Cells response under high dose rate and multi-bunch irradiation

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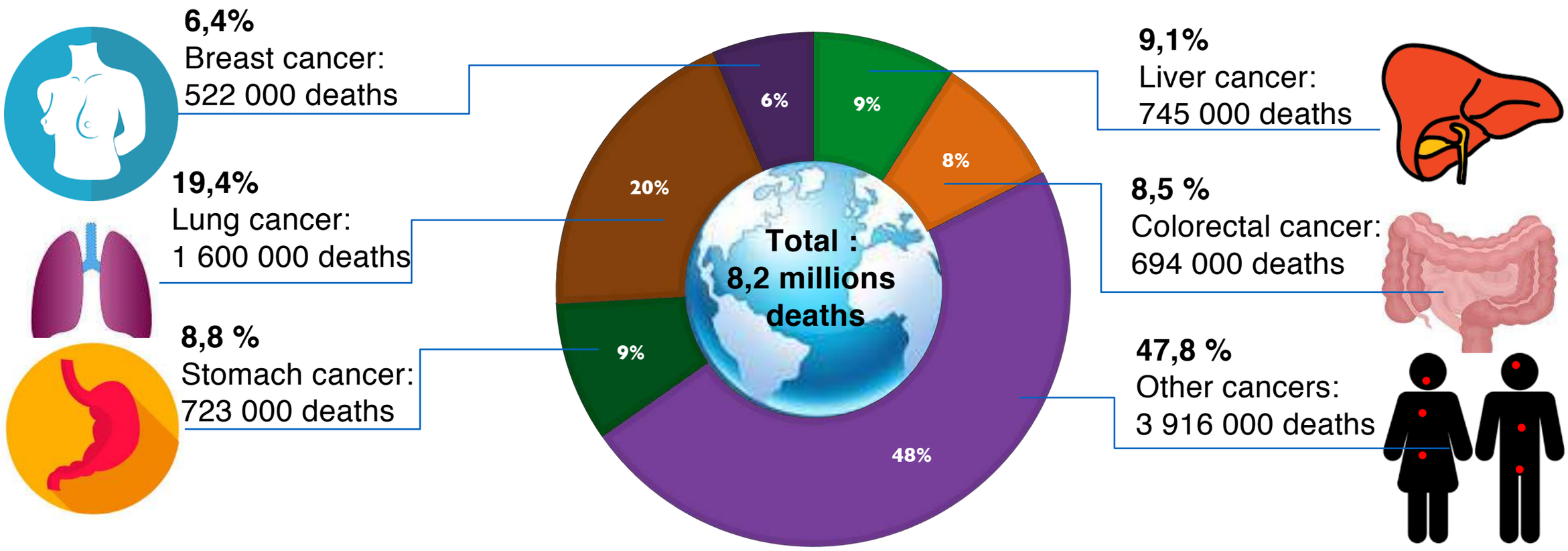
SPIE, Applying Laser-driven Particle Acceleration Workshop: Using Distinctive Energetic Particle and Photon Sources, Praha, Czech Republic, April 2-3 (2019)

Outline

- Motivation and context
- Ionizing radiation effects
- Experimental protocol
- Experimental results
- Conclusion and perspectives

Cancer : number one killer worldwide

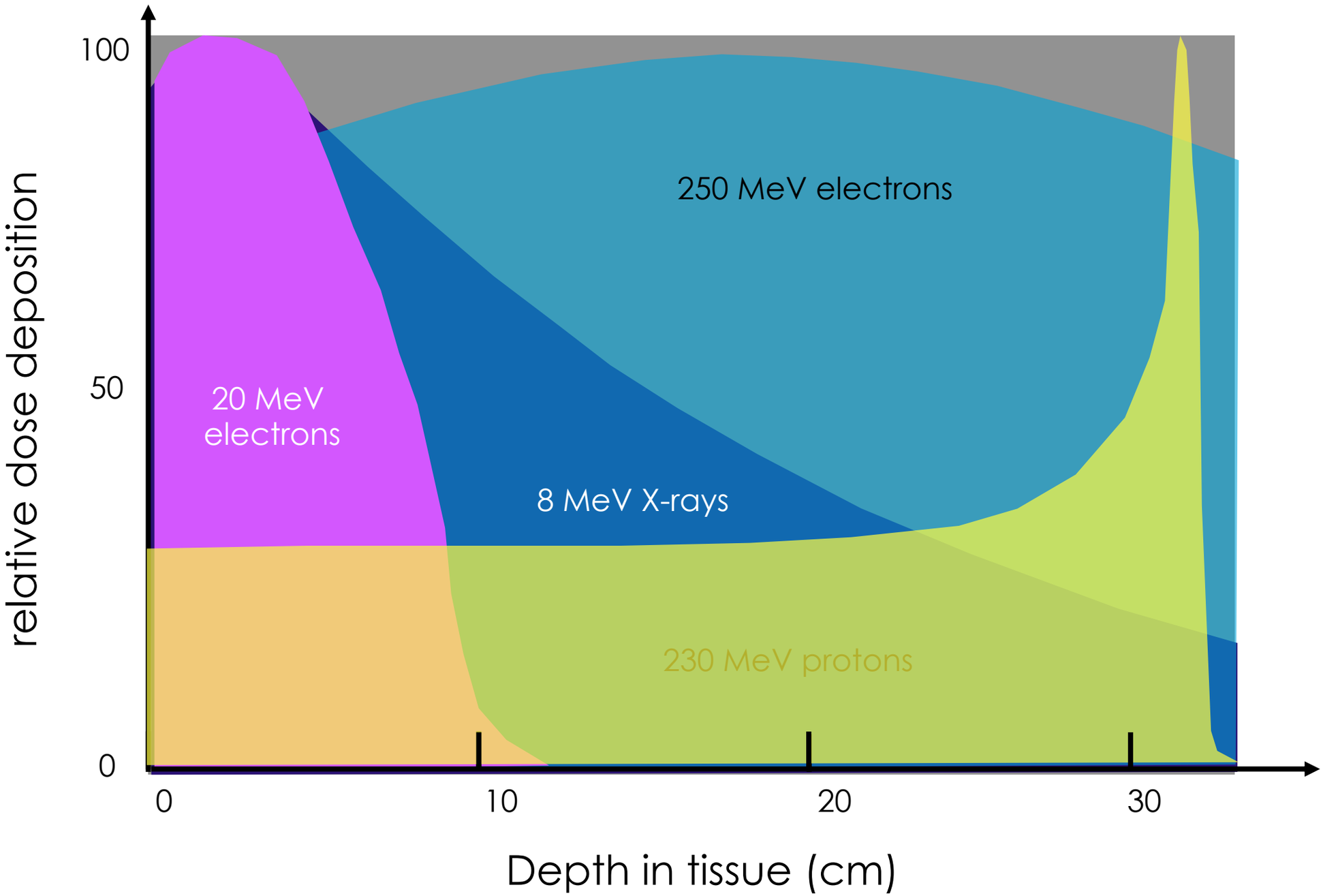
Responsible for nearly 13% of global mortality and growing epidemically with over 15 million new cancer patients per year. This rate is expected to double by 2035 due the global aging of the population.



Estimated number of deaths in 2018, all cancers, both sexes, all ages.

Source: International Agency for Research on Cancer

Radio-proton-electron-therapy

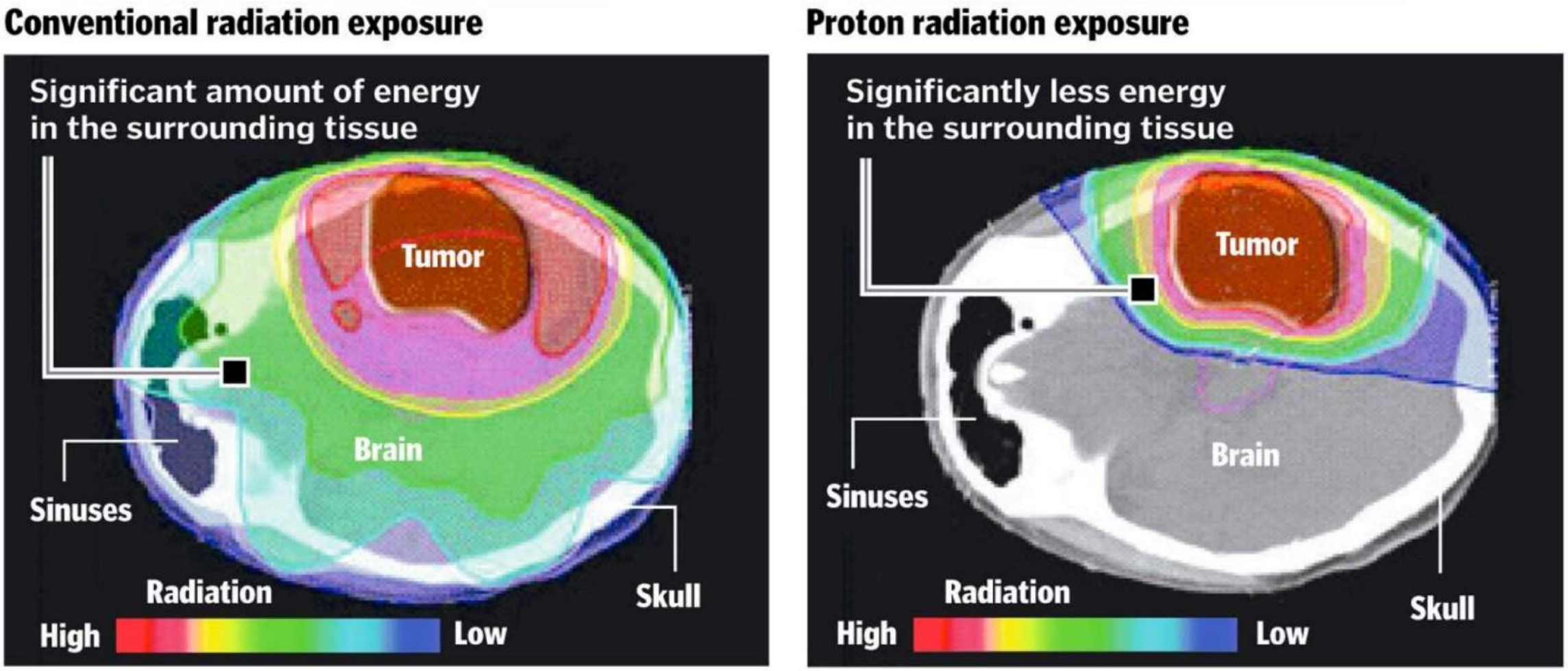


~50% of cancer are treated by radiation therapy

Favorable ballistic of hadrons (protons and heavy ions)

Dose "Focused" into the tumor.

