A biophysical model linking DNA damage, chromosome aberrations and cell death

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Outline

- Rationale

- Methods

    assumptions

    simulation of target & projectile

- Results

    comparison with experimental data

    DNA Cluster Lesions

    proximity effects

    potential applications for tumor therapy

- Conclusions and future developments

Rationale/Background

General

- the action of charged particles in biological targets needs further investigation, including cell killing by protons and carbon ions

  $\Rightarrow$ *hadrontherapy*

Specific

- role of DNA cluster damage (~nm scale)
- role of its spatial distribution in the cell nucleus (~μm scale)
- consequences in terms of cell death

…a biophysical model/MC code called

BIANCA

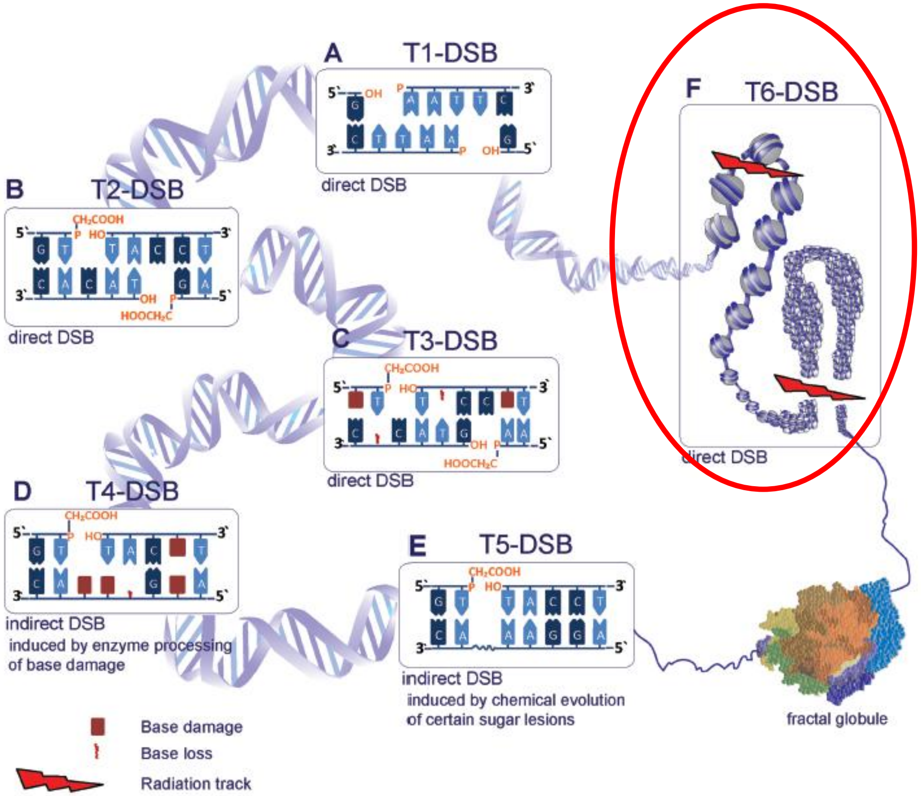
BIophysical ANalysis of Cell death and chromosome Aberrations

Assumption I

radiation $\Rightarrow$ DNA (cluster) damage

radiation induces DNA "Cluster Lesions" (CLs), and each CL produces two independent chromosome free-ends

(the CL yield is the 1st adjustable parameter, mainly dependent on the particle type and LET)



(Schipler and Iliakis 2013, Nuc Acids Res)

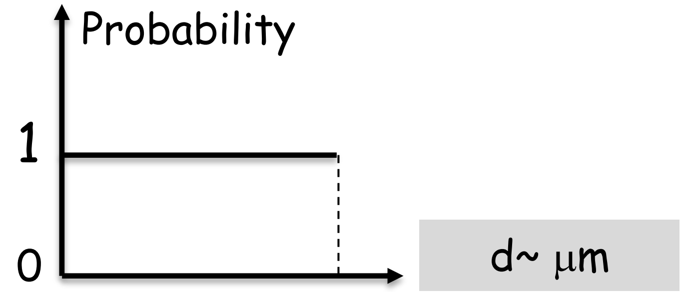
- Double Strand Breaks classified according to six levels of increasing complexity, from 'clean' DSBs to DSB clusters (=several DSBs in close proximity)
- authors concluded that the various repair pathways are likely to fail for DSB clusters, which are related with adverse biological endpoints including cell death

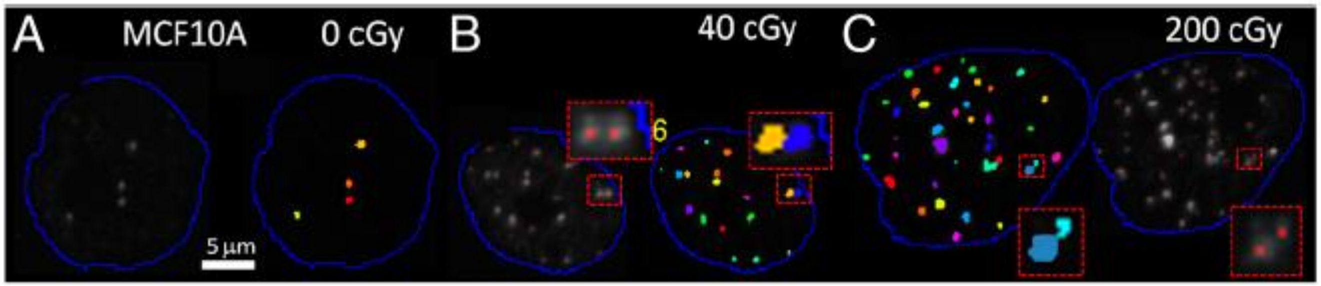
Assumption II

DNA cluster damage $\Rightarrow$ chromosome aberrations

only chromosome free-ends with initial distance $\leq d$ undergo end-joining, producing chromosome aberrations

(d is the 2nd, and last, adjustable parameter, dependent on the target cell features)





evidence for DSB repair centres, where multiple DSBs migrate for repair after travelling a few microns

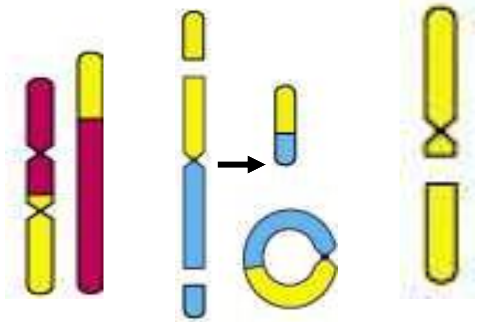
(Neumaier et al. 2012, PNAS)

