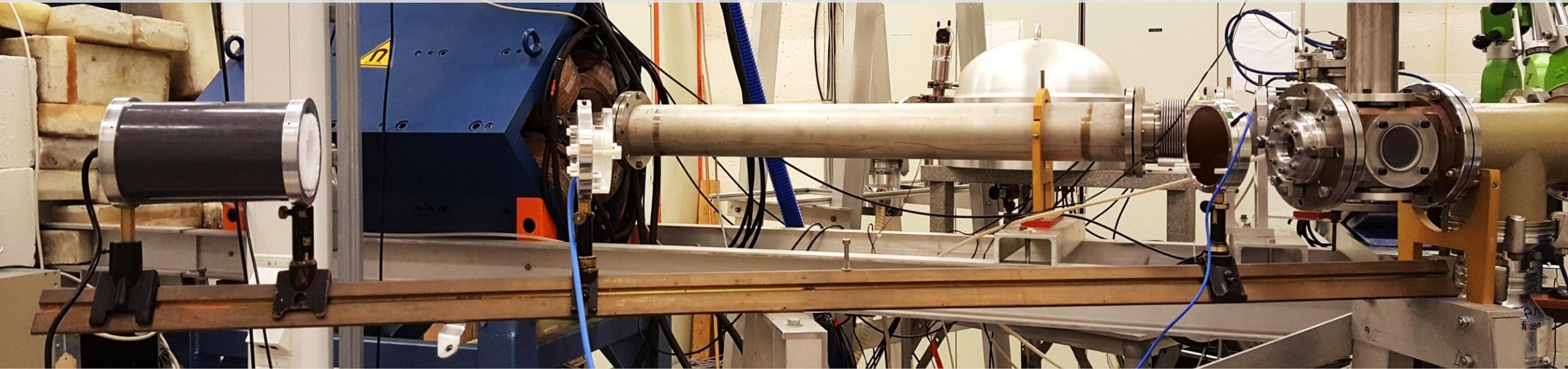


From Biological Hypothesis to Technological Realization

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OF OSLO**



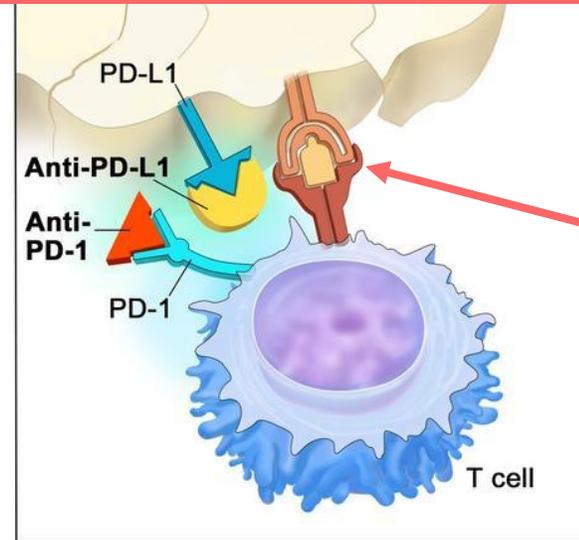
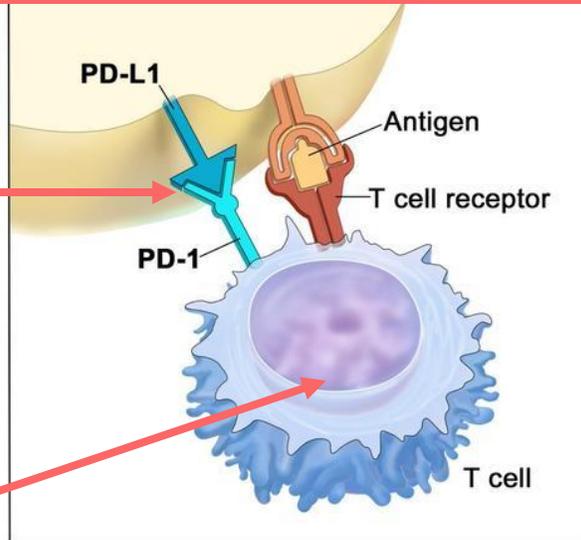
Immunotherapy is a very effective cancer treatment, but only for about 10-20% of the patients.

PD-L1 binds to PD-1 and inhibits T cell killing of tumor cell

Blocking PD-L1 or PD-1 allows T cell killing of tumor cell

Radiation can induce a type of cell death where the dying tumor cells send signals to the immune system that teaches the immune system to attack tumor cells (also metastases)

Protection against over-active immune system (Autoimmune response), stops the attack of the T cells



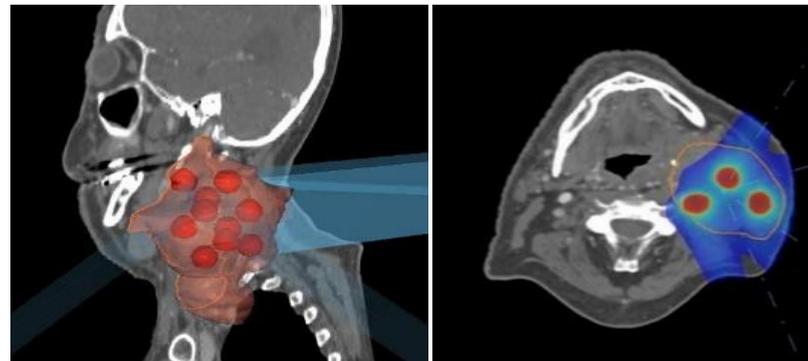
Immunotherapy only works if the T cell knows that it should attack the tumor cell, i.e. recognizes the antigen as foreign

Immune cell that can attack the tumor cell by binding to a tumor antigen

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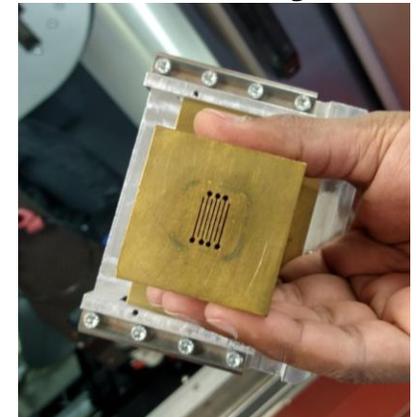
It is not enough to have the signal from the tumor cells; we also need the immune cells that are involved in the anti-tumor response to be present (not killed by radiation).