Preservation of neighboring tissues, sides effects decrease



SOURCES: University Hospitals Seidman Cancer Center; ProCure

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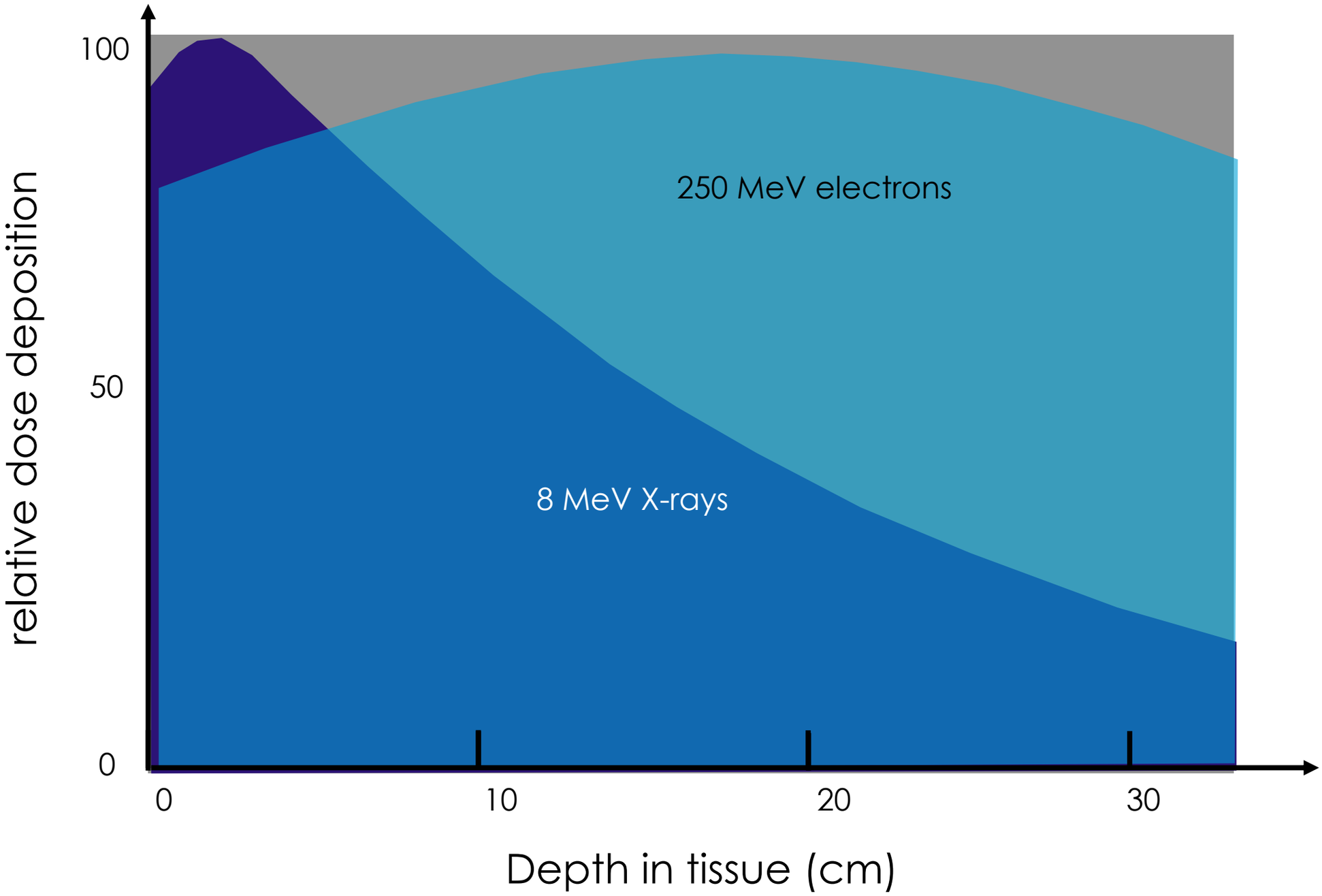
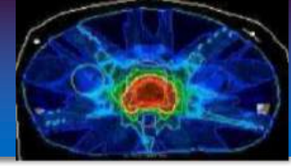
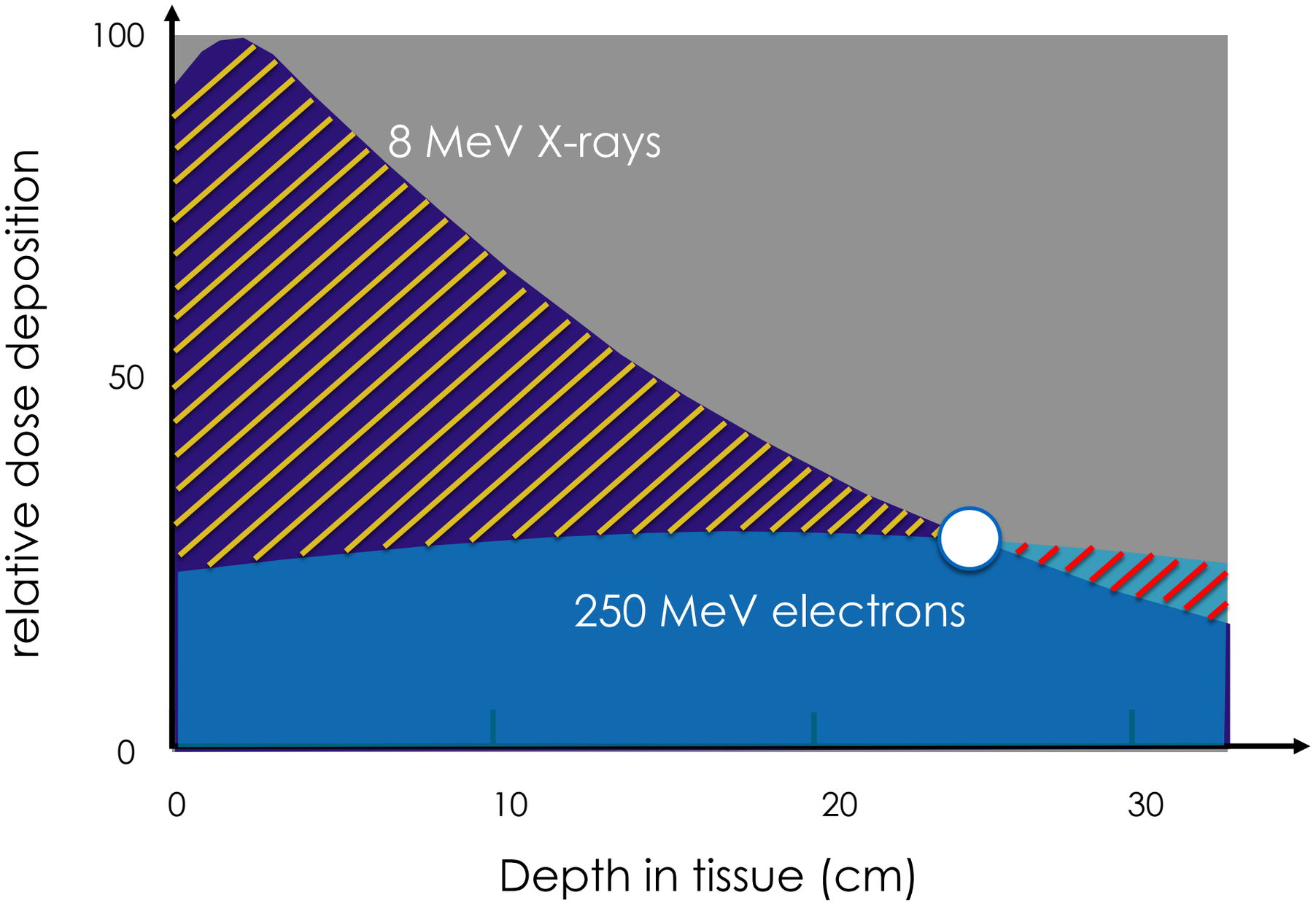
Radio-proton-electron-therapy

