Strategies to Exploit Damage Repair in IMRT

Rob Stewart, Ph.D.
Radiation and Biophysics Research
Pacific Northwest National Laboratory
Richland, WA 99352

Presented at

University of Maryland Medical School
Baltimore, MD 21201
December 3, 2001
PNNL-SA-35622

and

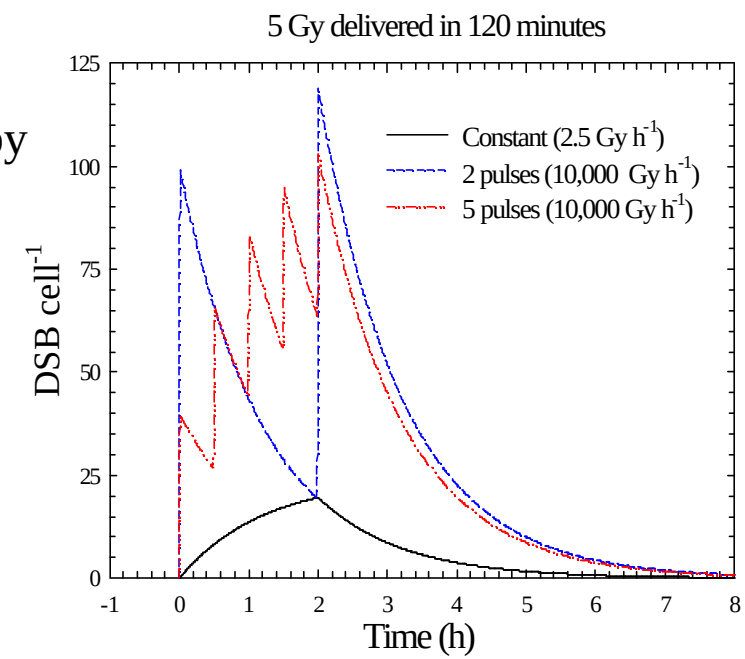
Memorial Sloan-Kettering Cancer Center
New York, NY. 10021
December 5, 2001
PNNL-SA-35623

Abstract

Biological optimization of radiation therapy treatment plans offers the potential for large gains in tumor-cell killing while simultaneously minimizing damage to normal tissue structures. This presentation will present results suggesting that DNA repair phenomena can impact cell killing in dose and dose-timing regimes relevant to intensity-modulated radiotherapy (IMRT). Results will be presented that suggest decreasing dose-fraction delivery times from 20 or 30 minutes to 5 or 10 minutes may shift tumor control probability (TCP) curves to lower doses by as much as 5 to 15%. To further exploit damage repair effects in IMRT, a biologically based scheme to optimize the intensity and sequence of the beams used to deliver daily dose fractions is presented and discussed.

Virtual Cell (VC) Radiobiology Software

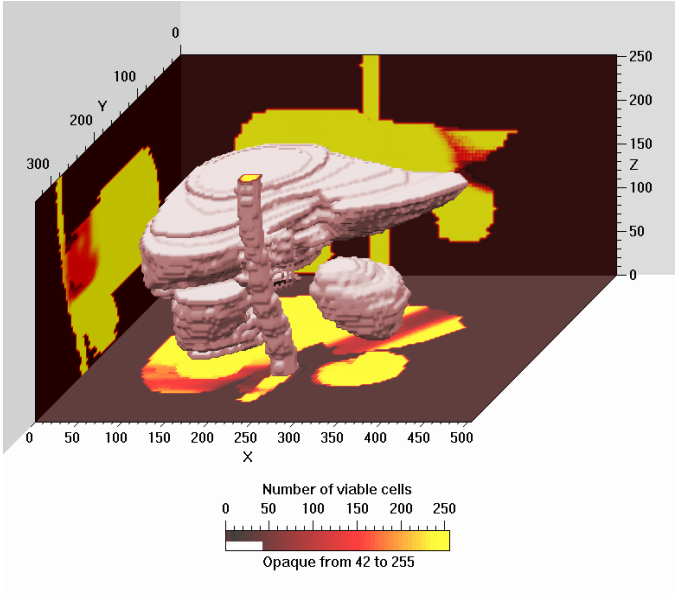
- **Complete computer program to simulate radiation effects in a homogeneous population of cells exposed to ionizing radiation.**
  - Double-strand break (DSB) rejoining kinetics.
  - Yield of lethal and non-lethal mutations by mechanism of action.
  - Genome instability and oncogenic (neoplastic) transformation (*prototype*).
  - Cell killing.
  - *Bystander effects (coming soon).*
- **Available repair models**
  - LPL Model (Curtis 1986)
  - RMR (Tobias 1985)
  - TLK (Stewart 2001)



R.D. Stewart, Virtual Cell (VC) Radiobiology Software. PNNL-13579, July 2001. Available at http://www.pnl.gov/berc/kbem/vc/

Virtual Life Form (VLF) software

- **Complete computer program to simulate radiation effects in three-dimensional tissue constructs.**
  - Same repair models as VC computer program.
    - ↗ LPL Model (Curtis 1986)
    - ↗ RMR (Tobias 1985)
    - ↗ TLK (Stewart 2001)
  - Import voxel-by-voxel dose distribution as a function of beam and/or leaf configuration. Constructs an instantaneous dose rate function for each voxel.
  - Poisson TCP model.
  - **Different regions of the tissue construct can be assigned different radiosensitivity parameters.**



Overview

- Some tests of models that link radiation damage to cell killing.
  - Lethal and potentially lethal (LPL) model.
    - S.B. Curtis, *Radiat. Res.* **106**(2):252-70 May 1986.
  - Two Lesion Kinetic (TLK) model.
    - R.D. Stewart, *Radiat. Res.* **156**(4), 365-378 October 2001.
- A practical TLK model calibration strategy
  - The Repair Capacity Strategy.
- Biological optimization.
  - Exploiting DNA repair effects in step-and-shoot IMRT.

Testing Mechanisms: A Perennial Challenge

- Cell survival data alone do not provide enough information to easily distinguish among alternate models.
  - LQ, LPL, RMR, TLK, … all yield very similar surviving fraction estimates as a function of dose and dose rate.
- So what's the big deal? Why not just use the LQ model?
- Two reasons:
  - The method(s) used to estimate model inputs (i.e., calibrate the model).
  - Accuracy and reliability of dose-effect *extrapolations* for difficult to measure exposure conditions.

Calibration of VC Repair Models

Adjust parameters in the LPL, RMR, or TLK models to minimize a figure of merit (FOM):

$$\text{FOM} = \frac{1}{N}\sum_{i=1}^{N} w_i \left[P_i - X_i\right]^2$$

$$P_i = X_i = \ln S(D)$$

The VC computer program uses the a quasi-Newton gradient method (local, non-linear optimization algorithm) to minimize the FOM.

Multi-Endpoint Approach to Model Testing

- Calibrate the models using cell survival data for a wide range of single-dose and split-dose exposure conditions.
  - Measured data from M.A. Stackhouse and J.S. Bedford, An ionizing radiation-sensitive mutant of CHO cells: irs-20. II. Dose-Rate Effects and Cellular Recovery Processes. *Radiat. Res.* 136(2), 250-254 (1993).
- Compare measured and calibrated-model predictions of the DSB rejoining rate.
  - Measured data from M.A. Stackhouse and J.S. Bedford, An ionizing radiation-sensitive mutant of CHO cells: irs-20. III. Chromosome aberrations, DNA breaks and mitotic delay. *Int. J. of Radiat. Biol.* **65**(5), 571-582 (1994).

Lethal and Potentially Lethal (LPL) Model

$$\frac{dL_{dsb}(t)}{dt} = \Sigma_{dsb}\dot{D}(t) - L_{dsb}(t)\left\{ l_{dsb} + e_{dsb} L_{dsb}(t) \right\}$$

$$\frac{dL_f(t)}{dt} = \Sigma_f \dot{D}(t) + e_{dsb} L_{dsb}(t) L_{dsb}(t)$$

$$L_f(0) = L_{dsb}(0) = 0 \qquad S = \exp(-L_f)$$

- Model has 4 “adjustable” parameters.
- DSB rejoining kinetics are a linear-quadratic function of $L_{dsb}(t)$.

SB Curtis, *Radiat Res* **106**(2):252-70 (1986). Published erratum appears in *Radiat Res* **119**(3):584 (1989).

Linear Quadratic (LQ) Model

$$S(D) = \exp\left\{-D\left(a + bGD\right)\right\}$$

a ~ 0.3 Gy^{-1}, b ~ 0.05 Gy^{-2}, and G is the Lea-Catcheside dose protraction factor

$$G = (2/D)^2 \int_{-\infty}^{\infty} dt\ \dot{D}(t) \int_{-\infty}^{t} dt'\ \dot{D}(t') \exp\left\{-l\,(t - t')\right\}$$

For two acute doses D_1 and D_2 separated by time interval T

$$G = \left\{D_1^2 + D_2^2 + 2D_1D_2e^{-lT}\right\}(D_1 + D_2)^{-2}$$

G = 1 for a single acute dose of radiation.

RK Sachs, P Hahnfeld, and DJ Brenner, *Int. J. Radiat. Biol.*, **72**, 351-374 (1997).

Relationship between LQ and LPL Models

For lower doses and dose rates,

$$l_{dsb} L_{dsb}(t) >> e_{dsb} L_{dsb}(t) L_{dsb}(t)$$

and

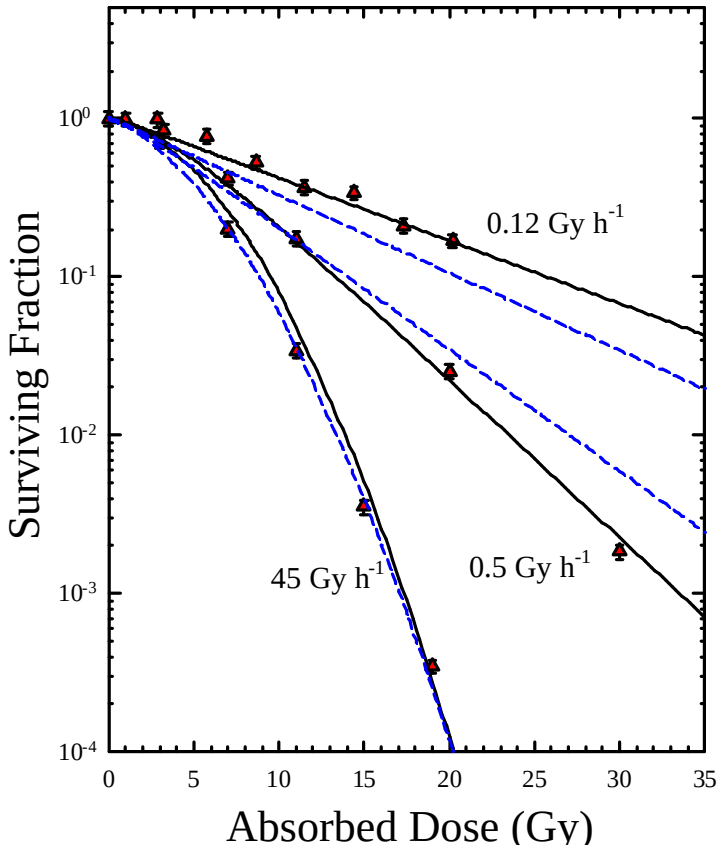
$$a = \Sigma_f + \Sigma_{dsb} \exp\{-l_{dsb} T_r\} \qquad Gb = \frac{e_{dsb} \Sigma_{dsb}^2}{2 l_{dsb}} \{1 - \exp(-l_{dsb} T_r)\}^2$$

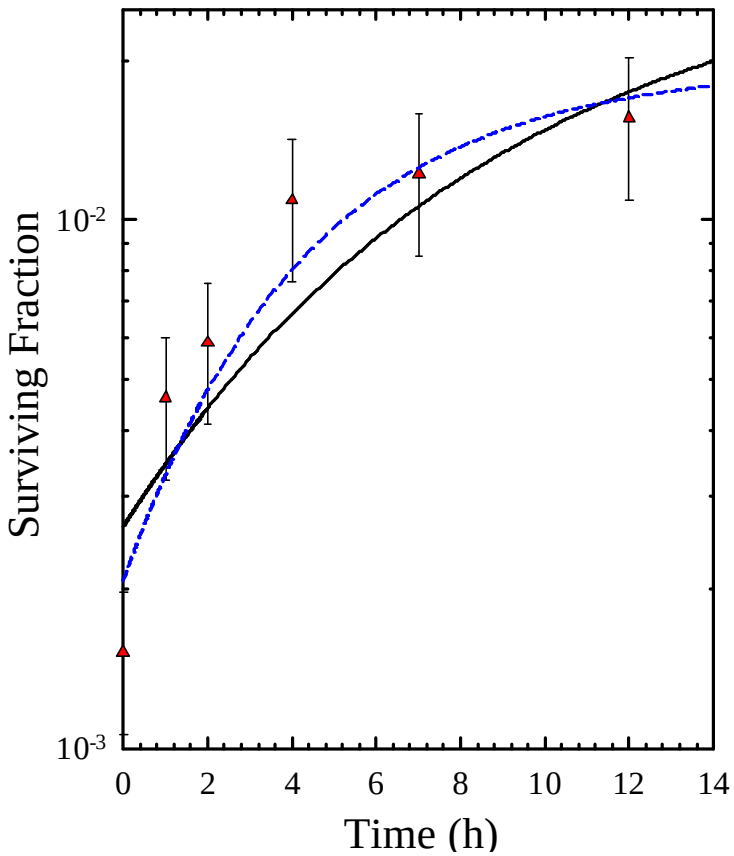
T_r is the effective time available for repair.

