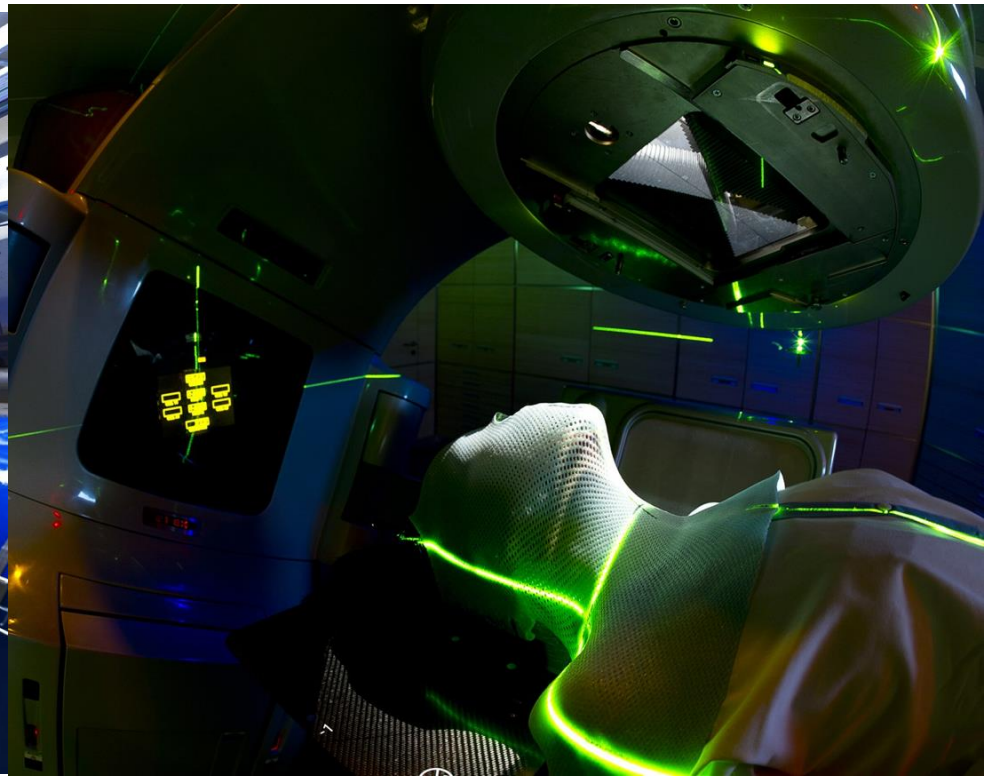
Introduction to Radiobiology



JAI Graduate Accelerator Physics Course, 19 February 2025

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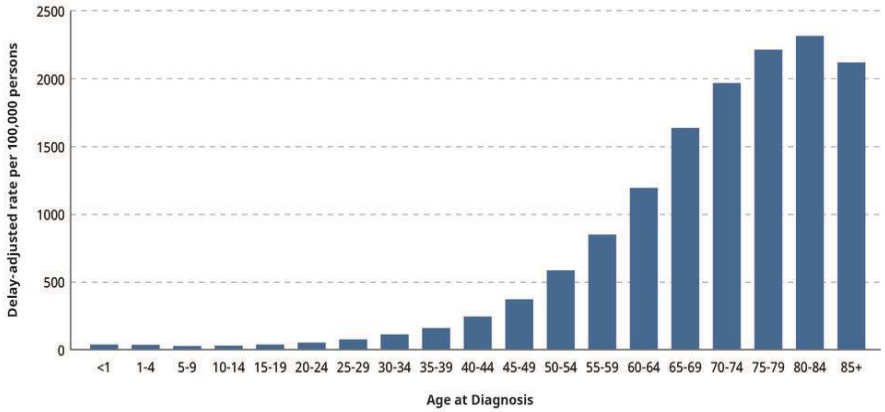
Radiation Dose

- Radiation effects depend on DOSE = Energy Deposited by Radiation per Unit Target Mass
- **Dose in biology/clinics is measured in Gray (Gy) (=1 joule / kg)**
- ...but different radiations have different effectiveness (Q)
- **Equivalent dose= QxD is measured in Sievert (Sv)**
- For x-, γ-rays and electrons: 1 Gy = 1 Sv
- But not alway equal, for example: 1 Gy α-particles= 20 Sv (Q=20)

- Average background radiation dose on Earth= 3 mSv / **year**
- CERN **1mSv** for non-professional and 20mSv/**year** professional exposure
- Occupational limit= 50 mSv/year
- Lethal dose= 4.5 Sv
- Radiotherapy= 60-70 Gy (to the tumour)
- Average background radiation dose in space = 1 mSv/day

Cancer is a growing global challenge

- 2020 Globally **19.3** million new cases per year diagnosed and **10** million deaths
- By 2040 this will increase to **27.5** million new cases and **16.3** million deaths
- **70% of these deaths** will occur in low-and-middle-income countries (LMICs)
- The complete spectrum of care is needed: screening, diagnosis, treatment
- Nearly 50-60% of all cancers can benefit from RT for cure or pain relief



- **Age** is the biggest factor
- More than nine out of 10 cancers are diagnosed in people >**45 and older**.
- Older >**70 make up almost quarter** of all new cases
- **1 in 4 cancer deaths** are caused by **smoking**

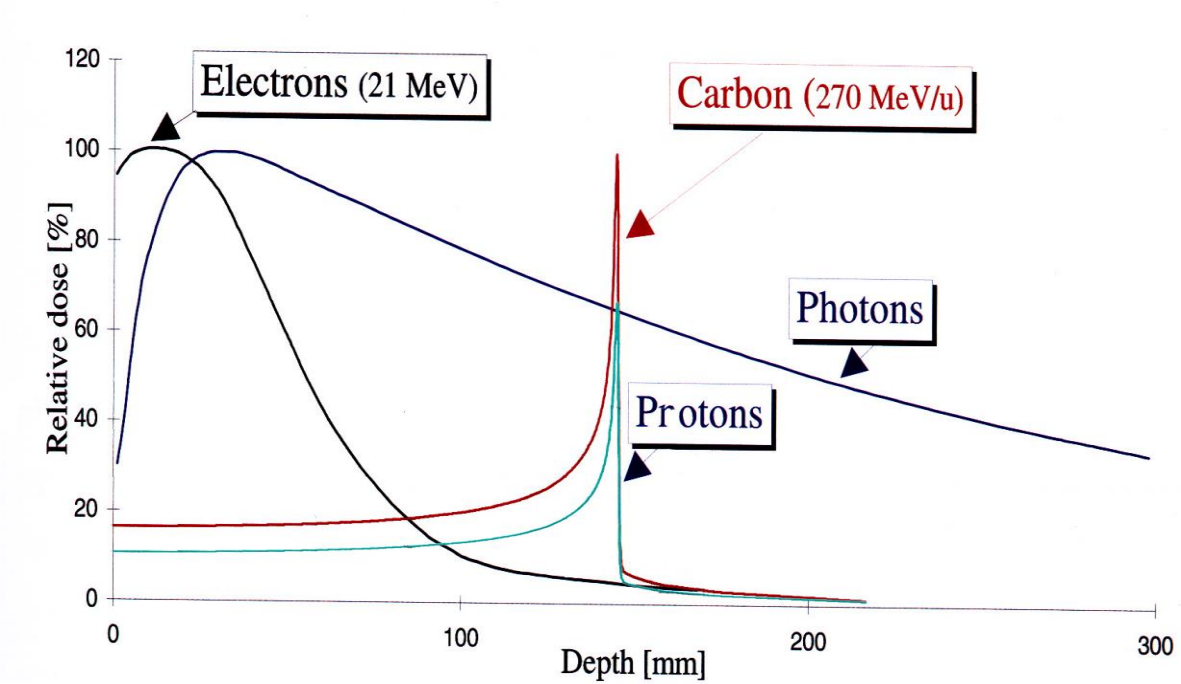
Radiation therapy is a key tool for cancer treatment

Aims of Radiotherapy:

- Irradiate tumour with sufficient dose to **stop cancer growth**
- **Avoid complications** and **minimise** damage to surrounding tissue

Current radiotherapy methods:

- MV photons
- 5 - 25 MeV electrons
- 50 - 300 MeV/u hadrons



Questions

- What is radiobiology?
- Why do we need biology for radiotherapy?
- What kinds of biology are important for radiotherapy?
- How do you investigate biological effects of particle beams?
- What do the data tell you?
- Do we know everything we need to know?

What is radiobiology?

- Radiobiology is a branch of science which tackles the action of radiation on animals, biological tissues and their cellular and the molecular components (Hall & Giaccia, 2006)
- When we deliver a known physical dose with a high degree of accuracy to similar cells/tumours - the effect on cells/tissues varies widely
- Radiobiology plays an important role in safe and effective application of radiation in imaging and radiation therapy for cancer treatment as well other applications such as radiation-protection.
- We need to understand the biological factors that influence the sensitivity of cells to deliver better radiation therapy

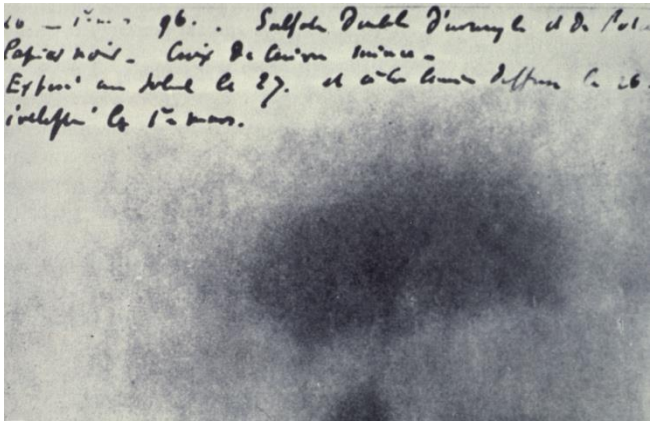
Radiobiology

- The response to radiation is different in normal tissues and cancer tissues:
  - at the molecular level
  - at the cellular level
  - at the tissue level
- These differences are due to the underlying biological properties of different tissues and cancers
  - What is the relevance of these differences?
  - How do they have to be taken into consideration?
  - How can we exploit them?
- Different types of ionizing radiation have different and differing effects on cells, tissues, tumours

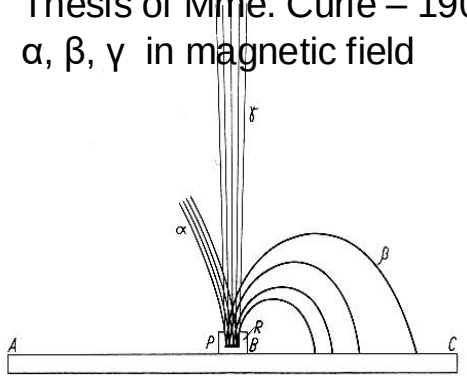
.....of radiation biology

Henri Becquerel (1852-1908)

1896:
Discovery of natural radioactivity

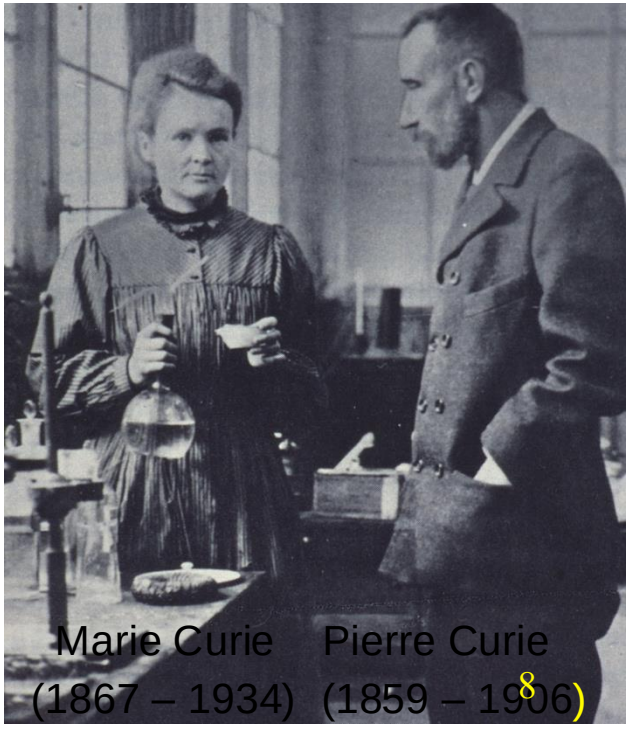


Thesis of Mme. Curie – 1904
α, β, γ in magnetic field



1898: Discovery of radium

used immediately for "Brachytherapy"



Marie Curie (1867 – 1934) Pierre Curie (1859 – 1906)

First Radiobiological Experiment

The first radiobiology experiment. Pierre Curie using a radium tube to produce radiation ulcer on his arm. Hall fig. 1-2

