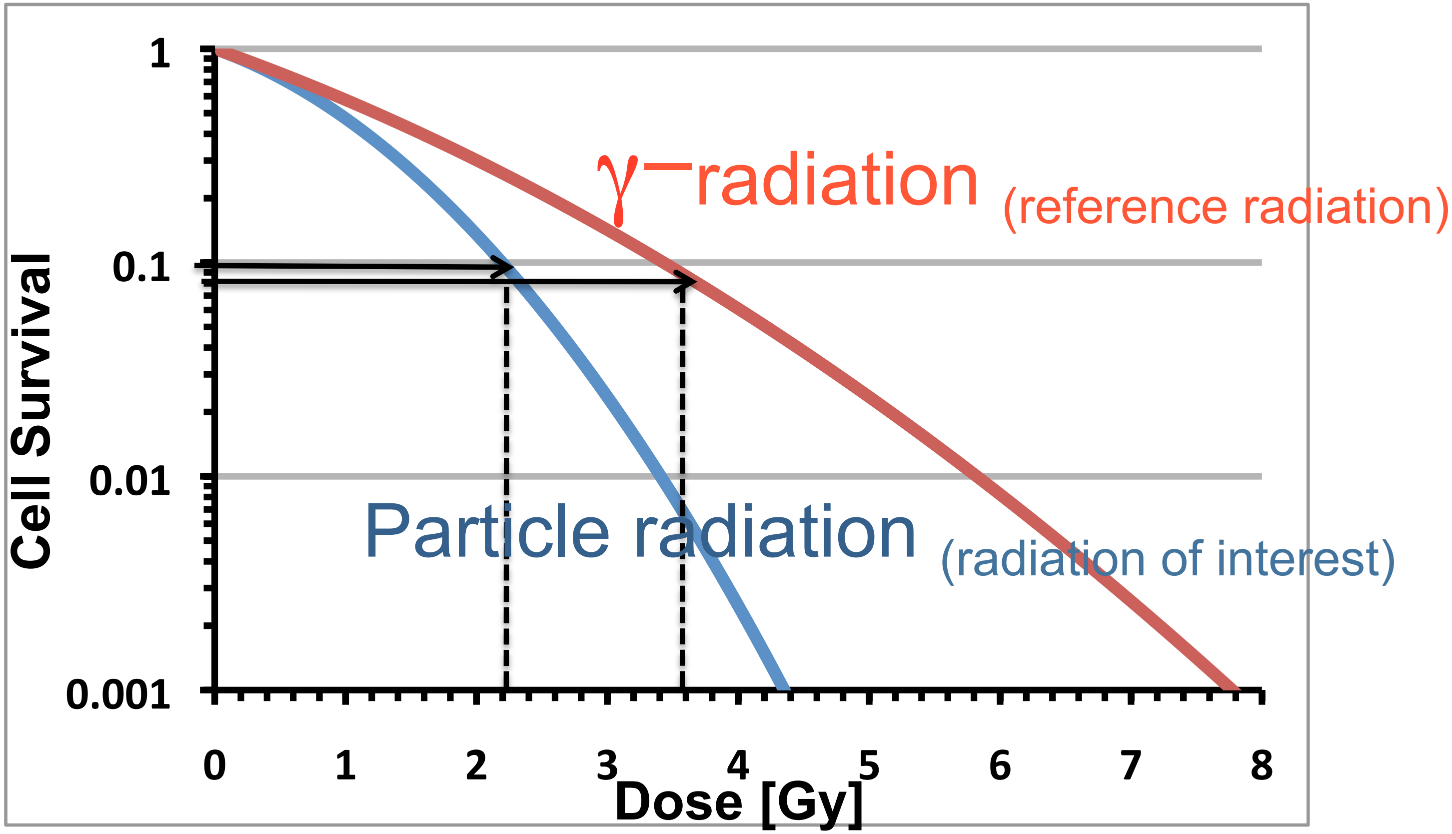
RBE: Relative Biological Effectiveness



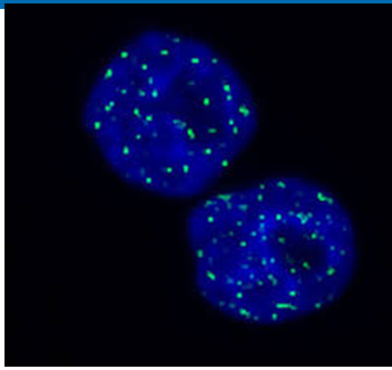
$$RBE = \frac{D_\gamma}{D_{particle}} \rangle effect \langle$$

The RBE is defined as the ratio of doses to reach the same level of effect when comparing two modalities, e.g. a reference radiation and proton radiation.

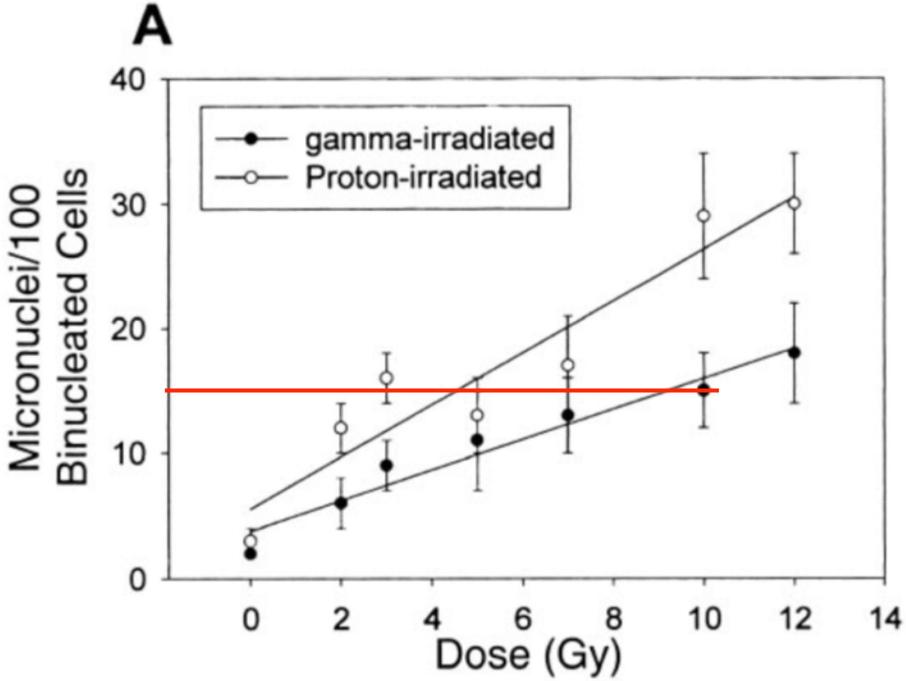
Definition of endpoint (effect) is relevant!

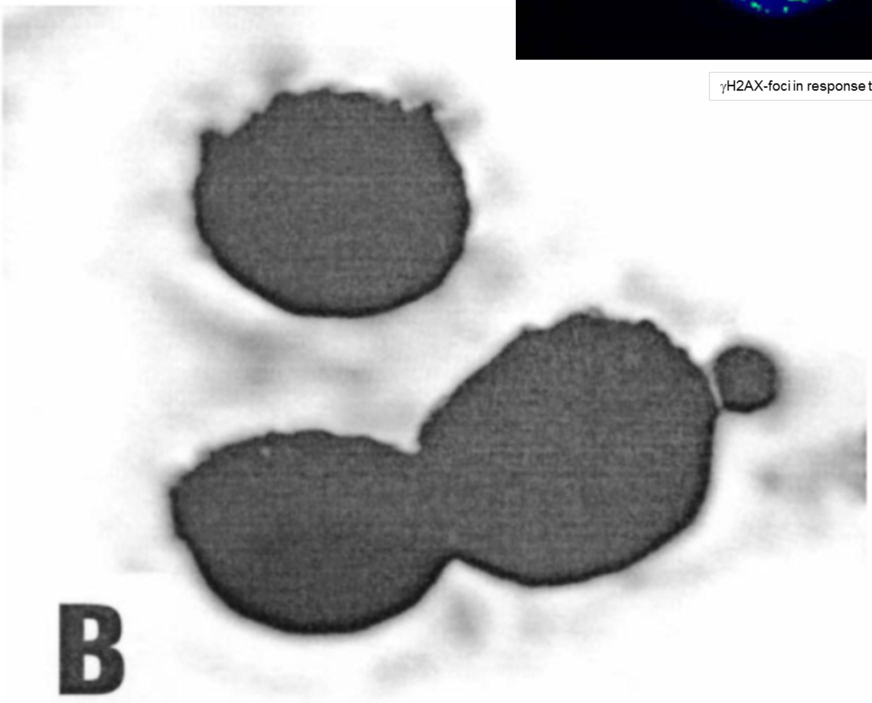
The RBE has no unique value

Mitotic Cell Death - Micronuclei Formation



γH2AX-foci in response to IR





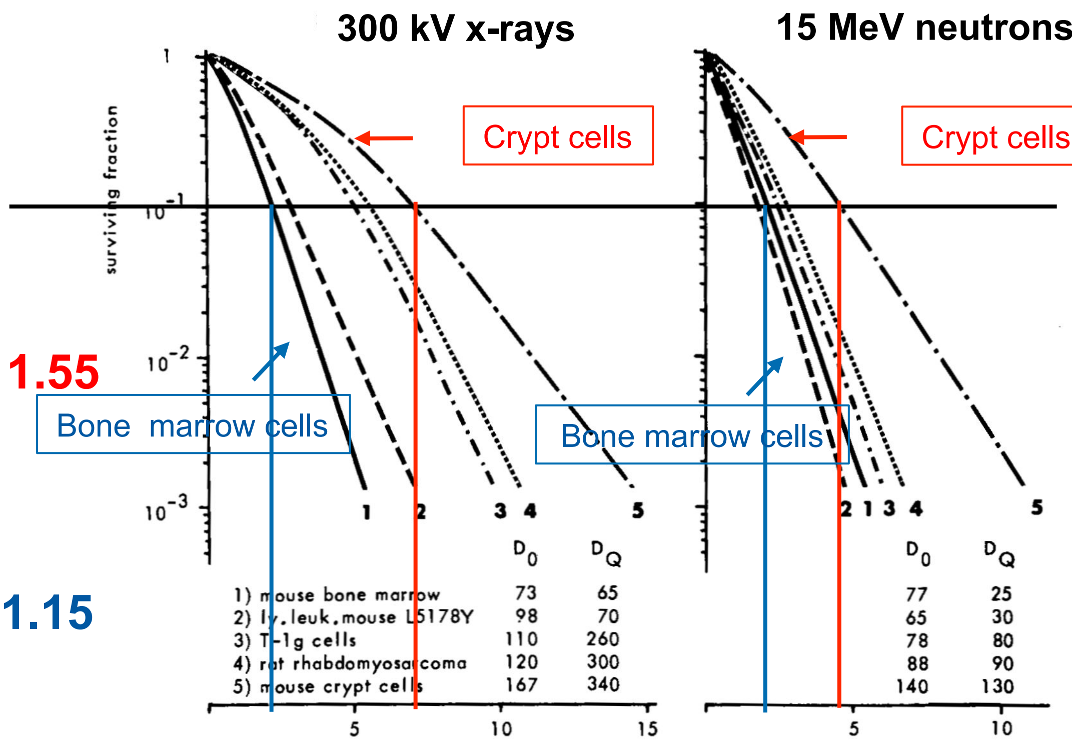
Micronuclei as measure of chromosomal damage

RBE $_{micronuclei}$: 1.7
Larger micronuclei: more severe damage
(Thyroid follicular cells
250MeV protons))

Green et al., Radiat Res. 155,32ff 2001

- Where do these differences derive from?
- Have they to be taken into consideration?
- Can we exploit them?

The RBE is different for each cell line / tissue



RBE 7 : 4.5 = 1.55

RBE 2.3 : 2 = 1.15

Cells characterized by an x-ray survival curve with a large shoulder, - indicating that they can accumulate and repair a large amount of sublethal radiation damage (sublethal damage repair) - show large RBEs for neutrons .

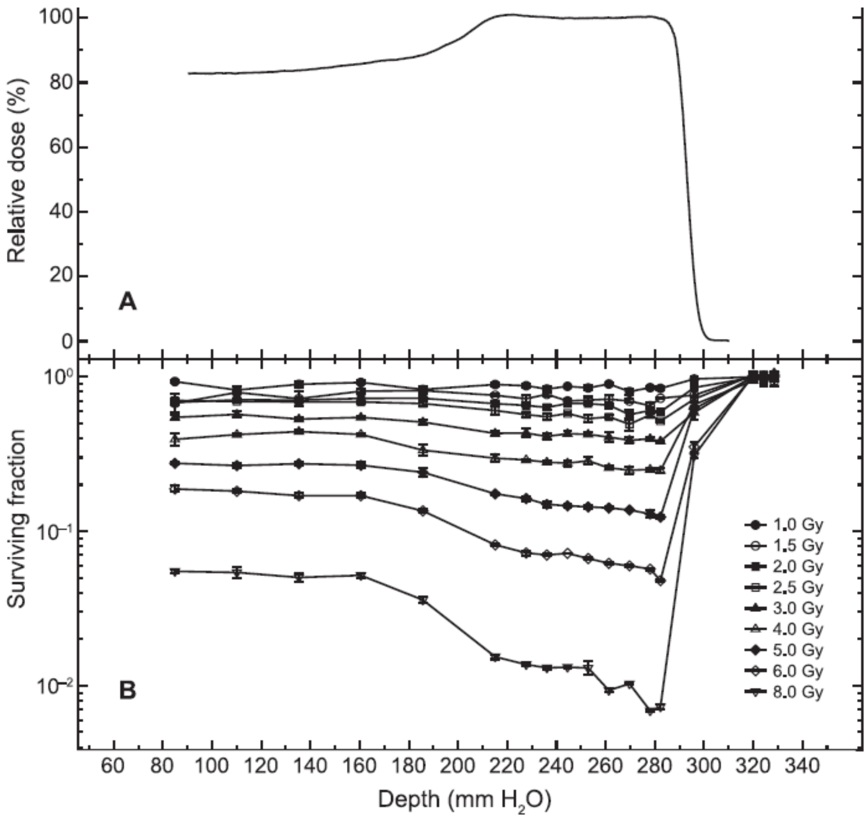
Conversely, cells for which the x-ray survival curve has little if any shoulder exhibit small neutron RBE values.

