Mapping RBE effects at the cellular level: relevance for fractionated proton radiotherapy

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Outline of presentation

- Radiation quality, dose and RBE for charged particles
- Studies comparing pristine and SOBP proton beams to set the baseline
  - Cell survival
  - DNA damage/repair
- Understanding clinically relevant treatment protocols at the cellular level

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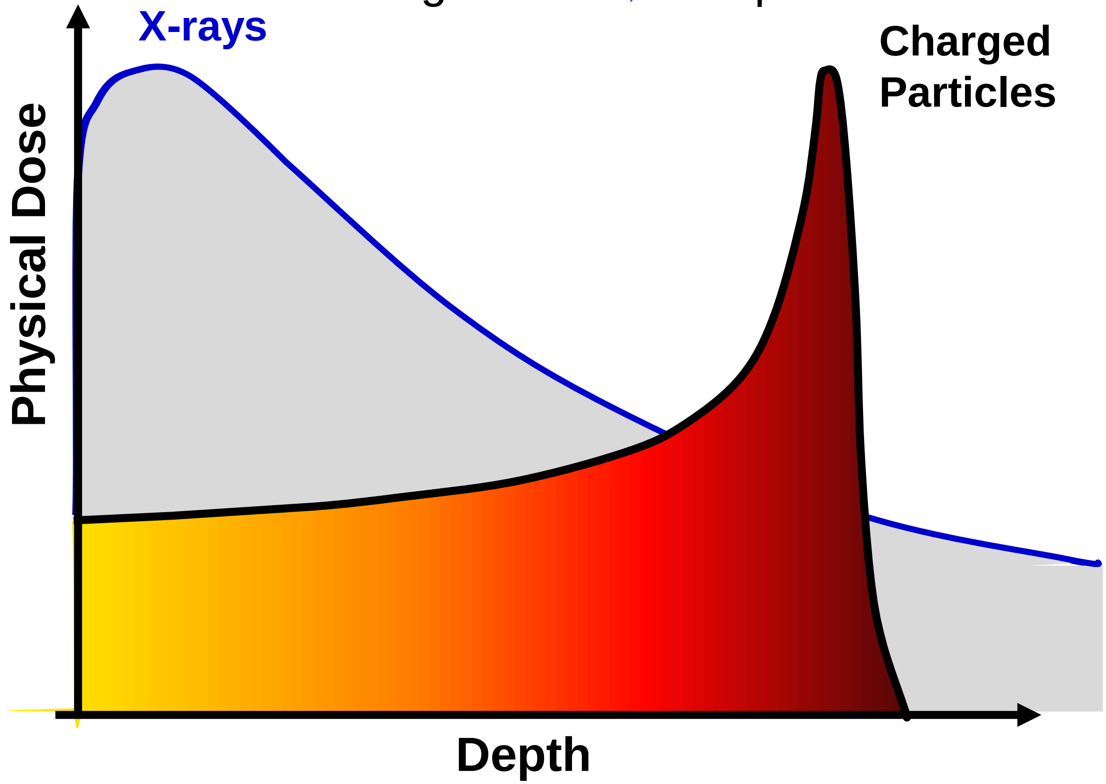
Background

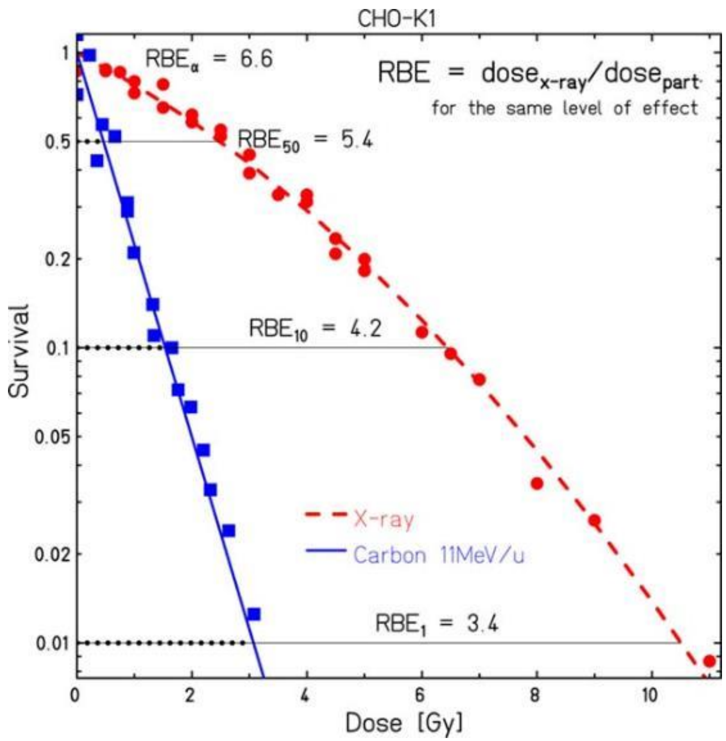
Charged particles are being increasingly used in cancer treatment
By the end of 2015, 154,097 patients had been treated, 131,134 with protons

- Inverse energy deposition → Selective dose localization
- Elevated RBE for cell killing → Improved tumour control



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

RBE: Relative Biological Effectiveness



RBE critically depends on both physical and biological parameters:

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

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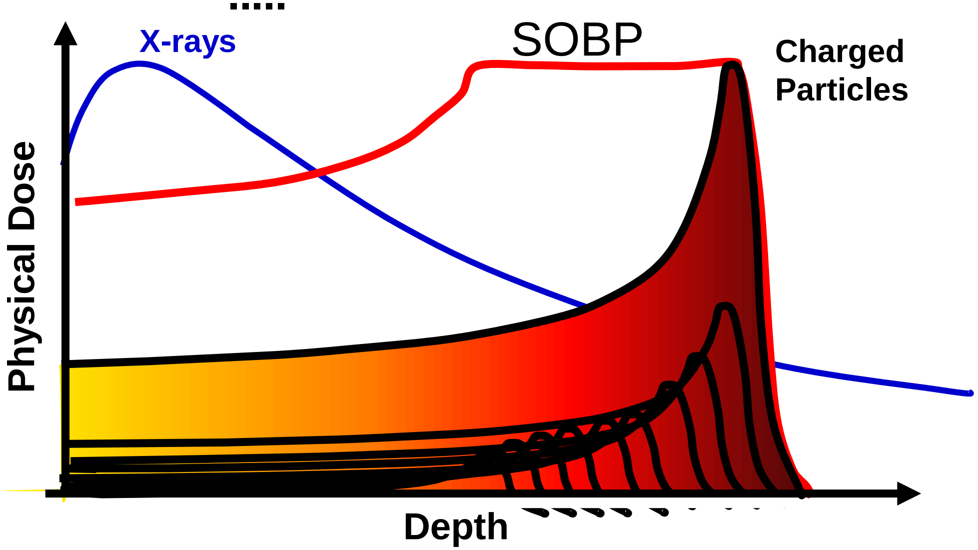

Currently fixed RBE values are used clinically and disregard any physical and biological dependency potentially limiting particle therapy effectiveness.

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

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Proton RBEs

- A **range of RBE values** *in vitro* and *in vivo* have been reported
- 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**

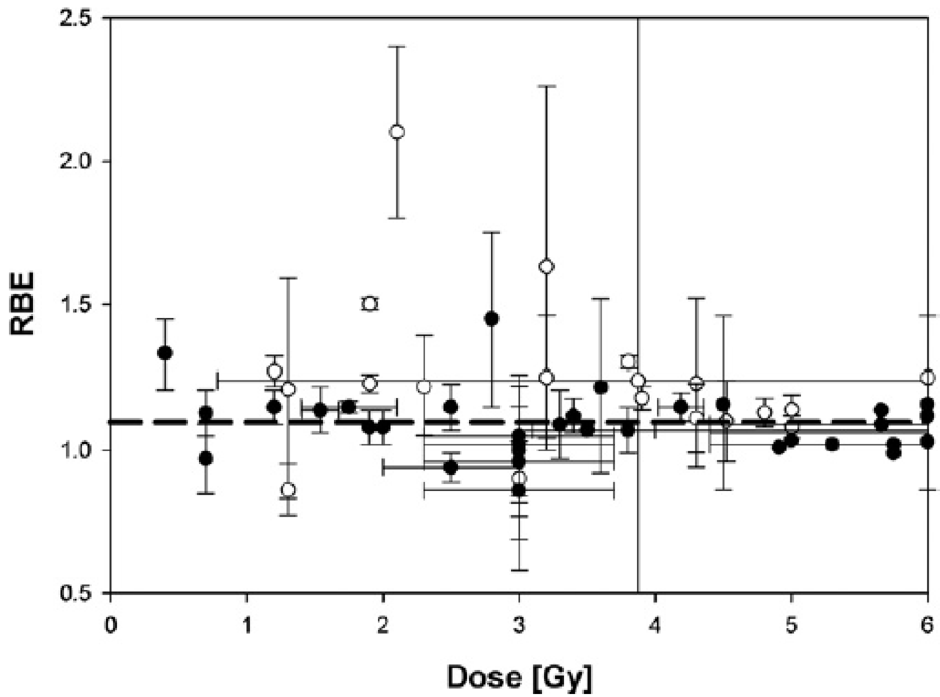

Figure 1 Experimental proton relative biological effectiveness (RBE) values (relative to ^{60}Co) as a function of dose/fraction for cell inactivation measured in vitro (open circles) and in vivo (closed circles). The thick dashed line illustrates an RBE of 1.1. Data taken from Paganetti et al.[15]

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

Proton RBEs

- Paganetti, H., 2014, *Phys Med Biol* **59,** R419-R452
- 367 datapoints from 100 publications
- Considerable uncertainty but increasing RBE with LET
- Friedrich *et al* 2013, *J Radiat Res* **54,** 494 online database

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)

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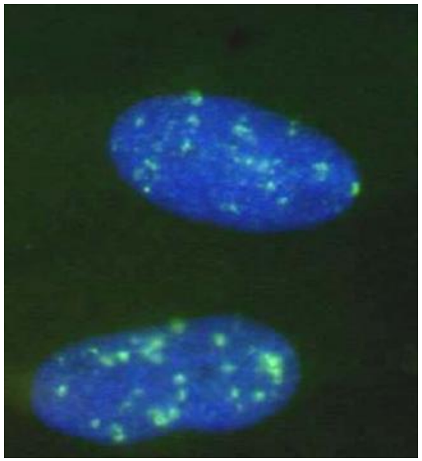
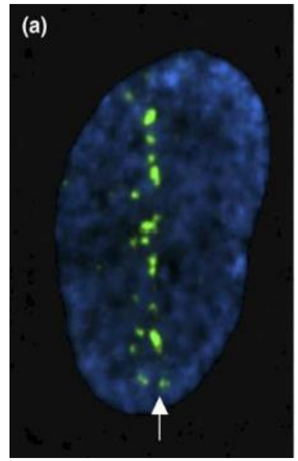
Dose, LET and RBE

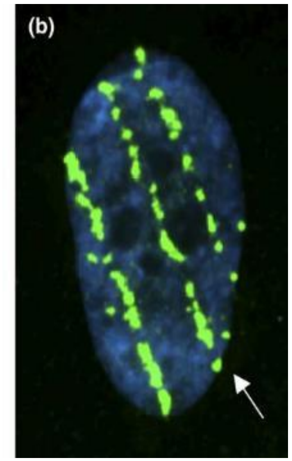
- Cellular response is determined by the level and quality of DNA damage, which reflects the energy deposition pattern.



X-rays



54 keV/µm Si ions



174 keV/µm Fe ions

- Severity of DNA damage depends on lesion proximity and repairability, hence it is not a constant value but depends on physical (particle type, LET, dose) and biological (cell type, oxygenation status, repair capacity) parameters.
- RBE varies with the particle energy and the change of the beam composition (SOBP and nuclear fragmentations): its distribution is not homogenous across a treatment field.
- Estimates of the RBE of each specific irradiation scenario and position along the ion path could be important inputs for the development of radiation treatment plans

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Overall aim

Combined assessment of early and late cellular response including DNA damage in a range of relevant cell lines to provide systematic high resolution information to develop a rigorous theory of ion radiation action at the cellular and molecular level.