Chronology of Radiation Effects

10^{-15}

10^{-12}

Energy deposition / Excitation and Ionization

10^{-9}

Formation of free radicals / chemical reactions

10^{-6}

10^{-3}

DNA damage

1 ms

10^{0}

10^{3}

1 s

Repair

10^{6}

Cell death / mutations

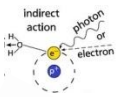
10^{9}

1 year

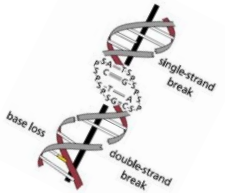
Promotion

Teratogenesis / Cancer inductions

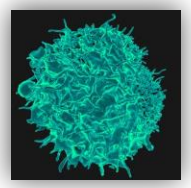
100 years



physical phase



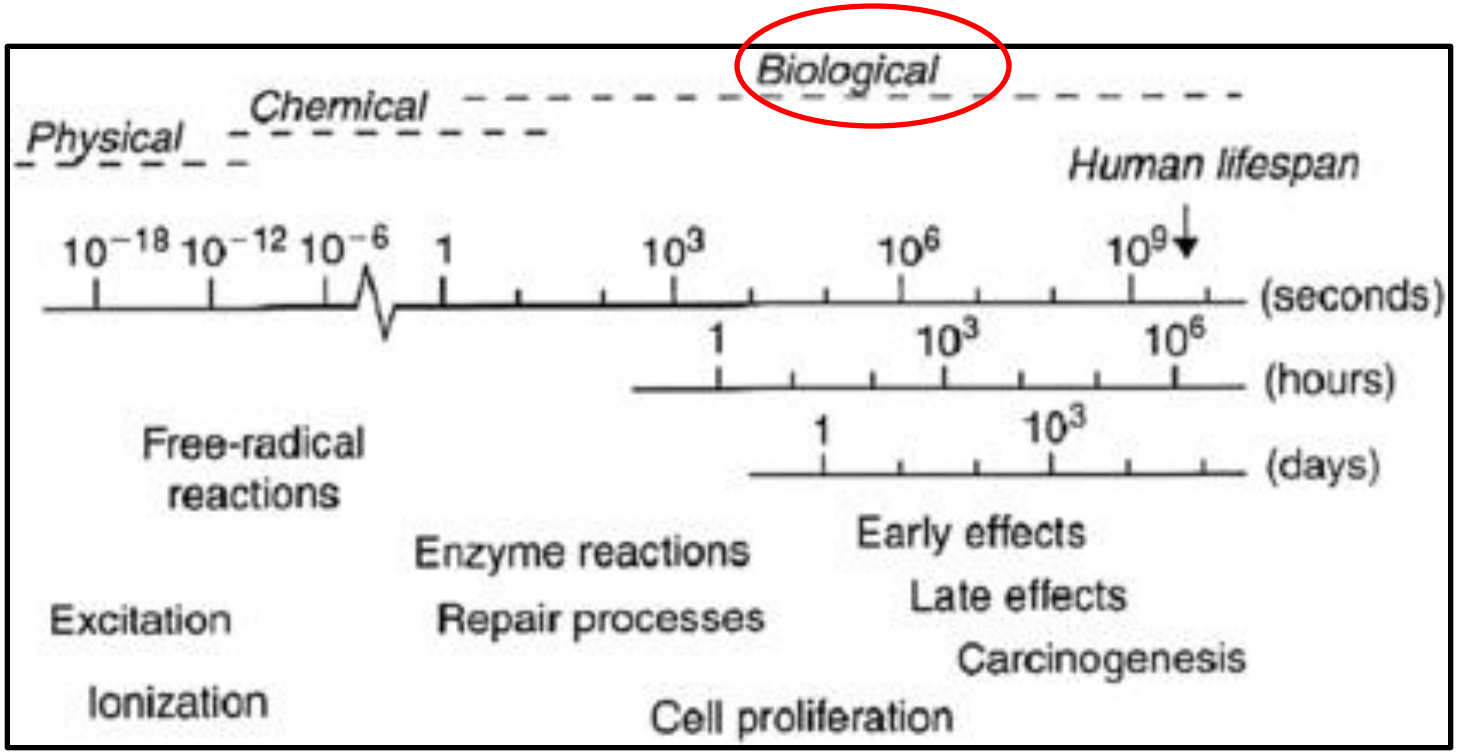
chemical phase



biological phase

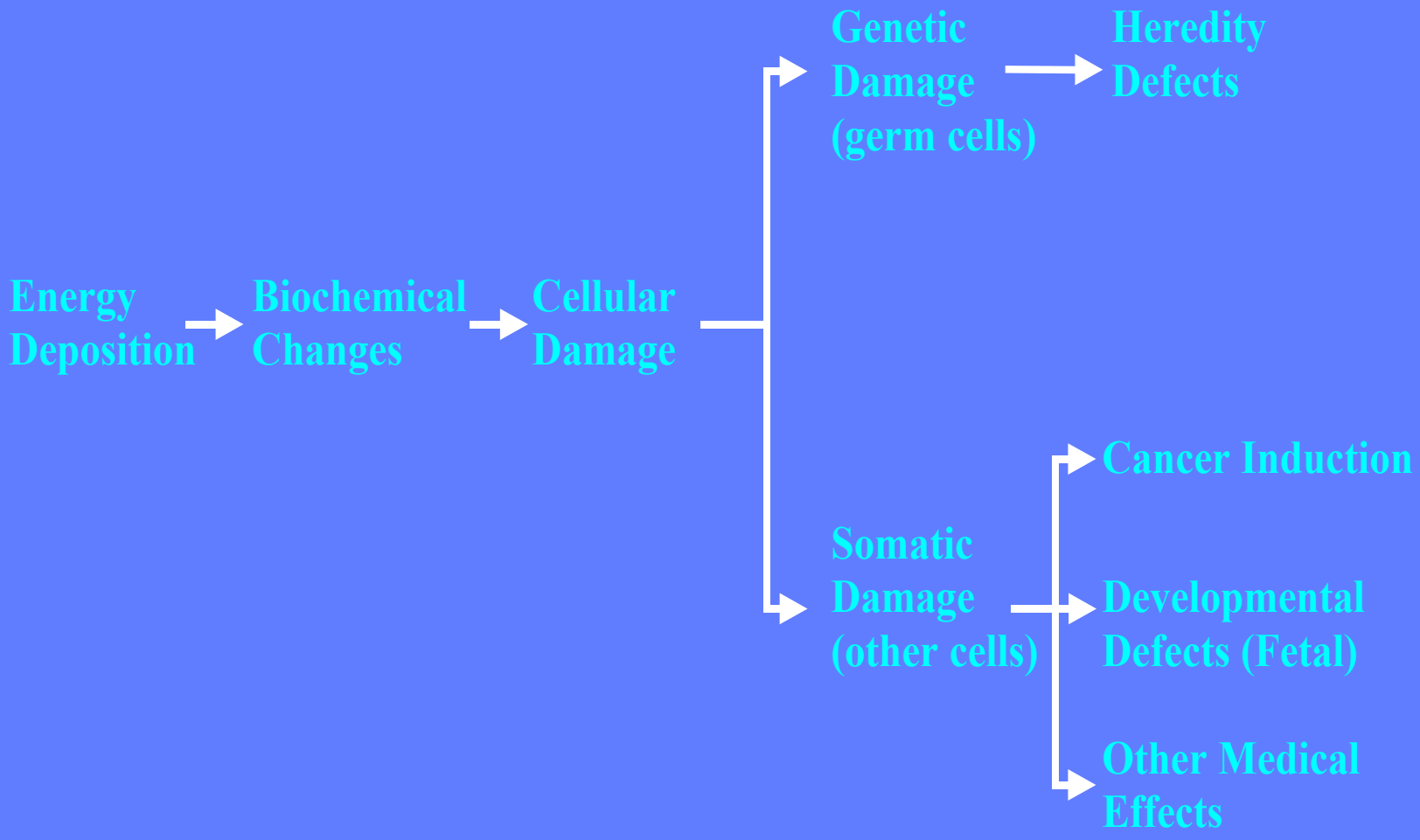
medical effects

Time-scale effects in radiation biology



Joiner and van der Kogel 2009, basic clinical radiobiology, 4th ed

Major Events Which Follow Energy Absorption From Ionizing Radiation



Effects of radiation damage on cells

Radiation causes damage to all cellular molecules, but DNA damage is most critical as most cellular and molecular components can be replaced/renewed

- Cells stops growing - cell cycle arrest:

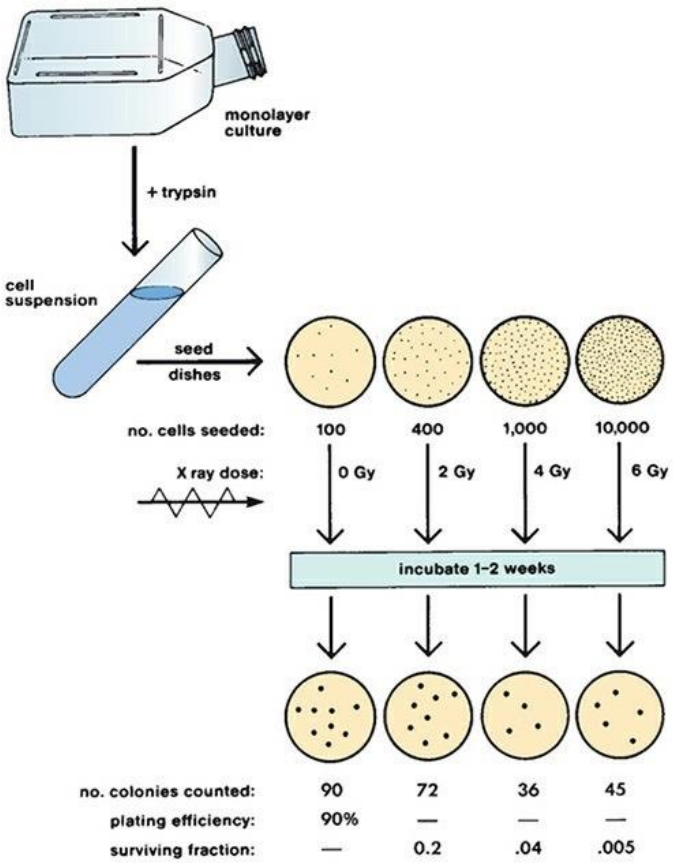
  DNA damage or blockage of replication by triggering "checkpoint" responses, which delay cell cycle progression, promote and give time to repair and protect genome integrity.

- DNA repair:

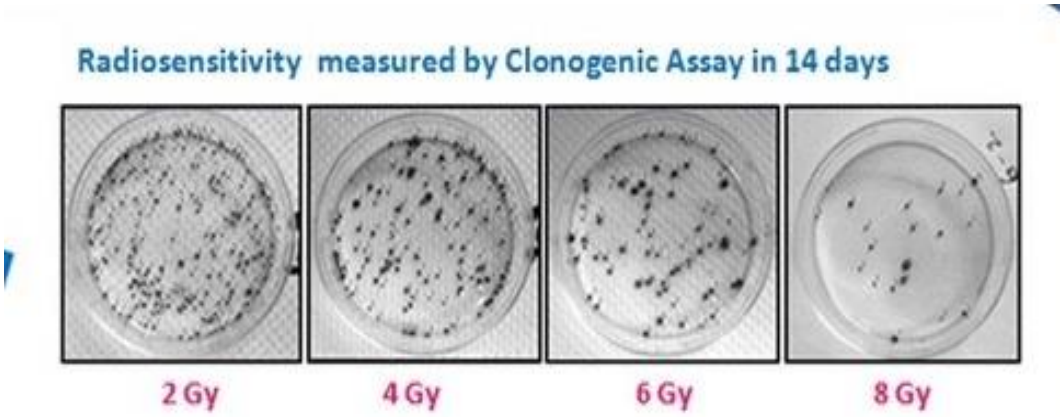
  DNA repair processes either remove the lesion(s)/damage or misrepairs the induced damage such that all surviving progeny of an irradiated cell carry the burden of radiation exposure, e.g. gene mutations

- Cell death

Cell culture techniques and cell survival curves



Puck and Marcus (1956) promoted the study of radiation on individual cells… using cell culture and cell survival ….



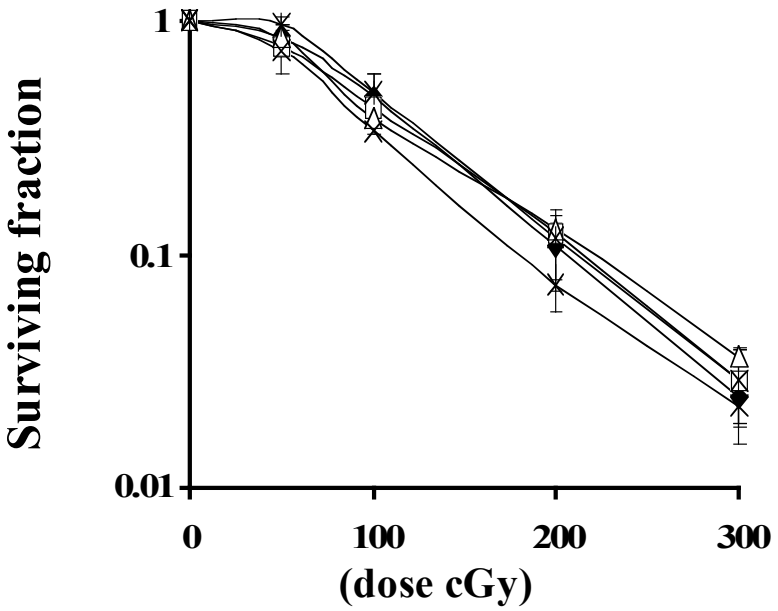
S/S_0 = colonies produced / cells plated * PE

PE = plating efficiency (correction factor derived from control samples)

Cell survival curves

- Describe the relationship between the radiation dose and the proportion of cells that survive reproductive integrity
- Cell survival as a function of radiation dose is graphically represented by plotting the surviving fraction on a logarithmic scale on the Y axis against dose on a linear scale on the X-axis.
- Cell surviving fractions are determined with in vitro or in vivo techniques.

1956: The first in vitro radiation survival curves on mammalian cells carried out by Puck & Marcus



Cell killing by different radiation types

Cell survival after exposure can be expressed in terms of a logarithmic curve of survival versus dose.

For X- or γ-rays (said to be sparsely ionizing), the dose-response curve has an initial slope, followed by a shoulder; at higher dose, the curve tends to become straight again.

For α-particles or low energy neutrons (said to be densely ionizing), the dose-response curve is a straight line from the origin (i.e., survival is an exponential function of dose).

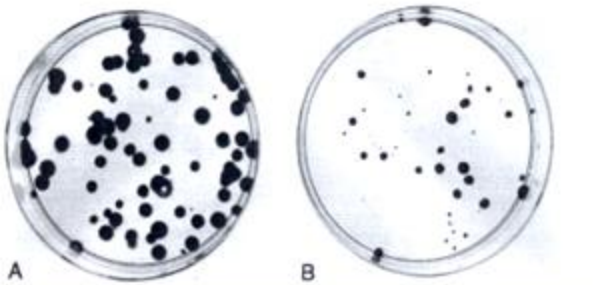
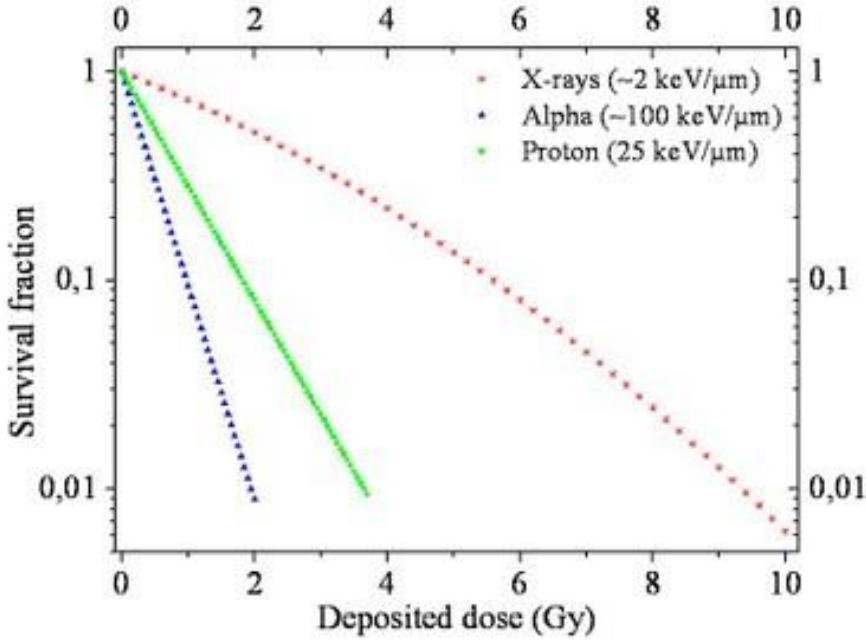
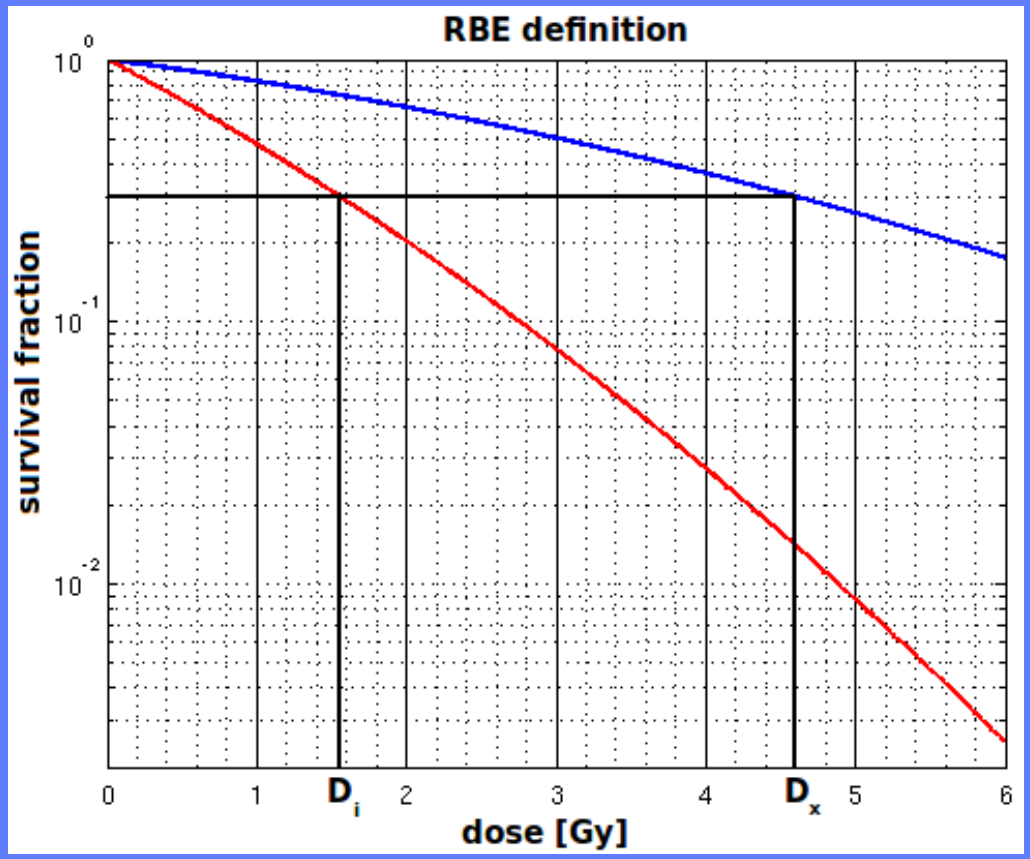


Figure 3.1. Colonies obtained with Chinese hamster cells cultured in vitro. **A**: In this unirradiated control dish 100 cells were seeded and allowed to grow for 7 days before being stained. There are 70 colonies; therefore the plating efficiency is 70/100, or 70%. **B**: Two thousand cells were seeded and then exposed to 800 rad (8 Gy) of x-rays. There are 32 colonies on the dish. Thus:
Surviving fraction = Colonies counted [colonies seeded x (PE/100)]
= 32/2000 x .7
= 0.023



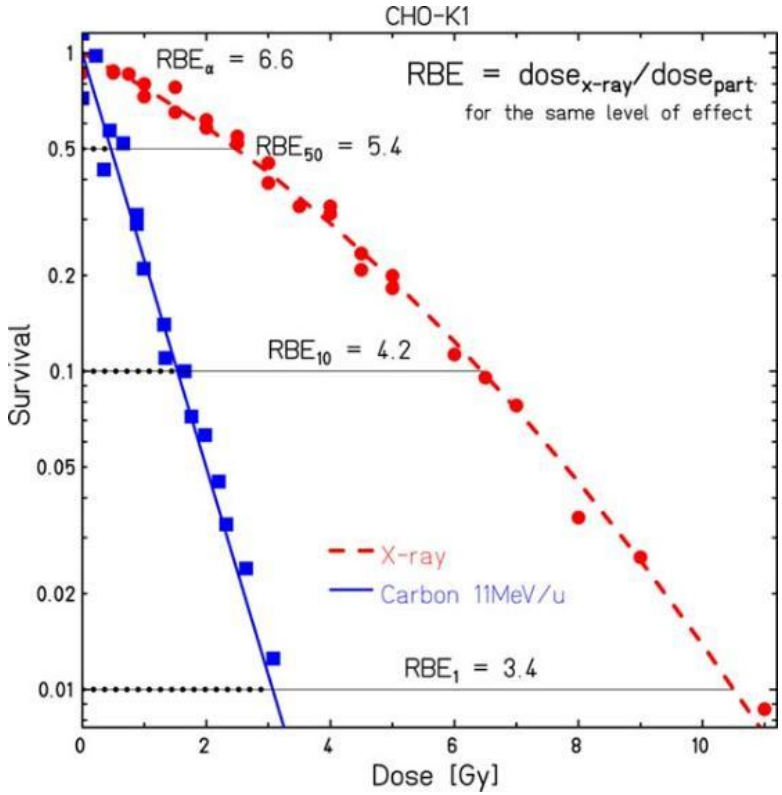
Cellular Survival Curves and Relative Biological Effectiveness

(reference vs test radiation)



$$\frac{D_x}{D_i} = \text{RBE}$$

RBE and how does it vary



RBE critically depends on both physical and biological parameters:

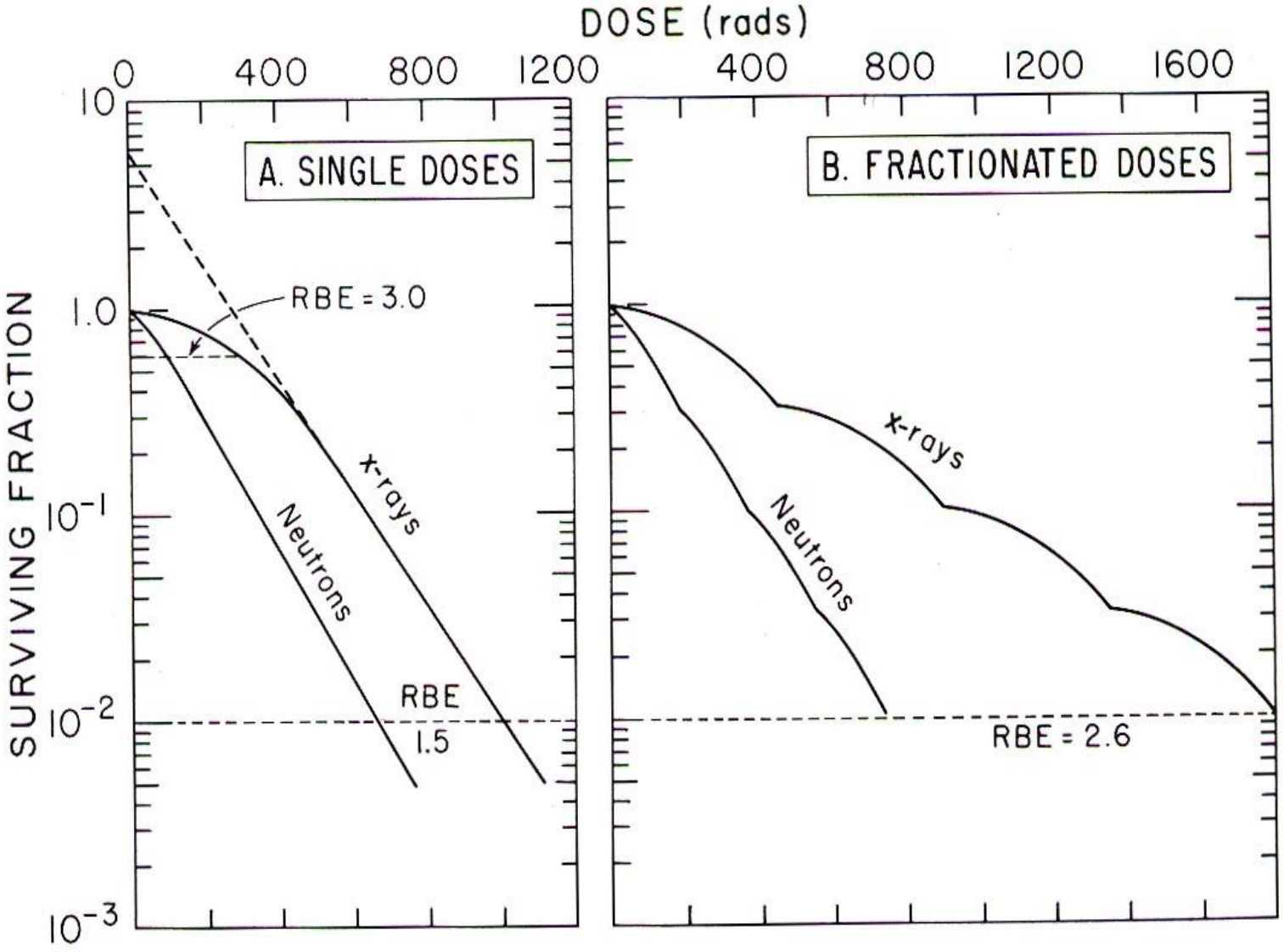
- Dose and Dose Rate
- Varies with types of radiation
- Varies with types of cell/tissue and radiosensitivity
- Varies with the biological effect under investigation
- Varies with dose rate and fractionation
- An increase in RBE in itself does not offer therapeutic advantage unless there is differential effect between normal and tumour tissues
- OER (oxygen enrichment ratio) effects RBE
- Effected by presence of other chemicals present

Kevin Prise, ENLIGHT 2017

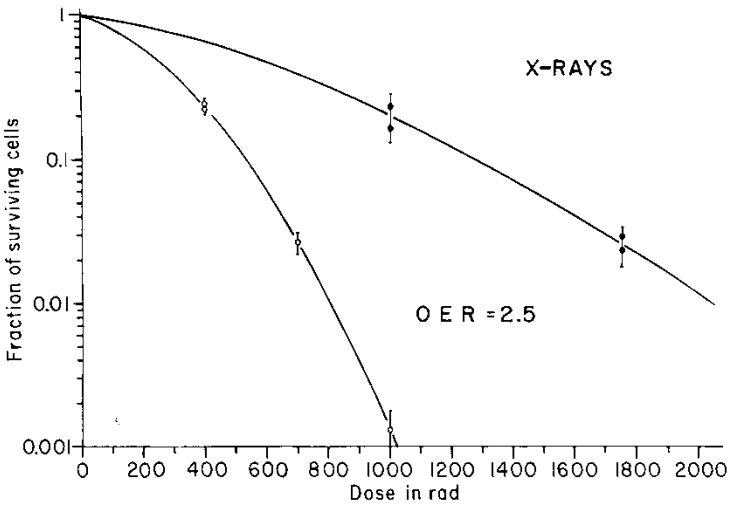
CCRCB

Fractionation

- Increased cell survival when a dose is split into two or more fractions separated by a time interval
- There is a point at which an increase in the number of fractions will no longer increase survival—plateau in the response

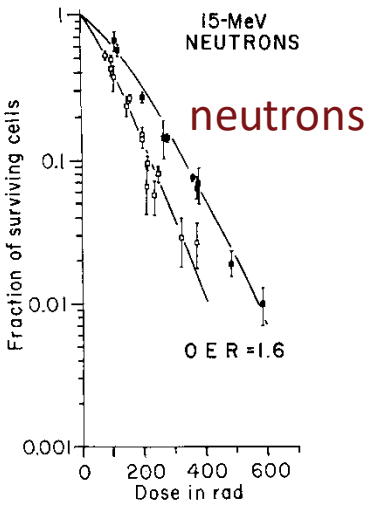

DOSE (rads)
0
400
800
1200
400
800
1200
1600
SURVIVING FRACTION
10^{0}
1.0
10^{-1}
10^{-2}
10^{-3}
A. SINGLE DOSES
B. FRACTIONATED DOSES
RBE = 3.0
x-rays
Neutrons
RBE
1.5
x-rays
Neutrons
RBE = 2.6

Oxygen for high and low LET radiations

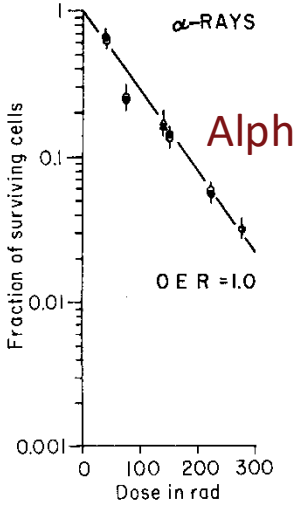


X-rays

OER varies with LET:
X-rays=2.5
Neutrons=1.6
Alpha particles=1.0



neutrons



Alpha particles

Figure 6.2. The oxygen enhancement ratio (OER) for various types of radiation. The OER for α particles is unity. X-rays exhibit a larger OER of 2.5. Neutrons (15-MeV $d^+ \rightarrow T$) are between these extremes, with an OER of 1.6. (Adapted from Barendsen GW, Koot CJ, van Kersen GR, Bewley DK, Field SW, Parnell CJ: Int J Radiat Biol 10:317, 1966; and Broerse JJ, Barendsen GW, van Kersen GR: Int J Radiat Biol 13:559, 1967, with permission.)

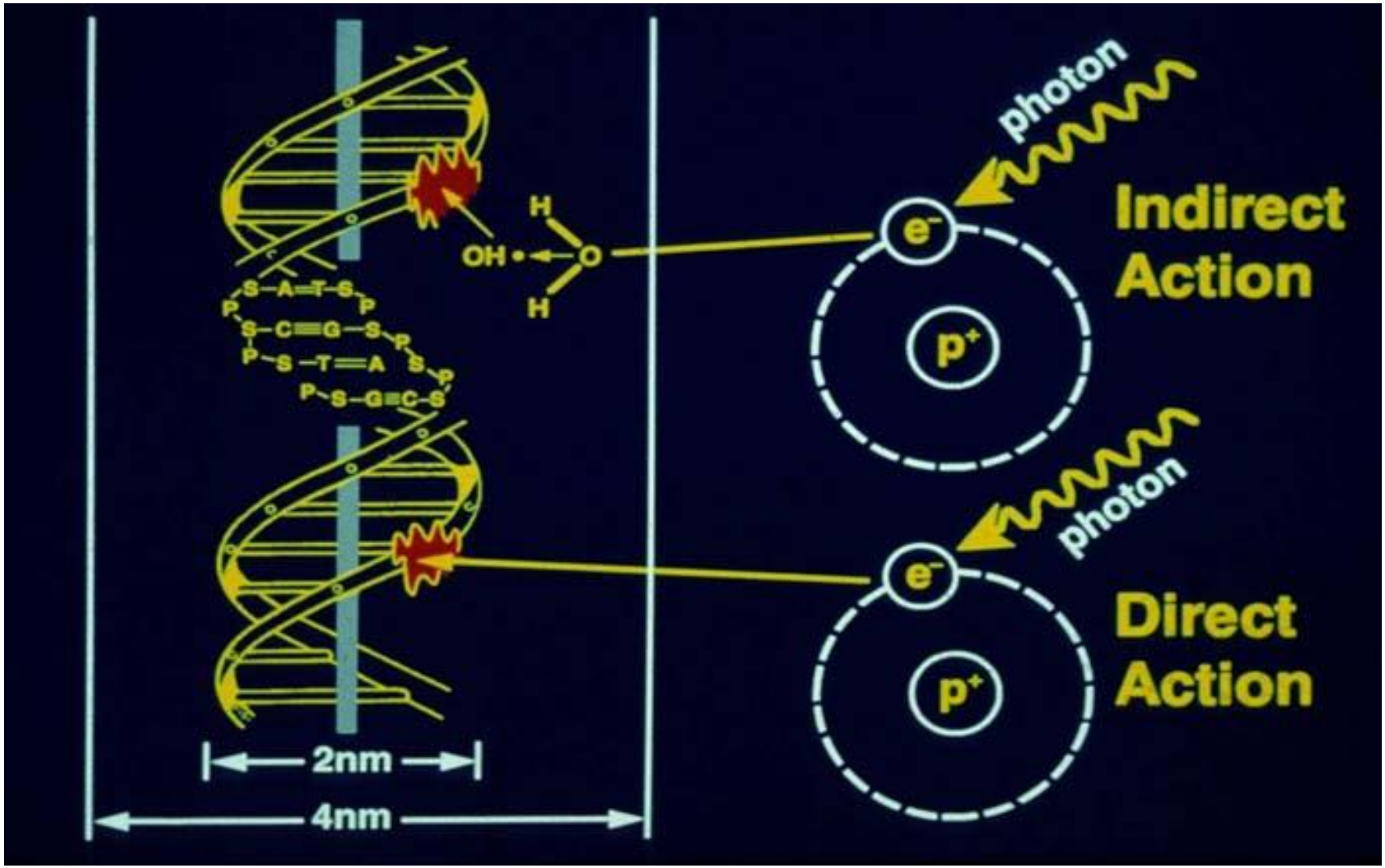
Re-oxygenation in Radiotherapy

- Hypoxia confers resistance to X-rays/gamma rays – also to chemotherapeutic drugs….tumours are normally hypoxic
- Human tumours that do not respond to radiotherapy may not re-oxygenate - radioresistance
- Optimal fractionation regimen depends on reoxygenation

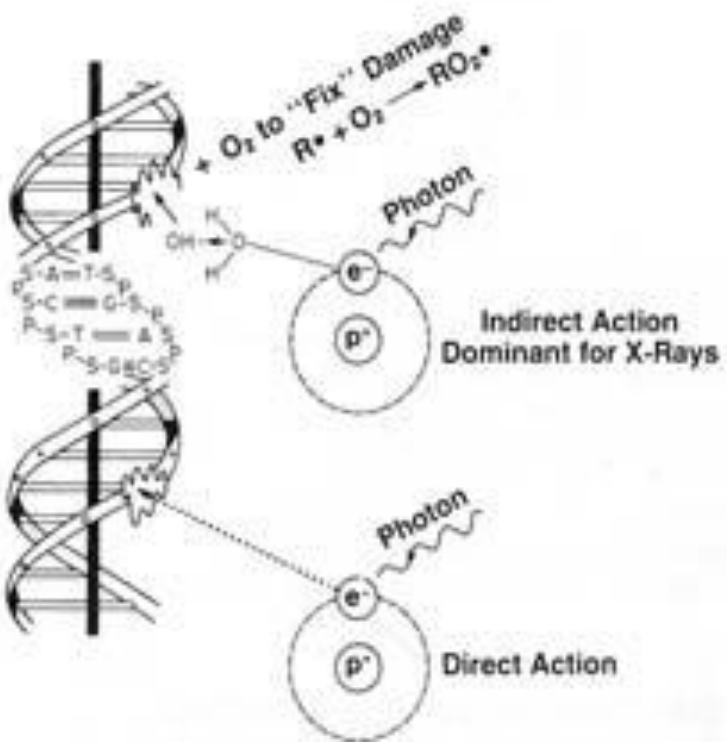
Is the measurement of cellular survival in vitro adequate?

- Which cell types are relevant?
- What about DNA damage and repair?
- What about molecular endpoints?
- What about acute effects in vivo?
- Are there late effects different from acute response?
- What about risk of secondary cancer?

Direct and Indirect Interactions

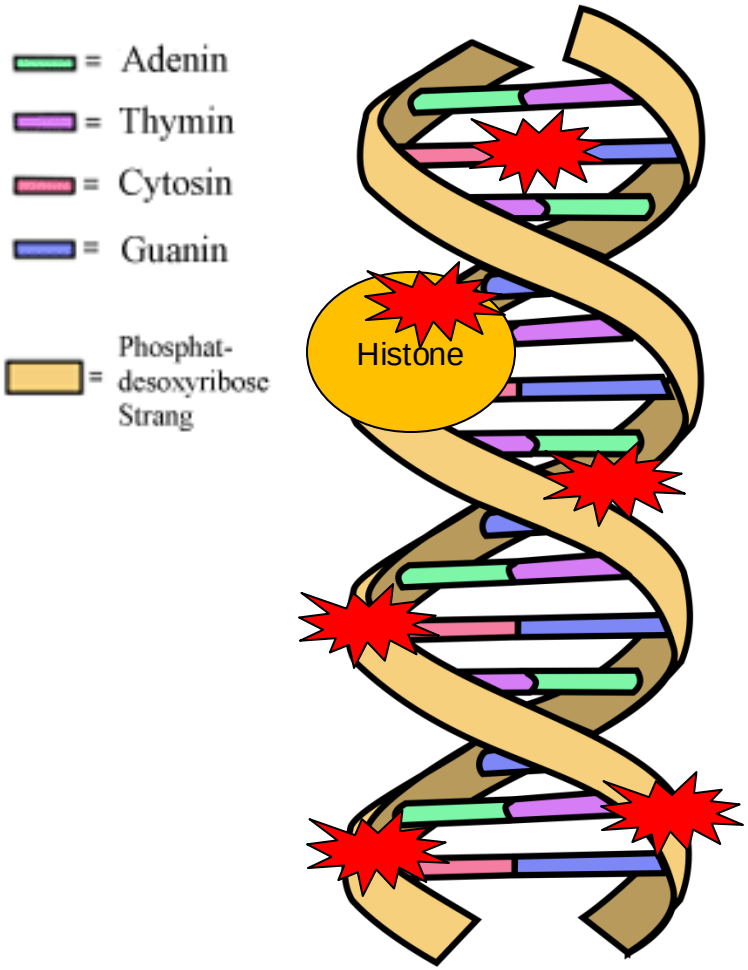


Indirect action in cell damage by radiation



- In indirect action the radiation interacts with other molecules and atoms (mainly water, since about 80% of a cell is composed of water) within the cell to produce free radicals, which can, through diffusion in the cell, damage the critical target within the cell.
- In interactions of radiation with water, short lived yet extremely reactive free radicals such as H2O+ (water ion) and OH• (hydroxyl radical) are produced. The free radicals in turn can cause damage to the target within the cell.
- The free radicals that break the chemical bonds and produce chemical changes that lead to biological damage are highly reactive molecules because they have an unpaired valence electron.
- About 2/3 of the biological damage by low LET radiations is due to indirect action.

DNA damage – 1 Gy



base damage

1000-2000 incidents

DNA-protein crosslinks

100-200 crosslinks

sugar changes

~ 1,000 changes

single strand breaks

~ 1000 SSB

double strand breaks

30- 50 DSB

DNA-DNA crosslinks

100-200 crosslinks

If cells are irradiated with x-rays, many breaks of a single strand occur. In intact DNA however single strand breaks are of little biological consequence because they are repaired readily using the opposite strand as template.

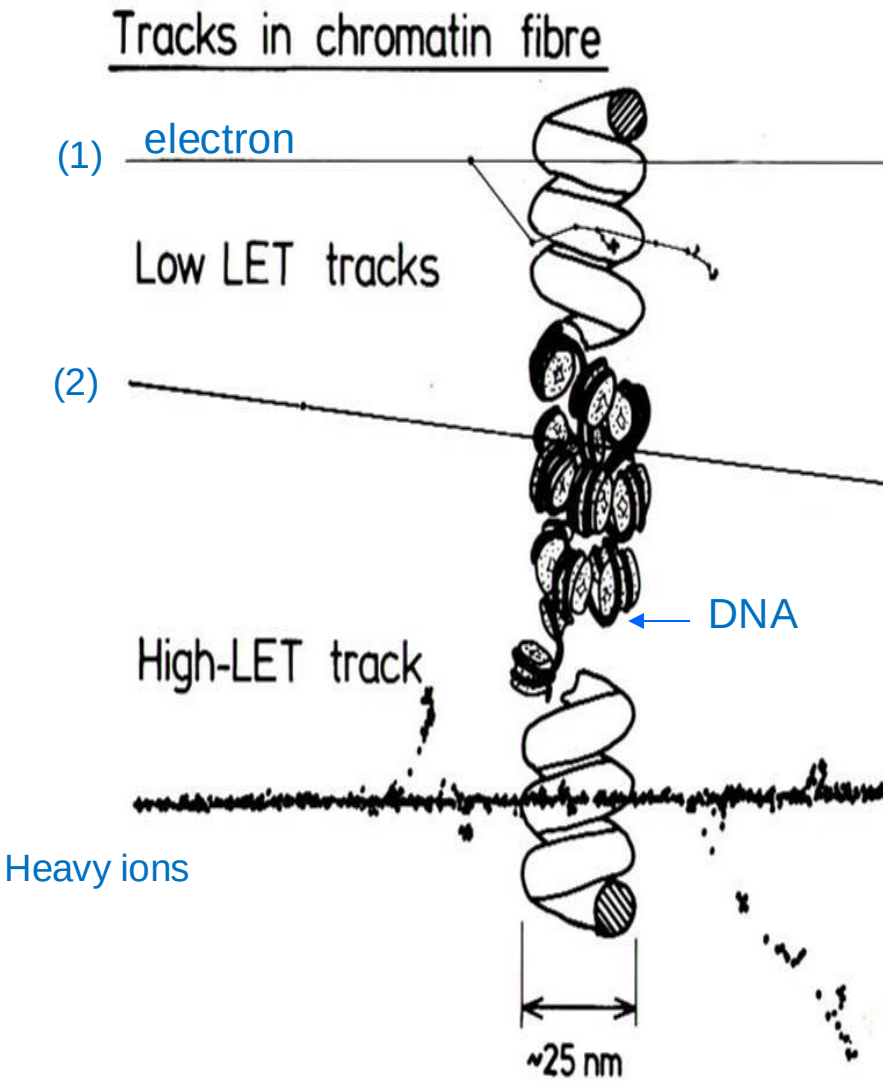
If the repair is incorrect (misrepair), it may result in a mutation.

