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THE LINEAR-QUADRATIC LOG-SURVIVAL RADIATION DOSE RESPONSE MODEL: CONFIDENCE ELLIPSES, DRUG-DRUG INTERACTIONS, AND BRACHYTHERAPEUTIC GAINS

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RESEARCH ARTICLE

ABSTRACT. A METHOD FOR DETECTING drug-drug interactions with respect to a linear-quadratic (LQ) log-survival radiation dose response curve has been proposed by Lindstrom et al. [Radiation Research 135: 269, 1993]. In this method, clonogenic survival data are first converted into estimates of the LQ cell killing parameters α and β, and two drugs are then defined as interacting if they cause significant deviations from an *a priori* expectation of additive effects in the parameter space. At least for α and β individually, this expectation leads naturally to definitions of synergy and antagonism. Our goal, however, is therapeutic gain. This can be achieved either through antagonism that is greater in normal tissue than in malignant tissue, or through synergism that is greater in malignant tissue than in normal tissue. Using clonogenic survival data in which mismatch repair deficient HCT116 cells serve as a model of malignant tissue and mismatch repair competent HCT116 3-6 cells serve as a model of the adjacent normal tissue, we compute expected brachytherapeutic gains (defined as either the ratio or difference of malignant and normal LQ α's) for various pretreatment scenarios. We suggest that the difference in α is preferable over the ratio as a metric of brachytherapeutic gain.

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1. INTRODUCTION

Lindstrom et al. [1] proposed that the impact of drugs on radiation-induced clonogenic cell death be quantified by their impact on the α and β parameters in the following linear-quadratic (LQ) log-survival radiation dose-response relationship,

$$S = ce^{-(\alpha D+\beta D^2)} \qquad \text{[Eq. 1]}$$

where S is the surviving fraction, c is the plating efficiency, and D is the dose in GRAY. Lindstrom et al. [1] further proposed that two drugs can be defined as "interacting" if they cause significant deviations from an *a priori* expectation of additive effects in the parameter space (α, β). Thus, if a drug combination causes an increase in α or β greater (or less) than that expected based on the sum of "observed" increases for each drug separately, the drugs are defined as synergistic (or antagonistic) for low (α) or high (β) dose rate therapy, respectively. Our interest, however, is not in synergy and antagonism per se, but rather therapeutic gain. If a drug pretreatment is found to be more antagonistic in normal tissue than in malignant tissue, or more synergistic in malignant tissue than in normal tissue, a therapeutic gain is expected [2,3]. To estimate expected therapeutic gains, two sets of experiments must therefore be performed in parallel, one in a model system of the malignant tissue, the other in a model system of the adjacent normal tissue. Taverna et al. [4] describe such a dataset. In their experiments, mismatch repair deficient HCT116 cells serve as a model of malignant tissue, and mismatch repair competent HCT116 3-6 cells serve as a model of the adjacent normal tissue. Using that dataset, we examine here the expected brachytherapeutic gain, defined as either the ratio or difference of malignant-to-normal LQ α's, i.e., defined as either α_m/α_n or α_m-α_n.

2. MATERIALS AND METHODS

2.1. DATA SET

The clonogenic survival data of Taverna et al. [4] used in this study is comprised of irradiated HCT116 cells and HCT116 3-6 cells pretreated with nothing (hereafter referred to as controls), methoxyamine (MX), iodinated deoxyuridine (IU), or a combination of MX and IU. This dataset is shown in TABLE 1. The drug pretreatment doses were 2.5 μM and 6 mM for IU and MX, respectively, and the radiation doses D were 0, 1, 2.5, and 5 GRAY.

