University of Strathclyde Glasgow

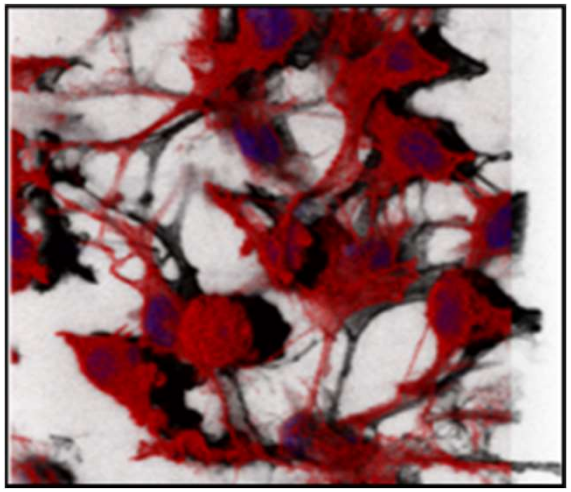
LhARA PoPLaR meeting

Laser-hybrid Accelerator for Radiobiological Applications

Radiobiology contribution

21 May 2024

Dr Natividad Gomez-Roman, PhD



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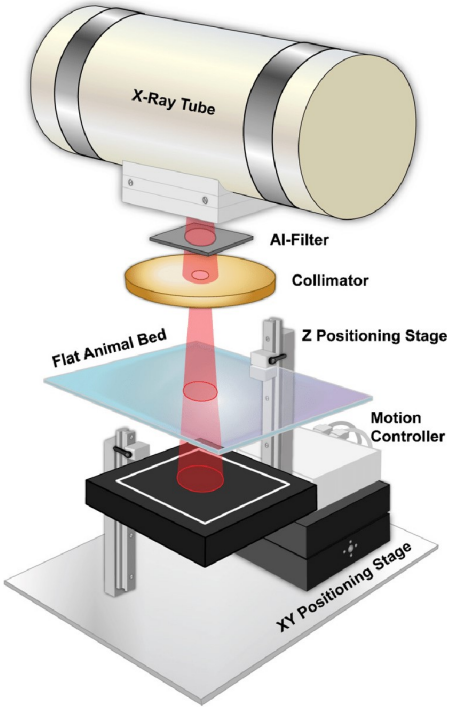
Current X-Ray Facilities in SIPBS



Dose Rate of 2.3 Gy/min
@ 50 cm height

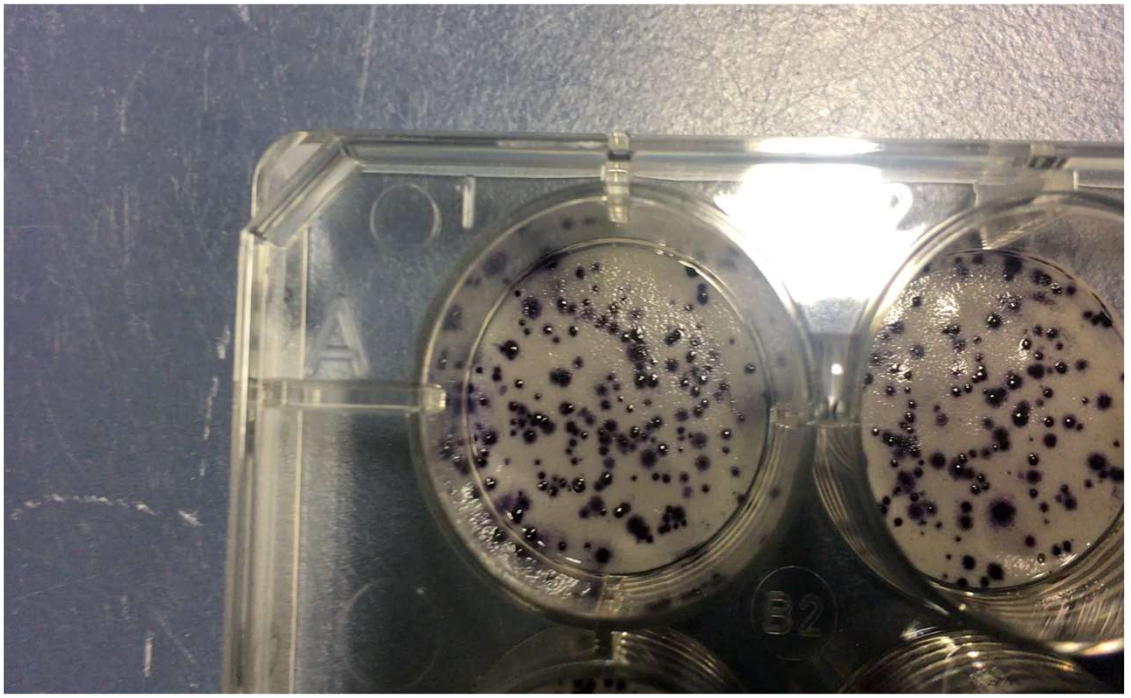
X-Rays delivered with an X-RAD 225
Device Maximum Voltage 225 kV; Device Maximum Amperage 13 mA

