UNIVERSITY OF BIRMINGHAM

LhARA PA2: WP7 - Radiobiology

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School of Physics and Astronomy

NIH National Institutes of Health
Turning Discovery Into Health

MRC Medical Research Council

north west cancer research
Putting our region's cancer needs first

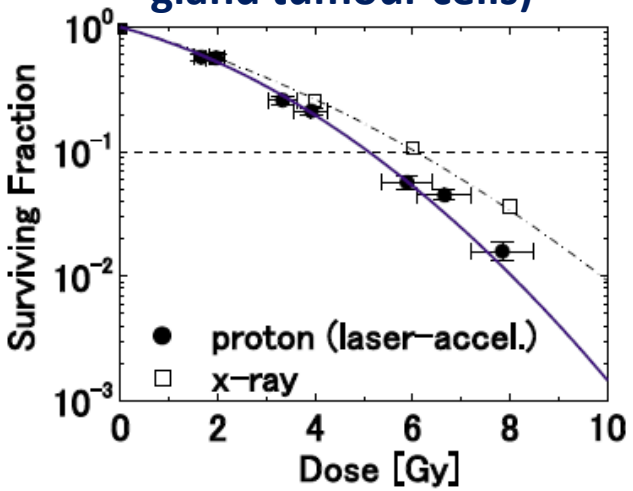
Aims of WP7 - Radiobiology

- *To establish a biology-based research programme which will enable analysis of LhARA in radiobiological experiments in vitro and in vivo.*

- To investigate the biology of laser-driven ions delivered at ultra-high dose rates (~10^9 Gy/s) in nanosecond pulses and different time structures.
- To compare the biology of laser-driven protons versus cyclotron accelerated protons using well characterised cellular models and biological end-points.

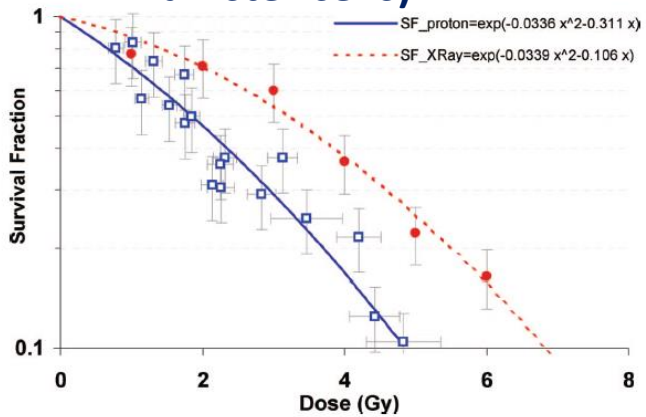
Previous evidence of laser-driven ion radiobiology

RBE=1.2 (Salivary gland tumour cells)



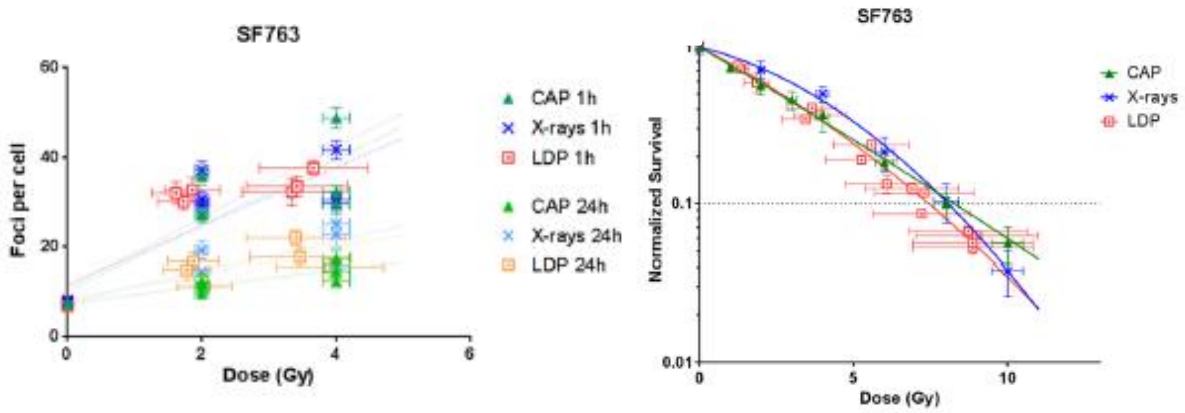
Yogo *et al.*, (2011) *Appl. Phys. Lett.*

RBE=1.4 (V79 Chinese Hamster cells)

