HITRI plus

Heavy Ion Therapy Research Integration

Radiobiology and General Introduction

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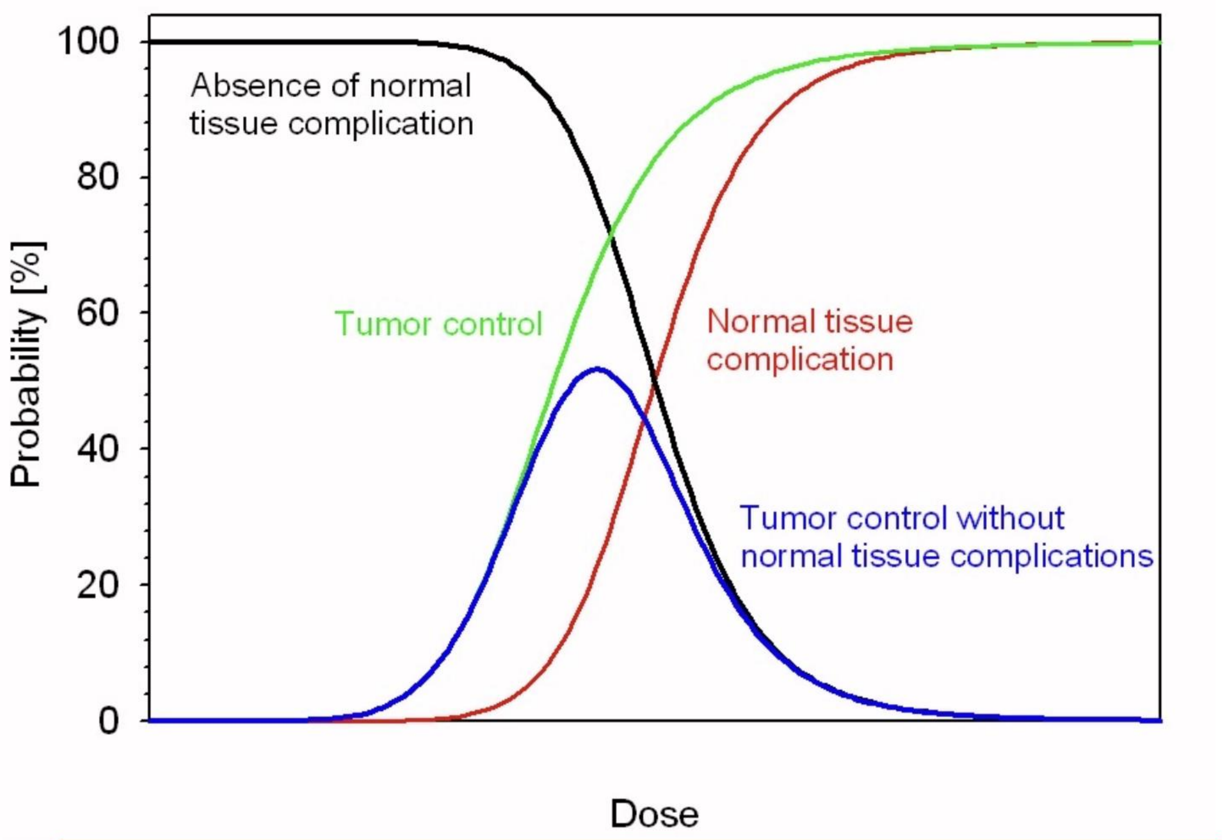
Heavy Ion Therapy Research Integration (HITRI)

3rd July, 2023

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

The therapeutic window in radiotherapy



Marco Durante presentation
PTCOG61- Educational Session

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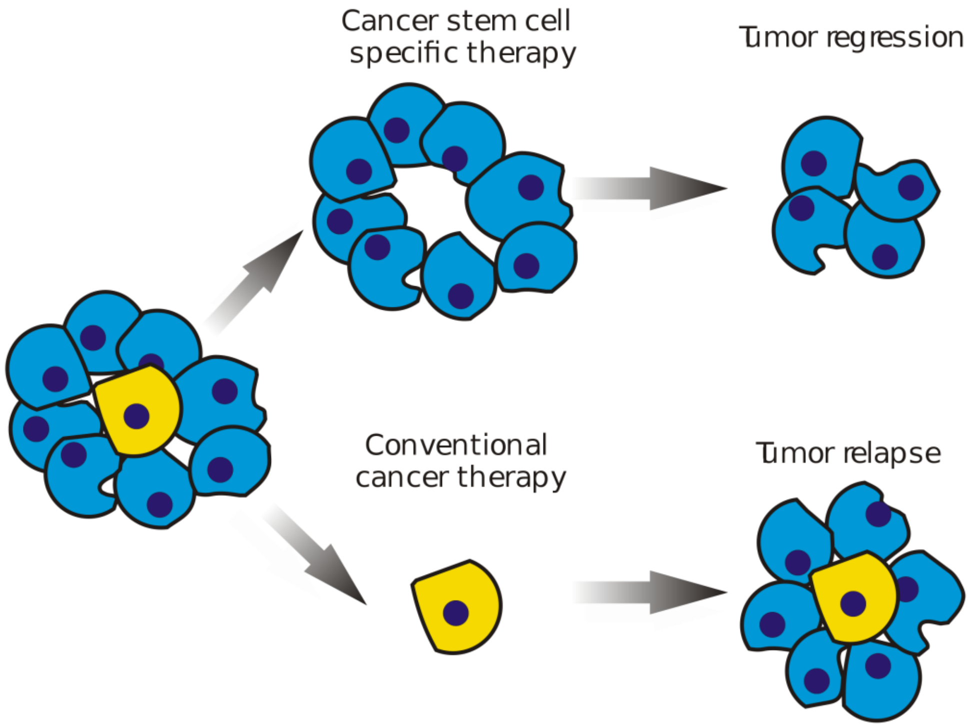
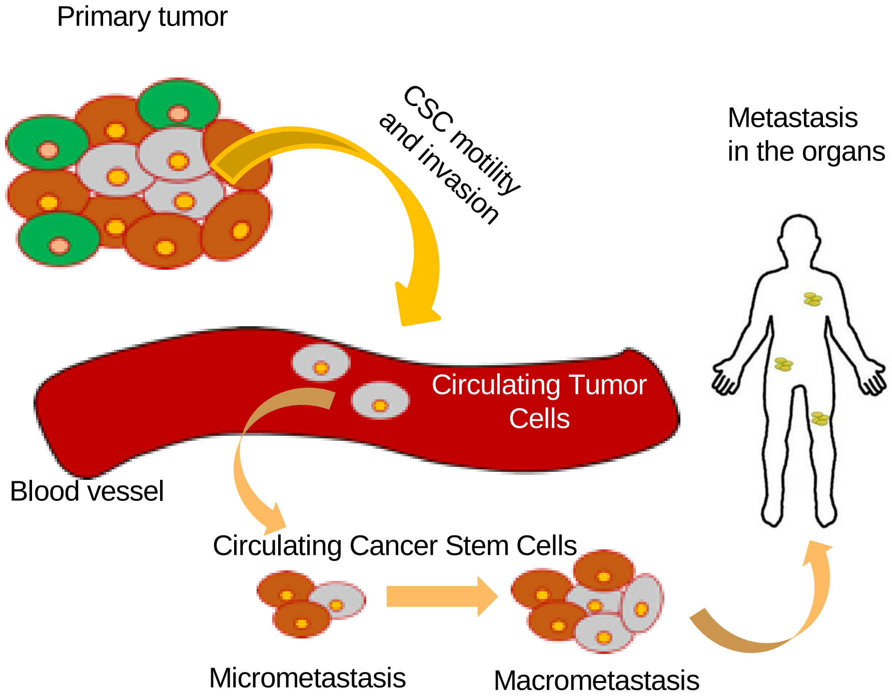
CANCER STEM CELLS and CIRCULATING CANCER STEM CELLS



Peter Znamenskiy from wikipedia



Self made picture.

Courtesy of: Martina Quartieri

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Types of 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.

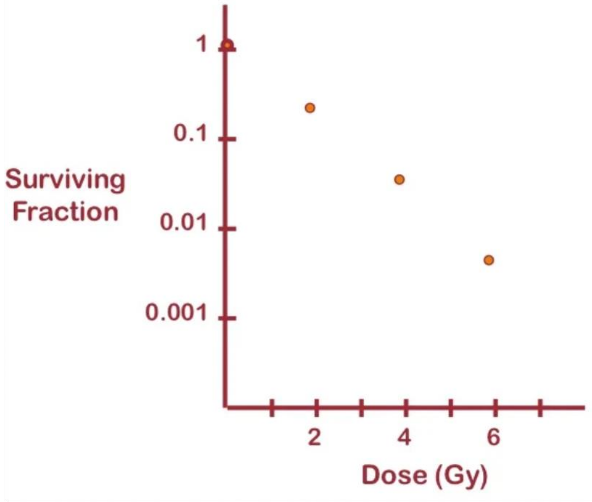
TUNEL; terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labelling.

Okada and Mak, *Nat. Rev. Cancer* 2004

Survival Curve/clonogenic assay

(Clonogenic survival is defined as the ability of a single cell to give rise to a colony)

Most modern radiobiology theory is based, to some extent, on the cells survival curve.
The survival describes relationship between radiation dose and the fraction of cells that “survive” that dose. Used to assess biological effectiveness of radiation.



Survival means:
Retention of reproductive integrity;
The capacity for sustained proliferation in cells that proliferate;

The proof of reproductive integrity is the capability of a sinlge cells to grow into a large colony, visible to the naked eye;

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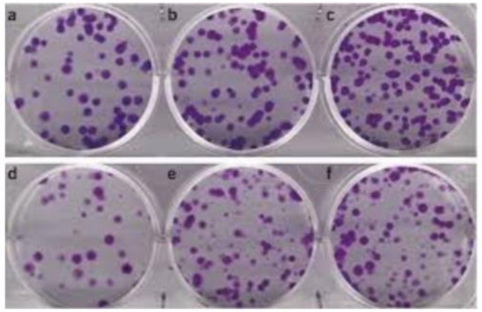
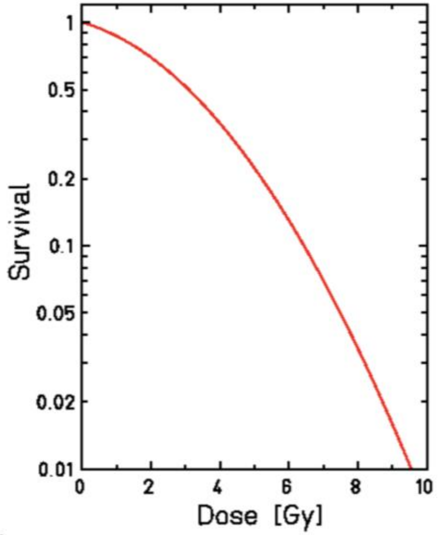
Survival after X- or γ-rays

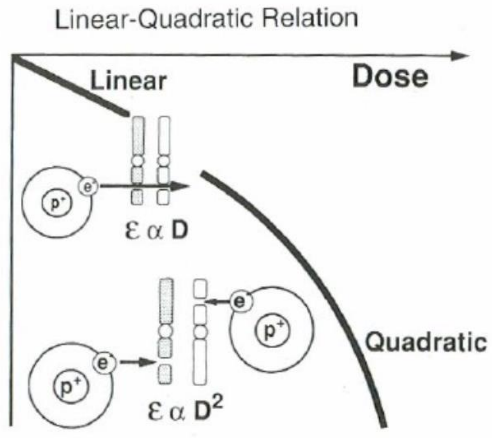




$$S = \frac{N_c}{PE \cdot N_0} = e^{-(\alpha D + \beta D^2)}$$

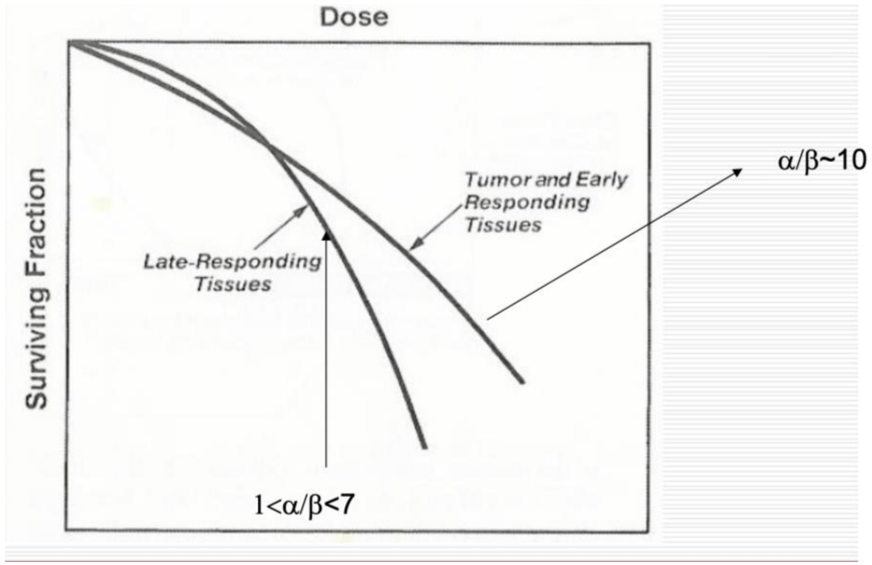
α[Gy^{-1}]: initial slope
β[Gy^{-2}]: bending of curve
α/β[Gy]: dose, at which contribution from linear term
= contribution from quad. term

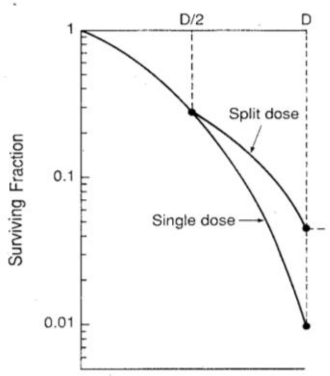
- linear component
  - low doses
  - breaks caused by a single electron
  - probability of interaction is proportional to dose
- quadratic component
  - higher doses
  - breaks caused by different electrons
  - probability of interaction is proportional to (dose)^2



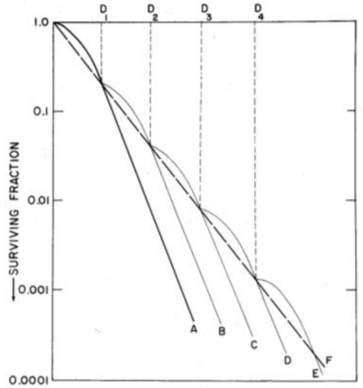
Kellerer and Rossi, *Curr. Top. Radiat. Res. Quart*. 1972

Marco Durante
Educational Session
PTCOG61





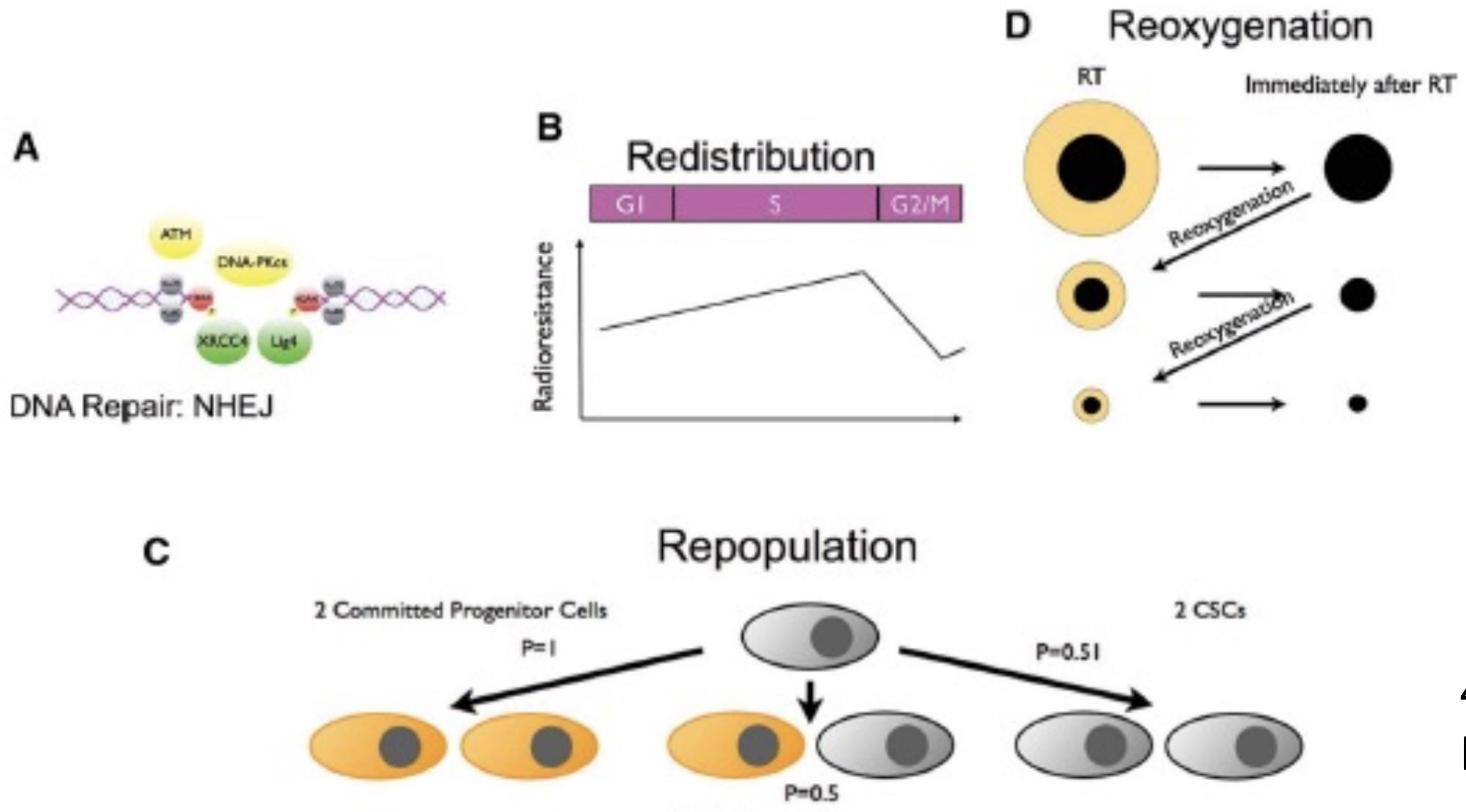
Surviving cells behave like unirradiated cells, if dose is split and time for recovery is given



Fractionation (Protraction) reduces the effect of total dose

Radiation Resistance of Cancer Stem Cells: The 4 R's of Radiobiology Revisited

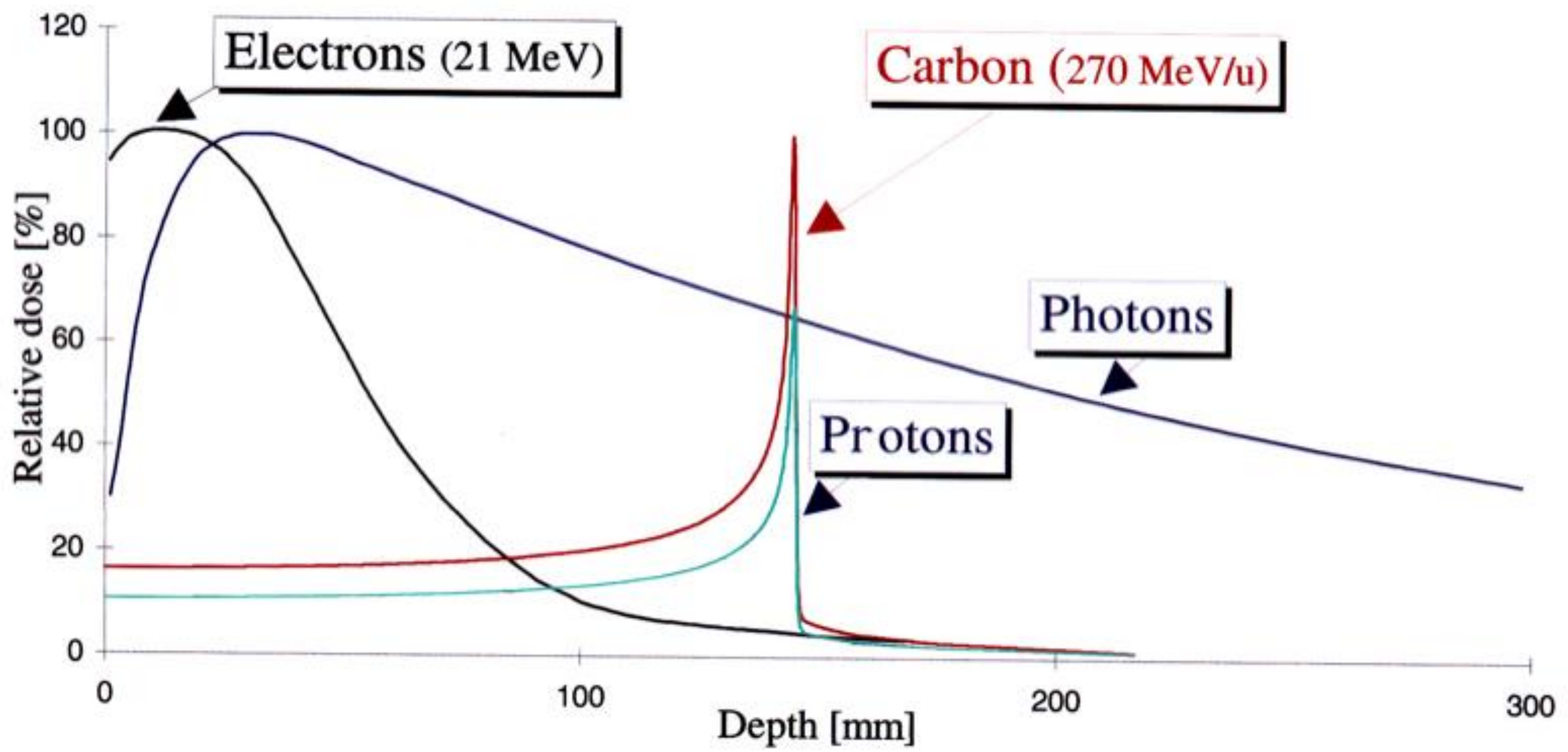
Frank Pajonk,[a,b] Erina Vlashi,[a] and William H. McBride[a,b]



4's Radiobiology
New R…

- Intrinsic radiosensitivity
- Radioimmunotherapy

Why the ions?



