Cell **Volume 156, Issue 3**

Supplemental Information

Mathematical Modeling of PDGF-Driven Glioblastoma Reveals Optimized Radiation Dosing Schedules

Kevin Leder, Ken Pitter, Quincey LaPlant, Dolores Hambardzumyan, Brian D. Ross, Timothy A. Chan, Eric C. Holland,* and Franziska Michor*

*Correspondence: eholland@fhcrc.org (E.C.H.), michor@jimmy.harvard.edu (F.M.)

Extended Experimental Procedures

Generation of tumors using RCAS/TVA

Df-1 cells were purchased from ATCC. Cells were grown at 39 °C according to ATCC instructions. Transfections with RCAS-PDGF-B-HA or RCAS-PDGF-B-HA-SV40-eGFPwas performed using Fugene 6 transfection kit (Roche # 11814443001) according to manufactures instructions. To generate gliomas we injected 1μl of transfected DF-1 cells (4×10^4) into the cortex area. Mice were monitored carefully for symptoms of tumor development (hydrocephalus, lethargy, head tilt).

Bioluminescence Radiation dose response

Tumor bearing *Nestin-tv-a;E2F1-Luc* mice were anesthetized with 3% isolflurane before retroorbital injection with 75mg/kg body weight D-luciferin (Xenogen). One minute after injection of the luciferin, images were acquired for 2 min with the IVIS 100 (Xenogen). A photographic image was taken onto which the pseudocolor image representing the spatial distribution of photon count is projected. We defined a circular region between the ears and used it as a standard in all experiments. From this region, photon counts are compared between different mice. Mice were imaged before and 24h post different doses of radiation. Total body irradiation (TBI) was delivered with a 137Cs irradiator (Shepherd Mark-I, model 68, SN 643) at a dose rate of 2.12Gy/min. Mice were sacrificed at 24h post radiation.

Survival Analysis

Adult *Nestin-tv-a, Ink4a-Arf*$^{-}/^{-}$ mice were anesthetized with isoflurane. One microliter of RCAS transfected DF1 cells (4×10^4 cells/μl) was delivered using a 30-gauge needle attached to a Hamilton syringe and stereotactic fixation device (Stoelting, Wood Dale, IL). Cells were injected to the right frontal cortex: coordinates anterior to bregma 1.5 mm, lateral 0.5 mm, and a depth 1.5 mm. Mice were monitored carefully and treatment began when they displayed neurological symptoms, such as lethargy or head tilt due to tumor burden. Following development of symptoms, mice were sedated with isoflurane and irradiation of the head was done using a X-RAD 320 from Precision X-Ray at 115 cGy/min. The rest of the mouse was shielded with

a lead jig to minimize radiation toxicity to normal tissues. The various 10 Gy dosing schemes are outlined in Table 2. For the 20 Gy treatment, radiation was dosed at 2 Gy/day for five days followed by 2 days off and again dosed for another 5 days. Animals were sacrificed upon recurrence of neurological symptoms, as defined by the Institutional Animal Care and Use Committee. Each survival arm was sufficiently powered to account for any baseline variability in response.

MRI Scans

MRI scans were performed on a 9.4T, 16 cm horizontal bore (Agilent Technologies, Inc., Santa Clara, CA) Direct Drive system using a mouse surface receive coil (m2m Imaging, Corp., Cleveland, OH) actively decoupled to a whole-body volume transmit coil (Rapid MR International, LLC., Columbus, OH). Throughout the MRI experiments, animals were anesthetized with a 1-2% isofluorane in air mixture, and body temperature was maintained using a heated air system (Air-Therm Heather, World Precision Instruments, Sarasota, FL). MR images were acquired prior to treatment initiation, daily during the first seven days and every other day until the animals were sacrificed or became moribund.
MRI experiments consisted of imaging mice for delineation of tumor volumes over time in order to assess treatment effects on growth rates and volumes. Delineation of tumor from healthy brain tissue was accomplished using a contrast-enhanced T1-weighted spin-echo images with the following parameters: Repetition time (TR)/echo time (TE) = 510/15 ms, field of view (FOV) = 20×20 mm^2, matrix size = 128×128, slice thickness = 0.5 mm, 25 slices and 2 averages. Total acquisition time was 2 minutes and 12 seconds. Contrast-enhancement was performed by i.p. administration of 50 μl of 0.5 M gadolinium-DTPA (Magnevist, Bayer Healthcare Pharmaceuticals, Wayne, N.J) at 5 minutes prior to image data acquisition.

Image Reconstruction and Analysis

Volumes of interest (VOIs) were manually contoured along the enhancing rim of the tumors on the contrast-enhanced T1-weighted images for tumor volume measurements. All image reconstruction and digital image analysis was accomplished using programs developed in Matlab (The Mathworks, Natick, MA, USA).

SP Analysis

Hoechst 33342 staining was performed as previously reported, [2]. Briefly, glioma-bearing mice were treated with standard, hyperfractionated, optimum-1 or optimum-2 schedules. 24 hours after the last treatment, mice were euthanized and tumor cells enzymatically dissociated and resuspended at 2×10^6 cells/ml. Cells were preincubated at 37 °C for 30 min with or without 100 mM verapamil (Sigma-Aldrich, St. Louis), to inhibit ABC transporters and were incubated for 90 min at 37 °C with 5 mg/ml Hoechst 33342 (Sigma-Aldrich). In order to focus the analysis on *bona fide* tumor cells, samples were gated on eGFP+ cells. Hoechst dye was excited at 407 nm by trigon violet laser, and its dual wavelengths were detected using 450/40 (Hoechst 33342-Blue) and 695/40 (Hoechst 33342-Red) filters. FITC was excited at 488 nm by an octagon blue laser, and fluorescence was detected using 530/30. Dead cells were excluded by gating on forward and side scatter and eliminating PI-positive population. The data were analyzed by FlowJo (Ashland, OR, USA).

Statistics

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA). Kaplan-Meier analysis was performed using the log-rank (Mantel-Cox) test. For Olig2 and SP quantification, standard and optimized protocols were compared using an independent sample Students t-test. $p < 0.05$ - * ; $p < 0.01$ - **; $p < 0.001$ - ***, $p < 0.0001$ - ****, absence of star=not significant.

PDGF Signalling

As this tumor model is catalyzed by high regional expression of PDGF, we wanted to ensure the observed survival benefit was not due to decreased PDGF production or signaling. To that end, we investigated the levels of the PDGF-B ligand and its tumor cell receptor, PDGFRα, in primary, residual, and recurrent tumors. The PDGF-B ligand has a c-terminal HA-epitope tag, and HA immunoreactivity can be used as a surrogate for PDGF-B production (Shih et al., 2004). Both ligand and receptor were strongly expressed in untreated tumors (Figure S1A,E). Due to radiation-induced tumor regression, both the standard and optimum-1 treated samples had fewer tumor cells

present on the sixth day, i.e. one day after the last dose of radiation (Figure S1B,C,F,G). However, many of the remaining cells were still strongly PDGF-B and PDGFRα. The recurrent tumors showed a similar expression pattern to the untreated tumors, suggesting that PDGF-B expression of residual tumor cells was sufficient to drive recurrence (Figure S1D,H). We further investigated downstream signaling events in a primary cell line derived from our mouse model. When activated, PDGFRα signals via a phosphorylation cascade resulting in increased levels of phosphorylated Akt (Fomchenko and Holland, 2007). Radiation did not inhibit the phosphorylation of either PDGFRα or AKT, whereas Imatinib, a potent PDGFRα inhibitor, was able to inhibit both (Figure S1I).

