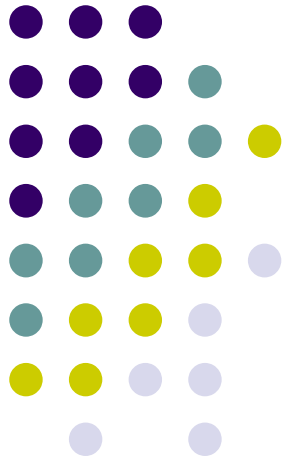
Curso de Radiobiología
UDELAR
Facultad de Ciencias
Unidad de Física Médica

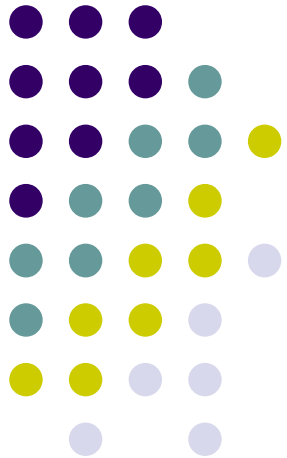
Dr. Eduardo Francisco Larrinaga Cortina



Curvas de supervivencia
Modelos matemáticos

Créditos:

Dr. Jerry Battista



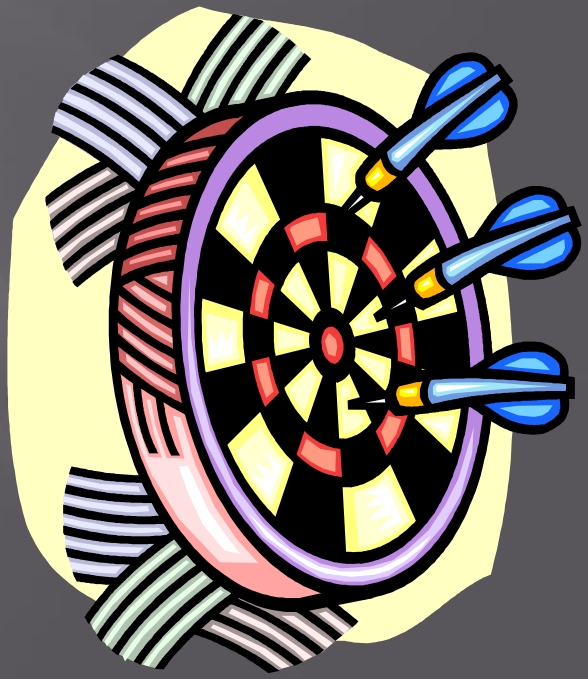
Chapter 1 and
everywhere else !

2011

CELL SURVIVAL CURVES
(AND TARGET THEORY)

J. Battista

(Scott Karnas, H. Fakir)



Learning Objectives

- Be able to sketch a typical cell survival curve with variable parameters
- Define the key characteristics of survival curves
- Apply different mathematical models to fit and interpret cell survival curves
- Use cell survival data to predict tumour control probability

Structure of the Lecture

Quick review

- Cell and DNA structure
- Radiation effects on cells (DNA damage)

Cell survival curves

Definition, measurements, interpretations

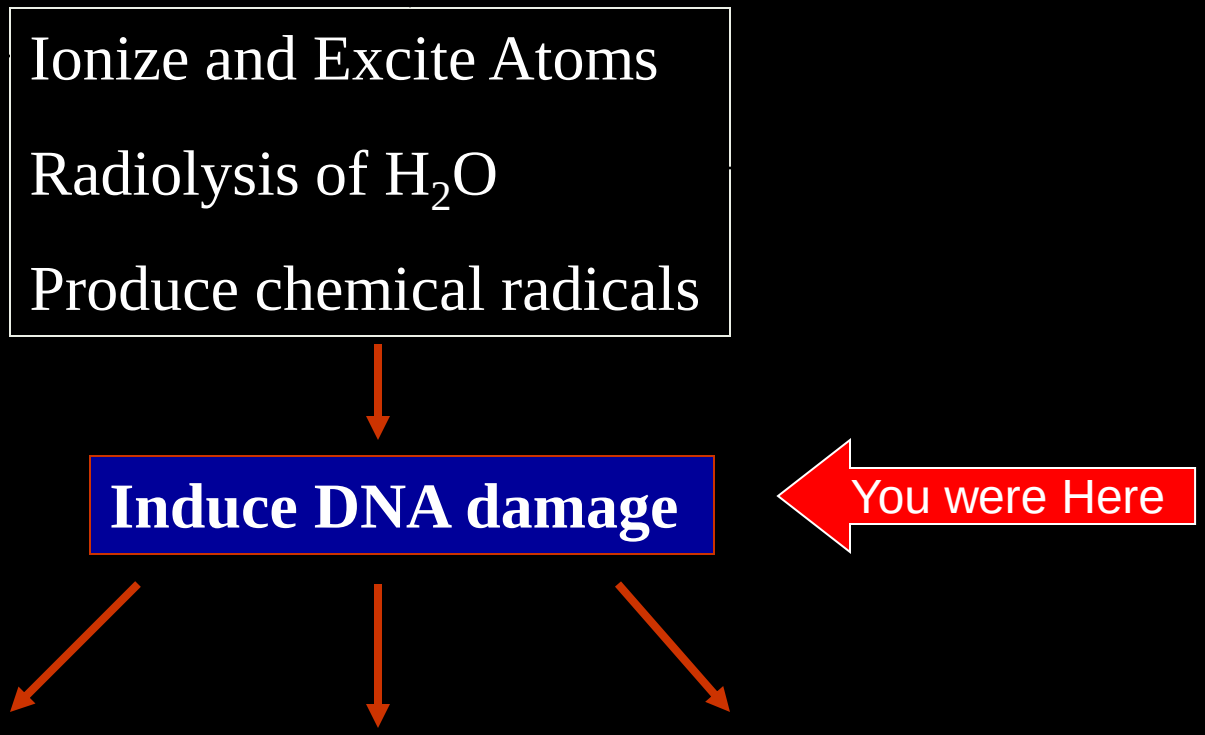
Explanation and modeling of survival curves

- Target Theory
- Linear quadratic model (dual radiation action)
- Biophysical models (repair models)

Tumour control probability (TCP)

- Calculations from survival data

My Famous Flow Chart



Radiobiology Timing

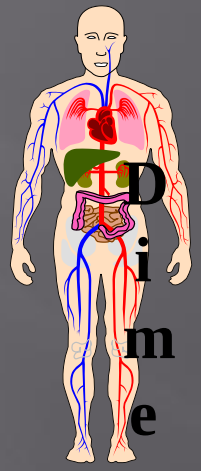
- Physics (10^{-18} sec)
  - photoelectric, Compton effects, etc.
  - Fast electrons in motion
- Radiation Chemistry (10^{-6} sec)
  - Ionization of H_2O
  - DNA damage
- Radiation Biology (> 10^{5} sec)
  - cell survival
  - "4 R's" of radiobiology

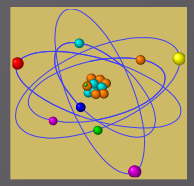
Time

You are Here

Radiobiology Sizing

- Physics (fm)
  - photoelectric, Compton effects
  - electrons in motion
- Radiation Chemistry (nm)
  - Ionization of H_2O
  - DNA damage
- Radiation Biology(>μm)
  - cell survival
  - "4 R's" of radiobiology
- Pathology and Physiology (cm to m)
  - organ, whole body, progeny

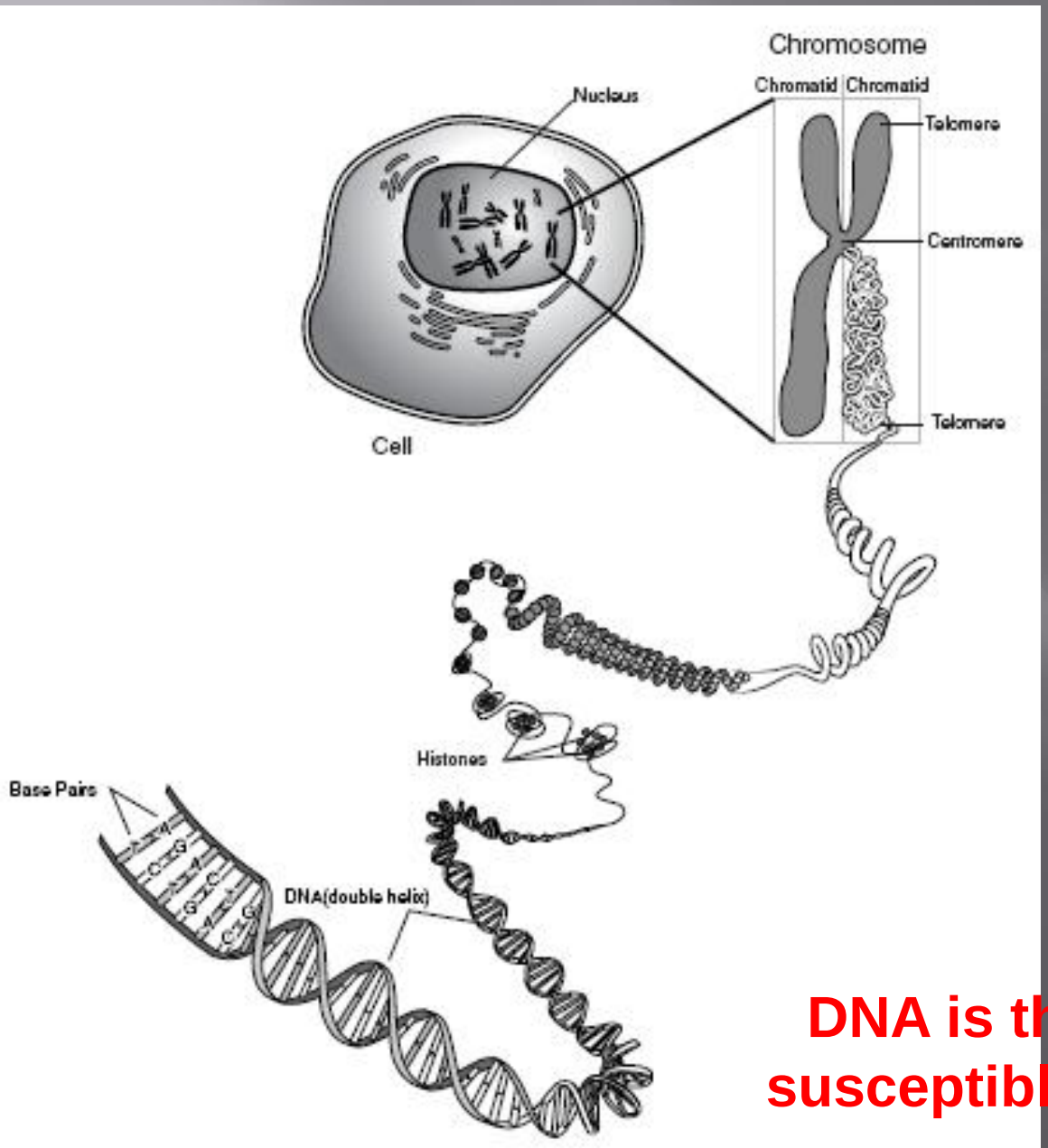




Dimension

And here

Cellular targets susceptible to radiation damage



The DNA contains the genetic information needed for the replication and the functioning of cells.

The **loss** or modification of some **specific genes** may lead to the loss of specific functions essential to cell survival

DNA is the part of a cell most susceptible to radiation damage

Evidence that DNA is a key Target

- DNA is sensitive to many types of chemical damage
  - Why not to radiation-induced radicals ?
- Micro-Irradiation of cell nucleus (alone) kills cells
  - micro-irradiation of cytoplasm is fare less effective
- Assays of DNA and chromosomes show clear damage
- Cell killing correlates with some types of chromosome aberrations
  - cell survival correlates with absence of such aberrations
- IUdR/BUdR experiments – DNA Base manipulation
  - Auger electrons within DNA space enhances damage
  - LET of radiation plays a role in “compacting” energy into DNA
- Computer simulations of radiation physics (tracks), chemistry, DNA damage predict experimental data

SSB's and DSB's

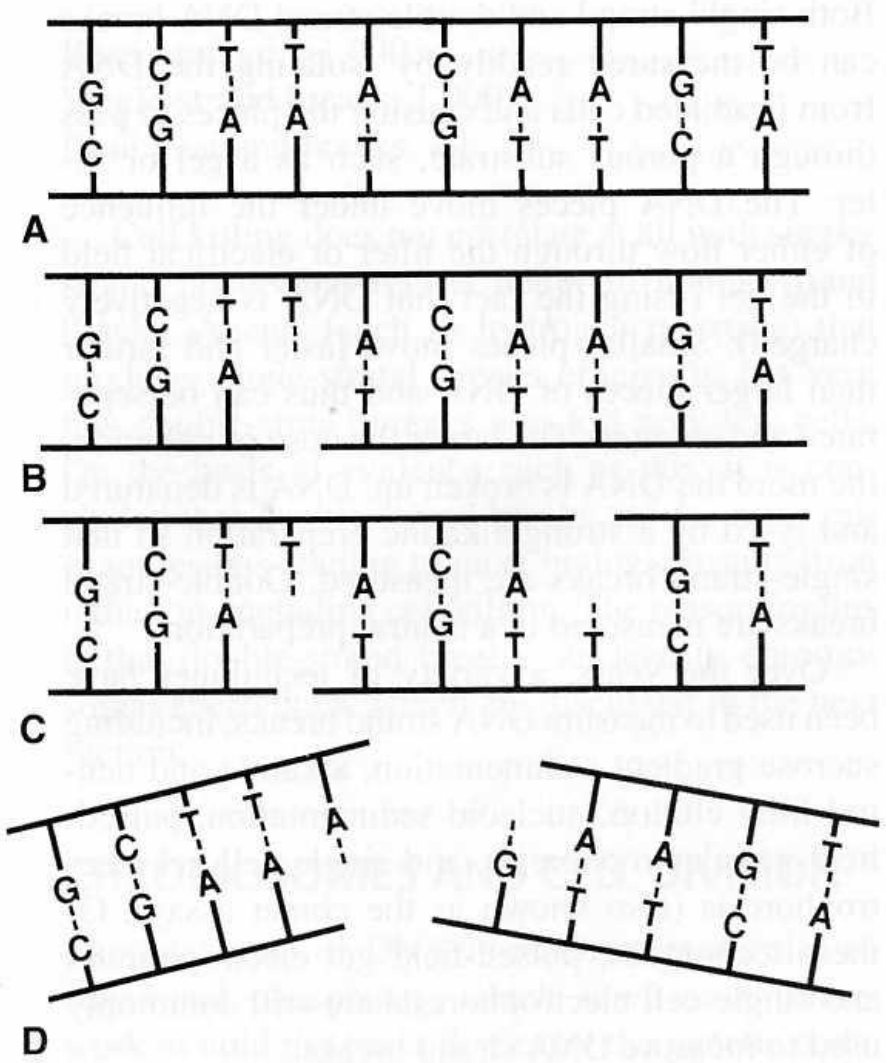


FIGURE 2.2 ● Diagrams of single- and double-strand DNA breaks caused by radiation. **A:** Two-dimensional representation of the normal DNA helix. The base pairs carrying the genetic code are complementary (i.e., adenine pairs with thymine, guanine pairs with cytosine). **B:** A break in one strand is of little significance because it is repaired readily, using the opposite strand as a template. **C:** Breaks in both strands, if well separated, are repaired as independent breaks. **D:** If breaks occur in both strands and are directly opposite or separated by only a few base pairs, this may lead to a double-strand break in which the chromatin snaps into two pieces. (Courtesy of Dr. John Ward.)

<10 base pairs

E. J. Hall and A. J. Giaccia. Radiobiology for the radiobiologist

Observing DNA Breaks

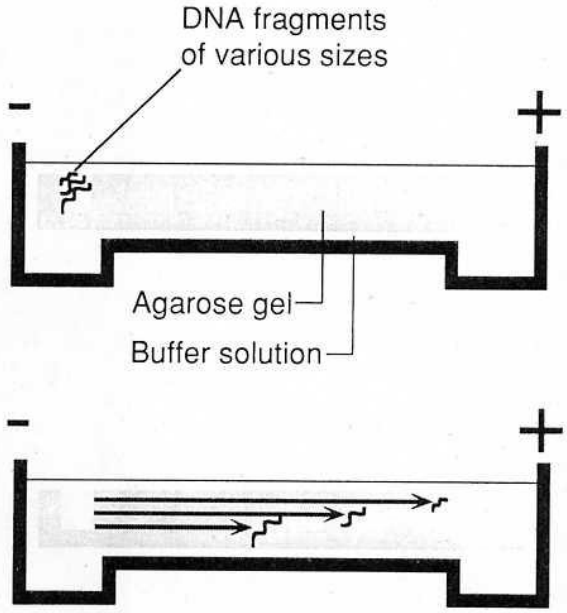


FIGURE 16.11 ● Illustration of agarose gel electrophoresis. DNA is negatively charged, so that under the influence of an electrical field, it migrates toward the anode. During electrophoresis, DNA fragments sort by size, small molecules moving farther than larger molecules. Because smaller molecules move farther than larger molecules in a given time, polyacrylamide gel electrophoresis often is employed to separate smaller DNA fragments with greater resolution than with agarose.

