The radiobiology of proton therapy: Accelerator and laser-based approaches

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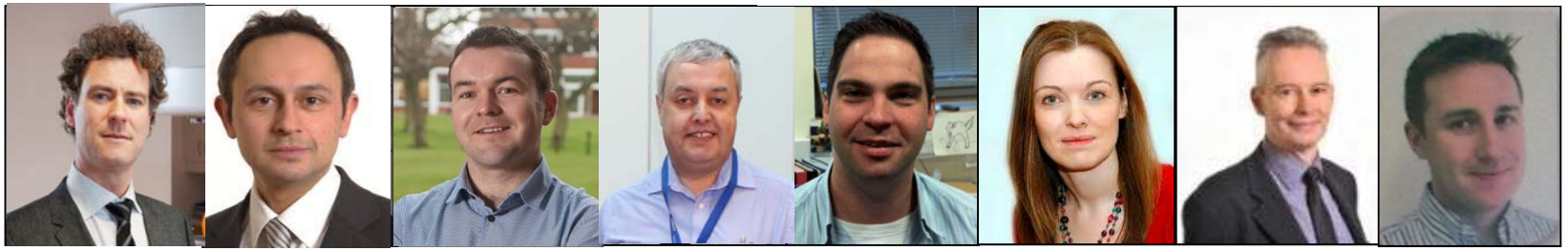
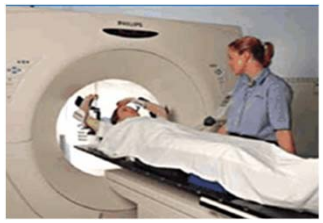
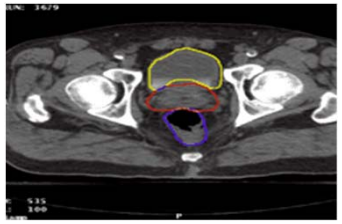
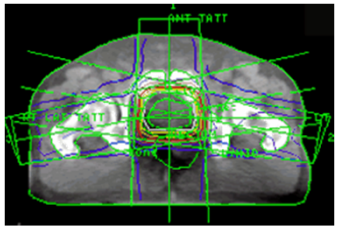
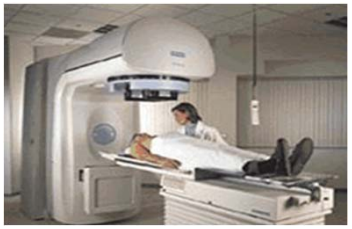
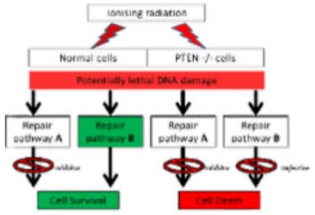


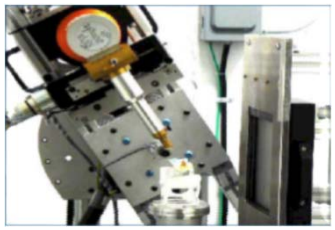
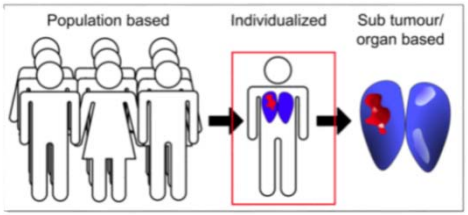
Multidisciplinary Advanced Radiotherapy Group

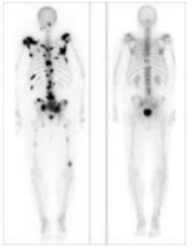
Translational Radiobiology | Experimental Physics | Radiotherapy Physics | Radiation Oncology

Preclinical research → Clinical trial → Improved standard of care





Science Questions ← Reverse Translation ← Clinical Questions

Delivering Advanced Radiotherapies Biologically Optimised to Individual Patients

Outline of presentation

- Introduction to Radiation quality, dose and RBE for charged particles
- Track structure and cellular DNA damage
- What we know from experimental studies
- Understanding clinically relevant treatment protocols at the cellular level
- Laser-based approaches – A-SAIL Project

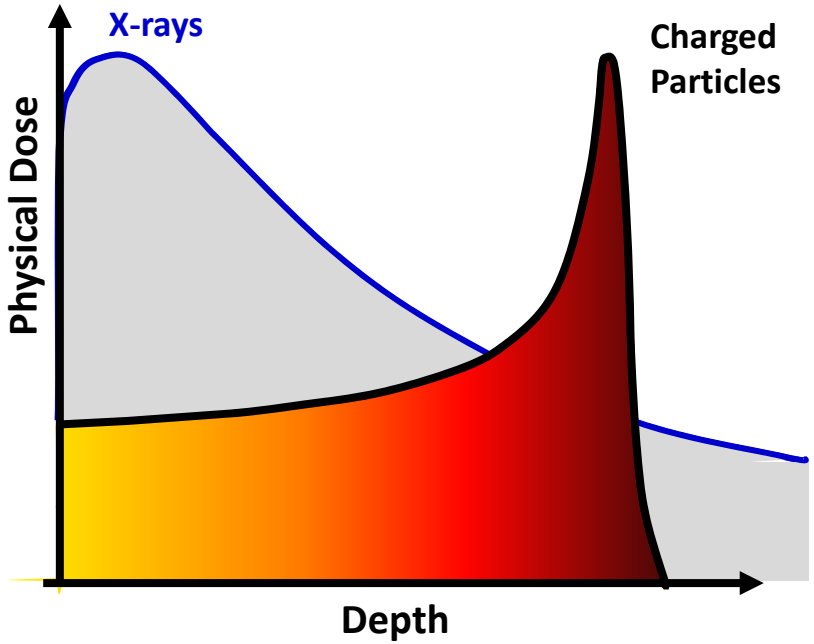
Background

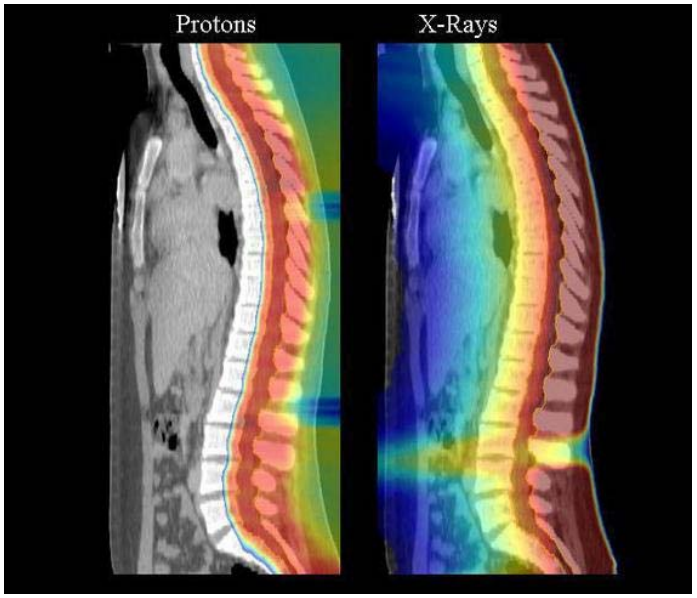
Charged particles are being increasingly used in cancer treatment

By the end of 2016, 174,512 patients had been treated, 149,354 with protons

- Inverse energy deposition
- Elevated RBE for cell killing

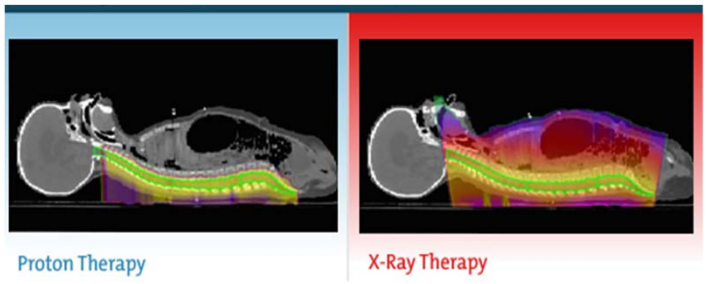
→ Selective dose localization
Improved tumour control

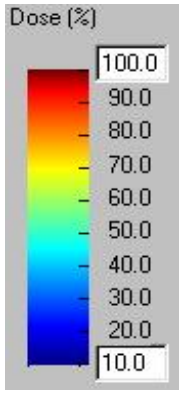




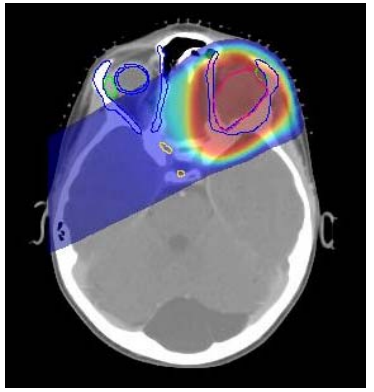
- The Bragg curve represents only the physical dose
  - Primary and secondary particles effects
  - Biological effects

Protons versus Photons



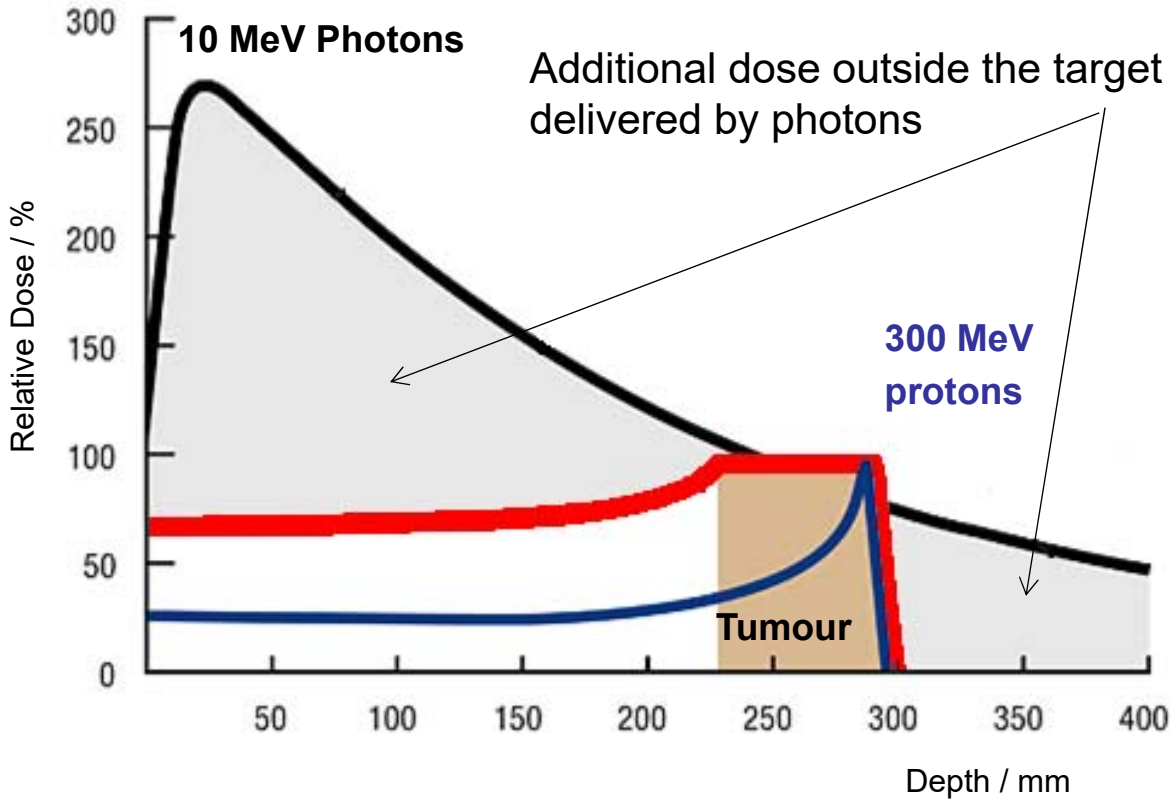


X-Rays



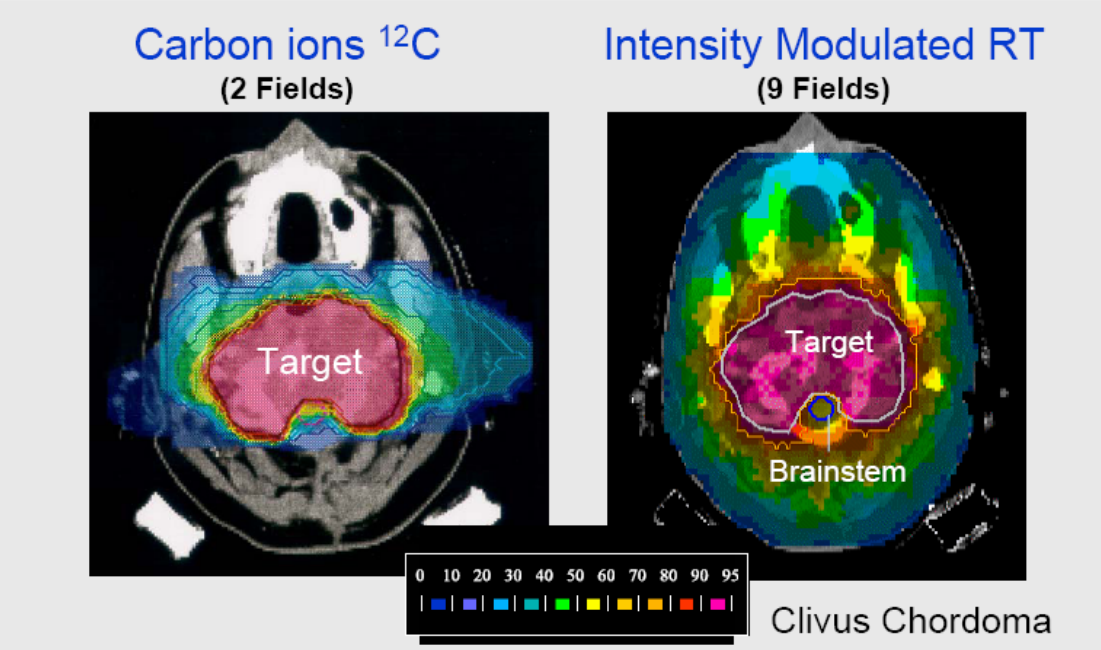
Protons

By producing a spread-out Bragg Peak (SOBP), uniform doses can be delivered at depth



Hadrontherapy treatment

Proton and Carbons from RF accelerators
are currently used for treating a number of tumours



Energies required:
60-250 MeV (protons)
or 100-450MeV/u (**C-ion**)

Typical dose fraction: 2-5 Gy
1 Gy ~ 10^{10} p+, ~10^{9} C in 5x5x5 cm^3
(delivered in few minutes)

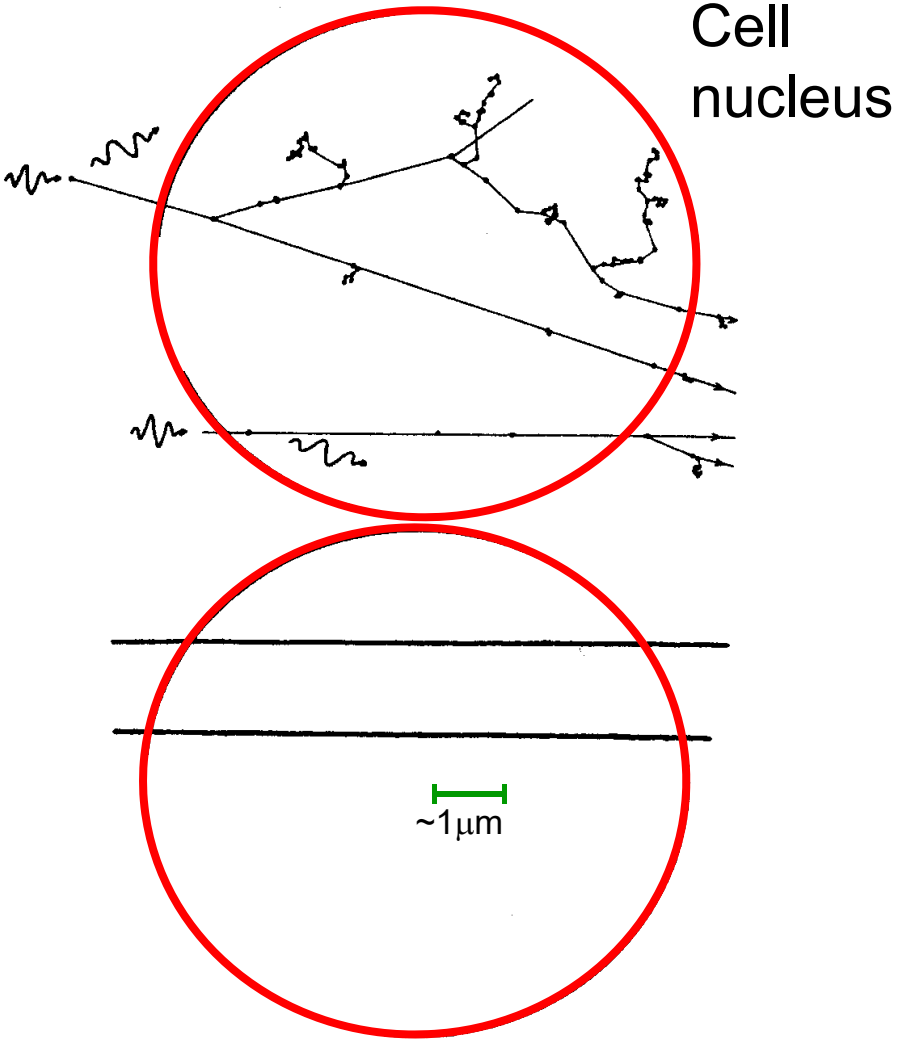
Better localization + increased biological effectiveness leads to improved clinical outcomes for many prescriptions
(~10% of cancer could be better treated by ions, only 0.1% are)

Track structure

Sparsely ionising
Low LET
– γ-rays, X-rays
1 Gy corresponds to
10^5 ionisations in
~ 1000 tracks

Densely ionising
High LET
– α-particles, carbon ions
1 Gy corresponds to
~ 4 tracks

LET = linear energy transfer

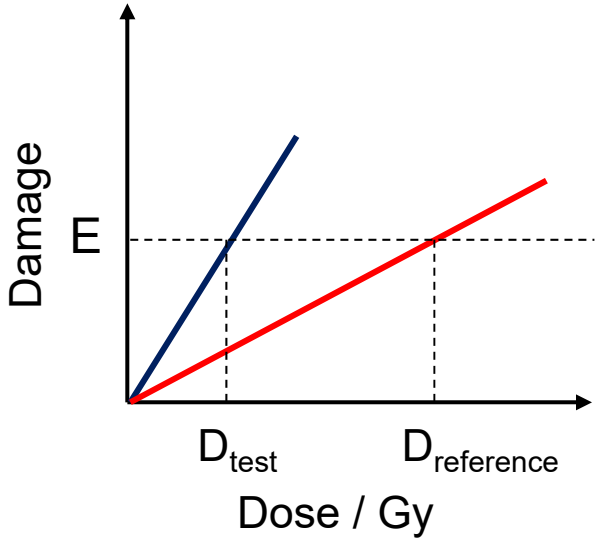


Definitions

LET (Linear Energy Transfer) = Energy deposited per unit length of the track. Normally quoted in kiloelectron volts per micrometer (keV/μm)

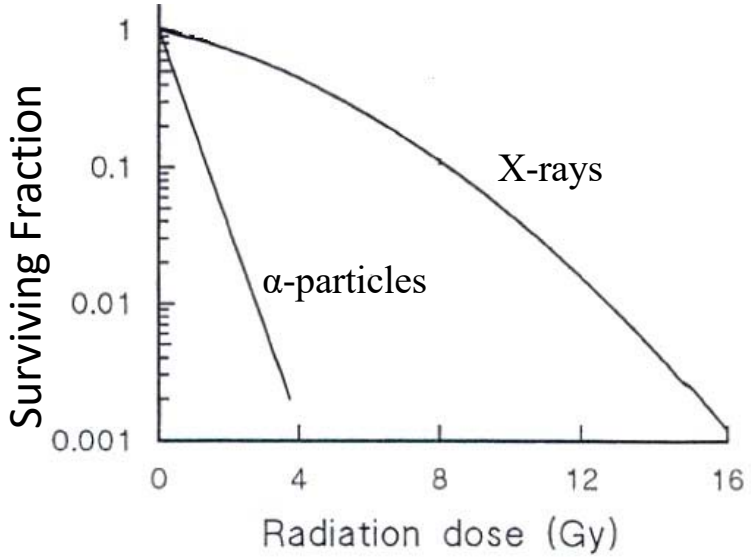
Track average  Equal track intervals

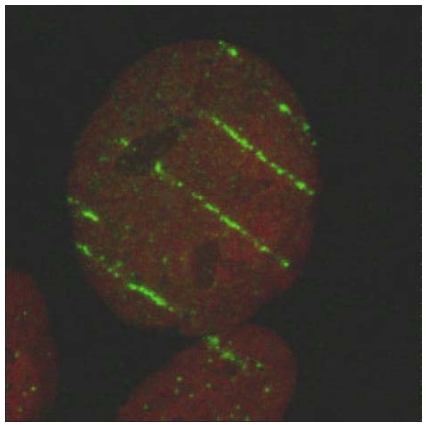
RBE (Relative Biological Effectiveness) = Ratio of the dose of a reference radiation ($D_{reference}$) to dose of a test radiation (D_{test}) producing equal effect (E)



Radiation type	LET	Quality factor (Q)
^{60}Co gamma (1.2 MeV)	0.3 keV/μm	1
250 kV X-ray	2 keV/μm	1
150 MeV protons	0.5 keV/μm	2
10 MeV protons	4.7 keV/μm	2
14 MeV neutron	12 keV/μm	5
2.5 MeV α-particle	170 keV/μm	20
2 GeV $^{56}Fe^{26+}$	1000 keV/μm	20

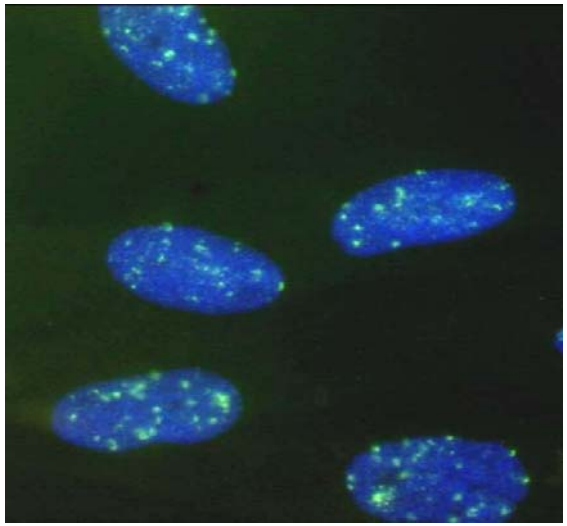
Track structure in cells





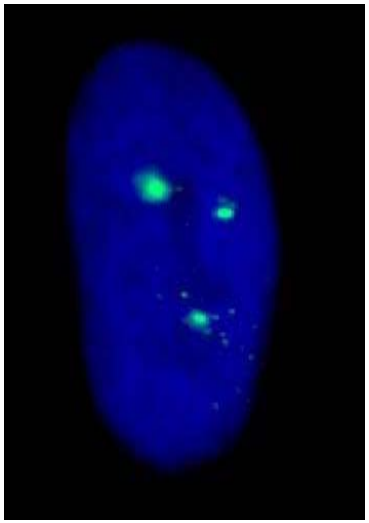
Pb-ions, 3.1 MeV/u, 3x10⁶/cm², 12,600keV/µm
B. Jakob et al., Radiat Res., 2000.

