Models and Mechanisms Connecting Physics and Biology at Multiple Scales in the Biological Hierarchy

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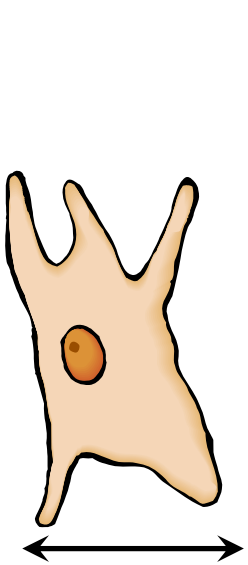
Session: TU-A-BRE-3 (*last number indicates order within session*)
Date and Time: Tuesday July 22, 2014 from 7:30 to 9:30 am
Location: Ballroom E
Abstract #23482

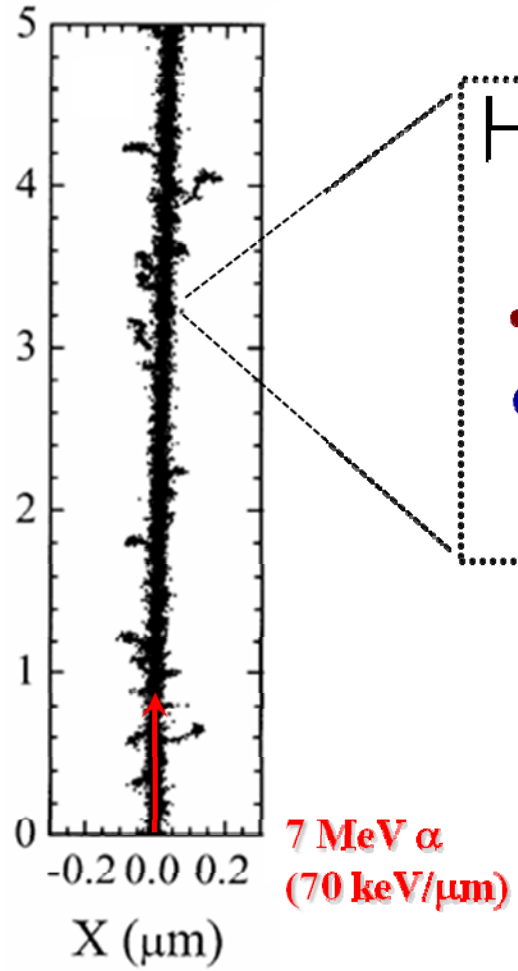
Learning Objectives

- **Review models and mechanisms connecting radiation biology at the molecular and cellular levels to radiation biology at the tumor and tissue level**
  - Focus on effects of particle linear energy transfer (LET)
- **Is the RBE for DNA damage useful for predicting cell survival?**
- **Is the RBE for cell survival useful for predicting the RBE for *clinical endpoints*?**

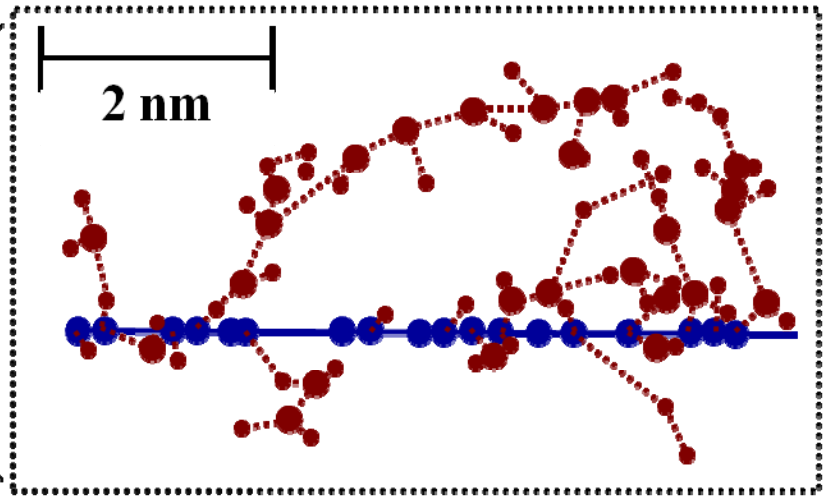
Spatial Pattern of Energy Deposits on the Molecular and Cellular Levels ("*Track Structure*")



10-15 μm



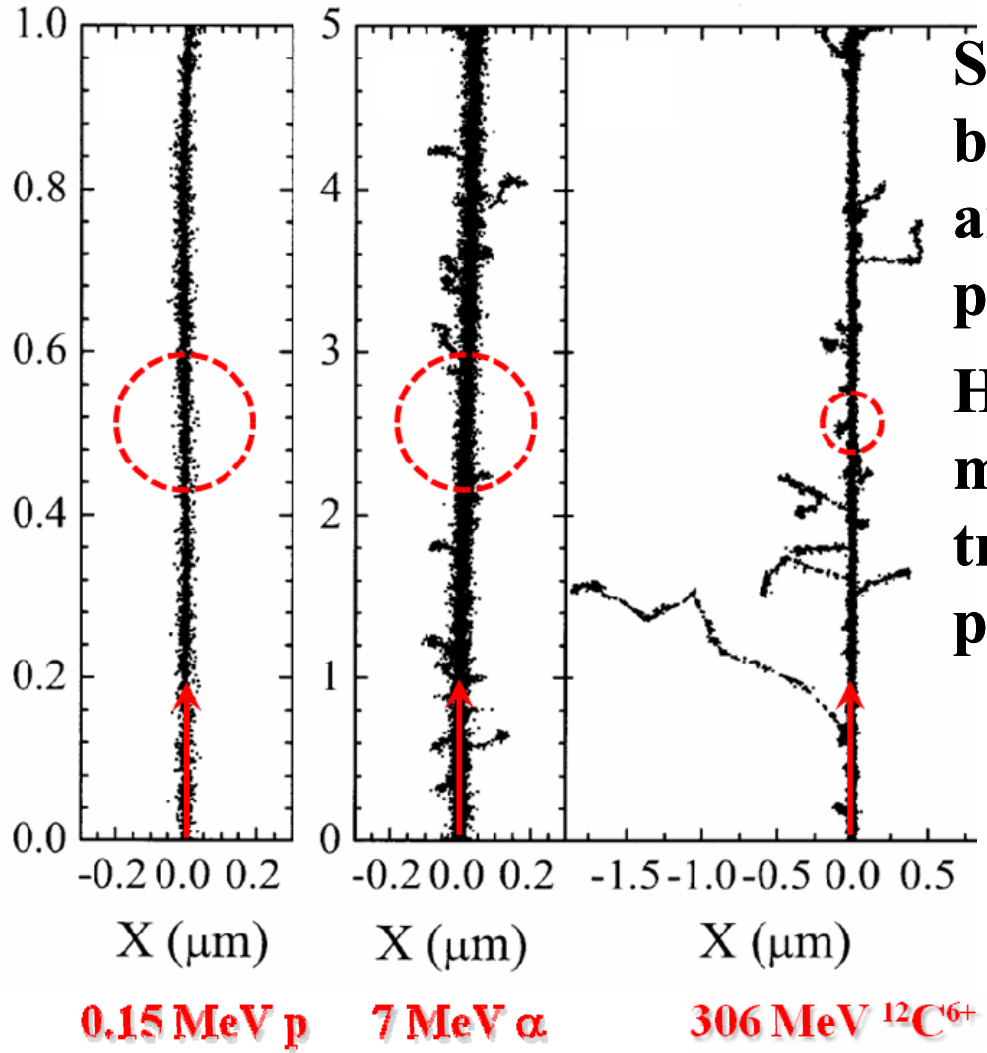
7 MeV α
(70 keV/μm)



Mostly "spurs" (< 100 eV), a few "blobs" (100-500 eV) and "short track segments"

Image adapted from Muroya Y, Plante I, Azzam EI, Meesungnoen J, Katsumura Y, Jay-Gerin JP. High-LET ion radiolysis of water: visualization of the formation and evolution of ion tracks and relevance to the radiation-induced bystander effect. *Radiat Res.* 165(4), 485-491 (2006).

Tracks formed by ions in water (70 keV/μm)



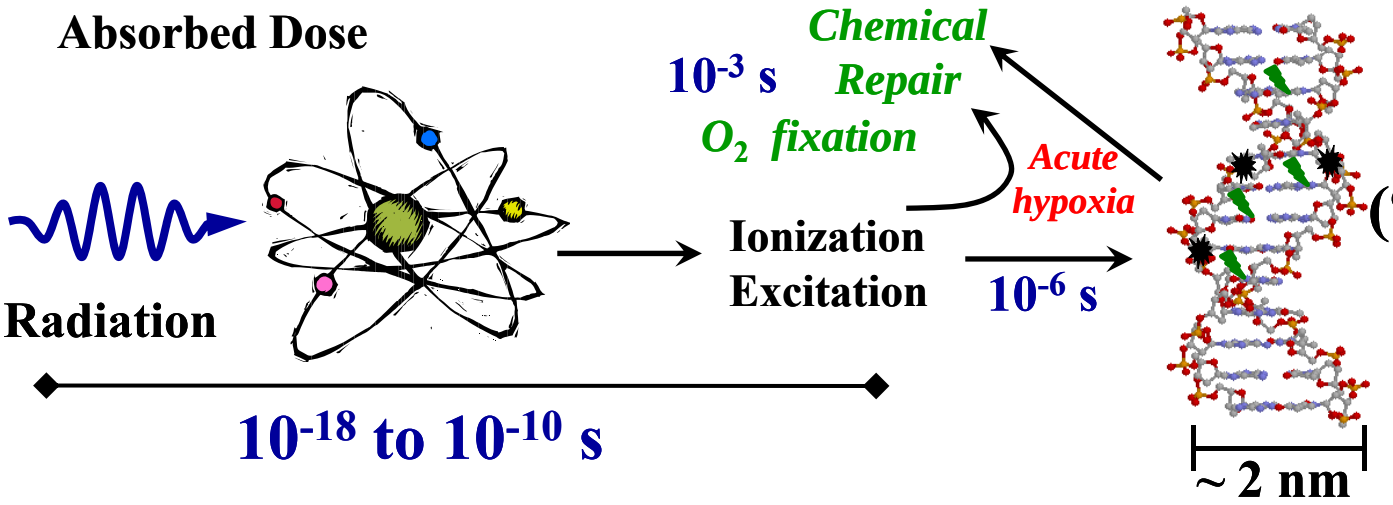
Structure of the tracks produced by particles with the same LET are not quite the same and can produce different biological effects

However if we "zoom out" to the macroscale (> 0.1 to 1 mm), the tracks of even very high LET particles look quite similar

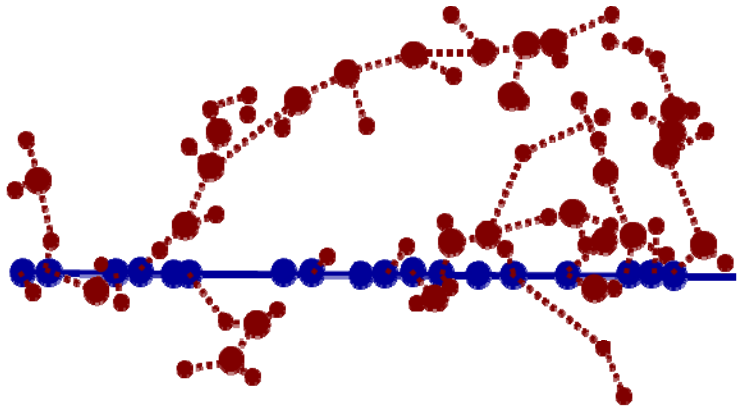
RBE effects must arise from the cellular and subcellular features of tracks – even for clinical endpoints!

Image adapted from Muroya Y, Plante I, Azzam EI, Meesungnoen J, Katsumura Y, Jay-Gerin JP. High-LET ion radiolysis of water: visualization of the formation and evolution of ion tracks and relevance to the radiation-induced bystander effect. *Radiat Res.* 165(4), 485-491 (2006).

Initial Damage to a Critical Molecule



Cluster of damaged nucleotides ("*lesions*") formed by passage of charge particle by DNA

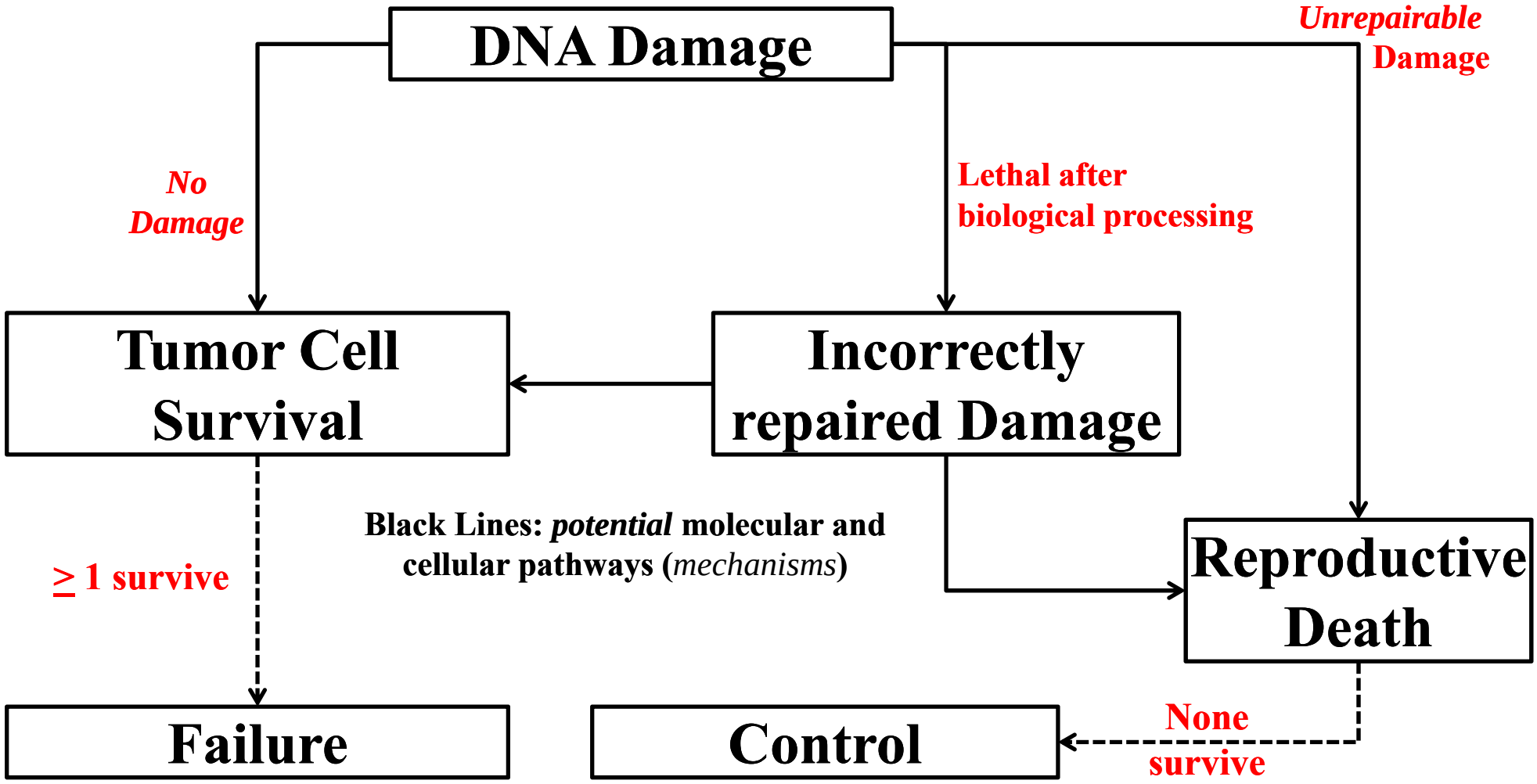


Segment of a 4 MeV α particle ($^4He^{2+}$)

Spatial pattern energy deposits determines local complexity of the cluster

Overall, a 1 Gy dose damages about 1 in 10^6 nucleotides.

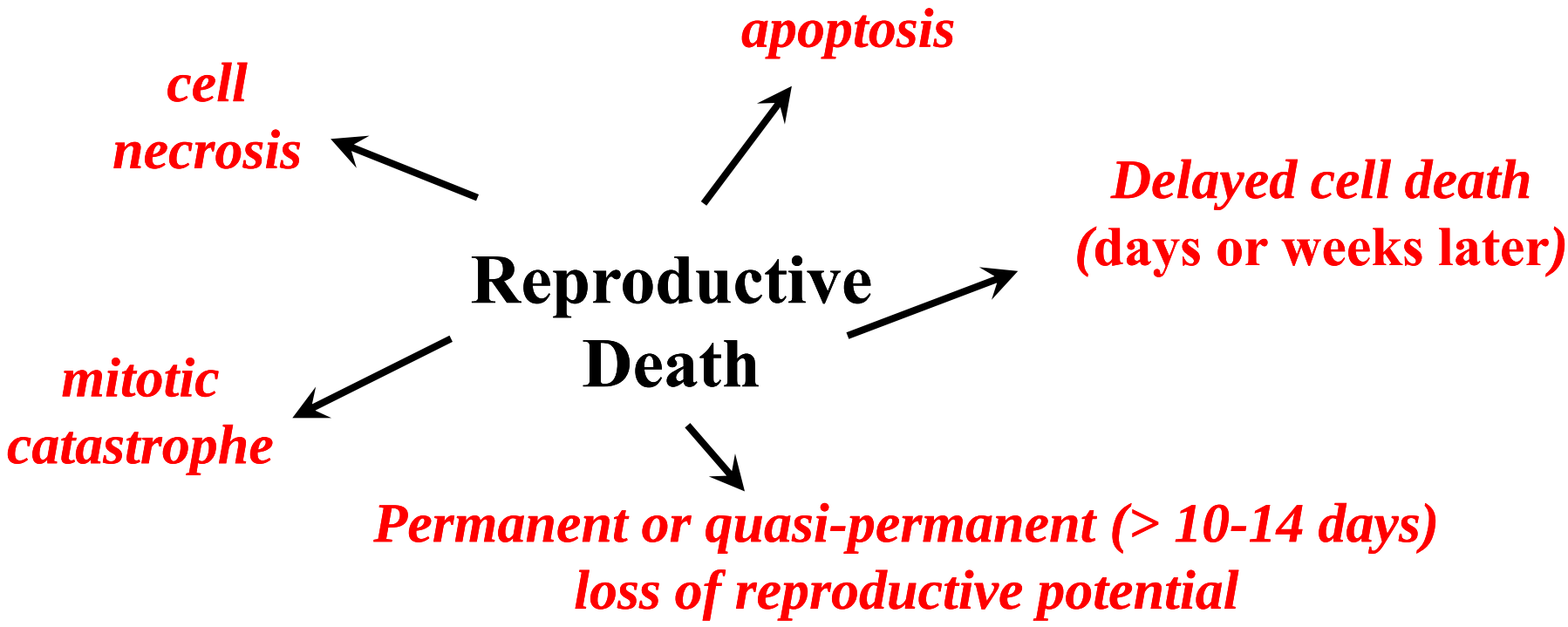
A Paradigm to Connect DNA Damage to Local Tumor Control



Black Dashed Lines: transition from cell to tissue-level biology

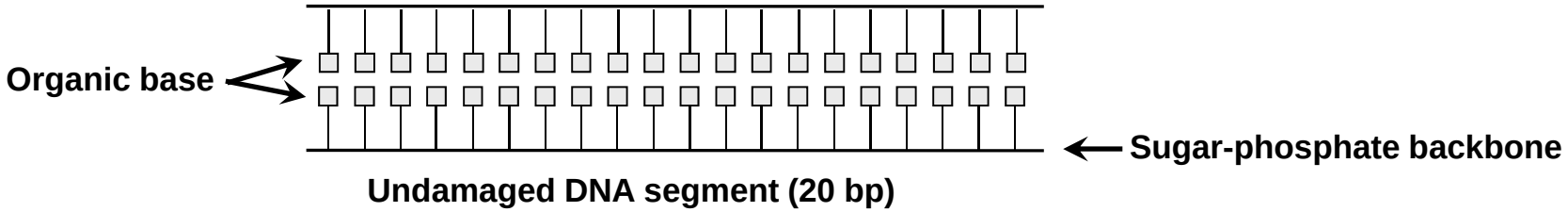
Reproductive Death?