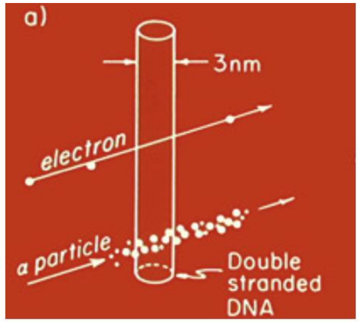
https://www.quantumdiaries.org/2012/02/15/the-hidden-face-of-cern/bragg-peak-3/

LINEAR ENERGY TRANSFER: The average amount of energy that is lost per unit path-length as a charged particle travels through a given material

LET: linear energy transfer

low LET

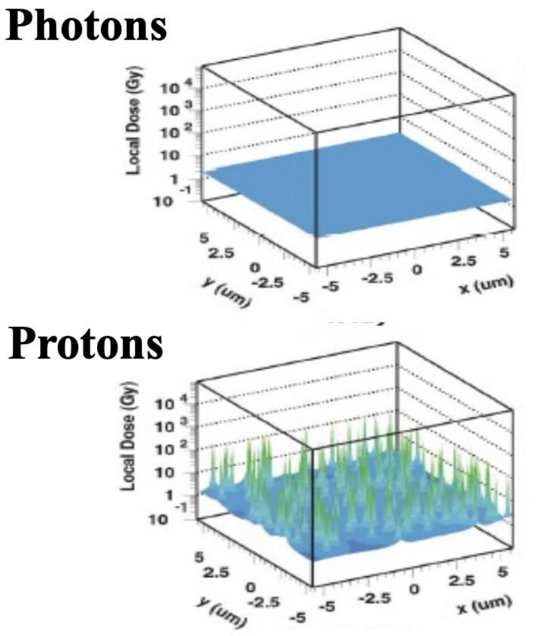
high LET



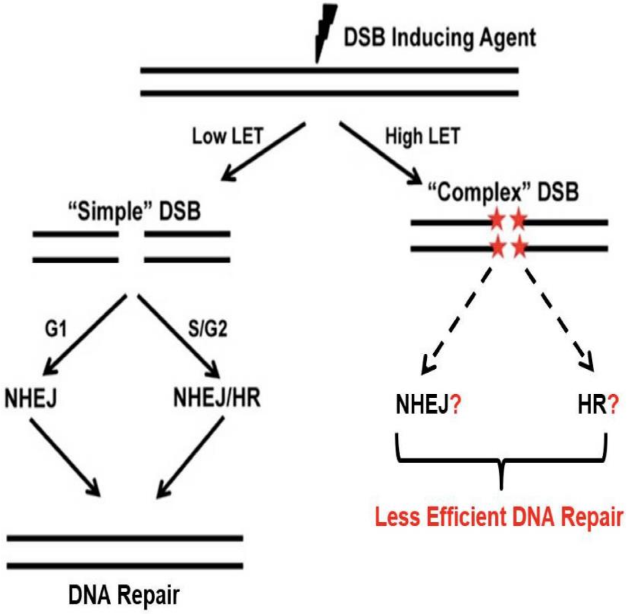
Dose = Fluence [1/cm^2] × LET [keV/cm] / ρ [g/cm^3]

Particles deposit more dose per lenght than photons (higher LET) path

Radiation is more effective when energy depositions are more concentrated in space



M. Krämer et al. Tech Cancer Res Treatm 2003



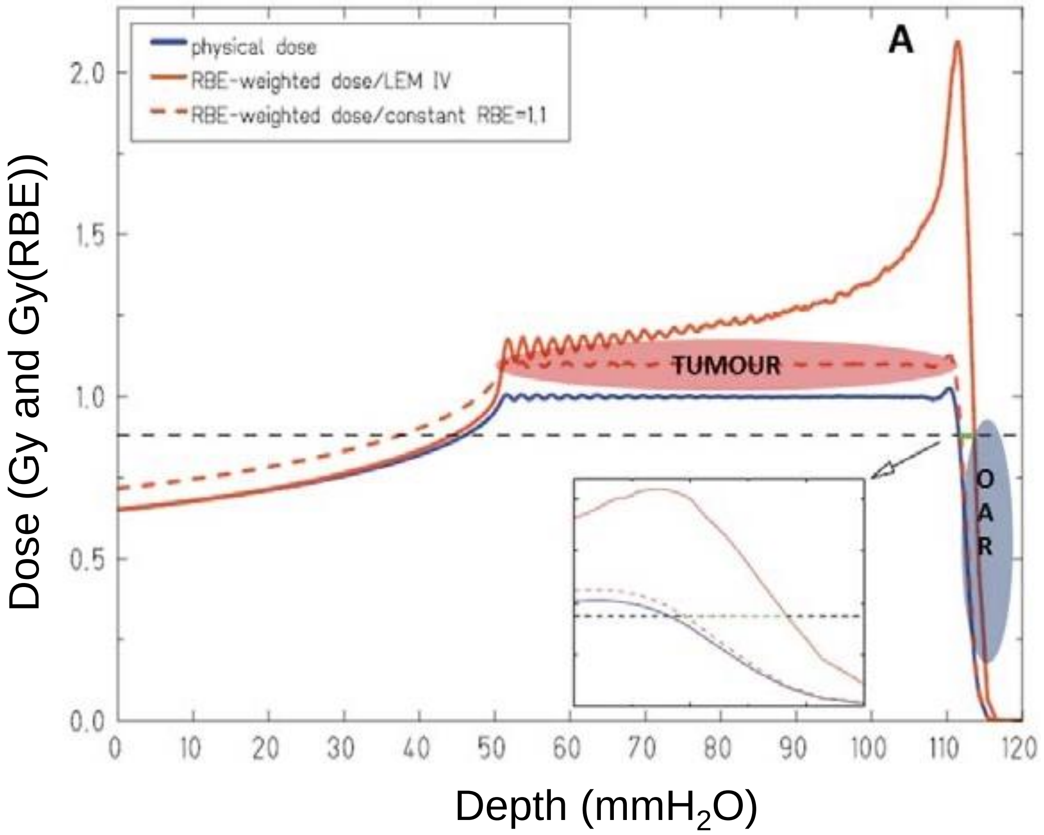
Mohamad et al Cancers 2017

Particle therapy: The beginning.

- Using heavier ions in cancer therapy was proposed and pursued by Cornelius A. Tobias at the Lawrence Berkeley Laboratory
- Patients in Berkley were treated in the period 1975-1992 with several ions:
  He, N, O, C, Ne, Si, and Ar.
- The goal of overcoming hypoxia justified using heavy ions like Argon. However, the toxicity in the entrance was too high and unacceptable for the patients' treatment.

PARTICLES:
-**Ability to more precisely target the tumor cells**.

PROTONS:
-**Pediatric patients**, given their risk of developing secondary cancers.
-A fixed **RBE of 1.1**
-**Enhanced biological effectiveness in cell killing**.

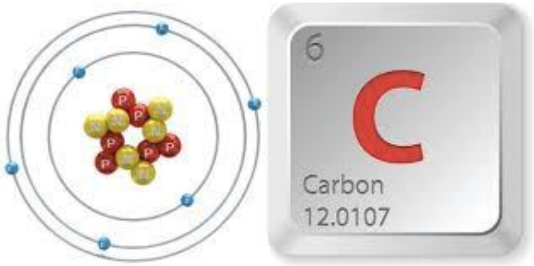


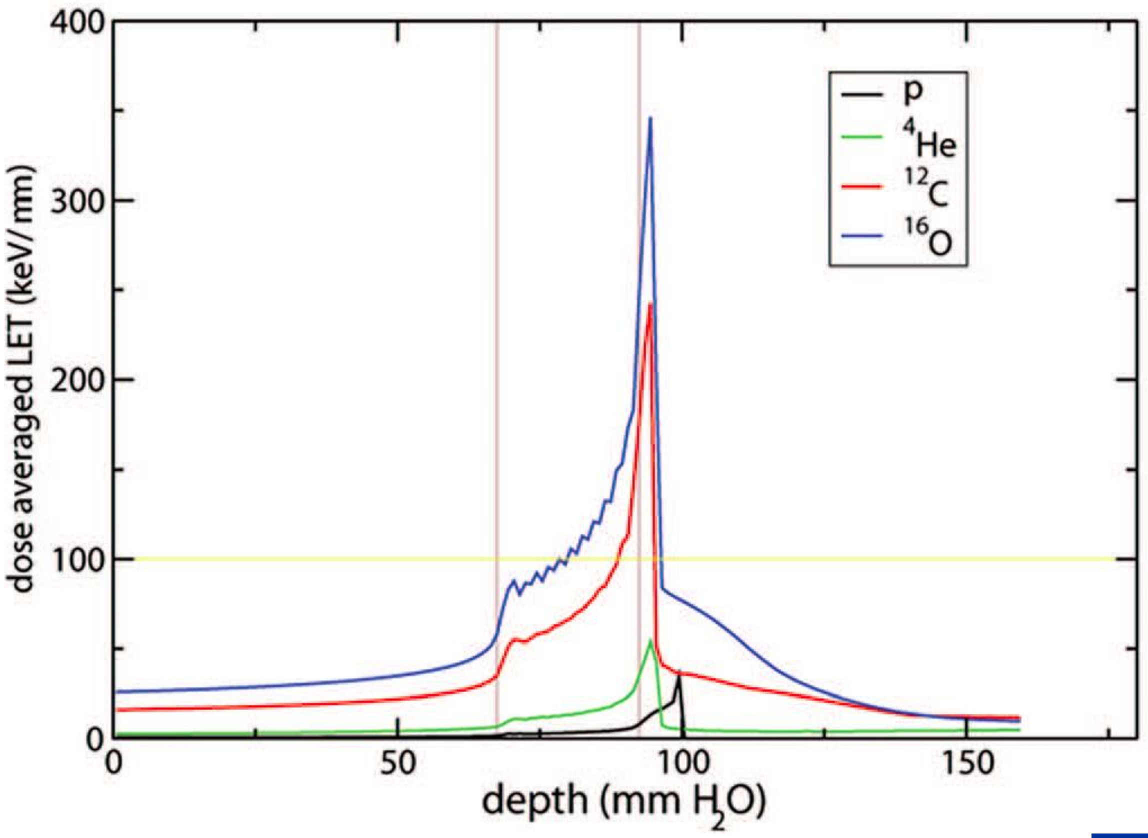
Proton Radiobiology

Francesco Tommasino [1] and Marco Durante [1,2,*]

Why heavier ions?

-It is well known that the peak of radiobiological effectiveness for several endpoints is around 100–200 keV/μm.





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This project has
research and inn

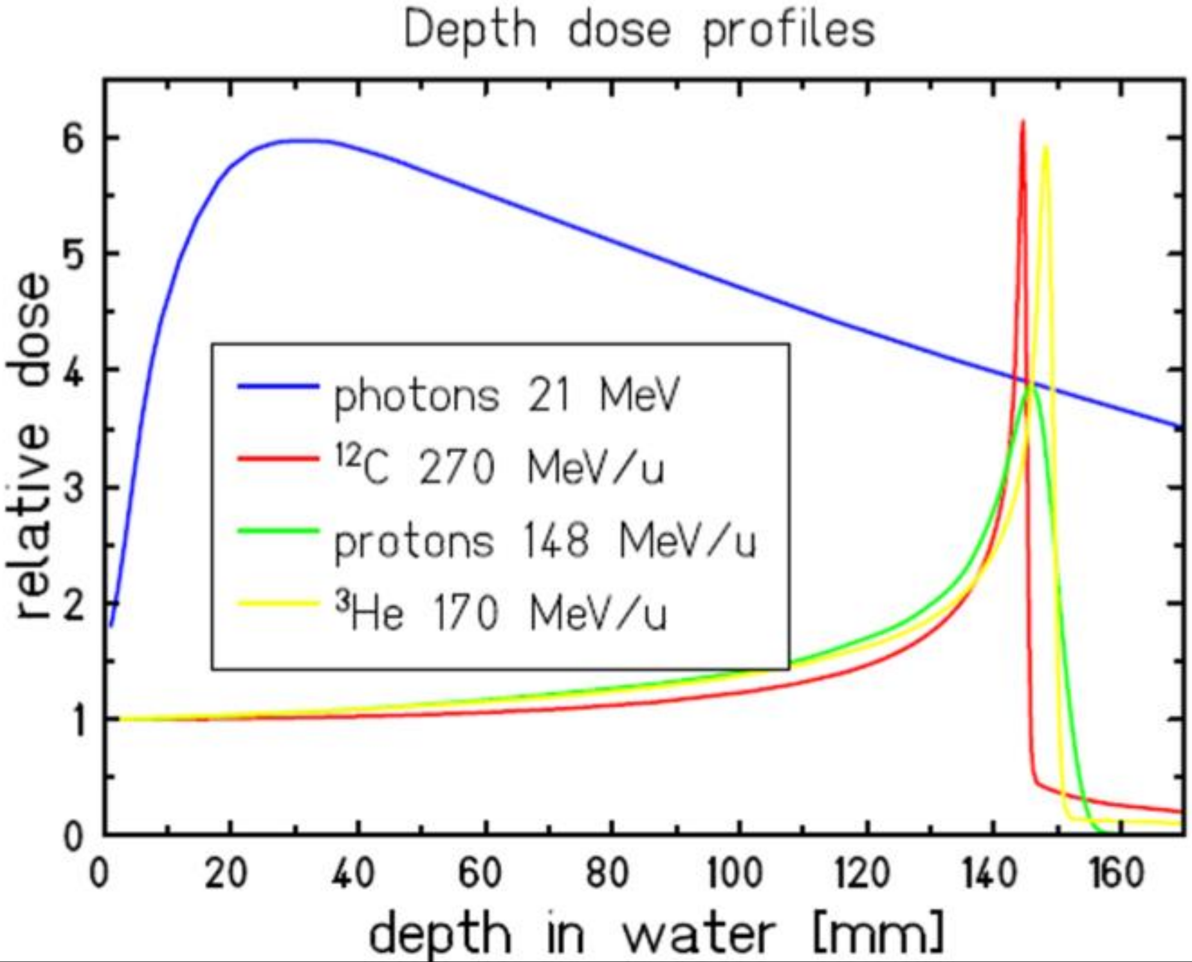
Carbon Ion Radiobiology

Walter Tinganelli [1], Marco Durante [1] [2]

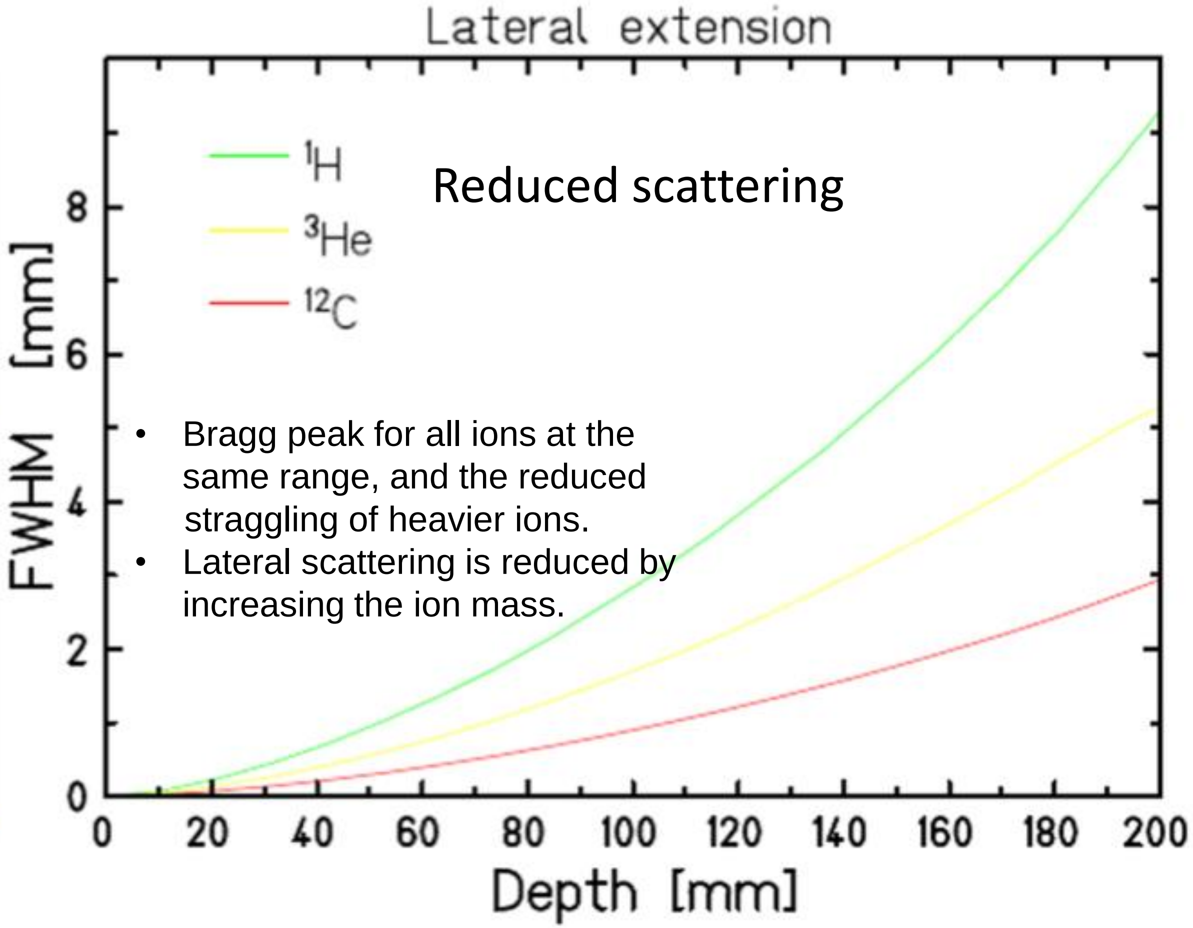
Carbon Ion Radiobiology

Walter Tinganelli [1], Marco Durante [1] [2]

Carbon is a good compromise for the more radioresistant tumors with an LET in the entrance channel between **11 and 13 keV/μm** and a farily high LET in the **SOBP between 40-80 keV/μm**.



Sharper peaks in PBS (7 vs. 25 mm)



Reduced scattering

- Bragg peak for all ions at the same range, and the reduced straggling of heavier ions.
- Lateral scattering is reduced by increasing the ion mass.

