HITRIplus
Heavy Ion Therapy Research Integration

Introduction to Radiobiology

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008548

Agenda

- Intro
- Linear quadratic model
- Cell cycle and cell death
- Relative Biological Effectiveness
- Biological Effective Dose
- Fractionation
- Oxygenation

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What is Radiobiology?

- [Radiobiology = Radiation Biology] is the study of action of ionizing radiation on living organisms
- Physics & Biology:
  - Different types of ionizing radiation: non-ionizing & ionizing radiation

    Ionizing radiation:
    - Directly ionizing radiation deposit dose straight away (charged particles): electrons, protons, alfa particles, heavy ions
    - Indirectly ionizing radiation interact with matter and cause ionization (neutral particles): photons, neutrons

  - Energy absorption at the atomic and molecular level → biological damage
- Radiobiological principles used in Radiotherapy aim to treat cancer by having minimal damage in healthy tissues

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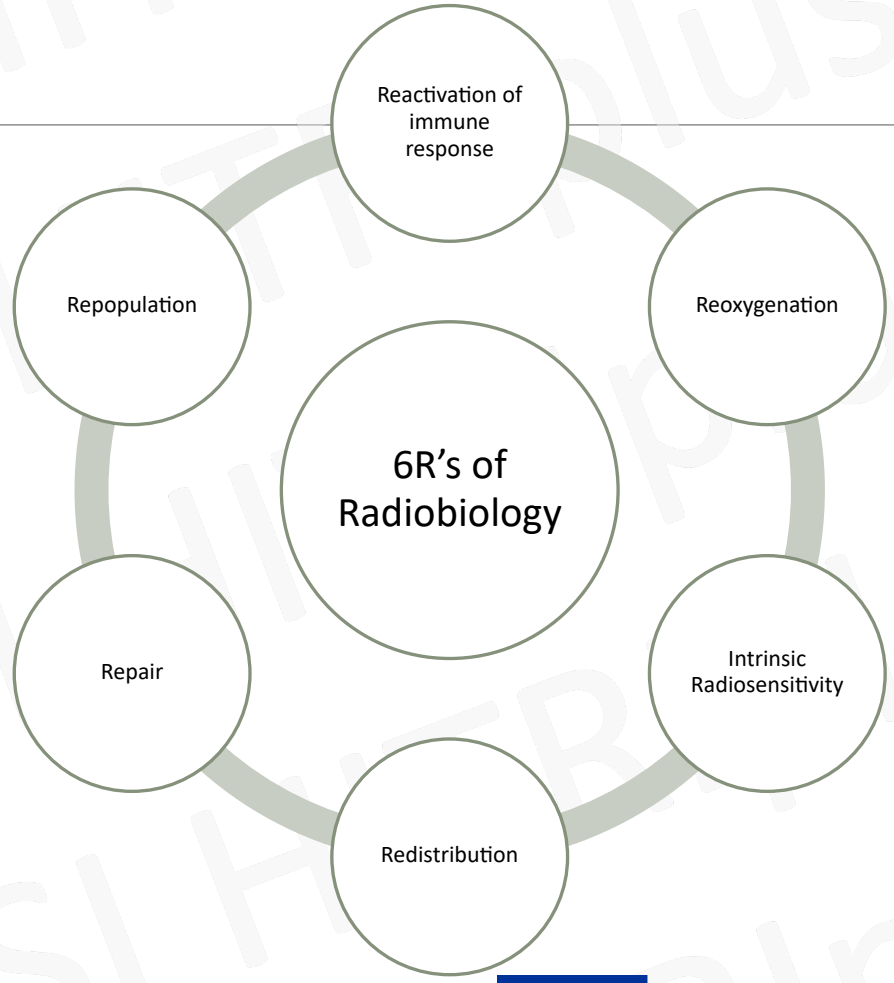
6R's of Radiobiology

All these processes determine the success of a radiotherapy and must be carefully considered during treatment



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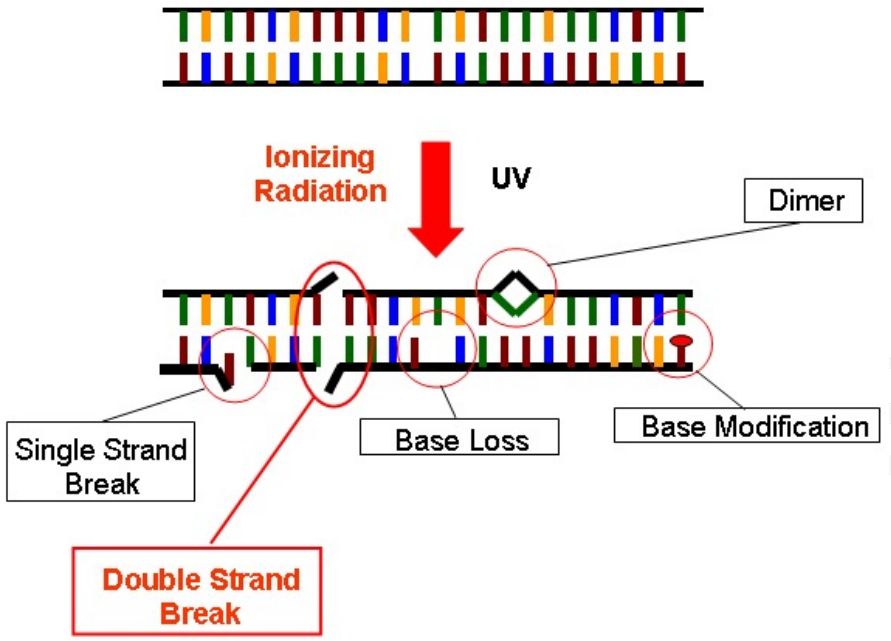
Basic concepts of radiation biophysics

- the DNA **Double Strand Break (DSB)** is considered the type of lesion most directly related to cell killing
- different radiation qualities produce the same spectrum of DNA lesions
- **BUT** the distribution of lesions inside the target can be very different



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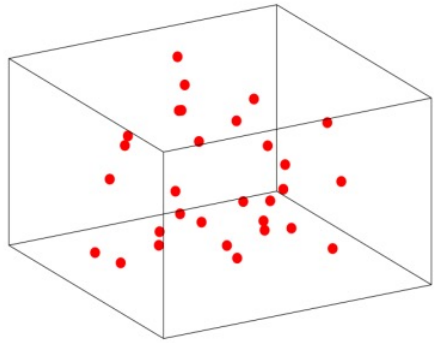
Photons

x-rays

Random

DSB distribution



- The distribution of lesions inside the target can be very different

-> this aspect strongly influences the biological effect!

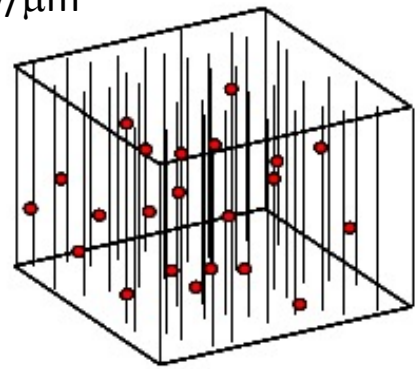
^{12}C Low LET

200 MeV/u, ≈ 16 keV/µm

Random

DSB distribution

(photon-like)



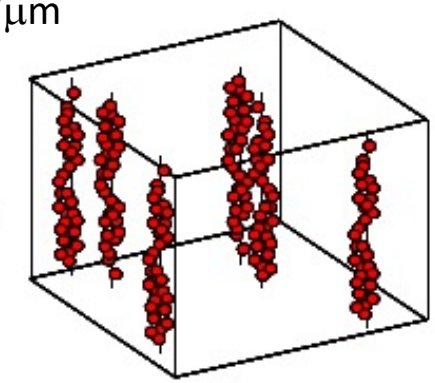
^{12}C High LET

1 MeV/u, ≈ 690 keV/µm

Non-random

DSB distribution

(RBE>>1)



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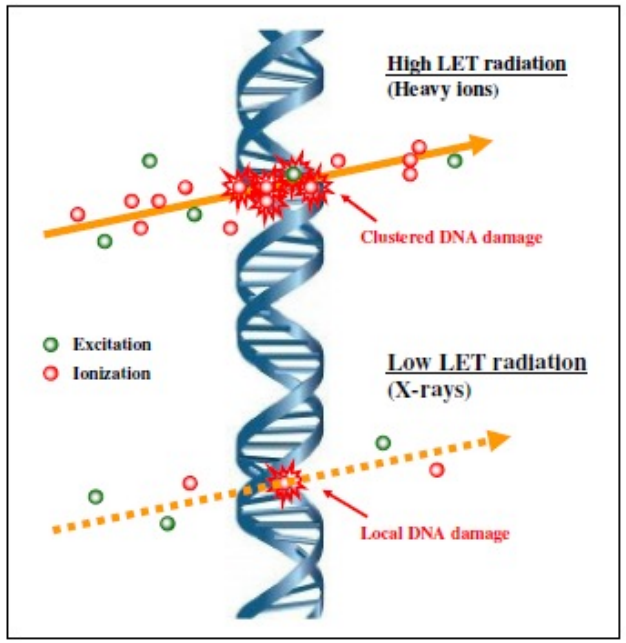
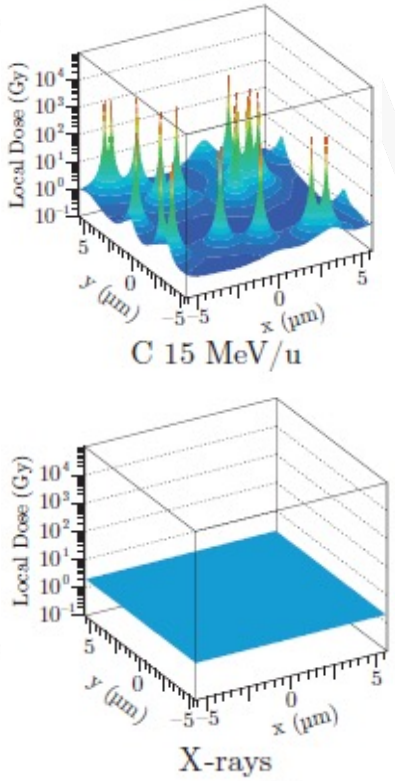
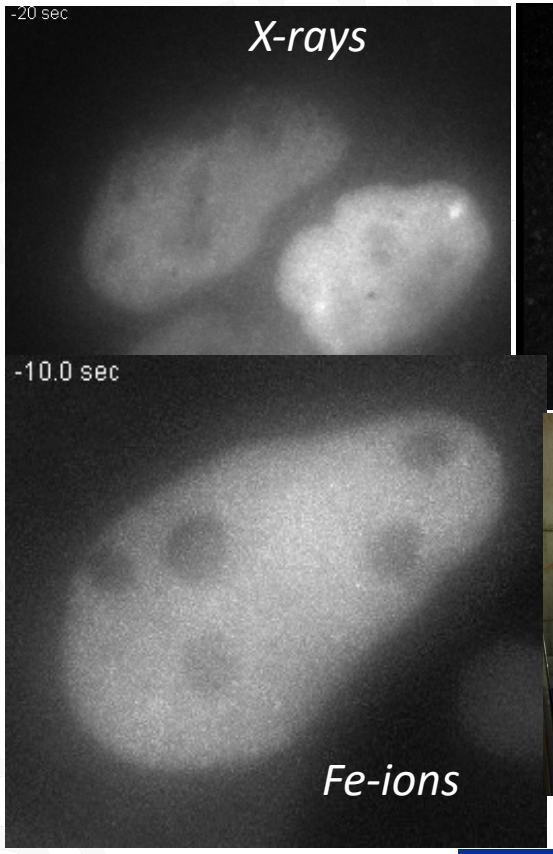
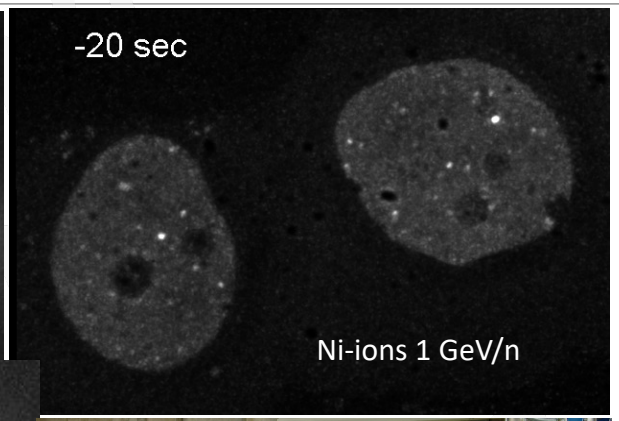
Heavy ion induced DNA damage