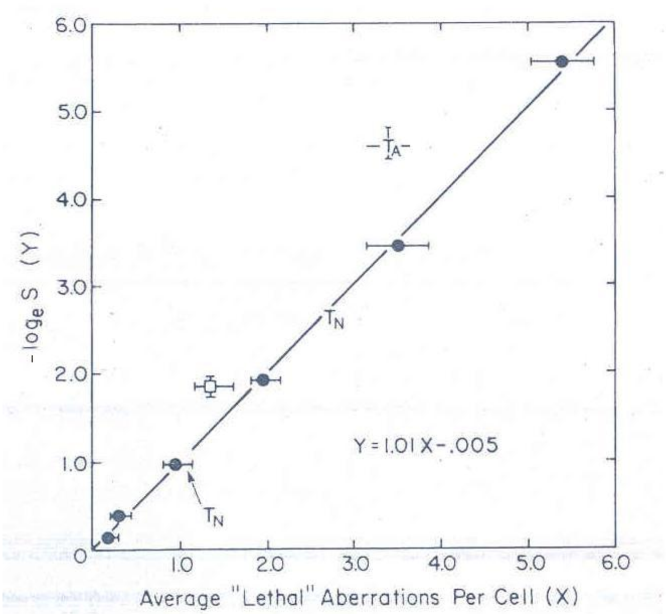
Assumption III

chromosome aberrations $\Rightarrow$ cell death

dicentrics, rings and large* deletions ("Lethal Aberrations") lead to cell death $\Rightarrow$ $S(D) = e^{-LA(D)}$

** visible in Giemsa (~ 3 Mbp)*





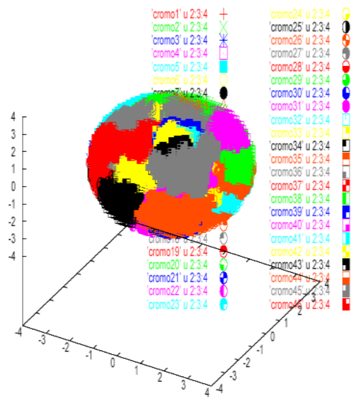
- *one-to-one relationship between lethal aberrations and –lnS for AG1522 human fibroblasts exposed to X-rays*

- *interpretation: these aberrations involve acentric fragments, which are lost during cell division $\Rightarrow$ intolerable genetic loss $\Rightarrow$ clonogenic cell death*

(Cornforth and Bedford 1987, Radiat Res)

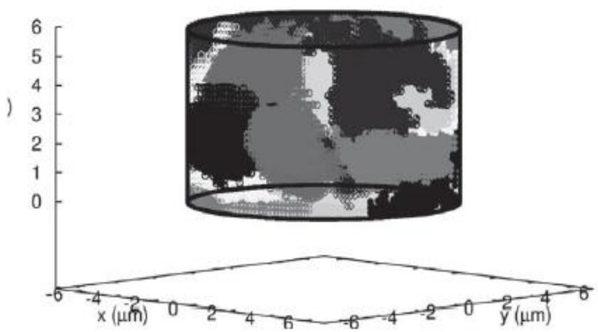
Simulation of target cell nucleus

spherical nucleus *(← cell suspensions)*

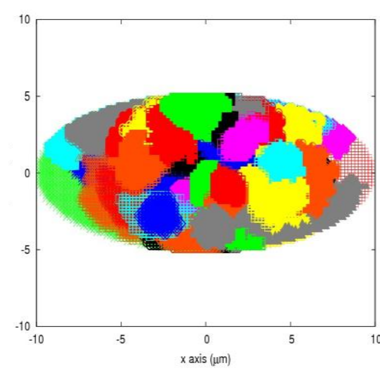


Interphase chromosome territories modelled by the union of cubic voxels (volume of chromosome territory proportional to chromosome DNA content)