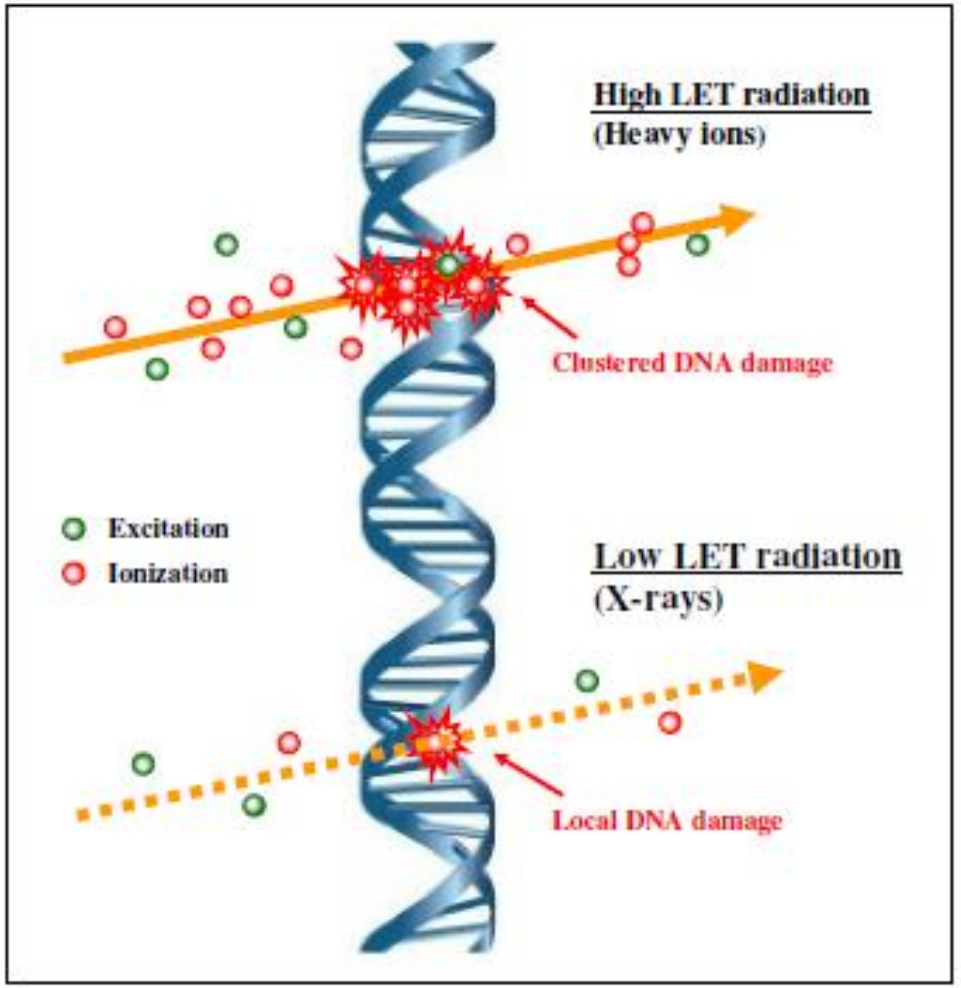
If both strands of the DNA are broken, and the breaks are well separated, repair again occurs readily because the two breaks are handled separately.

By contrast, if the breaks in the two strands are opposite one another, or separated by only a few base pairs, this may lead to a double strand break (DSB).

A DSB is believed to be the most important lesion produced in chromosomes by radiation

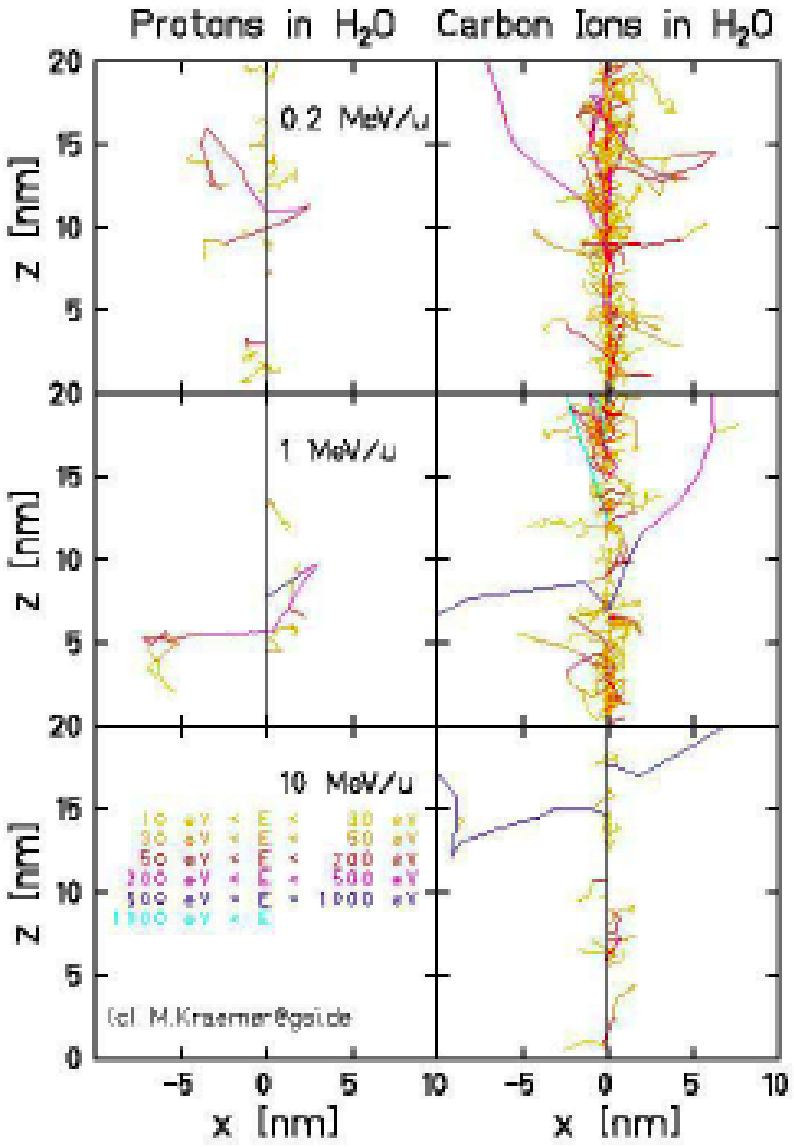
All radiation tracks are highly structured on the scale of DNA





Delta-ray electron

Track Structures of Proton vs. Carbon Ions



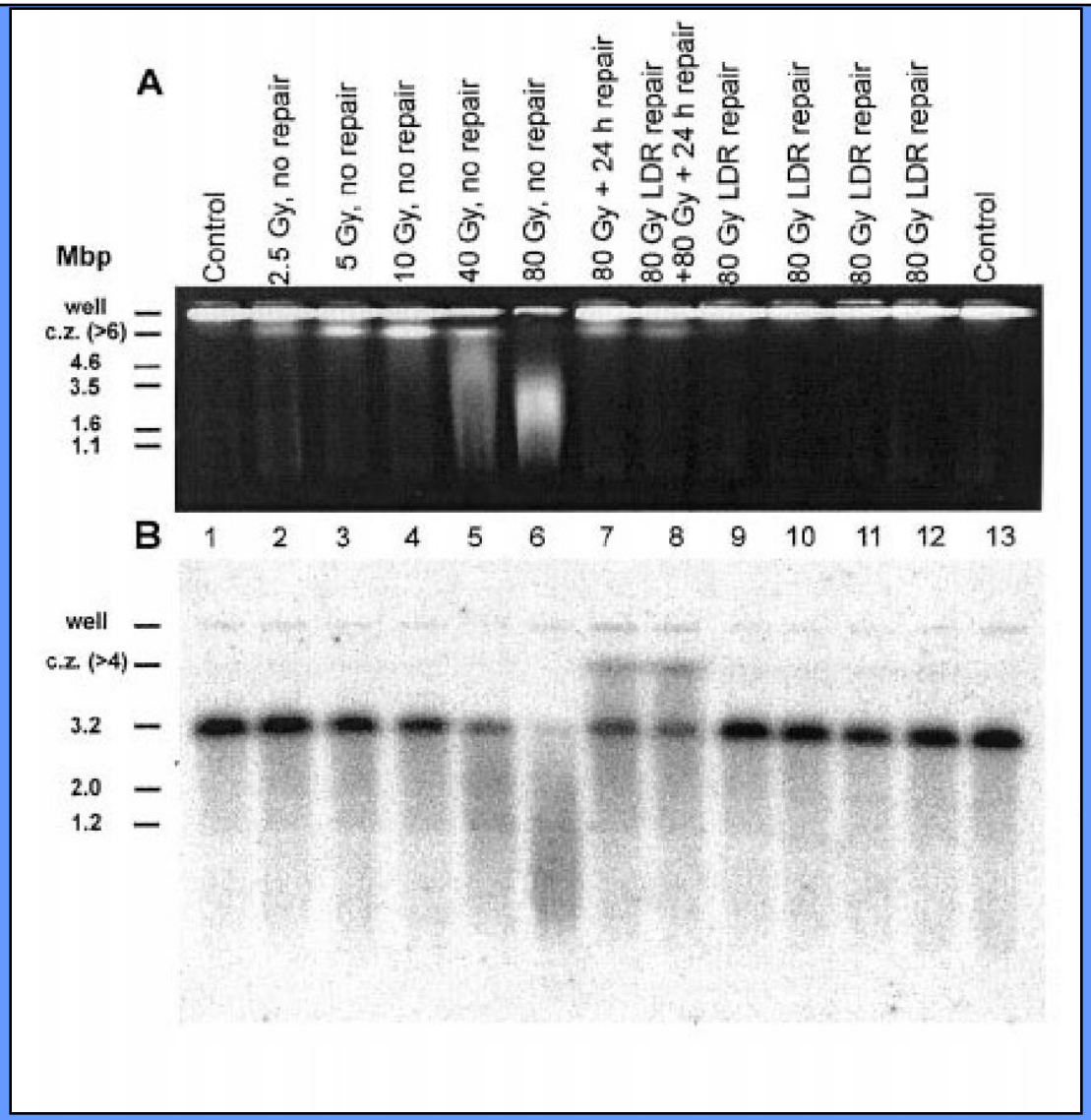
Linear Energy Transfer (LET) stands for the radiation energy deposited per unit length in tissue.

- X-rays and proton beams are low-LET radiations
- Heavy ion beams are high-LET radiation in Bragg peaks

Biological advantages:

- High LET to provide significant differences in DNA damages
- Suppression of radiation repair
- Yet avoids some complications with higher-Z ions

DSB induction and rejoining in normal human fibroblasts following X-irradiation

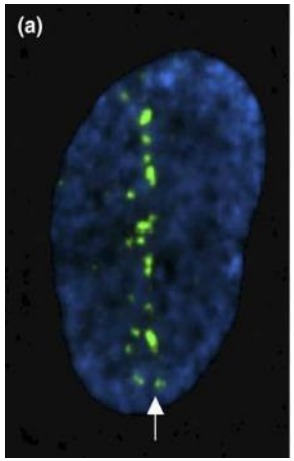


Rothkamm K. et al., Cancer Res., 2001.

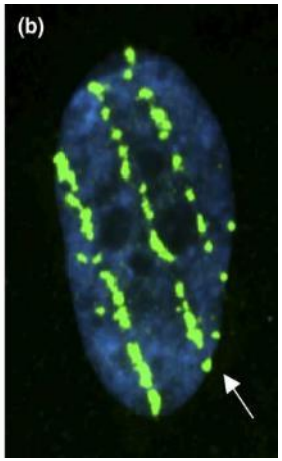
Dose, LET and RBE

- Cellular response is determined by the level and quality of DNA damage, which reflects the energy deposition pattern.

X-rays



54 keV/µm Si ions



174 keV/µm Fe ions

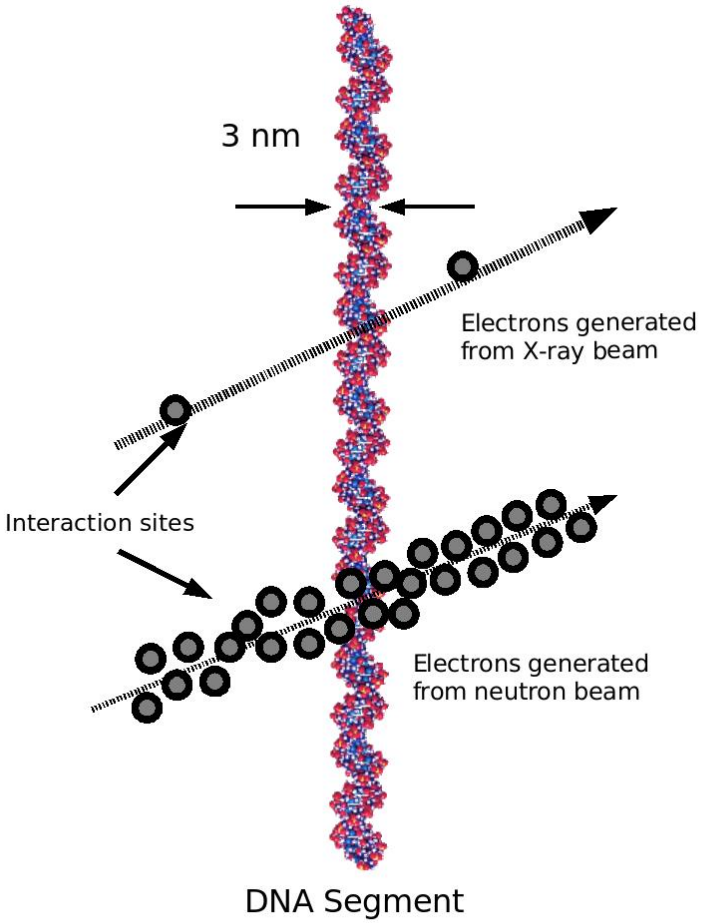
- Severity of DNA damage depends on lesion proximity and repairability, hence it is not a constant value but depends on physical (particle type, LET, dose) and biological (cell type, oxygenation status, repair capacity) parameters.
- RBE varies with the particle energy and the change of the beam composition (SOBP and nuclear fragmentations): its distribution is not homogenous across a treatment field.