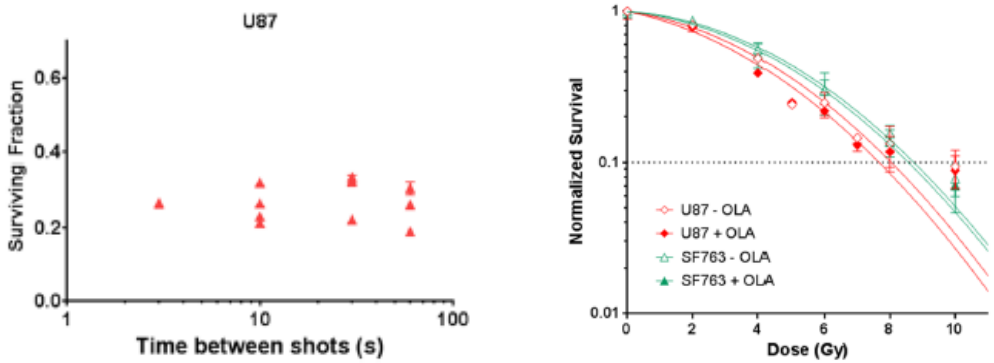


Doria *et al.*, (2012) *AIP Advances*

No apparent different in survival and DNA double strand break repair (GBM cells)

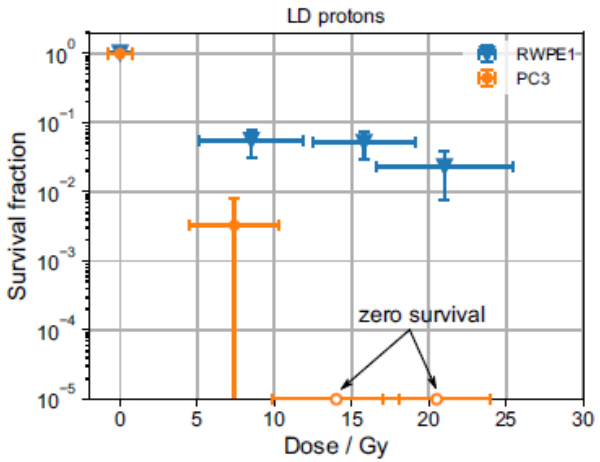


However, change in bunch repetition rate altered survival and can lead to a radiosensitive phenotype



Bayart *et al.*, (2019) *Scientific Reports*

Significant normal cell sparing compared to prostate cancer cell death



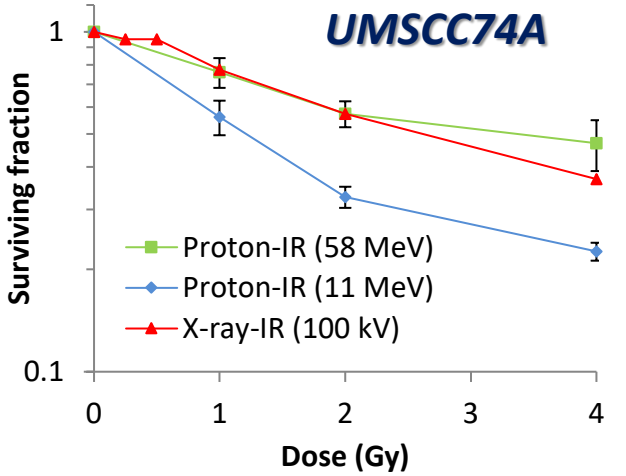
Bin *et al.*, (2022) *Scientific Reports*

Main objectives of WP7 - Radiobiology

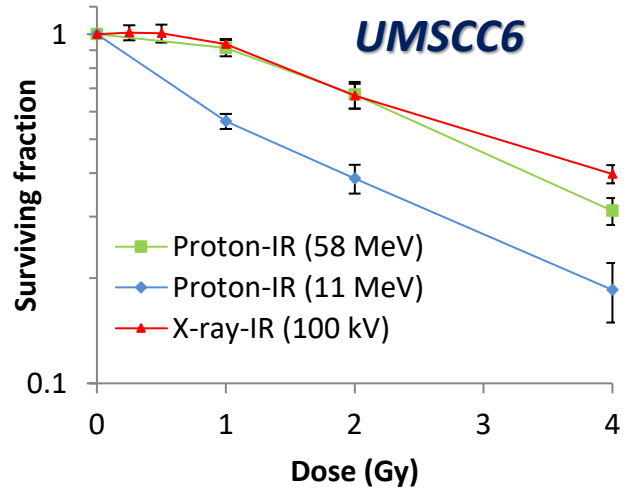
1. Identify an appropriate laser accelerator facility (e.g. SCAPA) that houses the appropriate equipment and resources for radiobiological research.
2. To perform experiments analysing the clonogenic survival of previously well characterised and established cell models, with laser-accelerated protons at different doses.
3. Compare RBEs of laser-accelerated protons versus pre-existing data using X-rays and cyclotron accelerated protons at both conventional (2-5 Gy/min) and FLASH (100 Gy/s) dose rates.
4. Analyse levels and repair of DNA damage with laser-accelerated protons at different doses, and compare with pre-existing data (X-rays, cyclotron accelerated protons at CONV/FLASH dose rates.
5. Compare generation of neoantigens formed by laser driven protons to cyclotron accelerated protons to determine the effects on the immunopeptidome and on T cell recognition.
6. Explore the potential for utilisation of more advanced *in vitro* cellular models, and of the experiments and resources necessary for *in vivo* examination of laser-accelerated ions.