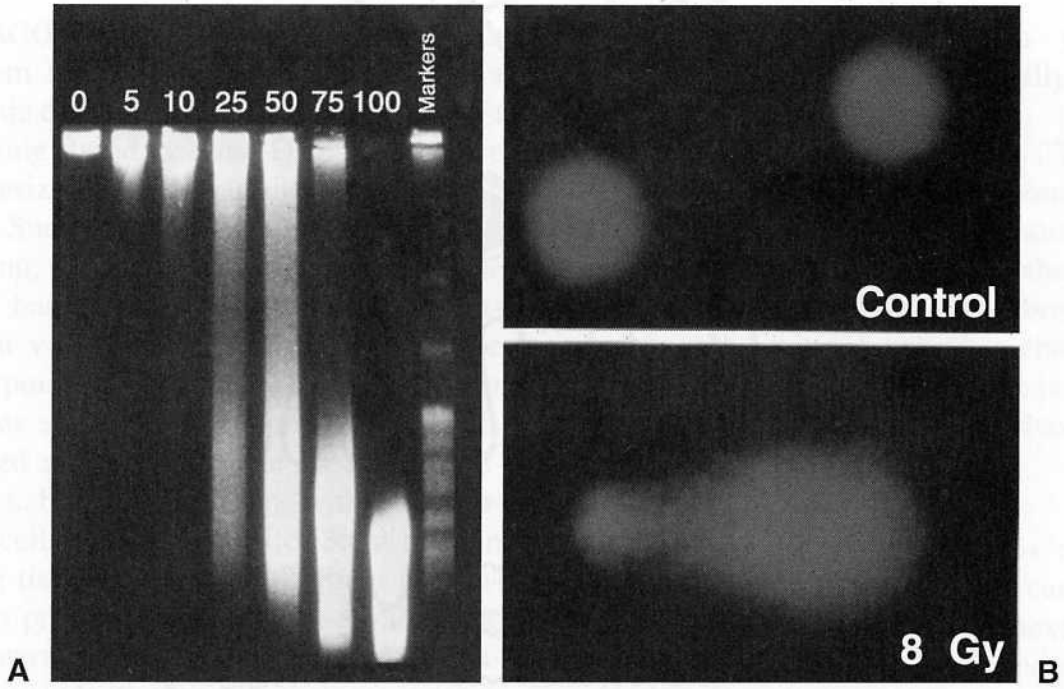
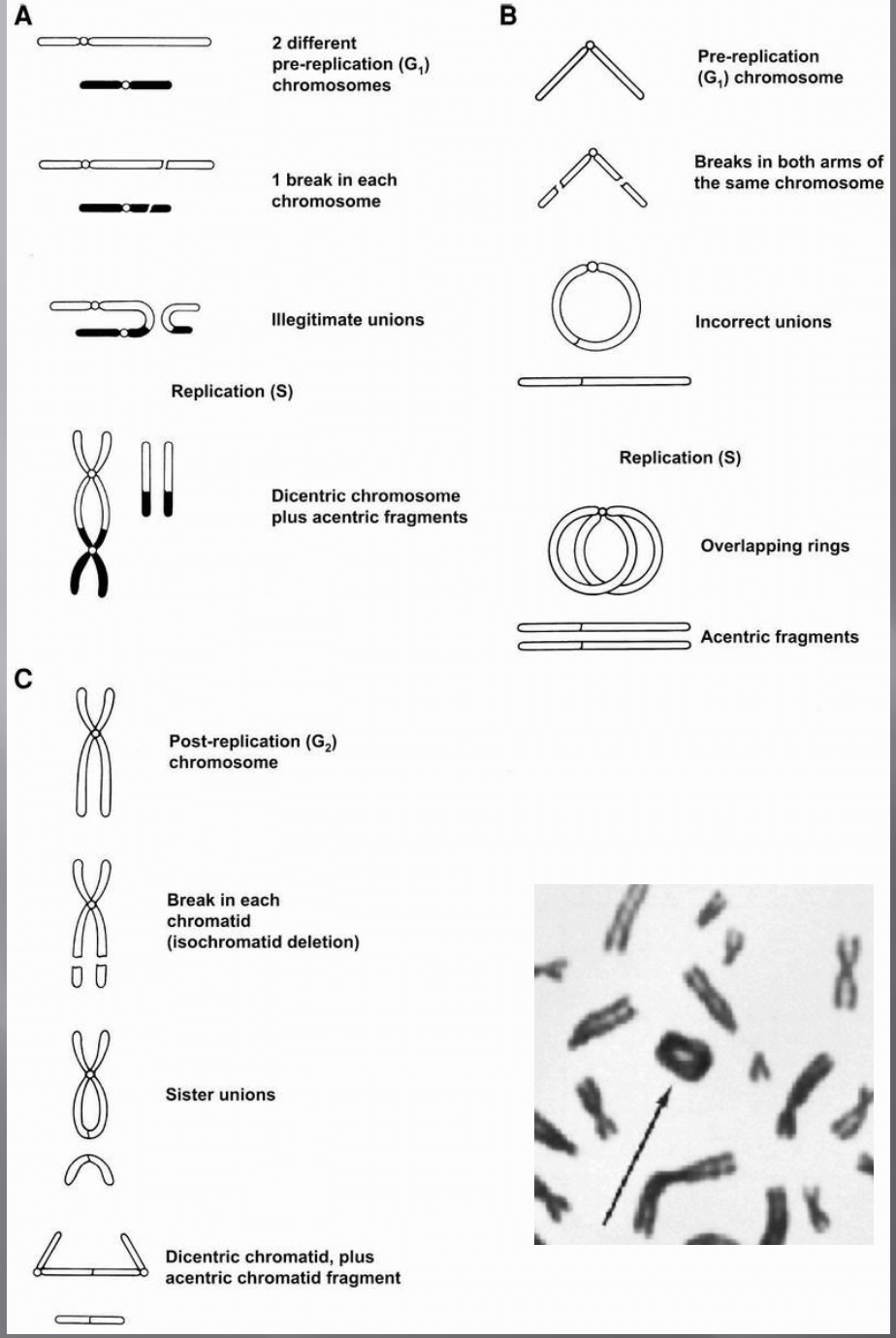
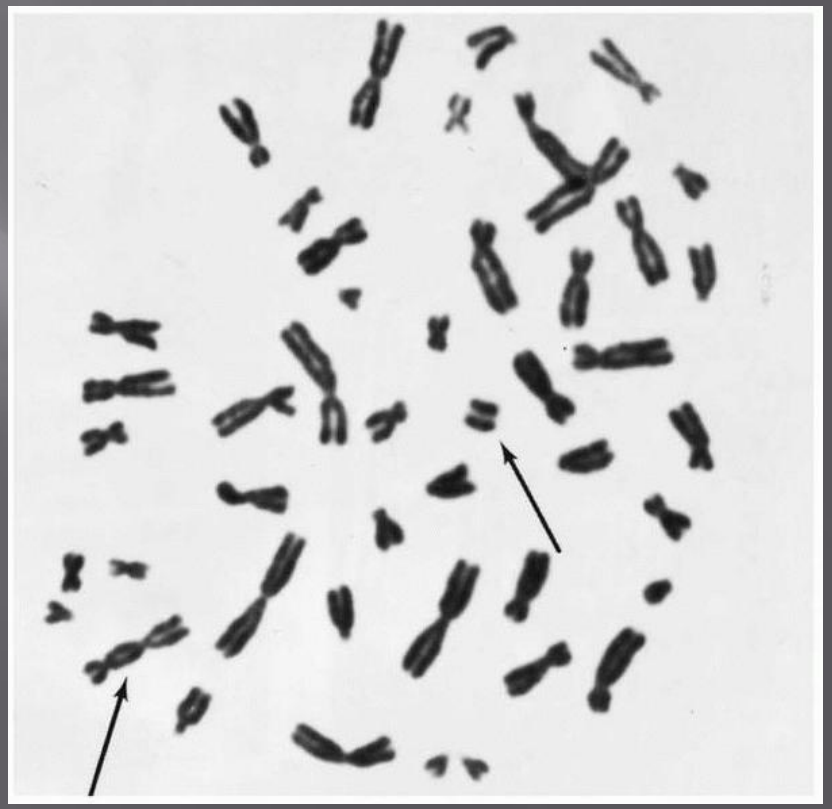


FIGURE 2.4 ● **A:** The effect of ionizing radiation on DNA strand break induction as measured by pulsed-field gel electrophoresis. As the dose of ionizing radiation increases from 5 to 100 Gy, the size of the DNA fragments as detected by ethidium brodime staining decreases. Thus, more DNA enters the gel with increasing dose of ionizing radiation. In these experiments, cells were embedded in agarose and irradiated on ice to eliminate the effects of repair. The number above each lane refers to the dose in Gy to which each group of cells was exposed. (Courtesy of Dr. Nicholas Denko.)
B: Photomicrograph of control and 8-Gy irradiated cells as detected by the comet assay. Unirradiated cells possess a near-spherical appearance, whereas the fragmented DNA in irradiated cells gives the appearance of a comet when stained with ethidium bromide. (Courtesy of Drs. Ester Hammond and Mary Jo Dorie.)

Chromosome aberrations resulting from DNA damage





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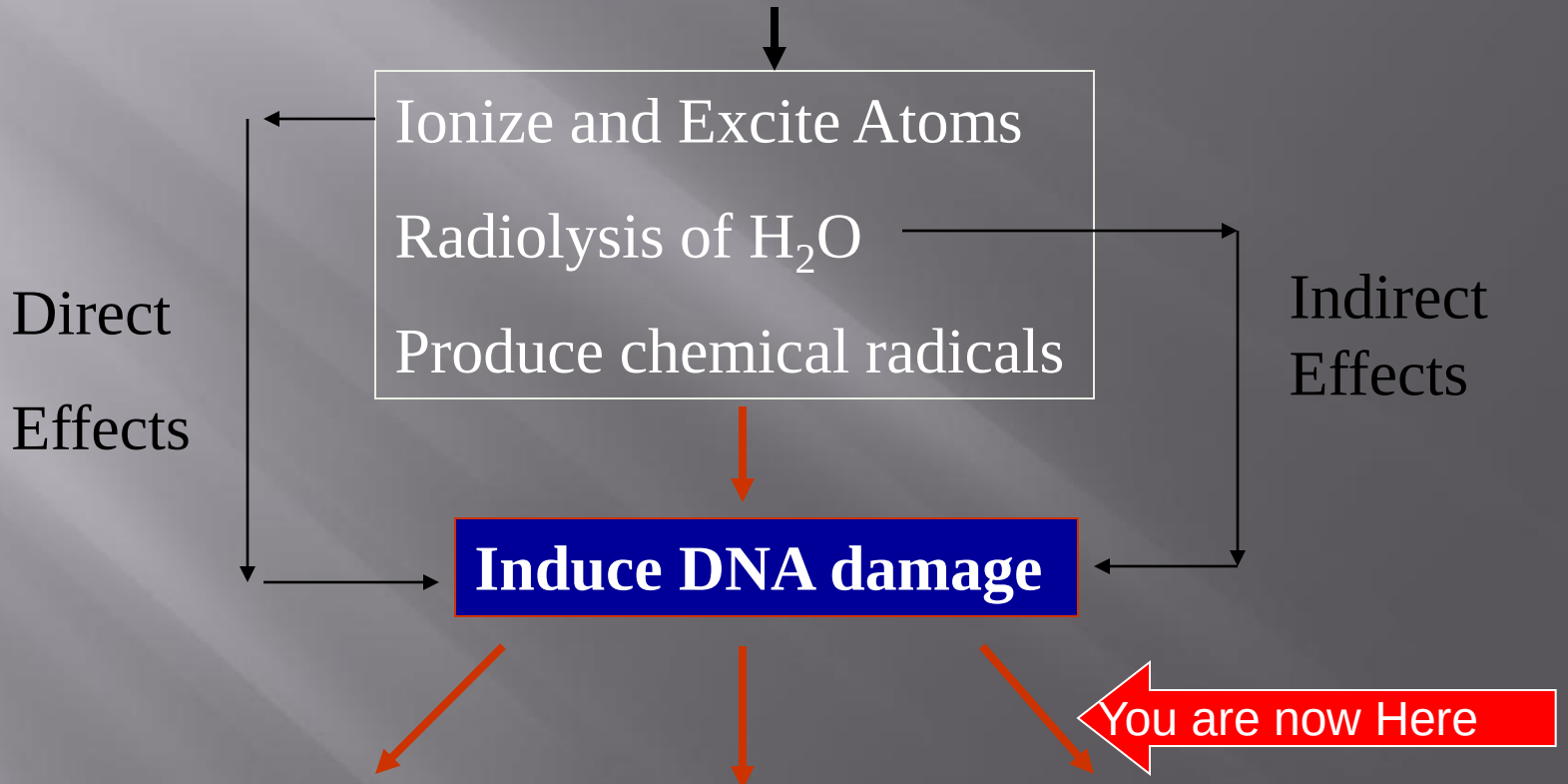
Flow Chart



Cell	Transformation	Mutation	Death
Organ	Carcinogeneis	Mutagenesis	Necrosis/Apoptosis

What is Cell Death?

Proliferating cells (stem cells and *in-vitro*),

Death = loss of reproductive ability

(cells loose the ability to exhibit unlimited cell division)

Non-proliferating cells (differentiated cells)

Death = loss of specific function

(interphase death)

Mechanisms of Cell Death

- Mitotic death: death of cells while attempting to divide. (Dominant after radiation, 2 Gy)
- Apoptosis (programmed cell death): programmed sequence of events controlled by specific genes. Part of the normal process.
- Other mechanisms: necrosis, senescence,...

What is Cell Survival?

In-Vitro Definition:

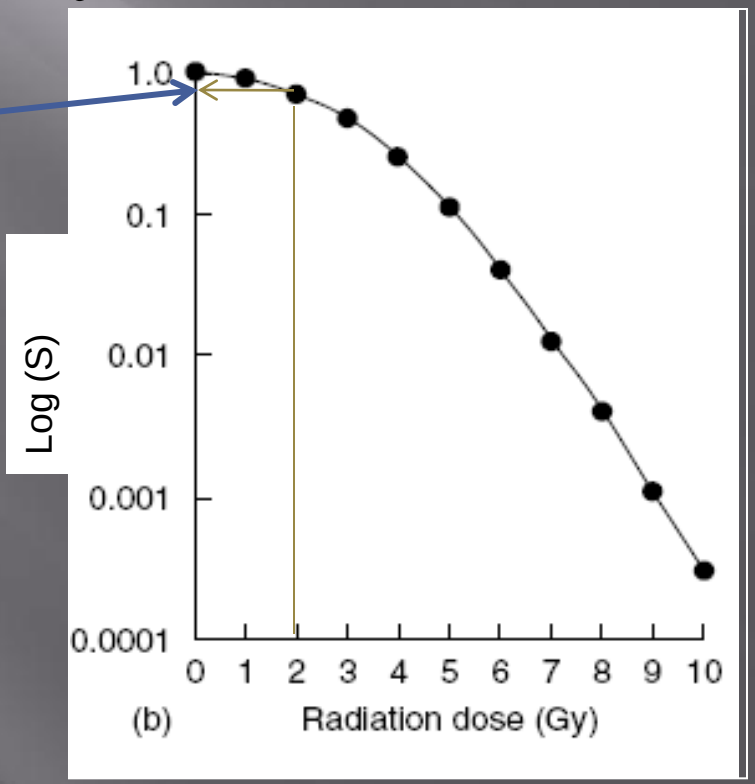
- The ability of a single cell to expand into a population of cells through repeated cell divisions and surpass the arbitrary colony size limit (e.g. 50 cells).
  - ~ 6 full generations of descendents

Definition can vary according to the end point of the assay (e.g. *in vivo tumour size*)

What is a Cell Survival Curve ?

- A survival curve describes the relationship between the radiation dose (x-axis) and the fraction of cells (S) (y-axis) that survive that dose level.
- SF_2

Survival Fraction for a dose of 2 Gray



What does it look like ?

- A survival curve describes the relationship between the surviving fraction of cells (fraction of cells that maintain their reproductive integrity) and the dose of "insult"

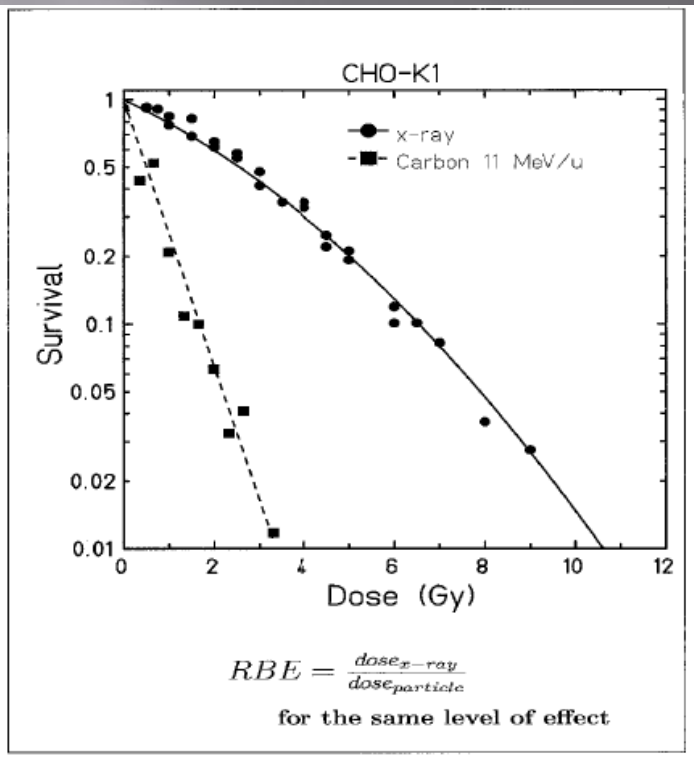


Fig. 1 Survival of CHO cells as a function of dose measured for x-rays and 11 MeV/u carbon ions. RBE values calculated from these curves as indicated depend strongly on the survival level [3].