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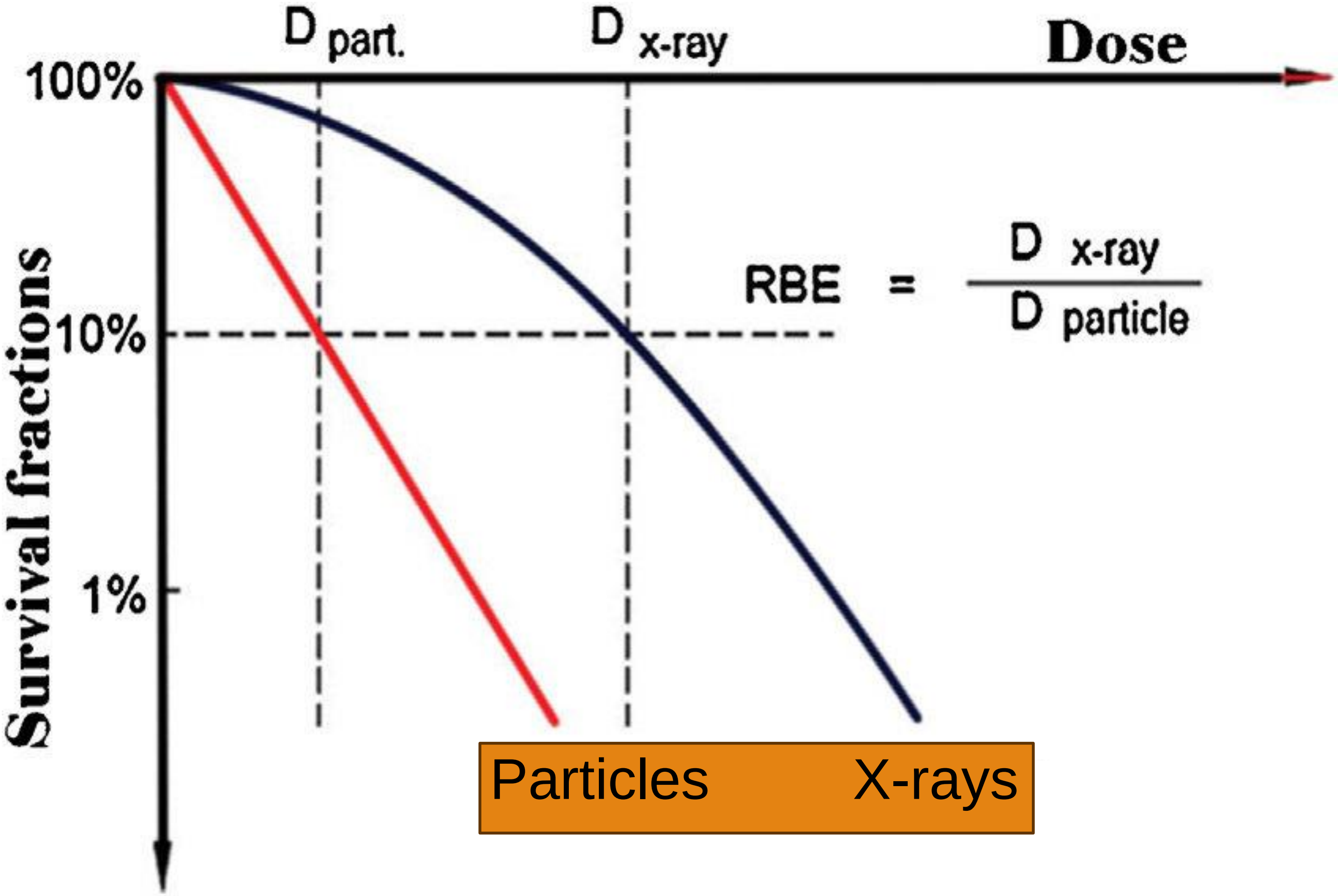
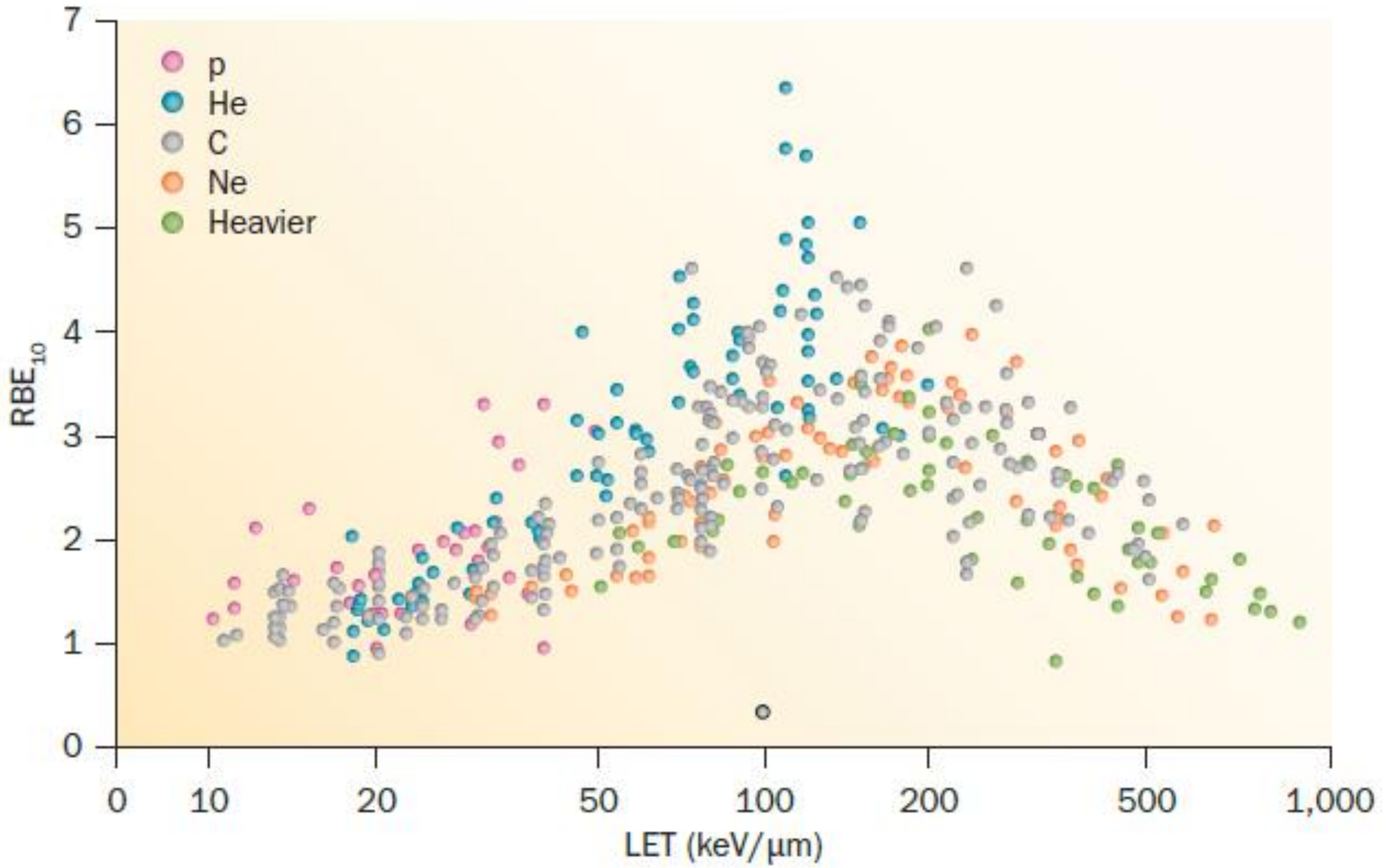
8548

Relative Biological Effectiveness (RBE)



RBE is function of the dose
RBE is function of the endpoint
RBE is function of the LET

Relative Biological Effectiveness (RBE)



PIDE database www.gsi.de/bio-pide

Friedrich *et al., J. Radiat. Res*. 2012

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

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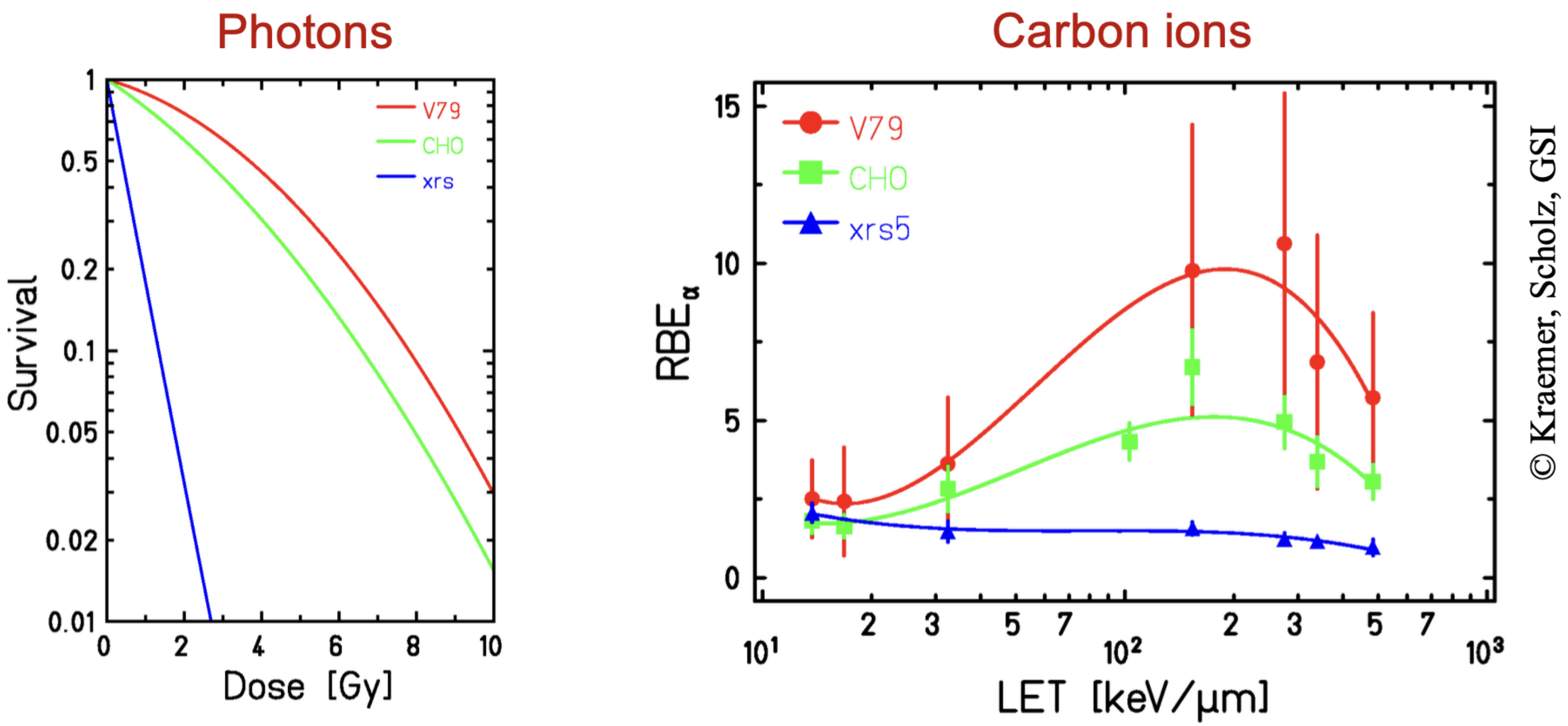
Cell line dependence of RBE

CELLS with higher repair capacity (low α/β) show a higher RBE (because of a larger shoulder in the cell survival curve)



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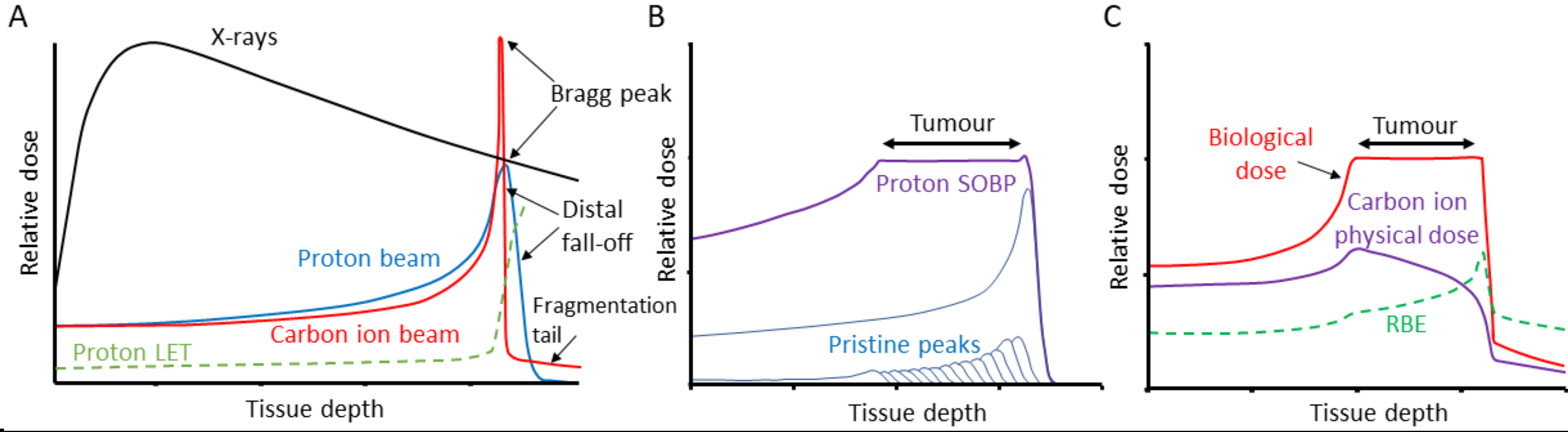
The Cellular Response to Complex DNA Damage Induced by Ionising Radiation

by Beth Wilkinson [1], Mark A. Hill [2] and Jason L. Parsons [3,*]

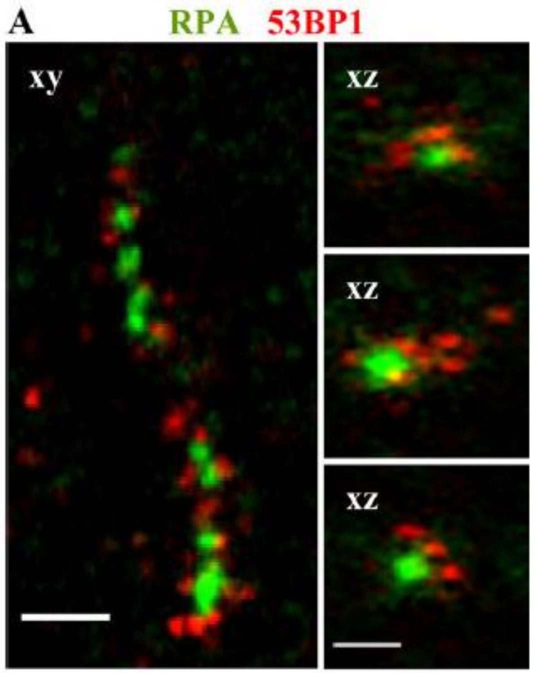
- Dose averaged LET for protons varying from ~1 keV/µm to ~17 keV/µm (the LET of carbon ions follows a similar trend, reaching a maximum of ~300–400 keV/µm).

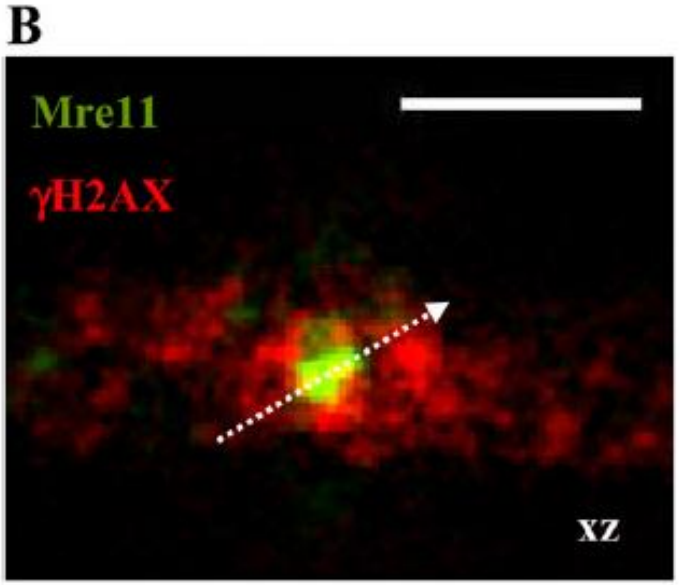
- Unlike for protons, the variation of RBE along the carbon ion beam is routinely accounted for using radiobiological models;

Biological Effective Dose: BED= RBE*DOSE



Clustered DNA breaks induced by heavy ions



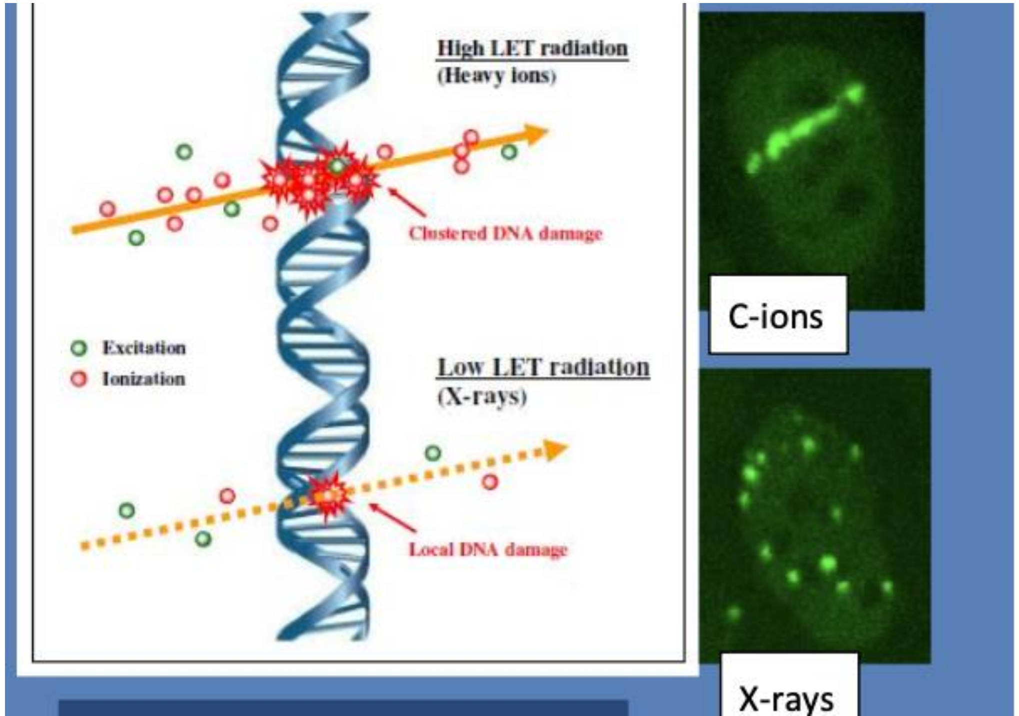


RPA: Replication protein A (RPA) is the major protein that binds to single-stranded DNA RPA is required in repair processes such as NER (nucleotide excision repair) and homologous recombination.

53BP1: The p53-binding protein 1 is a well-known DNA damage response (DDR) factor, which is recruited to nuclear structures at the site of DNA damage and forms readily visualized ionizing radiation (IR) induced foci.

Mre11: Component of the MRN complex, which plays a central role in double-strand break (DSB) repair, DNA recombination, maintenance of telomere integrity and meiosis.

γH2AX: is a type of histone protein from the H2A family encoded by the *H2AFX* gene. An important phosphorylated form is γH2AX (S139), which forms when double-strand breaks appear.