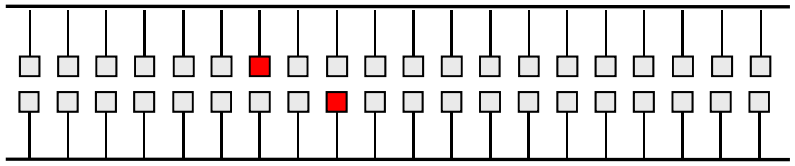
Reproductive **death is a general term that encompasses all** ***modes*** **of cell death,** ***including cells that remain metabolically active and intact but unable to divide…***



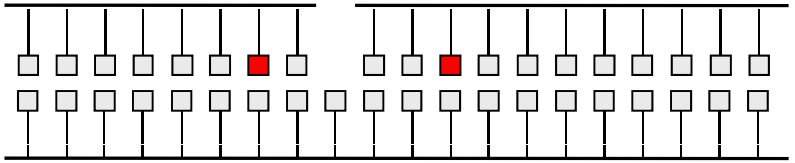
Clusters of DNA lesions

Groups of several DNA lesions within one or two turns of the DNA are termed a *cluster* or *multiply damaged site* (MDS)*

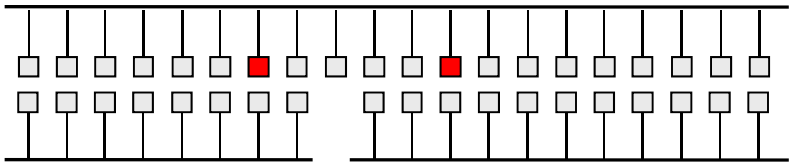




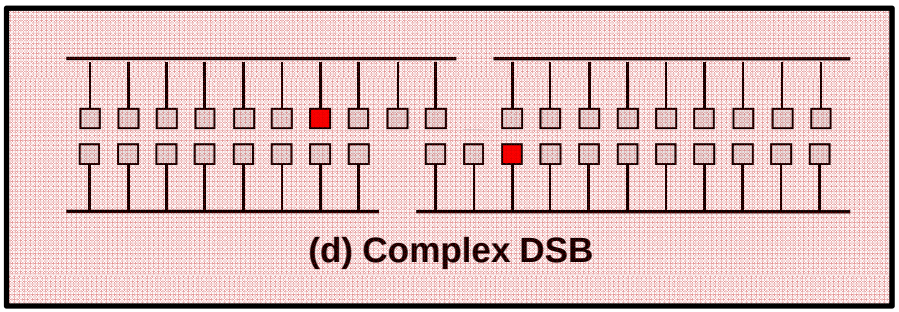
(a) Base damage in opposed strands



(c) complex SSB with adjacent base damage



(b) complex SSB with opposed base damage



Most critical category of DNA Damage

* Clustered lesions are also referred to as ***locally multiply damaged sites*** (LMDS)

Are all DSB Lethal? What about SSB?

After 1 Gy dose of low LET radiation, a typical human cell sustains 45 ± 10 DSB Gy^{-1} $cell^{-1}$ and 1000 ± 200 SSB Gy^{-1} $cell^{-1}$. If *all* DSB are lethal, the fraction of cells that will survive a 2 Gy dose is

$$S = \exp(-45\ \text{DSB Gy}^{-1} \cdot 2\ \text{Gy}) \sim 10^{-40}\ (10^{-31}, 10^{-48})$$

Only those cells that do not sustain a radiation-induced DSB survive
(Poisson distribution of DSB among irradiated cells)

For comparisons, *many* published studies indicate a surviving fraction of 0.1 (repair compromised**) to 0.9 (*repair proficient*) cells after a 2 Gy dose of radiation. Only way to reconcile observations is**

< 2% of initial DSB formed in a cell are lethal **and/or** **< 0.1% of initial SSB formed in a cell are lethal**

Cells are *really* good at repairing DNA damage, even DSB!

See also the classic review: DT Goodhead. Initial events in the cellular effects of ionizing radiations: clustered damage in DNA. IJRB 65(1): 7-17 (1994).

RBE for DSB induction

Σ = number of DSB Gy^{-1} $cell^{-1}$
= slope of line

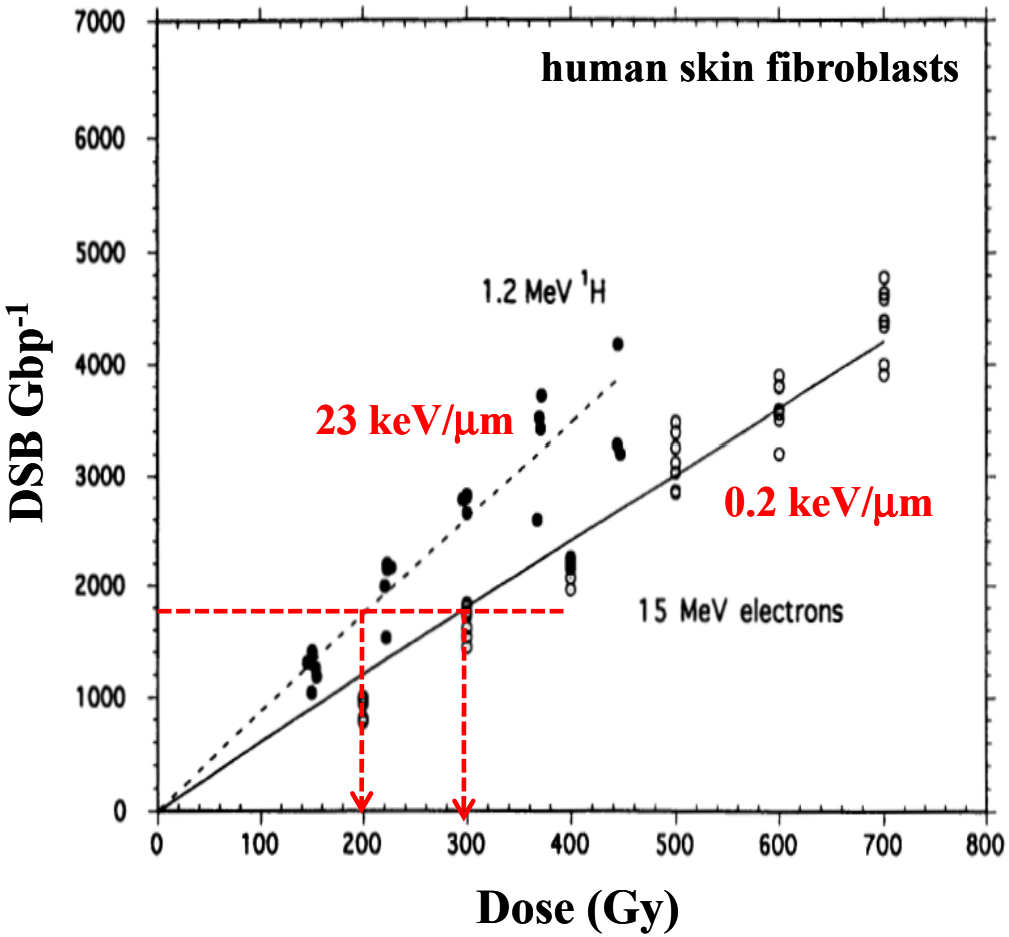
D_γ = dose of reference radiation

D_p = dose of proton

Want:

Number DSB = $\Sigma_\gamma D_\gamma = \Sigma_p D_p$

$$RBE_{DSB} \equiv \frac{D_\gamma}{D_p} = \frac{\Sigma_p}{\Sigma_\gamma} \cong 1.5$$



RBE is an example of an "isoeffect calculation"

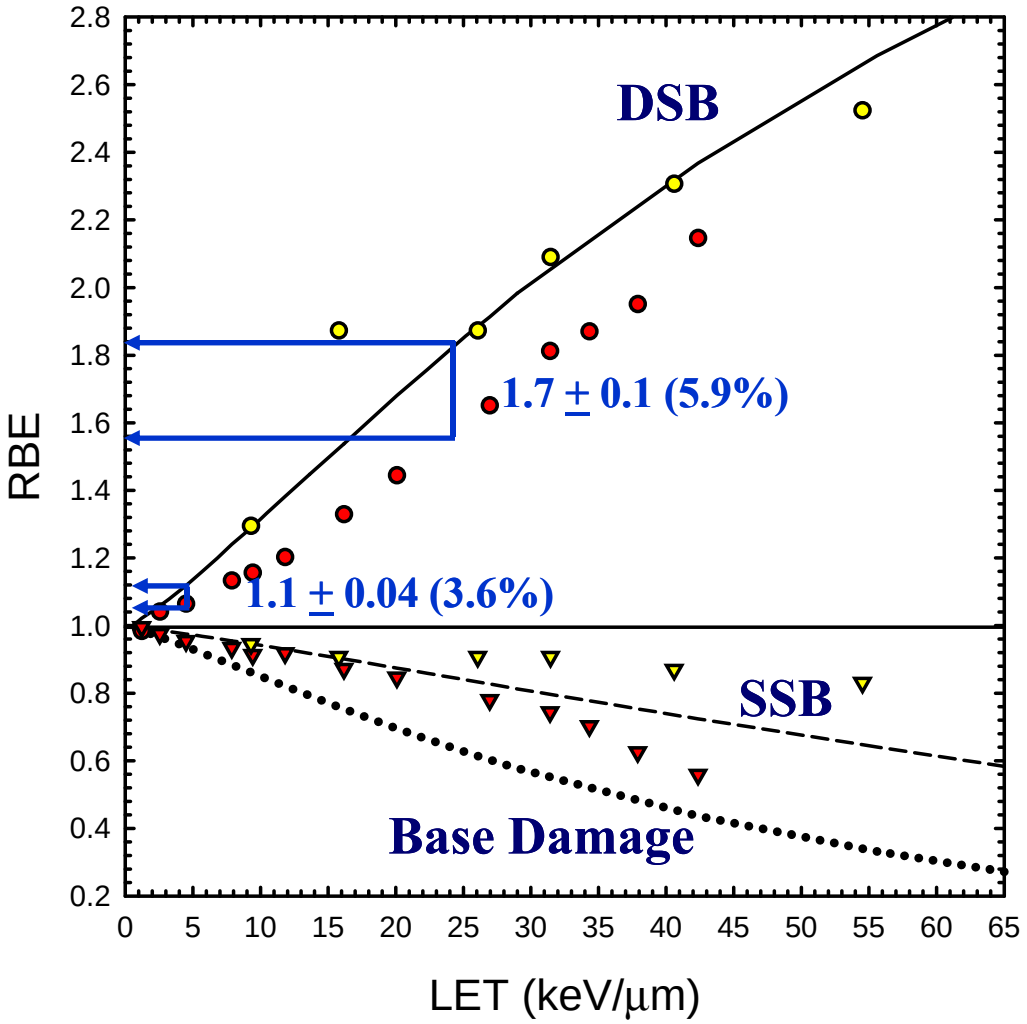
Measured data from Frankenberg D, Brede HJ, Schrewe UJ, Steinmetz C, Frankenberg-Schwager M, Kasten G, Pralle E. Induction of DNA double-strand breaks by ^{1}H and ^{4}He ions in primary human skin fibroblasts in the LET range of 8 to 124 keV/microm. *Radiat Res.* **151**(5), 540-549 (1999).

Trends in RBE_{DSB} with proton LET

Filled Yellow Symbols: ***Track Structure Simulation*** **(**Nikjoo *et al.* 1997, 2001, 2002**)**

Filled Red Symbols: ***Track Structure Simulation*** **(**Friedland *et al.* 2003**)**

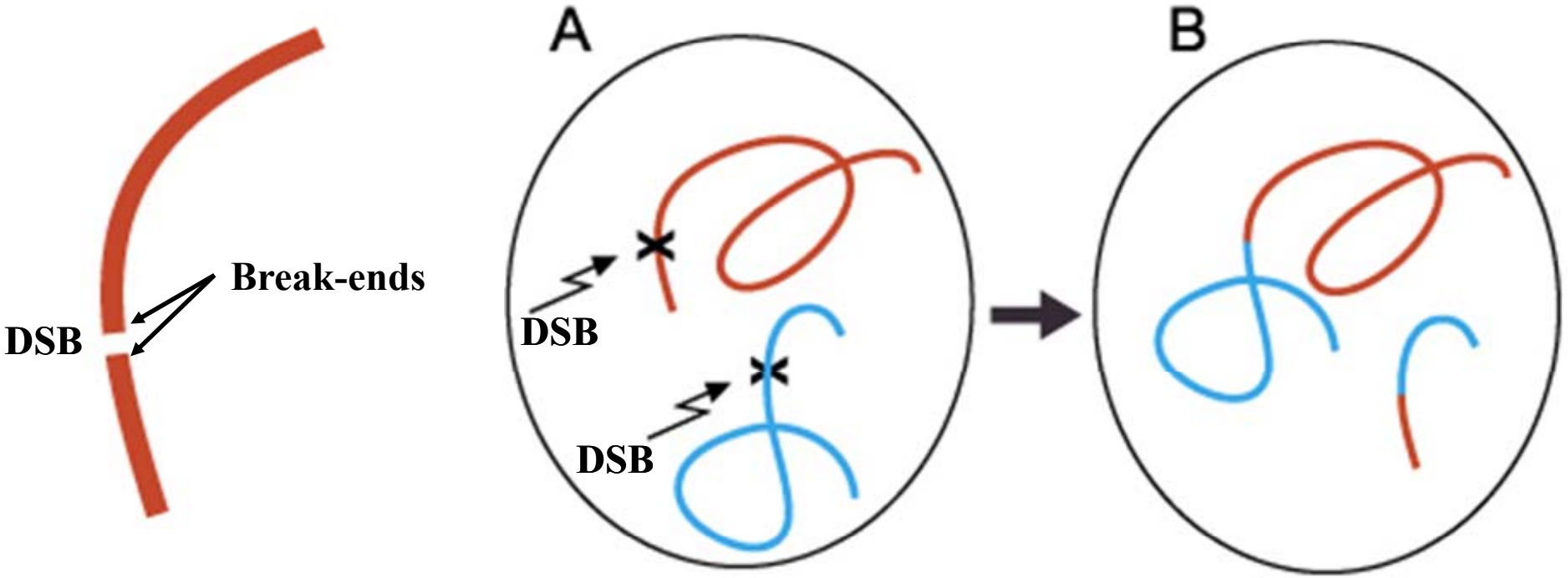
Lines: ***Monte Carlo Damage Simulation**** **(MCDS)**



DSB are *only* category of DNA damage that increases with increasing particle LET (*additional evidence SSB less critical form of DNA damage than DSB***)**

*** Semenenko and Stewart 2004, 2006, Stewart *et al.* 2011**

Why are DSB so effective at killing cells?
(*Breakage and Rejoining Theory*)

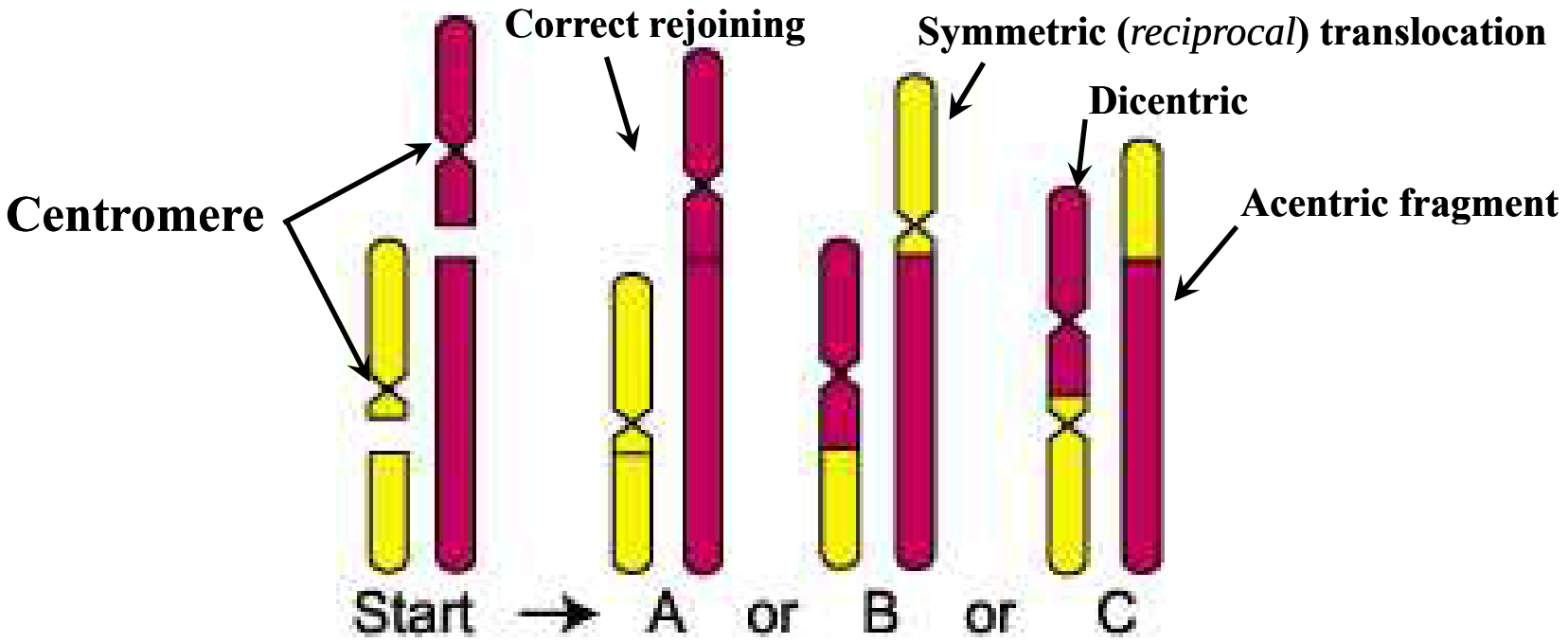


Break-ends associated with one DSB incorrectly rejoined to break-end associated with a *different* DSB

