Uncertainty and Issues in Biological Modeling for the Modern Medical Physicist

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Introduction

- Fundamental tumor/cell/cancer biology is becoming a more integral part of medical physics.
  - **More widespread soft tissue / functional imaging**
  - **Broader use of biological models in treatment planning.**
  - **Increased push for evidence based decision making.**

- Physics and biology groups are segregated.
- Physicists need to better understand biology studies and their sources of uncertainty.

Biological Models in RT

The use and QA of biologically related models for treatment planning: Short report of the TG-166 of the therapy physics committee of the AAPMa)
X. Allen Li, Markus Alber, Joseph O. Deasy, Andrew Jackson, Kyung-Wook Ken Jee, Lawrence B. Marks, Mary K. Martel, Charles Mayo, Vitali Moiseenko, Alan E. Nahum, Andrzej Niemierko, Vladimir A. Semenenko, and Ellen D. Yorke

Citation: Medical Physics **39**, 1386 (2012); doi: 10.1118/1.3685447

- Example: Use of radiobiological models in RT
- To properly design studies and assess the data and uncertainty need to understand:
  - **What is actually being modeled, under what conditions, and how this can affect the results.**
  - **Where the uncertainty lies in each step.**
  - **How to properly interpret and analyze the data.**

Example: (Simple) LQ Model

- "Simple" linear quadratic (LQ) model:

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

- S = surviving fraction of cells for a single fraction of radiation (D)
- LQ model forms the basis for BED, EQD, some TCP / NTCP models among others.
- S does not apply to patient, animal, or tissue survival – only cells.
- If your assay/experiment does not directly measure cell survival, it is either not applicable or there is additional uncertainty.

α, β, and α/β

- LQ model cell kill believed to be related to double strand DNA breaks (DSBs)
- α → Cell's sensitivity to lethal or irreparable DSBs
- β → Cell's sensitivity to potentially lethal or repairable DSBs.
- α/β → Describes how well a cell can repair damage.
- Low α/β (~3) = late responding tissue, high α/β (~10) = early responding tissue.

The LQ model would most directly describe the number of:

20% 1. Rats developing skin lesions following different levels of radiation
20% 2. Patients developing a complication after external beam radiation
20% 3. Cancer cells surviving irradiation with different dose levels
20% 4. Recurrences following patient radiation treatment for GBM

10

The LQ model would most directly describe the number of:

3. Cancer cells surviving irradiation with different dose levels

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

Source: Hall EJ and Giacca AJ. Radiobiology for the radiologist. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2011.

- LQ model: S is the surviving fraction of cells
- Does not apply to different outcomes.

Clonogenic Survival Assay

Plate and irradiate cells

Incubate for several cell cycles

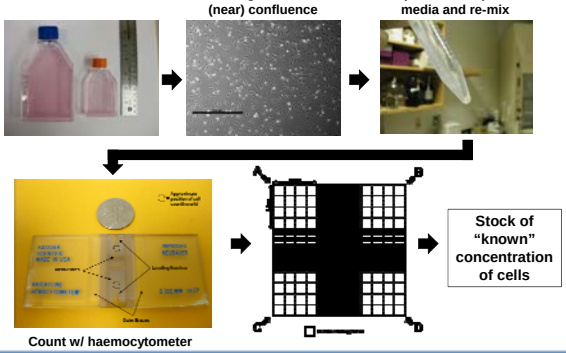
Wash and stain plates

Count Colonies

- "Gold standard" of cell survival assays in radiobiology.
- Direct measure of cell survival.
- "Survival" usually defined as cells which can survive *x* divisions (7 is typical)
- Can take 1-2 weeks to run.

Determining cell numbers

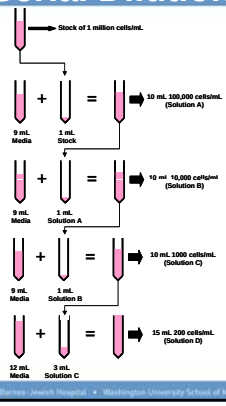
Fill container / media → Plate & grow cells to (near) confluence → Spin down, replace media and re-mix



Count w/ haemocytometer

Stock of "known" concentration of cells

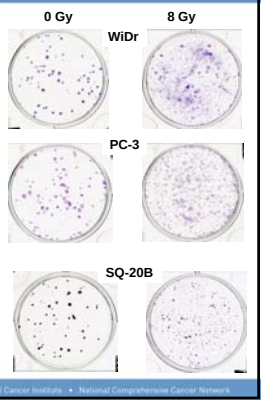
Serial Dilutions



- Start with "known" concentration.
- "Serial" dilutions: portions of subsequent stocks are taken to create desired new concentrations.
- Use these diluted concentrations to plate "known" numbers of cells.
- Each subsequent stock is an estimate of a previous estimate.
- Good mixing and experimental technique is imperative to minimize error.