Fabbrizi and Parsons (2022) *Expert Rev Mol Med*

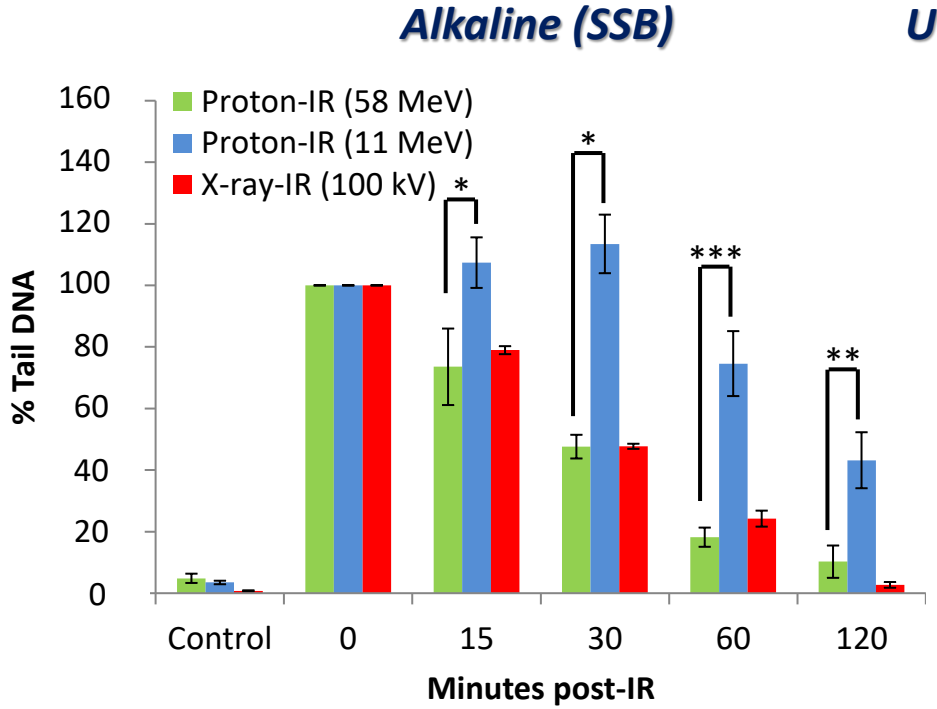
“Relatively” high-LET protons cause a decrease in cell survival due to CDD formation compared to low-LET protons