- **How does DNA damage and cell response vary across a pristine Bragg curve?**
- **How biological effectives of a pristine curve relates to a Spread Out Bragg Curve?**
- **What is the contribution of radial dose to heavy ion track structure?**
- **What other biological parameters play a role?**

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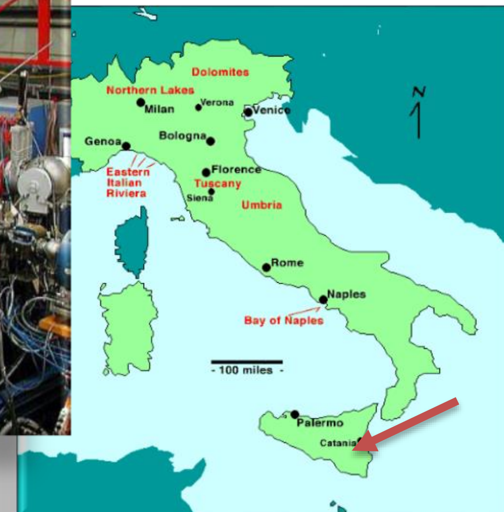
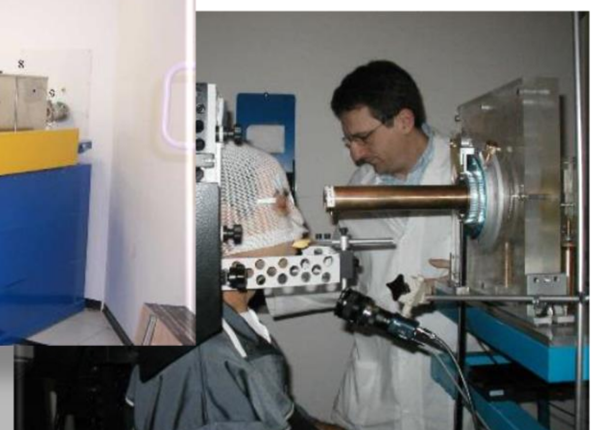
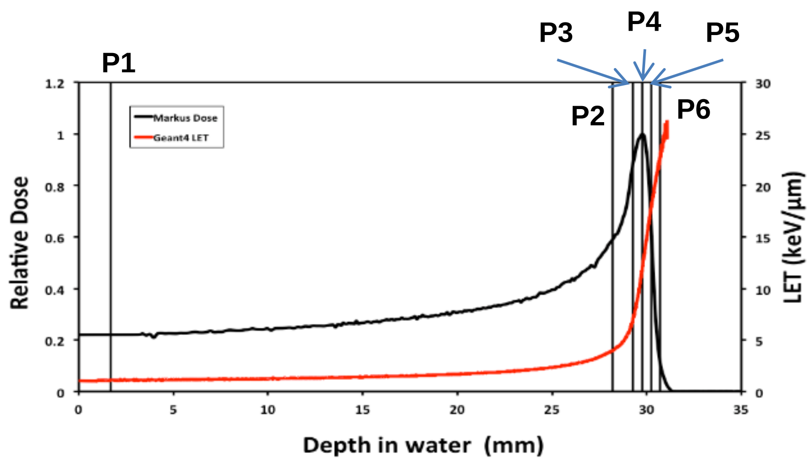
INFN Catania



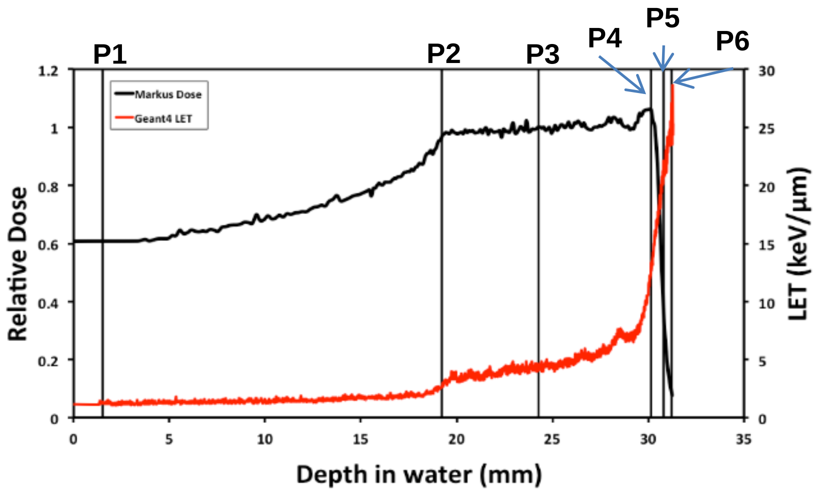


Catana Proton Therapy Facility

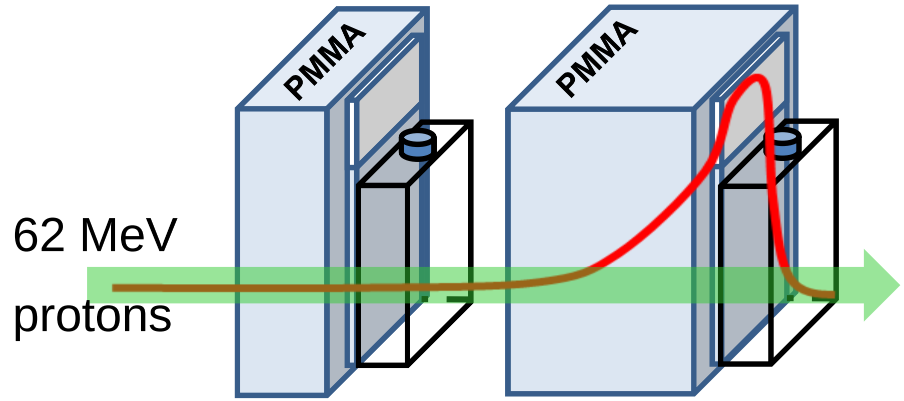
Irradiation Setup – INFN Catania

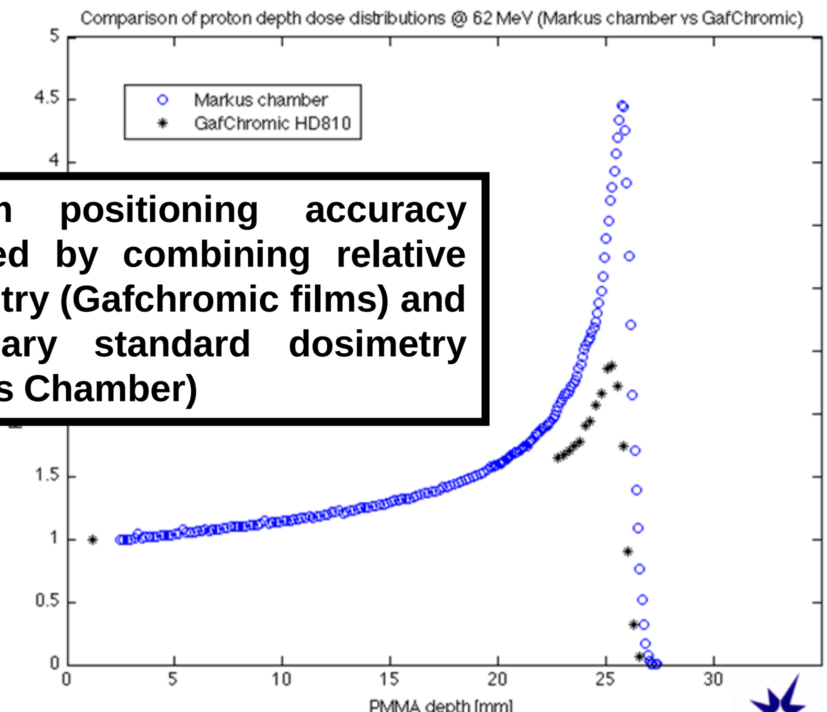


	P1	P2	P3	P4	P5	P6
Depth water [mm]	1.38	20.23	24.59	27.69	29.48	30.08
LET [keV/µm]	1.2	2.6	4.5	13.4	21.7	25.9



	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)

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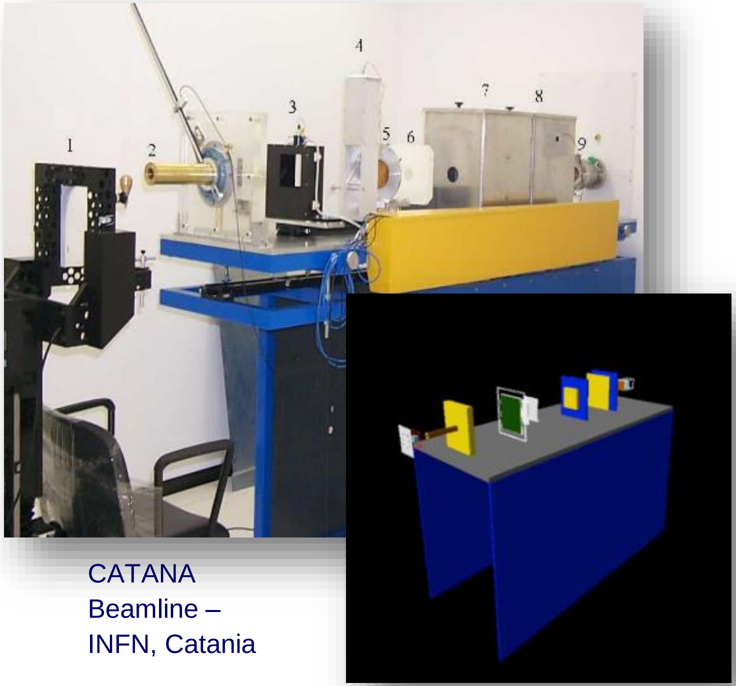
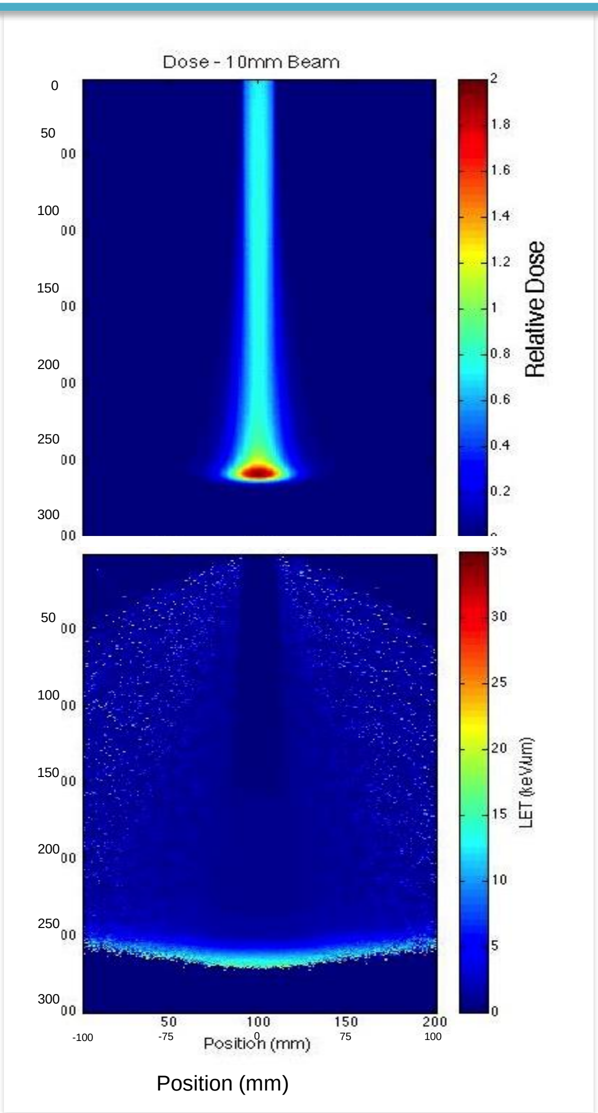
Geant4 Simulation



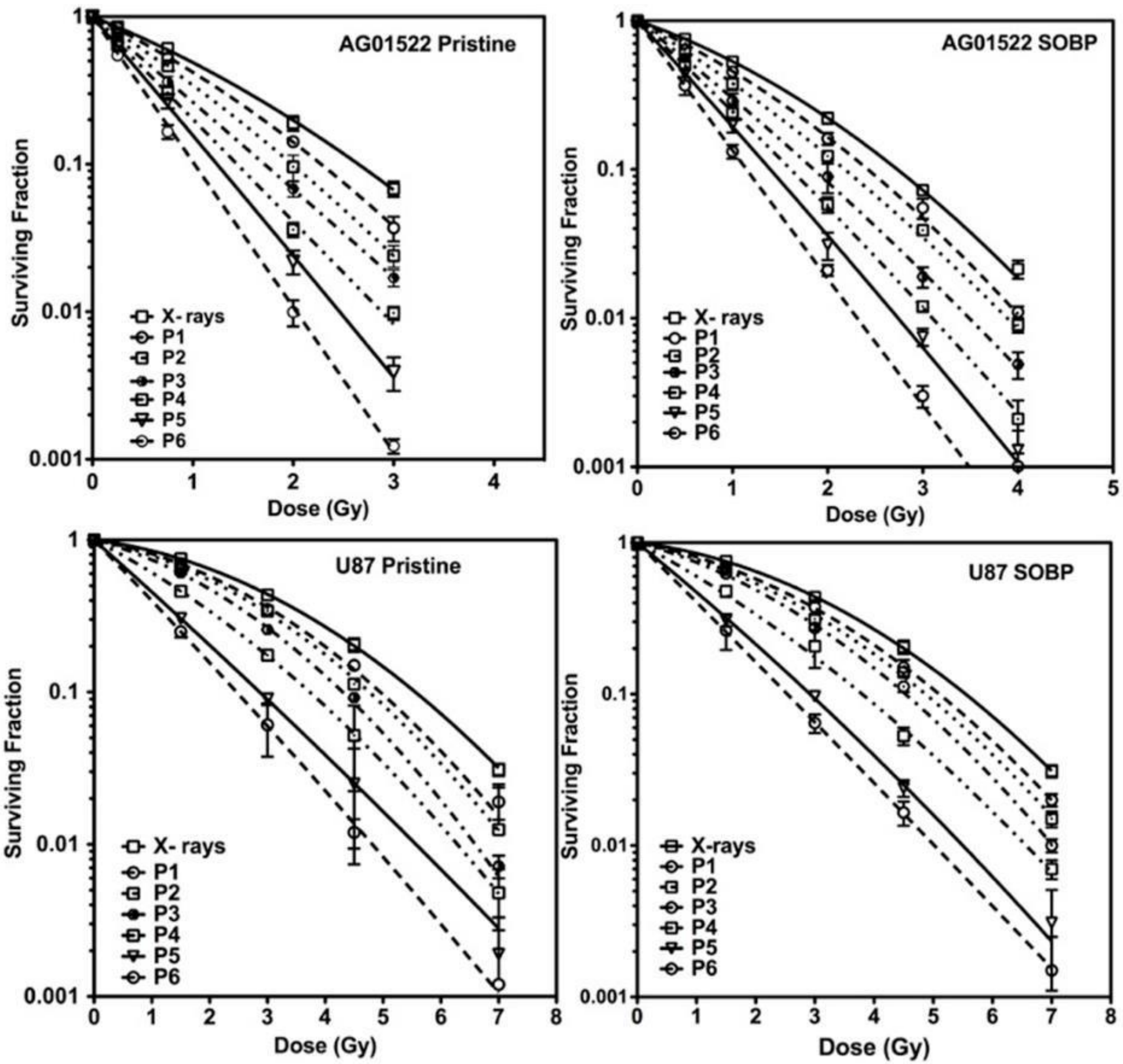
CATANA Beamline – INFN, Catania

- 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.