The mathematical model

Our model is based on the linear quadratic framework. We consider two separate populations of cells – stem-like resistant cells (SLRC) and differentiated/sensitive cells (DSC). SLRC are largely radio-resistant. After exposure to radiation, a fraction of the DSC revert to the SLRC state. The parameters of the model are as follows:

1. The parameters α_s and β_s characterize the response of SLRC to radiation.

2. The parameters α_d and β_d characterize the response of DSC to radiation.

3. The parameter γ denotes the fraction of DSC that revert to the SLRC state.

4. The rate ν denotes the rate at which DSC revert to the SLRC state.

5. The parameter L_d gives the minimum time it takes for DSC to return to cycle.

6. The parameter M_d specifies the minimum time for a newly created DSC to lead to clonal expansion.

7. The parameter L_s gives the minimum time it takes for SLRC to return to cycle.

8. The rate η_d represents the rate at which a newly created DSC leads to clonal expansion.

9. The rate λ_d gives the rate at which DSC exit quiescence.

10. The rate λ_s specifies the rate at which SLRC exit quiescence.

11. The rate r_d gives the rate at which DSC reproduce once they return to cycle.

12. The rate r_s specifies the rate at which SLRC reproduce once they return to cycle.

13. The parameter R denotes the initial ratio of DSC to SLRC.

14. The parameter a_s gives the rate at which SLRC produce DSC.

15. The parameter μ gives the time of maximal radiation-induced reversion potential after an initial radiation exposure to ionizing radiation.

16. The parameter σ^2 gives the width of radiation-induced reversion potential after initial radiation exposure.

For simplicity, we assume that $\alpha_s = \rho\alpha_d$ and $\beta_s = \rho\beta_d$ for $\rho \in [0, 1]$; this constant characterizes the level of radiosensitivity of SLRC. If $\rho = 0$, then they are completely immune to radiation, and if $\rho = 1$, they respond to radiation at the same level as the DSC. Also note that μ and σ^2 are not present in the initial model.

For the remainder of the Supplementary Material, we use the following notation: for two real numbers x, y, $x \vee y = \max(x, y)$; $(t - T)^+ = (t - T)1_{\{t>T\}}$; and

$$1_{\{t>T\}} = \begin{cases} 1, & \text{if } t - T > 0 \\ 0, & \text{if } t - T \leq 0. \end{cases}$$

The first dose of treatment

After the first dose of d Gy of radiation, the fraction of DSC that survives is given by $\exp(-\alpha_d d - \beta_d d^2)$, and the fraction of SLRC that survives is given by $\exp(-\alpha_s d - \beta_s d^2)$. Of those DSC that survive, a fraction γ revert to the SLRC state. Note that in the basic mathematical model, this fraction does

not depend on the radiation dose; however, that dependence can easily be incorporated into the model in future work. The conversion of DSC to the SLRC state occurs at a rate of ν. SLRC and DSC are in a quiescent state after a dose of radiation for a minimum of L_s and L_d hours, respectively. They exit from quiescence at respective rates λ_s and λ_d. In addition, there is a delay between the creation of a new DSC cell (i.e. progeny of a SLRC cell) and the time at which it begins to reproduce; this delay is a minimum of M_d time units and cells begin reproducing at rate η_d. For clarity we will first consider the scenario where $\lambda_s, \lambda_d, \eta_d \to \infty$, i.e., cells exit quiescence and begin reproducing synchronously at times L_s and L_d. We will consider the setting of finite rates in subsection .

In summary, if we start with a population of N^d DSC and N^s SLRC, then after the first dose of d Gy, there will be $N^d \exp(-\alpha_d d - \beta_d d^2)$ DSC and $N^s \exp(-\alpha_s d - \beta_s d^2)$ SLRC. Additionally, $\gamma N^d \exp(-\alpha_d d - \beta_d d^2)$ DSC are in the process of being converted to SLRC.

Let us now consider the cell numbers once t units of time have passed since the first treatment. At that time, the number of DSC is given by

$$\begin{aligned} & N^d e^{-\alpha_d d - \beta_d d^2} \left[(1-\gamma)\, e^{r_d (t-L_d)^+} + \gamma e^{-\nu t} \right. \\ & \quad \left. + \, a_s \gamma \nu \int_0^t e^{r_d (t-s-M_d)^+} \int_0^{(s-L_s)^+} e^{-\nu y} e^{r_s (s-y-L_s)^+} dy ds \right] \\ & \quad + a_s N^s e^{-\alpha_s d - \beta_s d^2} \int_{L_s}^{t \vee L_s} e^{r_s (s-L_s)} e^{r_d (t-s-M_d)^+} ds, \end{aligned}$$

where the first term in the brackets refers to the number of DSC that survived and do not have the potential to revert to the SLRC state, plus any growth that occurs if t is sufficiently large. The second term in the brackets represents the number of cells that have begun to revert but are still DSC at time t. The integral in the bracket represents the creation of new DSC from the newly created SLRC population, i.e. those SLRC that were created from the reversion of DSC. The integral outside the bracket represents the creation of DSC from the original SLRC cell population. Note that the two integral terms are zero unless $t > L_s$, because the SLRC population can only create DSC L_s hours after the dose of radiation. Then the number of SLRC is given by

$$N^s e^{-\alpha_s d - \beta_s d^2} e^{r_s (t-L_s)^+} + \gamma N^d e^{\alpha_d d - \beta_d d^2} \nu \int_0^t e^{-\nu s} e^{r_s (t-s-L_s)^+} ds.$$

The first term represents the population of SLRC that survived the dose of radiation plus any growth that has occurred since then. The second term represents the number of DSC that have reverted to the SLRC state plus any growth that has occurred in this population.

The ith dose of treatment

Let us now consider how treatment $i \geq 1$ affects the system. Suppose we know the number of SLRC and DSC, N_i^s and N_i^d, immediately prior to treatment i. In what follows, we describe how to use the knowledge of N_i^s, N_i^d,t (the time between treatments i and $i+1$), and d_i (the dose of treatment i) to determine the state of the population prior to treatment $i+1$.

Immediately after a treatment of d_i Gy, the number of DSC is given by

$$N_i^d e^{-\alpha_d d_i - \beta_d d_i^2}$$

with a fraction γ capable of converting to the SLRC state, and the number of SLRC is given by

$$N_i^s e^{-\alpha_s d_i - \beta_s d_i^2}.$$

Then the populations prior to treatment $i+1$ are given by

$$\begin{aligned} N_{i+1}^d = N_i^d e^{-\alpha_d d_i - \beta_d d_i^2} \Bigg[& (1-\gamma)\, e^{r_d (t - L_d)^+} + \gamma e^{-\nu t} \\ & + a_s \gamma \nu \int_0^t e^{r_d (t-s-M_d)^+} \int_0^{(s-L_s)^+} e^{-\nu y} e^{r_s (s-y-L_s)^+} dy ds \Bigg] \\ & + a_s N_i^s e^{-\alpha_s d - \beta_s d^2} \int_{L_s}^{t \vee L_s} e^{r_s (s-L_s)} e^{r_d (t-s-M_d)^+} ds \\ N_{i+1}^s = N_i^s e^{-\alpha_s d_i - \beta_s d_i^2} e^{r_s (t-L_s)^+} & + \gamma \nu N_i^d e^{-\alpha_d d_i - \beta_d d_i^2} \int_0^t e^{-\nu s} e^{r_s (t-s-L_s)^+} ds. \end{aligned} \tag{1}$$

The DSC, N_{i+1}^d, are comprised of those that have not yet started to revert, the cells that started to revert but have not yet reverted to the SLRC state, any growth that has occurred since the last treatment, and the creation of new DSC from the SLRC population. In the formula for the SLRC, N_{i+1}^s, the first term represents the SLRC that survived the dose of radiation while the second term is the result of any de-differentiation events that might have occurred.