- LQ model with first-order repair kinetics is a special case of the LPL model.
  - Tests of LPL model also apply to the LQ model with first order-repair kinetics.

SB Curtis, *Radiat Res* **106**(2):252-70 (1986). Published erratum appears in *Radiat Res* **119**(3):584 (1989).

Calibration of the LPL Model

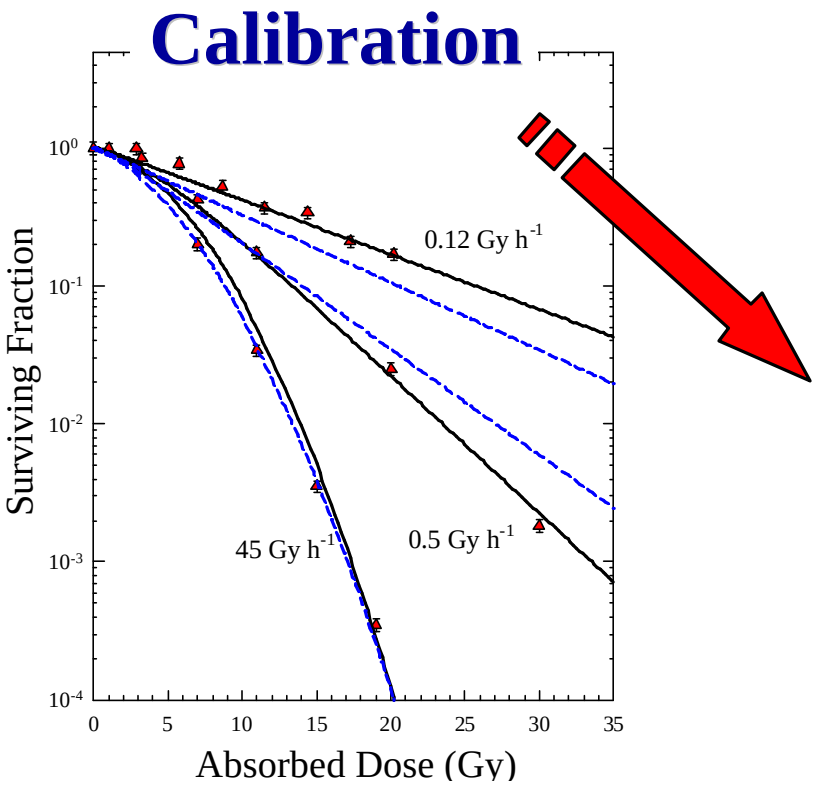




Survival of plateau phase CHO 10B2 cells irradiated by ^{137}Cs gamma-rays. ***Solid line:*** three-parameter LPL model fit ($2Y\Sigma_{dsb}$ = 25 cell^{-1} Gy^{-1}). ***Dashed line:*** two-parameter LPL model fit [$2Y\Sigma_{dsb}$ = 25 cell^{-1} Gy^{-1}, l_{dsb} = 0.231 h^{-1} (3 h DSB repair half-time)].

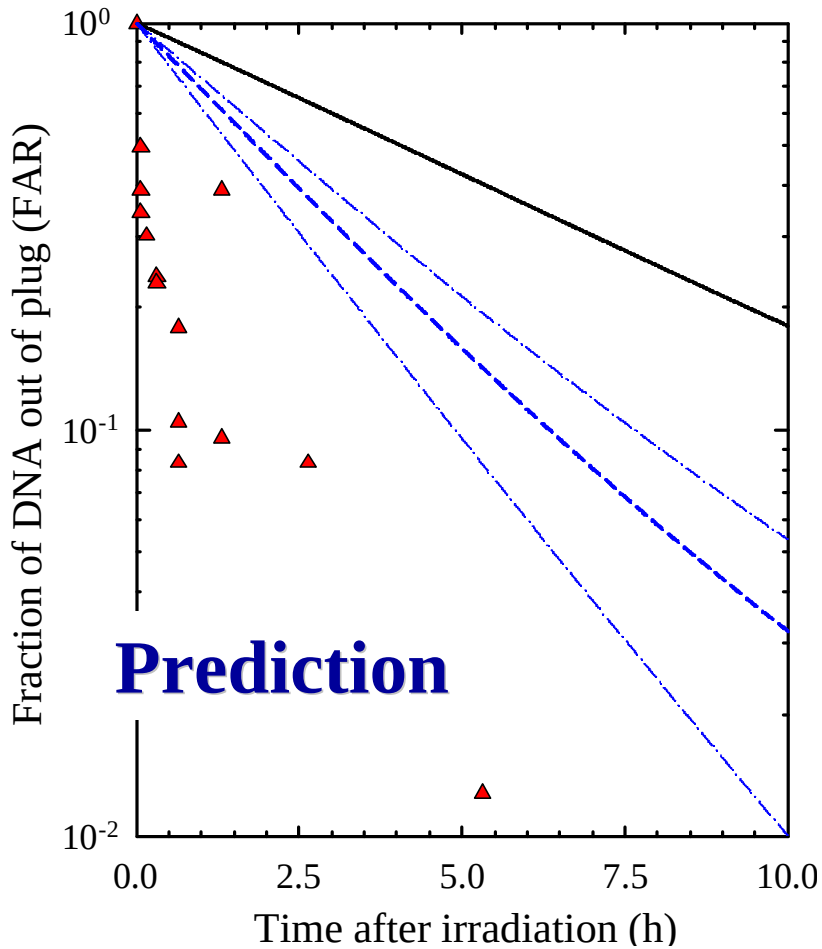
Adapted from R.D. Stewart, *Radiat. Res.* **156**(4), 365-378 October 2001.

A Test of the LPL Model



Solid line: three-parameter LPL model fit ($2Y\Sigma_{dsb}$ = 25 cell^{-1} Gy^{-1}).
Dashed line: two-parameter LPL model fit [$2Y\Sigma_{dsb}$ = 25 cell^{-1} Gy^{-1}, λ_{dsb} = 0.231 h^{-1} (3 h DSB repair half-time)].
Dash-dot lines: estimated lower and upper bounds on LPL model rejoining kinetics.

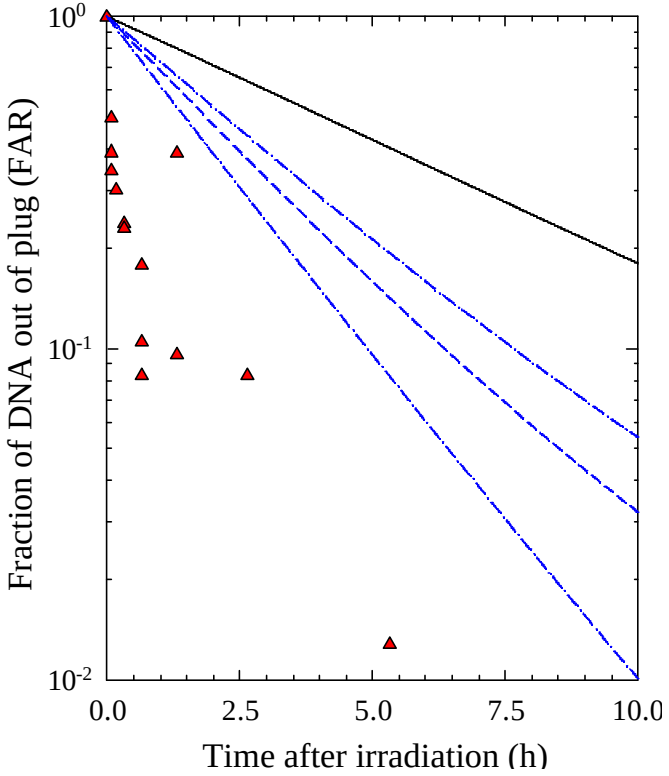
DSB rejoining kinetics in plateau phase CHO 10B2 cells following irradiation by 20 Gy dose delivered at 356.1 Gy h^{-1}.



Adapted from R.D. Stewart, *Radiat. Res.* **156**(4), 365-378 October 2001.

Conclusions from the LPL-Model Test

- Dataset contains sufficient information to “break” the LPL model.
- LPL model does not provide a satisfactory formalism to link biochemical processing of the DSB to cell killing.
- Major Consequence:
  - Model parameters cannot be reliably estimated from PFGE or other DSB assays.



Two-Lesion Kinetic Model

- Radiation creates “simple” and “complex” DSBs.
- Each kind of DSB may have its own unique rejoining kinetics and fidelity of repair.
- Break-ends associated with both kinds of DSB are allowed to interact in pairwise fashion to form exchange-type chromosome aberrations.

Simple DSB

Complex DSB

TLK Model Differential Equations

$$\text{DSBs}\begin{cases} \dfrac{d\bar{L}_1(t)}{dt} = 2\dot{D}(t)Y\Sigma_1 - \left\{ l_1 + h\left[\bar{L}_1(t) + \bar{L}_2(t)\right]\right\}\bar{L}_1(t) \\ \dfrac{d\bar{L}_2(t)}{dt} = 2\dot{D}(t)Y\Sigma_2 - \left\{ l_2 + h\left[\bar{L}_1(t) + \bar{L}_2(t)\right]\right\}\bar{L}_2(t) \end{cases}$$

$$\frac{d\bar{L}_f(t)}{dt} = b_1 l_1 \bar{L}_1(t) + b_2 l_2 \bar{L}_2(t) + gh\left[\bar{L}_1(t) + \bar{L}_2(t)\right]^2$$

$$\frac{d\bar{L}_m(t)}{dt} = (1 - b_1) l_1 \bar{L}_1(t) + (1 - b_2) l_2 \bar{L}_2(t) + (1 - g)h\left[\bar{L}_1(t) + \bar{L}_2(t)\right]^2$$

$b_1 \sim b_2$ $\qquad$ $\gamma \sim 0.25$

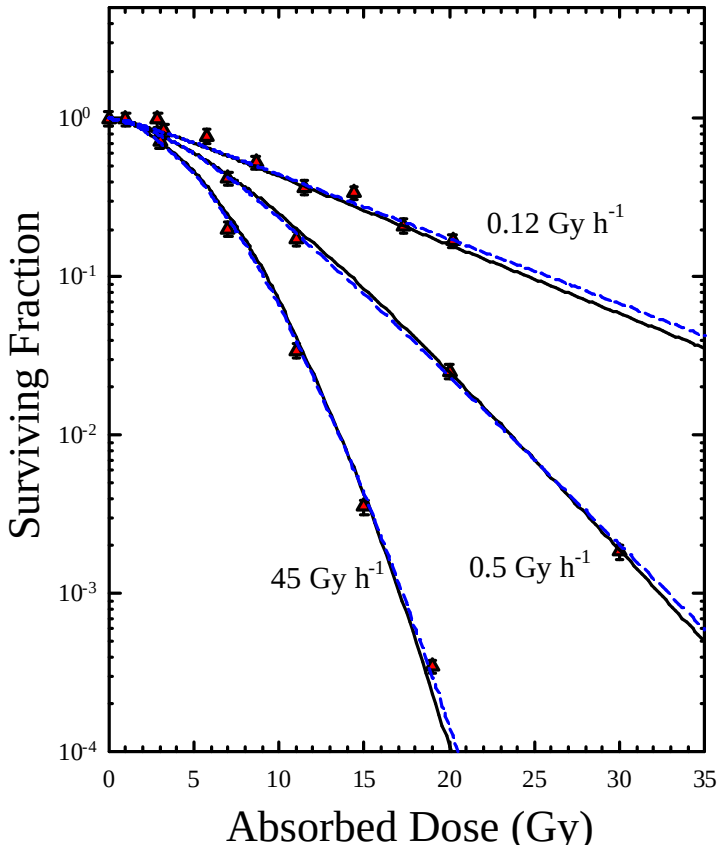
$2Y(\Sigma_1 + \Sigma_2) \sim 25$ to 40 Gy cell^{-1}