Clonogenic Survival Assay in 3D – Alvetex Scaffolds

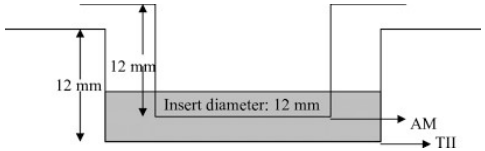
Comparing the response to different treatment modalities will require clonogenic survival assays

- Radiation is standard care of treatment for GBM
- **Clonogenic survival assay** is the **gold-standard assay** to assess cellular radiosensitisation
- Clonogenic survival assay readout is cell reproductive death
- 12 well plate dimensions:

Length: 127.76 +/- 0.15 mm width: 85.48 +/- 0.15 mm
Depth: 20 mm (10 mm covered in liquid media)



Polystyrene scaffolds



alvetex scaffold

6. Gomez-Roman n *et al*. (2017). A novel 3D human glioblastoma cell culture system for modeling drug and radiation responses. *Neuro-Oncology*, 19(2):229-241.

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Clonogenic Survival Assay

Cell culture

+ enzymatic digestion (cell membrane proteins)

Cell suspension

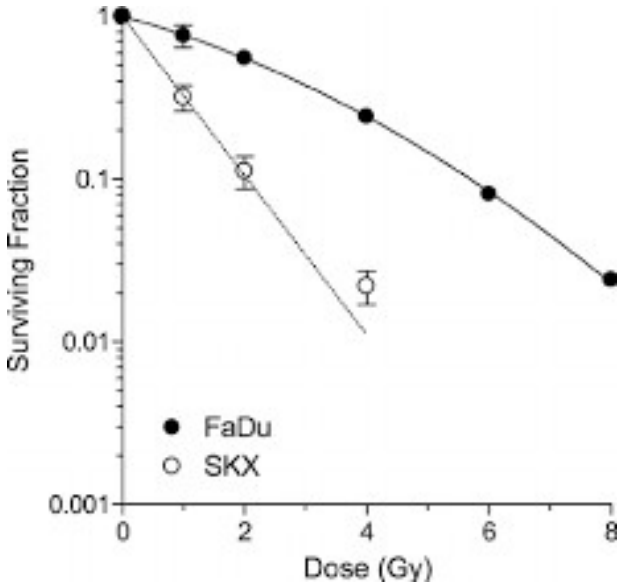
Seed dishes

No. cells seeded: 250 250 750 1250

X-Ray dose: 0 Gy 2 Gy 4 Gy 6 Gy

Incubate 2-3 weeks

No. colonies counted:	40	32	36	25
Plating efficiency:	40%			
Surviving fraction:		0.4	0.11	0.04



$$Plating\ efficiency = \frac{Number\ of\ colonies}{Number\ of\ cells\ seeded}\ x\ 100$$

$$Surviving\ fraction = \frac{Colonies\ counted}{Cells\ seeded\ x\ (\frac{PE}{100})}$$

6. Gomez-Roman n *et al*. (2017). A novel 3D human glioblastoma cell culture system for modeling drug and radiation responses. *Neuro-Oncology*, 19(2):229-241.

