Special Section on Quantitative Systems Pharmacology: A Foundation to Establish Precision Medicine

Radiation in Combination with Immune Checkpoint Blockade and DNA Damage Response Inhibitors in Mice: Dosage Optimization in MC38 Syngeneic Tumors via Modelling and Simulation

David Hodson, Hitesh Mistry, James Yates, Paul Farrington, Anna Staniszewska, Sofia Guzzetti, Michael Davies, Leon Aarons, and Kayode Ogungbenro
Division of Pharmacy and Optometry, Faculty of Biology, Medicine, and Health, University of Manchester, Manchester, United Kingdom (D.H., H.M., L.A., K.O.); DMPK (S.G., J.Y.) and Biosciences (P.F., A.S.), Research and Early Development, Oncology R&D, AstraZeneca, Cambridge, United Kingdom; and DMPK, Research and Early Development, Neuroscience R&D, AstraZeneca, Cambridge, United Kingdom (M.D.)

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ABSTRACT
Clinical trials assessing the impact of radiotherapy (RT) in combination with DNA damage response pathway inhibitors (DDRis) and/or immune checkpoint blockade are currently ongoing. However, current methods for optimizing dosage and schedule are limited. A mathematical model was developed to capture the impacts of RT in combination with DDRis and/or anti–PD-L1 (immune checkpoint inhibitor [ICI]) on tumor immune interactions in the MC38 syngeneic tumor model. The model was fitted to datasets that assessed the impact of RT in combination with the DNA protein kinase inhibitor (DNA PKi) AZD7648. The model was further fitted to datasets from studies that were used to assess both RT/ICI combinations as well as RT/ICI combinations followed by concurrent administration of the poly ADP ribose polymerase inhibitor (PARPi) olaparib. Nonlinear mixed-effects modeling was performed followed by internal validation with visual predictive checks (VPC). Simulations of alternative dosage regimens and scheduling were performed to identify optimal candidate dosage regimens of RT/DNA PKi and RT/PARPi/ICI. Model fits and VPCs confirmed a successful internal validation for both datasets and demonstrated very small differences in the median, lower, and upper percentile values of tumor diameters between RT/ICI and RT/PARPi/ICI, which indicated that the triple combination of RT/PARPi/ICI at the given dosage and schedule does not provide additional benefit compared with ICI in combination with RT. Simulation of alternative dosage regimens indicated that lowering the dosage of ICI to between 2 and 4 mg/kg could induce similar benefits to the full dosage regimen, which could be of translational benefit.

SIGNIFICANCE STATEMENT
This work provides a mixed-effects model framework to quantify the effects of combination radiotherapy/DNA damage response pathway inhibitors/immune checkpoint inhibitors in preclinical tumor models and identify optimal dosage regimens, which could be of translational benefit.

Introduction
Cancer is one of the leading causes of death in developed countries, where one in two people born since 1960 are projected to be diagnosed with cancer within their lifetime in the

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1Current affiliation: GlaxoSmithKline, Stevenage, United Kingdom.
2Current affiliation: Signature Discovery, Alderley Edge, United Kingdom.

Primary laboratory of origin: Division of Pharmacy and Optometry, University of Manchester (Manchester, UK).

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UK (Ahmad et al., 2015). It is estimated that approximately 50% of cancer patients are treated with radiotherapy (RT) at some stage during the course of their disease (Delaney et al., 2005). Radiotherapy offers the chance of both local and systemic control of tumors via the formation of reactive oxygen species, which induces DNA damage and can lead to apoptosis (Sia et al., 2020). This RT-mediated cell death leads to recruitment of the immune system, leading to activation of antitumor cytolytic CD8+ T cells (Zhu et al., 2021). However, radiotherapy also induces mechanisms within the tumor that enhance radioresistance, such as epithelial-mesenchymal transition (Kawamoto et al., 2012) and upregulation of immunosuppressive ligands on the surface of cells in the tumor, such as programmed death ligand 1 (PD-L1) (Gong et al., 2018).
Combinations of different pharmaceutical agents with radiotherapy may assist in delivering the antitumor effect of RT while to some extent inhibiting its immunosuppressive effects. As RT has been shown to induce immune checkpoint ligands such as PD-1/L1 (Han et al., 2020; Philippou et al., 2020), it has also been shown that the combination of RT with concurrent PD-L1 blockade [RT/immune checkpoint blockade (ICI)] is capable of inducing prolonged responses in various preclinical models (Deng et al., 2014; Grapin et al., 2019). Moreover, sequential administration of ICI after RT has been shown to improve responses in the clinic (Gray et al., 2020). Combining RT with DNA damage response pathway inhibitors (DDRis) has indicated that DDRi increases both the radiosensitivity of the tumor cells and also significantly lowers the surface expression of exhaustion markers on T cells. This leads to robust T-cell infiltration and complete responses (Nakamura et al., 2021). Although trials for RT/ICI, RT/DDRi, and RT/DDRi/ICI are currently ongoing, preclinical models have shown that a combination of RT/ICI and the ataxia-telangiectasia related (ATR) inhibitor AZD6738 significantly prolongs responses in syngeneic models of hepatocellular carcinoma. This improved efficacy was shown to be linked with increased T-cell infiltration and reduced T-cell exhaustion marker expression (Sheng et al., 2020).

Optimization of dosage for either combination treatment modality is likely to be tumor specific. Clinical evidence from the PACIFIC trial has implicated that sequential use of ICI after RT was capable of inducing prolonged responses (Gray et al., 2020). Preclinical evidence, however, suggests that concurrent administration of anti-programmed death 1 (PD-1) or anti–PD-L1 during a standard week-long fractionated dosage regimen of radiotherapy may be more appropriate compared with sequential administration (Dovedi et al., 2014). In addition, ICIs such as anti–PD-L1 have been shown to induce various immune-related adverse effects, such as colitis and thyroiditis (Su et al., 2020). Modification of the dosage regimen, which can maintain efficacy while reducing adverse effects when using these treatment modalities in combination with RT, could further reduce dropout and increase compliance (Czobor and Skolnick, 2011).