DNA damage distributions (foci)



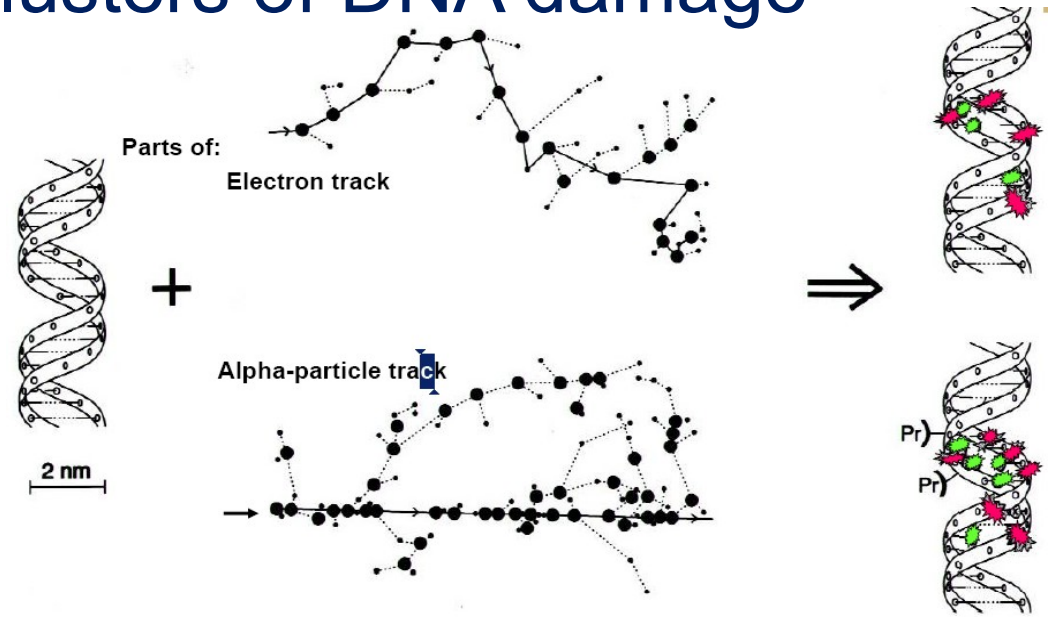
0.5 Gy X-rays

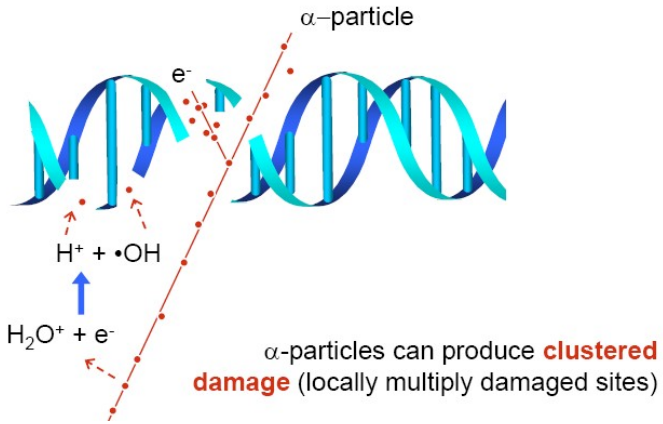
same dose

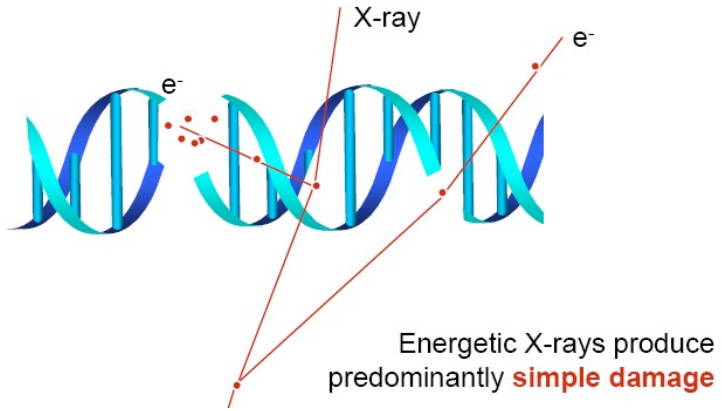


3 He ions (microbeam)
100keV/µm

Clusters of DNA damage





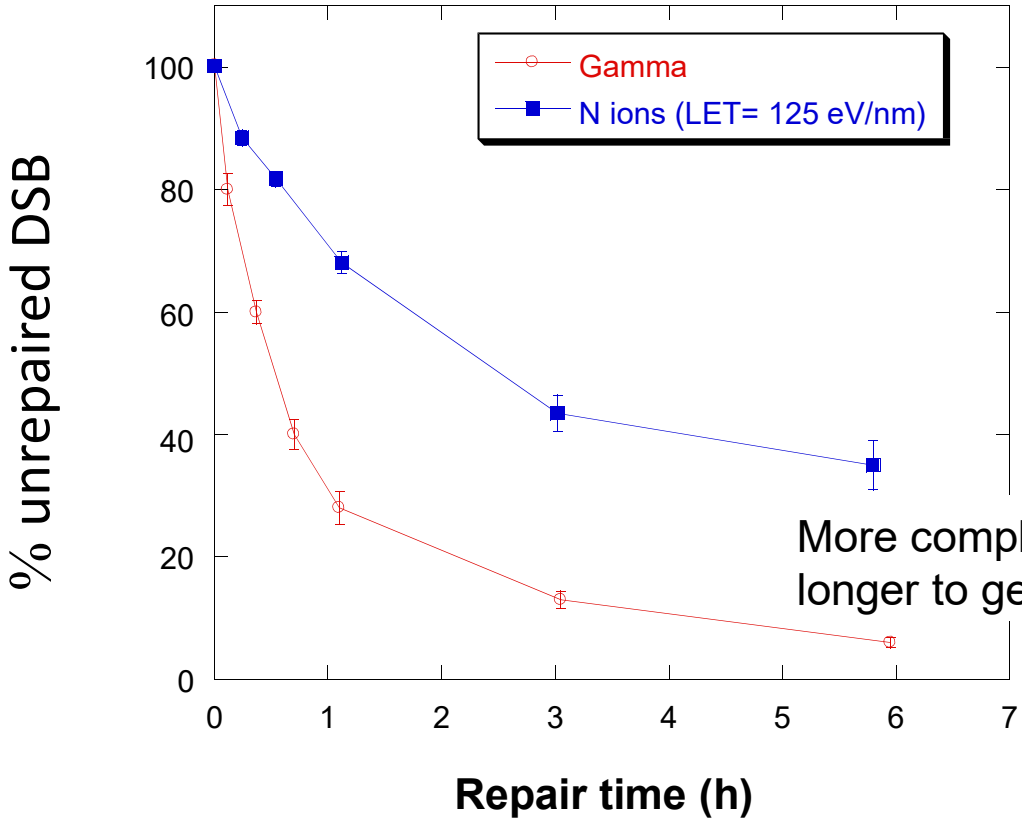


A single α-particle will deposit ~1-2 MeV in a cell, producing ~60,000 ionizations (~20 eV per ionization; 1-2 ionizations per nm)

A single X-ray will deposit ~6-10 keV in a cell, producing ~300 ionizations (~20 eV per ionization; 1 ionization every 4(

Complexity of DNA strand breaks

- Severity of the DNA damage impacts on DNA repair kinetics.
- Cells are able to easily and quickly repair “simpler” DNA damages.
- Observed experimentally for different LET radiations

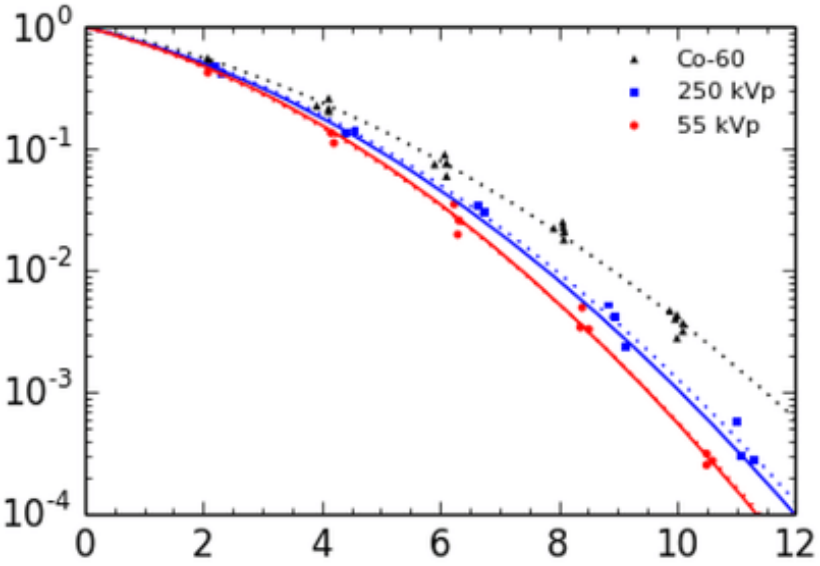


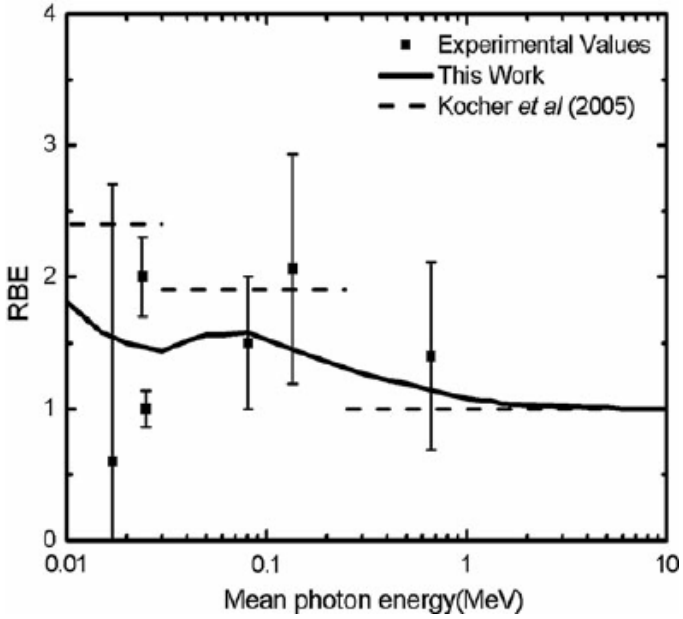
More complex damages may take longer to get repaired

Reference Radiation is important for RBE

- Photon energy used for reference radiation impacts on RBE calculations
- Most cellular studies have used gamma-rays (^{60}Co) or 250kVp X-rays
- Lower energy photons have a higher RBE
- Move to use MV photons as reference radiation for clinical relevance

Spadinger and Palcic, 1992
Bellamy *et al.,* 2015



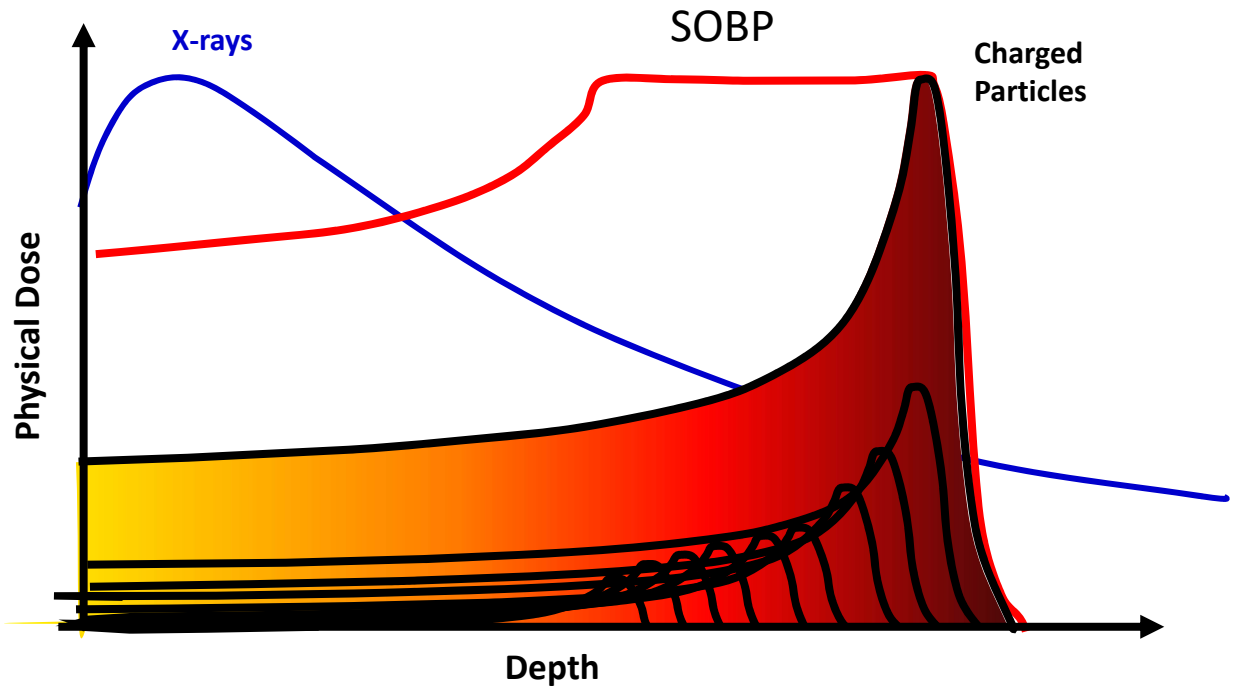


Studies with clinical beams

RBE critically depends on both physical and biological parameters:

- **Dose & Dose Rate**
- **Cell line radiosensitivity**
- **Ion mass**
- **Ion energy**
- **SOBP shape/size**
- **.....**

Clinical beams are delivered by a series of overlapping pristine monoenergetic beams



Currently fixed RBE values are used for protons clinically and disregard any physical and biological dependency potentially limiting particle therapy effectiveness

- Dose accuracy required in radiation therapy = 3.5 %
- Any uncertainty on the RBE will translate in the same uncertainty for biological effective dose

Proton RBEs

- A **range of RBE values** *in vitro* and *in vivo* have been reported over many years
- Average value at mid-SOBP over all dose levels of 1.2, **ranging from 0.9 to 2.1.**
- Studies using **human cells** show significantly **lower RBE** values compared with other cells owing to higher α/β ratios.
- The average RBE value at mid-SOBP **in vivo** is 1.1, ranging from **0.7 to 1.6**.
- The majority of RBE experiments have used ***in vitro* systems and V79 cells with a low α/β ratio**, whereas most of the ***in vivo*** studies were performed in **early-reacting tissues with a high α/β** ratio.
- A value of **1.1 is used clinically**

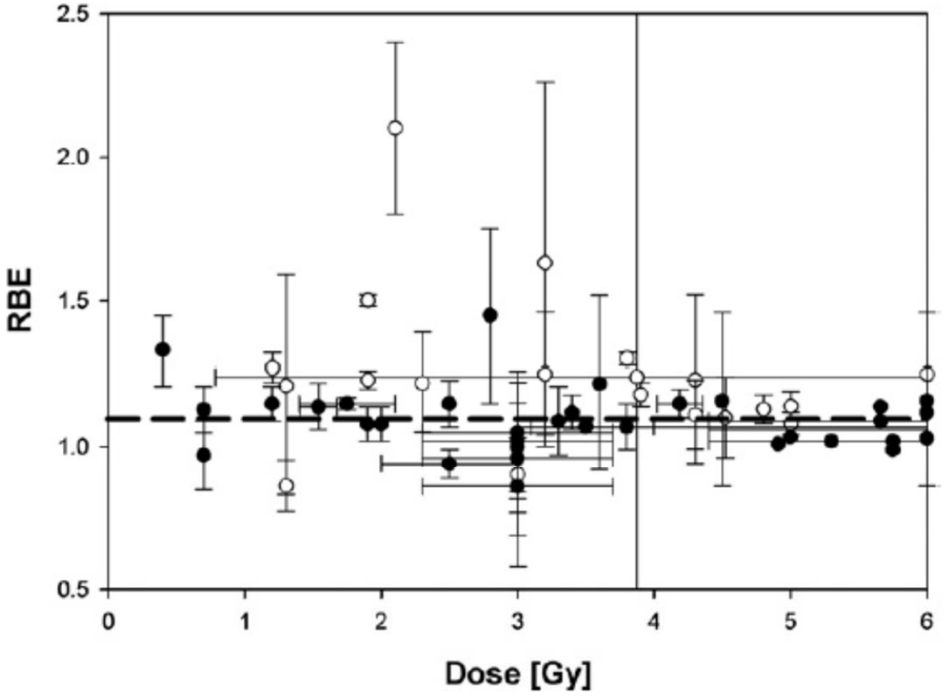


Figure 1 Experimental proton relative biological effectiveness (RBE) values (relative to ^{60}Co) as a function of dose/fraction for cell inactivation measured in vitro (open circles) and in vivo (closed circles). The thick dashed line illustrates an RBE of 1.1. Data taken from Paganetti et al.[15]

Paganetti and van Luijk, 2013, Sem Rad Oncol 23, 77-87

See also Friedrich *et al.,* 2013*, J Rad Res*, **54,** 494

Proton RBEs

- Paganetti, H., 2014, *Phys Med Biol* **59,** R419-R452
- 367 datapoints from 100 publications
- Considerable uncertainty but increasing RBE with LET

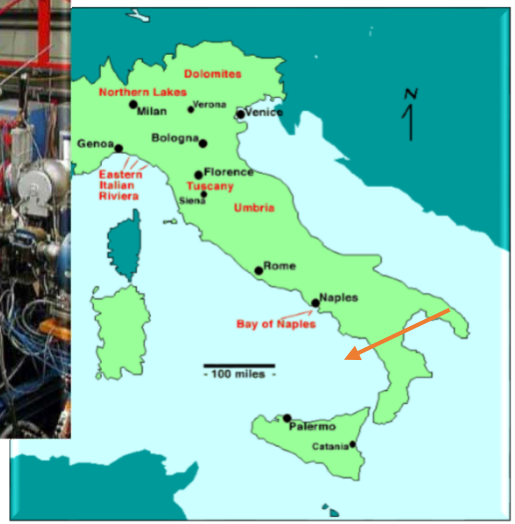
Table 1. Average RBE values based on the data shown in figure 8 considering all $(\alpha/\beta)_x$. LET_d values are given relative to the reference photon radiation. Uncertainties are based on 95% confidence intervals.

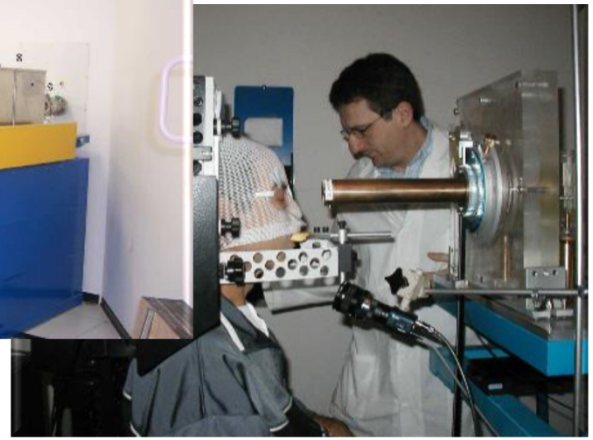
	Average RBE (2 Gy)	Average RBE (2 Gy); weights=1	Average RBE (6 Gy)	Average RBE (6 Gy); weights=1
LET_d = photon LET_d (from linear fit with $LET_d \leq 15\,keV\,\mu m^{-1}$)	1.02 (0.98, 1.06)	1.08 (1.02, 1.14)	0.99 (0.97, 1.02)	1.08 (1.03, 1.13)
$2 < LET_d < 3\,keV\,\mu m^{-1}$	1.12 (1.07, 1.16)	1.18 (1.13, 1.24)	1.09 (1.07, 1.12)	1.15 (1.11, 1.19)
$LET_d < 3\,keV\,\mu m^{-1}$	1.10 (1.07, 1.13)	1.15 (1.11, 1.19)	1.06 (1.04, 1.08)	1.13 (1.10, 1.15)
$3 \leq LET_d < 6\,keV\,\mu m^{-1}$	1.21 (1.16, 1.26)	1.38 (1.28, 1.49)	1.14 (1.11, 1.18)	1.33 (1.24, 1.41)
$6 \leq LET_d < 9\,keV\,\mu m^{-1}$	1.35 (1.25, 1.44)	1.38 (1.21, 1.55)	1.27 (1.19, 1.35)	1.36 (1.18, 1.54)
$9 \leq LET_d \leq 15\,keV\,\mu m^{-1}$	1.72 (1.54, 1.89)	1.74 (1.53, 1.95)	1.60 (1.36, 1.84)	1.53 (1.34, 1.72)

Key Questions

- How does cell response vary across a pristine Bragg peak?
- Clinical beams are delivered using a series of overlapping prisitine Bragg curves does this matter?
- How does the biological effectivenesss of a pristine peak relate to a Spread Out Bragg Curve for DNA damage and survival?
- What other biological parameters play a role?

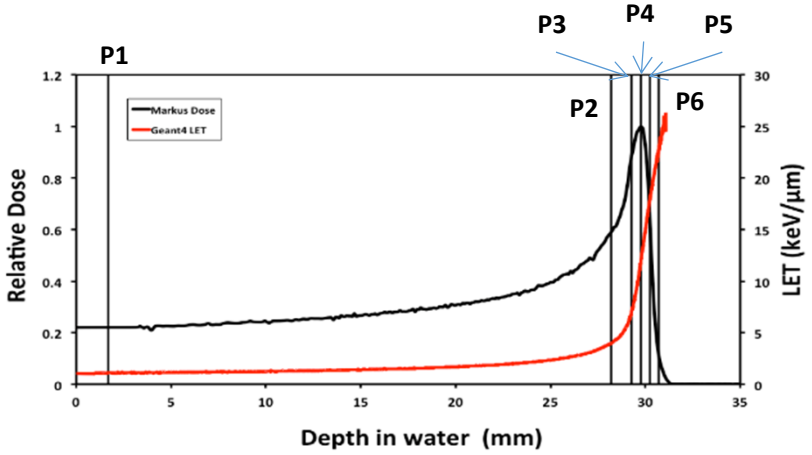
Example of an experimental study: INFN Catania



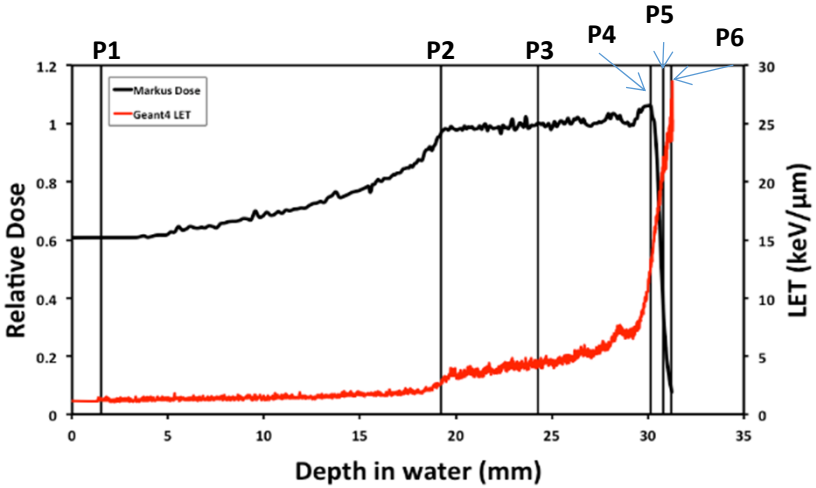


Catana Proton Therapy Facility

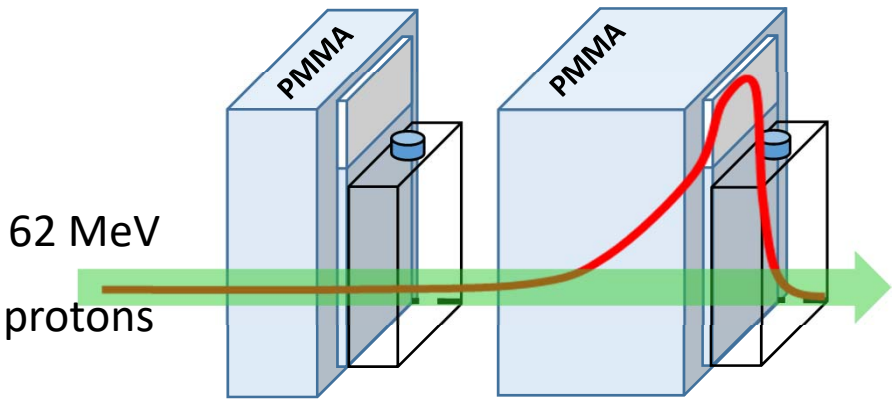
Irradiation Setup – INFN Catania

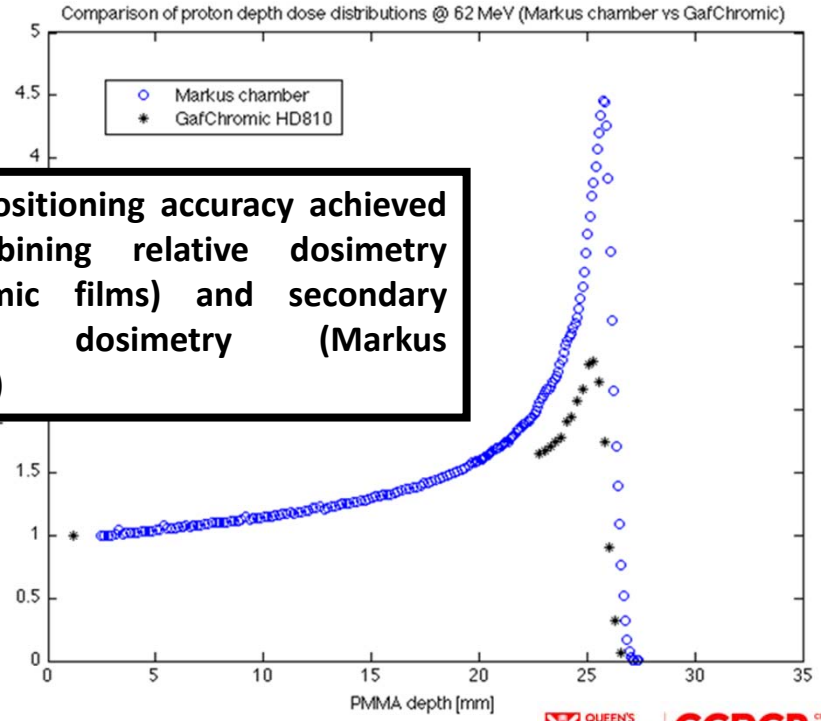


	P1	P2	P3	P4	P5	P6
Depth water [mm]	1.38	20.23	24.59	27.69	29.48	30.08
LET [keV/µm]	1.2	2.6	4.5	13.4	21.7	25.9



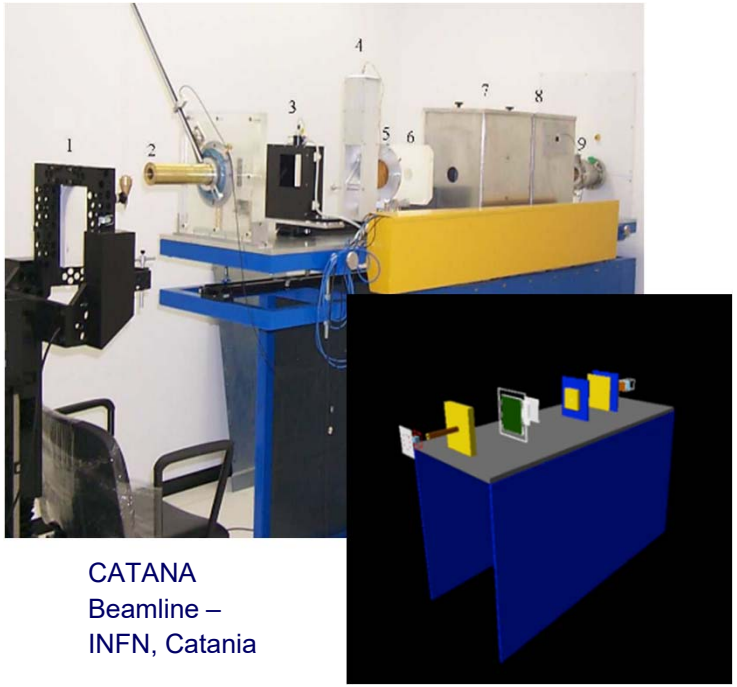
	P1	P2	P3	P4	P5	P6
Depth water [mm]	1.38	27.42	29.21	29.8	30.7	31.29
LET [keV/µm]	1.11	4.0	7.0	11.9	18.0	22.6





