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RBEs for Human Cancer Cells Exposed to Protons and Heavier Ions:
Implications for Clinical Use of Charged Particles in Cancer Therapy

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Disclosures

No Conflicts

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Research and Treatment Center from NIH (C06 CA059267)

RBEs of Human Cancer Cells

- Overview: Biological advantages of charged particles
- Quantification of RBEs for human tumors
- Role of genetic background on RBEs
- Hypofractionation

Why Charged Particles?

- Dose distribution advantage of protons and heavier ions
  - More dose in tumor while sparing normal tissues
- Biological advantages of heavier ions
  - Increased (tumor) cell killing
  - Decreased repair between fractions
  - Decreased effect of hypoxia

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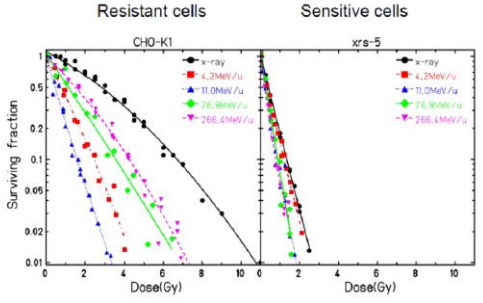
Increased Cell Killing with Heavy Ions



(from W. Kraft-Weyrather *et al.*, *Int. J. Radiat. Biol.* 1999)

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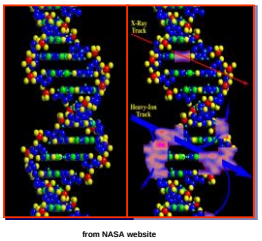
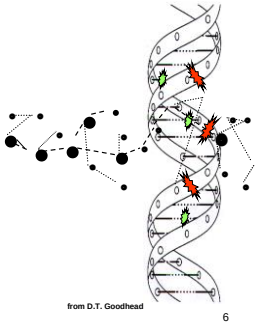
Increased Cell Killing: Charged Particle Tracks Through DNA



from NASA website

More complex DNA lesions, less repair (and less accurate repair), greater lethality.



from D.T. Goodhead

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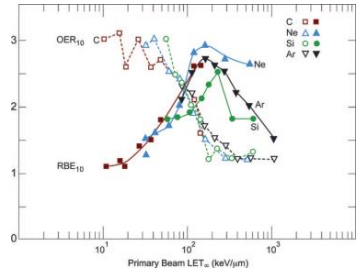
Optimum Cell Killing (RBE) at ~100 keV/μm



(from Blakely and Chang, 2009, based on pioneering work of Blakely *et al.* at Berkeley National Lab)

RBE = Relative Biological Effect (ratio of dose for effect with photons to dose for same effect with ions)

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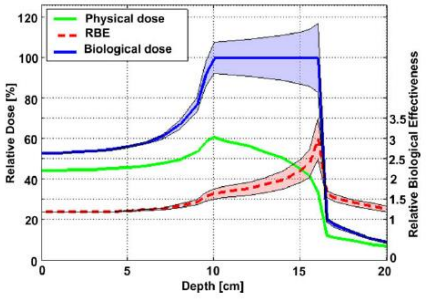
Greater Relative Biological Dose in Tumors over Normal Tissues Can Yield a Therapeutic Gain



(from Suit et al. 2010)

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Why Charged Particles?

- Dose distribution advantage of protons and heavier ions
  - More dose in tumor while sparing normal tissues
- Additional biological advantages of heavier ions
  - Increased (tumor) cell killing
  - Decreased repair/recovery between fractions
  - Decreased effect of hypoxia

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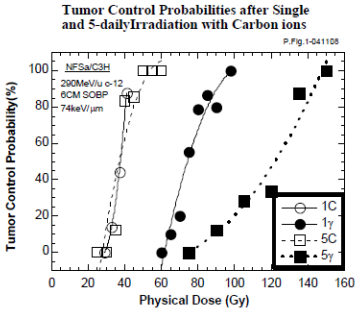
Decreased Repair Between Fractions



(from Ando on THREE website)

Why Charged Particles?