- **Differential Repair Capacity ; α/β values**

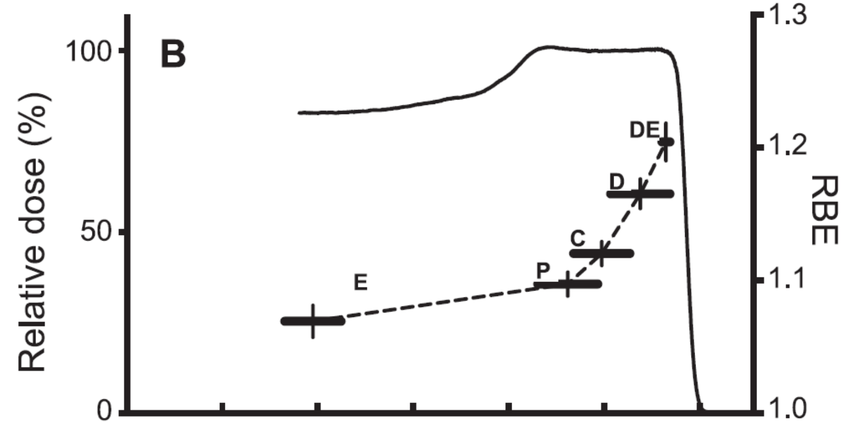
E.Hall:Radiobiology for the Radiologist

RBE: LET dependence

Radiobiological Intercomparison of the 160 MeV and 230 MeV Proton Therapy Beams at the Harvard Cyclotron Laboratory and at Massachusetts General Hospital



Chinese hamster lung cells (low α/β value)



E: entrance
P: proximal
C: central
D: distal
DE: distal edge

Wouters et al., Radiat Res, 2015,183, 174-187

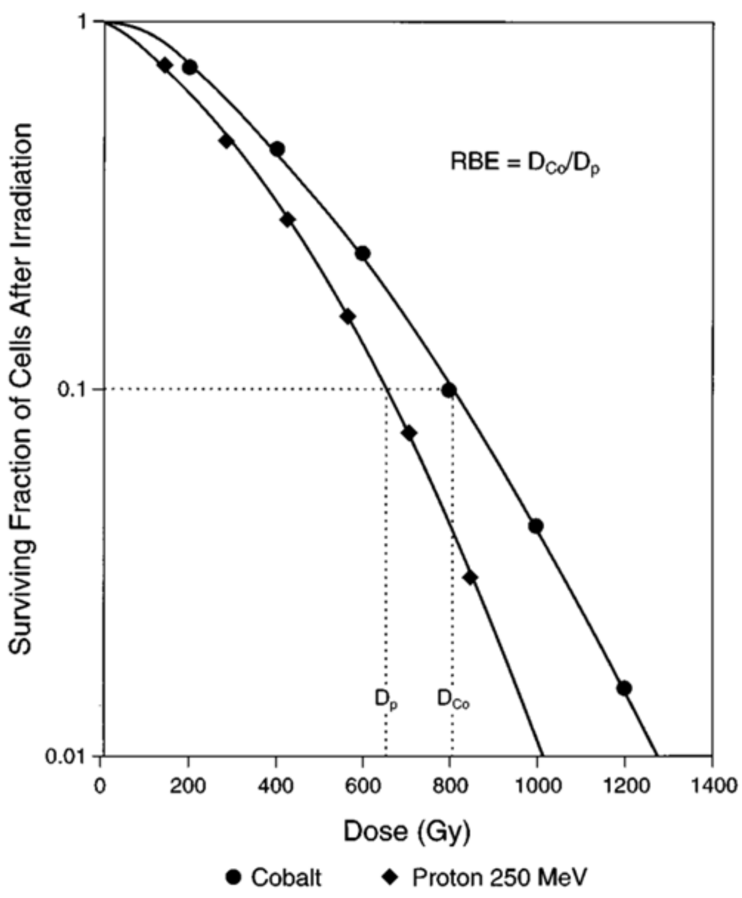
see also: Wouters et al. Radiat Res 1996; 146, 159-170

Uniform dose over SOBP (range-modulated beam), but non-uniform LET over SOBP, with increased LET at distal edge

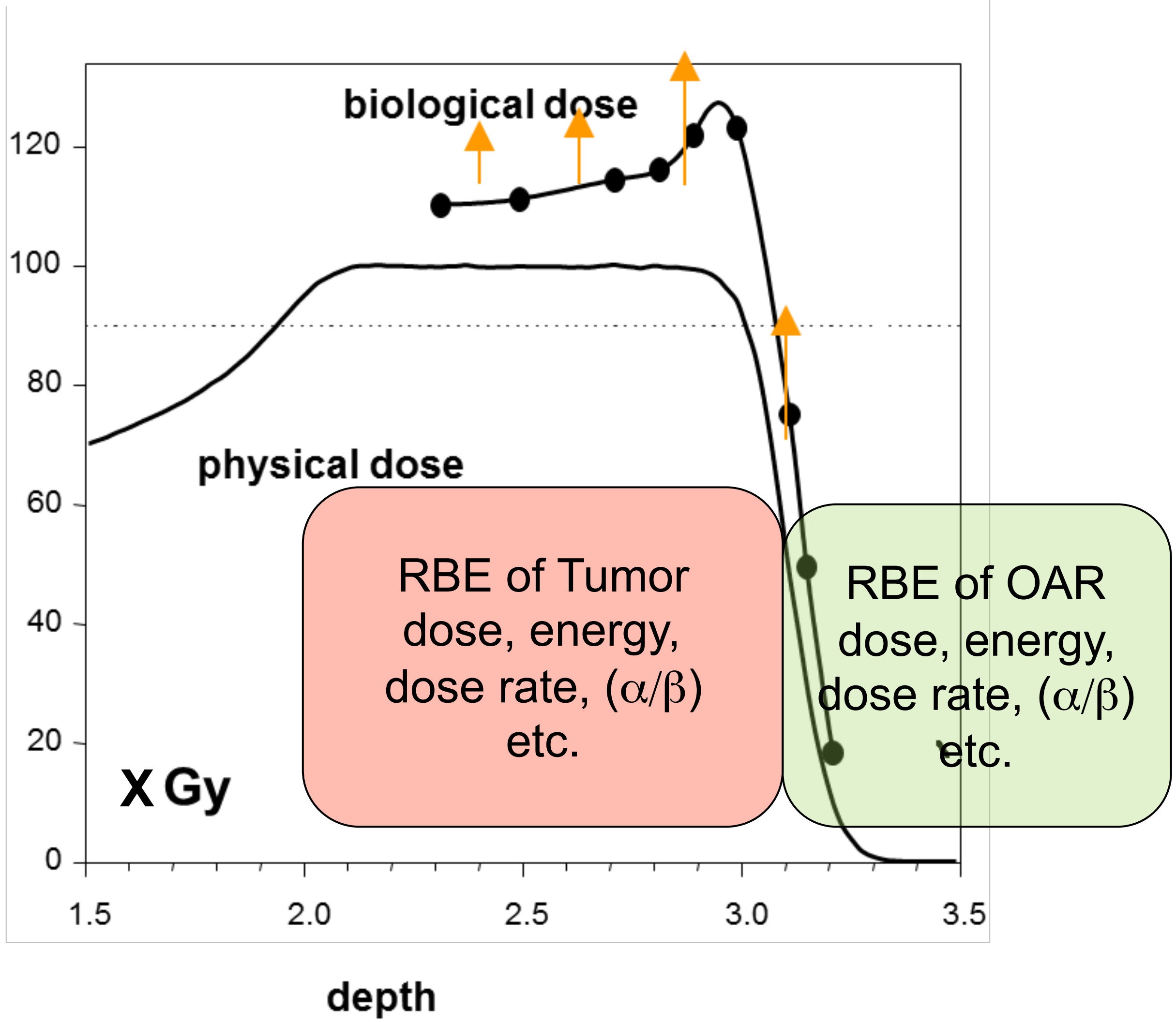
RBE is dependent on

- Cell line / Organ
- Endpoint
- α/β-ratio
- Recovery
- …

- Energie/LET
- Dose
- Dose rate
- Fractionation
- ….



RBE is not a unique value



- Uniform physical dose over SOBP, but non-uniform biological dose over SOBP
- Nevertheless, current clinical practice: use of an RBE 1.1

adapted from Paganetti, Med. Phys. 2000

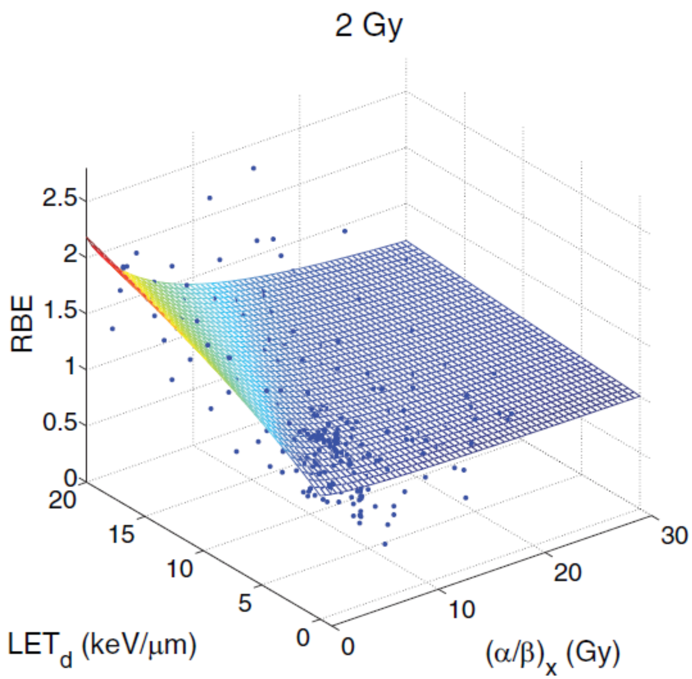
RBE is not a unique value

- RBE increases with decreasing dose
- The higher the LET, the larger the effect
- RBE increases for cells/tissues with smaller α/β ratios
- What does it mean for the RBE of OAR, at distal end of SOBP?
- Will treatment planning integrate flexible RBE?
- However, RBE's primarily based on (cell) survival studies

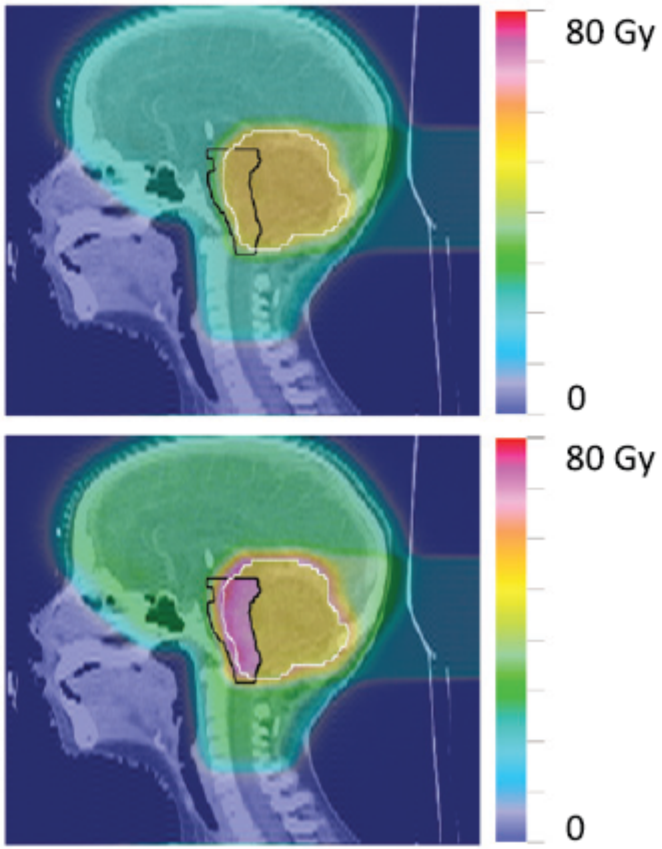
A phenomenological relative biological effectiveness (RBE) model for proton therapy based on all published *in vitro* cell survival data

Phys. Med. Biol. 60 (2015) 8399–8416

Aimee L McNamara, Jan Schuemann and Harald Paganetti

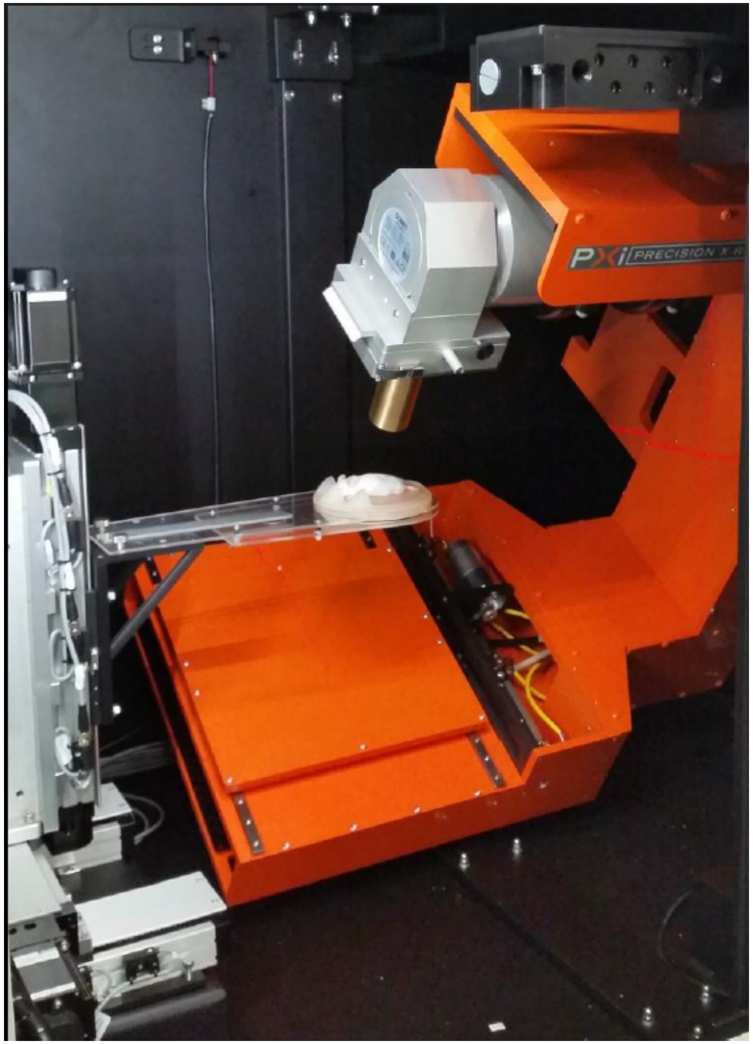


RBE for cell survival as a function of LET and α/β



Patient simulation studies (H&N; brain stem) with RBE 1.1 vs $RBE_{modeled}$

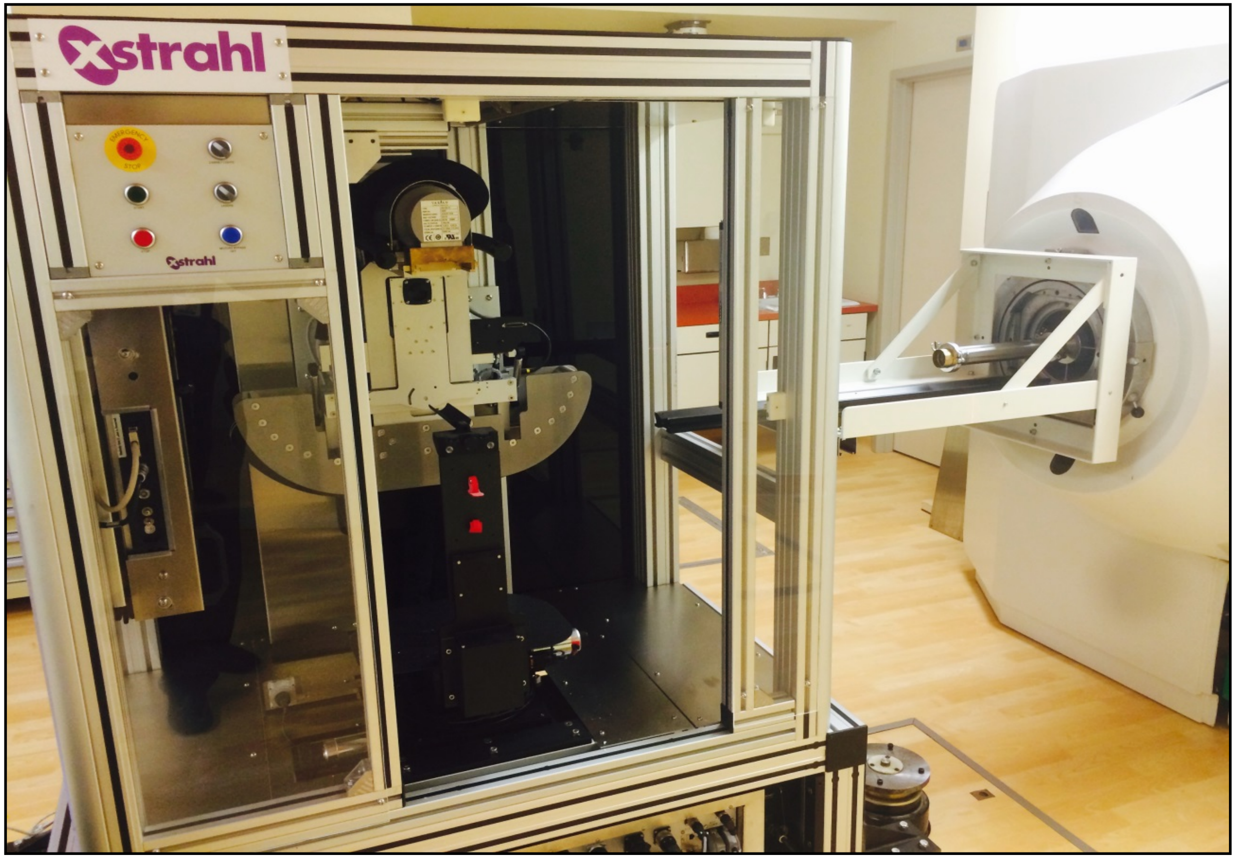
Small Animal Image-Guided Radiotherapy Platforms