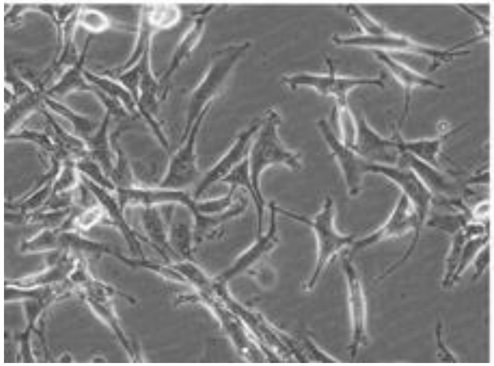
Top: Geant4 Depth - Dose distribution.
Bottom: Geant4 Depth - LET distribution.

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

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

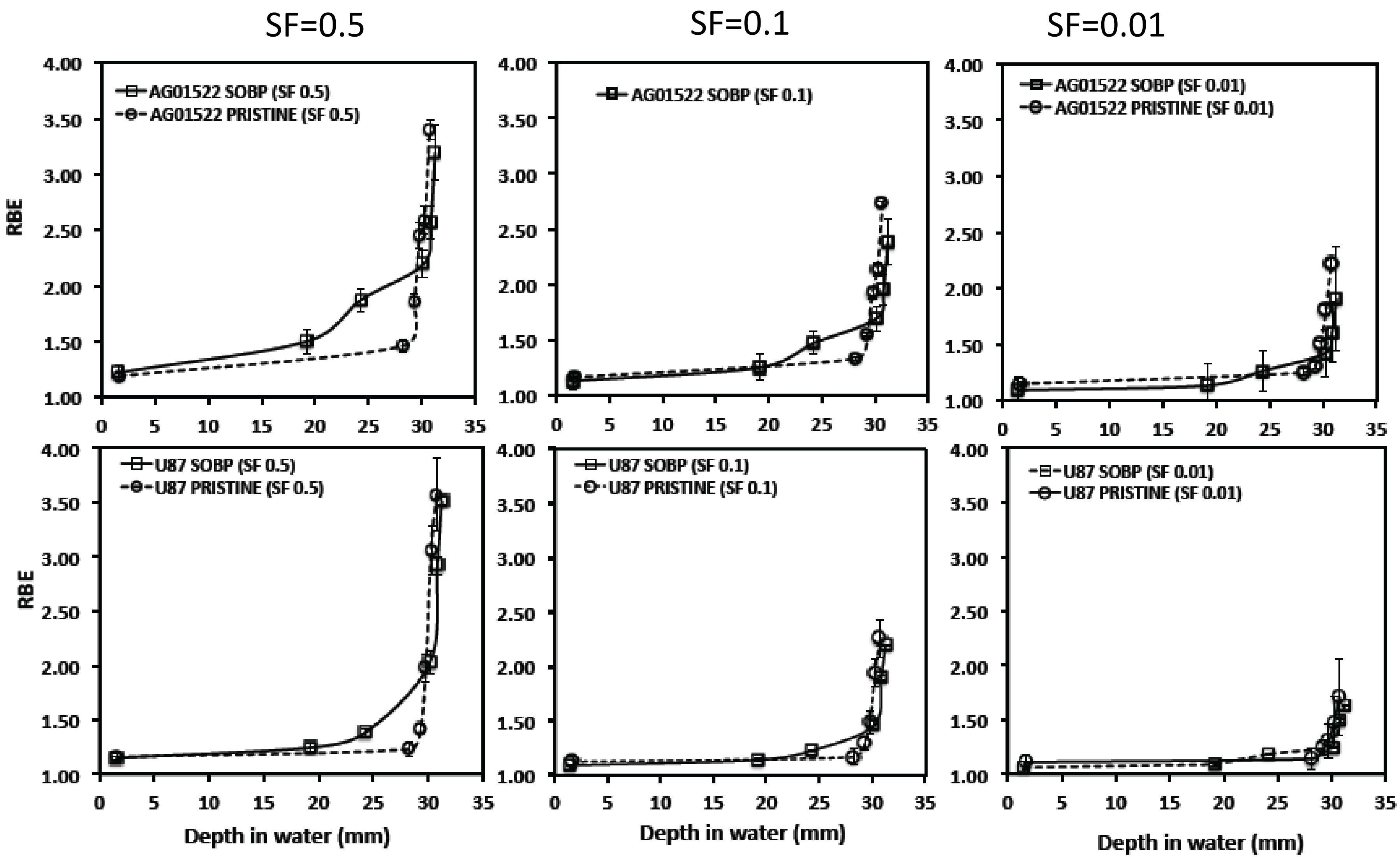
$$RBE = D_{X\text{-}ray} / D_{Proton} \; @ \; isoeffect$$

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

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



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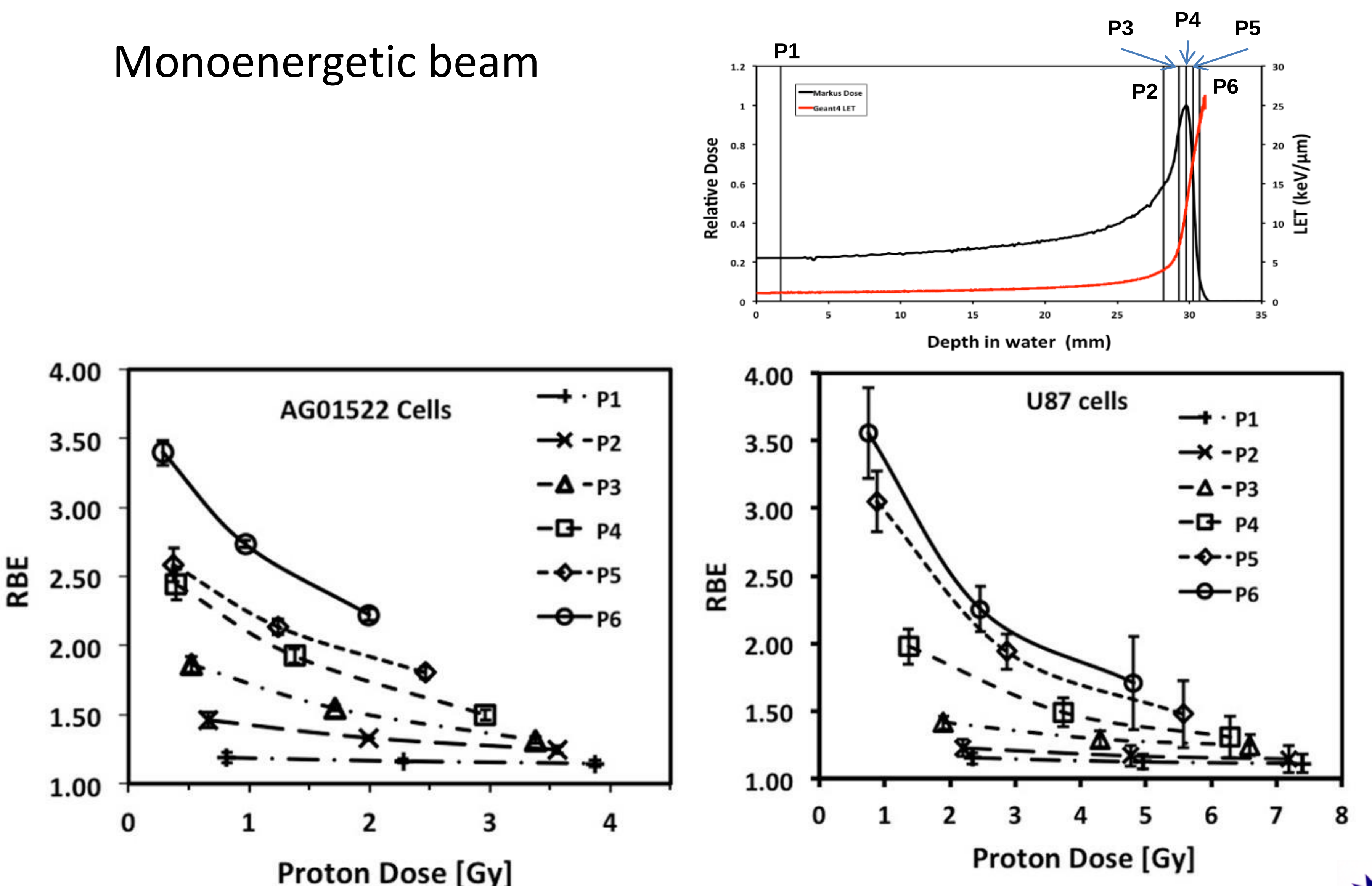
RBE versus Dose

Monoenergetic beam



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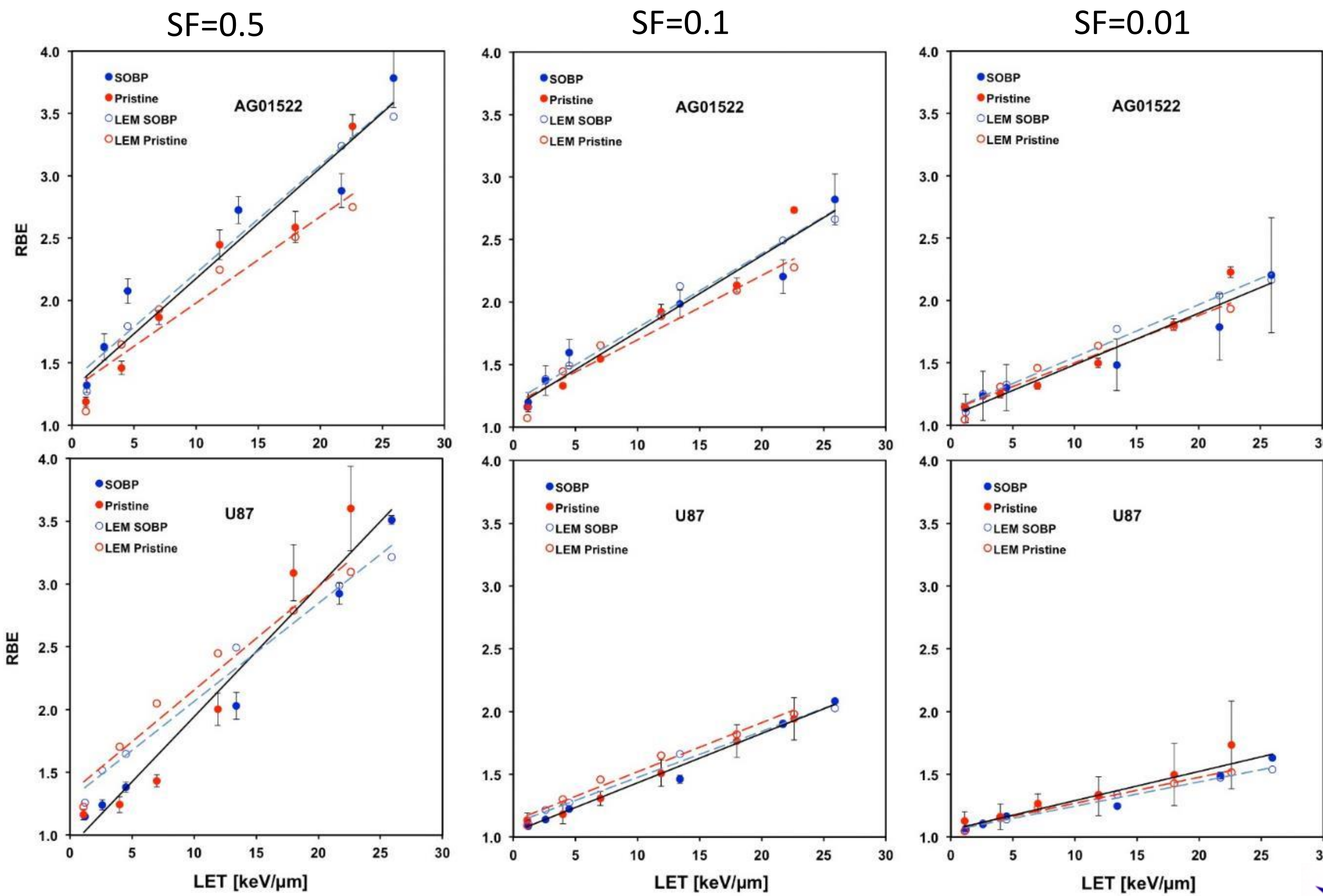
RBE versus LET