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  - More dose in tumor while sparing normal tissues
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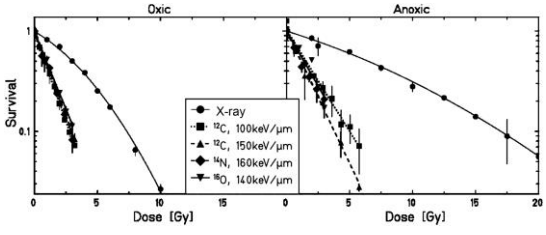
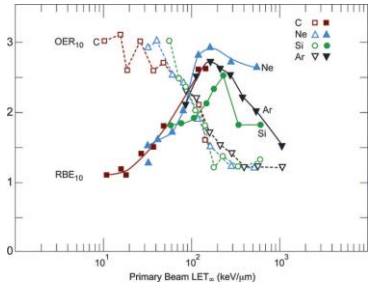
  - Increased (tumor) cell killing
  - Decreased repair between fractions
  - Decreased effect of hypoxia

Hypoxic Cells are Resistant to Photons, but Hypoxic Resistance is Decreased with Heavy Ions



(from Tinganelli et al. J Radiat Res 2013)

Maximal Loss of Hypoxic Resistance at ~100 keV/μm



(from Blakely and Chang, 2009, based on pioneering work of Blakely *et al*. at Berkeley National Lab)

OER = Oxygen Enhancement Ratio

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RBEs of Human Cancer Cells

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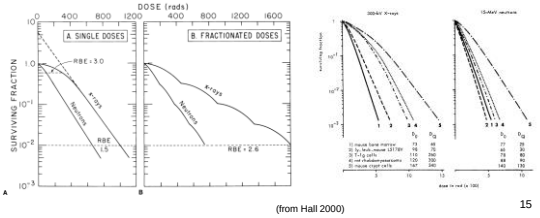
Heavy Ion RBEs: "Classic" Biology

- RBE is highly complex: depends on LET, ion species, dose, dose rate/fractionation, tissue/cell type, endpoint, oxygen concentration, microenvironment (and almost anything else you can think of!)



(from Hall 2000)

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Carbon Ion RBEs: Survival Curves with Human Cells

- Ando and Kase (2009): ~ 40 curves, mostly for human tumors; 3 for human fibroblasts

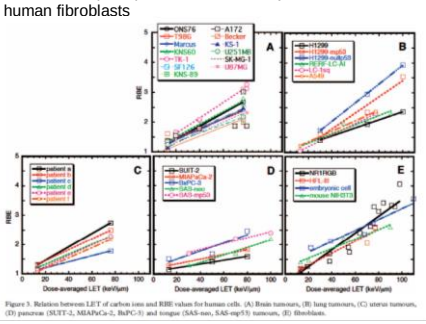

Figure 3. Relation between LET of carbon ions and RBE values for human cells. (A) Brain tumours, (B) lung tumours, (C) uterus tumours, (D) pancreas (SUIT-2, MIAPaCa-2, BxPC-3) and tongue (SAS-neo, SAS-mp53) tumours, (E) fibroblasts.

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Carbon Ion RBEs: Survival Curves with Human Cells

- Suit et al. (2010) – some of same studies as previous slide

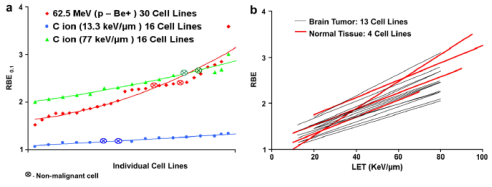

Fig. 7. RBE of tumor and normal cells in vitro. (a) Scattergrams of RBEs of 30 cell lines [two are normal cell lines] for 62 MeV neutron irradiation [33] and for 16 cell lines [two are normal cell lines] irradiated by ^{12}C ion beams at ~13 and ~80 keV/μm [34]. The large dots represent the normal cell lines. (b) RBE vs LET curves for 4 normal fibroblast cell lines and 13 brain tumor cell lines [35].

Conclusion from cell survival curves: Little evidence to support idea of consistently different RBEs for normal tissues than for tumors (if you think fibroblasts represent normal tissues).