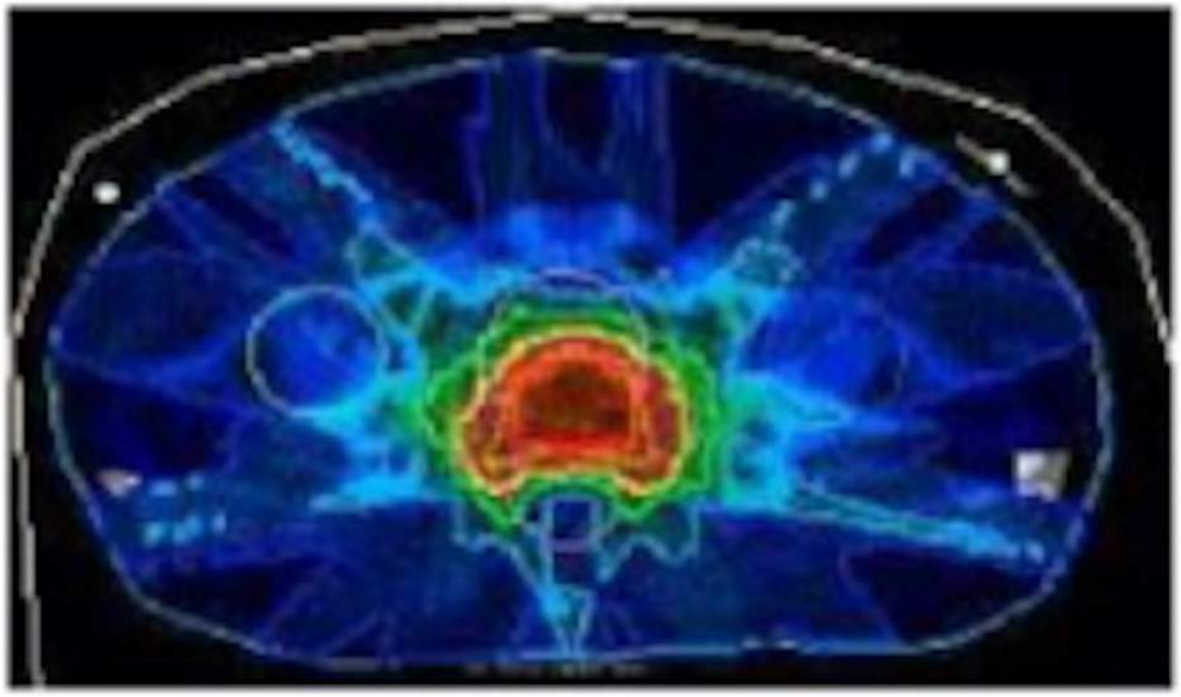


Dose deposition with high energy electrons

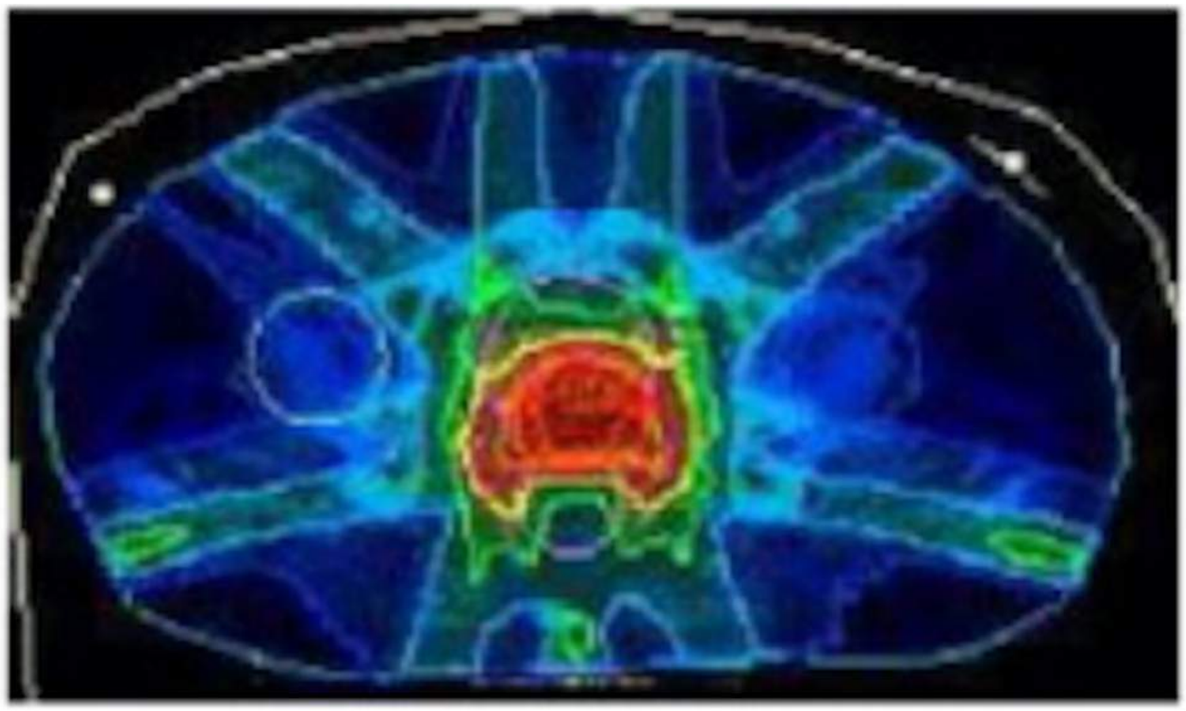




e-therapy: case of prostate cancer tumour



250 MeV electrons



6 MeV X ray

A comparison of dose deposition with 6 MeV X ray an improvement of the quality of a clinically approved prostate treatment plan. While the target coverage is the same or even slightly better for 250 MeV electrons compared to photons the dose sparing of sensitive structures is improved (up to 19%).

T. Fuchs *et al.*, Phys. Med. Biol. 54, 3315-3328 (2009), Patent

Y. Glinec *et al.*, Med. Phys. 33, 1, 155-162 (2006)

O. Lundh *et al.*, Medical Physics 39, 6 (2012)

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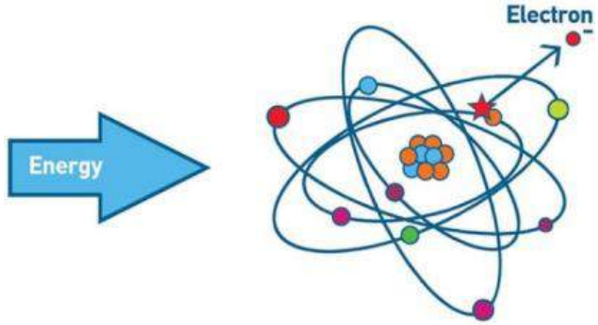
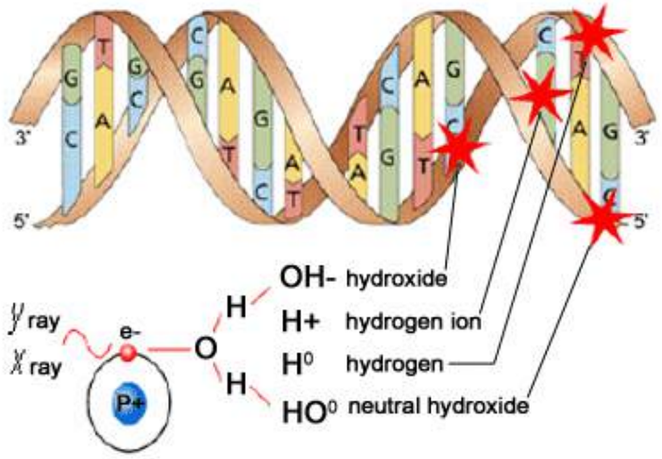
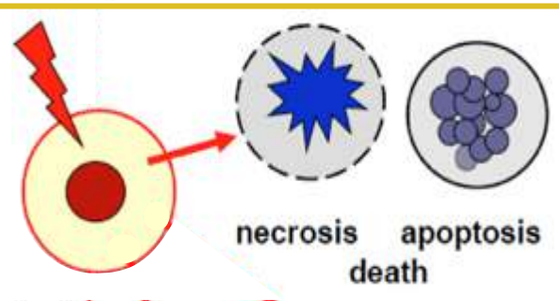
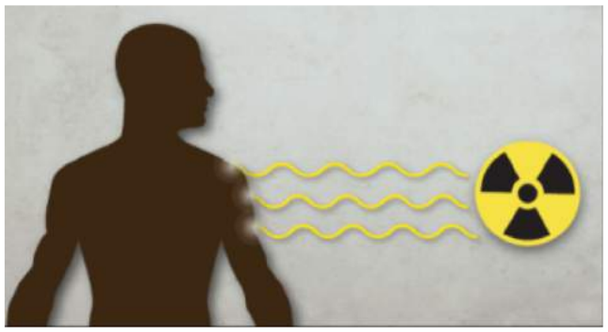
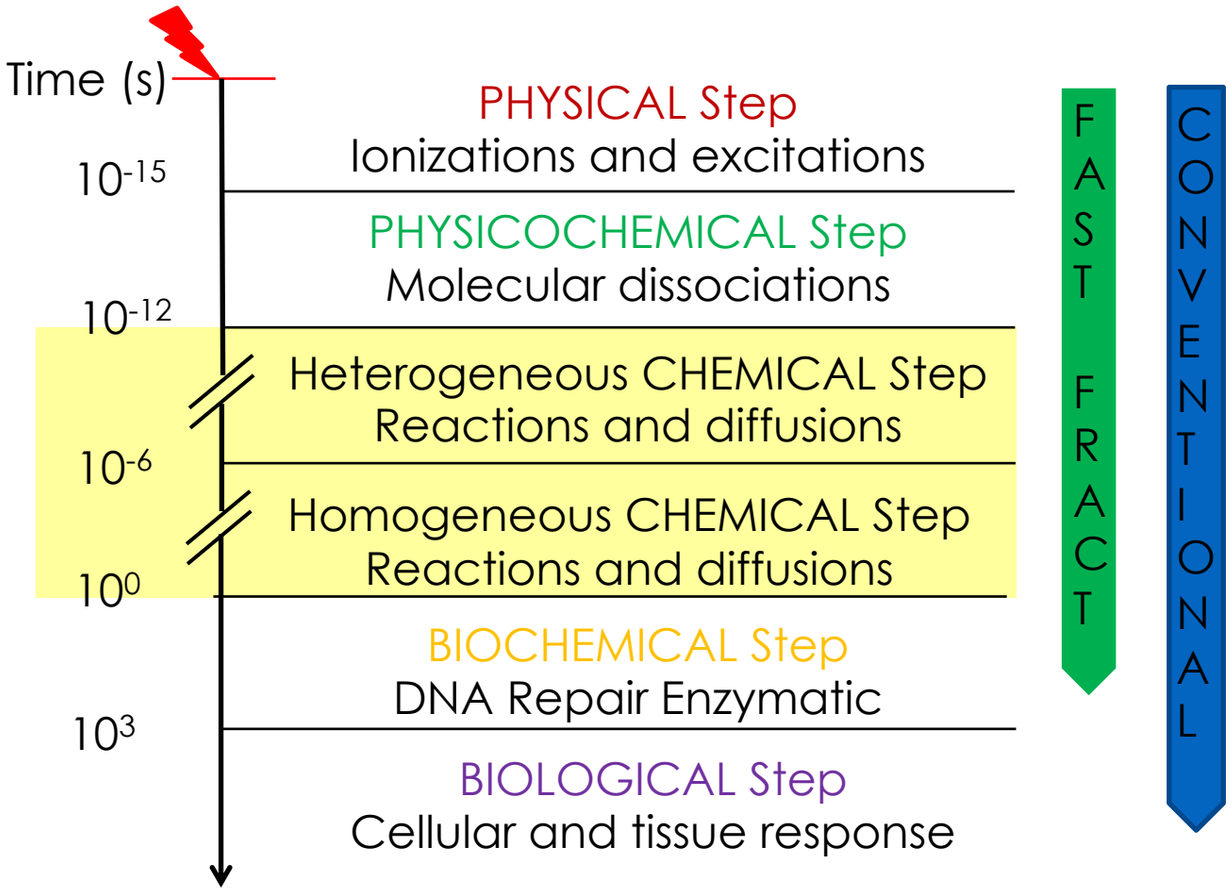
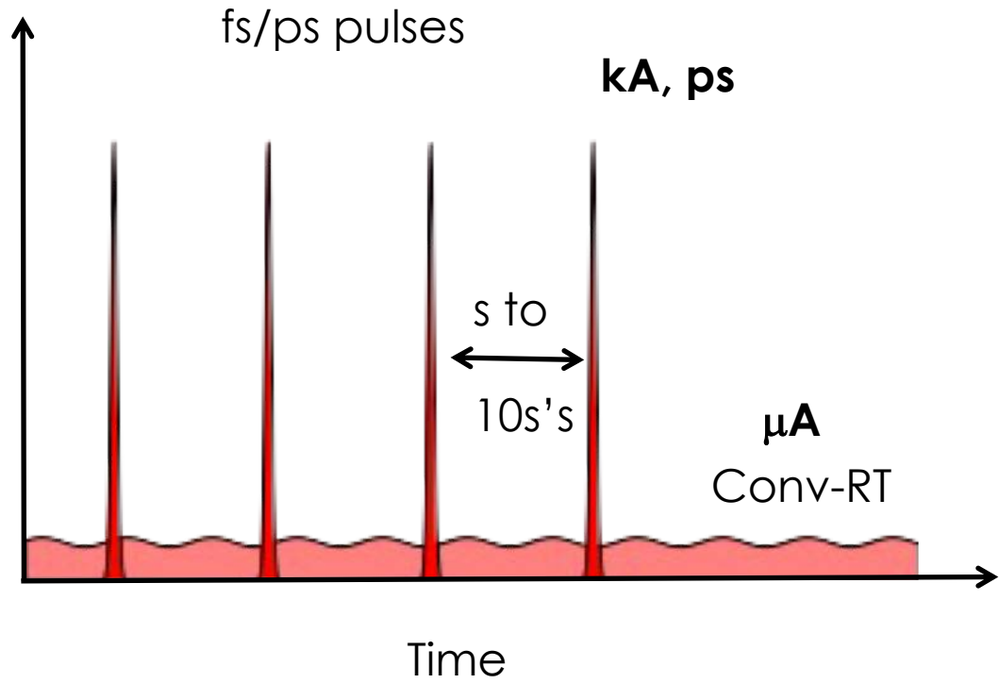
Effects of Ionizing Radiation

Atom		Excitation Ionization	fs to ns
Molecule		Radical production radiolysis DNA brain break DNA Damage	ns to seconds
Cell		Cell death	Days
Organism		Somatic disease & hereditary	Years