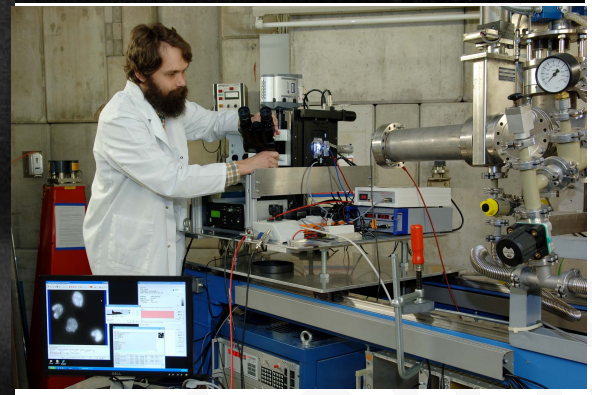




Courtesy of M. Scholz







B. Jakob *et al., Proc. Natl. Acad. Sci. USA* 2009;

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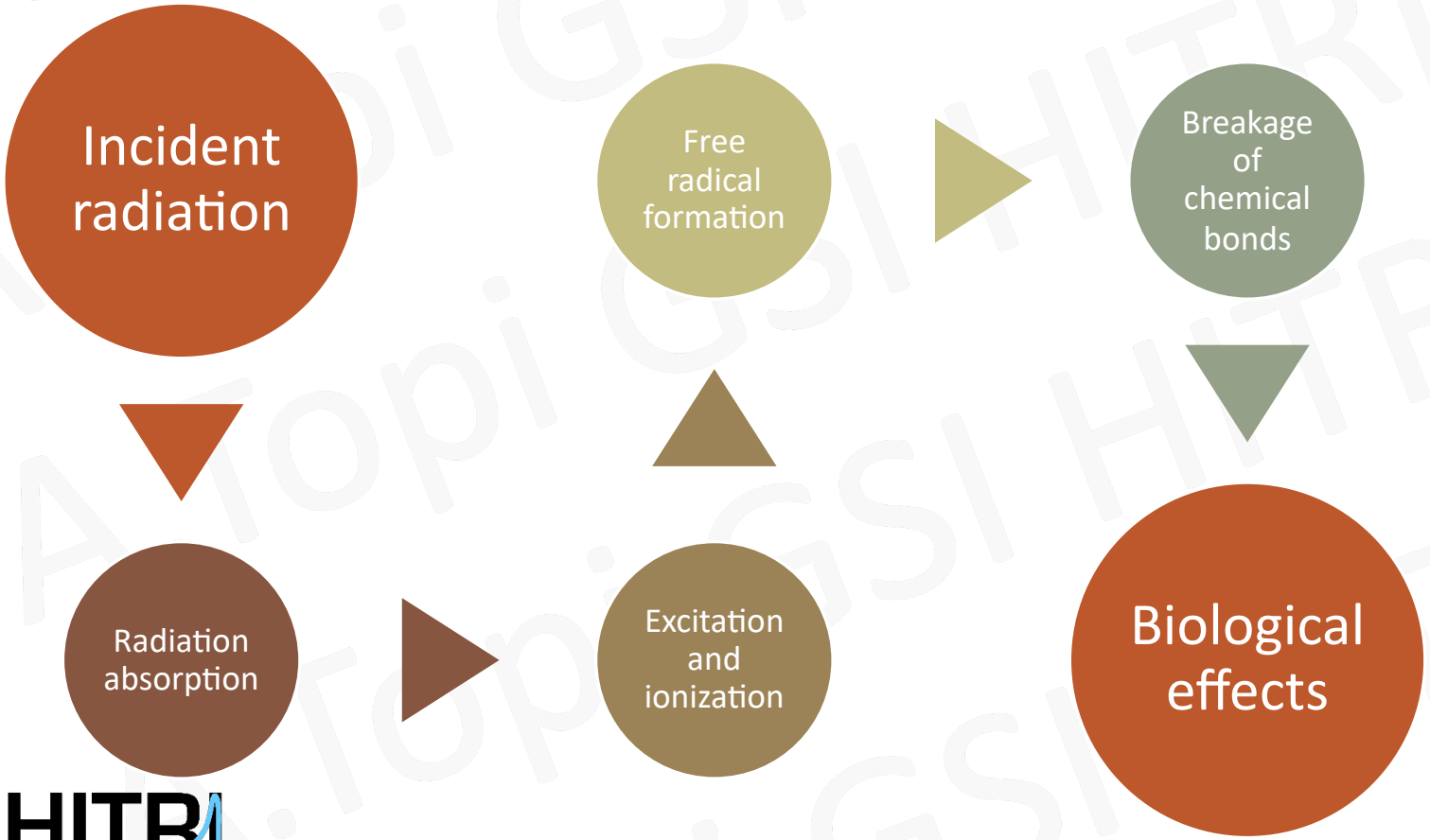
Radiobiological damage development



What happens with the irradiated medium?

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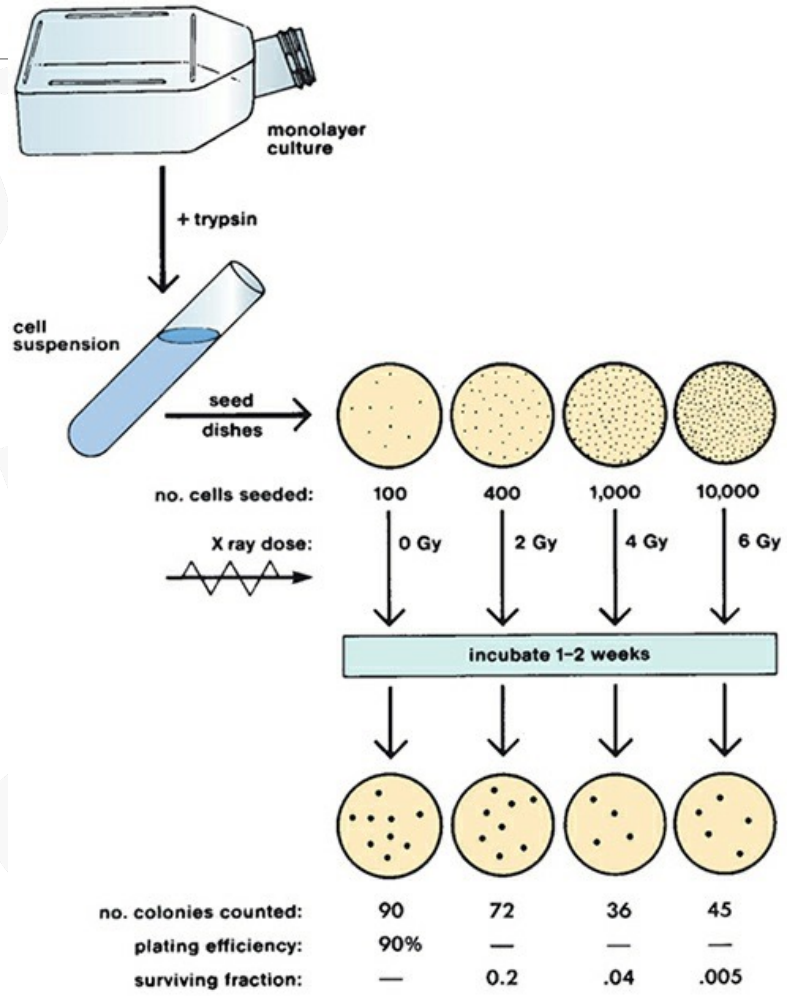
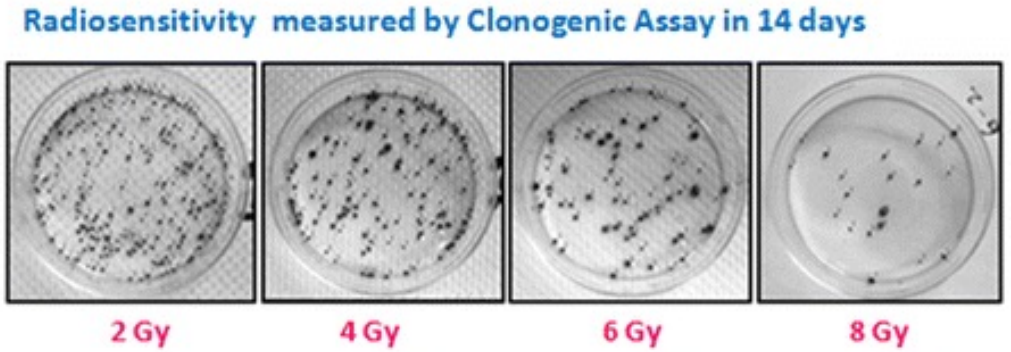
Clonogenic Cell Survival



Eric J. Hall and Amato J. Giaccia:
Radiobiology for the radiologist
8th edition, 2019



"Survivor" defined as:

1 cell (t=0) → 50 cell (t=7-10 days)

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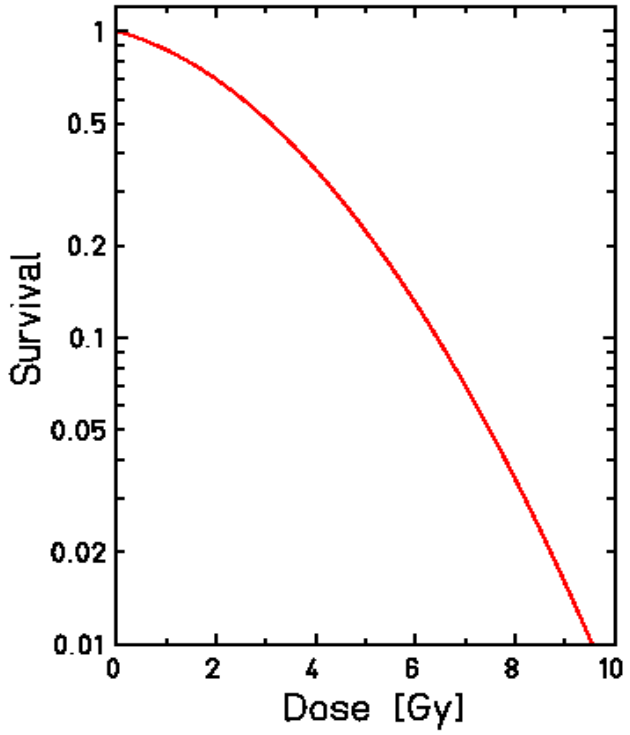
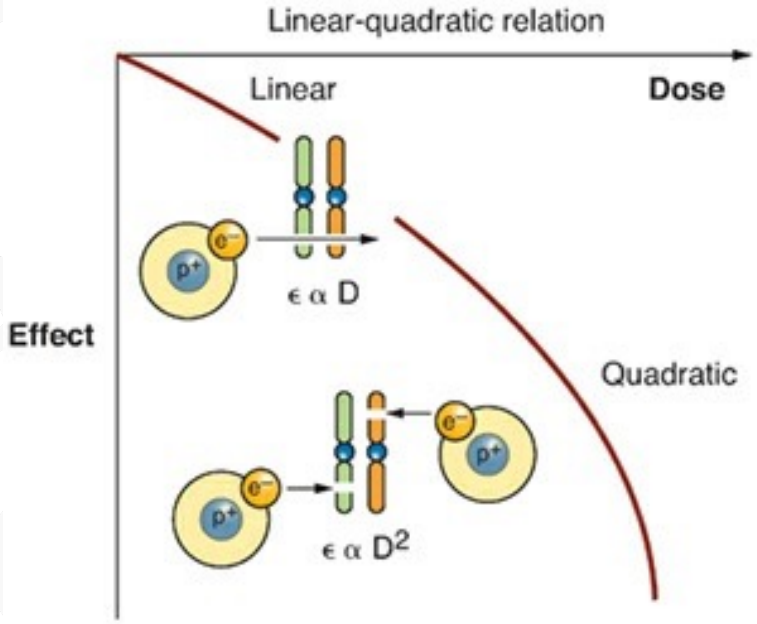
Linear quadratic model





- Linear component
  - Low dose
  - Breaks caused by a single electron
  - Probability of interaction is proportional to dose
- Quadratic component
  - High doses
  - Breaks caused by different electrons
  - Probability of interaction is proportional to (dose)^2

$$S = \frac{N_{col}}{N_{seed}} = e^{-(\alpha D + \beta D^2)}$$

S is the fraction of cells surviving a dose *D*
α and *β* are constants representing the linear and quadratic components of cell killing.