Proximity Effects: *pairs* of DSB formed in close spatial *and* temporal proximity are more likely to rejoin incorrectly than *pairs* of DSB separated in time and\or space (*dose rate* and *LET effects*)

R.K. Sachs and D.J. Brenner, Chromosome Aberrations Produced by Ionizing Radiation: Quantitative Studies
http://web.ncbi.nlm.nih.gov/books/bv.fcgi?call=bv.View..ShowTOC&rid=mono_002

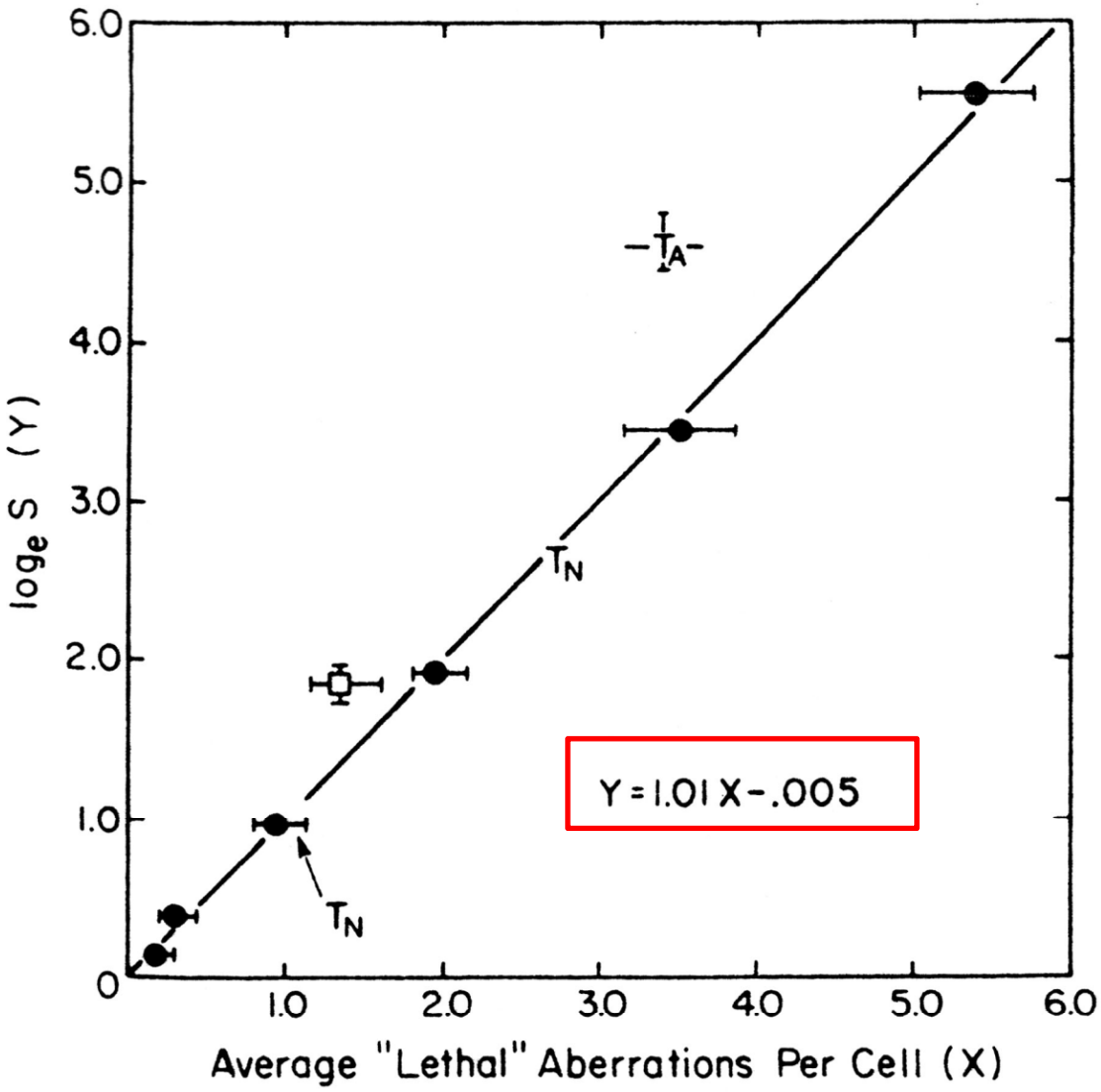
Lethal and Non-Lethal Aberrations



Dicentrics and acentric fragments are usually lethal in the reproductive sense because segregation of chromosomes at mitosis is disturbed. In contrast, correct DSB rejoining and symmetric (*reciprocal*) translocations are consistent with continued cell division

R.K. Sachs and D.J. Brenner, Chromosome Aberrations Produced by Ionizing Radiation: Quantitative Studies
http://web.ncbi.nlm.nih.gov/books/bv.fcgi?call=bv.View..ShowTOC&rid=mono_002.TOC&depth=10

Is there a 1:1 relationship?



AC 1522 normal human fibroblasts irradiated by x-rays

$$S = e^{-Y}$$

S = fraction that survive

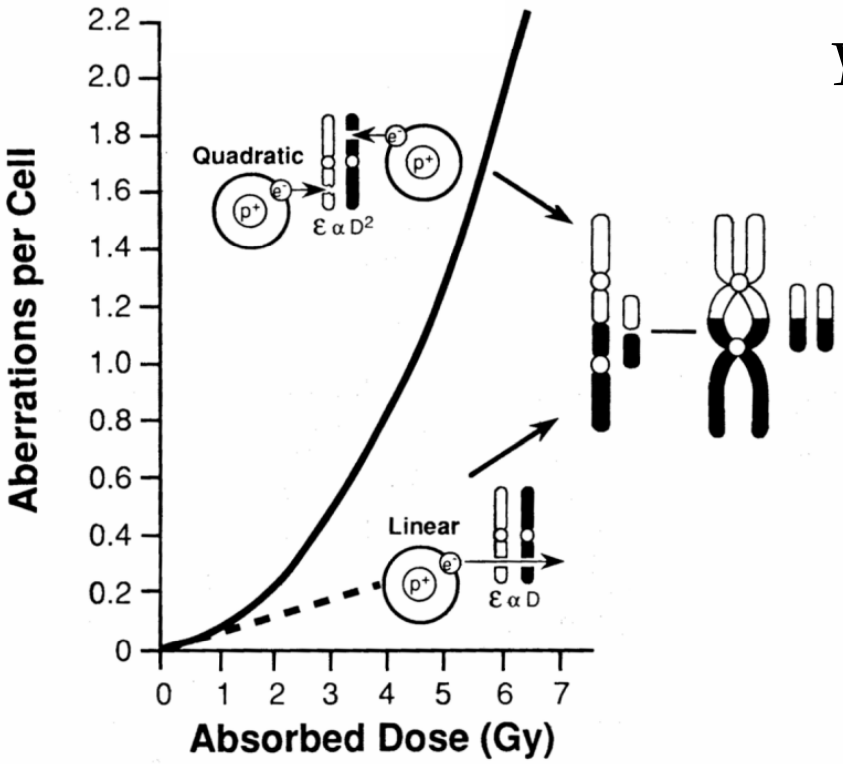
Y **= avg number of lethal aberrations per cell**

Source: Cornforth and Bedford, *Rad. Res.*, 111, p 385-405 (1987). See also Figure 3.4 in Hall (p. 37)

Linear-Quadratic (LQ) Model for Cell Survival

Pairs **of DSB formed by *same track* interact (*intra-track* *interactions*)**

$$Y(D) = \alpha D + \beta D^2 = \alpha D\left(1 + \frac{D}{\alpha/\beta}\right)$$



Pairs **of DSB formed by *different tracks* interact (*inter-track* *effect*)**

Cell survival after dose *D*

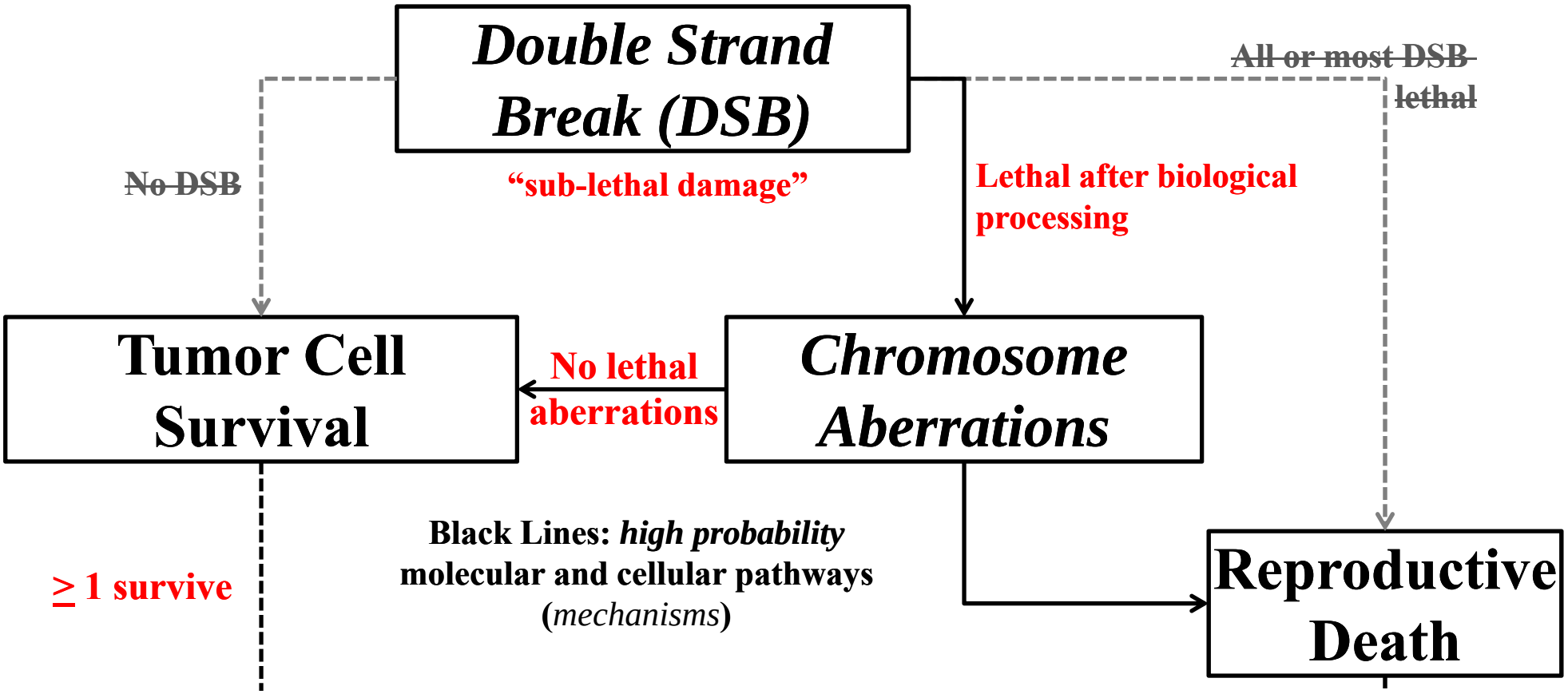
$$S(D) = e^{-Y(D)} = e^{-\alpha D - \beta D^2}$$

Only those cells without a lethal aberration in their DNA retain the ability to divide and produce viable progeny ("reproductive survival").

Figure 3.5 in EJ Hall and AJGiaccia, Radiobiology for the Radiologist, 6th Ed, Lippincott Williams & Wilkins (2006)

Connecting *DSB* to Local Tumor Control

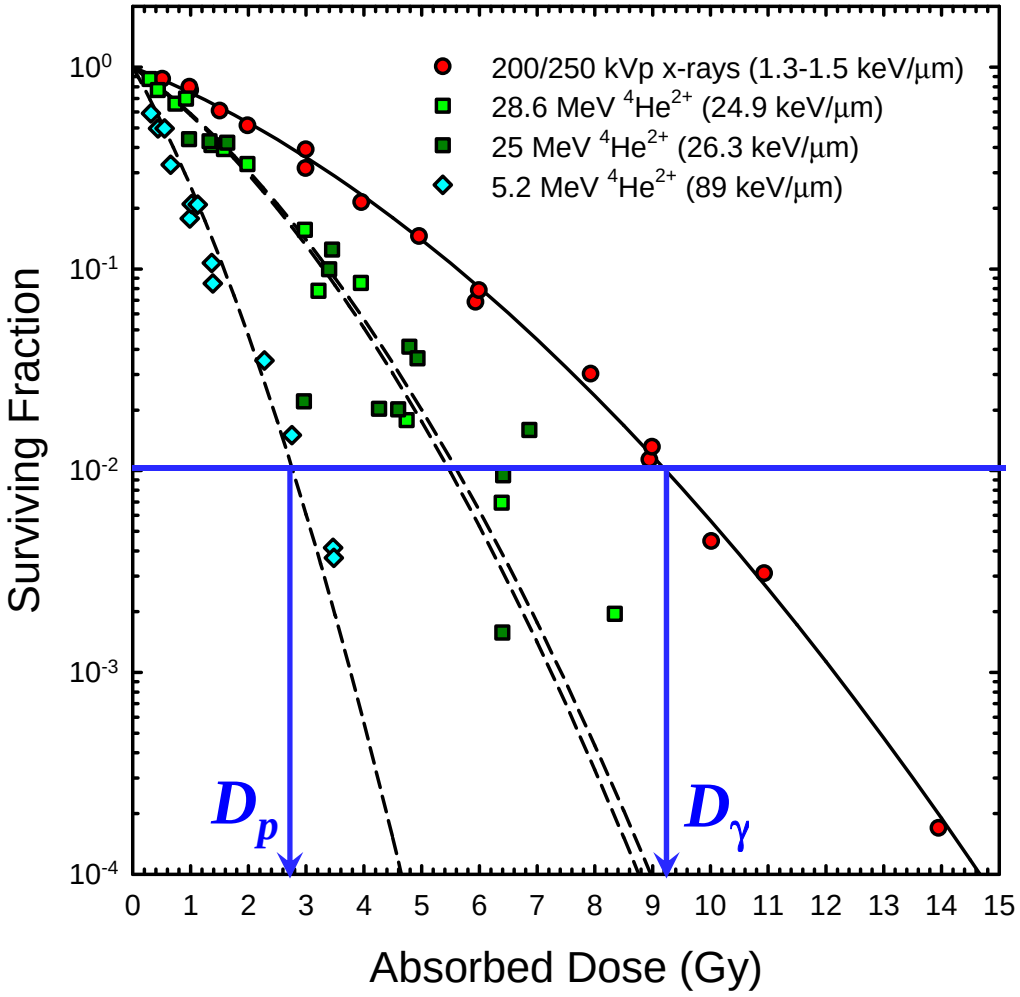
Grey Dashed Lines: *low probability* molecular and cellular pathways (*mechanisms*)



Are trends in the RBE for DSB induction qualitatively and quantitatively similar to the RBE for cell survival?