SF2, p<0.02; RBE=1.7

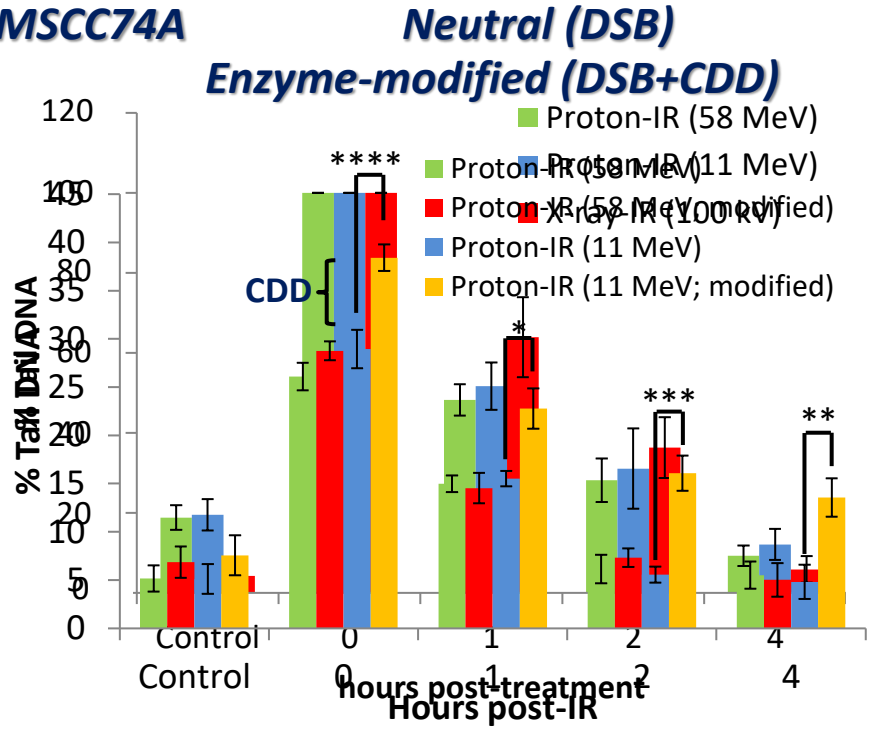


SF2, p<0.01; RBE=1.9



*p<0.005, **p<0.002, ***p<0.001

58 MeV (1 keV/µm); 11 MeV (12 keV/µm)



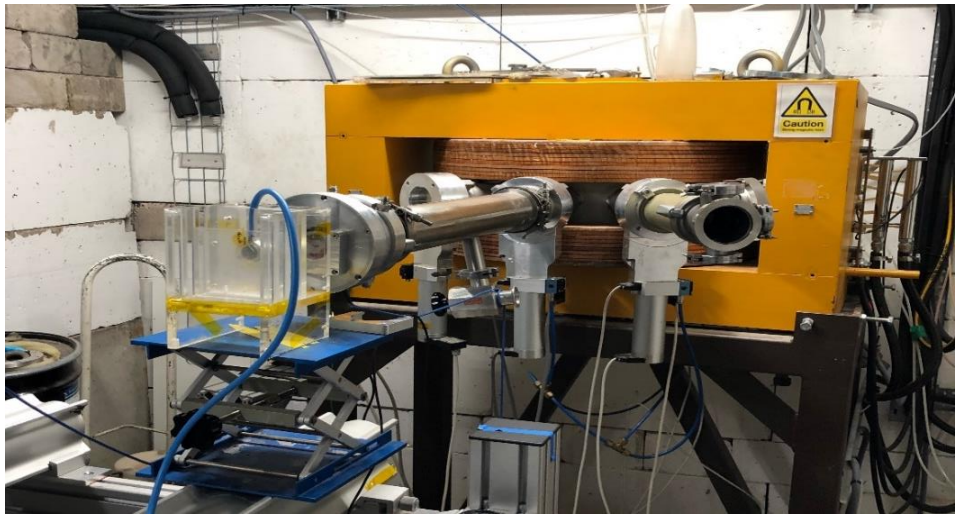
*p<0.02, **p<0.01, ***p<0.005, ****p<0.001

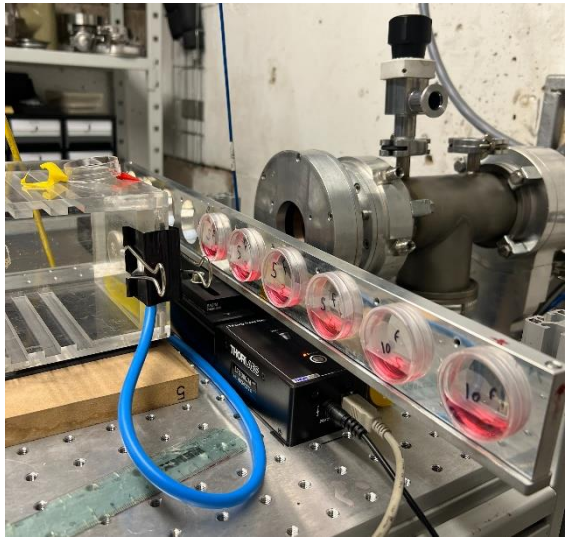
Nickson *et al.*, (2017) *Oncotarget*
Carter *et al.*, (2018) *Int J Rad Oncol Biol Phys*

Carter *et al.*, (2019) *Int J Rad Oncol Biol Phys*
Vitti *et al.*, (2020) *Cancers*

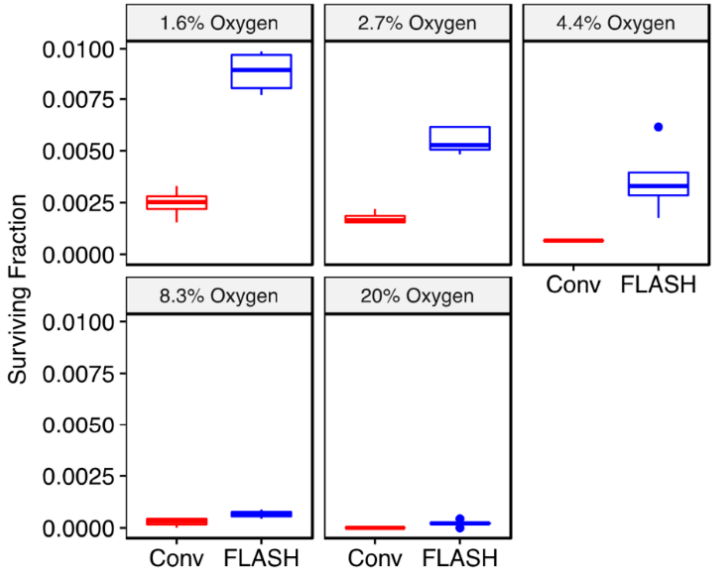
Nickson *et al.*, (2021) *Front Oncol*
Zhou *et al.*, (2022) *Front Oncol*