Instead of studying the total population of cells, let us work with the fraction of cells that remain viable. In particular, if N_1^d and N_1^s are the starting populations of DSC and SLRC, respectively, we examine the evolution of $F_i^d = N_i^d/N_1^d$ and $F_i^s = N_i^s/N_1^s$. Then, in lieu of (1), we have

$$\begin{aligned} F_{i+1}^d &= F_i^d e^{-\alpha_d d_i - \beta_d d_i^2} \Big[(1-\gamma)\, e^{r_d (t-L_d)^+} + \gamma e^{-\nu t} \\ &\quad + a_s \gamma \nu \int_0^t e^{r_d (t-s-M_d)^+} \int_0^{(s-L_s)^+} e^{-\nu y} e^{r_s (s-y-L_s)^+} dy ds \Big] \\ &\quad + a_s \frac{F_i^s}{R} e^{-\alpha_s d - \beta_s d^2} \int_{L_s}^{t \vee L_s} e^{r_s (s-L_s)} e^{r_d (t-s-M_d)^+} ds, \\ F_{i+1}^s &= F_i^s e^{-\alpha_s d_i - \beta_s d_i^2} e^{r_s (t-L_s)^+} + \gamma \nu R F_i^d e^{-\alpha_d d_i - \beta_d d_i^2} \int_0^t e^{-\nu s} e^{r_s (t-s-L_s)^+} ds. \end{aligned} \tag{2}$$

Recall that $R = N_1^d/N_1^s$.

For what follows it is useful to observe that

$$\nu \int_0^t e^{-\nu s} e^{r_s (t-s-L_s)^+} ds = \frac{\nu}{\nu + r_s} e^{r_s (t-L_s)^+} + \frac{r_s}{\nu + r_s} e^{-\nu (t-L_s)^+} - e^{-\nu t}, \tag{3}$$

and

$$\begin{aligned} &\int_0^t e^{r_d (t-s-M_d)^+} \int_0^{(s-L_s)^+} e^{-\nu y} e^{r_s (s-y-L_s)} dy ds \\ &= \frac{1}{\nu + r_s} \int_{L_s}^{L_s \vee t} e^{r_d (t-s-M_d)^+} \left(e^{r_s (s-L_s)} - e^{-\nu (s-L_s)} \right) ds, \end{aligned} \tag{4}$$

and for any z,

$$\begin{aligned} h(z,t,L_s,M_d) &= \int_{L_s}^{L_s \vee t} e^{r_d (t-s-M_d)^+} e^{z(s-L_s)} ds \\ &= \frac{1}{z - r_d} \left(e^{z(t-M_d-L_s)} - e^{r_d (t-M_d-L_s)} \right) 1_{\{t > L_s + M_d\}} \\ &\quad + \frac{1}{z} \left(e^{z(t-L_s)} - e^{z(t-L_s-M_d)} \right) 1_{\{t > L_s + M_d\}} + \frac{1}{z} \left(e^{z(t-L_s)} - 1 \right) 1_{\{L_s < t < L_s + M_d\}} \end{aligned} \tag{5}$$

Finite rates

Until now we have assumed that exit from quiescence induced by radiation is a synchronous phenomenon. In particular we have assumed that the rates

λ_d, λ_s η_d are infinite. However, in glioma there is no evidence to indicate that this is in fact the case. We will now adjust our model to incorporate the setting of finite rates, i.e., asynchronous exit from quiescence.

First define the coefficient functions,

$$\begin{aligned} a(t, L_s, L_d, M_d) &= (1-\gamma)\, e^{r_d(t-L_d)^+} + \gamma e^{-\nu t} \\ &\quad + \frac{a_s \gamma \nu}{\nu + r_s} \left(h(r_s, t, L_s, M_d) - h(-\nu, t, L_s, M_d)\right) \\ b(t, L_s, M_d) &= \frac{a_s}{R} h(r_s, t, L_s, M_d) \\ c(t, L_s) &= \gamma \nu R \int_0^t e^{-\nu s} e^{r_s(t-s-L_s)^+} ds \\ d(t, L_s) &= e^{r_s(t-L_s)^+}, \end{aligned} \tag{6}$$

where we used (4) and (5) to simplify the expressions for a and b. Then, in the setting of cells exiting quiescence simultaneously, equation (2) can be written in the compressed form

$$\begin{aligned} F^d_{i+1} &= F^d_i e^{-\alpha_d d_i - \beta_d d_i^2} a(t, L_s, L_d, M_d) + F^s_i e^{-\alpha_s d_i - \beta_s d_i^2} b(t, L_s, M_d) \\ F^s_{i+1} &= F^d_i e^{-\alpha_d d_i - \beta_d d_i^2} c(t, L_s) + F^s_i e^{-\alpha_s d_i - \beta_s d_i^2} d(t, L_s). \end{aligned}$$

Recall that this expression gives the remaining fraction of viable cells t units after a dose of d Gy of radiation.

In order to adapt this result to the setting of finite rate within each coefficient function a, b, c, d we need to replace L_s, L_d, and M_d with the independent random variables with respective densities

$$\begin{aligned} f_{L_s}(x) &= \lambda_s e^{-\lambda_s(x-L_s)},\ x \geq L_s \\ f_{L_d}(x) &= \lambda_d e^{-\lambda_d(x-L_d)},\ x \geq L_d \\ f_{M_d}(x) &= \eta_d e^{-\eta_d(x-M_d)},\ x \geq M_d. \end{aligned}$$