Solution: Spatial fractionation - minibeam radiation



Broadbeam vs minibeam **X-rays** w/wo anti-PDL1

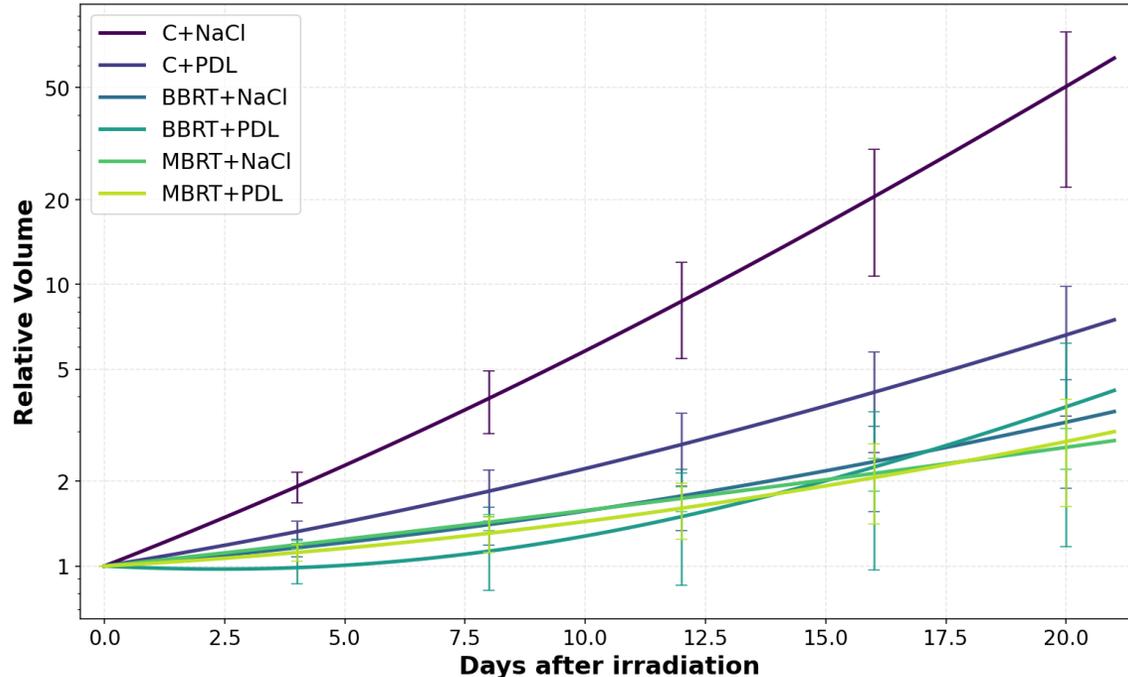
- Female C57BL/6Jrj mice (n=116)
- MOC1 (immunogenic) and MOC2 (non-immunogenic) cells; subcutaneous injections in the right flank
- Mean dose 15 Gy; broadbeam or minibeam, 220 kV x-rays
- Planar minibeam; width 700 μm , center-to-center spacing 1465 μm , **peak dose 40 Gy, valley dose 4 Gy**
- Intraperitoneal injection of anti-PDL1
- Caliper measurements of tumor volume
- Experiments done at Institut Curie, Orsay (Yolanda Prezado's group)