Examining the radiobiology of FLASH high-LET radiation

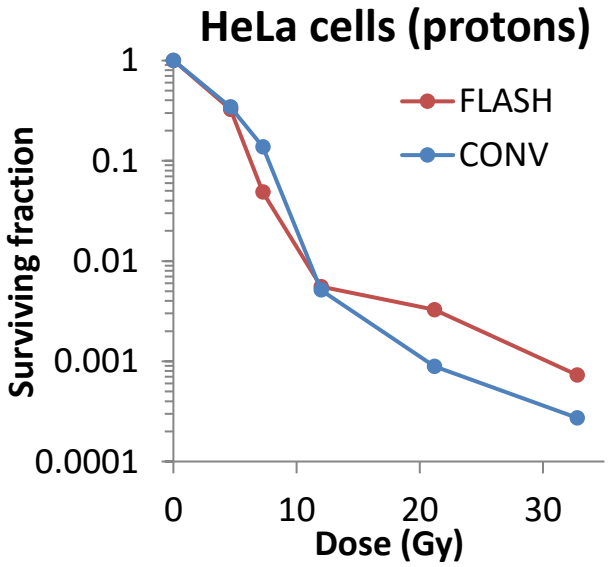


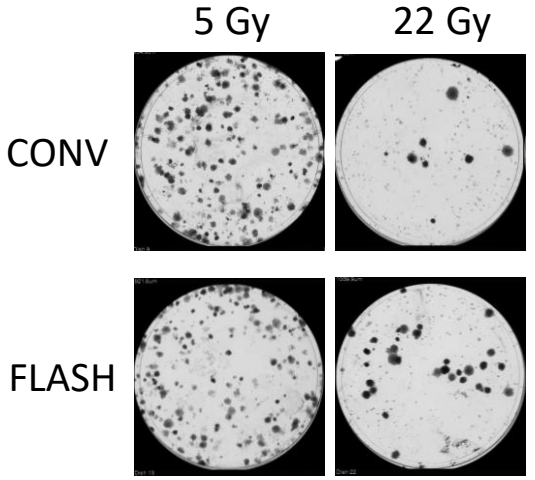


DU145 prostate cancer cells (18 Gy electrons)