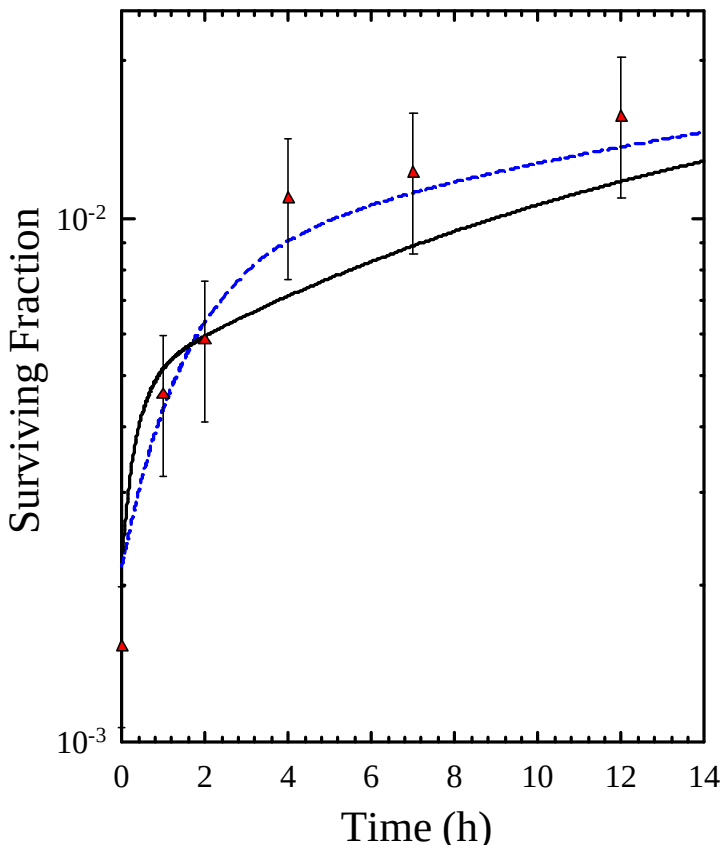
$$\bar{L}_1(0) = \bar{L}_2(0) = \bar{L}_f(0) = 0$$

$$S(t) = \exp\left\{-\bar{L}_f(t)\right\}$$

R.D. Stewart, *Radiat. Res.* **156**(4), 365-378 October 2001.

Calibration of the TLK model

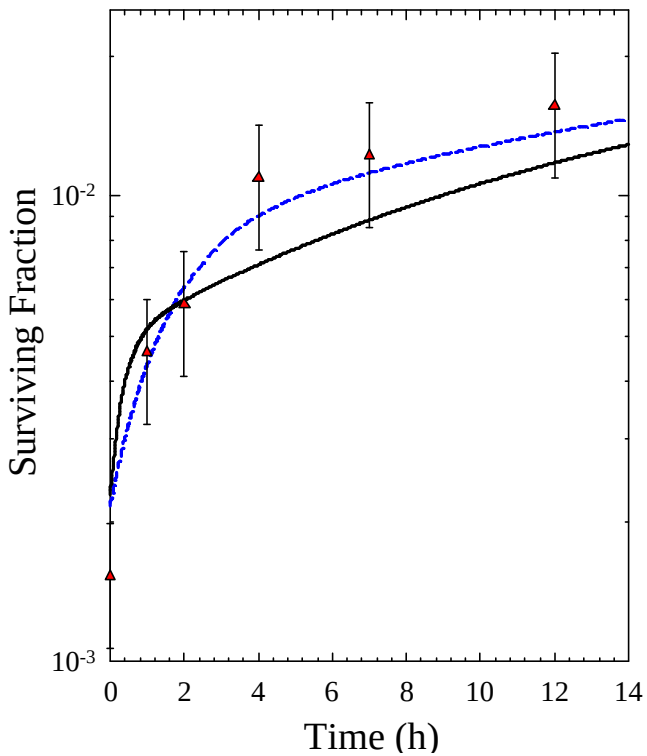




Survival of plateau phase CHO 10B2 cells irradiated by ^{137}Cs gamma-rays. ***Dashed lines:*** six-parameter TLK model fit [$\gamma = 0.25$, $2Y(\Sigma_1 + \Sigma_2) = 25$ cell^{-1} Gy^{-1}]. ***Solid lines:*** five-parameter TLK model calibration, $\gamma = 0.25$, $2Y(\Sigma_1 + \Sigma_2) = 25$ cell^{-1} Gy^{-1}, and $l_2 = 2.77$ h^{-1} (15-minute repair half-time).

Adapted from R.D. Stewart, *Radiat. Res.* **156**(4), 365-378 October 2001.

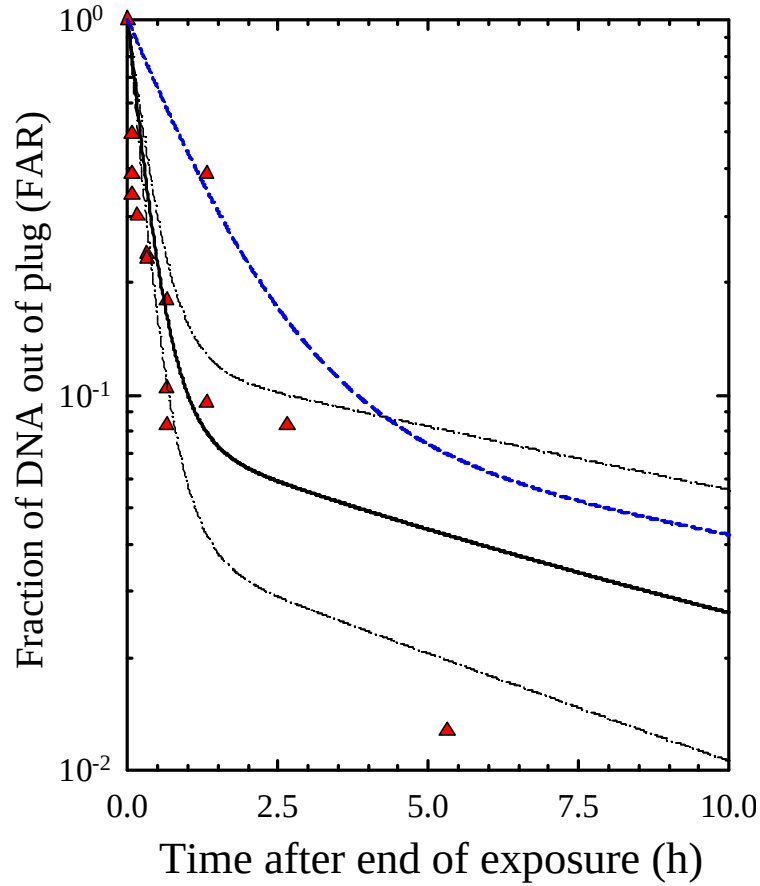
A Test of the TLK model



Dashed line: six-parameter calibration. $\gamma = 0.25$, $2Y(\Sigma_1 + \Sigma_2) = 25$ cell^{-1} Gy^{-1}.

Solid line: five-parameter calibration. $\gamma = 0.25$, $2Y(\Sigma_1 + \Sigma_2) = 25$ cell^{-1} Gy^{-1} and $l_2 = 2.77$ h^{-1} (15-minute repair half-time).

DSB rejoining kinetics in plateau phase CHO 10B2 cells following irradiation by 20 Gy dose delivered at 356.1 Gy h^{-1}.



Adapted from R.D. Stewart, *Radiat. Res.* **156**(4), 365-378 October 2001.

Conclusions from the TLK-Model Test

- TLK model provides a (more) satisfactory formalism to link biochemical processing of the DSB to cell killing.
  - It may be possible to directly estimate some TLK model inputs using data from PFGE or other DSB assays.
- But, models with more than 3 or 4 parameters are completely impractical…
- Or are they???

The Repair-Capacity Strategy

- Motivation: the rate and fidelity of DNA repair is more indicative of "intrinsic radiosensitivity" than the initial yield of damage.

Set

$2Y(\Sigma_1 + \Sigma_2) \sim 25$ to 40 Gy cell^{-1} $\quad 2Y\Sigma_1 \sim 2Y(\Sigma_1 + \Sigma_2)/5$

$b_1 = b_2 = 0$ Linear misrepair of a DSB is seldom lethal.

$g = 0.25$ Roughly ¼ of the DSBs mis-joined through the pairwise interaction process are lethal.

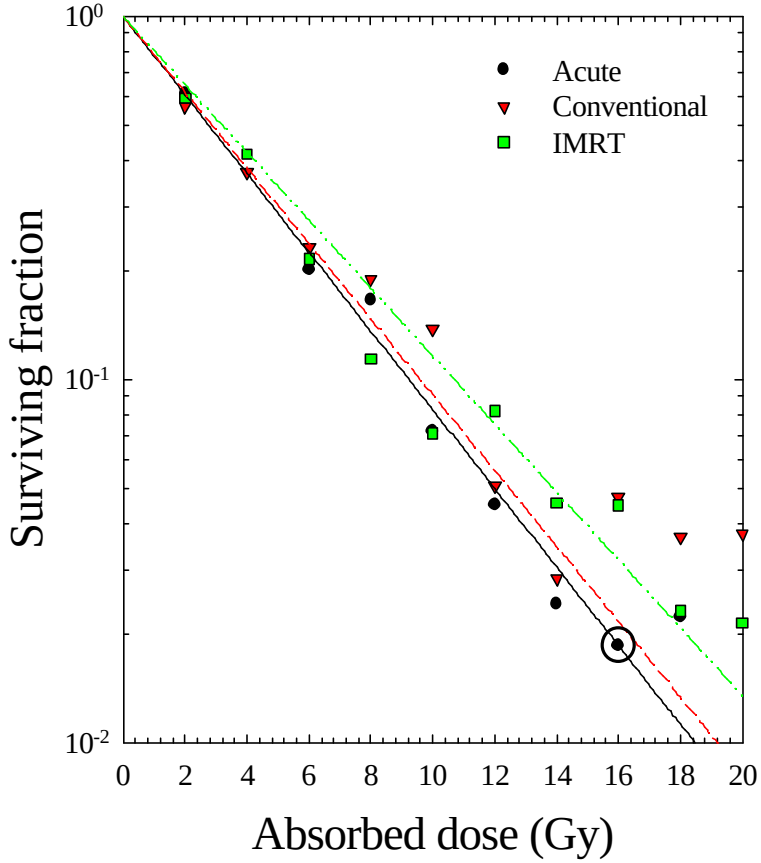
Adjust l_1, l_2, and/or the h TLK parameters.

CHOC4 Dataset

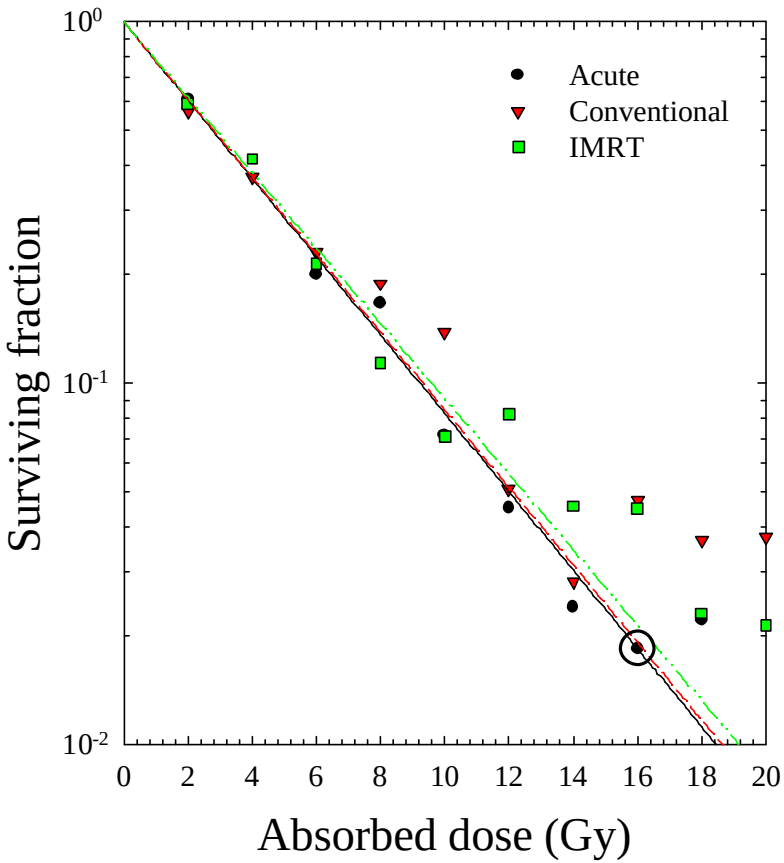
- Multi-fraction dataset. 2 Gy dose fraction delivered on sequential days.
  - Acute: 1 “pulse” of 2 Gy delivered at 60 Gy h^{-1}.
  - Conventional: 3 pulses of 66.67 cGy separated by 2 minutes.
  - IMRT: 7 pulses of 28.57 cGy separated by 3 minutes.
- Single dose dataset. Doses of 2, 4, 6, 8 Gy delivered at 60 Gy h^{-1}.

