Emerging trends in cellular response to proton irradiations at ultra-high dose rates

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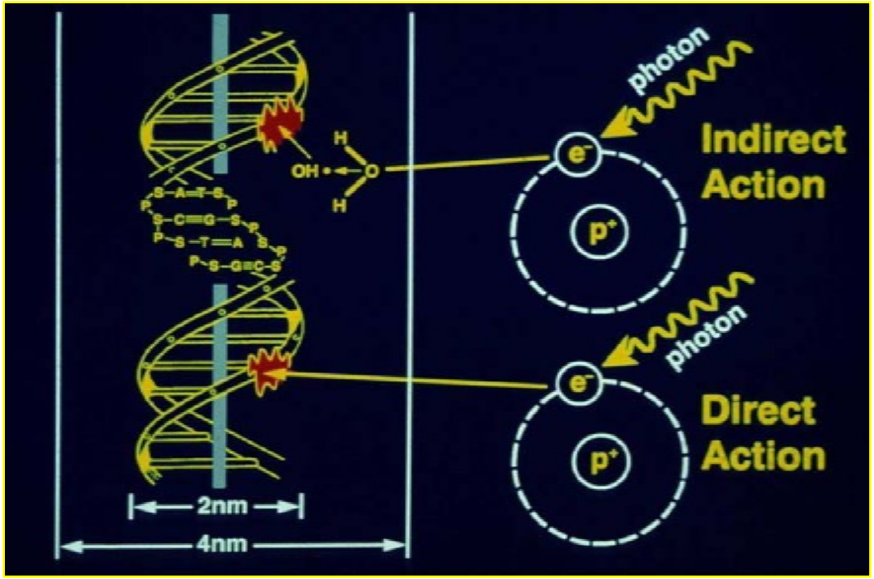
UHDpulse

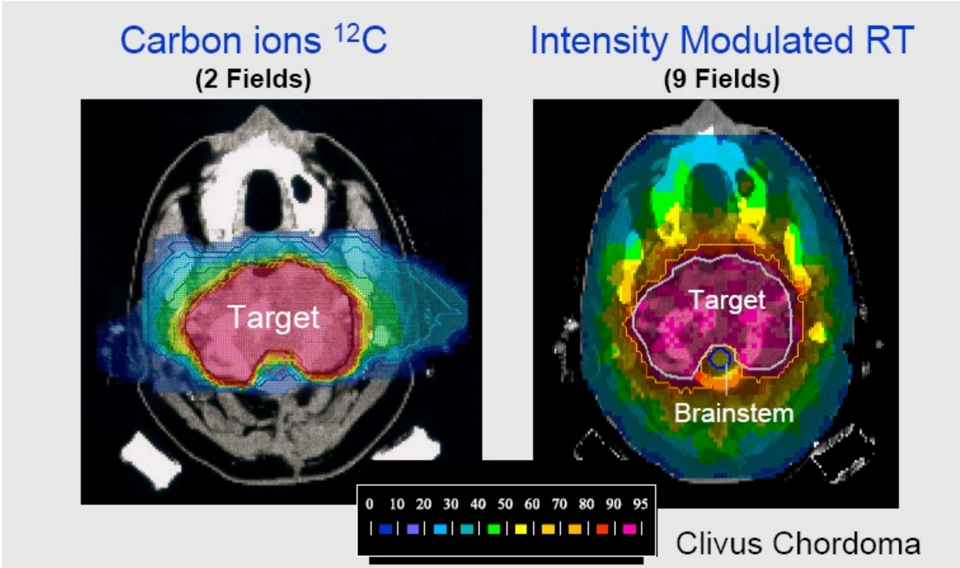
BRAIN TUMOUR INFORMATION & SUPPORT BRAINWAVES NI

IMPULSE

HORIZON 2020

Context: radiobiology/radiotherapy





Cancer radiotherapy :
use of radiation (x-ray photons, protons, electrons) for targeted damage to cancer cells

Typical radiation dose: 20-30 Gy (in several fractions)

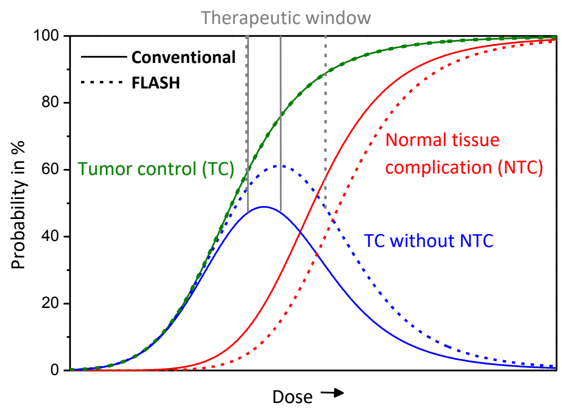
Proton (ion) therapy exploit favourable dose deposition profiles