Mathematical modeling can assist in identifying candidate dosage regimens, which could be of translational benefit. Quantitative systems pharmacology (QSP) models have previously predicted and verified differences in the effect of dosage and schedule on CT-26 tumor models treated with different RT/ICI regimens (Kosinsky et al., 2018). CT-26, however, is considered an immunologically hot tumor that readily responds to both of these treatments, even when incorporated as a monotherapy (Mosely et al., 2017). In this respect, mathematical models could be further used to capture potential differences in response across different tumor models and possibly explain some of the variability in responses to drugs observed within the clinic. Although there are mathematical models available that assess the impact of RT/DDRi (Checkley et al., 2015) and RT/ICI (Kosinsky et al., 2018) on tumor growth, a model describing the impacts of RT/DDRi/ICI on tumor growth is currently lacking.

The aim of this study was to develop a mathematical model of RT/DDRi/ICI combinations to identify optimal dosage regimens in the context of the solid syngeneic tumor model MC38. The model incorporates some fundamental aspects of the RT-induced immune response while also allowing DDRi and ICI to reduce negative feedback. Optimal dosage regimens were identified for studies involving RT in combination with the DNA protein kinase inhibitor (DNAPKi) AZD7648 or studies assessing RT in combination with anti–PD-L1 (ICI) and/or olaparib (poly ADP ribose polymerase inhibitor (PARPi)).

**Methods**

**The Mathematical Model**

**Tumor Compartments.** The full model schematic is shown in Fig. 1. The tumor is assumed to consist of a proliferating rim \( (P(t)) \) and a quiescent core \( (Q(t)) \). \( P(t) \) is assumed to grow logistically, where the competitive death term acts to transfer cells from \( P(t) \) into \( Q(t) \). \( P(t) \) can

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**Fig. 1.** Schematic representation of mathematical model of tumor and immune response fitted to tumor growth data in MC38 syngeneic mouse models. Fitted parameters are highlighted in bold with their respective roles within the model. Created with BioRender.com.
be targeted by T cells (T(t)) or natural killer (NK) cells (N(t)), which then leads to a release of cells from Q(t) back into P(t), where they are now susceptible to T-cell-mediated immunogenic cell death as described by eq. 1.

\[
p(t) = \lambda \cdot P(t) \cdot \left( 1 - \frac{P(t)}{K} \right) - K_{PT} \cdot P(t) \cdot (T(t) + N(t))
\]

\[
\frac{dP(t)}{dt} = \lambda \cdot P(t) \cdot \left( 1 - \frac{P(t)}{K} \right) - K_{PT} \cdot P(t) \cdot (T(t) + N(t))
\]

where \( \lambda, K, K_{PT}, K_{PTQ}, \) and \( K_R \) are the baseline tumor growth rate, maximum rim depth, rate of cytotoxic cell-mediated destruction of the rim, rate of core reoxygenation, and affinity of transfer from the core into the rim, respectively.

**DDRi-Associated Compartments.** It is assumed that the DDRi (Z(t)) can only function to augment the immunogenic effects of RT. The pharmacokinetics (PK) of both PARPi and DNAPKi suggest short half-lives of each drug, and that the drug elimination rate is linear (Sun et al., 2018; Fok et al., 2019). Empirical modeling describes multiple dosing of drugs with linear PK is derived from similar assumptions, which are used to formulate empirical PK models of drugs that are infused intravenously at a constant rate (Peck and Williams, 2008). As concentration measurements during the experiment were not available, the linear plasma concentration profile resulting from multiple dosing was approximated with the corresponding average steady-state concentration throughout the drug dosing schedule. This is represented as a Heaviside function active during the first 4 days of treatment (eq. 2). The Heaviside function is represented in NONMEM as in IF statement, which considers time as a time-varying covariate incorporated into the data file. To confirm that treatment is still active at day 4, the Heaviside function extends by 0.1 days beyond the final day of treatment (supplemental code for use in NONMEM; Supplemental data file). The DDRi is assumed to induce additional cell death (V(t)), which does not impact the expected measured tumor volume and in- treatment (supplemental code for use in NONMEM; Supplemental

\[
\frac{dZ(t)}{dt} = K_{Zt} \cdot \text{Heaviside}(4.1 - \text{TIME})
\]

\[
\text{Drug elimination rate} = K_{Zt} \cdot Z(t)
\]

\[
\frac{dV(t)}{dt} = K_{ZV} \cdot Z(t) \cdot \frac{V(t)}{K_{Z} + Z(t)}
\]

\[
\text{Removal of dying cells within the tumour} = K_{VT} \cdot V(t)
\]

Where \( K_{Zt}, K_{Zt}, K_{ZV}, K, \) and \( K_{VT} \) are the rate of DDRi infusion, DDRi elimination rate constant, rate of cell death induced by DDRi, cellular IC_{50} of the DDRi, and natural degradation rate of dying cells, respectively.

**Pharmacokinetics of Anti-PD-L1.** Deng et al. (2016) indicated that the PK of intravenously injected anti-PD-L1 in mice is linear for doses of 1 mg/kg; however, at doses of 10 mg/kg, the clearance of anti-PD-L1 is nonlinear. Assessment of cellular surface PD-L1 expression via flow cytometry indicated complete blockade of surface PD-L1 for between 7 and 14 days after a single 10 mg/kg dose of anti-PD-L1. Due to the lack of linearity the concentration of anti-PD-L1 (I(t)) is assumed to be Heaviside for 8 days before naturally declining (eq. 3). This attempts to coincide with the first week of dosing.

\[
\frac{dI(t)}{dt} = K_{I_0} \cdot \text{Heaviside}(8.1 - \text{TIME})
\]

\[
\text{Antibody elimination rate} = \frac{K_{I_0}}{K_{I_0} + \text{I}(t)}
\]

Where \( K_{I_0} \) and \( K_{I_0} \) are the ICI infusion rate and ICI elimination rate constant, respectively.