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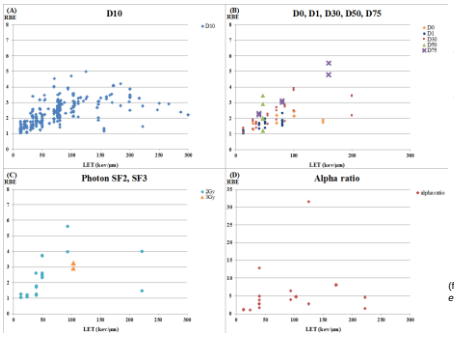
RBE vs LET for Human Tumor Cells: Various Parameters from Survival Curves

(Literature Summary)



Large spread in RBE values at any given LET

(from Held *et al.* 2016)

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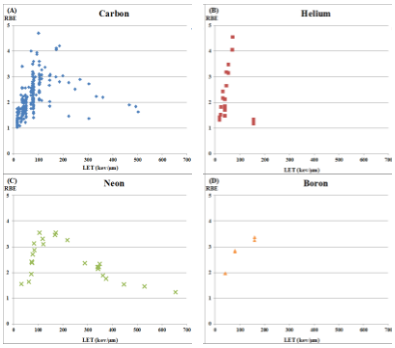
RBE_{10} vs LET for Human Tumor Cells: Various Charged Particles



Note the effectiveness of helium ions

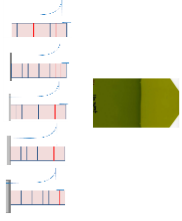
(from Held *et al.* 2016)

Our Recent Studies at NASA Space Radiation Laboratory (NSRL) at Brookhaven National Lab (BNL)

Experimental Set-up at NSRL to Explore Bragg Peak

Horizontal stack of water/media-filled T25 flasks containing cells aligned along beam axis; varying thicknesses of tissue equivalent polyethylene in front.

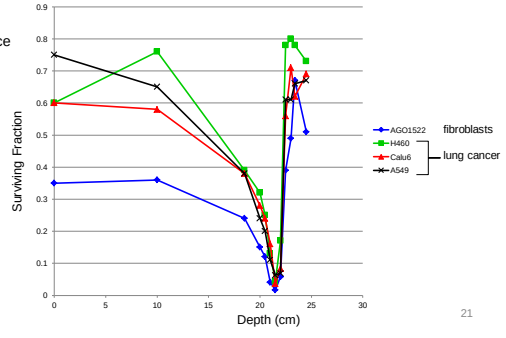
Ion beam

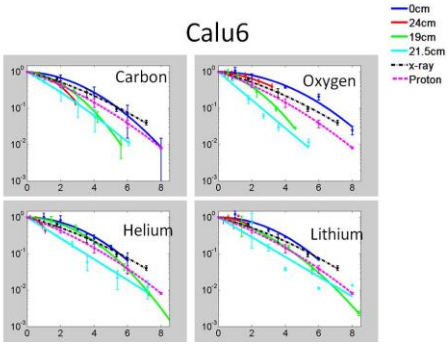


In physics measurements, Bragg peak at ~ 22.5 cm (surface of 10th flask in stack)

"Inverse" Bragg Curve with Human Cells: 355 MeV/n Carbon Ions

2 Gy entrance dose





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RBE (10% survival) Values (relative to 250 kVp X-rays) for Human Cells

		0 cm	19 cm	21.5 cm	24 cm
Carbon (355 MeV/n)	AG01522	1.0	1.3	1.4	1.8
	A549	1.5	1.2	1.7	(1.7)*
	H460	0.9	1.2	1.5	(1.3)
	Calu6	1.0	1.4	1.6	1.8
Oxygen (430 MeV/n)	AG01522	1.0	1.4	2.0	1.4
	A549	1.4	1.4	2.3	(1.4)
	H460	1.0	1.4	1.7	(1.6)
	Calu6	0.8	1.6	2.0	(1.1)
Helium (190 MeV/n)	AG01522	1.0	1.0	1.5	2.1
	A549	1.4	1.6	2.3	1.7
	H460	0.8	1.0	2.0	---
	Calu6	1.0	1.1	1.6	(1.6)
Lithium (215 MeV/n)	AG01522	1.0	0.8	1.3	1.9
	A549	0.9	1.0	1.7	1.0
	H460	0.9	1.0	1.3	---
	Calu6	1.0	1.2	1.4	(1.4)

*RBE values in () were estimated from extrapolation of data available only at higher survival levels. --- indicates insufficient data even to extrapolate.

