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Biophysical modeling of *in vivo* glioma response following whole brain radiotherapy in a murine model of brain cancer

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Abstract

Purpose—Individualized biophysical models of response to radiotherapy that can accurately characterize tumor response would dramatically assist in the design and optimization of individual radiotherapy plans potentially prolonging patient survival and reducing damage to normal tissue. Towards this goal, we have developed and investigated a set of biophysical models based upon a mechanically coupled reaction-diffusion model of the spatiotemporal evolution of tumor growth post-radiotherapy.

Methods—Post-radiotherapy response is modeled using a cell death model (M_d), a reduced proliferation rate model (M_p), and cell death and reduced proliferation model (M_{dp}). To evaluate each model, rats ($n = 12$) with C6 gliomas were imaged with diffusion-weighted magnetic resonance imaging (DW-MRI) and contrast enhanced MRI (CE-MRI) at seven time points over two weeks. Rats received either 20 or 40 Gy between the third and fourth imaging time point. DW-MRI was used to estimate tumor cell number within enhancing regions in CE-MRI data. Each

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model was fit to the spatiotemporal evolution of tumor cell number from time point 1 to time point 5 to estimate model parameters. The estimated model parameters were then used to predict tumor growth at the final two imaging time points. The model prediction was evaluated by calculating the error in tumor volume estimates, average surface distance, and voxel-based cell number.

Results—For both the rats treated with either 20 or 40 Gy, significantly lower error in tumor volume, average surface distance, and voxel-based cell number was observed for the M_{dp} and M_p models compared to the M_d model. The M_{dp} model fit, however, had significantly lower sum squared error compared to the M_p and M_d models.

Conclusions—The results of this study indicate that for both doses, the M_p and M_{dp} models result in accurate predictions of tumor growth, whereas the M_d model poorly describes response to radiotherapy.

Keywords

reaction-diffusion; C6 glioma; mathematical; pre-clinical

Introduction

For glioblastoma multiforme patients, radiotherapy is typically administered following surgical resection to target any residual or inoperable cancer [1]. Unfortunately, with the current standard-of-care (SOC) therapy, nearly all glioblastoma patients have progressive disease 7 to 10 months following adjuvant treatment [2]. Individualizing predictive models on a patient-specific [3] basis could optimize radiotherapy plans for the individual patient to both maximize tumor cell death and minimize exposure to healthy tissue. Several groups have studied incorporating patient-specific imaging information into biophysical models of tumor growth [4–14], and recently these models have begun to include response to radiotherapy [15].

Response to radiotherapy is commonly modeled using the linear quadratic (LQ) model and this formalism has been incorporated into several mathematical models of radiotherapy response and planning [16–21] that use medical imaging data to initialize and constrain patient-specific tumor simulations [3]. One such model by Rockne *et al* [16,17] utilizes magnetic resonance imaging (MRI) data acquired before and after the start of treatment to evaluate various dose schedules or estimate a patient's radiosensitivity. Corwin *et al* [18] expanded upon Rockne *et al*'s model to demonstrate an approach for individualizing intensity-modulated radiotherapy plans. The simulated optimized plans had a decreased exposure to normal tissue and increased time to progression by 63% to 93% and 21% to 105%, respectively, compared to the simulated SOC. Badoual *et al* [20] incorporated a model of edema in addition to radiotherapy response to recapitulate observations of post-radiotherapy growth delay. While these patient-specific radiotherapy models demonstrate the potential value modeling has for clinical radiotherapy, the accuracy and precision of these modeling approaches need to be validated with *in vivo* experiments. Towards this end, *in vivo* imaging measurements and histological sections are used in this study to assess the model prediction error of three models of response to radiotherapy.