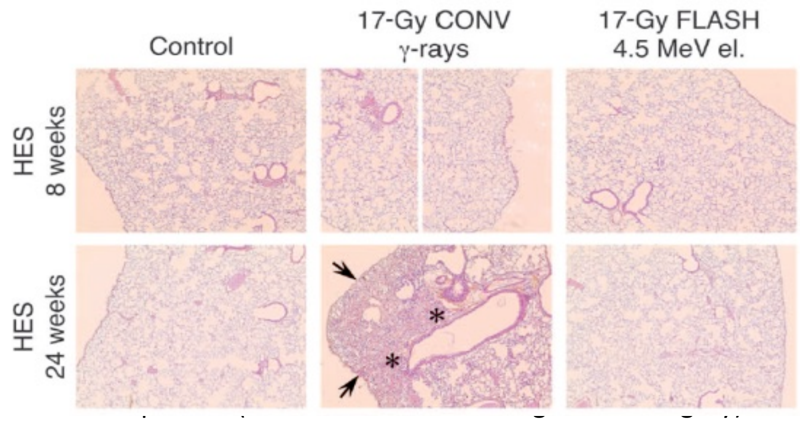
Radiobiology of suitable cell models underpins clinical use of radiation

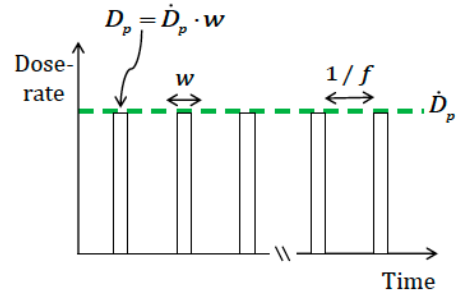
Growing interest in highly pulsed delivery (FLASH)

FLASH radiotherapy concept: prescribed dose delivered in a single or few pulses in a short time

FLASH **maintains tumour killing efficiency**, but leads to lower toxicity on healthy cells







FLASH parameters

- *1 – 10 Gy per pulse*
- *10^6 Gy/s per pulse*
- *40 – 1000 Gy/s mean dose rates*
- *100 ms dose delivery*

Examples:

mice lung and brain irradiations

(prevention of lung fibrosis , protection of blood vessels/bronchi, sparing of spatial memory)

Fauvadon, Science Transl. Med ,**16**, 245ra93 (2014)

Montay -Gruel , Clin Cancer Res, **27**, 775 (2021)

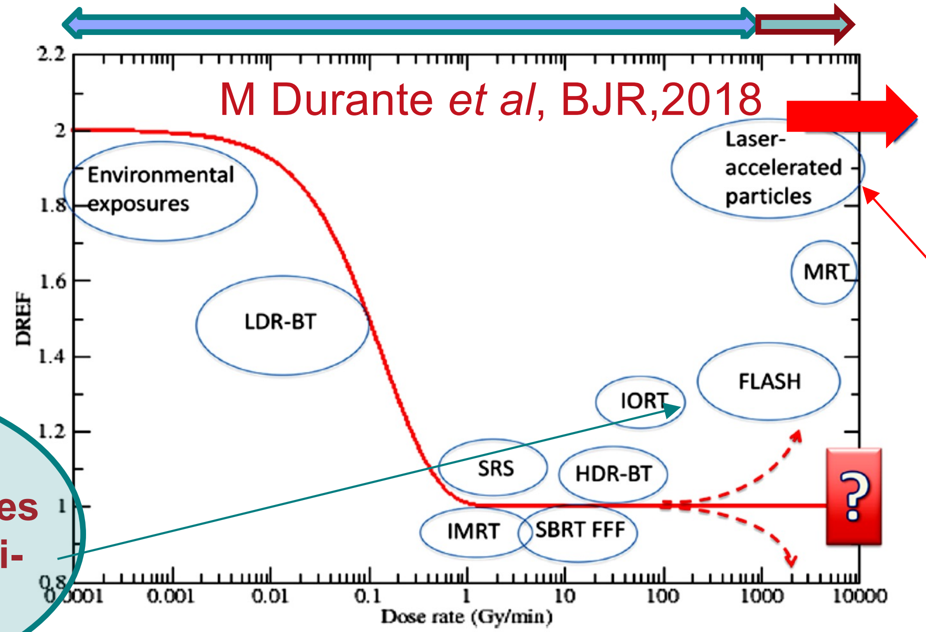
- Evidence mostly in-vivo
- Underlying mechanism still unclear
- Mostly using electrons, but also protons/carbons

Radiobiology at Ultra-High Dose Rate

Conventional dose-rate
0.01-0.1 Gy/s (1-10 Gy/min)

FLASH dose-rate
>40 Gy/s (>2400 Gy/min)

Laser-driven dose-rate
$>10^9$ Gy/s ($>10^{11}$ Gy/min)



Spare normal tissues preserving the anti-tumor activity

Possible effects

- Spatio-temporal overlap of independent tracks
- Local depletion of oxygen
- Potential commonalities with **FLASH effects**