We then integrate the resulting random variables with respect to these densities. Specifically the viable fraction of SLRC and DSC t units of time after d Gy of radiation in the setting of asynchronous exit from quiescence can be written as

$$\begin{aligned} F^d_{i+1} &= F^d_i e^{-\alpha_d d_i - \beta_d d_i^2} \bar{a}(t, L_s, L_d, M_d) + F^s_i e^{-\alpha_s d_i - \beta_s d_i^2} \bar{b}(t, L_s, M_d) \\ F^s_{i+1} &= F^d_i e^{-\alpha_d d_i - \beta_d d_i^2} \bar{c}(t, L_s) + F^s_i e^{-\alpha_s d_i - \beta_s d_i^2} \bar{d}(t, L_s), \end{aligned} \tag{7}$$

where

$$\begin{aligned}
\bar{a}(t, L_s, L_d, M_d) &= \gamma e^{-\nu t} + \lambda_d(1-\gamma)\int_{L_d}^{\infty} e^{r_d(t-x)^+} e^{-\lambda_d(x-L_d)} dx \\
&\quad + \frac{a_s \gamma \nu \eta_d \lambda_s}{r_s + \nu} \int_{M_d}^{\infty}\int_{L_s}^{\infty} h(r_s, t, x, y) e^{-\eta_d(y-M_d)} e^{-\lambda_s(x-L_s)} dx dy \\
&\quad - \frac{a_s \gamma \nu \eta_d \lambda_s}{r_s + \nu} \int_{M_d}^{\infty}\int_{L_s}^{\infty} h(-\nu, t, x, y) e^{-\eta_d(y-M_d)} e^{-\lambda_s(x-L_s)} dx dy \\
\bar{b}(t, L_s, L_d) &= \frac{a_s}{R} \int_{M_d}^{\infty}\int_{L_s}^{\infty} h(r_s, t, x, y) e^{-\lambda_s(x-L_s)} e^{-\eta_d(y-M_d)} dx dy \\
\bar{c}(t, L_s) &= \gamma \nu R \int_{L_s}^{\infty} \left(\int_0^t e^{-\nu s} e^{r_s(t-s-x)^+} ds \right) e^{-\lambda_s(x-L_s)} dx \\
\bar{d}(t, L_s) &= \int_{L_s}^{\infty} e^{r_s(t-x)^+} e^{-\lambda_s(x-L_s)} dx. \qquad (8)
\end{aligned}$$

Explicit formulas for these integrals are provided in Section .

Second iteration of the mathematical model

In order to better fit the observed mouse survival data, we introduce an iterated version of our mathematical model. The basic idea of the new version of the model is to assume that when a population of glioma cells is irradiated, the fraction of cells capable of reversion, γ, depends on how many doses of radiation have been administered and the time elapsed since the previous administration of radiation. The fraction of cells capable of reversion following the initial dose of radiation is given by γ_0. Subsequent doses of radiation will induce a different behavior. Specifically, if it has been t_0 time units since the last dose of radiation, then the fraction of cells capable of reversion following a dose of radiation is given by

$$\gamma(t_0) = \gamma_0 \exp\left[-(t_0 - \mu)^2/\sigma^2\right],$$

where γ_0, μ and σ are system parameters. The parameter $0 < \gamma_0 < 1$ represents the fraction of cells capable of reversion in response to the first dose of radiation, and thereafter represents a maximal possible fraction available for reversion. The parameters μ and σ^2 reflect the time dynamics of the reversion process. In particular, μ represents the dose spacing that leads to the maximal amount of cell reversion to the SLRC state, and σ represents

how sensitive this maximum is to changes in the spacing. For example, a larger value of σ^2 would mean that $\gamma(t_0)$ is less sensitive to the value of t_0, while smaller values of σ^2 mean an increased sensitivity to the value of t_0.

Based on this discussion, we can write the following expressions for the population of DSC and SLRC t units of time after the initial dose of d Gy of radiation as

$$\begin{aligned} & N^d e^{-\alpha_d d-\beta_d d^2}\left[(1-\gamma_0)\, e^{r_d(t-L_d)^+}+\gamma_0 e^{-\nu t}\right. \\ & \quad \left. +\, a_s\gamma_0\nu\int_0^t e^{r_d(t-s-M_d)^+}\int_0^{(s-L_s)^+} e^{-\nu y}e^{r_s(s-y-L_s)^+}dyds\right] \\ & \quad +\, a_s N^s e^{-\alpha_s d-\beta_s d^2}\int_{L_s}^{t\vee L_s} e^{r_s(s-L_s)}e^{r_d(t-s-M_d)^+}ds, \end{aligned}$$

and

$$N^s e^{-\alpha_s d-\beta_s d^2}e^{r_s(t-L_s)^+}+\gamma_0 N^d e^{\alpha_d d-\beta_d d^2}\nu\int_0^t e^{-\nu s}e^{r_s(t-s-L_s)^+}ds.$$

Analogous to (2) we report the fraction of viable cells immediately prior to the $i+1$ dose; however, we now stipulate that $i>1$ and that there are t_0 time units between dose $i-1$ and i. We then get that

$$\begin{aligned} F_{i+1}^d &= F_i^d e^{-\alpha_d d_i-\beta_d d_i^2}\left[(1-\gamma(t_0))\, e^{r_d(t-L_d)^+}+\gamma(t_0)e^{-\nu t}\right. \\ &\quad \left. +a_s\gamma(t_0)\nu\int_0^t e^{r_d(t-s-M_d)^+}\int_0^{(s-L_s)^+} e^{-\nu y}e^{r_s(s-y-L_s)^+}dyds\right] \\ &\quad + a_s\frac{F_i^s}{R}e^{-\alpha_s d-\beta_s d^2}\int_{L_s}^{t\vee L_s} e^{r_s(s-L_s)}e^{r_d(t-s-M_d)^+}ds, \\ F_{i+1}^s &= F_i^s e^{-\alpha_s d_i-\beta_s d_i^2}e^{r_s(t-L_s)^+}+\gamma(t_0)\nu R F_i^d e^{-\alpha_d d_i-\beta_d d_i^2}\int_0^t e^{-\nu s}e^{r_s(t-s-L_s)^+}ds. \end{aligned} \tag{9}$$

Similar to the original model we can incorporate asynchronous exit by treating the constants L_d, L_s, M_d as random variables and then integrating the previous display over the respective densities of these random variables. Doing so gives the formula

$$\begin{aligned} F_{i+1}^d &= F_i^d e^{-\alpha_d d_i-\beta_d d_i^2}\bar{a}(t_0,t,L_s,L_d,M_d)+F_i^s e^{-\alpha_s d_i-\beta_s d_i^2}\bar{b}(t_0,t,L_s,M_d) \\ F_{i+1}^s &= F_i^d e^{-\alpha_d d_i-\beta_d d_i^2}\bar{c}(t_0,t,L_s)+F_i^s e^{-\alpha_s d_i-\beta_s d_i^2}\bar{d}(t_0,t,L_s), \end{aligned} \tag{10}$$

where

$$\begin{aligned}
\bar{a}(t, L_s, L_d, M_d) &= \gamma(t_0)e^{-\nu t} + \lambda_d(1-\gamma(t_0))\int_{L_d}^{\infty} e^{r_d(t-x)^+}e^{-\lambda_d(x-L_d)}dx \\
&\quad + \frac{a_s\gamma(t_0)\nu\eta_d\lambda_s}{r_s+\nu}\int_{M_d}^{\infty}\int_{L_s}^{\infty} h(r_s,t,x,y)e^{-\eta_d(y-M_d)}e^{-\lambda_s(x-L_s)}dxdy \\
&\quad - \frac{a_s\gamma(t_0)\nu\eta_d\lambda_s}{r_s+\nu}\int_{M_d}^{\infty}\int_{L_s}^{\infty} h(-\nu,t,x,y)e^{-\eta_d(y-M_d)}e^{-\lambda_s(x-L_s)}dxdy \\
\bar{b}(t, L_s, L_d) &= \frac{a_s}{R}\int_{M_d}^{\infty}\int_{L_s}^{\infty} h(r_s,t,x,y)e^{-\lambda_s(x-L_s)}e^{-\eta_d(y-M_d)}dxdy \\
\bar{c}(t, L_s) &= \gamma(t_0)\nu R\int_{L_s}^{\infty}\left(\int_0^t e^{-\nu s}e^{r_s(t-s-x)^+}ds\right)e^{-\lambda_s(x-L_s)}dx \\
\bar{d}(t, L_s) &= \int_{L_s}^{\infty} e^{r_s(t-x)^+}e^{-\lambda_s(x-L_s)}dx. \qquad (11)
\end{aligned}$$

Explicit formulas for these integrals are provided in the section below, "Explicit formulas for asynchronous exit".