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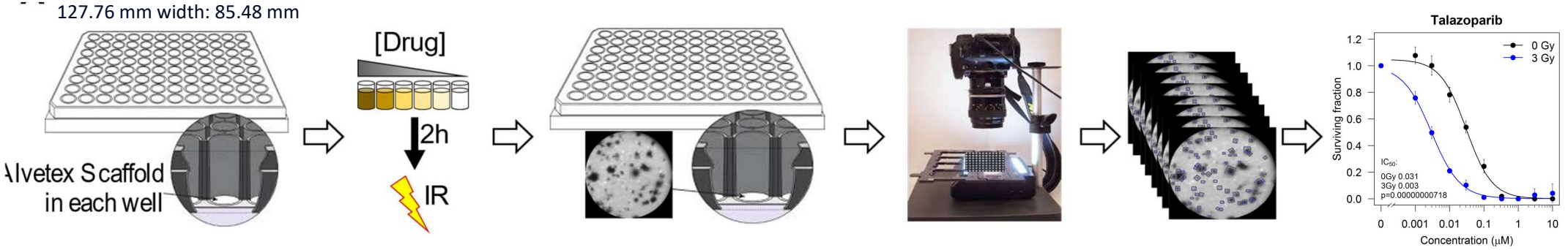
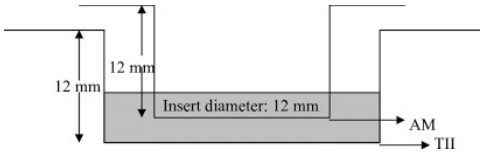
ClonoScreen3D platform for screening drug-IR combinations

96 well plate dimensions:
127.76 mm width: 85.48 mm



Day 1	**Day 2**	**Day 14**	**Acquire image**	**Image processing**	**Data analysis**
Seed cells	Drug cells for 2 hrs then IR (0 or 3 Gy)	Stain colonies (MTT) Fix	96-well plate	Well segmentation Quantification (colonies / well)	(DRC, RIR)

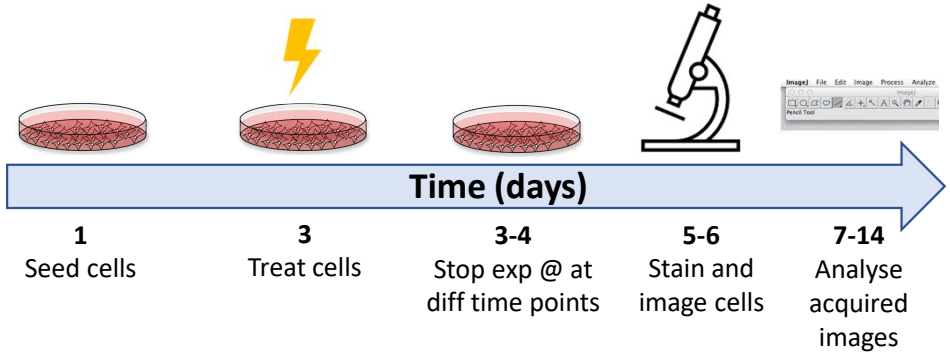


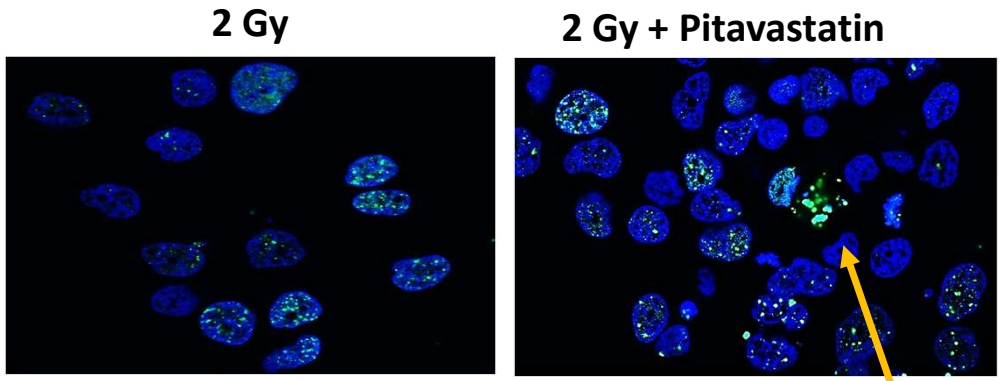
The 3D model provides much greater surface area in 96 well plate than 2D