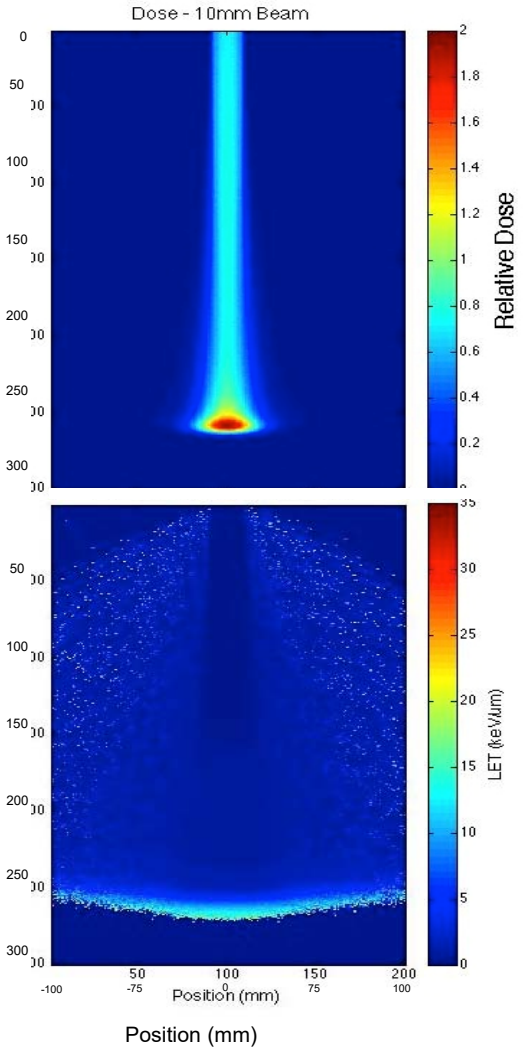
50 µm positioning accuracy achieved by combining relative dosimetry (Gafchromic films) and secondary standard dosimetry (Markus Chamber)

Geant4 Simulation



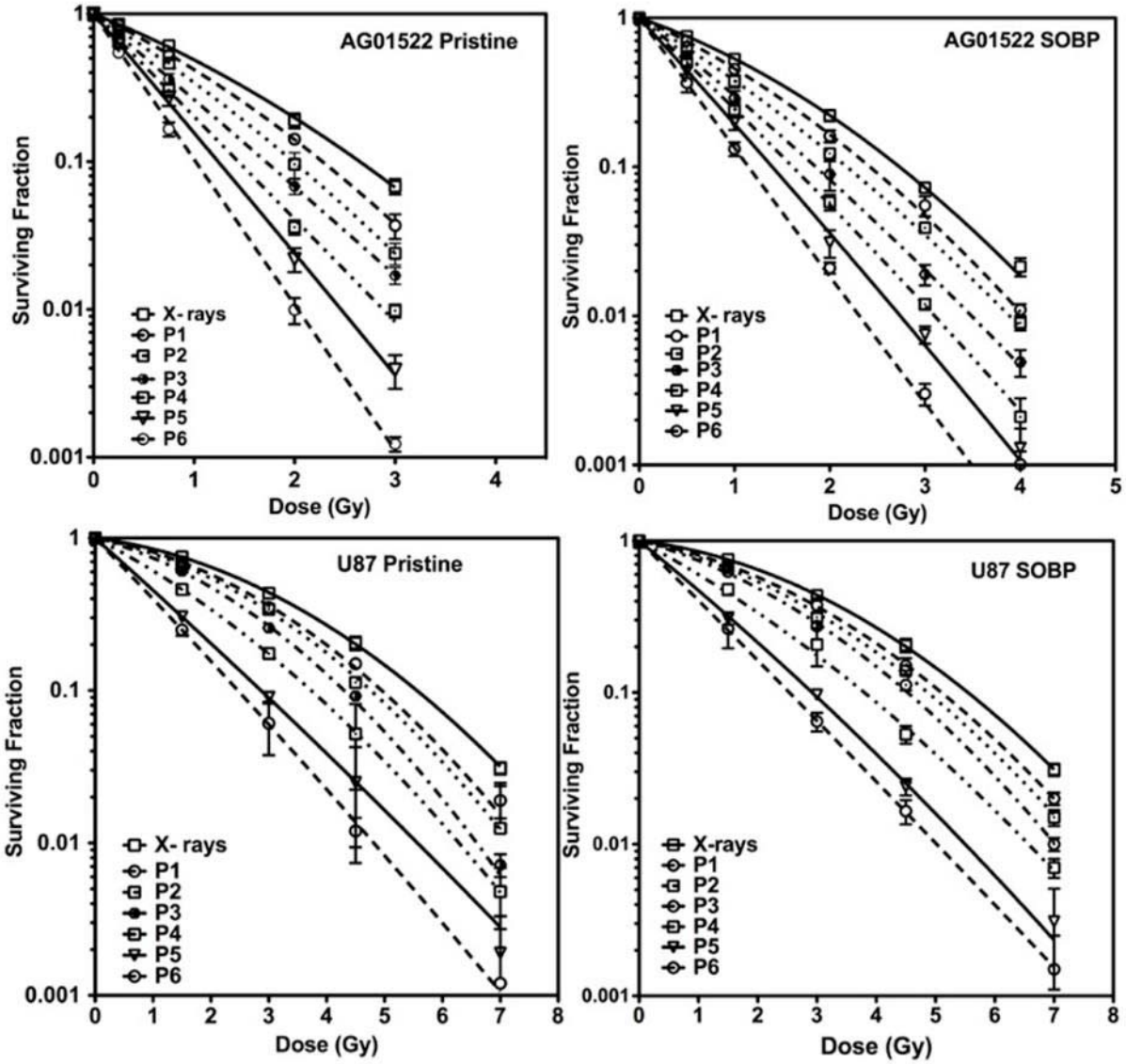
CATANA Beamline – INFN, Catania

- Not all quantities measurable experimentally ***e.g. LET***.
- The *Geant4* simulation toolkit allows us to model the experimental beam line to predict particle behaviour using the probability sampling *Monte Carlo* method.

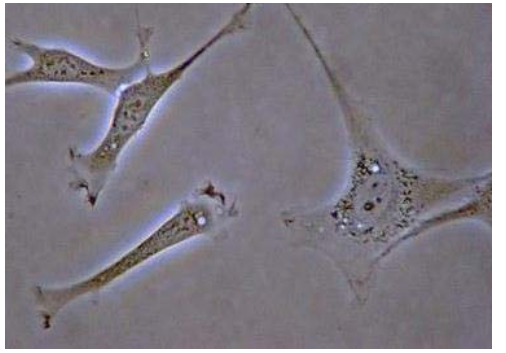


Top: Geant4 Depth - Dose distribution.
Bottom: Geant4 Depth - LET distribution.

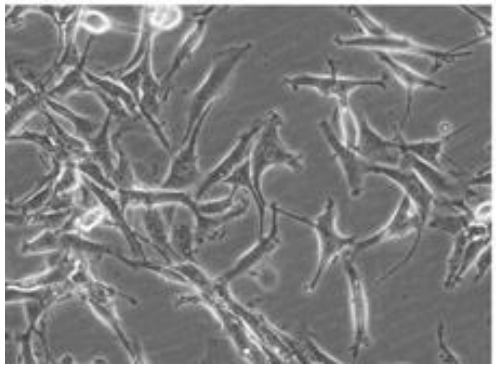
Survival data



AG01522 normal human fibroblast cell line



U87- human primary glioblastoma cell line with epithelial morphology, obtained from a stage four cancer patient



Chaudhary *et al.,*(2014) *Int J. Radiation Oncol Biol Phys,* **90**:27-35

Curve fitting and RBE Calculations

Linear quadratic equation

$$SF = e^{-\left(\alpha D+\beta D^2\right)}$$

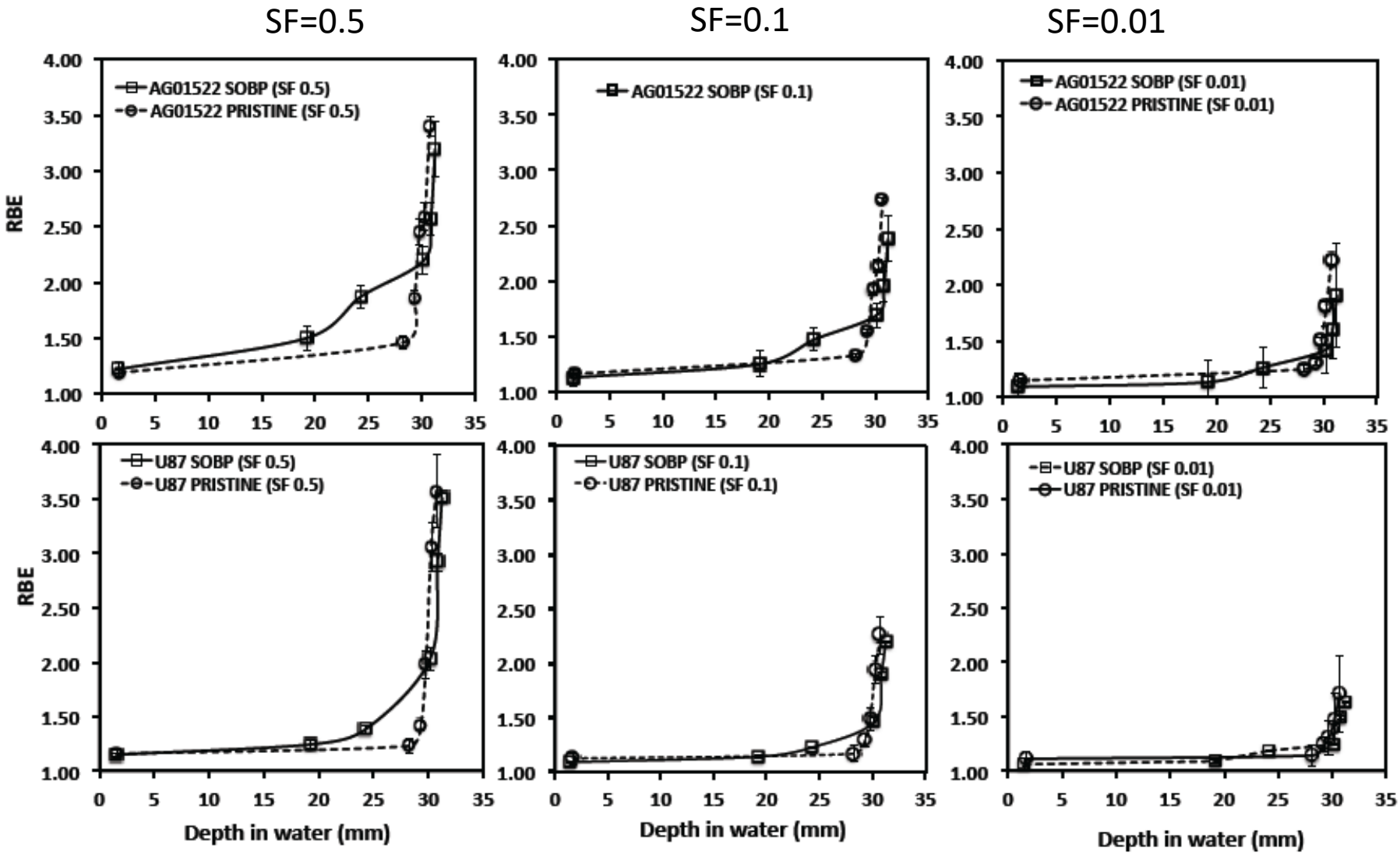
$$RBE = D_{X\text{-}ray} / D_{Proton} \text{ @ } isoeffect$$

$$RBE = \left(\left(\alpha_{x^2} + 4\beta_x D_p \left(\alpha_p + \beta_p D_p\right)\right)\text{^}(1/2) - \alpha_x \right) \Big/ \left(2\beta_x D_p\right)$$

Where α_x, β_x, α_p and β_p are the α and β parameter from the X-ray and proton exposure and D_p is the proton dose delivered

X-rays	α / Gy^{-1}	β / Gy^{-2}	α/β
AGO1522B	0.54 ± 0.06	0.062 ± 0.02	8.71
U87	0.11 ± 0.03	0.060 ± 0.01	1.83

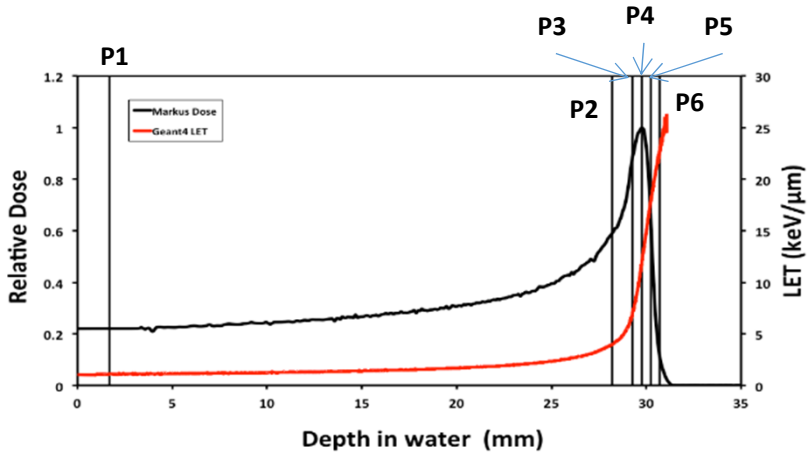
RBE versus Depth

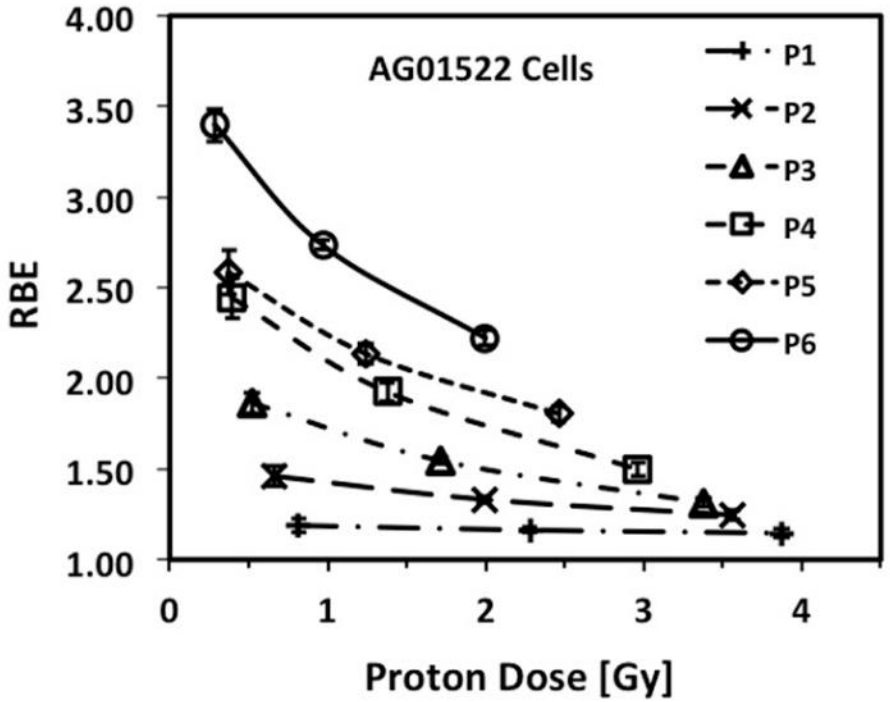


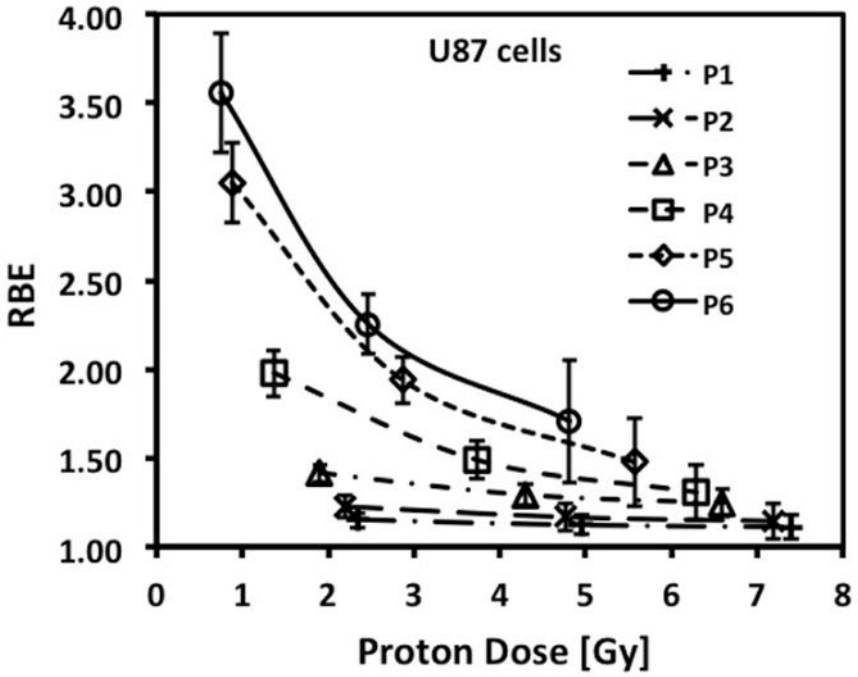
Chaudhary *et al.,*(2014) *Int J. Radiation Oncol Biol Phys,* **90**:27-35

RBE versus Dose

Monoenergetic beam

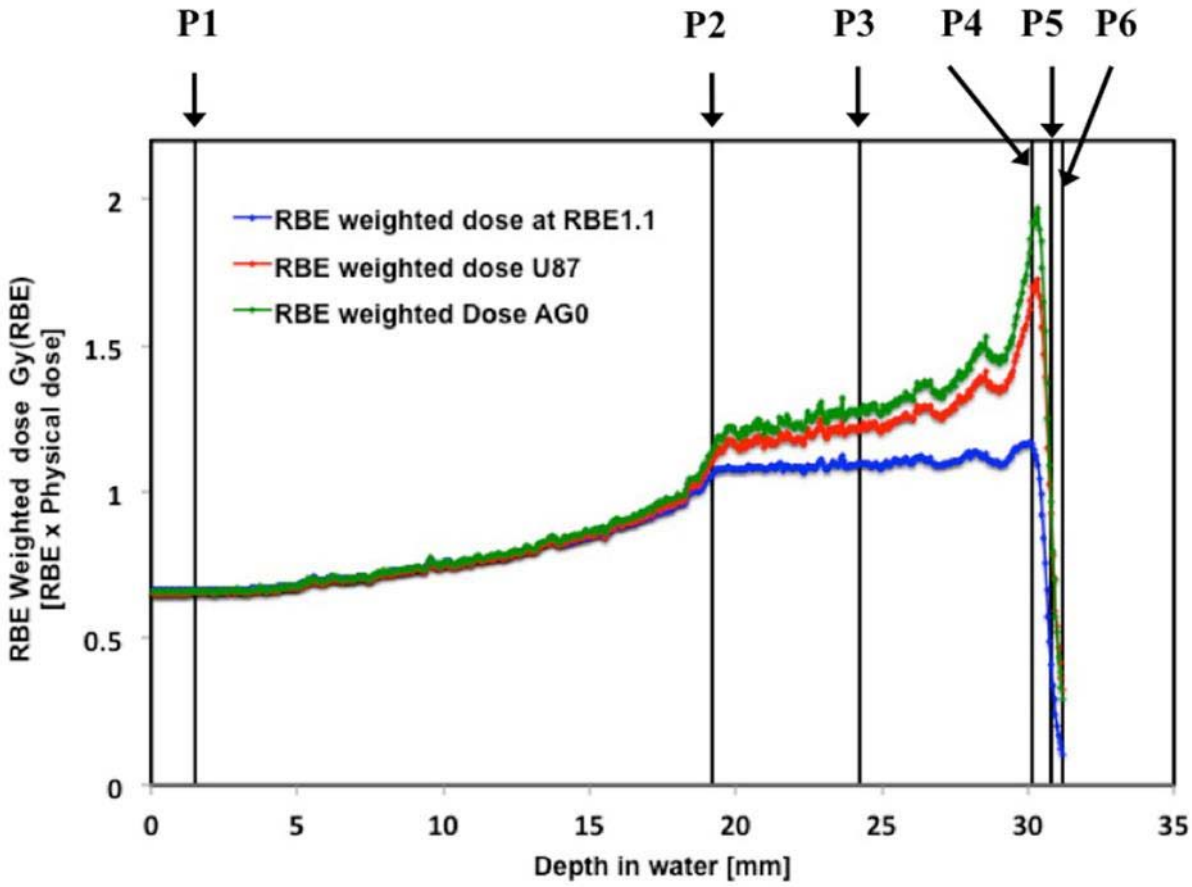






Chaudhary *et al.,*(2014) *Int J. Radiation Oncol Biol Phys,* **90**:27-35

Biological Effective Dose Profile



- A parameterised RBE model has been used
- In tumour region (SOBP) 17% and 18% increase in biological dose for AGO and U87 cells
- Extension of distal region by 130 and 150 μm respectively
- Physical dose or RBE 1.1 does not replicate the biological response

Chaudhary *et al.,*(2014) *Int J. Radiation Oncol Biol Phys,* **90**:27-35

QUEEN'S UNIVERSITY BELFAST | CCRCB CENTRE FOR CANCER RESEARCH AND CELL BIOLOGY

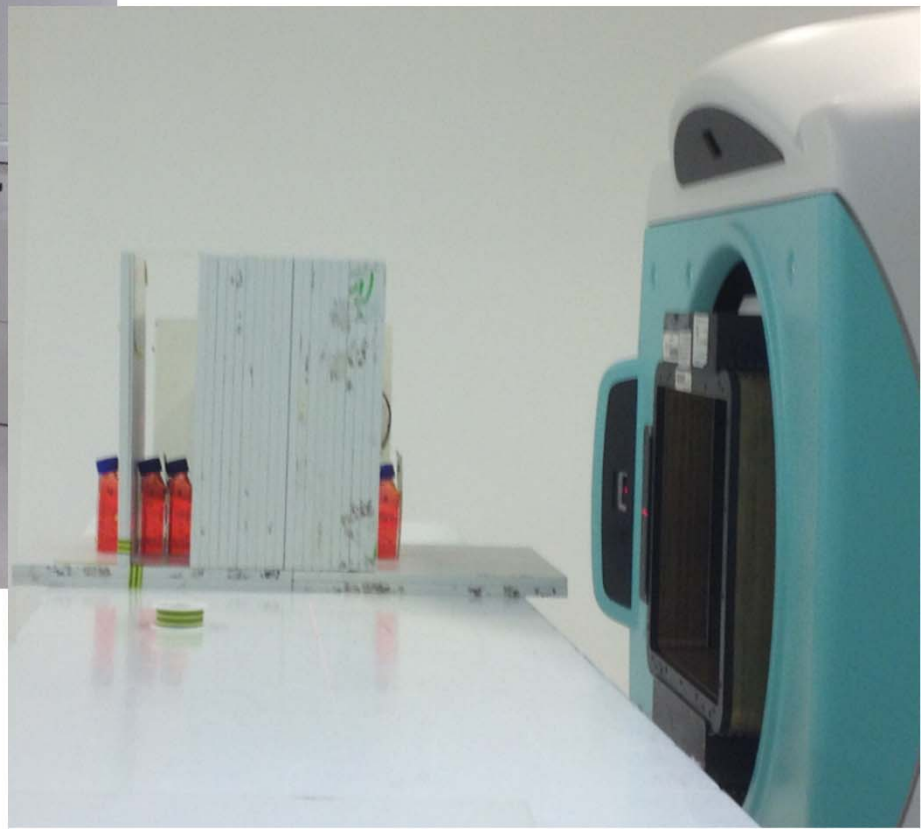
Proton Therapy Center, Prague

PROTON THERAPY CENTER

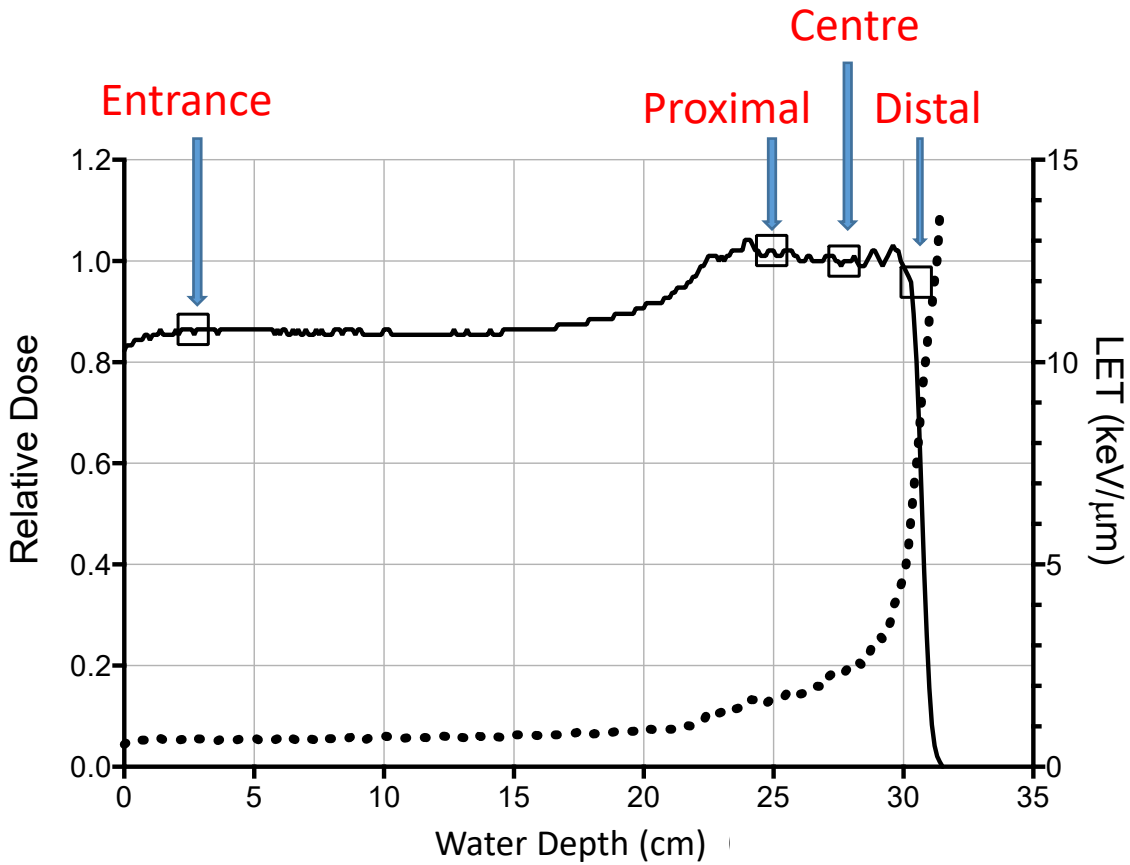
Marie Davidkova, Anna Michaelidesova, Vladimir Vondráček