Chronology of post-irradiation events

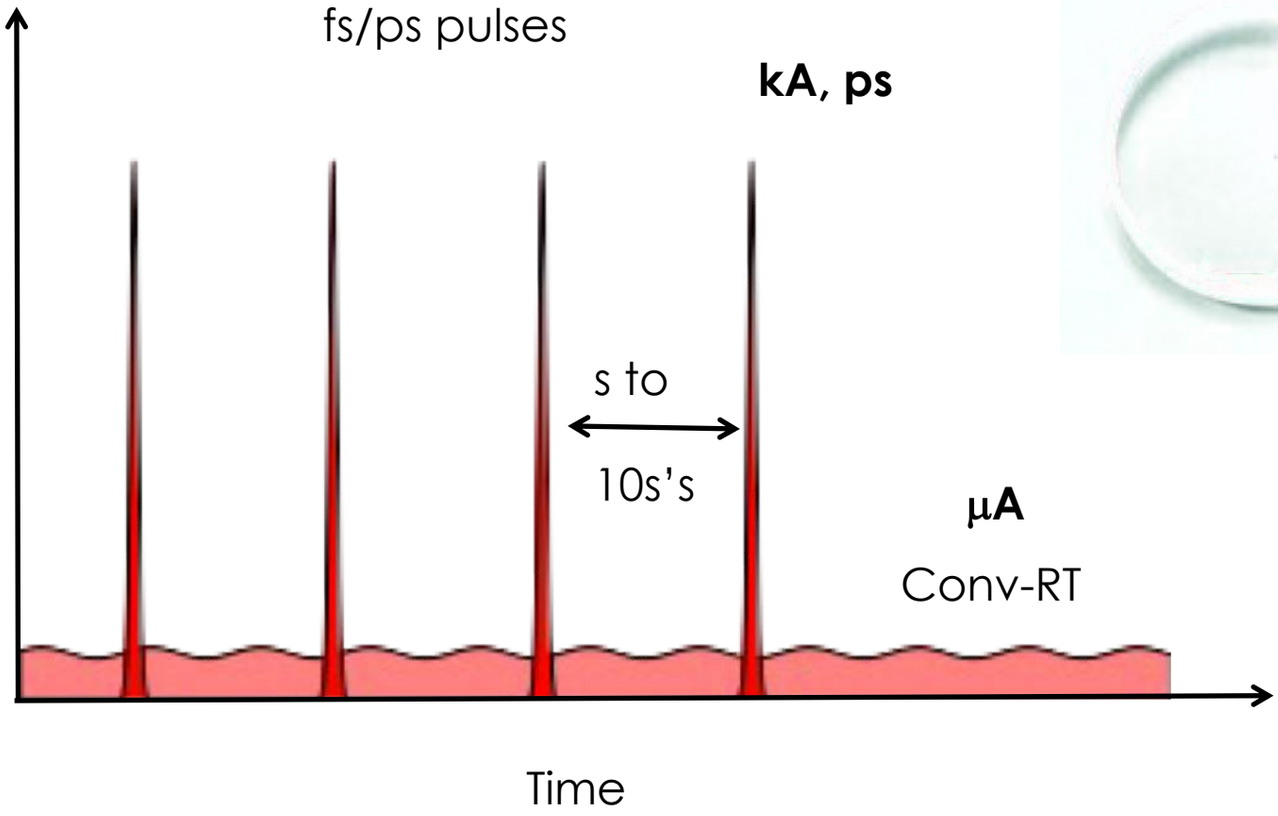




- Chronology of physical, physico-chemical, chemical, biochemical and biological events occurring after irradiation in the biological tissue.
- The difference between FF and RT delivered at a conventional dose rate (Conv-RT) is the duration of the exposure to the ionizing radiation during the chemical step of the cascade (in yellow).
- The chemical steps are highly dependent on the dioxygen concentration in tissues.

In vitro irradiation with protons

- Stability
- Repeatability
- Repetition rate
- Total dose: 2 Gy
- Irradiation surface
- Homogeneity
- And in air



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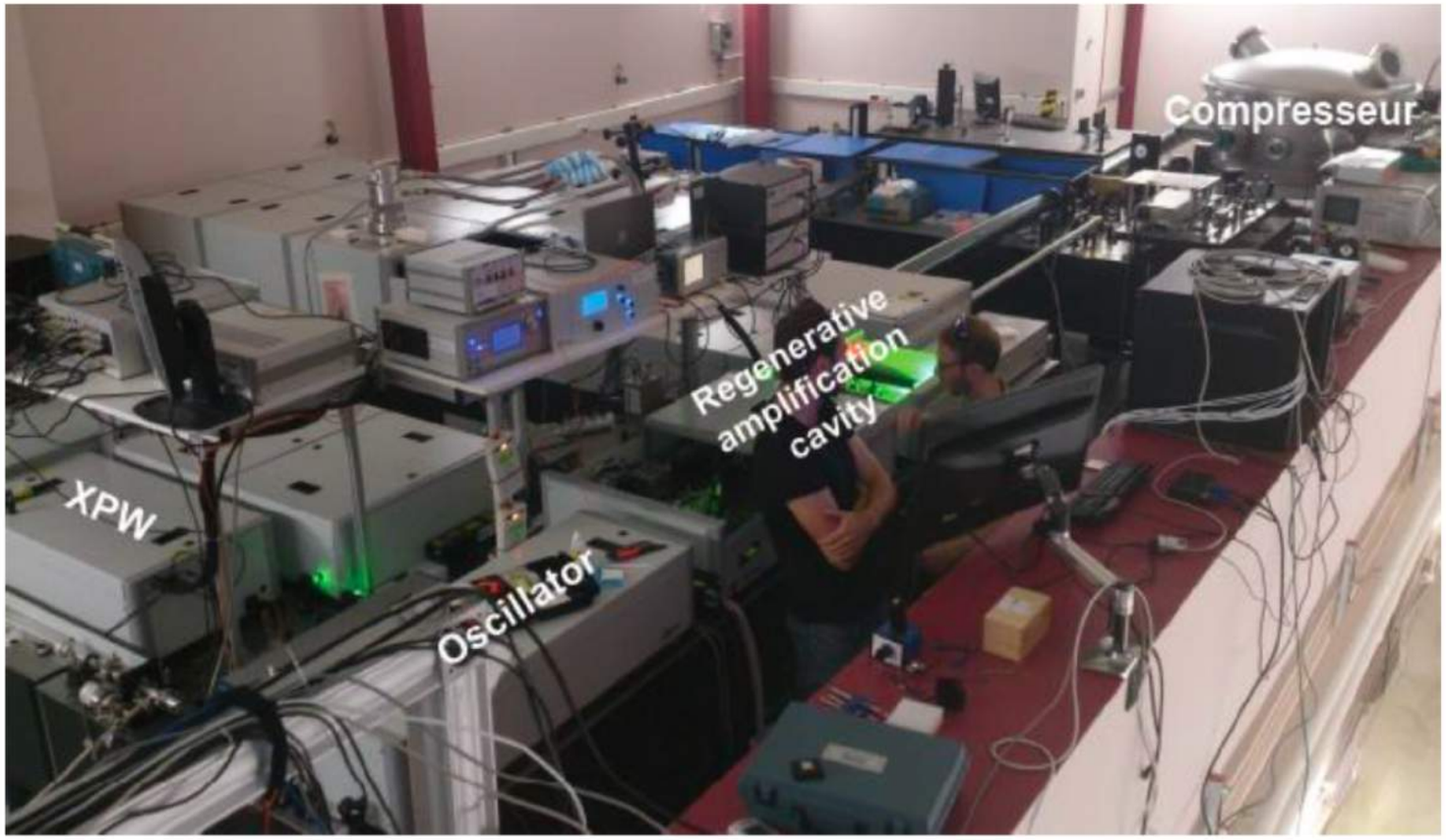
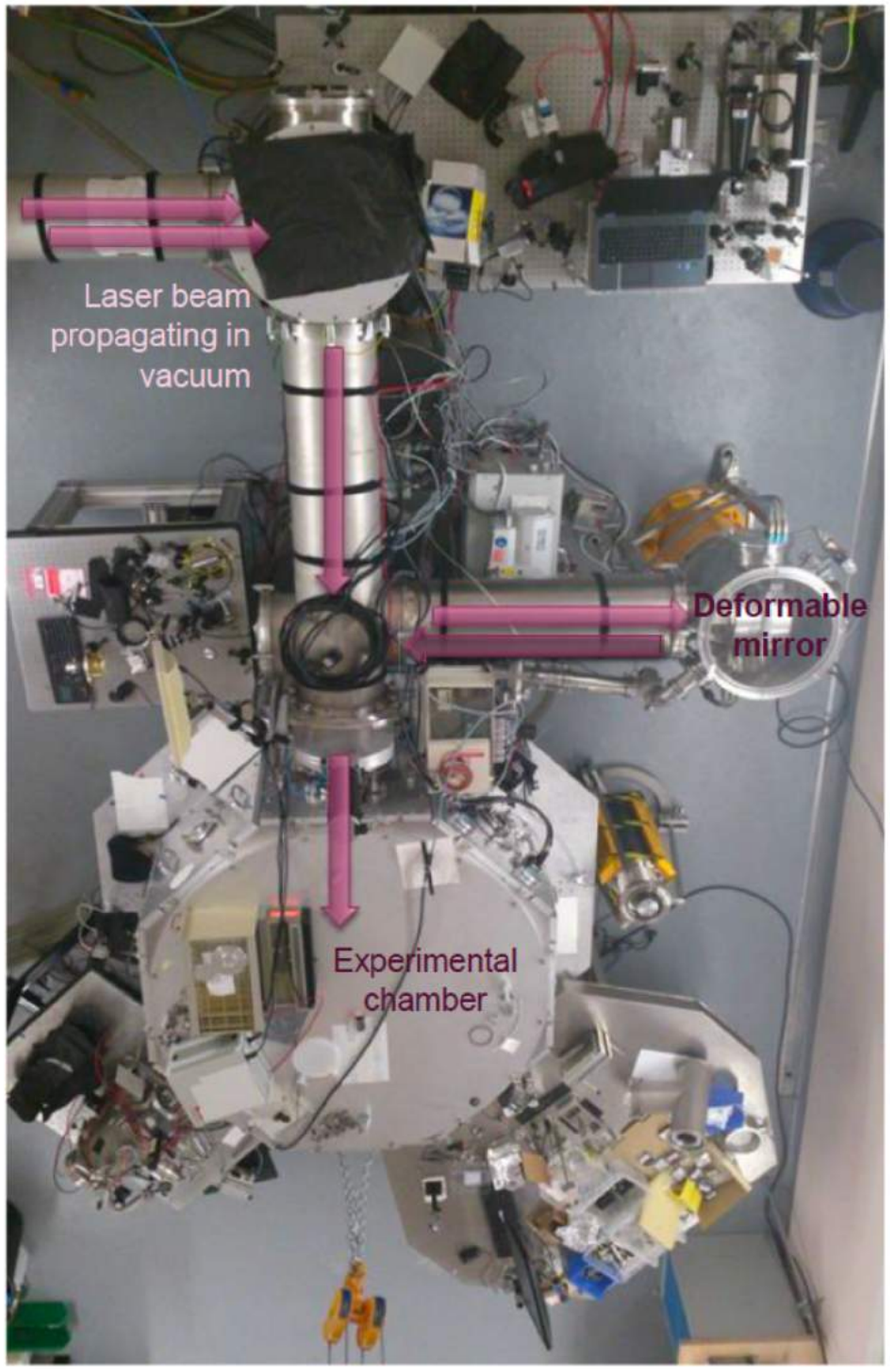
Experimental protocol