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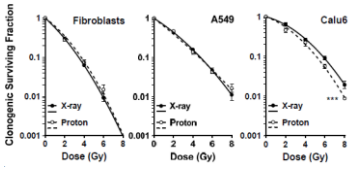
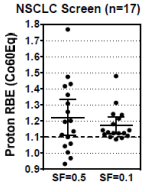
Some NSCLC Cell Lines Have Proton RBE > 1.1

H. Willers collaboration





(Liu et al. 2015)

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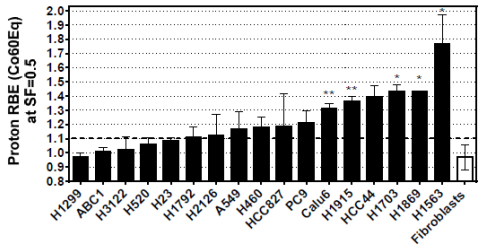

(Liu et al. 2015)

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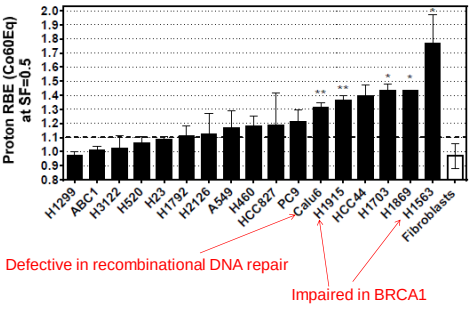
Cell Lines with Higher Proton RBEs May Be Defective In DNA Repair



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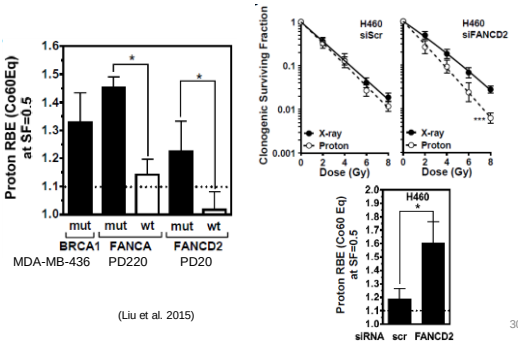
Cell Lines Defective in BRCA1 or Fanconi Anemia Pathway Are Sensitive to Protons



(Liu et al. 2015)

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Identification of Patients Whose Tumors are More Sensitive to Charged Particles

- Could guide selection of patients whose tumors might be treated more effectively with particles.
- Might allow de-escalation of charged particle dose if normal tissues of concern.
- Could facilitate use of biologically optimized tumor-directed therapy to improve tumor control.

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Clinical Trend with Carbon Ion Therapy Is To Hypofractionation

Examples of treatments at NIRS (Kamada *et al*. 2015)

- In peripheral stage I NSCLC, number of fractions reduced from 18 to 9 to 4 to 1.
- Prostate cancer treated in 16 fractions over 4 weeks, now reduced to 12 fractions over 3 weeks.
- In different protocols, hepatocellular carcinoma treated with 4 fractions or 2 fractions over 2 weeks.
- Pancreatic cancer treated with 12 fractions over 3 weeks.