Courtesy of Paul DeJean – Precision X-ray

UniversityHospital
Zurich

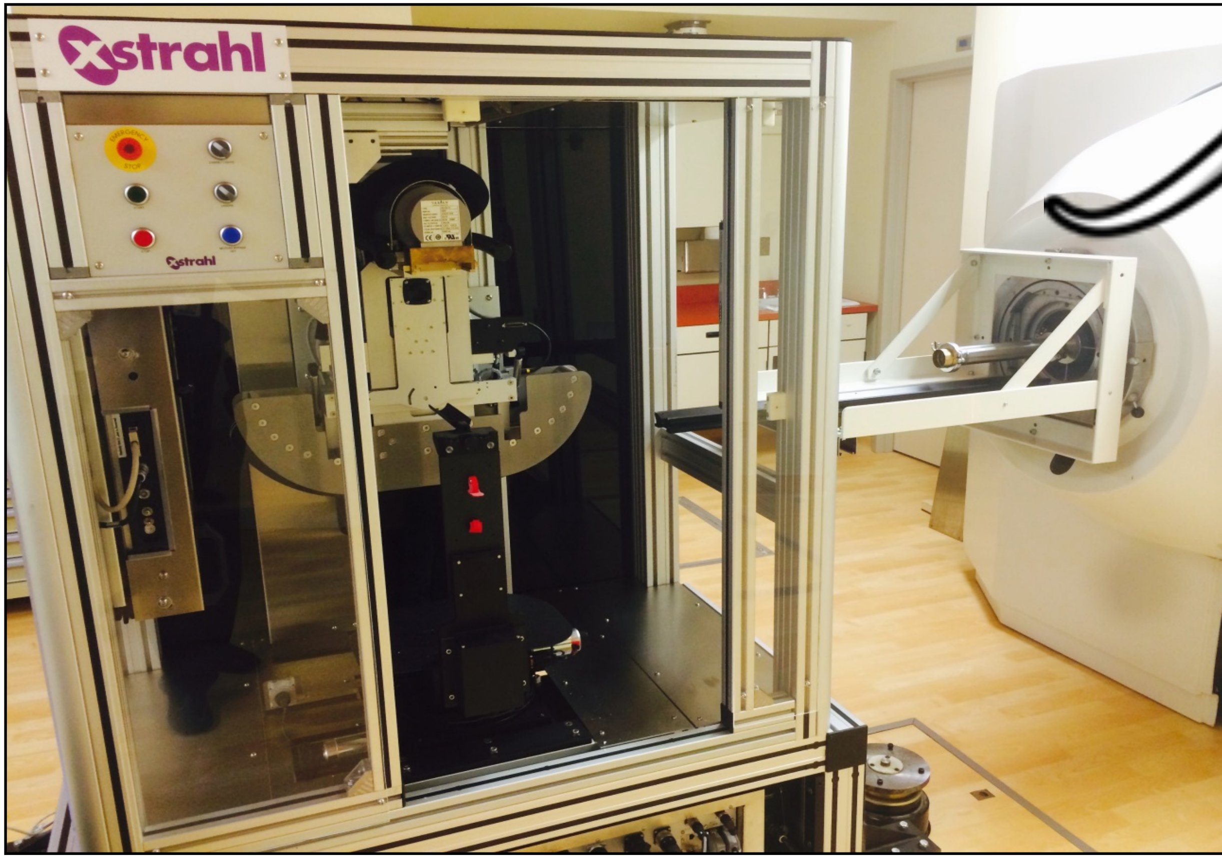
Small Animal Proton Radiotherapy Platform:



Courtesy of Eric Ford – University of Washington
Courtesy of Adrian Treverton – Xstrahl Inc

What are the relevant experiments to be performed?

Small Animal Proton Radiotherapy Platform:



Courtesy of Eric Ford – University of Washington
Adrian Treverton – Xstrahl Inc

- Confirmation of in vitro-derived results; in vivo RBEs; α/β-values, etc.: normal tissue
- Planning and treatment studies with small volumes; flexible RBE's

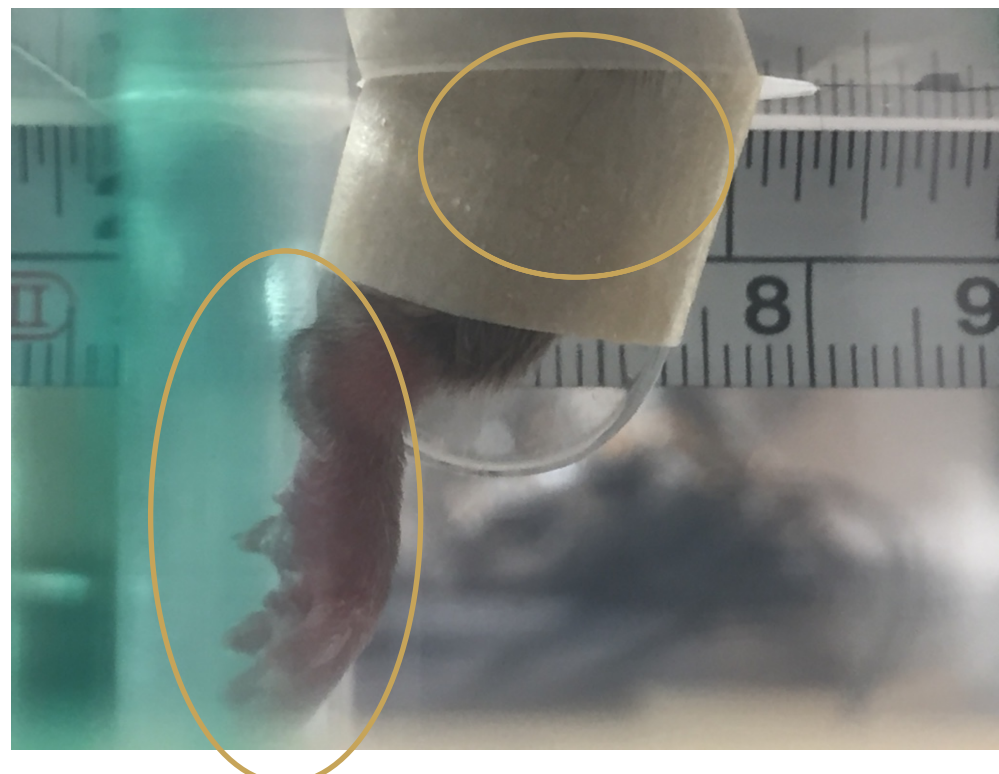
Radiobiological effects of proton radiation: normal tissue response

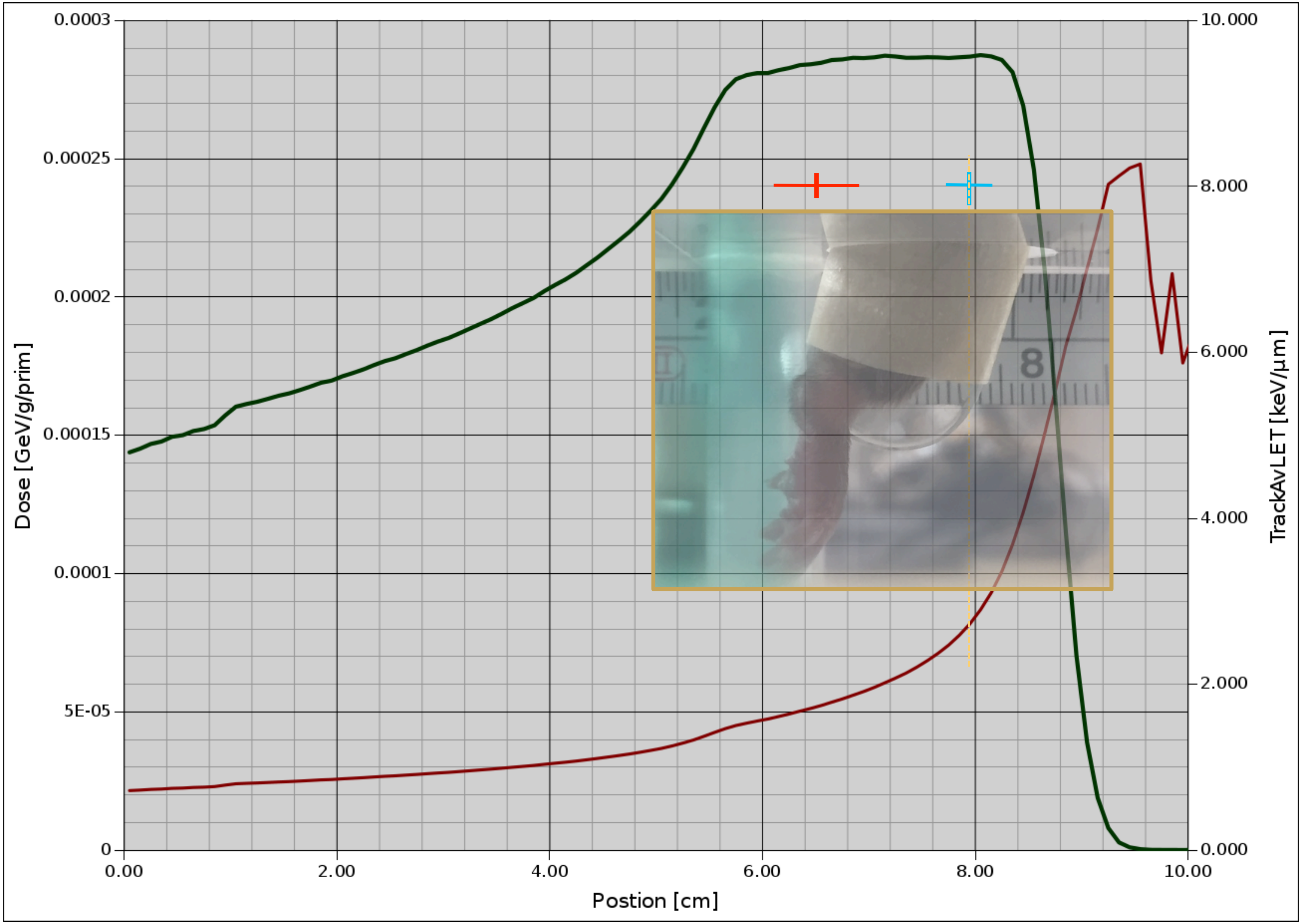
Brita Singers Sørensen: Aarhus, Denmark

CDF1 mice

Normal tissue damage

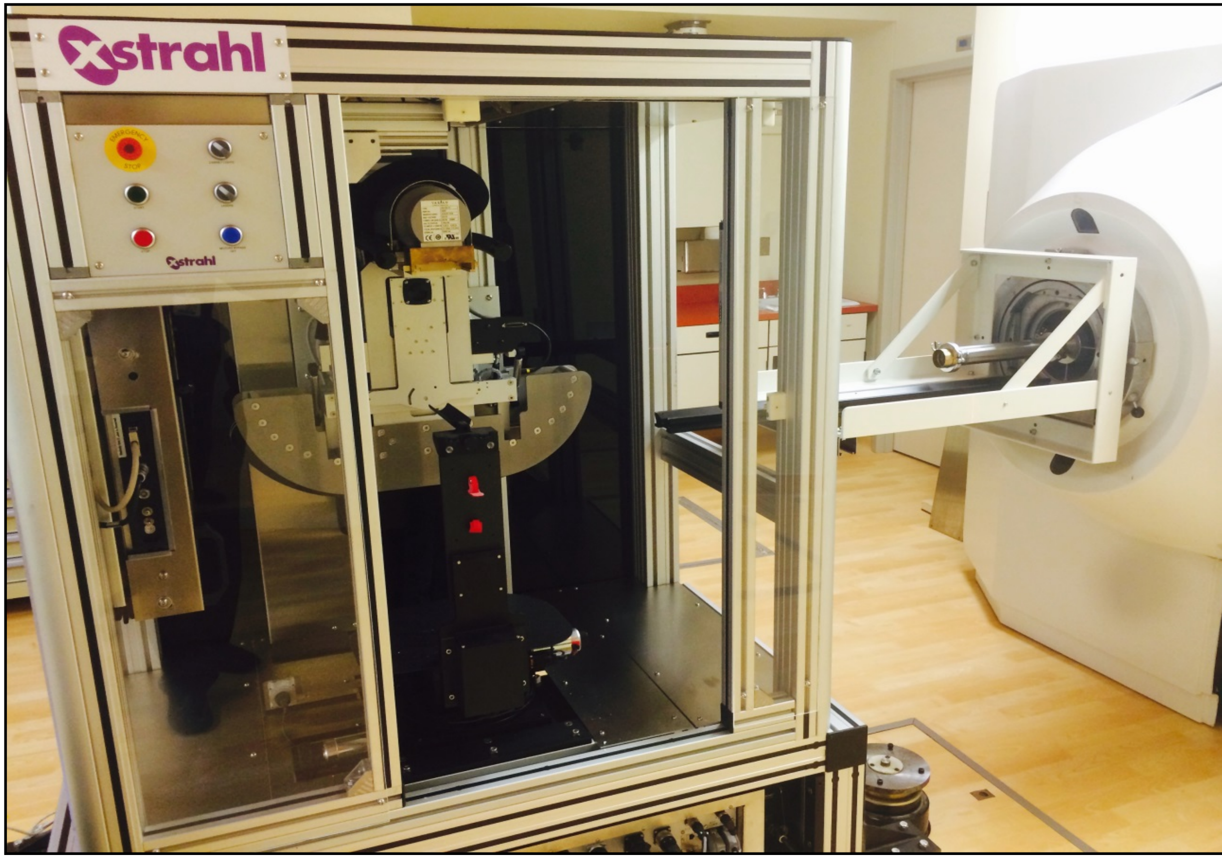
Acute effects

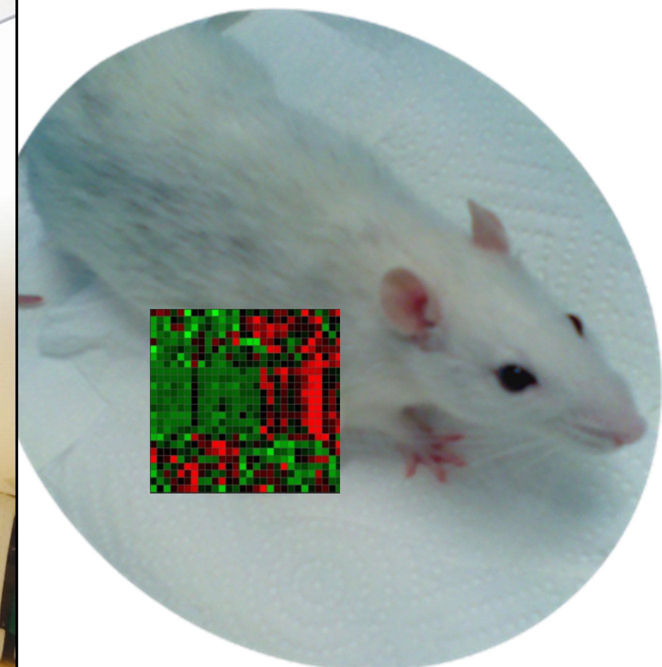




Courtesy of Brita Singers Sørensen

Small Animal Proton Radiotherapy Platform:



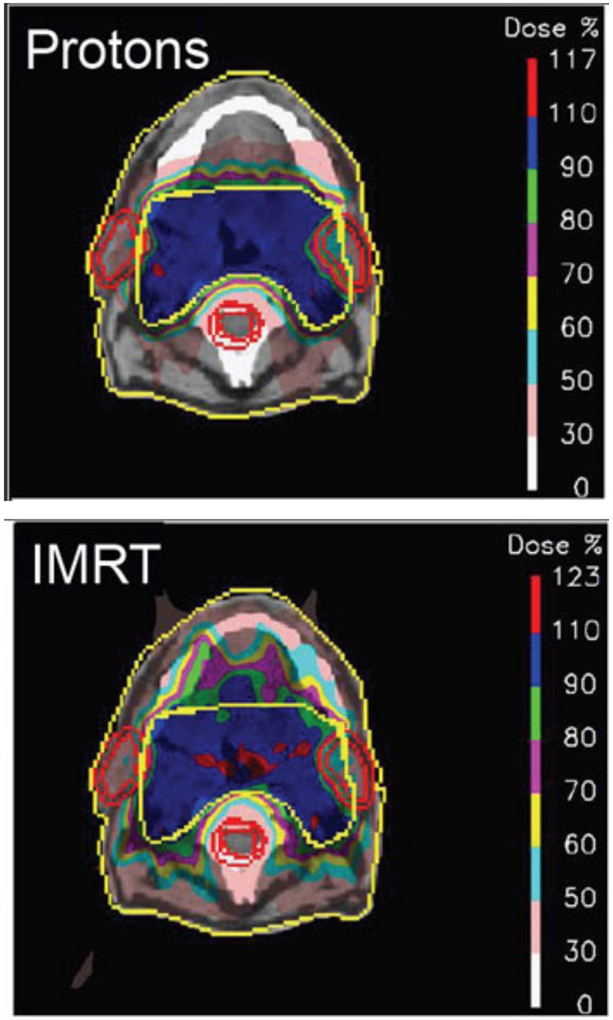


Courtesy of Eric Ford – University of Washington
Adrian Treverton – Xstrahl Inc

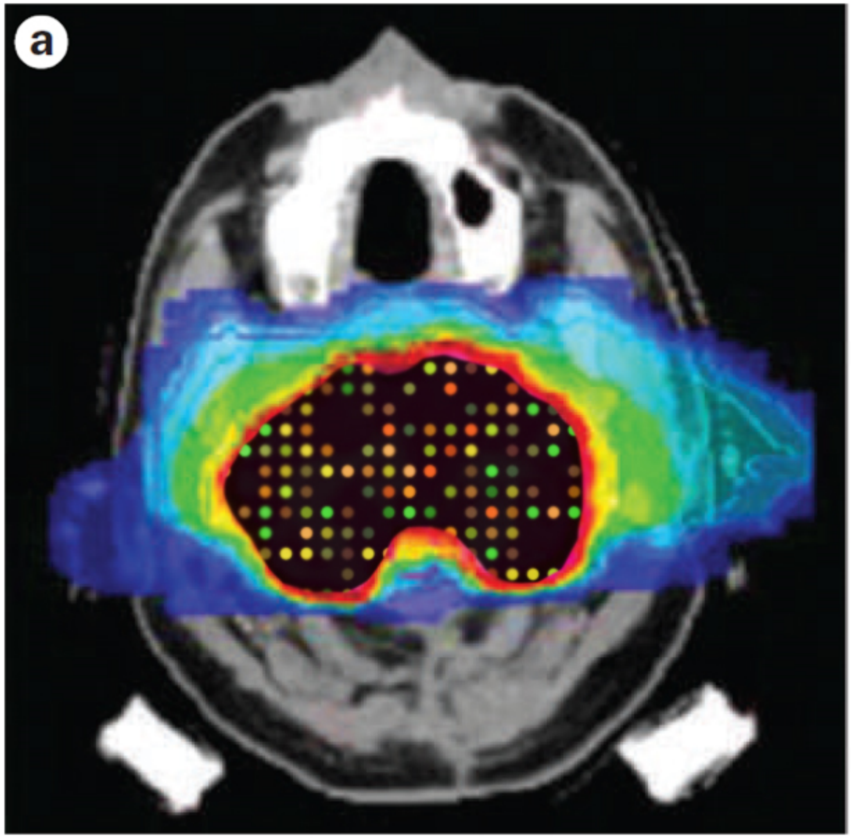
Additional challenges / sources of resistance:

- mutational status of the tumor
- tumor heterogeneity

Major Challenge: Personalized Treatment



The integral dose difference between protons and IMRT



- Integration of Biological Parameters
- Stratification not only based on Clinical Parameters

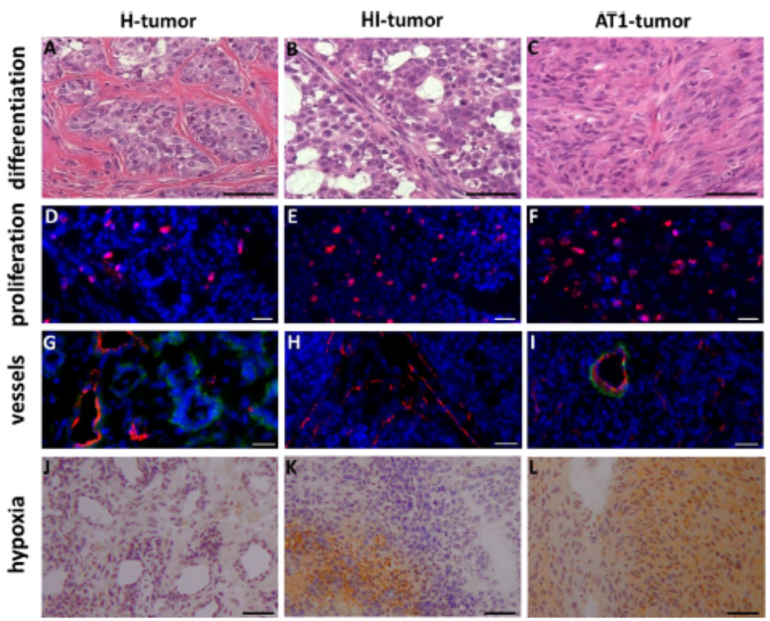
Original Articles

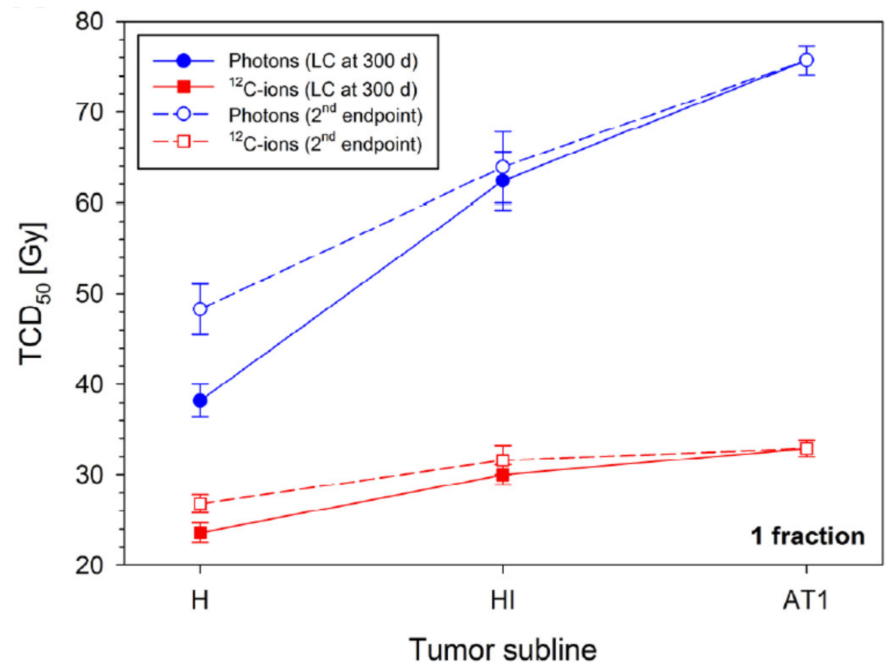
Carbon ion radiotherapy decreases the impact of tumor heterogeneity on radiation response in experimental prostate tumors

Christin Glowa [a,b,c,*], Christian P. Karger [b,c], Stephan Brons [c,d], Dawen Zhao [e], Ralph P. Mason [e], Peter E. Huber [a,c,f], Jürgen Debus [a,c], Peter Peschke [c,f]

[a] *Department of Radiation Oncology, University Hospital Heidelberg, Heidelberg, Germany*

Cancer Letters 378 (2016) 97–103





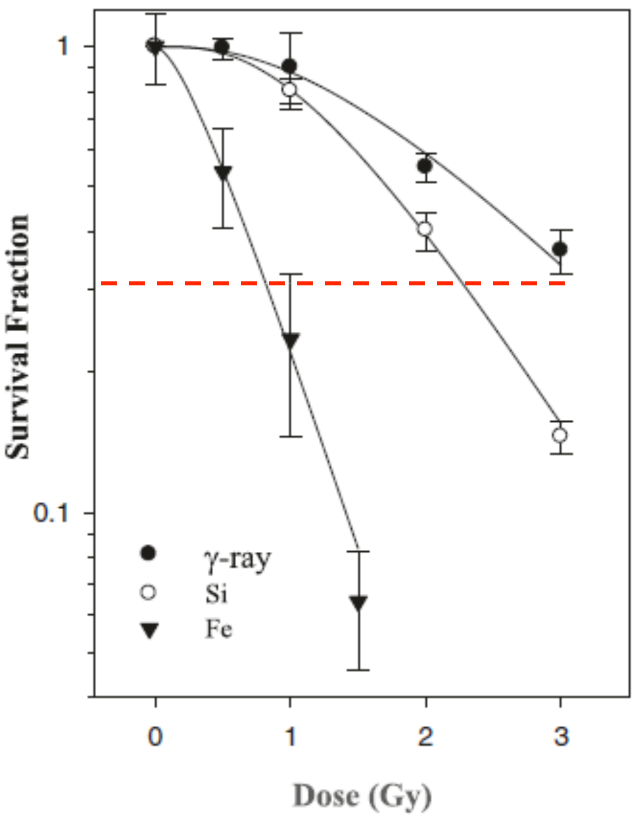
Rat prostate tumors derived from 3 different sublines treated with photon and carbon ion radiotherapy

Radiogenomics

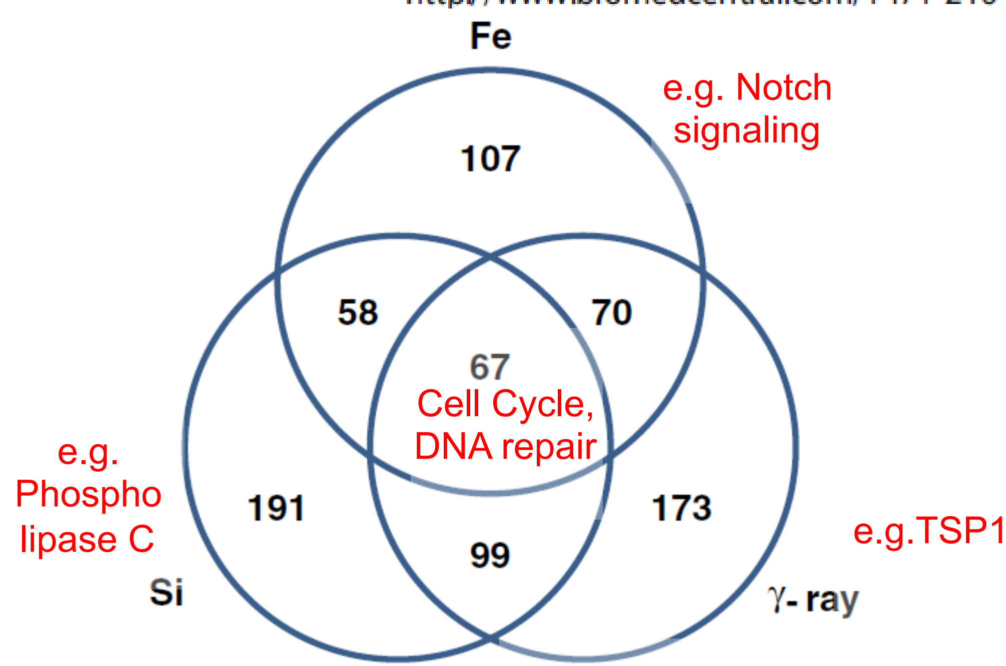
Distinct transcriptome profiles identified in normal human bronchial epithelial cells after exposure to γ-rays and different elemental particles of high Z and energy

Liang-Hao Ding[1], Seongmi Park[1], Michael Peyton[2], Luc Girard[2,3], Yang Xie[4,5], John D Minna[2,3,5,6] and Michael D Story[1,5*]

Ding *et al. BMC Genomics* 2013, **14**:372
http://www.biomedcentral.com/1471-2164/14/372



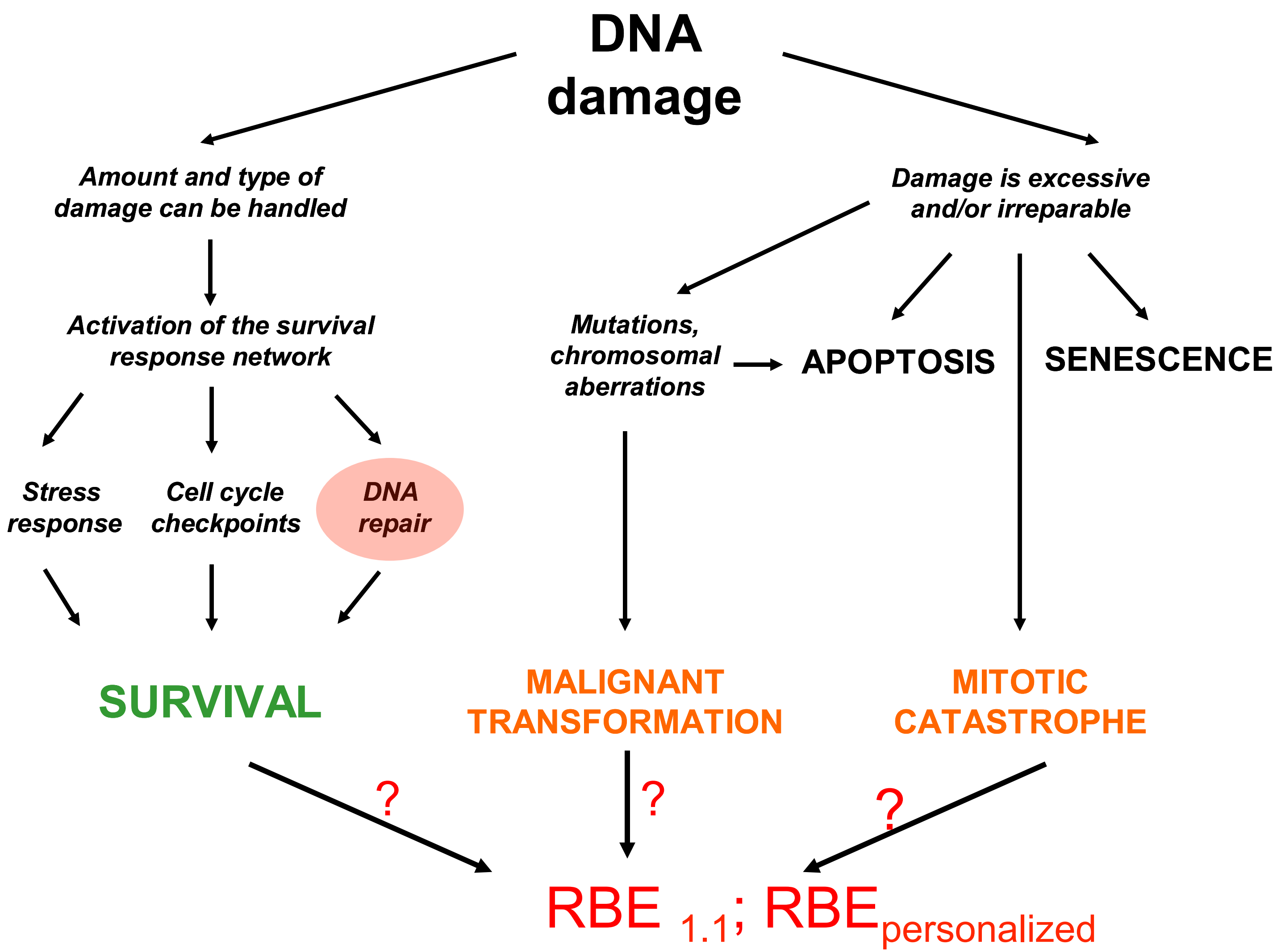
Clonogenic survival assay of HEBC3KT cells