**Immune Compartments.** The immune compartments consist of T(t) and N(t) as well as antigen-presenting cells (APCs) (A(t)) and exhaustion receptors (X(t)). A(t) is upregulated by RT (R(t)), which is assumed to be a Heaviside function active between days 0 and 4. A(t) then induces activation of T(t) and decays naturally. T(t) functions to target tumor cells but also induces activation of X(t). X(t) binds with T(t), which leads to T(t) degradation. X(t) is assumed to also decay naturally. Both V(t) and I(t) are assumed to noncompetitively inhibit the rate at which X(t) is synthesized by T(t). N(t) is assumed to be induced by DNAPKi explicitly (eq. 4). This DNAPKi-mediated induction of N(t) is assumed to be due to differences in the initial effects of RT/ PARPi compared with RT/DNAPKi on the observed tumor diameter, combined with recent data suggesting that RT/DNAPKi may induce higher levels of cytolytic enzymes in NK cells compared with RT during MC38 tumor challenge (Namakura et al., 2021).

\[
\frac{dA(t)}{dt} = K_{A_0} A_{\text{Baseline induction}} + \alpha \cdot P(t) \cdot R(t) - K_{A_0} A(t)
\]

\[
\frac{dT(t)}{dt} = K_A A(t) - K_{T_0} T(t) \cdot X(t)
\]

\[
\frac{dX(t)}{dt} = \frac{\beta \cdot T(t)}{1 + \gamma T(t)} - K_{X_0} X(t)
\]

\[
\frac{dN(t)}{dt} = -K_{N_0} N(t)
\]

\[
R(t) = 2 \cdot \text{Heaviside}(4.1 - \text{TIME})
\]

Where \( K_{A_0}, \alpha, K_{A_0}, K_A, K_{T_0}, \beta, \gamma, K_{X_0}, \delta \) and \( K_{N_0} \) are the baseline influx rate of APCs, RT-mediated influx rate of APCs, APC natural death rate, APC-mediated upregulation rate of T cells, T-cell exhaustion rate, T-cell exhaustion receptor synthesis rate, exhaustion receptor natural decline rate, DNAPKi-mediated NK cell upregulation rate, and NK cell death rate, respectively.

**Experimental Data.** The mathematical model was fitted separately to datasets containing pooled experiments designed to assess the impact of different combination therapies on tumor growth. Table 1 lists all experiments used in each dataset. The majority of these
datasets were derived from time-to-event (TTE) experiments that were designed to assess the efficacy of different treatment modalities and combinations on tumor growth in MC38 syngeneic models, where efficacy of each treatment was measured by the amount of time for the tumor to reach 1 cm³. Mice were sacrificed when the tumor reached over 1 cm³ or when the tumor was presenting as a wet ulcer-pooled experiments. Two experiments (KN2005 and DNAPK1908) were designed to assess the efficacy of RT in combination with 2.5 mg/kg ATM with ICI (DDRIO1824 and DDRIO1835). Due to sparsity of data, heterogeneity of cohort sizes, and interstudy variability, any cohorts incorporating ATMi into the dosage regimen were removed from the dataset and this analysis. Three (DDRIO1815, DDRIO1821, and DDRIO1824) of the four datasets consisted of 99 mice split over eight cohorts. Fifteen (15) mice were in the control cohort and were given no therapy or mock oral and intraperitoneal treatments (control group and cohort 1). Twelve (12) mice were used in each of the other cohorts. Three cohorts (cohorts 2–4) of 12 mice each were given either RT (RT group), DDRi, or ICI as monotherapies; three cohorts (cohorts 5–7) of 12 mice each were given either ICI plus DDRi (ICI/DDRi cohort) or RT plus DDRi (RT/DDRi cohort) or RT plus ICI (RT/ICI cohort); and one final cohort was given all three treatments (RT/DDRi/ICI cohort). However, one experiment (DDRIO1835) involving ATMi inhibition only had 12 control mice, with six mice per cohort, which were treated with RT/ATMi or RT/ICI. Data points below the limit of quantification (2.4 mm) as well as data points that were recorded as “no tumor” (1 mm) were included before model fitting.

**Model Fitting and Simulation.** A list of the fixed model parameters, definitions, values, and sources is shown in Table 2. Datasets 1 and 2 were fitted using NONMEM version 7.4.3 using FOCE followed by SAEM. Log likelihood values (−2LL_{\text{SAEM}}) and relative standard errors were calculated using importance sampling with an expectation-only step, followed by a final covariance step. Interindividual variability (IIV) was incorporated into the baseline size of the tumor’s quiescent core ($Q_0$), the baseline tumor growth rate ($\lambda$), and the rate of exhaustion receptor synthesis ($\beta$). IIV was assumed to be log-normally distributed and is represented by a $3 \times 3$ block matrix. Random unexplained variance was assumed to be additive. As there was no improvement in survival or tumor control after the administration of either DDRi or ICI as monotherapy (not shown), the data were excluded from the analysis prior to fitting.

One hundred simulations of datasets 1 and 2 were performed using the model output results produced from the model fit; these were used to plot visual predictive checks (VPCs) using R version 3.6.3 (https://www.R-project.org/), overlaying the 2.5%, 50%, and 97.5% percentiles of the observed data with the corresponding areas from simulations.
DNAPIK was given at doses that lead to significantly higher free plasma concentrations than the cellular free in vitro IC\(_{50}\) (Fok et al., 2019; Goldberg et al., 2020). Similarly, PARPi was given at doses where the free PARPi concentration is two orders of magnitude larger than the cellular free in vitro IC\(_{50}\) (Norris et al., 2014) while radiation is being administered. This relationship between average drug concentration and the IC\(_{50}\) was incorporated into the model.

This analysis aims to address the impact of the number of days that the DDRi is given concurrently with the RT on tumor growth. The function, assuming a full 5-day course of DDRi, is shown in eq. 2. Equation 5 shows the Heaviside function describing DDRi administration for different lengths of time, where (d) represents the number of days DDRi is given concurrently with RT. Simulations of different DDRi dosage regimens were performed by modifying d between 1 and 5 in increments of 1.

\[
\frac{dZ(t)}{dt} = K_{\text{Heaviside}} \left( \frac{d}{5} \times \text{TIME} \right) - K_{\text{DDRi}} \cdot Z
\]

As this model fits an estimate for the pharmacodynamic IC\(_{50}\) of the drug dosage given a 10 mg/kg dose of ICI, model simulations assessing different dosage regimens of ICI were produced via modifications of the peak concentration during the Heaviside phase of the drug concentration curve. These were performed by multiplying \(K_{\alpha}\) (eq. 3) by values between 0.1 and 1 in increments of 0.1.