Relative tumor volume with time

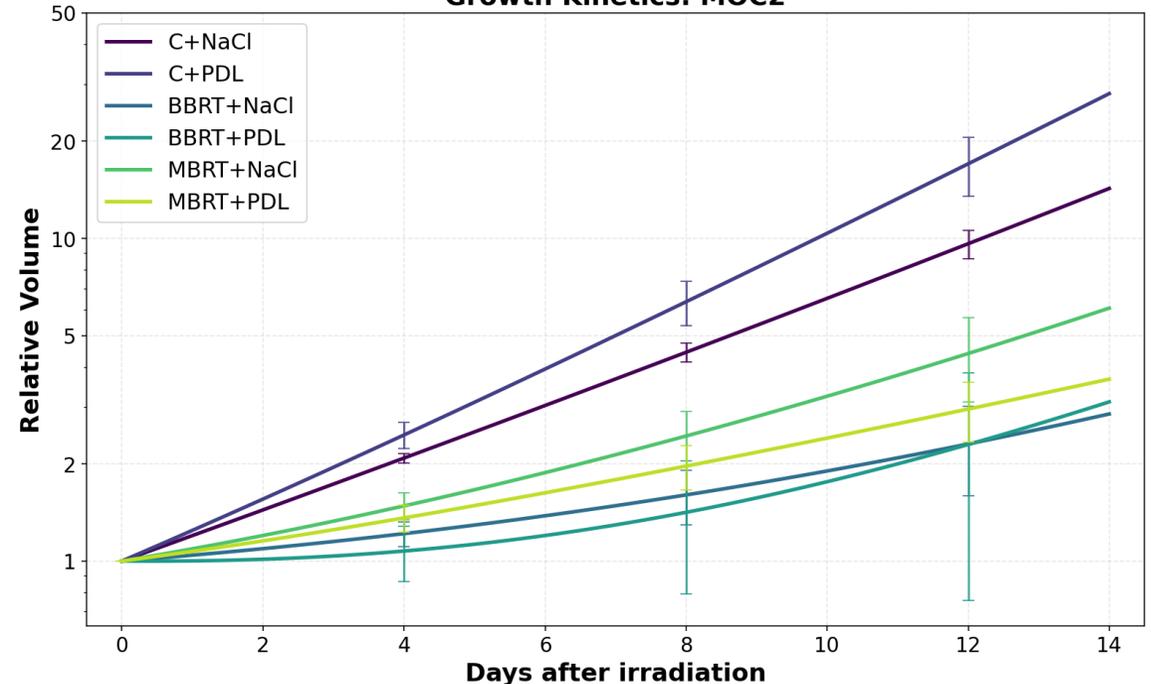
MOC1

Growth Kinetics: MOC1



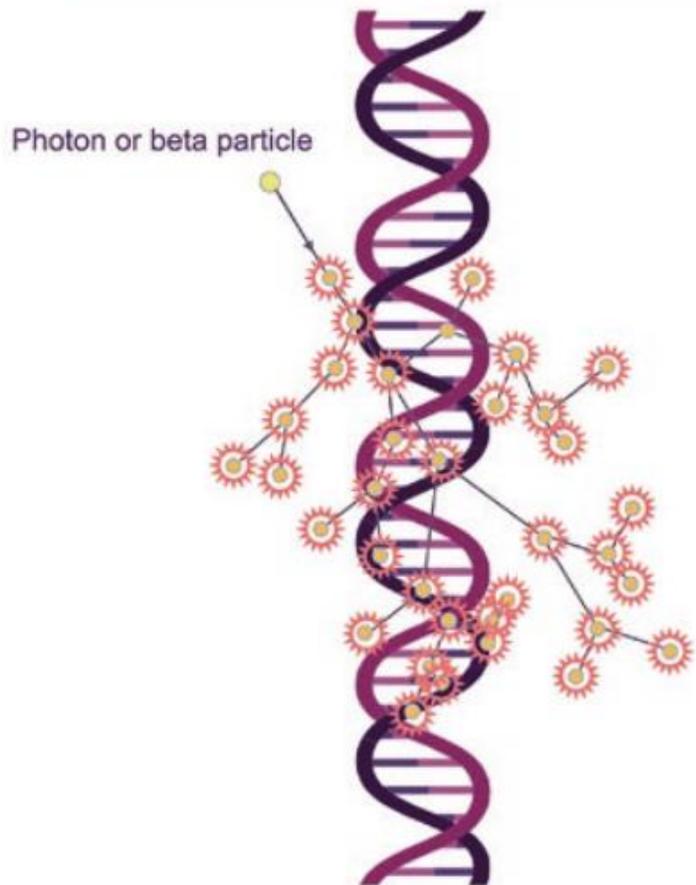
MOC2

Growth Kinetics: MOC2



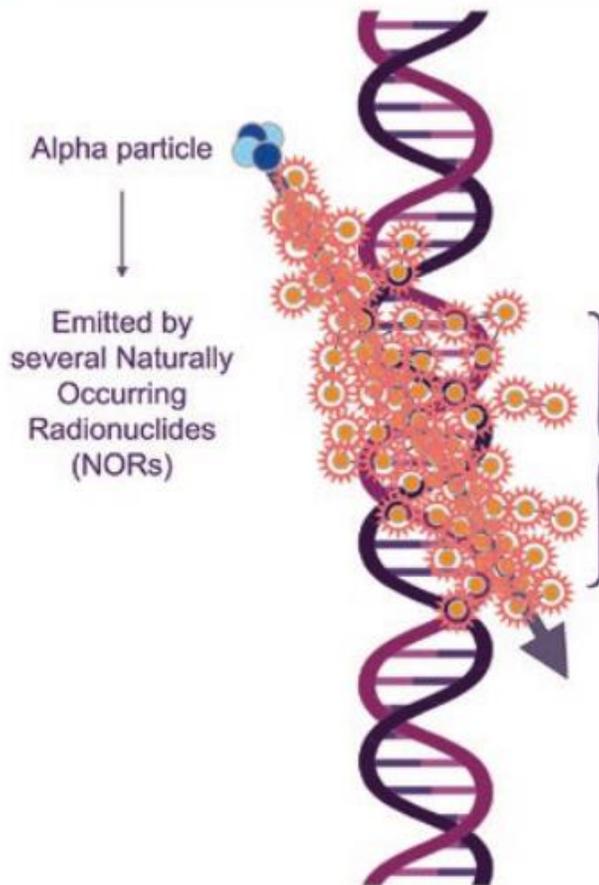
- For MOC1, anti-PDL1 alone had a large effect. Radiation dose was too high, giving small chance to demonstrate synergy
- For MOC2, there was a tendency for synergy between irradiation and immunotherapy

Low LET radiation DNA damage distribution



 Ionizations

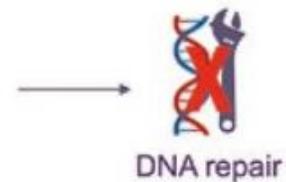
High LET radiation DNA damage distribution



Alpha particle
↓
Emitted by
several Naturally
Occurring
Radionuclides
(NORs)

Complex DNA damage
(closely spaced DNA
lesions that form
clusters)

DNA lesions can
be from the same
or different types



DNA Double strand breaks



DNA Single strand breaks



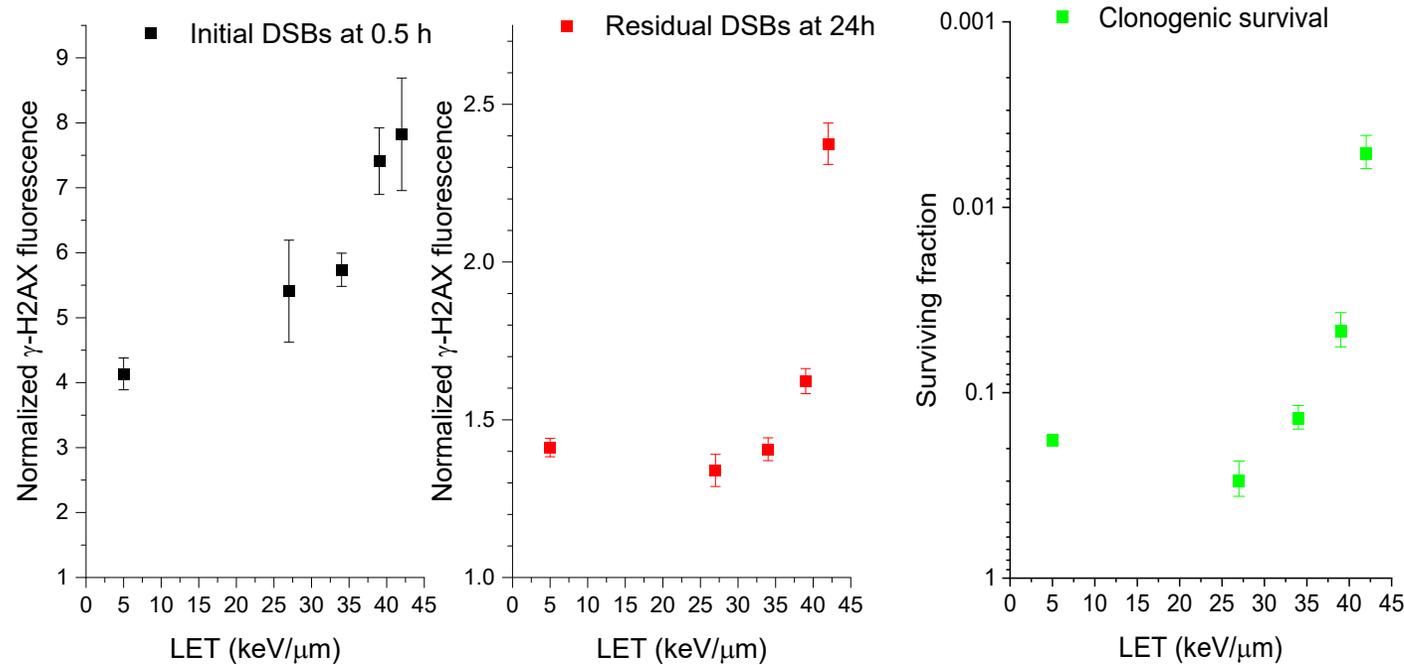
Apurinic/Apyrimidinic sites



Oxidised base lesions

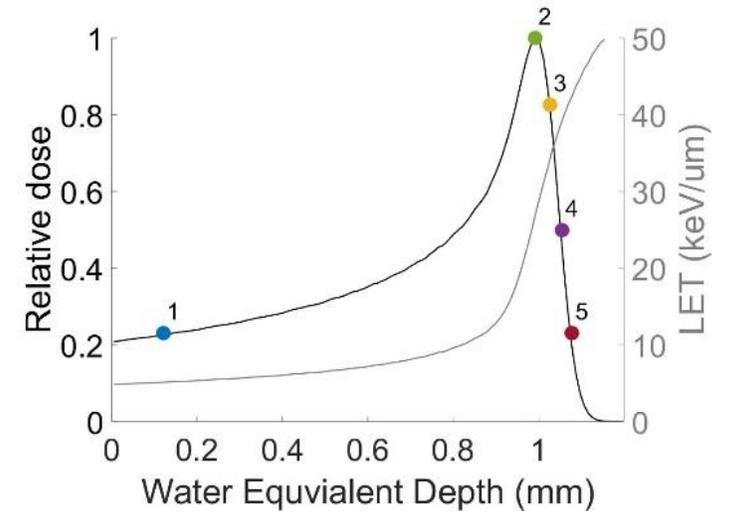


DNA double strand breaks and surviving fraction after 5 Gy 15.5 MeV protons with different LET in T98G glioblastoma cells