W. K. Weyrather, G. Kraft. *RBE of carbon ions: Experimental data and the strategy of RBE calculation for treatment planning*, Radiother Oncol. 2004 Dec;73 Suppl 2:S161-9.

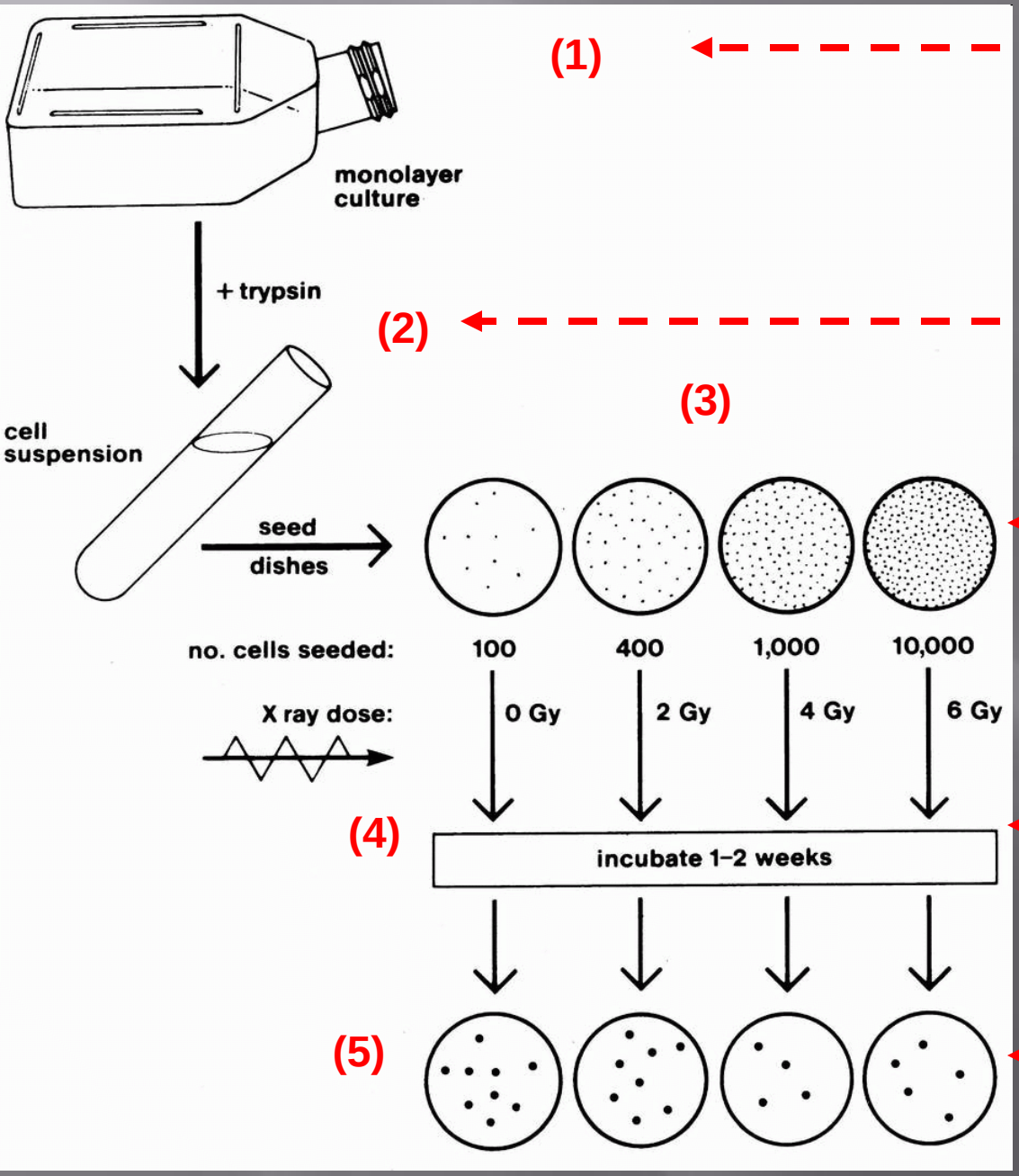
HOW IS CELL SURVIVAL MEASURED?

Clonogenic assays

Basic Method

Remove cells from the tumor (or any tissue), place them in a defined growth environment, and test their ability to produce a viable colony of descendents

- Start with actively growing culture of cells. **(1)**
- Prepare single cell suspension by adding trypsin (enzyme) to detach cells from the surface of the flask. **(2)**
- Count the number of cells per unit volume. **(2)**
- Seed a known number of cells into new Petri dishes.
- Flasks are irradiated at different doses, including a control with no dose. **(3)**
- Incubate for 1-2 weeks to allow colony formation. Each single cell may divide several times and ultimately form a macroscopic colony that can be counted if it exceeds a threshold size. **(4)**
- Count colonies containing ≥ 50 cells (5-6 generations of proliferation) **(5)**



Actively growing culture of cells.

- Prepare single cell suspension by adding trypsin (enzyme) to detach cells from the surface of the flask.
- Count the number of cells per unit volume.

Seed a known number of cells into new Petri dishes. Some flasks are irradiated at different doses and others are used as control.

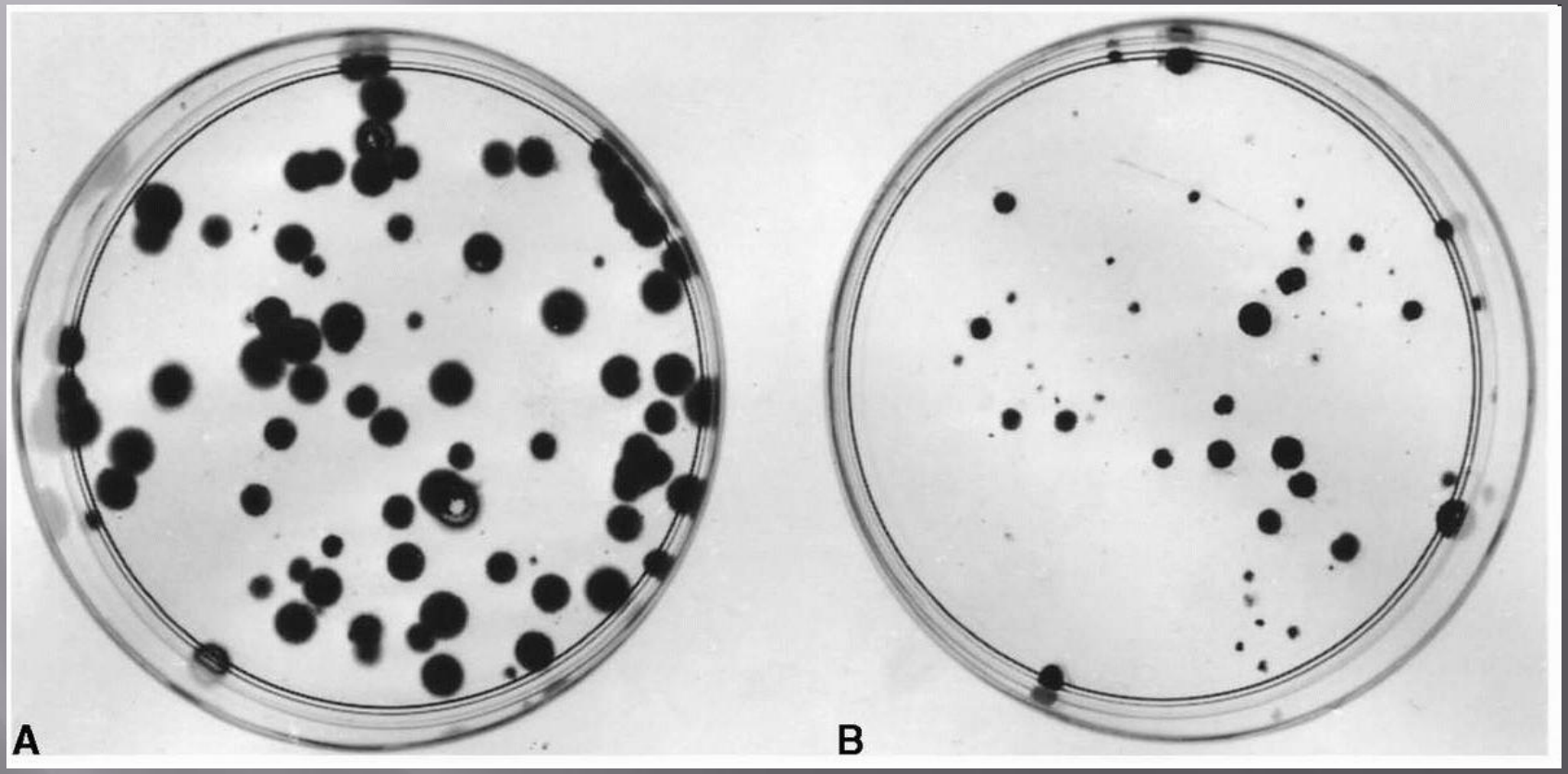
Allow for growth. Each single cell may divide several times and ultimately form a macroscopic colony that can be counted.

Count colonies containing ≥ 50 cells (5-6 generations of proliferation)

In-Vitro Colonies are “spots” of Specified Size

Fig. 3.1. Colonies of *V79-1* Chinese hamster cells (9 cm Pyrex petri dishes) stained with methylene blue (½ ml of a 1% water solution per 10 ml of growth medium; 45 minutes at room temperature): upper left, 0 rad (12-days growth); upper right, 542 rad (14-days growth); lower left, 1083 rad (16-days growth); lower right, two 542 rad exposures separated by 8 hours at 37°C (16-days growth). (Courtesy of Elkind and Sutton, *Radiation Res.*, Ref. 3.)

Cell Colony Math



Colonies obtained with Chinese hamster cells cultured in vitro.
A: In this unirradiated control dish, 100 cells were seeded and allowed to grow for 7 days before being stained. There are 70 colonies; therefore, the plating efficiency is 70/100, or 70%.
B: Two thousand cells were seeded and then exposed to 8 Gy of x-rays. There are 32 colonies on the dish. Thus:
Surviving fraction = Colonies counted/[Cells seeded × (PE/100)]
= 32/(2,000 × 0.7) = 0.023

Plating Efficiency Correction

Ideal case:
If we seed 100 cells (not irradiated) into each dish, how many colonies should we expect?

100 ???

In practice, we don't measure a 100% growth efficiency.
Not all cells plated out will form colonies.

Cells can die for several reasons (independent on radiation):
growth medium, damage and stress during trypsinization,….
"Plating efficiency" is a coefficient that accounts for this extraneous effect

$$Plating\ \ Efficiency\ = PE = 100 \times \frac{Number\ of\ \ colonies\ \ counted\ (@\ ZeroDose)}{Number\ of\ \ cells\ \ seeded}$$

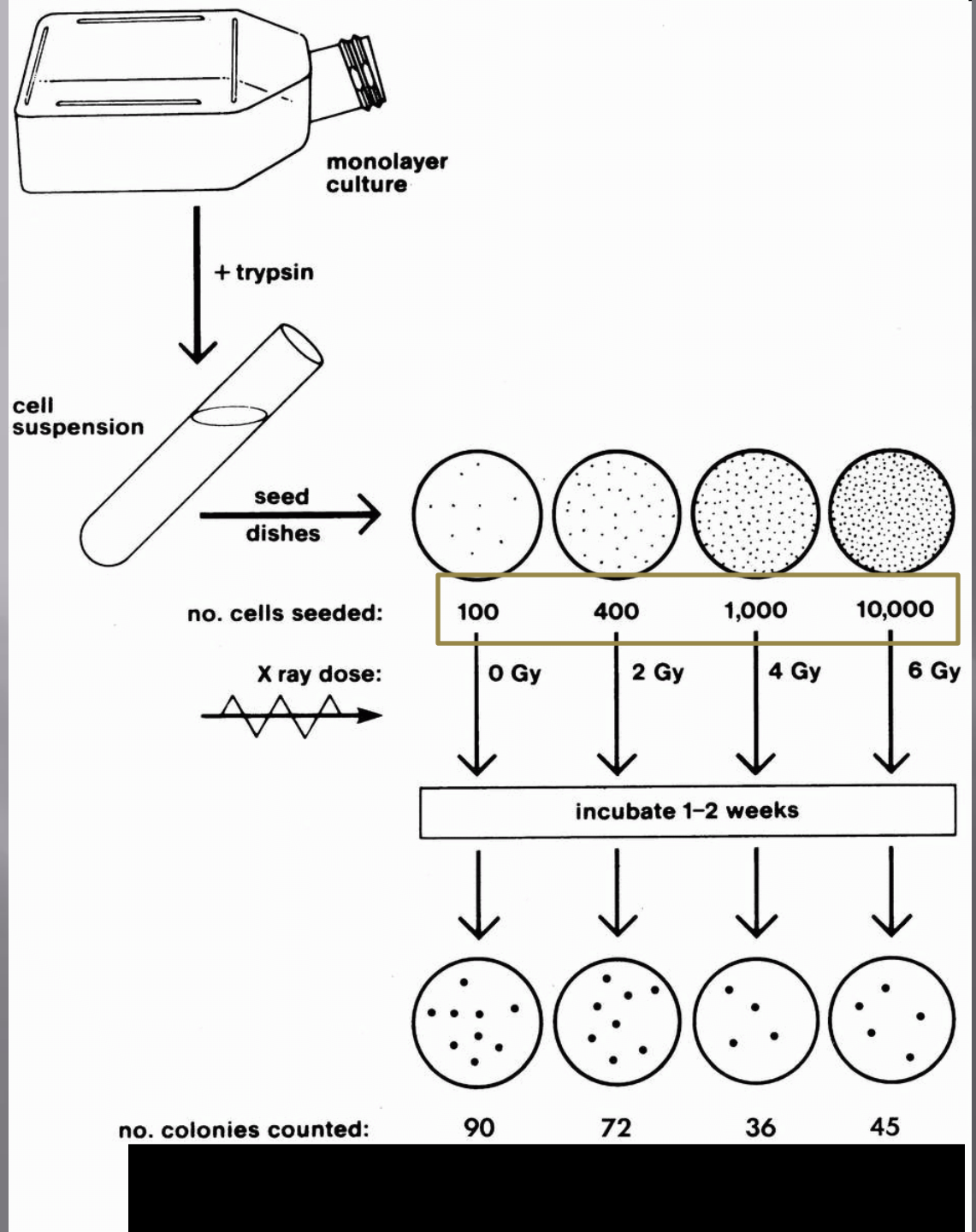
Net Survival Fraction as a function of Dose

Seed different dishes with different number of starting cells exposed to a range of radiation

The survival fraction as a function of dose is given by:

$$SF(D) = \frac{Colonies\ counted}{Cells\ seeded \times (PE/100)}$$

NOTE: Number of starting cells seeded per dish needs to be adjusted so that a countable number of colonies is obtained with statistical significance.



Quiz

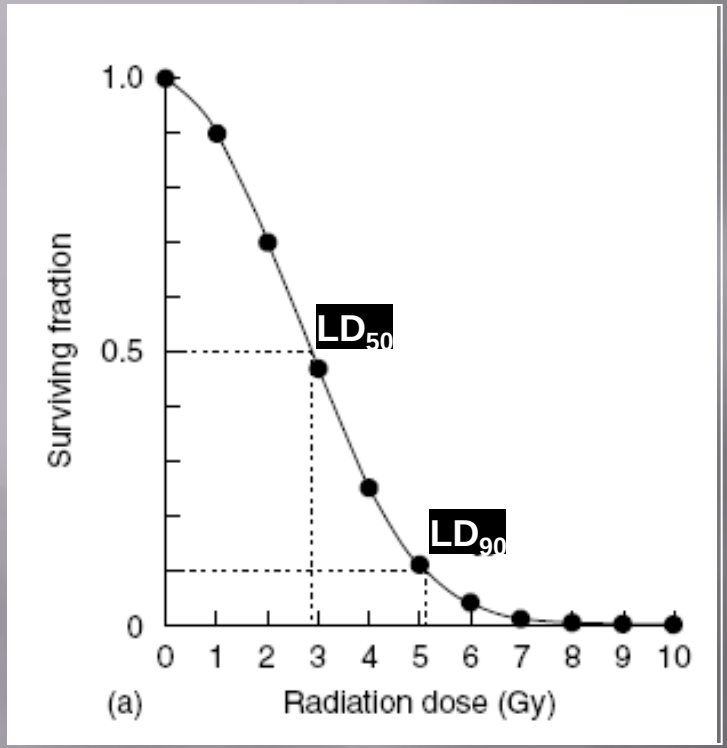
What is the plating efficiency?