Counting Colonies

- Grow for 10-14 days after treatment.
- Always create non-irradiated controls.
- Stain cells with crystal violet or some other similar agent.
- Counts done by first finding and denoting smallest colony w/ appropriate # of cells (~50, for example) under microscope.
- Manually count all dots on a plate which are that size or larger.



Inter-observer error

	Observer	A	B	C	D	B+Micro	A	B	C	D
		Counts					% Difference in count vs B+Micro			
WiDr	**6 Plate Average:**									
	0 Gy	64.7	58.7	69.2	55.2	66	-2.00%	-11.10%	4.80%	-16.40%
	6 Gy	97.8	93.8	110.3	120.5	149.8	-34.70%	-37.40%	-26.40%	-19.60%
	8 Gy	86.7	73.5	152.3	131.2	125.2	-30.80%	-41.30%	21.70%	4.80%
PC-3	**6 Plate Average:**									
	0 Gy	72.8	67.7	76.3	75	73.7	-1.10%	-8.10%	3.60%	1.80%
	6 Gy	84.5	74.8	111	100	86. 3	-2.10%	-13.30%	28.60%	16.00%
	8 Gy	61.8	57.5	50	46.2	57	8.50%	0.90%	-12.30%	-19.00%
SQ-20B	**6 Plate Average:**									
	0 Gy	64.7	60	58.5	57.2	62. 7	3.20%	-4.30%	-6.60%	-8.80%
	6 Gy	70.3	72.8	74.5	50.5	65. 3	7.70%	11.50%	14.00%	-22.70%
	8 Gy	93.8	92.7	96.7	102	107. 3	-12.60%	-13.70%	-9.90%	-5.10%

- Cell appearances, colony size vary by cell line, dose.
- Human factors → differences in the counting numbers achieved by observers.
- Do not mix results from multiple observers without further analysis.

Intra-observer error

- Inexperienced Observer, 8 Gy WiDr Plate (the "hardest") of the one on hand – most variability between observers

		Count 1st	2nd	3rd	Plate Average	%Standard Error
Plate Number	1	146	168	164	159.3	4.25%
	2	184	170	136	163.3	8.73%
	3	163	211	192	188.7	7.40%
	4	157	138	120	138.3	7.72%
	5	196	169	163	176	5.77%
	6	179	192	180	183.7	2.27%
Averages		170.8	174.7	159.2	168.2	6.02%

- Human factors also give rise to intra-observer error.
- With a single observer, all of the above errors fall into the "rule-of-thumb" of around 10% variation in the end point of the assay.

Statistics of Survival

- PE is the plating efficiency, the experimental control which describes how many cells die with no dose or action applied.

WiDr		# cells counted		
Gy	# Cells Plated	A	B	C
0	100	69	65	71
0.5	100	63	63	64
1	100	57	60	56
2	200	78	95	77
4	500	95	76	83
8	5000	54	51	45

$$PE = S(D=0) = \frac{1}{3}\cdot\sum\frac{69}{100}+\frac{65}{100}+\frac{71}{100} = 0.683$$

- Average surviving fraction is the ratio of the number of cells counted (t) to the "known" number plated (n), accounting for the PE:

$$S(D=2\ Gy) = 0.683\cdot\frac{1}{3}\cdot\sum\frac{78}{200}+\frac{95}{200}+\frac{77}{200} = 0.284$$

- Standard error propagation techniques could then be used to characterize the uncertainty in PE and cell survival:

$$\frac{\sigma_{PE}}{PE} = \frac{\sigma(\bar{t}(D=0))}{\bar{n}(D=0)} \qquad \frac{\sigma_{\bar{t}(D)}}{\bar{t}(D)} = \sqrt{\left(-\frac{1}{PE^2\cdot\bar{n}(D)}\sigma(PE)\right)^2+\left(\frac{1}{PE\cdot\bar{n}(D)}\sigma(\bar{t}(D))\right)^2}$$

A Different Approach

- Gupta et al. Radiat Res. 1996 show a potential way to statistically account for the uncertainty the survival data.
- Can use a binomial distribution basis or Poisson statistics for calcuations →
  - Use of Poisson considers n (plating number) has uncertainty
- Plate different n values to characterize error in PE. (c = control, t = irradiated).

$$\hat{PE}_c = \frac{t_c}{n_c} \qquad \hat{PE}_t = \frac{t_t}{n_t} \qquad \hat{SF} = \frac{\hat{PE}_t}{\hat{PE}_c}$$

- Fieller's theorem allows for confidence intervals from ratios of two means:

$$\left(\hat{SF}+\left(\frac{g}{1-g}\right)\cdot\hat{SF}\right)\pm\left(\frac{z}{\hat{PE}_c(1-g)}\right)\cdot\sqrt{\hat{SF}^2V_c+V_t(1-g)} \qquad z=\left(\frac{Z}{\hat{PE}_c}\right)^2V_c$$