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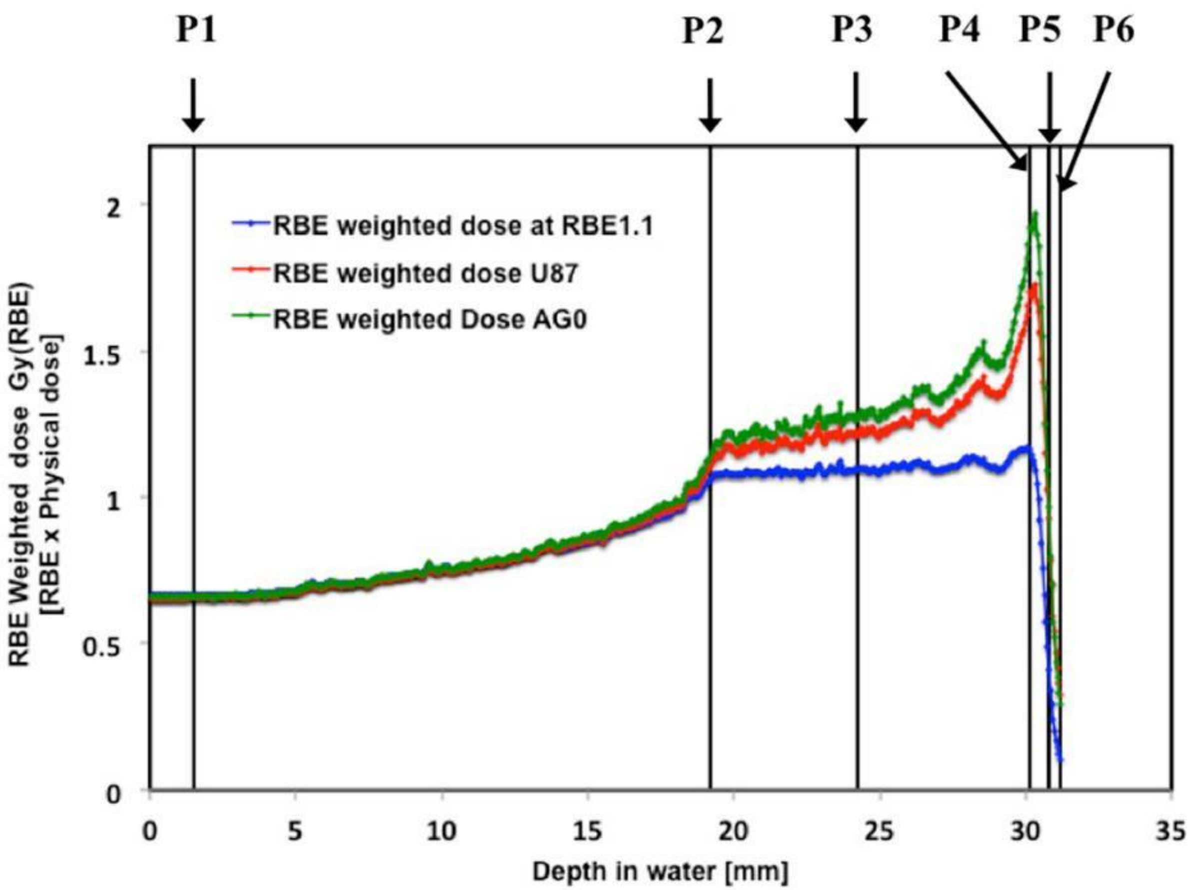
Biological Effective Dose Profile



- 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

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

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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?

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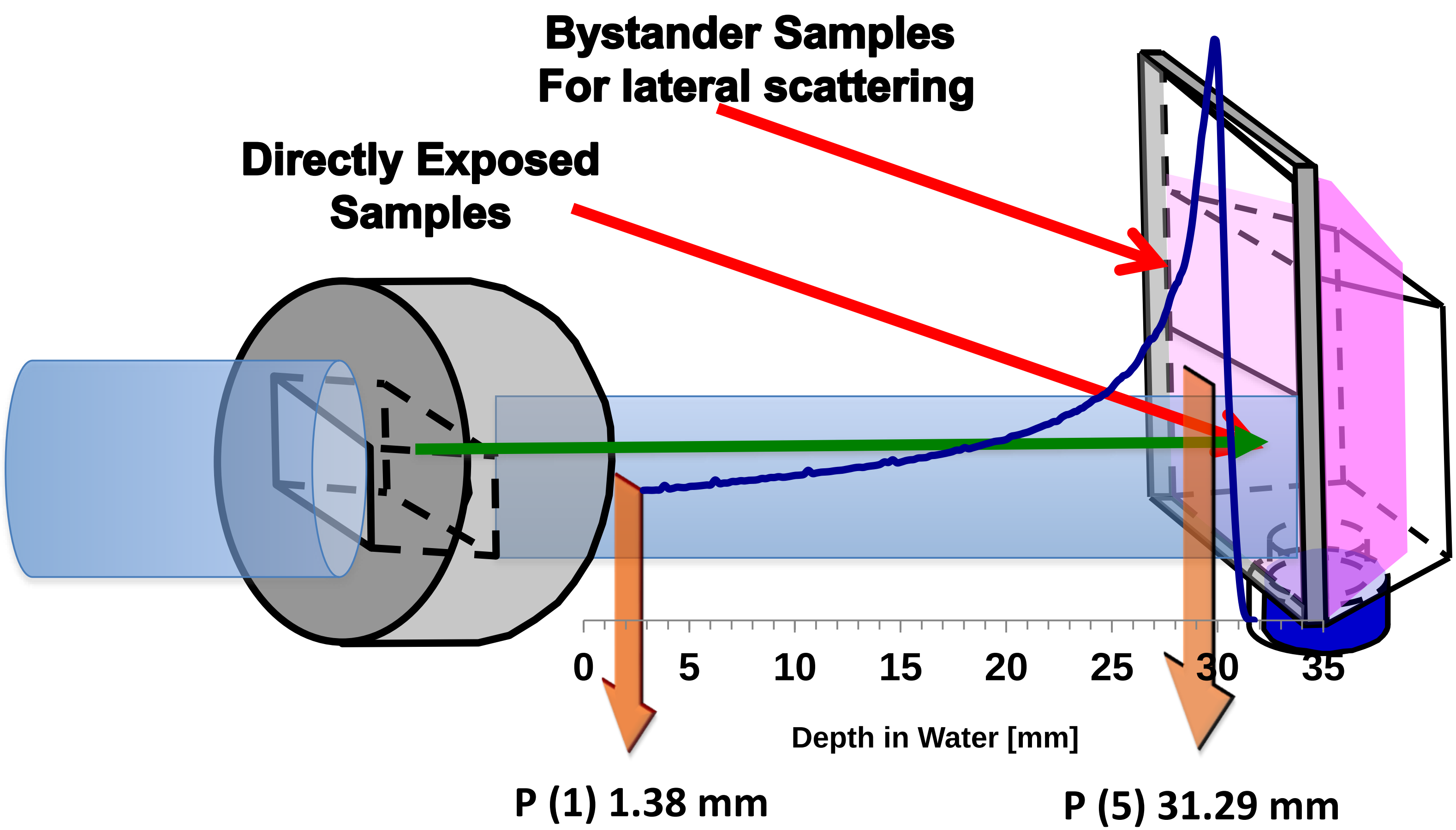
Measurement of direct and bystander DNA damage

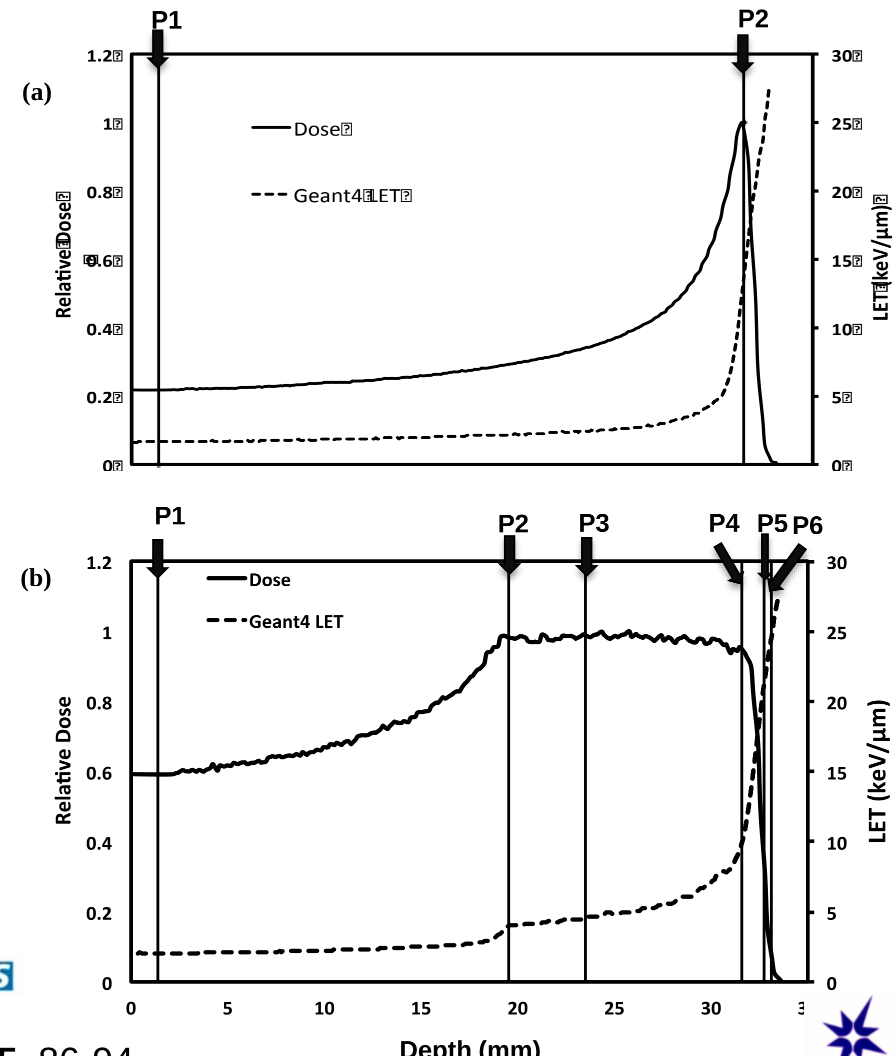
62 MeV protons INFN Catania and Clatterbridge



Beam profile – DNA damage studies

- Douglass Cyclotron Clatterbridge Oncology Centre 60 MeV
- Dose, depth and LET profiles for (a) monoenergetic and (b) modulated SOBP proton beams.
- Relative dose across the depth as measured using diode dosimetry is shown using solid lines.
- Dashed line indicate LET values as calculated using the GEANT4 toolkit.

The Clatterbridge Cancer Centre NHS
NHS Foundation Trust



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

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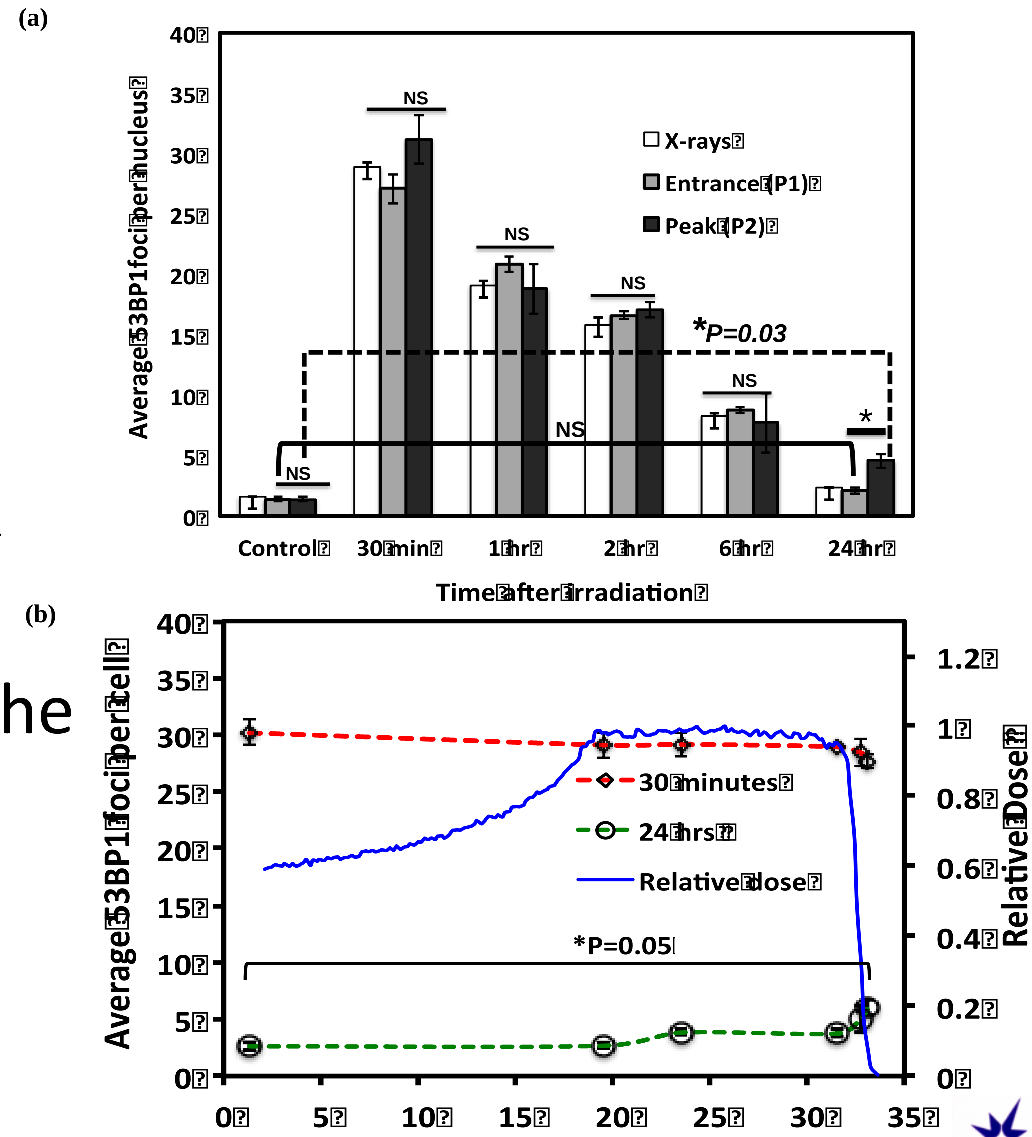
Proton – DNA damage and repair