2.2. MODEL

The linear-quadratic log-survival low-LET dose response model (Eq. 1) was fitted to the data in TABLE 1 as a binomially distributed generalized linear model [5] with the logarithmic link, i.e.,

$$\log_e(S) = \log(c) + \alpha(-D) + \beta(-D^2) \qquad \text{[Eq. 2]}$$

where $S = y/N$ and y is the number of colonies and N is the number of cells plated. Estimates of the parameter triplet [$\log_e(c)$, α, β] were obtained using Matlab's glmfit routine — zero entries in TABLE 1 deterred us from using standard linear regression. Parameter estimate confidence intervals (CIs) shown in TABLES 2 and 3 are Wald CI, computed as $b_i \pm 1.96*stdev_i$ where b_i and $stdev_i$ are the means and standard deviations of the i-th parameter, i = 1, 2 or 3 corresponding respectively to $\log_e(c)$, α, and β. The CI of c is obtained by exponentiating the CI of $\log_e(c)$.

2.3. CONFIDENCE ELLIPSES

FIG. 1 shows joint confidence ellipses of α and β obtained as contour plots of

$$(b - E(b))\Sigma^{-1}(b-E(b)) = 2F_{2,n-3}(0.95) \qquad \text{[Eq. 3]}$$

where b is now the two parameter vector (α, β) [shown as the locus of points on the ellipse], $E(b)$ is the maximum likelihood estimate of b [shown as the center of the ellipse], Σ^{-1} is the inverse of the lower-right 2 x 2 block of the estimated 3 x 3 (including $\log_e(c)$) parameter covariance matrix and n = 24, 33, 24 and 33 for control, IU-pretreated, MX-pretreated, and MX + IU-pretreated cells, respec-

TABLE 1. RAW CLONOGENIC SURVIVAL DATA

Pretreatment	Dose of IR	No. of Cells/Plate	HCT116 Colonies	HCT3-6 Colonies	Pretreatment	Dose of IR	No. of Cells/Plate	HCT116 Colonies	HCT3-6 Colonies
Controls	0	250	190	183	MX 6 mM	0	250	175	149
	0	250	171	170		0	250	170	160
	0	250	180	168		0	250	168	148
	0	250	193	180		0	250	158	171
	0	250	205	170		0	250	170	171
	0	250	204	180		0	250	160	185
	1	250	176	143		1	250	183	161
	1	250	172	168		1	250	171	163
	1	250	165	150		1	250	165	167
	1	250	168	156		1	250	136	162
	1	250	182	162		1	250	145	132
	1	250	159	158		1	250	150	
	2.5	500	234	243		2.5	500	236	244
	2.5	500	243	238		2.5	500	231	221
	2.5	500	235	247		2.5	500	218	
	2.5	500	190	270		2.5	500	183	198
	2.5	500	210	280		2.5	500	194	210
	2.5	500	202			2.5	500	187	205
	5	1000	217	273		5	1000	138	143
	5	1000	218	265		5	1000	141	153
	5	1000	210	254		5	1000	150	
	5	1000	183	212		5	1000	124	211
	5	1000	202	204		5	1000	133	197
	5	1000		205		5	1000	134	194
IUDR 2.5 µM	0	250	107	126	MX 6 mM	0	1000	69	76
	0	250	105	136	+	0	1000	62	75
	0	250	102	129	IU 2.5 µm	0	1000	62	70
	0	250	82	91		0	500	23	37
	0	250	76	100		0	500	20	32
	0	250	90	101		0	500	23	33
	1	250	53	83		1	750	7	25
	1	250	59	79		1	750	10	28
	1	250	62	81		1	750	10	24
	1	250	45	74		1	1000	18	35
	1	250	47	76		1	1000	24	28
	1	250	47	82		1	1000	23	36
	1	250	52	77		1	1000	16	34
	1	250	43	82		1	1000	19	36
	1	250	50	89		1	1000	20	31
	2.5	500	36	76		2.5	2000	7	25
	2.5	500	26	74		2.5	2000	10	27
	2.5	500	27	62		2.5	2000	7	23
	2.5	500	29	64		2.5	2000	6	26
	2.5	500	26	63		2.5	2000	11	
	2.5	500	32	63		2.5	2000	6	
	2.5	500	28	60		2.5	1500	5	14
	2.5	500	32	52		2.5	1500	4	12
	2.5	500	34	51		2.5	1500	1	18
	5	1000	7	21		5	3000	1	2
	5	1000	3	25		5	3000	1	3
	5	1000	3	16		5	3000	1	4
	5	1000	5	16		5	4000	0	1
	5	1000	7	14		5	4000	0	2
	5	1000	4	12		5	4000	0	0
	5	1000	4	13		5	4000	2	2
	5	1000	5	17		5	4000	0	4
	5	1000	4	13		5	4000	0	4