Black Dashed Lines: transition from cell to tissue-level biology

Low and High Dose RBE (*cell survival*)



αD dominates
($D << \alpha/\beta$)

βD² dominates
($D >> \alpha/\beta$)

RBE for a specific dose (*cell survival level*)

$$RBE = \frac{D_\gamma}{D_p}$$

$$\cong \frac{9.2\ \text{Gy}}{2.8\ \text{Gy}} = 3.3\ (1\%\ \text{survival})$$

Low Dose RBE: $-\ln S \cong (\alpha D)_\gamma = (\alpha D)_p$

$$\text{low dose } RBE_{SF} = \frac{D_\gamma}{D_p} = \frac{\alpha_p}{\alpha_\gamma}$$

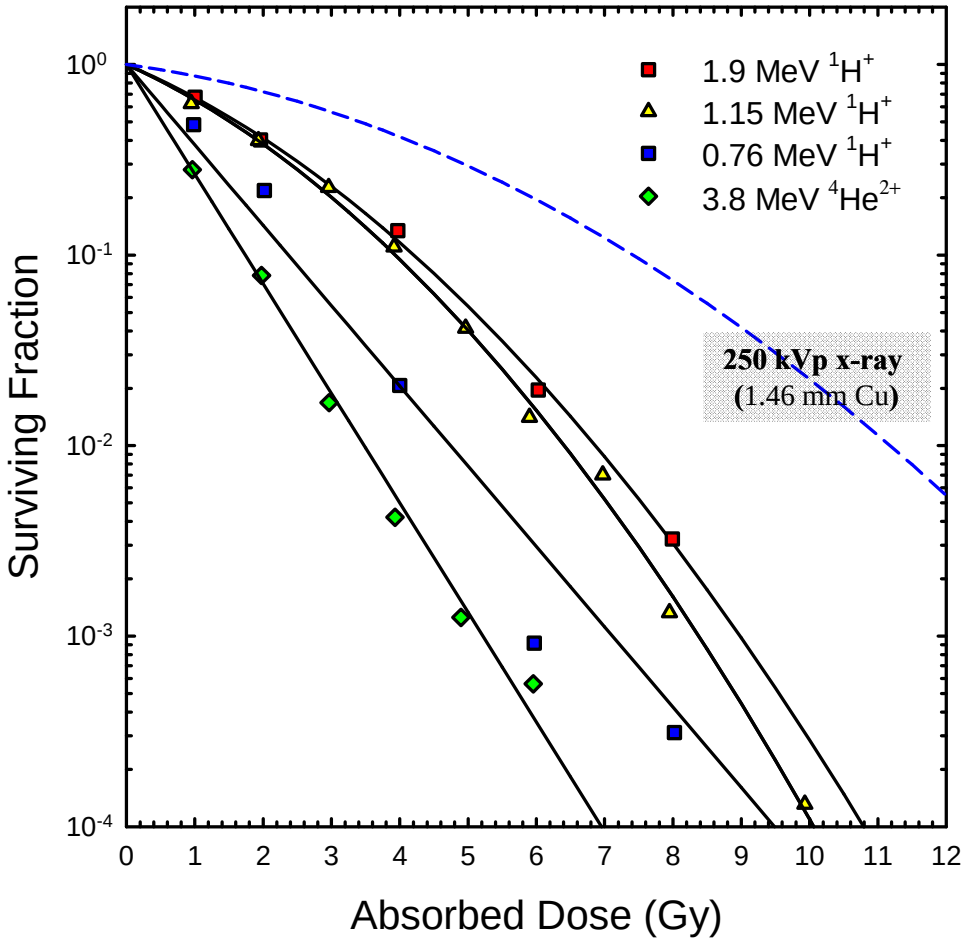
"RBE_{max}"

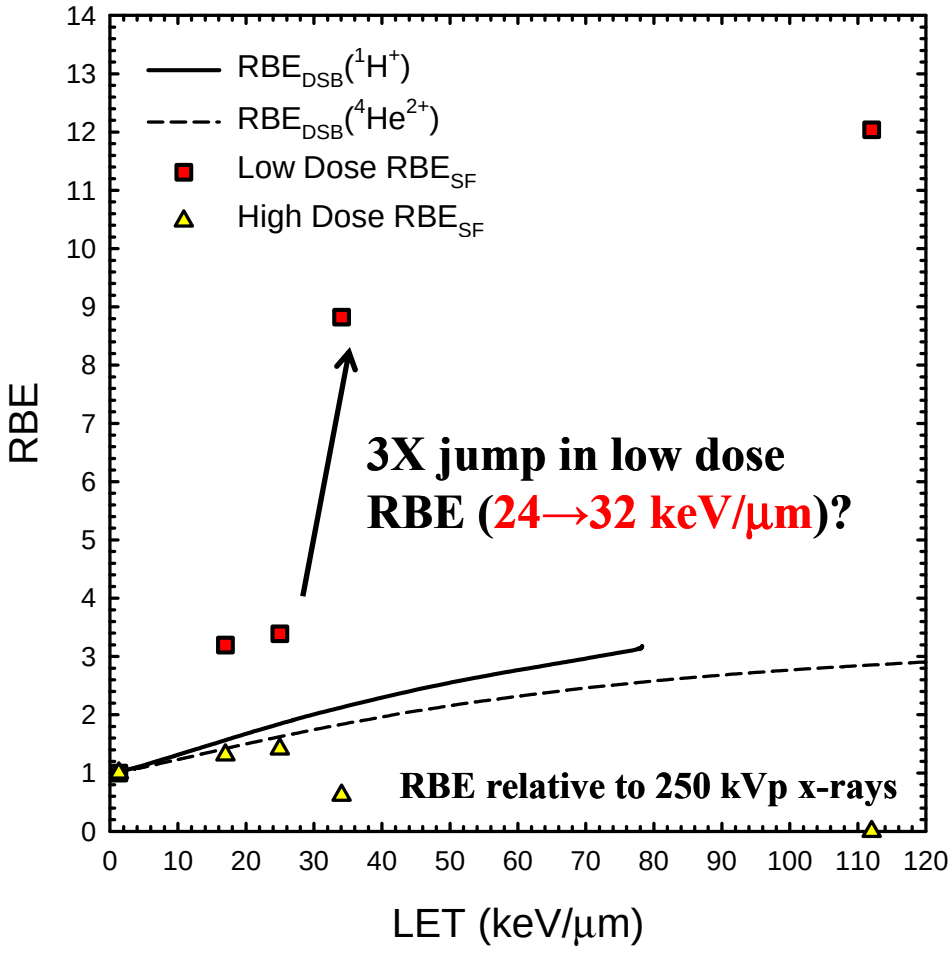
High Dose RBE: $-\ln S \cong (\beta D^2)_\gamma = (\beta D^2)_p$

$$\text{high dose } RBE_{SF} = \frac{D_\gamma}{D_p} = \sqrt{\frac{\beta_p}{\beta_\gamma}}$$

"RBE_{min}"

Is RBE_{DSB} predictive of RBE_{SF}?





Little if any evidence RBE_{SF} ($^1H^+$ and $^4He^{2+}$) is related to RBE_{DSB}, or so it seems…

Measured data from Prise *et al.* IJRB, 58, p 261-277 (1990)

* Semenenko and Stewart 2004, 2006, Stewart *et al.* 2011

A Mechanistic Model for α and α/β

The Repair-Misrepair-Rixation (RMF) model (*Carlson et al. 2008*) predicts, in the limit when the *D* is small compared to α/β, that

***Intra*-track chromsomal aberrations**

$$\alpha = \theta\Sigma + \kappa\bar{z}_F\Sigma^2$$

Unrepairable and misrepaired

$$\beta = \frac{\kappa}{2}\Sigma^2$$

***Inter*-track aberrations**

$$\frac{\alpha}{\beta} = \frac{2}{\Sigma}(\theta/\kappa) + 2\bar{z}_F$$

θ, κ are *adjustable cell- or tissue-specific* parameters related to biological processing of DNA damage (*independent of LET and O_2 concentration*)

Σ is the number of DSB Gy^{-1} Gbp^{-1} (or per cell); estimate using the MCDS (*strong function of LET and O_2 concentration*)

$\bar{z}_F$ is the frequency-mean specific energy (in Gy) delivered to the cell nucleus (*strong function of LET but independent of O_2 concentration*) – estimate with the MCDS or other Monte Carlo code(s)

D.J. Carlson, R.D. Stewart, V.A. Semenenko and G.A. Sandison, Combined use of Monte Carlo DNA damage simulations and deterministic repair models to examine putative mechanisms of cell killing. *Rad. Res.* **169**, 447-459 (2008)

How is RBE_{DSB} related to RBE_{SF}?

With the RMF-motivated formulas for α and β, the low and high dose RBE_{SF} is

$$\alpha = \theta\Sigma + \kappa\bar{z}_F\Sigma^2 \qquad \beta = \frac{\kappa}{2}\Sigma^2$$

DSB Gy^{-1} Gbp^{-1}

"RBE_{min}" high dose $RBE_{SF} = \sqrt{\frac{\beta_p}{\beta_\gamma}} = RBE_{DSB}$

DSB Gy^{-1} Gbp^{-1} reference radiation

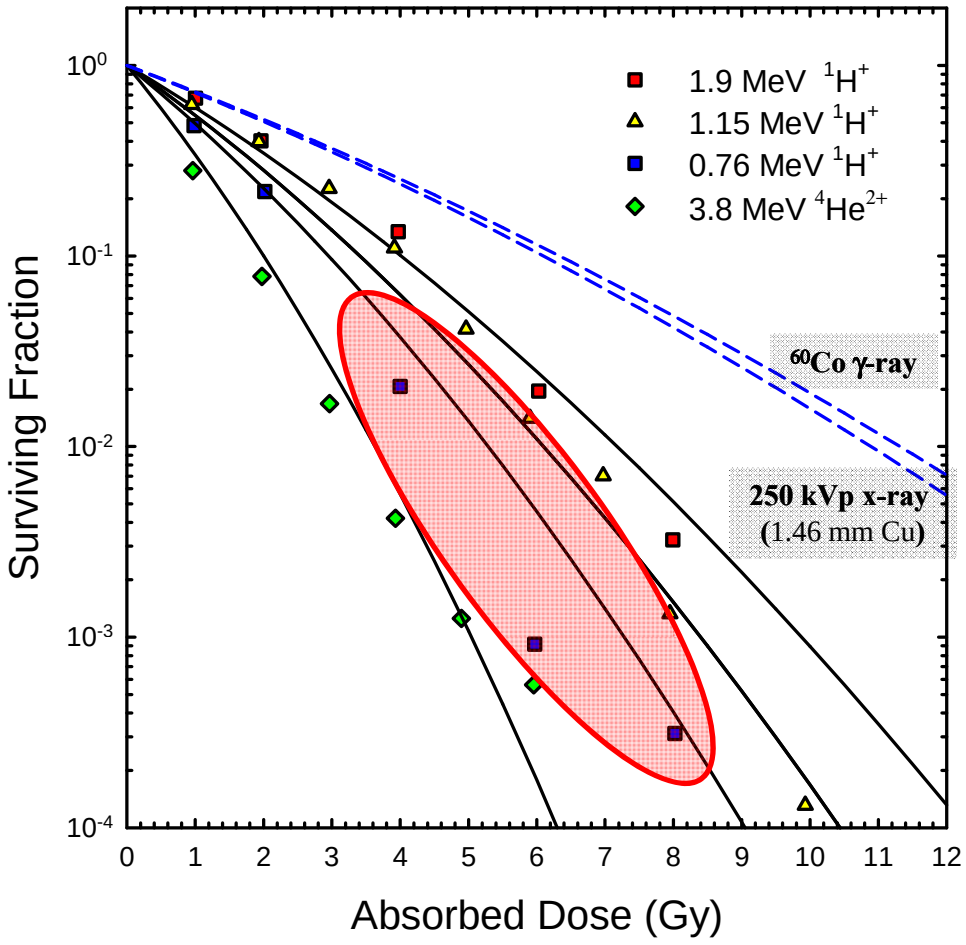
"RBE_{max}" low dose $RBE_{SF} = \frac{\alpha_p}{\alpha_\gamma} \cong RBE_{dsb}\left\{1 + RBE_{dsb}\frac{\bar{z}_F\Sigma_\gamma}{\theta/\kappa}\right\} \geq RBE_{dsb}$

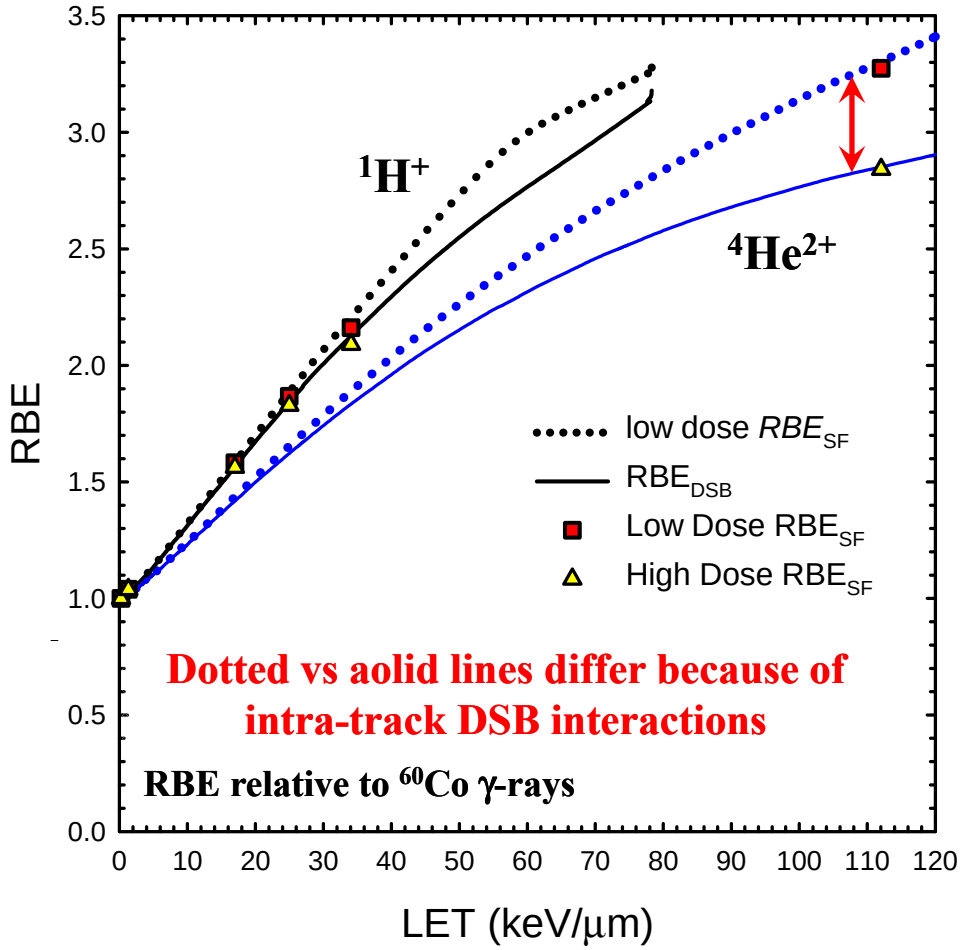
***D* is "*small*" compared to α/β**

Intra-track* DSB interactions increase with increasing LET because of *proximity effects

$$\frac{\bar{z}_F\Sigma_\gamma}{\theta/\kappa} \propto \frac{\Sigma_\gamma}{\theta/\kappa}\cdot LET$$

Is RBE_{DSB} predictive of RBE_{SF}? *Version 2.0*





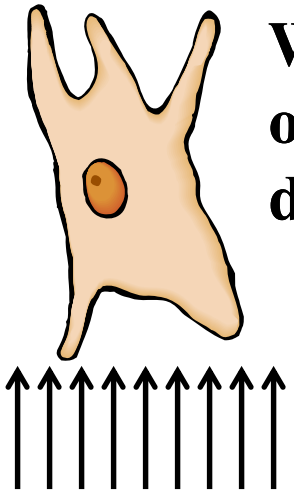
Reasonable fit to cell survival data for all energies.

Low dose $RBE_{SF} \geq RBE_{DSB}$

Measured data from Prise *et al.* IJRB, 58, p 261-277 (1990)

* Semenenko and Stewart 2004, 2006, Stewart *et al.* 2011

Dosimetry of Short-Range Particles is Tricky…



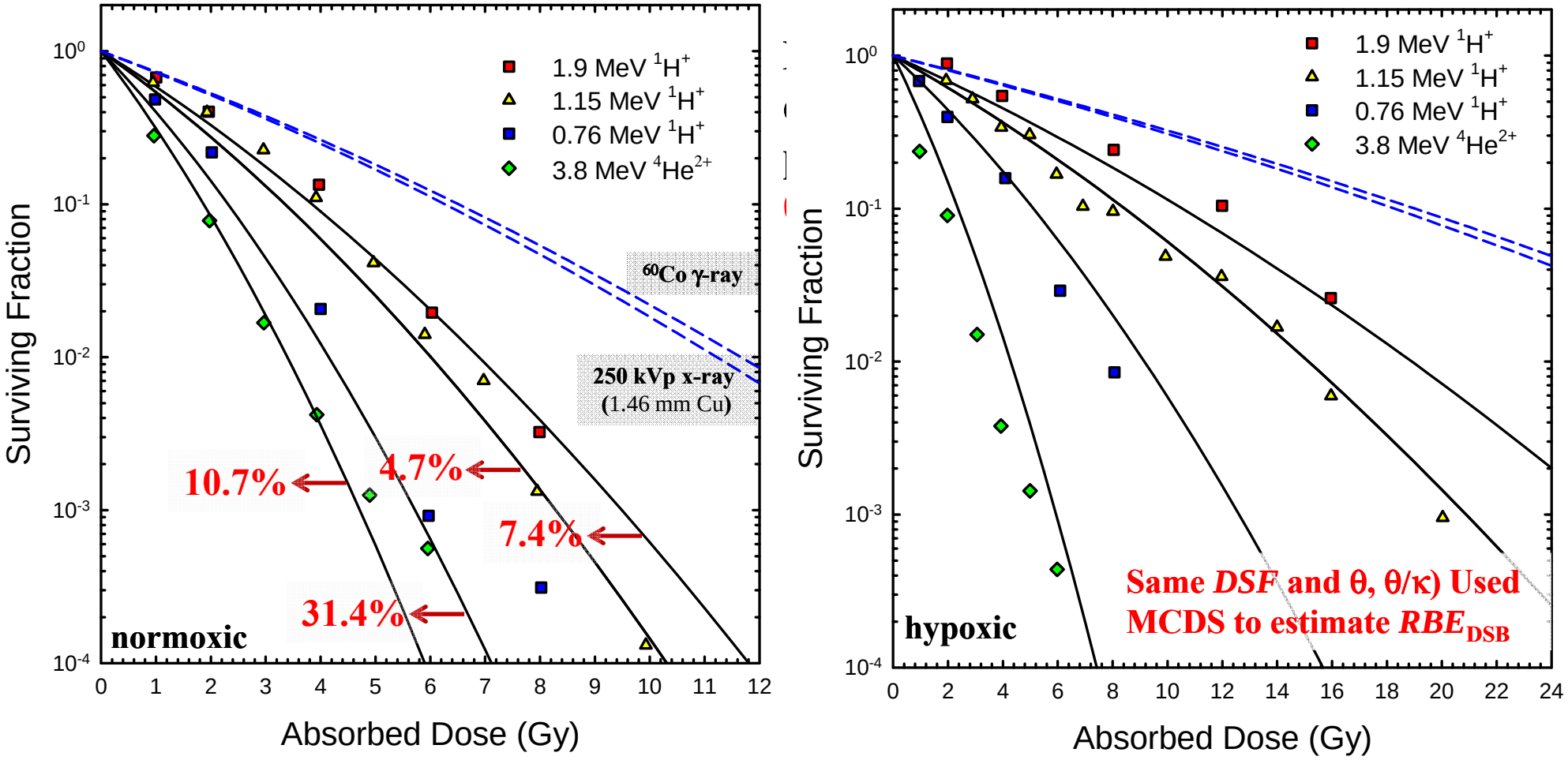
When the CSDA range of a charge particle is of the same order of magnitude as the dimensions of the biological target, dosimetry needs to be corrected for

- **Change in stopping power within target**
- **Energy and path length straggling**
- **Finite particle range ("stoppers"), energy and angular distribution of particles incident on target**

For a monoenergetic particle incident on a 5 μm target

Particle	Range (μm)	LET (keV/μm)			D/Φ (nGy-cm^2)		
		Reported	Entrance	Exit	Entrance	Avg. over Target	% difference
1.9 MeV $^1H^+$	67.7	17.0	16.8	17.4	26.9	27.3	1.30
1.15 MeV $^1H^+$	29.9	24.0	24.4	26.5	39.1	40.3	3.06
0.76 MeV $^1H^+$	15.9	32.0	32.5	38.6	52.1	55.3	6.12
3.8 MeV $^4He^{2+}$	25.3	110.0	108.7	120.9	174.2	180.9	3.89