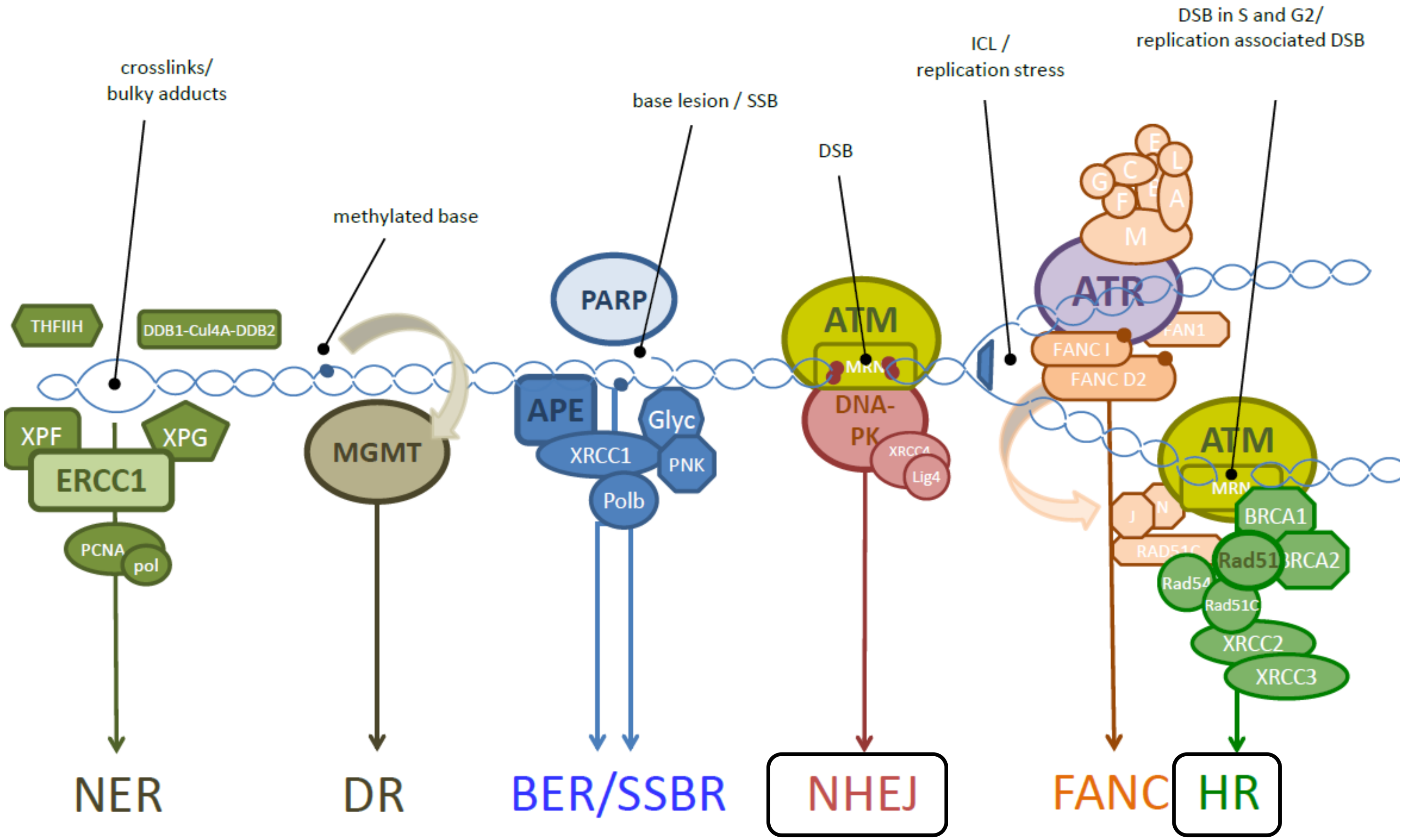
Differentially expressed genes in response to ^{56}Fe, ^{28}Si and γ-ray irradiations

We need more radiogenomic data in the field of particle vs photon-irradiation
e.g. Ghirdani et al., 2012; Suetens et al., J Radiat Res, 2015

Photon vs Proton Irradiation: The cellular response to radiation damage



Differential Demands on DNA Double Strand Break Repair Machineries after Proton- and Photon-Irradiation?



DNA Damage Response and Repair

Proton versus Photon Irradiation

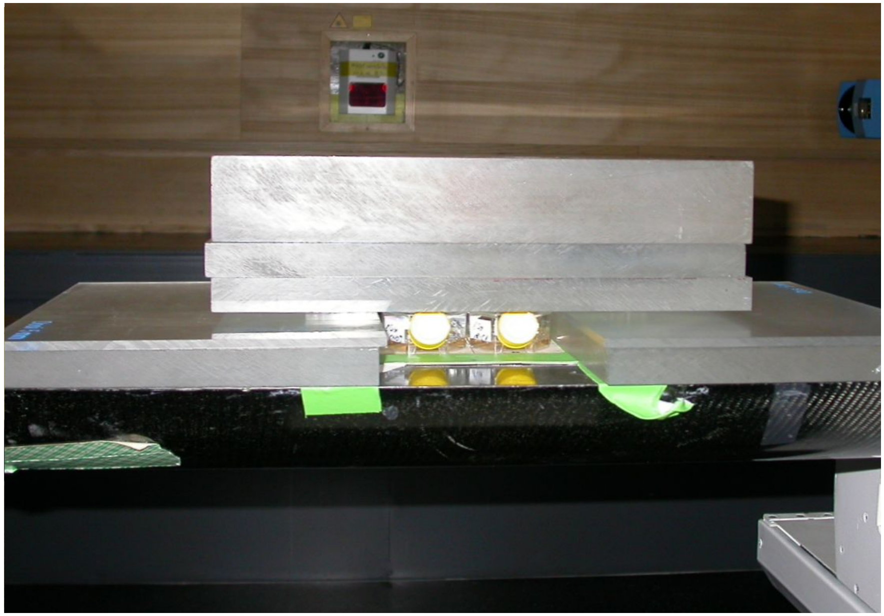
PAUL SCHERRER INSTITUT

Indirect Approach:

Radiosensitivity screening of different CHO cell lines → differential sensitivities could indicate differences in the amount/quality of the DNA damage

Direct Approach:

- Quantification of initial γH2AX foci
- Kinetics of γH2AX, Rad51, pDNA-PKcs foci appearance/disappearance
- Cell cycle distribution analysis
- Quantification of chromosomal aberrations



Gantry 1 at PSI Cell flasks at the Gantry table

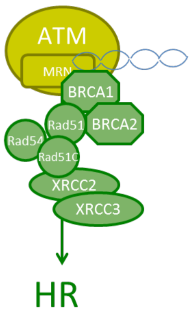
Photon Irradiation: 200 kV X-ray unit at 1 Gy/min

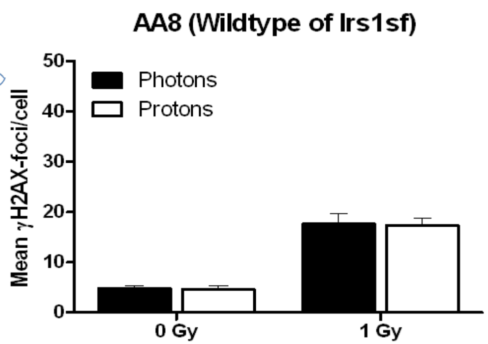
Proton Irradiation: in the middle of SOBP; max energy 138 MeV

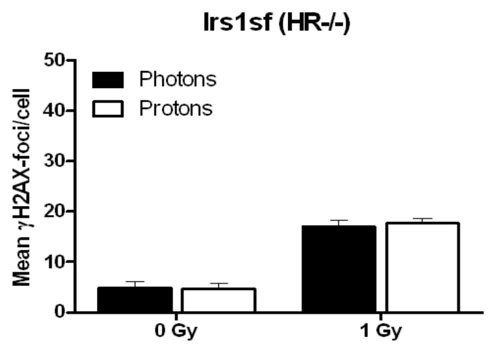
UniversityHospital Zurich

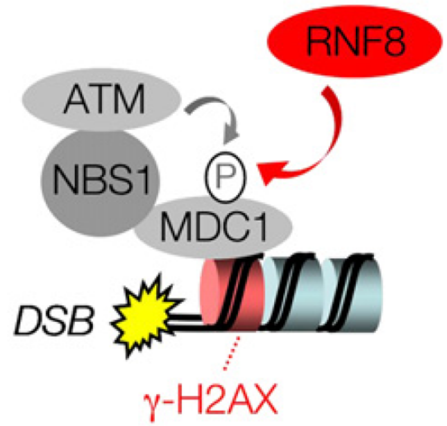
Grosse, Pruschy et al., IJROBP, 2014

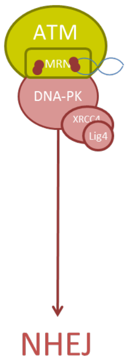
Quantification of the Initial Amount of DNA DSBs

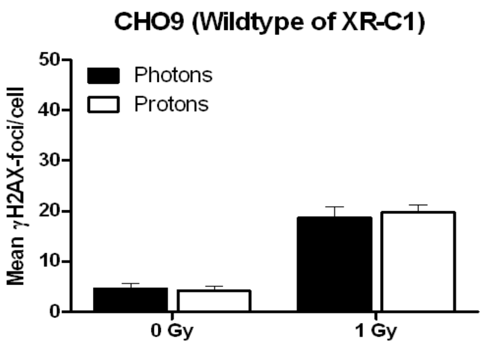


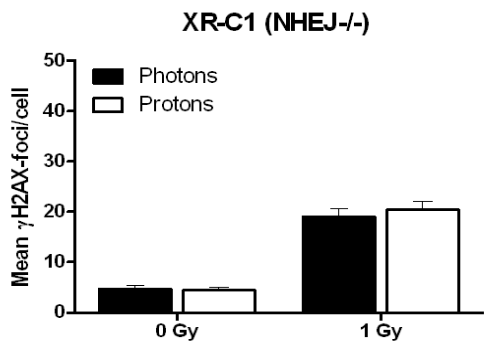






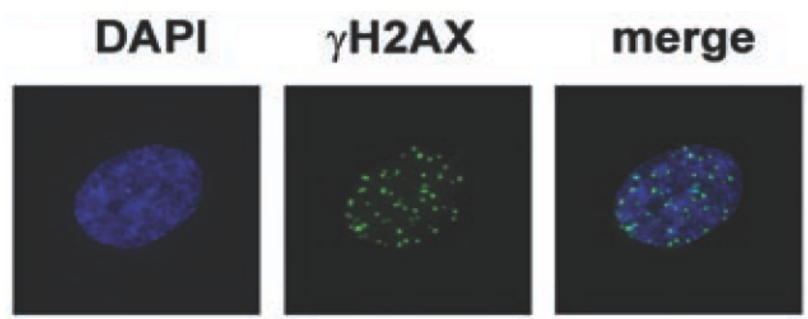




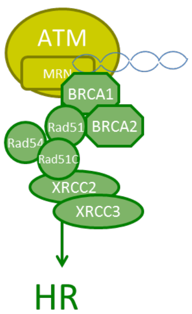


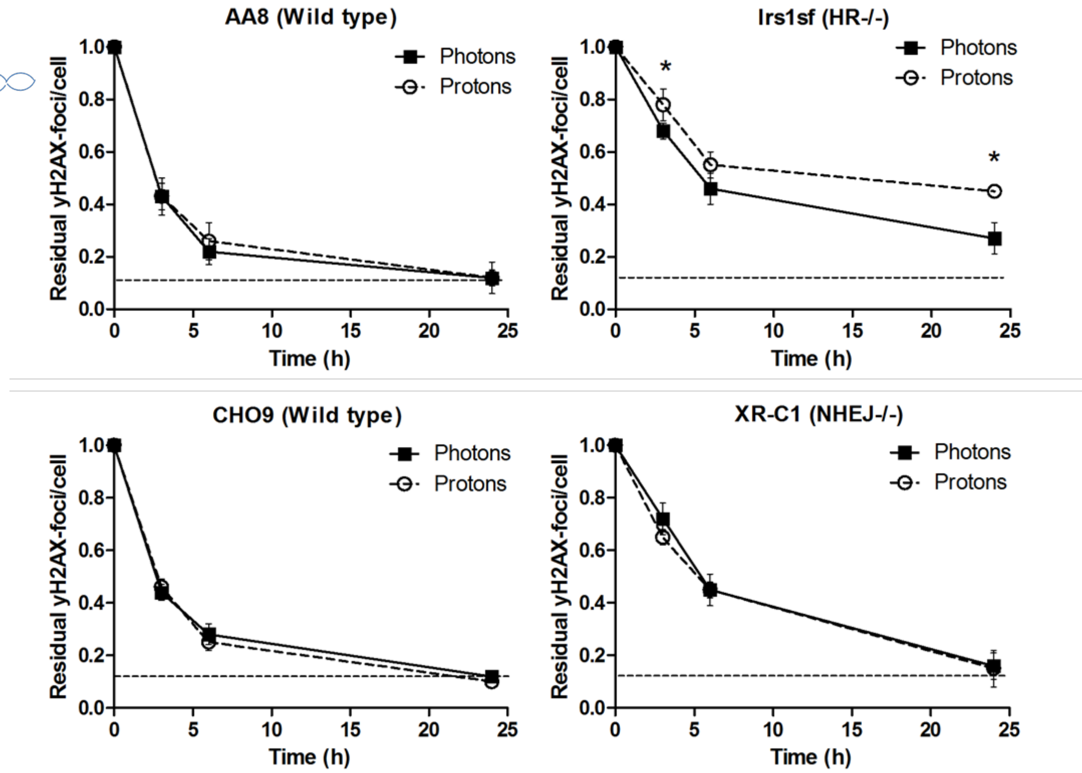
visualized

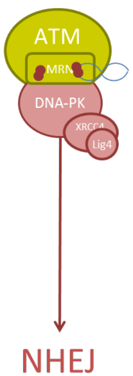
No difference in the initial amount of DNA damage after Proton vs Photon Irradiation