tively. Note that the numerator degrees of freedom in Eq. 3 correspond to the dimensionality of the collapsed parameter space (i.e., 2) and that the denominator degrees of freedom (i.e., n-3) correspond to those of the full (3 parameter) model. Also note that each of the ellipses has the exact same orientation. This results because the experimental design matrices (determined by the choice of

TABLE 2. HCT116 CELLS.

	Control Cells	IU-Treated Cells	MX-Treated Cells	MX + IU-Treated Cells
C	0.773 (0.753, 0.793)[a]	0.377 (0.354, 0.401)	0.676 (0.654, 0.699)	0.057 (0.051, 0.064)
α	0.156 (0.122, 0.191)	0.569 (0.471, 0.667)	0.046 (0.007, 0.086)	1.124 (0.918, 1.330)
β	0.022 (0.015, 0.029)	0.063 (0.037, 0.089)	0.055 (0.047, 0.063)	0.008 (-0.052, 0.069)

NOTE: [a] 95% CI computed as the mean ± 1.96**stdev*.

TABLE 3. HCT116 3-6 CELLS.

	Control Cells	IU-Treated Cells	MX-Treated Cells	MX + IU-Treated Cells
C	0.694 (0.672, 0.716)	0.462 (0.439, 0.487)	0.665 (0.643, 0.688)	0.070 (0.063, 0.077)
α	0.043 (0.007, 0.080)	0.336 (0.267, 0.405)	0.061 (0.020, 0.102)	0.601 (0.456, 0.745)
β	0.034 (0.027, 0.042)	0.068 (0.052, 0.084)	0.041 (0.032, 0.049)	0.062 (0.026, 0.097)

TABLE 4. 10TH, 50TH AND 90TH PERCENTILES OF TWO NOTIONS OF LOW DOSE RATE THERAPEUTIC GAIN.[a]

	Control Cells	IU-Treated Cells	MX-Treated Cells	MX + IU-Treated Cells
$\alpha_{116} / \alpha_{3\text{-}6}$	(2.21, 3.58, 7.93)	(1.43, 1.69, 2.03)	(0.32, 0.76, 1.55)	(1.54, 1.87, 2.30)
$\alpha_{116} - \alpha_{3\text{-}6}$	(0.08, 0.11, 0.15)	(0.16, 0.23, 0.31)	(-0.05, -0.01, 0.02)	(0.36, 0.52, 0.69)

NOTE: [a] Based on 5000 samples from the underlying normal parameter estimates.

radiation doses) are identical in each case. COMMENT: The confidence ellipse projections yield slightly larger CI than those in TABLES 2 and 3 because the CI in FIG. 1 are joint CI, similar to Bonferroni CIs used in multiple comparisons, in contrast to the CI's in TABLES 2 and 3 which are to be interpreted "one-at-a-time," see reference [6].