Acute and fractionated survival data courtesy of Drs. M. Bose, W.F. Morgan, S. Naqvi, and L. Huang. University of Maryland Medical School. Personal communication Dr. M. Guerrero (U. of Maryland). November 2001.

CHOC4 Multi-Fraction: Attempt #1

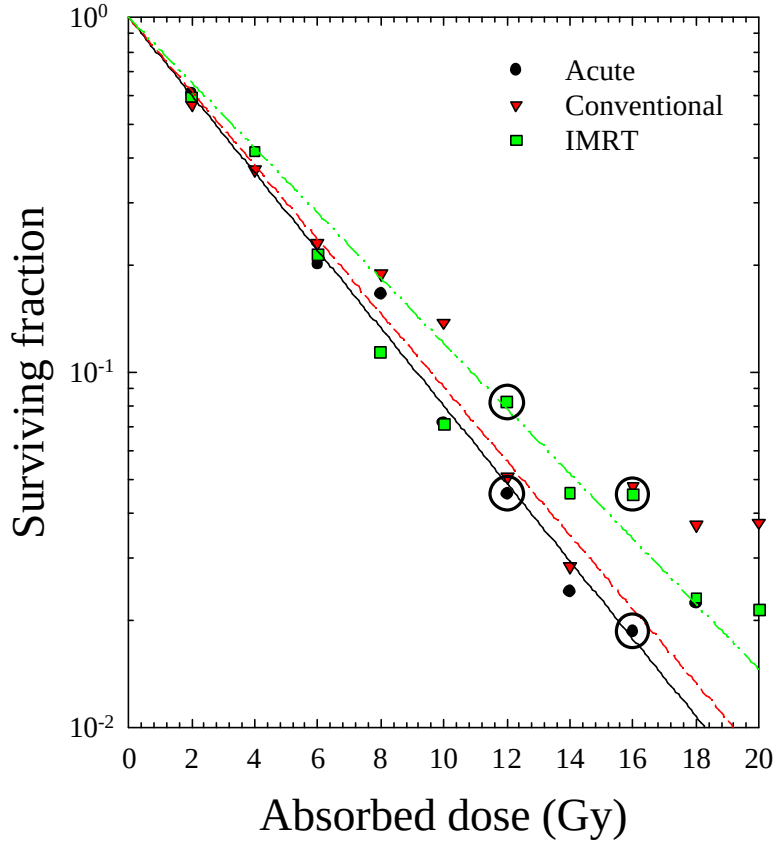


Set l_1 and l_2 = 1.386 h^{-1}
(0.5 h repair half time)



Set l_1 and l_2 = 0.347 h^{-1}
(2 h repair half time)

CHOC4 Multi-Fraction: Attempt #2

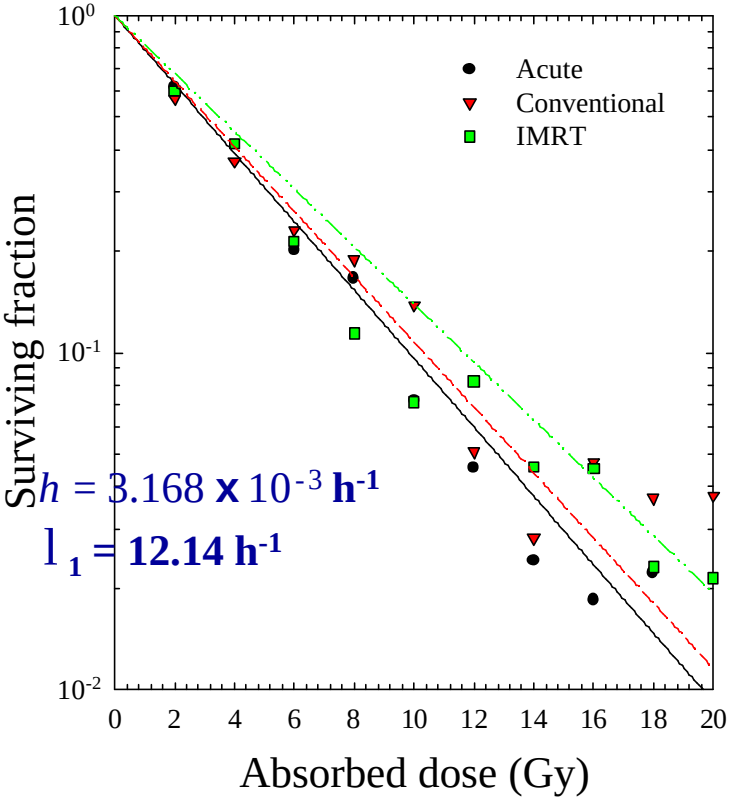


Set l_1 and l_2 = 1.386 h^{-1}
(0.5 h repair half time)

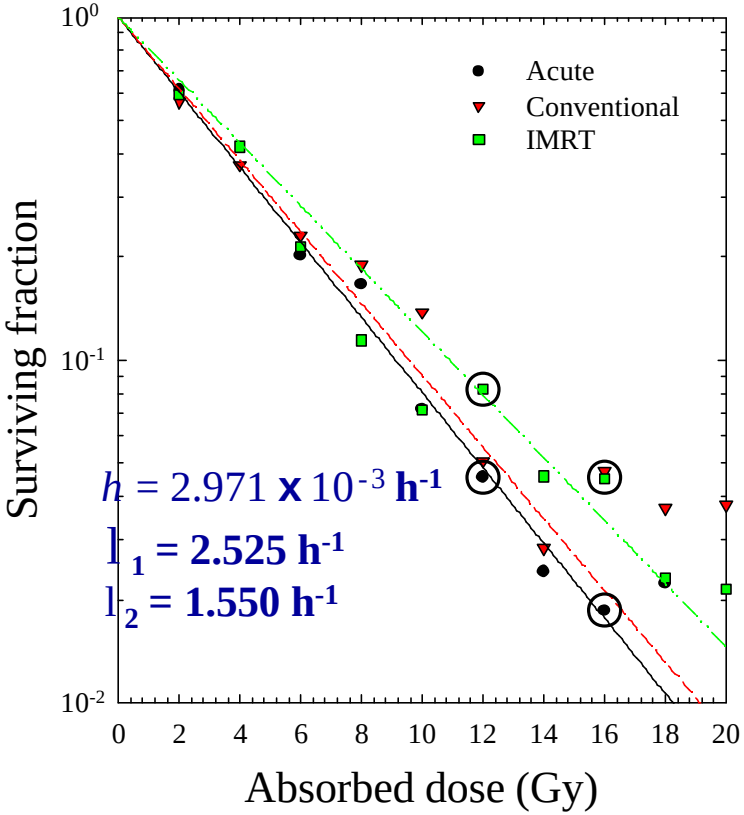
Summary

l_1 and l_2	Half Time (h)	$10^3 \times \eta$	FOM
1.3863	0.5	2.197	0.0651
0.6931	1.0	1.058	0.0686
0.3466	2.0	0.518	0.0717
0.1733	4.0	0.251	0.0739
0.0866	8.0	0.106	0.0775

CHOC4 Multi-Fraction: Best Guess

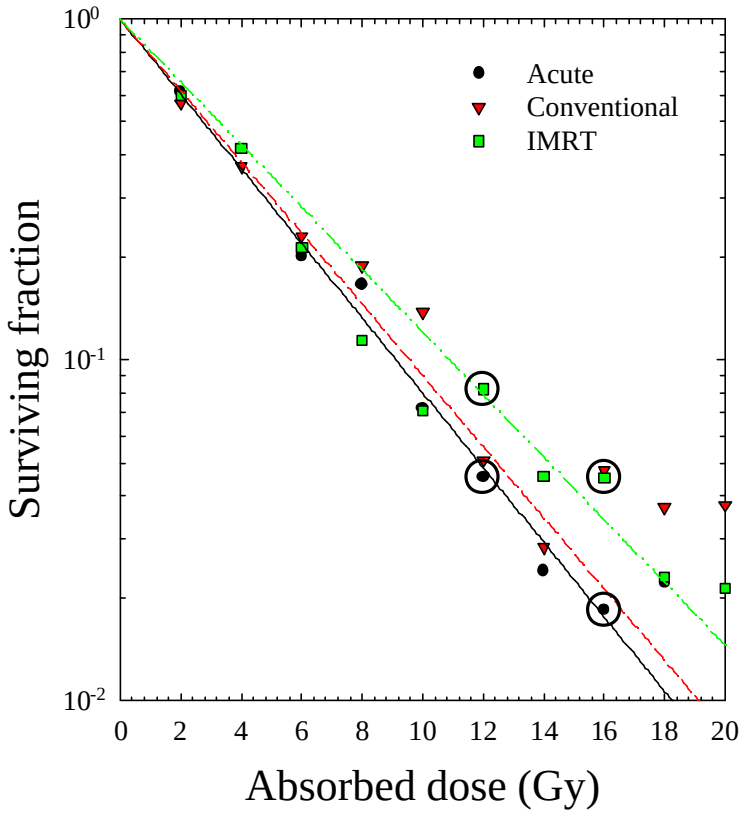


Adjust l_1 and h.
$l_2 = 1.386\ h^{-1}$

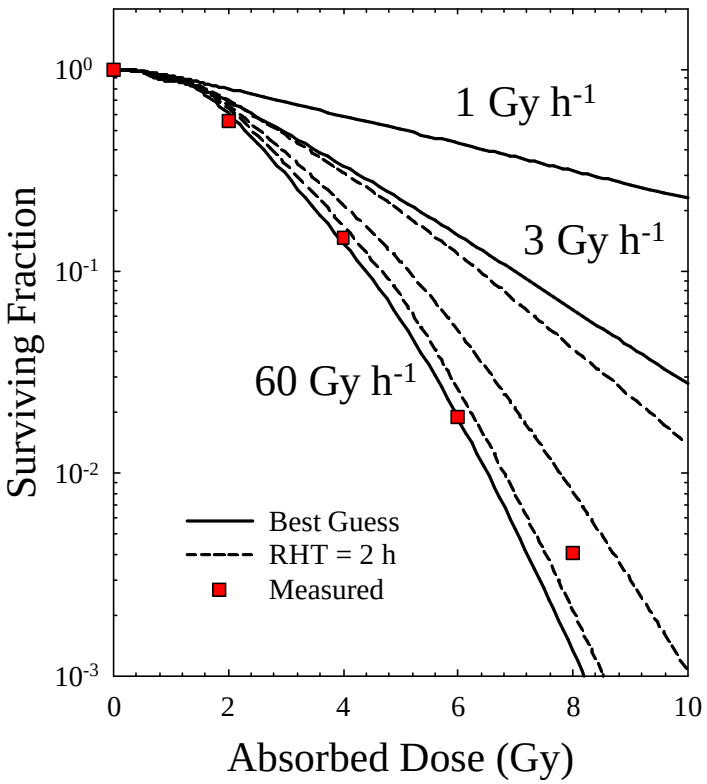


Adjust l_1, l_2 and h.

Prediction of Single-Dose Survival



Calibrate by adjusting l_1, l_2 and h.



Prediction of single dose data

Conclusions from the CHOC4 Analysis

- The repair capacity strategy (RCS) appears to work reasonably well for this dataset.
  - Can use the multi-fraction data to identify a “good enough” model calibration to accurately predict the acute, single-dose data.
  - Can go the other way too (acute -> multi-fraction).
- Acute, conventional, and IMRT dosing schemes produce slightly different levels of cell killing.