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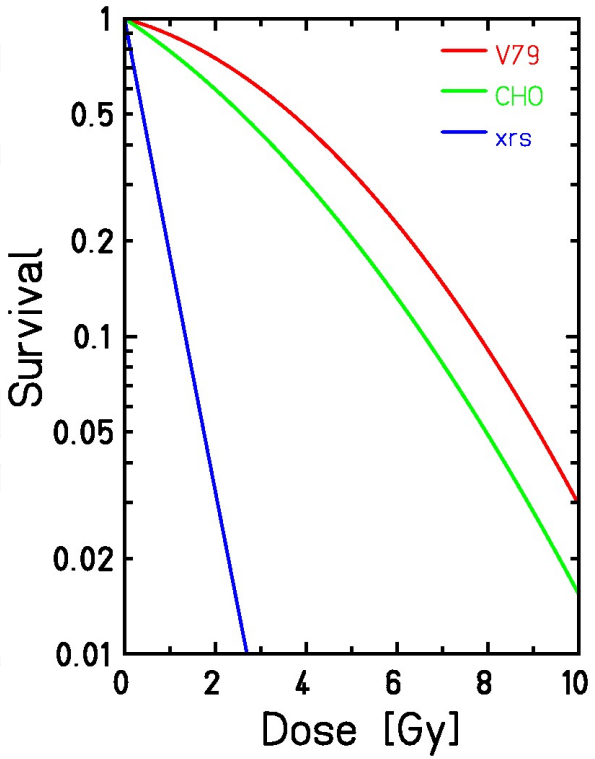
Sensitivity of different (tumour) cell lines



Typical cell lines in the lab:

V79 – hamster lung
CHO – chinese hamster ovary
XRS - repair deficient CHO
(X-ray sensitive) mutant cells
Deficient DSB repair
mechanisms

-> shoulder depends on repair

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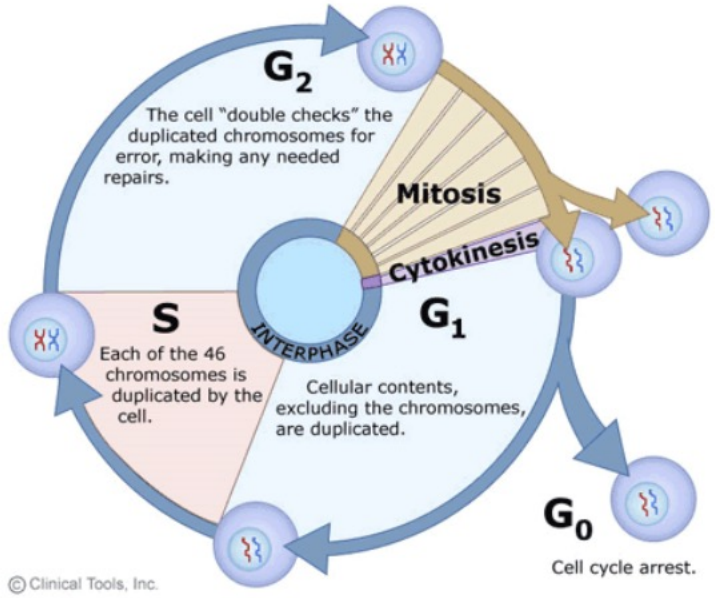
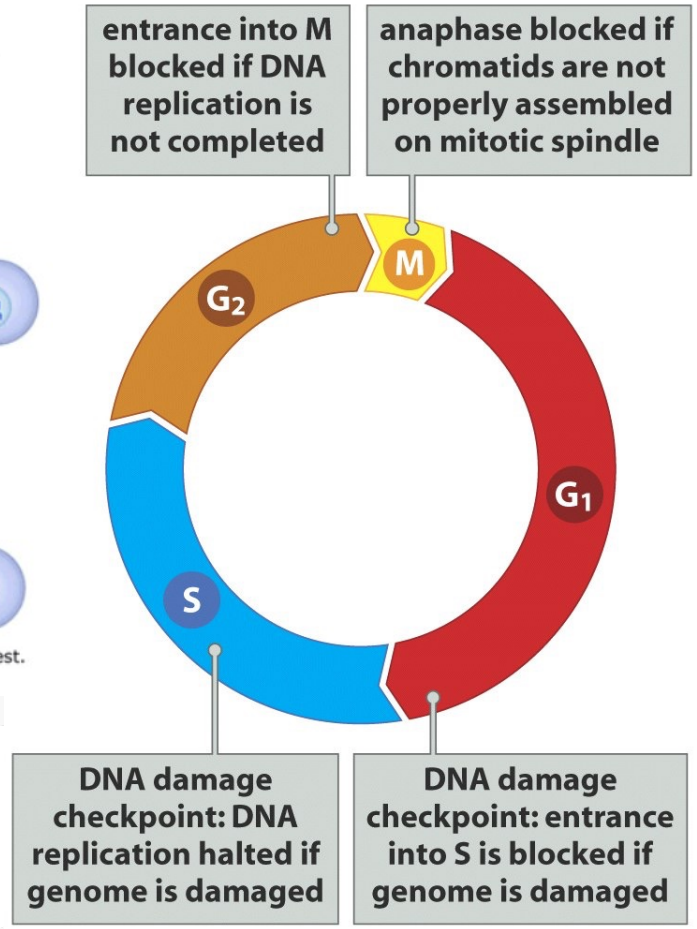
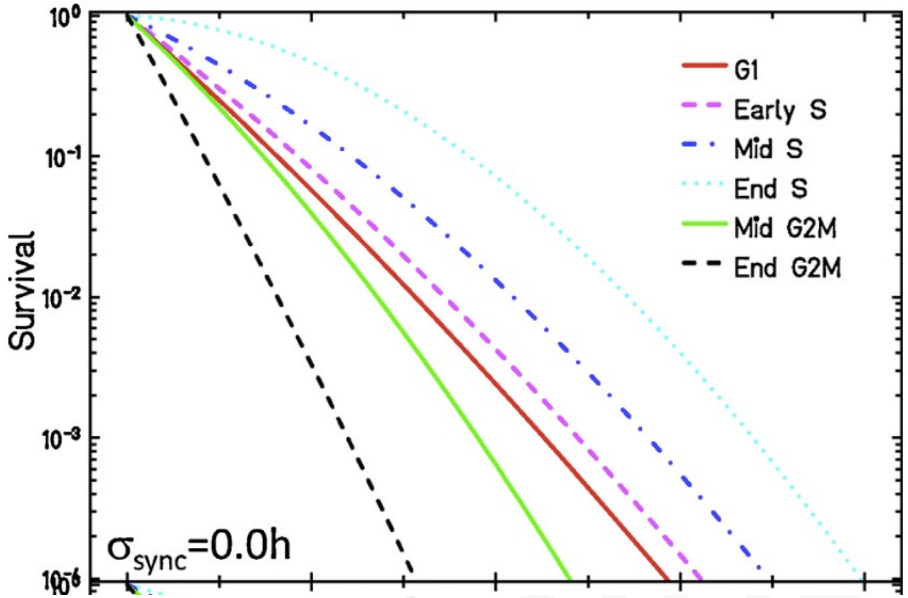
Cell cycle dependence







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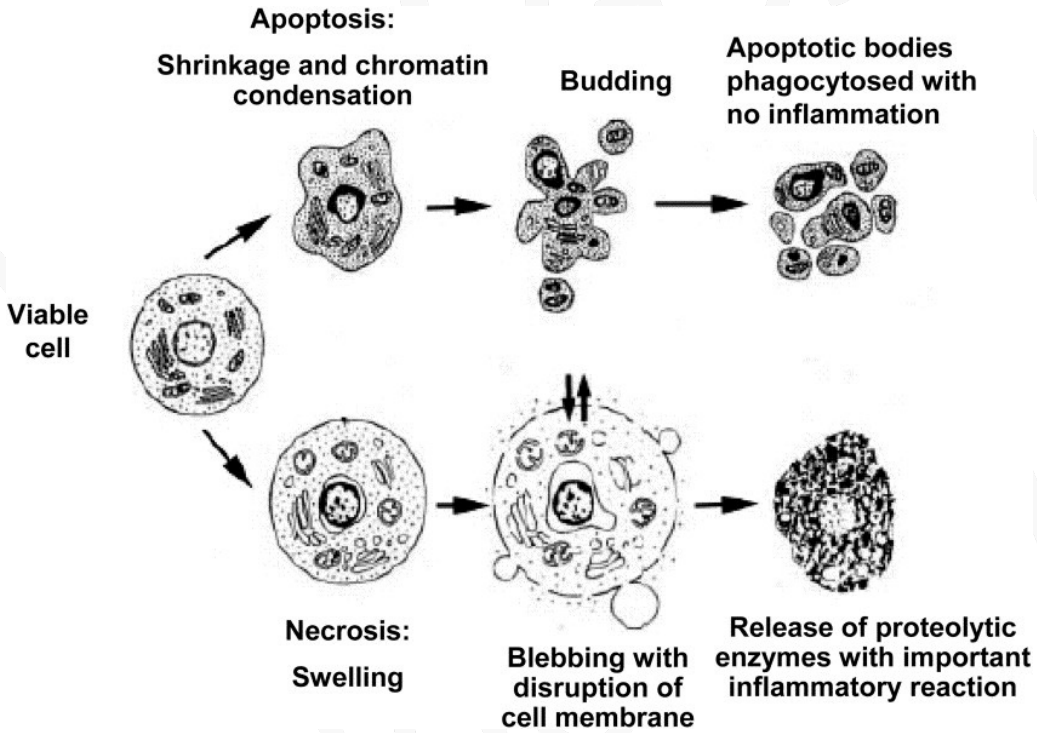
Cell death



necrosis: passive cell death

affects a group of cells

exhaustion of oxygen or nutriment	→ exhaustion of ATP in the cell	→ impairment of the cell membrane	→ enzyme release causes inflammation

apoptosis: active self-destruction

affects an isolated cell

triggering of the death program	→ intracellular signaling (caspases)	→ fragmentation of the cell into vesicles	→ phagocytosis by neighbor cells

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Cell death

Table 1 | **Types of cell death observed following treatment of cells with DNA-damaging agents**

Mode of death	General characteristics of death	Detection methods
Apoptosis	Cells visibly shrink and have condensed chromatin with nuclear margination and DNA fragmentation. Blebbing of cell membrane is often seen.	TUNEL staining; annexin-V staining; DNA laddering; caspase activation; electron microscopy; flow cytometry to detect cells with sub-G1 content.
Necrosis	Cells visibly swell and there is an early breakdown of the cell membrane. Cells have an atypical nuclear shape with vacuolization, non-condensed chromatin and disintegrated cellular organelles along with mitochondrial swelling. Typically not genetically determined.	Early permeability to vital dyes such as trypan blue; electron microscopy; flow cytometry for vital dye staining.
Mitotic catastrophe	Typically occurs after or during mitosis and is probably caused by mis-segregation of chromosomes and/or cell fusion. Cells often have micronuclei and it is common to see giant-cell formation or multinucleate cells. This can lead to apoptosis and is typically p53-independent.	Presence of micronuclei after mitosis; multinucleated cells detected by light or electron microscopy.
Senescence	Senescent cells are metabolically active but non-dividing and show an increase in cell size. These cells express senescence-associated β-galactosidase and this process is generally p53-dependent.	Staining for senescence-associated β-galactosidase.
Autophagy	This is a genetically regulated form of programmed cell death in which the cell digests itself. It is characterized by the formation of double-membrane vacuoles in the cytoplasm, which sequester organelles such as mitochondria and ribosomes. Autophagy is caspase and p53 independent.	Exclusion of vital dyes until late stages; prominent cytoplasmic vacuoles detected with monodansylcadaverine; lack of marginated condensed nuclear chromatin by electron microscopy.