QUEEN'S UNIVERSITY BELFAST | CCRCB CENTRE FOR CANCER RESEARCH AND CELL BIOLOGY

Treatment room



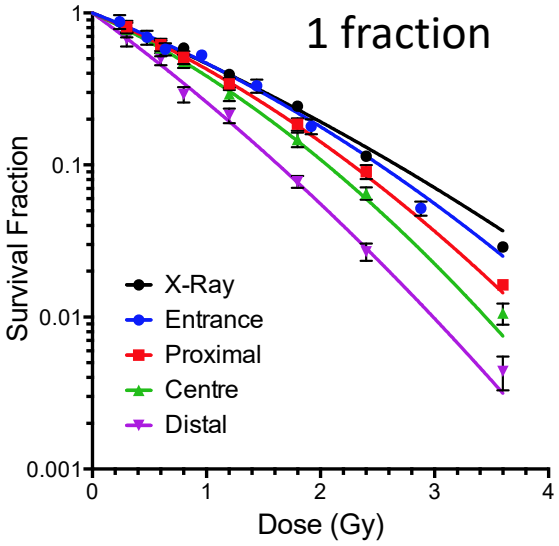
Prague Proton - uniform exposures

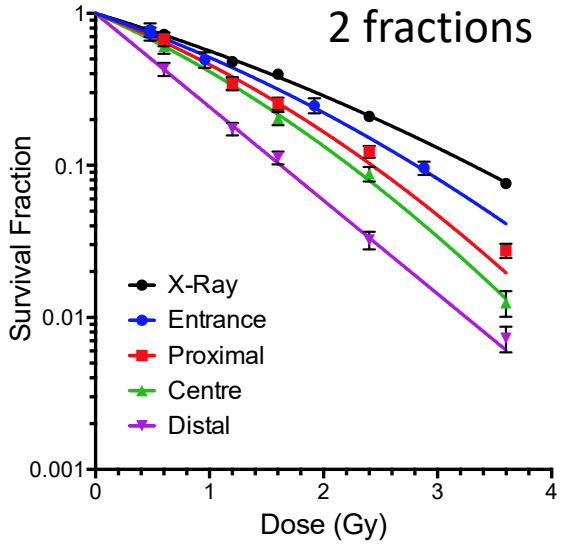


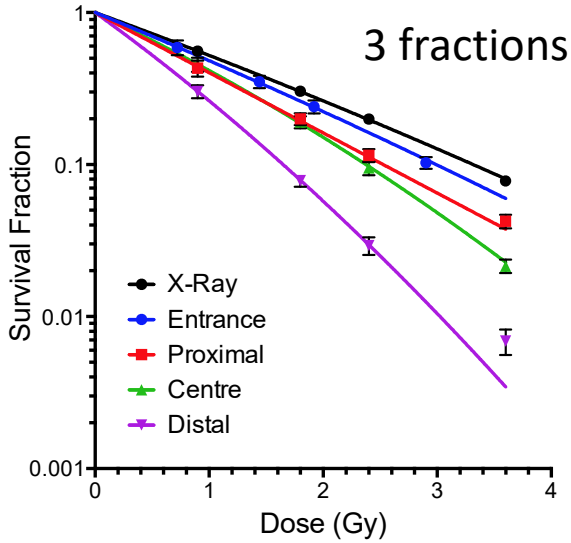
Dose and LET profiles for actively scanned modulated proton beam with maximum energy 219.65 MeV. Vertical lines mark the four cell irradiation positions at the Entrance, Proximal, Centre and Distal positions. Relative dose and GEANT4 derived dose averaged LET values are indicated in dashed and solid black lines respectively.

Fractionated protons exposures – total dose

- AGO1522 fibroblasts irradiated with X-rays or protons at entrance, proximal, centre or distal positions with either 1, 2 or 3 fractions, 24 hours apart



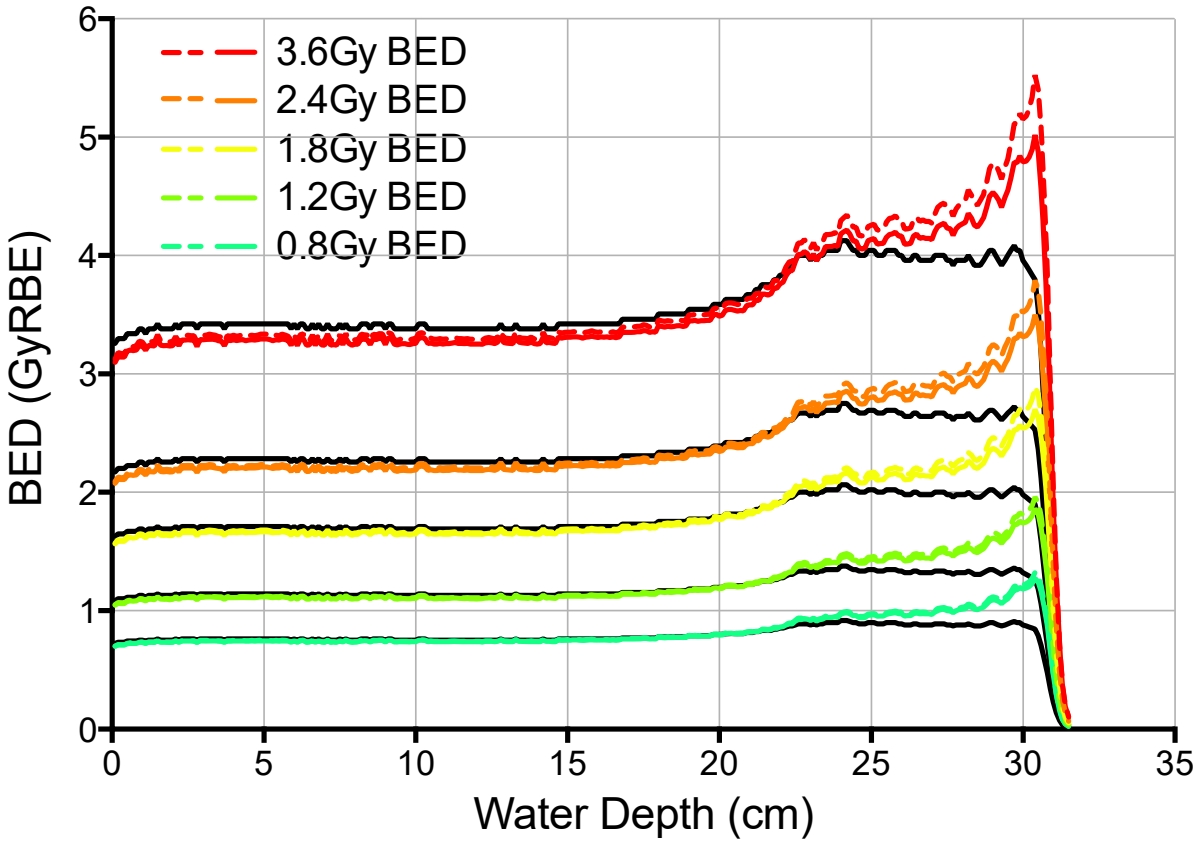




Marshall *et al.,*(2016) *Int J. Radiation Oncol Biol Phys,* **95,** 70-7.

SOBP – Biologically effective dose

- SOBP Biologically Effective Dose (BED) profile comparing analytically obtained BED values (RBE x Physical Dose (Gy)) when delivering a plateau dose of 3.6, 2.4, 1.8 and 0.8 Gy in both acute (solid colour) and fractionated (dashed colour) regimes.
- Fractionation can be seen to further increase this effect in the plateau region, seeing increases of 8.3 – 12.1 % in integral BED over the clinical case in comparison to 4.6 – 10.6 % for the acute delivery of the same doses.



Marshall *et al.,*(2016) *Int J. Radiation Oncol Biol Phys,* **95,** 70-7.

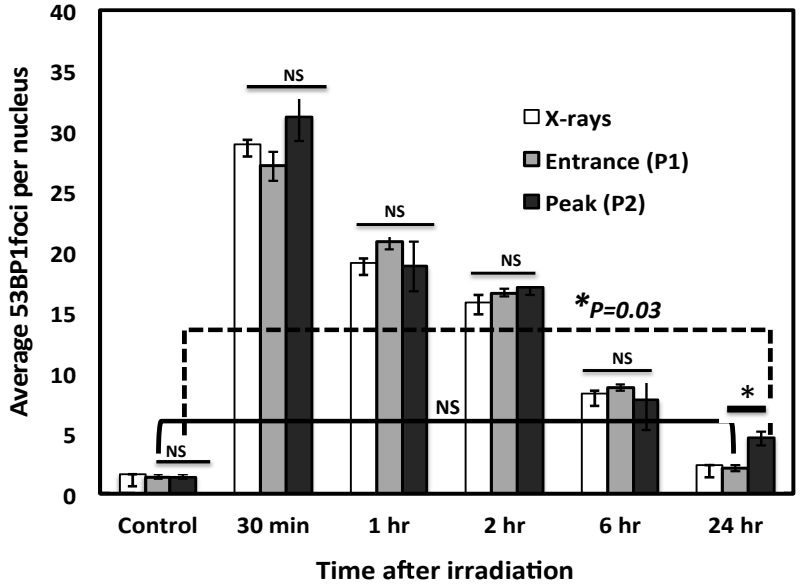
Do DNA damage and repair rates change predictably in clinically relevant ion-beam dose distributions?

- What is the relationship between DNA damage/repair and lethality along a SOBP?
- What are the implications of non-targeted effects for particle radiotherapy where high RBE and steep dose patterns are expected?

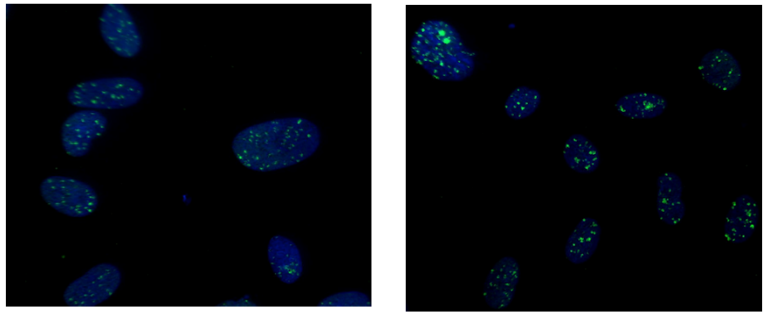
Proton – DNA damage and repair

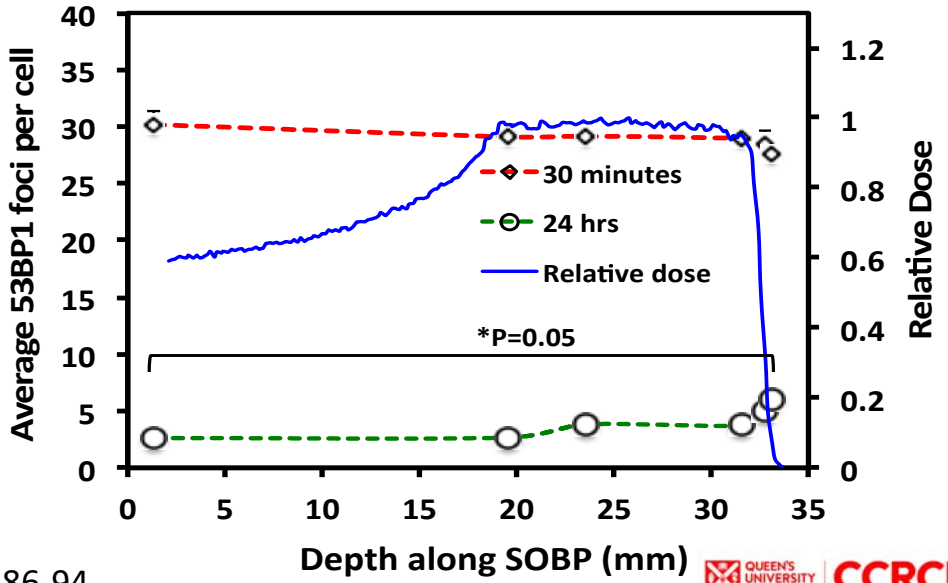
- Pristine versus SOBP 53BP1 1Gy X-rays or 60 MeV protons
- Increased residual damage at pristine peak
- Gradual increase in residual damage along the SOBP

(a)



(b)

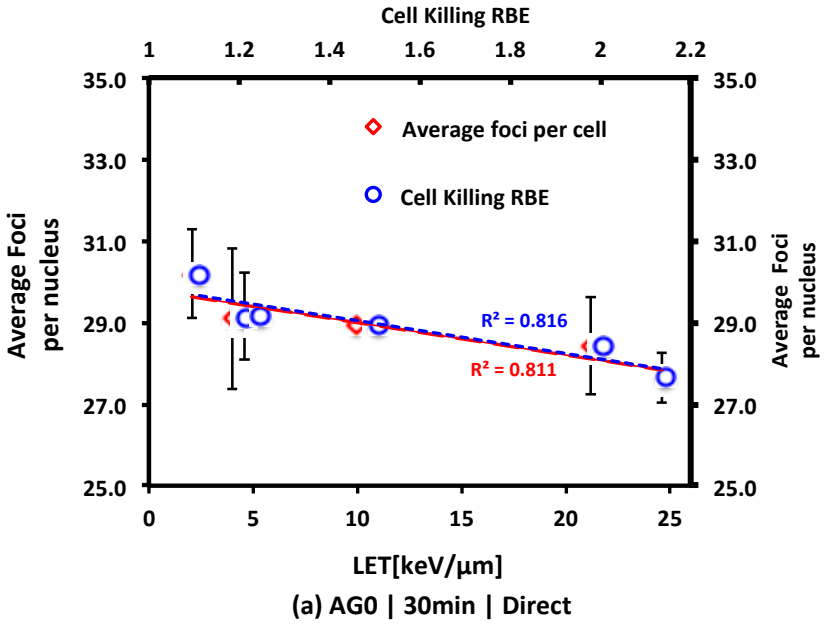


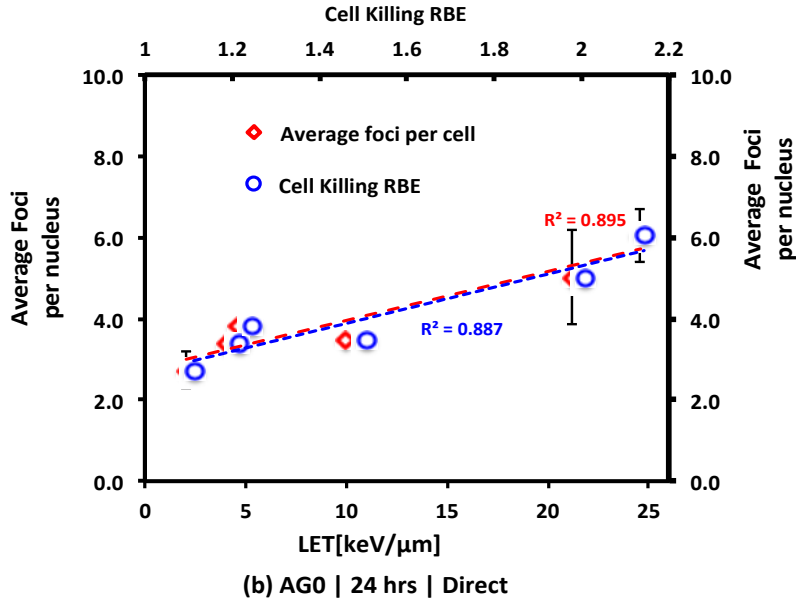


Chaudhary *et al.*,(2016) *Int J. Radiation Oncol Biol Phys*, **95,** 86-94

Cell killing and DNA damage

- Comparing foci per nucleus with survival RBE data shows an inverse correlation with initial damage
- Good correlation between residual foci and LET/RBE

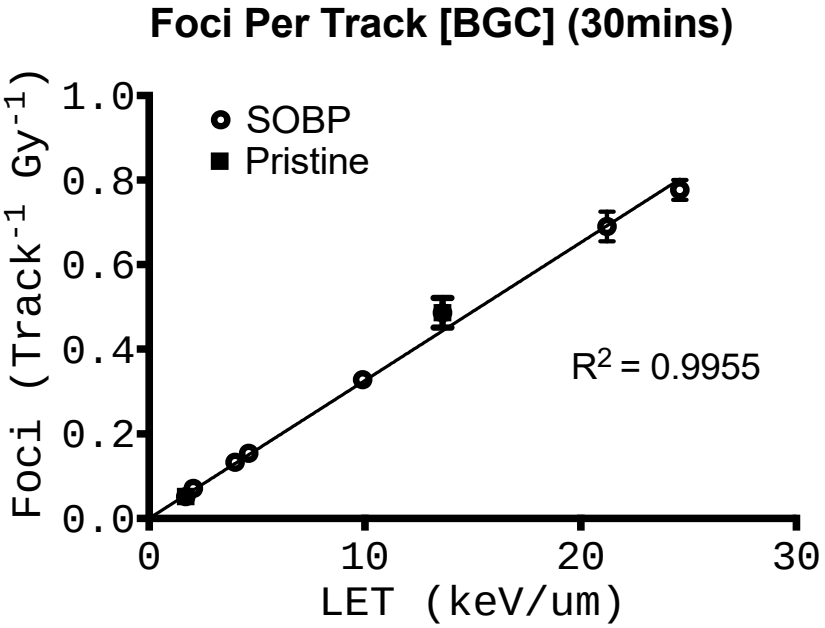


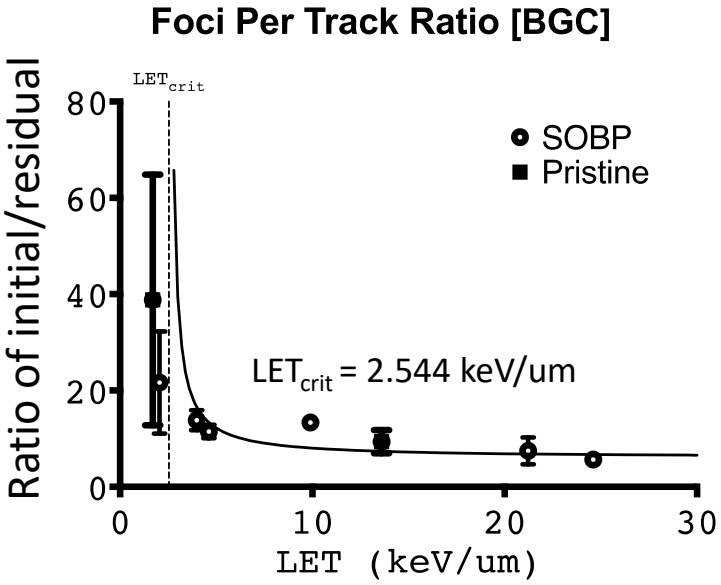


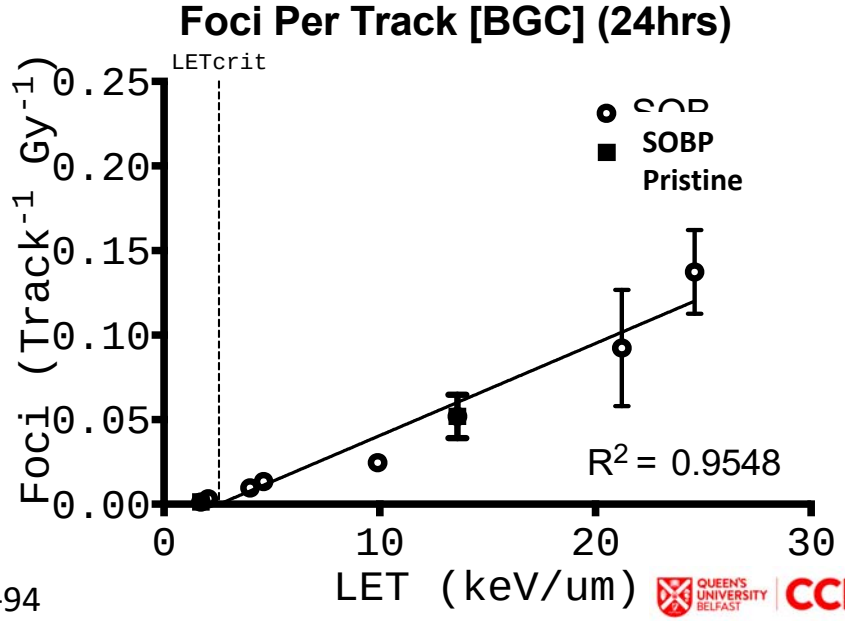
Chaudhary *et al.,*(2016) *Int J. Radiation Oncol Biol Phys,* **95,** 86-94

Fluence – DNA damage per track

- Direct proportionality between foci per track and LET
- 24 hour data predict a minimal LET for producing residual foci of 2.5 keV/μm







Chaudhary *et al.,*(2016) *Int J. Radiation Oncol Biol Phys,* **95,** 86-94

DNA damage versus LET for other ions

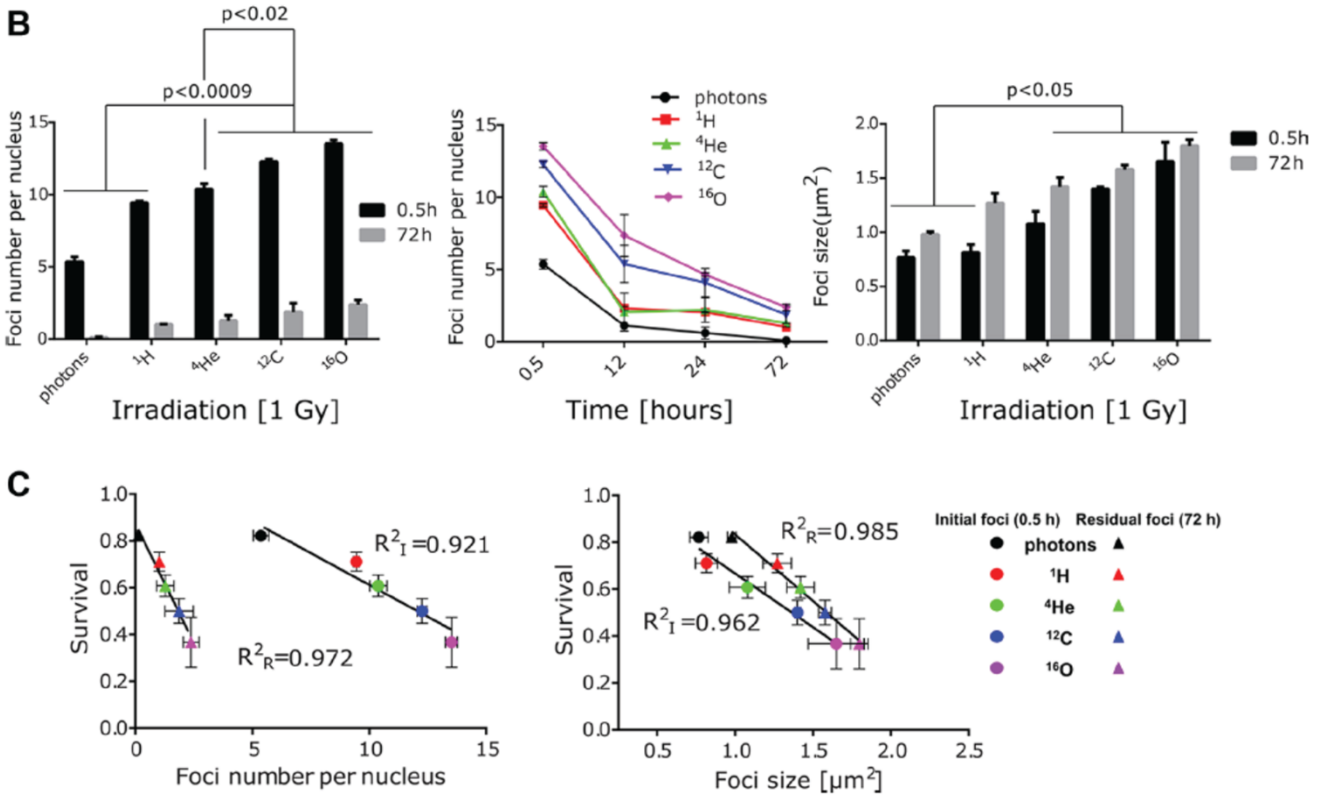
- For protons, helium, carbon and oxygen ions
- Increased yield of residual foci and foci size with LET