- 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



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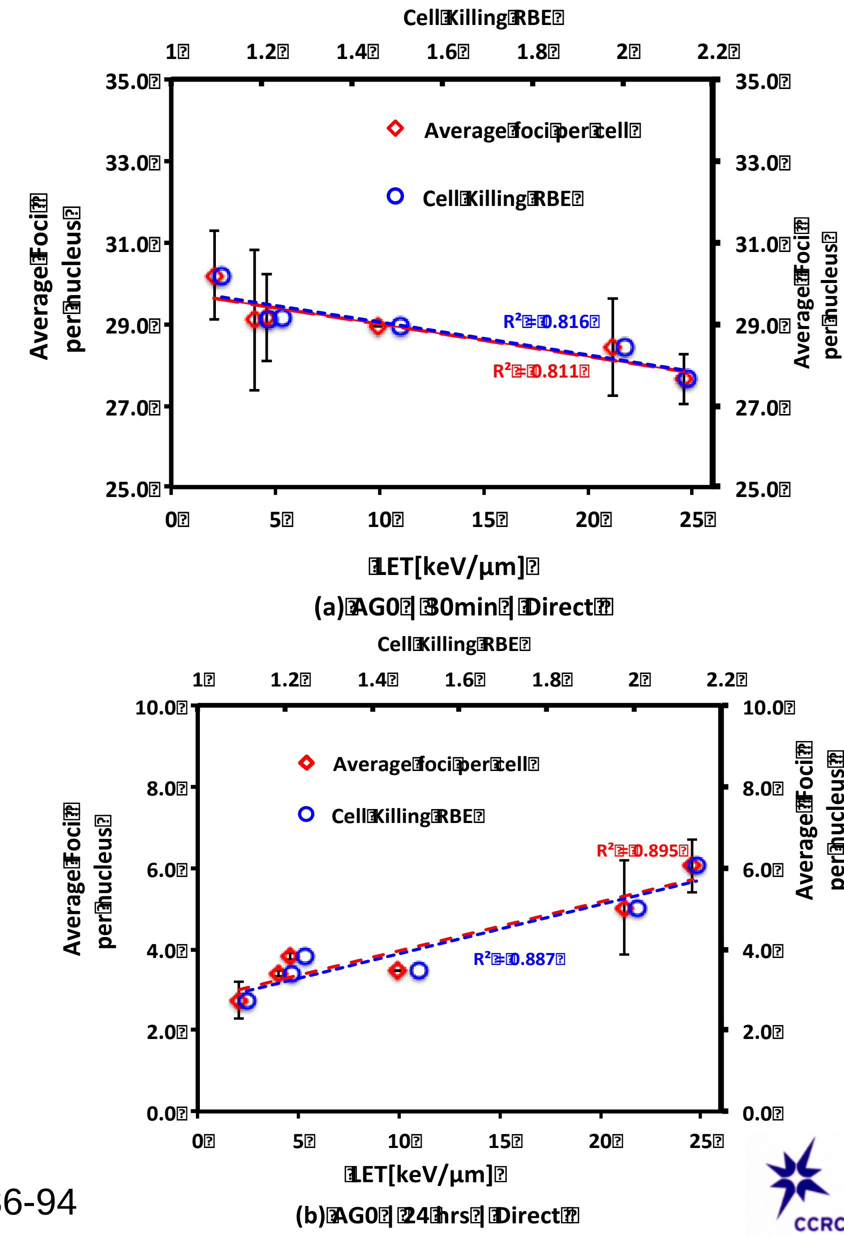
Cell killing and DNA damage

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



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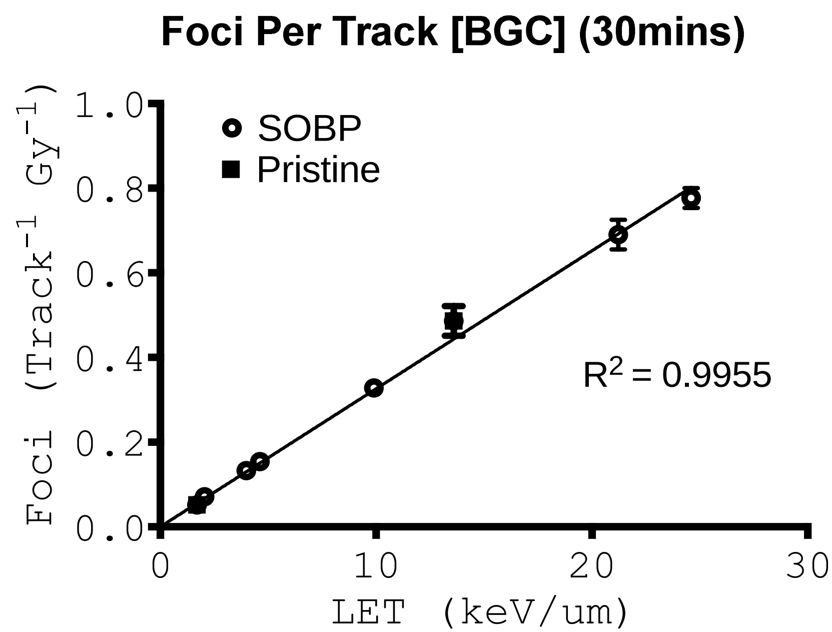
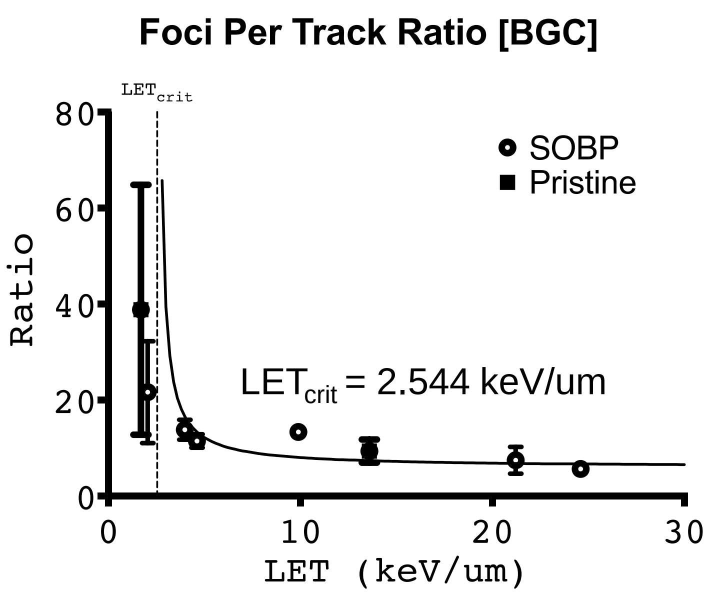
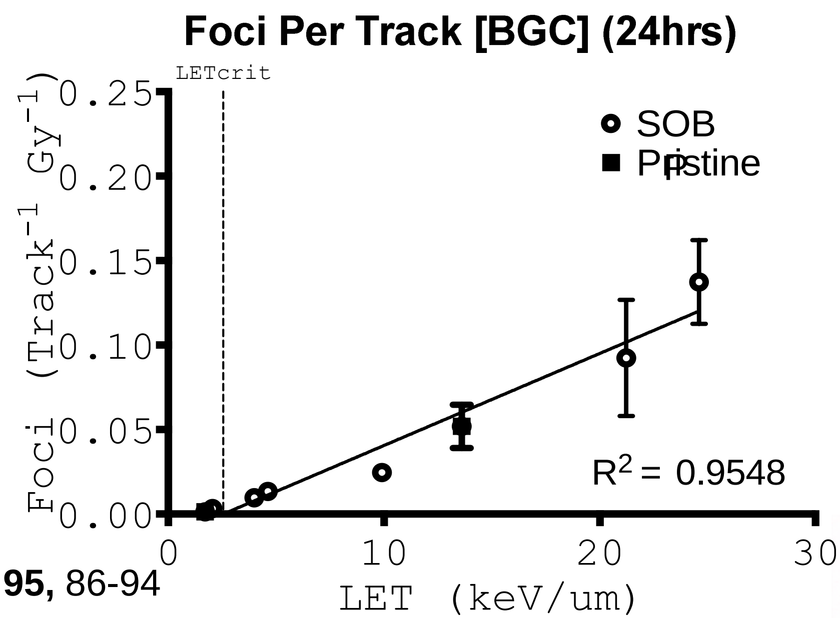
Fluence – DNA damage per track

- 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 – other ions

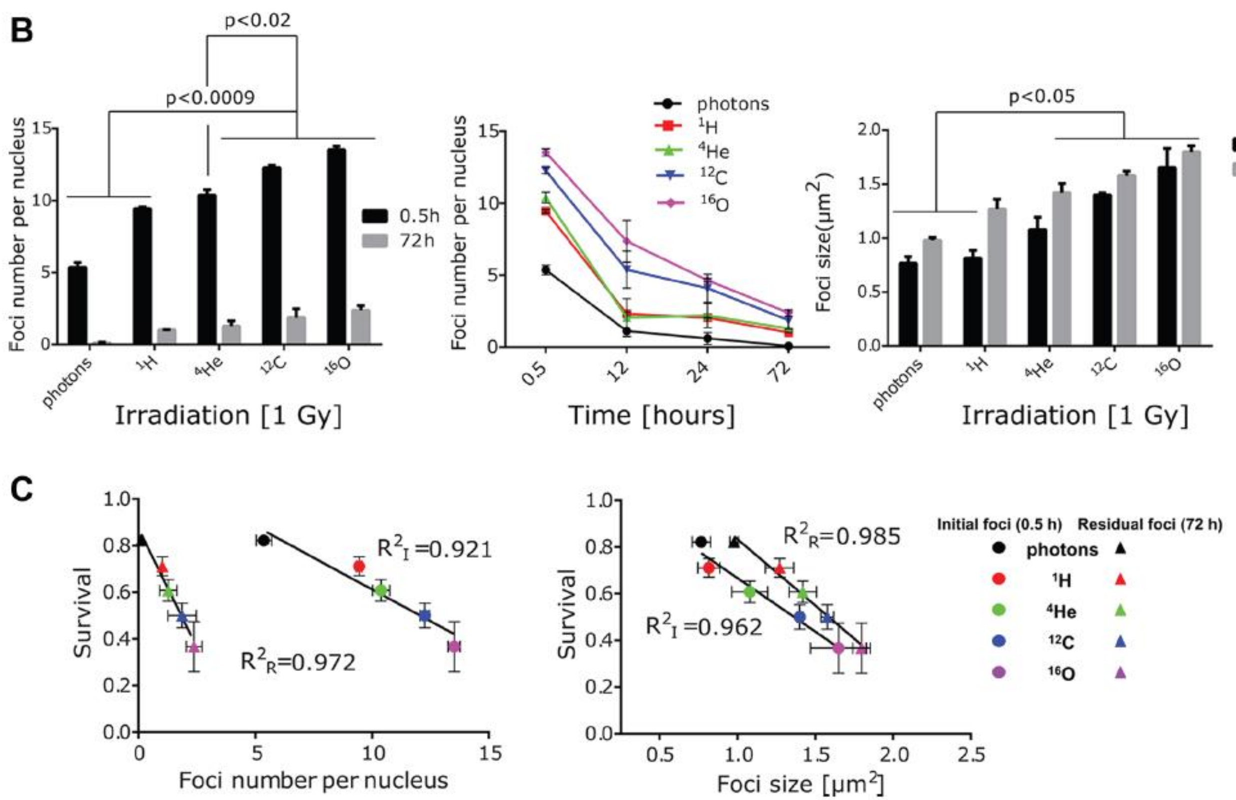
- For protons, helium, carbon and oxygen ions
- Increased yield of residual foci and foci size with LET

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]



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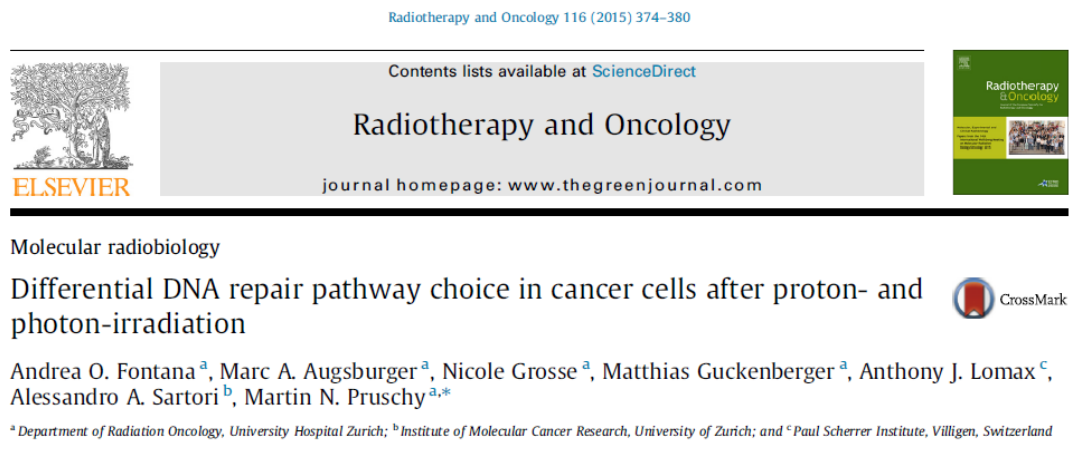
Protons and DNA repair pathway

- 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 were demonstrated.