Flow cytometry measurements of DSBs

Cell survival



Protons induce more immunogenic signaling than photons

Cancer cells that are already immunogenic (visible to the immune system)

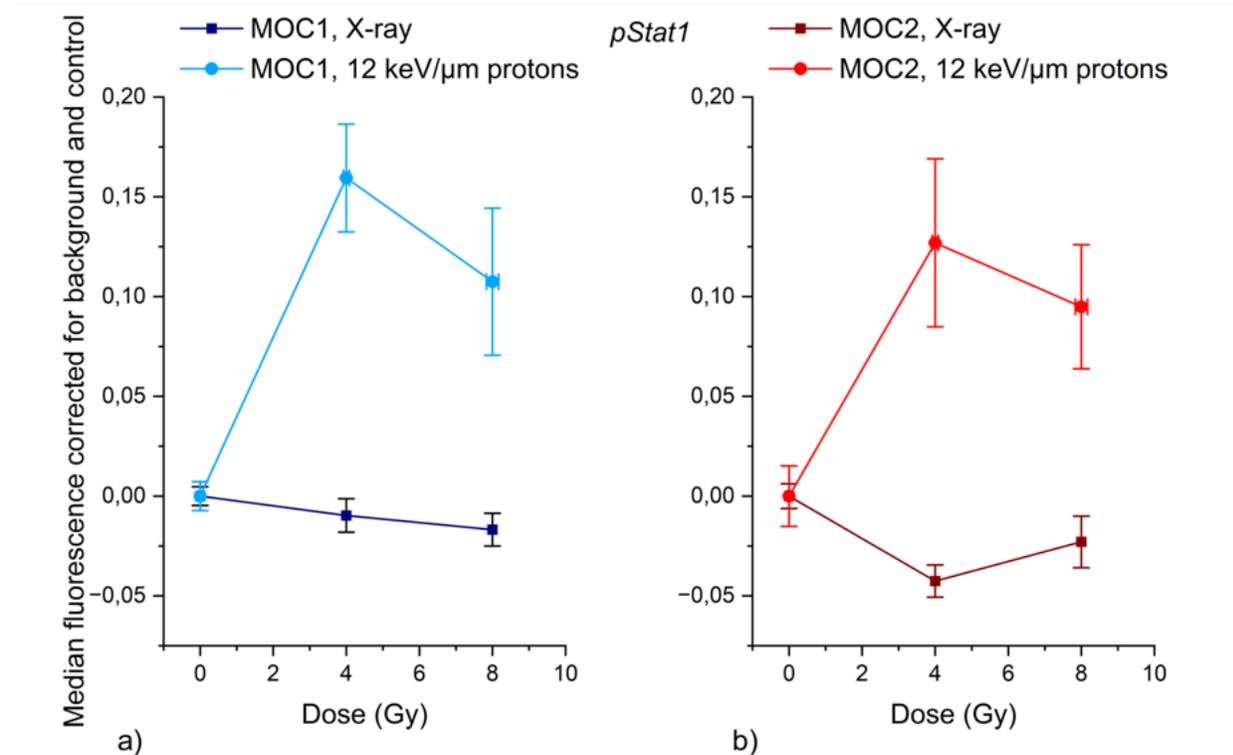


Figure 2. pStat1 measured by flow cytometry in MOC1 (a) and MOC2 (b) three days after 220 kV X-rays and 12 keV/μm protons. Signal corrected for background and unirradiated control. Mean ± SEM is shown.

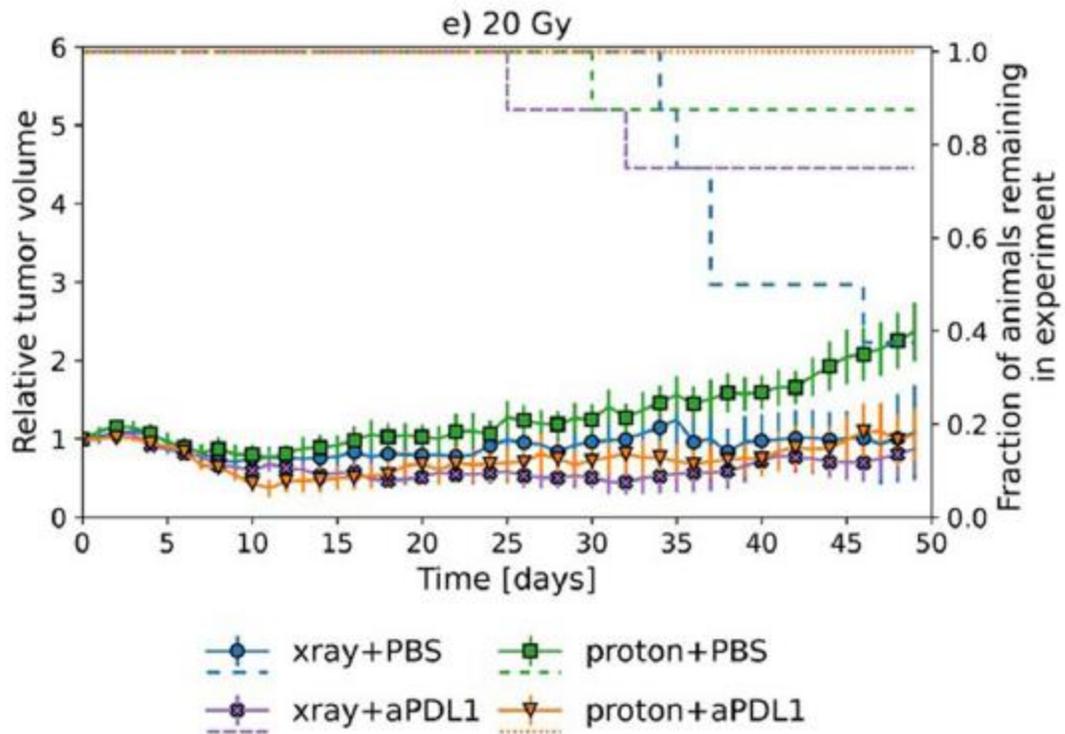
Cancer cells that are not immunogenic (visible to the immune system)