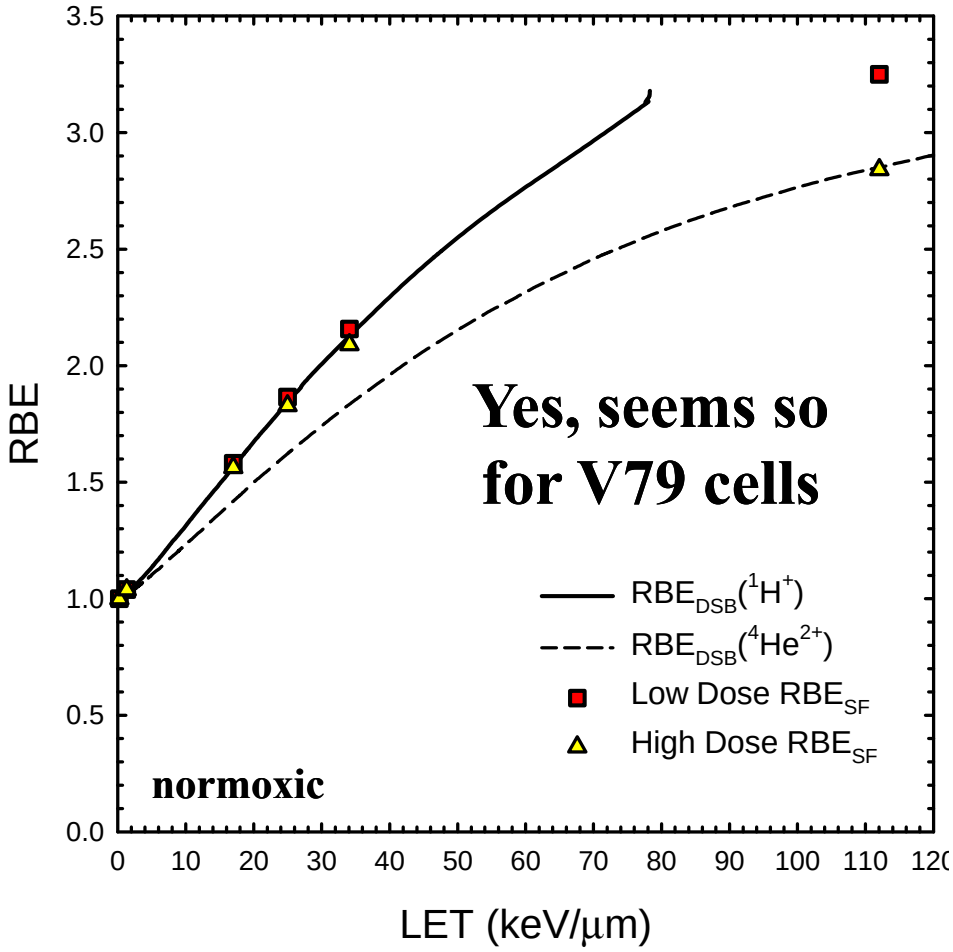
Fit with "Corrected" Dosimetry

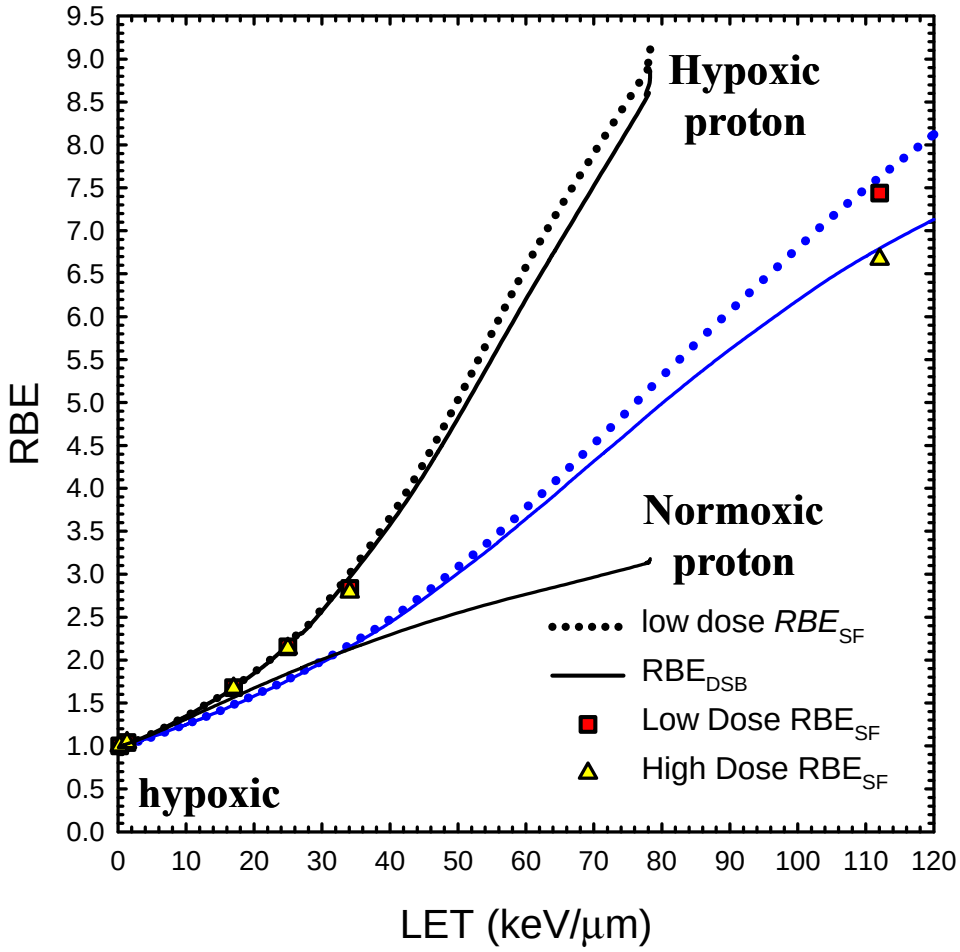


Improved fit to measured data with small (*quite plausible*) changes in the mean particle energy (< 10 μm shift)

Measured data from Prise *et al.* IJRB, 58, p 261-277 (1990)

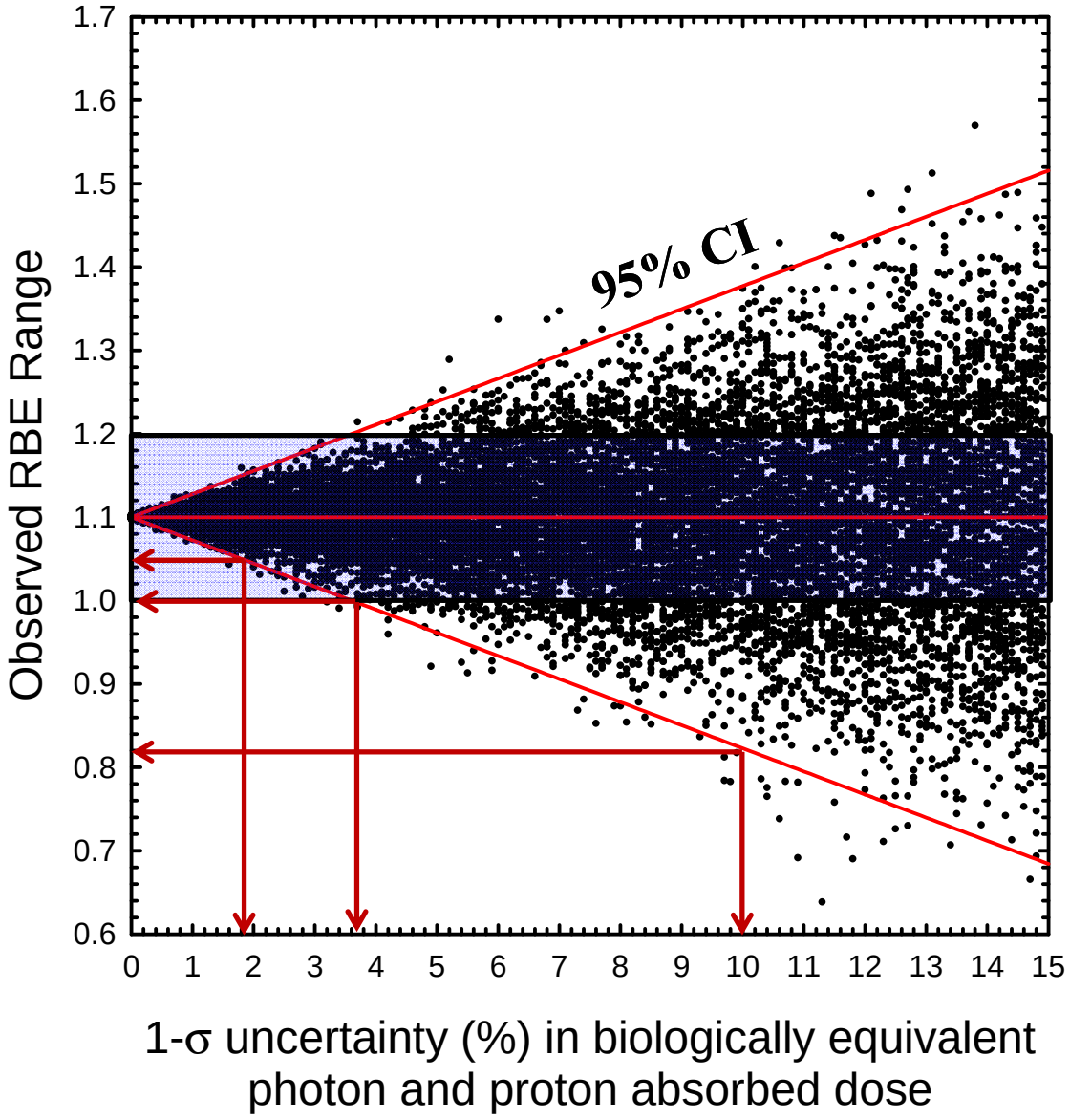
Is RBE_{DSB} predictive of RBE_{SF}? *Version 2.1*





For protons with an LET ≤ 20 keV/µm (≥ 2 MeV), RBE is about the same in cells irradiated under normoxic and anoxic conditions (no change in OER from ^{60}Co γ-rays**).**

Impact of Uncertainties on "observed" RBE



RBE is ratio of doses that produce same biological effect

$$RBE = \frac{D_\gamma \pm \sigma_\gamma}{D_p \pm \sigma_p}$$

RBE 1.1 ± 0.1 (*blue shaded region*)

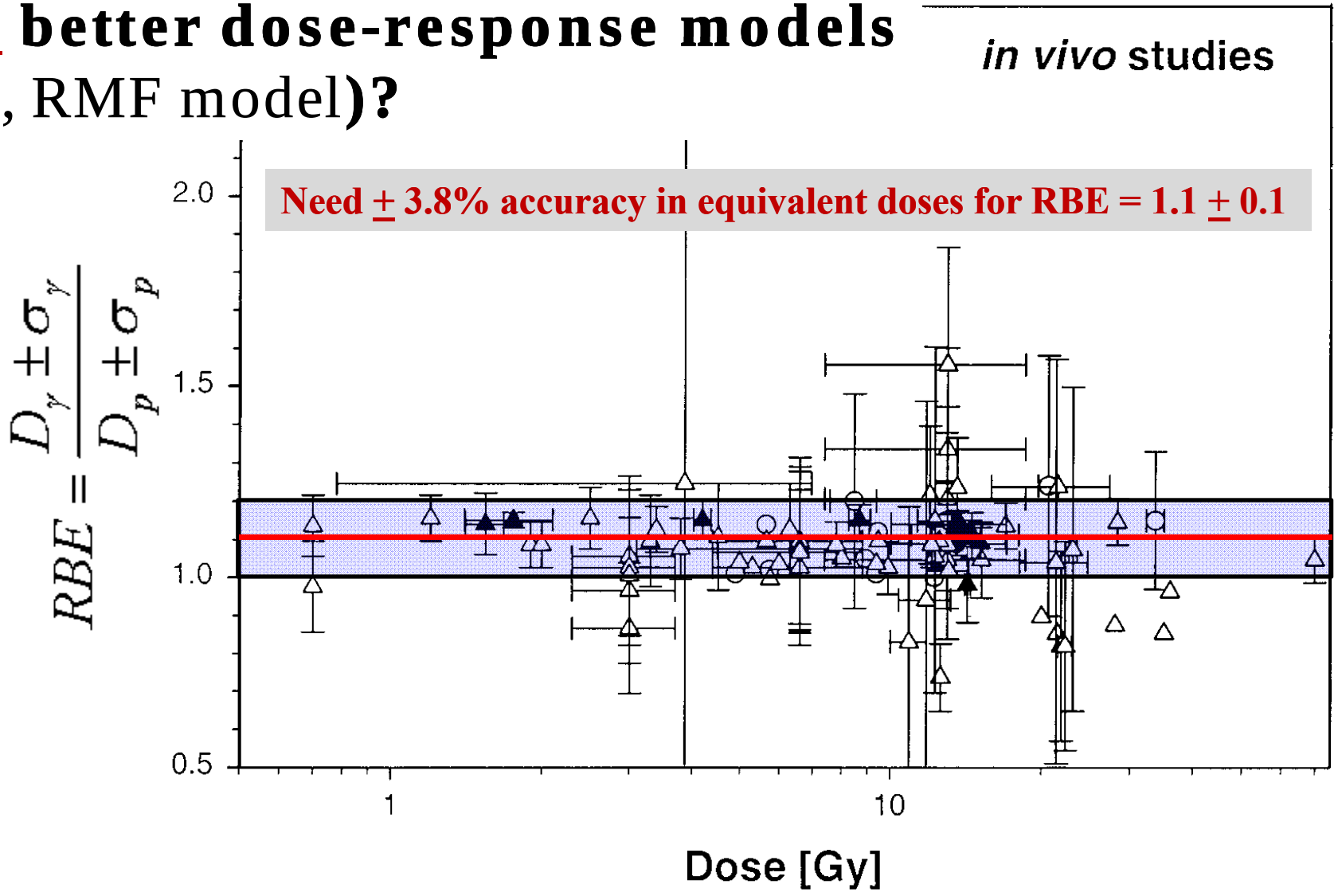
1.8% uncertainty in equivalent physical dose: RBE = 1.1 ± 0.05

3.8% uncertainty in equivalent physical dose: RBE = 1.1 ± 0.1

10% uncertainty in equivalent physical dose: RBE = 1.1 ± 0.3

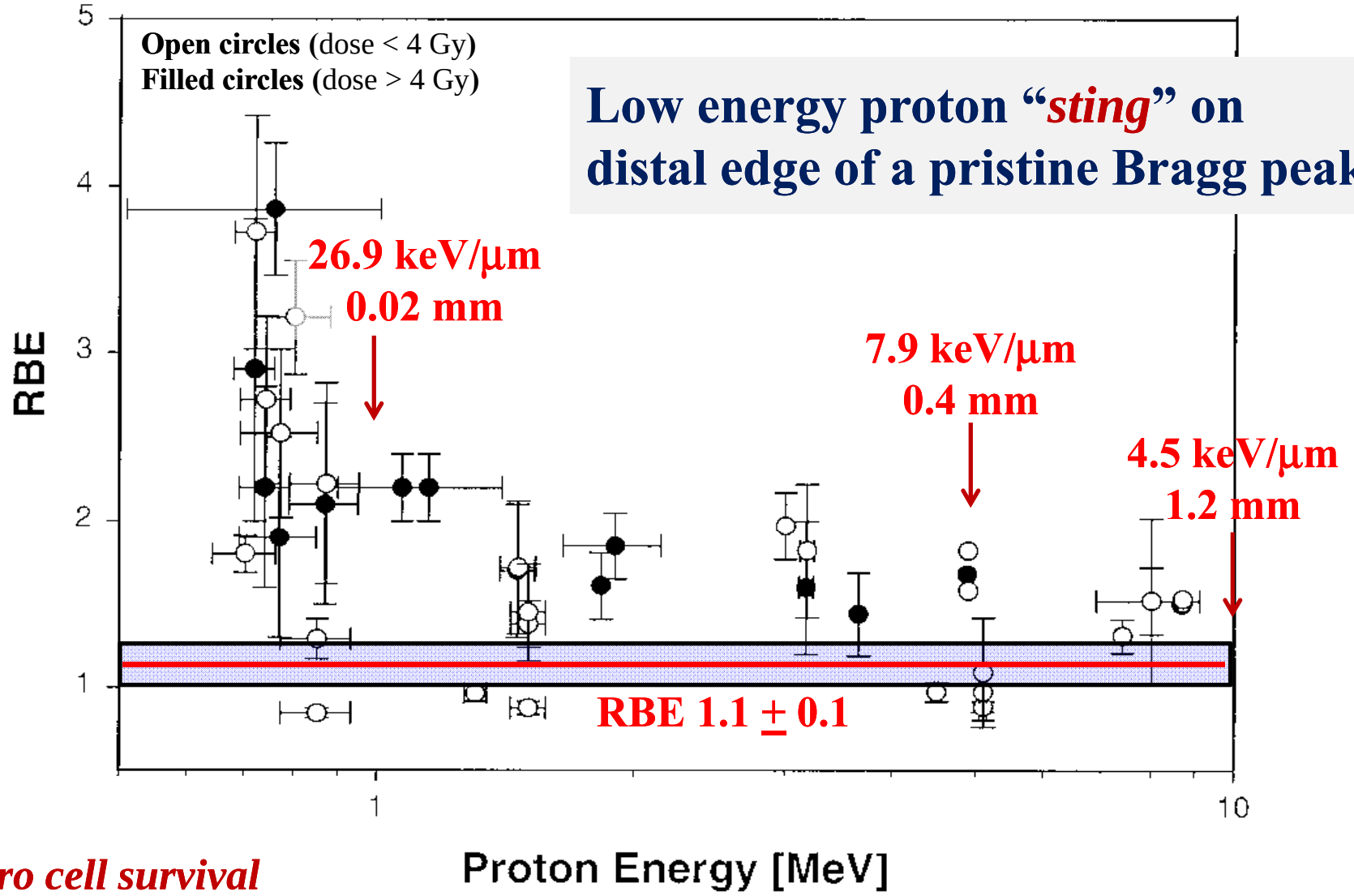
Do we just need more accurate dosimetry

and better dose-response models (e.g., RMF model)?