Carbon Ion Radiobiology

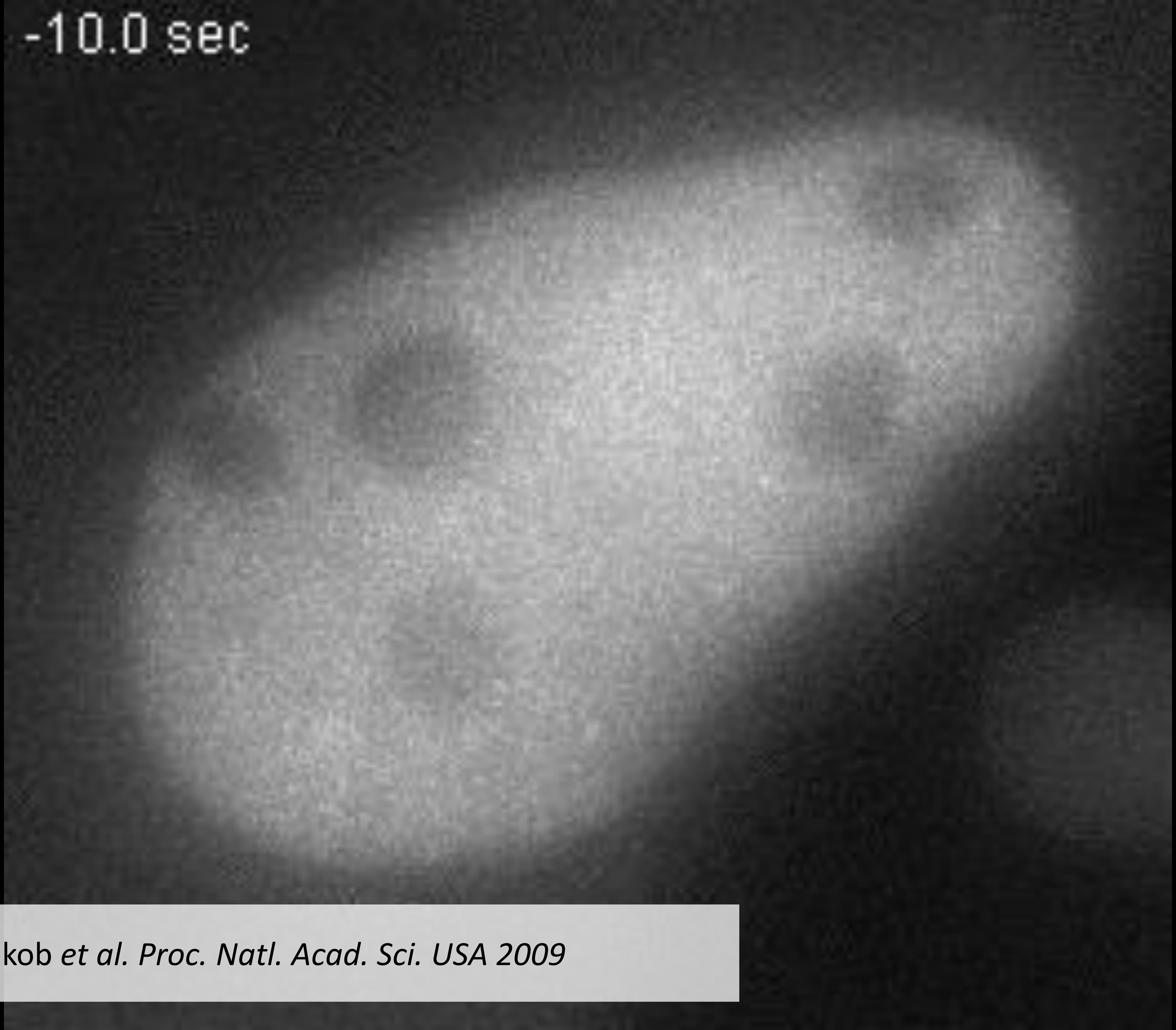
Walter Tinganelli [1], Marco Durante [1 2]

Biological dose estimation of UVA laser microirradiation utilizing charged particle-induced protein foci

J. Splinter[1], B. Jakob[1,*], M. Lang[2], K. Yano[3], J. Engelhardt[2], S. W. Hell[2], D. J. Chen[3], M. Durante[1,4] and G. Taucher-Scholz[1]

[1]Department of Biophysics, GSI Helmholtz Center for Heavy Ion Research, Planckstrasse 1, D-64291 Darmstadt, Germany, [2]Department of High Resolution Optical Microscopy, German Cancer Research Center, D-69120 Heidelberg, Germany, [3]Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA and [4]Institute for Condensed Matter Physics, Technical University Darmstadt, D-64289 Darmstadt, Germany.

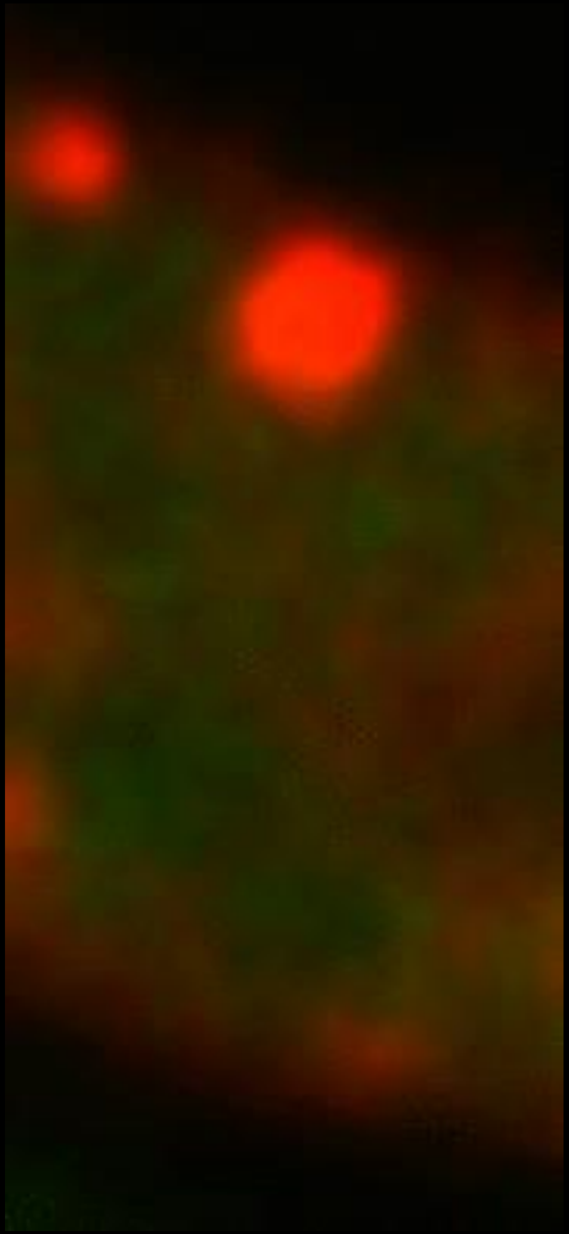
Mutagenesis pp. 1–9, 2010



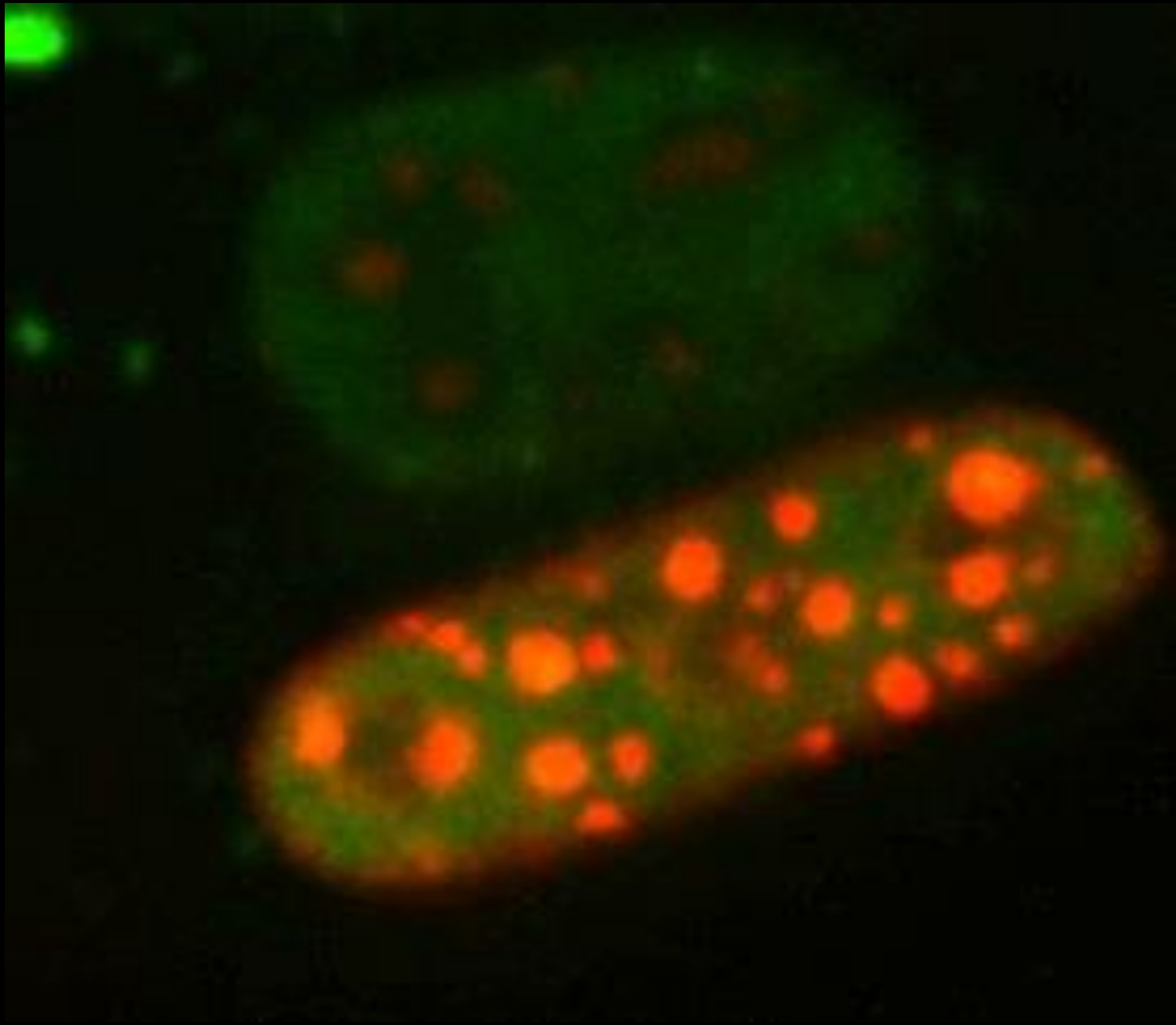
Jakob *et al. Proc. Natl. Acad. Sci. USA 2009*

NSBS1

Repair in eu- and hetero-chromatin



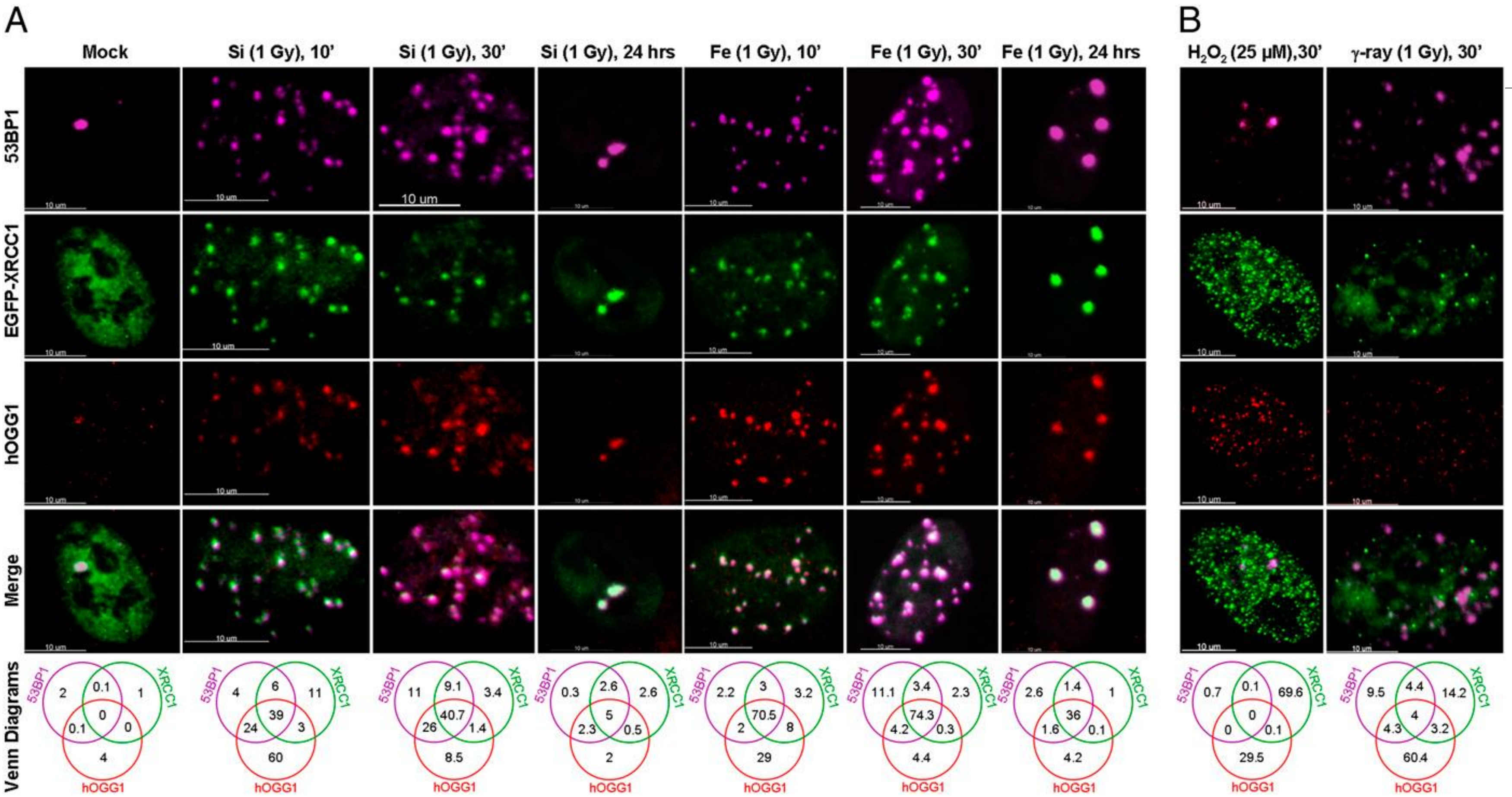
XRCC1



Jakob *et al., Proc. Natl. Acad. Sci. USA* 2009; *Nucl. Acids Res*. 2011

Unrepaired clustered DNA lesions and heavy ions

UTSouthwestern Medical Center



53BP1: The p53-binding protein 1 is a well-known DNA damage response (DDR) factor, which is recruited to nuclear structures at the site of DNA damage and forms readily visualized ionizing radiation (IR) induced foci.

hOGG1: It is responsible for removing genotoxic lesions caused by oxidative damage in the presence of reactive oxygen species (ROS)

XRCC1: DNA repair protein, X-ray repair cross-complementing protein 1, is involved in DNA repair. It complexes with the DNA ligase III

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Chen *et al.*, *Proc. Natl. Acad. Sci.* USA 2011

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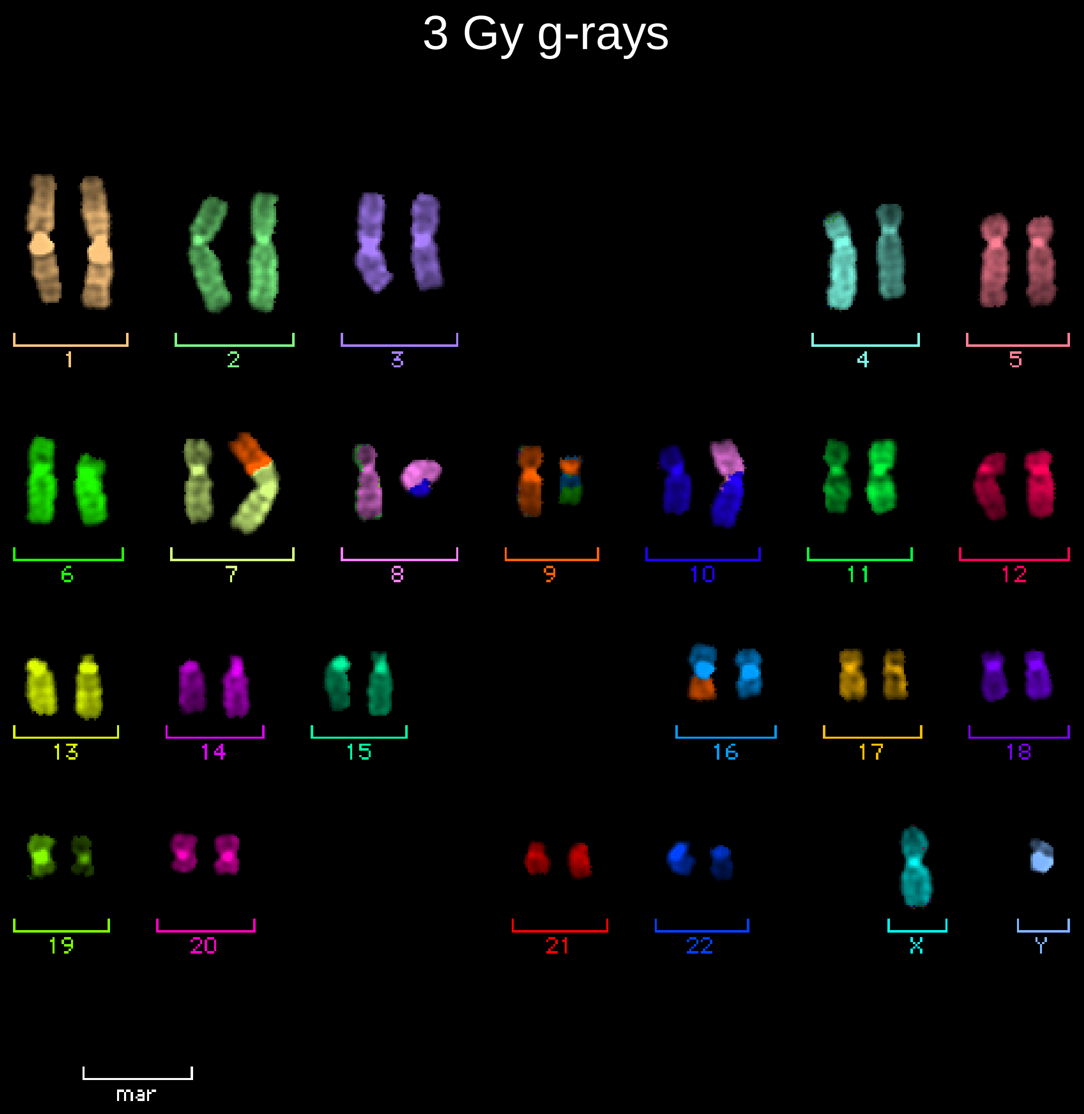
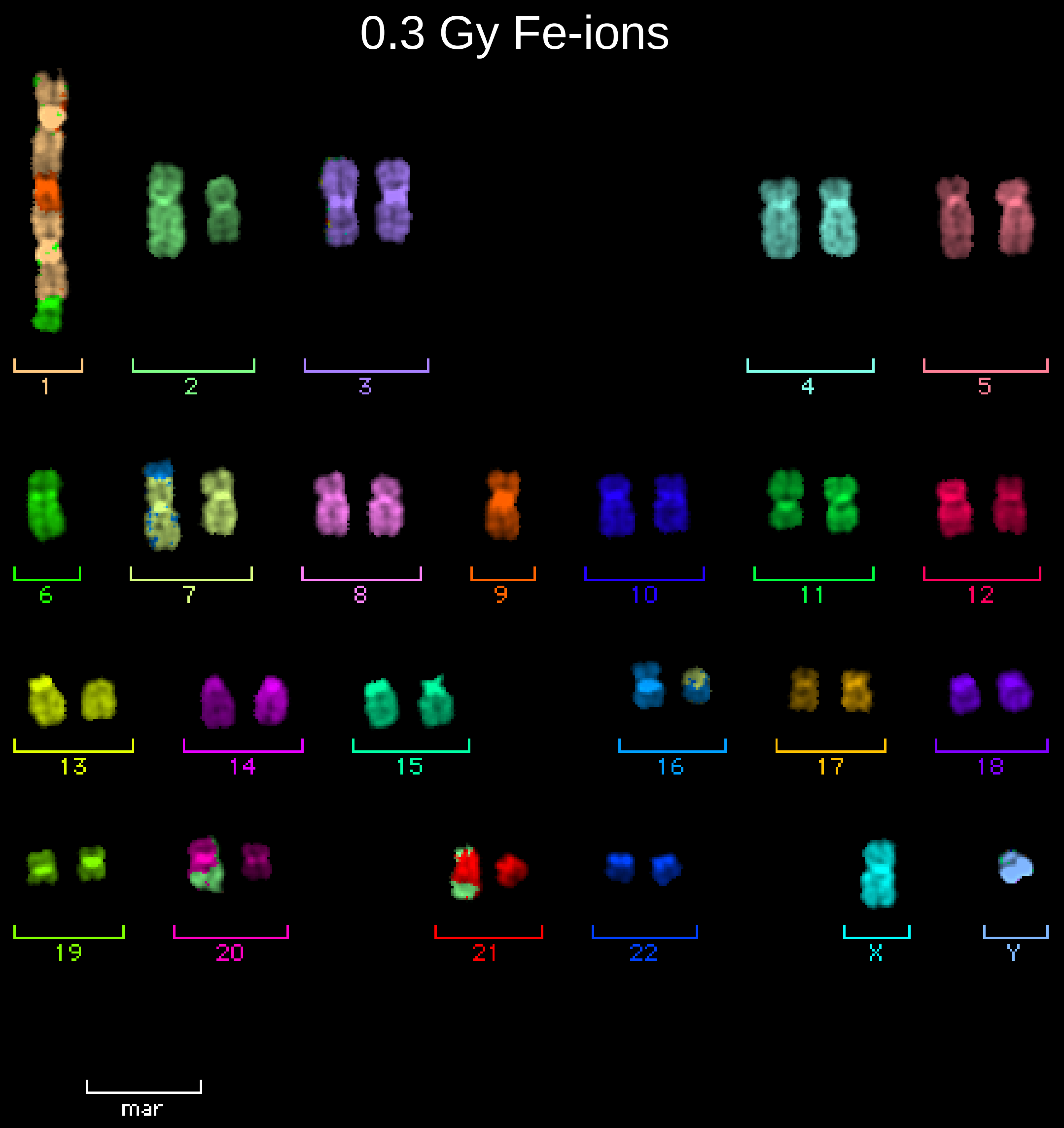
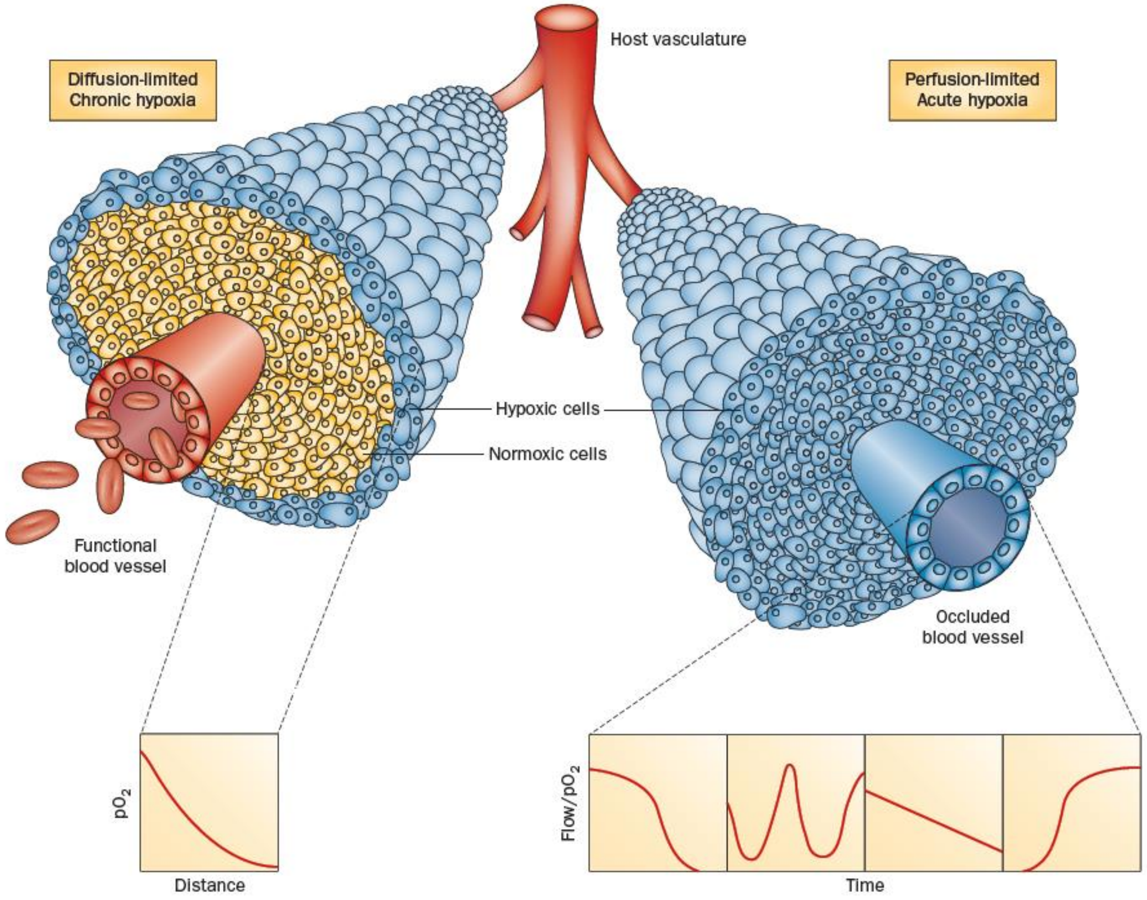
Chromosomal aberrations induced by heavy ions