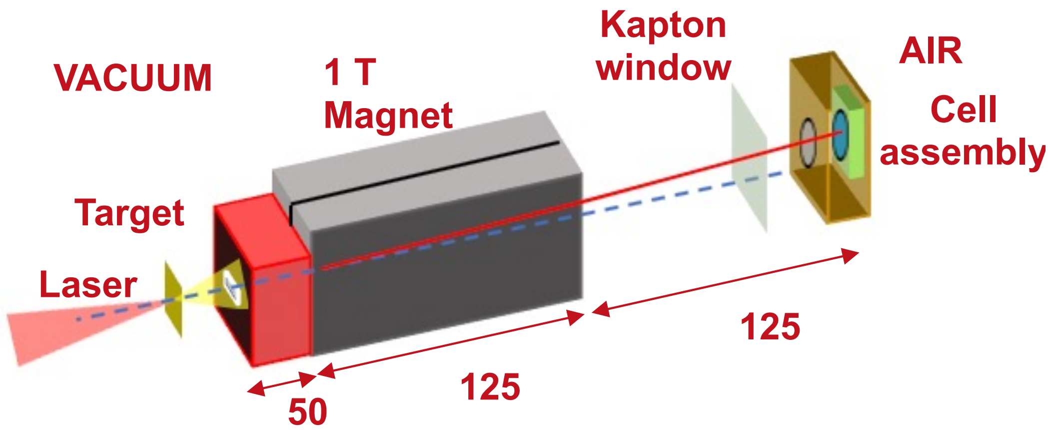
A Novel regime of radiobiology

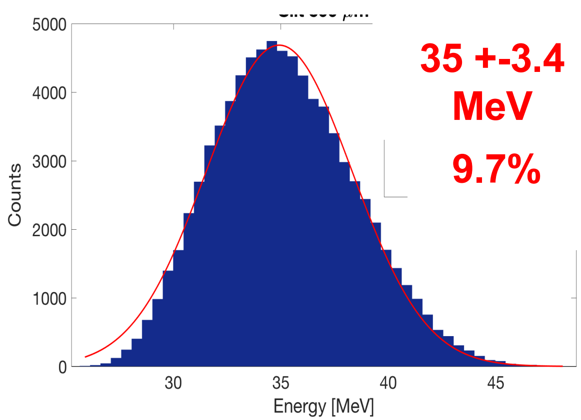
Recent review: Chaudhary P, et al (2021), *Front. Phys.* **9**:624963.

Single-shot experimental arrangement for UHDR beam delivery

Compact and simple setup

- Multi-Gy dose **in a single pulse**
- Energy resolution at the cell position
- High dose-rate at the cell plane
- Easy implementation in physics research lab





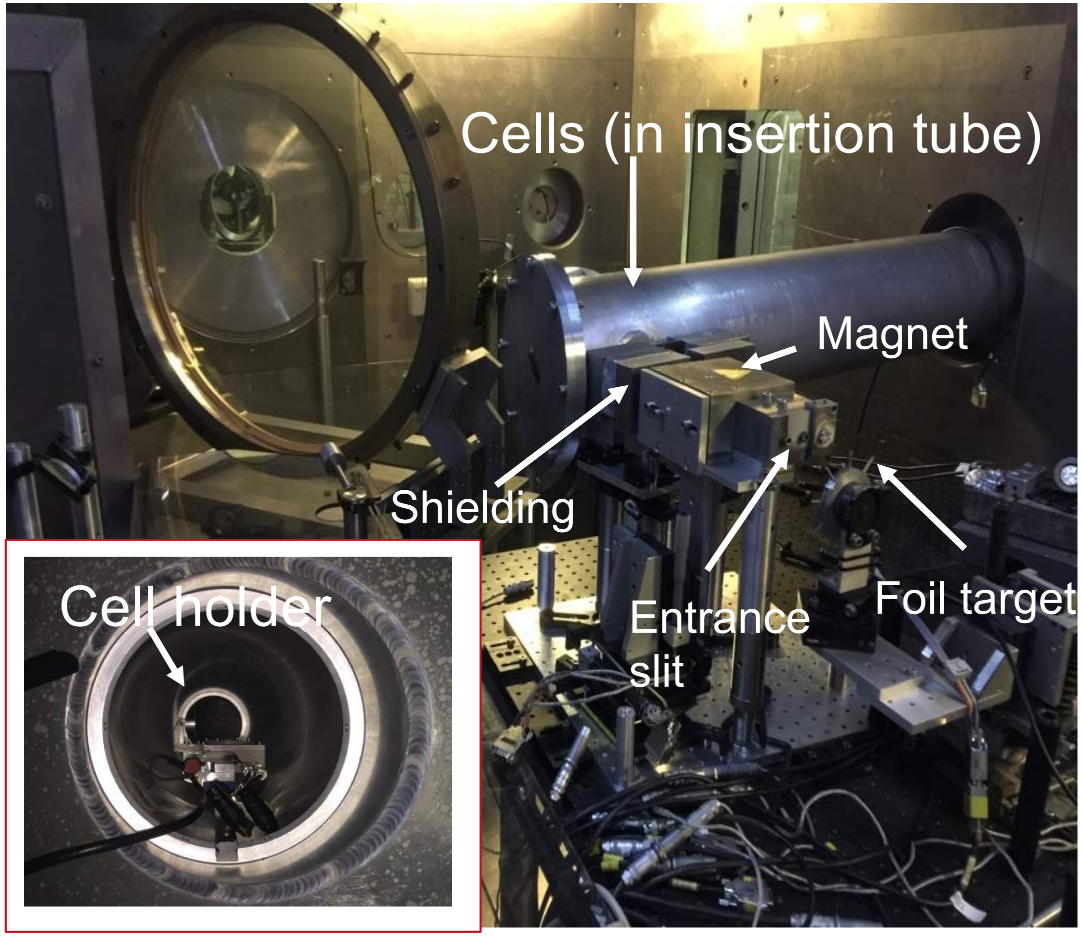
Proton energy distribution at cell location (35 MeV)

