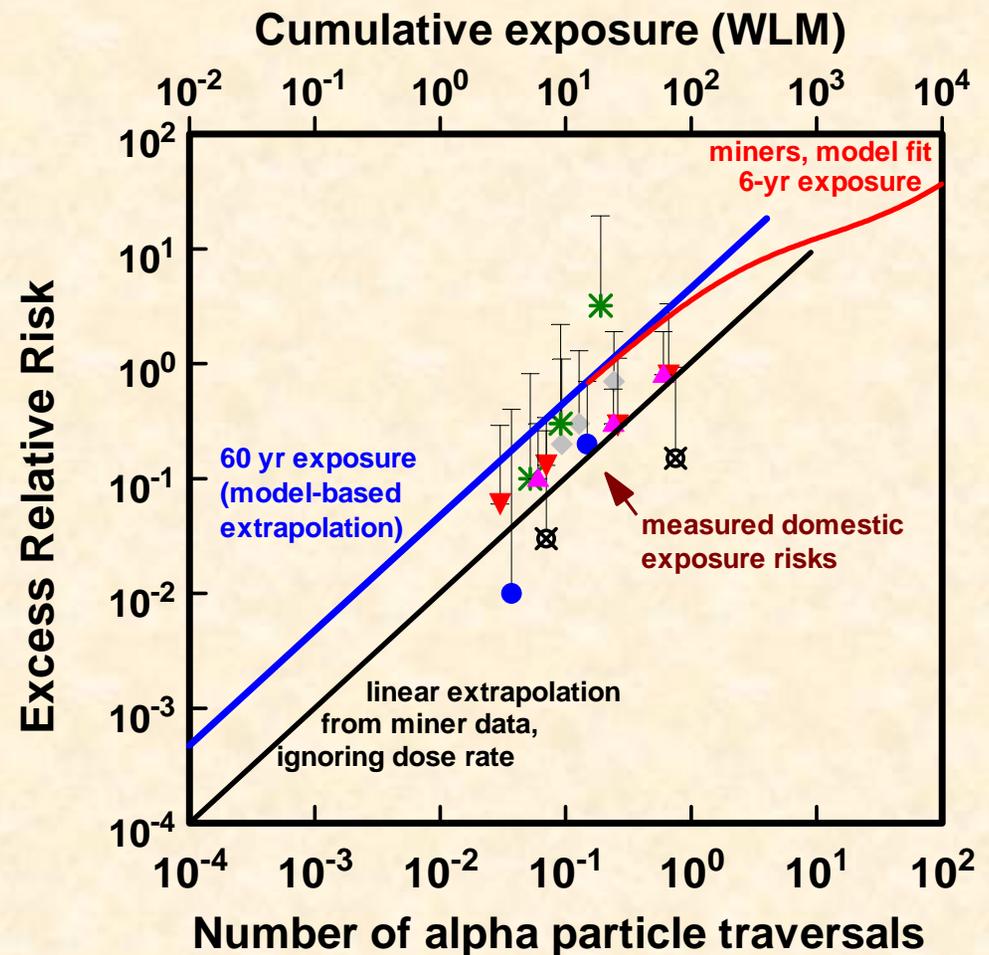


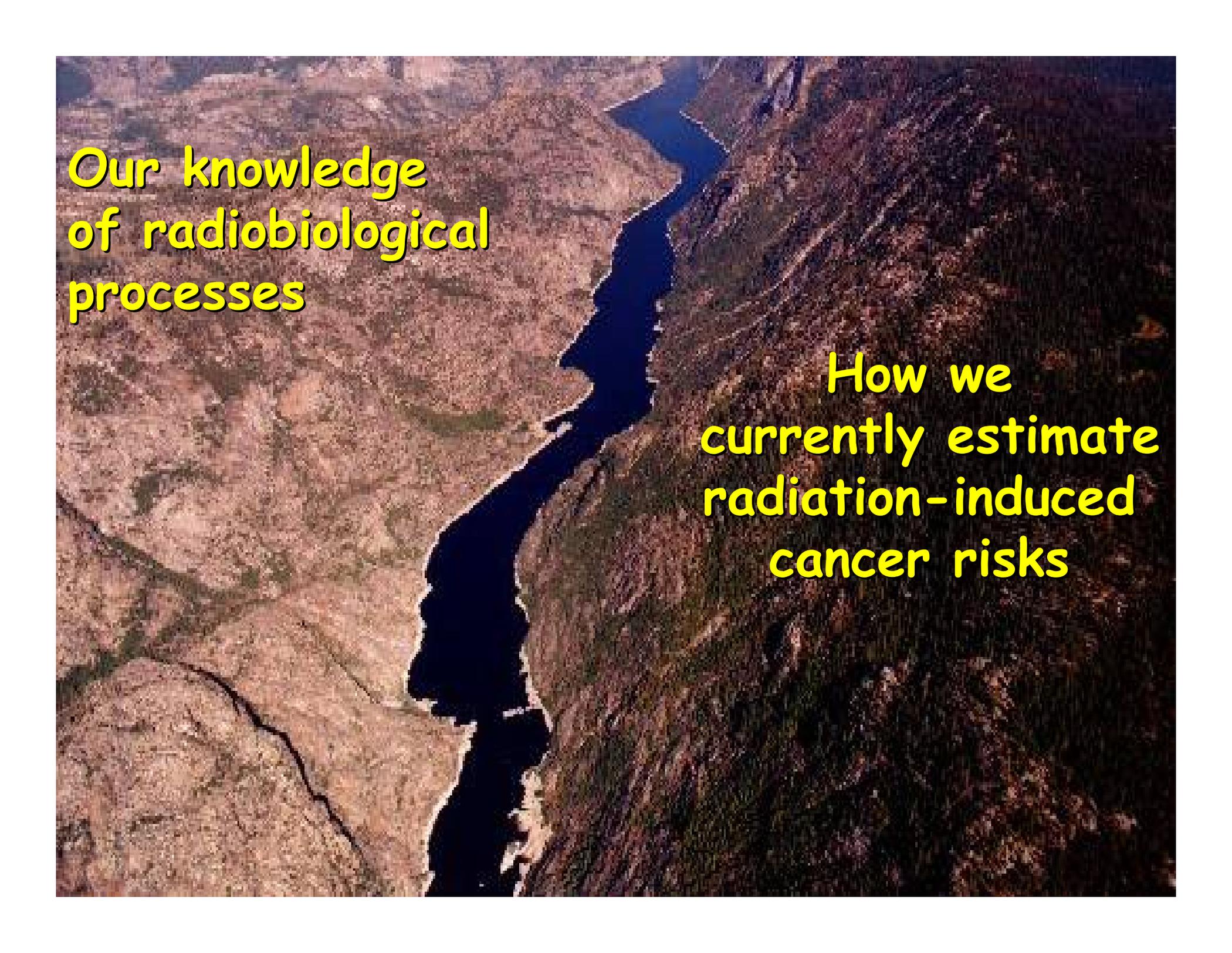
So beware thy neighbor...

Does this mean that we are currently underestimating domestic radon risks?

The BEIR-VI approach, roughly, was to linearly extrapolate from miner data, and then to increase the risk estimate by an “inverse dose-rate related” factor of about 4.

The current more mechanistic approach came up with about that same factor of 4.