A simplified version of the model

The formulas in (9) and (10) are unwieldy and difficult to analyze and optimize. We therefore now simplify them by considering realistic values of some of the parameters. We will focus on simplifying (9), any resulting formulae can then be integrated to give a simplified version of (10).

We can numerically evaluate our model and learn that if we assume $L_s > 200$ and $M_d < 1$, our model still provides a good fit to the survival data. To further simplify that model we then replace M_d with 0. Another observation is that the model essentially gives the same prediction for all values of ν larger than 2 or 3, and furthermore these predictions match well with experimental results. We therefore simplify our model by taking the limit $\nu \to \infty$. The version of the model after sending $\nu \to \infty$ (without assumptions on L_s) is

$$\begin{aligned}
F^d_{i+1} &= F^d_i e^{-\alpha_d d_i - \beta_d d_i^2}\left[(1-\gamma(t_0))\,e^{r_d(t-L_d)^+} + a_s\gamma(t_0)\int_{L_s}^{t\vee L_s} e^{r_d(t-s)}e^{r_s(s-L_s)^+}ds\right] \\
&\quad + a_s\frac{F^s_i}{R}e^{-\alpha_s d - \beta_s d^2}\int_{L_s}^{t\vee L_s} e^{r_s(s-L_s)}e^{r_d(t-s)}ds, \\
F^s_{i+1} &= F^s_i e^{-\alpha_s d_i - \beta_s d_i^2}e^{r_s(t-L_s)^+} + \gamma(t_0)RF^d_i e^{-\alpha_d d_i - \beta_d d_i^2}e^{r_s(t-L_s)^+}.
\end{aligned}$$

Next if we assume that L_s is greater than the spacing between any two doses during the course of therapy, then the two integrals in the previous display will vanish for all but the final dose of radiation. Therefore if we have K total doses of radiation administered then for $i < K$ we have the much simplified form

$$\begin{aligned} F^d_{i+1} &= (1-\gamma(t_0))\, e^{r_d(t-L_d)^+} F^d_i e^{-\alpha_d d_i - \beta_d d_i^2} \\ F^s_{i+1} &= F^s_i e^{-\alpha_s d_i - \beta_s d_i^2} + \gamma(t_0) R F^d_i e^{-\alpha_d d_i - \beta_d d_i^2}. \end{aligned} \tag{12}$$

If we denote the fraction of viable cells x days after conclusion of therapy by (F^d_{K+x}, F^s_{K+x}) then we have (assume $24x \geq L_s$), using the substitution $t = 24x$,

$$\begin{aligned} F^d_{K+x} &= F^d_K e^{-\alpha_d d_i - \beta_d d_i^2} \left[(1-\gamma(t_0))\, e^{r_d(t-L_d)^+} + a_s \gamma(t_0) \int_{L_s}^t e^{r_d(t-s)} e^{r_s(s-L_s)} ds \right] \\ &\quad + a_s \frac{F^s_K}{R} e^{-\alpha_s d - \beta_s d^2} \int_{L_s}^t e^{r_s(s-L_s)} e^{r_d(t-s)} ds, \\ F^s_{K+x} &= F^s_K e^{-\alpha_s d_i - \beta_s d_i^2} e^{r_s(t-L_s)} + \gamma(t_0) R F^d_K e^{-\alpha_d d_i - \beta_d d_i^2} e^{r_s(t-L_s)}. \end{aligned}$$

Explicit formulas for asynchronous exit

In this section, we evaluate the integrals from (8).

We begin by calculating $\bar{a}(t)$, where we first will need the following

$$\begin{aligned} J^d_1(t,z) &= \lambda_d \int_{L_d}^\infty e^{z(t-t_d)^+} e^{-\lambda_d(t_d - L_d)} dt_d \\ &= \left(\frac{\lambda_d}{z+\lambda_d} e^{z(t-L_d)} + \frac{z}{z+\lambda_d} e^{-\lambda_d(t-L_d)} \right) 1_{\{t>L_d\}} + 1_{\{t<L_d\}}, \end{aligned} \tag{13}$$

for $z + \lambda_d \neq 0$. If $z + \lambda_d = 0$, then

$$J^d_1(t,z) = \left(\lambda_d e^{zt + \lambda_d L_d}(t - L_d) + e^{-\lambda_d(t-L_d)} \right) 1_{\{t>L_d\}} + 1_{\{t<L_d\}}.$$

Looking at the next term in $\bar{a}$ we see that we will need to calculate

$$\lambda_s \eta_d \int_{L_s}^\infty \int_{M_d}^\infty h(z,t,x,y) e^{-\lambda_s(x-L_s)} e^{-\eta_d(y-M_d)} dy dx,$$

for arbitrary z. Looking at the definition of h in (5) we see that it will be necessary to know the following integral for arbitrary z,

$$J_2(t,z) = \lambda_s\eta_d \int_{L_s}^{\infty}\int_{M_d}^{\infty} e^{z(t-t_d-t_s)}e^{-\lambda_s(t_s-L_s)}e^{-\eta_d(t_d-M_d)}1_{\{t_s+t_d<t\}}dt_d dt_s \quad (14)$$
$$= \frac{\lambda_s\eta_d}{\lambda_s+z}\left(\frac{e^{z(t-L_s)}e^{\eta_d M_d}\left(e^{-M_d(z+\eta_d)} - e^{-(t-L_s)(z+\eta_d)}\right)}{\eta_d+z}\right.$$
$$\left. - \frac{e^{-\lambda_s(t-L_s)}e^{\eta_d M_d}\left(e^{(t-L_s)(\lambda_s-\eta_d)} - e^{M_d(\lambda_s-\eta_d)}\right)}{\lambda_s-\eta_d}\right)1_{\{t>L_s+M_d\}}$$

for $\lambda_s + z \neq 0$. If $\lambda_s + z = 0$ then we have

$$J_2(t,z) = 1_{\{t>L_s+M_d\}}\lambda_s\eta_d e^{-\lambda_s t}e^{\lambda_s L_s+\eta_d M_d}\int_{M_d}^{t-L_s} e^{-t_d(z+\eta_d)}(t-t_d-L_s)dt_d$$
$$= 1_{\{t>L_s+M_d\}}\frac{\lambda_s\eta_d e^{-\lambda_s t}e^{\lambda_s L_s+\eta_d M_d}}{z+\eta_d}\left[(t-L_s)\left(e^{-M_d(z+\eta_d)} - e^{-(t-L_s)(z+\eta_d)}\right)\right.$$
$$\left. - M_d e^{-M_d(z+\eta_d)} + (t-L_s)e^{-(t-L_s)(z+\eta_d)} - \frac{e^{-M_d(z+\eta_d)} - e^{-(t-L_s)(z+\eta_d)}}{z+\eta_d}\right]$$