In this contribution, we systematically evaluate the ability of three biophysical models to describe and predict the *in vivo* spatio-temporal development of C6 glioma growth following radiotherapy differentiated by: 1) cell death immediately after radiotherapy (or M_d model), 2) a reduction of proliferation rate post-radiotherapy (or M_p model), and 3) the combination of cell death and reduced proliferation rate (or M_{dp} model). Diffusion weighted magnetic resonance imaging (DW-MRI) and contrast enhanced MRI (CE-MRI) data acquired before and after radiotherapy are used to estimate tumor cell count. The M_d model is related to the LQ model resulting in immediate cell death; that is, following irradiation some fraction of the cells lose their clonogenic survival eventually resulting in predominantly apoptosis or necrosis. The M_d model assumes that the effect of radiotherapy (i.e., cell death) occurs over a relatively short period. The M_p model assumes that consequences of irradiation predominantly result in a reduced net proliferation of tumor cells. A dose dependent reduction in proliferation rate has been observed in the C6 line [22] and may be due to cell cycle arrest [23] or senescence. The M_p model, however, assumes that the effect of radiotherapy is a long-term alteration of growth kinetics. The M_{dp} model incorporates the effects of both reduced proliferation and cell death to model post-radiotherapy growth providing a balance between the short and long term effects of radiotherapy. The M_d , M_p , and M_{dp} models are then fit to the measured 3D tumor cell count time courses. The estimated model parameters are then used to predict future tumor growth and response. The discrepancy between the model prediction and the measured data is assessed at the post-radiotherapy time points. The model prediction is also compared to post-mortem histological analysis.

Methods and Materials

In vivo Experiments

The experimental procedures were approved by our Institutional Animal Care and Use Committee. Rats were anesthetized with 2% isoflurane in 98% oxygen for all imaging, surgical, and irradiation procedures. Twelve Female Wistar rats (257 ± 9 g, mean $\pm$ 95% confidence interval) were anesthetized and inoculated intracranially with 10^5 C6 glioma cells *via* stereotaxic injection on day 0. On day 8, permanent jugular catheters were placed in each rat for injection of an MRI contrast agent. Rats were imaged three times prior to treatment (days 10, 12, 14, or t_1 through t_3) and four times after treatment (days 16.5, 18.5, 20.5, 22.5, or t_4 through t_7). MR images were acquired using a 9.4 T horizontal-bore magnet (Agilent, Santa Clara, CA, USA). A pulsed gradient fast spin echo DW-MRI [24,25] sequence was used to measure the apparent diffusion coefficient (ADC) which was then used to estimate tissue cellularity as previously described [8,9,26–28]. A T_1 map acquired using an inversion-recovery snapshot experiment was used to identify anatomical regions. A spoiled gradient echo CE-MRI experiment was used to identify tumor regions-of-interests (ROI) following the injection of a 200 μ L bolus ($0.05 \text{ mmol kg}^{-1}$) of gadolinium-diethylenetriamine-pentaacetic acid (Gado-DTPATM, BioPal, Worcester, MA). MR images were acquired over a $32 \times 32 \times 16 \text{ mm}^3$ field of view sampled with a $128 \times 128 \times 16$ matrix ($250 \times 250 \times 1000 \mu\text{m}$ voxel resolution). Additional MRI experimental details are reported in the Supplemental Material.

Rats were irradiated with 20 Gy ($n = 5$) or 40 Gy ($n = 7$) at a dose rate of 2.3 Gy/min with a Therapax DXT 300 x-ray machine (300 kVp/10 mA, Pantak Inc., East Haven, CT, USA) on day 14.5 (t_{rt}). Large single fraction doses were selected for this initial study to elicit distinct responses for model development and validation. During the irradiation protocol, rats were shielded to minimize exposure outside of the brain. At the conclusion of the last imaging study, animals were sacrificed and the brain tissue was prepared for histological sectioning and staining with H&E and Ki-67. Additional histology details are reported in the Supplemental Material.