cylindrical nucleus, with circular or elliptical base *(← cell monolayers)*



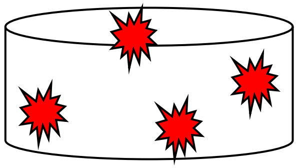
front view



top view

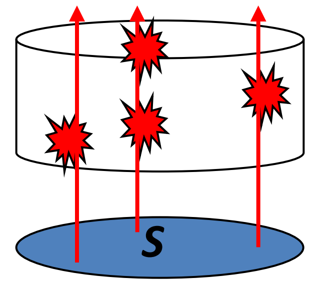
Simulation of irradiation

photons



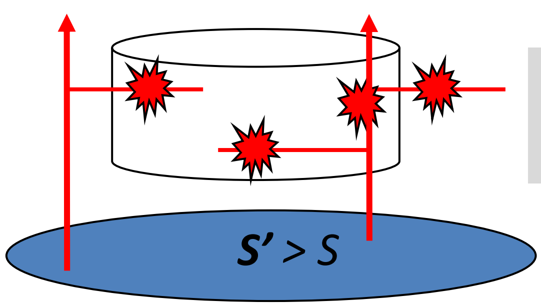
CLs distributed randomly

light ions



CLs along the primary tracks

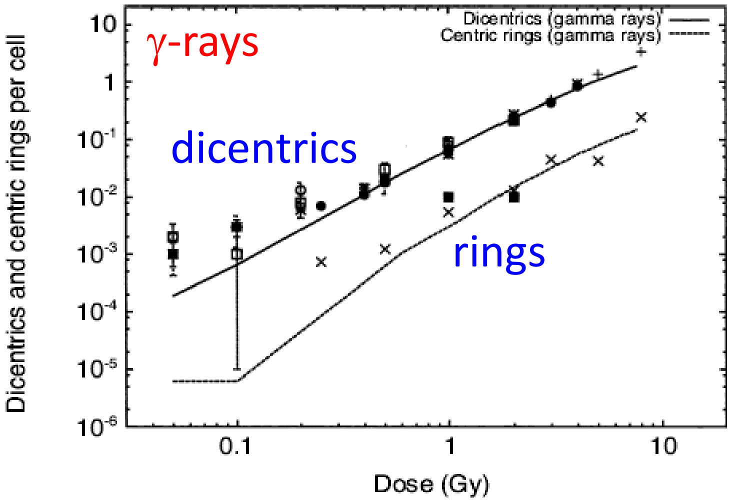
heavy ions



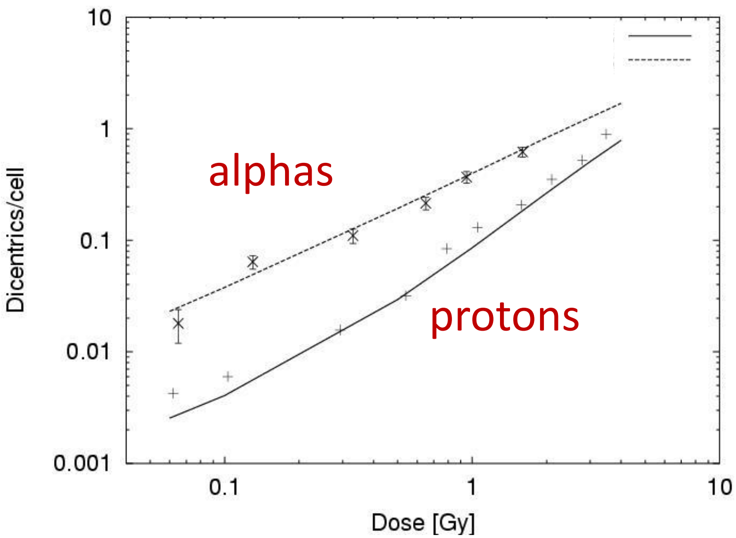
CL also outside the track core

(old) comparisons with aberration data

human lymphocytes

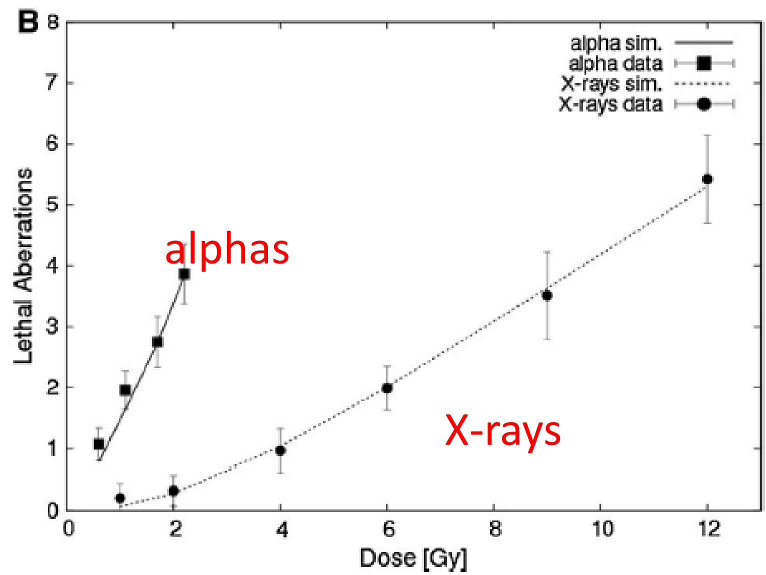


(Ballarini et al 2002)



(Ballarini and Ottolenghi 2003)

human fibroblasts



(Ballarini et al 2014 Radiat Environ Bio)

- *the model can predict chromosome aberrations by different radiations in different cells*

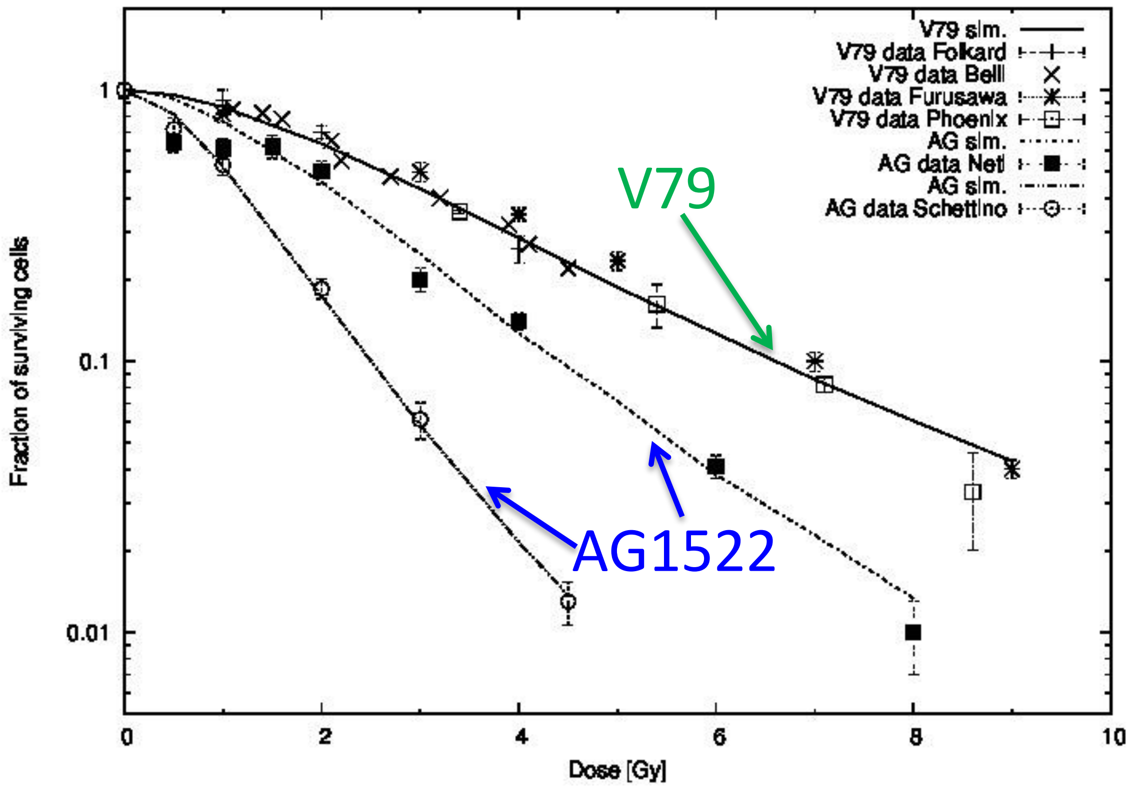
Comparison with survival data - general

Cell lines:

- **V79**, which are radioresistant and have been used to characterize many hadron therapy beams, including those at PSI (Switzerland, eye tumors), GSI (Germany), NIRS (Japan), CNAO (Pavia, Italy)…

- **AG1522**, which are normal human fibroblasts and have been used by Cornforth and Bedford to find the relationship between chromosome aberrations and cell death applied in our model

Comparison with survival data - photons



exp. data from:

Folkard et al 1996 (V79)
Belli et al 1998 (V79)
Furusawa et al (V79)
Phoenix et al 2009 (V79)
Neti et al 2004 (AG)
Chaudhary, Prise, Schettino et al, Int J. Radiat. Oncol Biol Phys in press (AG X rays)