Broadbeam protons vs X-rays w/wo anti-PDL1

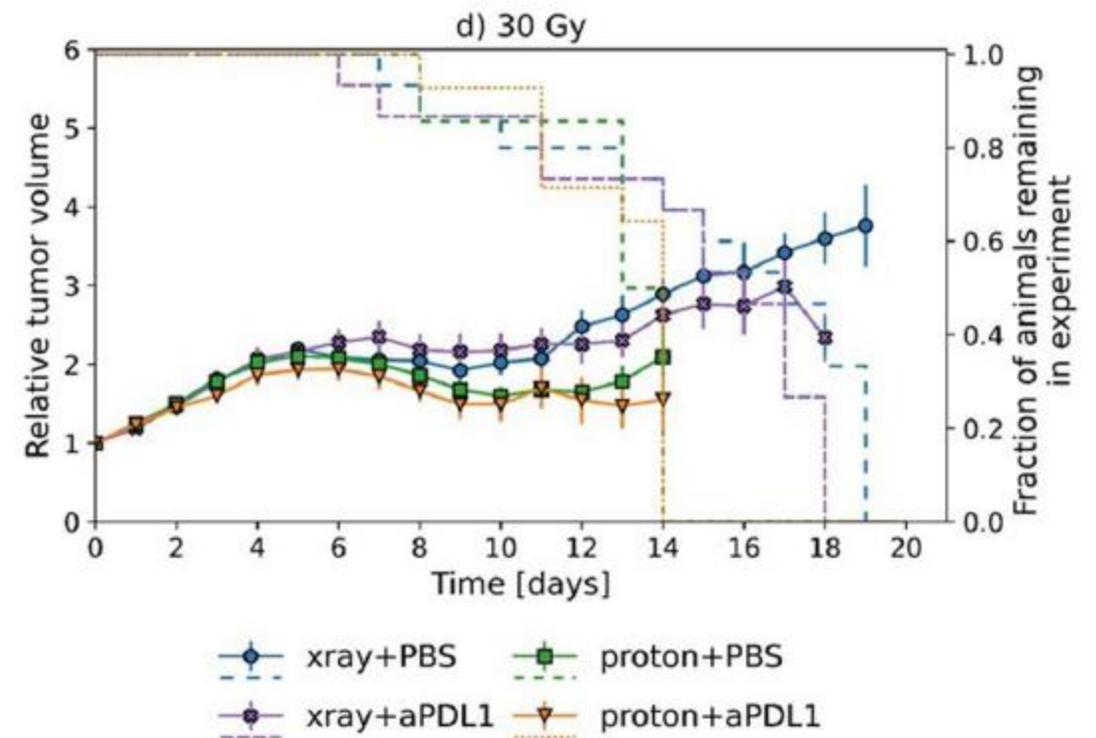
- Female C57BL/6Jrj mice (n=398)
- MOC1 (immunogenic) and MOC2 (non-immunogenic) cells; subcutaneous injections in the right leg
- Doses 5-20 Gy (MOC1) and 10-30 Gy (MOC2)¹⁵; broadbeam, 280 kV x-rays or protons (spread-out Bragg peak)
- Intraperitoneal injection of anti-PDL1
- Caliper measurements of tumor volume
- Experiments done at DCPT in Aarhus, Denmark (Mike Horsman's and Brita S. Sørensen's group)

Broadbeam protons vs. X-rays w/wo anti-PDL1

MOC1



MOC2



- For MOC2 (non-**Next step proton minibeam** inducing immune response

Radiation also induce immunosuppressive signals

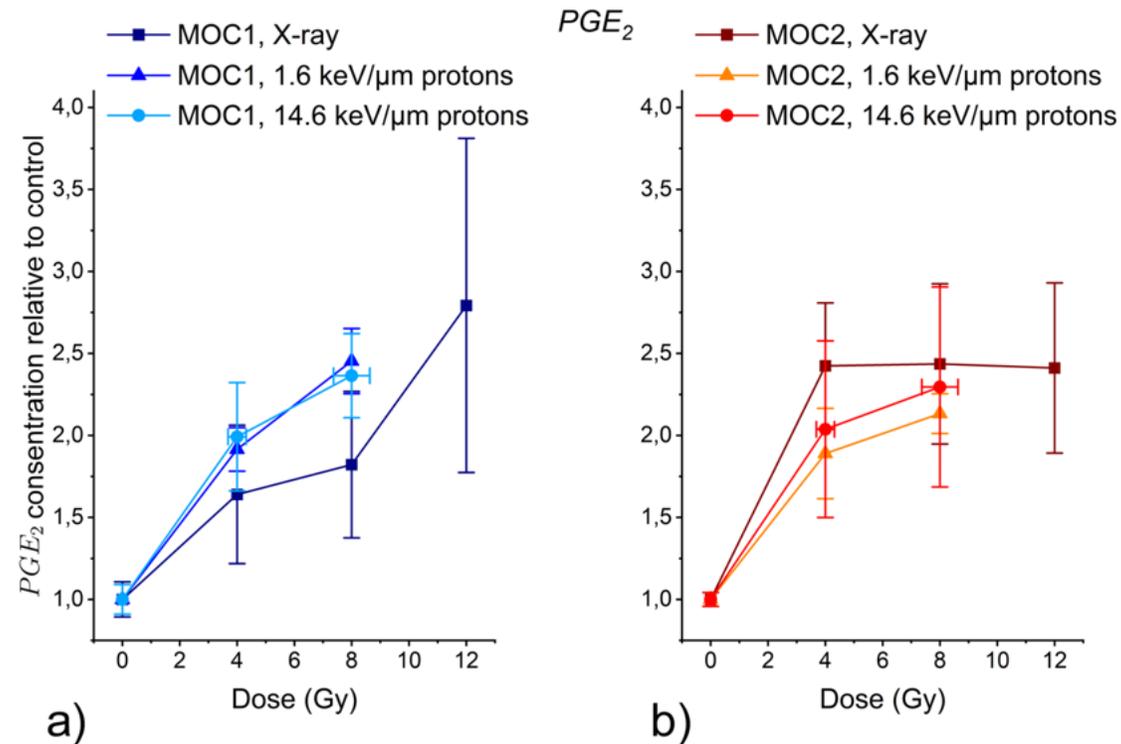
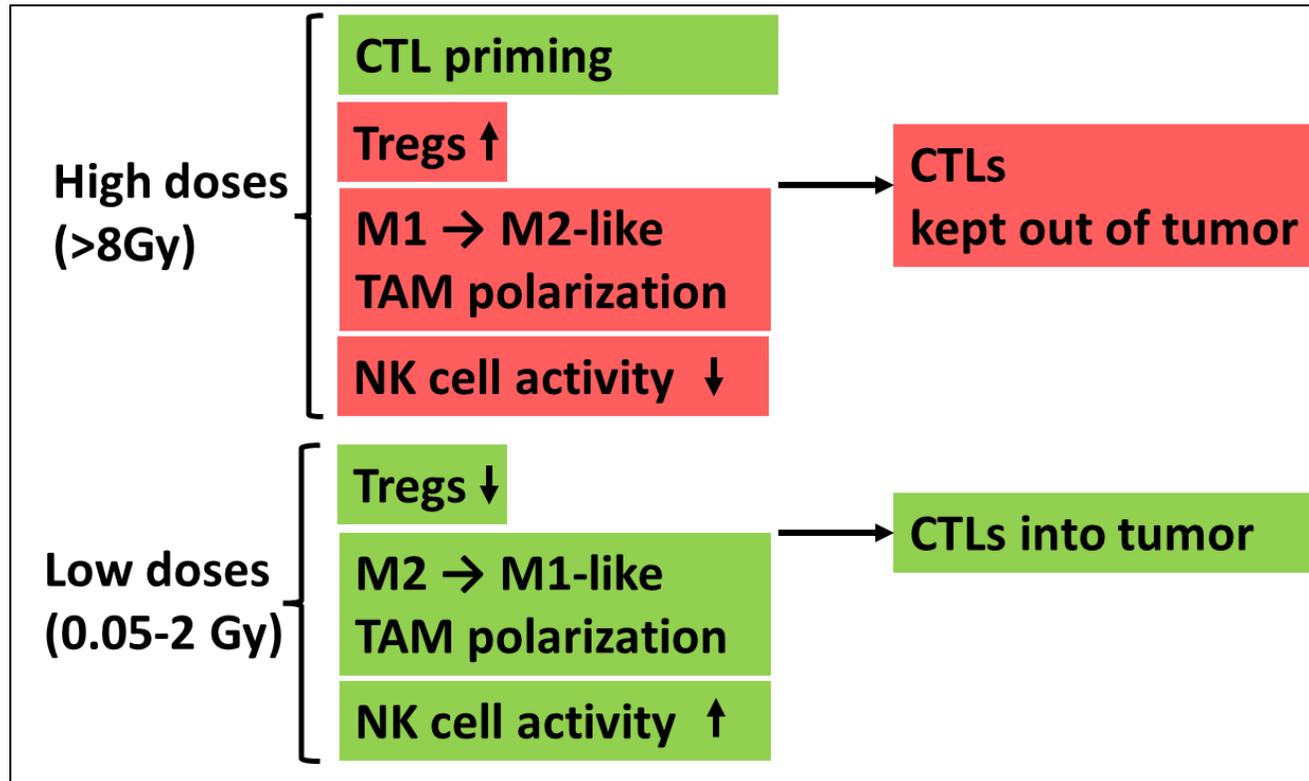