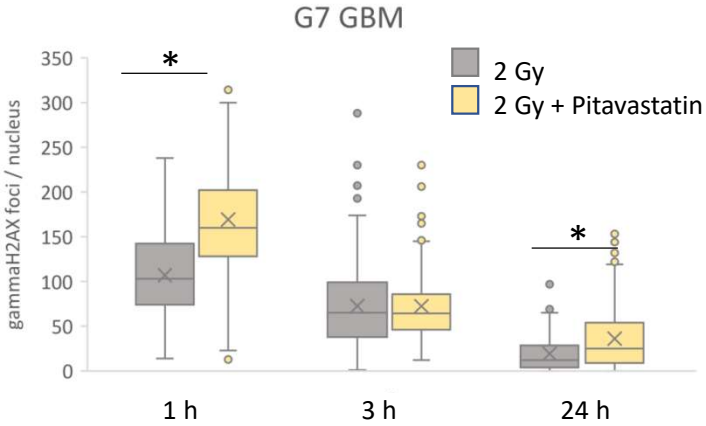
Dr Mark Jackson
Dr Nati Gomez-Roman

University of Strathclyde Glasgow

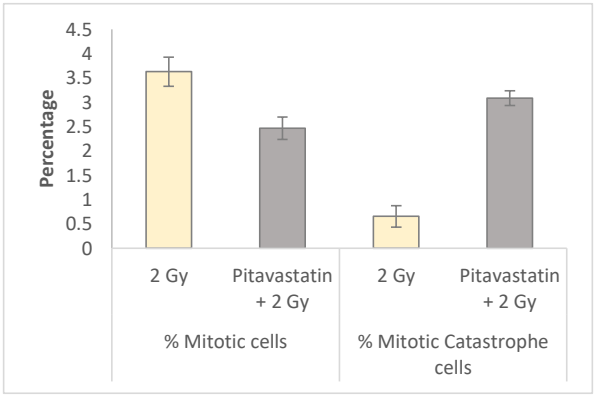
Characterisation of radiation response



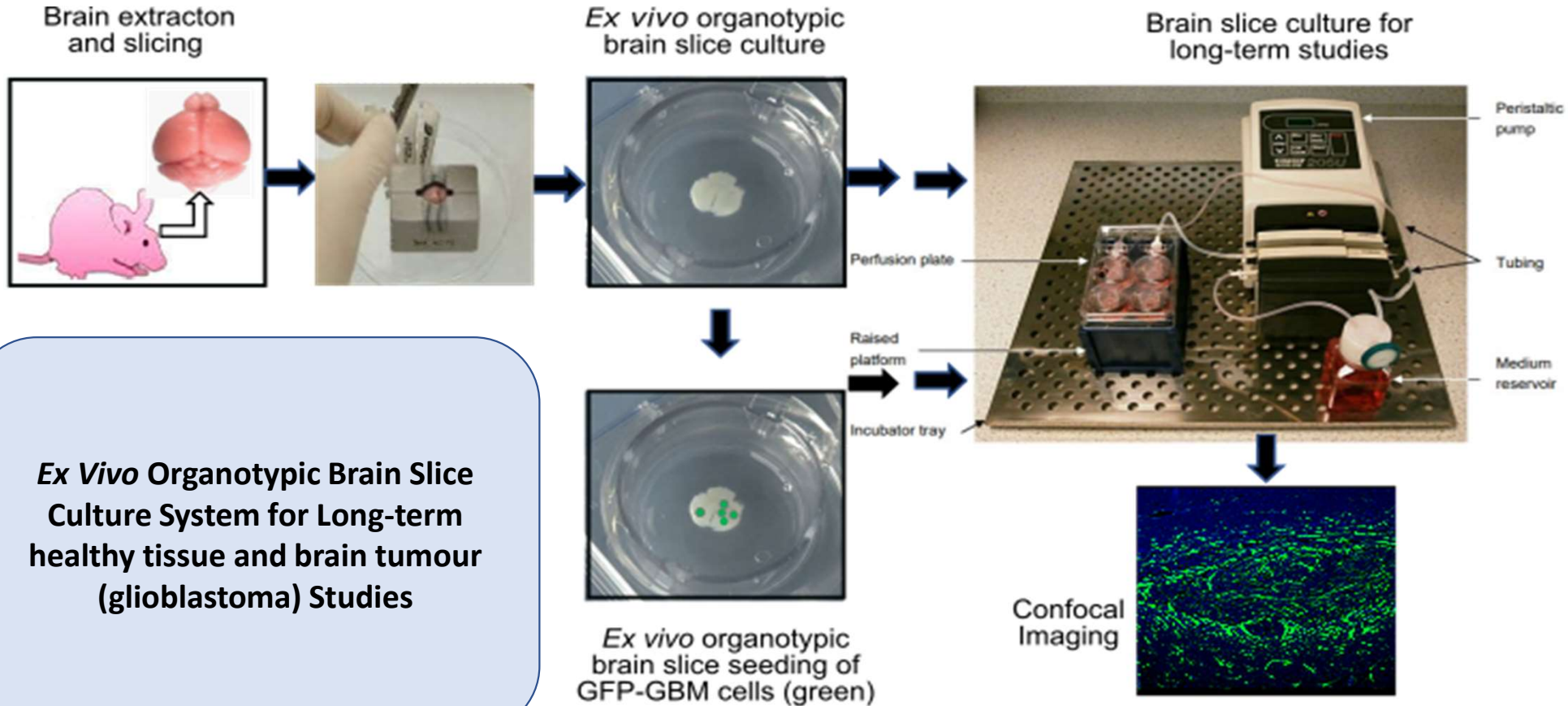




Quantification of DNA double strand breaks (DSB) using H2AX phosphorylation (γH2AX) as a DSB marker