One hundred simulations of each candidate dosage regimen were performed in NONMEM \(v\) 7.4.3. One hundred simulations of dataset 1 were used to assess different RT/DNAPKi dosage schedules. 97.5%, 50%, and 2.5% percentiles of each simulated dataset were plotted in R \(v\) 3.6.3 to assess the impact of different RT/DNAPKi dosage schedules on tumor diameters. For assessment of different RT/PARPi/ICI schedules, 100 simulations of a dataset for a virtual study that assesses 12 mice per cohort, with tumor sizes observed every day between days 0 and 34, was used. Simulated tumors that were below 2.4 mm at day 34 were considered complete responders. Overall response was characterized by tumors that achieved a tumor diameter reduction of 1.39 mm from day 0 at any point during the simulated time course. To assess T-cell infiltration, the area under the curve of the expected T-cell profile was calculated. Heat maps of each metric with respect to dosage regimen were plotted in R \(v\) 3.6.3.

### Local Sensitivity Analysis

Parameters were assessed for their impact on the expected tumor trajectory by day 21 individually. Simulations of the model, with each parameter modified by adding or subtracting 20% from the model fit outputs calculated when fitting for RT/PARPi, were performed using MATLAB R2019. Plotting of the expected change in tumor size by day 21 was then performed in R \(v\) 3.6.3.

### Results

The mathematical model was fitted to dataset 1 to observe whether the model could appropriately capture the effect of RT and RT/DNAPKi on the observed tumor volume in the context of MC38 syngeneic tumor models. Final parameter estimates are listed and described in Table 3 with relative standard errors for each estimated parameter; IIV is represented as the coefficient of variation. The model fit estimated the NK cell influx rate (\(b\)) to be 73.8 cells day\(^{-1}\). This indicated that over the initial 5 days of RT/DNAPKi treatment, the baseline growth rate was impeded by approximately 0.07 mm day\(^{-1}\). The effect of DNAPKi on T-cell exhaustion (\(u\)) was estimated to be approximately 0.0181 cells; when accounting for the half-life of dying cells and the rate of cell death, this indicates a 99% reduction of exhaustion receptor synthesis on average over the expected time course when dying cells are

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**TABLE 2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Units</th>
<th>Value</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P_0)</td>
<td>Initial rim depth</td>
<td>mm</td>
<td>2.4</td>
<td>Salem et al., 2019*</td>
</tr>
<tr>
<td>(A_0)</td>
<td>Initial DC concentration</td>
<td>Cells mm(^{-1})</td>
<td>0</td>
<td>Fixedb</td>
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<tr>
<td>(T_0)</td>
<td>Initial active CD8 concentration</td>
<td>Cells mm(^{-1})</td>
<td>0</td>
<td>Fixedb</td>
</tr>
<tr>
<td>(X_0)</td>
<td>Initial exhaustion receptor level</td>
<td>Receptors</td>
<td>0</td>
<td>Fixedb</td>
</tr>
<tr>
<td>(V_0)</td>
<td>Initial amount of dying cells</td>
<td>Cells</td>
<td>0</td>
<td>Fixedb</td>
</tr>
<tr>
<td>(Z_0)</td>
<td>Initial DDRi concentration</td>
<td>(\mu)M</td>
<td>3.25</td>
<td>Fok et al., 2019*</td>
</tr>
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<td>(I_0)</td>
<td>Initial ICI concentration</td>
<td>(\mu)g ml(^{-1})</td>
<td>100</td>
<td>Deng et al., 2016*</td>
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<td>(K)</td>
<td>Rim-carrying capacity</td>
<td>mm</td>
<td>2.4</td>
<td>Salem et al., 2019*</td>
</tr>
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<td>(K_{\text{Heaviside}})</td>
<td>CD8 rim killing rate</td>
<td>Cell(^{-1}) day(^{-1})</td>
<td>0.001</td>
<td>Kosinski et al., 2018*</td>
</tr>
<tr>
<td>(K_{\text{Heaviside}})</td>
<td>CD8 transfer of core to rim</td>
<td>Cell(^{-1}) day(^{-1})</td>
<td>0.001</td>
<td>Fixedd</td>
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<td>(K_{\alpha})</td>
<td>Transfer affinity from the core</td>
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<td>Fixedd</td>
</tr>
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<td>(K_{\text{Heaviside}})</td>
<td>Baseline influx of APCs</td>
<td>Cells day(^{-1}) mm(^{-1})</td>
<td>0.001</td>
<td>Fixedd</td>
</tr>
<tr>
<td>(K_{\text{Heaviside}})</td>
<td>APC natural death rate</td>
<td>Day(^{-1})</td>
<td>0.648</td>
<td>Kronik et al., 2010e</td>
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<td>(K_{\text{Heaviside}})</td>
<td>NK cell natural death rate</td>
<td>Day(^{-1})</td>
<td>0.648</td>
<td>Fixedd</td>
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<tr>
<td>(K_{\text{Heaviside}})</td>
<td>DDRi elimination rate</td>
<td>Day(^{-1})</td>
<td>9.12</td>
<td>Kronik et al., 2010e</td>
</tr>
<tr>
<td>(K_{\text{Heaviside}})</td>
<td>T-cell exhaustion rate</td>
<td>Day(^{-1}) receptor(^{-1})</td>
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<td>Fixedd</td>
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<td>DDRi elimination rate</td>
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<td>(K_{\text{Heaviside}})</td>
<td>Dying cell induction rate</td>
<td>Cells day(^{-1})</td>
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<td>(K_{\text{Heaviside}})</td>
<td>Dying cell elimination rate</td>
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<td>0.17</td>
<td>Kroniski et al., 2018*</td>
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<td>ICI infusion rate</td>
<td>(\mu)g ml(^{-1}) day(^{-1})</td>
<td>69</td>
<td>Deng et al., 2016*</td>
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<td>(K_{\text{Heaviside}})</td>
<td>ICI elimination rate</td>
<td>Day(^{-1})</td>
<td>0.69</td>
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<td>(K_{\text{Heaviside}})</td>
<td>Exhaustion marker elimination rate</td>
<td>Day(^{-1})</td>
<td>0.099</td>
<td>Nakamura et al., 2021f</td>
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<td>(K_{\text{Heaviside}})</td>
<td>DDRi IC(_{50})</td>
<td>(\mu)M</td>
<td>0.091</td>
<td>Goldberg et al., 2020g</td>
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</table>

*Calculated from data in citation.  
**Assumes baseline immune response is negligible.  
†Direct reference.  
‡Fixed to ensure that the core is released into the rim at the same rate at which rim is removed by the immune system.  
§Fixed to assume NK cell death scales with APC cell death.  
¶Fixed to assume that exhaustion scales with death in the rim per unit receptor.  
#Fixed arbitrarily.
present within the tumor. This reduction in exhaustion marker synthesis to near baseline levels during RT/DNAPKi treatment is consistent with results observed by Nakamura et al. (2021).