Proton pulse **duration** on cells: ΔT~ 350 ps

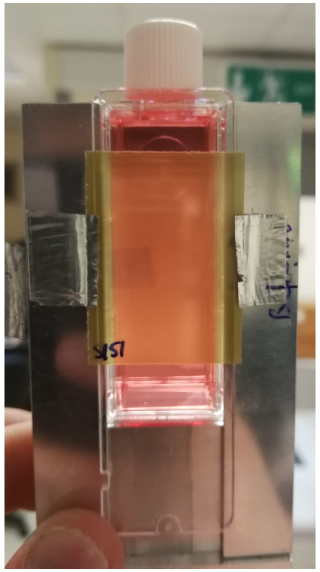
Dose ~ 2 Gy

Dose-rate ~ 6 10^9 Gy/s

Example: irradiation set-up @ VULCAN PW (CLF-RAL)

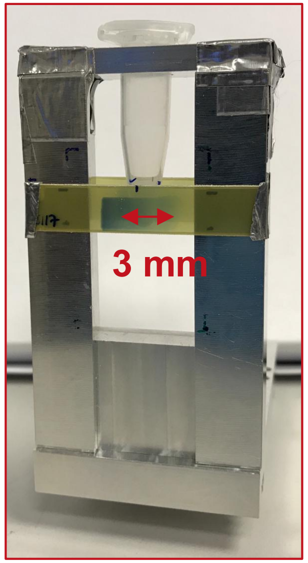


Slide flask



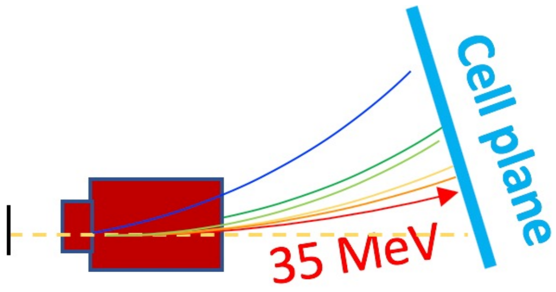
Monolayer (10 um) cells

Eppendorf tubes

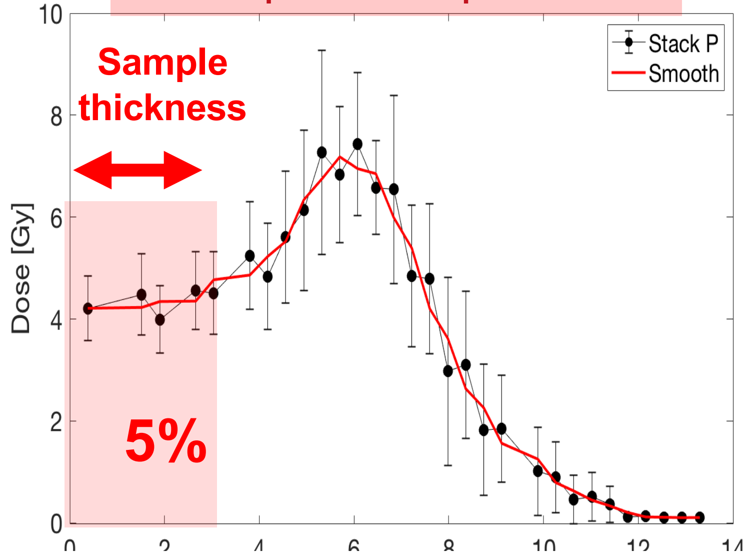


3D cell model in medium

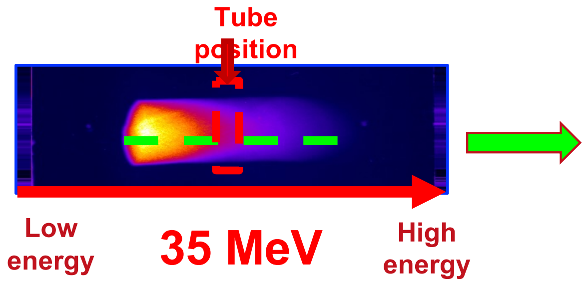
Beam transport and dosimetry



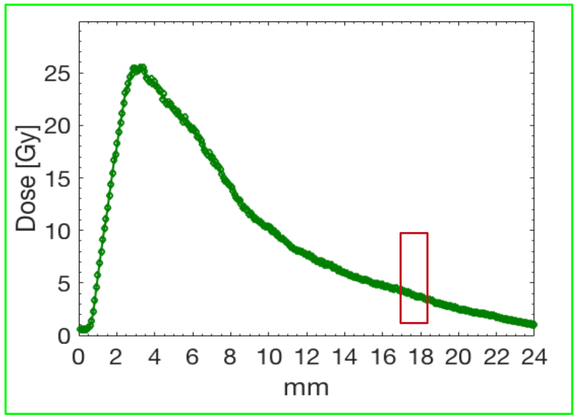
Depth-dose profile



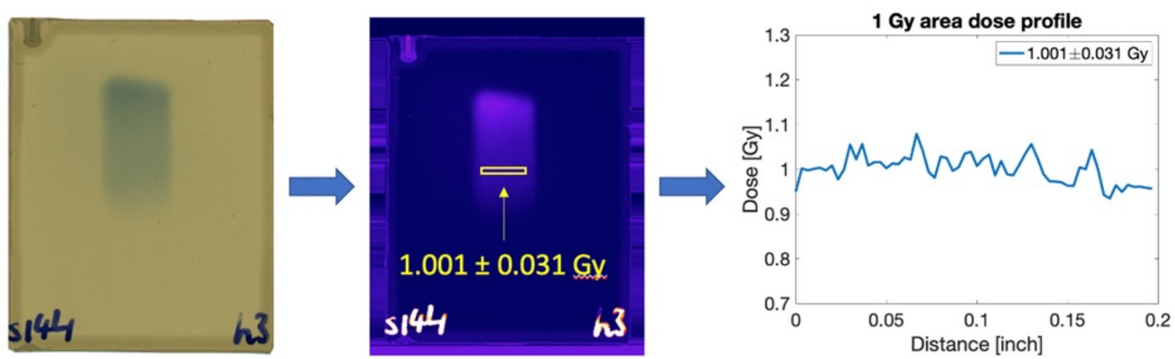
Dose distribution in RCF @ cell plane



Dose along dispersion direction



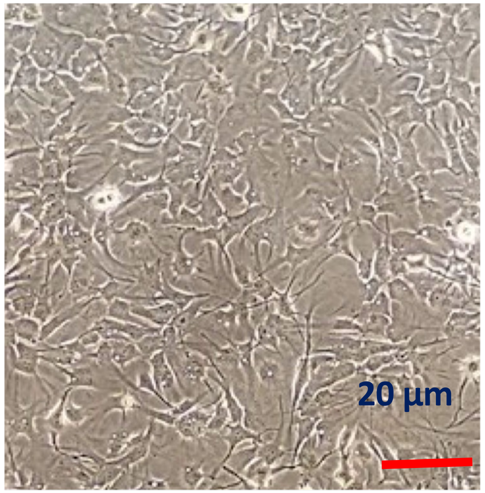
Dose reconstruction from calibrated RCF