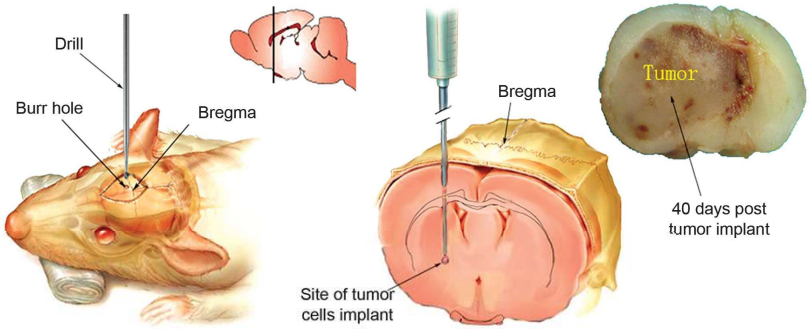
Quantification of cell death via mitotic catastrophe

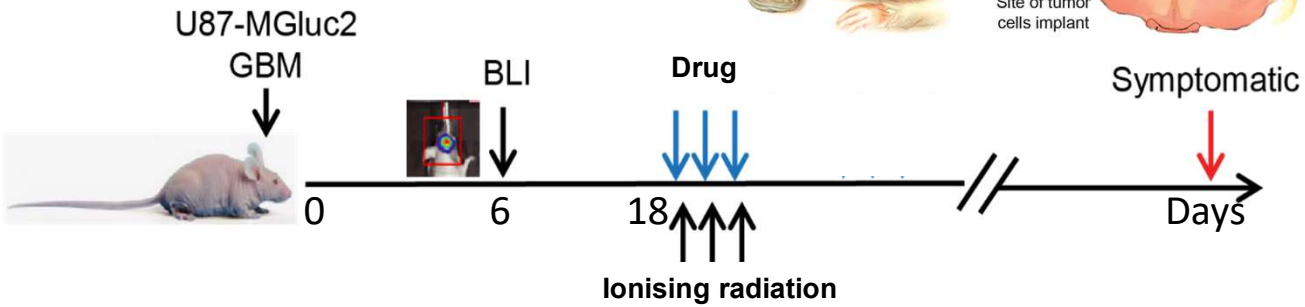


***Ex Vivo* Organotypic Brain Slice Culture System for Long-term healthy tissue and brain tumour (glioblastoma) Studies**