Potential Advantages of the TLK Approach

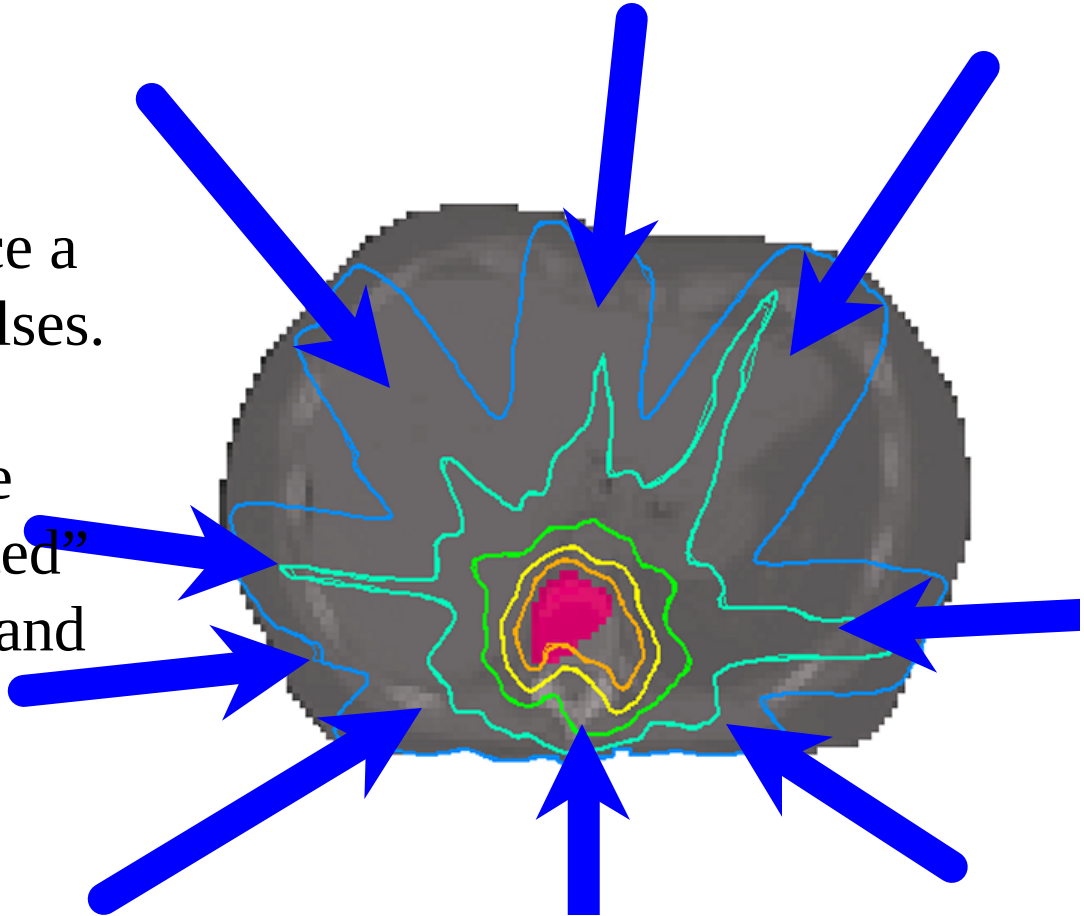
- Focuses on the "repair capacity" of the cell as the primary determinate of intrinsic radiosensitivity.
  - Less critical parameters are assigned biologically plausible values.
- Room to grow.
  - Adjust 1 or 2 parameters for expediency. May only need results from a single experiment to generate a rough calibration (e.g., SF2).
  - Adjust 4 to 6 parameters for maximum accuracy (probe for subtle, difficult-to-measure effects).
- Reduces "gracefully" to the LQ, LPL, and RMR model(s)
  - LQ model with *two* first-order repair rates for lower doses and dose rates (M. Guerrero, U. of Maryland). Publication in progress.
  - RMR and ~ LPL model when $l_1 = l_2$ and $b_1 = b_2$ (LQ model at lower doses and dose rates).

IMRT Optimization: Working Hypothesis

- In step-and-shoot IMRT, cells experience a series of radiation “pulses” separated by a few minutes.

- Different regions of the tumor and surrounding normal tissues experience a different sequence of pulses.

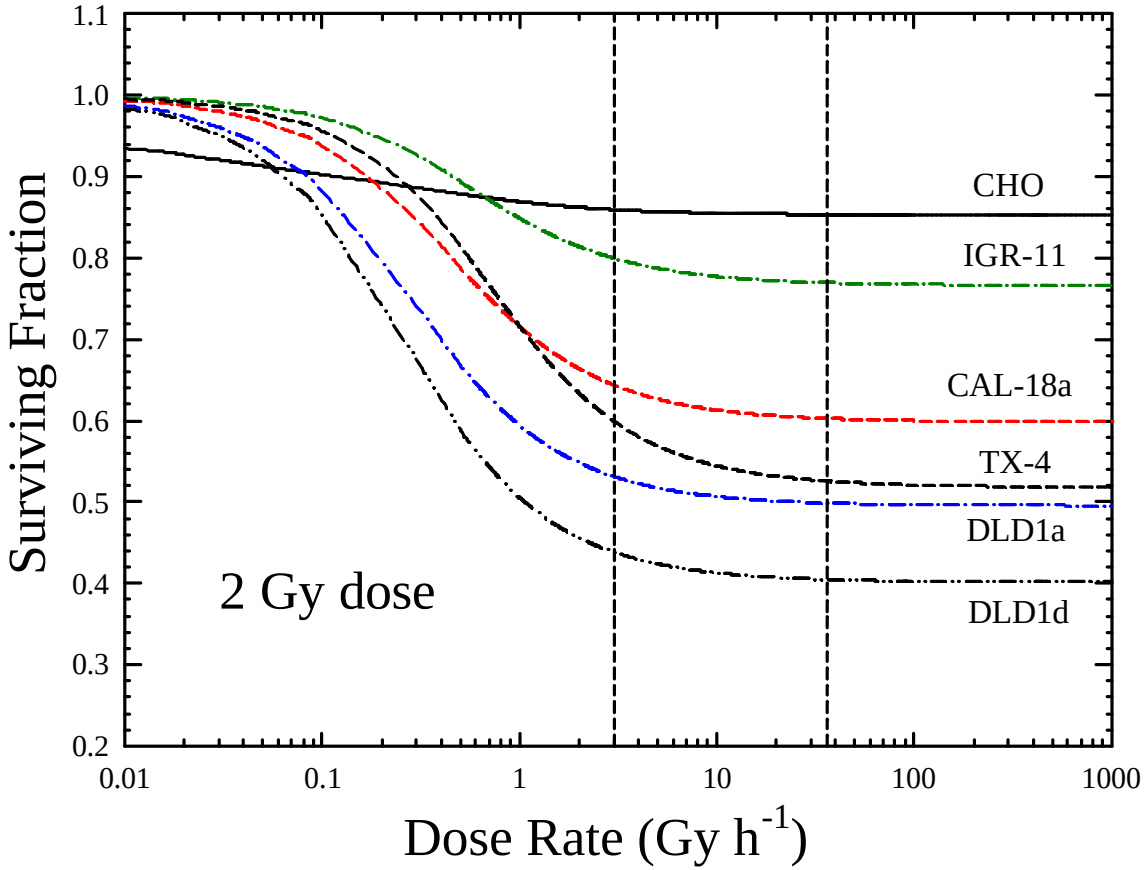
- Tumor and normal tissue responses can be “sculpted” by varying the intensity and timing of the beams.



Questions of Interest

- How significant are DNA repair effects for dose-timing regimes relevant to IMRT?
- How are dose rate effects influenced by intrinsic radiosensitivity?
- What are some strategies to sculpt tissue responses by manipulating the delivery of radiation in time and space?

Effect of Dose Rate



Cells that exhibit rapid DSB rejoining kinetics tend to be more sensitive to changes in dose rate than cells that repair damage slowly.

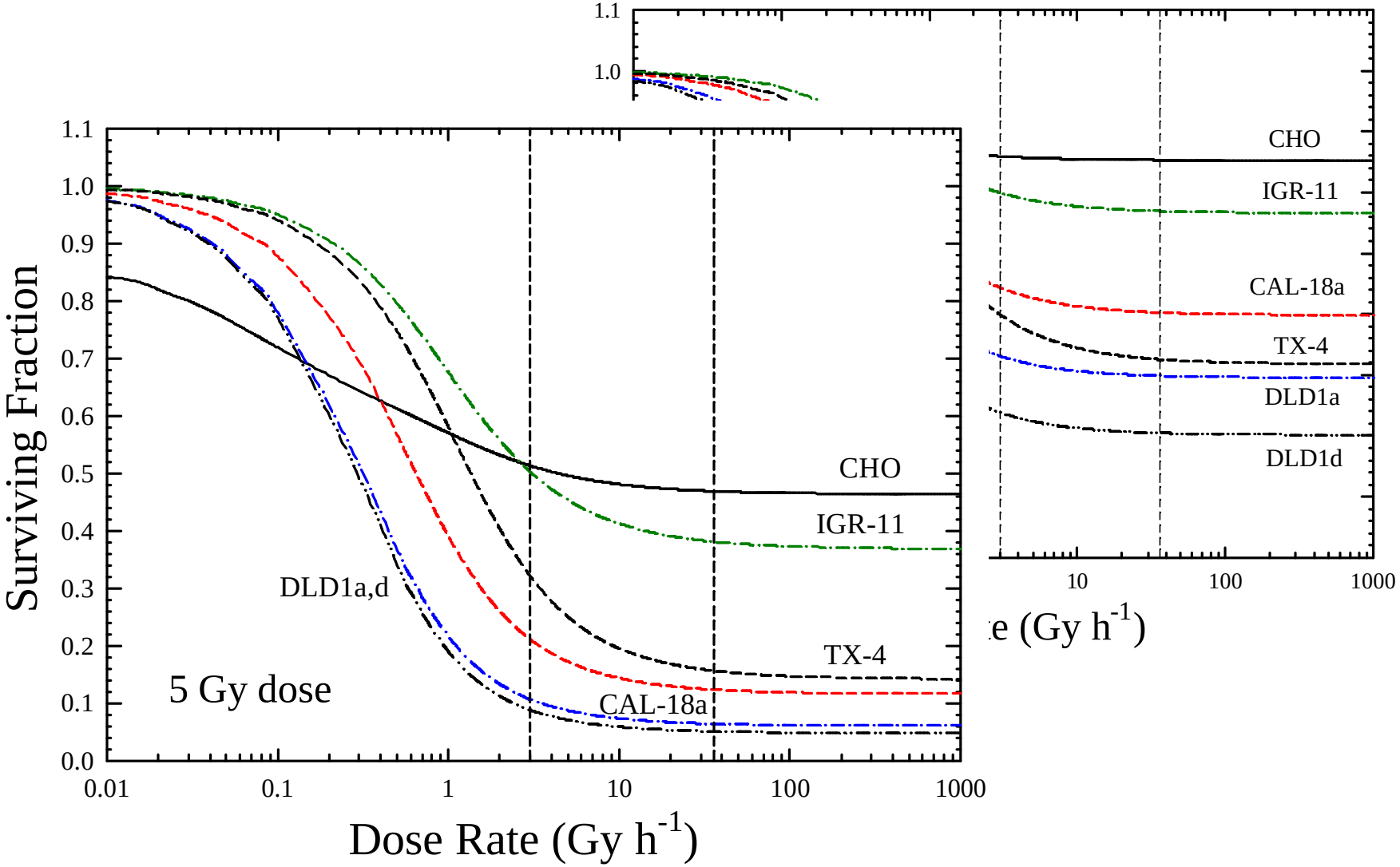
Radiosensitivity data derived from P.J. Deschavanne, B. Fertil, N. Chavaudra, E.P. Malaise. The relationship between radiosensitivity and repair of potentially lethal damage in human tumor cell lines with implications for radioresponsiveness. *Radiat. Res.* **122**(1):29-37 (1990).