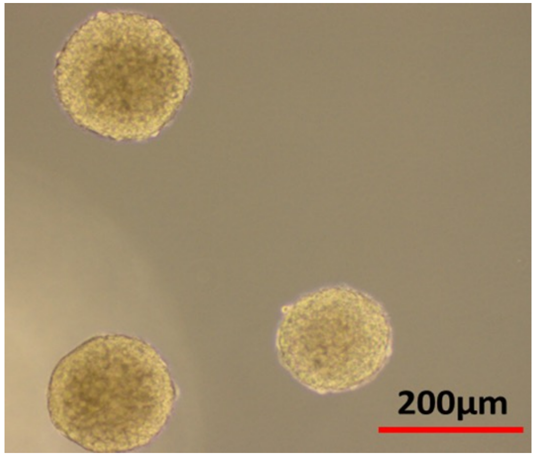
Cellular Models

Cells :

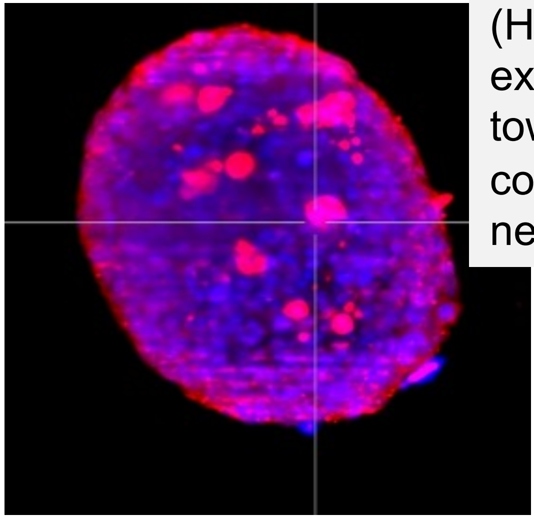
1) Human normal skin Fibroblasts –AG01522
2) Patient derived GBM stem cells 2D and 3D models



Cells grown as **2D** monolayers



3D neurospheres developed from patient derived 2D GBM stem cells



Hypoxia marker (HIF-1α) expression (red) towards the inner core of a neurosphere.

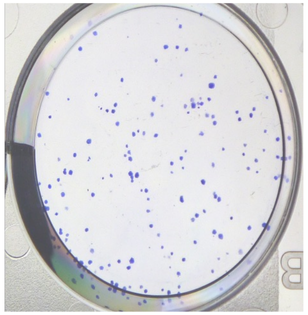
Hypoxic regions within core of neurospheres are a cause of **radioresistance**

Main assays employed

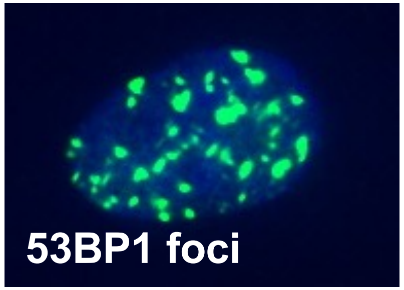
Clonogenic assays (cell survival studies):

1 week

Cell irradiation → Cell culturing → Counting of colonies → Survival fraction



DNA damage and repair (53BP1 immunofluorescence):