Durante *et al.*, *Radiation Research* 2002

Chronic and acute hypoxia / Oxygen Enhancement Ratio (OER)



Horsman et al., Nature Rev. Clin. Onc. 2012

$$OER = \left.\frac{D_{\text{hypoxic}}}{D_{\text{oxic}}}\right|_{\text{isoeffect}}$$

Review

Cell PRESS

Passing the baton: the HIF switch

Mei Yee Koh and Garth Powis

Department of Experimental Therapeutics, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA

poral and functional roles: HIF-1 drives the initial response to hypoxia (<24 h) and HIF-2 drives the chronic response (>24 h). Here, we review the significance of the HIF switch and the relation between HIF-1 and HIF-2 under both physiological and pathophysiological conditions.

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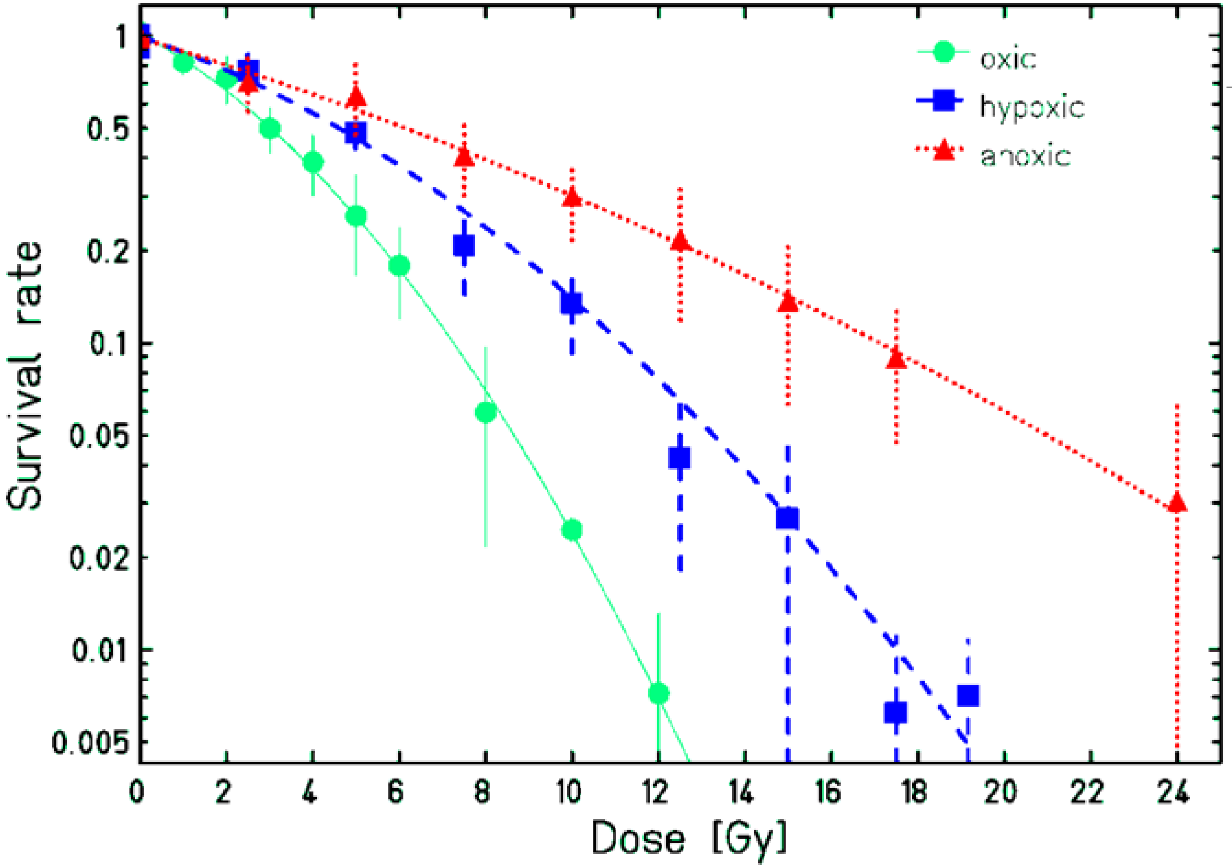
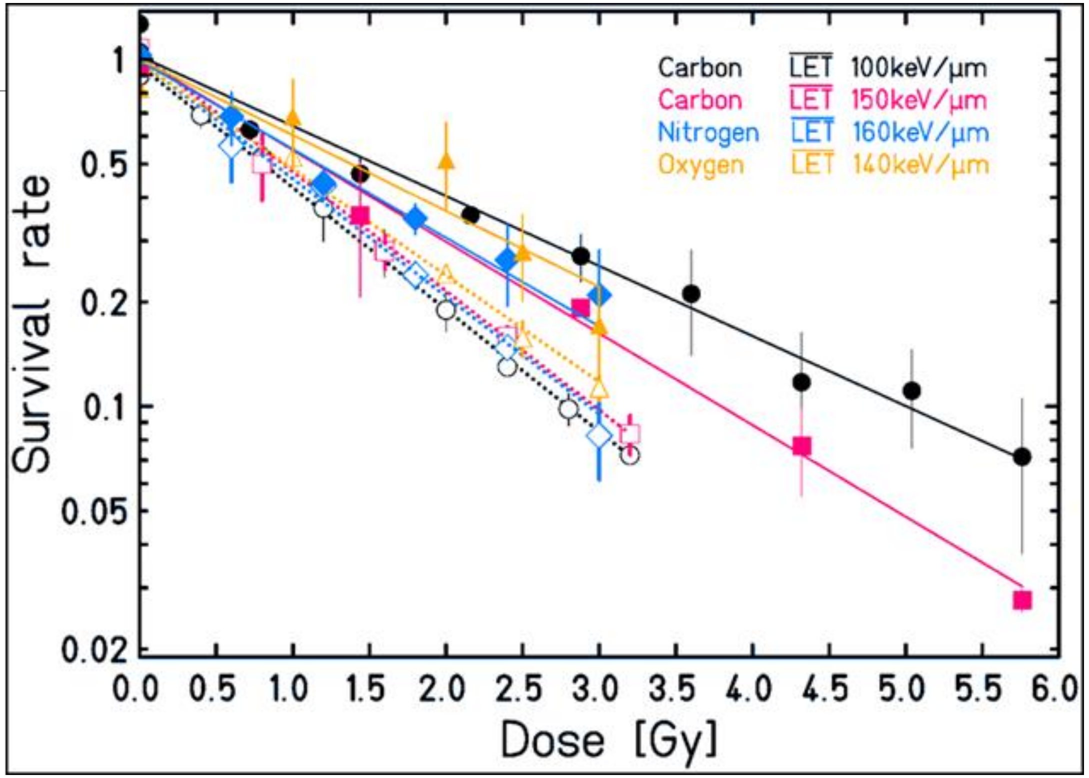
Oxygen Enhancement Ratio: CHO cells experiments

Tinganelli et al. J Radiat Res. 2013





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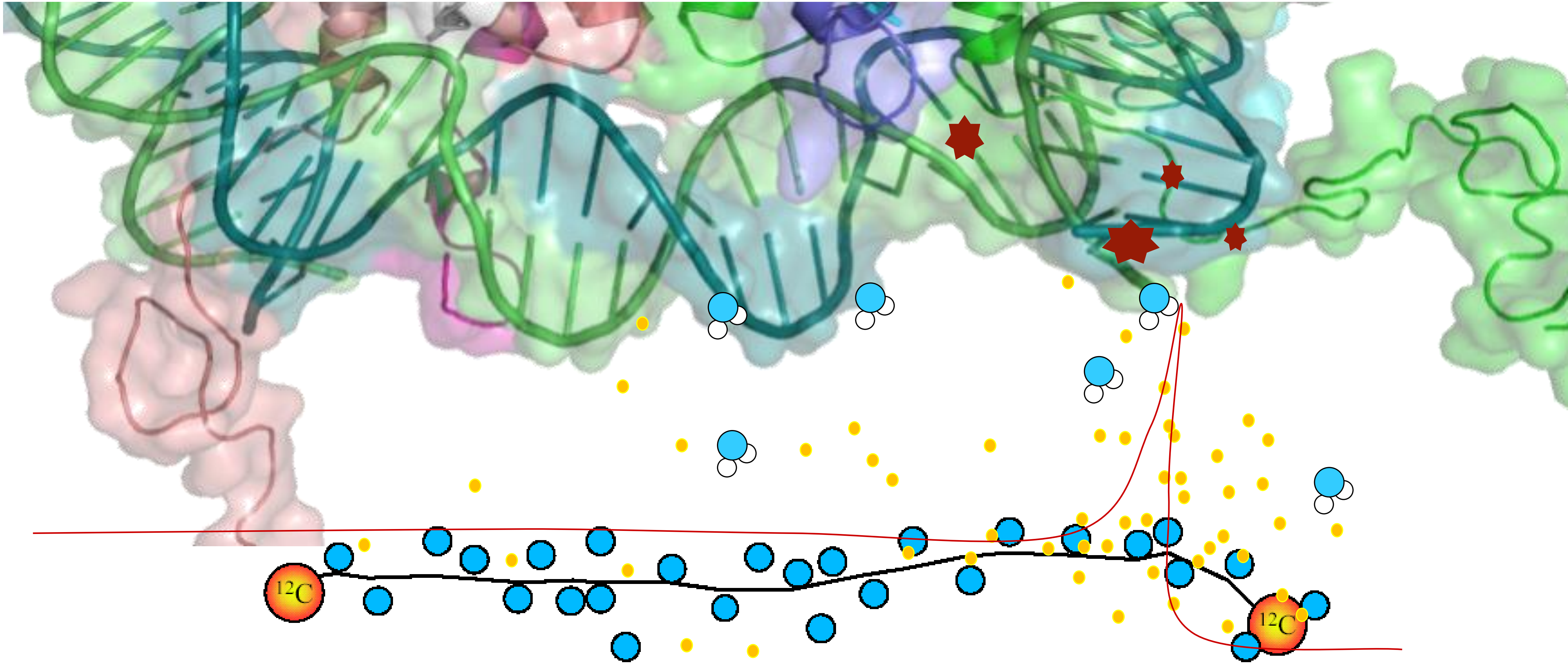
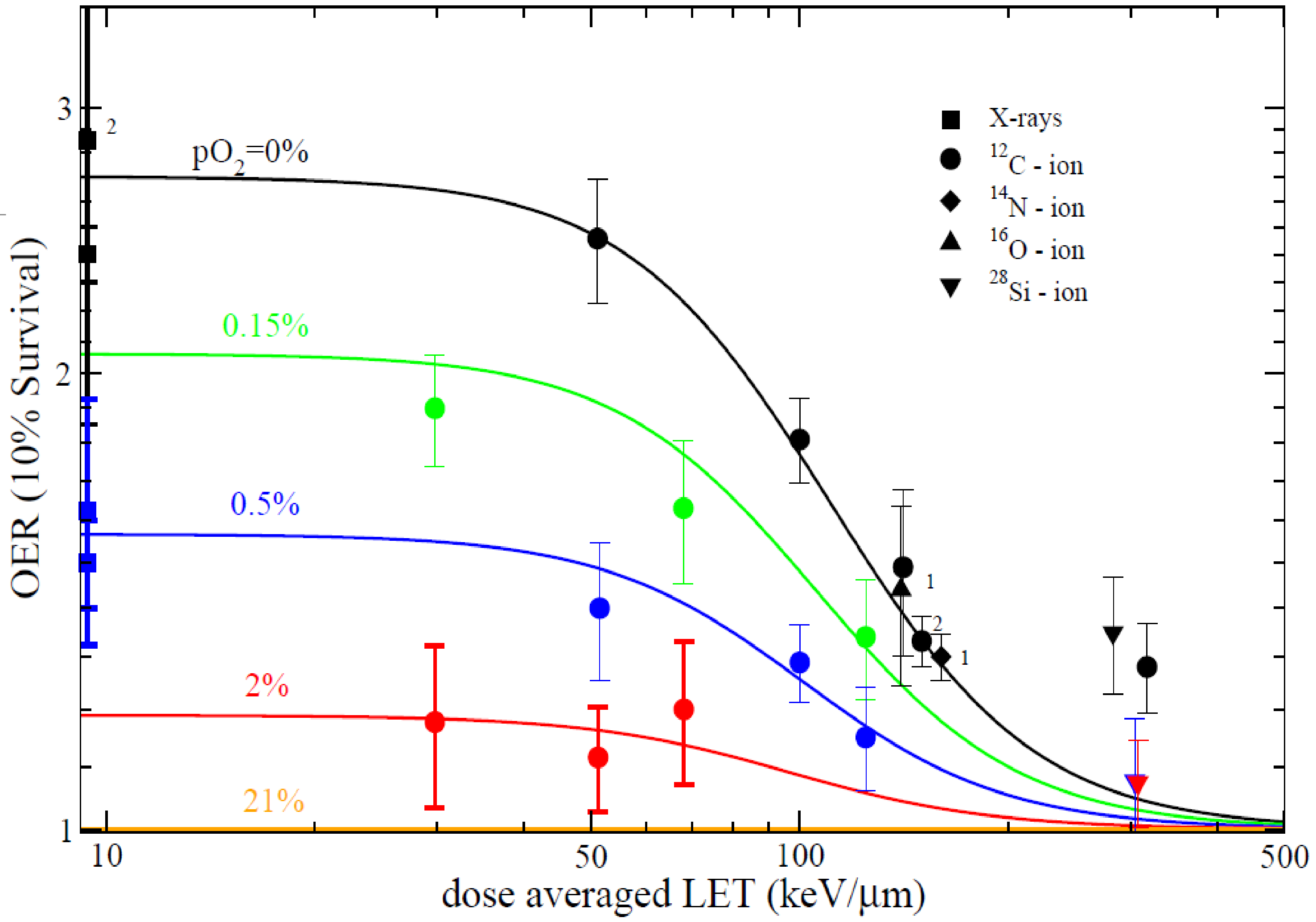
OER reduction at high LET are related to decrease of indirect effect