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Experimental Data on RBEs with Fractionated Heavy Ions Are Limited

Growth delay in NFSa tumor and skin reactions after carbon ion irradiation

(from Ando *et al.* 2005)

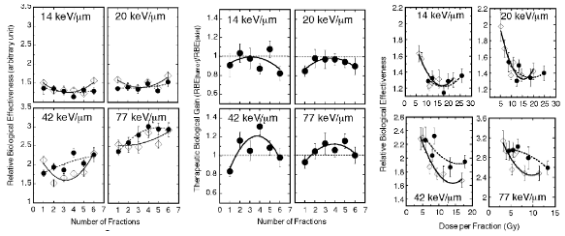
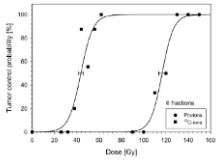


Fig. 3. Biological effectiveness of carbon ions relative to γ rays. The ratio of the isoeffect dose for γ rays to that for carbon ions at a given number of fractions, i.e., RBE, is plotted against the number of fractions (Fig. 3A) and the dose per fraction (Fig. 3C). The symbols and bars are the same as those in Fig. 2. The therapeutic gain is the ratio of the tumor RBE to the normal tissue (skin) RBE, and means positive when the RBE ratio exceeds 1.0 (Fig. 3B).

Carbon Ion RBEs for a Prostate Tumor



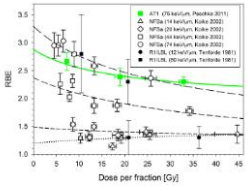


Fig. 2. Dose-response curves for 6 fractions of photons and carbon ions. The uncertainty (1 SD) of dose at 50% tumor control probability is indicated.

Table 2 TCD_{50} and RBE values measured in this study (6 fractions) and previous study (1 and 2 fractions) (9), including single standard errors and 90% confidence intervals

Study	TCD_{50} ± SE (90% CI) (Gy)		RBE ± SE (90% CI)
	Photons	Carbon ions	
1 Fraction*	75.7 ± 1.6 (69.9-78.6)	32.9 ± 0.9 (30.8-34.9)	2.30 ± 0.08 (2.17-2.44)
2 Fractions*	90.6 ± 2.3 (85.6-95.4)	37.9 ± 2.3 (33.6-42.6)	2.39 ± 0.16 (2.15-2.68)
6 Fractions	116.6 ± 3.0 (109.9-122.8)	43.7 ± 2.3 (39.1-47.5)	2.67 ± 0.15 (2.43-2.94)

(from Karger *et al.* 2013)

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Decreasing Number of Fractions (increasing dose/fraction) with Charged Particles

- Will measuring more RBE values in experimental systems help treat patients?
- Changing two parameters at once:
  - Fractionation
  - LET

- What's the Biology?

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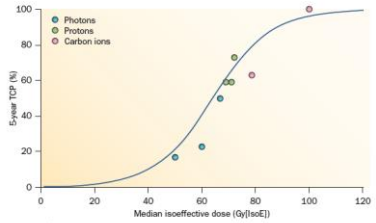
Is Hypofractionation with Carbon Ions "Just" More Dose or "Different" Dose?



(from Loeffler and Durante 2013)

Figure 1 | Dose-escalation for skull-base chordomas. The local control (tumour control probability, TCP) is plotted against the isoeffective dose (for the reference of 2 Gy/fraction) to the target for many clinical trials, using X-rays, protons, or carbon ions. Carbon ions isoeffective doses are calculated using the latest version (IV) of the local effect model (LEM).[8] Data points are reported and discussed in the paper by Schulz-Ertner *et al.*[60] Adapted with permission from IOP Publishing © Grün, R. *et al. Phys. Med. Biol.* 57, 7261–7274 (2012).

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Decreasing Fraction Number with Ions What's the Biology?

- 4 (5) R's
  - Repopulation
  - Redistribution
  - Reoxygenation
  - Repair
  - (Inherent Radiosensitivity)
- "New" biology

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Are "R"s Important?

	Conventional photons	Hypofractionation photons	Carbon Ions
Repopulation	yes	no	less?
Redistribution	maybe	no	little
Hypoxia	yes	yes	less
Reoxygenation	yes	no	maybe less
Repair	yes	less	less

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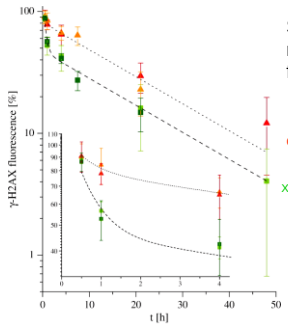
Molecular Mechanisms: Uniqueness of Track Structure