Kevin Prise, ENLIGHT 2017

Linear Energy Transfer (LET)

It is the measurement of the number of ionisations which radiation causes per unit distance as it traverses the living cell or tissue

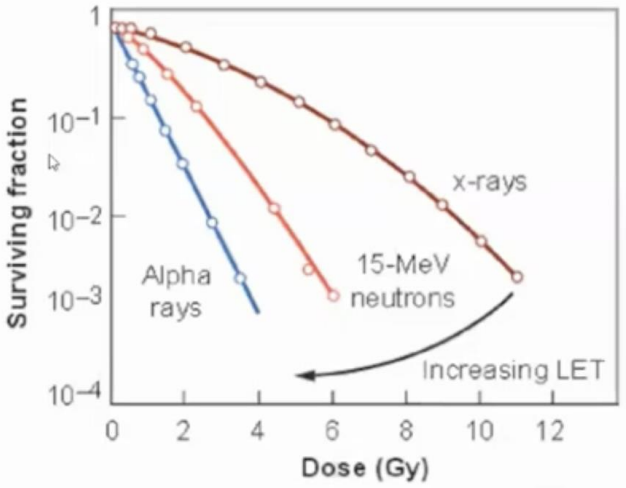
The LET depends on the charge and velocity of the ion: fast moving, light ions have low LET, and their biological effectiveness is close to that of X-rays; slow, heavy ions have high LET, and are more effective than X-rays for killing cells, as well as for other end points



Effect of LET on clonogenic cell survival

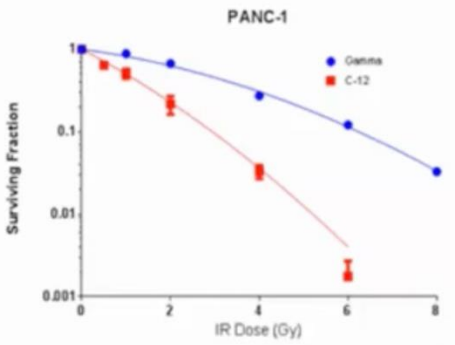
Effect of LET on clonogenic cell survival

→ As the LET of the radiation increases, the clonogenic survival changes: **the slope of the curves gets steeper**



https://www.slideshare.net/nileshkucha/oer-rbe-amp-let

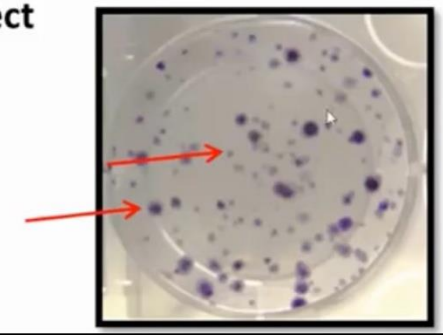
Radiobiological Effect (RBE)

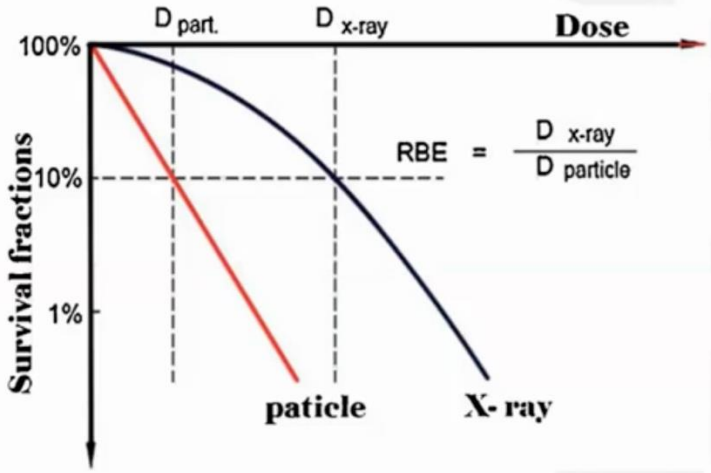


Equal doses of different types of radiation do not produce equal biologic effect

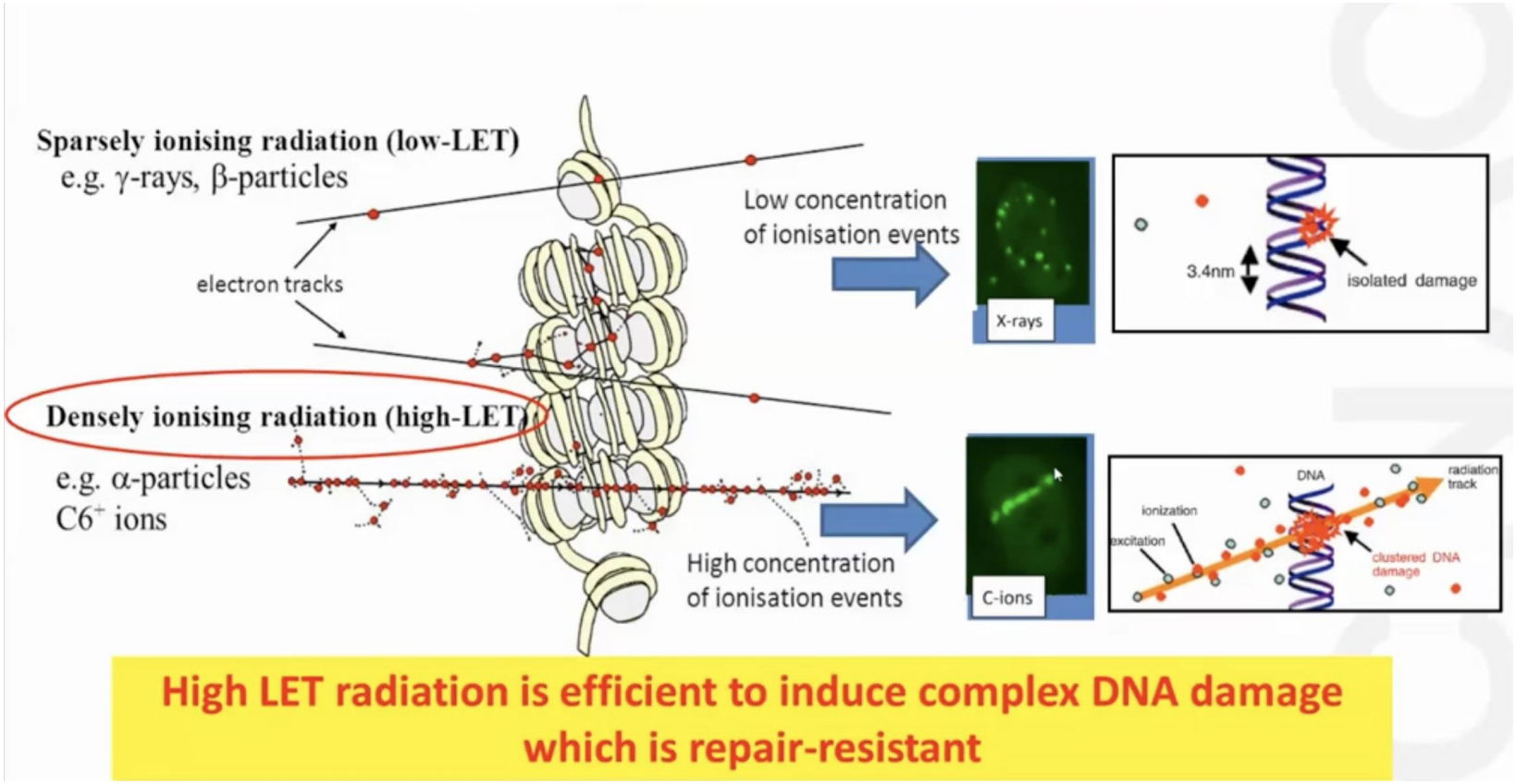
Relative biological effectiveness (RBE)

The **RBE** is defined as the ratio of absorbed dose of a reference beam of photons to the absorbed dose of any other radiation to produce **the same biological effect**





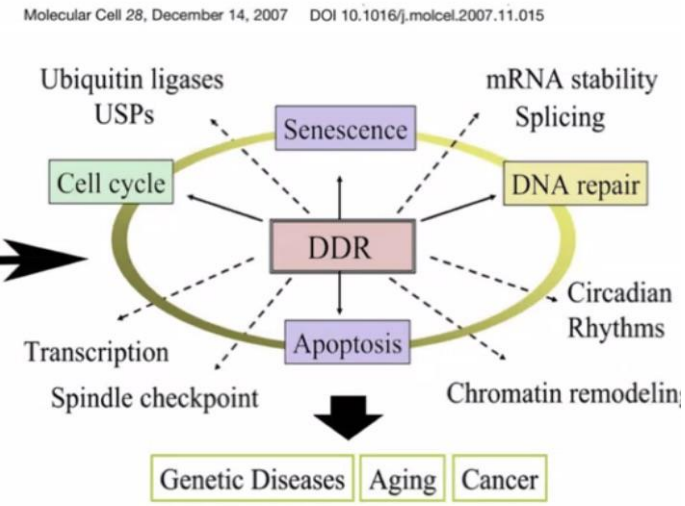
Carbon ions are densely ionising radiations inducing complex DNA damage



DNA Damage Response after recognition by damage sensors

c) repair begins with the triggering of the **DNA Damage Response (DDR)**, a tightly-regulated series of mol events that together not only repair the damage (if possible), but also coordinate the repair with other important cell activities

- Damage recognition by sensors
- Recruitment of repair proteins
- Coordination of repair with other cellular processes (cell cycle, cell survival/death, gene expression, metabolism, etc.)
- Repair of damage
- ...and hopefully, restoration of genomic stability



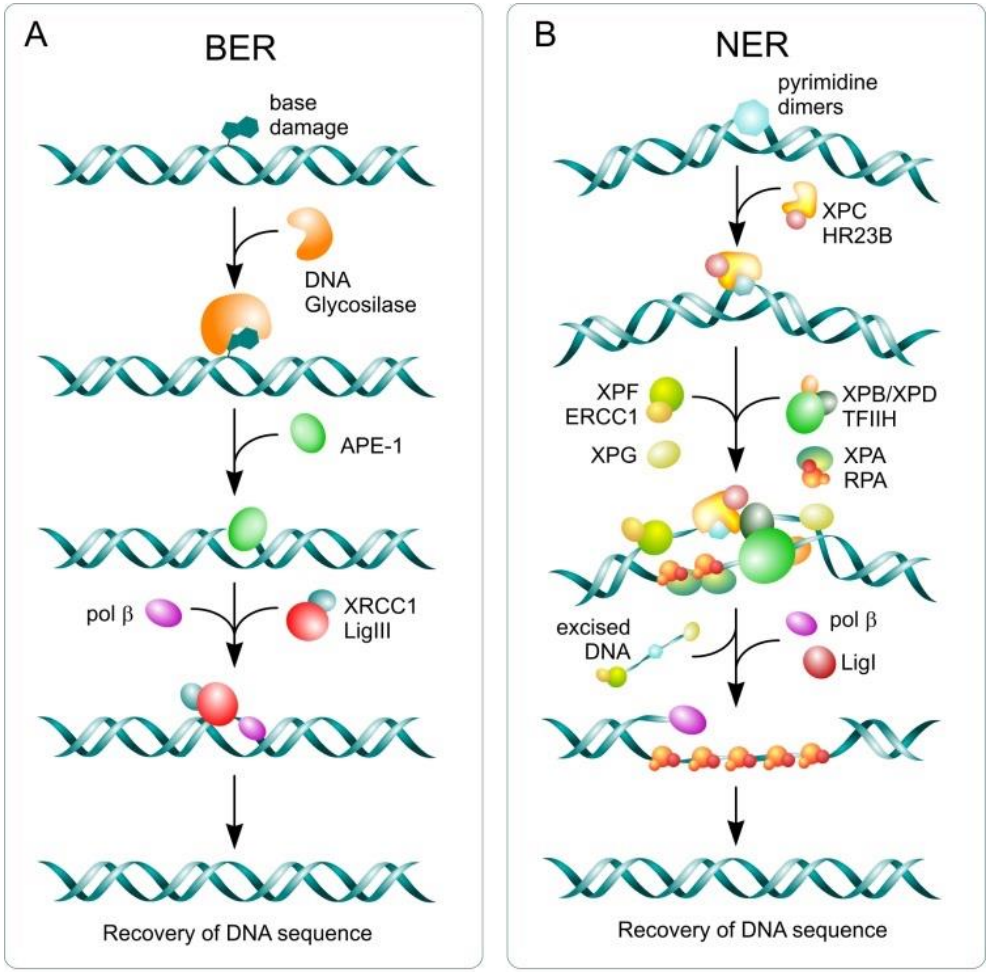
DNA damage repair – Single Strand Breaks

Base excision repair

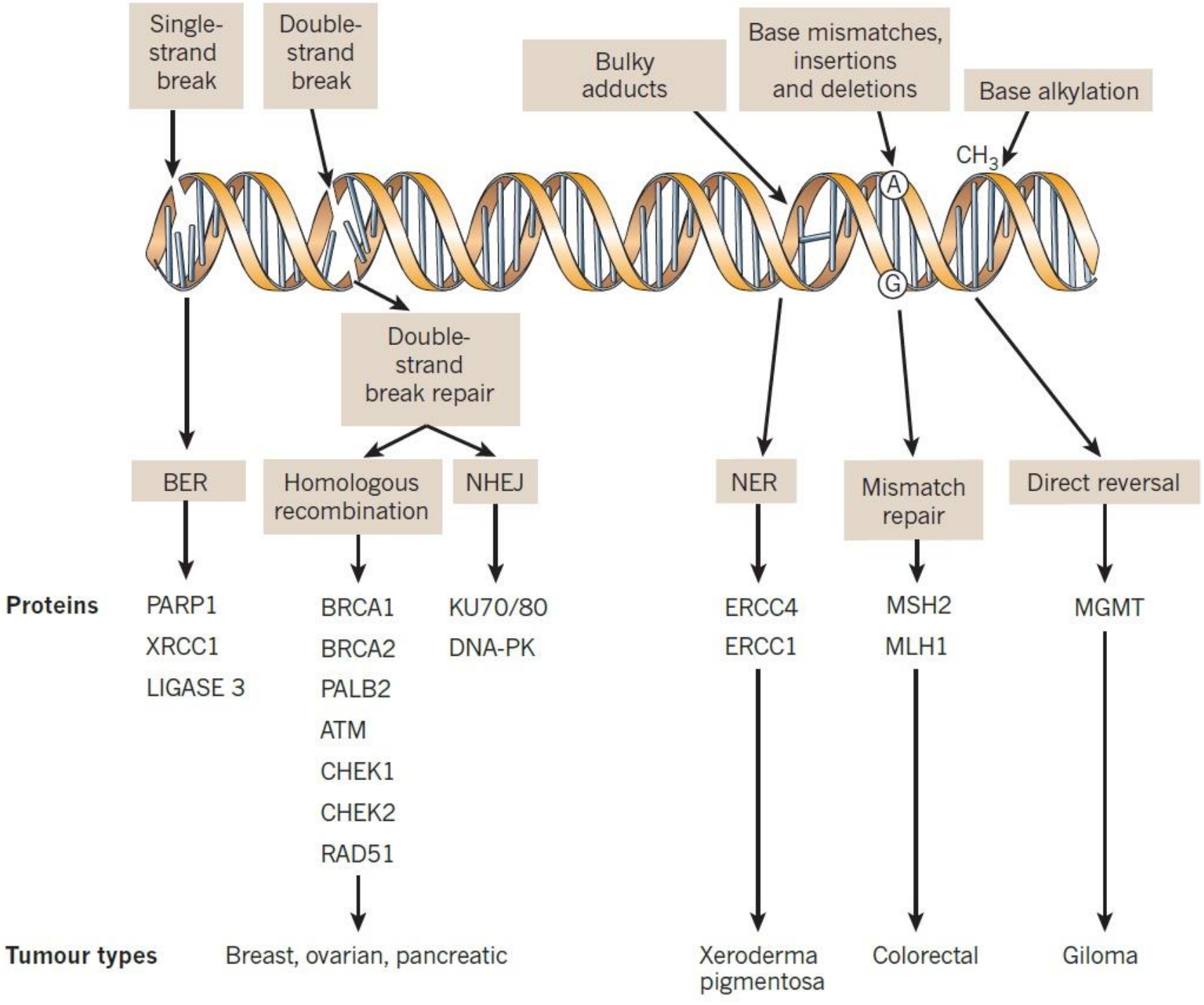
- base damage
- induction of SSB
- synthesis of missing bases
- annealing

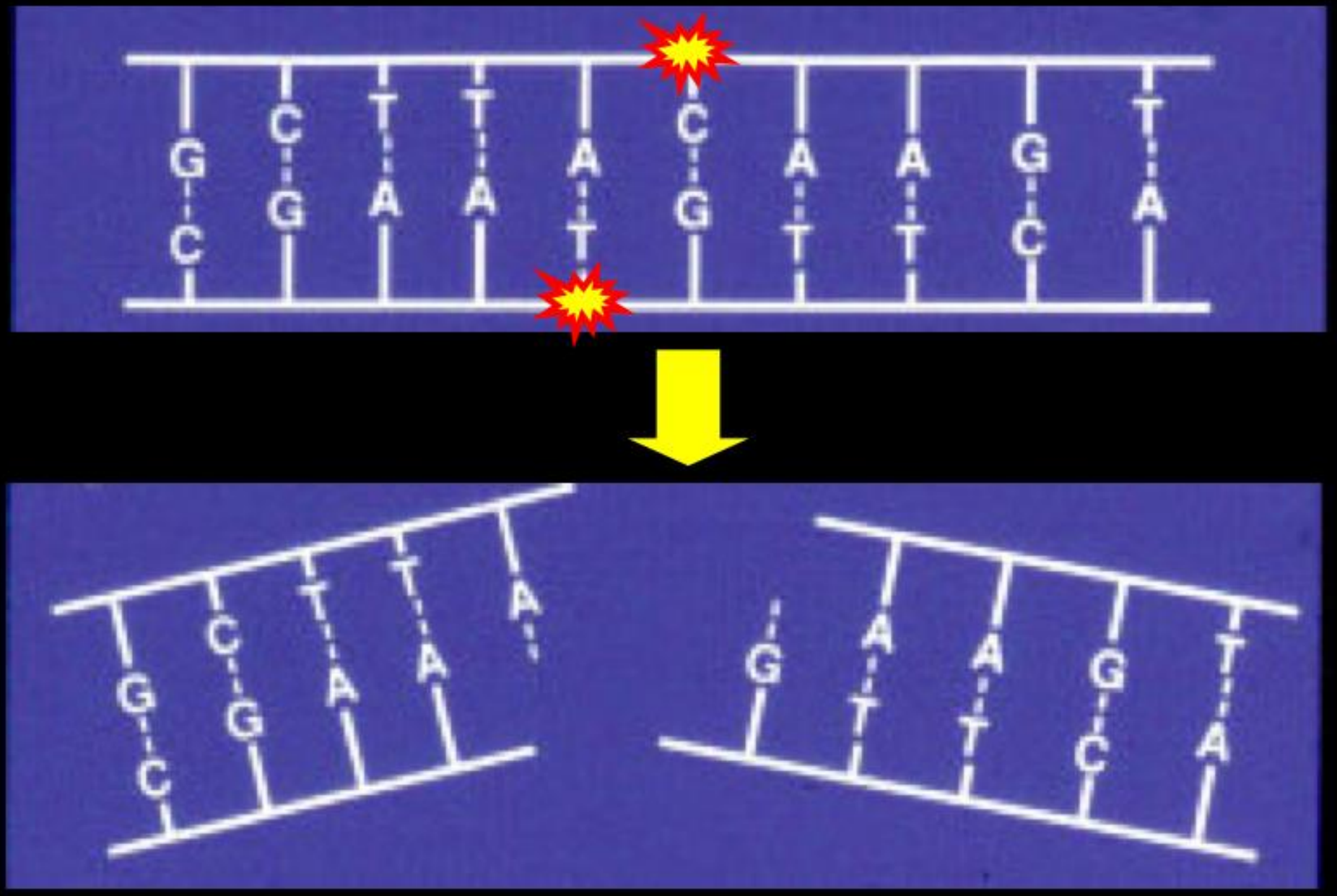
Nucleotide excision repair

- bulky lesions
- induction of SSB
- excision of 20-30 BP
- synthesis of missing bases
- annealing



The Journal of Biological Chemistry, 285, 9762-9769, March 26, 2010


Single-strand break
Double-strand break
Bulky adducts
Base mismatches, insertions and deletions
Base alkylation
CH_3
A
G
Double-strand break repair
BER
Homologous recombination
NHEJ
NER
Mismatch repair
Direct reversal
Proteins
PARP1
XRCC1
LIGASE 3
BRCA1
BRCA2
PALB2
ATM
CHEK1
CHEK2
RAD51
KU70/80
DNA-PK
ERCC4
ERCC1
MSH2
MLH1
MGMT
Tumour types
Breast, ovarian, pancreatic
Xeroderma pigmentosa
Colorectal
Giloma