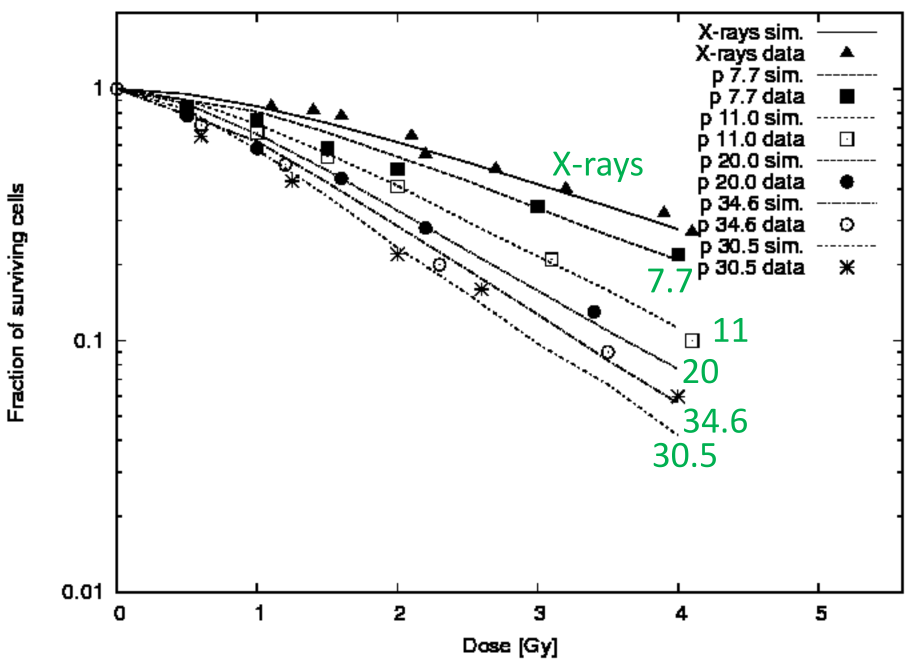
parameters:

d = 5 μm (not changed from now on)
$\sim$2-4 CLs$\cdot$Gy$^{-1}\cdot$cell^{-1}

- the relationship between lethal aberrations and cell death holds not only for AG1522 cells, but also for V79 (and possibly others)
- important to take into account the specific experimental scenario

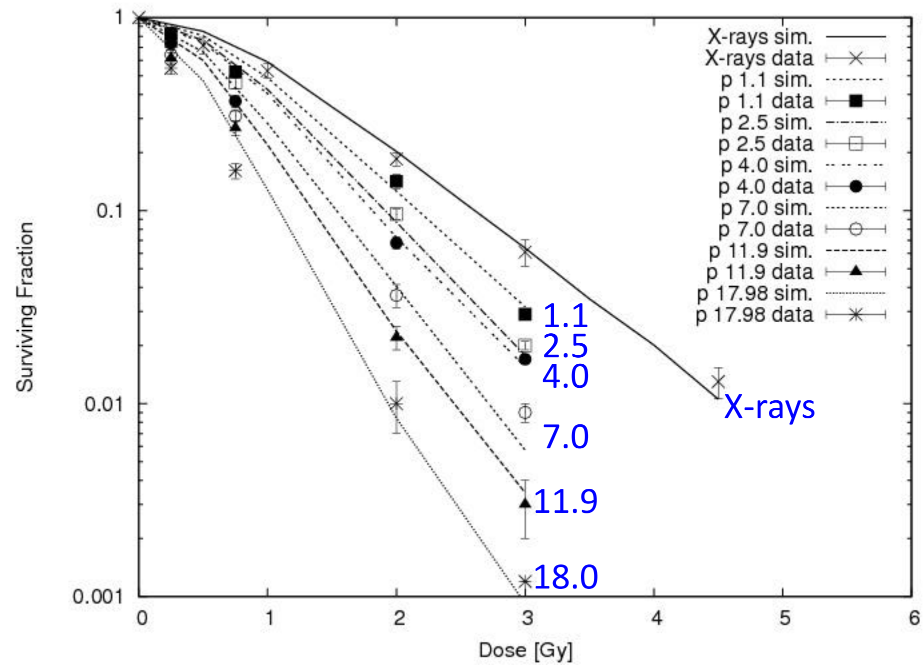
Comparison with survival data - protons

V79



(Ballarini et al 2013, Rad Res; data from Belli et al. 1998)