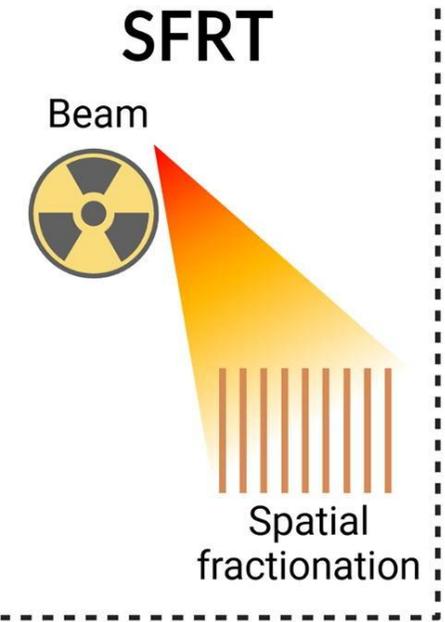
Figure X. PGE_2 concentration relative to unirradiated control in irradiated MOC1 (a) and MOC2 (b) cells measured in the supernatant one day after treatment.



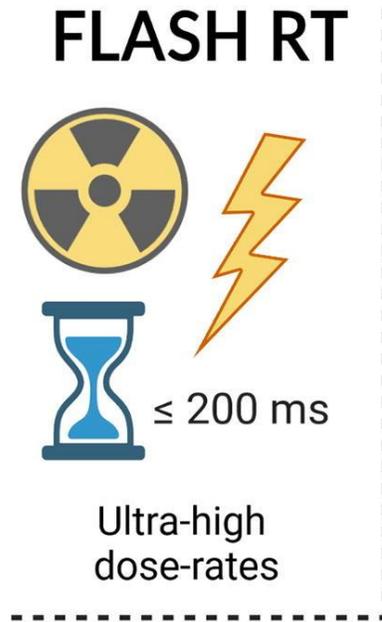
What if you combine high and low doses?

Design minibeam patterns with optimal peak and valley doses?

Combination of minibeam (SFRT) with FLASH?



- ↑ *Anti-tumor immunity*
- ↑ *Abscopal effects*
- ↓ *Vascular toxicity*
- + *Bystander effects*