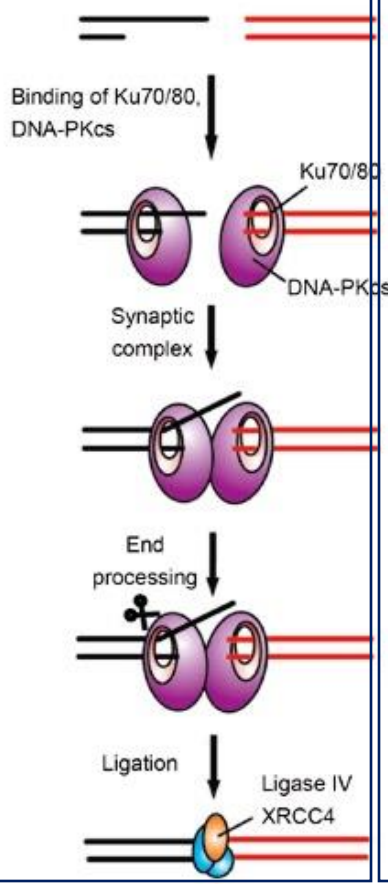
DNA double break triggers cell death

DNA Damage Repair – Double Strand Breaks

Nonhomologous endjoining

- >80 % of DSB
- G0 and G1 cell cycle phase

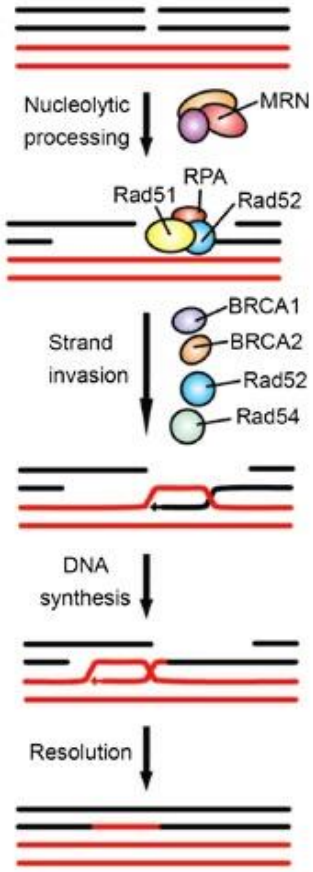
error prone



Homologous recombination

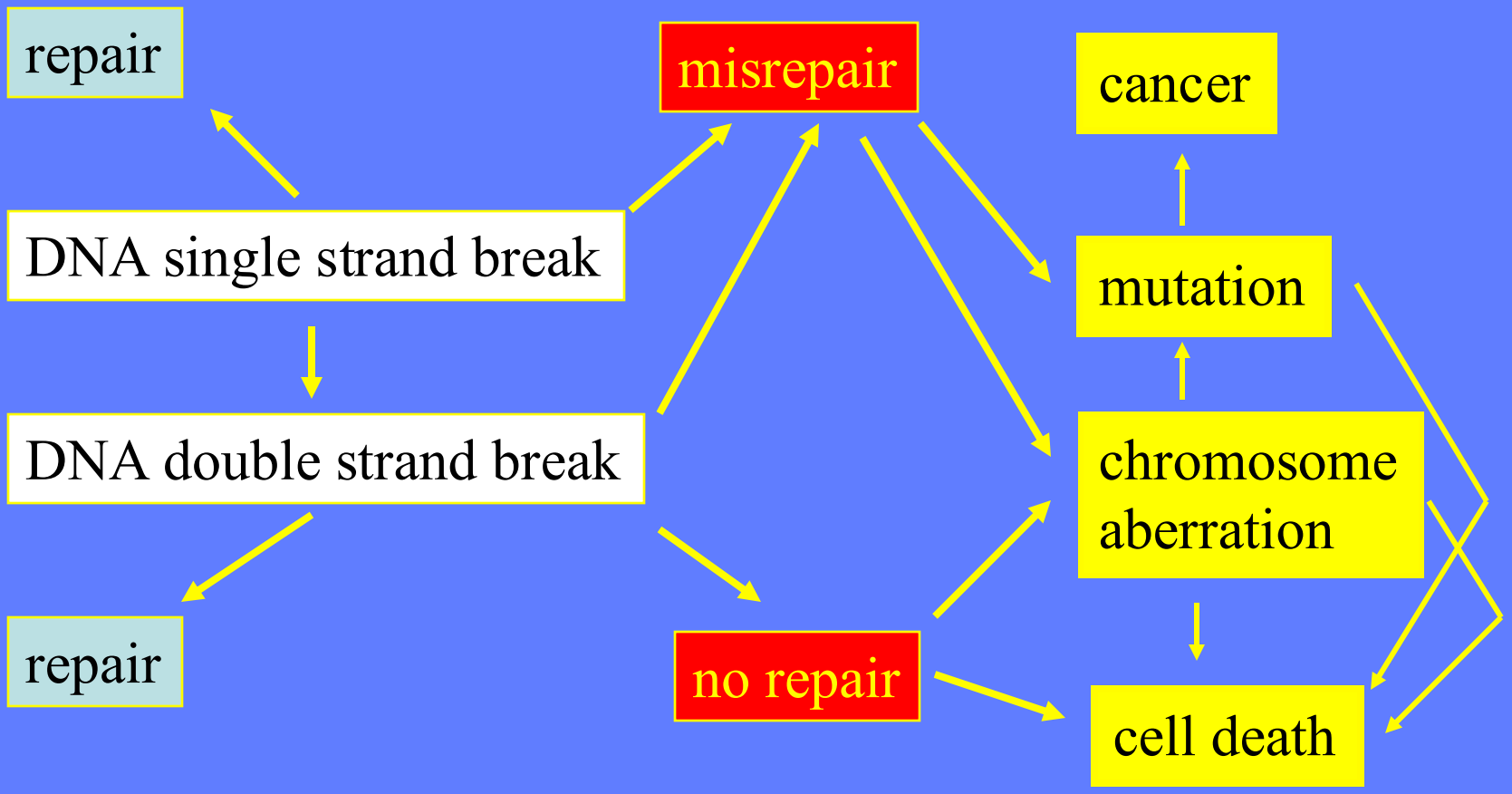
- < 20% of DSB
- S and G2 cell cycle phase

error free



Brandsma, I., van Gent, D.C. Genome Integrity ***3,*** *9 (2012).*

DNA damage and its consequences



What happens to cells that still have residual DNA Damage after all the repair possible has occurred?

1. Cells have another important decision to make at this point: Can I tolerate this damage and survive, or do I die?

a) if the damage is survivable, the cell risks a permanent mutation (which may or may not have measurable consequences) plus it still could die later

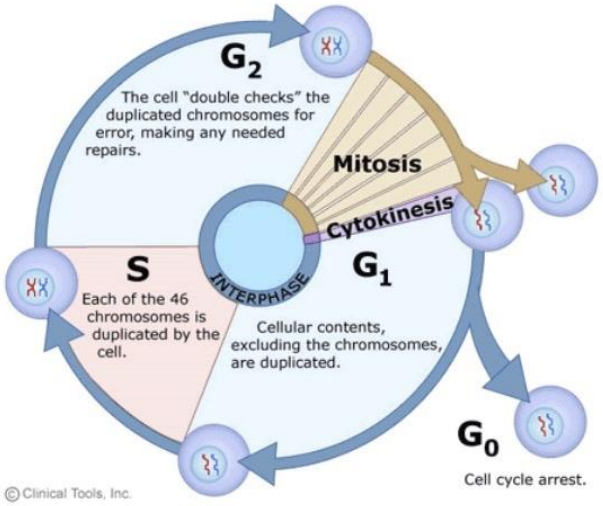
b) however, if the damage is lethal, then the cell needs to decide how it wants to die

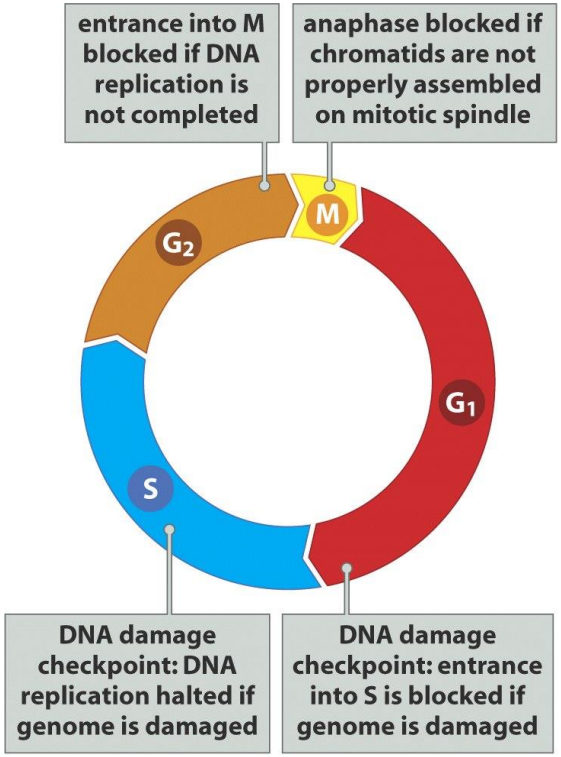
2. Modes of cell death

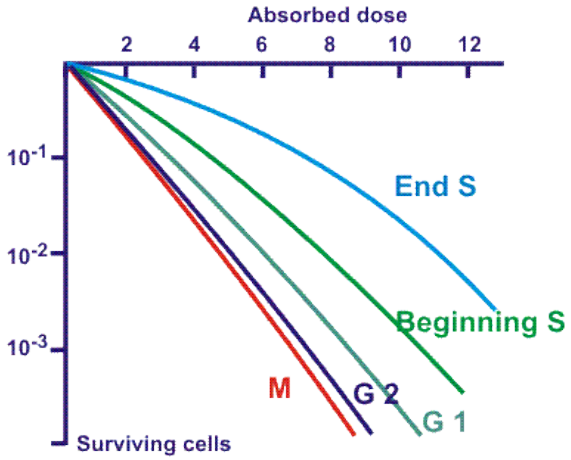
a) the types of cell death recognized today - not all of which are directly elicited by radiation exposure (but could be indirectly)

- include: Mitotic Catastrophe, Apoptosis, Senescence

Cell Cycle Dependence Radiosensitivity



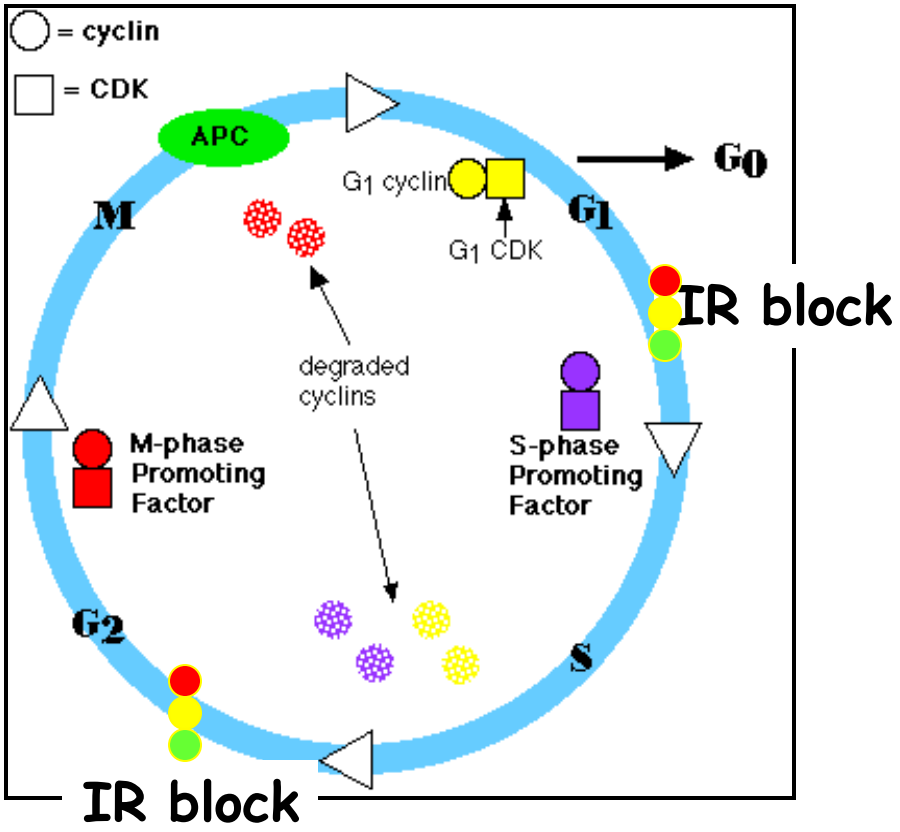




M>G2>G1>early S>late S for sensitivity

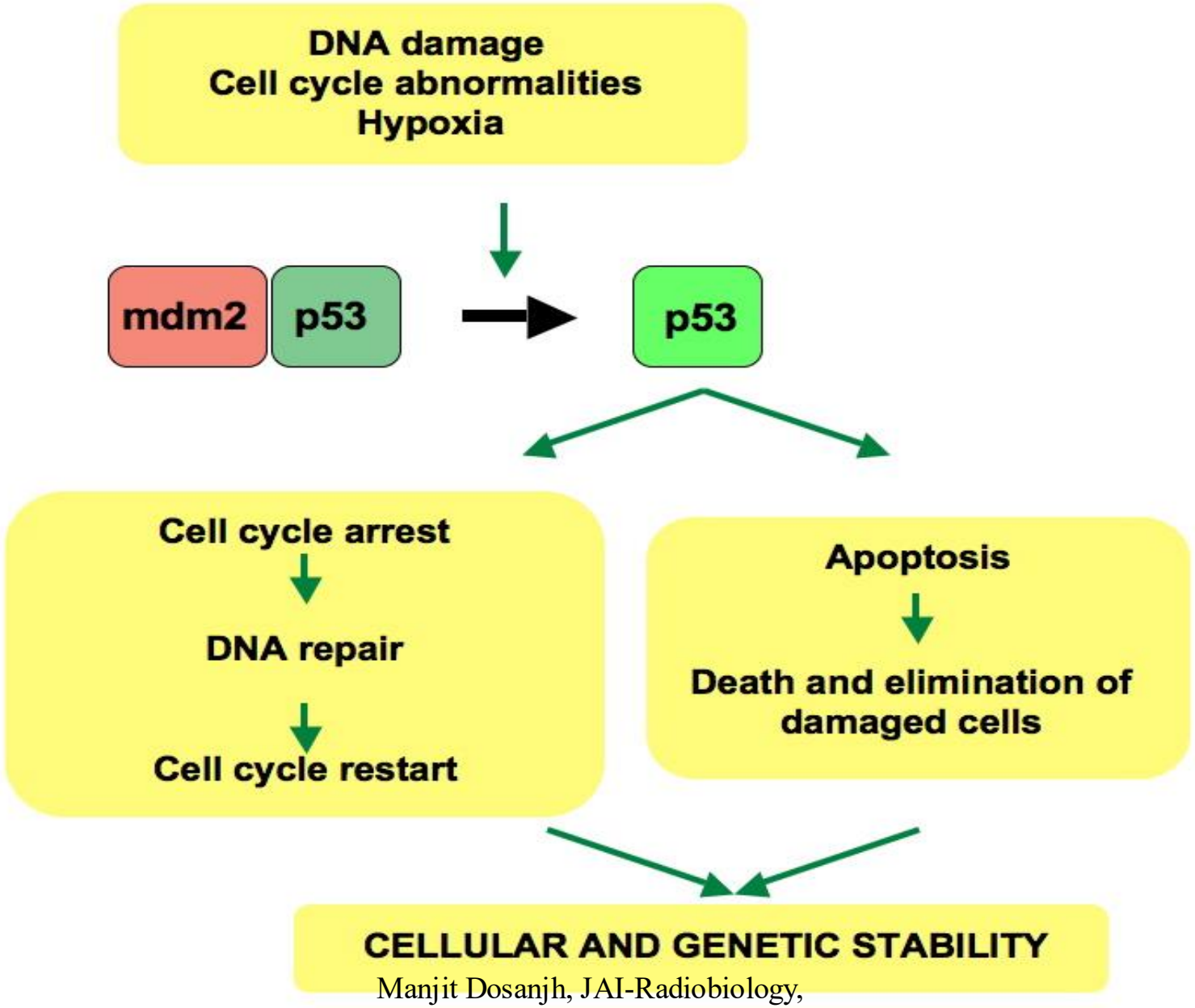
Difference caused by cell cycle are similar to difference caused by Oxygen effect

Regulation of the cell cycle



Cell cycle arrest can occur in response to DNA damage (e.g. IR) in order to allow for DNA repair.

p53-guardian of the genome…..Lane,1992



4 Rs of radiotherapy now 6 Rs

- Repair (few hours)
- Redistribution (few hours)
- Repopulation (5-6 weeks)
- Reoxygenation (hours to days)

Recently also added

- Intrinsic Radiosensitivity
- Reactivation of immune Response

Redistribution (sensitivity during cell cycle)

- Cells are most sensitive to radiation at or close to M
- Cells are most resistant to radiation in late S
- For prolonged G1 → a resistant period is evident early G1 followed be a sensitive period in late G1
- Cells are usually sensitive to radiation in G2 (almost as sensitive as in M)

Implications for Radiotherapy

Single dose:

- Tends to synchronize cell population
- More radiosensitive phases of cell cycle are killed / more resistant survive
- Majority of cells left are in resistant phase of the cell cycle

Multiple Doses:

- With multiple doses, cells progress through to a new phase of the cell cycle, get ↑ sensitive cells
- "Sensitization due to reassortment and redistribution" causes therapeutic gain

Repair and Repopulation

DNA damage and repair

1. Lethal - irreversible, irreparable, leads to cell death
2. Sublethal - repaired in hours; if a second dose is given, can interact with more damage to create lethal damage; represents shoulder on cell survival curve
3. Sublethal Damage Repair - increase survival when a dose is fractionated over time
4. Potentially Lethal Damage - can be modified by the post-irradiation environment