Courtesy of E. Scifoni

OER-LET

Tinganelli *et al.*, *Sci. Rep*. 2015



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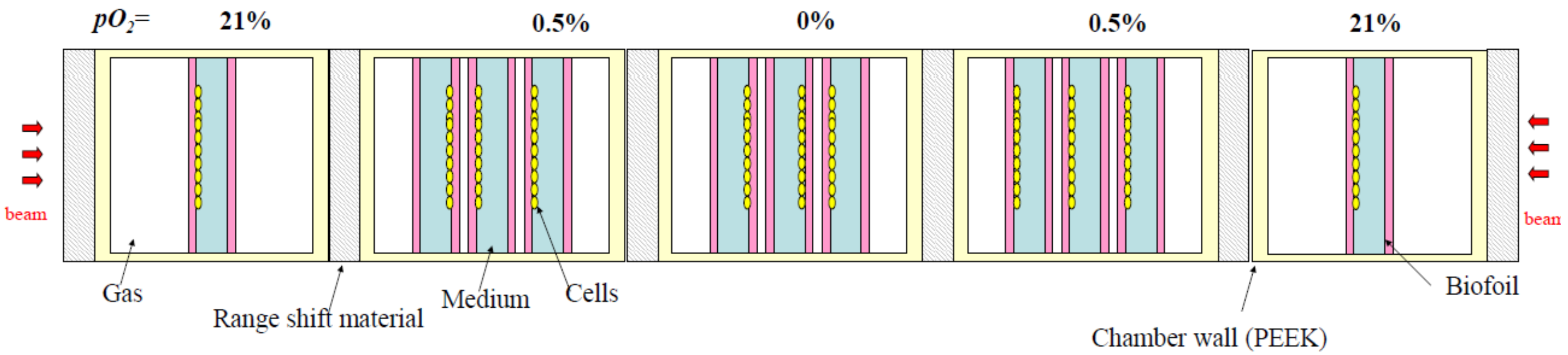
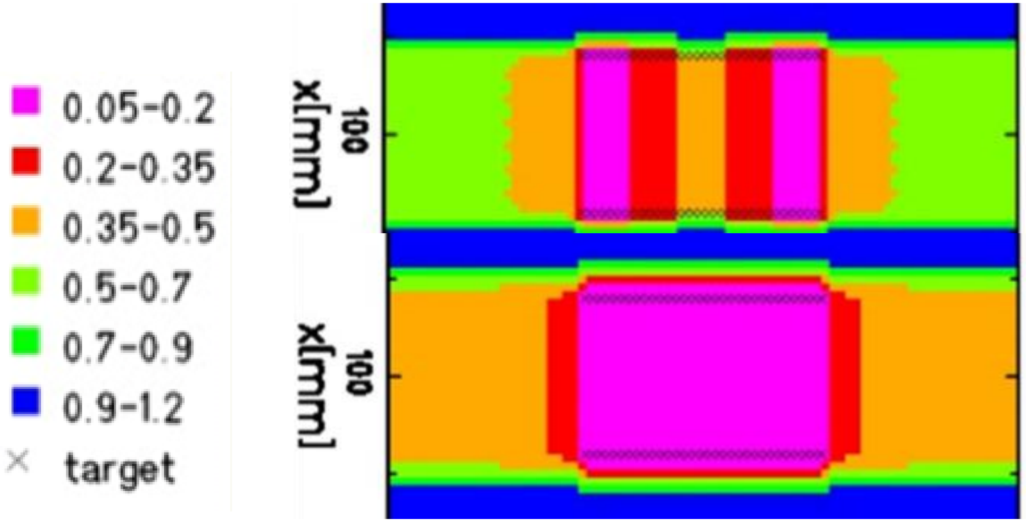
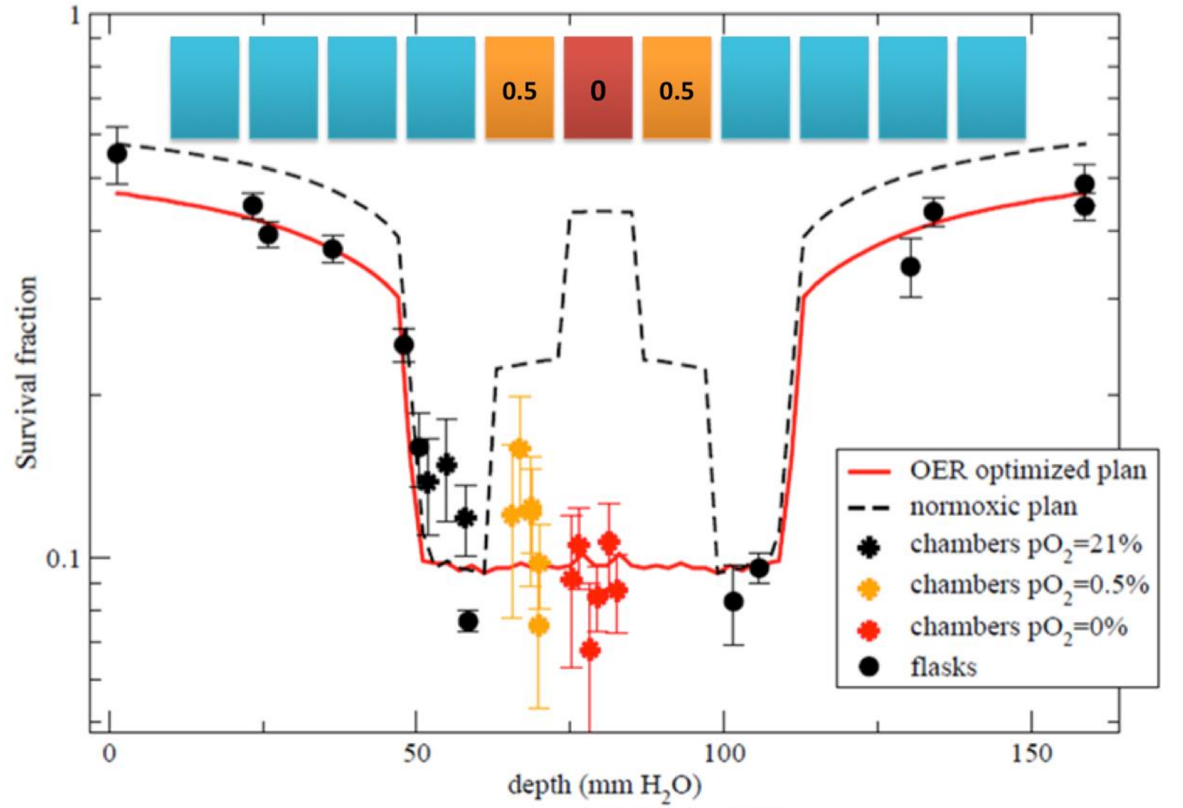
Experimental tests

Tinganelli *et al.*, *Sci. Rep.* 2015





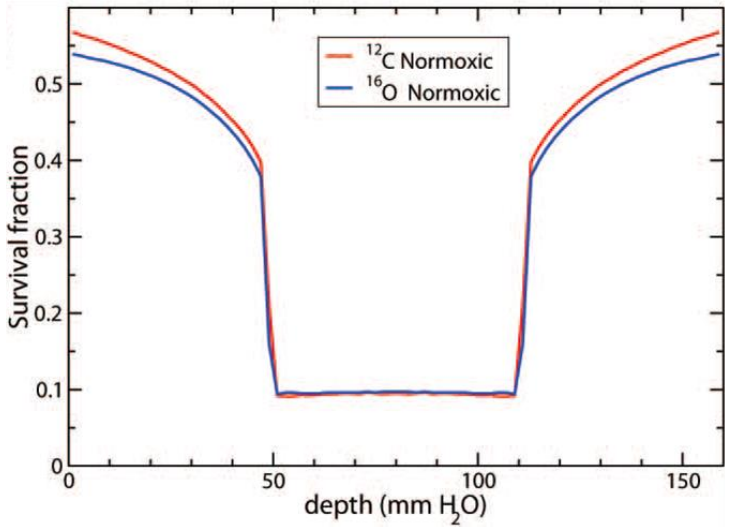


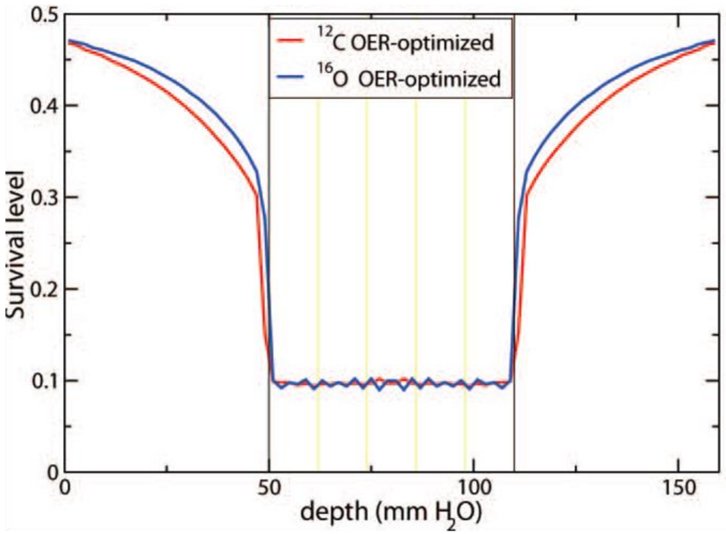
New Ions for Therapy

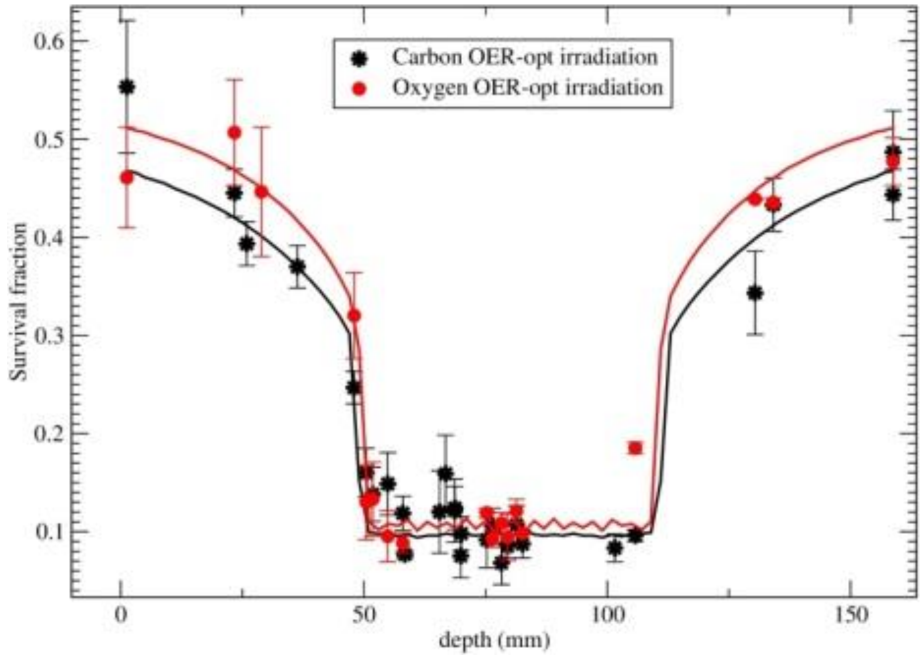
Francesco Tommasino, PhD[1,2]; Emanuele Scifoni, PhD[1]; Marco Durante, PhD[1,2]

[1] Biophysics Department, GSI Helmholtzzentrum für Schwerionenforschung, Darmstadt, Germany
[2]Trento Institute for Fundamental Physics and Applications (TIFPA), National Institute for Nuclear Physics (INFN), Department of Physics, University of Trento, Povo, Italy

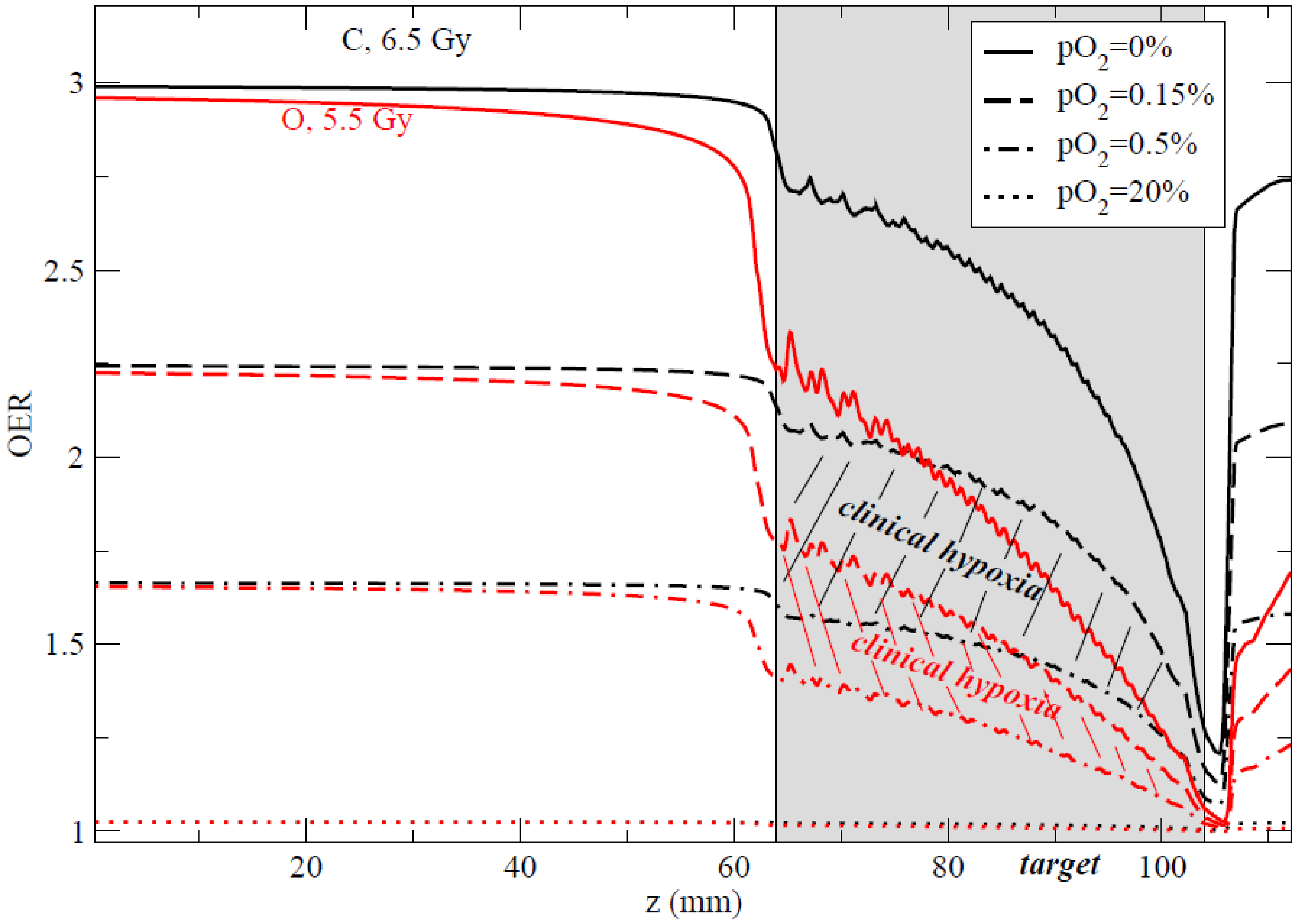
Tinganelli *et al., Sci. Rep*. 2015







Carbon vs. Oxygen



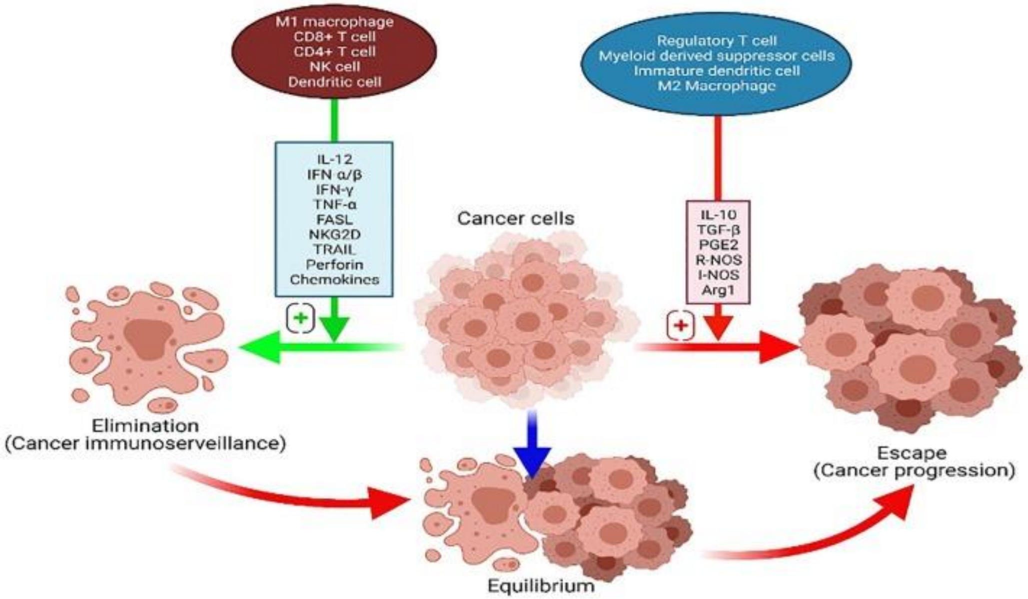
- **OER along the irradiation depth for different ion and pO2;**

-Selective advantage of O beam;

-Lower prescribed dose in the target in order to have the same survival fraction in the entrance channel.

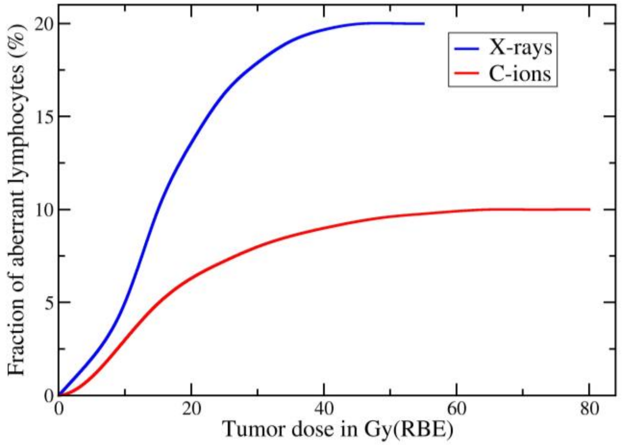
Scifoni et al PMB 2013

Do heavy ions elicit a stronger immune response?

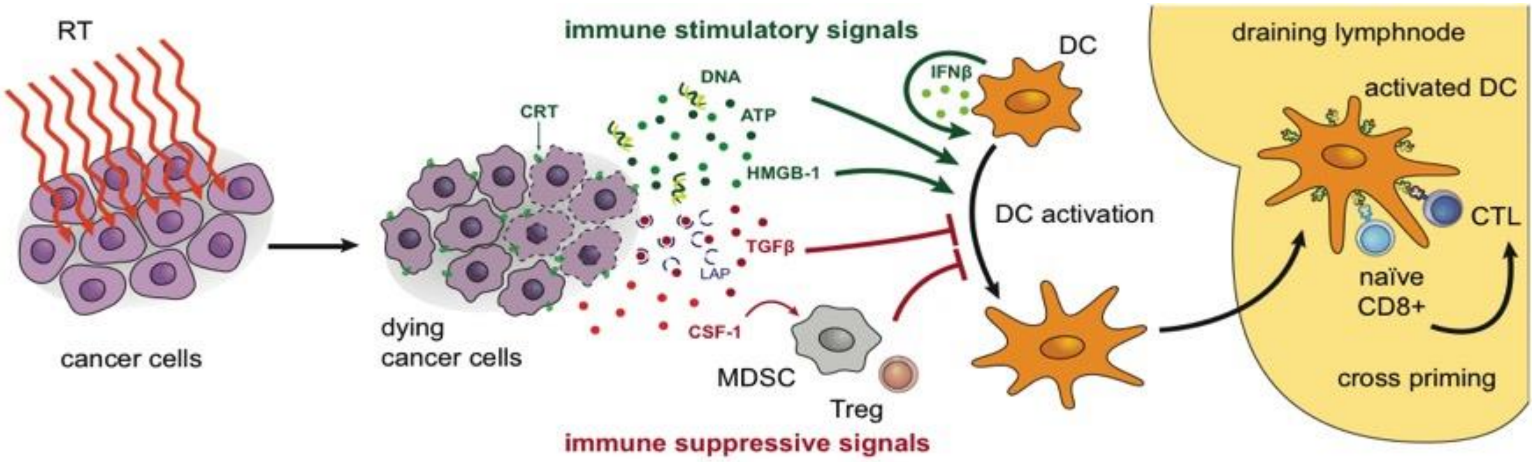


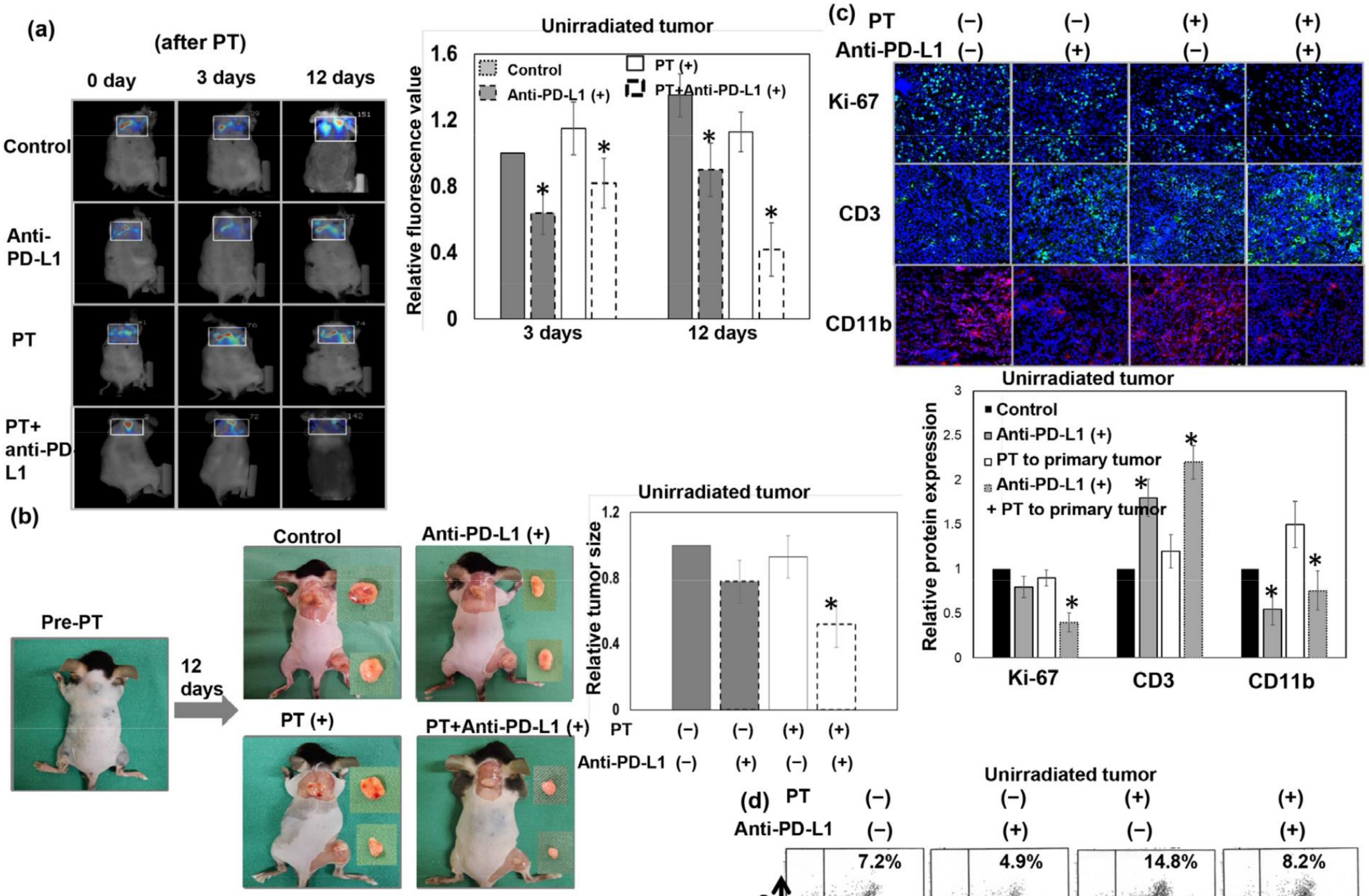
Rahul Mallick Asim Duttaroy Med Hypotheses, 2021