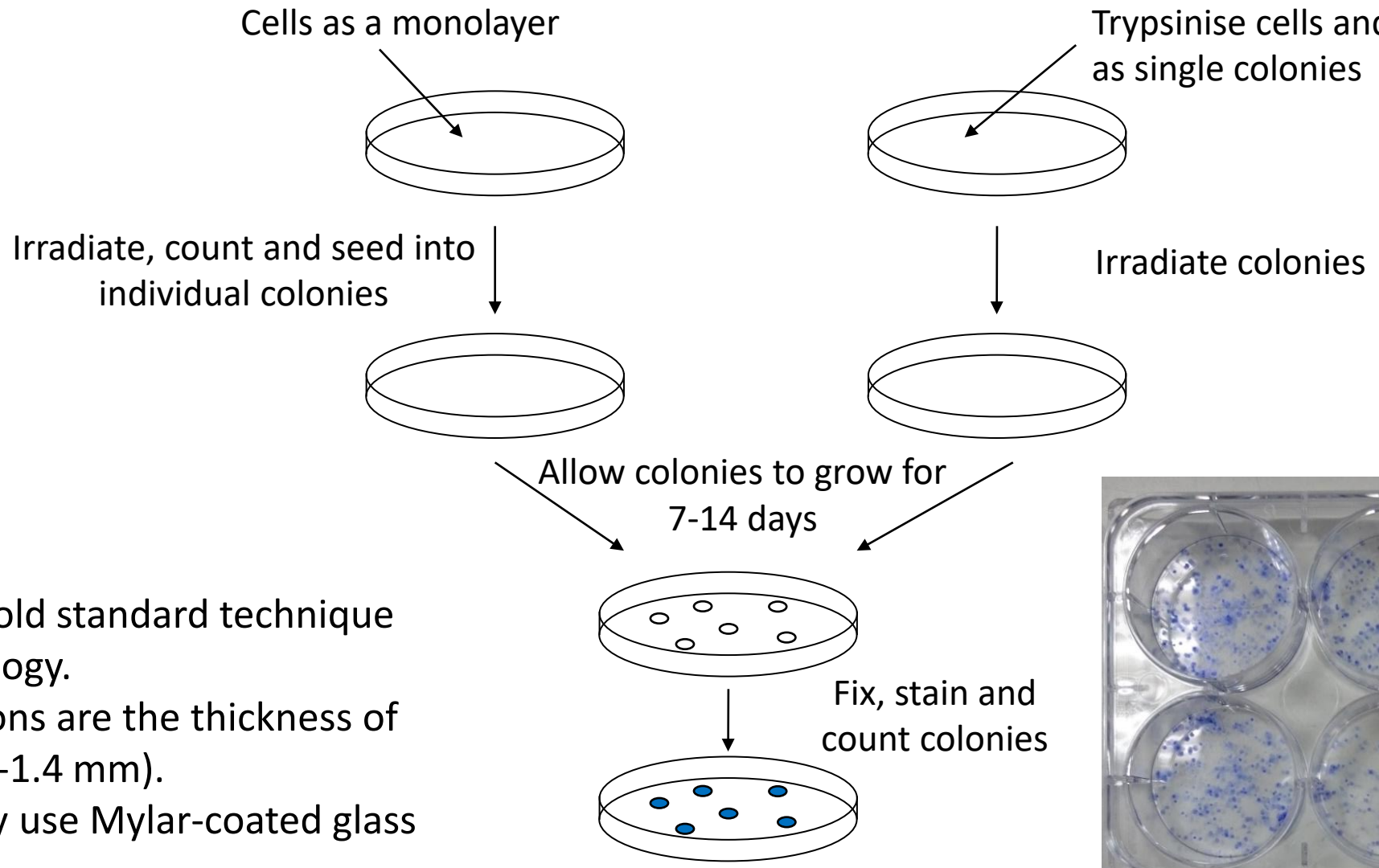
Courtesy of Kristoffer Petersson



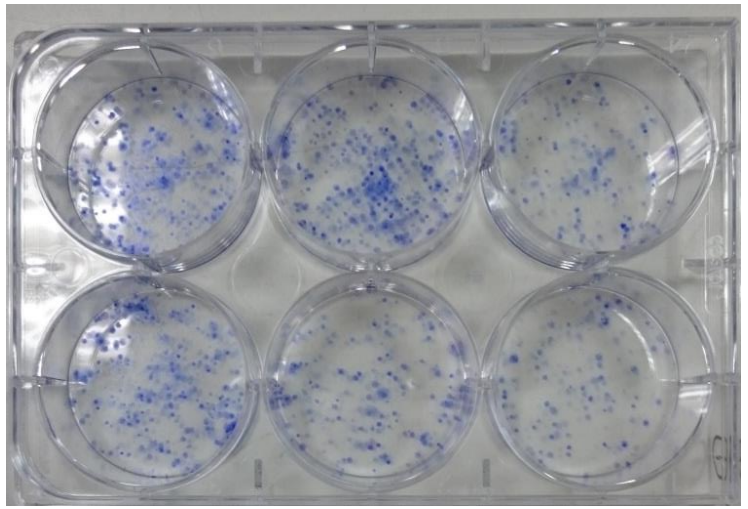


Collaborations with Stuart Green, Tzany Wheldon, Tony Price, Ben Phoenix and Kristoffer Petersson

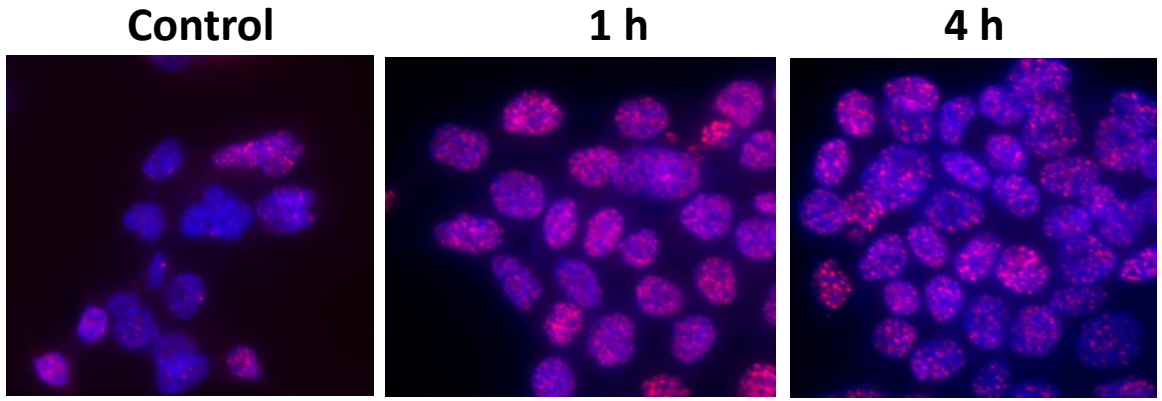
Clonogenic assays for analysis of IR-induced cell sensitivity