Effective (average) dose rate

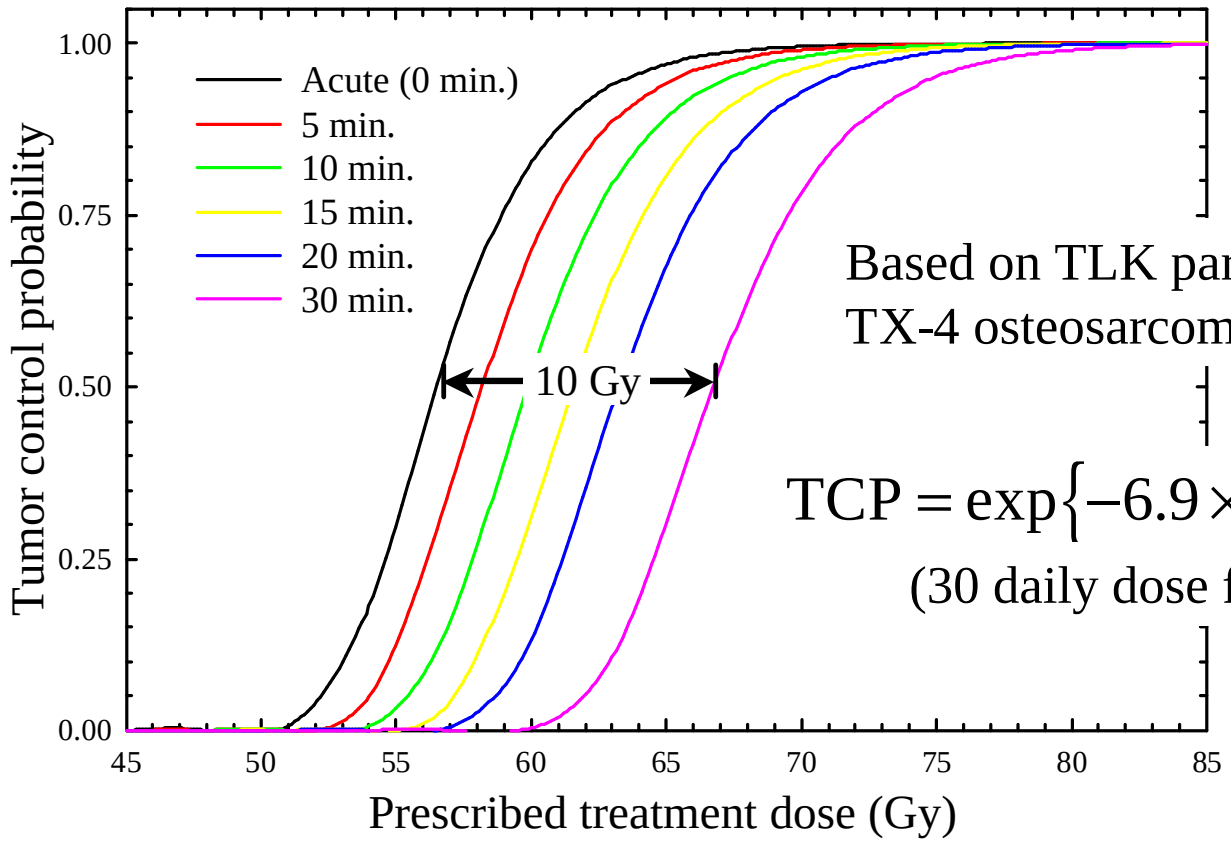
Dose fraction delivery time (min.)	Dose fraction size (Gy)				
	1.0	**1.5**	**2.0**	**2.5**	**3.0**
1	60.00	90.00	120.00	150.00	180.00
2	30.00	45.00	60.00	75.00	90.00
5	**12.00**	**18.00**	**24.00**	**30.00**	**36.00**
8	**8.00**	**12.00**	**16.00**	**20.00**	**24.00**
10	**6.00**	**9.00**	**12.00**	**15.00**	**18.00**
15	**4.00**	**6.00**	**8.00**	**10.00**	**12.00**
20	**3.00**	**4.50**	**6.00**	**7.50**	**9.00**
30	2.00	3.00	4.00	5.00	6.00
45	1.33	2.00	2.67	3.33	4.00
60	1.00	1.50	2.00	2.50	3.00

Effective dose rate (EDR) is the absorbed dose to a region of tissue divided by the total time to delivery the dose fraction.

Interplay Between Repair Capacity, Dose, and Dose Rate



Effect of Dose-Fraction Delivery Time



Based on TLK parameters for TX-4 osteosarcoma cells.

$$\mathrm{TCP} = \exp\left\{-6.9\times10^{7}\,S(D)^{30}\right\}$$

(30 daily dose fractions)

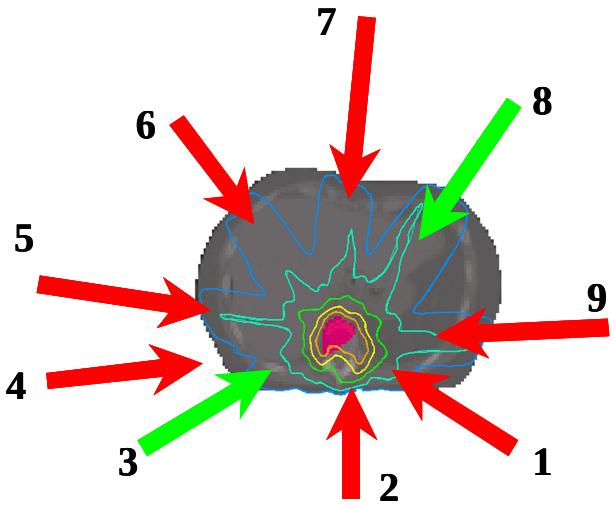
- Uniform cell density and radiosensitivity properties throughout the GTV.

Strategies to Exploit Damage Repair

a. Maximize tumor-cell killing by minimizing the dose delivery time.

b. Minimize normal tissue damage by increasing the dose delivery time.

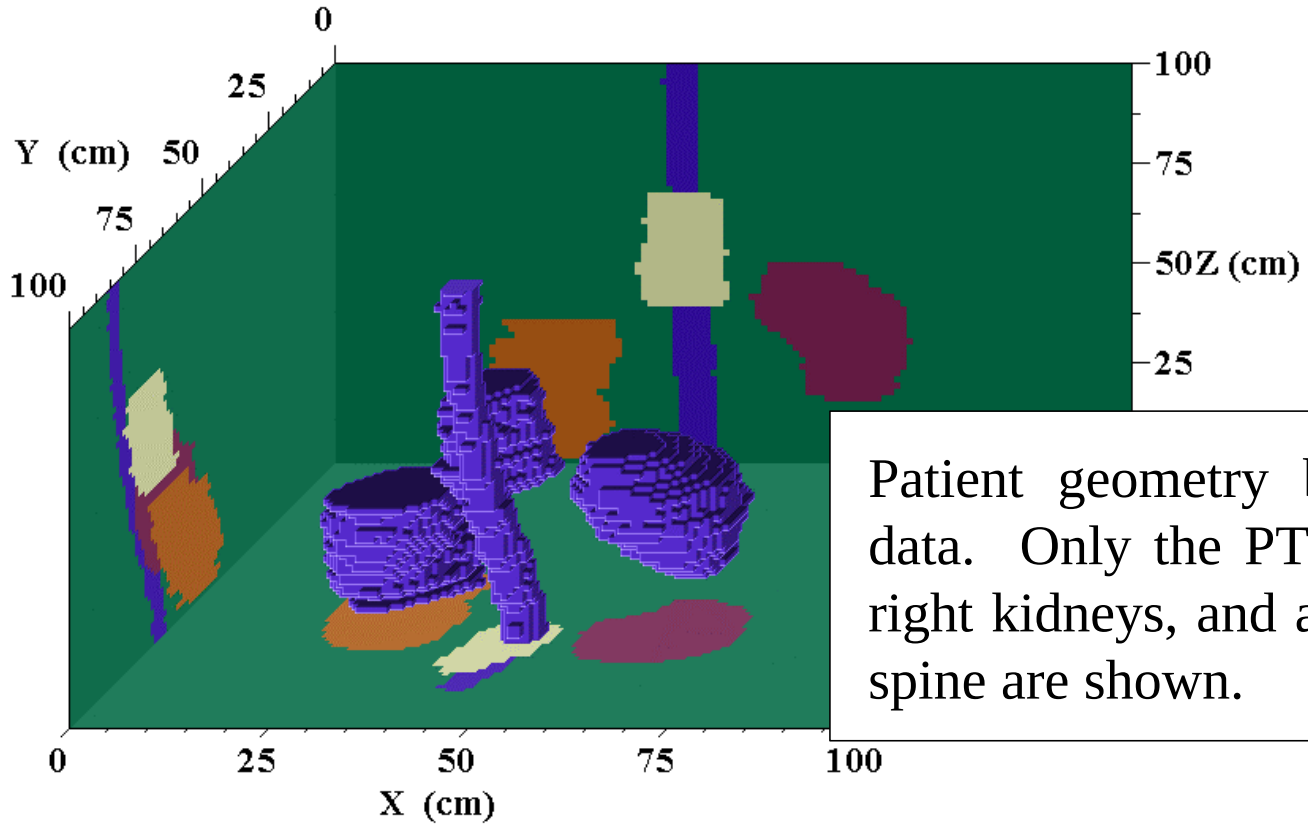
Need to increase or decrease of the dose delivery time by at least 5 or 10 minutes to have much impact on cell killing.



c. Use biological models to optimize the sequence, timing, and intensity of the beams.

- Paired-Beam Approach (PBA).

A Representative 3-D Tissue Construct



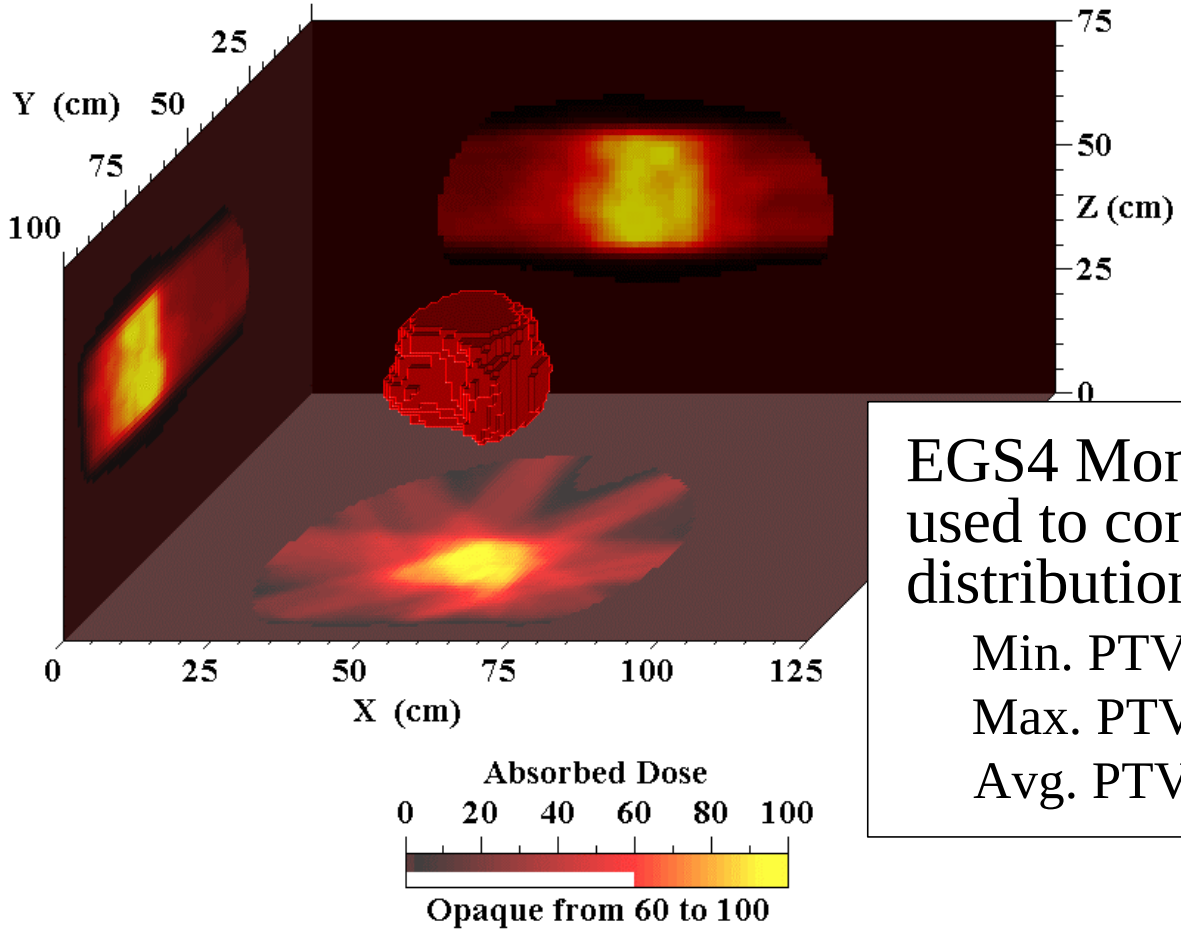
Patient geometry built from CT data. Only the PTV, the left and right kidneys, and a portion of the spine are shown.