Slower and less complete repair of DNA damage from carbon ions

(from Schmid *et al*. 2010)

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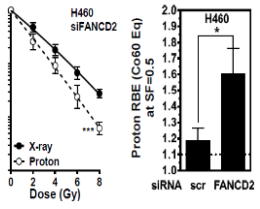
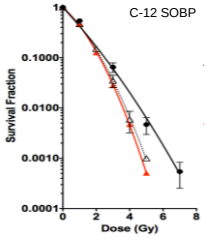
DNA repair following protons or ^{12}C requires homologous recombination



siRNA knockdown on FANCD2 (and others)

Liu *et al*. 2015



HR inhibitor

Aroumougame and Chen

(slide from M. Story)

Decreasing Fraction Number with Ions What's the Biology?

- 4 (5) R's
- "New" (different) biology – high dose or high LET?
  - More apoptosis
  - Damage to vasculature
  - Changes in reoxygenation
  - Immune effects
  - More killing of cancer stem cells
  - Changes in tumor cell migration
  - Suppression of angiogenesis factors
  - Altered gene expression patterns
  - Altered intra- and inter-cellular signaling

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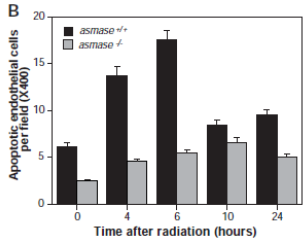
Some Data Suggest High Doses Increase Vascular Damage

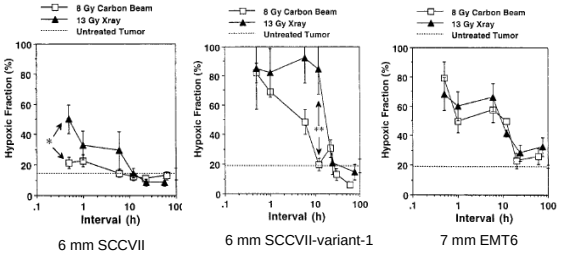
- Kolesnick, Fuks *et al*. have shown substantial radiation-induced apoptosis in tumor endothelial cells.



(from Garcia-Barrios *et al*., 2003)

MCA/129 fibrosarcoma; 4 h after 15 Gy

Reoxygenation Can Be Different after Carbon Ions in Some Tumors



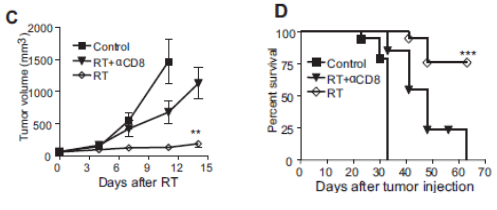
6 mm SCCVII | 6 mm SCCVII-variant-1 | 7 mm EMT6

(from Oya *et al*., 2001)

High Doses May Increase Immune System Effects on Tumor

- In some systems reduction of tumor burden after ablative RT depends largely on T-cell response.

B16 melanoma treated with 20 Gy



(from Lee *et al*. 2009)

Decreasing Fraction Number with Ions
What's the Biology?

- 5 R's
- "New" (different) biology – high dose or high LET?
  - More apoptosis (both but maybe different cell types)
  - Damage to vasculature (both)
  - Faster reoxygenation (maybe both, but LET more)
  - Immune effects (both, but LET more)
  - More killing of cancer stem cells (LET)
  - Tumor cell migration (inhibited with high LET)
  - Angiogenesis factors (suppressed with high LET)
  - Altered gene expression patterns (both, but different)
  - Altered intra- and inter-cellular signaling (both, but different)

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Summary

- Biological advantages of ions heavier than protons include increased cell killing, decreased repair, and decreased OER.
- RBE values depend on many, many factors; differences between tumors and normal tissues not clearly evident, although normal tissue data are limited.
- How high are RBE values beyond the Bragg peak?
- Are helium ions useful?
- Genetics, especially of tumors, may be useful to exploit.
- Hypofractionation of ions is increasing, but biology is less understood and may involve complex interplay of "different" biology at high doses and with high LET.

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THANK YOU