2.4. DRUG-DRUG INTERACTIONS

Lindstrom et al. [1] suggest that drug-drug interactions with respect to radio-sensitization be quantified using α and β of Eq. 2 as follows: two agents are defined as non-interacting if the expected sum of the difference between IU and controls, added to the difference between MX and controls, differs insignificantly from that observed for the combination of MX and IU. To test this, we began by taking the difference between the observed MX + IU parameter estimates (thick solid arrows in FIG. 1) and those expected (thick dashed arrows in FIG. 1), i.e. we began by forming the two parameter random vector $b = b_{(1,1)} - b_{(1,0)} - b_{(0,1)} + b_{(0,0)}$ where the subscripts indicate the presence or absence of drug pretreatment. Because the experiments are independent, the 2 x 2 covariance matrices underlying this expression can be summed to form the covariance matrix of b, hereafter called Σ. To estimate the P value for interaction, we used the large sample approximation of Eq. 3,

$$(b\text{-}E(b))\ \Sigma^{-1}\ (b\text{-}E(b)) \sim \chi^2\ (2) \qquad \text{[Eq. 4]}$$

with $E(b)$ equal to the origin (i.e., a vector of zeros).

3. RESULTS AND DISCUSSION

3.1. DRUG-DRUG INTERACTIONS

We used the definition of drug-drug interactions given by Lindstrom et al. [1] and Eq. 4 to deduce P values $<<0.0001$ for both the HCT116 cell line and HCT116 3-6 cell line, i.e. highly significant drug-

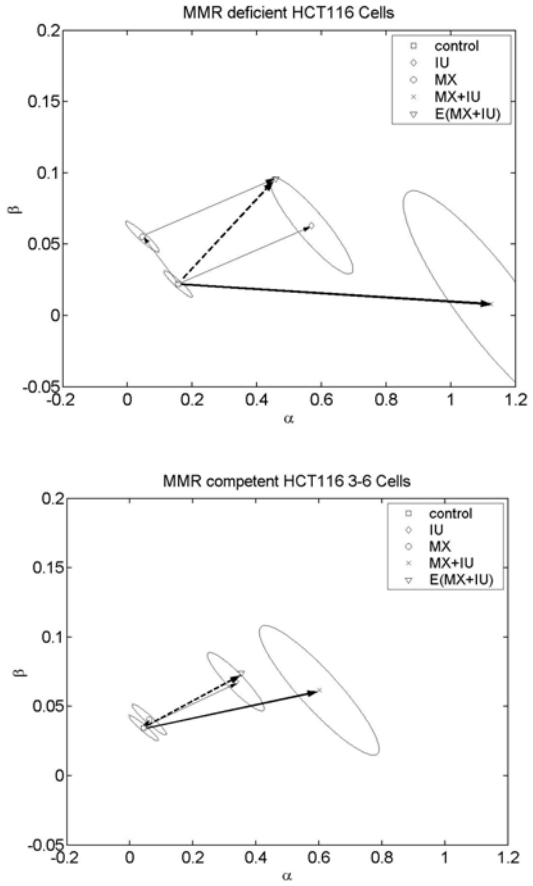

FIGURE 1. THE α-β 95% JOINT CONFIDENCE ELLIPSES CORRESPONDING TO TABLES 2 (LEFT) AND 3 (RIGHT). These plots suggest that drug-drug interactions are highly statistically significant, consistent with E (MX + IU) (dashed arrows,▽) lying well outside the 95% confidence ellipsoids for the observed MX + IU parameter estimates (thick solid arrows, x).