AG1522

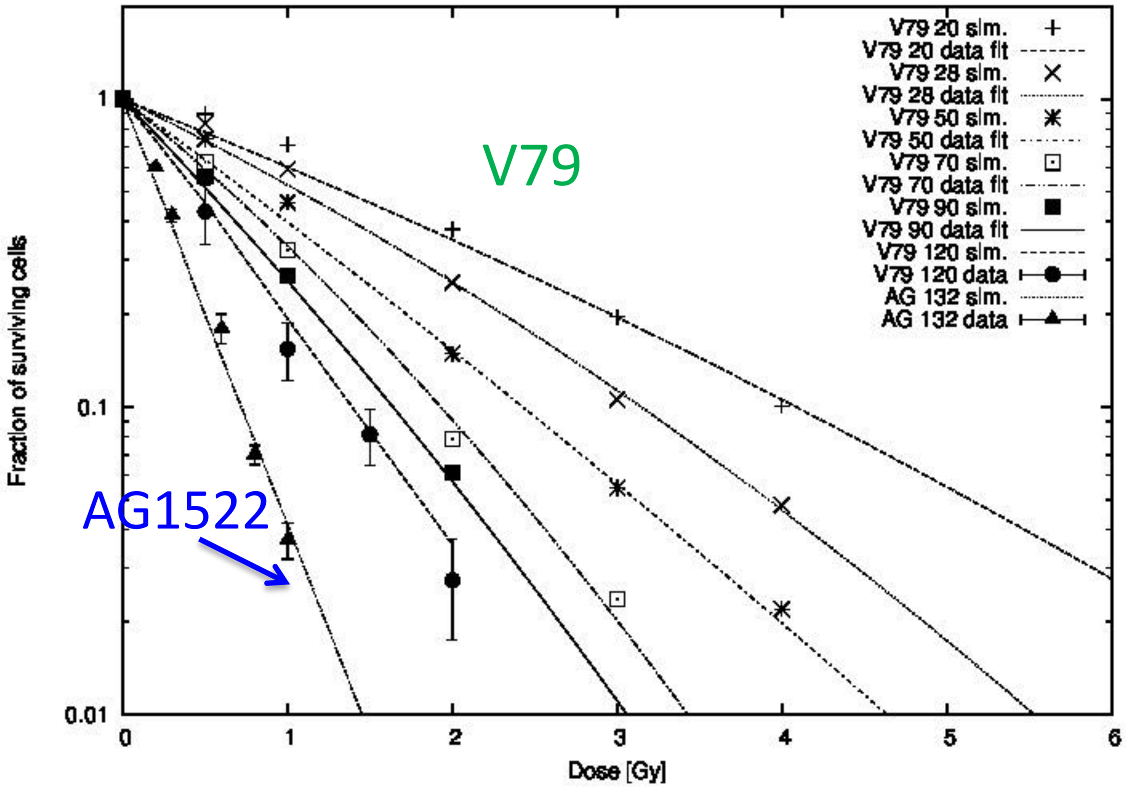


(unpublished; data from Chaudhary, Prise, Schettino et al., Int J Radiat Oncol Biol Phys, in press

- the approach works not only for photons but also for protons
- confirmed that low-energy protons are more effective than photons *(→ proton therapy)*

(parameters: ~2-4 CLs · Gy^{-1} · $cell^{-1}$ for V79, ~5-12 CLs · Gy^{-1} · $cell^{-1}$ for AG)

Comparisons with survival data – α particles



unpublished

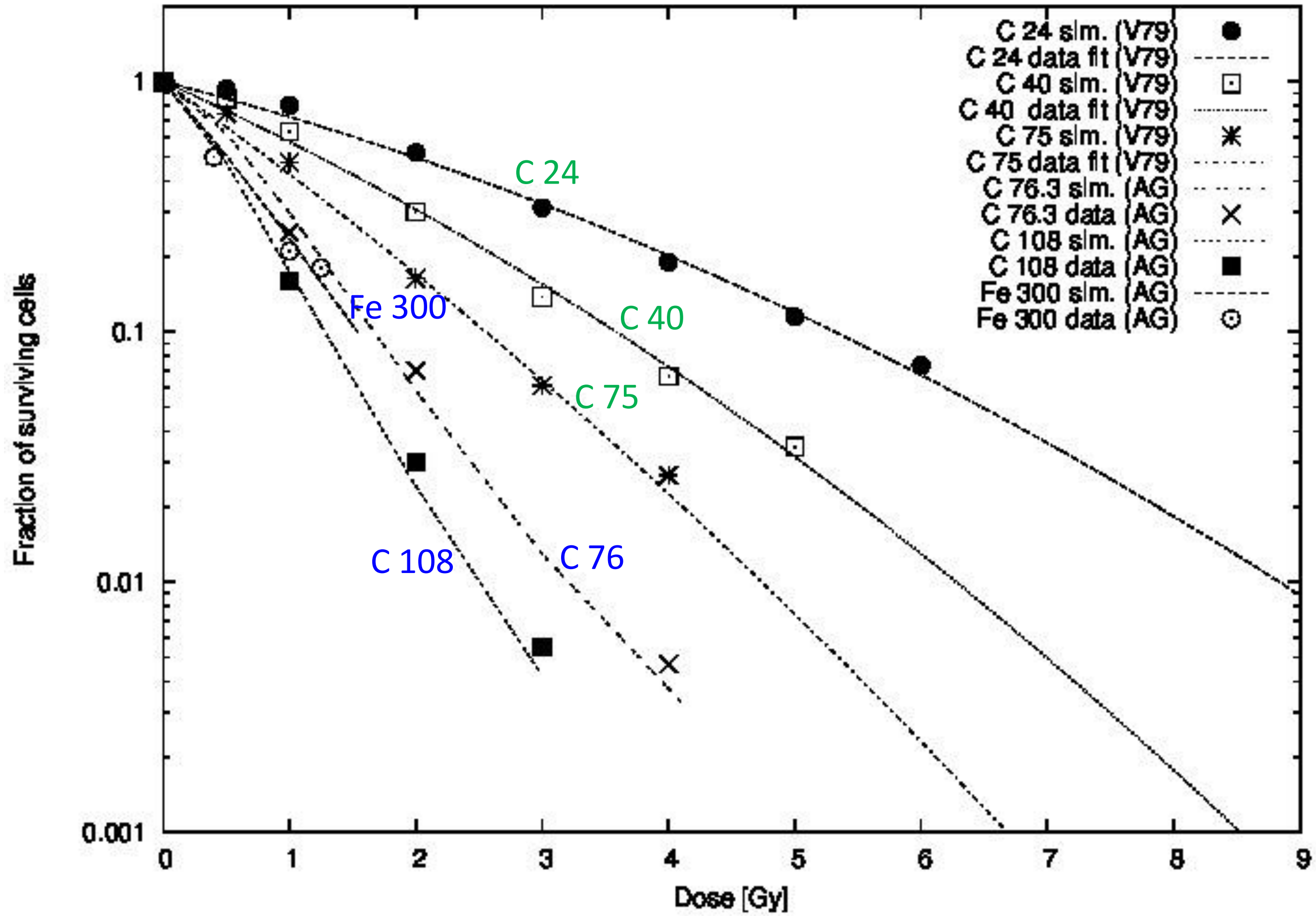
exp. data from:

Cox et al 1977 (V79)
Phoenix et al 2009 (V79)
Neti et al 2004 (AG)

parameters:

$d = 5\ \mu m$
$\sim 3\text{-}18\ CLs \cdot Gy^{-1} \cdot cell^{-1}$

Comparisons with survival data – heavy ions



exp. data from:

Belli et al 2008 (C V79)
Hamada et al 2006 (C AG)
Tsuboi et al 1992 (Fe AG)

parameters:

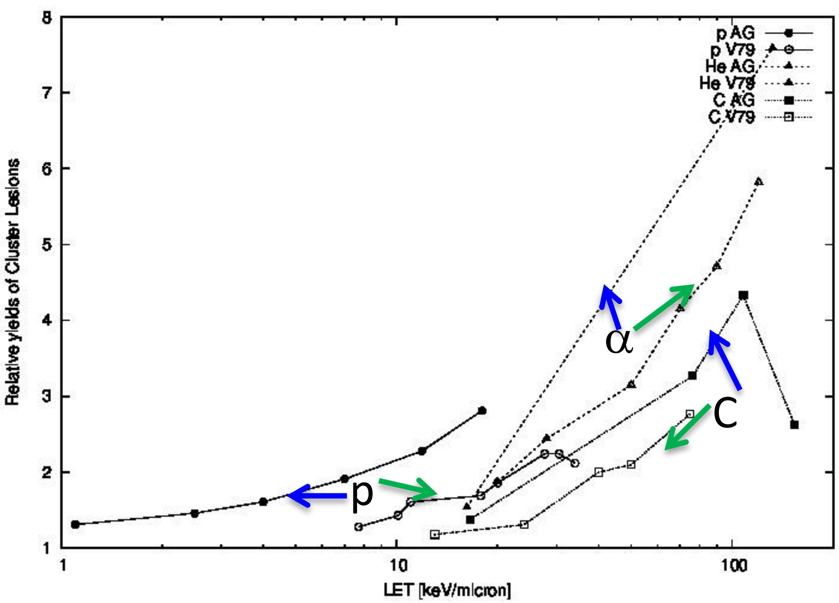
d = 5 μm
$\sim$*2-9 CLs*$\cdot$*Gy*$^{-1}\cdot$*cell*$^{-1}$

(Ballarini et al 2014, Radiat Environ Biophys)

DNA Cluster Lesions – dependence on radiation quality

CL relative yields

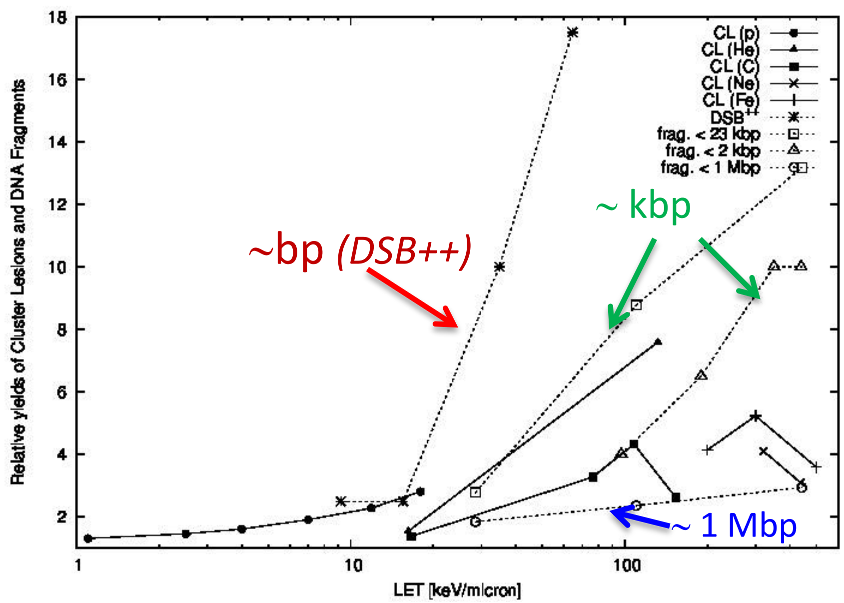
(adjusted after comparison with survival data)



• *CLs increase with LET (~2-20 CL· Gy^{-1}·$cell^{-1}$), consistent with the hypothesis of cluster damage*
• *light particles were more effective than heavier particles of the same LET*
• *more CLs for AG1522 cells than for V79*

comparison with DNA fragmentation data

(AG1522)

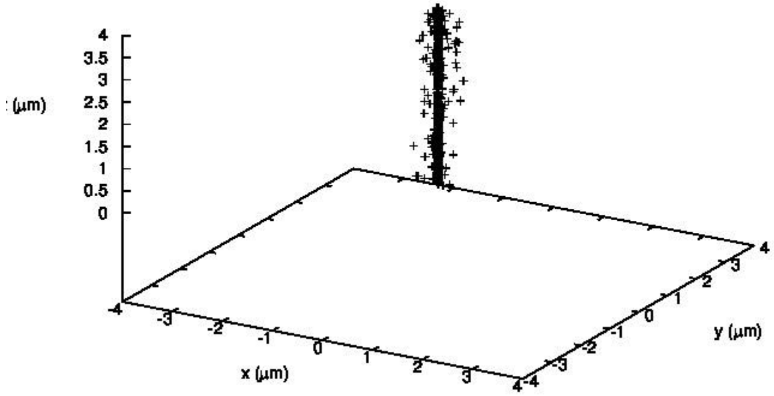


CLs showed a similar LET-dependence as ~ kbp fragments ⇒ better candidates as critical DNA damage, with respect to very small (~ bp) or very large (~ Mbp) fragments

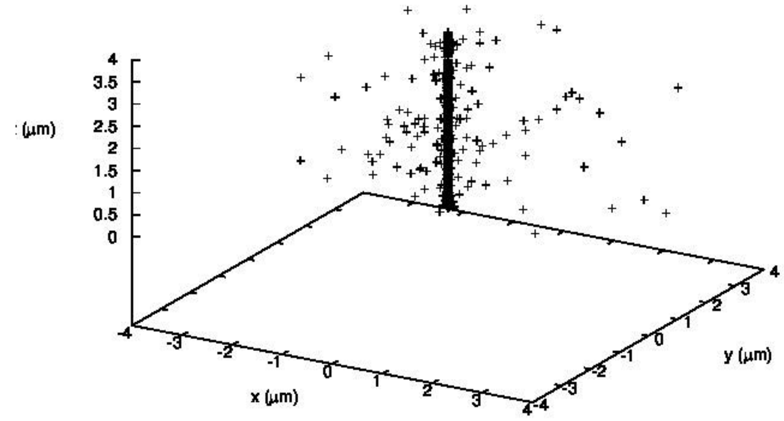
[fragment data: Nikjoo et al 2001 (DSB++), Friedland and co. 2005 and 2007 (< 23 kbp and < 1 Mbp), Rydberg 1994 (< 2 kbp)]