90%

What is the surviving fraction?

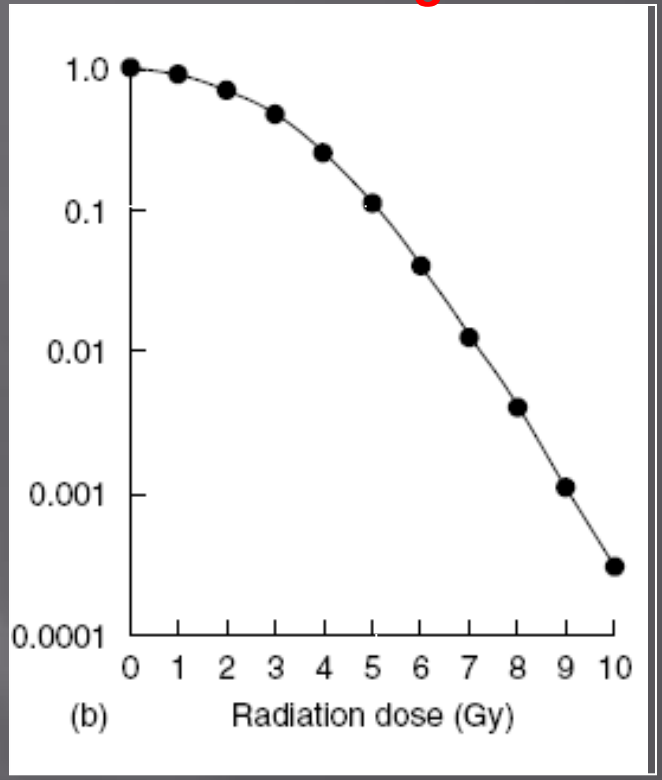
Cell survival curve Plotting

Linear



Cell survival against dose is graphically represented by plotting the surviving fraction S(D) on a logarithmic scale on the ordinate against dose D on a linear scale on the abscissa.

Logarithmic



Handbook of radiotherapy physics: theory and practice

Why a logarithmic scale?

1. Effects at very low survival levels can be shown and compared.
2. The cure of a tumor requires many orders of magnitude of cell killing.
3. If cell kill is the result of a single-hit radiation event, then survival will be exponential function of dose

Practical uses of cell survival curves - RBE

⇒ **measurement of LET effects**

⇒**relative biological effectiveness (same cell line, different radiations)**

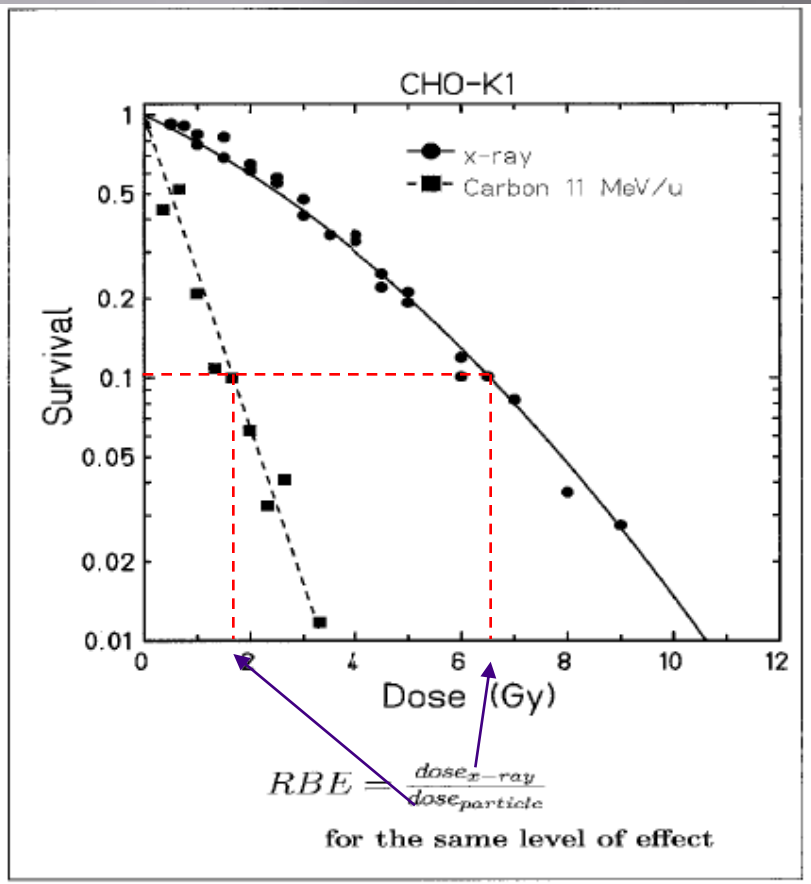
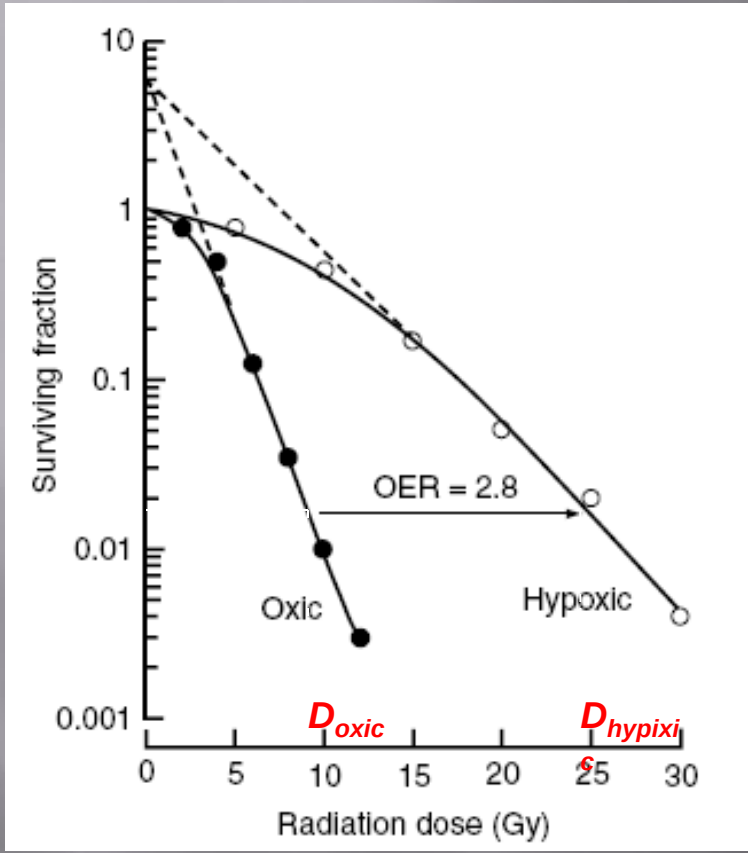


Fig. 1 Survival of CHO cells as a function of dose measured for x-rays and 11 MeV/u carbon ions. RBE values calculated from these curves as indicated depend strongly on the survival level [3].

- Densely irradiated cells (high LET): Exponential function of dose
- Sparsely irradiated cells (low LET): Initial slope followed by a shoulder region and a straight line at high doses

Practical uses of cell survival curves - OER

⇒ **Compare sensitivities of different or altered cell lines (same radiation source)**



Example: investigate the effect of oxygenation.

The response of cells to ionizing radiation is strongly dependent upon oxygen.

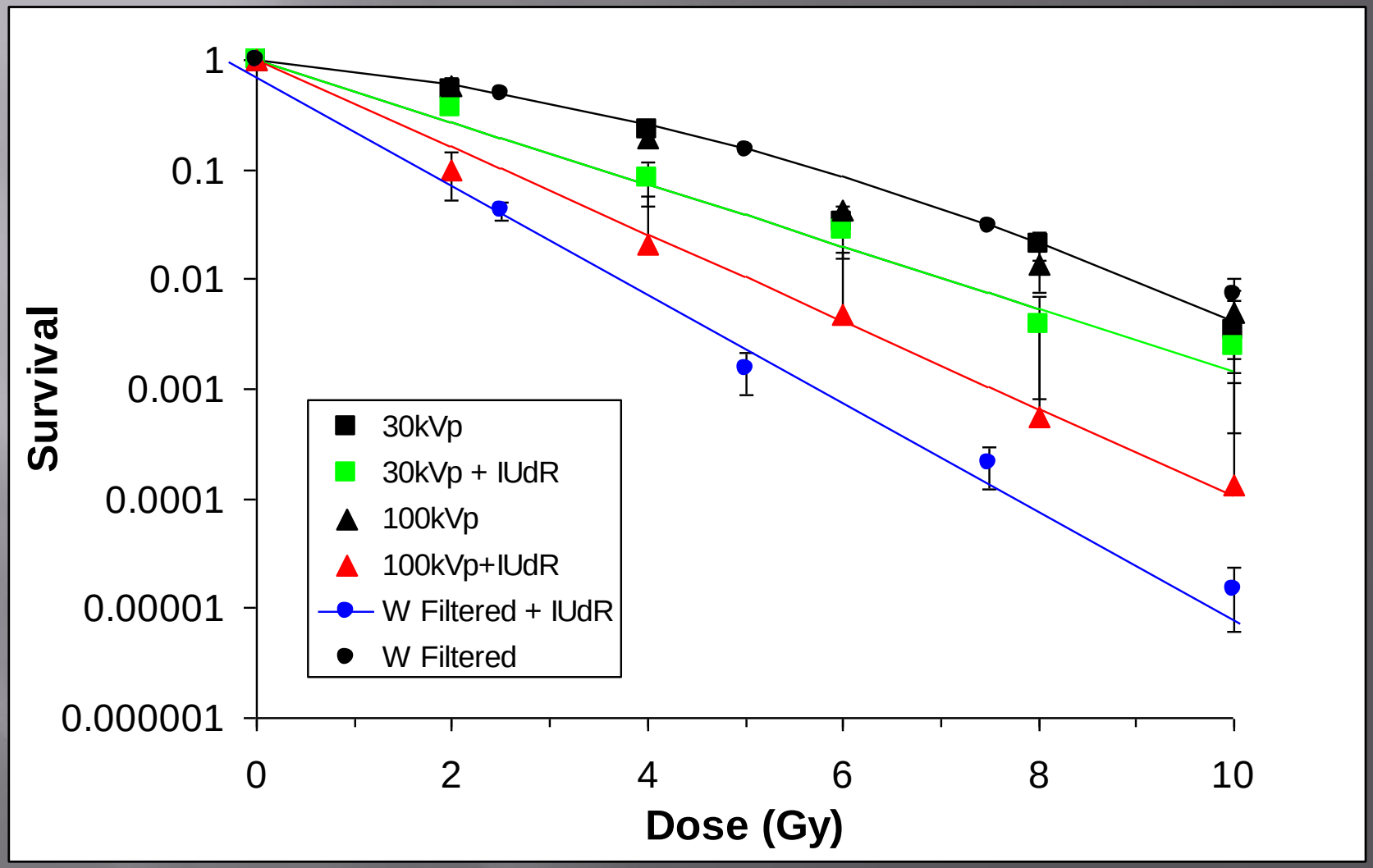
OER: oxygen enhancement ratio = ratio of the radiation dose in hypoxia to dose in air needed to achieve **the same biological effect**

$$OER = \frac{D_{hypoxic}}{D_{oxic}}$$

⇒ **measurement of effects of radio-sensitizers/ protectors (same radiation)**

SER: Sensitizer Enhancement Ratio $$SER = \frac{D_{without}}{D_{with}}$$

Practical uses of cell survival curves - Sensitizers



Karnas et al.

Cell Survival Observations

- Survival (or clonogenic) assays examine the effect of radiation (or drugs) on the ability of a cell to proliferate.
- Thus - survival is defined as the ability of a cell to retain long term proliferative potential.
- Conversely cell killing is regarded as inhibition of proliferative potential. It may or may not result in cell death (i.e. lysis) and removal from the population.
- Following low doses of irradiation (< 15-20 Gy single dose) many cells do not show morphological evidence of damage unless they attempt mitosis.
- At higher doses cells may die in interphase (usually by apoptosis)
- Exceptions include lymphocytes, spermatocytes, oocytes, endothelial cells which undergo apoptosis following lower doses (1-2 Gy for lymphocytes)

"Law" of Bergonie and Tribondeau

"***The radiosensitivity of a tissue is directly proportional to its reproductive capacity and inversely proportional to its degree of differentiation***" 1906

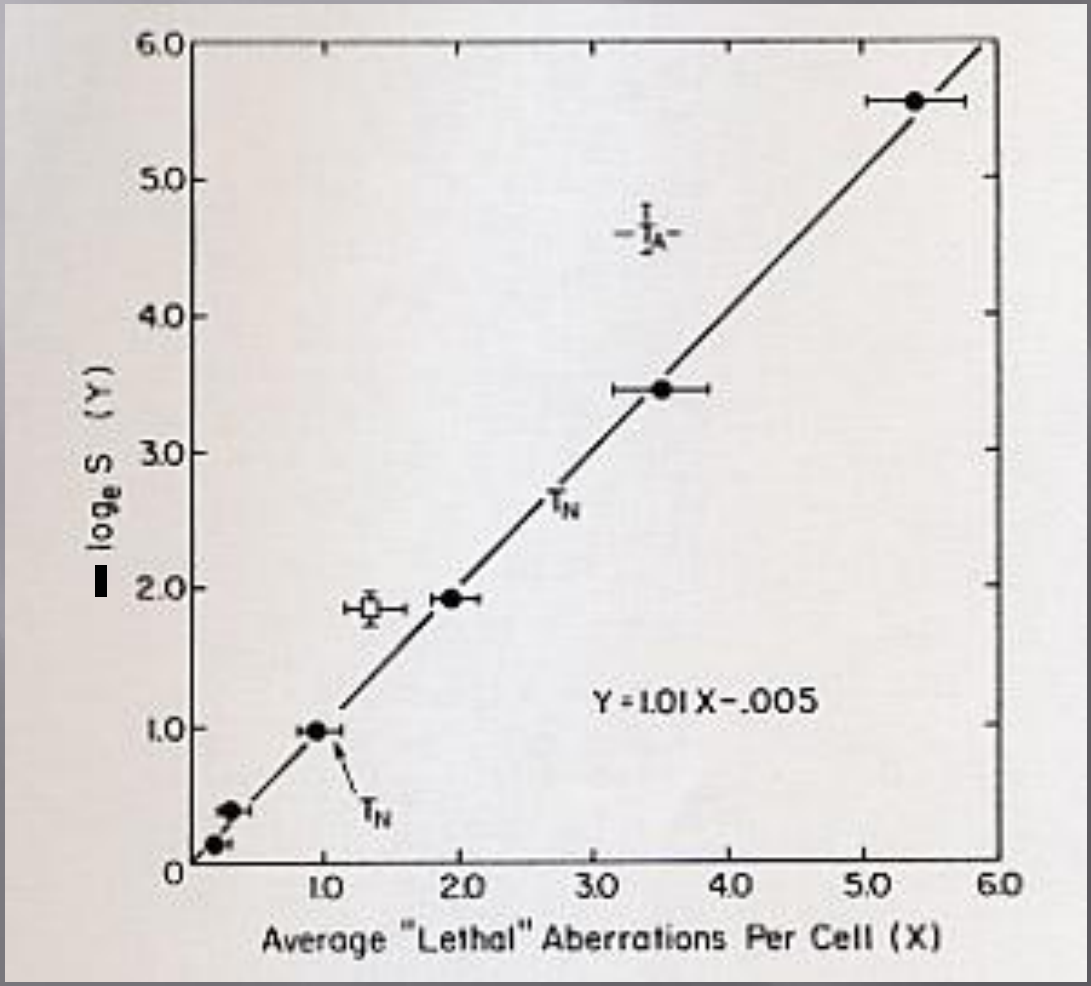
Cells tend to be more radiosensitive if they have some key properties:

- High division rate (Proliferation).
  - Long dividing future (Longevity).
- Undifferentiated type (Young, Stem).
- High metabolic rate (Active).
  - Access to nutrition.

- **Radiosensitive Tissues:**
  - Germinal cells of the ovary and testis.
  - Haematopoietic tissues: red bone marrow, spleen, lymph nodes and thymus.
  - Epithelium of the skin.
  - Epithelium of the GIT.