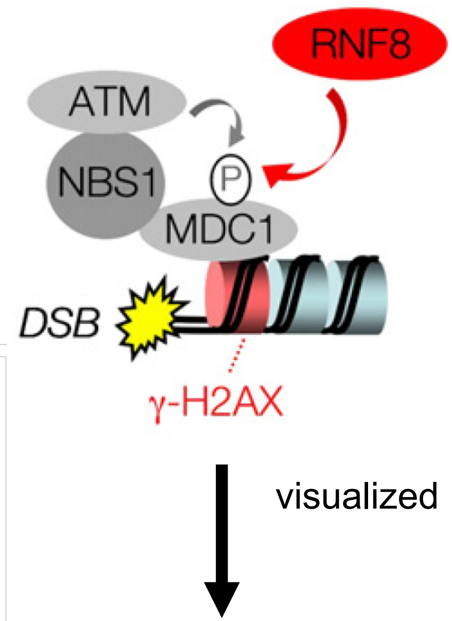


Quantification of the Residual Amount of DNA DSBs

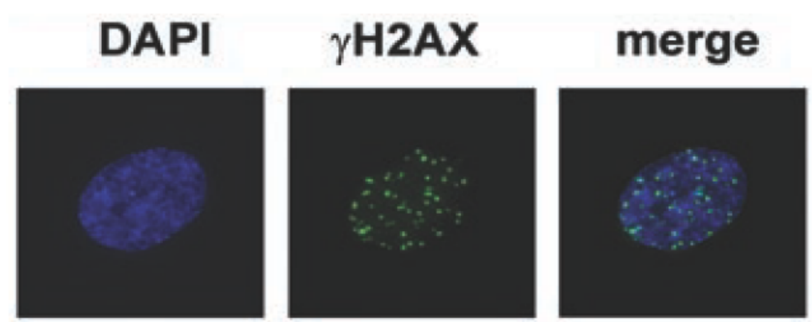




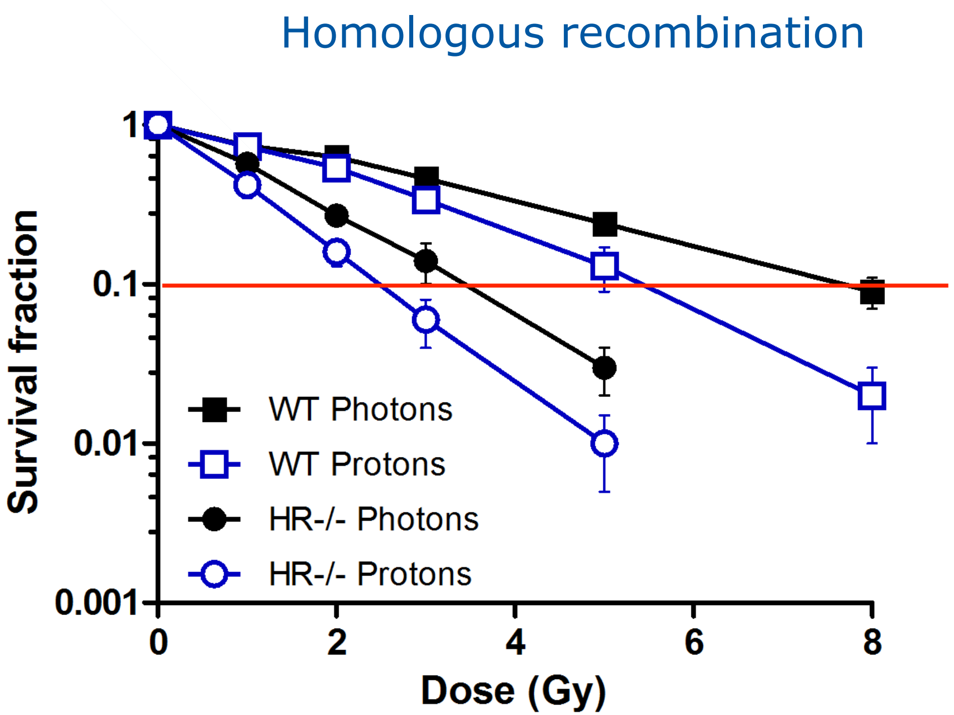


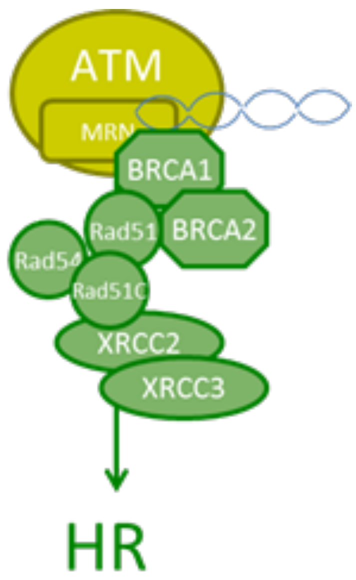


DNA DSB repair is significantly slower in HR-deficient cells after Proton Irradiation



Clonogenic survival in wildtype and HR-deficient cells in response to photon and proton irradiation

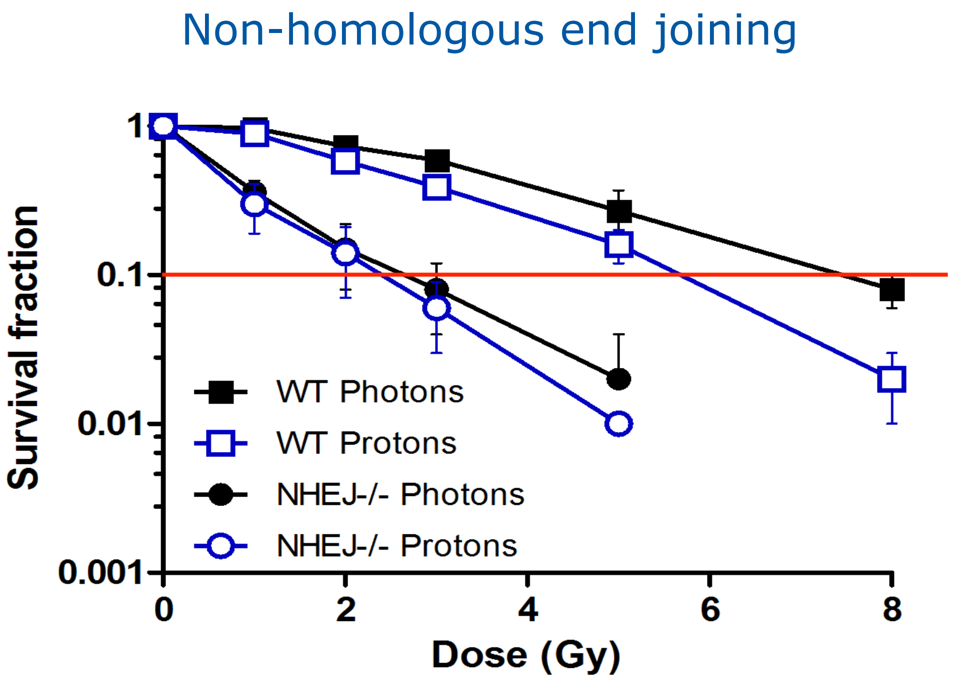


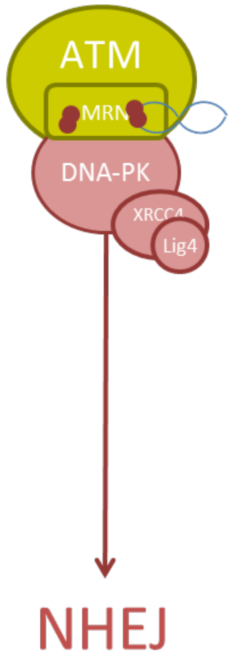


% survival	HR-wt log	HR -/- log	p-value
37	1.25±0.05	1.54±0.10	0.02
10	1.29±0.04	1.44±0.06	0.03

Grosse et al., IJROBP, 2014

Clonogenic survival in wildtype and NHEJ-deficient cells in response to photon and proton irradiation

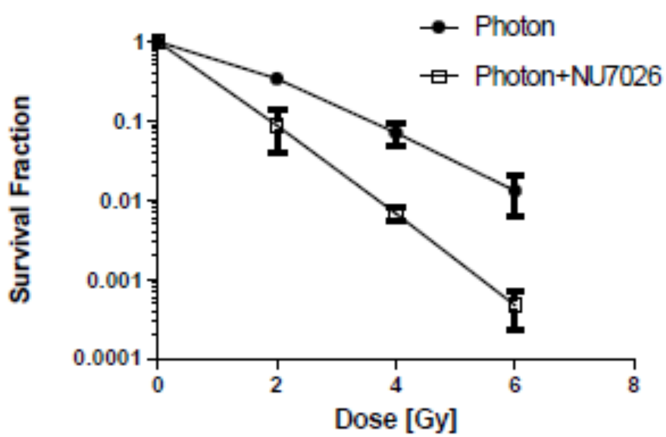


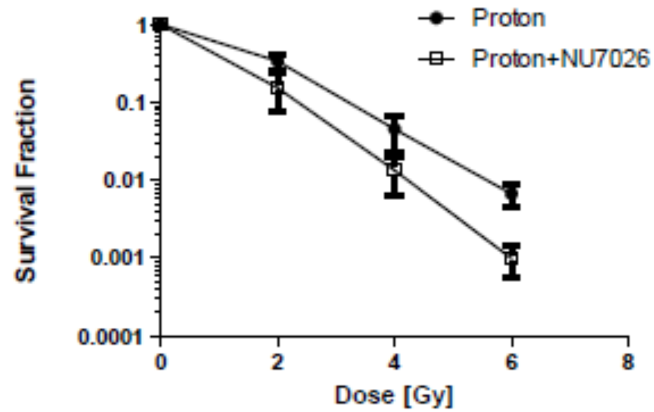


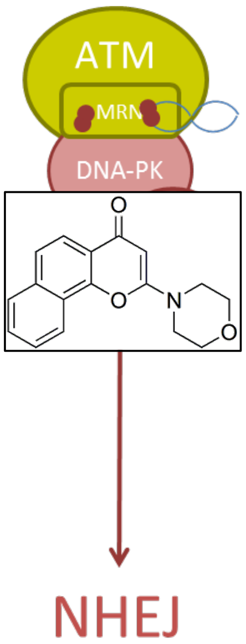
% survival	NHEJ-wt log	NHEJ-/- log	p-value
37	1.32±0.12	1.08±0.07	0.11
10	1.20±0.05	1.09±0.08	0.38

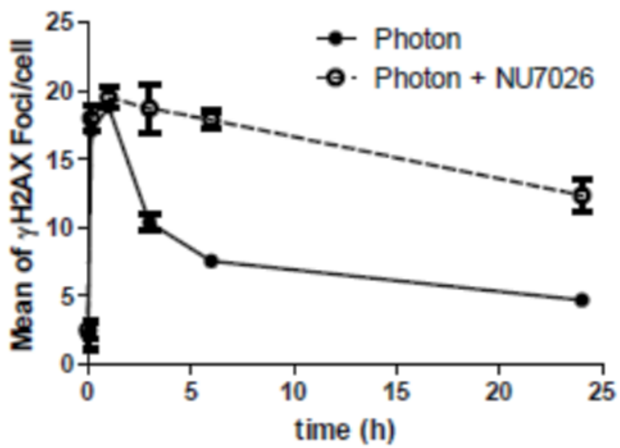
What about tumor cells?

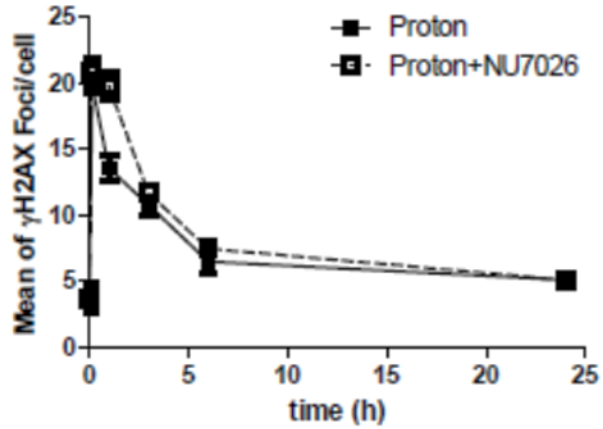
Inhibitor of NHEJ more strongly sensitizes for Photon-Irradiation (lung carcinoma cells)







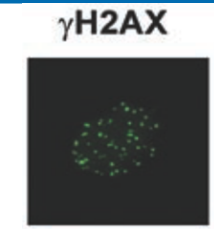


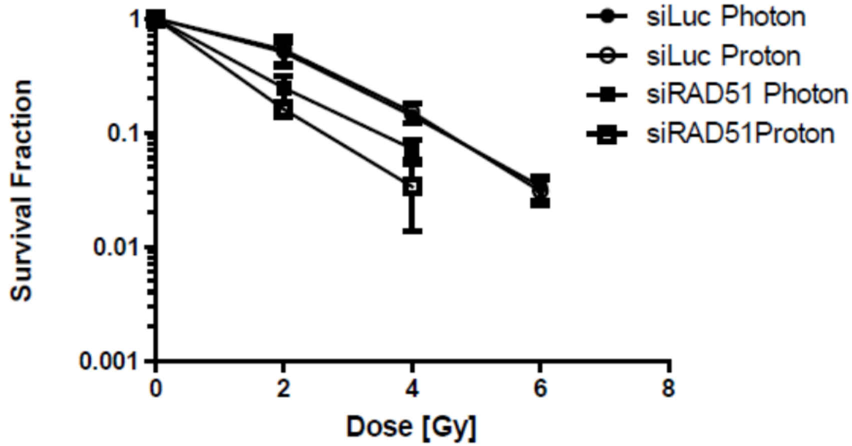


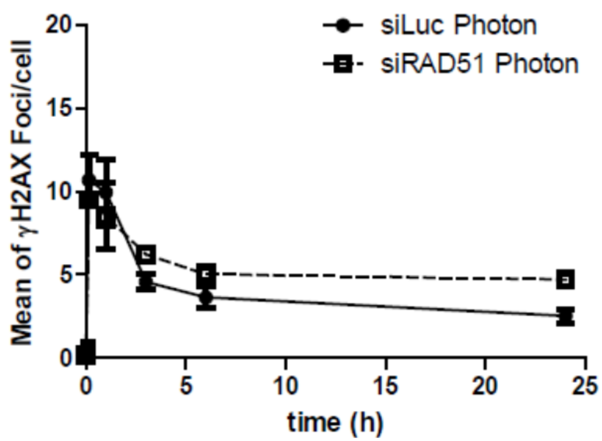
(also in glioblastoma cells)

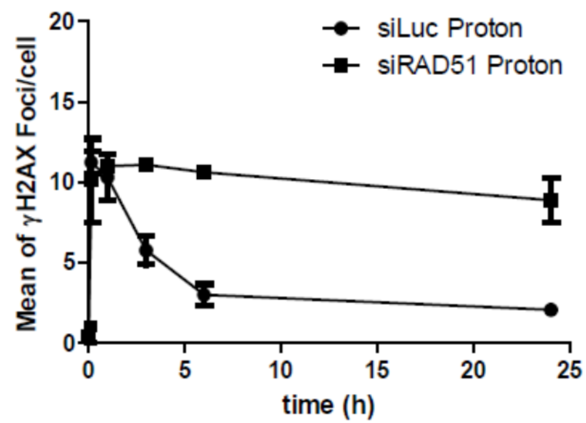
Fontana, Pruschy et al., Radiother Oncol. 2015;116:374-80

HR-knockdown renders cells more sensitive to Proton Irradiation



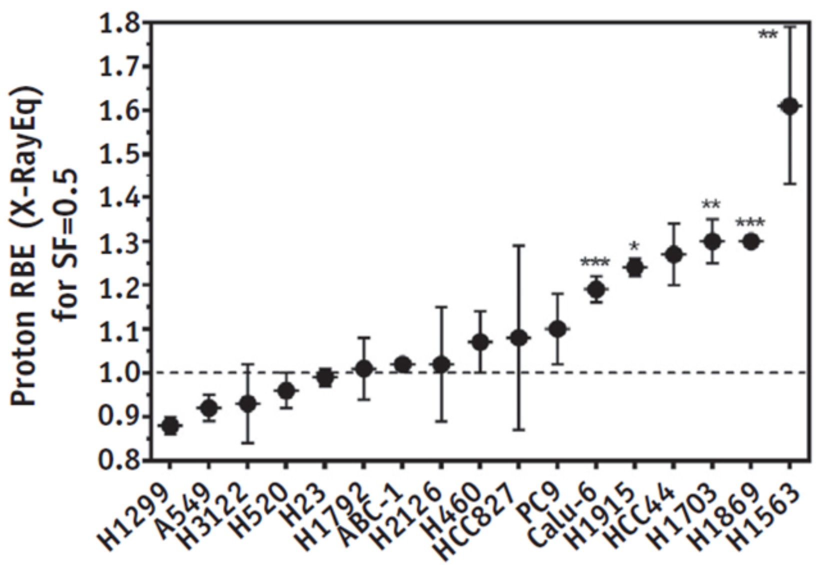


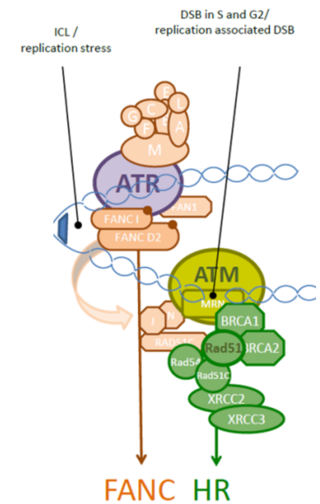




Genetic background defines differential sensitivities to photon vs proton irradiation: options for combined treatment modalities