Adapted from Figure 2 in Paganetti, Niemierko, Ancukiewicz, Gerweck, Goitein, Loeffler, and Suit, Relative Biological Effectiveness (RBE) Values for Proton Beam Therapy, IJROBP 53(2) 407-421 (2002).

Strong evidence for spatial (*and LET*) variations in proton RBE despite uncertainties …

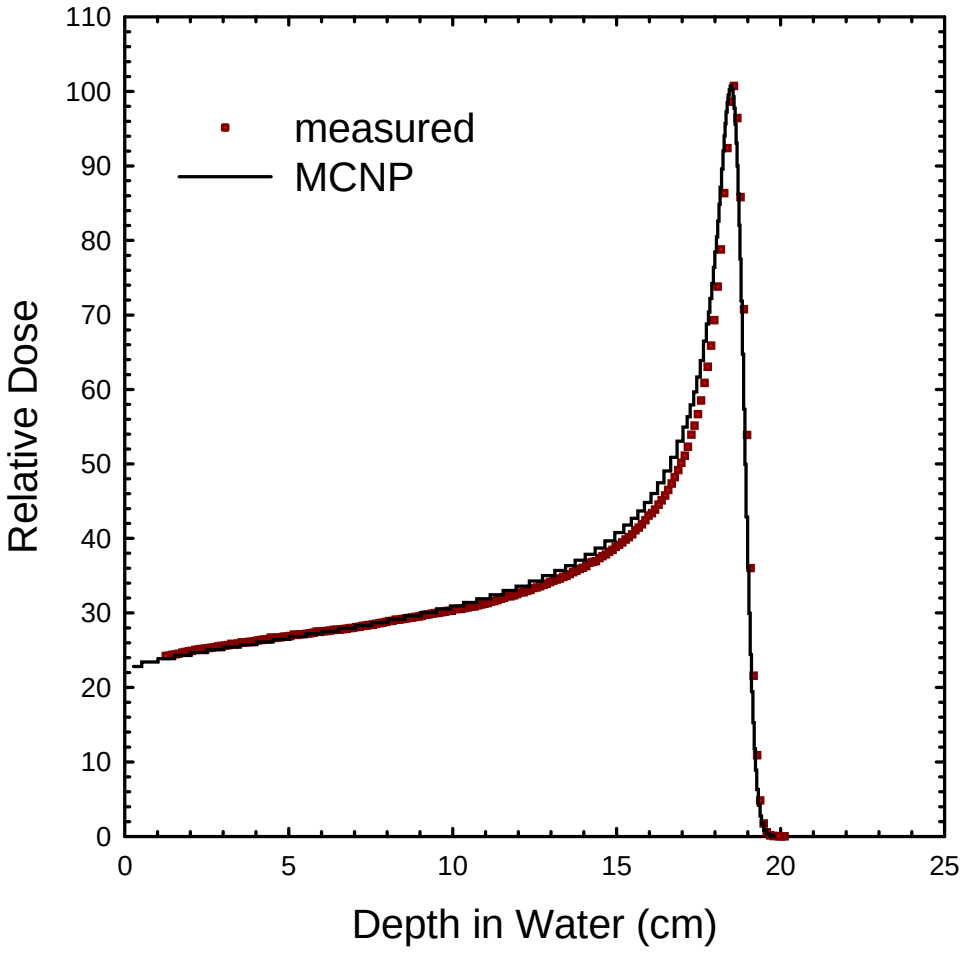


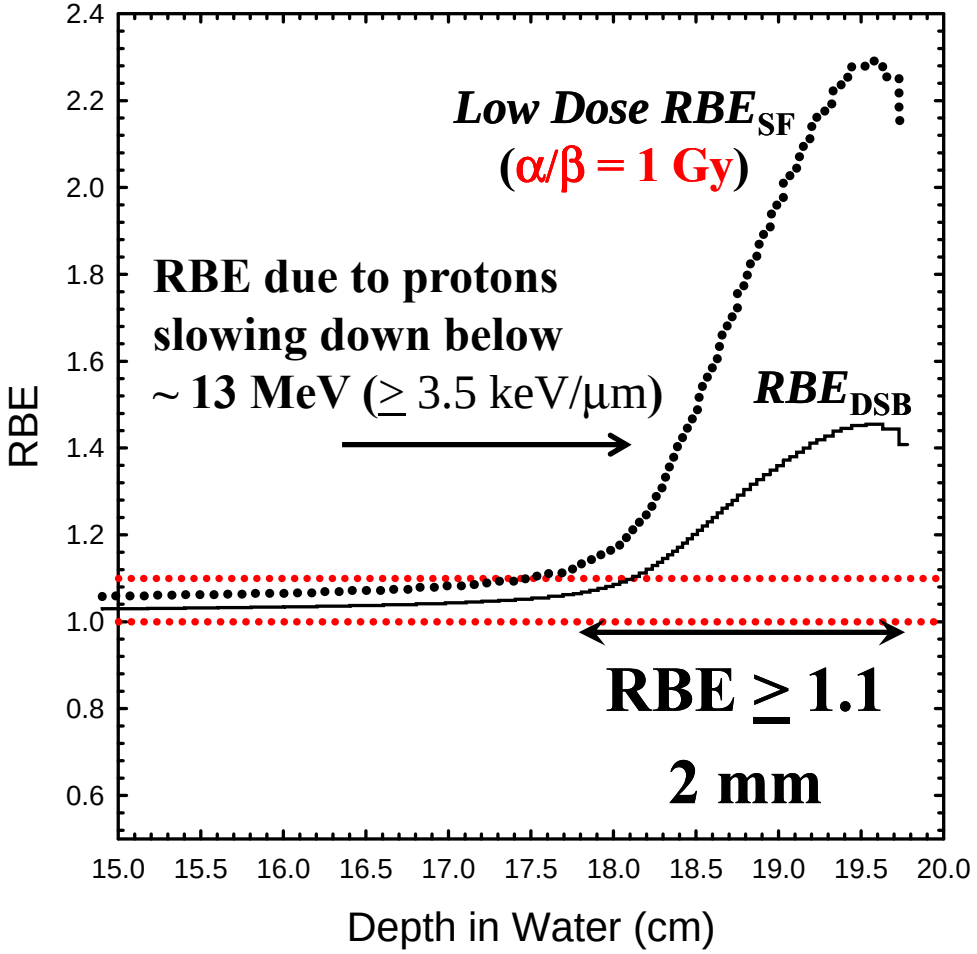
in vitro cell survival

Adapted from Figure 3 in Paganetti, Niemierko, Ancukiewicz, Gerweck, Goitein, Loeffler, and Suit, Relative Biological Effectiveness (RBE) Values for Proton Beam Therapy, IJROBP 53(2) 407-421 (2002).

RBE effects in a 163 MeV pencil beam

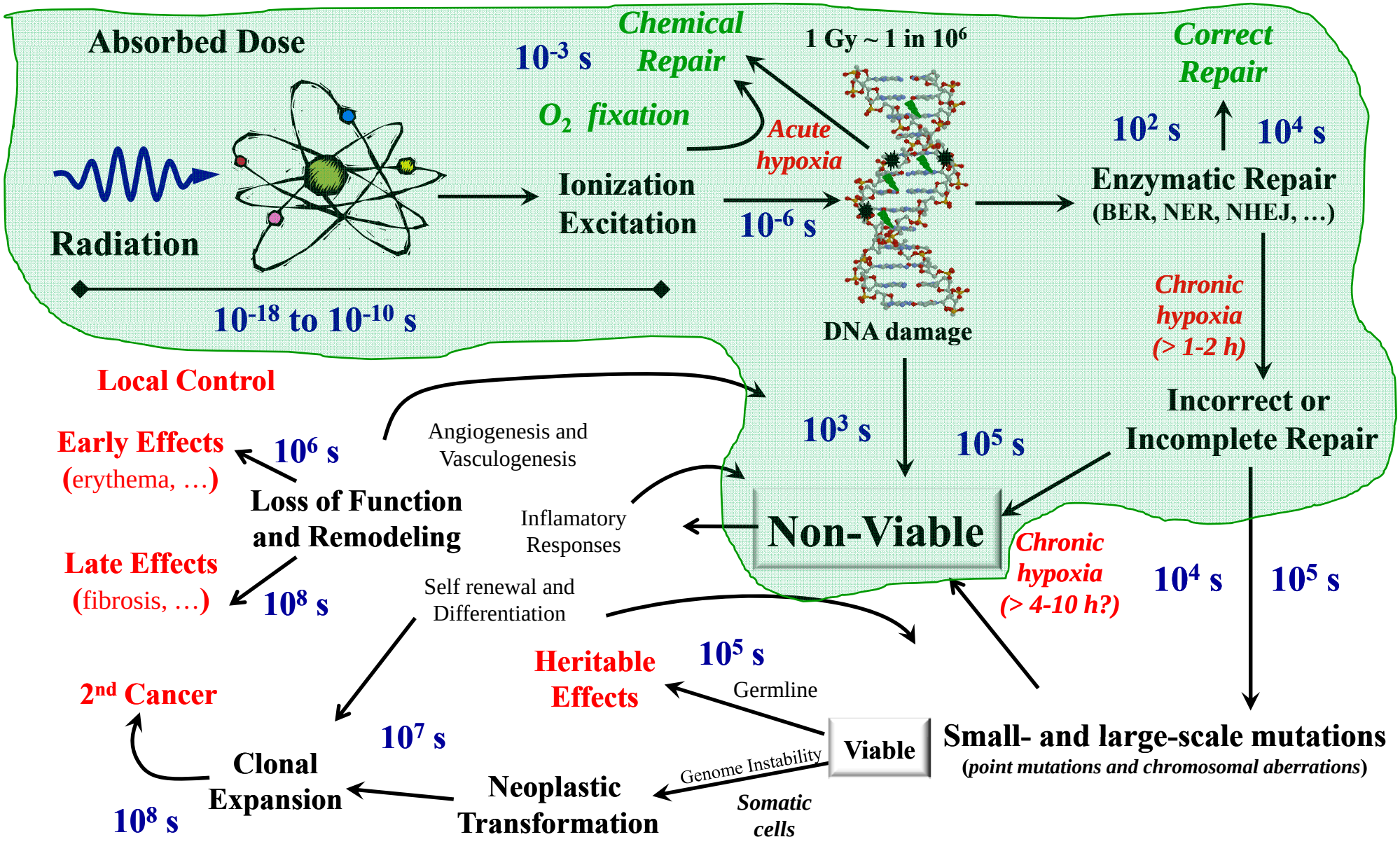
Tuned MCNP 6.1 model of 163.25 MeV pencil beam to match measured depth-dose profile (SCCA proton facility, Seattle, WA**).**



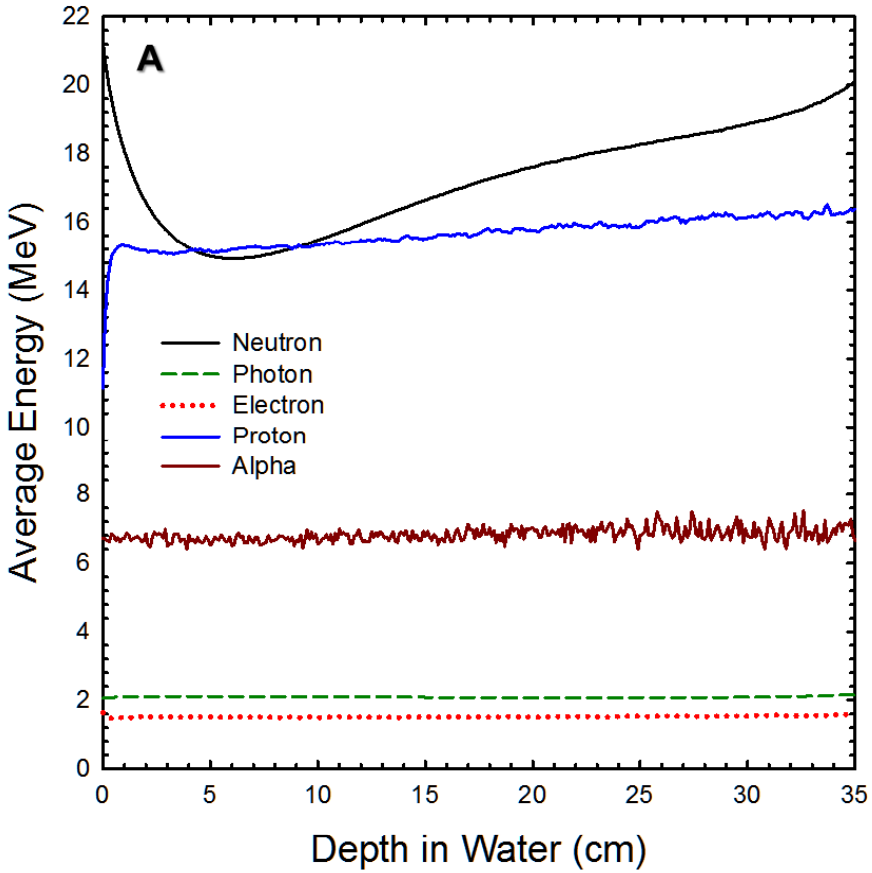


*** Really easy way to generate dose-averaged RBE in MCNP. See supplemental slides.**

Proton RBE for Healthy Organs and Tissues?



UW Experience with Fast Neutrons



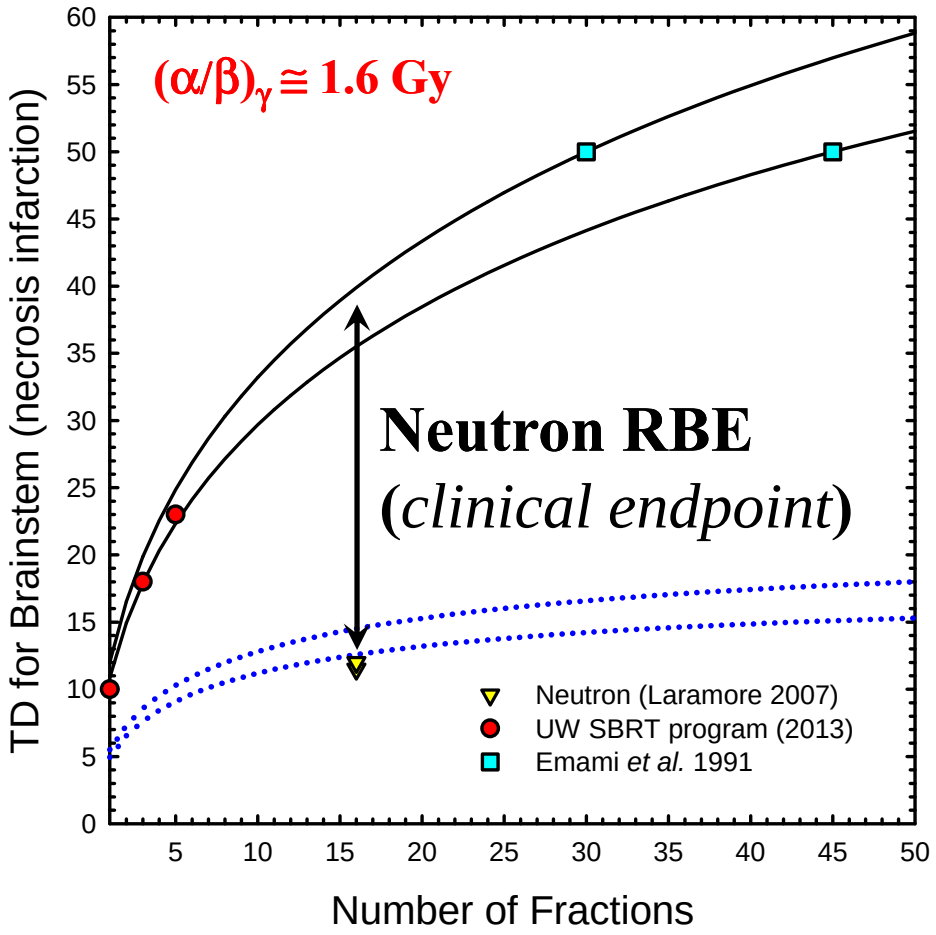
80-90% of absorbed dose to patient is from lower energy protons ($E_{avg} \cong 16$ MeV)

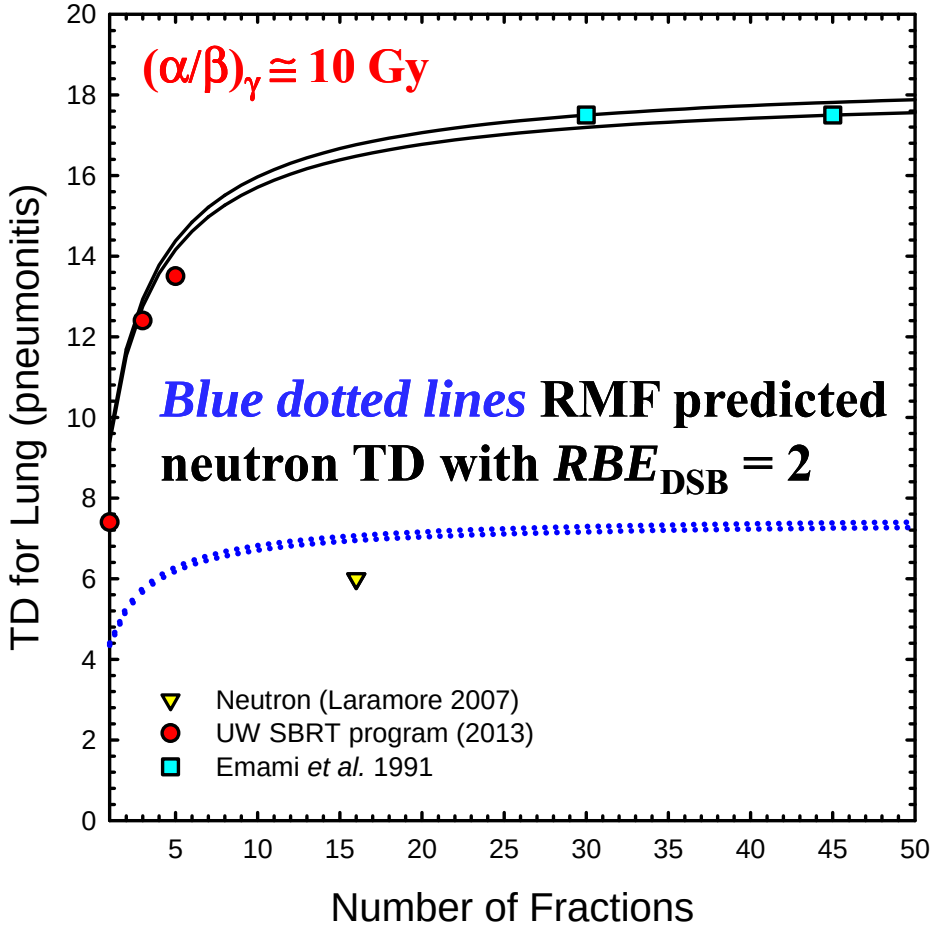
Tolerance doses derived from over 25+ years of clinical experience

Used as a guide for tolerance doses in carbon ion therapy

What can fast neutron therapy tell us about RBE effects in a proton Bragg peak?