Biophysical Models of Tumor Growth

Tumor growth is modeled using Eq. (1), which describes the change in the distribution and number of tumor cells due to the random movement of tumor cells (first term on the right hand side), the proliferation of cells (second term on the right hand side), and the death of cells due to radiotherapy (third term on right hand side):

$$\frac{\partial N(\bar{x}, t)}{\partial t} = \nabla \square \left[\theta_{max} D(\bar{x}, t) \nabla \left(\frac{N(\bar{x}, t)}{\theta(\bar{x})} \right) \right] + k_p(\bar{x}) \cdot RT_p(\bar{x}, t) \cdot N(\bar{x}, t) \left(1 - \frac{N(\bar{x}, t)}{\theta(\bar{x})} \right) - RT_d(\bar{x}, t) \cdot N(\bar{x}, t), \quad (1)$$

where the number of tumor cells at three-dimensional position $\bar{x}$ and time t is $N(\bar{x}, t)$, θ_{max} is the maximum number of cells that can physically fit in a voxel (50,970 cells), $D(\bar{x}, t)$ is a mechanically-coupled tumor cell diffusion coefficient [8,9,29,30] (details in the Supplemental Material), $\theta(\bar{x})$ is the local carrying capacity, $k_p(\bar{x})$ is the local proliferation rate, $RT_p(\bar{x}, t)$ is a post-radiotherapy proliferation function, and $RT_d(\bar{x}, t)$ is a post-radiotherapy death function. $RT_p(\bar{x}, t)$ is represented by a piecewise function, Eq. (2):

$$RT_p(\bar{x}, t) = \begin{cases} f_{p,rt}(\bar{x}) & t \geq t_{rt} \\ 1 & t < t_{rt} \end{cases}, \quad (2)$$

where $f_{p,rt}$ is a reduced proliferation fraction. In this implementation, we are assuming that the long-term effects of radiation will simply reduce the number of proliferating cells or the effective proliferation rate. Thus, this model does not consider long-term cell death or delayed cell death. Similarly, $RT_d(\bar{x}, t)$ is also represented by a piecewise function, Eq. (3):

$$RT_d(\bar{x}, t) = \begin{cases} k_{d,rt}(\bar{x}) & t = t_{rt} \\ 0 & t \neq t_{rt} \end{cases}, \quad (3)$$

where $k_{d,rt}$ is the post-radiotherapy death rate. The cell death term is assumed to occur instantaneously over a single time step. We note that, while the LQ model is not explicitly used, the M_d model plays an equivalent role. Specifically, the model parameter $k_{d,rt}$ could be replaced with a LQ formulation of cell survival (as done in [17]). Instead of fitting for a spatially varying $k_{d,rt}$, we could fit for a spatially varying α (and assume a fixed α/β ratio and uniform dose distribution). The end result would still be a fraction of cells that do not survive radiation therapy. We decided to then simplify the model to capture this effect as a single parameter that implicitly takes into account dose and radiosensitivity. These three models represent natural extensions of the LQ model to incorporate the instantaneous and delayed effects of radiotherapy and to do so using imaging data that is available in the clinical setting.

Measured values of $N(\bar{x}, t)$, $N_{meas}(\bar{x}, t)$, are obtained using DW-MRI before and after radiotherapy [27,28,30]. Pre-treatment (t_1 through t_3) measurements of $N(\bar{x}, t)$ are used to solve an inverse problem [28,30] to return estimates of $\theta(\bar{x})$, $k_p(\bar{x})$, and tumor cell diffusion. Similarly, post-treatment measurements (t_4 through t_5) are then used to solve an inverse problem to estimate $k_{d,rt}$ and $f_{p,rt}$ voxel-wise within the tumor. Model parameters were calibrated using a Levenberg-Marquardt least squares algorithm [31,32] which minimizes the error between the modeled and measured cellularity. For the M_d model, $k_{d,rt}$ is estimated and $f_{p,rt}$ is set to 1. For the M_p model, $f_{p,rt}$ is estimated and $k_{d,rt}$ is assigned to 0. For the M_{dp} model both $k_{d,rt}$ and $f_{p,rt}$ are estimated. Literature values used for mechanical tissue properties (discussed in Supplemental Material) were assigned from excised rat brain samples [33,34], though it would be preferable to measure these parameters on an individual basis (potentially through MR elastography [35]). Literature values were also used to assign cell packing density [36] and the average cell size [37]. We further assume that the packing density and cell size remain constant throughout the duration of the experiment. (Future efforts could employ advanced diffusion approaches to measure, for example, changes in cell size [38,39]). The estimated model parameters are then used in a forward evaluation of the model system, $N_{model}(\bar{x}, t)$, to predict tumor growth at t_6 and t_7 . The finite difference method was used to solve Eq. (1) using a three dimension in space ($250 \times 250 \times 1000 \mu\text{m}$ grid spacing) and fully explicit in time simulation (time step of 0.01 days). (The Supplementary Materials expand upon the *in vivo* and computational details.)