- TX-4 parameters.
- Uniform cell density

Courtesy Dr. C.-M. Ma, Fox Chase Cancer Institute.

IMRT Dose Distribution

Nine 15 MV photon beams.

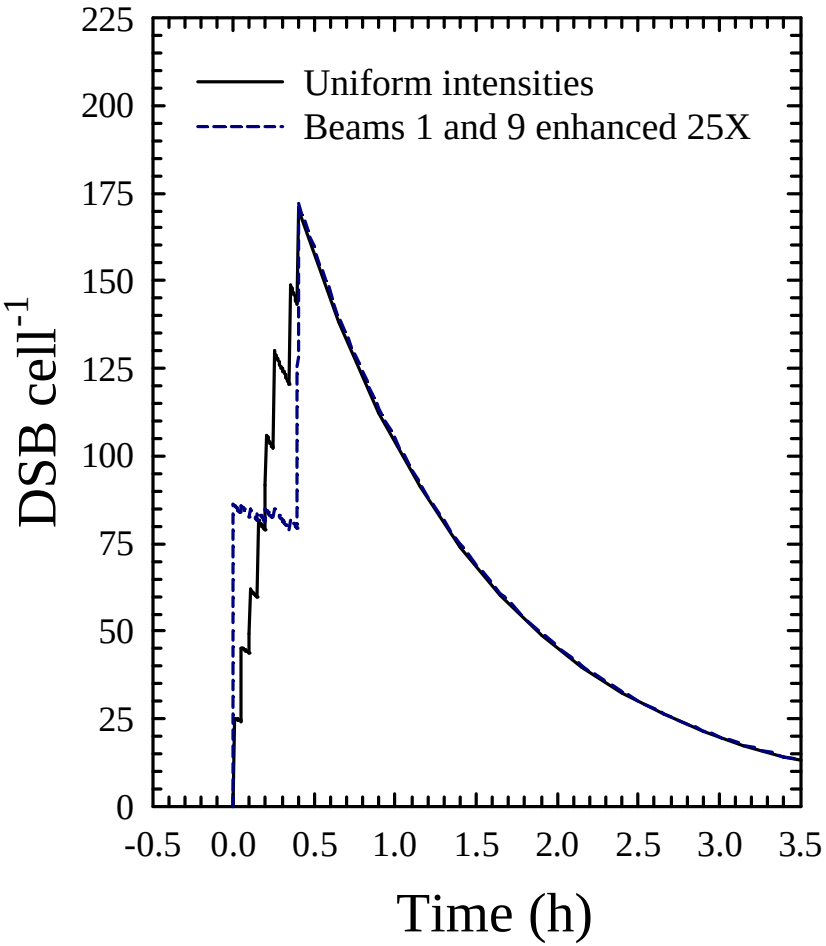


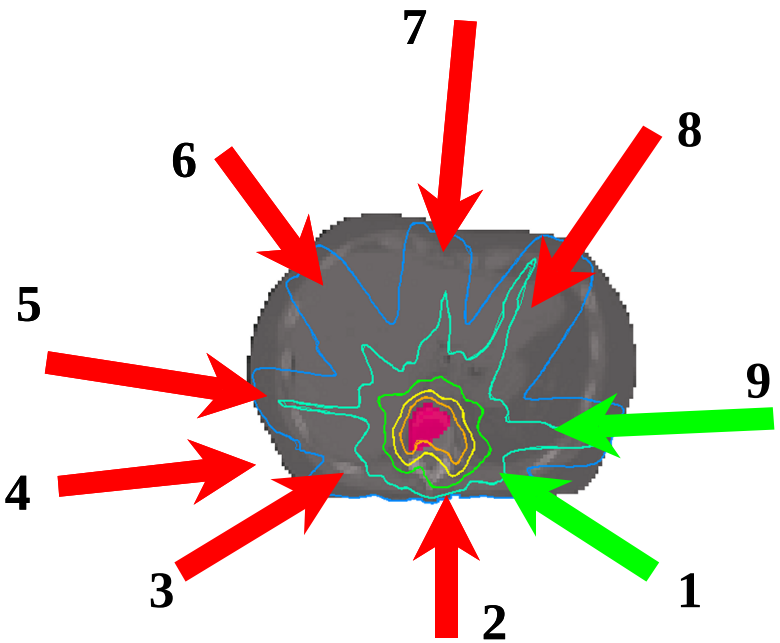
EGS4 Monte Carlo code was used to compute the dose distribution.

- Min. PTV dose: 1.01 Gy
- Max. PTV dose: 2.20 Gy
- Avg. PTV dose: 1.82 Gy

Dose distribution courtesy of Dr. C.-M. Ma, Fox Chase Cancer Institute.

DSB Formation and Repair at D_{max}





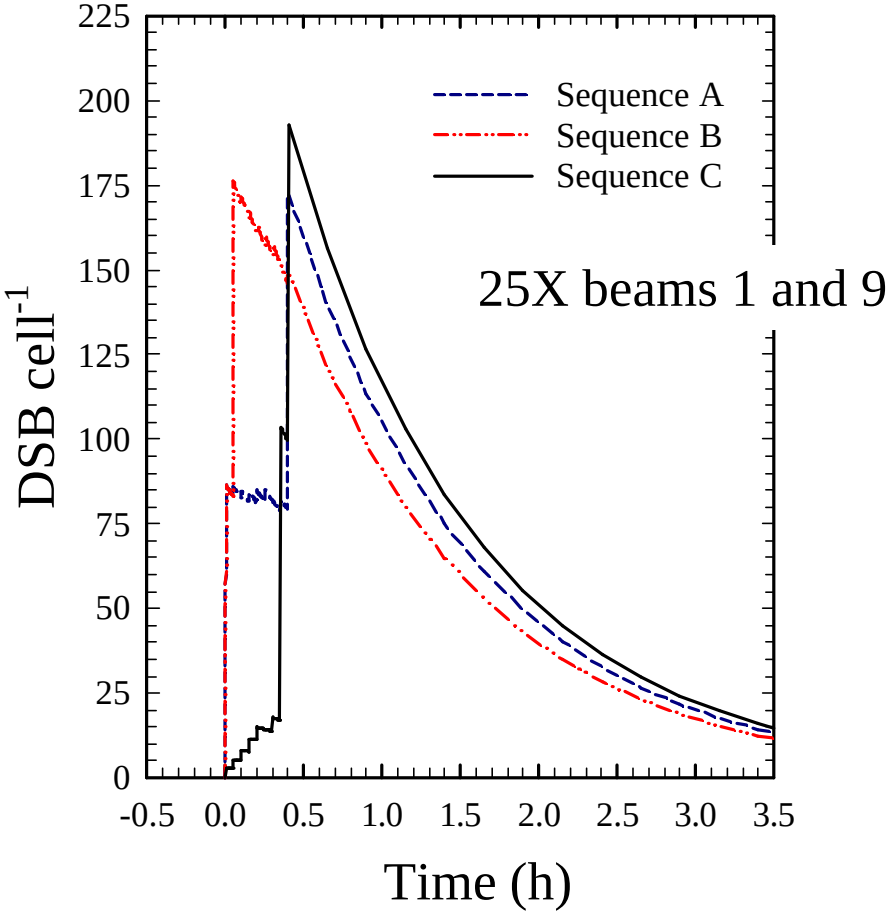
Sequence A (**1,** 2, 3, 4, 5, 6, 7, 8**, 9**)

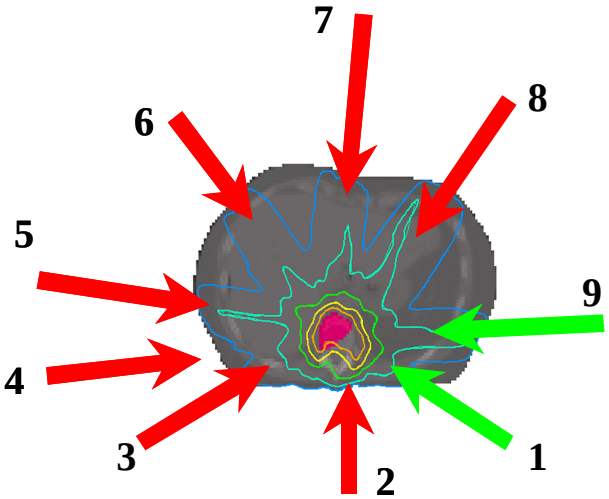
SF_{uni}: 0.185
SF_{25x}: 0.185

5 Gy dose fraction delivered in 24 minutes.

DSB Formation and Repair at D_{max}

5 Gy dose fraction delivered in 24 minutes.





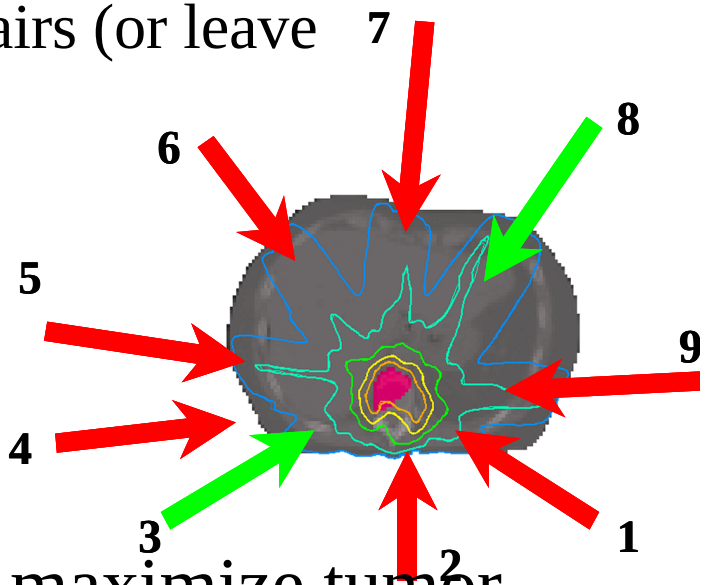
- Sequence A. (**1,** 2, 3, 4, 5, 6, 7, 8**, 9**)
- Sequence B. (**1, 9,** 2, 3, 4, 5, 6, 7, 8)
- Sequence C: (2, 3, 4, 5, 6, 7, 8, **1, 9**)

SF_A: 0.185
SF_B: 0.206
SF_C: 0.153

21% difference in cell killing

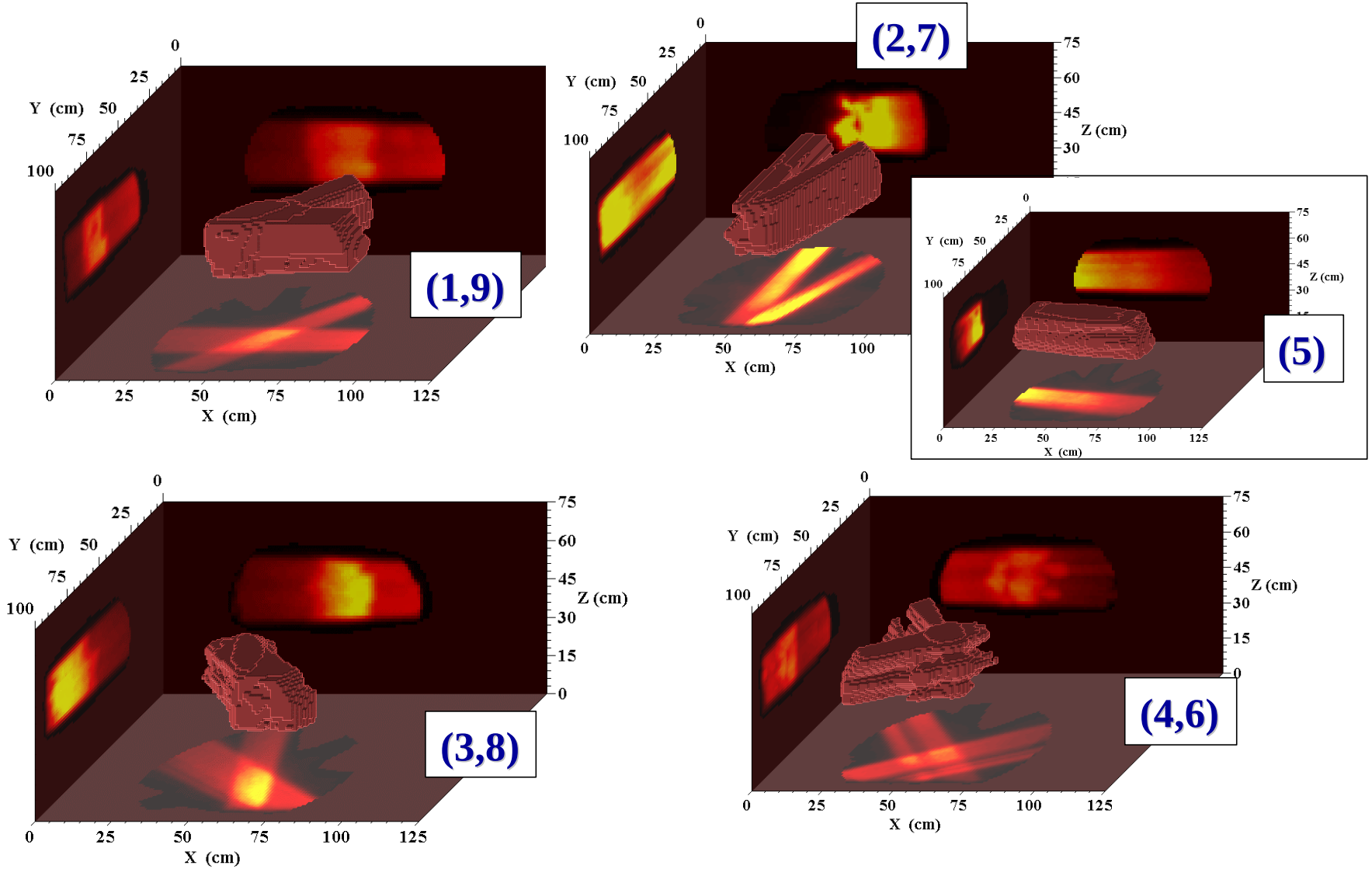
Paired-Beam Approach (PBA)