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 7, No. 35

Research Paper

Next generation multi-scale biophysical characterization of high precision cancer particle radiotherapy using clinical proton, helium-, carbon- and oxygen ion beams

Ivana Dokic[1,2,3,4,*], Andrea Mairani[3,5,*], Martin Niklas[1,2,3,4], Ferdinand Zimmermann[1,2,3,4], Naved Chaudhri[3], Damir Krunic[6], Thomas Tessonnier[4,7], Alfredo Ferrari[8], Katia Parodi[3,7], Oliver Jäkel[3,9], Jürgen Debus[1,2,3,4], Thomas Haberer[3], Amir Abdollahi[1,2,3,4]



Protons and DNA repair pathway

- A differential DNA damage response to protons versus photons
- Enhanced susceptibility of HR-deficient tumour cells to proton-irradiation
- increased sensitivity of photon-irradiated tumour cells to NHEJ inhibitors

Radiotherapy and Oncology 116 (2015) 374–380

Contents lists available at ScienceDirect

Radiotherapy and Oncology

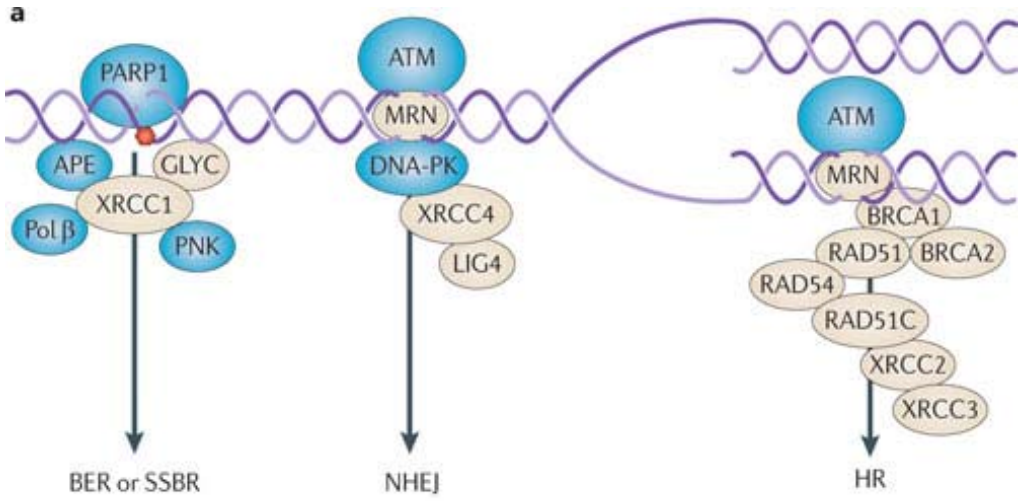
journal homepage: www.thegreenjournal.com

Molecular radiobiology

Differential DNA repair pathway choice in cancer cells after proton- and photon-irradiation

Andrea O. Fontana [a], Marc A. Augsburger [a], Nicole Grosse [a], Matthias Guckenberger [a], Anthony J. Lomax [c], Alessandro A. Sartori [b], Martin N. Pruschy [a,*]

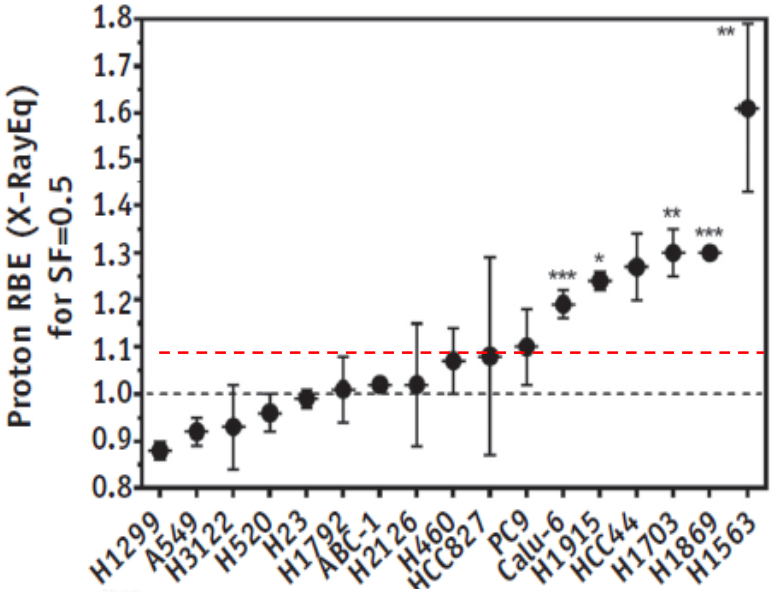
[a] Department of Radiation Oncology, University Hospital Zurich; [b] Institute of Molecular Cancer Research, University of Zurich; and [c] Paul Scherrer Institute, Villigen, Switzerland

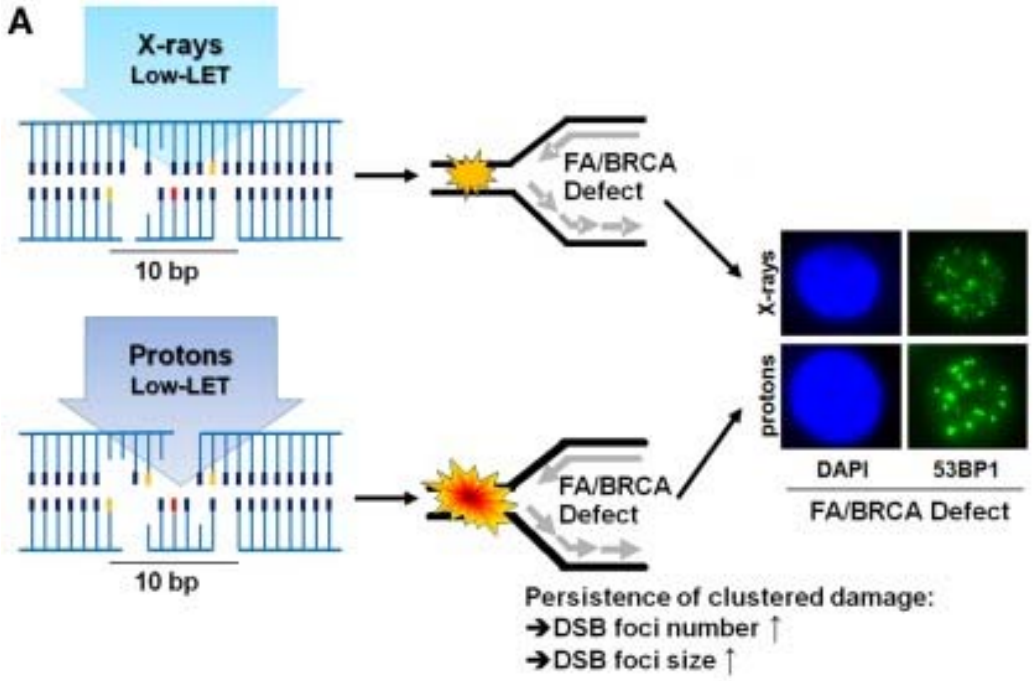


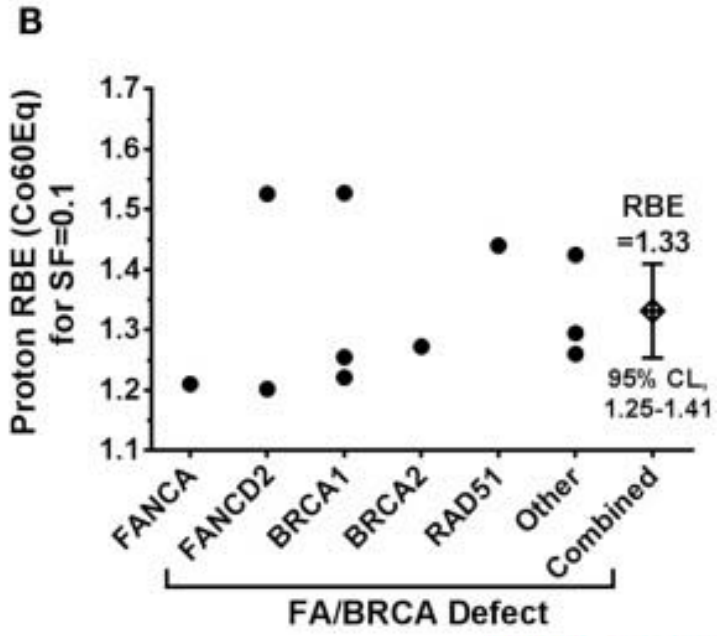
RBE for different lung tumour cells

- Variations in proton RBE in 17 human lung cell lines (1.31 – 1.77 in a subset)
- Correlated with defects in the Fanconi anemia/BRCA pathway of DNA repair

Liu *et al.,* 2015, IJROBP, 91, 1081; Held *et al.,* 2016, Front Oncol., 6, 23







In vivo studies

- *In vivo* data limited to intestinal crypt assay
- Normal tissue end points required
  - Spinal cord, parotid gland, lung etc
- Several Groups with *in vivo* studies underway
- Bespoke preclinical systems (SARRP etc)
- Clinical systems adapted for pre-clinical use

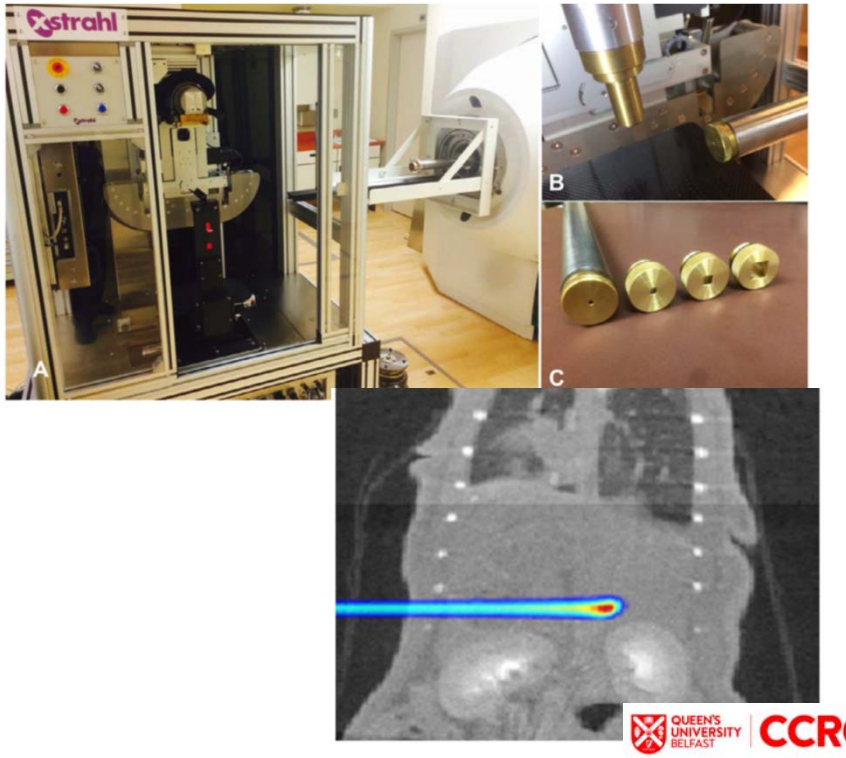
IOP Publishing | Institute of Physics and Engineering in Medicine Physics in Medicine & Biology

Phys. Med. Biol. **62** (2017) 43–58 doi:10.1088/1361-6560/62/1/43

An image-guided precision proton radiation platform for preclinical *in vivo* research

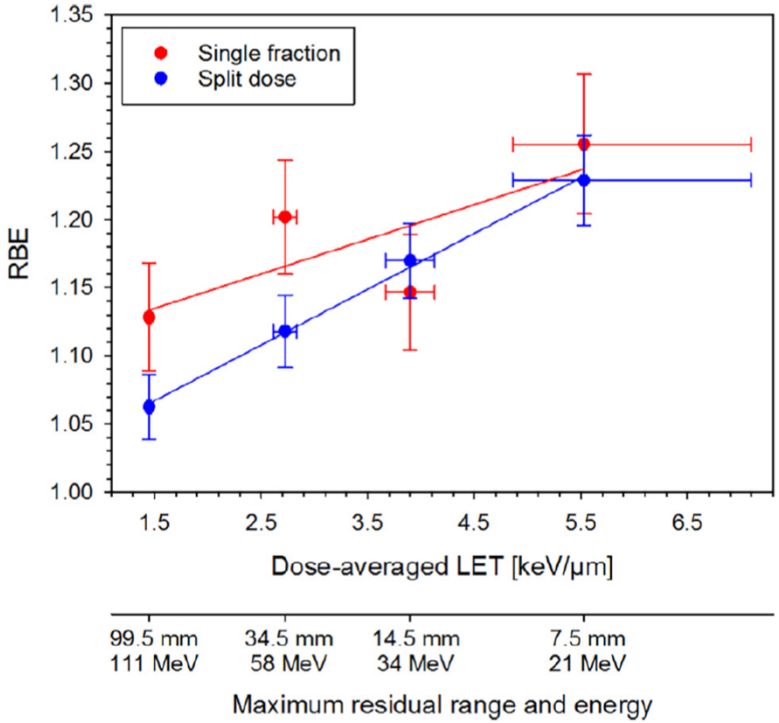
E Ford[1], R Emery, D Huff, M Narayanan, J Schwartz, N Cao, J Meyer, R Rengan, J Zeng, G Sandison, G Laramore and N Mayr

Department of Radiation Oncology, University of Washington Seattle, WA, USA



In vivo proton studies

- Rat spinal cord irradiated with single or two equal fractions at four positions (LET 1.4–5.5 keV/µm) along spread-out Bragg peak (SOBP).
- RBE-values for myelopathy increased from 1.13 ± 0.04 to 1.26 ± 0.05 (1F) and from 1.06 ± 0.02 to 1.23 ± 0.03 (2F).



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Radiotherapy and Oncology xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Radiotherapy and Oncology

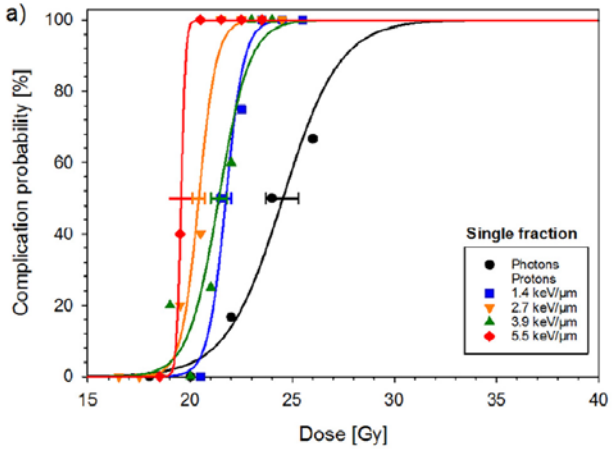
journal homepage: www.thegreenjournal.com

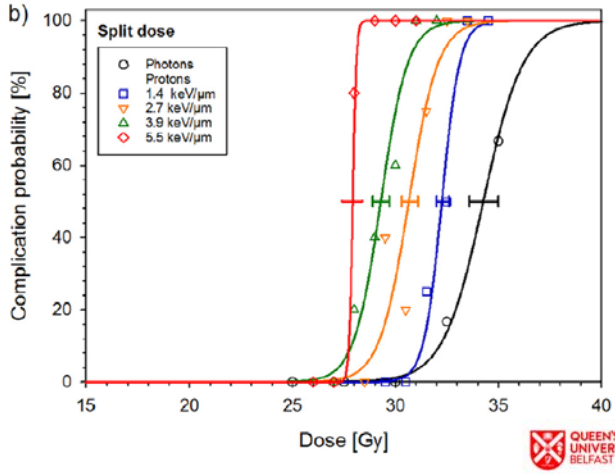
Original article

Determination of the proton RBE in the rat spinal cord: Is there an increase towards the end of the spread-out Bragg peak?

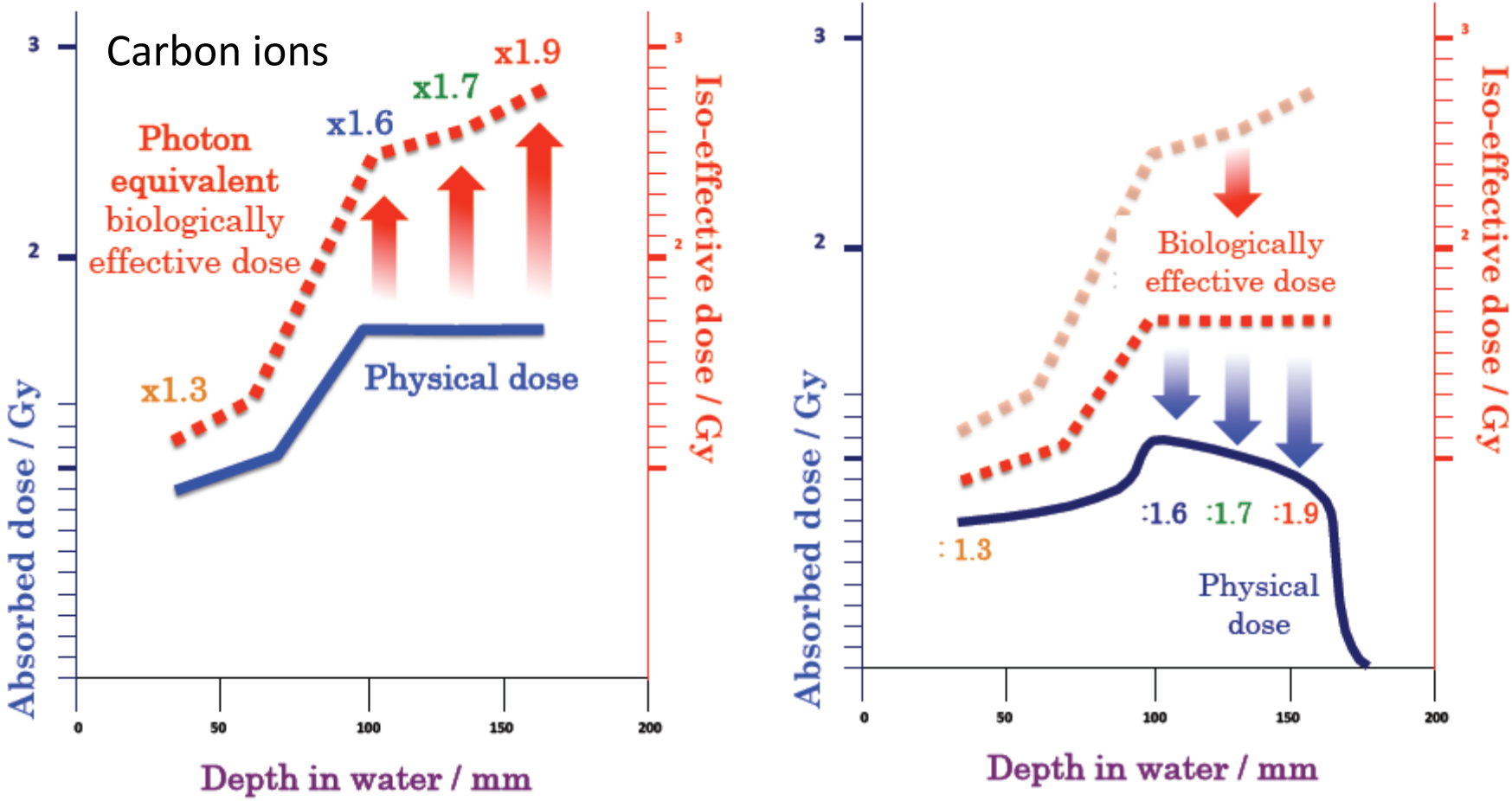
Maria Saager[a,d,*], Peter Peschke[a,d], Stephan Brons[b,d], Jürgen Debus[c,d], Christian P. Karger[a,d]

[a] Dept. of Medical Physics in Radiation Oncology, German Cancer Research Center (DKFZ); [b] Heidelberg Ion Beam Therapy Center (HIT); [c] Dept. of Radiation Oncology, University Hospital of Heidelberg; and [d] National Center for Radiation Research in Oncology (NCRO), Heidelberg Institute for Radiation Oncology (HIRO), Heidelberg, Germany





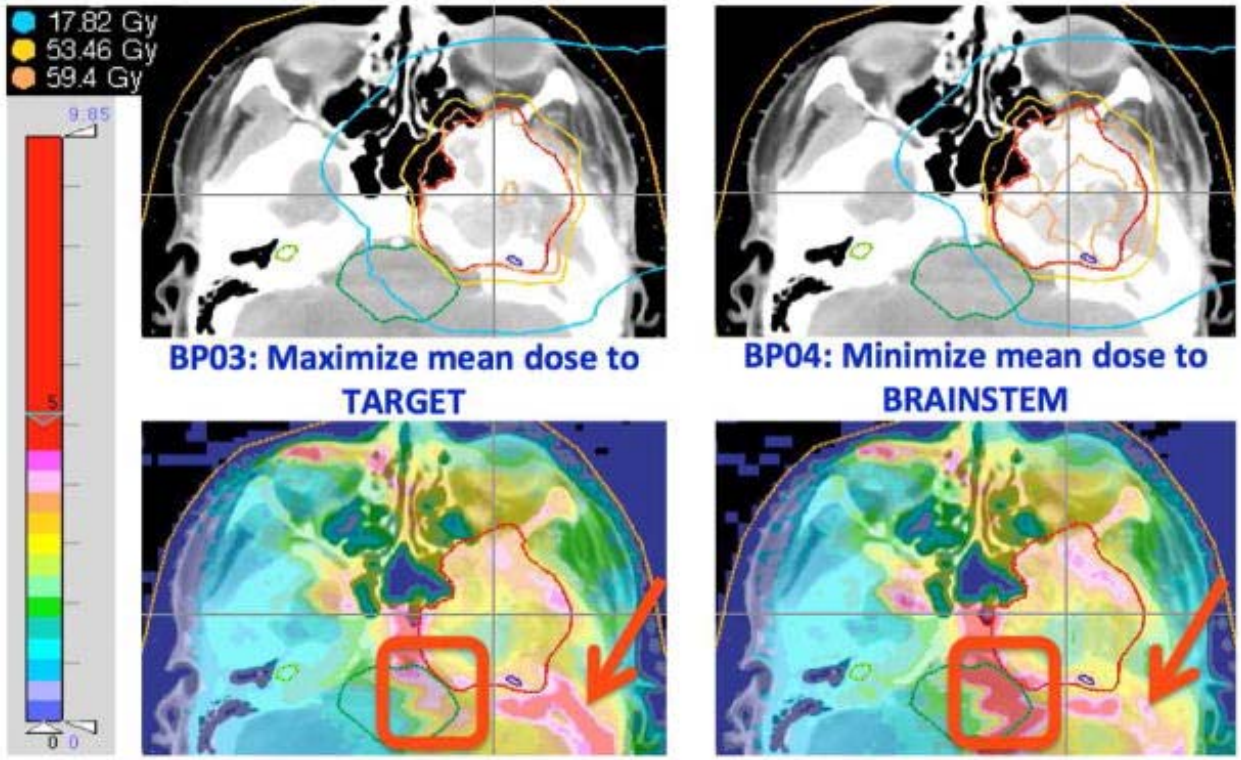
RBE – consequences for treatment planning



Gueulette *et al* 2010

- **A homogeneous biologically effective dose requires an inhomogeneous physical dose distribution – even for protons**
- **Biological factors maybe important for individualising therapy?**

Optimized RBE?



- Optimising to dose alone can lead to LET hotspots

Giantsoudi *et al.,* 2013 IJROBP, **87,** 216

Gaps in Knowledge (Pre-clinical)

- Limited models used for cell studies
  - RBE influenced by DNA repair
  - Oxygenation
  - High dose per fraction biology (immune responses?)
  - Other biology?
- Limited *in vivo* studies
  - Late tissue effects (e.g. spinal cord, parotid, lung)
  - Defined genetic models
- Definition of suitable parameters for treatment planning
  - Dose, LET, Dose*LET, CWD….

Summary

- The **RBE** of charged particles depends on a **range of parameters** including:
  - Cell type, dose, LET, fractionation and radiosensitivity

- For clinical beams the fixed RBE of 1.1 for protons **underestimates the dose delivered to the tumour volume**

- RBE variation for ion beams is driven by lesion complexity and **is dependent on repair pathways available**

- There is a significant body of *in vitro* data underpinning our understanding but this needs further ***in vivo* data** to validate clinical relevance

- Can future treatment planning systems input **biological parameters** to personalise the delivery of radiotherapy?