TUNEL; terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labelling.

NATURE REVIEWS | CANCER

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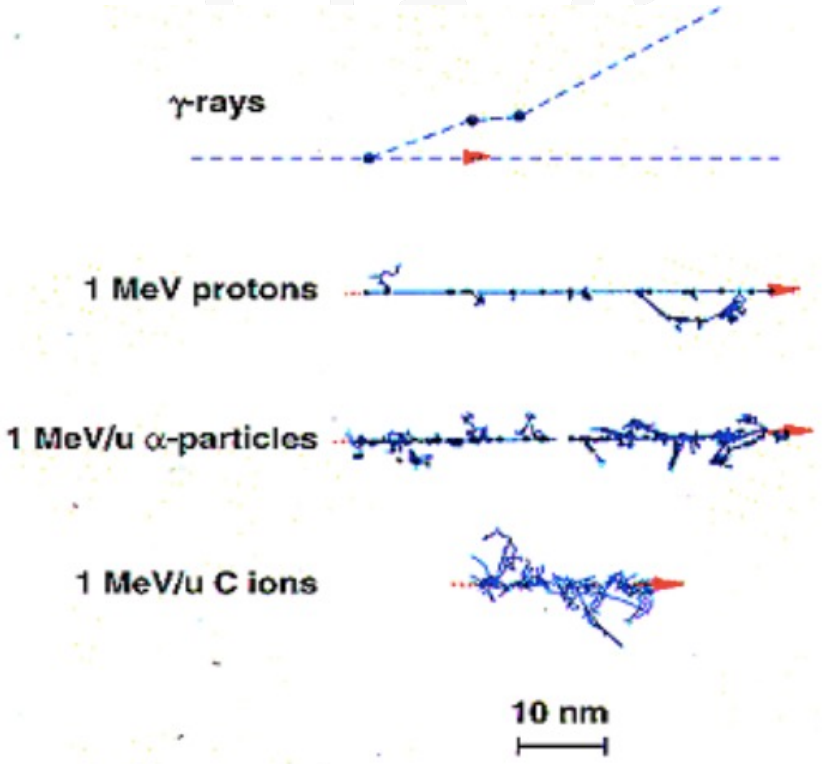
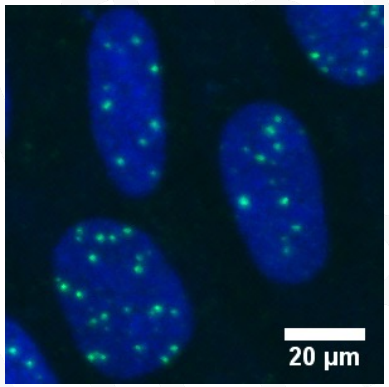
Towards RBE…

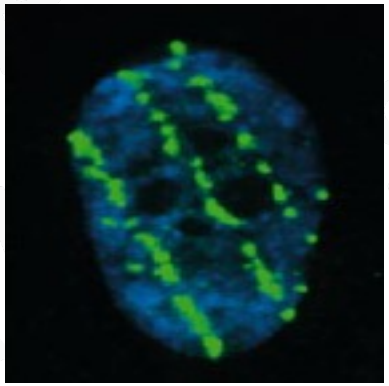


Linear Energy Transfer (LET): dE/dx
where dE is the energy locally imparted to the medium by a charged particle of specified energy in traversing a distance dx (ICRU)

γH2AX/DNA



0.8 Gy
X-rays



0.5 Gy
Iron Ion 176 keV/µm

Desai et al. 2005 Rad Res

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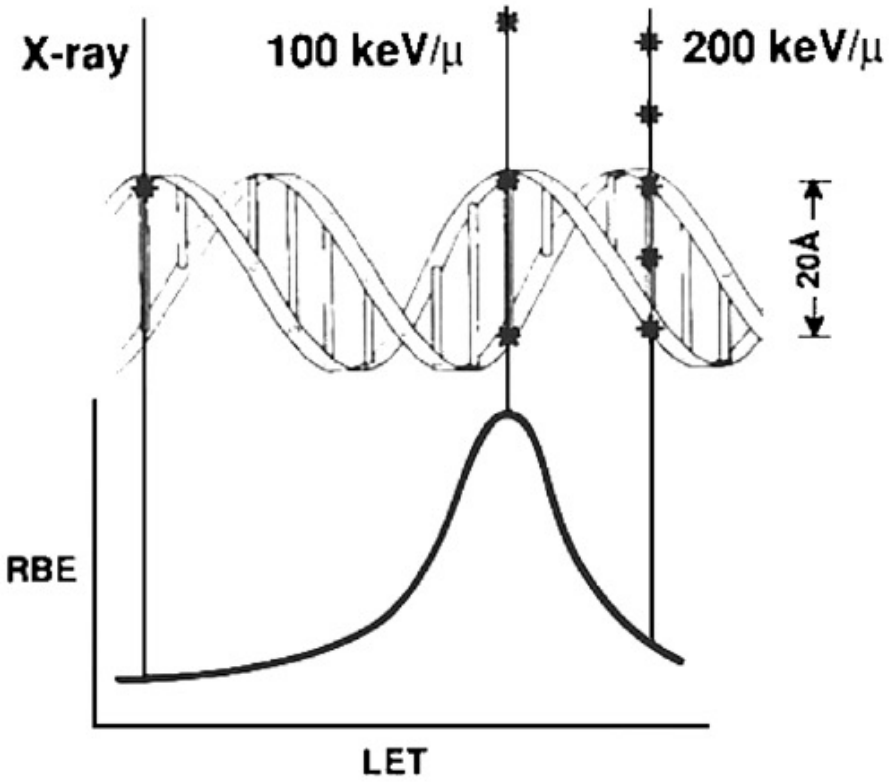
LET and DNA damage



Type of Radiation	LET (keV/µm)
Cobalt-60 gamma radiation	0.2
250 keV X-radiation	2.0
10 MeV protons	4.7
150 MeV protons	0.5
2.5 MeV α particles	166

Optimal LET at approx. 100 keV/µm
-> average distance of consecutive ionizations ≈ nm
-> comparable with width of DNA molecule: DSB induction!

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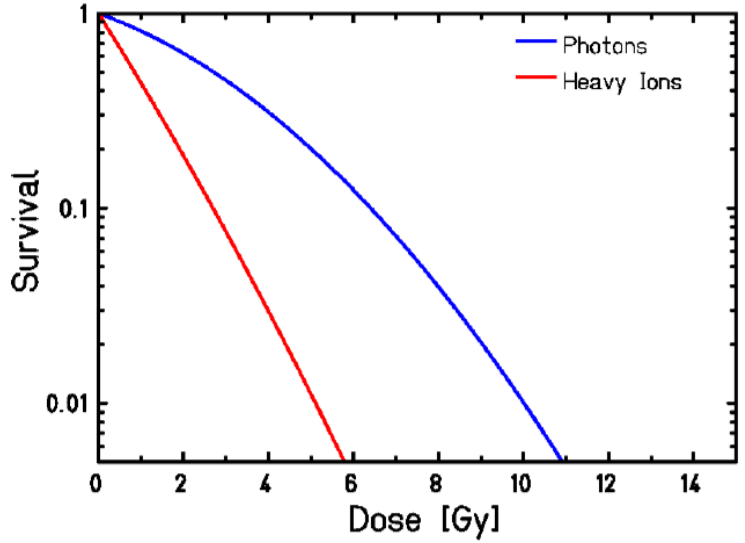
Relative Biological Effectiveness RBE

Increased effectiveness of ion beam radiation

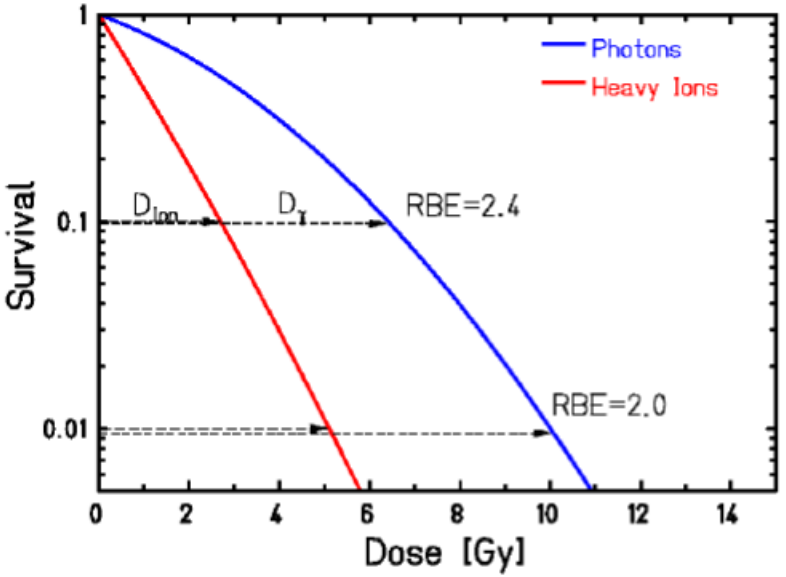


$$RBE = \frac{D_\gamma}{D_{Ion}}\Big|_{Isoeffect}$$

RBE depends on:

- Physical parameters (dose, LET, fractionation).
- Biological parameters (cell cycle, oxygenation, end-point).