Cell irradiation → Cell fixing → Cell staining → Counting of foci

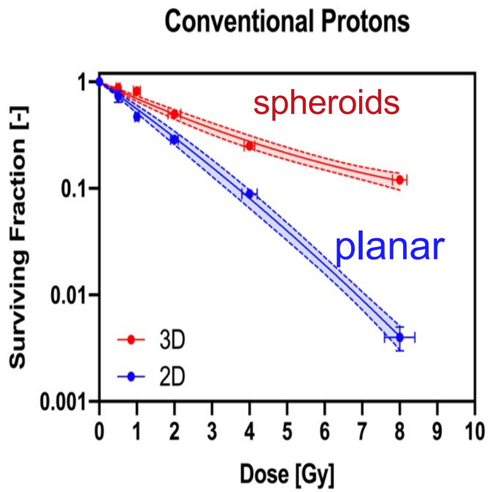
0.5- 24 hr

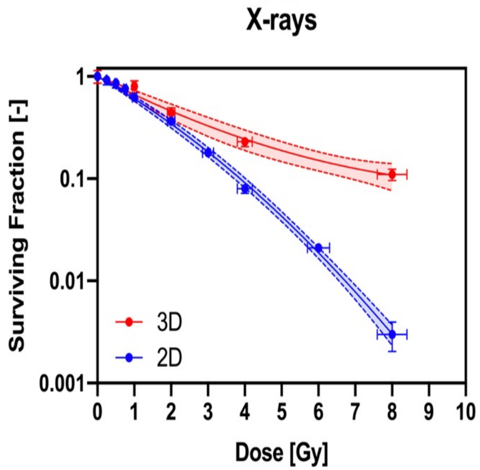
Process involving an agent which binds to DSBs

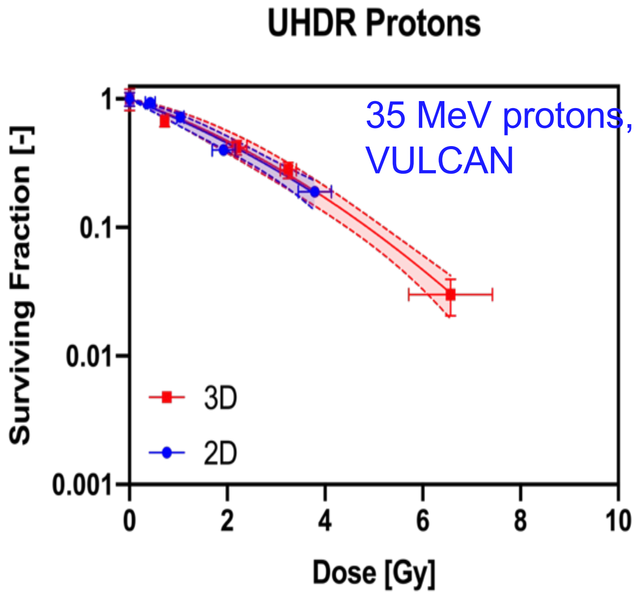
Foci (regions of accumulation of the agent) are highlighted , e.g. by fluorescence

Cell survival: 2D vs 3D cell models

P.Chaudhary et al, in preparation (2023)





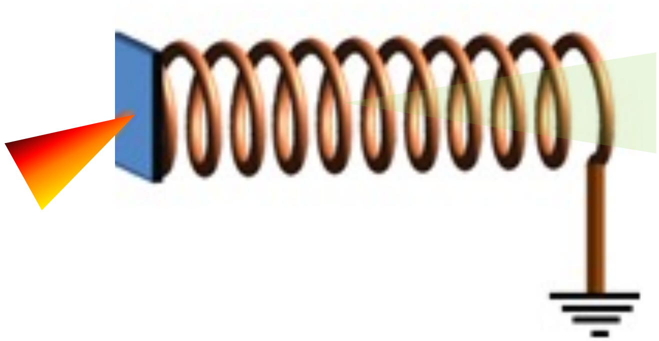


- Enhanced cell killing in 3D model @UHDR
- UHDR overcomes hypoxia-induced radioresistance
- Some analogy with new in-vivo FLASH results?

3D	Cyclotron	UHDR
RBE_{50}	0.65±0.05	0.94±0.30
RBE_{10}		1.79±0.49

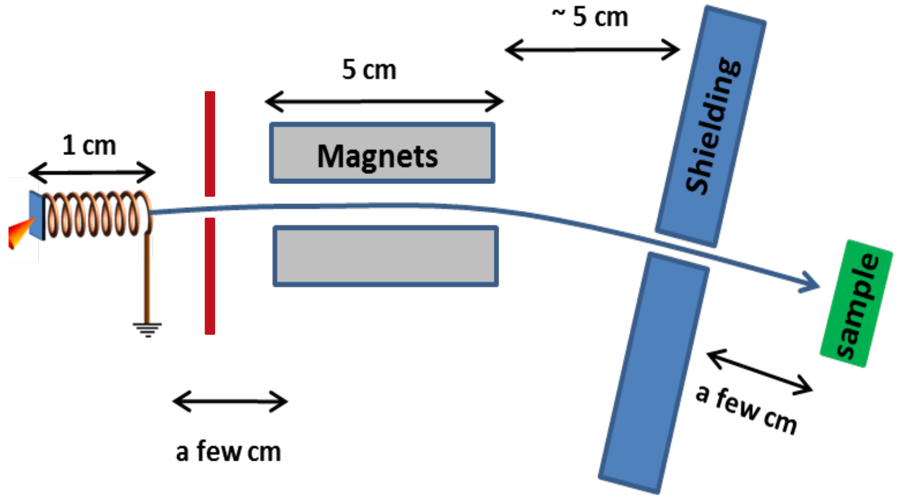
R.J.Leavitt *et al*, bioRxiv
Hypoxic tumors are sensitive to FLASH radiotherapy
doi: https://doi.org/10.1101/2022.11.27.518083