The A-SAIL project

Queen's University Belfast
University of Strathclyde
Imperial College London
CLF RAL - STFC

EPSRC
Pioneering research and skills

PROGRAMME GRANT
(2013-2020)

A-SAIL
ADVANCED STRATEGIES FOR ACCELERATING IONS WITH LASERS

Investigators:

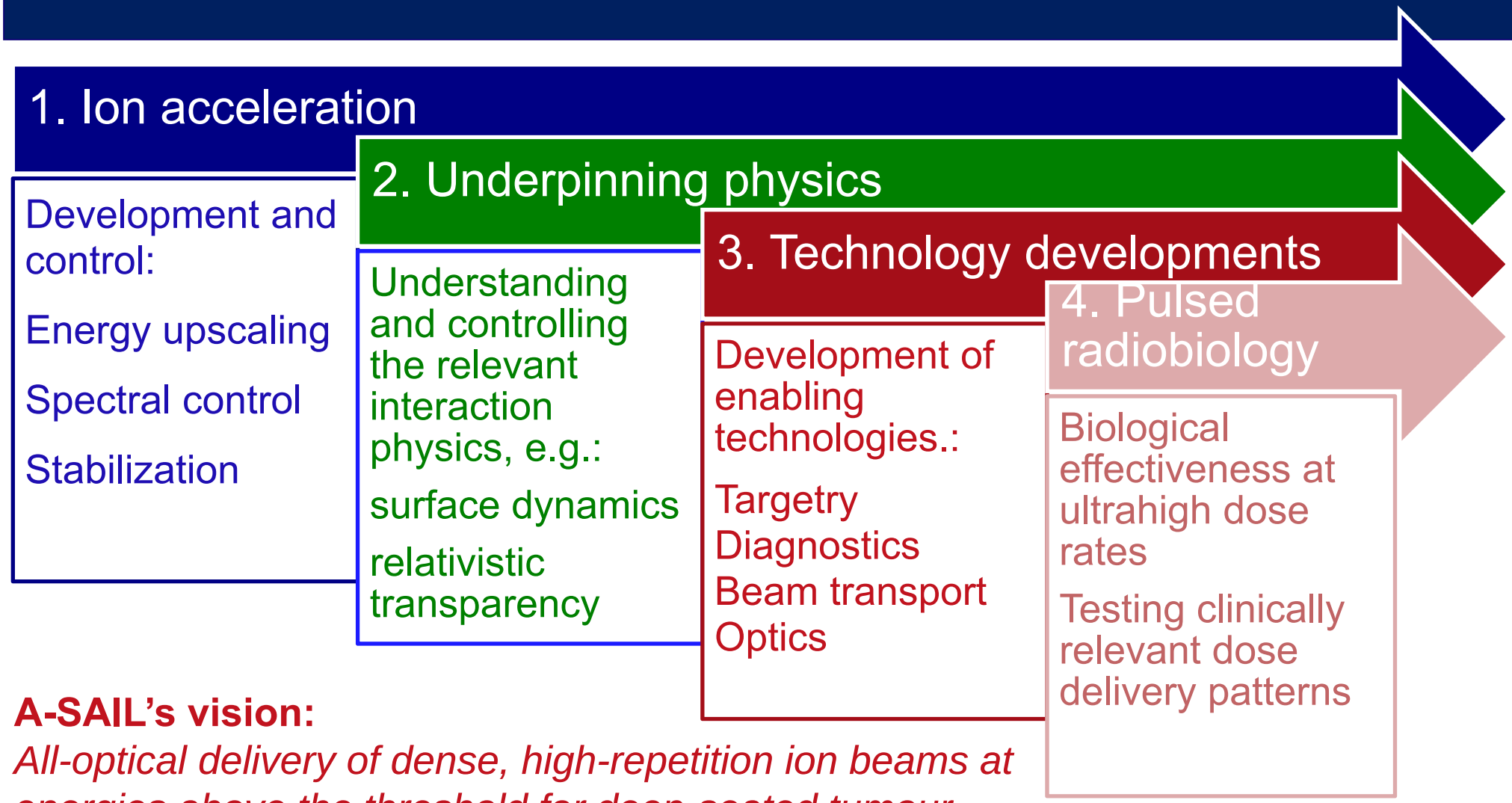
M.Borghesi, M. Zepf, K. Prise, S.Kar
The Queen's University of Belfast

P.McKenna, Strathclyde University

Z.Najmudin, Imperial College

D. Neely, Rutherford Appleton Lab

A-SAIL Vision & Structure



A-SAIL's vision:

All-optical delivery of dense, high-repetition ion beams at energies above the threshold for deep-seated tumour treatment and diagnosis (~200 MeV/nucleon).

Two classes of lasers are mainly used for this work

High energy CPA systems

- Nd: Glass technology
- 100s J energy, up to PW power
- Low repetition rate
- 100s fs duration
- I_{max}~ 10^{21} Wcm^2

VULCAN, RAL (UK)
Phelix, GSI (De)
Trident, LANL (US)
Texas PW, Austin (US)
…..

E_{max}~ 70 MeV

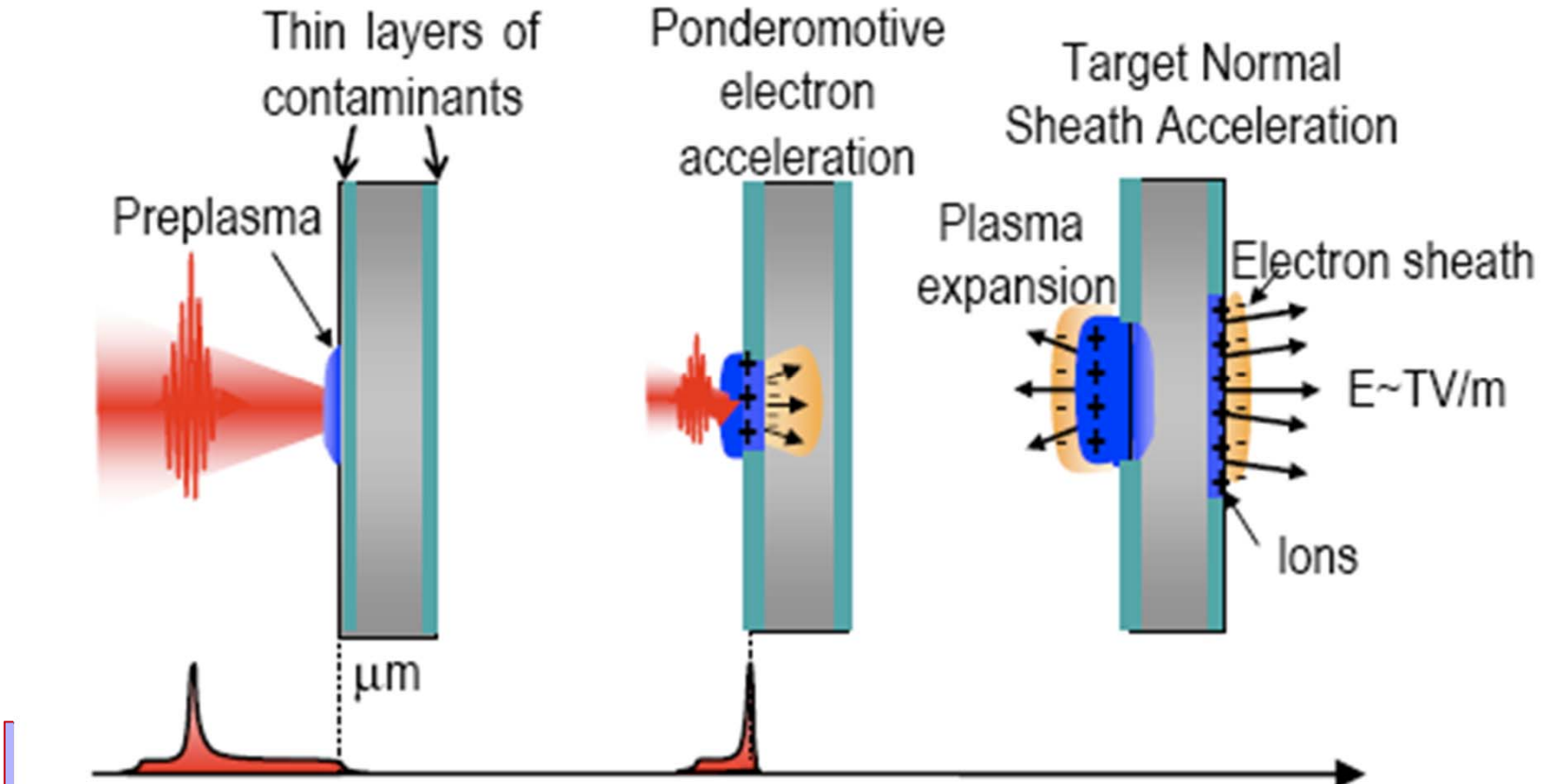
Ultrashort CPA systems

- Ti:Sa technology
- 10s J energy, up to PW power
- 1-10 Hz repetition
- 10s fs duration
- I_{max}~ 10^{21} Wcm^2

GEMINI, RAL (UK)
Draco, HZDR (De)
Pulser I, APRI (Kr)
J-Karen, JAEA (J)
…..

E_{max}~ 40 MeV

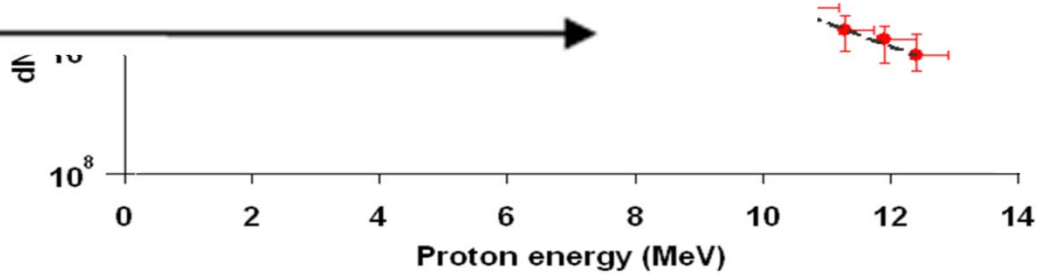
The established mechanism: Sheath Acceleration (TNSA)



E ~10 J on target in 10 µm spot

Intensity: ~10^{19} W/cm^2, duration : 500 fs

Target: Al foil 10um thickness



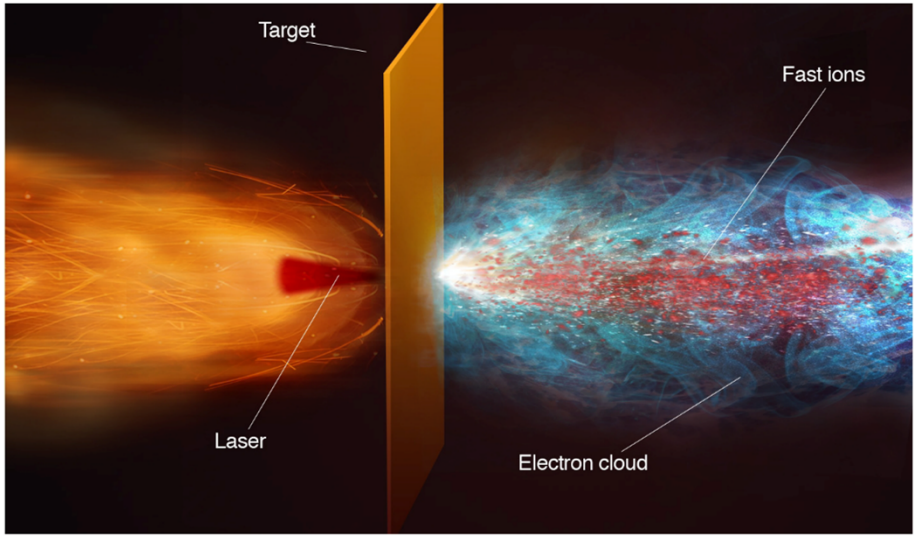
Laser-driven ion acceleration: in 2018

First reports of multi-MeV ion acceleration:

Clark *et al*, PRL, **84** ,670 (2000)

Maksimchuk *et al*, PRL, **84**, 4108 (2000)

Snavely *et al*, PRL, **85**,2945 (2000)



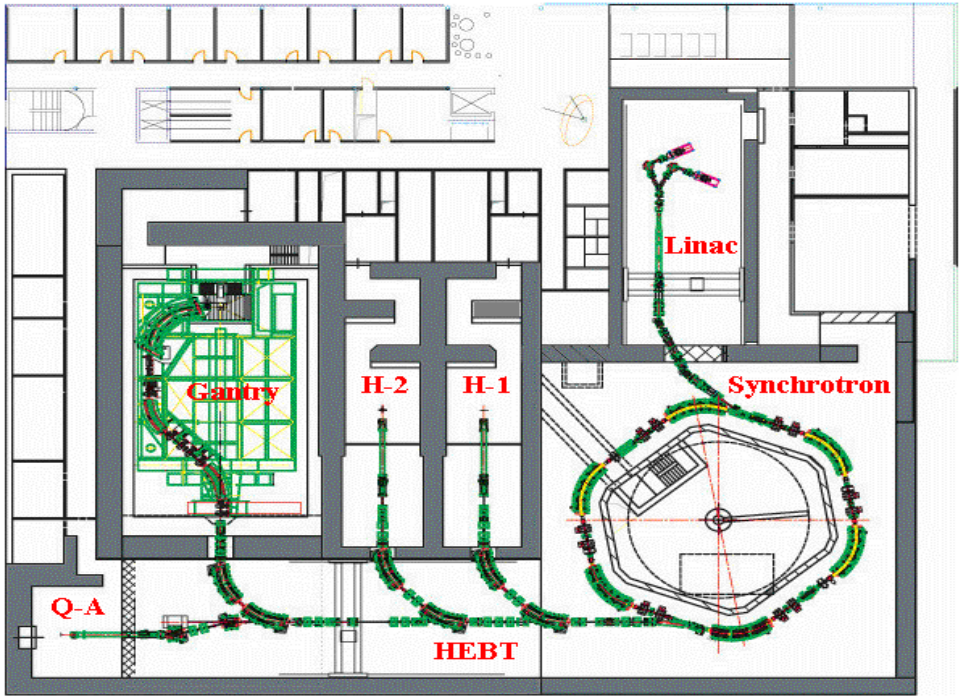
State of the art (2018):

- up to 100 MeV nucleon (protons-published)
- > 10^{13} protons, > 10^{11} C ions accelerated in single shots in whole beam
- very low emittance measured (< 0.1π mm mrad)
- proofs-of-principle of spectral manipulation and beam focusing

Higginson et al., Nature Communications, **9**, 724 (2018)

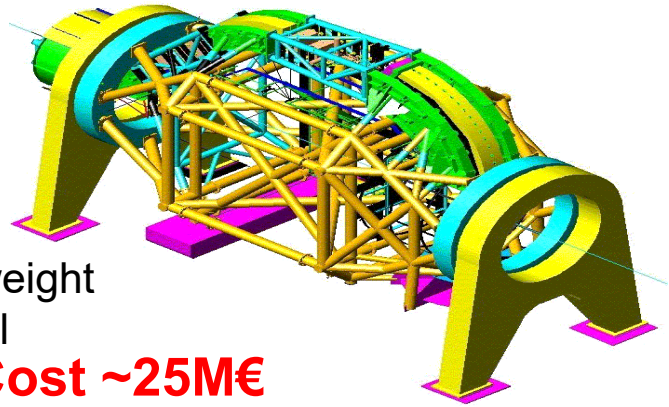
Ion Therapy costs

Heidelberg Ion Therapy Centre



3m thick walls and roof shielding

Ion gantry:
13m diameter
25m length
600ton overall weight
420ton rotational



Cost ~25M€

Accelerator
4m diameter
60 tons
500nA, 250MeV

Cost ~10-20M€

Demand for treatment much higher than offer -
scope for investigating alternative approaches for future therapy

...cility

...70%)

Is there scope for a laser-driven approach?

Reduced cost/shielding:

- Laser transport rather than ion transport (vast reduction in radiation shielding)
- Possibility to reduce size of gantry

***Vision* first proposed in** :

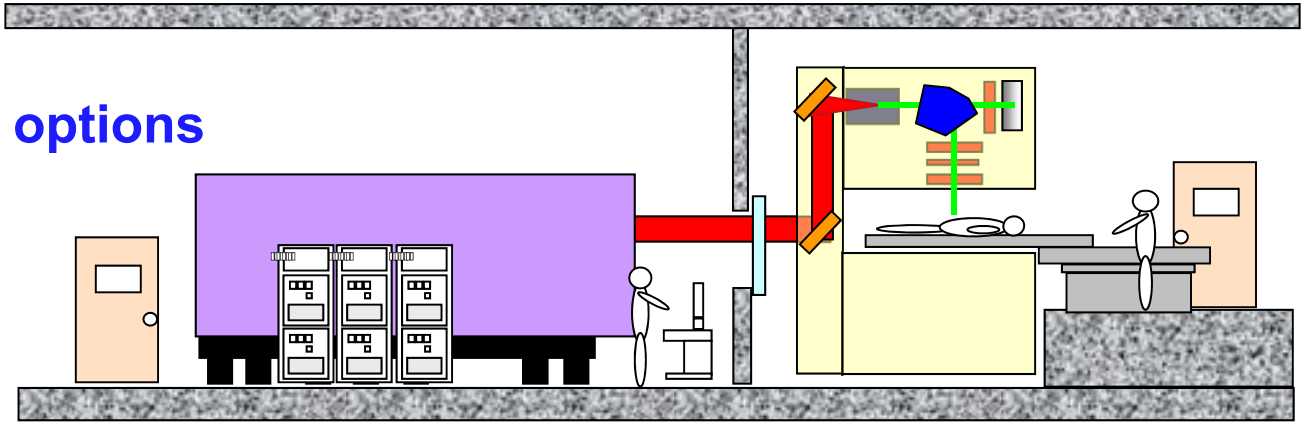
S.V. Bulanov *et al*, Phys. Lett. A, **299**, 240 (2002)
E. Fourkal et al, Med Phys., **30**, 1660 (2003)
V. Malka, *et al*, Med. Phys., **31**, 1587 (2004)

Flexibility:

- Possibility of controlling output energy and spectrum
- Possibility of varying accelerated species
- Spectral shaping for direct “painting” of tumour region

Novel therapeutic/diagnostic options

- Mixed fields: x-ray + ions
- In-situ diagnosis
- Proton radiography/PET…



Challenges of current research

- **Demonstrate feasibility of ion beam production**
  - High energy
  - Natively narrow energy distribution
  - High repetition, stability
- **Develop methods of beam transport/ delivery**

  - magnetic based or target based
- Assess the **biological effectiveness** of ultrashort ion bunches
- **Development of appropriate dosimetry**

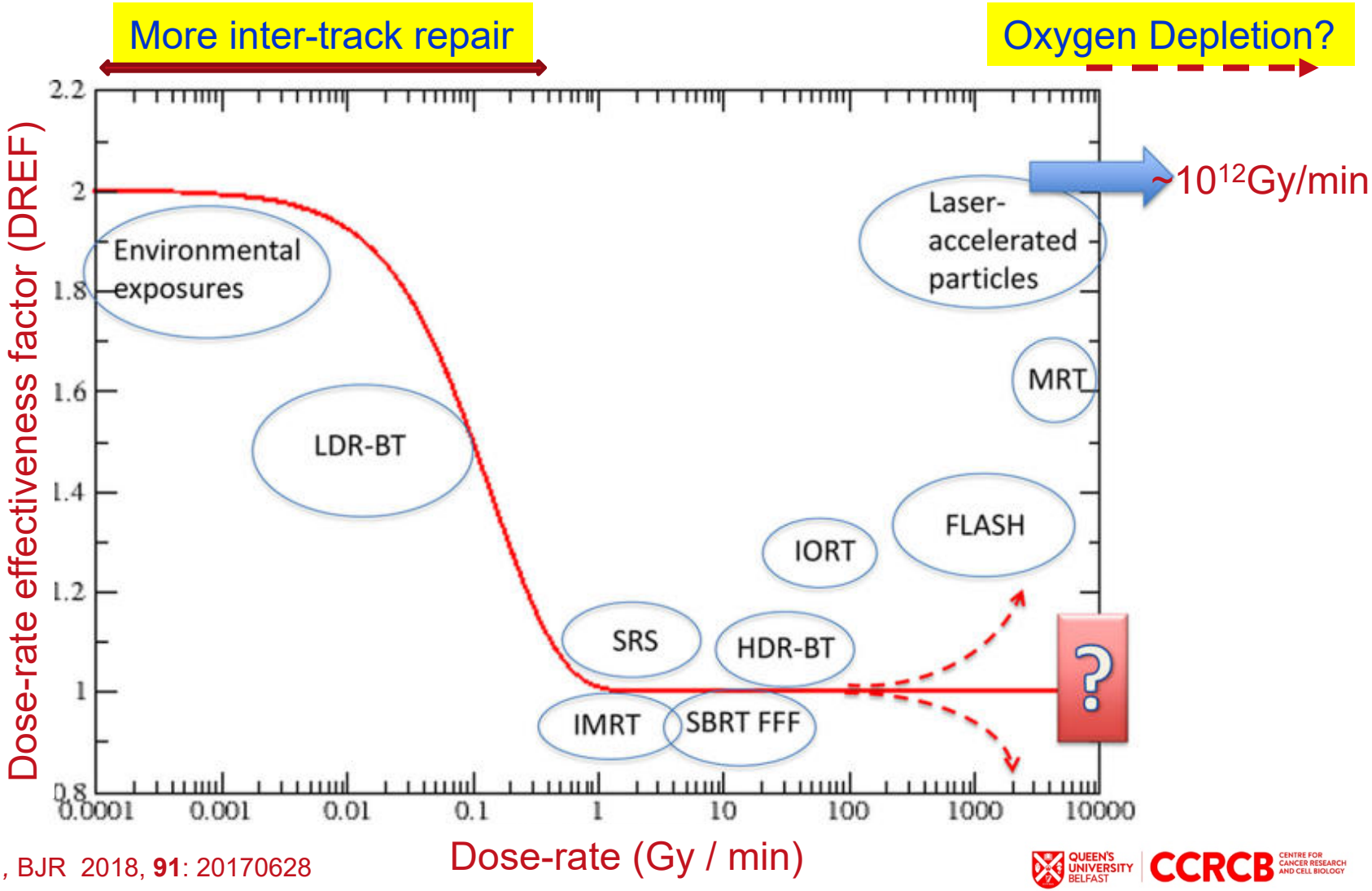
What will it take for laser driven proton accelerators to be applied to tumor therapy?

Ute Linz[1,*] and Jose Alonso[2,†]
[1]*Forschungszentrum Jülich, D-52425 Jülich, Germany*
[2]*Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA*
(Received 27 April 2007; published 24 September 2007)

In addition to having to develop an entirely new technology for effective beam delivery and dose conformation, the following challenges must be faced by the laser community: (i) verifying scaling laws for proton energy with laser power, (ii) improving proton flux by at least an order of magnitude, (iii) improving shot-to-shot reproducibility to the few-percent level, (iv) development of suitable dose-monitoring devices, (v) development of techniques for accurate dose control and cutoff, and (vi) addressing quality-assurance and patient-safety aspects. This is not to say that one should not work towards solving these tremendous problems! After all, it was realized over 100 years ago that orthovoltage x rays could be used for treating malignancies, but it took many decades—plus the

PRSTAB, 2007

Dose-rate effects



Durante *et al.*, BJR 2018, **91**: 20170628

High Dose-Rate Radiobiology

- Dose-rates higher than 10^9 Gy/s and 5 – 10 Gy deplete cellular oxygen
- Some data suggesting changes at lower dose-rates (10^2 Gy/s for *in vivo* studies (FLASH Radiotherapy Normal tissue sparing)
- No data for high LET radiations

Authors (dates)	Experimental system	Oxygen depletion dose, etc.	Radiation type	Dose rate	Pulse duration
Town et al (1967) [2]	HeLa S-3 cells	Above 9 Gy exposure; effect lost for second pulse 2.5×10^{-3} s later	15 MeV electrons	3.5×10^7 Gy s^{-1}	1.3 μs
Prempree et al (1969) [3]	Human lymphocyte chromosomal aberrations	Reduction in yield described	X-rays	4.8×10^8 Gy s^{-1}	n/a
Nias et al (1969) [4]	HeLa	7 Gy	8–14 MeV electron	$<1.8 \times 10^7$ Gy s^{-1}	1 μs
Berry et al (1969) [5]	HeLa S-3oxi and CHL-F	5–10 Gy for short pulses	2 MV X-rays up; 3.7 MV X-rays:	10^9 Gy s^{-1}, up to 10^{10} Gy s^{-1}	7 ns pulse, 50 ns pulse
Berry et al (1972) [6]	2 HeLa lines and murine leukaemia	5–10 Gy; partly hypoxic cells develop radiological hypoxia above 5 Gy	400 KeV electrons at dose rate	10^9 Gy s^{-1}	3 ns
Purrot et al (1977) [7]	Chromosomal aberrations in human lymphocytes	No increase in yield	15 MeV electrons	5×10^6 Gy s^{-1}	1 μs
Ling et al (1978) [8]	CHO cells	12 Gy depletion dose; oxygen diffusion to single cells significant after 3×10^{-3} s	Electrons	10^9 Gy s^{-1}	3 ns
Watts et al (1978) [9]	Cultured V-79 cells	Oxygen diffusion to single cells significant after $1–2 \times 10^{-3}$ s	400 keV electrons	10^9 Gy s^{-1}	n/a

CHO, Chinese hamster ovary; n/a, not available.