- **Radioresistant Tissues:**
  - Bone Liver Kidney
  - Cartilage Muscle Nervous System

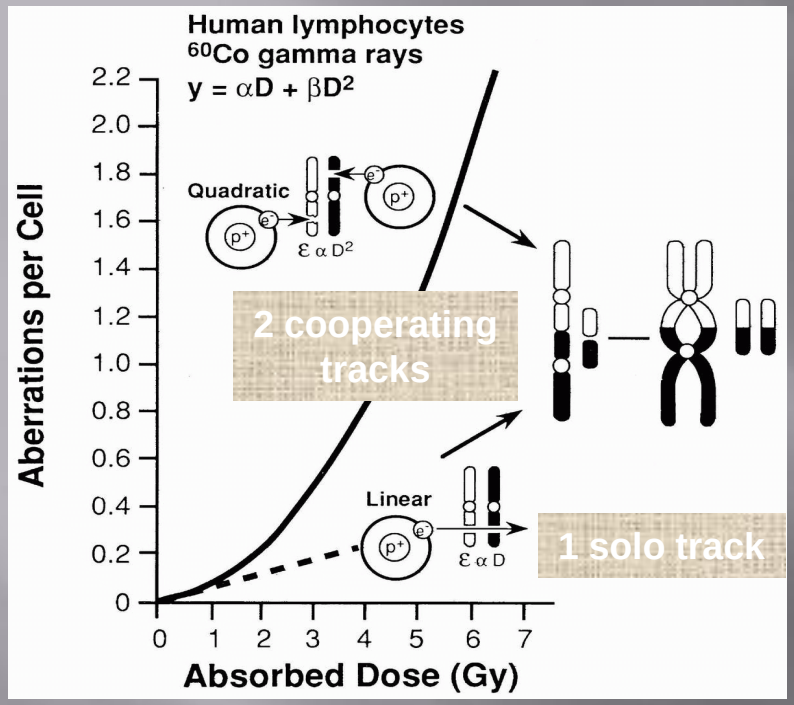
Linking Survival back to Chromosome Damage



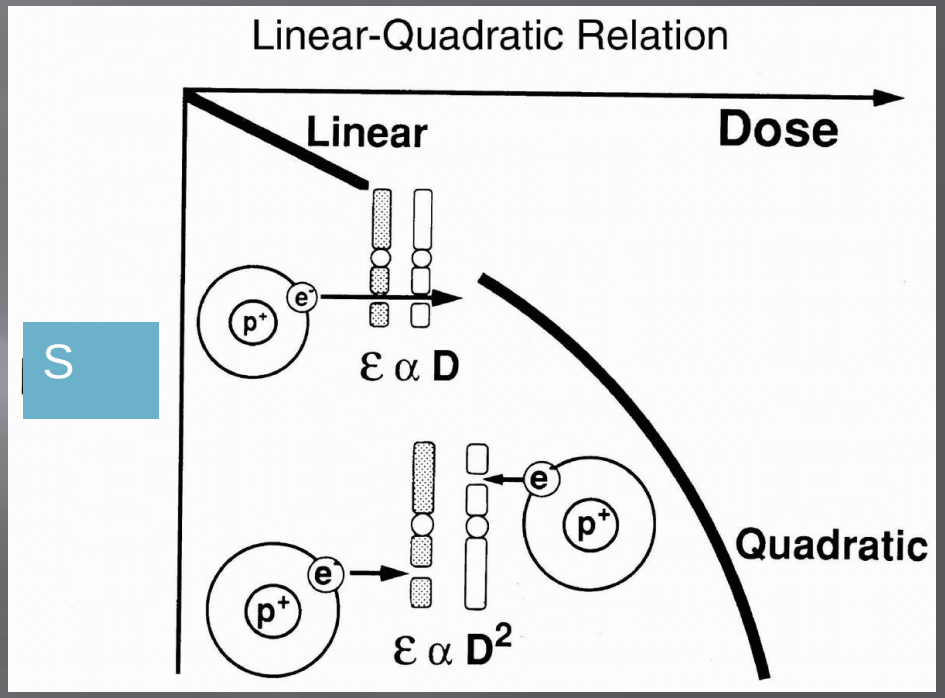
This plot clearly corelates the number of unstable CA (n) and cell survival (S)

S = Exp (-n)

RECAP on Linear Quadratic (LQ) Model



$$S(D) = e^{-\alpha D - \beta D^2}$$

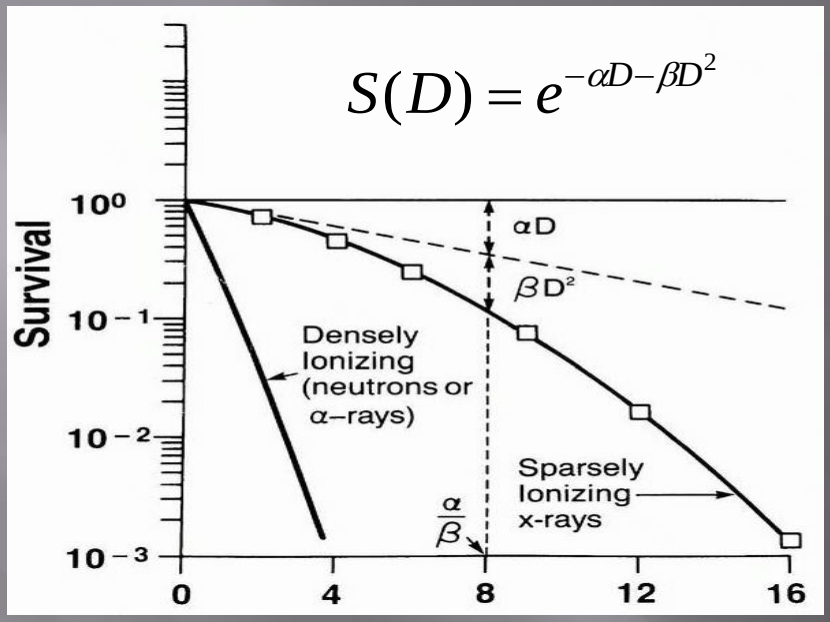


α – Initial slope at low Dose
susceptibility to single-track damage

β – Adds curvature to the final slope (larger Dose)
susceptibility to dual-track damage.
affected by dose rate and *interim* chromosome Repair

α/β – Dose at which the two cell-killing effects are equal.
Measure of "Insensitivity" to dose fractionation/rate scheme

LQ Parameters



- There are two components to cell kill by radiation: linear and quadratic.
- α describes the initial slope.
- β is a smaller constant describing the quadratic component.
- α/β gives the dose at which the linear and quadratic components are equal

α/β ≈ 10Gy for Tumour Cells; α/β ≈ 3Gy for Normal Cells - curvier

Advantage: Only a couple of parameters to fit
Disadvantage: Continuous downturn does not fit experimental data at large D

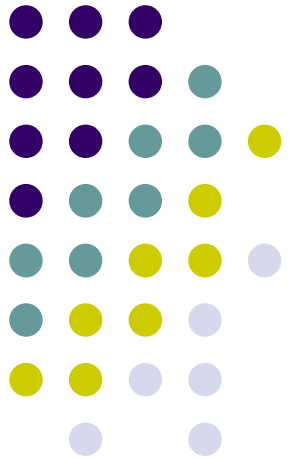
α/β ratios from lab data
(From Thames and Hendry)

Early Reactions	High α/β: ~10	α/β
skin		9.4 - 21.0
hair follicles		5.5 - 7.7
lip mucosa		7.9
jejunum		7.1
colon		8.4
testis		13.9
spleen		8.9
Late Reactions	**Low α/β: mostly ~3**	
spinal cord		2.1 - 5.2
brain		2.1
eye		1.2
kidney		0.4 - 4.1
bladder		7.2 - 7.8
lung		2.1 - 4.3
bowel		3.0 - 5.0

Table 9.1 Fractionation sensitivity of human normal tissues and tumours

Tissue/organ	Endpoint	α/β (Gy)	95% CL (Gy)	Source
Early reactions				
Skin	Erythema	8.8	6.9; 11.6	Turesson and Thames (1989)
	Erythema	12.3	1.8; 22.8	Bentzen *et al.* (1988)
	Dry desquamation	~8	N/A	Chogule and Supe (1993)
	Desquamation	11.2	8.5; 17.6	Turesson and Thames (1989)
Oral mucosa	Mucositis	9.3	5.8; 17.9	Denham *et al.* (1995)
	Mucositis	15	−15; 45	Rezvani *et al.* (1991)
	Mucositis	~8	N/A	Chogule and Supe (1993)
Late reactions				
Skin/vasculature	Telangiectasia	2.8	1.7; 3.8	Turesson and Thames (1989)
	Telangiectasia	2.6	2.2; 3.3	Bentzen *et al.* (1990)
	Telangiectasia	2.8	−0.1; 8.1	Bentzen and Overgaard (1991)
Subcutis	Fibrosis	1.7	0.6; 2.6	Bentzen and Overgaard (1991)
Breast	Cosmetic change in appearance	3.4	2.3; 4.5	START Trialists Group (2008)
	Induration (fibrosis)	3.1	1.8; 4.4	Yarnold *et al.* (2005)
Muscle/vasculature/ cartilage	Impaired shoulder movement	3.5	0.7; 6.2	Bentzen *et al.* (1989)
Nerve	Brachial plexopathy	<3.5*	N/A	Olsen *et al.* (1990)
	Brachial plexopathy	~2	N/A	Powell *et al.* (1990)
	Optic neuropathy	1.6	−7; 10	Jiang *et al.* (1994)
Spinal cord	Myelopathy	<3.3	N/A	Dische *et al.* (1981)
Eye	Corneal injury	2.9	−4; 10	Jiang *et al.* (1994)
Bowel	Stricture/perforation	3.9	2.5; 5.3	Deore *et al.* (1993)
Bowel	Various late effects	4.3	2.2; 9.6	Dische *et al.* (1999)
Lung	Pneumonitis	4.0	2.2; 5.8	Bentzen *et al.* (2000)
	Lung fibrosis (radiological)	3.1	−0.2; 8.5	Dubray *et al.* (1995)
Head and neck	Various late effects	3.5	1.1; 5.9	Rezvani *et al.* (1991)
Head and neck	Various late effects	4.0	3.3; 5.0	Stuschke and Thames (1999)
Supraglottic larynx	Various late effects	3.8	0.8; 14	Maciejewski *et al.* (1986)
Oral cavity + oropharynx	Various late effects	0.8	−0.6; 2.5	Maciejewski *et al.* (1990)
Tumours				
Head and neck				
Various		10.5	6.5; 29	Stuschke and Thames (1999)
Larynx		14.5*	4.9; 24	Rezvani *et al.* (1993)
Vocal cord		~13	'wide'	Robertson *et al.* (1993)
Buccal mucosa		6.6	2.9; ∞	Maciejewski *et al.* (1989)
Tonsil		7.2	3.6; ∞	Maciejewski *et al.* (1989)
Nasopharynx		16	−11; 43	Lee *et al.* (1995)
Skin		8.5*	4.5; 11.3	Trott *et al.* (1984)
Prostate†		1.1	−3.3; 5.6	Bentzen and Ritter (2005)
Breast		4.6	1.1; 8.1	START Trialists Group (2008)
Oesophagus		4.9	1.5; 17	Geh *et al.* (2006)
Melanoma		0.6	−1.1; 2.5	Bentzen *et al.* (1989)
Liposarcoma		0.4	−1.4; 5.4	Thames and Suit (1986)

CL, confidence limit.
*Re-analysis of original published data.
†Several more estimates are available from comparisons of outcome after brachytherapy versus external-beam therapy.
Reference details are available from Søren Bentzen. See also Thames *et al.* (1990) and Table 13.2.



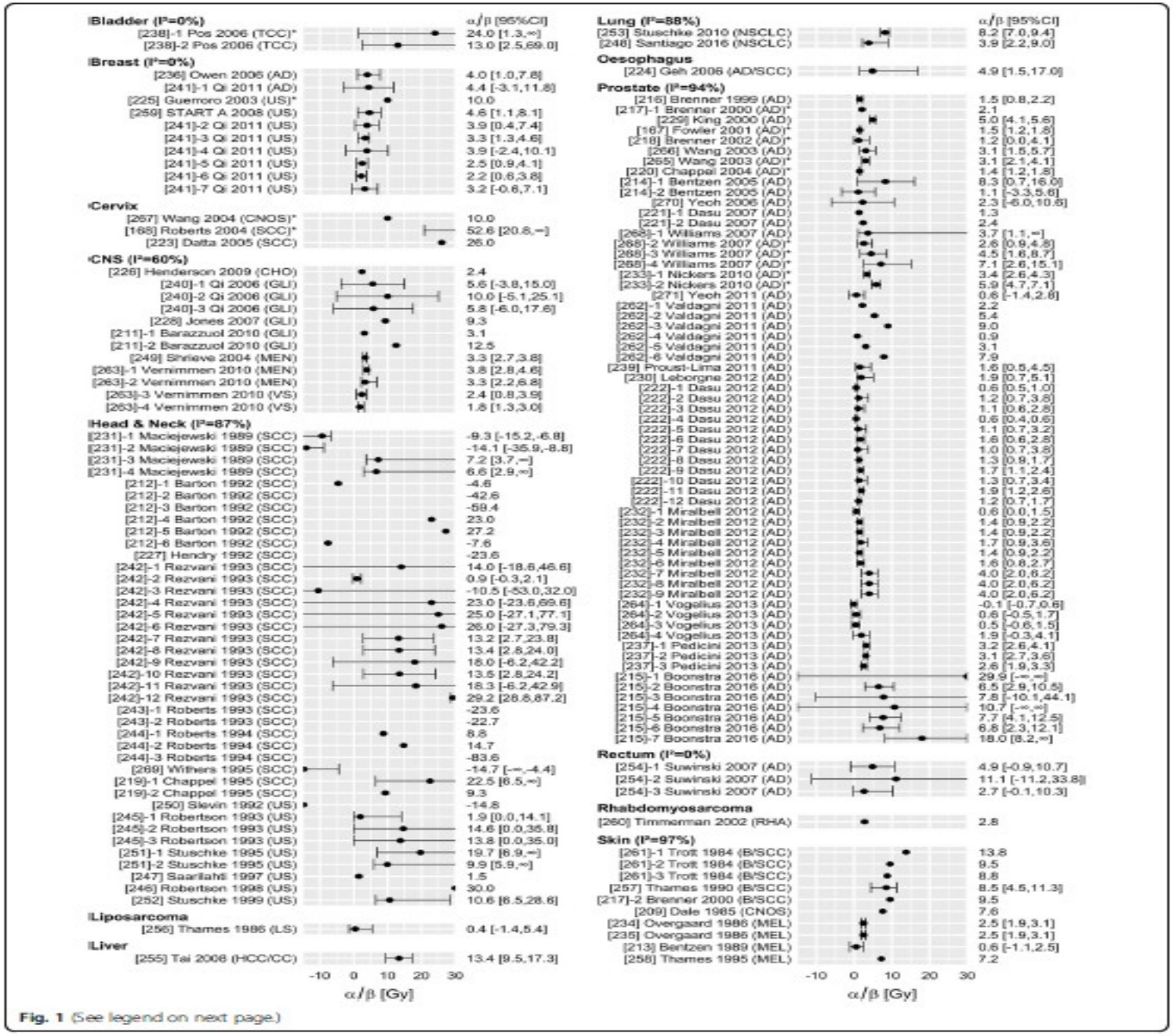


Fig. 1 (See legend on next page.)

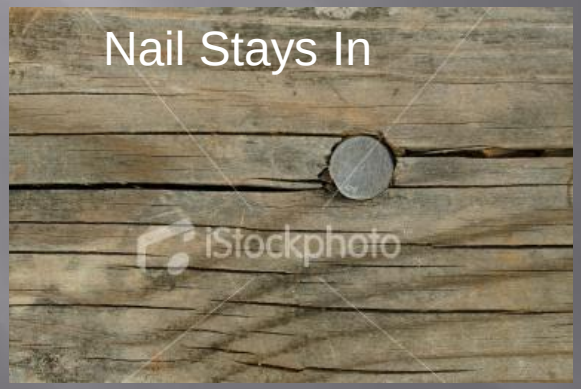
The alfa and beta of tumours: a review of parameters of the linear-quadratic model, derived from clinical radiotherapy studies. van Leeuwen et al. Radiation Oncology (2018) 13:96

Ski Analogy

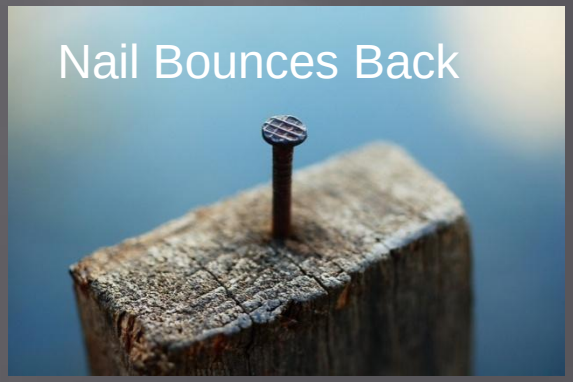
http://www.hicker-stock-photography.com/images/600/alpine-skier-whistler-mountain-bc-316.jpg

http://images.google.ca/imgres?imgurl=http://www.silverliningchalets.co.uk/userimages/downhill-skiing-1.jpg&imgrefurl=http://

Nailing Down the α/β Concept



α/β = 10



α/β = 3

Introduction to
Tumor Control Probability (TCP)

Objective of radiation therapy:
Eradicate the tumor without causing complications in the normal tissue

Idealized model:

- No variability between tumours and between patients, no host response (e.g. immunology, inflammation) against residual cancer cells.
- Tumor control is achieved when the last clonogenic cell is sterilized.
- Poisson statistics apply:

TCP models are used to make outcome predictions and to optimize treatment plans based on biological models

We need to understand:

- The effect of varying tumor characteristics on outcome (radiosensitivity, heterogeneity,…).
- The effect of different treatment modalities on outcome (prescribed dose, fractionation,….)