- On each treatment day, select a set of beam pairs that preferentially target some region of the tumor.
  - (1,9), (2,7), (3,8), (4,6), (5). Increase or decrease the intensity of the selected beam pairs (or leave unchanged).
  - Cumulative dose for a complete cycle is the same as the "standard" IMRT dose distribution.



- Adjust the sequence of beams to maximize tumor-cell killing (and/or minimize normal tissue damage.

Beam-Pair Dose Distributions

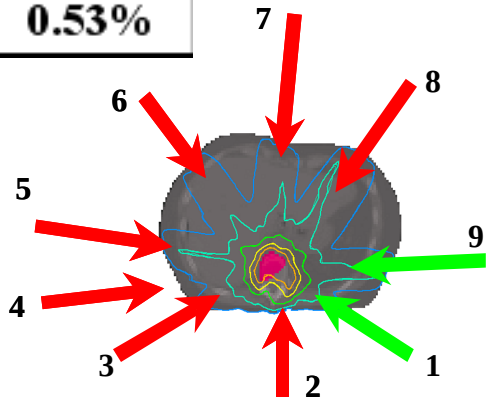


Unaltered Beam Intensities (2 Gy fraction)

Surviving fraction of tumor

	Beam Pair				
Sequence	**1 and 9**	**2 and 7**	**3 and 8**	**4 and 6**	**5**
A	0.6015	0.6015	**0.6015**	**0.6015**	0.6015
B	**0.6049**	**0.5947**	**0.6045**	**0.6026**	**0.6035**
C	**0.5993**	**0.6071**	0.6023	0.6011	**0.6003**
Min.	0.5993	0.5947	0.6015	0.6011	0.6003
Max.	0.6049	0.6071	0.6045	0.6026	0.6035
(Max-Min)/Min	**0.93%**	**2.09%**	**0.50%**	**0.25%**	**0.53%**

- Sequence A. (**1,** 2, 3, 4, 5, 6, 7, 8**, 9**)
- Sequence B. (**1, 9,** 2, 3, 4, 5, 6, 7, 8)
- Sequence C: (2, 3, 4, 5, 6, 7, 8, **1, 9**)

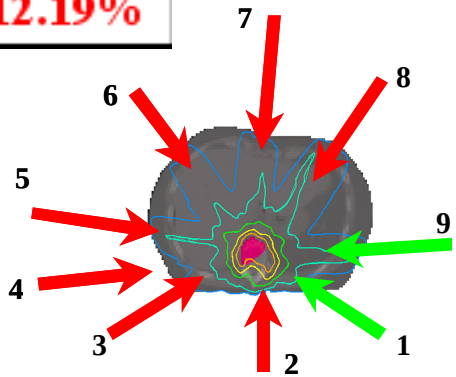


25X Enhanced Beam Intensities (2 Gy fraction)

Surviving fraction of tumor

	Beam Pair				
Sequence	**1 and 9**	**2 and 7**	**3 and 8**	**4 and 6**	**5**
A	0.5846	**0.6524**	0.5845	0.6176	0.5920
B	**0.6178**	**0.6604**	**0.6235**	**0.6486**	**0.6251**
C	**0.5501**	0.6167	**0.5609**	**0.5858**	**0.5572**
Min.	0.5501	0.6167	0.5609	0.5858	0.5572
Max.	0.6178	0.6604	0.6235	0.6486	0.6251
(Max-Min)/Min	**12.31%**	**7.09%**	**11.16%**	**10.72%**	**12.19%**

- Sequence A. (**1,** 2, 3, 4, 5, 6, 7, 8**, 9**)
- Sequence B. (**1, 9,** 2, 3, 4, 5, 6, 7, 8)
- Sequence C: (2, 3, 4, 5, 6, 7, 8, **1, 9**)



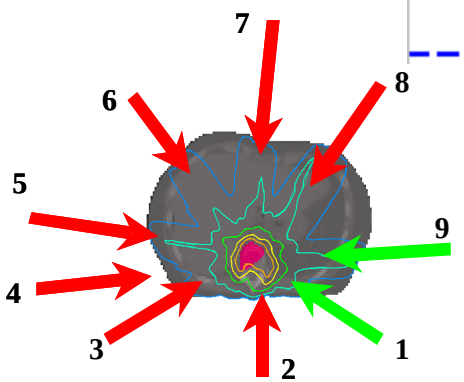
Comparison of Paired-Beam Strategies

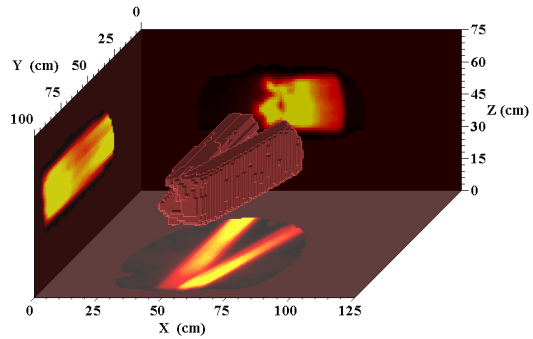
Uniform

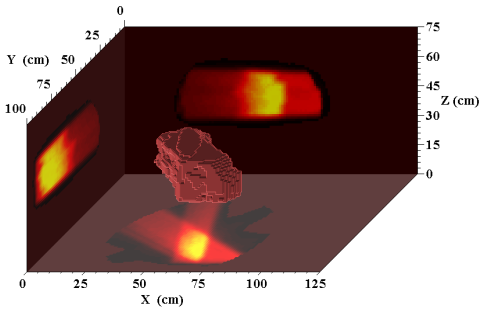
	Beam Pair				
Sequence	1 and 9	2 and 7	3 and 8	4 and 6	5
A	0.6015	0.6015	0.6015	0.6015	0.6015
B	0.6049	0.5947	0.6045	0.6026	0.6035
C	0.5993	0.6071	0.6023	0.6011	0.6003

25X Enhanced

	Beam Pair				
Sequence	1 and 9	2 and 7	3 and 8	4 and 6	5
A	0.5846	0.6524	0.5845	0.6176	0.5920
B	0.6178	0.6604	0.6235	0.6486	0.6251
C	0.5501	0.6167	0.5609	0.5858	0.5572







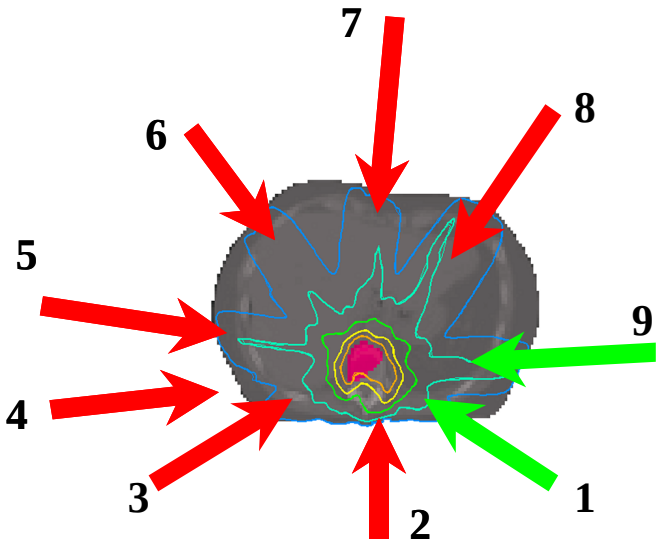
Cautionary Notes and Questions

- Dose to the skin may place limits on how much relative beam intensities can be increased or decreased.
- The biologically optimal beam intensities and sequences may depend on radiosensitivity factors.
  - What about tumor hypoxia or other factors that produce differential cell-killing effects in different regions of the tumor?

Alternatives and Refinements

- Approach could be improved by
  - Searching through all possible beam-pair configurations and by treating beam intensities as adjustable parameters (2X, 5X, …).
  - Designing beams to target specific portions of the PTV.
  - Use a "tri" or "quad" beam approach instead of the PBA.

- Deliver each daily dose fraction using a subset of the beams.
- Multiple daily fractions using different beam subsets.
- Could try non-uniform dose distributions.



Conclusions

- For some dose-fraction sizes and delivery times, damage repair phenomena impact tumor-cell killing by as much as 10 to 30% per fraction.
  - Reducing dose-fraction delivery times from 20 minutes to 5 minutes could shift the TCP curve to lower doses by as much 10%.
  - Dynamic IMRT has an edge over step-and-shoot IMRT in this respect.
- Dose rate effects may be a source of inter-patient variability in treatment effectiveness.

Speculation

- The effectiveness of step-and-shoot IMRT methods could be improved by selective targeting of different regions of the PTV and/or by reducing the effective dose rate experienced by normal tissues.

Acknowledgements

- Dr. C.-M. Ma, Fox Chase Cancer Institute.
- Drs. M. Bose, M. Guerrero, A. Li, W.F. Morgan, S. Naqvi, and L. Huang. University of Maryland Medical School.

Pacific Northwest National Laboratory operated by Battelle for the US Department of Energy under contract DE-AC06-76RLO 1830. Some aspects of this research are supported by a grant from the U.S. Department of Energy (DOE) Office of Biological and Environmental Research (OBER) to Dr. R.D. Stewart.

Additional Information

- Biological Effects of Radiation and Chemicals
  - http://www.pnl.gov/berc/
- Virtual Cell Radiobiology Software
  - http://www.pnl.gov/berc/kbem/vc/
- Rob Stewart
  - http://www.pnl.gov/berc/staff/rds.html

DSBs ↔ Fractional Activity Released (FAR)

- Random breakage model is used to convert unrepaired DSBs into an estimate of the fractional activity released out of well (FAR).

$$\mathrm{FAR} = F_{max}\left\{1-\left[1+K\bar{N}_{dsb}\left(1-\frac{K}{M_0}\right)\right]\exp^{-K\bar{N}_{dsb}}\right\}$$

F_{max} = maximum fraction of the cellular DNA that can enter the gel.
M_0 = average chromosome size (base pair).
K = gel exclusion size (DNA fragments larger than K do not move out of the well).

$$\bar{N}_{dsb} = \bar{L}_{dsb}(t)/Y \text{ or } [\bar{L}_1(t)+\bar{L}_2(t)]/Y$$

Belli M, Cherubini R, Dalla Vecchia M, Dini V, Moschini G, Signoretti C, Simone G, Tabocchini MA, Tiveron P. DNA DSB induction and rejoining in V79 cells irradiated with light ions: a constant field gel electrophoresis study. *Int. J. Radiat. Biol.* **76**(8):1095-104 (2000).

Tumor Control Probability (TCP)

Probability the tumor is eradicated can be expressed as

$$\mathrm{TCP} = \exp\left\{-\sum_{i=1}^{Q} N_i S_i(D)\right\}$$

$Q =$ Number of voxels.

$N_i =$ Initial number of tumor cells in the *i*th voxel.

$S_i(D) =$ Surving fraction of tumor cells in the *i*th voxel after dose *D*.