Laser Installation



Experimental chamber



Laser Parameters

Gain medium : **Ti:Sapphire crystal**

Output energy : **6 J** (uncompressed)

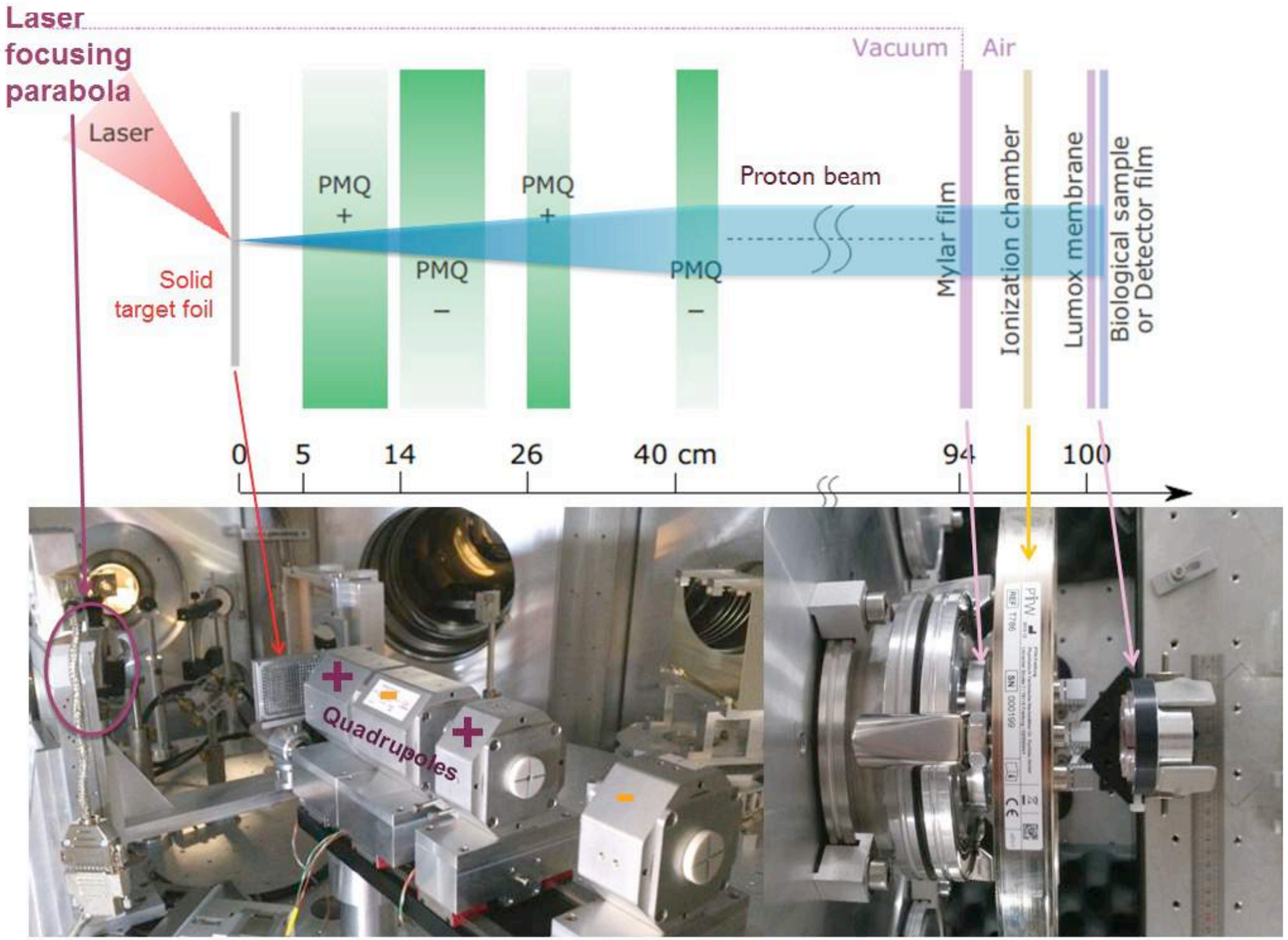
Pulse duration : **25 fs**

Focal spot diameter : **10 µm**

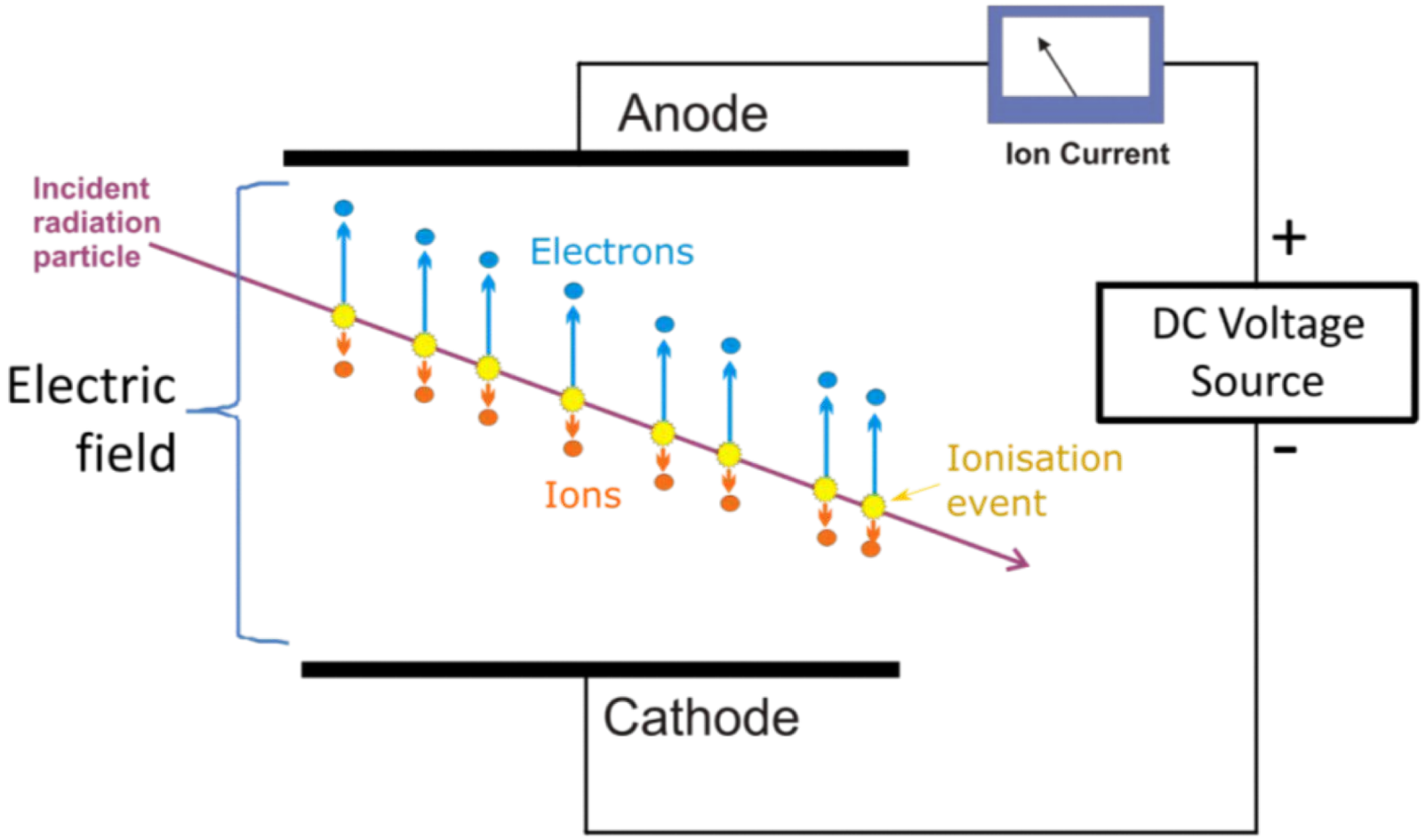
Intensity on target : **2×10^{20} W.cm-2**