Durante *et al.*,
Int. J. Radiat. Oncol. Biol. Phys. 2000



Formenti *et al., JAMA Oncol*. 2015
Durante, Brenner & Formenti*, Int. J. Radiat. Oncol. Biol. Phys*. 2016





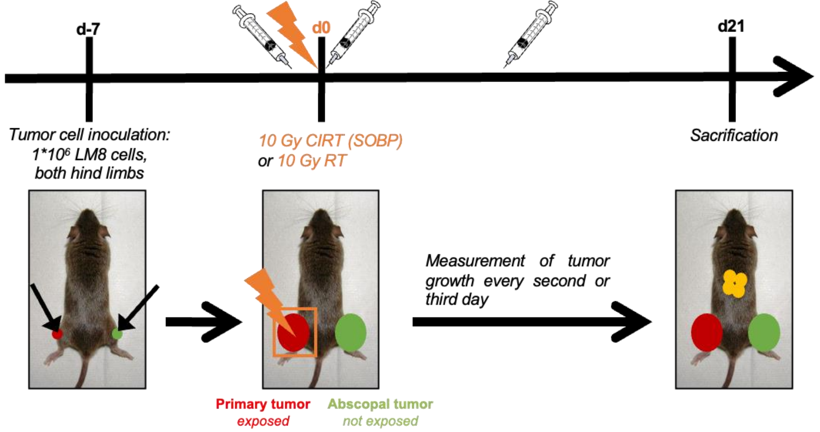
Effect of Proton Therapy on Tumor Cell Killing and Immune Microenvironment for Hepatocellular Carcinoma.
Miao-Fen Chen, Ping-Tsung Chen, Ching-Chuan Hsieh, and Chih-Chi Wang. *Cell* **2023;** 12, 332.

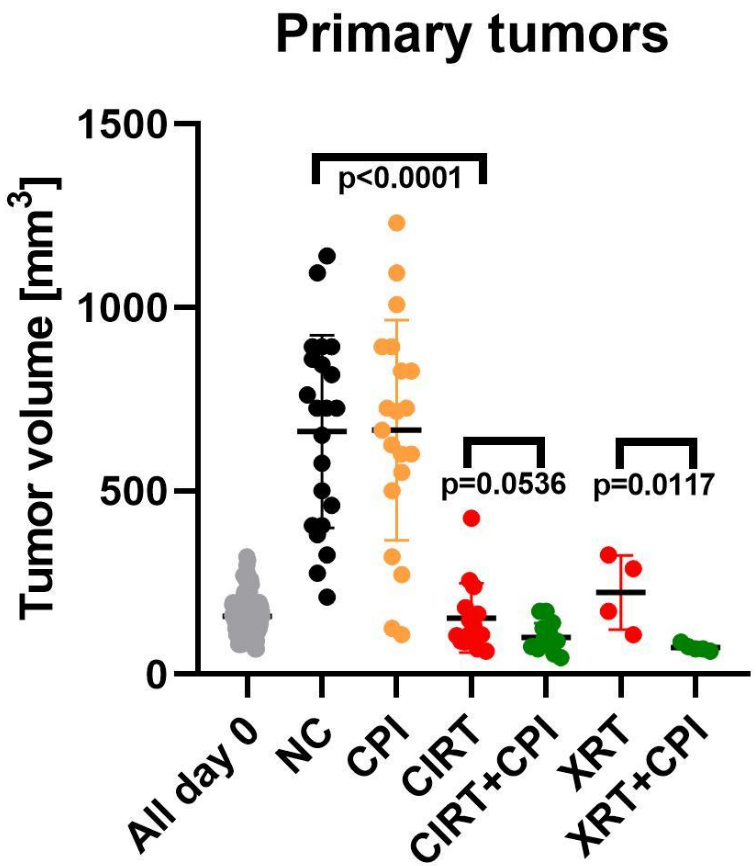
- **KI-67** is a nuclear protein that is associated with cellular proliferation.
- **CD3** is a T cell co-receptor that is involved in activating both the cytotoxic T cell (CD8+ naive T cells) and T helper cells (CD4+ naive T cells)
- **CD11b** is expressed on the surface of many leukocytes involved in the innate immune system. It icreases the migration, adhesion and transmigration through the blood vessels.

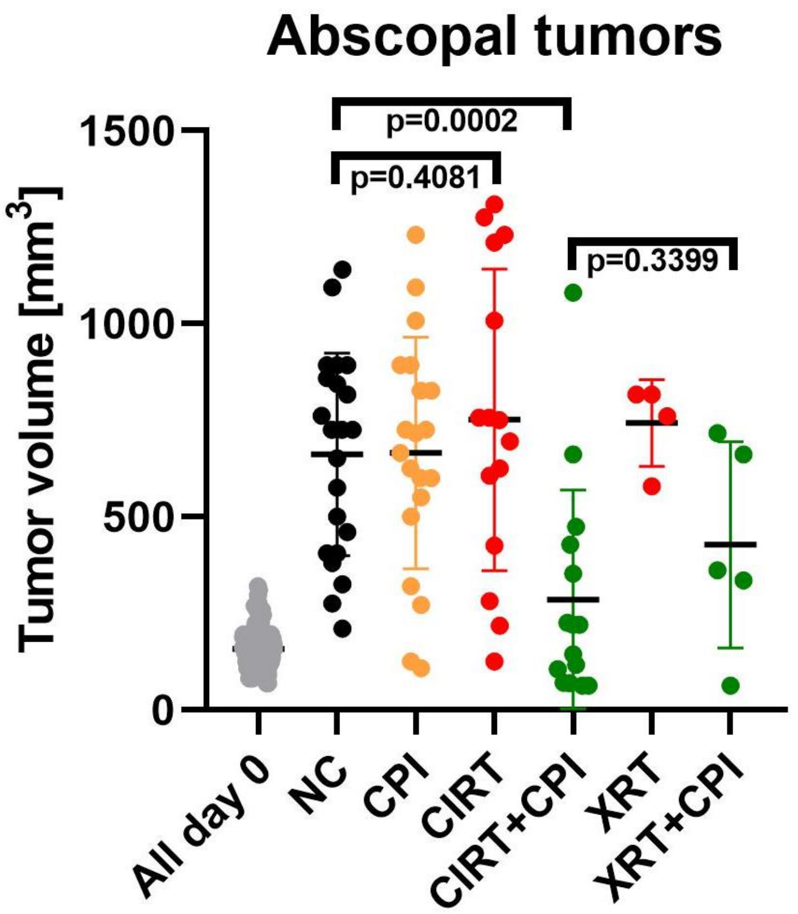
- **In addition to the biological effect on cancer cells, PT also has an immune modulation effect on the tumor microenvironment.**
- **anti-PD-L1 elicited anticancer immunity and subsequently augmented the response of primary and distant tumors to PT irradiation**

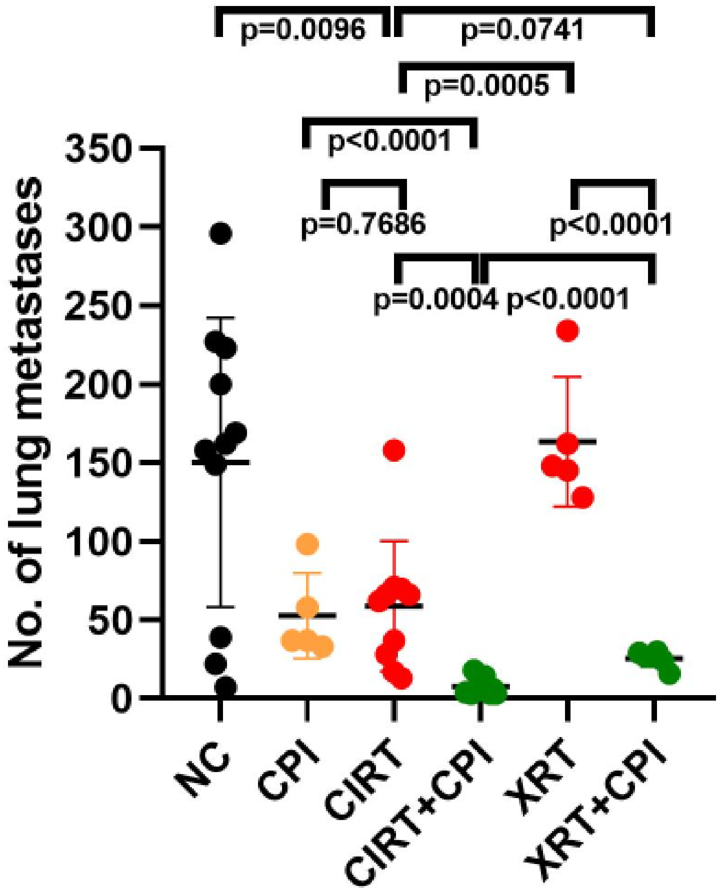
Reduction of Lung Metastases in a Mouse Osteosarcoma Model Treated With Carbon Ions and Immune Checkpoint Inhibitors

Alexander Helm [1], Walter Tinganelli [1], Palma Simoniello [2], Fuki Kurosawa [3], Claudia Fournier [1], Takashi Shimokawa [3], Marco Durante [4]









Radioimmunotherapy

«carbon ions can be considered as a different "drug" in oncology, and may elicit favorable responses such as an increased immune response and reduced angiogenesis and metastatic potential»

Carbon Ion Radiobiology

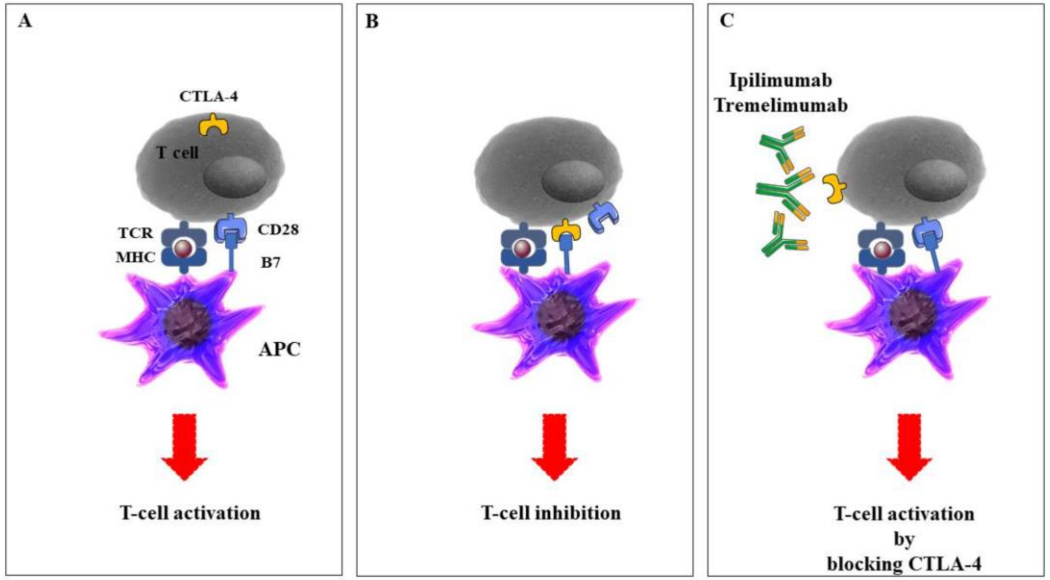
Walter Tinganelli [1], Marco Durante [1 2]

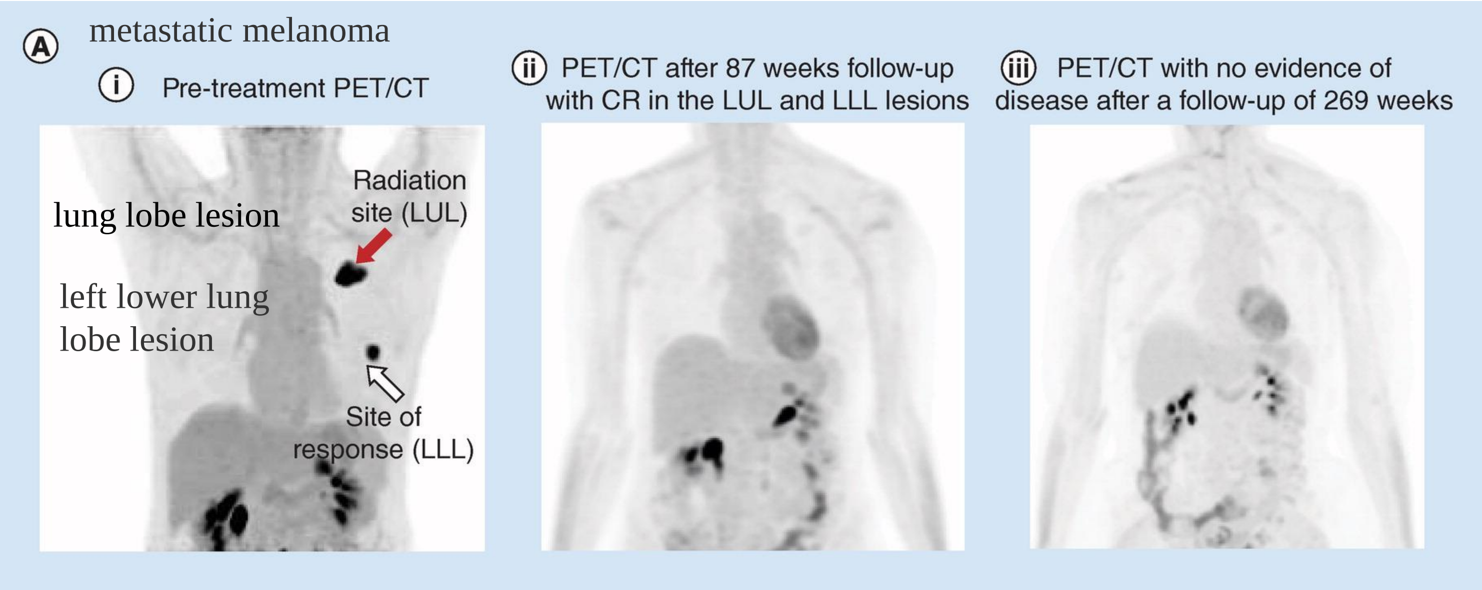
MELANOMA MANAGEMENT, VOL. 7, NO. 1 | SHORT COMMUNICATION

Durability of response in metastatic melanoma patients after combined treatment with radiation therapy and ipilimumab

Quaovi H Sodji, Paulina M Gutkin, Susan M Swetter, Sunil A Reddy, Susan M Hiniker‡ & Susan J Knox‡

Kooshkaki et al. 2020





FLASH radiotherapy not a new story… 7° R'?

- Dewey and Boag in 1959:

  Ultra-high dose-rate 1.5-MV X-rays were used to irradiate a **bacterium**, *Serratia marcescens*.

  The profile of radioresistance to ultra-high dose rates was similar to that observed under hypoxic conditions, in which bacteria have the greatest resistance to radiation. *Dewey, D. L. & Boag, J. W. Nature 183, 1450–1451 (1959).*

- Berry et al. showed similar results in **hamster cells and HeLa cells** using ultra-high dose-rate (1,000 rads for the 15-ns pulse) irradiation. A series of experiments showed that the flash effect is related to oxygen consumption.

- In the 2010s, a paradigm-shifting set of experiments was performed in the frame of a collaboration between Institut Curie, Institut Gustave Roussy (Paris) and Centre Hospitalier Universitaire Vaudois (CHUV, Lausanne): **Ultra-high dose rate irradiation can widen the therapeutic window killing tumours while sparing non-malignant tissues.**

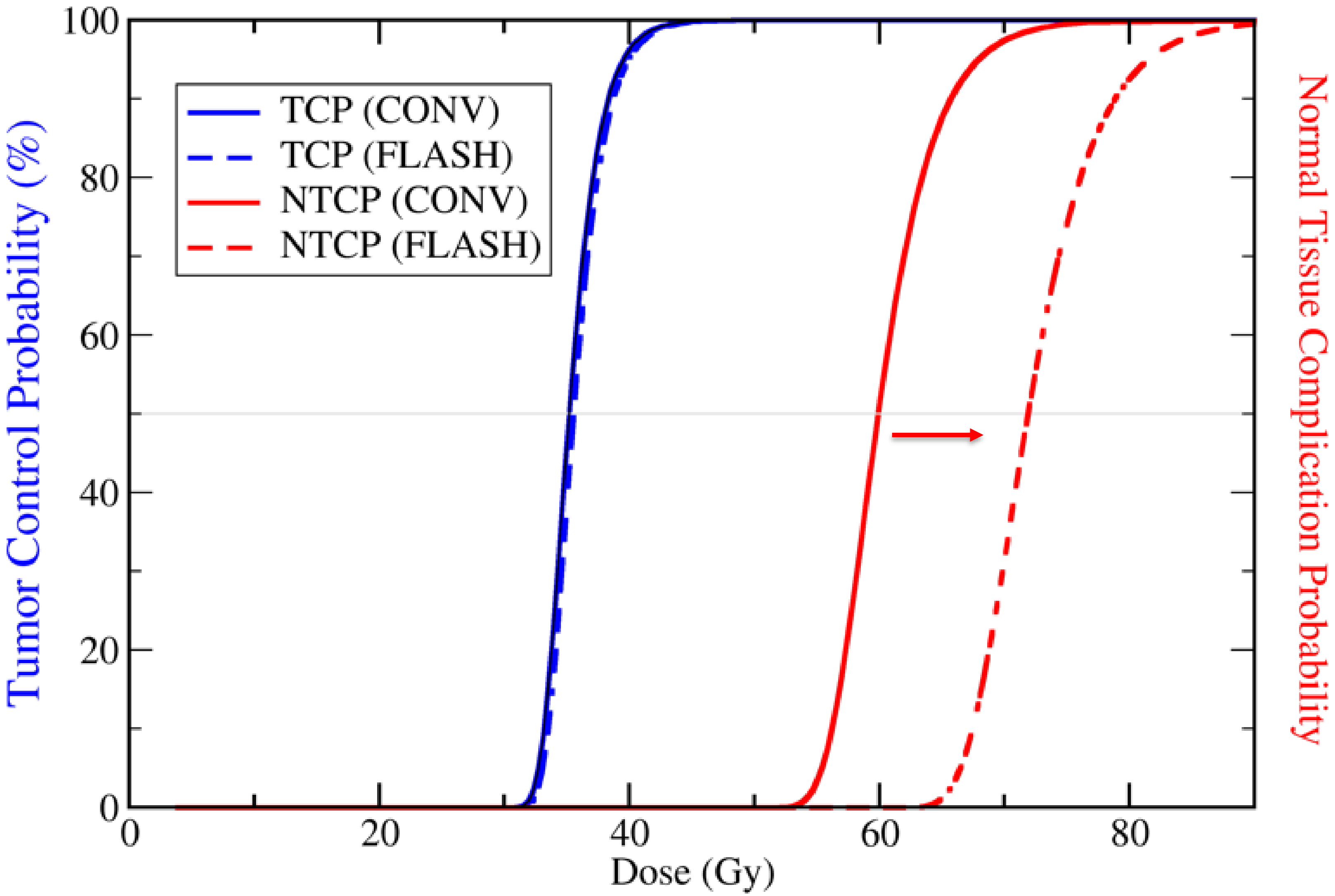
  The study was performed using the Kinetron LINAC, a linear accelerator (linac) delivering 4.5 MeV electrons originally built to investigate pulsed radiolysis and, thus, able to reach extremely high dose intensities.