Radiotherapy and Oncology 116 (2015) 374–380

Contents lists available at ScienceDirect

Radiotherapy and Oncology

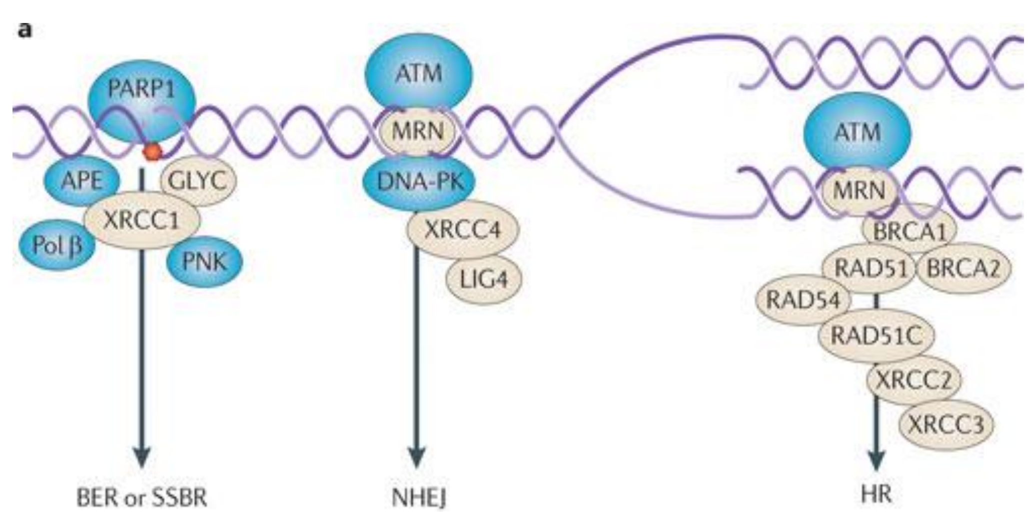
journal homepage: www.thegreenjournal.com

Molecular radiobiology

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

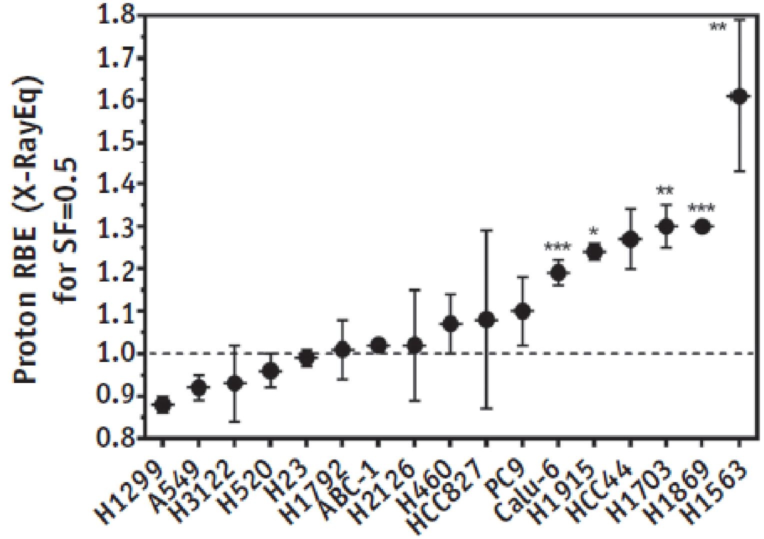
Andrea O. Fontana [a], Marc A. Augsburger [a], Nicole Grosse [a], Matthias Guckenberger [a], Anthony J. Lomax [c], Alessandro A. Sartori [b], Martin N. Pruschy [a,*]

[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 cell types

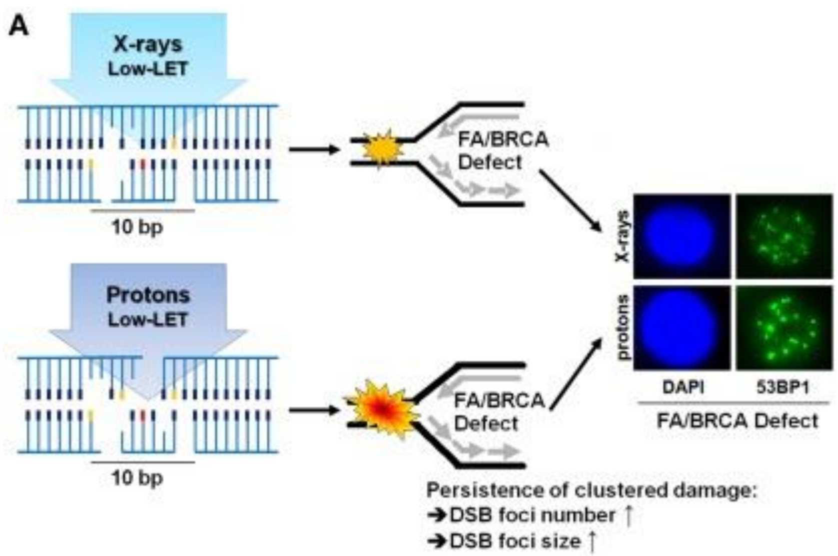
- Variations in proton RBE in 17 human lung cell lines (1.31 – 1.77 in a subset)
- Correlated with defects in the Fanconi anemia/BRCA pathway of DNA repair

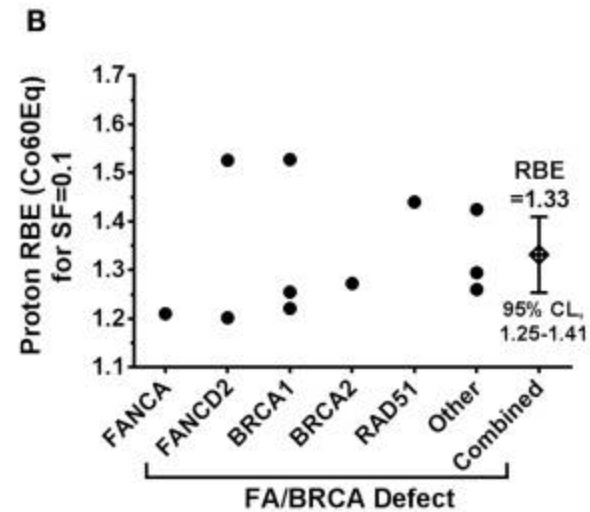


Biology Contribution

Lung Cancer Cell Line Screen Links Fanconi Anemia/BRCA Pathway Defects to Increased Relative Biological Effectiveness of Proton Radiation

Qi Liu, PhD,* Priyanjali Ghosh, BA,* Nicole Magpayo, BS,* Mauro Testa, PhD,† Shikui Tang, PhD,† Liliana Gheorghiu, MS,* Peter Biggs, PhD,† Harald Paganetti, PhD,† Jason A. Efstathiou, MD, DPhil,* Hsiao-Ming Lu, PhD,† Kathryn D. Held, PhD,* and Henning Willers, MD*





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

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Do RBE effect impact on the response to fractionated proton exposures?

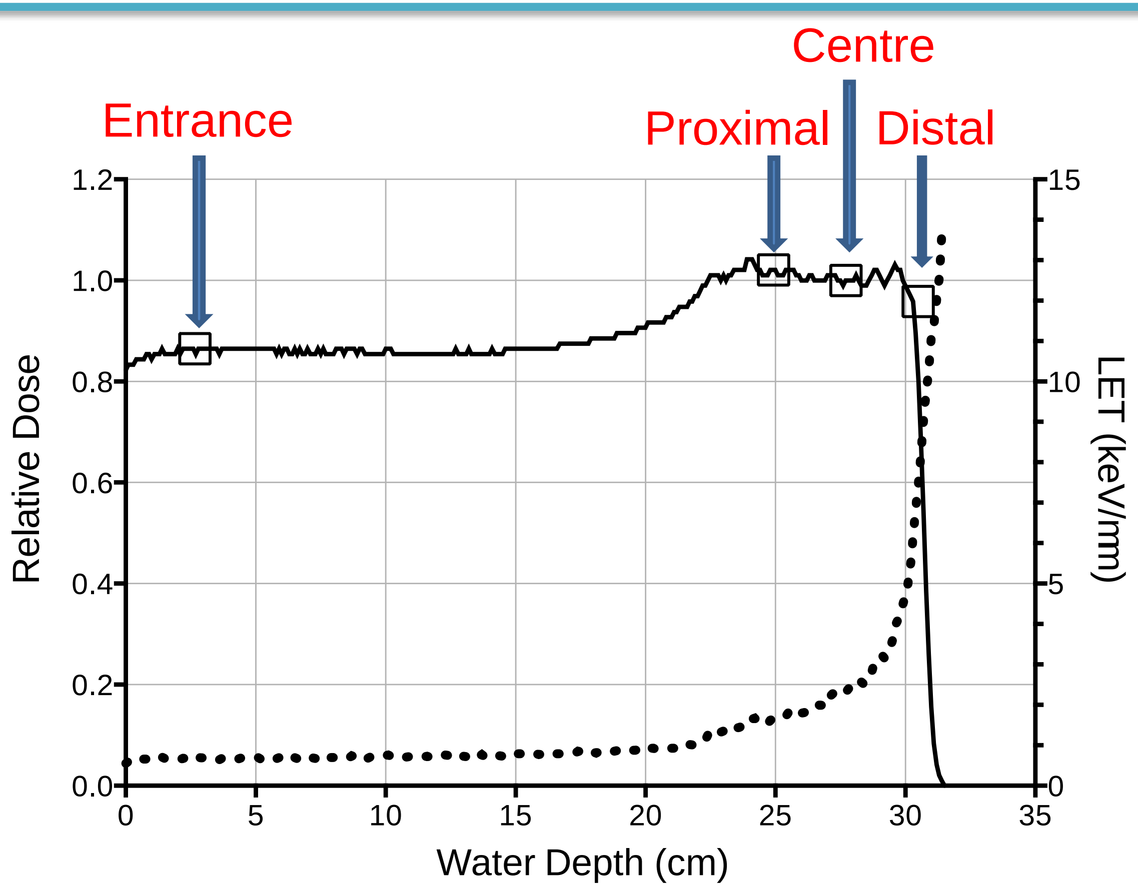
- What is the relationship between survival and lethality along a SOBP for fractionated exposures?
- What are the implications of RBE for high dose?

Proton Therapy Center, Prague

Marie Davidkova, Anna Michaelidesova, Vladimir Vondráček

Treatment room

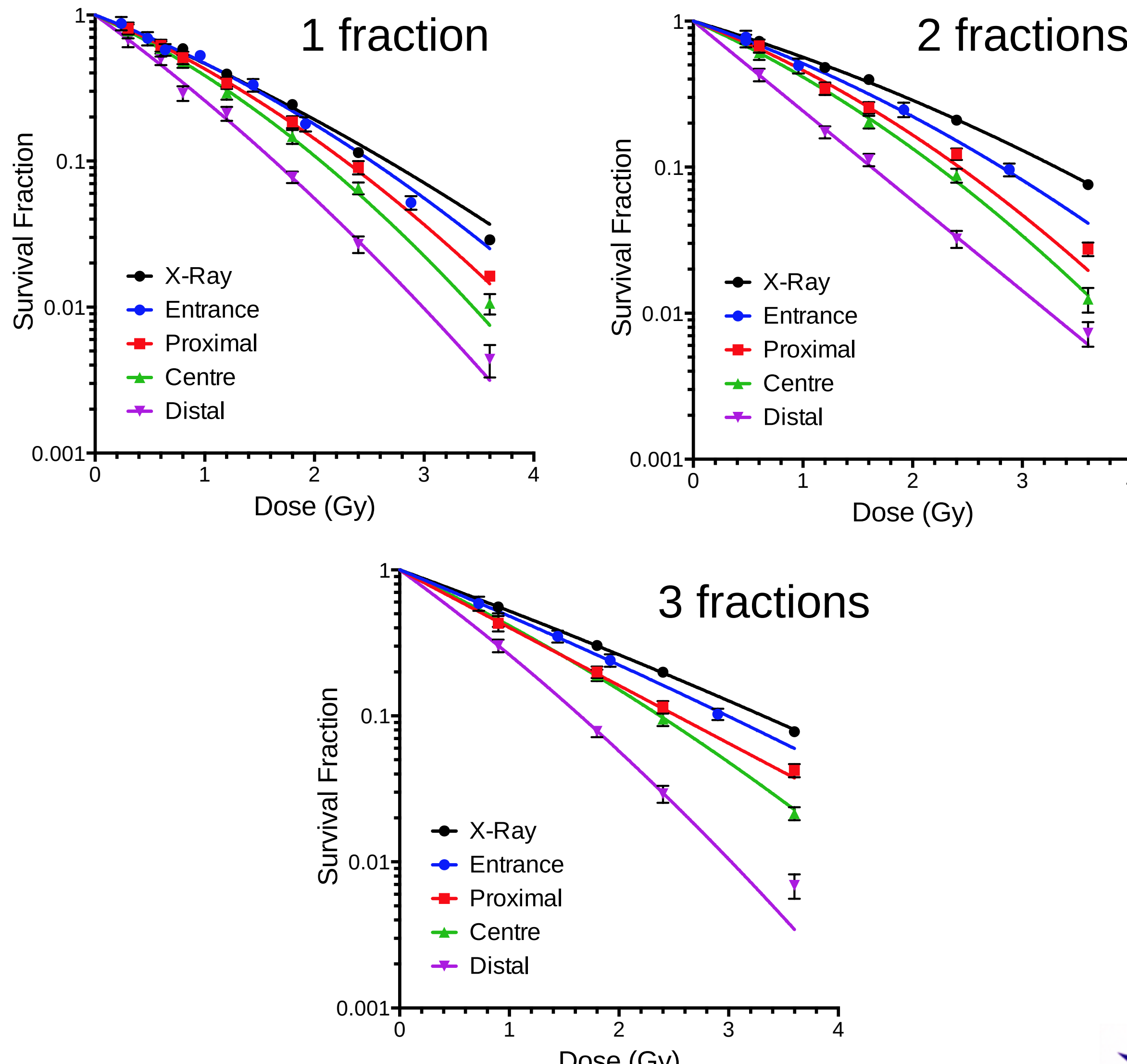
Prague Proton - uniform exposures



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.