Lung Cancer Cell Line Screen Links Fanconi Anemia/BRCA Pathway Defects to Increased RBE





FANC-pathway: replication fork maintenance

	H1299	ABC1	H3122*	H520	H23	H1792	H2126	A549	H460	HCC827	PC9*	Calu-6*	H1915	HCC44	H1703	H1869	H1563
RBE(Co60Eq)	0.97	1	1.02	1.06	1.09	1.11	1.12	1.17	1.18	1.18	1.21	1.31	1.36	1.39	1.43	1.43	1.77
BRCA1			Not available								Not available	Not available	***				MUT
BRCA2																	
FANCD2																	MUT
FANCE																	
FANCF																	
FANCG																	
FANCI					MUT												
BRIP1					MUT												
FANCL																	
FANCM		MUT															
SLX4																	
RAD51	**																
RAD52																	
RAD51D																	
XRCC2																	
XRCC3																	
MMC-Survival				ND	ND											ND	
CDDP-Survival				ND												ND	
PARPi-Survival	ND	ND	ND	ND	ND				ND					ND		ND	

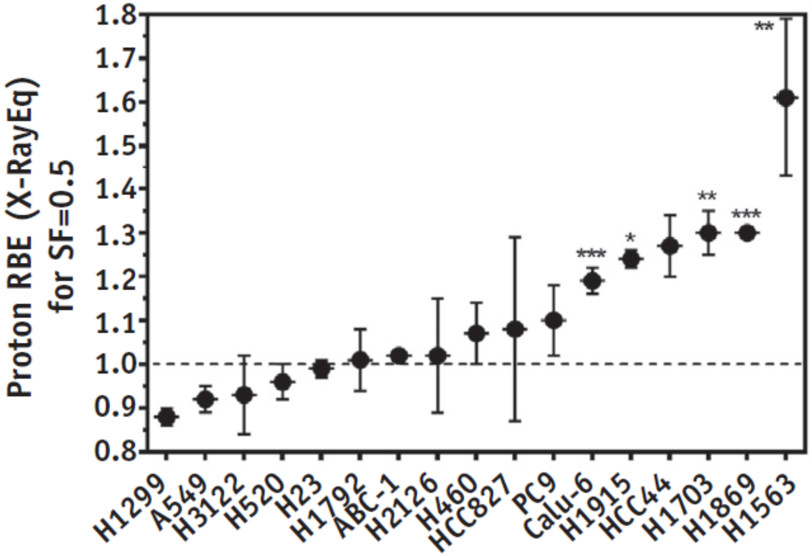
Survival MMC/CDDP <1%, PARPi <10%
Survival MMC/CDDP 1-10%, PARPi 10-25%
Survival MMC/CDDP 10-100%, PARPi 25-100%

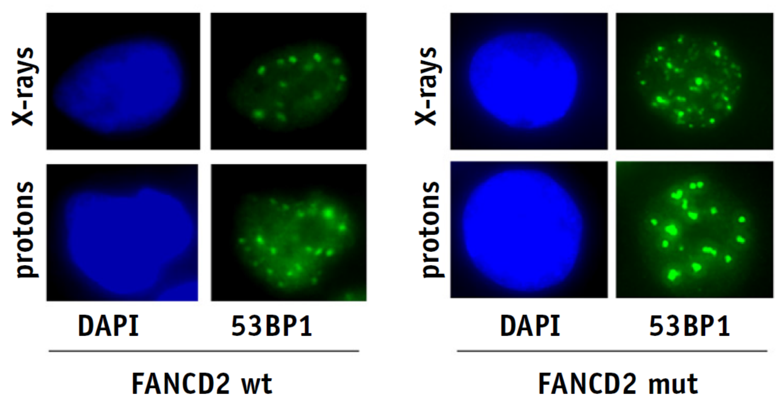
homozygous deletion
amplification

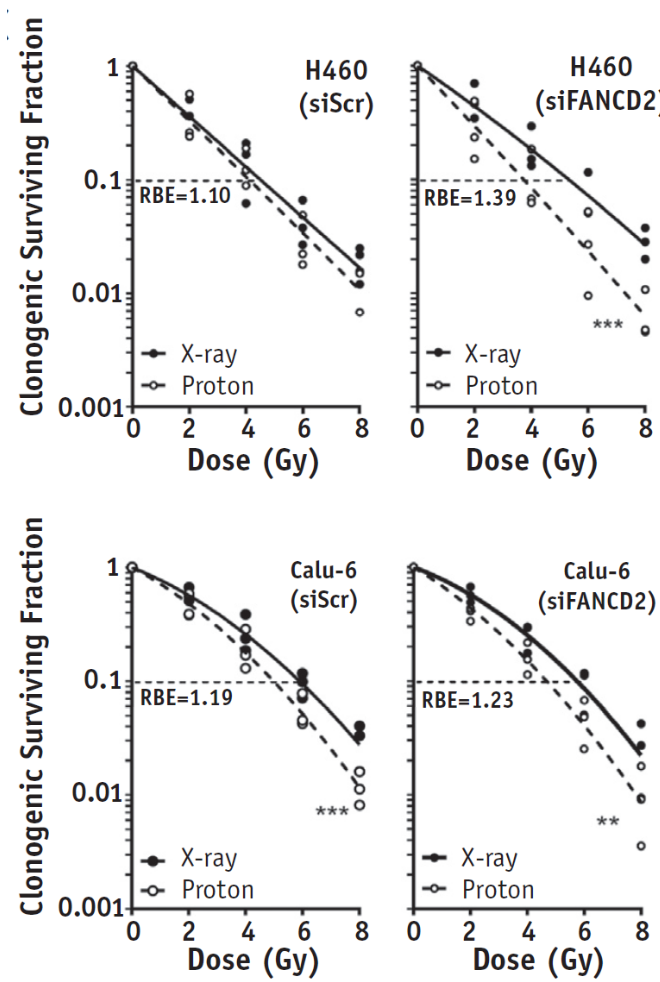
mRNA downregulation
mRNA upregulation

Liu, Willers et al, IJROBP, 2015

Lung Cancer Cell Line Screen Links Fanconi Anemia/BRCA Pathway Defects to Increased RBE







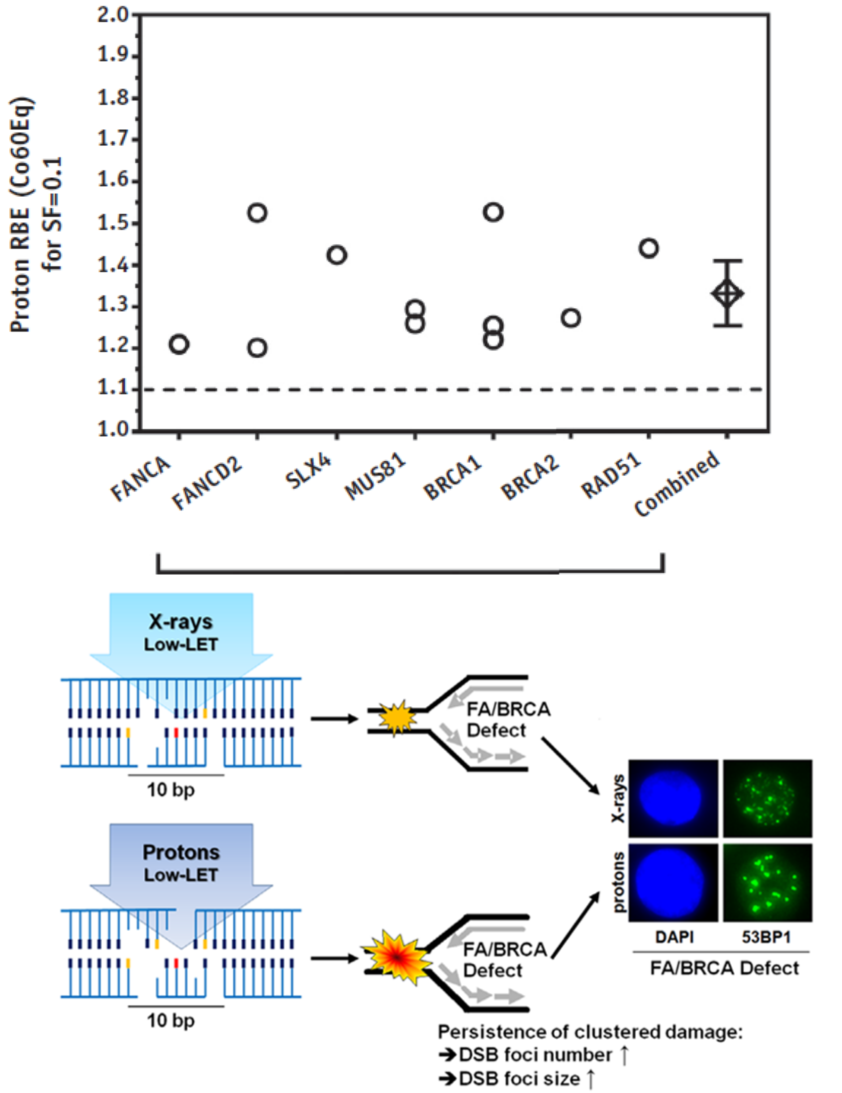
53BP1-Foci-size as putative Biomarkers

Disruption of the FANCP-scaffold increases the RBE

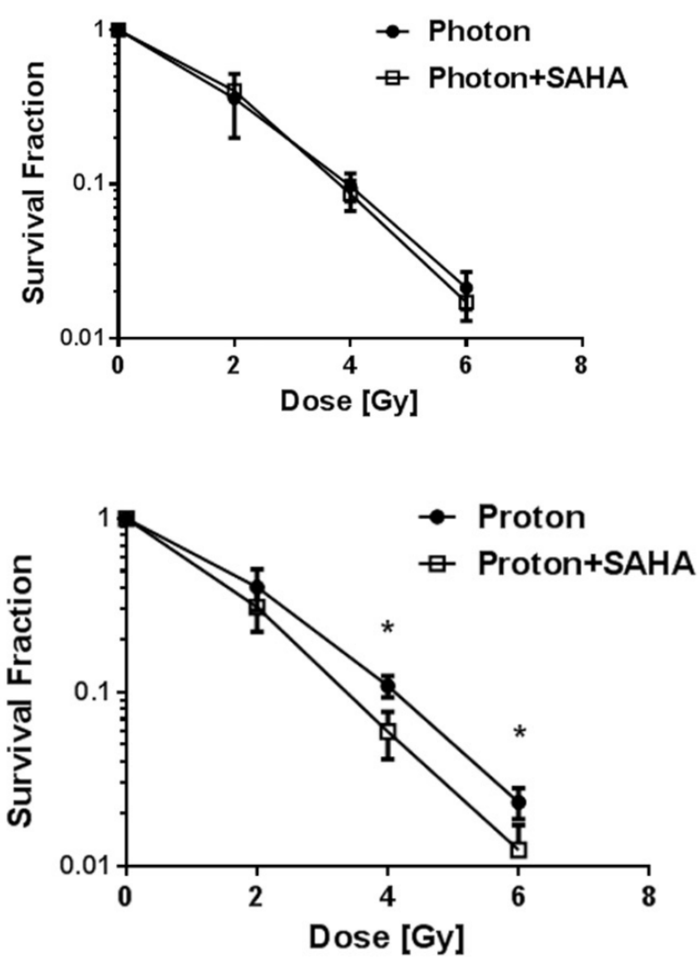
Liu et al, IJROBP, 2015

Liu et al, IJROBP, 2016

RBE’s in dependence of gene defects

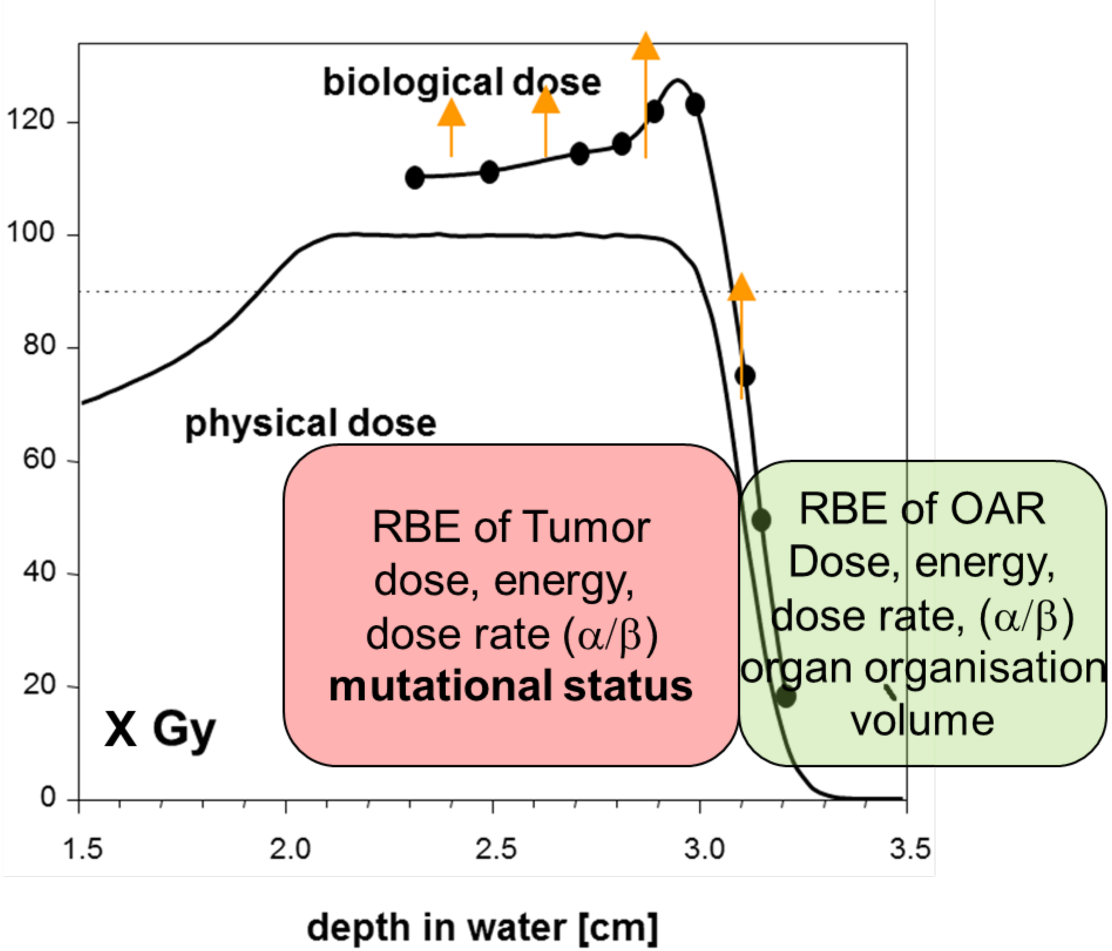


Courtesy of K. Held



Fontana, Pruschy et al.,2015

What do we see – from the biological point of view?