Validating *in vitro* results *in vivo*: GLIOBLASTOMA INTRACRANIAL MODEL

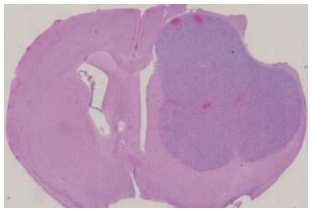
- Intracranial injection in CD1 nude mice
- Experiments range from 4 weeks (U87) to 12-20 weeks (patient derived cell lines)



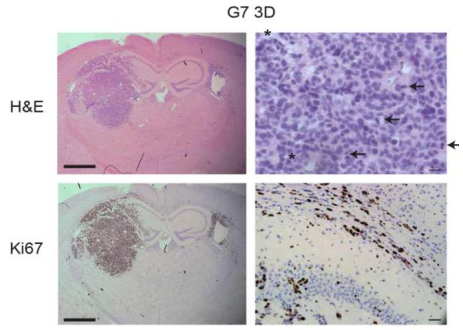


Non-Invasive

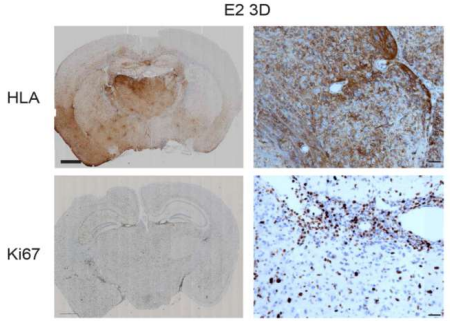
U87-MGLuc2



Invasive

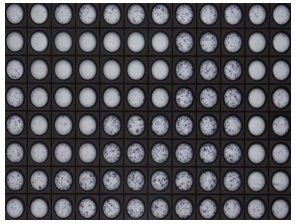


Highly invasive



GLIOBLASTOMA INTRACRANIAL MODEL – Preclinical Pipeline

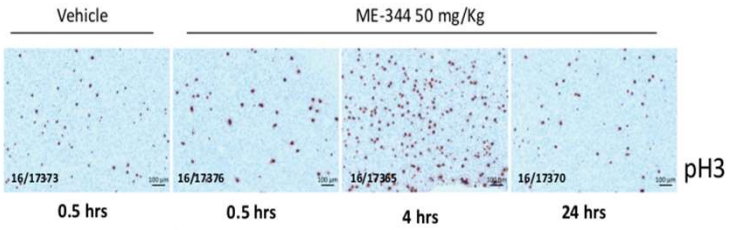
Hit(s) selected from Omics screen

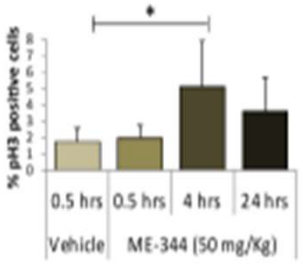


3D assay +/- IR

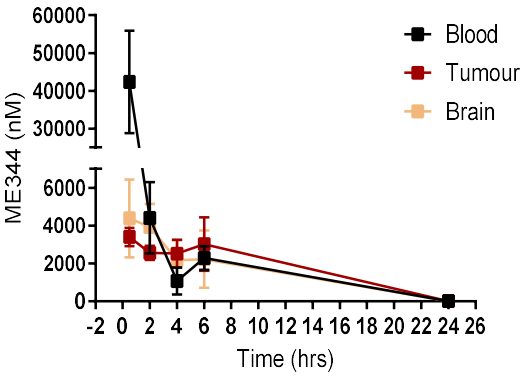
Single agent or drug/IR combination activity