Example: investigate the therapeutic advantage of increasing dose

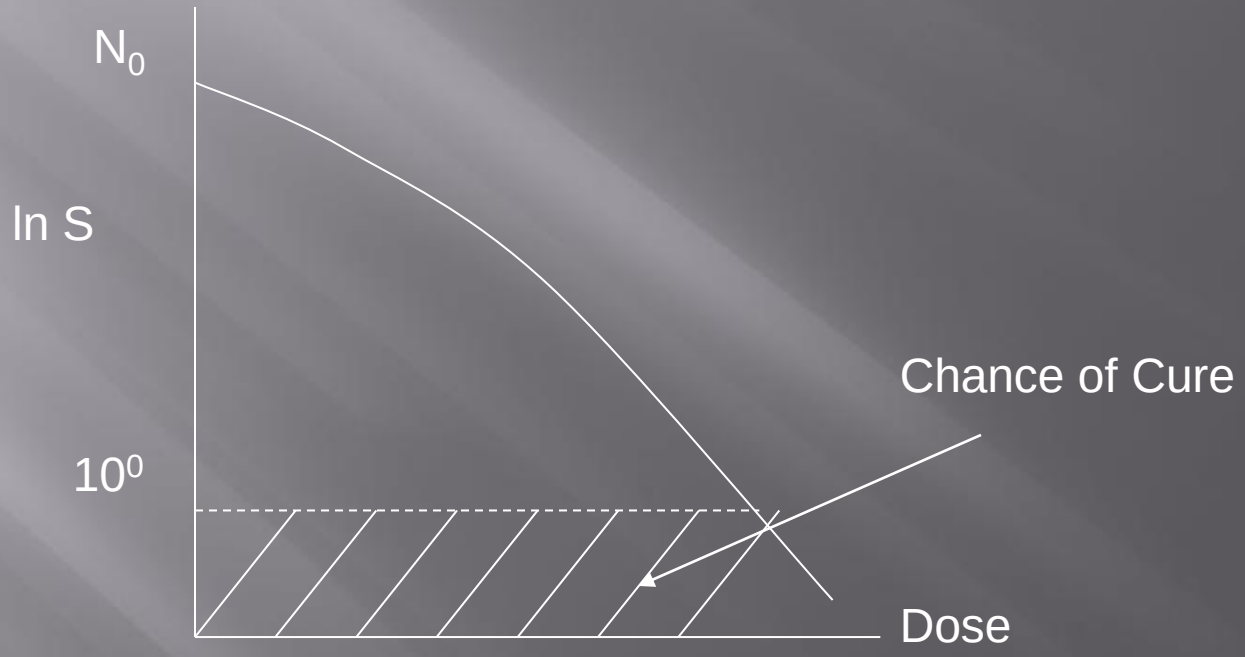
$$TCP = e^{-m}$$

m: mean number of clonogenic cells that survive the treatment.

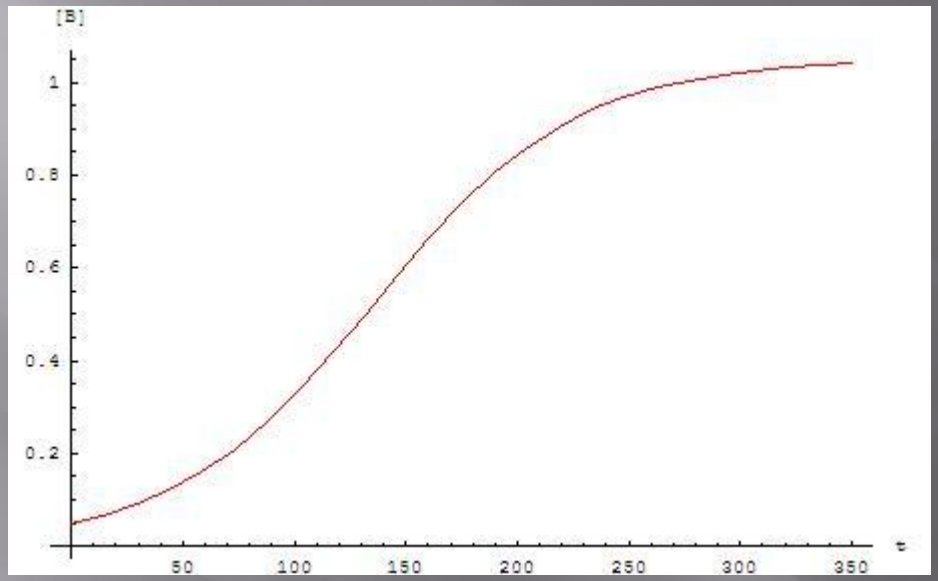
How does m relate to our previous sections?

Can Survival Predict Tumour Cure?

- For a "cure", we need cell survival to be <<<1 cell
- What is the probability of this occurring ? TCP



TCP Curves

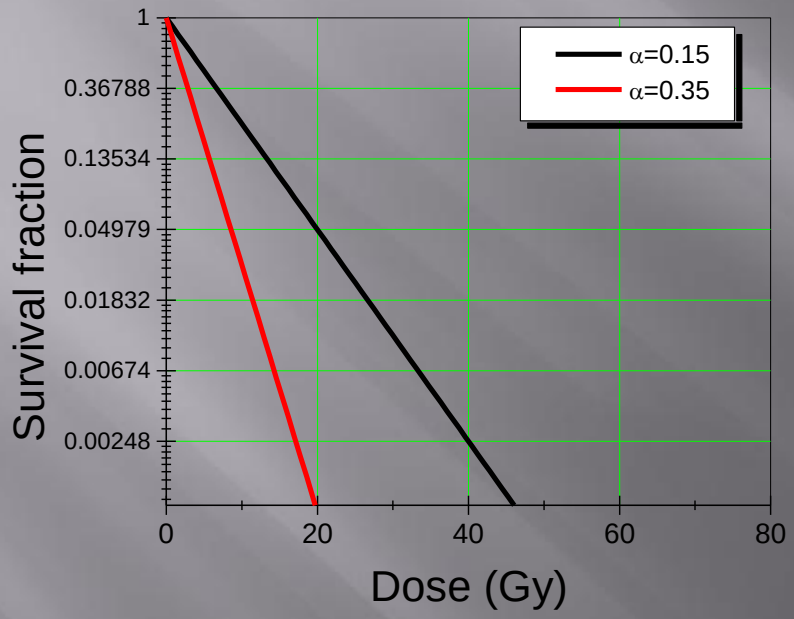


- Dose response curve is sigmoidal in shape.
- At lower doses, the curve is shallow and there is only a slow increase in the effects with radiation dose.
- The steepness reaches a max at 50% (0.5) response.
- The same is true at high doses (saturation).

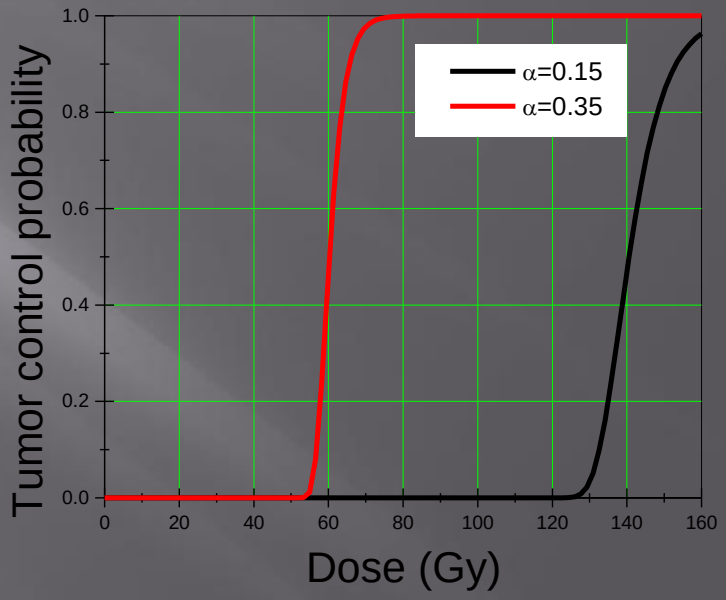
http://upload.wikimedia.org/wikipedia/commons/9/98/Sigmoid_curve_for_an_autocatalytical_reaction.JPG

Two tumours with the same number of cells (N_0) and different sensitivity (α) values

$$S = e^{-\alpha D}$$

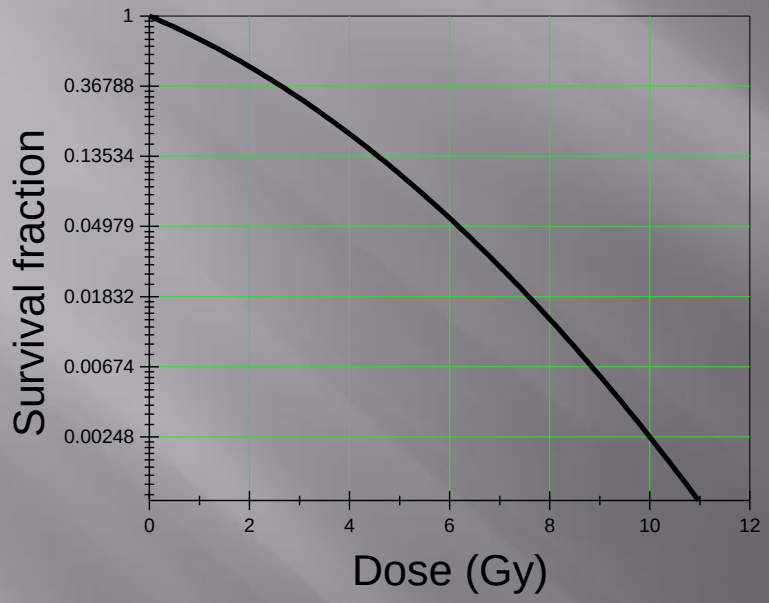


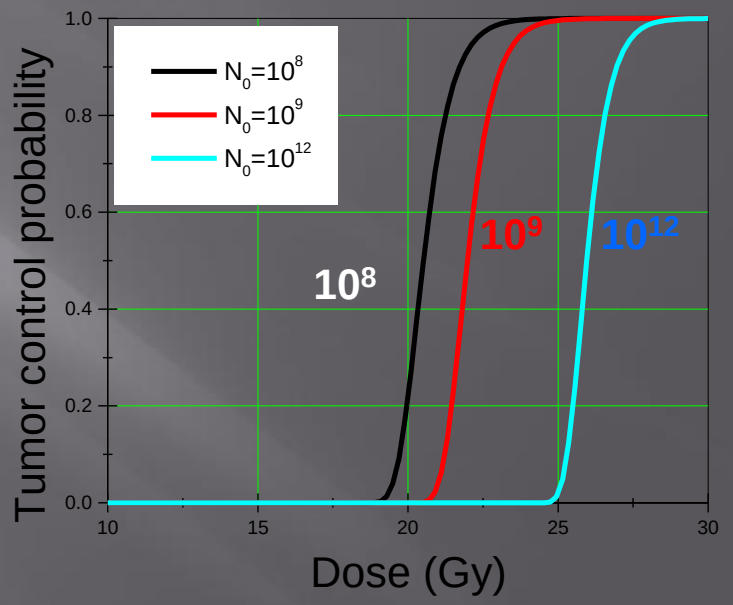
$$TCP = e^{-N_0 S}$$



$N_0=10^9$, $\beta=0$

Same α and β values, but tumors of different sizes





α=0.3, β=0.03

Multi-Fraction Cell Survival Curves: Dose Fractionation – Assignment #2

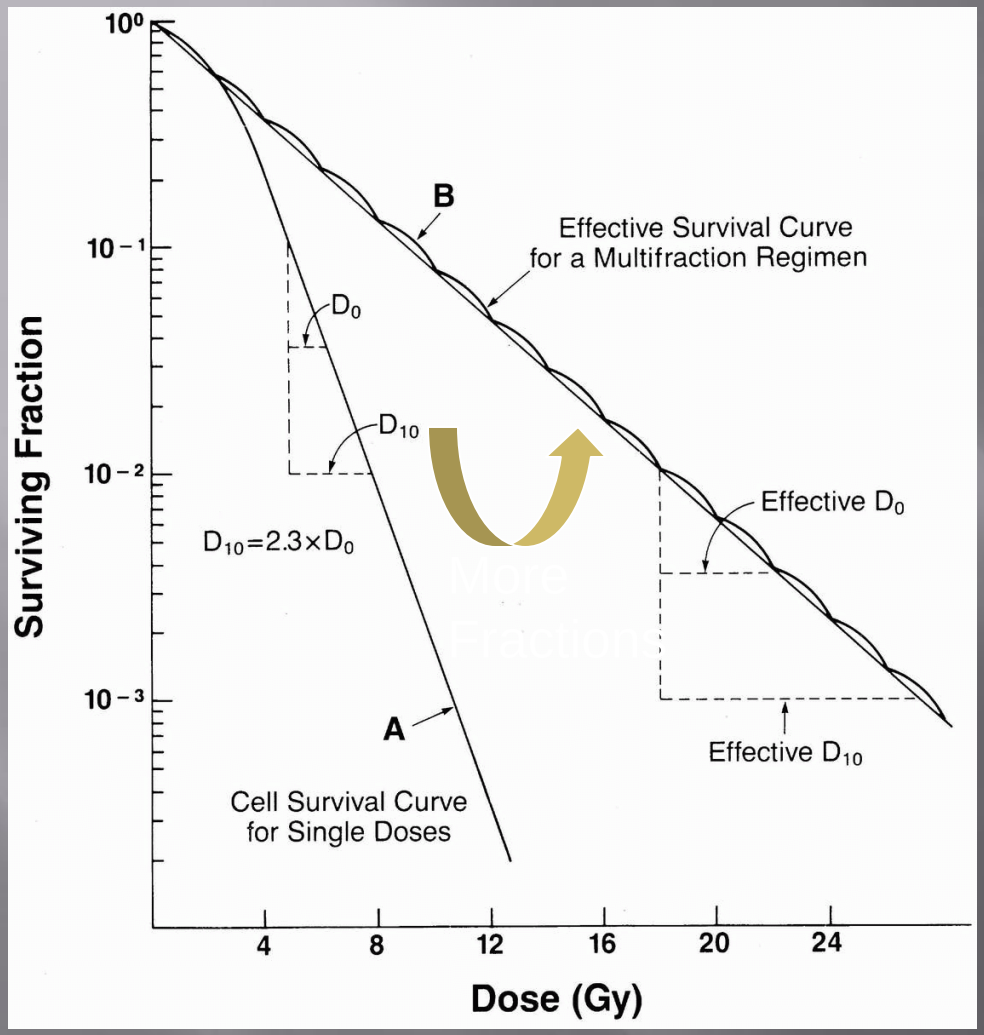


Figure 3.9

α – Initial linear slope at small D
Greater for tumours
Early-reacting tissue

β – Curviness parameter
Greater for Normal Tissue
Late-reacting tissue

α/β = 10 for Tumours (typical)
α bigger, β smaller
= 3 for Normal Tissue
α smaller, β bigger

This differential in , plus the ability to give a lower Dose per fraction to Normal Tissues vs Tumour, yields the therapeutic advantage.

Summary

- Double Strand DNA breaks lead to a break in chromosome “arms” /”legs”
- Pairs of chromosome breaks lead to re-arrangements that can be stable (cell transformation) or unstable (cell death)
- Cell survival curves provide insight into the effects of radiation ± drugs
- The Linear Quadartic (LQ) Model links chromosome damage to cell survival nicely !

Part II - Mathematical models of cell survival

Why do we need mathematical models?

- **Understand** and **explain** the shape of survival curves
- **Predict** survival for exposure situations for which no data are available

Two types of mathematical models have been developed:

Target theory models: Physics

- Single-Hit Multi-target model
- Linear quadratic model (Dual Action)

Biophysical models: Biology

- include bioprocesses (4Rs)
- e.g. DNA repair

Target Theory

- Assumptions:
  - ≥1 sensitive volume in a cell
  - Identical sensitivity
  - A "Hit" causes cell inactivation
    - A Hit is a physical event **in a sensitive volume (e.g. DNA space in nucleus)**
- Method:

  Cell Death is due to the **discrete** and **random** nature of radiation

  ↓

  Track Statistics

  ↙ ↘

  Binomial Distribution Of 'Hits' Following N 'trials'

  Poisson Distribution If Probability of "hits" is small, And the # of trials is Large.