Error Analysis

The model prediction error was assessed using two metrics on days t_6 and t_7 . First, the differences between the predicted and measured tumor size and shape was assessed by calculating the percent error in tumor volume and the average surface distance (ASD), respectively. Second, within overlapping regions of the predicted and measured tumors, the percent error in cell number was calculated. The percent error in tumor volume was determined by calculating the percent difference between the predicted and the measured estimates of tumor volume. The ASD reports the average minimum distance between a voxel on the surface of the model tumor volume and a voxel on the surface of the measured tumor volume. The error in model fit post-radiotherapy was calculated using a weighted sum squared error (wSSE), described in the Supplemental Materials, to equally weight each time

point based on the total number of cells at each time point. At the voxel level, the percent difference in cell number was calculated between the model and measurement wherever $N_{meas}(\bar{x}, t)$ was greater than 0. All results are reported as the mean and 95% confidence interval when appropriate. A one-way analysis of variance was used to evaluate the differences in global and local errors between the model fits within treatment groups. Tukey's honest significant difference test was then used for pair-wise comparisons. A p value less than 0.05 was considered significant. Pearson's correlation coefficient (r) was calculated between the histology measurements and model parameters.

Results

Figures 1 and 2 show measured and modeled number of tumor cells as well as the percent difference between the two from the central tumor slice for representative rats from the 20 Gy and the 40 Gy groups, respectively. For the rat irradiated with 20 Gy, the predictions of the M_d model (row (d)) overestimates tumor size (error greater than 55.7%) on t_6 and t_7 compared to the M_p and M_{dp} models (error less than 17.0% and 9.6%, respectively). No significant differences were observed between the models at the voxel level (mean error ranged from 10.2% to 13.2%). Importantly, the M_d and M_p models fail to capture the developing low cell density region on days t_6 through t_7 (row (c)), resulting in greater than 50% error in these regions (rows (e,g,i)), while the M_{dp} model exhibits lower error (less than 30%) in these regions.

For the rat irradiated with 40 Gy, the predictions of the M_d model had high error in tumor volume (greater than 114.9%), while the M_p and M_{dp} had lower error in tumor volume (less than 23.5% and 10.5%, respectively). At the voxel level, no significant difference was observed between the three models (average error less than 5.1%).

Figures 3–4 compare the central imaging slice to histological sections from the same representative rats in Figure 1 and 2. For the rat irradiated with 20 Gy, low cell density regions (2281 ± 238 cells/mm²) in $N_{meas}(\bar{x}, t_7)$, panel (a), are also present in the H&E slice (panel (b), inset A), while a second low density region (panel (b), inset B) is not visible in this imaging slice. A high level of positive stained Ki-67 cells ($73.95 \pm 4.56\%$) are observed throughout the tumor region. For the rat irradiated with 40 Gy, an average cell density of 6336 ± 334 cells/mm² was observed in the tissue sections. High cell density is observed in both inset A and B. Although there are a few low cell density regions (inset A) that appear throughout the tumor. Fewer positive Ki-67 stained cells ($64.53 \pm 2.90\%$) were observed compared to the rat irradiated with 20 Gy. The necrosis observed in the rat irradiated with 20 Gy, but not the one irradiated with 40 Gy, is likely due to inter-animal variability in tumor growth and response.