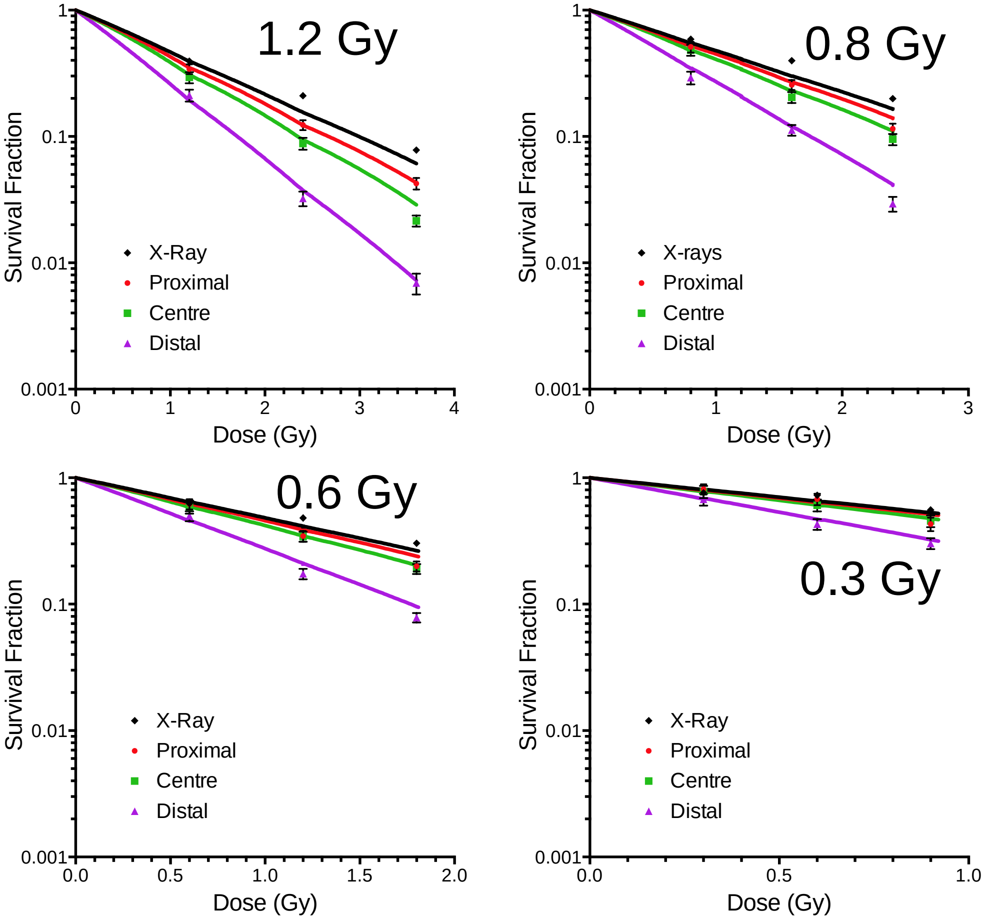
Fractionated protons exposures – total dose

- 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.

Fractionated exposures – dose per fraction



Fits obtained using the Linear Quadratic Model to estimate survival based on repeated acute response.

$$SF = \exp(-\alpha nD - \beta nD^2)$$

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

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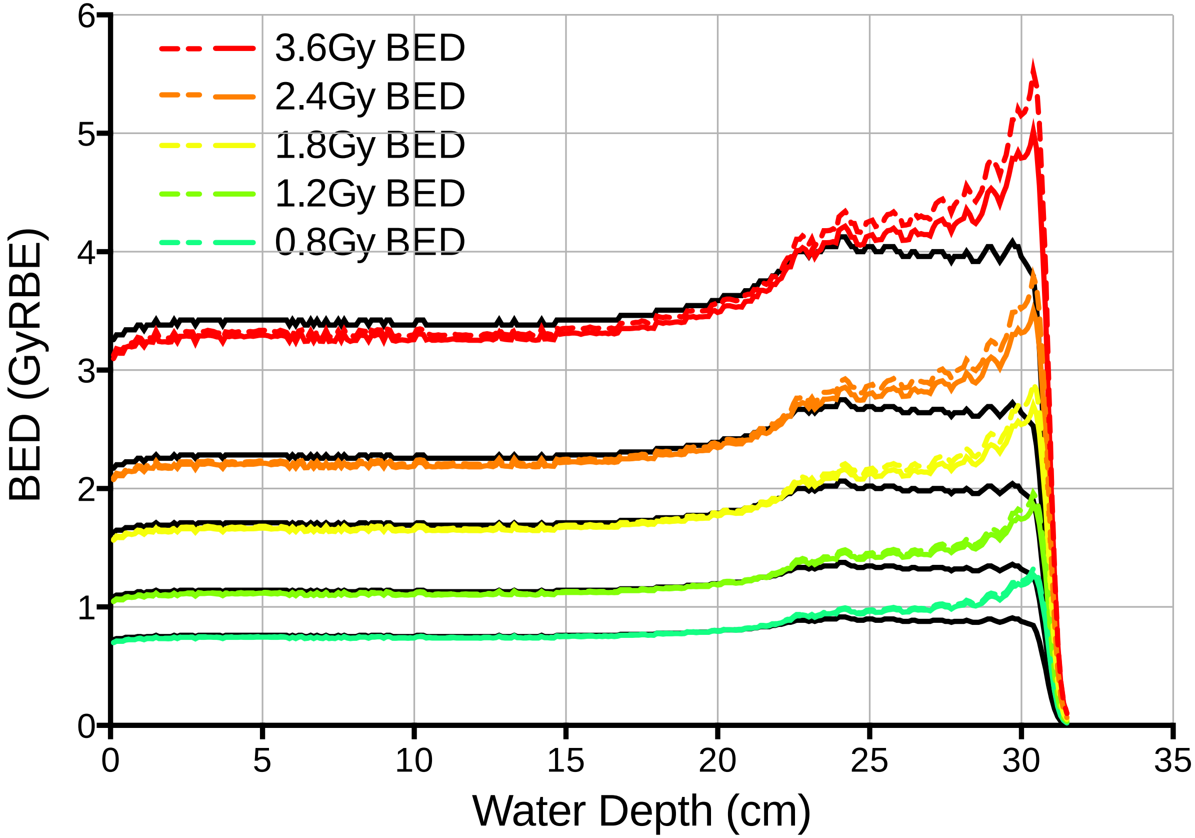
SOBP – Biologically effective dose

- 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.

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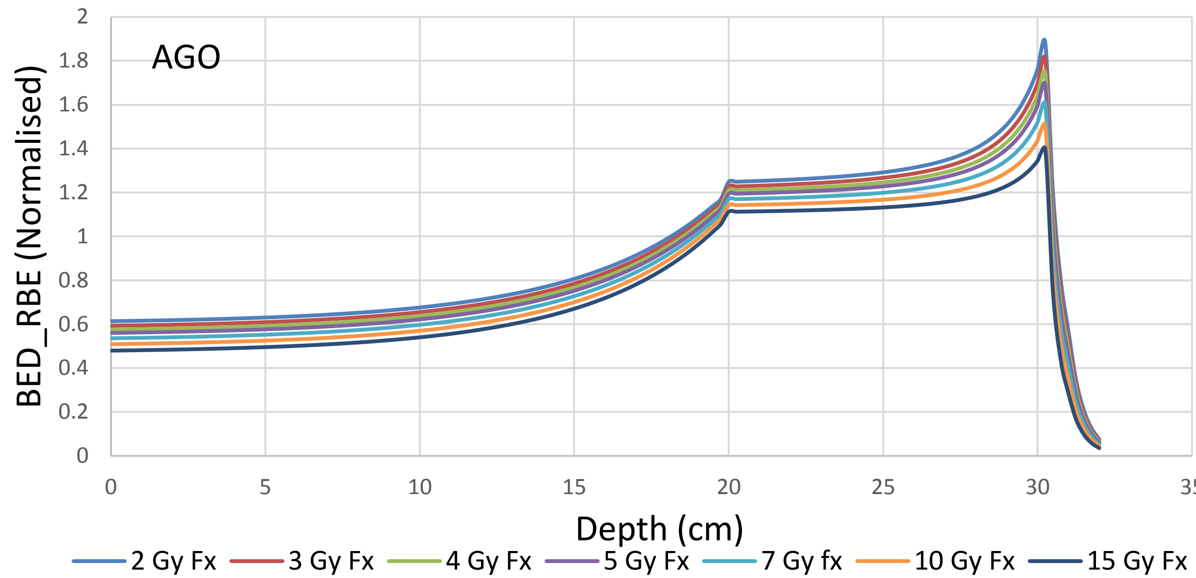
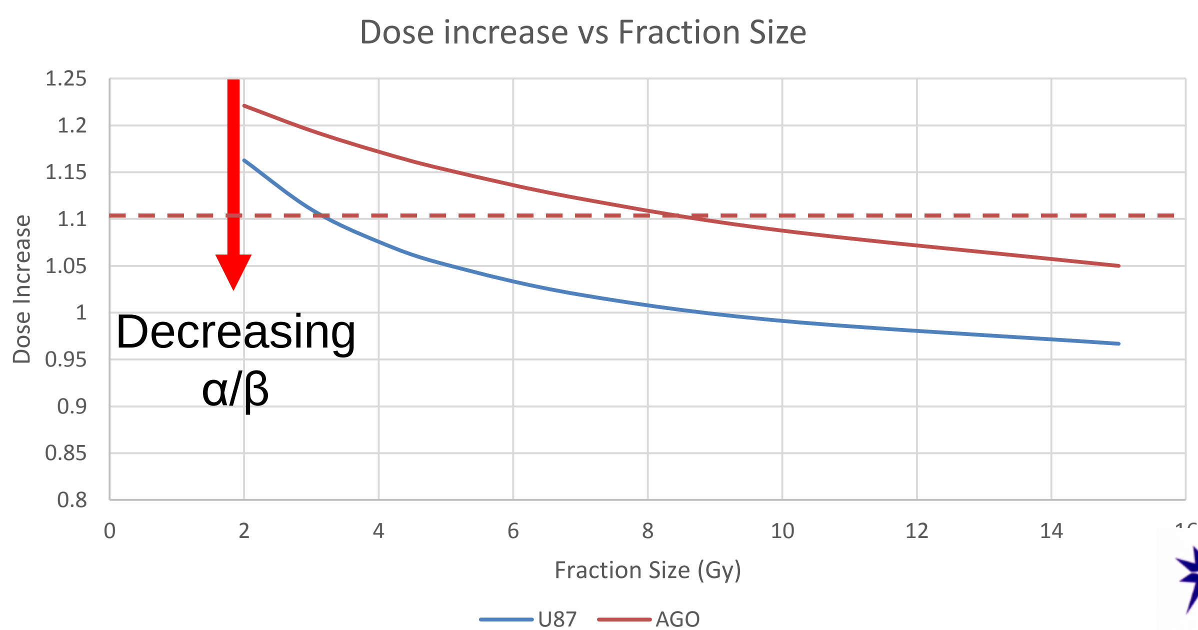
Fractionation predictions