DNA Cluster Lesions – spatial distribution by heavy ions

108 keV/μm carbon *(1000 tracks)*

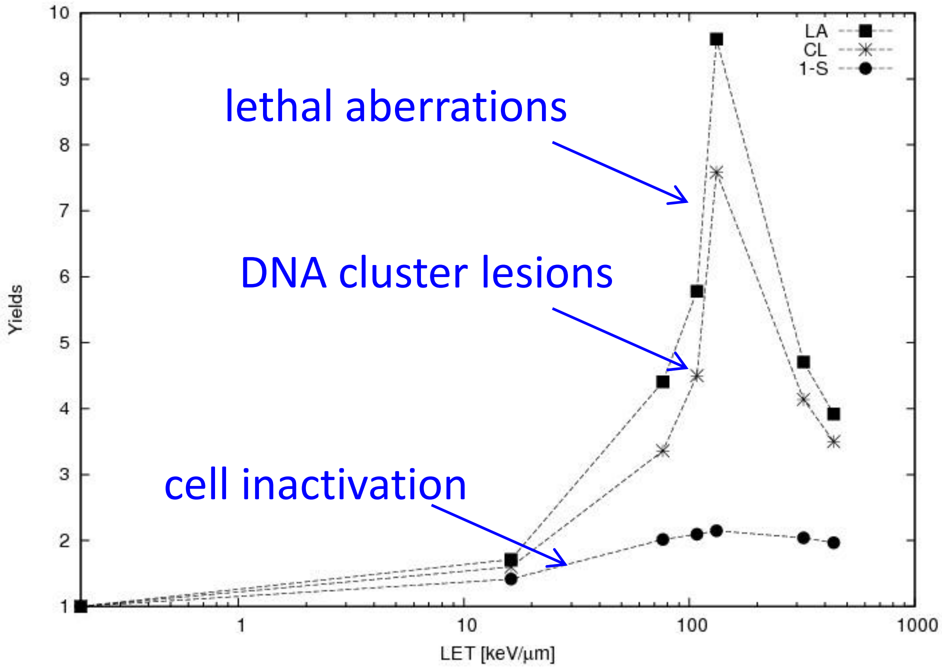


300 keV/μm iron *(1000 tracks)*

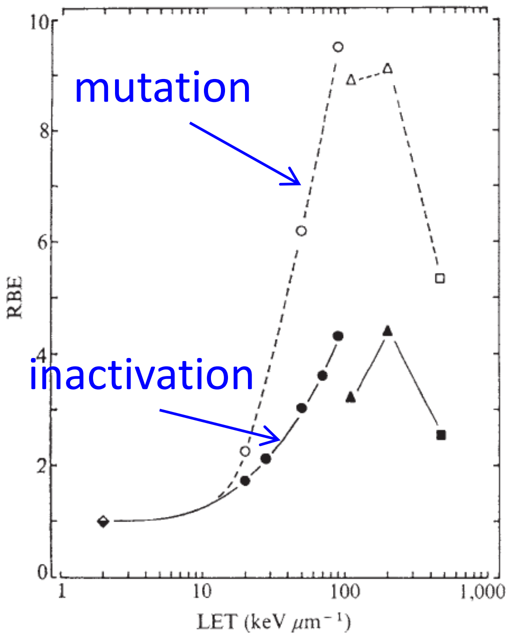


LET dependence of different endpoints

DNA Cluster Lesions, Lethal Aberrations and cell death *(relative yields after 2 Gy)*



experimental RBE for mutation and inactivation *(Cox et al. 1977 Nature)*



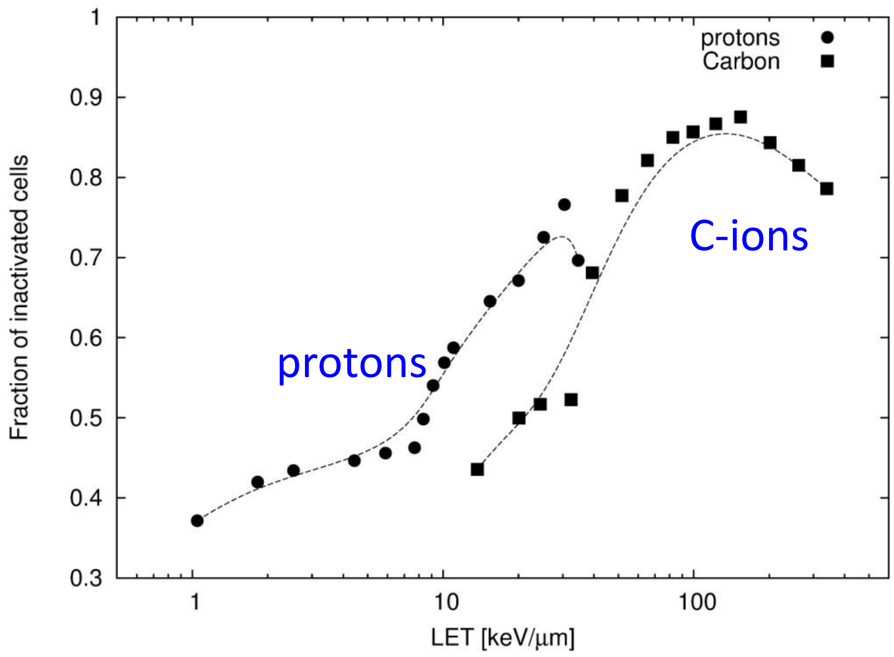
• increase in effectiveness with LET, with maximum around 100-200 keV/µm, followed by a decrease

• in agreement with experimental data, the increase in DNA/chromosome damage was much more pronounced than the increase in cell death *(which can be explained by the relationship S=exp(-LA))*

Potential applications for hadron therapy

(Ballarini et al 2013 Rad Res)

predicted cell death for 2 Gy protons and carbon

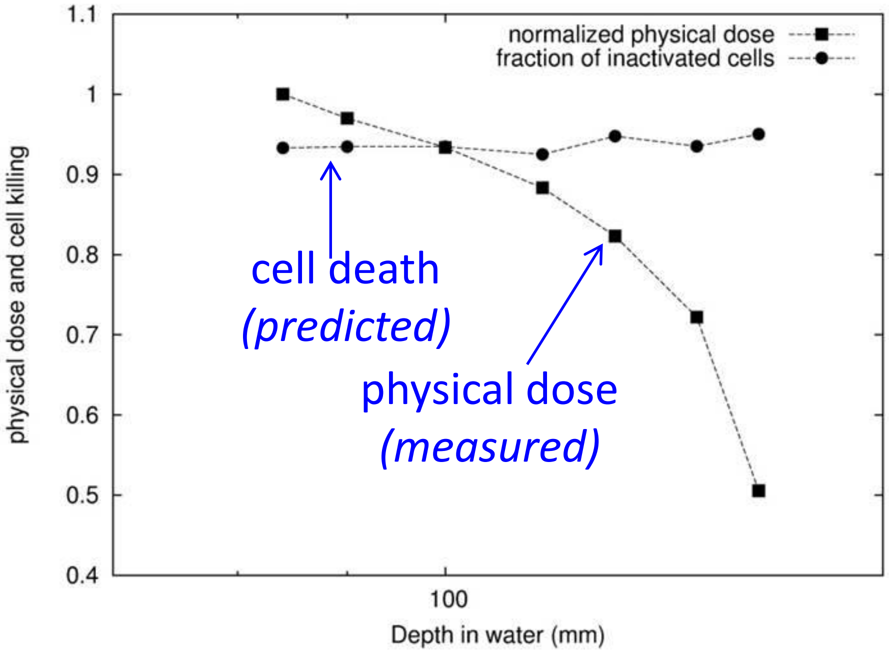


- *cell death predicted for many LET values (also interpolating CL yields)*

Potential applications for Carbon therapy

(Ballarini et al 2013 Rad Res)