Graphical Model Diagnostic with VPC

To simulate the impact of drop out, only simulated tumor diameters of 11.44 mm or smaller were included in the VPCs, corresponding to tumors that reach 1.5 cm\(^3\). Parameter estimates in Table 2 were used for this purpose. The median, upper, and lower percentiles of the raw dataset were generally well captured within the range of simulated upper, median, and lower percentiles in control-, RT-, and RT/DNAPKi-treated cohorts (Fig. 2). The model simulations capture the lack of variability in tumor sizes that are observed in RT/DNAPKi cohorts throughout the course of the experiment, which is consistent with the strong effects of RT/DNAPKi on MC38 syngeneic tumor models. The VPCs indicate that the lower-bound and median estimates are less well estimated; this is partially due to the lack of variability in the data points due to the strength of the response.

Simulation of a Modified Dosage Schedule of RT/DNAPKi Treatment

Although simulations indicated that the median and lower quantile values are likely to be underestimated, it was considered appropriate to assess alternative dosage regimens of the model with these caveats in mind. A larger variability in observed tumor responses would likely be beneficial when assessing the impact of different biomarkers on tumor growth in the preclinical setting. Therefore, the model was used to investigate whether simulations of an alternative dosage regimen would correspond to a sufficient increase in the variability of tumor trajectories. Upper, median, and

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (RSE %)</th>
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<tbody>
<tr>
<td>(Q_0)</td>
<td>Initial core depth (mm)</td>
<td>3.12 (2)</td>
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<tr>
<td>(\lambda)</td>
<td>Baseline tumor growth rate (d(^{-1}))</td>
<td>0.221 (3)</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>RT-mediated APC recruitment rate (cells d(^{-1}) G(^{-1}))</td>
<td>1.52 (2)</td>
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<tr>
<td>(\delta)</td>
<td>T-cell–mediated exhaustion receptor recruitment rate (d(^{-1}))</td>
<td>1.08 (13)</td>
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<tr>
<td>(\delta)</td>
<td>DNAPKi-mediated NK cell recruitment rate (cells d(^{-1}))</td>
<td>73.8 (9)</td>
</tr>
<tr>
<td>(\gamma_v)</td>
<td>Dying cell exhaustion receptor IC(_{50}) (cells)</td>
<td>0.0181 (32)</td>
</tr>
<tr>
<td>(\eta_{Q_0})</td>
<td>IV in (Q_0) (CV%)</td>
<td>18.1 (16)</td>
</tr>
<tr>
<td>(\eta_\lambda)</td>
<td>IV in (\lambda) (CV%)</td>
<td>25.5 (17)</td>
</tr>
<tr>
<td>(\eta_\delta)</td>
<td>IV in (\delta) (CV%)</td>
<td>141 (38)</td>
</tr>
<tr>
<td>(\text{Cov}(\eta_{Q_0}, \eta_\lambda))</td>
<td>Covariance between (Q_0) and (\lambda) (unitless)</td>
<td>-0.0171 (34)</td>
</tr>
<tr>
<td>(\text{Cov}(\eta_{Q_0}, \eta_\delta))</td>
<td>Covariance between (Q_0) and (\delta) (unitless)</td>
<td>0.00716 (207)</td>
</tr>
<tr>
<td>(\text{Cov}(\eta_\lambda, \eta_\delta))</td>
<td>Covariance between (\lambda) and (\delta) (unitless)</td>
<td>-0.0436 (177)</td>
</tr>
<tr>
<td>(\sigma)</td>
<td>RV (mm(^2))</td>
<td>0.197 (5)</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; RSE, relative standard error; RV, Random Unexplained Variability.

Fig. 2. VPCs of model fit output from dataset 1. The 2.5, 50, and 97.5 percentile tumor diameters of the observed datasets (dashed lines) are overlaid with the corresponding range of 2.5, 50, and 97.5 percentile tumor diameters from simulated datasets (shaded regions) from (A) Control cohorts, (B) RT-treated cohorts, (C) RT/DNAPKi-treated cohorts.
lower percentile estimates from simulations of the full dosage regimen (Fig. 3A) indicate that tumors are expected to be below the limit of quantification by day 15, with minimal likelihood of relapse. However, simulations of a single RT/DNAPKi dose followed by four consecutive doses of RT indicated that the median tumor size is expected to be around the limit of quantification at approximately day 15. In addition, comparison of the simulated 97.5% percentiles from day 15 onwards shows that the 97.5% percentile is expected to show growing tumors from this time point. This would potentially be indicative of tumors that would have sufficient levels of variability at day 15 to observe candidate biomarkers implicated in the response to RT/DNAPKi treatment.

Model Fitting to RT/PARPi/ICI-Treated Cohorts

The results of the model fit following RT/PARPi/ICI treatments (Table 4) indicated sufficiently identifiable parameter values for both fixed effects and random effects. Parameters describing the effects of PARPi indicated that PARPi reduces the rate of exhaustion receptor expression by approximately 66% on average while dying cells were present within the tumor. The results also indicated that the ICI dose administered at the current schedule reduces the rate of exhaustion receptor expression by 96% during the infusion period. This is indicative of a significantly larger difference in expected tumor responses to ICI compared with PARPi at the given dosages and schedules.

Graphical Model Diagnostic with VPC

The upper, lower, and median bound of VPC for control-, RT-, RT/PARPi-, RT/ICI-, and RT/PARPi/ICI-treated cohorts from model simulations all had strong overlap with each of the observed percentile estimates from respective treatment cohorts. In addition, the model captures how the upper, median, and lower percentile estimates of RT/ICI are comparable to the corresponding percentile estimates following RT/PARPi/ICI treatments. This indicates that at the given dosage and schedule, there is no observable benefit of incorporating PARPi into an RT/ICI dosage regimen in the context of MC38 syngeneic tumor models as the effects of ICI dominate the response.