Figure 5 summarizes the error analysis for both groups of irradiated with 20 (panels (a–c,g)) and 40 Gy (panels (d–f,h)). A statistically significant ($p < 0.03$) reduction in error was observed at all time points for the M_p (error in tumor volume, ASD, error in voxel-cell number) and M_{dp} (error in tumor volume, ASD) models compared to the M_d model. Similarly, for the rats irradiated with 40 Gy, a statistically significant ($p < 0.04$) reduction in error was observed at all time points for the M_p and M_{dp} (error in tumor volume, ASD, error

in voxel-cell number) models compared to the M_d model. For both treatment groups, significantly increased wSSE ($p < 0.05$) was observed for the M_p and M_{dp} models compared to the M_{dp} . Additionally, the M_p model for the rats irradiated with 20 Gy had significantly decreased wSSE ($p < 0.01$) compared to the M_d model.

Table 1 reports the mean estimated model parameters and their correlation to the percent positive stained Ki-67 cells within tumor regions for both treatment groups. Significant differences were observed between doses for M_p and M_{dp} model parameters. Significant ($p < 0.05$) and strong correlation was observed for $f_{p,rt}$ for the rats irradiated with 40 Gy ($r = 0.99$) and $k_{d,rt}$ for the rats irradiated with 20 Gy ($r = -0.98$) parameters estimated for the M_{dp} model and Ki-67 staining.

Discussion

The M_d model is a variation of the LQ model that has been previously applied [16,40–42] to clinical datasets following fractionated radiotherapy. The M_d model reflects some of the short-term effects of radiotherapy including cell death and shrinking of the tumor. Rather than assigning the death rate as the fraction of cells that die due to radiotherapy [16,17], we fit the models to time course data to estimate a spatially-varying death rate. The negative (although not statistically significant) correlation between $k_{d,rt}$ and percent positive stained Ki-67 cells suggests $k_{d,rt}$ may reflect observed tumor biology. The varied response between the 20 and 40 Gy group suggests that the M_d model may be more valid at low doses where post-radiotherapy growth kinetics more closely match the untreated growth kinetics (Supplemental Figure 1).

The M_p model is another variation of the LQ model where the loss of proliferative ability is modeled as a decrease in the net proliferation rate compared to the pre-treatment proliferation rate. Generally, the M_p model provided better model predictions compared to the M_d model. Based on the reduced doubling time observed following radiotherapy (Supplemental Figure 1), it was hypothesized that this model may be able to better describe the post-radiotherapy growth kinetics. The positive (although not statistically significant) correlation between $f_{p,rt}$ and percent positive stained Ki-67 cells, as well as the dose dependency of the mean $f_{p,rt}$, suggests $f_{p,rt}$ may reflect the observed decrease in tumor proliferation. However, the M_p model results in high error in cases where the tumor volume shrinks or indicates no growth following radiotherapy. The M_p model may more accurately describe growth at lower doses; however, it fails to capture decreases in tumor volume post-therapy or areas of necrosis.

The M_{dp} model combines both short-term and long-term effects [22,43]. Both the M_p and M_{dp} resulted in decreased error (i.e., in tumor volume, ASD, and voxel cell number) in model predictions, but the M_{dp} model may provide a better overall description due to the decrease in wSSE compared to the M_p model. The statistically significant differences between M_{dp} and M_p model for the wSSE metric, but not the other metrics, is likely to be due to reduced error in both the calibration and prediction phases for the M_{dp} model which only the wSSE metric assesses. The M_{dp} model may also provide improved predictions of intra-tumor heterogeneity (Figures 1 and 3). Model parameters ($f_{p,rt}$ and $k_{d,rt}$) appeared to be dose

specific suggesting that a more explicit relationship between dose and $f_{p,rt}$ and $k_{d,rt}$ may exist; this is the subject of ongoing efforts. Comparison of model parameters to histological staining indicated an inverse relationship between $k_{d,rt}$ and the percent positive stained Ki-67 cells and a positive relationship between $f_{p,rt}$ and Ki-67 staining. The histological correlation for both parameters suggests that they may provide insight into the underlying tumor biology post-radiotherapy (*i.e.*, delayed or altered proliferation, reduced number of proliferating cells). The M_{dp} model is a natural extension of previous modeling efforts [16,17] of response to radiation therapy. The main benefit of the M_{dp} model is the incorporation of both single time-point cell kill and a long term effect, which more accurately recapitulates *in vivo* observations of the persistent effect of radiation therapy.