In addition we need the following

$$J_3(t,z) = \lambda_s\eta_d \int_{L_s}^{\infty}\int_{M_d}^{\infty} e^{z(t-t_s)}e^{-\lambda_s(t_s-L_s)}e^{-\eta_d(t_d-M_d)}1_{\{t_s+t_d<t\}}dt_d dt_s \quad (15)$$
$$= \frac{\lambda_s e^{zt+\lambda_s L_s}}{\lambda_s+z}\left(e^{-L_s(z+\lambda_s)} - e^{-(t-M_d)(z+\lambda_s)}\right)1_{\{t>L_s+M_d\}}$$
$$- \frac{\lambda_s e^{zt+\lambda_s L_s}e^{-\eta_d(t-M_d)}}{z+\lambda_s-\eta_d}\left(e^{-L_s(z+\lambda_s-\eta_d)} - e^{-(t-M_d)(z+\lambda_s-\eta_d)}\right)1_{\{t>L_s+M_d\}},$$

and lastly,

$$J_4(t,z) = \lambda_s\eta_d \int_{L_s}^{\infty}\int_{M_d}^{\infty} e^{z(t-t_s)}e^{-\lambda_s(t_s-L_s)}e^{-\eta_d(t_d-M_d)}1_{\{t_s<t<t_s+t_d\}}dt_d dt_s \quad (16)$$
$$= \lambda_s e^{zt}e^{\lambda_s L_s+\eta_d M_d}\left[\frac{e^{-\eta_d t}}{z+\lambda_s-\eta_d}\left(e^{-L_s(z+\lambda_s-\eta_d)} - e^{-(t-M_d)(z+\lambda_s-\eta_d)}\right)\right.$$
$$\left. + \frac{e^{-\eta_d M_d}}{\lambda_s+z}\left(e^{-(\lambda_s+z)(t-M_d)} - e^{-t(\lambda_s+z)}\right)\right]1_{\{t>L_s+M_d\}}$$
$$+ \frac{\lambda_s e^{zt}e^{\lambda_s L_s}}{\lambda_s+z}\left(e^{-L_s(\lambda_s+z)} - e^{-t(\lambda_s+z)}\right)1_{\{t<L_s+M_d\}}1_{\{t>L_s\}}.$$

With these formulas in place we can now write down an explicit expression for the integral of the function h,

$$\lambda_s \eta_d \int_{L_s}^{\infty} \int_{M_d}^{\infty} h(z,t,x,y) e^{-\lambda_s(x-L_s)} e^{-\eta_d(y-M_d)} dy dx = \frac{1}{z-r_d} \left(J_2(t,z) - J_2(t,r_d) \right) \tag{17}$$
$$+ \frac{1}{z} \left(J_3(t,z) - J_2(t,z) \right) + \frac{1}{z} \left(J_4(t,z) - J_4(t,0) \right).$$

Combining the formula for $\bar{a}$ from (8) with the previous display displays (13),(14),(15),(16) we arrive at

$$\begin{aligned} \bar{a}(t) = \gamma e^{-\nu t} + (1-\gamma) J_1^d(t,r_d) + \frac{a_s \gamma \nu}{r_s + \nu} \Bigg[& \frac{1}{r_s - r_d} \left(J_2(t,r_s) - J_2(t,r_d) \right) \\ & + \frac{1}{r_s} \left(J_3(t,r_s) - J_2(t,r_s) \right) + \frac{1}{r_s} \left(J_4(t,r_s) - J_4(t,0) \right) \Bigg] \\ & + \frac{a_s \gamma \nu}{r_s + \nu} \Bigg[\frac{1}{\nu + r_d} \left(J_2(t,-\nu) - J_2(t,r_d) \right) \\ & + \frac{1}{\nu} \left(J_3(t,-\nu) - J_2(t,-\nu) \right) + \frac{1}{\nu} \left(J_4(t,-\nu) - J_4(t,0) \right) \Bigg] \end{aligned} \tag{18}$$

Moving onto $\bar{b}$ we combine (8) with (17) to get

$$\begin{aligned} \bar{b}(t) &= \frac{a_s \eta_d \lambda_d}{R} \int_{L_s}^{\infty} \int_{M_d}^{\infty} h(r_s,t,x,y) e^{-\lambda_s(x-L_s)} e^{-\eta_d(y-M_d)} dy dx \\ &= \frac{a_s}{R(r_s - r_d)} \left(J_2(t,r_s) - J_2(t,r_d) \right) + \frac{a_s}{R r_s} \left(J_3(t,r_s) - J_2(t,r_s) \right) \\ &\quad + \frac{a_s}{R r_s} \left(J_4(t,r_s) - J_4(t,0) \right). \end{aligned} \tag{19}$$

In order to write down an expression for $\bar{c}(t)$ and $\bar{d}(t)$ we need to define the following

$$\begin{aligned} J_1^s(t,z) &= \lambda_s \int_{L_s}^{\infty} e^{z(t-t_s)^+} e^{-\lambda_s(t_s - L_s)} dt_s \\ &= \left(\frac{\lambda_s}{z+\lambda_s} e^{z(t-L_s)} + \frac{z}{z+\lambda_s} e^{-\lambda_s(t-L_s)} \right) 1_{\{t > L_s\}} + 1_{\{t < L_s\}}. \end{aligned}$$

Then from (8) and (3) we know that

$$\begin{aligned}\bar{c}(t) &= \gamma\nu\lambda_s R \int_0^\infty \int_{L_s}^\infty e^{-\nu s} e^{r_s(t-s-x)^+} e^{-\lambda_s(x-L_s)} dx ds \\ &= \frac{\gamma R}{\nu + r_s} \left(\nu J_1^s(t, r_s) + r_s J_1^s(t, -\nu)\right) - \gamma R e^{-\nu t}.\end{aligned} \tag{20}$$

Finally we have that

$$\bar{d}(t) = J_1^s(t, r_s). \tag{21}$$

Initial parameter estimate

Parameter values

In order to estimate net growth rate of the tumor bulk, we utilized the data displayed in Supplementary Table 1. The images this data is based on can be seen in Supplementary Figure S1.

Parameters used for schedule search

We then determined parameter values (see Supplementary Table 2) to identify optimized and alternative treatment schedules.

Optimization techniques

We assume that the quality of a given dosing regime is determined by measuring the population of tumor cells x days after treatment is concluded. There are of course multiple methods by which we can search for the optimal schedule. However, since this problem can be viewed as an integer programming problem it is an NP hard problem and it is therefore in general not feasible to find the absolute optimal therapy. Therefore, we settled for finding schedules that are near optimal, or at least significantly outperform a standard schedule. There are many approaches to solving such a problem, e.g., discretized dynamic programming, solving the problem without the integer constraints, and lastly a stochastic search method. Due to speed of computation, we chose the approach of a stochastic search algorithm, specifically the simulated annealing algorithm.

In order to carry out our optimization procedure it is necessary to specify the constraints on the radiation schedules we are considering. As mentioned earlier we are considering schedules implemented over a continuous five day period. Furthermore each dose of radiation can only be administered at 10 hourly appointments between 8am and 5pm. For simplicity we assume that the hourly appointments are on the hour. An additional constraint is that the total amount of radiation administered over the course of the schedule must be less than 10Gy. This amount is chosen because it has been shown that additional radiation provides little benefit in additional tumor reduction in the mouse model under consideration. In addition we do not consider the

effects of BED because the observed value of β_d is so negligible, and since this is a survival study we are not interested in long term effects of the schedule. The last constraint that we impose on our schedules is that we only allow a maximum of three treatments per day. This constraint was chosen to recreate conditions in the clinic whereby a schedule with over 3 treatments in a day would be judged as too onerous for a patient.