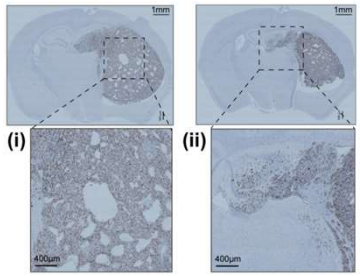
Mitotic cells

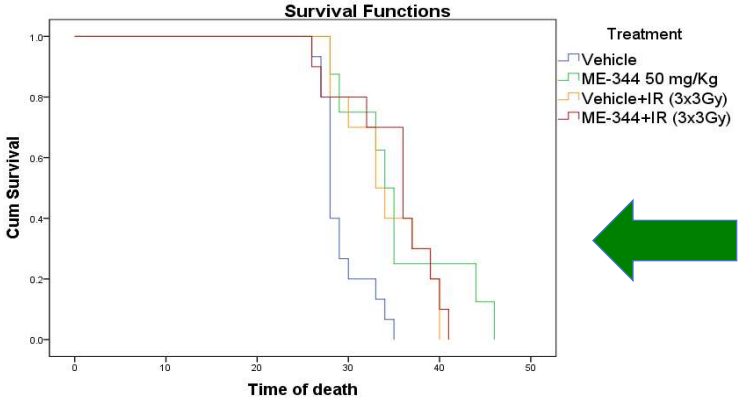




In vivo PK/PD







In vivo efficacy

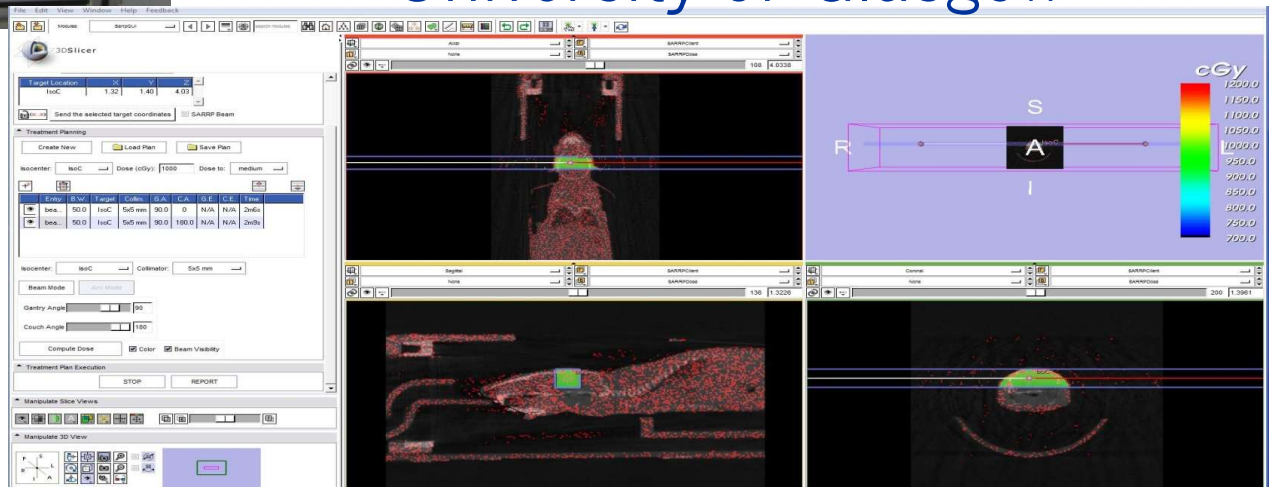
Phase I trial with PK/PD

CANCER RESEARCH UK | RadNet Glasgow

SMALL ANIMAL RADIOTHERAPY RESEARCH PLATFORM – SARRP

Beatson Institute for Cancer Sciences
University of Glasgow

- CT scan
- 3D image guided micro irradiator
- image acquisition, reconstruction, and treatment planning
- target localization
- dose validation
- targeted radiation treatment
- Dose rate 4.8 Gy/min
- 220 kV (peak) X-Ray beams, parallel opposed
- 5 x 5 mm collimator



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Conclusions

- Comparison between current standard of care therapies using X-ray vs proton and ion will require extensive assessment using clonogenic survival assays
- Characterisation of response to treatment will require *in vitro* assays including:
  - DNA damage and repair assays (γH2AX foci, mitotic catastrophe, Comet assay, etc)
  - Cell cycle distribution (cell cycle distribution)
  - DNA repair pathways analysis (activation of DNA repair pathways via immunofluorescence, protein analysis, etc)
- *In vivo* validation of the findings will need to be performed in animal cancer models