There are several limitations to the current study. One limitation is in the interpretation of imaging measurements following radiation. In this work, we use *ADC* measurements to estimate cell number. Several pre-clinical and clinical studies have demonstrated a strong correlation between histological estimates of cellularity in human brain tumors [44], breast cancer [45], extracranial lesions [46], small animal models of breast cancer [47], and *in vitro* studies [26]. There is, however, other factors (cell membrane permeability [48], cell size, tortuosity [49], edema [50], necrosis [50]) which can also effect the measured *ADC* before and after treatment. This approach to transform *ADC* to cell number must, therefore, be regarded as a first order approximation to the true tumor cellularity. CE-MRI enhancement may also potentially indicate false volume increases or decreases following radiotherapy [51]. Pseudoprogression is often a result of increased inflammation, edema, and vessel permeability and can be challenging to distinguish from actual progression and separating these two phenomena is an active area of research [52–55]. A second limitation is that there is a lack of histology for untreated rats to which the treated groups can be compared, as well as a lack of histology at the time of treatment. However, the dose dependent decreases in Ki-67 have been observed by others [43]. A third limitation is the use of single large doses over the whole brain. Radiotherapy is more commonly delivered in small doses over several fractions with a more focal dose. Large single fraction doses were selected for this initial study to provide two distinct treatment responses from which to test and validate the mathematical models. Ongoing studies will investigate smaller, fractionated dose response. A fourth limitation is the lack of a validation of the pre-treatment parameters. An additional imaging time point could be used to verify the pre-treatment parameters, however, that will limit the number of post-treatment time points we could acquire. When applied to a cohort of untreated rats, this modeling approach resulted in less than 2.20% error in tumor volume, average surface distance less than 0.38 mm, and average percent error in voxel cell number less than 13.25% [30]. Based on those results we have confidence in the ability of this framework to capture untreated tumor growth. Finally, in a data limited setting (*e.g.*, standard-of-care clinical studies) this approach may not be tenable. However, with the advent of MRI guided external beam radiation treatment [56], functional or anatomical data could be acquired on a per-fraction basis. This scenario would produce measurements that can be used to calibrate model parameters (and correct parameters as more data becomes available) that can be used to adapt the remaining treatment plans [57].

Conclusion

The models developed and analyzed within this study are an encouraging step towards the development of mathematically rigorous, individualized radiotherapy plans. After receiving 20 Gy, growth can be accurately described using the M_p or M_{dp} model. At higher doses, the M_p and M_{dp} models both resulted in lower error in tumor volume, ASD, and voxel cell number, while the M_d model poorly described tumor response at high doses. Overall, the M_{dp} model provides a more complete characterization of the post-radiotherapy growth kinetics, although further model development is needed to more accurately characterize the heterogeneous response to radiotherapy within the tumor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Summary

In this experimental and computational study, three biophysical models are developed and evaluated for their ability to accurately describe *in vivo* growth and response following whole brain radiotherapy. Models that include reduced proliferation post-radiotherapy more accurately predicted future tumor growth.

Figure 1. Measured and Simulated $N(\bar{x}, t)$ for a rat irradiated with 20 Gy

The measured (rows (a–c)) and simulated (rows (d,f,h)) $N(\bar{x}, t)$ are shown for a rat irradiated with 20 Gy. The black lines in rows (d,f,h) represent the boundaries of $N_{meas}(\bar{x}, t)$. Percent error between the measured and simulated $N(\bar{x}, t)$ are also shown in rows (e,g,i), with the white space indicating areas where $N_{meas}(\bar{x}, t) = 0$. The tumor size was overestimated for the M_d and M_{dp} models at t_6 and t_7 compared to the M_p model. The M_p and M_d models had greater than 60% error (rows (e,g,i)) in low cell density areas observed in $N_{meas}(\bar{x}, t)$ (row