Simulation of Alternative Dosage Regimen

Given the lack of PK data available within the current datasets, PK parameters were fixed for all subjects to typical

TABLE 4
List of parameters estimated when dataset 2 was fitted

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition (units)</th>
<th>Value (RSE %)</th>
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<tr>
<td>Q₀</td>
<td>Initial core depth (mm)</td>
<td>3.72 (2)</td>
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<tr>
<td>λ</td>
<td>Baseline tumor growth rate (d⁻¹)</td>
<td>0.212 (3)</td>
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<tr>
<td>α</td>
<td>RT-mediated APC recruitment rate (cells⁻¹ Gy d⁻¹)</td>
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</tr>
<tr>
<td>β</td>
<td>T-cell-mediated exhaustion receptor recruitment rate (d⁻¹)</td>
<td>1.67 (17)</td>
</tr>
<tr>
<td>γ_v</td>
<td>Dying cell exhaustion receptor IC₅₀ (cells)</td>
<td>0.727 (31)</td>
</tr>
<tr>
<td>γ_I</td>
<td>ICI exhaustion receptor IC₅₀ (µg ml⁻¹)</td>
<td>3.65 (30)</td>
</tr>
<tr>
<td>η_q₀</td>
<td>IV in Q₀ (CV%)</td>
<td>21.9 (16)</td>
</tr>
<tr>
<td>η_I</td>
<td>IV in λ (CV%)</td>
<td>28 (20)</td>
</tr>
<tr>
<td>η_6</td>
<td>IV in 6 (CV%)</td>
<td>285 (23)</td>
</tr>
<tr>
<td>Cov(Q₀, λ)</td>
<td>Covariance between Q₀ and λ (unitless)</td>
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</tr>
<tr>
<td>Cov(Q₀, β)</td>
<td>Covariance between Q₀ and β (unitless)</td>
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</tr>
<tr>
<td>Cov(β, η_6)</td>
<td>Covariance between β and η_6 (unitless)</td>
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</tr>
<tr>
<td>σ</td>
<td>Random Unexplained Variability</td>
<td>0.5 (4)</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; RSE, relative standard error; RV, Random Unexplained Variability.
individual values. In addition, given that ICI is given at doses that drive exposures significantly higher than the expected dissociation constants and that PARPi is given at doses achieving plasma concentrations two orders of magnitude higher than the expected IC₅₀, the effects observed for ICI and PARPi were considered the maximum effects observable within these treatments. Simulations were then performed to assess the impact of theoretically lower dosage regimens, with lower exposure to both drugs (see Methods). The aims were to assess whether there could be doses of ICI and PARPi that could implicate significant differences in efficacy between RT/ICI and RT/PARPi/ICI, as well as identify doses of RT/PARPi/ICI that could lead to sufficiently high levels of T-cell responses, comparable to those simulated in the current study. Comparisons of complete response rate (CRR), overall response rate (ORR), and T-cell area under the curve (AUC) were used to infer differences in efficacy.

![Fig. 4. VPCs of model fit output from dataset 2. The 2.5, 50 and 97.5 percentile tumor diameters of the observed datasets (dashed lines) are overlaid with the corresponding 2.5, 50 and 97.5 percentile tumor diameters from simulated datasets (shaded regions) from (A) Control cohorts, (B) RT-treated cohorts, (C) RT/PARPi-treated cohorts, (D) RT/ICI-treated cohorts, (E) RT/PARPi/ICI-treated cohorts.](image)

![Fig. 5. Simulations of alternative RT/PARPi/ICI dosing regimens and their respective impacts on (A) ORR, (B) CRR, and (C) total T-cell infiltration over the projected time course [area under the curve (AUC)].](image)
between different candidate dosage regimens. When assessing CRR (Fig. 5A), assuming the full PARPi dosage schedule is given, the difference in the percentage of CRR between RT/ICI and RT/PARPi/ICI treatments increases between 0–5 mg/kg of ICI. However, after 5 mg/kg ICI, there is no net increase in CRR between RT/ICI and RT/PARPi/ICI larger than 15%. Similarly, differences between ORR (Fig. 5B) in RT/ICI-treated mice and RT/PARPi/ICI-treated mice, assuming the full PARPi dosage is given, indicate that doses of between 1–3 mg/kg of RT/ICI lead to the largest observable differences in ORR between RT/ICI and RT/PARPi/ICI, whereas doses above 4 mg/kg exhibit no changes in the percentage of difference in ORR between RT/ICI and RT/PARPi/ICI. When assessing T-cell influx (Fig. 5C), doses of 4 mg/kg RT/ICI in combination with a full PARPi regimen appear to induce similar T-cell influxes over a 34-day period to the full RT/ICI dosage regimen, which is in line with the similar expected CRR rates observed in this treatment cohort compared with the 10 mg/kg RT/ICI CRR rate. Collectively, this indicates that the doses of approximately 4 mg/kg given over the same time frame as observed during these experiments could be a more appropriate dosage to observe the benefits of RT/PARPi/ICI treatments compared with RT/ICI treatments.

**Local Sensitivity Analysis.** A local sensitivity analysis was performed on all parameters of each model compartment (Fig. 6); these included all fitted parameters. The majority of parameters modestly impacted the expected tumor size (Supplemental Table 1). The baseline tumor growth rate was found to have the largest impact on observed tumor size, with $K_{RT}$ having the second-largest impact on tumor size. Interestingly, the model was not very sensitive to the T-cell rate of exhaustion receptor expression. However, in the non-linear mixed-effects model, this was the parameter that IVV was fitted to that led to the largest improvement in model fit (not shown). This may, in fact, be one of the major reasons for IVV in $\beta$ being larger in the RT/PARPi/ICI model fit compared with the RT/DNAPKi model fit to account for this moderate lack of sensitivity.

**Discussion**

Dosage optimization for both preclinical and clinical experiments requires extensive experimental data. Mathematical modeling and simulation have the potential to identify dosage regimens that may be optimal when assessing efficacy of combination therapies or assessing pharmacodynamic biomarkers. Simulation can also assist in avoiding the overuse of animal subjects when finding these optimal regimens, in line with the principles listed within the three Rs of animal research (reduction, refinement, and replacement), which minimize the use and suffering of animals (Blattner, 2019). This study describes a successful model formulation, validation, and simulation of RT/DDRi and RT/DDRi/ICI combinations in a preclinical context, with the potential for the model to be used when optimizing dosing schedules for pharmacodynamic study design as well as minimizing dosage while retaining efficacy.