Before describing the algorithm, it is important to point out that simply finding an 'optimal' schedule is not sufficient for the problem at hand. In any animal population, the animals' response to radiation therapy will vary significantly from individual to individual. In the context of the mathematical model we have developed, this means that each individual will have its own unique set of parameters for the model. Thus simply finding the optimal schedule for the 'normal' parameters will not deliver the best possible schedule to every patient. However, it is possible to improve survival results by finding schedules that significantly outperform commonly used schedules across a wide region of parameter space. A direction for future research will be to develop methodologies that enable the estimation of parameters for individual patients based on the dynamic process of how their tumor responds to radiation therapy.

As mentioned above, the quality of a schedule is judged by the fraction of original cells that are present x days after K days of therapy. If the number of viable SLRC and DSC at the end of the course of treatment is given by $N^s_{1,K+x}$ and $N^d_{1,K+x}$, respectively, then we would be interested in

$$\frac{N^s_{1,K+x}+N^d_{1,K+x}}{N^s_{1,1}+N^d_{1,1}} = \frac{F^s_{1,K+x}N^s_{1,1}+F^d_{1,K+x}N^d_{1,1}}{N^s_1(1)+N^d_{1,1}} = \frac{N^d_{1,1}\left(\frac{1}{R}F^s_{1,K+x}+F^d_{1,K+x}\right)}{N^d_{1,1}\left(\frac{1}{R}+1\right)}$$
$$= \frac{\frac{1}{R}F^s_{1,K+x}+F^d_{1,K+x}}{\frac{1}{R}+1} = f\left(F^s_{1,K+x},F^d_{1,K+x}\right).$$

For any fixed schedule s we can easily find $c(s) = f\left(F^s_{1,K+x},F^d_{1,K+x}\right)$, but of course one needs to find an optimal (or in our case an improved) schedule. This is carried out via the method of simulated annealing (SA). Note that when using this optimization approach it is impossible to know whether the returned schedule is in fact the optimal schedule. However, one does know that the returned schedule satisfies some criteria such as a minimum level of improvement over a standard schedule. In fact we use this as a stopping criteria in our algorithm. We start the algorithm with the standard schedule

of 2 Gy once per day 5 days a week for w weeks, denote this schedule by s_0, and the 'cost' incurred by this schedule as c_0.

The intuitive idea of the algorithm is that one performs a random walk on the space of feasible schedules, but does not always accept random walk steps that result in a poorer performing schedule. Specifically, the random walk always accepts changes that improve the performance and only accepts those that diminish the performance with a probability (defined below). This probability starts high and shrinks to zero as the system runs for longer periods of time, see e.g. [5]. In order to describe the SA we need to define an acceptance probability and how we choose 'neighboring' schedules in our random walk. The probability of accepting a schedule with cost c' given the state of the current schedule is c on step k of the algorithm is given by

$$P(c, c', k, \phi) = \begin{cases} 1, & c' < c \\ e^{(c-c')k\phi}, & , c' > c, \end{cases}$$

where ϕ is a small positive constant.

Any implementation of simulated annealing depends closely on the generation of neighboring states. Here the state space is the space of feasible schedules. Given a feasible schedule x we generate a neighboring schedule x' as follows. We randomly select one of the time slots where radiation is administered and remove a single Gy of radiation from that slot. We then search over all feasible slots and choose one of those slots to move this Gy of radiation. Slots that are closer to the slot that we removed the Gy from are given a heavier weight. Therefore, the neighbor algorithm can be decomposed into two steps, choosing a 'donor' slot and a 'recipient' slot. The algorithm we use is described as follows

Data: Initial Schedule, $nday$, $nslot$,k
Result: Neighboring Schedule
Create vector day; (where $day(i)$ is total Gy administered on day i)
Renormalize day into probability vector $\rightarrow$ $pday$;
Choose $donor\_day$ at random according to $pday$;
Create vector $slot$; (where $slot(i)$ is Gy given during slot i on day $donor\_day$)
Renormalize $slot$ into probability vector $\rightarrow$ $pslot$;
Choose $donor\_slot$ at random according to $pslot$;
Set $j_* = donor\_day - 5\text{floor}(donor\_day/5)$; (That is j_* corresponds to the day of the week that $donor\_day$ lies on).
Create 5 dimensional vector rec_day as

$$dayw(i) = \begin{cases} k^{0.4}, & i = j_* \\ 1/|i - j_*|, & i \neq j_* \end{cases}$$

Renormalize $dayw$ into probability vector $\rightarrow$ $prec\_day$;
Choose i_* at random according to $prec\_day$;
Set $rec\_day = i_* + 5floor(donor\_day/5)$;
Create vector $cslot$ with $nslot$ entries as

$$cslot(i) = \begin{cases} k^{.125}, & i = donor\_slot \\ 1/|i - donor\_slot|, & i \neq donor\_slot \end{cases}$$

Renormalize $clsot$ into probability vector $\rightarrow$ $prec\_slot$;
Choose s_* at random according to $prec\_slot$;
If $s_* = donor\_slot$ and $rec\_day = donor\_day$ redraw s_*;

Sensitivity analysis

In order to identify important parameters and parameters that can be ignored, we performed a sensitivity analysis of the model. Specifically for all parameters we plotted model predictions as we varied a parameter. The result of this is shown in Figure S4. In order to ensure that we performed our sensitivity analysis in a physically meaningful regime, we adjusted the remaining 16 parameters as we varied the parameter of interest to ensure that the model predictions still matched the experimental observations.

In order to do this we had to develop a mathematical framework for studying the discrepancy between our model predictions and the experiment observations. For any given parameter set $\mathbf{x} = (x_1, \ldots, x_{17})$, define the function $E_1(\mathbf{x})$ to be the discrepancy between the model prediction based on parameter set $\mathbf{x}$ and the time series observation data. Specifically, if we denote our time series data by $f_1, \ldots, f_n$ and our corresponding predictions of fractional population change under parameter set $\mathbf{x}$ and two weeks of standard therapy by $f_1(\mathbf{x}), \ldots, f_n(\mathbf{x})$, then we define

$$E_1(\mathbf{x}) = \sum_{i=1}^{n} \left(f_i - f_i(\mathbf{x})\right)^2.$$

In addition we need to incorporate the survival studies into the error associated with a parameter set $\mathbf{x}$. Thus for any schedule s, denote the observed median survival date by m_s, and denote the predicted fractional population change on day m_s after exposure to schedule s under parameter set $\mathbf{x}$ by $m_s(\mathbf{x})$. Then define the function

$$E_2(\mathbf{x}) = \sum_{s \in \mathcal{S}} \left(1.75 - m_s(\mathbf{x})\right)^4,$$

where $\mathcal{S}$ is the set of schedules considered. Note that we used $\mathcal{S} = \{$hyperfractionated, optimum-1, standard, single dose, and scramble$\}$. We did not include optimum-2 because we did not have the data at the time of the analysis, and we did not include hypofractionated because it caused difficulties with our optimization algorithm. We assume that 1.75 is the fractional change associated with onset of recurrence induced sacrifice of the mouse. Note that we have used the fourth power in this formula to add further weight to the survival studies since $|\mathcal{S}| \ll n$, i.e., there are fewer data points in the survival studies than in the time series. We then define the total discrepancy associated with the parameter set $\mathbf{x}$ as