Comparison of dose values at **Isoeffect-Level!**



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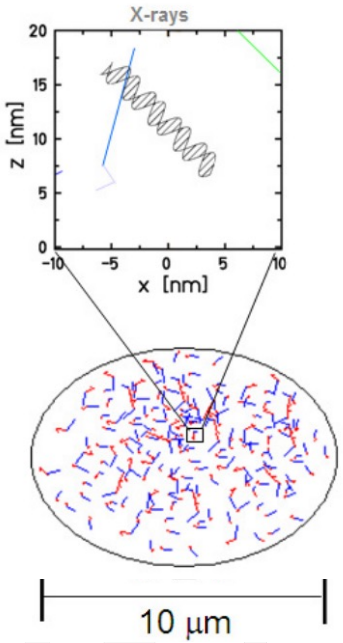
RBE

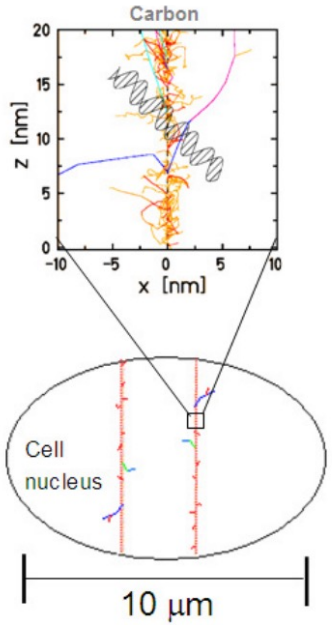
Photons and charged particles are characterized by a **different pattern of energy release**:

Photons



- secondary electrons mainly produced via Photoelectric and Compton effect
- sparsely ionization pattern
- random spatial distribution of energy deposition

Charged particles



- release of energy by secondary electrons concentrated around the particle track
- very localized ionization pattern
- a single charged particle can release a high amount of dose

M. Krämer

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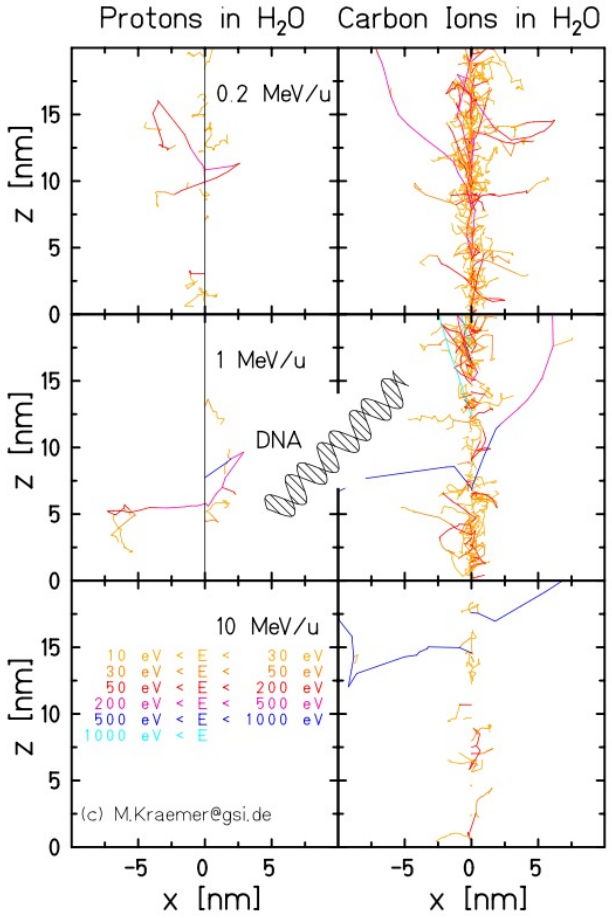
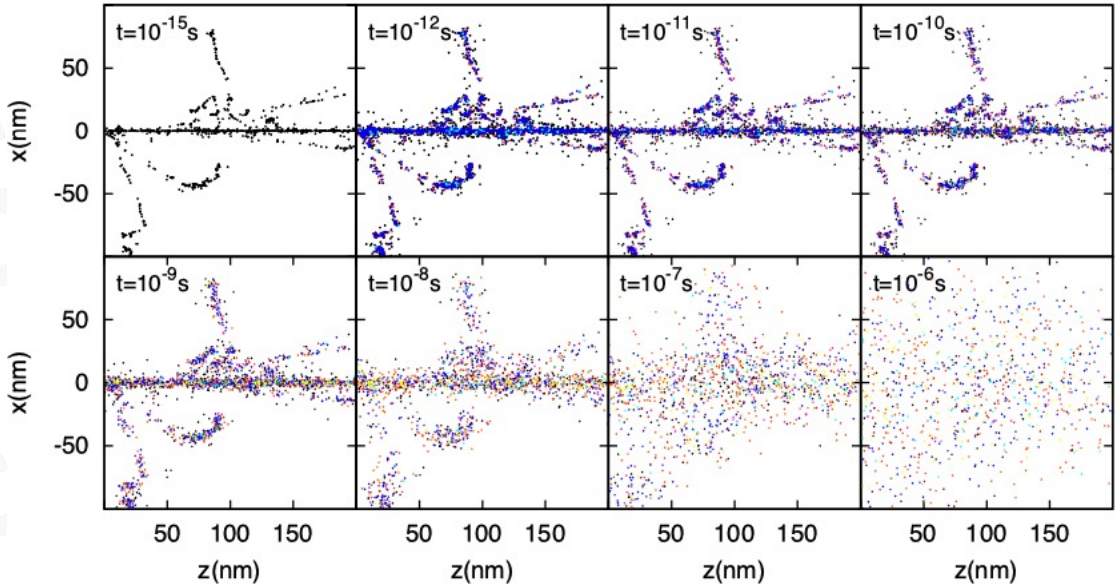
Microscopic simulation

TRAX MC code



Courtesy of Michael Krämer



Daria Boscolo PhD Thesis 2018

TRAXCHEM – TRAX extension with radicals

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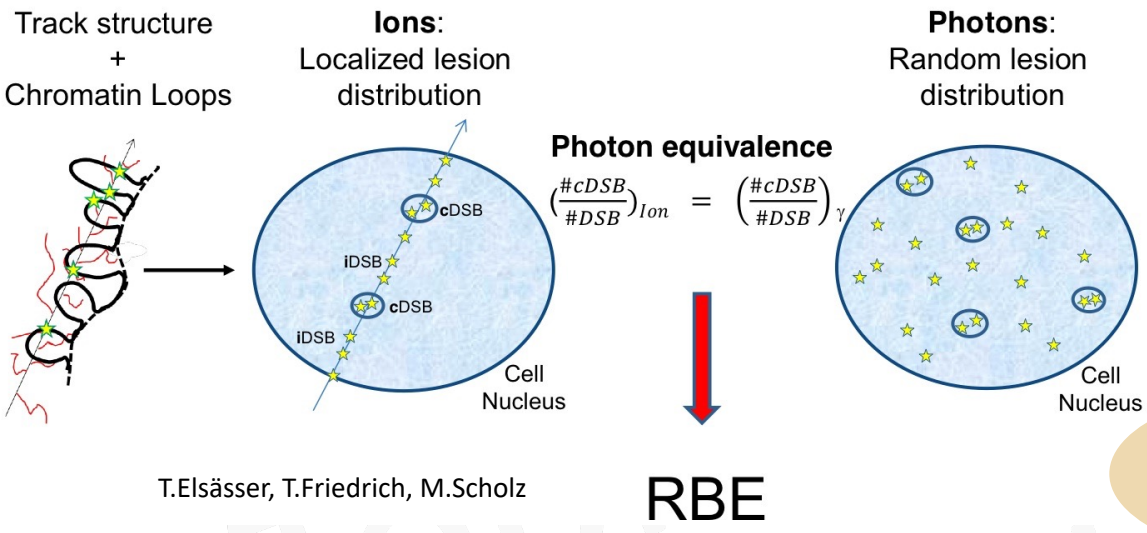
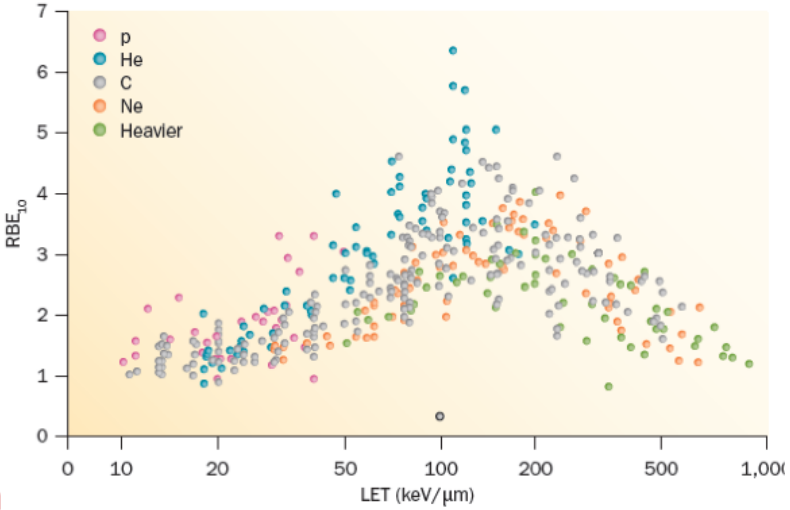
Local Effect Model (LEM)



Developed during GSI pilot project with 12C ion

Input parameters: α, β



PIDE database – http://www.gsi.de/bio-pide
Friedrich *et al., J. Radiat. Res*. 2013

On microscopic scale (local level) the same local dose produce equal biological effect, independent of radiation type.
→Microscopic energy deposit pattern of radiation can be used together with the biological system to low-LET radiation to estimate the macroscopic biological effect (RBE)

More info: www.gsi.de
https://www.gsi.de/en/work/forschung/biophysik/forschungsfelder/radiobiological_modelling

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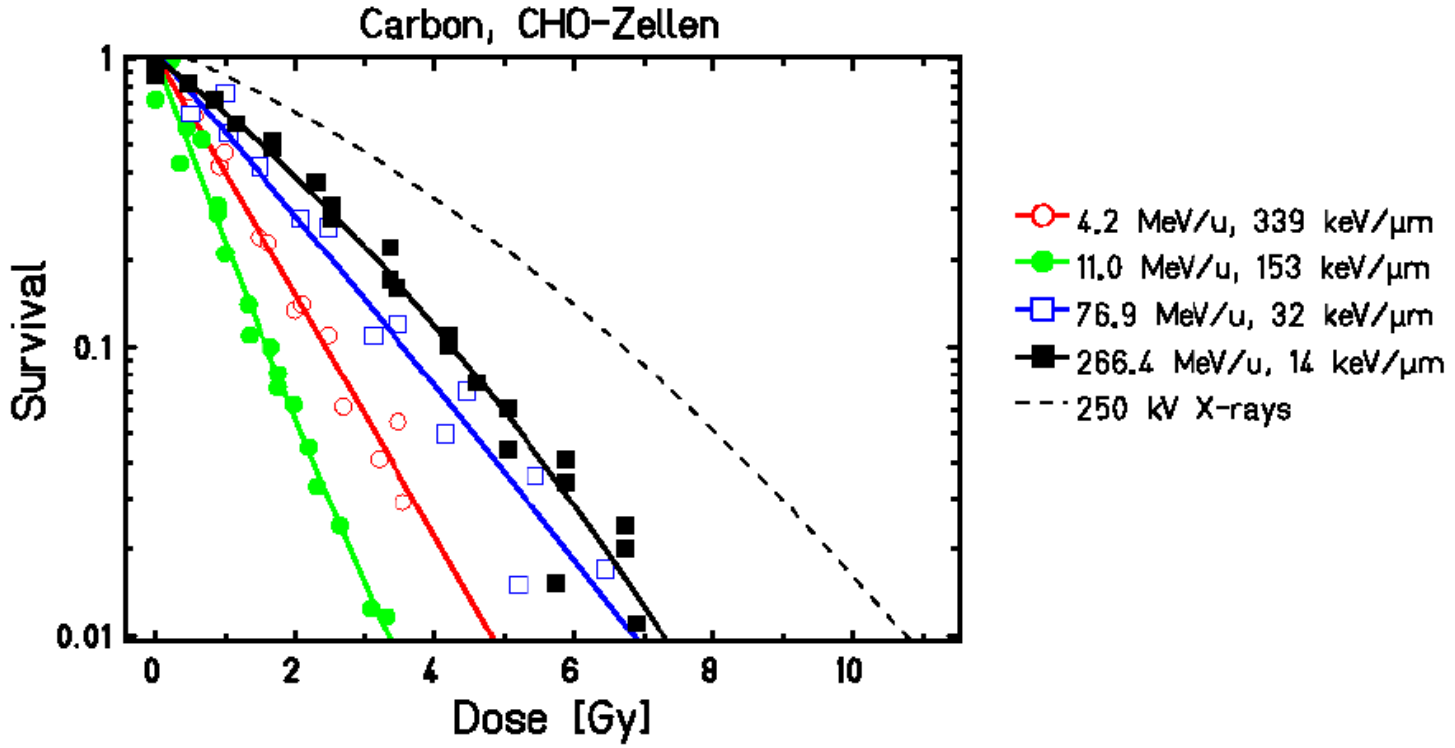
Energy/LET dependence of RBE