Reaching higher doses needs improvement in ion transport

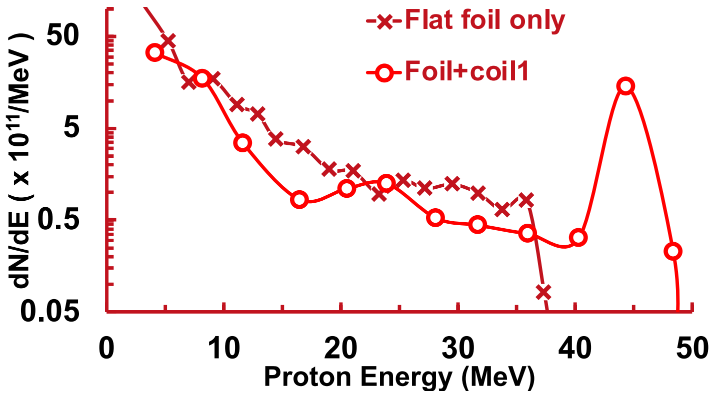


Coil targets for beam collimation

S.Kar *et al*, Nature Comm., **7**, 10792 (2016)



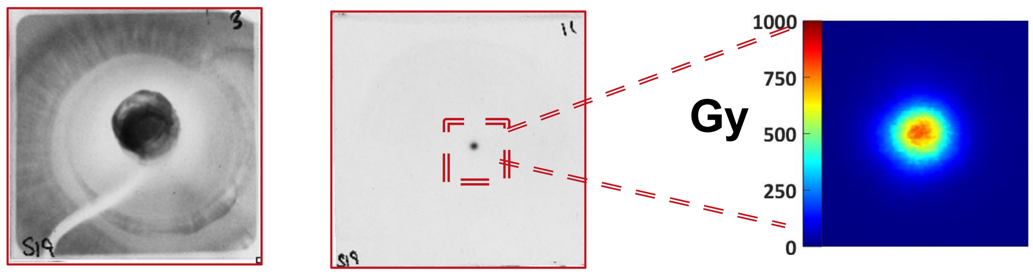
H.Ahmed *et al*, Sci. Rep., **11**, 699 (2021)