FLASH effect

- **40 Gys-1 at total doses of ≥10 Gy**;

- Pre-clinical evidence:
  Reduce normal tissue toxicities,
  Same tumor response as conventional RT, **improving the therapeutic ratio**;

- **The FLASH effect:**
  Virtually all radiation modalities used in radiotherapy.



Ultra-high dose rate (FLASH) Radiotherapy

FLASH Radiotherapy, is a novel approach of radiotherapy using ultra-high dose rate:

>40 Gy/s overall dose rate, and over 8-10 Gy

9 mo post-RT

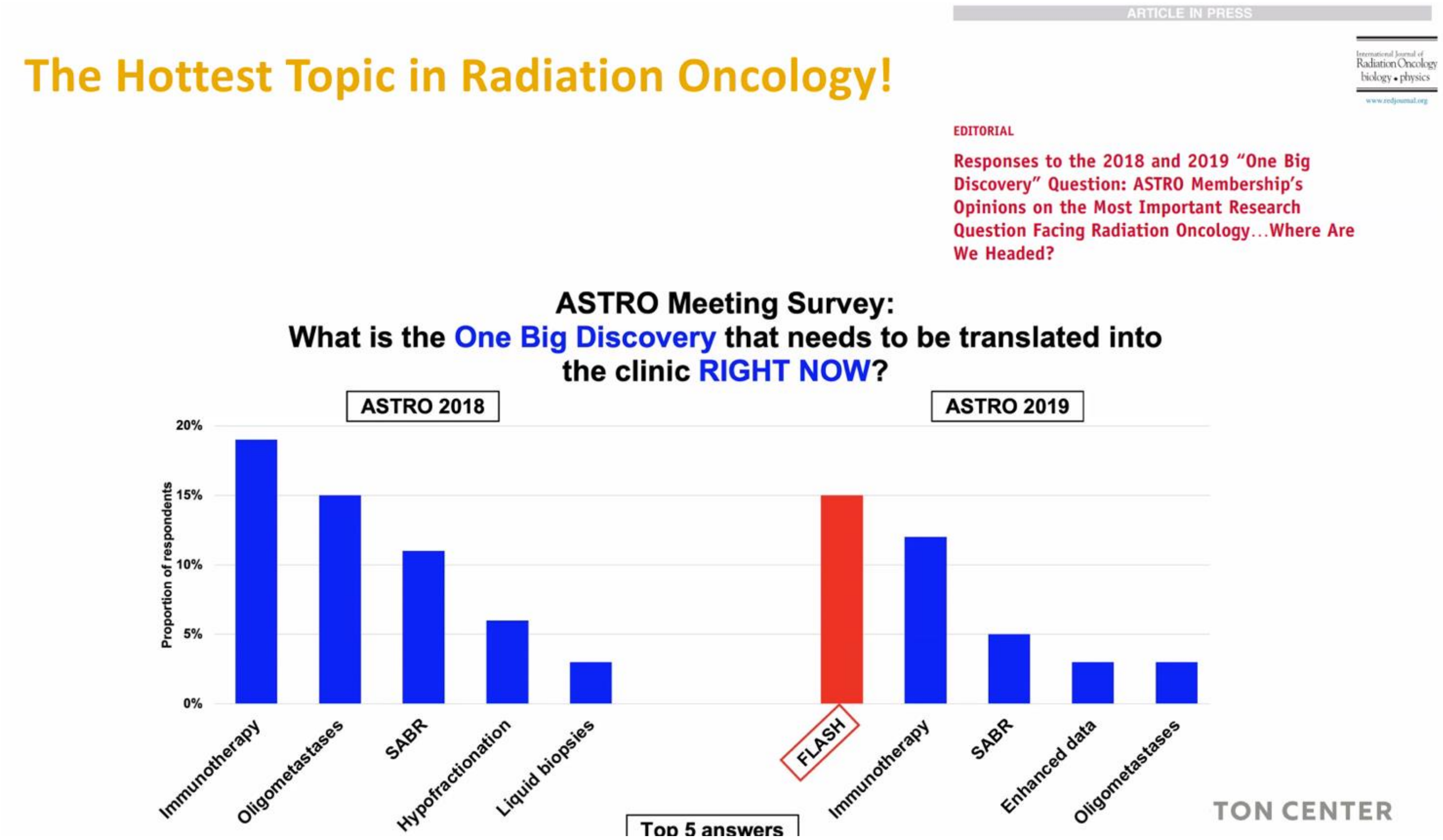
34Gy 31Gy 28Gy

3 years post-RT

34Gy 31Gy 28Gy

Vozenin *et al., Clin. Cancer Res*. 2019

FLASH „boom“



HITRI plus
Heavy Ion Therapy Research Integration

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

Why are we interested in HI-FLASH?

Widening the therapeutic window in C-ion therapy (12 centers in operation worldwide, many more in planning stage)

Exploiting the reduced toxicity to use heavier ions such as ^{20}Ne or ^{40}Ar (LBNL pilot trial)

Understanding the FLASH mechanisms: most of the current hypothesis would predict a *decreased* sparing effect at high-LET

HITRI plus
Heavy Ion Therapy Research Integration

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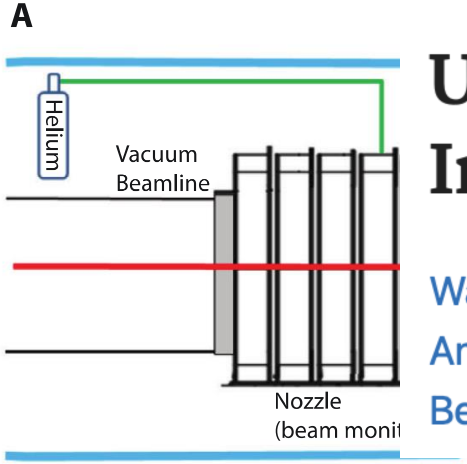
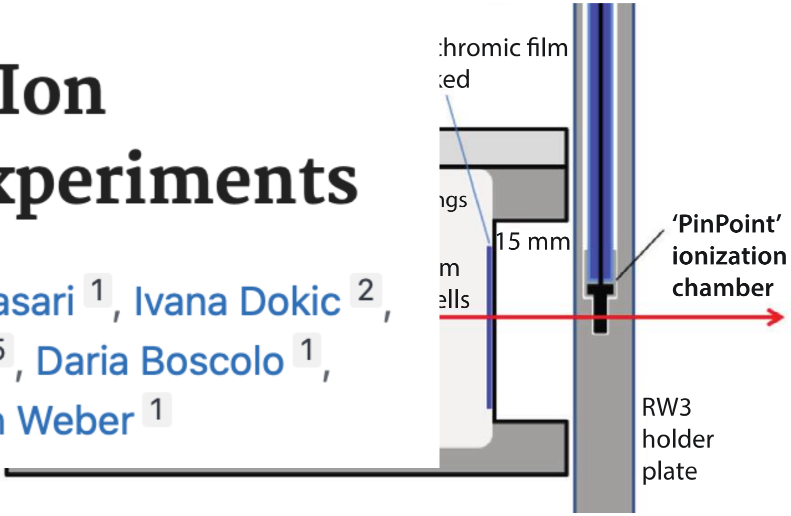
FLASH irradiation: First Carbon ion in vitro experiment

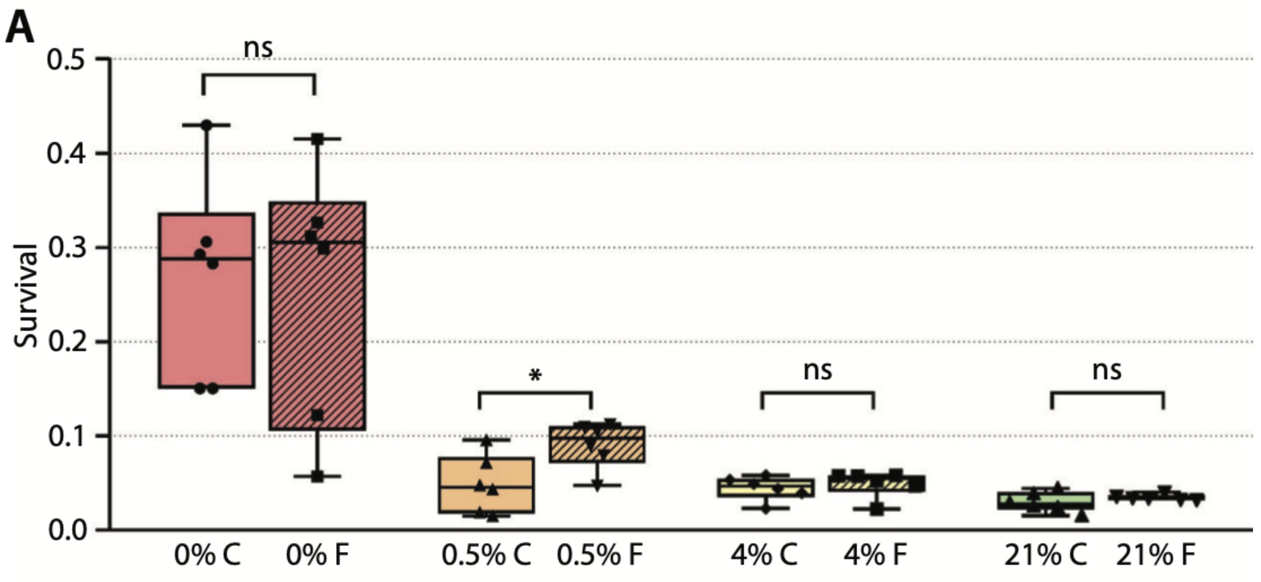
GERMAN CANCER RESEARCH CENTER
IN THE HELMHOLTZ ASSOCIATION

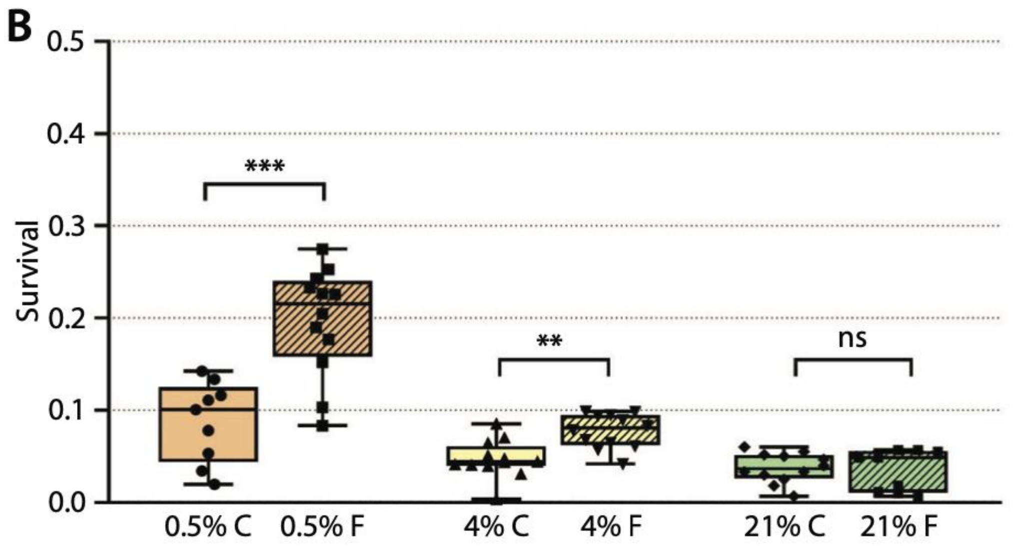


Ultra-High Dose Rate (FLASH) Carbon Ion Irradiation: Dosimetry and First Cell Experiments

Walter Tinganelli [1], Olga Sokol [1], Martina Quartieri [1], Anggraeini Puspitasari [1], Ivana Dokic [2], Amir Abdollahi [2], Marco Durante [3], Thomas Haberer [4], Jürgen Debus [5], Daria Boscolo [1], Bernd Voss [1], Stephan Brons [4], Christoph Schuy [1], Felix Horst [1], Ulrich Weber [1]







FLASH heavy Ion radiotherapy and immune effects

Preservation of lymphocytes through the mitigation of radiation damage to lymphoid organs and **circulating lymphocytes** is crucial for advancing radiotherapy.

The potential benefit of particle therapy, **attributable to its smaller dose bath**, is the sparing of the immune system.

Radioimmunotherapy efficacy depends on the health of the immune system. **Immunotherapy after FLASH radiotherapy (particles) could be greater than after conventional radiation.**

- **Even more sparing effect on circulating immune cells**
- **Less Inflammatory (TGFBeta) (at least for protons)**

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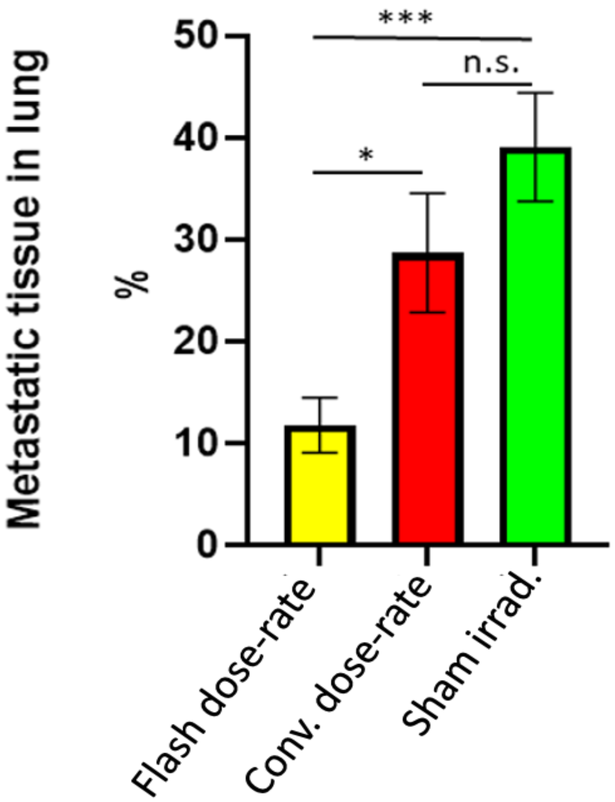
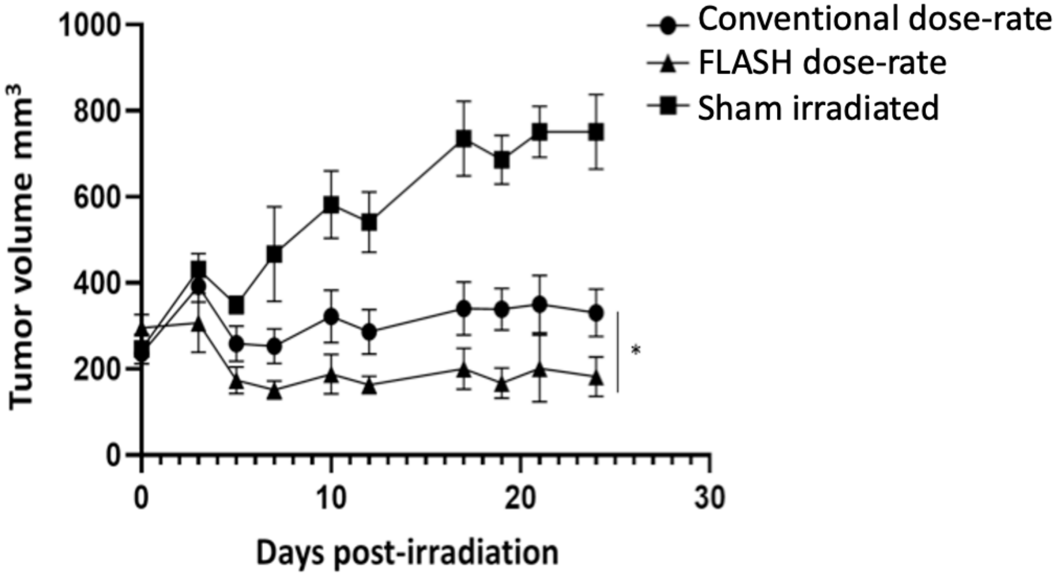
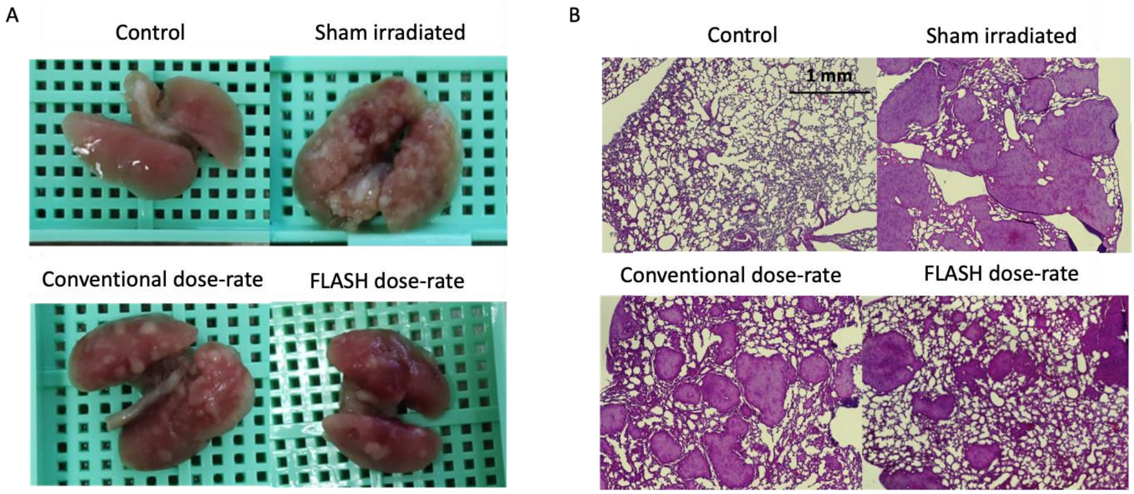
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008548

FLASH with carbon ions: Tumor control, normal tissue sparing, and distal metastasis in a mouse osteosarcoma model

Walter Tinganelli [a], Uli Weber [a], Anggraeini Puspitasari [a], Palma Simoniello [b], Amir Abdollahi [c], Julius Oppermann [a], Christoph Schuy [a], Felix Horst [a], Alexander Helm [a], Claudia Fournier [a], Marco Durante [a, d]







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Take Home Message

- Radiobiology research is essential in particle therapy, especially radiobiology of heavy ions;
- Heavy ion biological effects are qualitatively different from X-rays;
- Heavy ions are more efficient against radioresistant/hypoxic tumors;
- Heavy ions seem to be more effective in activating an immune response / higher immunogenic cell death;
- FLASH effect is also observed with carbon ions *in vitro* and *in vivo*;
  - FLASH is promising for the future of radiation oncology, but its comprehension is limited;
  - More experiments at high LET are necessary for mechanistic understanding
  - The suppression of lung metastases, maybe a unique feature of particle beams.

HITRIplus
Heavy Ion Therapy Research Integration

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Thank you for your attention

Heavy Ion Therapy Research Integration

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