Weyrather et al. 1999

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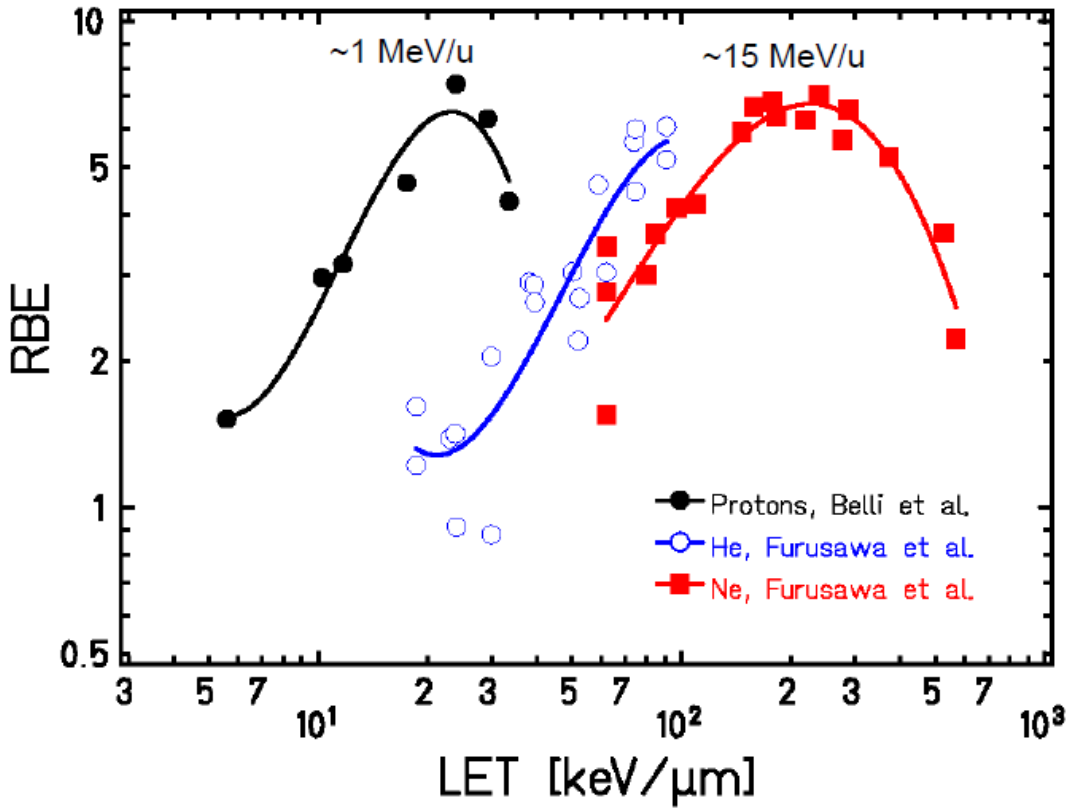
LET and particle dependence of RBE



RBE for a given endpoint cannot be single-value dependent on LET alone, but also on particle species, due to the different dose deposition profiles on microscopic scale

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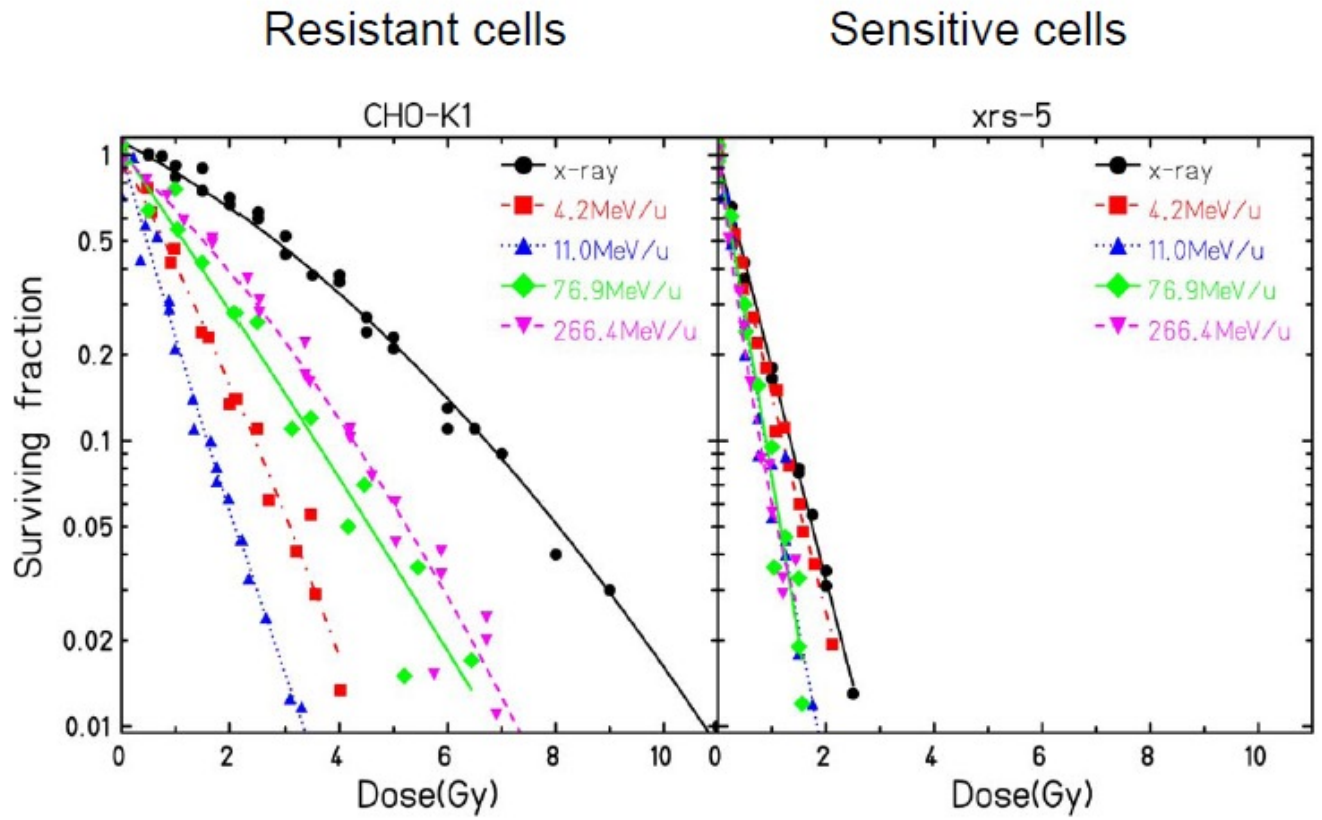
Cell line dependence of RBE



☺️ Same Physics

🤔 Different radiobiology

W. Kraft-Weyrather et al. 1999

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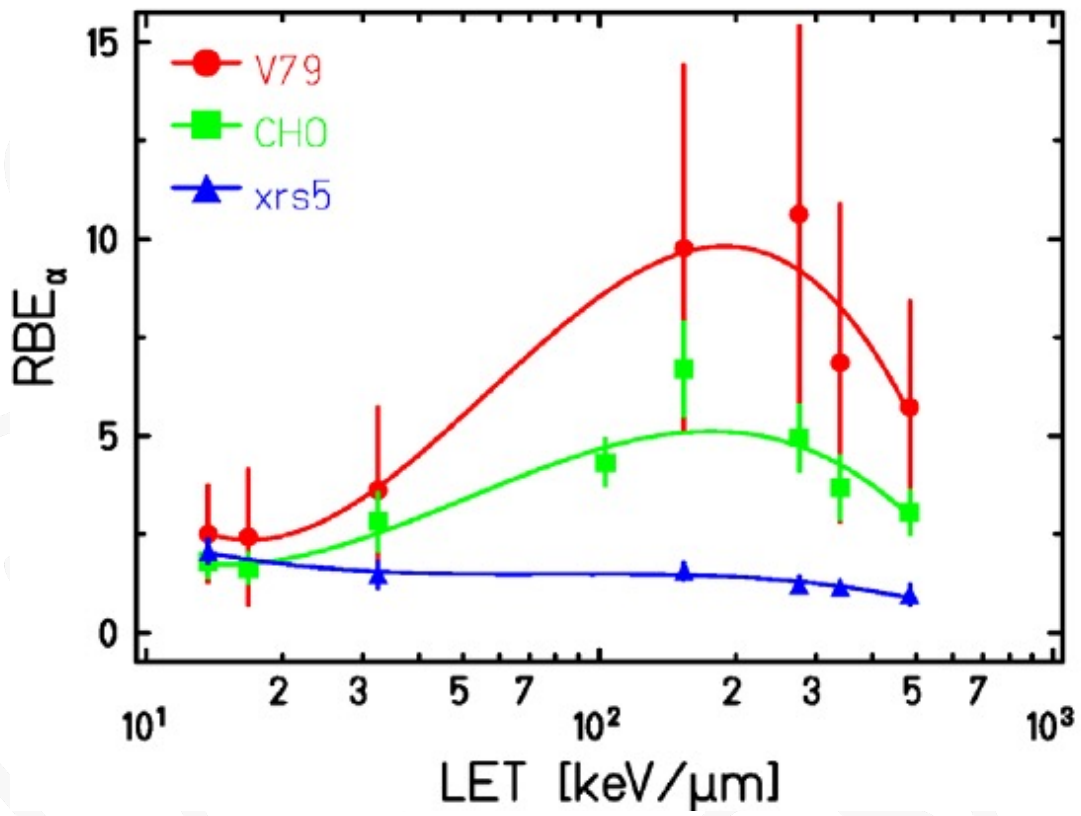
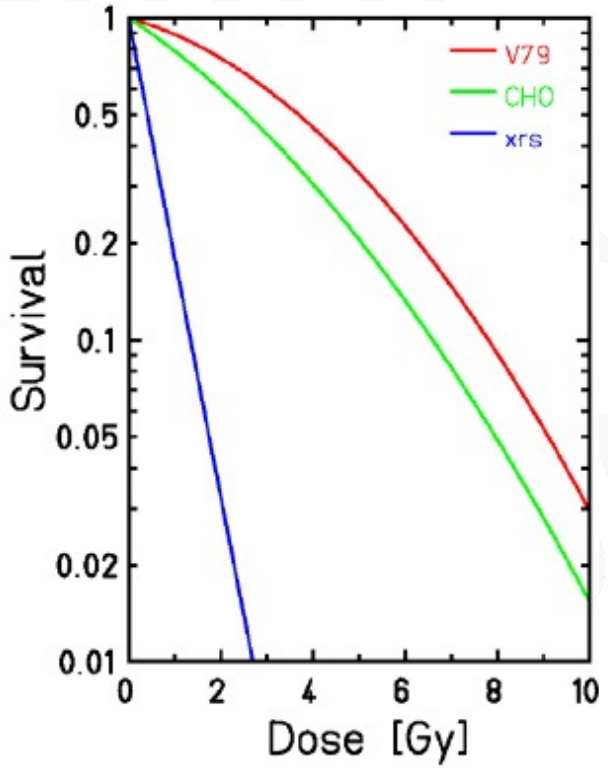
Cell line dependence of RBE