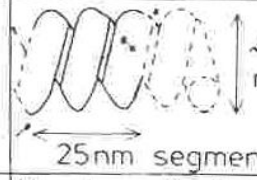
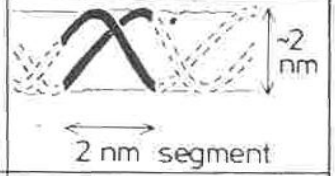
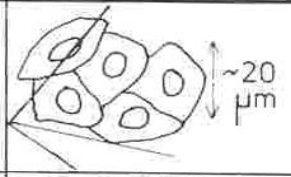
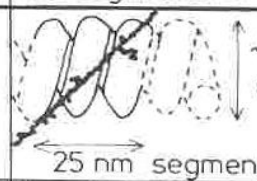
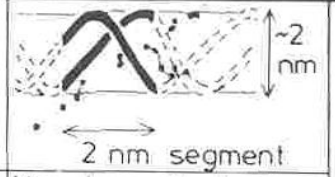
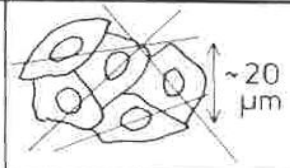
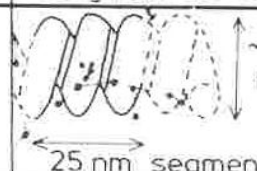
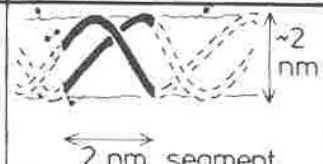
  Packets of Energy Along Track (~10's eV)

  ↙ ↘

  Direct Action

  Indirect Action

Tracks and Lethal Lesions for different LET Radiation and Volumes

	Whole tissue	Individual cells	Chromatin fibre (total ~5 cm per cell)	DNA (total ~2 m per cell)	Mean number lethal lesions per cell
External γ rays					~0·001
Dose uniformity	Uniform Dose = 1 cGy	~ Uniform Dose ≈ 1 cGy	Very large fluctuations Doses = 0 to ~10^3 Gy	Very large fluctuations Doses = 0 to ~10^6 Gy	
Mean number of tracks	10^9 gram^{-1}	~ 50 cell^{-1} No cells unirradiated	~10^{-6} segment^{-1} ~20 segments hit cell^{-1}	~10^{-8} segment^{-1} ~10 segments hit cell^{-1}	
Internal ^{220}Rn (3 α's)					~0·01
Dose uniformity	Variable Doses = 0 to ~2 cGy	Large fluctuations Doses = 0 to ~30 cGy	Very large fluctuations Doses = 0 to ~10^4 Gy	Very large fluctuations Doses = 0 to ~2×10^6 Gy	
Mean number of tracks	~10^7 gram^{-1}	~ 0·1 cell^{-1} ~90% of cells unirrad.	~6×10^{-7} segment^{-1} ~1 segment hit cell^{-1}	~10^{-8} segment^{-1} ~10 segments hit cell^{-1}	
External 10 MeV neutrons					~0·005
Dose uniformity	Uniform Dose = 1 cGy	Large fluctuations Doses = 0 to ~5 cGy	Very large fluctuations Doses = 0 to ~5×10^3 Gy	Very large fluctuations Doses = 0 to ~10^6 Gy	
Mean number of tracks	~10^7 gram^{-1}	~ 1 cell^{-1} ~37% of cells unirrad.	~4×10^{-6} segment^{-1} ~8 segments hit cell^{-1}	~10^{-8} segment^{-1} ~10 segments hit cell^{-1}	

LET

cm's

nm's

DNA damage depends on the "intersection" of chromatin structure and the radiation track structure.

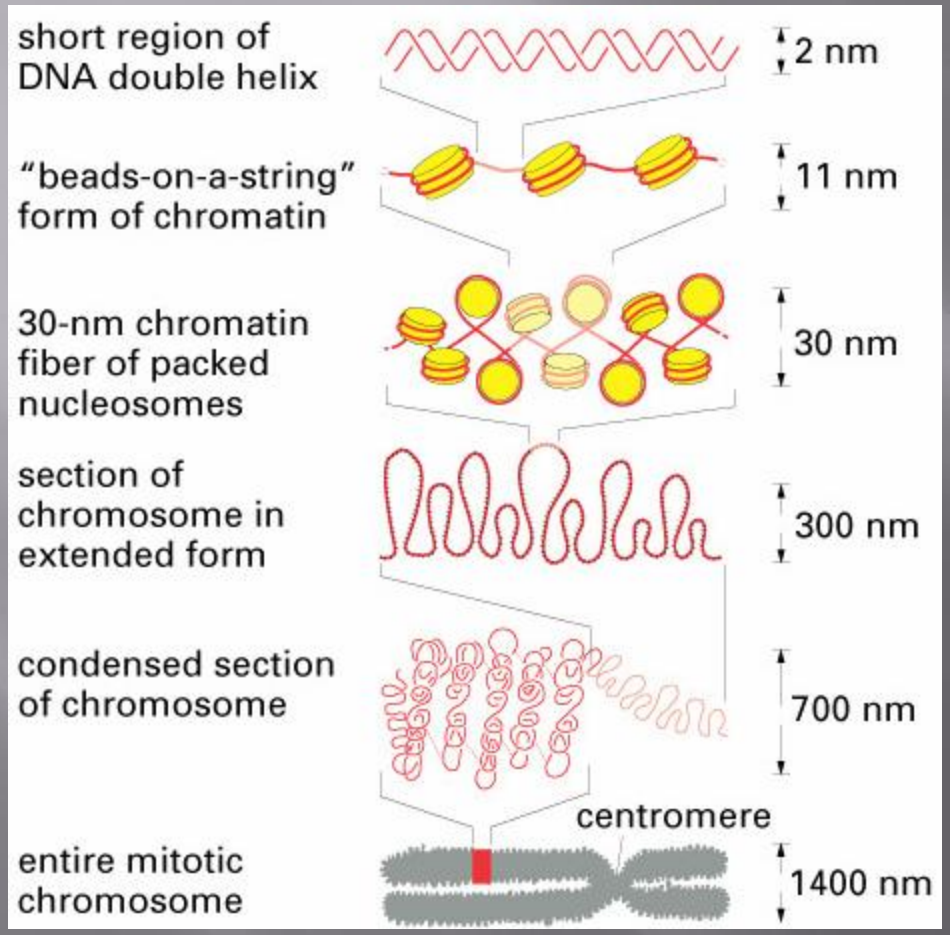
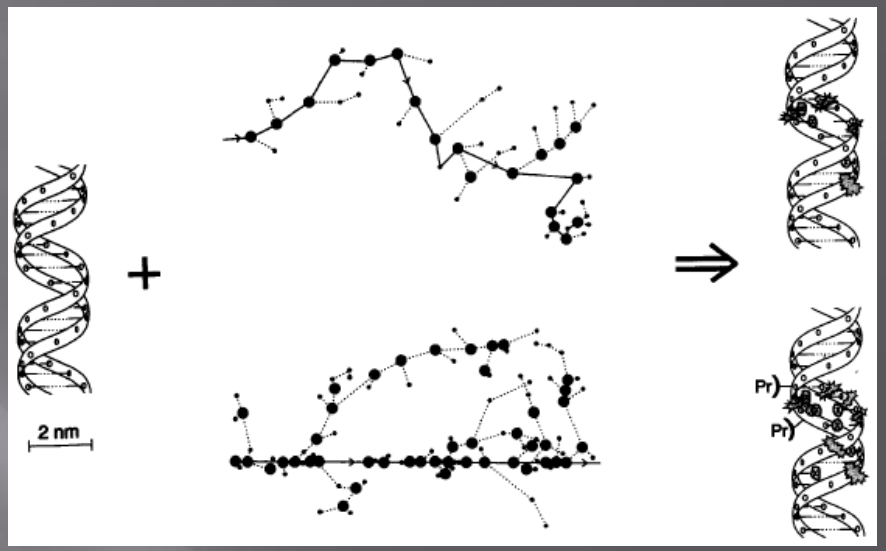


Figure 5-24 Essential Cell Biology, 2/e. (© 2004 Garland Science)



D. T. Goodhead. *Mechanisms for the biological effectiveness of high-LET radiations*. J. Radiat. Res., 40: Suppl., 1-13 (1999).

Target Theory

Assumptions:

1. Sensitive volumes (**targets**) are uniformly distributed within the cell nucleus
2. Targets have identical sensitivity to radiation damage
3. A **hit** in a given number of targets causes cell inactivation

A "Hit" is defined as a physical event (energy transfers) in a sensitive target volume leading for instance to the inactivation of a gene critical to cell survival

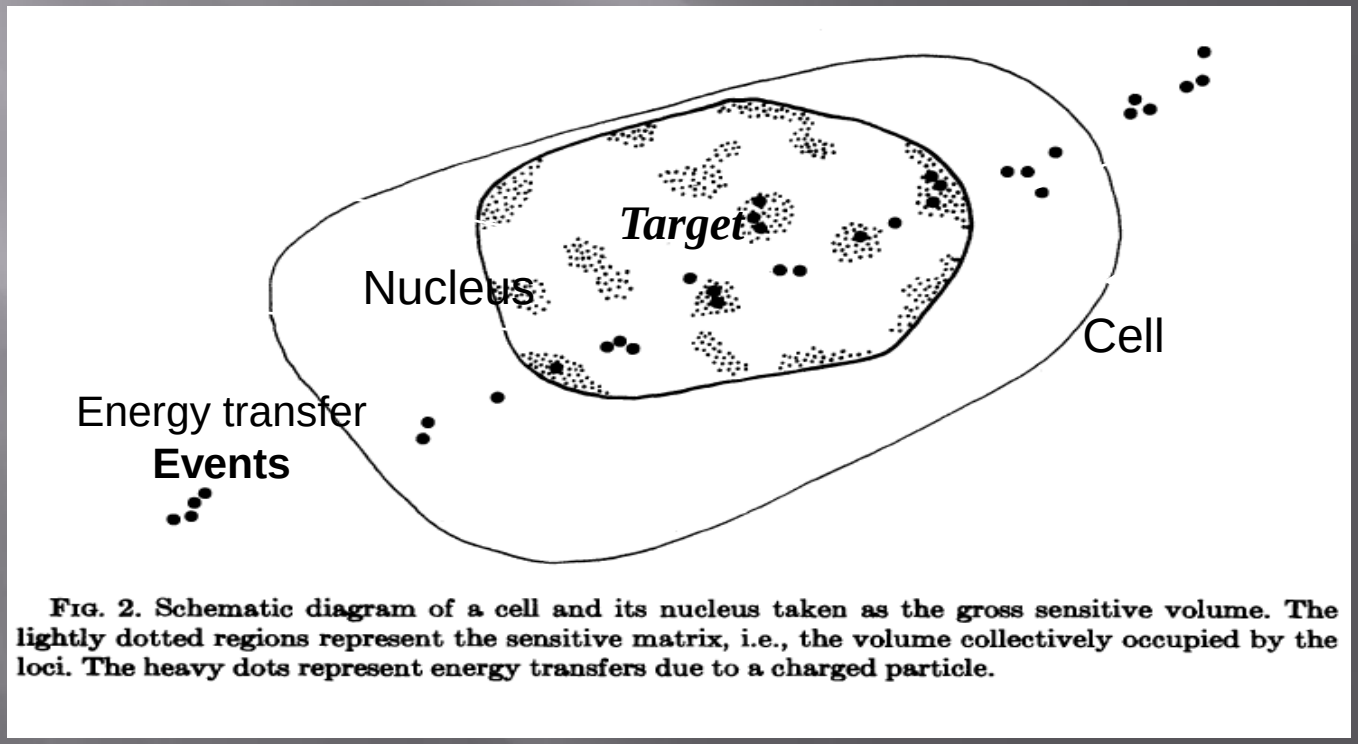


FIG. 2. Schematic diagram of a cell and its nucleus taken as the gross sensitive volume. The lightly dotted regions represent the sensitive matrix, i.e., the volume collectively occupied by the loci. The heavy dots represent energy transfers due to a charged particle.

Kellerer A. and Rossi. A generalized formulation of dual radiation action. Radiat. Res. 75, 471-488 (1978)

General Model

- Consider an exposure of a tissue to a given type of radiation. D [Gy] is the dose.
- The exposure yields *N* "energy deposition events" per unit volume and unit dose

$\rho_e = N \times D$ [ions/cm^3] : density of "events"

The targets are uniformly distributed. The probability to "hit" a target is:

$$p = \frac{\#of\ events\ in\ V_S}{\#of\ events\ in\ V_G} \quad \Rightarrow \quad p = \frac{\rho_e V_S}{\rho_e V_G} = \frac{V_S}{V_G}$$

V_G: is a "gross" volume of interest
- e.g. the volume of the cell

What is the chance that a sensitive volume in the cell is hit "*h*" times?

The number of events in the cell (Trials) is $\boldsymbol{\rho_e V_G}$
If the target is hit "h" times, then it is missed ($\boldsymbol{\rho_e V_G - h}$) times.
Assuming independent events, the chance of getting hit exactly "h" times, **in any order**, is:

$$P_h = p^h (1-p)^{\rho_e V_G - h} \frac{\rho_e V_G !}{h!(\rho_e V_G - h)!}$$

Binomial Distribution

Case of Single Target, Single Hit

Assume that cell death can be caused if a single target is hit just once.

$\Rightarrow$ Cell survival is only possible when the sensitive volume (target) cell is **NOT** hit (set h=0) at all.

Therefore:

$$S = P_0 = p^0 (1-p)^{\rho_e V_G - 0} \frac{\rho_e V_G !}{0!(\rho_e V_G - 0)!} = (1-p)^{\rho_e V_G}$$

$$S = \exp[\ln[(1-p)^{\rho_e V_G}]] = \exp[\rho_e V_G \ln(1-p)]$$

For small values of p (use Taylor Expansion)

$$S = e^{-\rho_e V_G p}$$

$$S = e^{-NV_S D} = e^{-KD} = e^{-D/D_0}$$

$$S = e^{-KD} = e^{-D/D_0}$$

Example

Consider a sample irradiation
1cGy = 10^{-2}J/kg = 10^{-2}mJ/g = 6.2x10^{13}eV/g

An average ionization event ~30eV

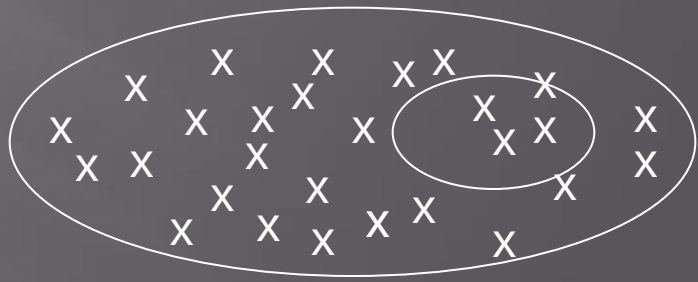
N = 2 x 10^{12} [ionizations /gram per cGy]

ρ = 2 x 10^{12} D [ionizations /gram]

If we assume 10^9 cells/gram and ~6μm cell radius

N' = 2000 ionizations/cell per cGy

Dose=1cGy



2000 Ionizations in cell !

If you measure K or D_0, can estimate the size of the target volume V_S

Survival curves (Experimental data) $\Rightarrow K \Rightarrow$ Then $V_S = K / N'$

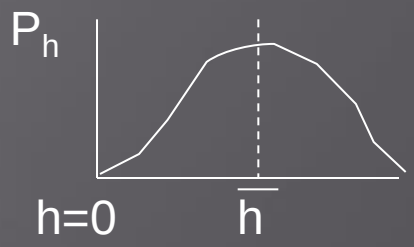
What is the biophysical interpretation of K ?

Expected average number of hits in the cell per unit dose

Alternatively, for rare events and many trials, you can use **Poisson statistics** where the probability of "h" hits is given by:

$\bar{h}$ Expected number of hits= K x D = D/D_0

$$P_h = \frac{\bar{h}^h e^{-\bar{h}}}{h!}$$



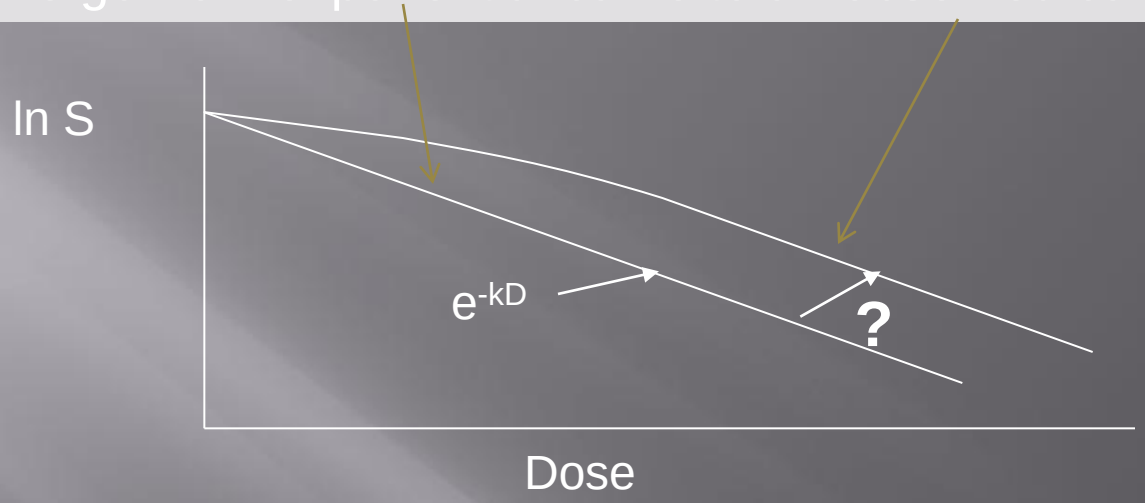
$$S = P_0 = e^{-\bar{h}}$$

Note: If h = 1 (i.e. average of 1 hit/target)

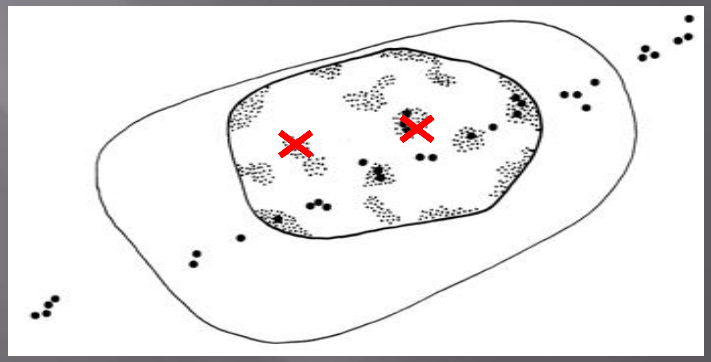
Then: $S = e^{-1} = 0.37$

$= e^{-Do/Do}$ -> 1 hit/target on average when D = Do

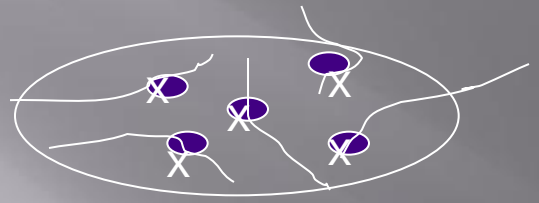
How do we go from exponential curve to an observed curve?