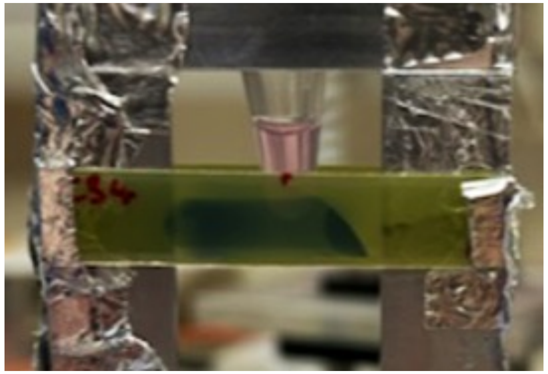
15 MeV

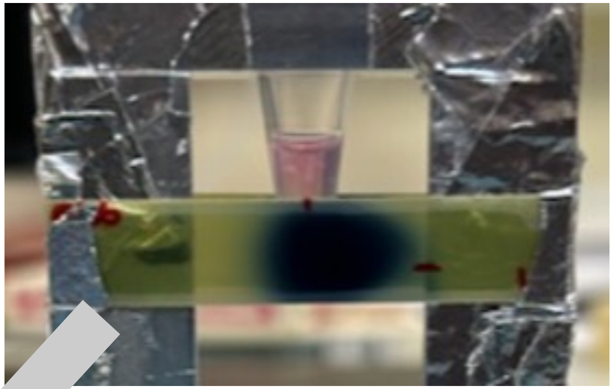
45 MeV

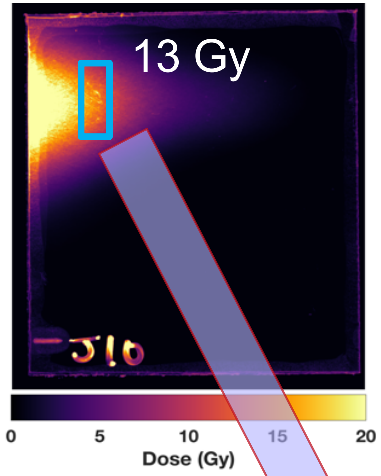
Divergence < 0.5 °



Larger doses demonstrated in recent VULCAN experiment

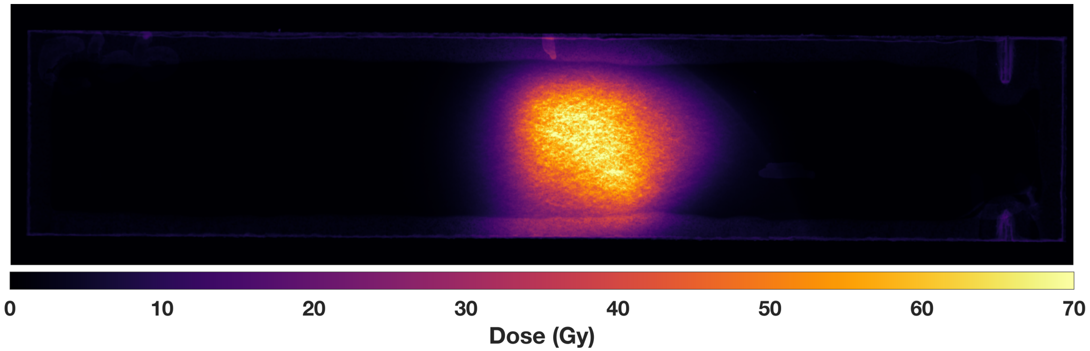


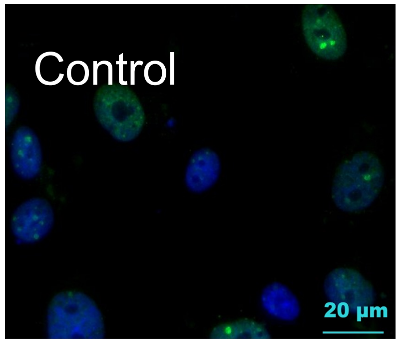




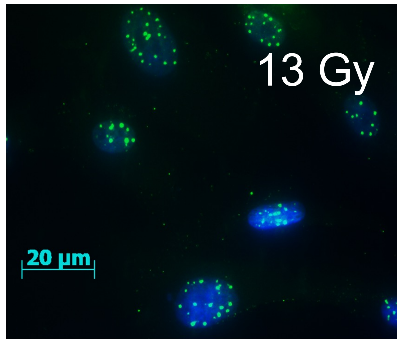
Flat foil dose @ 35 MeV
~ 1 Gy

Foil/coil: dose @ 35 MeV
~ 70 Gy





AG01522 Control 24 hrs



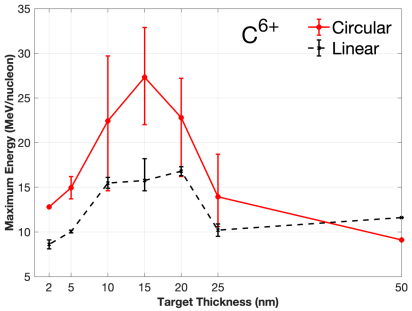
AG01522 Shot 118 – 13 Gy 24 hrs

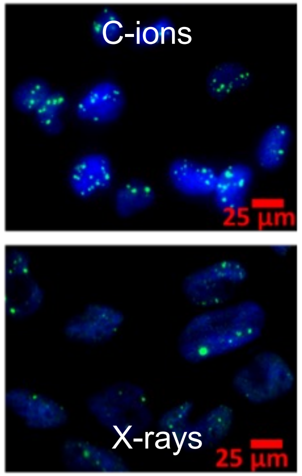
UHDR carbon radiobiology

P. Chaudhary *et al*, Phys Med Biol. **68**, 025015 (2023)

Laser-driven carbons @ GEMINI

Efficient C6+ acceleration from ultrathin foils (10s nm)
(e.g. A. McIlvenny et al, PRL, 127194801)



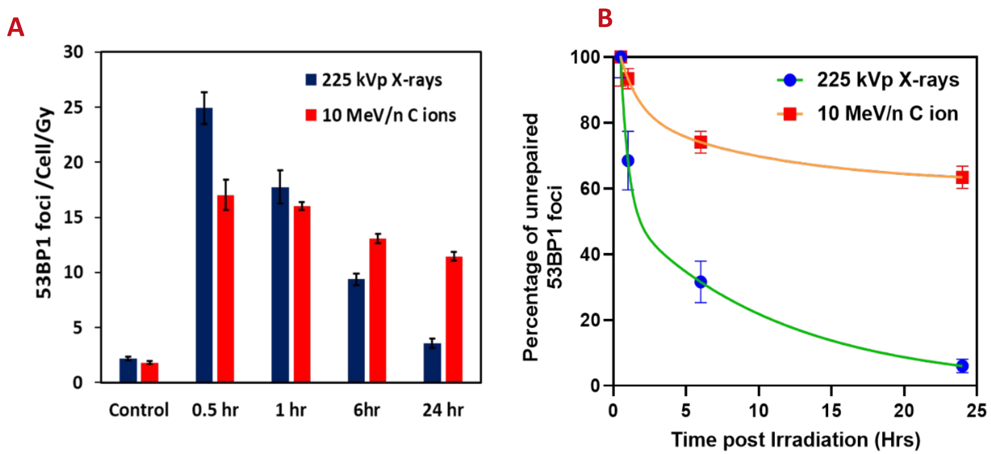


Interest of carbon ions

- ✓ More complex damages to the cell DNA
- ✓ Higher LET > 100 KeV/um
- ✓ Higher efficiency for the treatment of radioresistant tumours
- ✓ Growing interest in HI-FLASH

10 MeV/n C^{6+} pulses
1 Gy at 10^9 Gy/s

Carbon damage is persistent,
large fraction is unrepaired after 24h



Conclusions

Emerging trends in cellular response to proton irradiations at ultra-high dose rates

Laser-driven ion acceleration:

- Intrinsically short bursts, high flux
- Proton + carbon sources

UHDR radiobiology

Compact set-up
Multi-Gy doses, >10^9 Gy/s
Beyond FLASH regimes

Emerging evidence of non-standard cell response at UHDR

- UHDR overcomes radioresistance in 3D models
- Differential cell response (normal vs cancer)
- Potential communalities with FLASH observations

Need for new models and new understanding