(c)). The pre-treatment parameters D_0 and mean k_p were $1.19 \mu\text{m}^2/\text{day}$ and 2.16 day^{-1} , respectively. The mean post-treatment parameters were: $f_{p,Mp} = 0.38 \pm 0.06$, $f_{p,Mdp} = 0.36 \pm 0.01$, $k_{d,Md} = 0.47 \pm 0.06$, and $k_{d,Mdp} = 0.34 \pm 0.02$.

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Figure 2. Measured and Simulated $N(\bar{x}, t)$ for a rat irradiated with 40 Gy

(The data is presented in an identical fashion to Figure 1.) The M_d model overestimated tumor size on days t_6 through t_7 (row d). The M_p and M_{dp} models more closely matched the tumor shape and size at all time points (rows f, h)). The pre-treatment parameters D_0 and mean k_p were $2.01 \mu\text{m}^2/\text{day}$ and 4.23 day^{-1} , respectively. The mean post-treatment parameters were: $f_{p,Mp} = 0.06 \pm 0.01$, $f_{p,Mdp} = 0.25 \pm 0.01$, $k_{d,Md} = 0.72 \pm 0.04$, and $k_{d,Mdp} = 0.63 \pm 0.03$.

Figure 3. Model and measured $N(\bar{x}, t)$ compared to histology for a rat irradiated with 20 Gy MRI measured and simulated $N(\bar{x}, t)$ (panel (a)) are compared to equivalent H&E (panel (b)) and Ki-67 (panel (c)) stained sections for a rat irradiated with 20 Gy. Insets A and B indicate areas of low cell density and low positive Ki-67 stained cells. In panel (c), positive staining (brown) was seen throughout the tumor with the exception of the low cell density regions (insets A and B). The low cell density regions in $N_{meas}(\bar{x}, t_7)$ are present in inset A from the H&E slide.

Figure 4. Model and measured $N(\bar{x}, t)$ compared to histology for a rat irradiated with 40 Gy (The data is presented in an identical fashion to Figure 3.) Very few areas of low cell density (column b, inset A) were observed throughout the tumor. Column (c) shows a Ki-67 stained tissue section in which clusters of proliferating cells were observed throughout the tumor.

Figure 5.

Global and local error results for the 20 Gy (panels a-c) and 40 Gy (panels d-f) groups are reported as the mean and 95% confidence interval from day t_4 through t_7 . wSSE is also reported in panels (g-h) for the 20 and 40 Gy rats, respectively. Generally, high global-level errors were observed for the M_d model for both treatment groups, while the M_{dp} model resulted in low global-level errors. All models resulted in low local-level errors for the 20 Gy group (panel c), while the M_d and M_{dp} models had lower error for the 40 Gy group (panel f).

Table 1

Estimated model parameters and correlation to histology

Model	Parameter	Estimated Value		Model Parameter vs. Percent Positive Stained Ki-67 Cells	
		Mean $\pm$ 95% CI	r	p	
20 Gy	M_d	$k_{d,\pi}$	0.66 ± 0.24	-0.77	0.23
	M_p	$f_{p,\pi}$	$0.52 \pm 0.27^*$	0.84	0.16
	M_{dp}	$k_{d,\pi}$	$0.31 \pm 0.10^*$	-0.98	0.02
		$f_{p,\pi}$	$0.30 \pm 0.09^*$	0.90	0.10
	M_d	$k_{d,\pi}$	0.74 ± 0.12	-0.73	0.25
	M_p	$f_{p,\pi}$	$0.12 \pm 0.07^*$	0.73	0.27
40 Gy	M_{dp}	$K_{d,\pi}$	$0.61 \pm 0.02^*$	-0.80	0.20
		$f_{p,\pi}$	$0.22 \pm 0.09^*$	0.99	0.01

Statistically significant differences ($p < 0.05$) between treatment groups indicated by*.