It was observed from preliminary analyses that there was not a large degree of interstudy variability between response rates, growth rates, or baseline tumor size within either the RT/DNAPKi or RT/PARPi/ICI dataset. Consequently, a study effect was not incorporated into the final model. The results of model fitting to dataset 1 (Fig. 2) indicated that the parameter estimations from the fitted RT/DNAPKi dataset captured the lack of variability in responses at the given dosage and schedule. The lack of variability and strength of the observed responses to RT/DNAPKi makes the task of producing models capable of fitting the observed data more difficult. However, the lack of interstudy variability would also mean that fitted models would more likely capture observed responses in external datasets. In a recent study on development of a QSP model that described how antibody-mediated blockade of cytotoxic T lymphocyte antigen 4 (CTLA-4) impacted CT-26 syngeneic tumors, models required refitting the external dataset in accordance with the baseline growth rate to improve model validation (Qiao et al., 2022). Thus, dosage modification to increase the variability in response, in combination with a richer dataset, may provide an avenue for more improved model fitting. However, experimental protocols need to be robust enough to ensure sufficiently low levels of interstudy variability, which will aid external validation.

Simulations of alternative dosing regimen of RT/DNAPKi indicated that a single dose of RT/DNAPKi, followed by four doses of RT, would sufficiently increase the variability in the response observed, which would facilitate identification of candidate biomarkers implicated in the MC38 tumor response to RT/DNAPKi. The model specification with built-in negative feedback would suggest continuous repeated dosing of RT before administering doses of RT/DNAPKi would be less efficient due to higher expression of exhaustion markers. However, this model ignores the direct effects that RT has on tumor cells. RT functions to directly kill tumor cells, which leads to changes in the distribution of oxygen within the tissue (Crook et al., 2005), as well as changes in the distribution of cells in different phases of the cell cycle (Pajonk et al., 2010). These direct effects would further be augmented during concurrent treatment with DDRis. In addition, direct comparison of 2 Gy RT and 2 Gy RT/DNAPKi on the cell cycle distributions of A459 cells in vitro have indicated significant increases in the percentage of cells in the G2/M cell cycle phase. This effect was also indicated to be dose dependent (Fok et al., 2019). Furthermore, fractionated radiotherapy also leads to repopulation of the tumor, with changes in the distribution of cells that are radiosensitive and radioresistant, which also impacts the effects of...
each subsequent fractionated dose of RT (Baskar et al., 2014). A model specification that describes both the direct and indirect effects of radiation may imply an alternative optimal dosing schedule.

Identifying the appropriate schedule to give RT/DDRi will likely require additional data, a different mathematical analysis, and an alternative model specification than what is discussed within the scope of this report. Dosage optimization strategies such as genetic algorithms (McCall, 2005) could be employed, which attempt to compromise between the beneficial effects of each fractionation step on tumor control in terms of direct cell killing and immuno-activation, with the consequent induced immunosuppression. Alternatively, identification of the appropriate dosage could be formulated as an optimal control problem, which could be solved using nonlinear optimization algorithms (Jarrett et al., 2020). Studies performed in Cardilin et al. (2022) attempt to use exposure-response modeling of RT in combination with unspecified radiosensitizers to tune dosing schedules; however, this analysis was performed using data from immunocompromised mice and thus removes the impact that the immune system has on local tumor control. Furthermore, the description of how RT induces cell death within the tumor is purely dependent on dose and does not take into consideration other aspects of the tumor microenvironment. A sophisticated description of how RT/DDRi impacts the tumor in terms of reoxygenation (Salem et al., 2019) and redistribution of cell cycle phases (Checkley et al., 2015) would also be beneficial when attempting to couple the links between cell death, immunogenicity, and immunosuppression. Now that a model describing how RT/DDRi/ICI impacts the immune response has been developed, additional data that describes how RT/DDRi induces direct cell death, reoxygenation, and redistribution could be coupled with this model to further assess the interplay between radiation dosage and response.

The current datasets assess dosages achieving exposures typically much larger than their potency or dissociation constants (Norris et al., 2014; Deng et al., 2016; Fuk et al., 2019), making it potentially more difficult to view the differential effects of PK (drug exposure) on efficacy. The simplified PK of DNAPKi, PARPi, and ICI could be improved with additional data showing how doses of ICI and DDRi impact the response in preclinical tumor models, followed by full PK-pharmacodynamics modeling. Datasets incorporating alternative schedules of RT/DDRi could also have been useful for model parameterization, and the results of both the RT/DNAPKi and RT/PARP/ICI simulations need to be validated experimentally to confirm or refine the model accordingly.

The results of the RT/PARP/ICI cohorts (Fig. 4) indicated that the parameter estimates captured the upper, median, and lower percentiles of each cohort. The VPCs also successfully captured how at the given dosage and schedule, incorporation of PARPi into an RT/ICI dosage regimen does not significantly improve the response rates observed within these tumors. Simulations of RT/PARP/ICI indicated that based on T-cell influx, a 4 mg/kg dose of ICI in combination with a full PARPi dosage regimen would be capable of inducing a similar immune response as a 10 mg/kg dose of ICI (Fig. 5), which was shown to induce a large response in these cohorts. These results indicate the potential for dosage reduction while maintaining efficacy in these cases. The results of this data could be of translational benefit if appropriately validated in preclinical experiments.

The results of the local sensitivity analysis implicated the baseline tumor growth rate to have the most significant impact on the response. This appears to be consistent with the results of the local sensitivity analysis in Kosinsky et al. (2018) and may reflect similarities in model specification as both models are comprised of an immune system–mediated negative feedback mechanism. However, individual parameters assessed within the local sensitivity analysis indicated that numerically, significant fluctuations in parameter values did not change the model output significantly and that significant changes in model outputs may require interactions between different parameters. Another QSP model (Qiao et al., 2022) also indicated that differences in partial and complete responses to anti–CTLA-4 were not sufficiently explained by changes in one parameter. In this respect, global sensitivity analysis may provide more insight on the interdependency of model parameters and its impact on the total variability of the overall tumor response to RT and RT/DDRi combination.