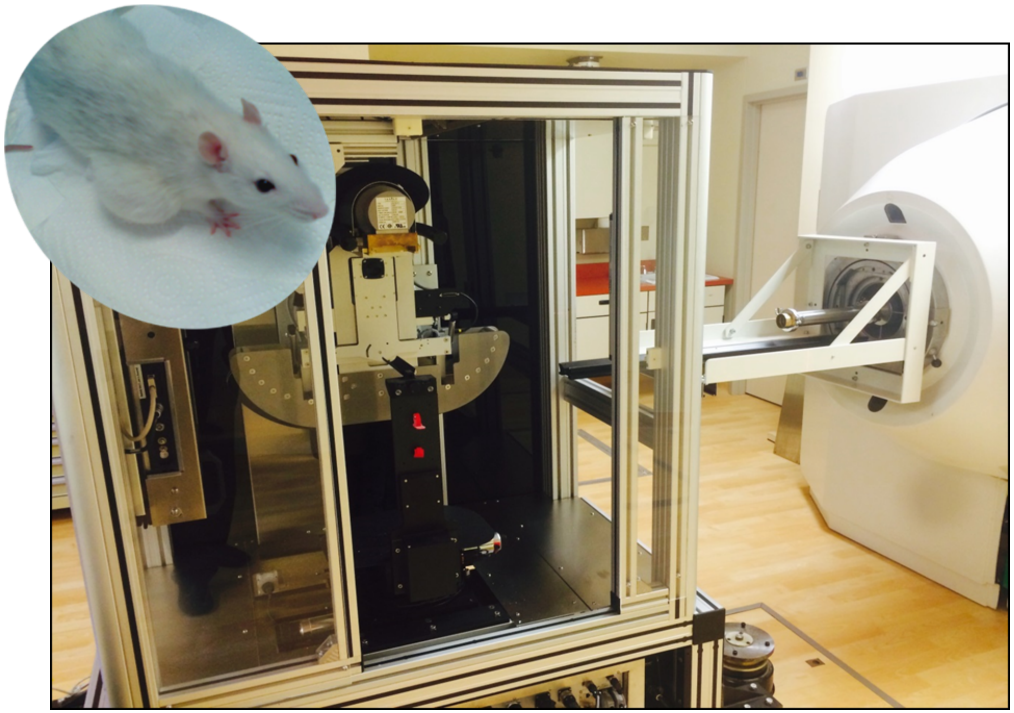


- Uniform physical dose over SOBP, but non-uniform biological dose over SOBP
- Mutational status of the tumor strongly influences response to biological dose:
  - ➢ personalized approaches

CHALLENGES (from biological point of view):

- RBE is variable, is dependent on multiple factors
- Where does the RBE exactly derive from (on the biological level)? Differential biological responses?
- To which extent will mutational status affect RBE?
- Long term goal: Stratification along biological parameters; genetic background → personalized medicine?

Summary: What do we need?



more in vivo
work

more translational
work

WP6: Radiobiology, RBE

Progress report

2nd European Particle Therapy Group Meeting

Brussels, May 18, 2016

Coordinators: Jan Alsner, Aarhus, Denmark

Manjit Dosanjh, CERN

Bleddyn Jones, Oxford, UK

Jörg Pawelke, Dresden, Germany

Martin Pruschy, Zurich, Switzerland

WP6: Radiobiology, RBE

Aim

Form a network of the distributed therapy facilities

- Standardisation of radiobiological experiments
  - designated cell lines
  - sharing and exchange of animal models
  - standardisation radiobiological assays and procedures
  - radiation quality (clinical beam) for control irradiation
- Dosimetric intercomparisons and standard QA protocols
- Questionnaire to all collaborators
  - definition of beam characteristics & aspects is in progress
  - biological experiments and beam time)
  - etc.

WP6: Radiobiology, RBE

Next steps

- Integrate more interactive biological questions
  - Combined treatment modality (e.g. drug modification)
  - Advanced molecular biology
- Dedicate a symposium at the next ESTRO meeting (PREVENT track, ESTRO 36, May 2017) to WP6 and related work
  - running title: Novel Approaches in Particle Biology
  - details will be discussed soon
- Research beyond comparison of X-rays, protons, carbon ions as pointed out in recent meetings: ICTR-PHE, Divonne, NCI workshop

➢ Please contact us if interested to join

Literature - Review

Proton Radiobiology

Francesco Tommasino and Marco Durante

Cancers 2015, 7, 353-381

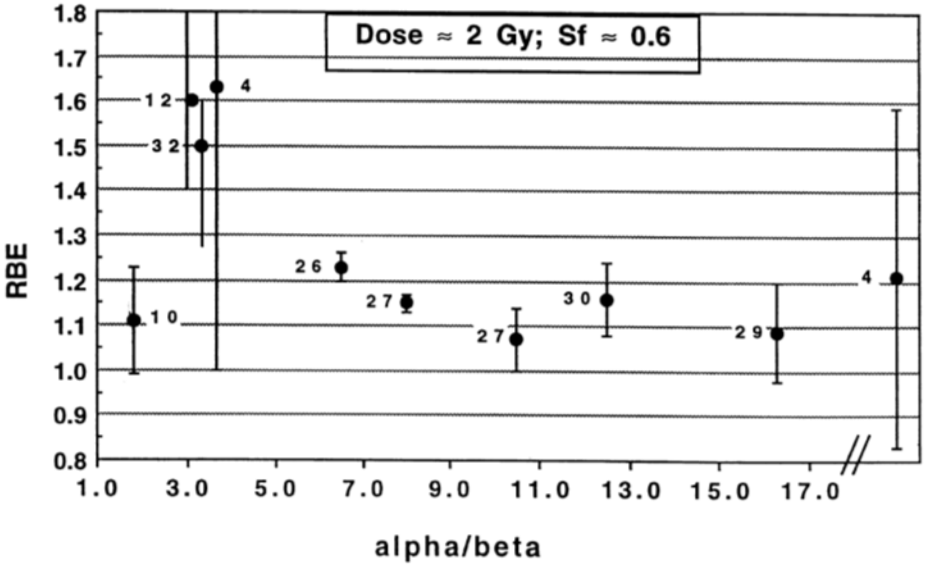
Relative biological effectiveness (RBE) values for proton beam therapy. Variations as a function of biological endpoint, dose, and linear energy transfer

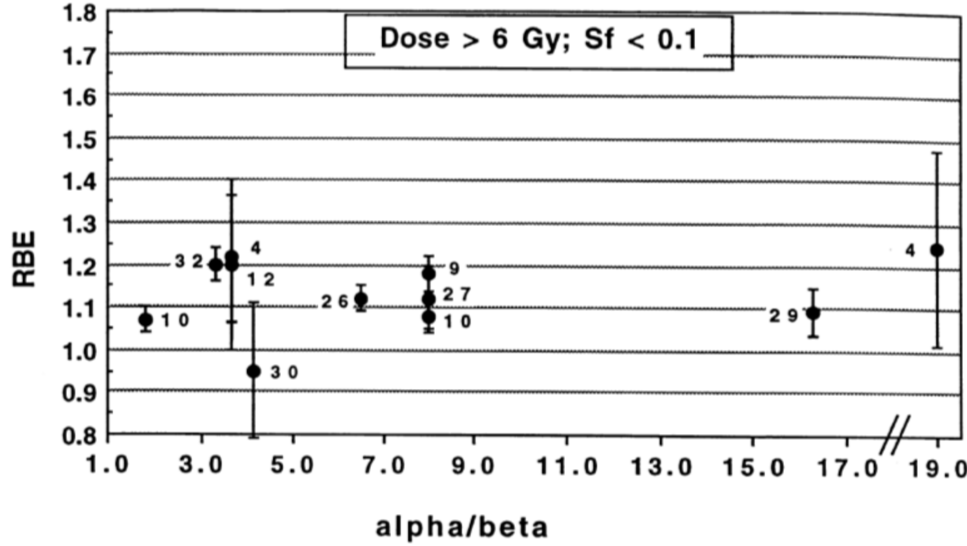
Harald Paganetti; Physics in Medicine& Biology, 2014, R419-R472

Effects of Charged Particles on Human Tumor Cells

Kathryn Held et al., Frontiers of Oncology, 2016

RBE values as a funtion of α/β



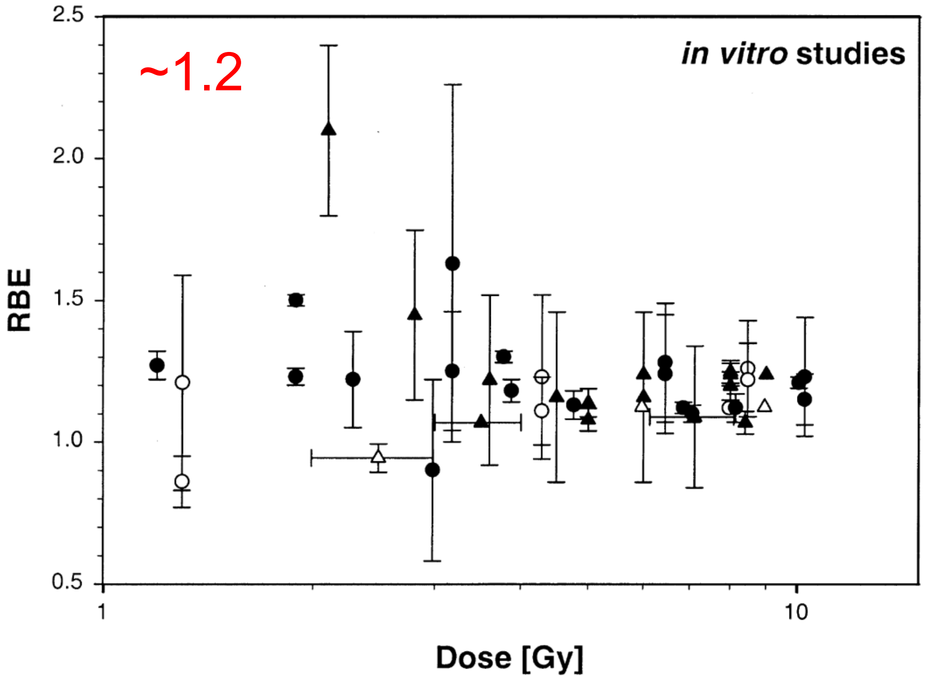


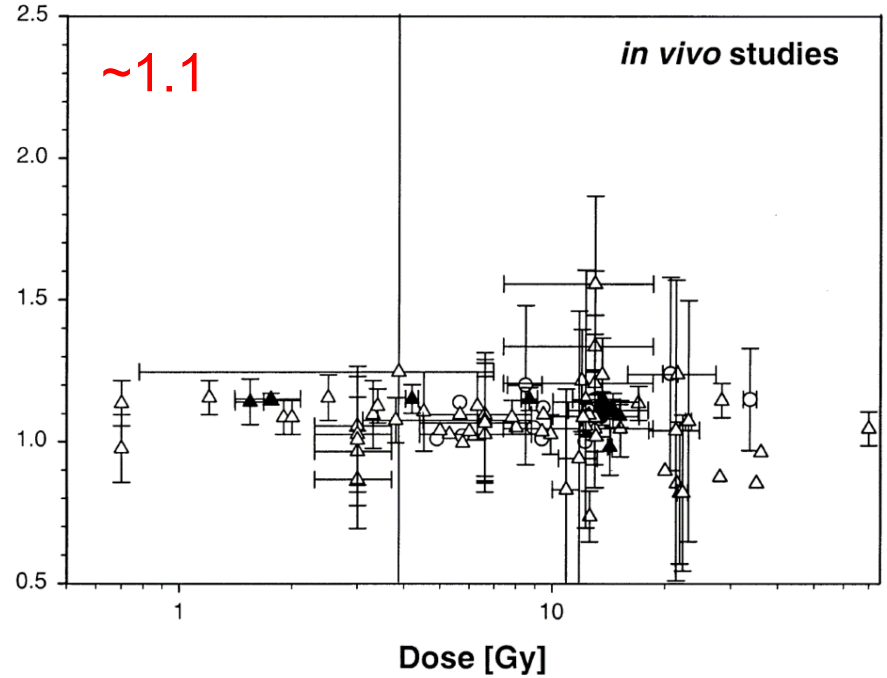
- A tendency for increasing RBE for cells/tissues with smaller α/β ratios
- RBE (typically) increases with decreasing dose, particularly for low α/β
- Low α/β: late responding (healthy) tissue
- High α/β: early responding (tumor) tissue

Gerweck et al., Green Journ., 50, 135ff, 1999

Paganetti et al., IJROBP, 2002, 53, 407ff

RBE versus dose: *in vitro* and *in vivo* studies



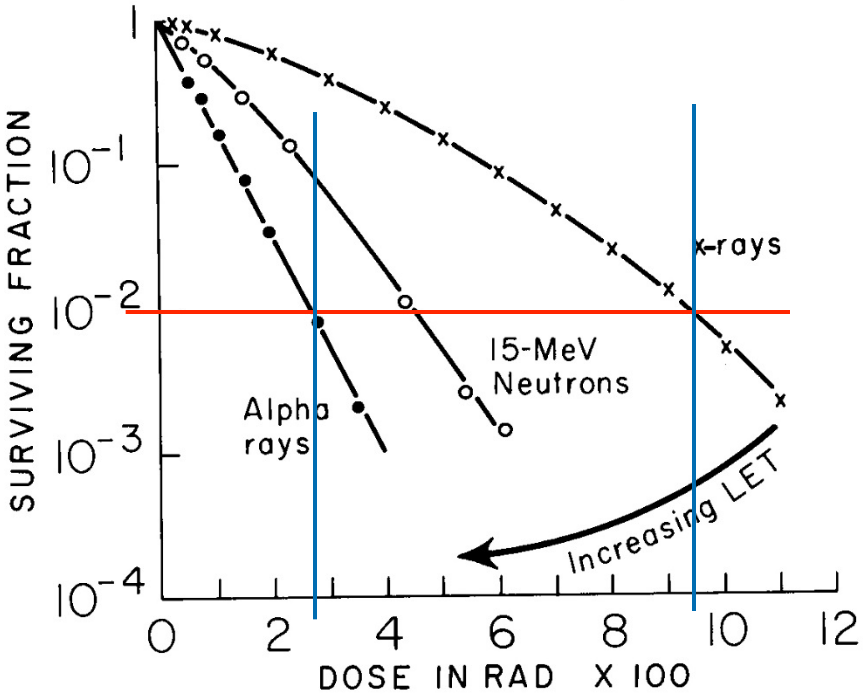


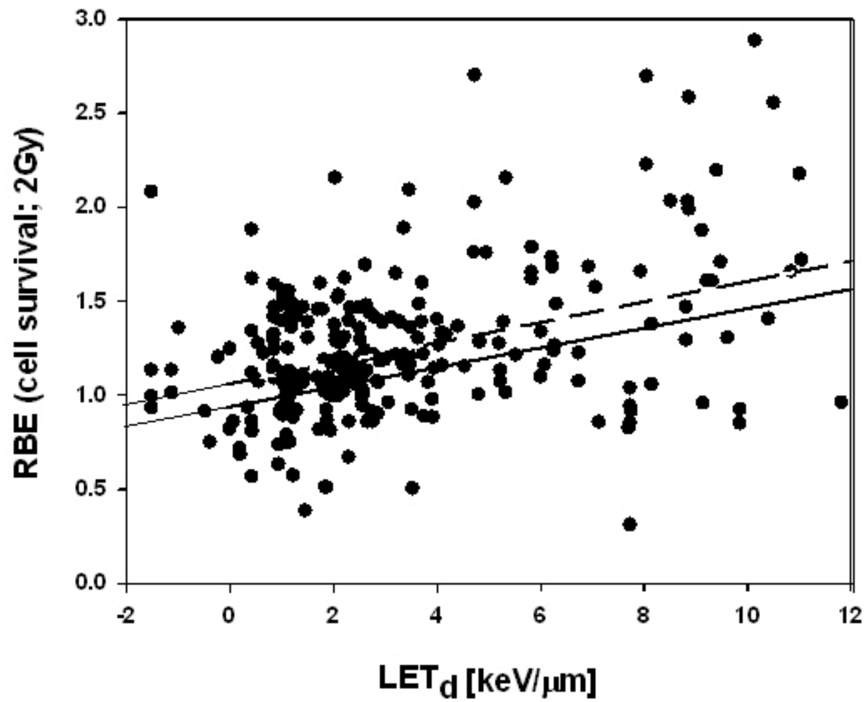
- as a function of dose
- CHO, CoCa, V79 lung fibroblast; diff. cell lines, etc

- acute and late-reacting tissue systems (jejunal crypt cells, lung, skin, etc)

Paganetti et al., IJROBP, 2002, 53, 407ff

LET dependence:





As the LET increases from about 2 keV/µm for x-rays up to 150 keV/µm for α-particles, the survival curve changes in two important respects:

1. the survival curve becomes steeper.
2. the shoulder of the curve becomes progressively smaller as the LET increases.

9.5Gy:2.8Gy = 3.4

RBE increases for cell survival as a function of LET at a proton dose of 2 Gy

Paganetti H: Phys Med Biol 2014 59: R419-R472

LET: descriptor of energy transferred from the beam to the irradiated material per units of particle path length (e.g. keV/µm)