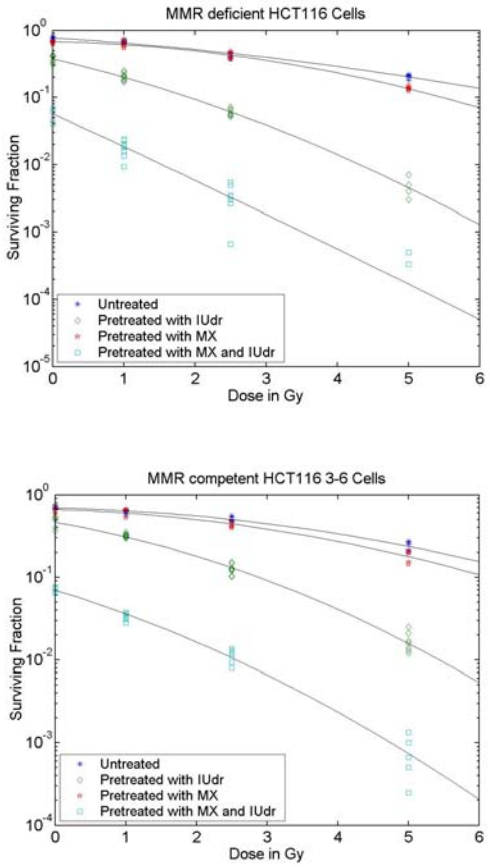

FIGURE 2. DOSE RESPONSE DATA AND CURVE FITS TO Eq. 2 CORRESPONDING TO TABLES 2 (LEFT) AND 3 (RIGHT).

drug interactions are present in both panels of FIG. 1. In Lindstrom et al. [1], estimates of α and β were generated for each dose-response experiment and subsequently analyzed using multivariate analysis of variance (*MANOVA*) [6]. Here, the number of dose-response experiments is so small (see TABLE 1 and note that replicates are in blocks of 3) that the second step in the Lindstrom approach (the *MANOVA* step in the parameter space) would have had at most 2 to 3 points per group. Lindstrom's *MANOVA* step also would have led to unnecessary losses of second moment parameter estimate information. We therefore replaced Lindstrom's *MANOVA* step with a large sample covariance matrix approximation. Since the large sample approximation is equivalent to assuming zero uncertainty in the variance estimate, in general, the present approach leads to *P* values for drug-drug interactions that are slightly small.

3.2. OTHER STATISTICAL CONSIDERATIONS

The number of cells plated per dish (TABLE 1) is treated here as being known exactly. Accounting for this uncertainty would broaden CI ellipses and thus drive expected therapeutic gains (in TABLE 4) toward unity (for ratios) or zero (for differences).

The MX + IU confidence ellipse in the left panel of FIG. 1 is consistent with $\beta = 0$. One might therefore be inclined to reduce the LQ model by removing the β term for this case. The uncertainty in α would then decrease to approximately the cross section of the ellipse at $\beta = 0$. Such an uncertainty estimate would, however, be conditional on the

model $\beta = 0$ being correct, which, based on the radiobiological literature, is unlikely to be true [7]. A null hypothesis of $\beta = 0.06$ is also quite consistent with this ellipse, and more importantly, also consistent with the literature [7]. Since estimates of α can differ substantially between these default assumptions, we advocate that either β be retained in all survival dose-response models, or that null hypothesis assumptions be guided by biologic plausibility (e.g., $\beta = 0.06$) rather than mathematical simplicity (e.g., $\beta = 0.00$).

3.3. BRACHYTHERAPEUTIC GAIN

Brachytherapeutic gain can be defined as the ratio of α for malignant cells divided by α for normal cells, $\alpha_{116} / \alpha_{3\text{-}6}$, or as the difference, $\alpha_{116} - \alpha_{3\text{-}6}$. For each of the four drug pretreatment conditions we calculated the 10th, 50th and 90th percentiles of these measures by forming 5000 random samples of (α_{116}, $\alpha_{3\text{-}6}$) from their normal parameter estimate distributions. Though the results (see TABLE 4) suggest based on α differences that pretreatment with MX + IU is best, they also suggest based on α ratios that no pretreatment is best. Which of these metrics of brachytherapeutic gain is preferable? From a statistical perspective, differences of normal distributions are normal and are thus preferred to ratios of normal distributions which must be treated using Monte Carlo methods. From a biophysical perspective, differences in α correspond to differences in log survival and thus survival ratios, imparting a clear interpretation relative to ratios of α. Finally, from a biological perspective, observations that DNA mismatch repair (MMR) deficient cells incorporate more IU than MMR competent cells, and that this difference is potentiated by MX [4], are much more consistent with differences in α compared to ratios, see TABLE 4. These arguments collectively favor the use of differences in α over ratios as metrics of brachytherapeutic gain.

4. ACKNOWLEDGEMENTS

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