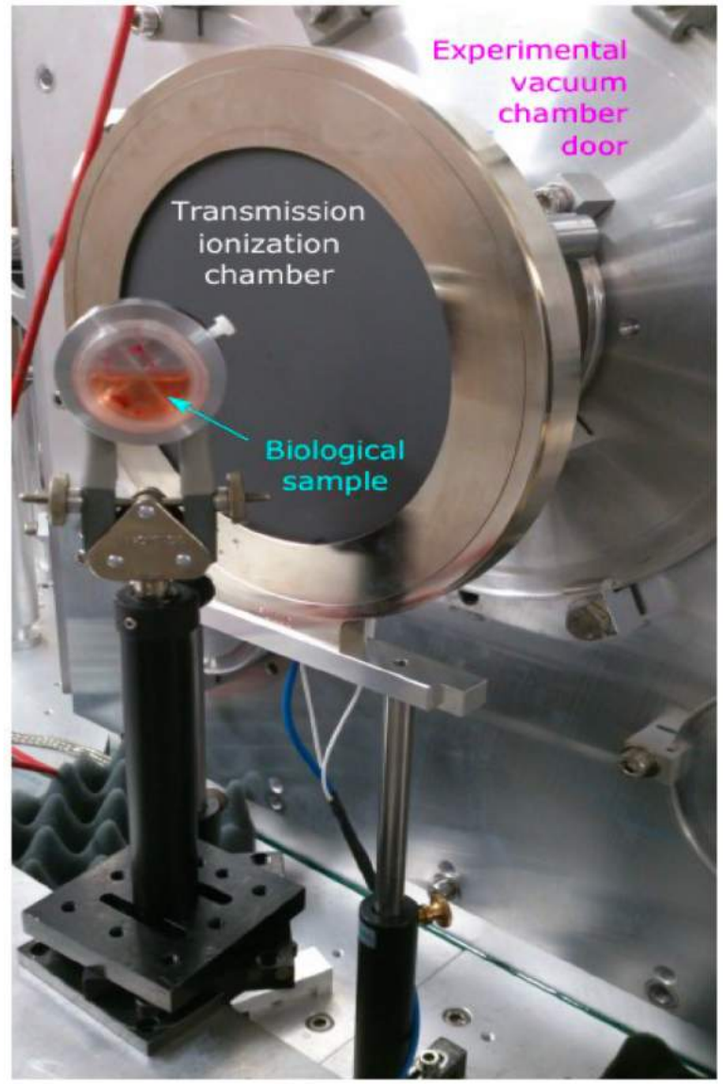
Contrast with XPW : **10^{-10}**

Experimental protocol : beam transport



Experimental protocol : dosimeter



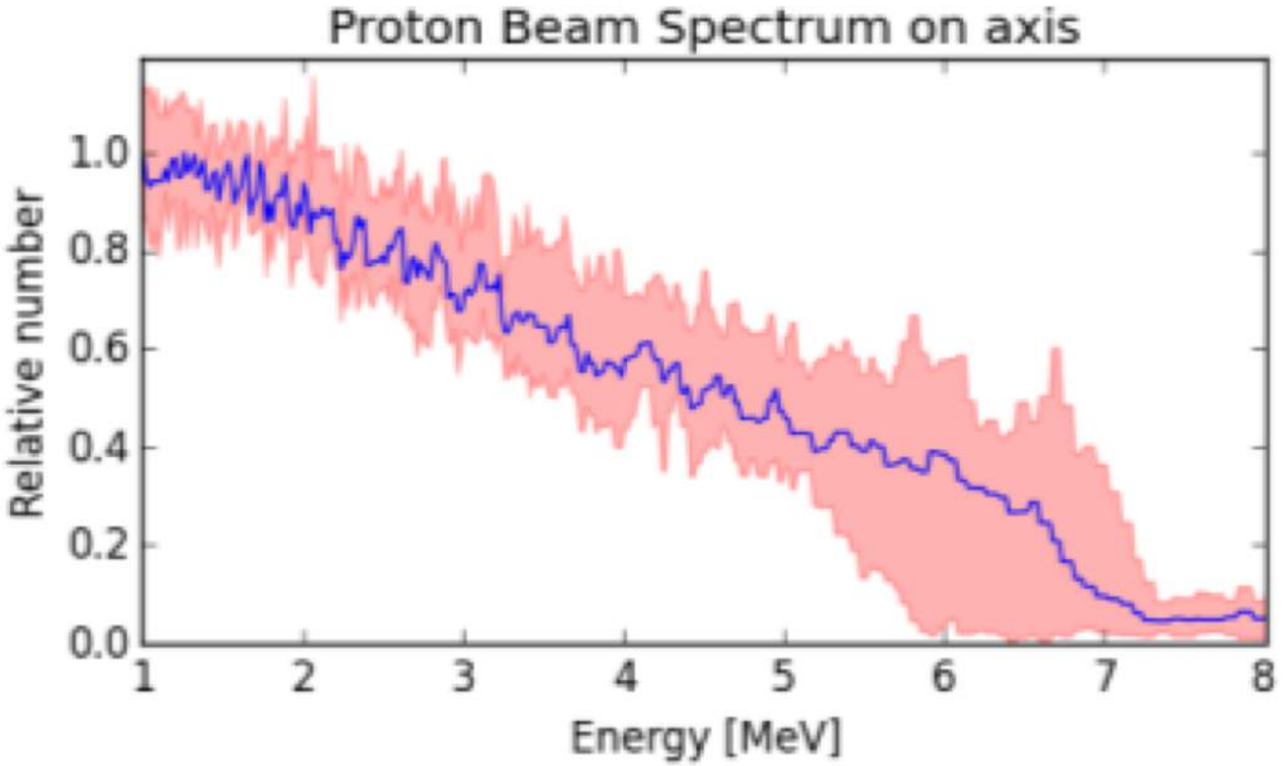


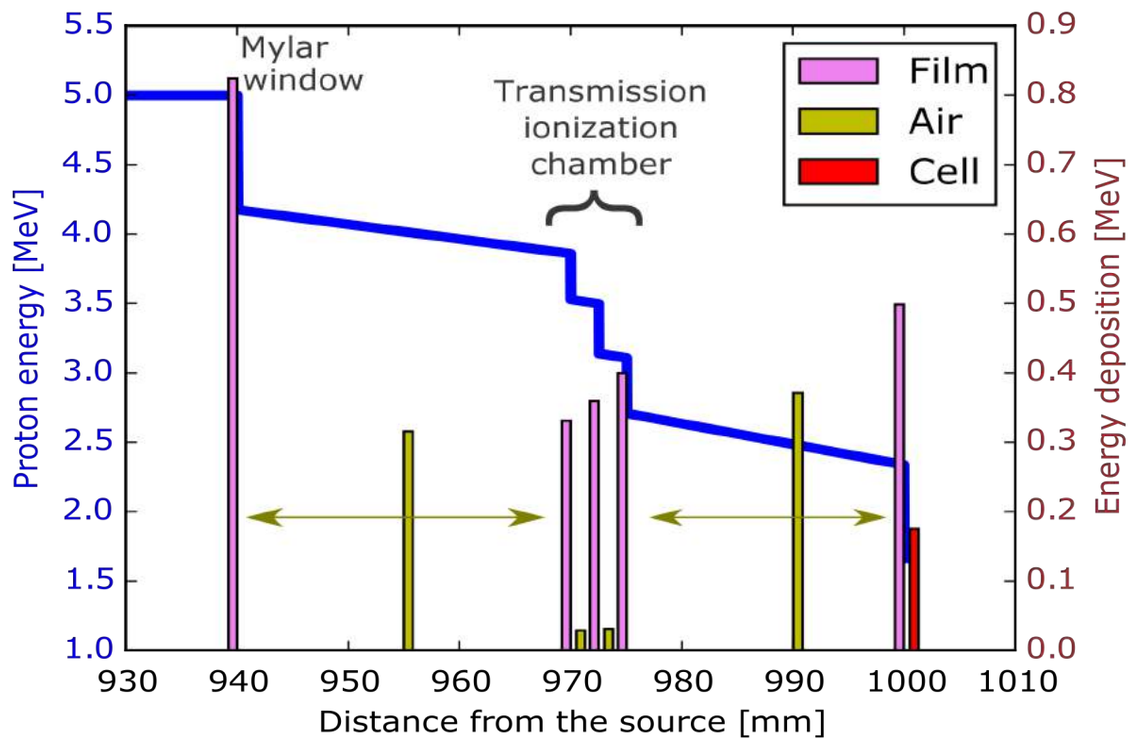
Transmission Ionization chamber

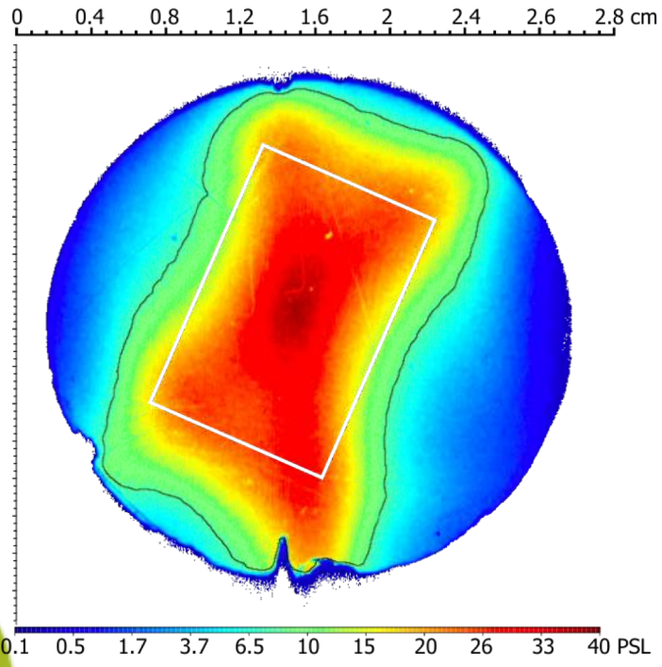
Large diameter: 15cm, thickness: 110um H_2O, Linear response

Absolute calibration at CPO

Irradiation condition *in vivo*

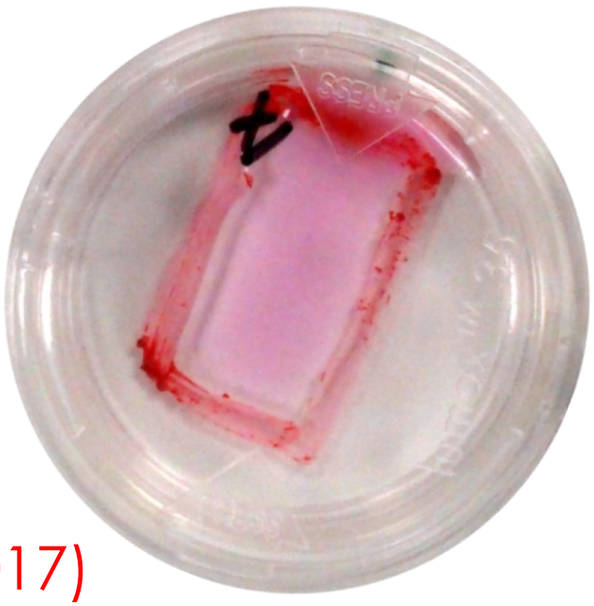






- 0.7 Gy/shot (in vitro 2D)
- stability : 6 % rms
- uniformity : 20 % rms
- duration : ~ ns
- dose rate : > 10^8 Gy/s
- repetition : 1/1.5 s (quasi-automatic)