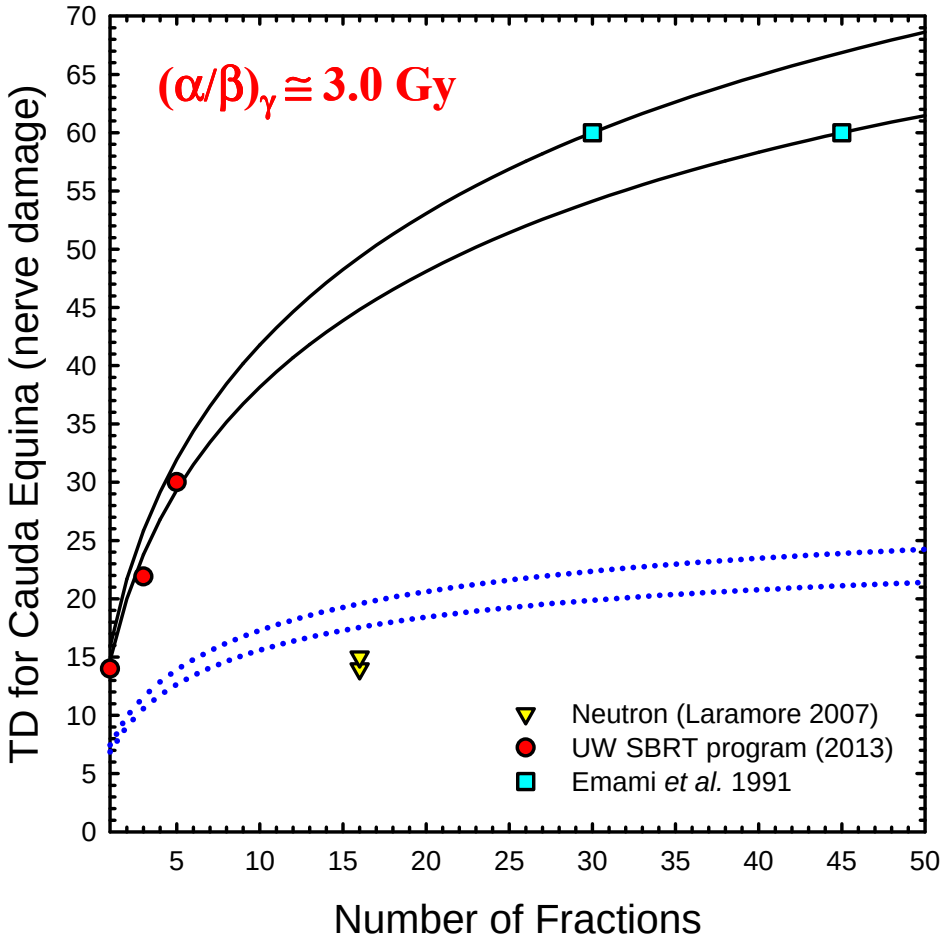
Neutron TD5/5 Dose (*Brainstem and Lung*)

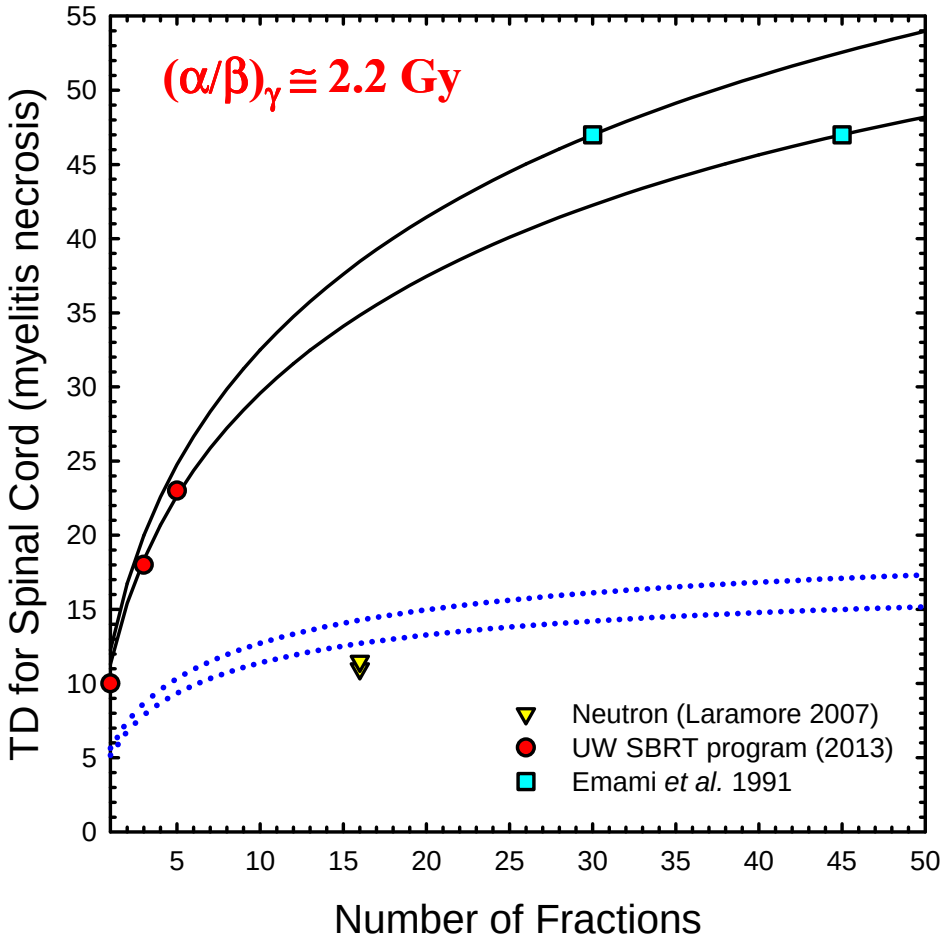




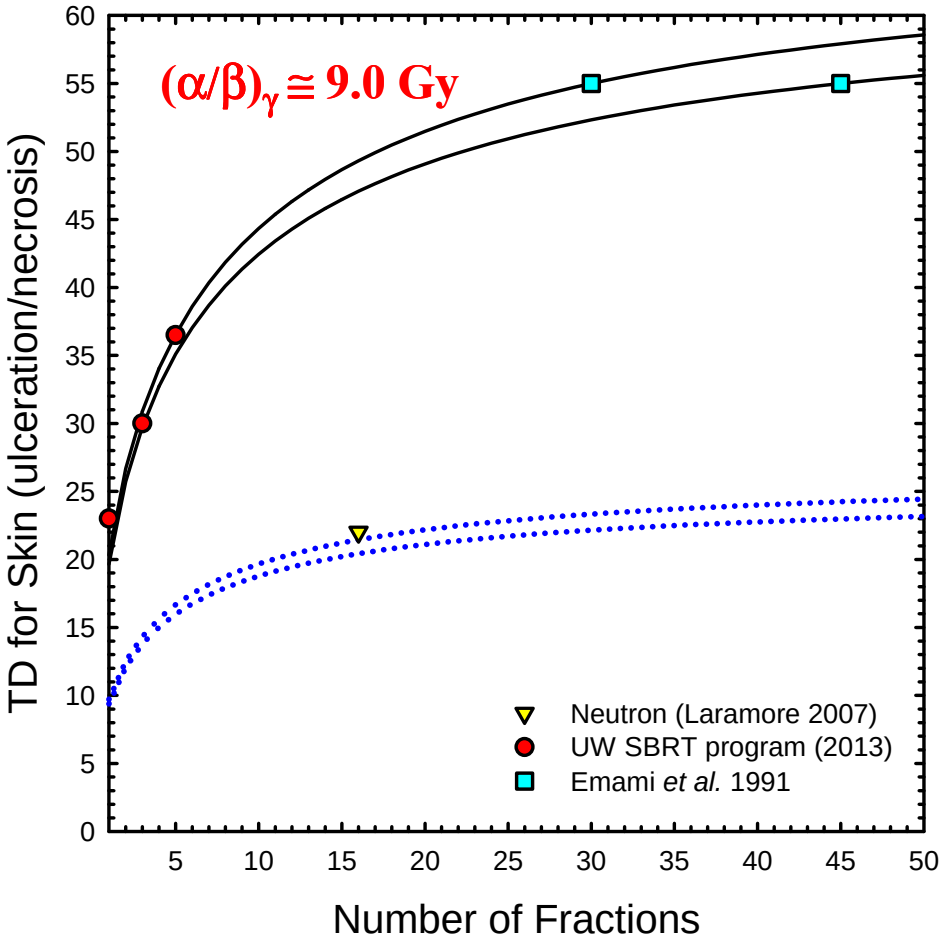
Usual caveats apply about accuracy of tolerance dose estimates

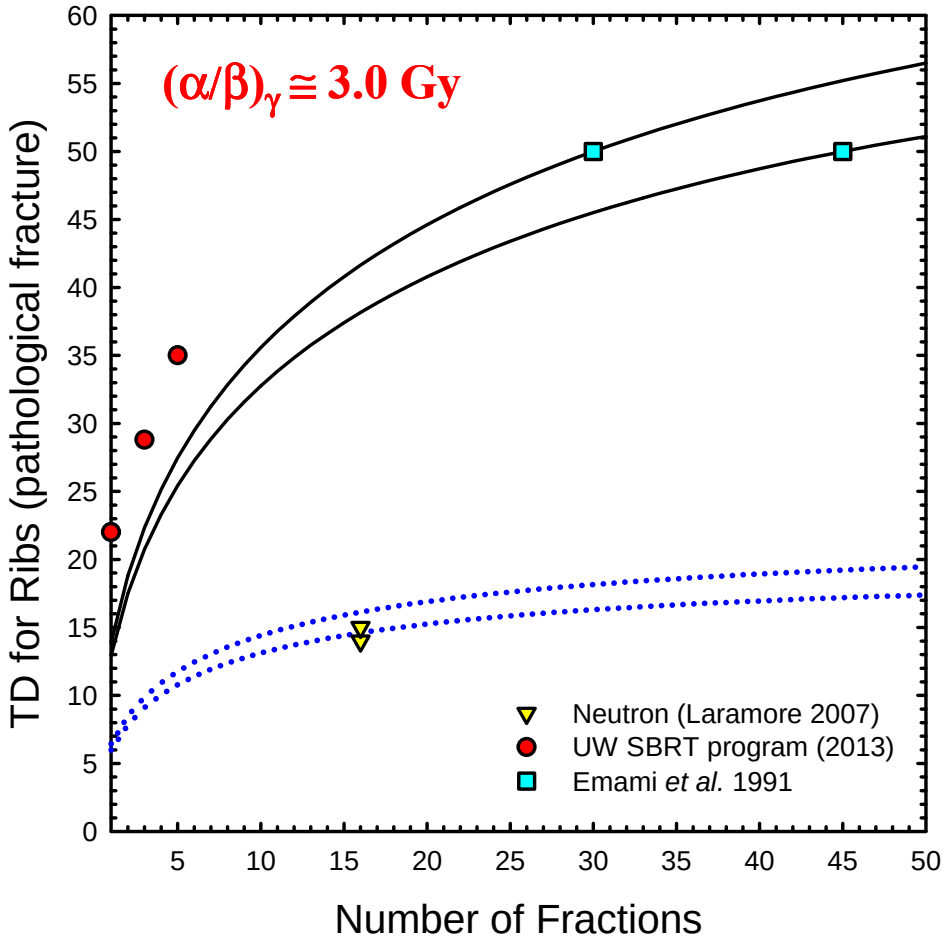
Neutron TD5/5 Dose (*Cauda Equina and Cord*)



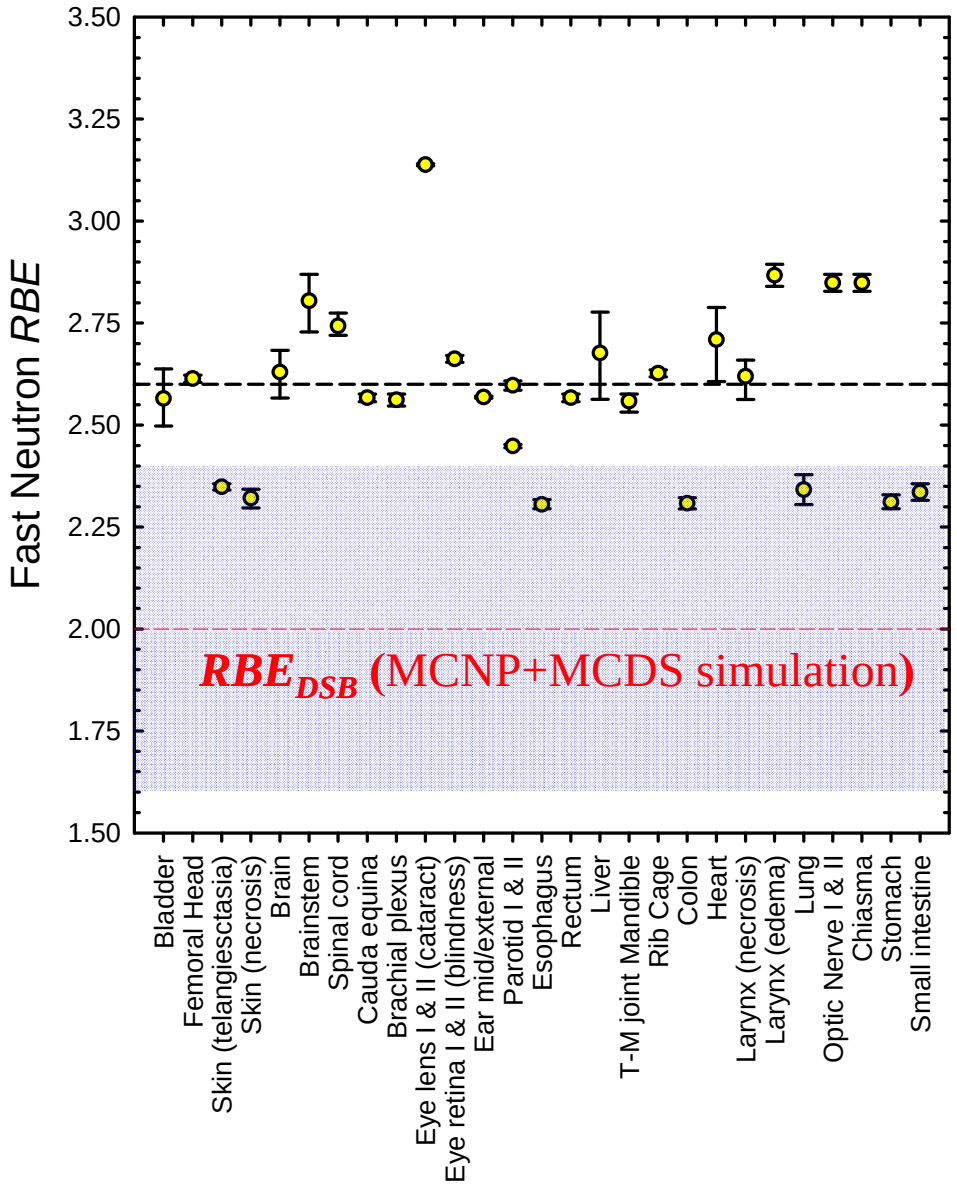


Neutron TD5/5 Dose (*Skin and Ribs*)





Fast Neutron RBE for Selected Tissue



Filled circles: RBE(n = 16) = TD_γ/TD_n

Avg RBE = 2.6 ± 0.3

27 tissues/endpoints

Red Dashed line: Monte Carlo ("*first principles*") simulation of neutron RBE for DSB induction.

Blue Shaded Region: Estimate of RBE for DSB induction derived from analysis of *in vitro* cell survival data for 30 human tumor cell lines (Warenius *et al.* IJROBP 1994**).**

Clinical RBE ≥ RBE_{DSB}, as predicted by the RMF model

Summary and Conclusions

- **Much of the uncertainty in proton RBE due to**
  - Uncertainty in dosimetry of the reference radiation and proton beam
  - Need for mechanistic dose-response models to guide the interpretation and analysis of measured data
- **RBE_{DSB} (RBE_{min}) and low dose RBE_{SF} (RBE_{max}) are relevant biological endpoints for (*1*) local tumor control and (2) tolerance doses for healthy organs and tissues**
- **Ample (*very strong*) evidence that spatial\LET variations in proton RBE are real and clinically relevant (*exploitable*)**
  - **Sticking with a constant $RBE = 1.1$ is a missed opportunity to enhance the therapeutic ratio!**

Thank You!

Supplemental Slides…

- Acknowledgements
- Example of an easy way to setup dose-weighted RBE calculations in MCNP and MCNPX (*DE DF modified F6 tally*)
- Is dose-averaged LET a could surrogate for RBE_{DSB} and\or the RBE_{SF}?
- Approximate formula linear and linear-quadratic formulas to estimate RBE_{DSB} as a function of proton LET
- Why does RBE_{DSB} increase with increasing LET and the RBE for SSB and base damage decrease with increasing LET?
- Why are some DSB more lethal than others?

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NOTE: “trawets” = “stewart” spelled backwards.