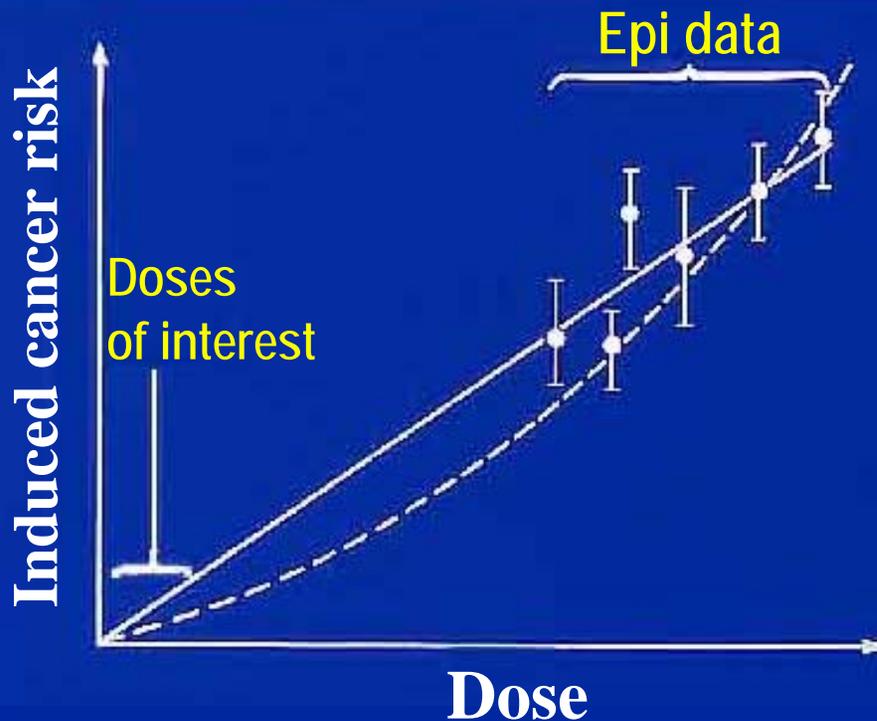
An aerial photograph of a winding river flowing through a rugged, hilly landscape. The river is dark blue and meanders through the terrain, which is covered in brownish, rocky soil and sparse vegetation. The hills are steep and eroded, with visible gullies and ridges. The overall scene is one of a natural, somewhat desolate environment.

**Our knowledge
of radiobiological
processes**

**How we
currently estimate
radiation-induced
cancer risks**

Purely empirical (descriptive) approaches

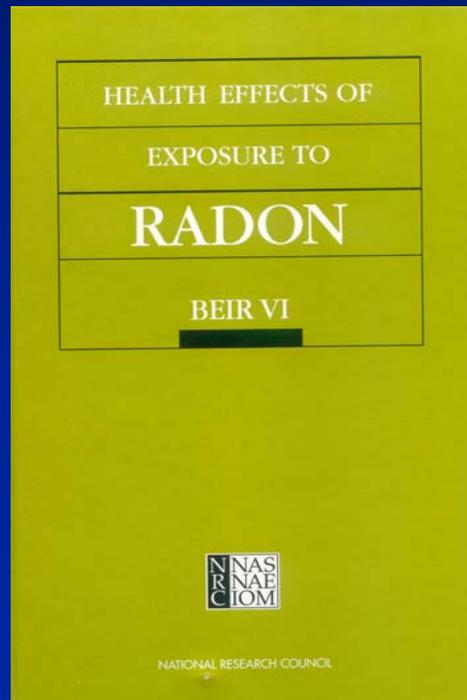
Limited value for extrapolation



Purely phenomenological or statistical approaches to dose-effect relations have probably gone about as far as they can go....

BEIR VI (1999)

“State of the art” evaluation of the human health consequences of low levels of radon



- *500 pages long*
- *Molecular genetics discussed on pp 37-43*
- *Molecular genetics not used in risk estimation*

Molecular genetics & risk estimation

One day, molecular techniques will help us to directly quantify the risks to human health of low levels of radiation.



That day is probably a long way in the future.