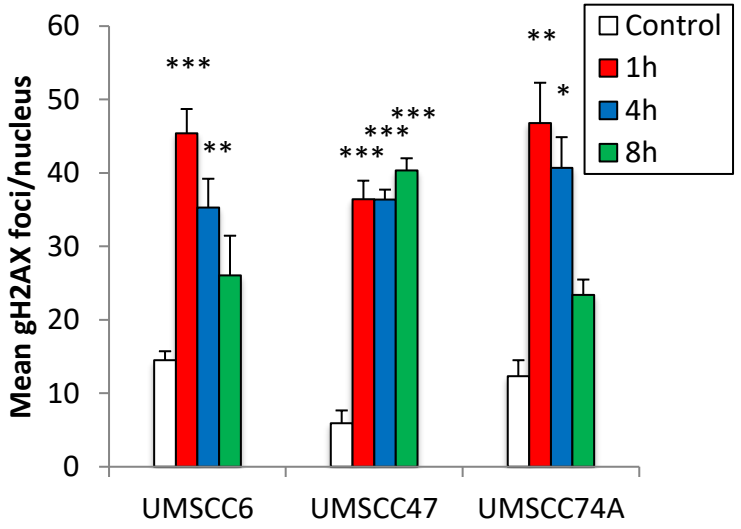
- This is the gold standard technique for radiobiology.
- Considerations are the thickness of dishes (~1.2-1.4 mm).
- Alternatively use Mylar-coated glass rings.

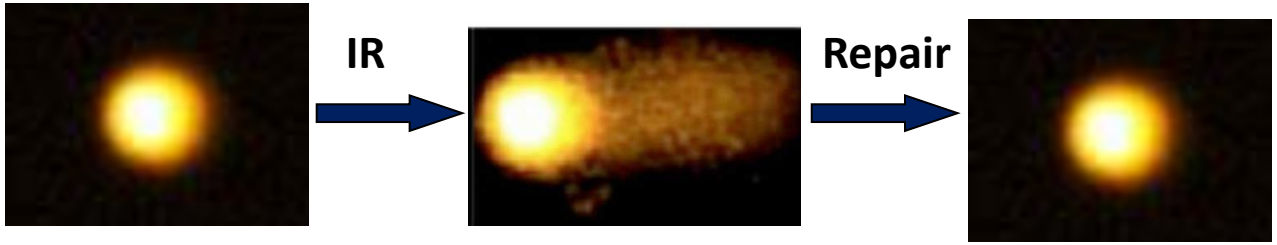


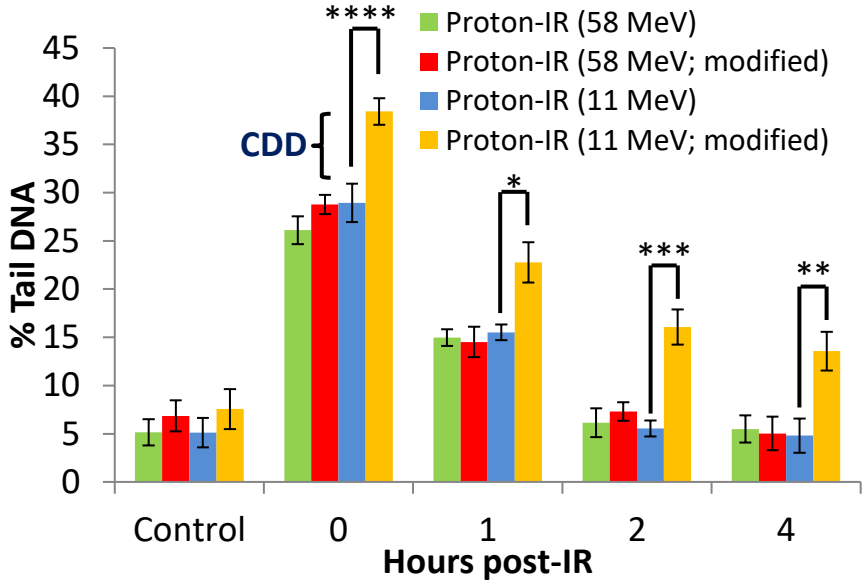
Analysis of DNA damage: γH2AX foci as a surrogate DNA double strand break (DSB) marker, and comet assays



Head and neck cancer cells (4 Gy)