Reoxygenation in Radiotherapy

Hypoxia confers resistance to X-rays/gamma rays – also to chemotherapeutic drugs

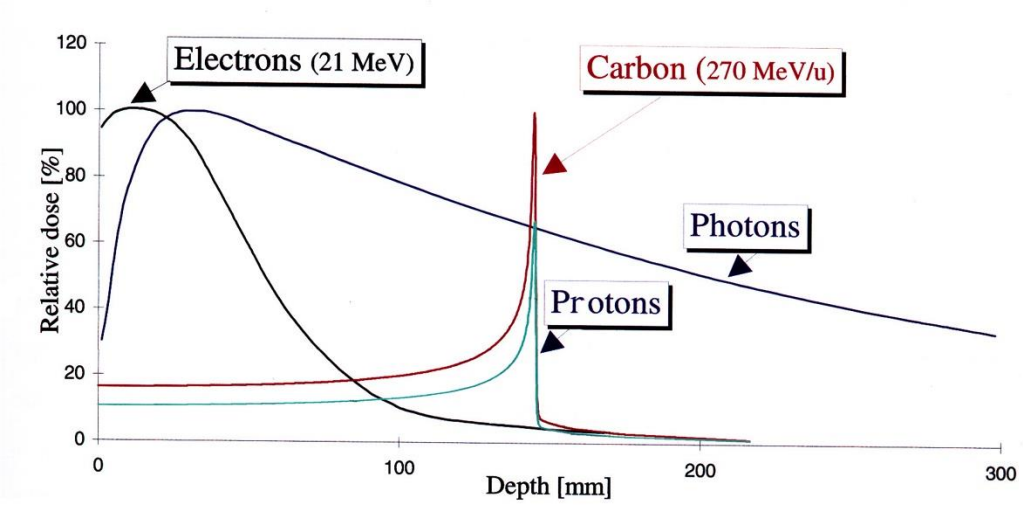
Human tumours that do not respond to radiotherapy may not re-oxygenate

Optimal fractionation regimen depends on reoxygenation

SUMMARY: Role of Radiobiology in Therapy

- Radiobiology research is essential in improving therapy
- The RBE is defined as the ratio of doses to reach the same level of effect when comparing two modalities and depends on multiple physics- and biology related parameters
- RT-induced DNA-damage and its repair is key in achieving better outcomes
- Role of 4Rs which are now 6Rs in treatment: repair, repopulation, redistribution, reoxygenation, intrinsic radiosensitivity and now reactivation of immune response
- New emerging modalities such as VHEE and FLASH

Hadrontherapy vs. radiotherapy



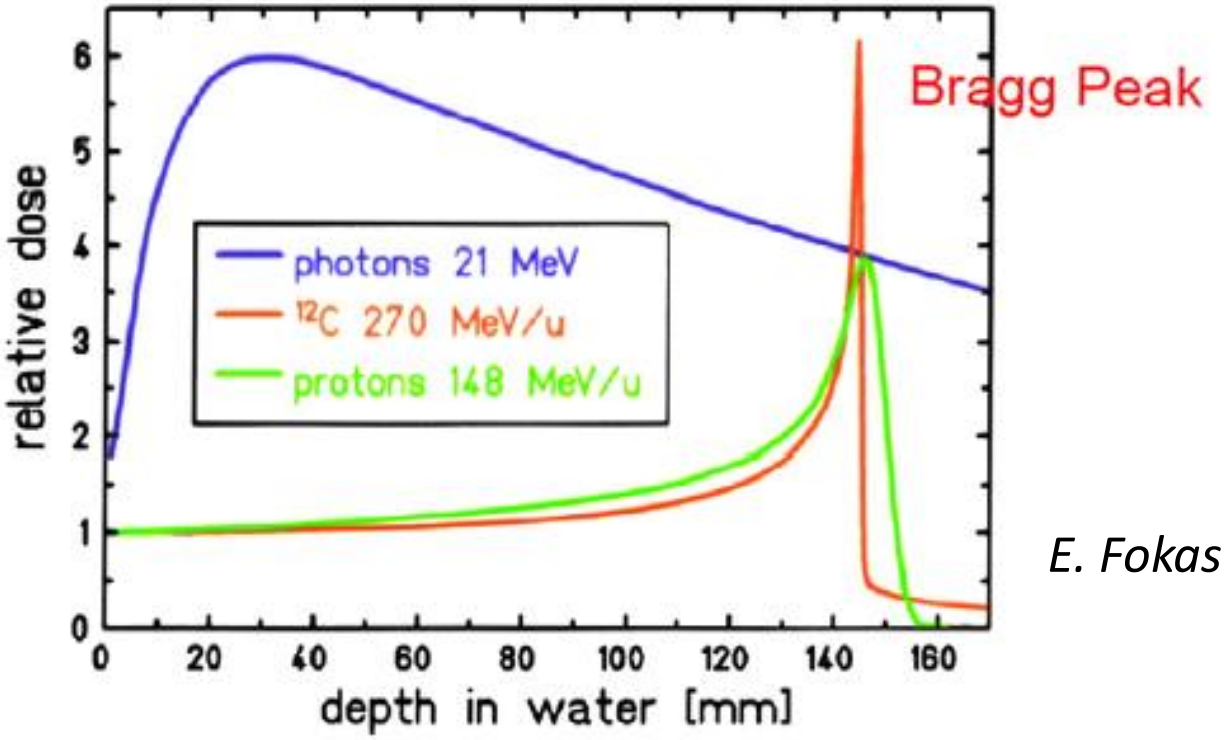
- Tumours close to critical organs
- Tumours in children
- Radio-resistant tumours

Photons and Electrons vs. Hadrons

- Physical dose high near surface
- DNA damage easily repaired
- Biological effect lower
- Need presence of oxygen
- Effect not localised

- Dose highest at Bragg Peak
- DNA damage not repaired
- Biological effect high
- Do not need oxygen
- Effect is localised

Hadron Therapy vs classical RT



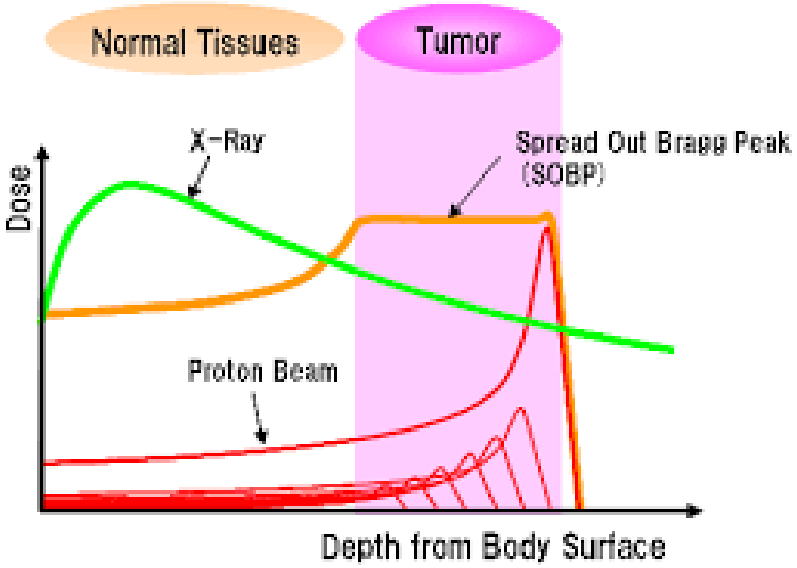
E. Fokas et al, 2009

In tumour therapy, hadrons, such as carbon produce a better depth-dose profile than protons.

The essential advantage of carbon ions is the higher biological effectiveness at the end of their range in the tumor. In the entrance channel the RBE is only slightly elevated.

In combination with the low dose in the entrance channel, as well as less more easily repairable damage is produced in normal tissue.

HT increasingly used due to better tumour targeting



Hadrons

Dose highest at Bragg Peak

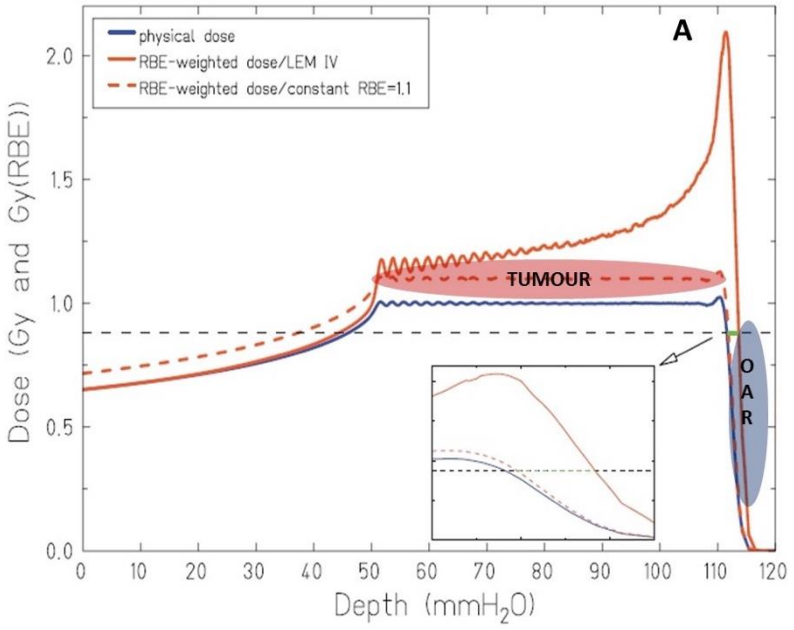
DNA damage not repaired

Biological effect high

Do not need oxygen

Effect is localised

Biological Range Uncertainity and actual RBE



Distal SOBP

- Decrease of dose and increase in LET
- RBE is highly uncertain
- RBE can be greater than 1 in OAR
- Biological optimization needed

FLASH: a new way of delivering Radiotherapy for treating cancer?

CERN

UNIVERSITY OF OXFORD

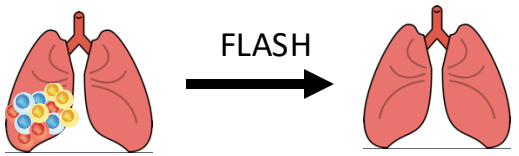
FLASH radiotherapy is based on the observation that healthy tissue is less damaged if treatment occurs very fast

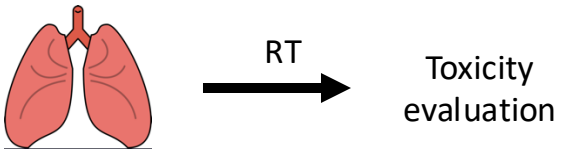
RESEARCH ARTICLE

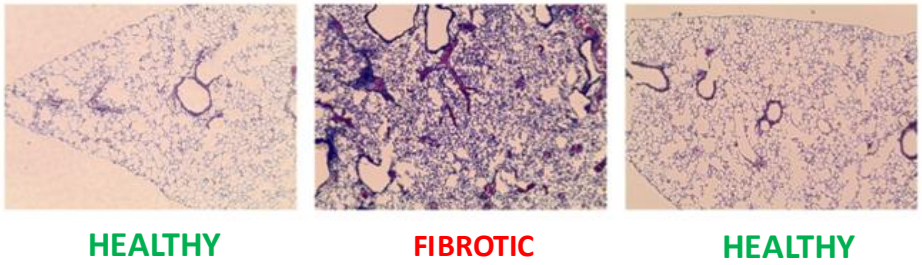
RADIATION TOXICITY

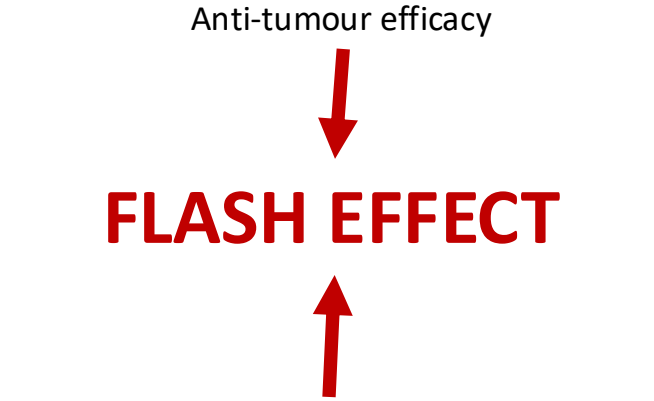
Ultrahigh dose-rate FLASH irradiation increases the differential response between normal and tumor tissue in mice

Vincent Favaudon,[1,2]* Laura Caplier,[3†] Virginie Monceau,[4,5‡] Frédéric Pouzoulet,[1,2§] Mano Sayarath,[1,2¶] Charles Fouillade,[1,2] Marie-France Poupon,[1,2‖] Isabel Brito,[6,7] Philippe Hupé,[6,7,8,9] Jean Bourhis,[4,5,10] Janet Hall,[1,2] Jean-Jacques Fontaine,[3] Marie-Catherine Vozenin[4,5,10,11]

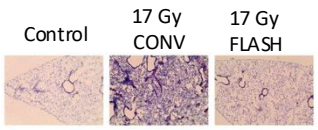






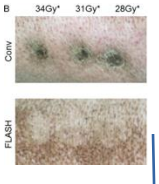


First observation of 'normal tissue sparing' at high dose rates (1969)



Favaudon et al, FLASH effect confirmed for the first time in mice (July 2014)

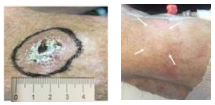
Stanford-Clinac modified ultra-high doses



Vozenin et al. - electron FLASH mini-pigs

1969 — 2014 — 2015 — 2016

First patient treated with electron FLASH therapy at CHUV (2018)



First Patient treated with transmission proton FLASH at Cincinnati Children's Hospital (Nov 2020)

2017 — 2018 — 2019 — 2020 — 2021

Proton FLASH toxicity study Maryland (2017)

Proton FLASH tumour control study on Cincinnati (2018)

Much research needed

Treatment of a first patient with FLASH-radiotherapy

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original Article

Treatment of a first patient with FLASH-radiotherapy

Jean Bourhis [a,b,*], Wendy Jeanneret Sozzi [a], Patrik Gonçalves Jorge [a,b,c], Olivier Gaide [d], Claude Bailat [c], Fréderic Duclos [a], David Patin [a], Mahmut Ozsahin [a], François Bochud [c], Jean-François Germond [c], Raphaël Moeckli [c,1], Marie-Catherine Vozenin [a,b,1]

[a] Department of Radiation Oncology, Lausanne University Hospital and University of Lausanne; [b] Radiation Oncology Laboratory, Department of Radiation Oncology. Lausanne University Hospital and University of Lausanne; [c] Institute of Radiation Physics, Lausanne University Hospital and University of Lausanne; and [d] Department of Dermatology, Lausanne University Hospital and University of Lausanne, Switzerland

5.6 MeV linac adapted for accelerating electrons in FLASH mode

15 Gy with 10 pulses **in 90 ms**

3.5 cm diameter tumour, multiresistant cutaneous

Appears that instantaneous dose Induces a massive oxygen consumption and a transient protective hypoxia in normal issues

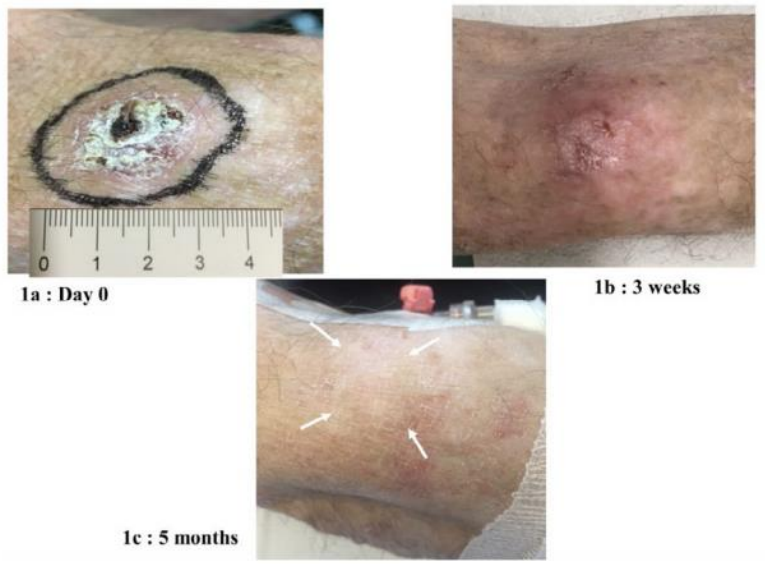


Fig. 1. Temporal evolution of the treated lesion: (a) before treatment; the limits of th PTV are delineated in black; (b) at 3 weeks, at the peak of skin reactions (grade 1 epithelitis NCI-CTCAE v 5.0); (c) at 5 months.