$$E(\mathbf{x}) = E_1(\mathbf{x}) + k * E_2(\mathbf{x}),$$

where $k > 0$ is chosen to add further weight to the survival data. This function can be minimized over the vector $\mathbf{x}$ to obtain a minimal amount of discrepancy, we denote this as

$$E = \min_{\mathbf{x}} E(\mathbf{x}),$$

and denote the minimizing parameter set by $\mathbf{x}^*$. We are further interested in the following minimal values for $1 \leq j \leq 17$,

$$\hat{E}_j(x) = \min\{E(\mathbf{x}) : x_j = x\},$$

that is the the minimal error when we constrain our parameter set so that parameter j takes the value of x. Denote the minimizing vector by $\hat{\mathbf{x}}_j^*(x)$. With this mathematical framework in place it is now possible to perform a sensitivity analysis of our parameter set while ensuring that we only consider parameter regimes that give predictions that are sufficiently close to our experimental observations. In particular, we generate a sensitivity plot as the jth parameter varies over the interval $[a_j, b_j]$ as follows.

1. For each value $x \in [a_j, b_j]$, create the vector $\mathbf{x}_j^*(x)$ by replacing the jth coordinate of $\mathbf{x}^*$ with x.

2. If $E(\mathbf{x}_j^*(x)) < 2 * E$, then we say that the parameter set $\mathbf{x}_j^*(x)$ is acceptable. If not we test $\hat{\mathbf{x}}_j^*(x)$. Specifically, if $E(\hat{\mathbf{x}}_j^*(x)) < 2 * E$ then we use the parameter set $\hat{\mathbf{x}}_j^*(x)$. If $E(\hat{\mathbf{x}}_j^*(x)) > 2 * E$ then we declare the value x as infeasible.

3. For each feasible value $x \in [a_j, b_j]$, we generate model predictions based on the acceptable parameter set.

Parameter Ranges

Based on our sensitivity analysis procedure we were able to identify feasible ranges for several of our model parameters. However, the parameters η_d, λ_d, L_s and M_d gave feasible answers for all values tested. This was a partial motivation for excluding these parameters in the simplified model. The relevant ranges for the remaining parameters are reported in Supplementary Table 3.

We then investigated which parameters in the model most sensitively affect its predictions (Figure 5C, Figure S4, Supplemental Information). To this end, we investigated the relative efficacy of standard therapy to optimum-1 as we varied each parameter. Predictably, the top two most important parameters are the initial radiosensitivity (α) and the proliferation rate (r_d) of DSCs, as they control the surviving fraction and rate of tumor regrowth, respectively. However, the next three most sensitive parameters are novel

components of our model: the fraction of cells capable of reversion, γ_0, the time of maximal reversion after radiation, μ, and the width of the window of reversion after radiation, σ^2. These parameters are vital for incorporating the dynamic nature of rapidly acquired resistance, and are essential to defining the relative effectiveness of the various schedules tested (Figure 5C, S4).

In panels (C) and (D) of figure S4 we observe important constraints on the parameters μ and σ^2. If the peak occurs too quickly after radiation (less than roughly 1.6 hours), then the model predicts that the scrambled control will outperform the optimum-1 schedule. Similarly, if the peak reversion occurs too long after radiation (roughly 4 hours), then the model predicts that hyperfractionated therapy will outperform the optimum-1 schedule. Since these predictions contradict our experimental observations, we conclude that a reasonable range for peak reversion (μ) is 1.6 to 4 hours. In addition, if the width of the window for reversion, σ^2, is too large (roughly 2), then the model predicts that the scrambled control will outperform the optimum-1 schedule. We therefore obtain an upper bound on reasonable values for σ^2.

Supplemental References

[1] S. Bao, Q. Wu, and et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*, 444:756–760, 2006.

[2] A. Bleau, D. Hambardzumyan, and et al. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. *Cell Stem Cell*, 4:226–235, 2009.

[3] N. Charles, T. Ozawa, and et al. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. *Cell Stem Cell*, 6:141–152, 2010.

[4] Karim Helmy, John Halliday, Elena Fomchenko, Manu Setty, Ken Pitter, Christoph Hafemeister, and Eric C. Holland. Identification of global alteration of translational regulation in glioma *in vivo*. *PLoS ONE*, 7:e46965, 10 2012.

[5] P.J. van Laarhoven and E.H. Aarts. *Simulated Annealing: Theory and Applications*. Springer, 1987.

Mouse	Day 1 Volume (mm^3)	Day 2 Volume (mm^3)	Day 3 Volume (mm^3)
M1	8.3	21.7	93.8
M2	9.2	30.5	125.9

Table S1. Growth Results for Mouse Gliomas. Related to Figure 1.

Parameter Value	Justification
$\alpha_d = .0987$	Fit from figure 1B
$\beta_d = 1.14 * 10^{-7}$	Fit from figure 1B
$\rho = 0.4$	Previous lab experience, model is robust, e.g., figure S4.
$\nu = 1.15$	[3]
$r_d = .0088$	Fit from Supplementary Table 1
$r_s = a_s = 0.0001$	Difficult to measure, model is robust, e.g., figure S4.
$L_d = 24$, $L_s = 36$	Estimate from previous experience, [4]
$\lambda_d = 0.5$, $M_d = 24$	Estimate from previous experience, [4]
$\eta_d = 0.5$, $\lambda_s = 0.35$	Estimate from previous experience, [4]
$R = 20$	[2] and [1]
$\gamma = 0.15$	Not known how to measure, used a conservative estimate.

Table S2. Parameters Used for Schedule Search. Related to Figure 2.

Parameter Value	Range	Units
α_d	$[0.005, 0.22]$	$1/Gy$
β_d	$[0, 0.0025]$	$1/Gy^2$
ρ	$[0, 1]$	Non-dimensional ratio
γ	$[0.15, 1]$	Non-dimensional ratio
ν	$[0.015, \infty)$	1/hour
r_d	$[0.0028, .0045]$	1/hour
r_s	$[0, 0.0015]$	1/hour
a_s	$[0, 0.0025]$	1/hour
L_d	$[0, 160]$	hour
λ_d	$[0.023, \infty)$	1/hour
μ	$[1.6, 4]$	hour
σ^2	$[0, 2]$	hour

Table S3. Parameter Ranges. Related to Figure 5.

Supplemental References

[1] S. Bao, Q. Wu, and et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*, 444:756–760, 2006.

[2] A. Bleau, D. Hambardzumyan, and et al. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. *Cell Stem Cell*, 4:226–235, 2010.

[3] N. Charles, T. Ozawa, and et al. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. *Cell Stem Cell*, 6:141–152, 2010.

[4] Karim Helmy, John Halliday, Elena Fomchenko, Manu Setty, Ken Pitter, Christoph Hafemeister, and Eric C. Holland. Identification of global alteration of translational regulation in glioma *in vivo*. *PLoS ONE*, 7:e46965, 10 2012.

[5] P.J. van Laarhoven and E.H. Aarts. *Simulated Annealing: Theory and Applications*. Springer, 1987.