W. Kraft-Weyrather et al. 1999

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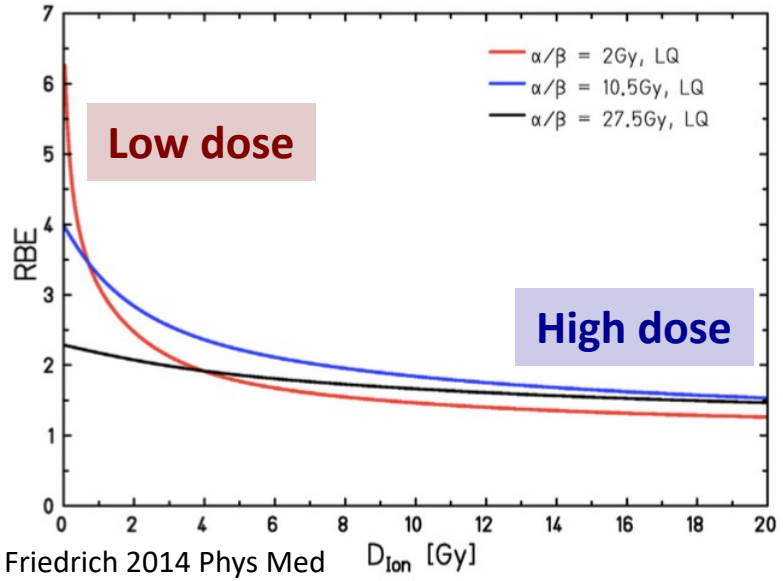
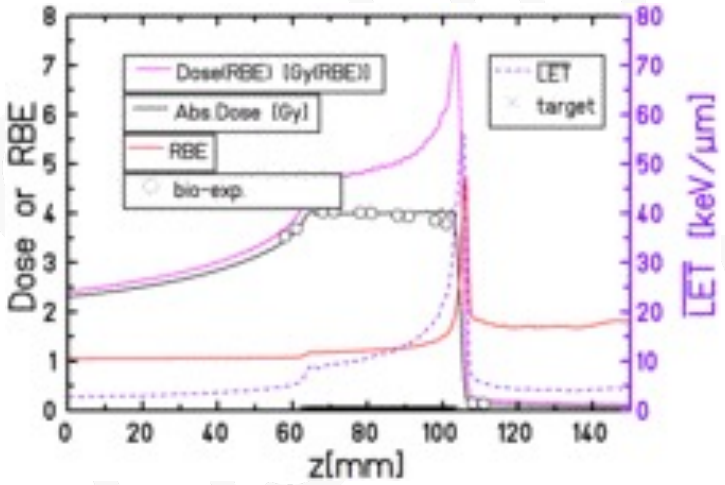
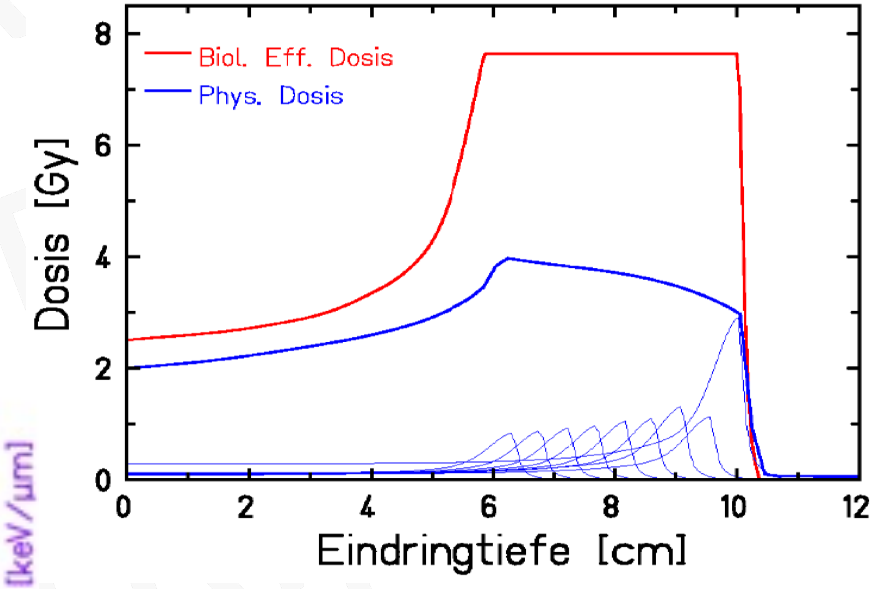
Biological effective dose - BED



Friedrich 2014 Phys Med

BED = RBE * Dose





- Dependence on dose
- Dependence on α/β ratio

-> inversion at high doses!

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Fractionation

Radiotherapy is fractionated to spread the treatment over several weeks.

Why?
Advantage of repair abilities of normal and malignant tissues.

The multifraction regimens commonly used in radiation therapy are a consequence largely of radiobiologic experiments performed in France in the 1920s and in the 1930s.

Regaud and The French Ram

- A single radiation dose can sterilize but with significant skin toxicity
- Dose delivered in several fractions sterilized the ram without skin toxicity

Regaud -> extended treatment time for uterine cancer improved the outcome
Coutard -> used the benefit of fractionation for head and neck cancer

Eric J. Hall and Amato J. Giaccia: Radiobiology for the radiologist 8th edition, 2019

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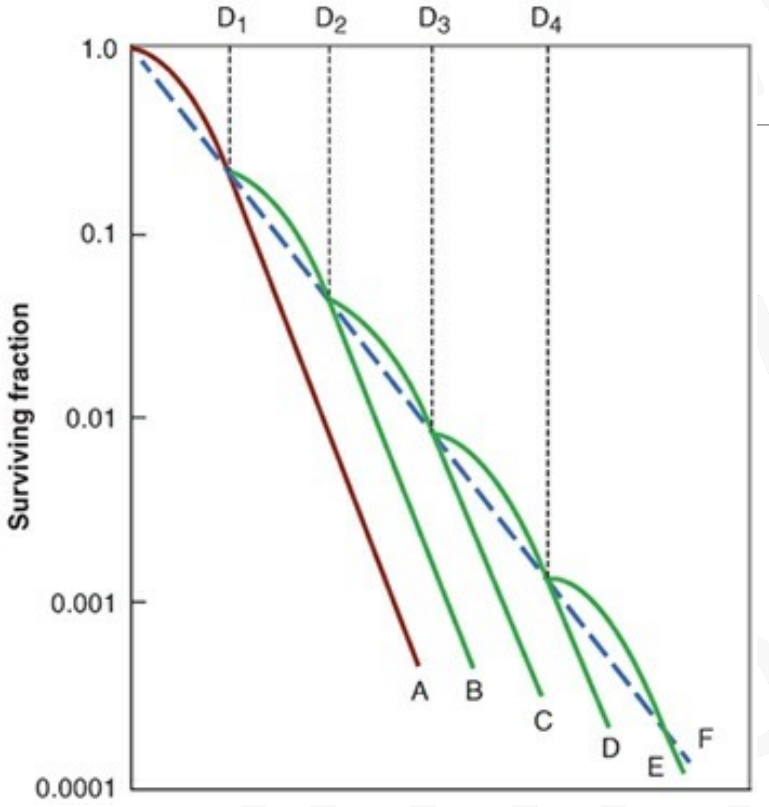
Fractionation

In using the LQ model, it is assumed that each fraction has an equal effect.
For a fractionated regime
(n fractions of size D):

$SF = [\exp -(\alpha D+\beta D^2)]_n$

or

$-\ln SF = n(\alpha D+\beta D^2)$



Idealized fractionation experiment.

Eric J. Hall and Amato J. Giaccia: Radiobiology for the radiologist 8th edition, 2019

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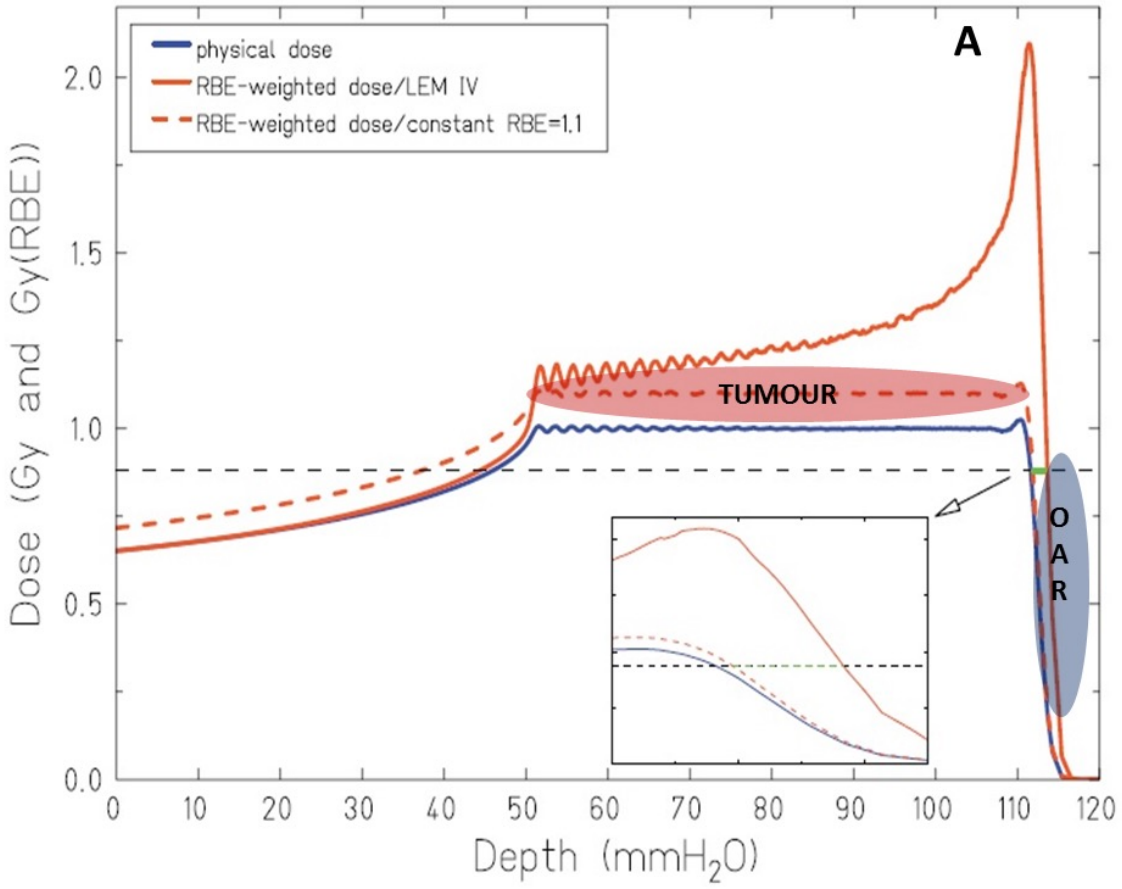
Biological Range Uncertainty

(sums up to physical range uncertainty!)