Acknowledgements

Entire UW physics group **but especially D. Argento, G. Sandison, C. Bloch, E. Ford, F. Yang; other UW faculty: J. Schwartz, G. Laramore, U. Parvatheni**

Many Other (non-UW***) Collaborators,*** **especially V. Semenenko, D. Carlson, C. Kirkby, N. Cao, G. Moffitt, S. Streitmatter, T. Jevremovic, Y. Hsaio, V.K. Yu, J.H. Park, M. Frese, R. Rockne, K. Swanson**

Example of *D* × RBE tally in MCNP

```
FC1026   RBE-weighted proton (1H+) dose; DSB induction (aerobic)
F1026:H  3
FM1026   0.1602
DE1026   1.000E-03 2.000E-03 3.000E-03 4.000E-03 5.000E-03 6.470E-03
         7.500E-03 1.000E-02 2.000E-02 3.000E-02 4.000E-02 5.000E-02
         6.000E-02 7.000E-02 8.000E-02 9.000E-02 1.000E-01 2.000E-01
         3.000E-01 5.000E-01 9.000E-01 1.000E+00 1.100E+00 1.300E+00
         1.500E+00 2.000E+00 2.500E+00 3.000E+00 3.500E+00 4.000E+00
         5.000E+00 6.000E+00 7.500E+00 1.000E+01 1.500E+01 2.500E+01
         5.000E+01 7.500E+01 1.000E+02 1.500E+02 2.000E+02 2.500E+02
         5.000E+02 1.000E+03
DF1026   3.375E+00 3.367E+00 3.368E+00 3.363E+00 3.359E+00 3.352E+00
         3.348E+00 3.340E+00 3.317E+00 3.290E+00 3.264E+00 3.242E+00
         3.216E+00 3.193E+00 3.168E+00 3.143E+00 3.122E+00 2.889E+00
         2.687E+00 2.370E+00 1.986E+00 1.916E+00 1.860E+00 1.760E+00
         1.685E+00 1.542E+00 1.451E+00 1.386E+00 1.336E+00 1.297E+00
         1.244E+00 1.204E+00 1.164E+00 1.123E+00 1.083E+00 1.051E+00
         1.026E+00 1.016E+00 1.012E+00 1.004E+00 1.004E+00 1.003E+00
         1.001E+00 9.995E-01
```

KE of proton ←

***RBE*$_{DSB}$ from MCDS 3.10A** ←

Above tally will record *D* × *RBE*$_{DSB}$. Divide by dose (*separate tally*) to get dose-averaged RBE.

See http://faculty.washington.edu/trawets/ for additional (*downloadable*) examples for protons and other particles

Analytic way to estimate an approximate RBE_{DSB}×Dose for proton beams?

Recall

$$\frac{D(x)}{\Phi(x)} = \frac{S(x)}{\rho} = \frac{LET_{\infty}(x)}{\rho} \qquad \therefore \frac{D(x)/\Phi(x)}{D(x_r)/\Phi(x_r)} = \frac{LET_{\infty}(x)/\rho}{LET_{\infty}(x_r)/\rho}$$

If we know the LET and dose per unit fluence at a reference location x_r, LET at other locations along depth-dose curve computed from

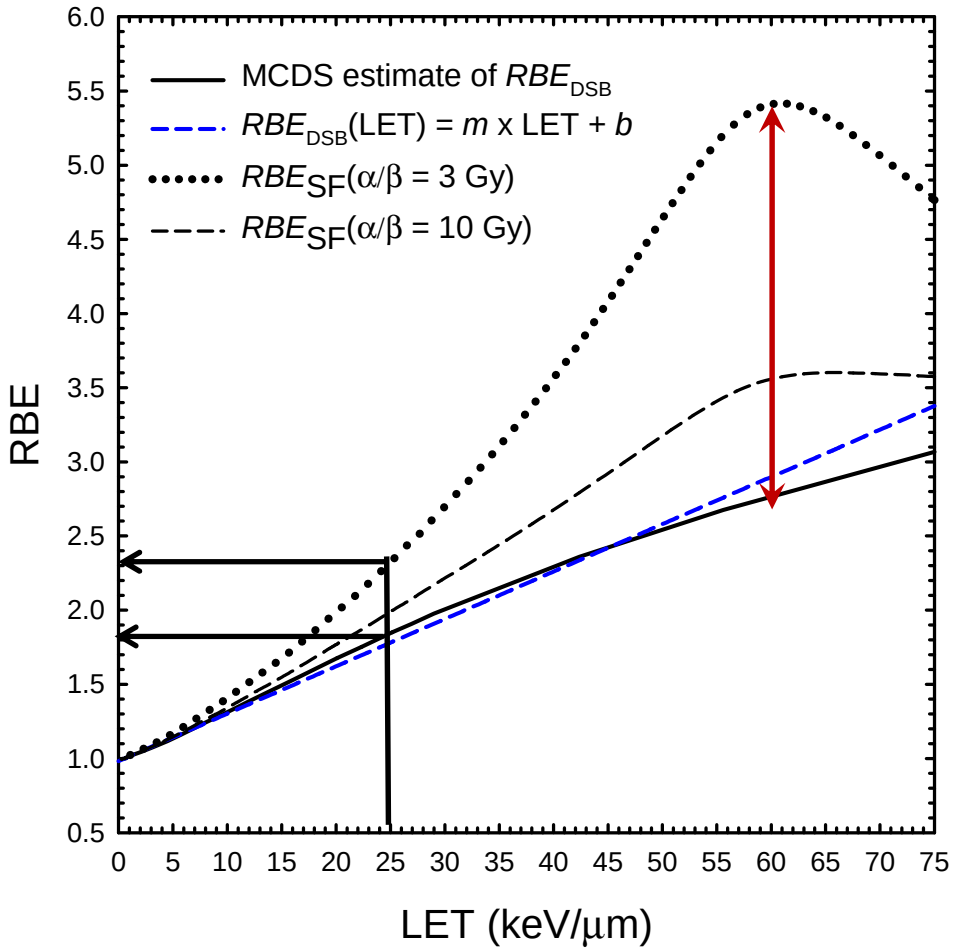
$$LET_{\infty}(x) = LET_{\infty}(x_r)\frac{D(x)/\Phi(x)}{D(x_r)/\Phi(x_r)}$$

$$RBE_{DSB}(x)D(x) = \left(mLET_{\infty}(x) + b\right)D(x)$$

m = 0.03193 μm/keV and b = 0.98274

Simple formulism to connect patient-specific QA measurements of dose-average LET to RBE_{DSB} and (*hence*) the RBE_{SF} (via RMF model)?

LET_D instead of RBE_{DSB} or RBE_{SF}?



For a clinically relevant*range of proton energies (LET < 25 keV/μm), dose-averaged LET is a good surrogate for RBE_{DSB} and the low dose RBE_{SF}.

m = 0.03193 RBE/(keV/μm)
b = 0.98274

For tumors and\or tissues with a low α\β, RBE_{SF} may be larger than RBE_{DSB} by 25-30% larger at 25 keV/μm.

*** Caveat: ± 2 mm of the Bragg peak *really* low energy protons (< 1-5 MeV) contribute in a substantial way to dose and RBE.**

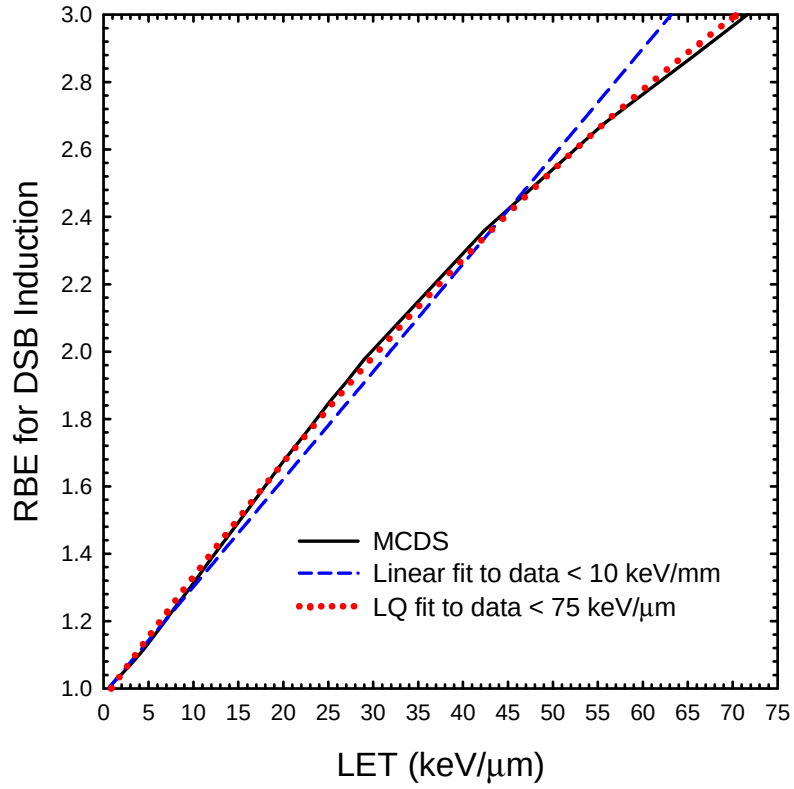
LQ fit to proton RBE_{DSB} as function of LET

To better capture trends in the RBE for DSB induction as a function of LET, a linear-quadratic (LQ) fit is highly recommended.

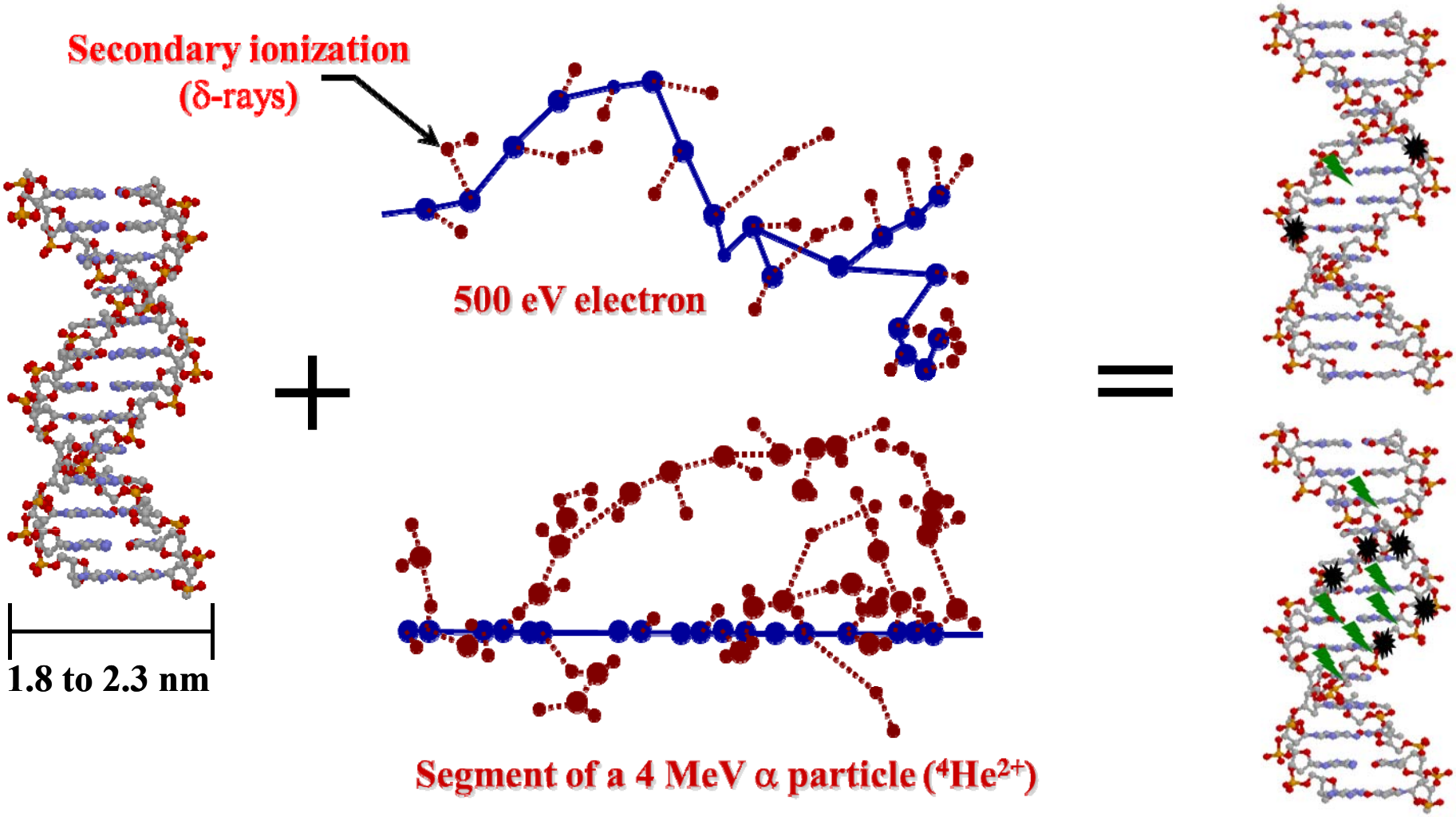
$$RBE_{DSB} = a \cdot LET^2 + b \cdot LET + c$$

$$a = 6.771 \times 10^{-3}$$
$$b = 2.553 \times 10^{-2}$$
$$c = 9.969 \times 10^{-1}$$

*** Coefficients for fit may need to be adjusted slightly to correct for uncertainties in proton LET.**



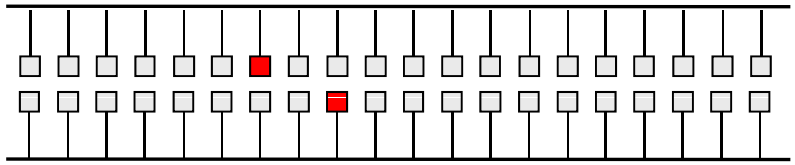
Ionization Density and Cluster Complexity



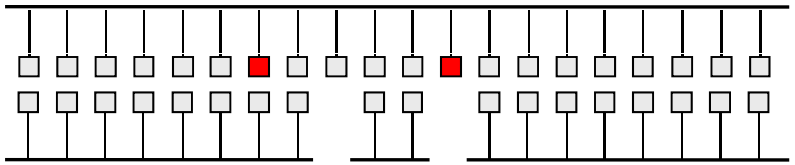
Number of DNA lesions per cluster tends to increase with increasing particle LET.

Why does RBE_{DSB} ↑ and RBE_{SSB} and RBE_{Bd} ↓

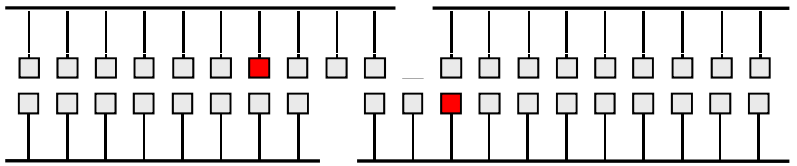
A cluster categorized as a DSB cannot *also* be categorized as a (simple or complex) SSB or as a (*simple or complex*) cluster of nucleotides with base damage – *mutually exclusive categories of DNA damage*



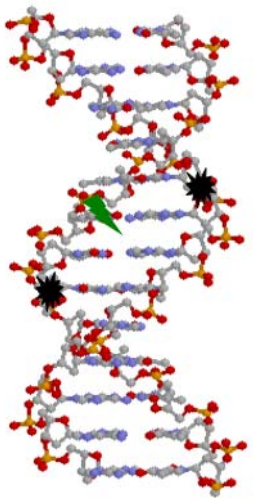
Cluster = 2 nucleotides with base damage

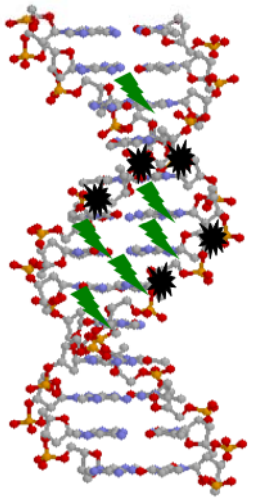


Cluster = complex SSB



Cluster = complex DSB





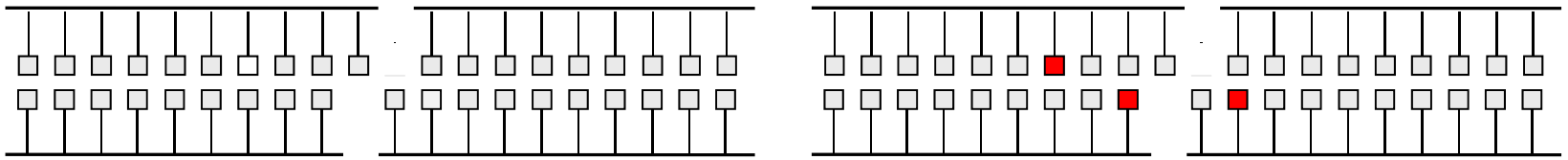
Chance a cluster will contain a pair of opposed strand breaks within about 10 bp increases (*i.e.*, *be a simple or complex DSB*) as LET and the number of DNA lesions per cluster increases.

Why are some DSB (*more*) lethal and others not?

Answer: *we don't know for sure…*

Hypotheses:

(*1*) Some DSB are unrepairable (*unrejoinable*) *or* more slowly rejoined than others because of the local (*spatial*) complexity of DNA lesions



(*2*) DSB formed in close spatial and temporal proximity to other DSB are more often mis-rejoined to form chromosome aberrations than DSB separated in time and/or space (*breakage and reunion theory*)

(*3*) Combination of mechanisms (*1*) and (*2*)

RMF model (Carlson *et al.* 2008**) tends to emphasize mechanism 2**

Critical MCDS and RMF Literature Citations

- V.A. Semenenko and R.D. Stewart. A fast Monte Carlo algorithm to simulate the spectrum of DNA damages formed by ionizing radiation. *Radiat Res.* **161**(4), 451-457 (2004)
- V.A. Semenenko and R.D. Stewart. Fast Monte Carlo simulation of DNA damage formed by electrons and light ions. *Phys. Med. Biol.* **51**(7), 1693-1706 (2006)
- D.J. Carlson, R.D. Stewart, V.A. Semenenko and G.A. Sandison, Combined use of Monte Carlo DNA damage simulations and deterministic repair models to examine putative mechanisms of cell killing. *Rad. Res.* **169**, 447-459 (2008)
- R.D. Stewart, V.K. Yu, A.G. Georgakilas, C. Koumenise, J.H. Park, D.J. Carlson, Effects of Radiation Quality and Oxygen on Clustered DNA Lesions and Cell Death, *Radiat. Res.* **176**, 587-602 (2011). **MCDS Version 3.10A developed and tested in this one.**
- M.C. Frese, V.K. Yu, R.D. Stewart, D.J. Carlson, A Mechanism-Based Approach to Predict the Relative Biological Effectiveness of Protons and Carbon Ions in Radiation Therapy, *Int. J. Radiat. Oncol. Biol. Phys.*, **83**, 442-450 (2012).
- • C Kirkby, E Ghasroddashti, Y Poirier, M Tambasco, RD Stewart, Monte Carlo Simulations of Relative DNA Damage From KV CBCT Radiation. *Phys. Med. Biol.* **58**, 5693-5704 (2013)
- A.G. Georgakilas, P.O'Neill, R.D. Stewart, Induction and Repair of Clustered DNA Lesions: What Do We Know So Far? *Radiat. Res.* **180**,100-109 (2013)