Jones *et al.*, 2012, BJR, 35, e933

WP4 – Radiobiology at extreme dose-rates

- 4.1. Biological response of cells to ultrashort ion bursts
- 4.2. Testing models of oxygen enhancement at high dose-rate
- 4.3. Testing clinically relevant dose-distributions

Hypothesis: *Ultra-high dose-rate (> 10^9 Gy/s), laser produced ion beams, being developed in this program will have a significant impact on the biological response to relative to conventional ion beams due to both spatial and temporal differences in their delivery*

Cellular response at these high dose rates is virtually unknown.
Possible effects:

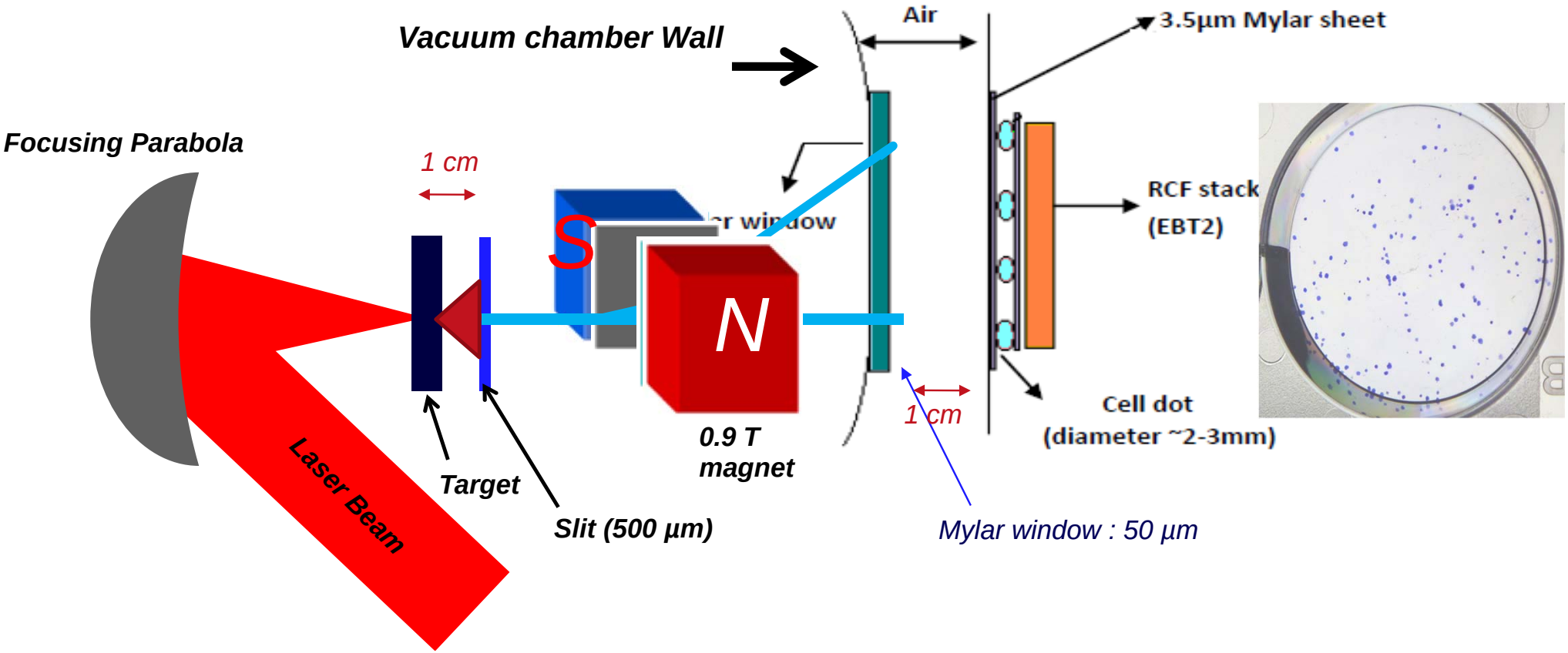
- **Spatio-temporal overlap of independent tracks** causing collective effects and enhancing Linear Energy Transfer
- **Local depletion of oxygen** causing a reduction in cell radiosensitivity
- **Lack of interaction between prompt DNA lesions and indirect lesions** (caused by radicals with diffusion times of μs)

Survival studies (clonogenic assay)

QUB TARANIS experiment , V79 (Chinese hamster) cells

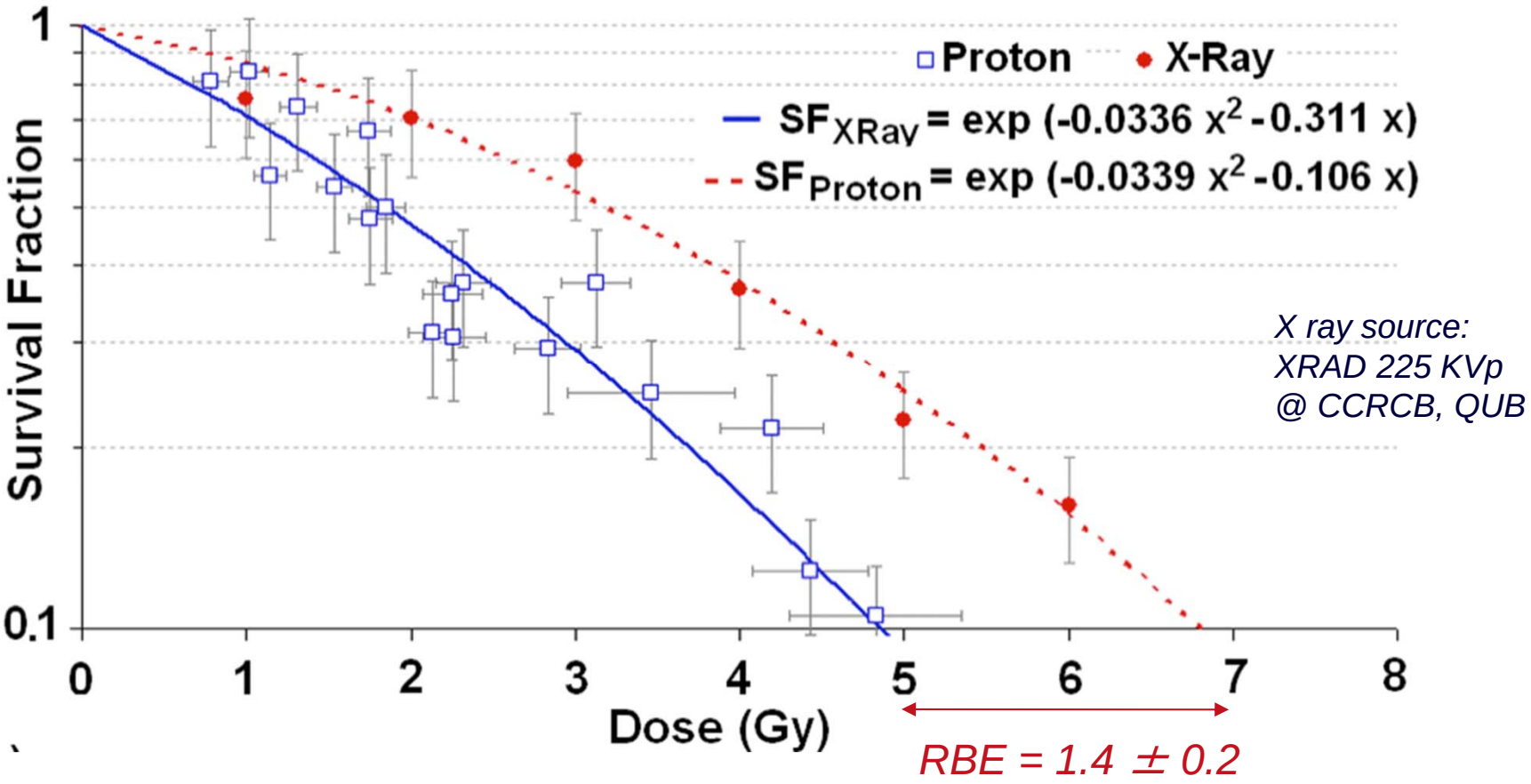
1 week

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



Single shot clonogenic survival curve

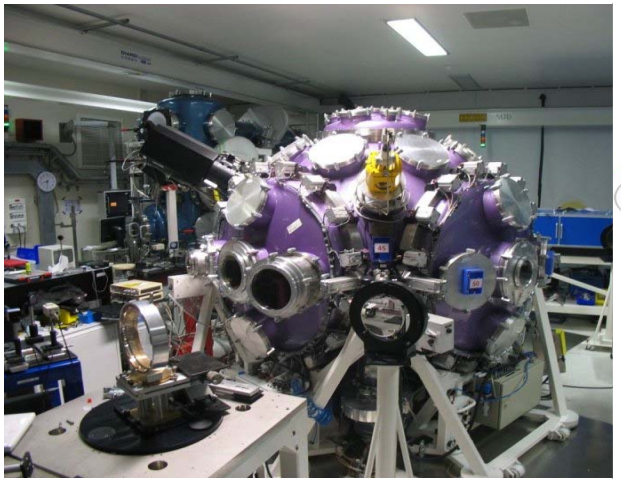
D.Doria et al, AIP Advances, **2**, 011209 (2012)

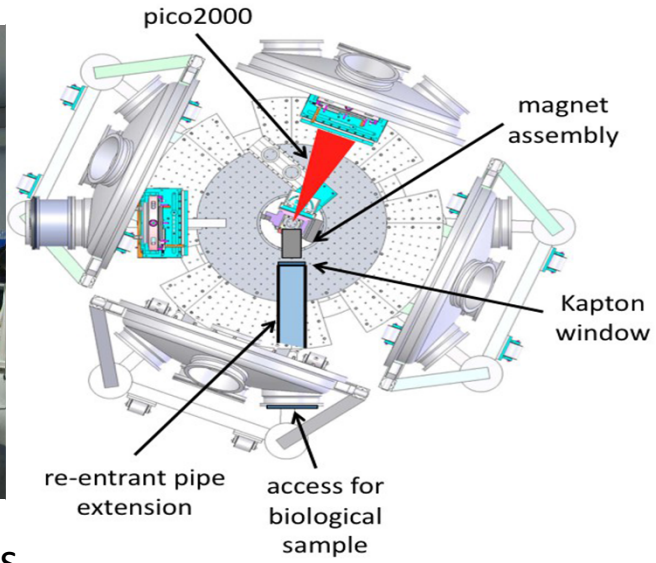


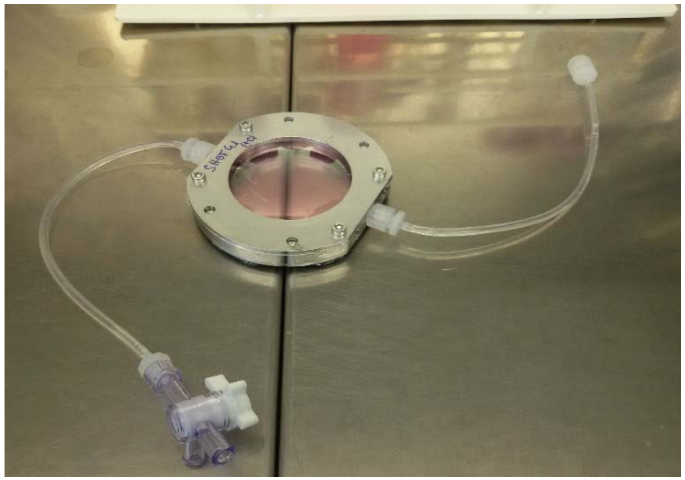
Dose rate > 10^9 Gy/s

In line with "standard" results with V79 cells
e.g. Folkard et al, Int.Jour. Rad. Biol., 69, 729 (1996)
Same RBE with LET=17.8 Kev/µm

LULI – MILKA LASER FACILITY

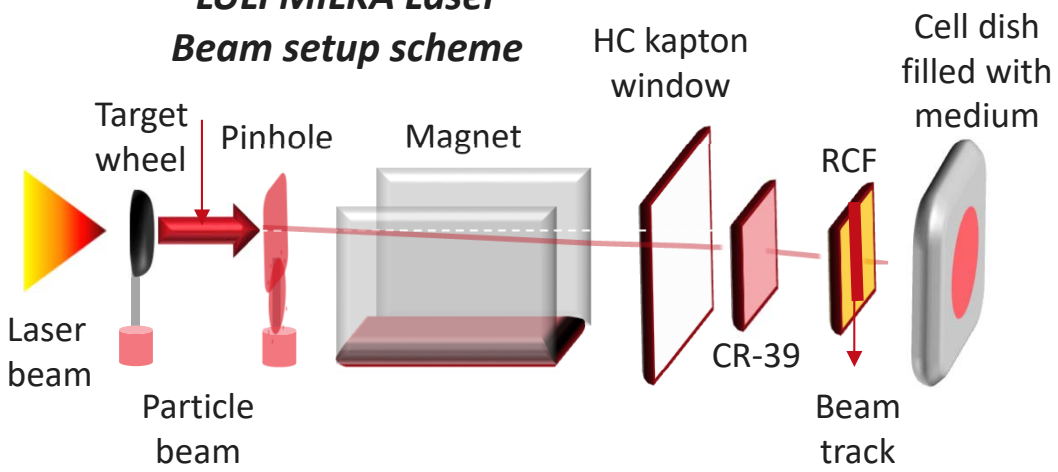






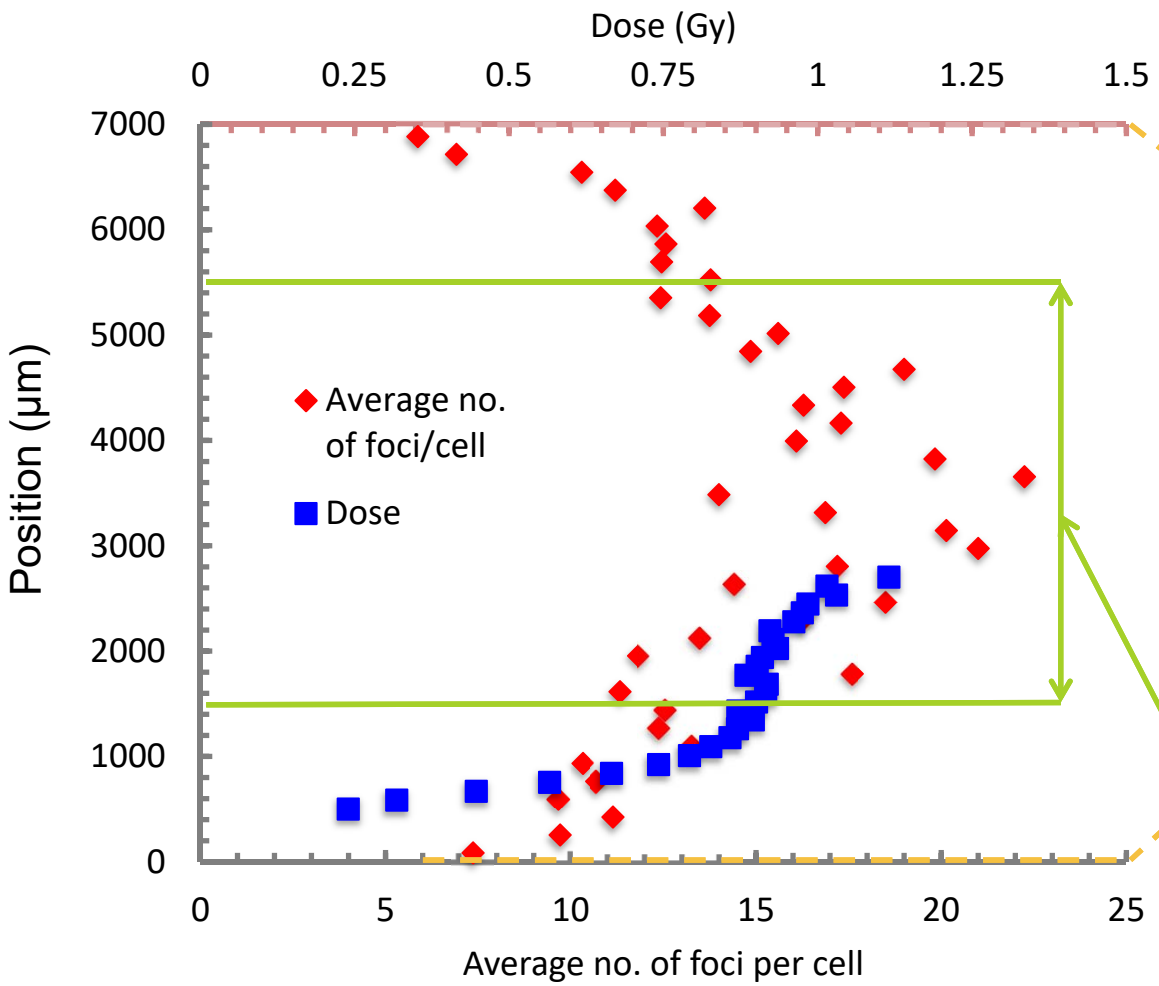
- Targets: 5-100µm gold foils.
- LULI pico2000 laser at standard operating parameters of 80J in 1ps at 1ω.
- Angle of incidence approximately 22.5° using the f=800mm, f/4 off axis parabola.

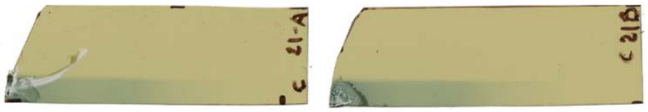
LULI MILKA Laser Beam setup scheme



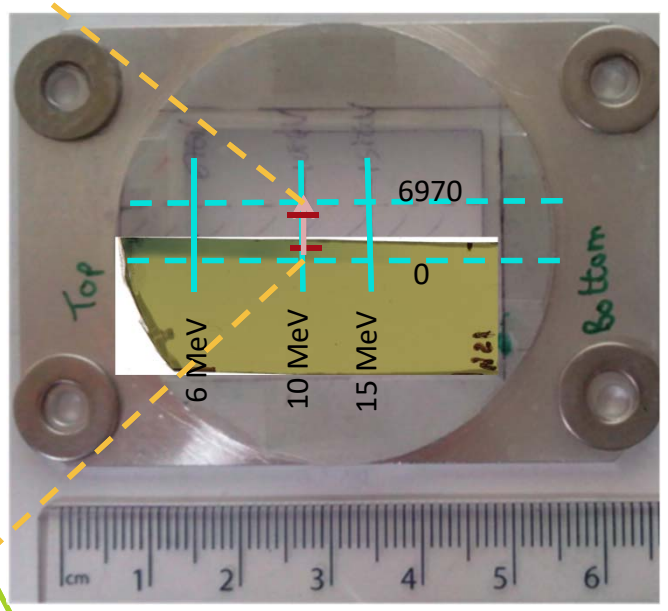
Data Acquisition Scheme

Alignment of dose and average no. of foci per cell.





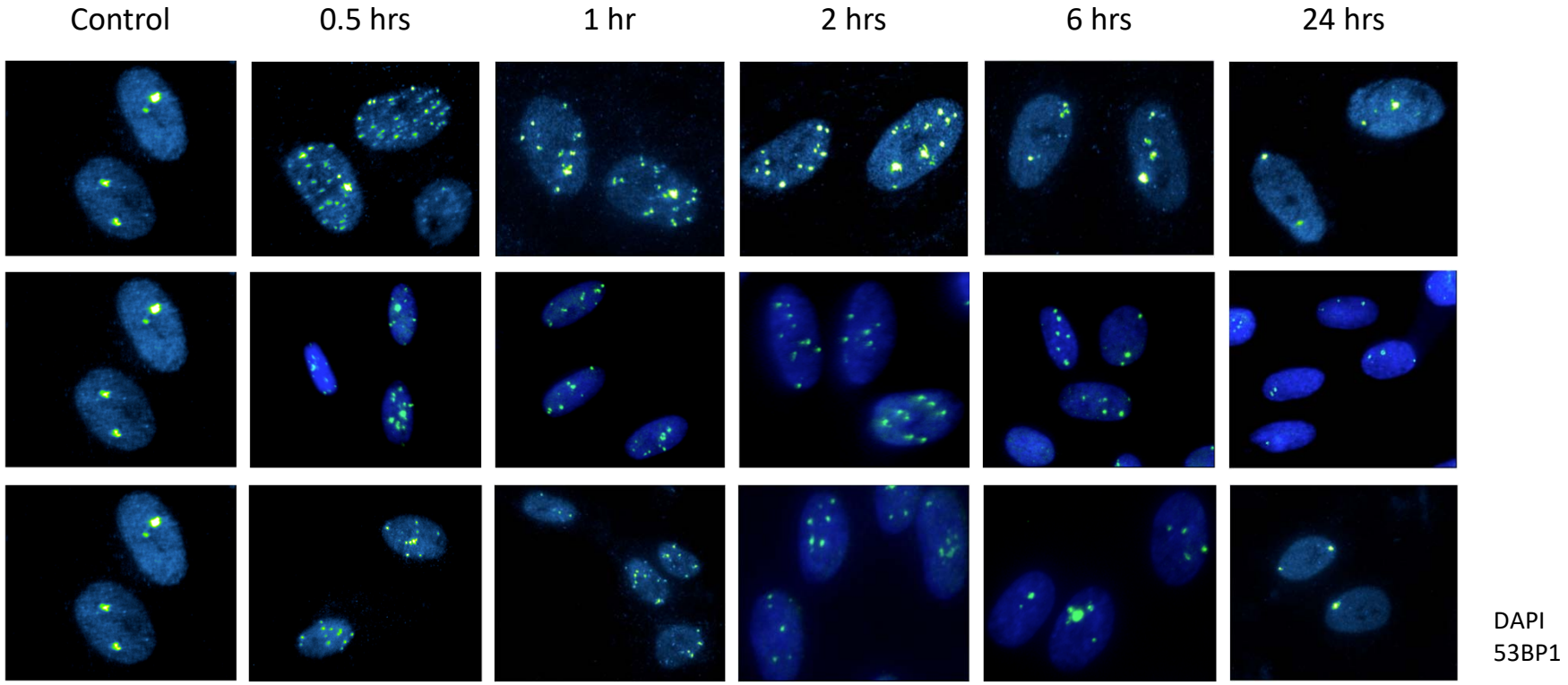
2 customised and 1 normal EBT3 film were placed behind the cell dish.



Foci counted in this region for foci kinetics

DNA DSB Repair Kinetics LULI

End point staining – 53BP1



Example images of the 53BP1 foci taken at the positions irradiated by 10MeV, ~13MeV and 15MeV protons, at the 5 different time points of 0.5, 1, 2, 6 and 24hrs post irradiation and the control.

Experimental setup for VULCAN

The cells are irradiated by the proton beam generated by focusing the VULCAN beam, at native contrast and intensities above 10^{20} W/cm^2, on thin (μm scale) low-Z foils. A 1T magnet will disperse the protons, spatially selected by a collimator to achieve a dispersion of order MeV/mm on the cell plane. The protons will reach the cells by crossing a flange-mounted, thin (~ 50 μm) mylar window, as in our previous measurements.

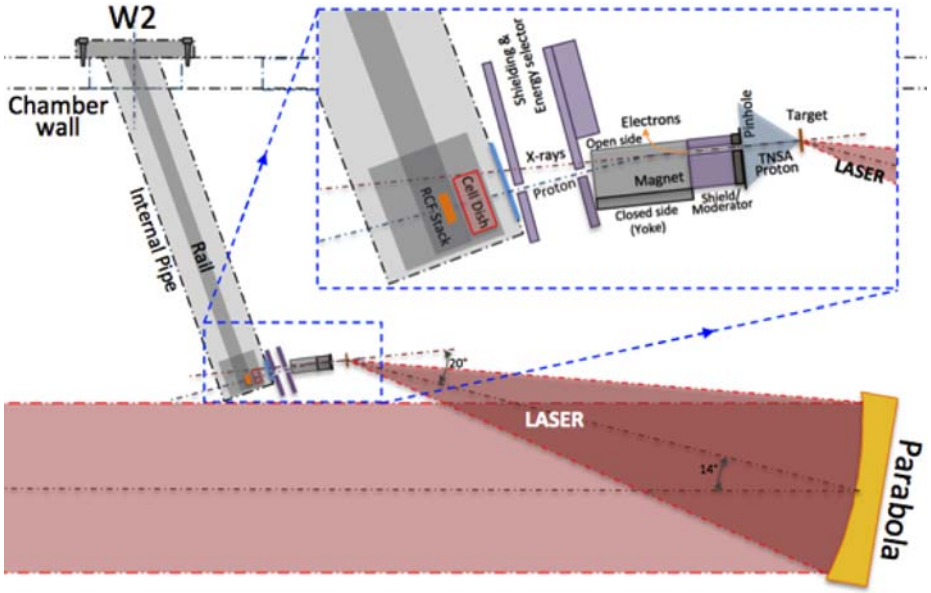


Table 1 – Indicative on-cell beam parameters estimated for the set-up in fig.1, with entrance slit 25 mm wide, placed at 5 cm from the target, and with target-cell distance of 30 cm. 1 T magnet, 10 cm long. Calculation for a typical TNSA spectrum (in this case from a 10 mm Al target)

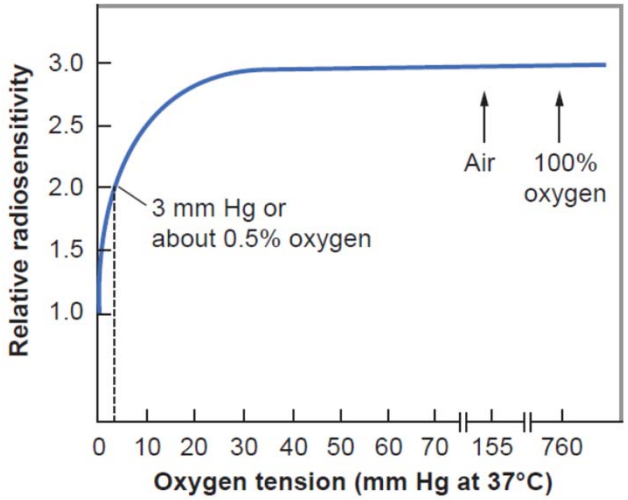
Proton energy MeV	ΔE MeV	Duration ps	Proton flux #p/um2	Track radius um	#p in track section	#p in track section in 1 ps	Dose Gy	Dose rate 10^{10} Gy/s
5	0.03	46	0.46	0.86	1	0.02	0.57	1.2
15	0.15	43	0.81	5.4	74	1.7	0.43	1
20	0.26	43	0.67	9	170	3.9	0.28	0.65
30	0.48	44	0.31	18.8	340	7.7	0.09	0.21

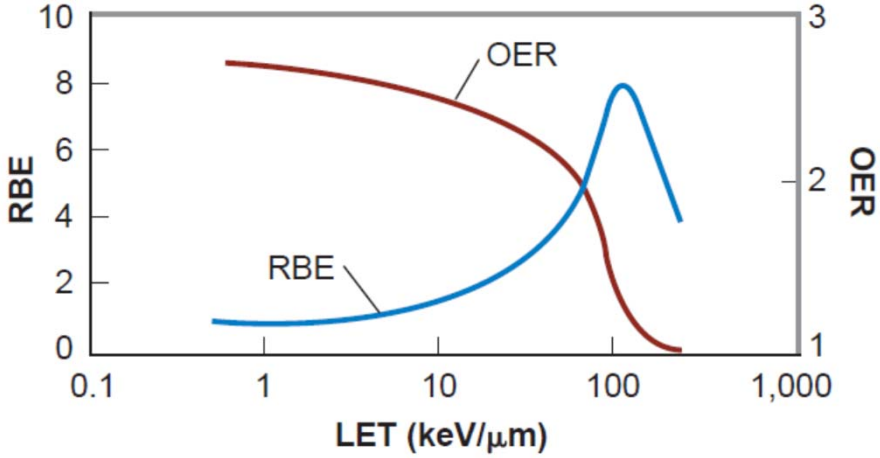
Vulcan exposure details

- Average Energy on Target Per shot : 595 J
- Average Pulse Duration : 809 Femto seconds
- Target used on Cells 10 nm Aluminium
- Average Dose on Cells per shot 1-3 Gy
- Energy on Cells : 14-19 MeV
- Ions: Protons, Carbon, X-rays needs further analysis
- Average Flux : CR39 Etching results
- Dose rate: 10^{10} Gy per second