3 years of experiment



L. Pommarel *et al.*, PRAB 20, 032801 (2017)

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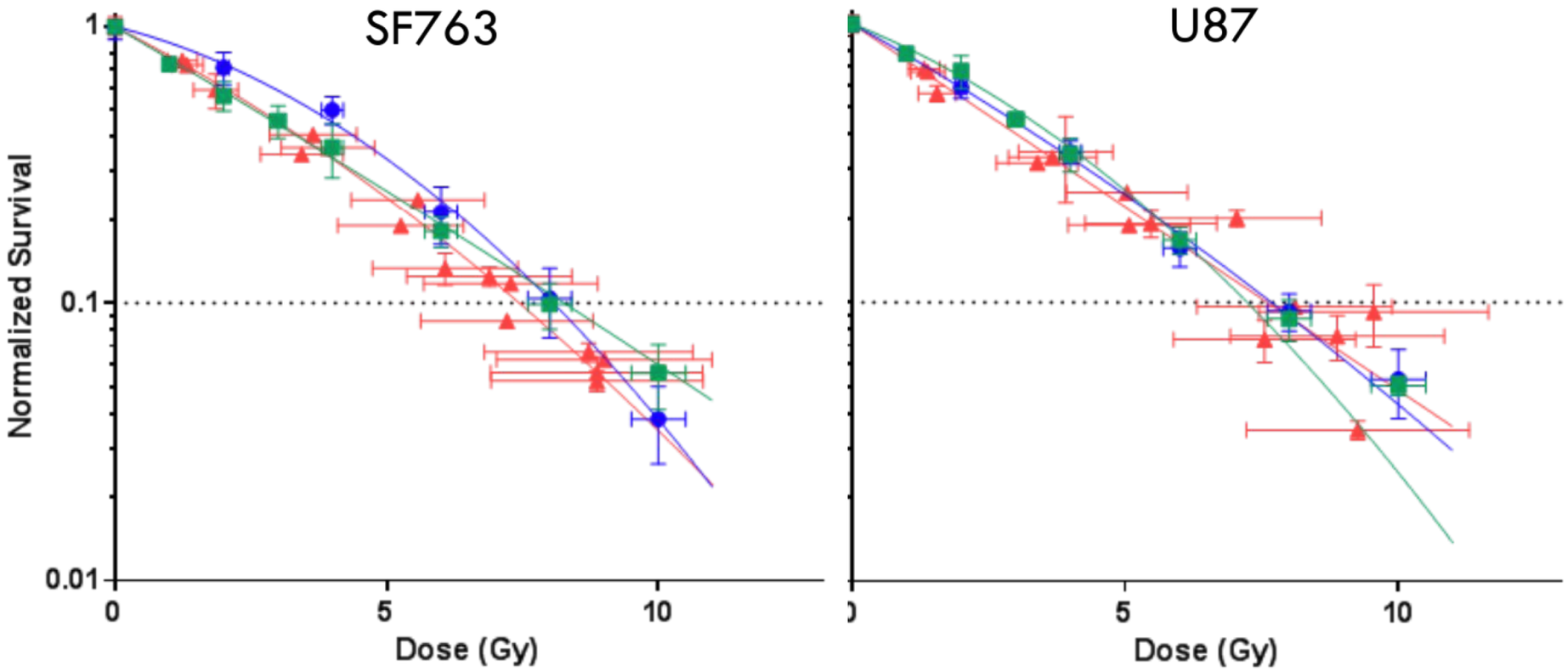
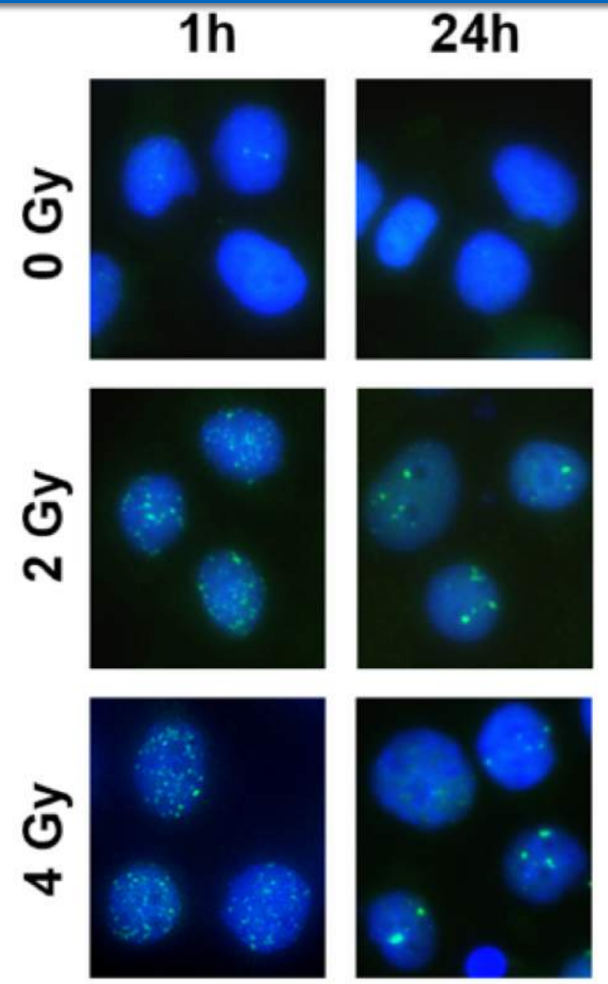
No difference in DNA damage foci irradiated by p-Laser, p-Conv, and X rays

Highly resistant human glioblastoma cell lines SF763/U87



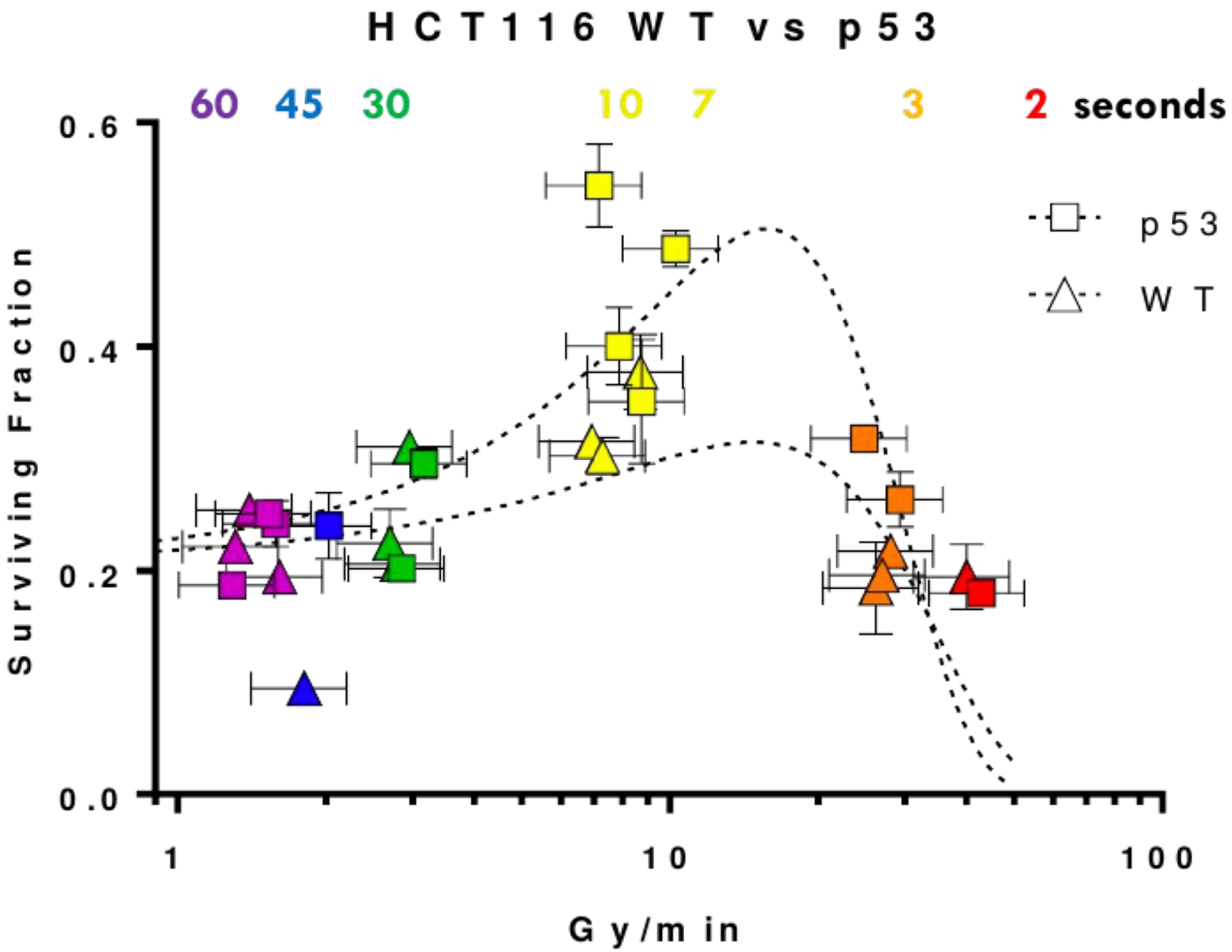


Dose responses of DNA damage foci formation and of cell survival. (A) Representative immune-fluorescent images of cells obtained 1h and 24h after exposure to the indicated doses of laser driven protons (LDP, dotted square), conventional accelerated protons (CAP, triangles) and X-rays (x cross). The negative controls (0 Gy) were sham-irradiated.

Average dose rate : 2.1 Gy/min (Δt = 20 s)

Fast Fractionation effects on radiosensitive cells

radiosensitive colorectal cancer cells HCT116WT and
Its radioresistant counterpart HCT116 $p53^{-/-}p53$



*Cell lines were exposed to a fixed number of LDP bunches with a variable delay between shots, from 60 to 2 seconds.

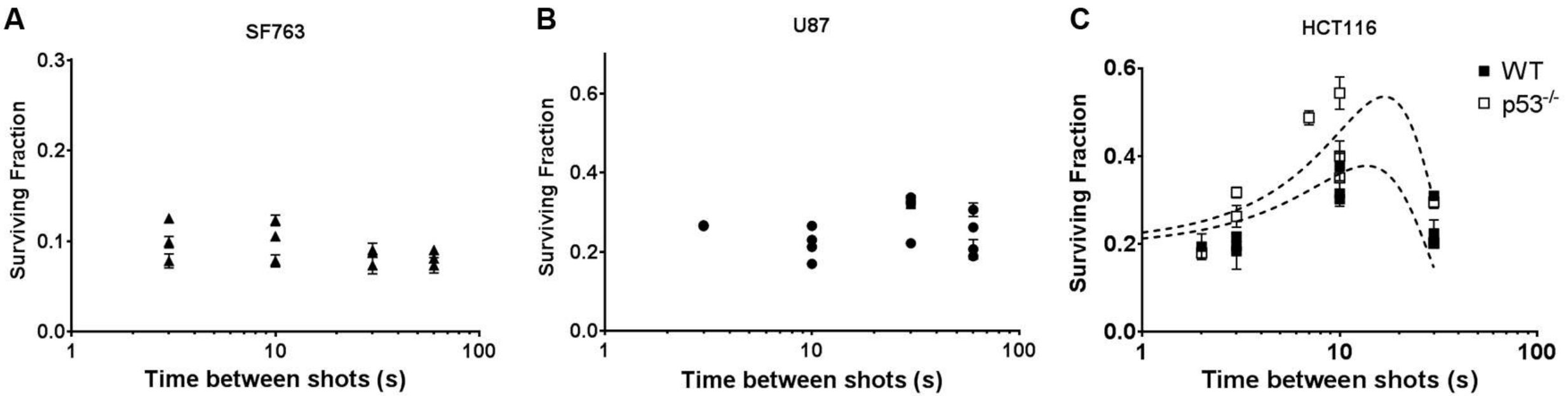
*Hence the total irradiation time varied from 15 to 540 seconds, which changes the overall "average" dose rate.

E. Bayart *et al.*, submitted

FF effects radioresistant/radiosensitive cells

Radioresistant Glioblastoma cell lines SF763 (triangles)

radiosensitive colorectal cancer



Dependency of variation of the repetition rate of bunches on cell survival. Normalized survival fraction resulting from exposure for a given dose of LDP bunches delivered with different delay ranging from 3 to 60 seconds. Radioresistant Glioblastoma cell lines SF763 (triangles) (A) and U87-MG (dots) (B) were submitted to a dose corresponding to nine bunches of LDP (6.3±1.39 Gy). (C) radiosensitive colorectal cancer HCT116 cells, WT (white squares) and $p53^{-/-}$ (black squares) were exposed to five bunches of LDP (3.5±0.77 Gy). Each data point represents the mean of three replicates obtained in at least three independent experiments.