Binomial: $V_{t,t} = \frac{PE_{t,t}(1-PE_{t,t})}{n_{t,t}}$

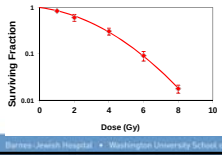
Poisson: $V_{t,t} = \frac{PE_{t,t}}{n_{t,t}}$

Determining Parameters

- Results give you errors at each survival point.
- Fit data to the model to get parameters.
- Ex: Solve LQ model to make it a polynomial: $-\ln(S) = \alpha D + \beta D^2$

WiDr		# colonies		
Gy	# Cells Plated	A	B	C
0	100	69	65	71
0.5	100	63	63	64
1	100	57	60	56
2	200	78	95	77
4	500	95	76	83
8	5000	54	51	45

	β	α	α/β
	0.046	0.158	3.40
Std Err	0.001	0.008	0.20
R^2	0.9999		



- Linear regression analysis or other similar tools can fit the survival data.
- Generate parameters (α, β) along with standard errors from the fit.

Other Issues

- Multiple (≥ 3) plates for each condition are recommended → biological variation
- Repeat experiments multiple (≥ 3) times → similar results show an effect is real.
- Probabilistic nature of radiation induced cell death + human factors in counting → plates with low colony counts (below 30-50) could skew results.
- "Calibrate" the plating number to yield appropriate numbers of colonies at each dose level.
- Plating too many cells can result in minimal or no survivors.

Dilutions in the clonogenic assay introduce what type of uncertainty?

20% 1. Estimation in numbers of cells plated.
20% 2. Uncertainty in dose delivered
20% 3. Yield inter-observer counting errors
20% 4. Increase the number of plates needed
20% 5. Do not add any uncertainty

Dilutions in the clonogenic assay introduce what type of uncertainty?

1. Estimation in numbers of cells plated.

Source: Gupta N, Lamborn K, Deen DF. "A Statistical Approach for Analyzing Clonogenic Surival Data." *Radiat Res*. 145 636-640 (1996)

- Stir then draw a certain amount of liquid with a "known" concentration of cells.
- Don't truly know how many cells you have plated.
- Each dilution → introduce additional uncertainty.

External Estimates

- Some will use model parameters acquired from literature or "generalized" estimates.
- Experimentally determined parameters can vary by cells , technique, equipment used, experiment performer, etc.
- Example generalized estimates: α/β = 3 for normal tissue, =10 for tumors.
- Use either technique with caution.
- Survey the literature, and attempt to account for the error or uncertainty involved in such assumptions.

Parameter Estimation Issues

- Parameters for the same line or tissue can vary widely in the literature.
- Example: SQ-20B (human head-and-neck cancer cells).

SQ-20B Parameters

Source	Energy	Technique	α	β	α/β
Beuve *et al.*, IJROBP (2008)	10 MV	Monolayer	0.058	0.047	1.2
"	250 kVp	Monolayer	0.11	0.037	3.0
Belli *et al.*, J. Rad Res (2008)	^{60}Co / ^{137}Cs	Monolayer	0.16	0.012	13.3
Dahlberg *et al.* Can Res (1999)	160 kVp	Monolayer	0.252	0.023	11.0
Altman *et al.* IJROBP (2009)	6 MV	Monolayer	0.14	0.016	8.7

- Besides experimental variations, cells can also mutate between stocks in different places.

Parameter Estimation Issues

- Values can differ between cell line/types and from individual to individual, or from outcome to outcome:

Estimates of LQ model parameters.

	K	10 × alpha (Gy^{-1})	100 × Beta (Gy^{-2})	Alpha/beta (Gy)
Weight loss > 15%, X-rays	357.8 [149.9,845.6]	1.13 [0.95,1.31]	0.57 [0.44,0.69]	19.9 [15.2,27.0]
Lethality before 2 months X-rays	61.5 [34.1,110.0]	0.50 [0.40,0.60]	0.37 [0.29,0.46]	13.4 [9.3,19.5]
Lethality after 2 Months X-rays	24.0 [13.2,44.3]	0.41 [0.29,0.54]	0.60 [0.45,0.75]	6.9 [4.2,10.8]
Short feces > 25%, X-rays	7.6 [4.4,13.1]	0.11 [- 0.02,0.24]	0.77 [0.54,1.00]	1.4 [- 5.4,4.5]

The estimates [95% confidence interval] for alpha and beta were multiplied by 10 and 100 for legibility purpose, respectively.
[N.A.] = program failed to calculate confidence limits.

Excerpted from: Gasinska A, *et al.* Early and late injuries in mouse rectum after fractionated X-ray and neutron irradiation. *Radiother Oncol* 1993;26:244-253.

Summary

- Biological experiments and data are an increasingly important part of medical physicists professional lives.
- Biological experiments >> variability vs. physics-based ones.
- For model based studies, know what is being modeled, under what conditions, and the uncertainties in each step.
- Standard error analysis may be applicable, but effort must be made to characterize the errors of all the elements used.

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