In summary, this analysis describes a successful development of a mathematical model that captures tumor immune interactions in response to RT/DDRi/ICI and suggests modification of RT/DNAPKi dosage regimens, with the aim of increasing the variability in responses within the RT/DNAPKi–treated cohorts, as well as modification of the ICI dosage in RT/PARP/ICI cohorts to observe additional benefits of tritherapy, which could be of translational relevance.

Acknowledgments

The authors would like to acknowledge Elaine Cadogan and Kyoko Nakamura for the generation of the experimental data that has been used in this paper.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authorship Contributions

Performed data analysis: Mistry, Yates, Aarons, Ogungbenro.

Conducted experiments: Farrington, Staniszewska.

Contributed new reagents or analytic tools: Mistry, Guzzetti, Davies.

Performed data analysis: Hudson.

Wrote or contributed to the writing of the manuscript: Hudson, Guzzetti, Aarons, Ogungbenro.

References


Address correspondence to: Kayode Ogungbenro, Division of Pharmacy and Optometry, Faculty of Biology, Medicine and Health, Stoford Building, Room 3.123, Oxford Road, University of Manchester, Manchester, UK, M13 9PT. E-mail: kayode.ogungbenro@manchester.ac.uk
Supplementary code for use in NONMEM.

$PROBLEM    MC38 IMMUNE MODEL XX ALL DATA
$INPUT      VOL ID TRT TIME DV STUDY
$SUBROUTINE ADVAN13 TRANS1 TOL=6
$MODEL      NCOMPARTMENTS=8
$DATA       SIMULATEDPOPULATION.CSV
$PK

Q0 = THETA(1)*EXP(ETA(1))
LAMBDA = THETA(2)*EXP(ETA(2))
ALPHA = THETA(3)
BETA = THETA(4)*EXP(ETA(3))
GAMMA = THETA(5)
ZETA = THETA(6)

KDTL = 0.027*24
KDL = 0.027*24
KDLCD8 = 0.38*24
KCD8lt = 0.06

;tumour ics

A_0(1) = 2.4
;dc ics
A_0(2) = Q0
A_0(3) = 0
; cd8 ics
A_0(4) = 0
A_0(5) = 3.5*3.25
A_0(6) = 0
A_0(7) = 0
A_0(8) = 100

; mdsc ic
; pdl1 ic

$DES

HEAV1 = 0
RT1 = 0
RT2 = 0
PARP = 0
ATM1 = 0
IO1 = 0
DDR = 0

IF (TIME.LT.4.1) HEAV1 = 1
IF (TIME.LT.8.1) HEAV2 = 1
IF (TRT.GT.1) RT1 = 1
IF (TRT.EQ.3.OR.TR1.EQ.6) PARP = 1
IF (TRT.EQ.4.5.OR.TR1.EQ.7) ATM1 = 1
IF (TRT.EQ.3.OR.TR1.EQ.6.OR.TR1.EQ.4.5.OR.TR1.EQ.7) DDR=1
IF (TR1.GT.5.4) IO1=1

; tumour compartments
FQ = A(2)/(0.1 + A(2))
DADT(1) = (LAMBDA)*A(1)*(1 - A(1)/(2.4)) + 0.001*A(4)*A(1)*FQ - 0.001*A(4)*A(1)
DADT(2) = ((LAMBDA)*A(1)**2)/(2.4) - 0.001*A(4)*A(1)*FQ
; dendritic cell compartments
DADT(3) = 0.001 + ALPHA*A(1)*(HEAV1*RT1*2) - KDTL*A(3)

; pd1 compartment solved

; cd8 cell compartments

DADT(4) = KDLCD8*A(3) - 0.0024*A(4)*A(7)
DADT(5) = 3.25*3.5*HEAV1*(DDR) - 3.5*A(5)
DADT(6) = A(5)*(DDR)/(0.091 + A(5)) - 0.17*A(6)
DADT(7) = 0.099*BETA*A(4)*(1 - A(6)*DDR/(GAMMA + A(6)))*1 - A(8)*IO1/(ZETA + A(8))) - 0.099*A(7)
DADT(8) = 100*HEAV2*IO1*0.69 - 0.69*A(8)

A4 = A(4)

$ERROR

Y = (A(1) + A(2)) + EPS(1)

; estimation based theta initial values

$THETA (0,4,6)
(0,0.20)
(0.15,5)
(0.16)
(0,0.02)
(0.2,100)
$\Omega$ Block(3)

0.0468
-0.02 0.0747
-0.01 -0.01 2.5

$\Sigma$ 0.45

$\text{ESTIMATION METHOD}= 1 \ \text{PRINT}= 100 \ \text{MAXEVAL}= 9999$

$\text{ESTIMATION METHOD}= \text{SAEM} \ \text{NITER}= 500 \ \text{AUTO}= 1 \ \text{PRINT}= 50$

$\text{ESTIMATION METHOD}= \text{IMP} \ \text{EONLY}= 1 \ \text{PRINT}= 1 \ \text{NITER}= 5 \ \text{ISAMPLE}= 1000 \ \text{MAPITER}= 0$

$\text{COVARIANCE SLOW MATRIX}= R \ \text{UNCONDITIONAL}$

: estimation output values for simulation

: $\Theta$ 3.74 0.212 1.38 1.60 0.958 4.46

: $\Omega$ Block(3) 0.0434 -0.0133 0.068 -0.0667 0.0813 2.3

: $\Sigma$ 0.504

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Supplementary file: SIMULATEDPOPULATION.CSV – simulated RT/PARPi/ICI dataset.
Radiation in Combination with Immune Checkpoint Blockade and DNA Damage Response Inhibitors in Mice: Dosage Optimisation in MC38 Syngeneic Tumours via Modelling and Simulation.

David Hodson, Hitesh Mistry, James Yates, Anna Staniszewska, Paul Farrington, Sofia Guzzetti, Michael Davies, Leon Aarons, Kayode Ogungbenro.

Journal of Pharmacology and Experimental Therapeutics.


Supplementary Table 1. Full local stability analysis estimates using 20% perturbations in each parameter.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>+20%</th>
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