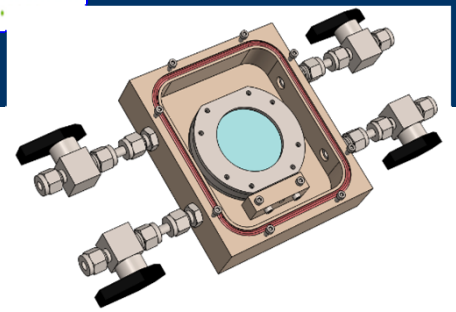
Impact of Hypoxia

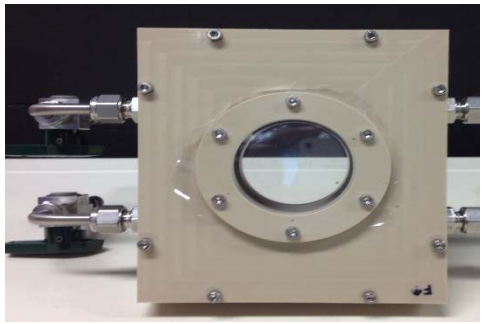
- Cells and tissues are 3 times more radioresistant in the absence of oxygen
- This is a major limiting factor in the treatment of solid tumour with hypoxic regions by photon radiotherapy
- With increasing ionization density (LET) the modulation by oxygen (OER) decreases
- This is one of the rationales for using ion beams with higher LETs for therapy

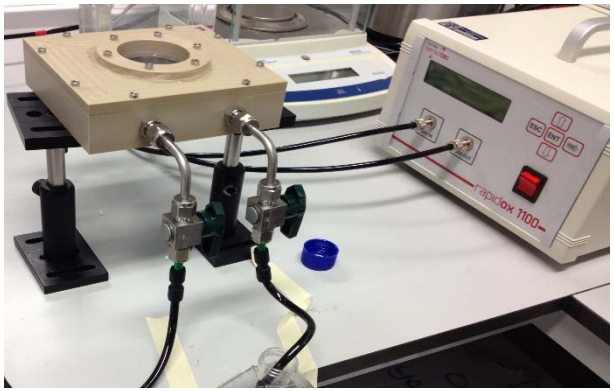




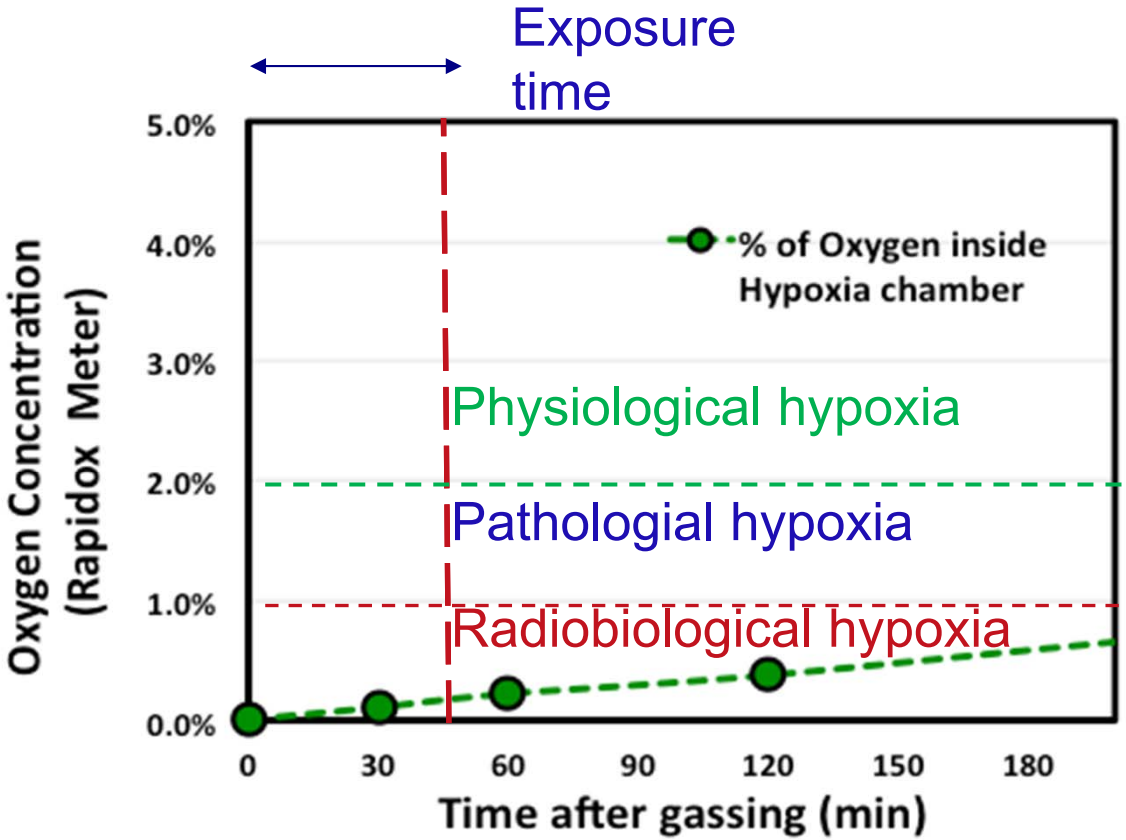
Hypoxia Chamber Testing





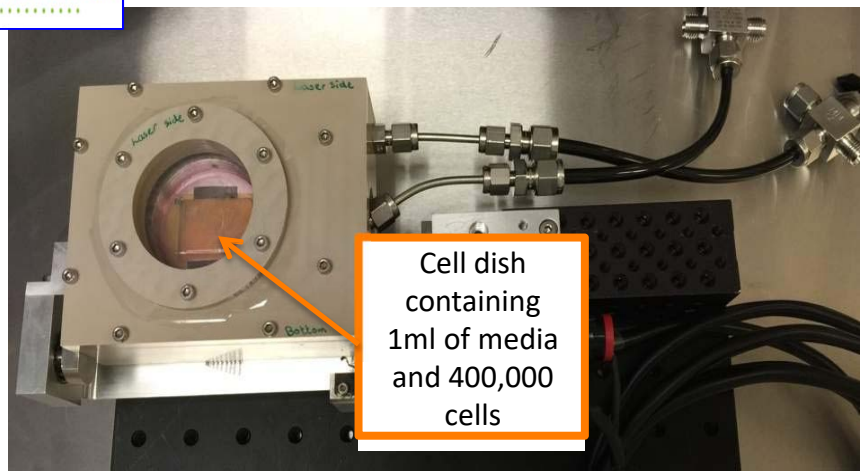


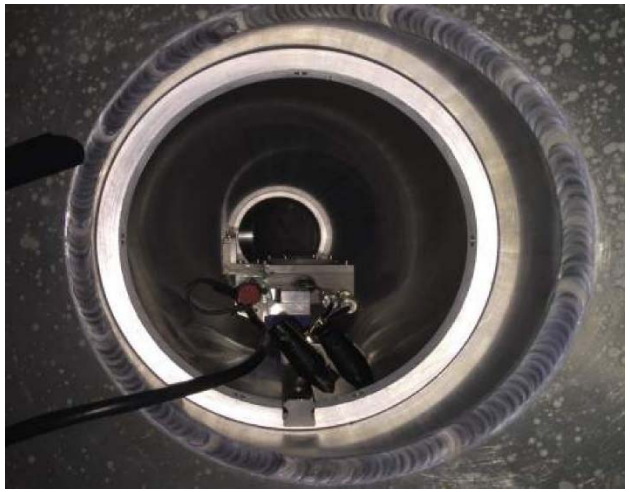
Hypoxia induction through gassing with 5% CO_2, 95% N_2



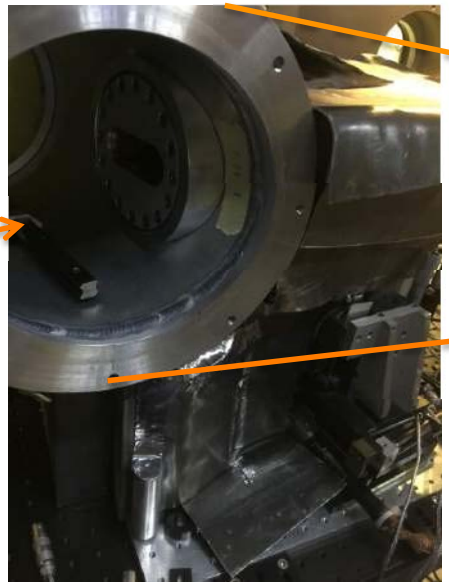
Total Time needed for each shot after gassing is about 45 minutes maximum

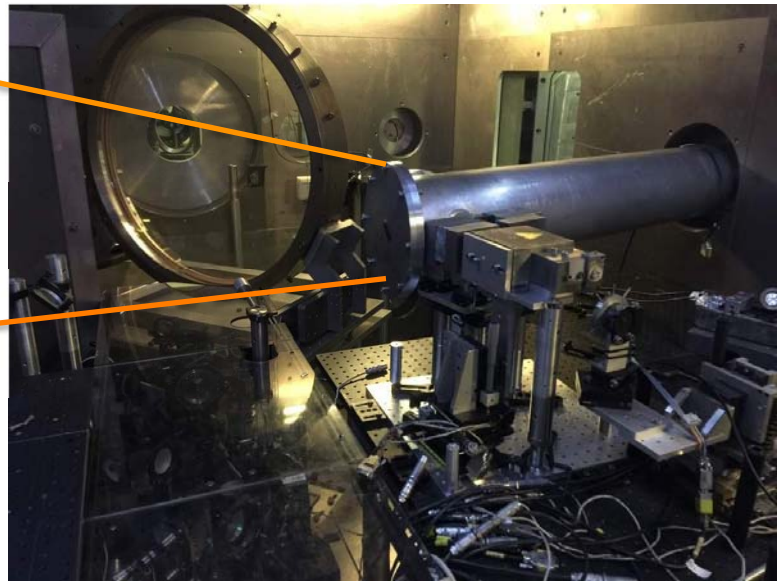
Placement of Hypoxia Chamber Inside Interaction Chamber





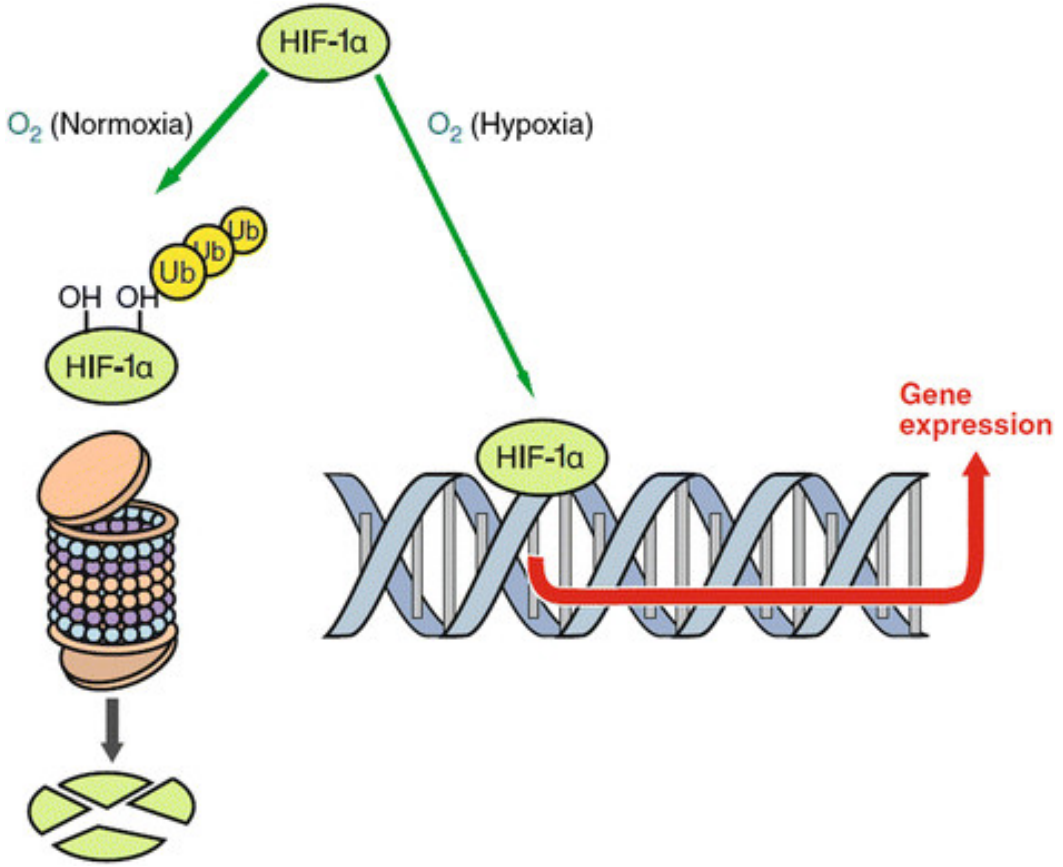
Position of hypoxia chamber during irradiations



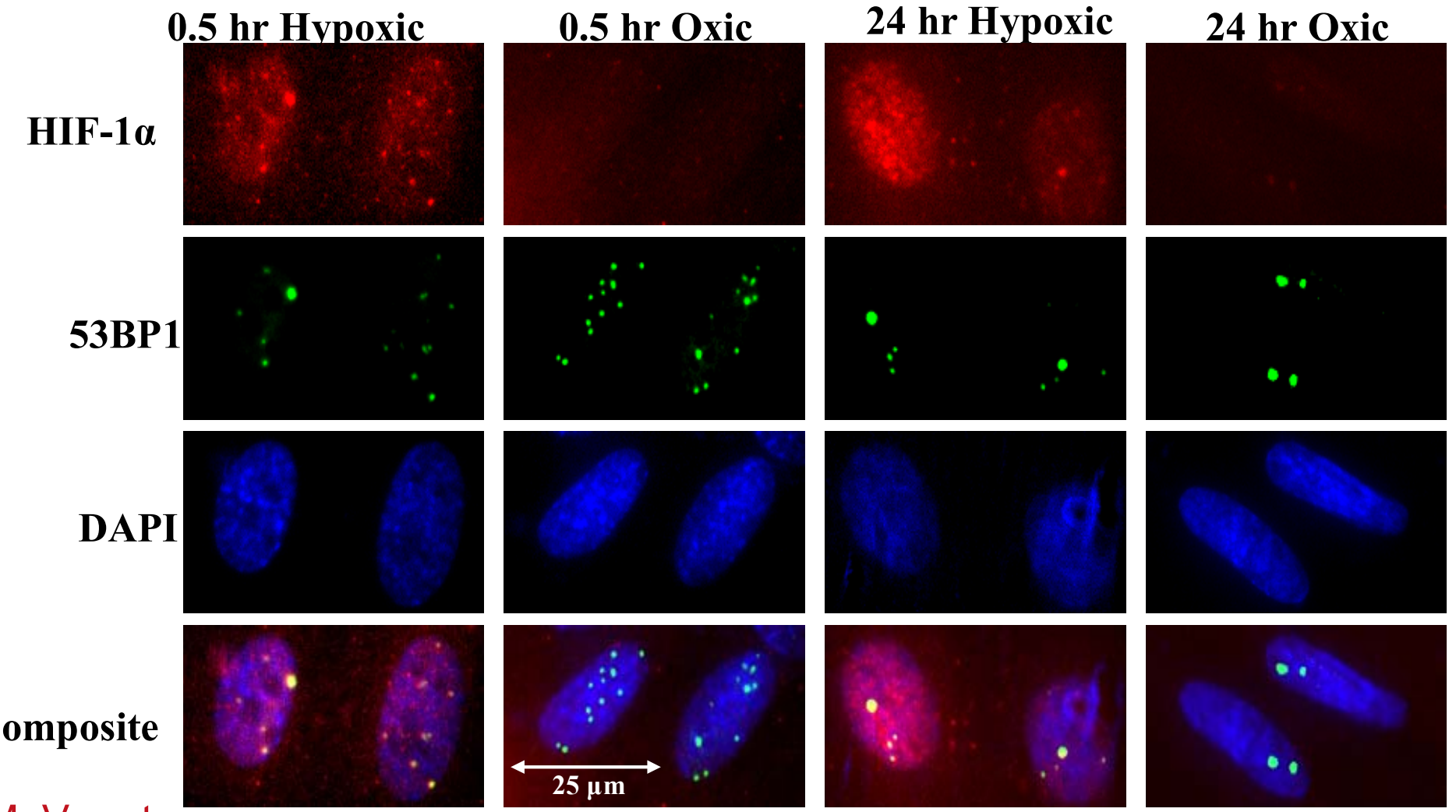


Markers of Hypoxia

- Hif-1α is a major biomarker of hypoxia
- In the presence of oxygen it is degraded by the proteasome system
- In the absence of oxygen it activates multiple genes down stream

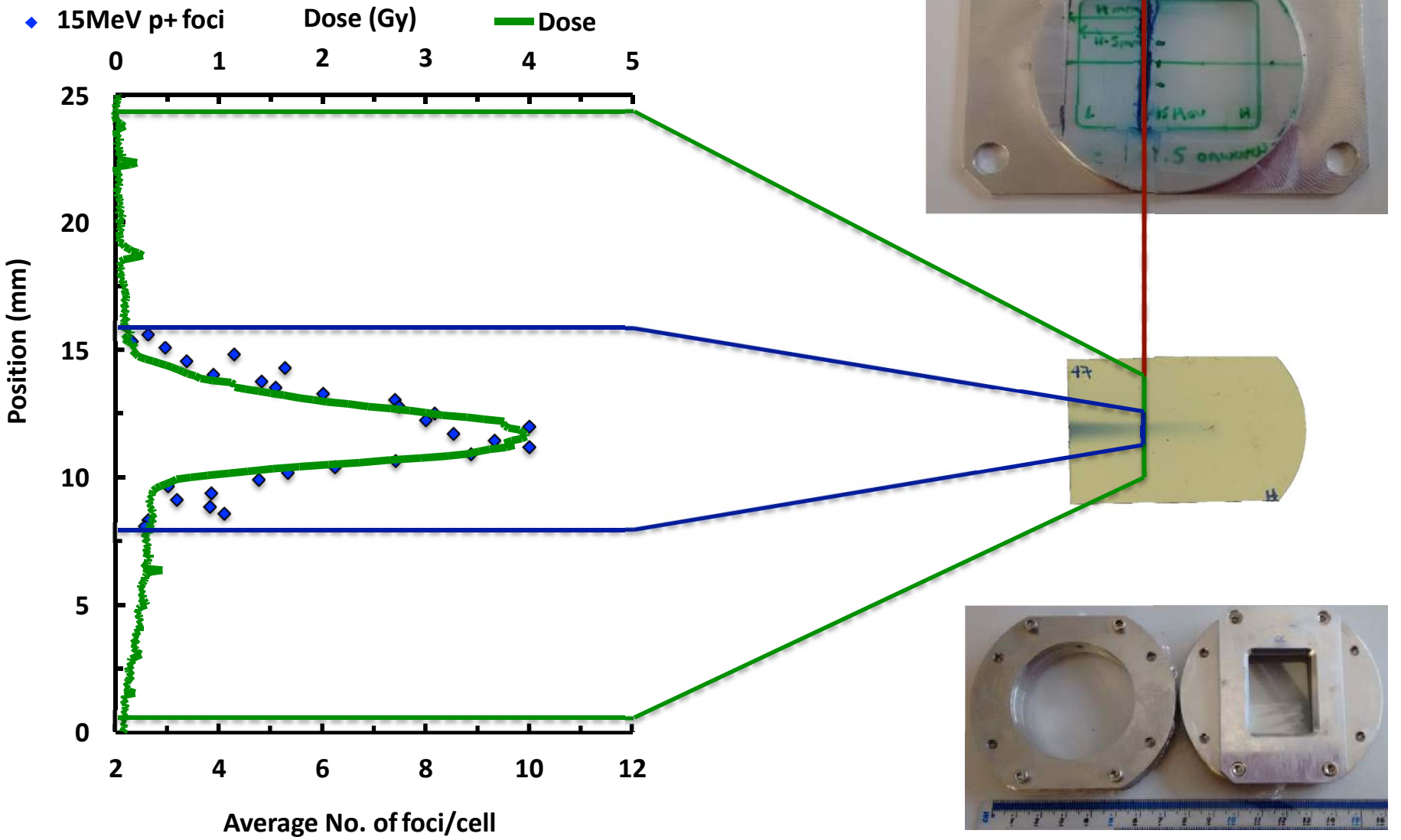


Immunofluorescent staining of 53BP1 foci and HIF-1 α in human skin fibroblasts



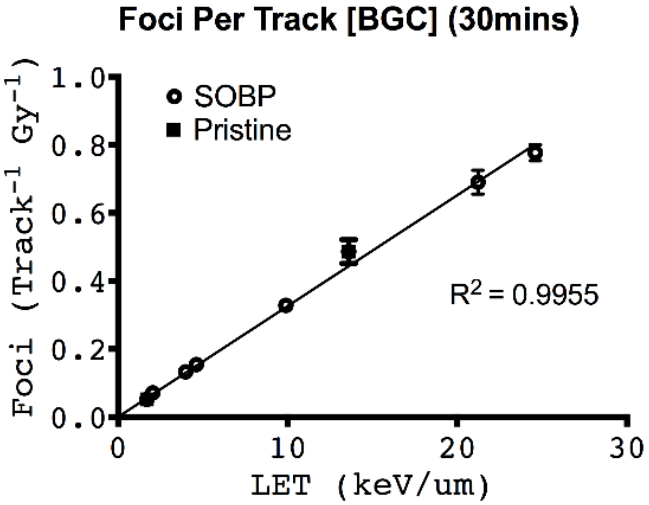
15 MeV protons

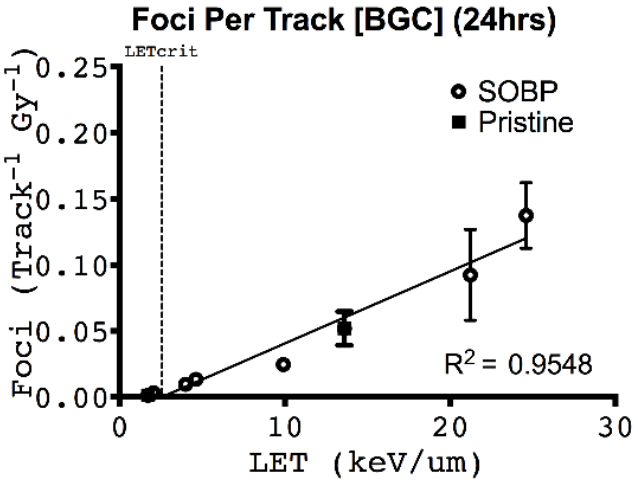
Preliminary Results

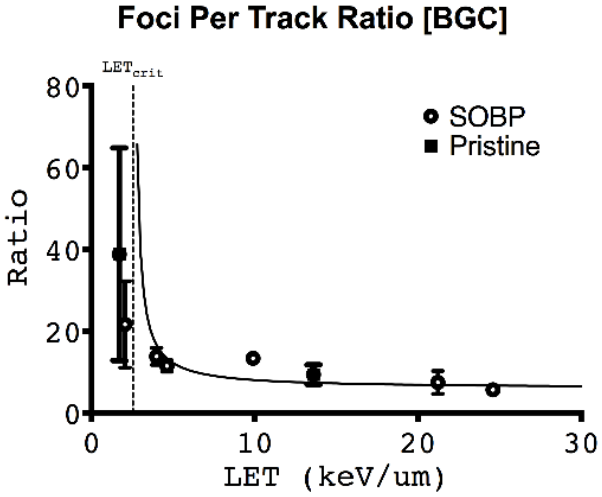


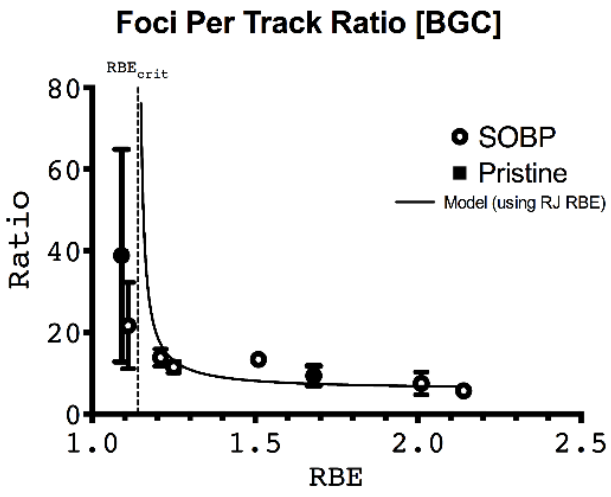
Calibrations of DNA damage - protons

- Conventional protons delivered at ~ 2Gy/min
- Linear relationship between foci per track and LET
- Slope dependent on repair time









Chaudhary *et al.* 2016
Int J. Radiat Oncol Biol Phys, **95,** 86

Summary

- A-SAIL has been performing key studies on TARANIS, GEMINI, LULI and VULCAN laser facilities to characterise DNA damage and survival response at ultra-high dose-rates
- Preliminary data for hypoxic response obtained
- Calibration data for defined proton energies being used to benchmark data
- Other biological models and endpoints being characterised
- Further work to define impact of dose-rate effects

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Prague Proton Therapy Centre

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