- ↑ *Anti-tumor immunity*
- ↓ *Vascular toxicity*
- + *Oxygen dependance*

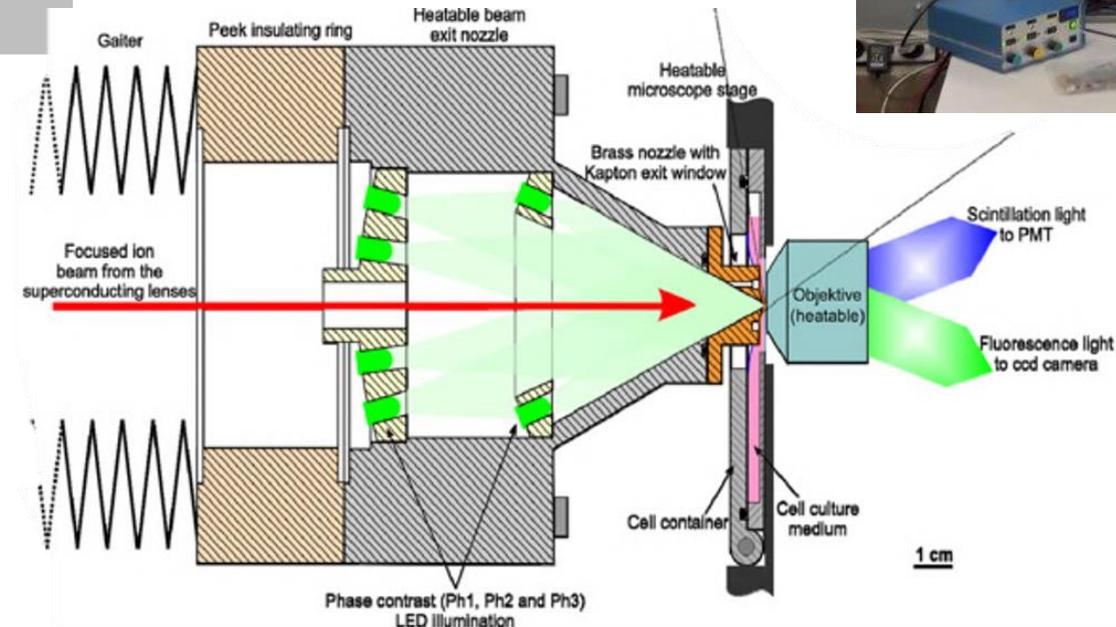
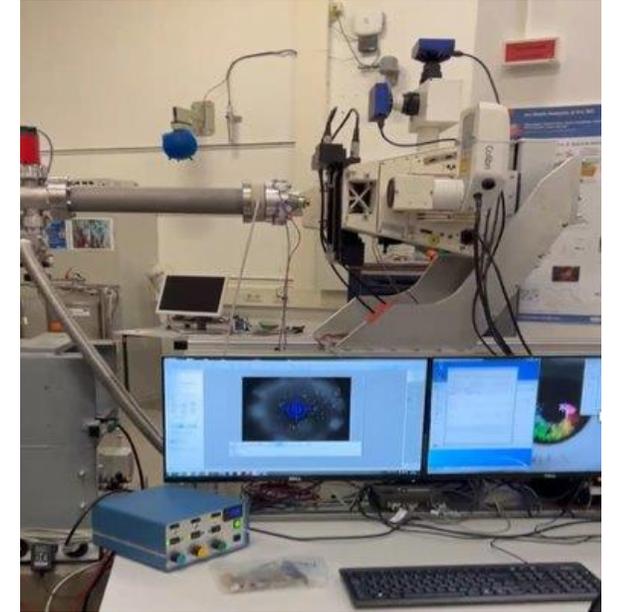
The underlying mechanisms are (most likely) different – potential for synergy

What do we need for a technical realization?

Dedicated particle irradiation
platforms

Millipede and BIOMICRO @ HZDR, Dresden, Germany

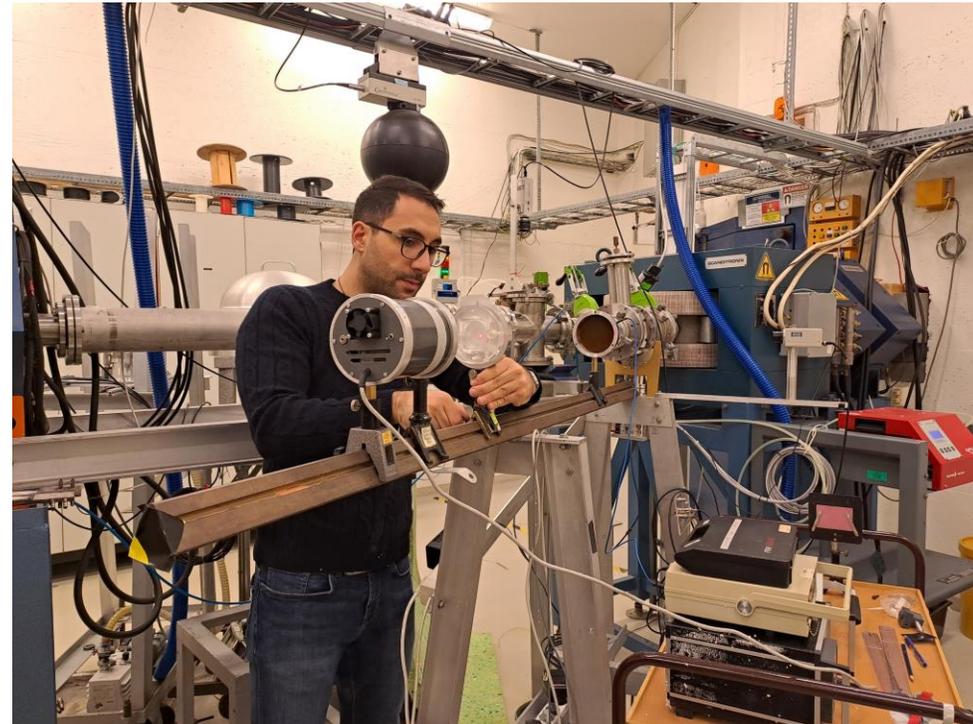
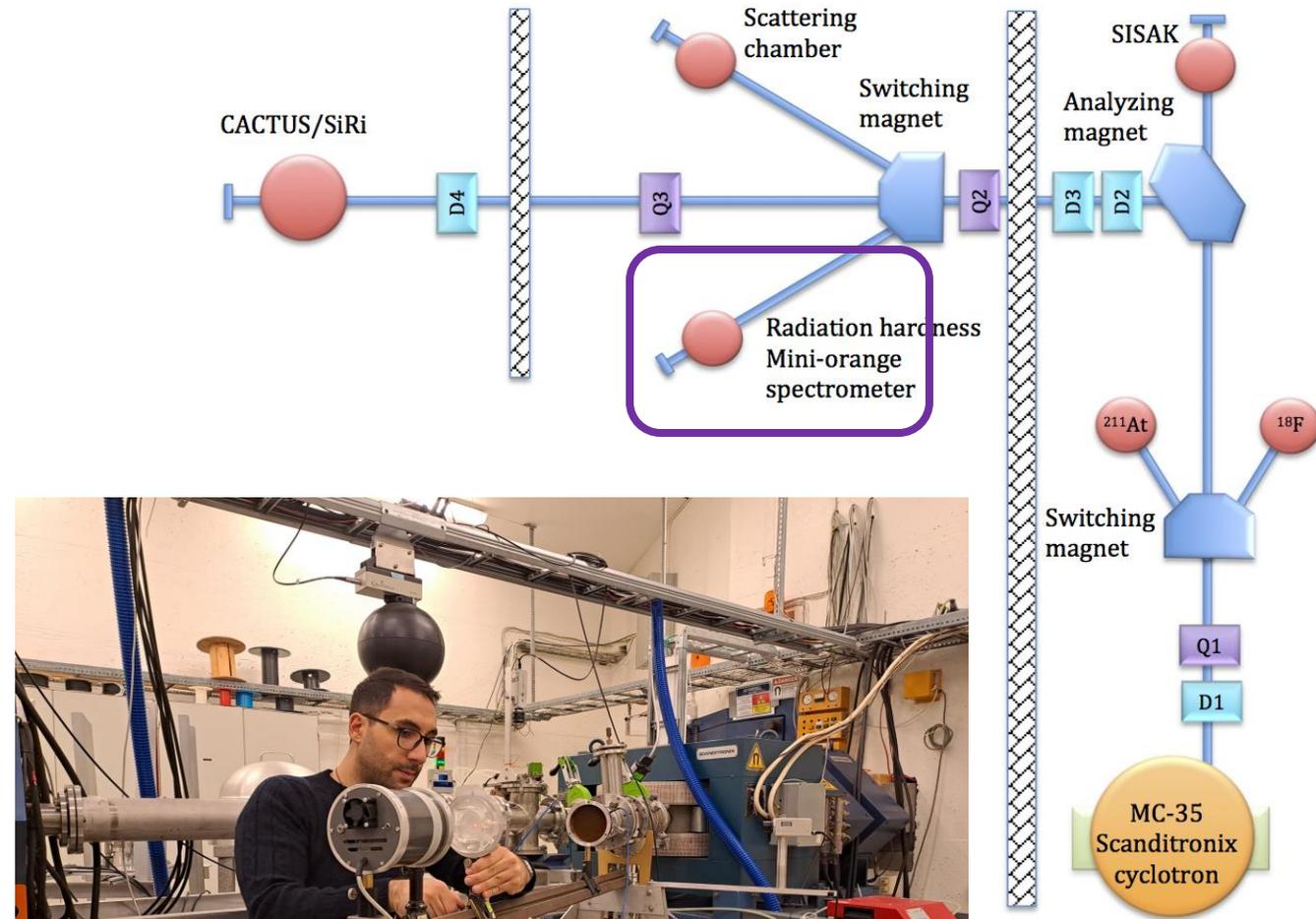
Parameter	
Ion species	p, He, Li, B, C, O
Ion energy	Up to 12 MeV/u
Dose rate	Single ion irradiation – 1 kGy/s
Beam size	~500 nm (BIOMICRO), 70 – 250 μm (Millipede) and Broadbeam