Grün 2013 Med Phys

Distal SOBP:

- Parallel decrease of dose and increase in LET
- RBE is highly uncertain
- **RBE can be >>1 in OAR!!**
- **Better characterization -> more accurate treatments**
- **Biological optimization strongly advised**

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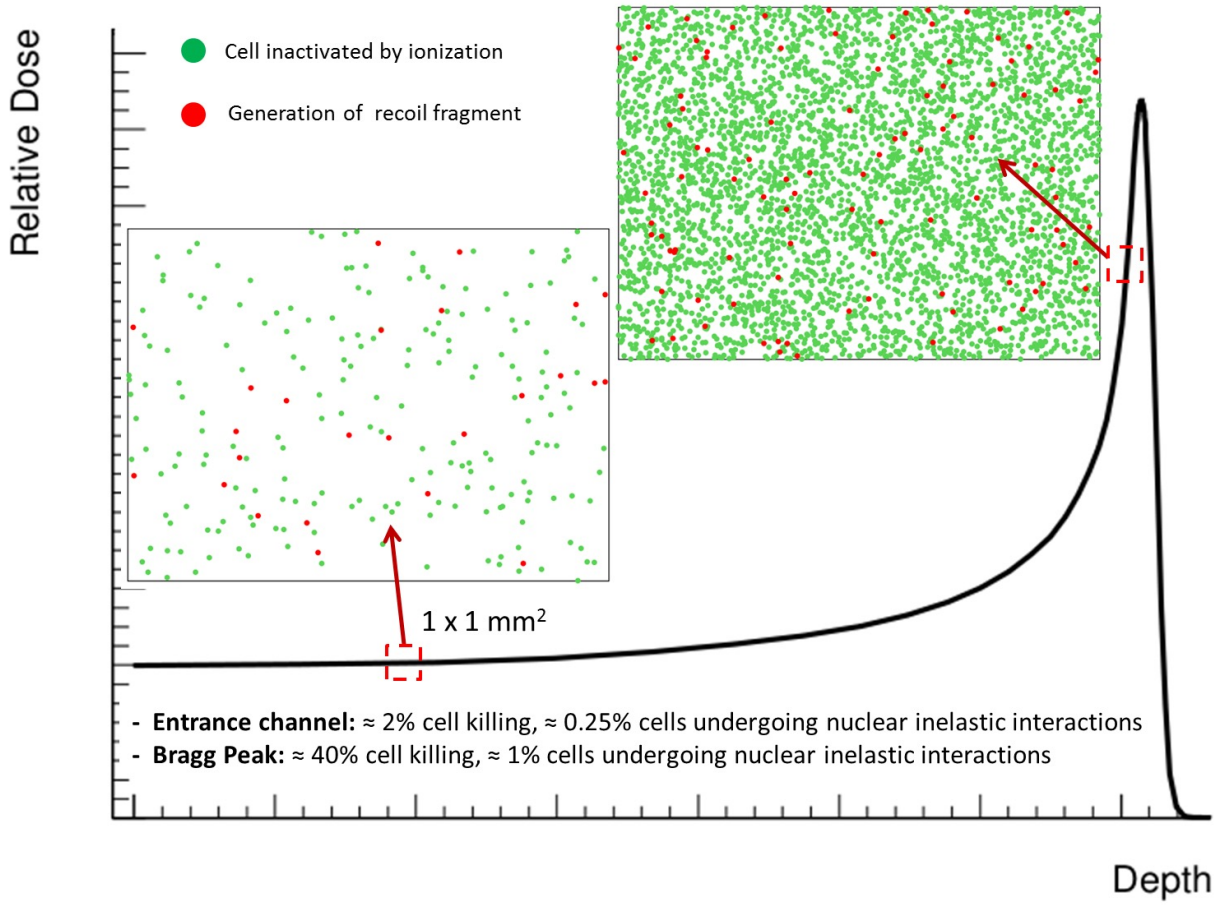
Target fragmentation in proton therapy



About 10% of biological effect in the entrance channel due to secondary fragments

Largest contributions of recoil fragments expected from **He, C, Be, O, N**

See also dedicated MC studies:
- Paganetti 2002 PMB
- Grassberger 2011 PMB

Courtesy of Francesco Tommasino

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Impact of target fragmentation in clinical practice

- **Currently the contribution of target fragments is implicit (RBE=1.1)**
- **Improved description would be needed for:**
  - Better definition of peak-to-entrance ratio
  - Side effects in the entrance channel (NTCP) and dose to target (TCP)
  - Prediction of secondary cancer risks
  - Implications for space radiation research

→**FOOT experiment** (TIFPA-INFN) for the measurement of target fragments in proton therapy

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Quickly dividing tumor cells are generally more sensitive than the majority of body cells.

This is not always true → Hypoxia

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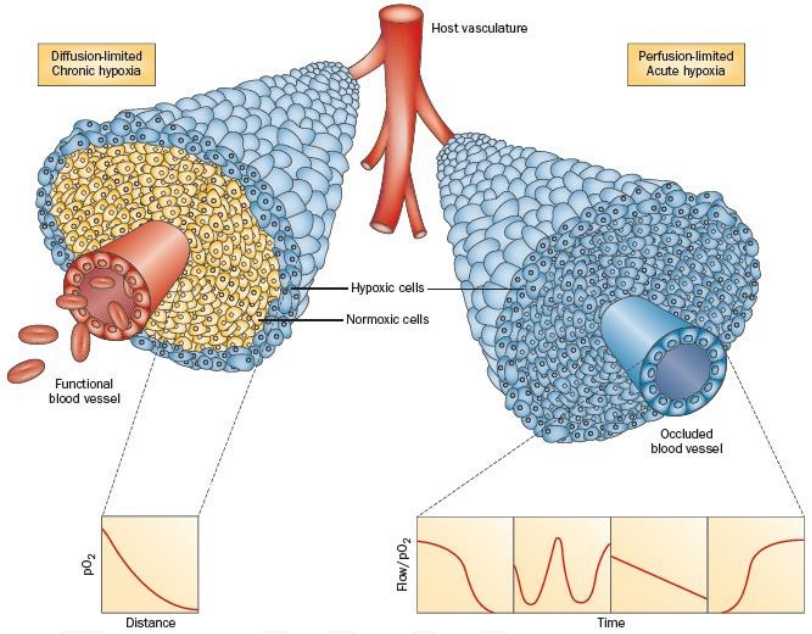
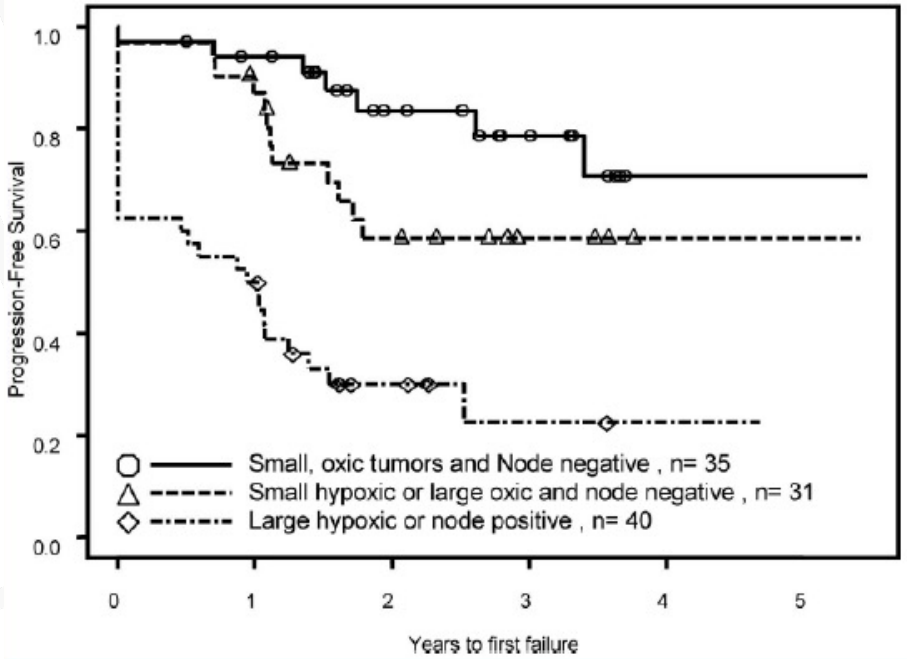
Hypoxia

↘ O_2 pressure ➡️ ↘ Radiosensitivity



Horsman et al Nat. Rev. Clin. Oncol. (2012)

😱 Significantly reduced patient survival rate



Fyles *J Clin Oncol.* (2003)

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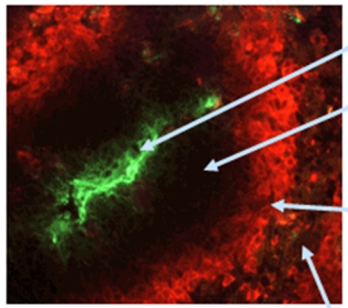
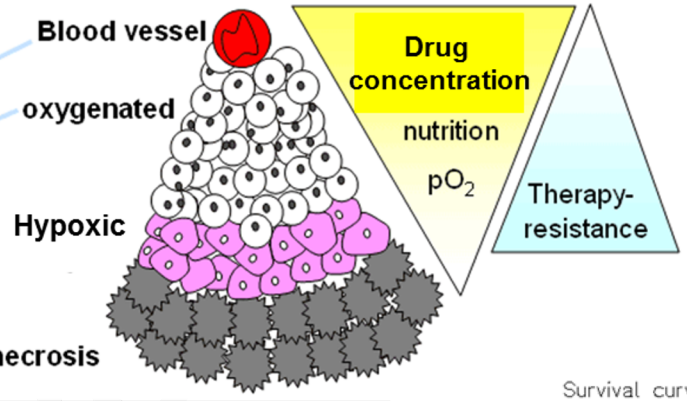
Oxygen Enhancement Ratio



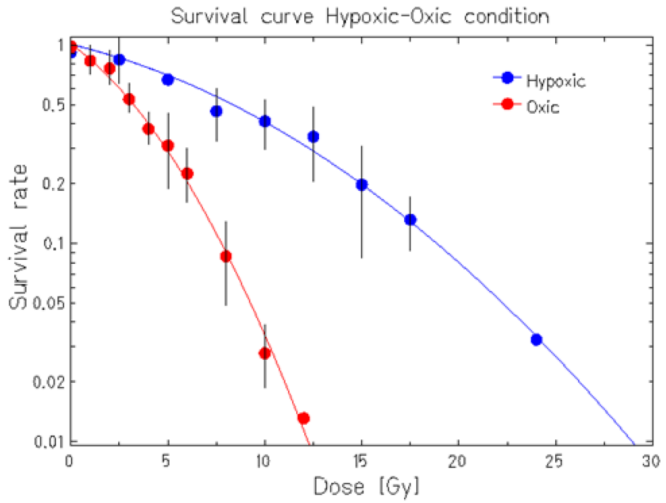
Green: CD31
Red: anti-Pimonidazole

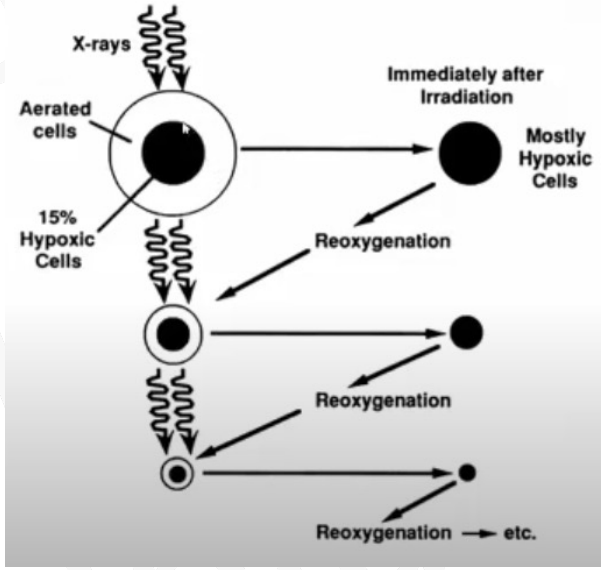


Research Institute, Osaka Medical Center for Cancer and Cardiovascular Diseases

OER-> Ratio of doses without and with oxygen to produce the same biological effect

$$OER = \frac{D_{hypoxic}}{D_{normoxic}}\Big|_{sameeffect}$$





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Take home message

- Radiobiology research is essential in particle therapy
- Heavy ion biological effects are qualitatively different from X-rays
- Biological optimisation in treatment planning is crucial for a proper planning.
- Fractionation gives better tumor control for a given level of heathy tissue toxicity than a single large dose.
- Oxygenation level in tissues plays an important role in radiotherapy outcome.

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Thank you

This material was prepared and presented within the HITRIplus Heavy Ion Therapy MasterClass school, and it is intended for educational purposes to facilitate students; people interested to use any of the material for any other purposes (such as other lectures, courses etc) are kindly requested to please contact the authors

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Credits:

Marco Durante, Michael Krämer, Wilma Kraft-Weyrather, Walter Tinganelli, Thomas Friedrich, Michael Scholz, Burkhard Jakob … and many others.

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