- Decreased effectiveness with increased dose per fraction

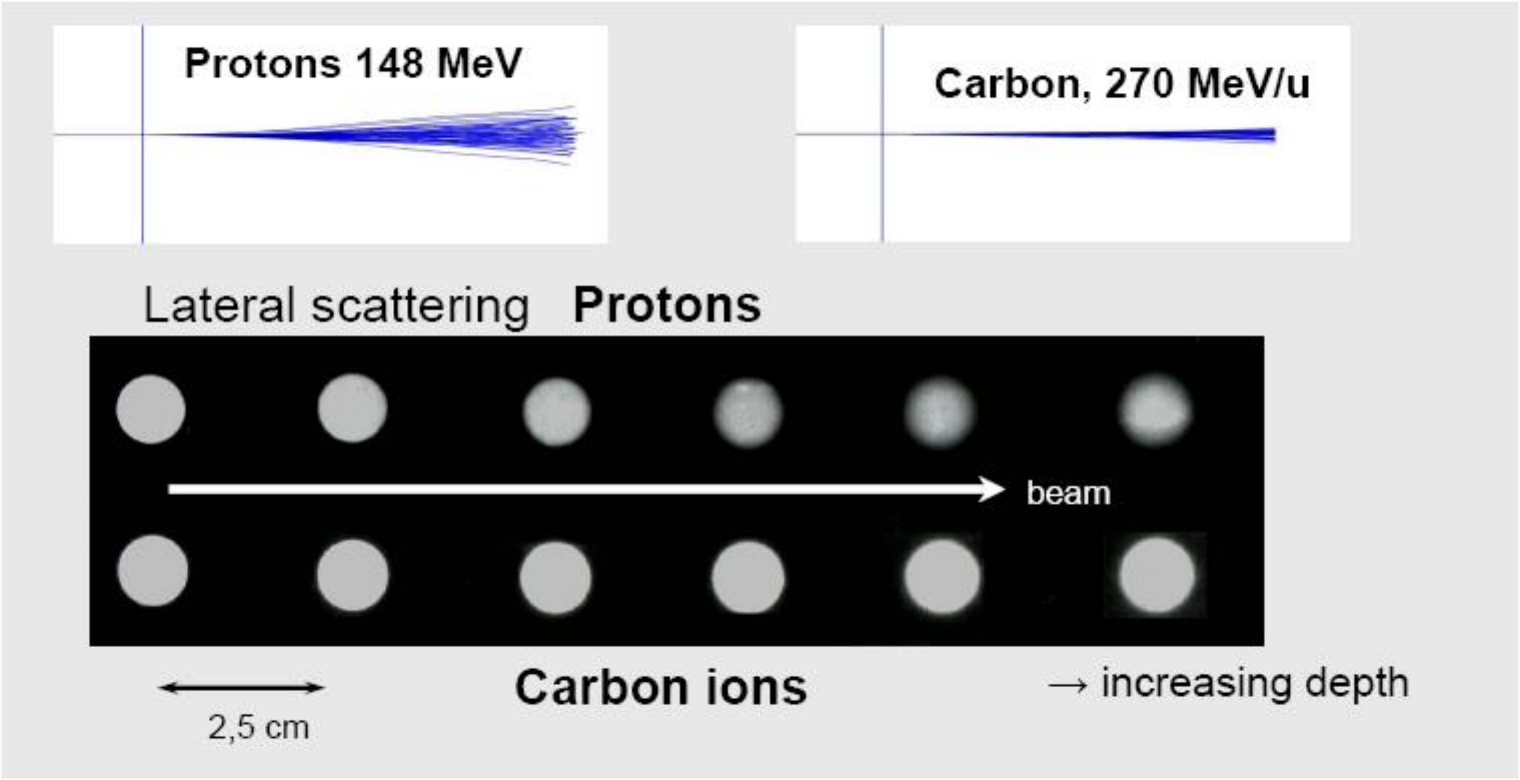


- For the target area RBE can decrease below 1.1 dependent on α/β ratio



McMahon *et al.*, in preparation

Lateral Straggling



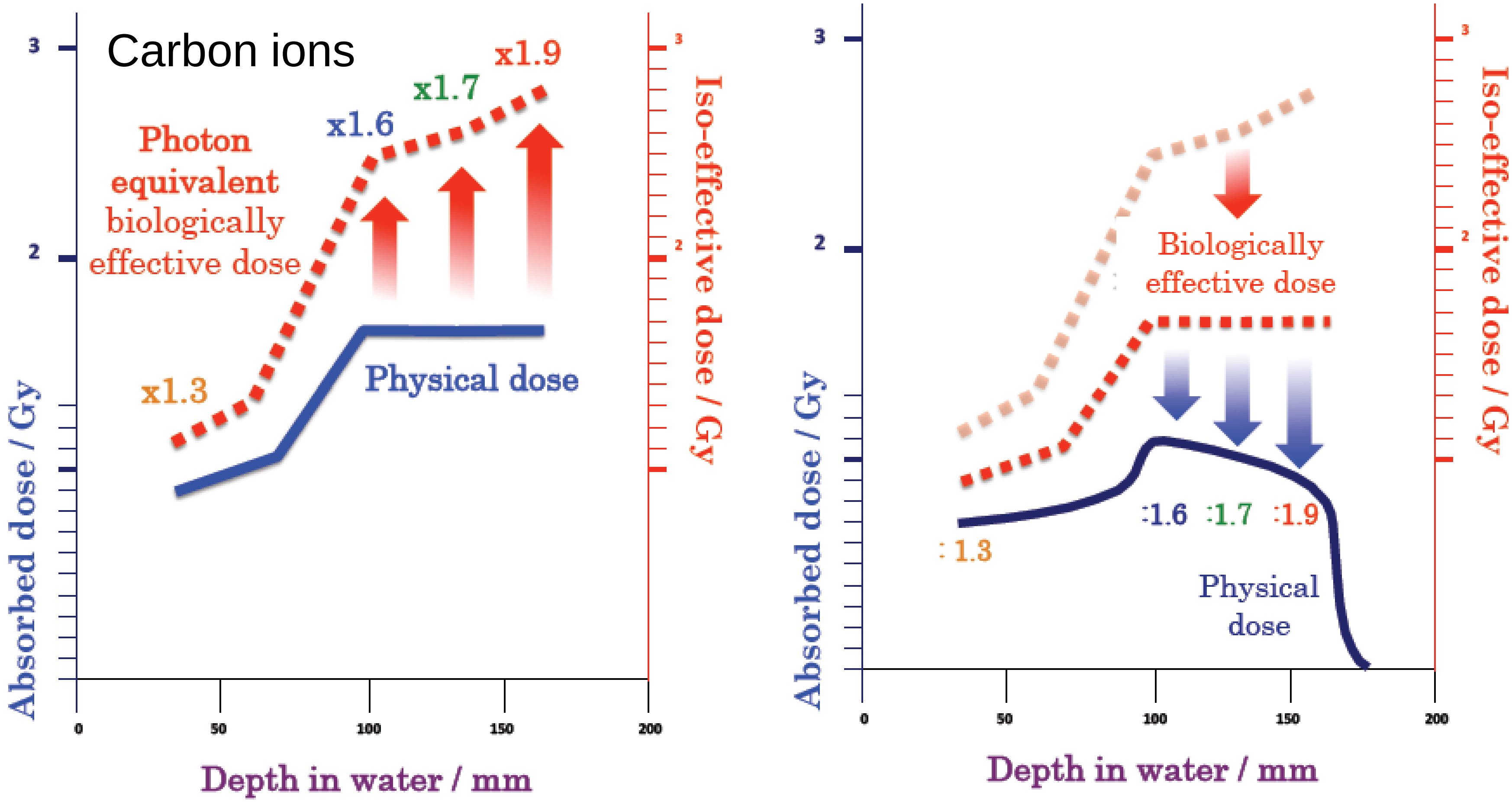
Using Carbon beams:

- Smaller dose gradient perpendicular to beam path

- High dose delivery near critical organs

Blakely et al, LBL Berkeley

RBE - painting

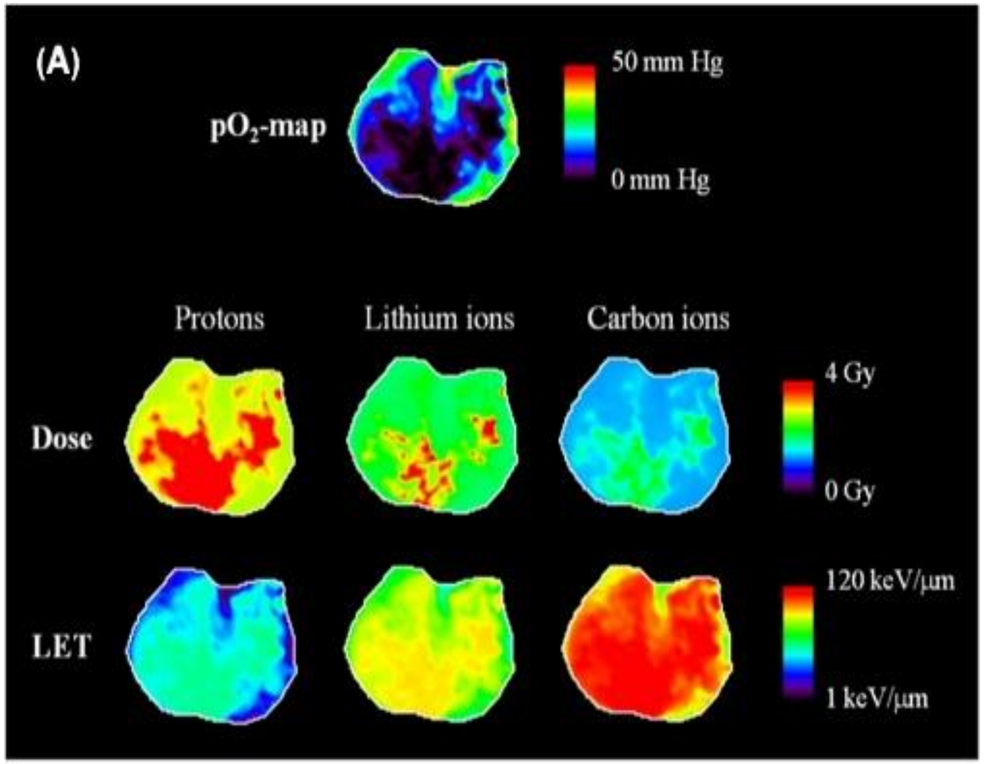


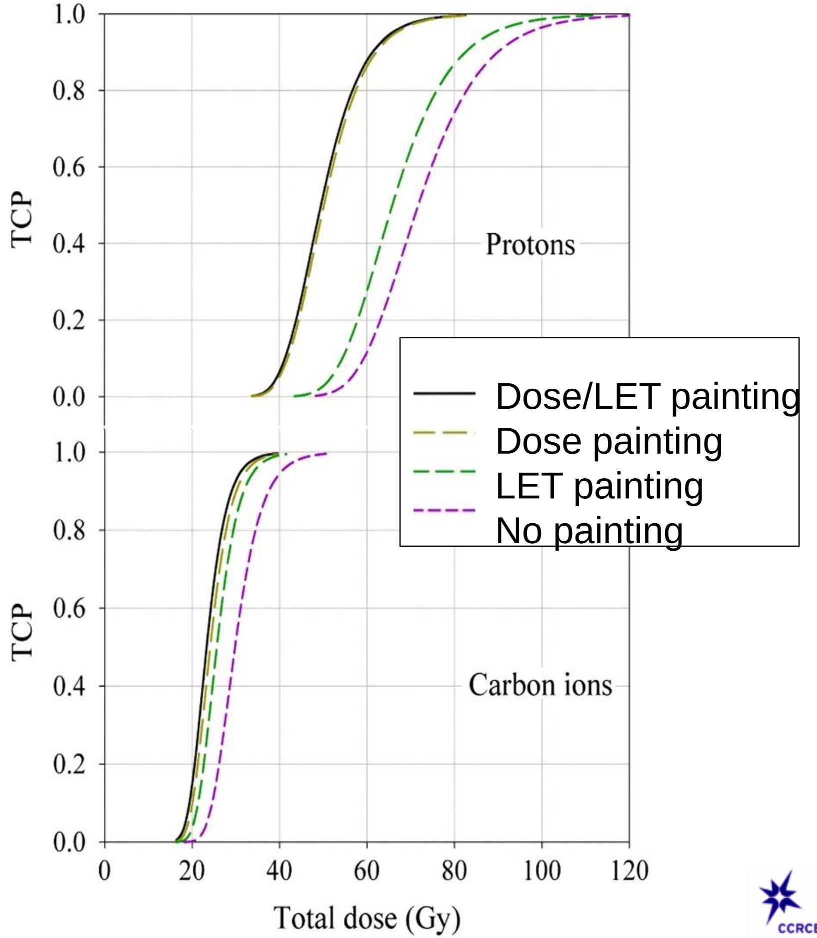
Gueulette *et al* 2010

- **A homogeneous biologically effective dose requires an inhomogeneous physical dose distribution – even for protons**

Optimising Proton Therapy plans

- Optimised plans on the basis of dose/LET/RBE
- Oxygenation is important for protons
- Need to define the impact of fractionation for plan optimisation





Malinen 2015 *Acta Oncol* **54,** 1614

Conclusions

RBE varies significantly across the Bragg curve with strong dependency on LET, Dose, and Radiosensitivity

- RBE variation for proton beams **does not significantly extend the range** of the SOBP (compared to fixed RBE = 1.1)
- Fixed RBE of 1.1 for protons **underestimates the dose delivered to the tumour volume**
- **Residual DSB foci** increase along the SOBP
- Different cell models can have different RBE values related to **defects in DNA repair**
- Future combined chemo-radiation studies with protons need to consider RBE effects
- **Biophysical models** need to be optimised for advanced radiotherapies to include clinically relevant exposure scenarios **including fractionation**
- Future treatment planning systems will input **biological parameters** to personalise the delivery of radiotherapy

CCRCB

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