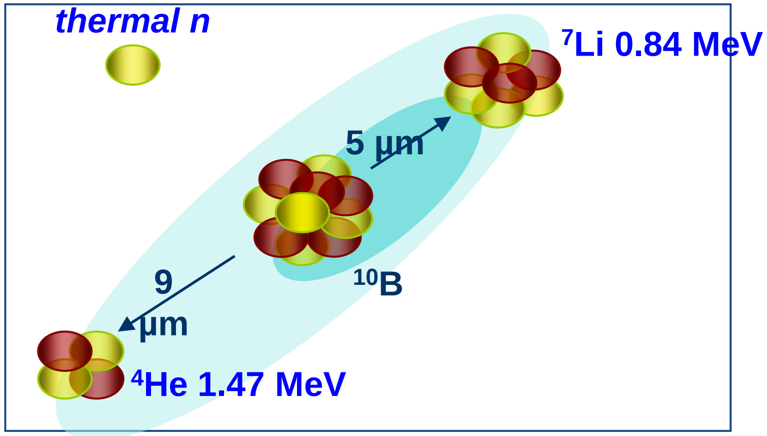
predicted cell death along a carbon SOBP used for hadrontherapy in Chiba, Japan *(V79)*



• predicted cell death along a therapeutic carbon SOBP was ~constant, in agreement with data

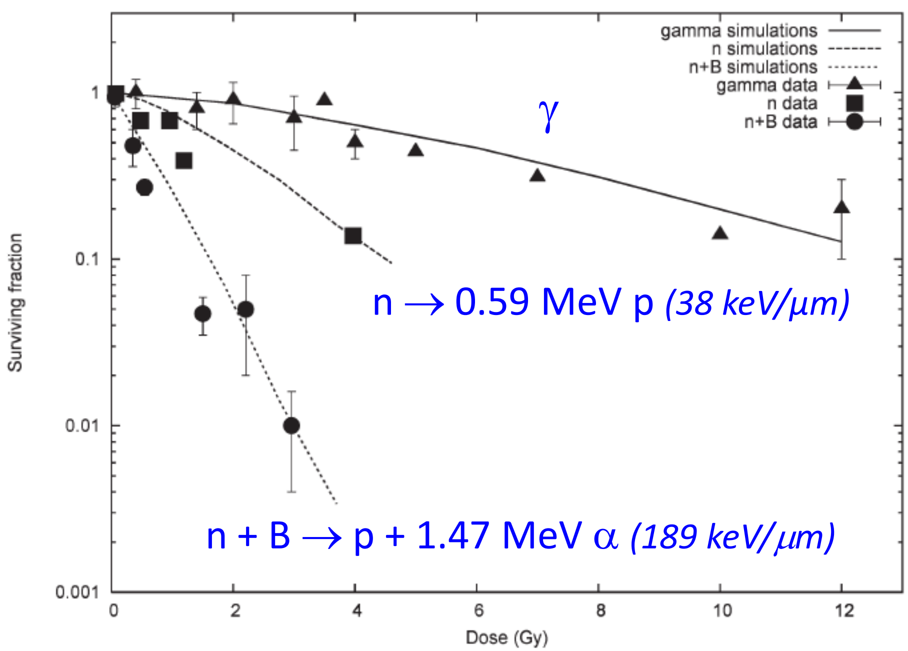
Potential applications for BNCT

(Boron Neutron Capture Therapy)



$^{10}B(n,\alpha)^{7}Li$

$^{14}N(n,p)^{14}C$



(Ferrari et al 2013 Rad Res)

α-particles and protons starting from random positions in the cell nucleus, with random directions

Weak points...  ...& strong points

- not mechanistic “enough”
- radiosensitivity not modelled explicitly
- not suitable for cells exposed during S- or G2-phase
- (maybe) not suitable for cells that undergo apoptosis
- … … …

- only two parameters
- no use of experimental RBE values
- works for V79 cells *(used for the characterization of many therapeutic beams)*

Concluding remarks…

General

- model/MC code of cell killing that is mechanism-based but uses only 2 adjustable parameters *(yield of DNA Cluster Lesions and threshold distance for chromosome fragment end-joining)*

- the model predicted the survival of normal (AG1522) and radioresistant (V79) cells exposed to photons, protons, alpha particles and heavy ions (including carbon), with yields of cluster lesions in the range ~2-20 CLs $\cdot Gy^{-1} \cdot cell^{-1}$ (depending on radiation quality) and a threshold distance for fragment rejoining at the micrometer scale

Specific

- fundamental role of DNA cluster damage *(@kbp level?)*, modulated by proximity effects at the micrometre scale

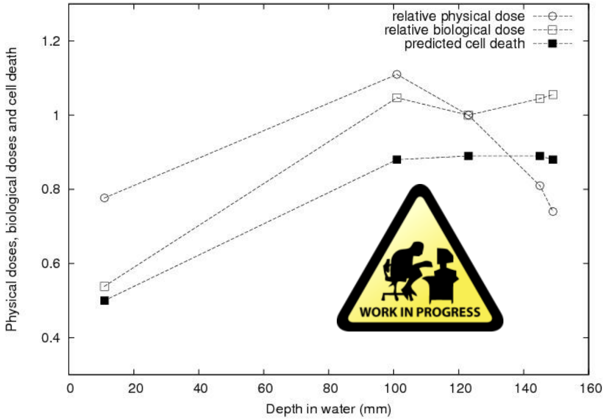
- cell death explained by lethal chromosome aberrations (dicentrics, rings and large deletions)

- possibility to calculate cell survival for hadron therapy beams

…and future developments

• extend to other cell types, including tumor cells

HSG cells, therapeutic carbon beam (Hyogo)



• non-lethal aberrations in surviving cells *(⇒ possible implications for the risk of normal tissues)*

• include explicitly radiosensitivity and apoptosis
• describe more explicitly the main steps of repair
• … … …

Acknowledgements

- Info/data sharing: M. Cornforth, M. Durante, K. Prise, G. Schettino, A. Tabocchini, W. Weyrather…

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- …and the audience☺