Key feature:
High precision spatial fractionation and single cell irradiation for basic radiobiology and space applications

Cyclotron @ UiO, Oslo, Norway

Parameter	
Ion species	p, He
Ion energy	10 to 30 MeV
Dose rate	2 Gy/min – ? kGy/s
Beam size	Broad Beam

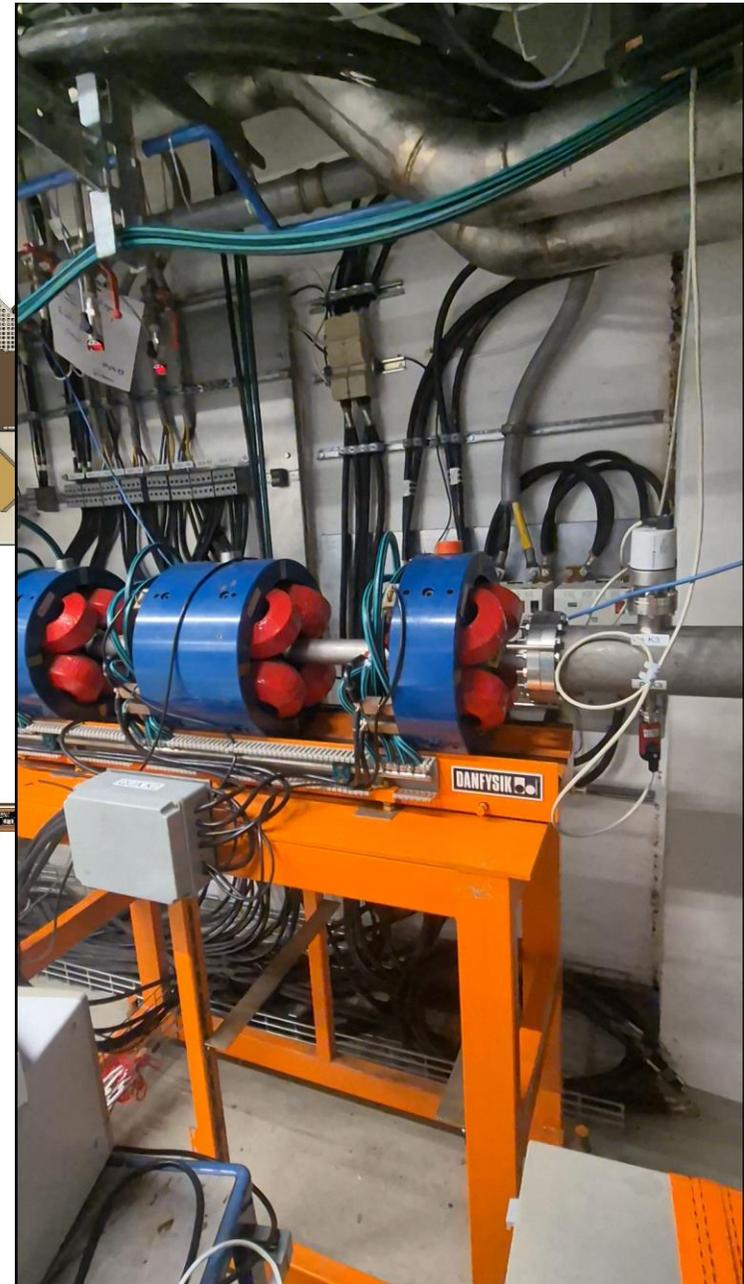
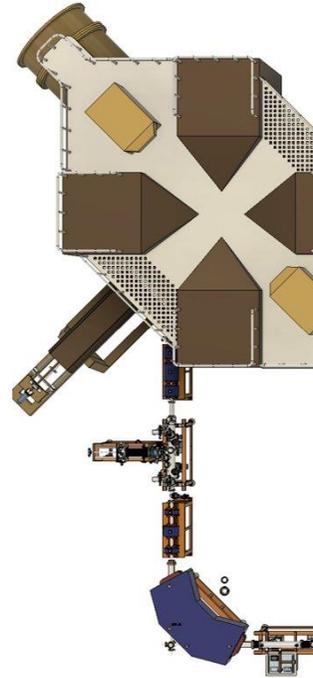


Key feature:
In-house availability, new setup design with a chopper system for systematic studies on FLASH irradiation planned

MiniBEE @ HZB, Berlin, Germany

Parameter	
Ion species	p, (He planned)
Ion energy	35 to 68.5 MeV
Dose rate	2 Gy/min – 1 kGy/s
Beam size	Down to 50 μ m

Key feature:
Ultra-flexible beam shaping, specifically
designed for pre-clinical work



Research Beamline @Proton Therapy Center OUS, Oslo, Norway

Parameter	
Ion species	p
Ion energy	70 to 210 MeV
Dose rate	Several Gy/min
Beam size	Broad Beam, Focused Ion Beam (~500 μ m)



Key feature:
Patient relevant ion beams, suitable for
technology development for translational
research



Irradiation technology at Section for Biophyscis and Medical Physics @UiO, Oslo

Unique combination of platforms across **scales**

subcellular → **cellular** → **tissue** → **clinical**

Access to wide range of

ion species, energies, dose rates, spatial resolutions

Enables

mechanistic understanding + translational validation