Multi-target single-hit theory:
multiple targets must be hit a least once in order to induce cell inactivation



Single Hit of Multiple Targets



n targets hit ⇨ 1 cell kill

The probability of a target not being hit is equivalent to the single target/single hit survival.

$$S = e^{-kD}$$

Therefore, the probability of being hit is: $1-e^{-kD}$

And the probability of all "n" targets being hit is: $(1-e^{-kD})^n$

Therefore, survival is the probability of NOT all targets being hit:

$$S = 1-(1-e^{-kD})^n$$

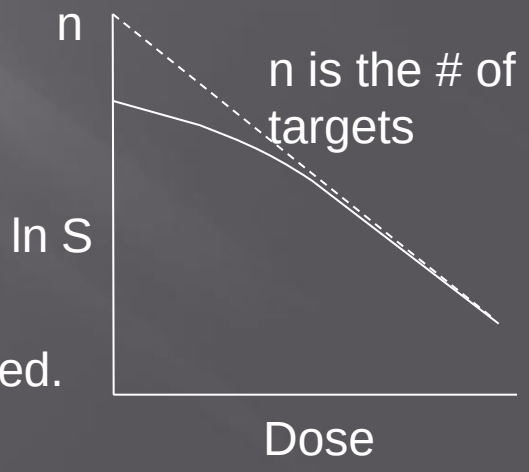
For large D ↓

$$S = ne^{-kD}$$

Therefore:

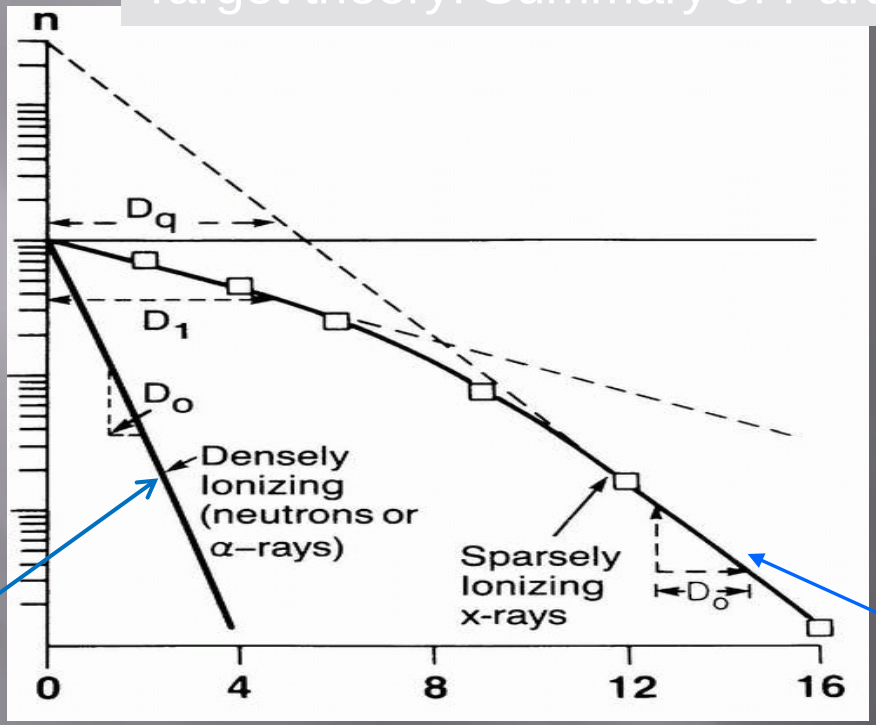
$$\ln S = \ln n - kD$$

Intercept Slope



Disadvantage: predicts zero initial slope, which is not observed.

Target theory: Summary of Parameters



Single hit, single target

$$S = e^{-D/D_0}$$

Single hit, multiple targets

$$S = 1-(1-e^{-D/D_0})^n$$

$S = ne^{-D/D_0}$ for Large D

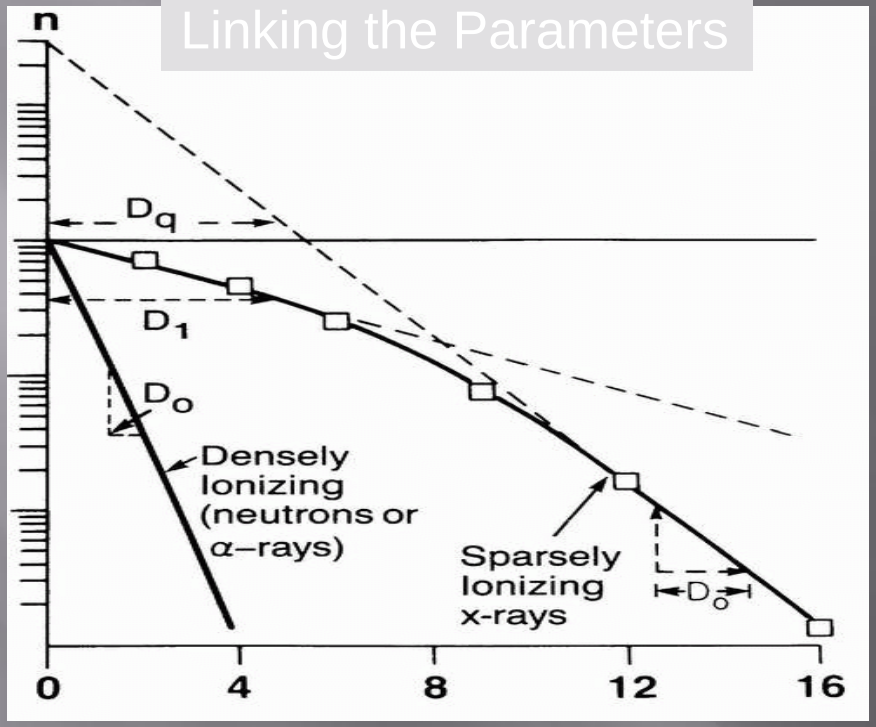
The multi-target single-hit model describes the slop of the survival curve by:

D_0 characteristic dose (mean lethal dose): reduces the population of cells from N_{cells} to $0.37N_{cells}$ (1/e) on parts of the survival curve that are exponential.

$D_0 \equiv$ dose required to deliver one inactivating event (on average) per cell.

Extrapolation number n: extrapolated point of intersection of the final slope onto the log survival axis

Linking the Parameters



The shoulder portion can be described either by the extrapolation number (n targets) or the quasi-threshold, D_q.

$$D_q = D_0 \ln(n)$$

D_q: quasi-threshold dose is obtained as the offset at intersection of S_0 line with the final slope line (Do) .

For D >> Dq,

$$S \approx e^{-(D-Dq)/D_0}$$

Adding Biology: a Repair/Recovery Term

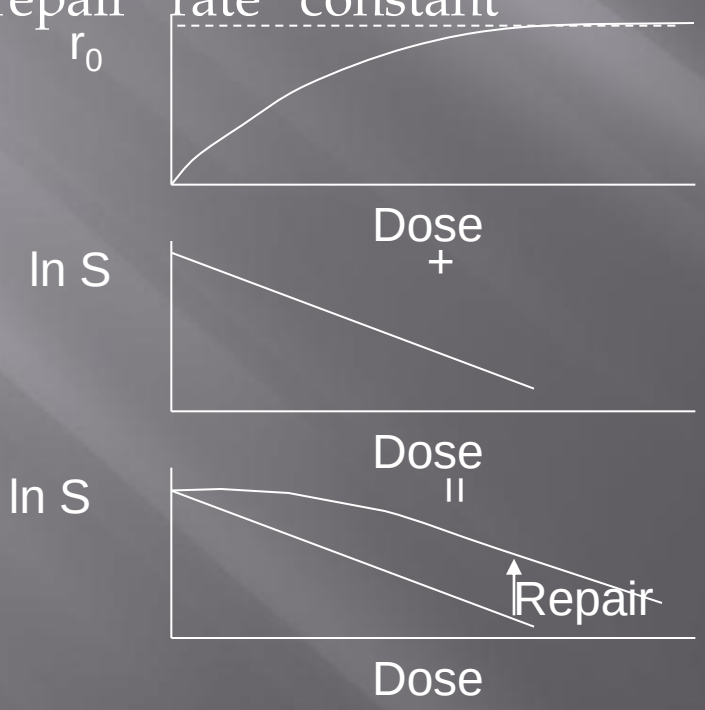
DNA repair term added to single-hit, single-target model

$$S = e^{-D/Do}\, e^{+ro[1-e(-D/r)]}$$

kill repair

r_0 = maximal survival gain due to repair (saturation)

r = repair "rate" constant



More about the dual action: spatial distributions

A Generalized Formulation of Dual Radiation Action[1]

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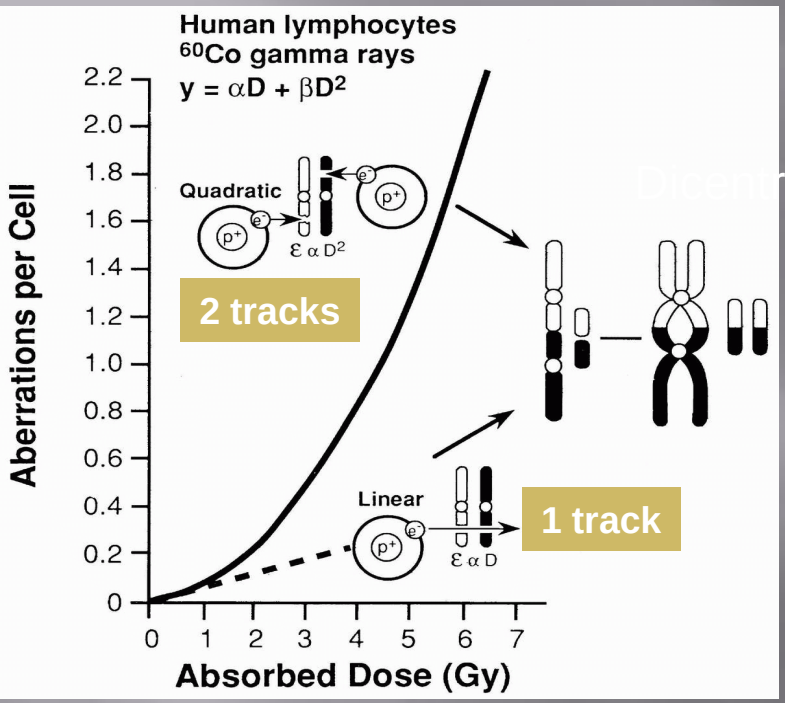
KELLERER, A. M., AND ROSSI, H. H. A Generalized Formulation of Dual Radiation Action. *Radiat. Res.* **75**, 471–488 (1978).

Dual radiation action is a process in which cellular lesions are produced as a result of the interaction of pairs of sublesions that are molecular alterations produced by ionizing radiation. Previous formulations of this process have employed a number of simplifying assumptions that limit the accuracy and the range of application of theoretical analysis. The formulation presented here removes some of these restrictions by introducing three functions that describe the geometry of the sensitive material in the cell, the geometry of the pattern of energy deposition, and the interaction probability of sublesions as a function of their separation. The relation derived is similar to that obtained previously, in that lesion production is found to depend on two terms that are proportional to the first and the second power of the absorbed dose. However, the coefficients of these terms are now derived on the basis of a more realistic treatment.

Back to Linear Quadratic (LQ) Model



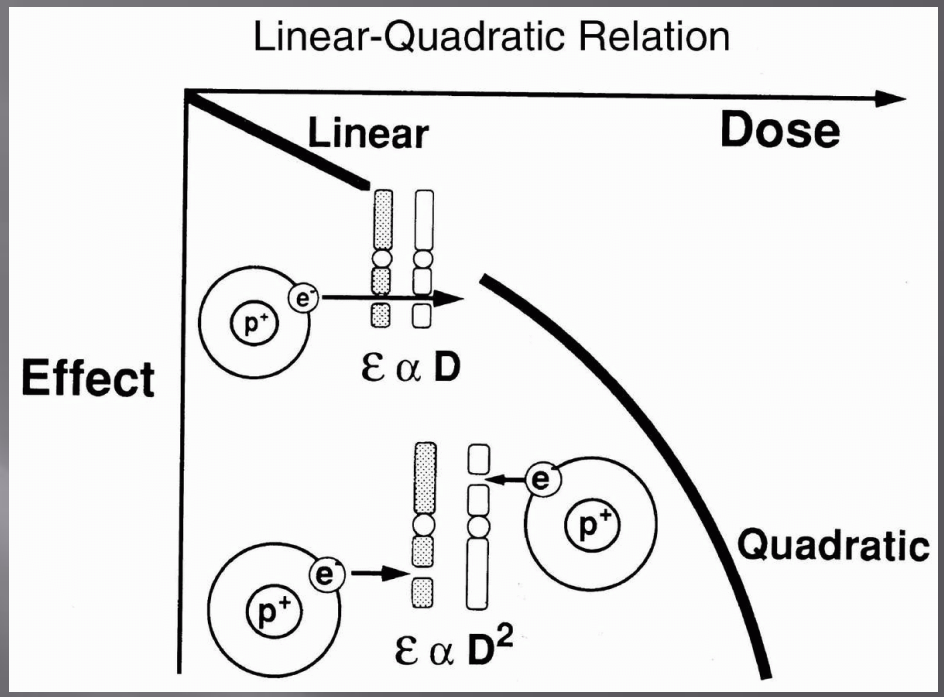


FIGURE 2.11 • The frequency of chromosomal aberrations (dicentrics and rings) is a linear-quadratic function of dose because the aberrations are the consequence of the interaction of two separate chromosome breaks **(a pair of DSBs**). At low doses, both breaks may be caused by the same electron; the probability of an exchange aberration is proportional to dose (D). At higher doses, the two chromosome breaks are more likely to be caused by separate electron tracks. The probability of an exchange aberration is proportional to the square of the dose (D^2).

$$S = e^{-\#of\_lethal\_aberrations}$$

FIGURE 3.5 • Relationship between chromosome aberrations and cell survival. Cells that suffer exchange-type chromosome aberrations (such as dicentrics) are unable to survive and continue to divide indefinitely. The survival curve bends if the quadratic component dominates as the dose increases

Original Dual Action Idea

(Lea, *Action of Radiation on living cells*)

Single track event

Two uncorrelated track events
Close in time-space

$$S(D) = e^{-\alpha D - \beta D^2}$$

Basic Concept :

Two energy deposition events are involved.

A sublesion is produced by one energy deposit.

A pair of sublesions could interact to produce a lesion - if within a given permissible distance for interaction

The yield of sublesions is proportional to D

The yield of lesions is proportional to D^2

More Refined Models:

- More specific definitions of "sublesion" and "lesion" (observables)
- The interactions of sublesions related to temporal and spatial energy distributions (distance models). *It's all a complex matter of "space and time"*
- Compound dual radiation action theory

Mathematical Models

Reality is:

All models except the single-hit, single-target fit the experimental data reasonably well within error bars.

The biological data are not sufficiently precise nor are the predictive theoretical curves sufficiently different to allow the selection of a ‘CORRECT’ model. It is curve-fitting !

They do not directly and uniquely identify the underlying physical or biological mechanisms !

Overall Summary

Cell Survival Curves can be measured

For mammalian cells, the curves are not simply exponential

The LQ Model fits most observation very well
- *lethal unstable chromosome yield linked to*
- *cell survival (on semi-log plot)*

Underlying cell killing actions are described by target statistics and dual action theories
- *sublesions, lesions*
- *what are these biological entities ?*

Tumour control can be predicted from cell survival
(if cell parameters are known *in vivo !)*