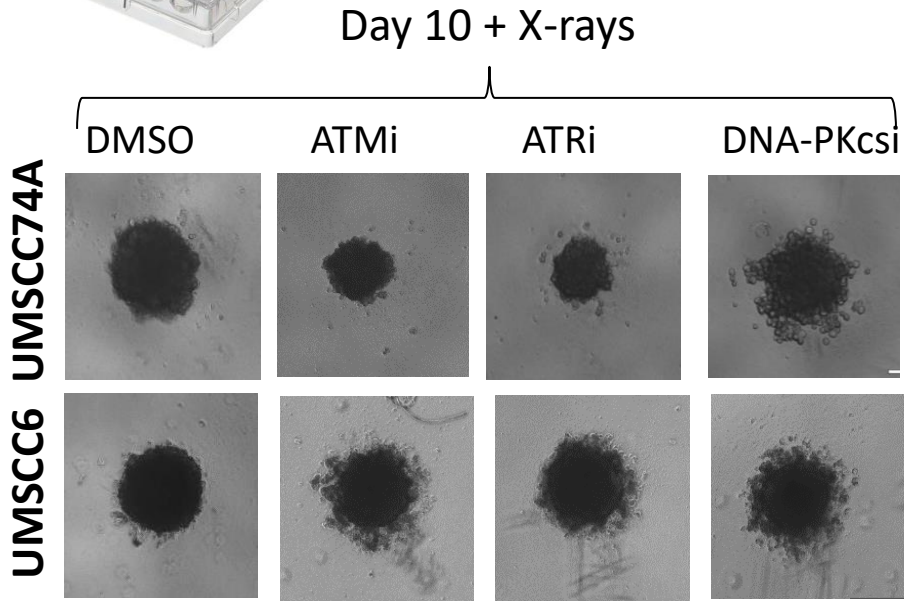


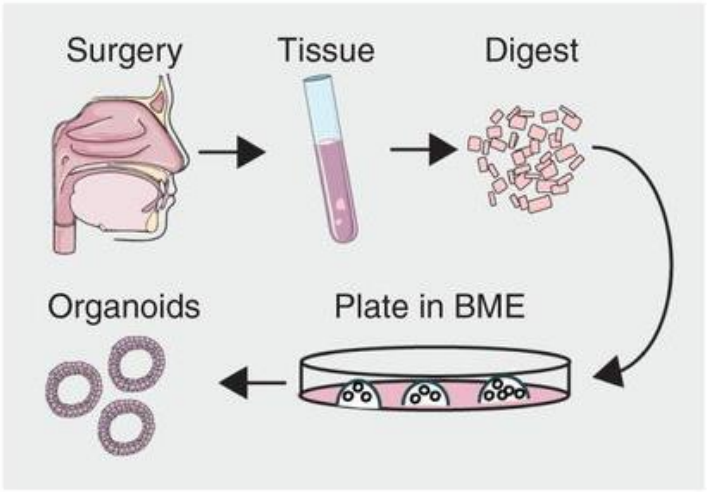


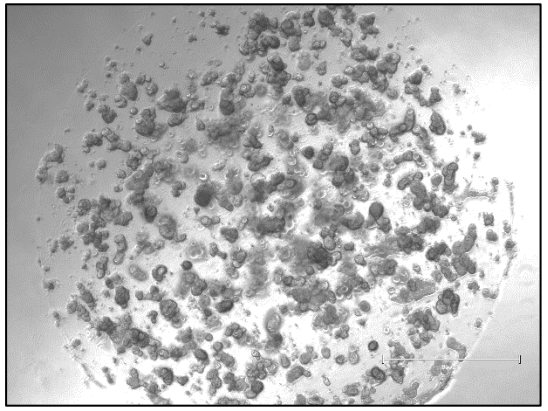


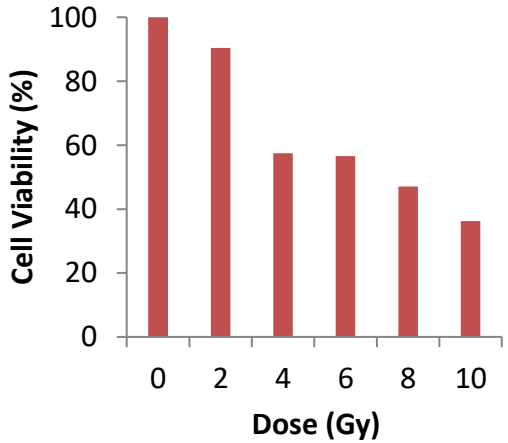
- Reveals the kinetics of repair of DSBs (1-24 h), but note that γH2AX is a signalling marker and doesn't directly measure the damage itself (unlike comet assays).
- Considerations are the thickness of dishes (~1.2-1.4 mm) plus glass coverslips (~0.13-0.16 mm) for γH2AX foci, and additional resources/equipment needed for comet assays.

Radiobiology using more advanced 3D models *in vitro* and *in vivo*









- Reveals the growth of tumour cells in 3D as a more accurate translational model.
- Considerations are for spheroids that these are grown in U-well shaped plates (different thicknesses at base and sides) and in suspension (free floating and not necessarily at a defined depth). Organoids are more fixed but within a basement membrane matrix (with a certain depth/volume).