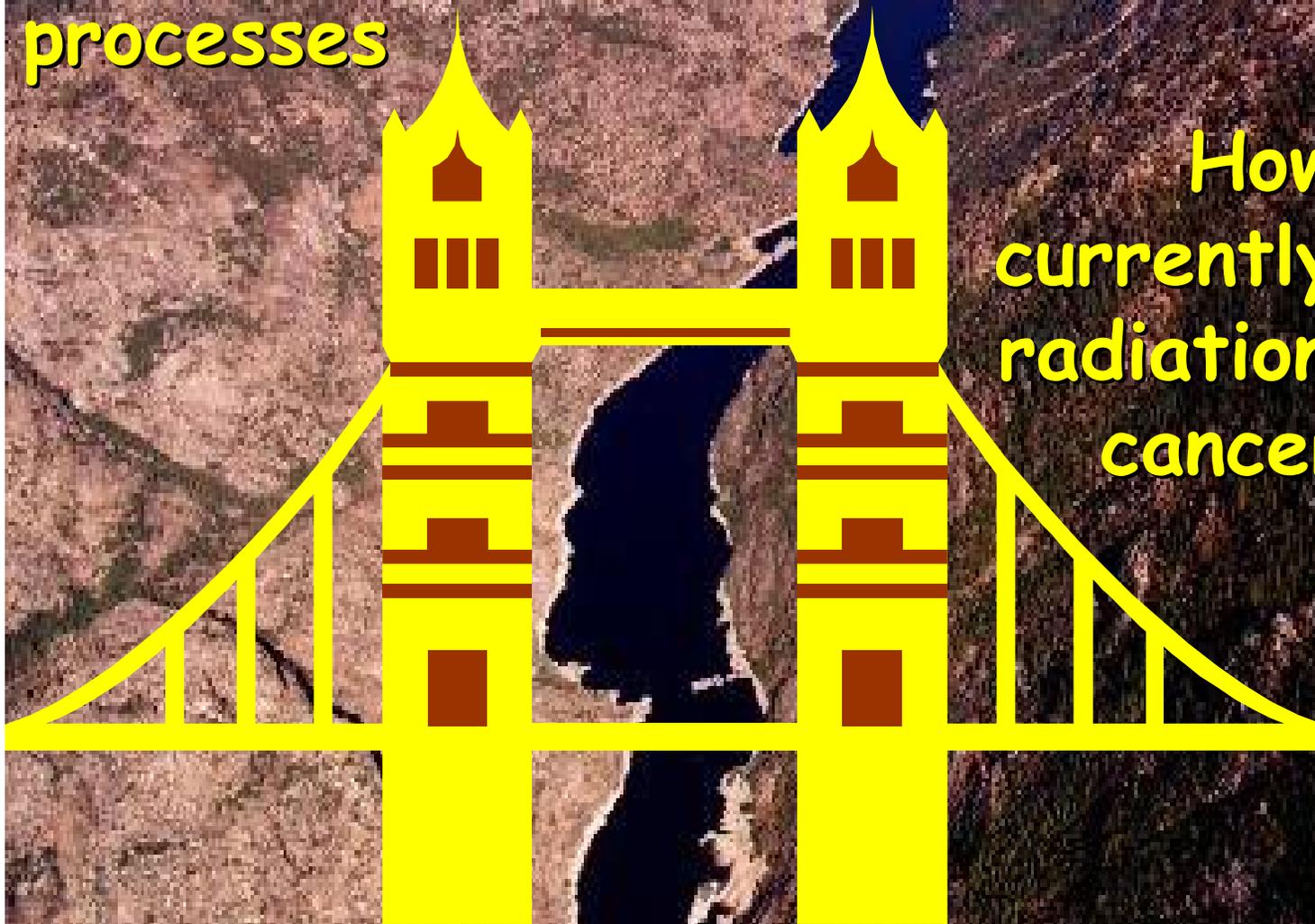
Purely mechanistic, biologically-motivated approaches

We do not yet know the
complete mechanistic picture -
paradigms are changing rapidly



Our knowledge
of radiobiological
processes

How we
currently estimate
radiation-induced
cancer risks



Radiobiology has the potential to provide **relative** information concerning cancer risks, such as

- high dose vs. low dose,
- wild-type vs. heterozygote,
- acute vs. fractionated
- low-LET vs. high LET

This **relative** information can be applied to modify radiation risk estimates that are originally based, for example, on A-bomb survivor data.

This “relative” approach minimizes our dependence on the details of the particular models we use.

Radiobiology

can guide

**empirical epidemiological
analyses**

*in specific areas where there
is uncertainty*

A hybrid approach:

**Empirical modeling
supplemented with relevant
mechanistic information**

NIH 2004

Interactions between radiation epidemiologists and radiation biologists are going to become increasingly important as the field focuses more and more on the effects of low doses.

NCI 2004

Radiobiology



Radiation epidemiology