First Patient Treated in FAST-01 FLASH Proton Therapy (November 2020)

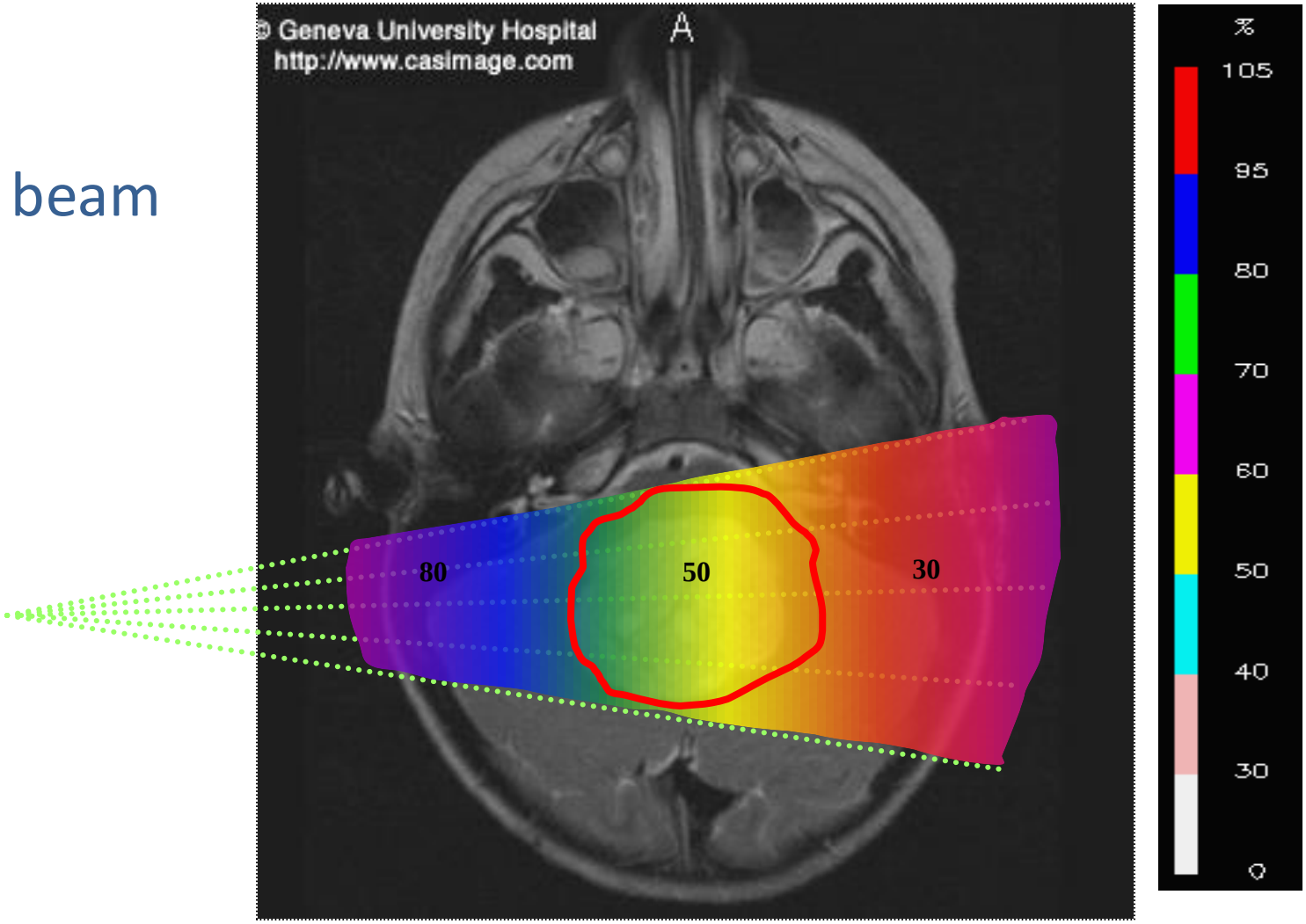
Transmission-shoot through

FeAsibility Study of FLASH Radiotherapy for the Treatment of Symptomatic Bone Metastases). The clinical trial involves the investigational use of Varian's ProBeam particle accelerator modified to enable radiation therapy delivery at ultra-high dose rates (dose delivered in less than 1 second) and is being conducted at the Cincinnati Children's/UC Health Proton Therapy Center with John C. Breneman M.D.

The study will assess Varian's ProBeam particle accelerator modified to deliver an advanced non-invasive treatment for cancer patients. *(Credit: Bokskapet from Pixabay)*

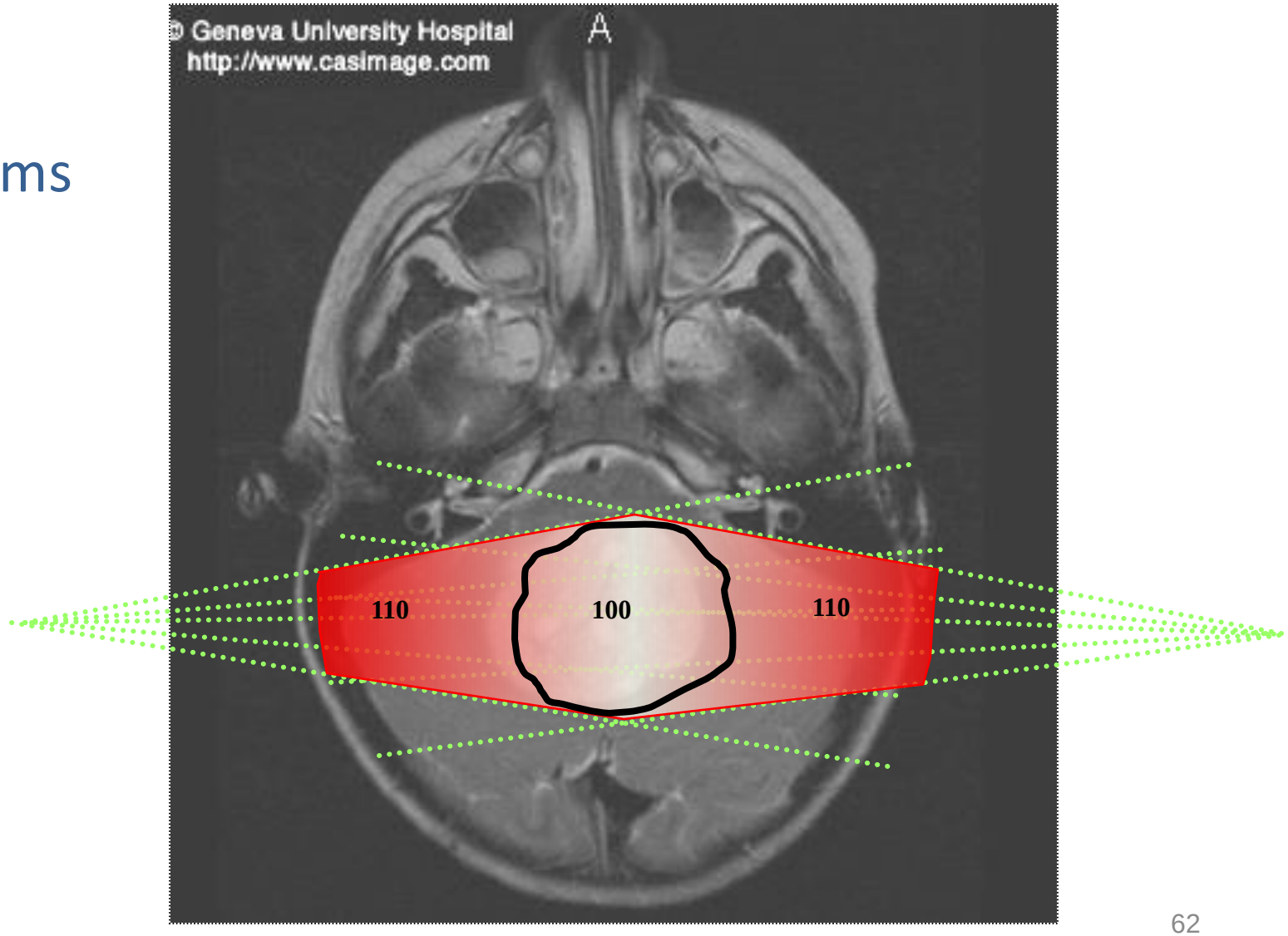
Classical Radiotherapy with X-rays

single beam

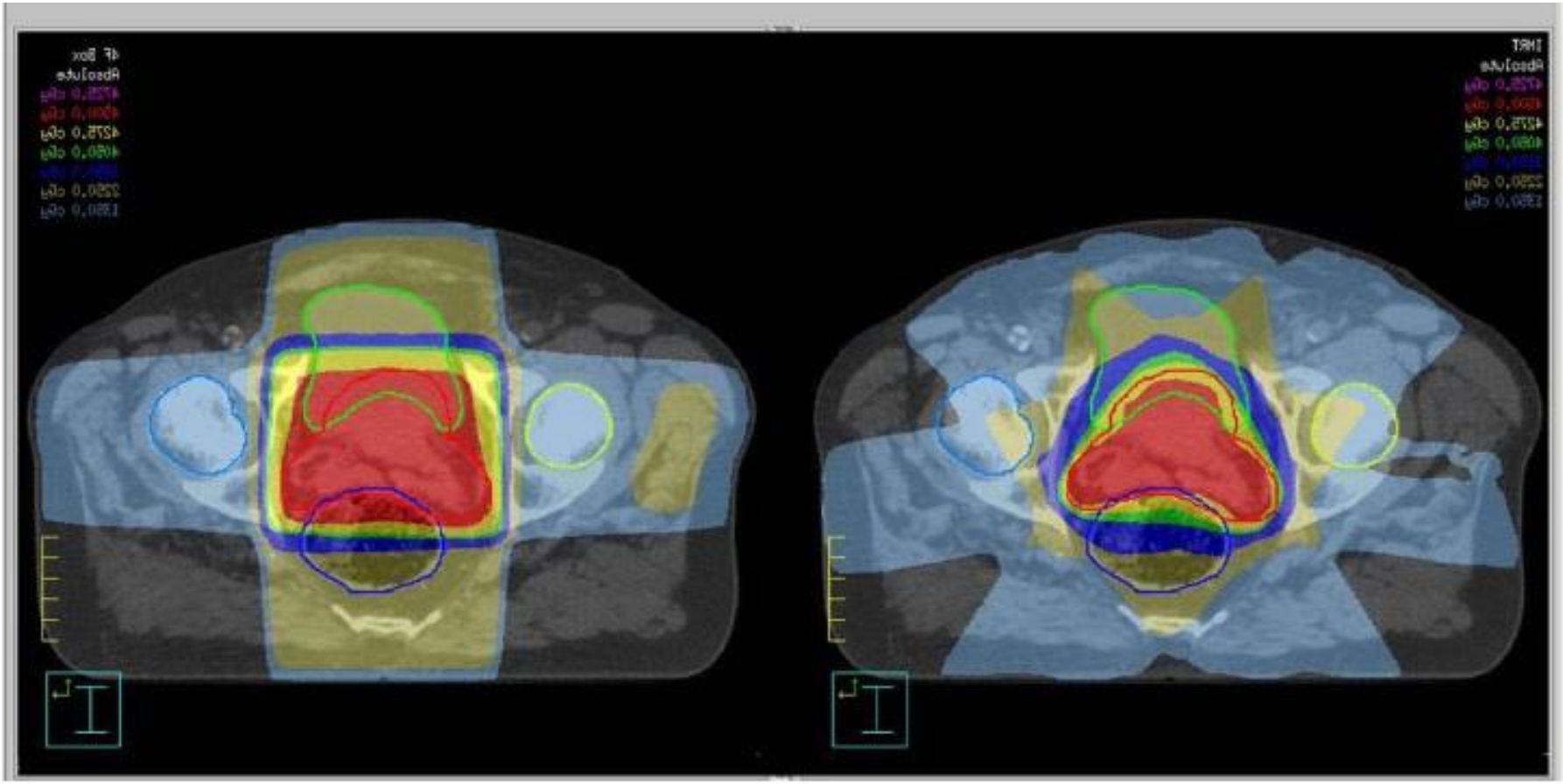


Radiotherapy with X-rays

two beams



Improved Delivery

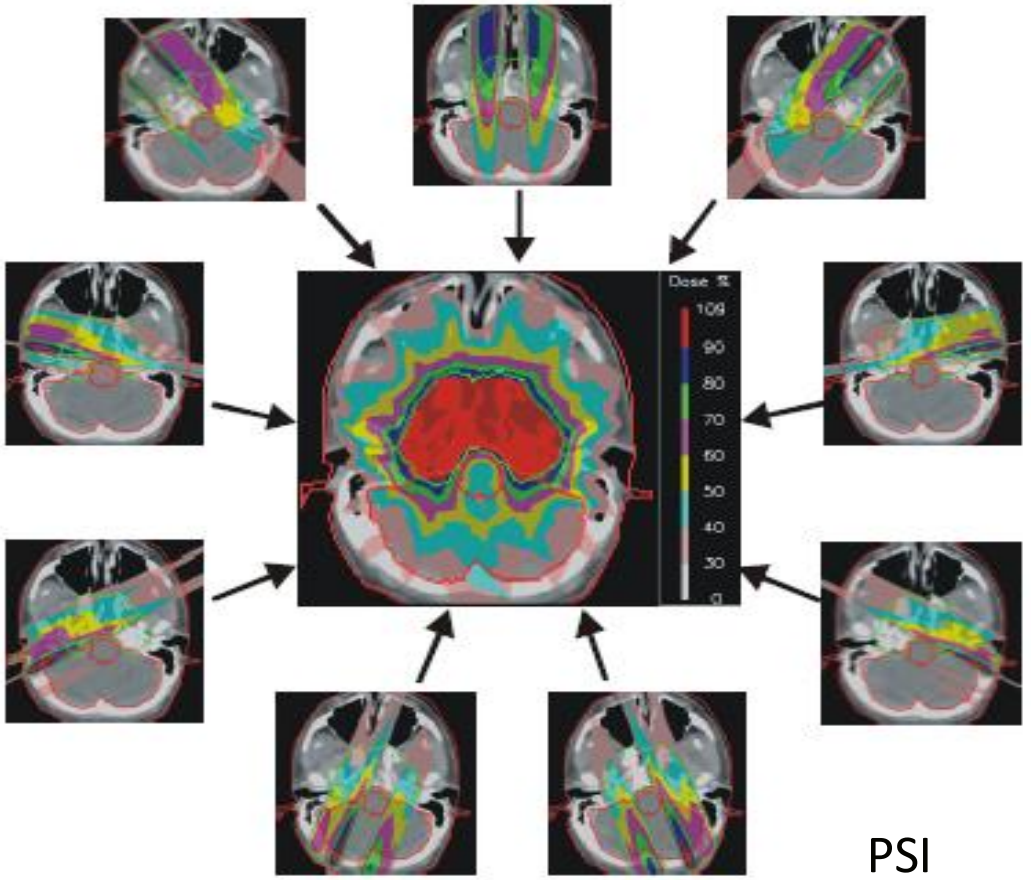


1990s: 4 constant intensity fields

Current state of RT: Intensity Modulated Radiotherapy (IMRT) – Multiple converging field with planar (2D) intensity variations

Intensity Modulated Radiation Therapy

9 NON-UNIFORM FIELDS



PSI

60-75 grays (joule/kg) given in 30-35 fractions (6-7weeks)

to allow healthy tissues to repair:

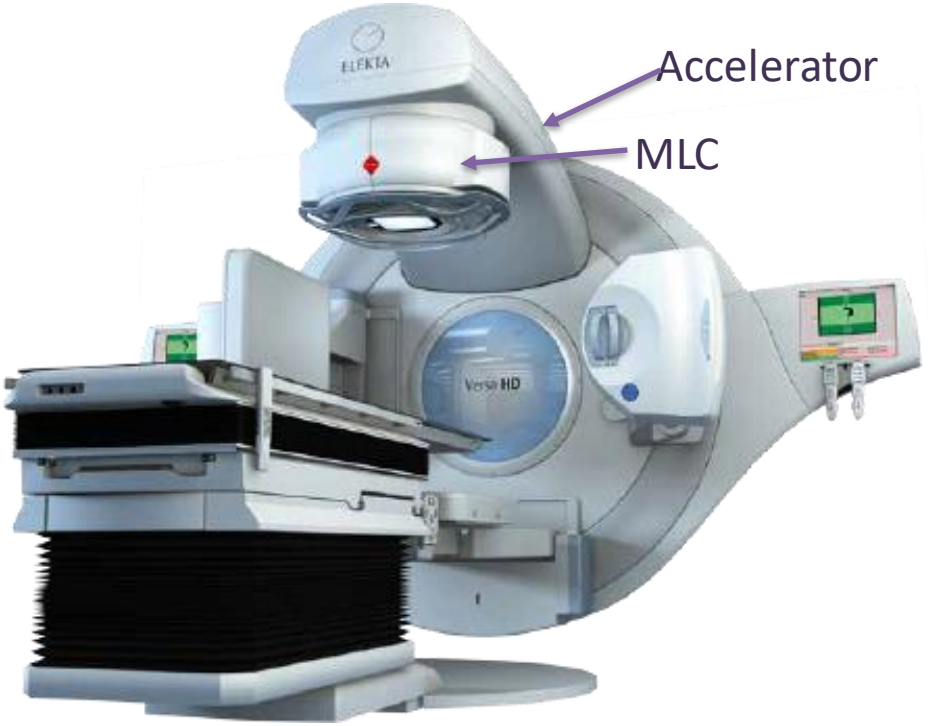
90% of the tumours are radiosensitive

Ugo Amaldi, TERA

The most widely available accelerator

Electron Linac (linear accelerator) for radiation therapy treatment of cancer)

More than >18,000 in use



Widely available in all major hospitals in specially in high income countries (HIC)

Advances in Radiation Therapy

In the past two decades due to:

- improvements in imaging modalities, multimodality
- technology, powerful computers and software and delivery systems have enabled:
  - Intensity Modulated Radiotherapy (IMRT),
  - Image Guided Radiotherapy (IGRT),
  - Volumetric Arc Therapy (VMAT) and
  - Stereotactic Body Radiotherapy (SBRT)
  - MRI-guided Linac therapy
- Is Hadron/Particle Therapy the future?
- FLASH??

Much more still needs to be done

- Treat the tumour and only the tumour
  - ⇒ Imaging and dose delivery: control and monitor the ideal dose to the tumour
  - ⇒ Minimal collateral radiation "outside" the tumour
  - ⇒ Minimal radiation to nearby critical organs
  - ➢ Even if the tumour is moving
- Compact: Fit into a large hospital
  - ⇒ Accelerator: smaller, simpler, cheaper
  - ⇒ Gantry: compact, cheaper, energy efficient
- Be affordable
  - ✓ Capital cost ?
  - ✓ Operating costs ?
  - ✓ Increased number of treated patients per year ?
- Wish list from community
  - ✓ Improve patient through-put
  - ✓ Increase effectiveness
  - ✓ Decrease cost
- New ideas being explored

Many thanks to:

- U. Amaldi, CERN &TERA
- E. Blakely, LBNL, USA
- M Durante, GSI, Germany; Kevin Prise, Queens, UK
- K. Prise, Queens
- A. Faocetti, CNAO, Italy
- Martin Pruschy, Zurich, Switzerland
- HIT, CNAO, MedAustron, PSI,
- ENLIGHT collaborators and colleagues