➢ FF effects seems to be cell type dependent

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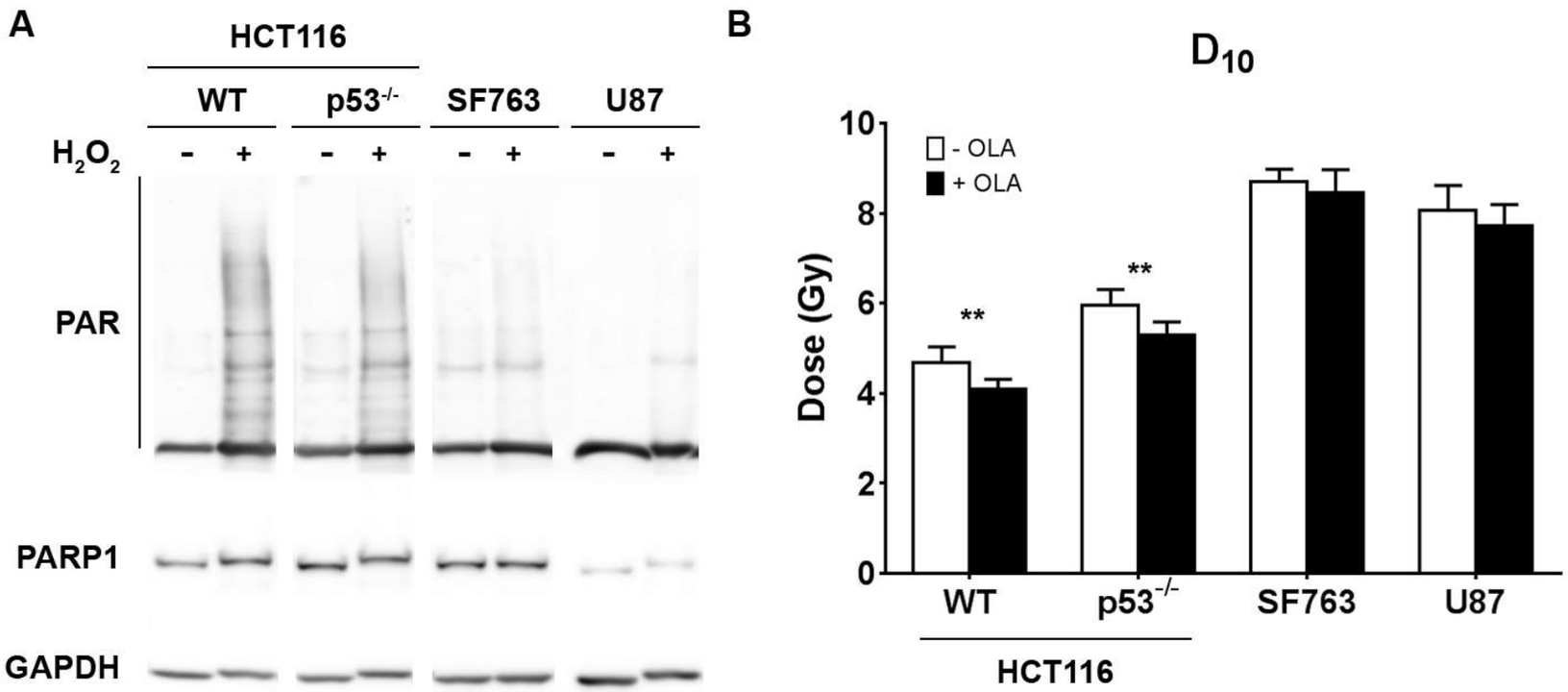
FF effects sensitive to parp1/parylation protein

Oxidative stress (with hydrogen peroxide) promotes the formation of DNA breaks, which induces the over-activation of PARP1 and leads to protein parylation.



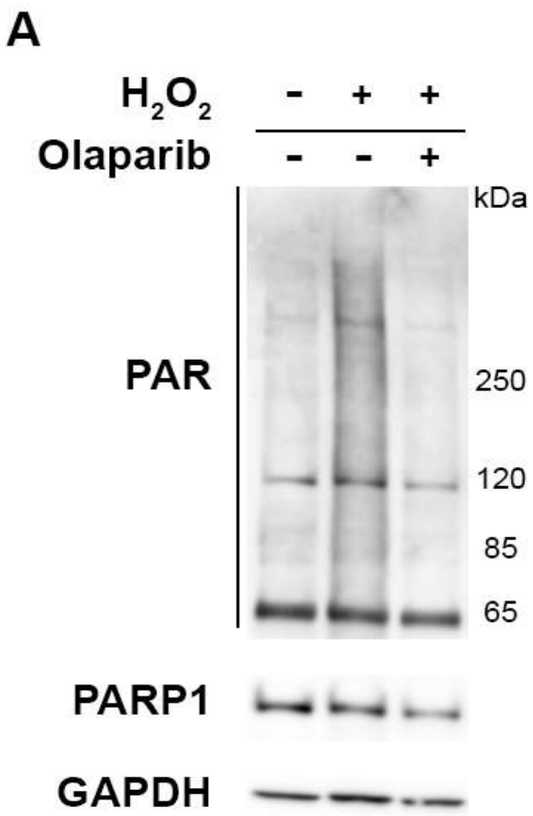
(A) Western Blot detection of the PARP1 protein and parylation for HCT116 WT, HCT116 p53$^{-/-}$, SF763 and U87-MG cells left untreated or treated. (B) D_{10} values determined from survival curves obtained after exposure to increasing doses of X-rays of HCT116 WT, HCT116 p53$^{-/-}$, SF763 and U87-MG cells left untreated or treated with Olaparib (PARP1 inhibitor).

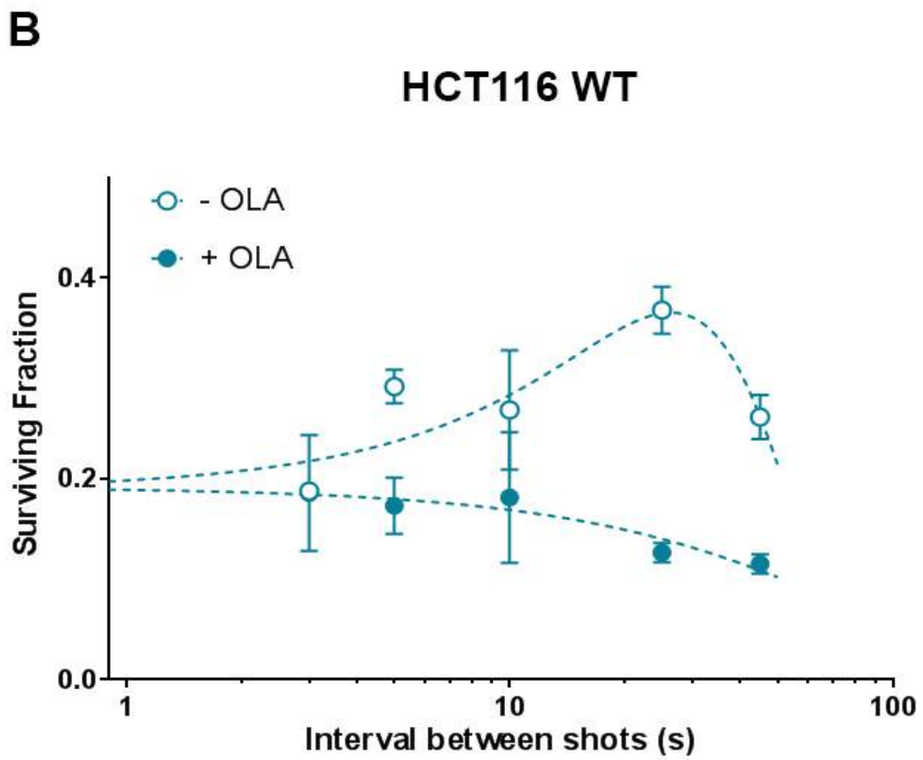
Inhibition of PARP1 in cells produces an increased sensitivity to genotoxic stress, particularly in response to ionizing radiations.

To exploit this advantage for the treatment of cancer, PARP1 inhitibors, such as Olaparib have been developed.

Results suggest that the cell survival variation with the proton bunch cadency is related to the presence of a functional PARP1 protein

Parp1 inhibition on cells survival in Fast Fractionation





Effect of PARP1 inhibition on cell survival behavior in response to the variation of the delay between LDP bunches. (A) Western Blot detection of the PARP1 protein and parylation (B) HCT116 WT cells were left untreated or treated one hour with 200 nM Olaparib, and were then exposed to five bunches of LDP (3.5±0.77 Gy).

As PARP1 protein is a crucial actor in DNA damage signaling, it suggests that cell survival variation induced by proton bunch repetition rate could be due to a specific impact of this dose deposition modality on DNA damage signaling and repair.
The increase of the proton bunch cadency led cells exhibiting a functional PARP1 activity to the same decrease of cell survival level as combining LPD to PARP1 inhibition: this result suggests that only using specific LDP dose deposition modality could be as efficient as drug-combined radiotherapy.

E. Bayart *et al.*, submitted

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Conclusion

- Fast fractionation of the dose has an additional impact on the resulting cell survival at constant dose

- The FF effect is the consequence of a particular fast dose fractionation condition, with the lowest cell survival for a bunch delay of about 2-3 seconds.

- Interestingly, at this repetition rate, the cell survival of the radioresistant HCT116 $p53^{-/-}$ cells converged with that of HCT116 WT cells, which was similar to the case when Olaparib was added. This result suggests that, depending on the bunch repetition rate, cell killing obtained with laser pulsed particles could be as efficient as combined therapy using PARP inhibitors.

- This particular point highlights a therapeutic advantage that laser plasma accelerators could provide compared to continuous therapeutic beams.

- These results supported the great therapeutic potential of laser plasma technology and pulsed beams of ionization radiation to provide innovations in radiotherapy for cancer treatment.

Perspectives

High Dose rate (10^8-10^{13} Gray/s) pulsed ionizing beam will open new horizons in modern radiobiology with *time* as a new parameter

- What are the fundamental mechanisms of radiation damage*?
- What are the early biological responses of living tissues?
- How the cells communicate after such huge stress?
- Do the cancer cells recover differently that the safe ones?,
- How those effects scale with the dose and the dose rate ?

More References

•Challenges of dosimetry of ultra-short pulsed very high energy electron beams, Subiel et al, Physica Medica 2017, May 11.

•Ultra short laser accelerated proton pulses have similar DNA damaging effectiveness but less immediate nitroxadative stress than conventional proton beams, S. Rasche et al., Sci. Rep. 6, 32441(2016)

•Very high-energy electron (VHEE) beams in radiation therapy; Treatment plan comparison between VHEE, VMAT, and PPBS, Schuler et al, Med Phys 2017

•Assessment of the quality of very high-energy electron radiotherapy planning, Palma, et al, Radiother Oncol. 2016 Apr;119(1).

•Treatment planning for radiotherapy with very high-energy electron beams and comparison of VHEE and VMAT plans, Magdalena Bazalova-Carter, Med. Phys. 42, 2615 (2015)

•Comparison of film measurements and Monte Carlo simulations of dose delivered with very high-energy electron beams in a polystyrene phantom, Magdalena Bazalova-Carter, Med Phys. 2015 Apr;42(4):1606-13

•Dosimetry of very high energy electrons (VHEE) for radiotherapy applications: using radiochromic film measurements and Monte Carlo simulations, Subiel et al, Phys Med Biol. 2014 Oct 7;59(19):5811-29

•Pluridirectional Very High Electron Energy Radiation Therapy Systems and Processes, Patent # US 20130231516 A1

•Laser-Driven Very High Energy Electron/Photon Beam Radiation Therapy in Conjunction with a Robotic System, Kazuhisa Nakajima, Appl. Sci. 2015, 5, 1-20

Acknowledgements

B. Vauzour, L. Pommarel, O. Delmas, D. Levy, M. Cavallone, Ph. Zeitoun, D. Adjej, A. Flacco, S. Sebban

F. Schillaci, F. Romano, V. Scuderi, G. A. P. Cirrone

E. Bayart (now at LOA), F. Megnin-Chanet, E. Deutsch, F. Goujil, C. Nauraye