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

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DDR – DNA Damage Response Pathway

DDRi – DNA Damage Response Pathway inhibitor

DNAPK – DNA Protein Kinase

DNAPKi – DNA Protein Kinase inhibitor

Gy – Gray

ICI – Immune Checkpoint Blockade

IIV – Inter-Individual-Variability

ORR – Overall Response Rate

PARP – Poly Adenylyl Ribose Polymerase

PARPi – Poly Adenylyl Ribose Polymerase inhibitor

PD – Pharmacodynamics

PD-1 – Programmed Death 1

PD-L1 – Programmed Death Ligand 1

PK – Pharmacokinetics

QSP – Quantitative Systems Pharmacology

ROS – Reactive Oxygen Species

RSE – Relative Standard Error

RT – Radiotherapy

RV – Residual Variability

TTE – Time-To-Event

VPC – Visual Predictive Check
Recommended section: Inflammation, Immunopharmacology, and Asthma
Abstract.

Clinical trials assessing the impact of Radiotherapy (RT) in combination with inhibitors of the DNA Damage Response Pathway (DDRi) and/or immune checkpoint blockade are currently ongoing. However, current methods for optimising dosage and schedule are limited. A mathematical model was developed to capture the impacts of RT in combination with DDRi and/or anti PD-L1 (Immune Checkpoint Inhibitor, ICI) on tumour immune interactions in the MC38 syngeneic tumour model. The model was fitted to datasets which assessed the impact of RT in combination with the DNA Protein Kinase inhibitor (DNAPKi) – 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 modelling was performed followed by internal validation with Visual Predictive Checks (VPC). Simulations of alternative dosage regimen and scheduling were performed to identify optimal candidate dosage regimen of RT/DNAPKi 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 tumour 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 to ICI in combination with RT. Simulation of alternative dosage regimen indicated that lowering the dosage of ICI to between 2-4mg/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
RT/DDRi/ICI in preclinical tumour 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 1 in 2 people born since 1960 are projected to be diagnosed with cancer within their lifetime in the 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 tumours via the formation of reactive oxygen species (ROS), 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 anti-tumour cytolytic CD8+ T cells (Zhu et al., 2021). However, radiotherapy also induces mechanisms within the tumour that enhance radioresistance, such as epithelial-mesenchymal transition (Kawamoto et al., 2012) and upregulation of immunosuppressive ligands on the surface of cells in the tumour such as Programmed Death Ligand 1 (PD-L1) (Gong et al., 2018).

Combinations of different pharmaceutical agents with radiotherapy may assist in delivering the anti-tumour effect of RT while to some extent inhibiting its immunosuppressive effects. As RT has been shown to induce immune checkpoint ligands such as PD-L1 (Han et al., 2020; Philippou et al., 2020), it has also been shown that combination of RT with concurrent PD-L1 blockade (RT/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 Inhibitors (RT/DDRi) have indicated that DDRi increases both the radiosensitivity of the tumour 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). While 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).

Optimisation of dosage for either combination treatment modality is likely to be tumour 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 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 would further reduce dropout and increase compliance (Czobor and Skolnick, 2011).

Mathematical modelling can assist in identifying candidate dosage regimen 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 tumour models treated with different RT/ICI regimen (Kosinsky et al., 2018). CT-26 however is considered an immunologically hot tumour which 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 utilized to capture potential differences in response across different tumour models and possibly explain some of the variability in responses to drugs observed within the clinic. While there are mathematical models available which assess the impact of RT/DDRi (Checkley et al., 2015) and RT/ICI (Kosinsky et al., 2018) on
tumour growth, a model describing the impacts of RT/DDRi/ICI on tumour growth is currently lacking.

The aim of this study was to develop a mathematical model of RT/DDRi/ICI combinations in order to identify optimal dosage regimens in the context of the solid syngeneic tumour 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 (PARPi).
Methods

The mathematical model.

Tumour compartments.

The full model schematic is shown in Figure 1. The tumour 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 be targeted by T cells \( T(t) \) or 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 Equation 1.

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

\[
\frac{dQ(t)}{dt} = \lambda \cdot \left( \frac{P(t)^2}{K} \right) - K_{PTQ} \cdot P(t) \cdot (T(t) + N(t)) \cdot \frac{Q(t)}{K_R + Q(t)}
\]

where \( \lambda, K, K_{PT}, K_{PTQ} \) and \( K_R \) are the baseline tumour 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 (Fok et al., 2019; Sun et al., 2018). Empirical modelling describing multiple dosing of drugs with linear PK is derived from similar assumptions which are used to formulate empirical PK models of drugs which 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 in...
from multiple dosing was approximated with the corresponding average steady state concentration ($C_{AV}$) throughout the drug dosing schedule. This is represented as a Heaviside function active during the first 4 days of treatment (equation 2). The Heaviside function is represented in NONMEM as in IF statement which considers time as a time varying covariate (TIME) incorporated into the data file. In order to confirm that treatment is still active at day 4, the Heaviside function extends by 0.1 days beyond the final day of treatment (Supplementary code for use in NONMEM, Supplementary data file). The DDRi is assumed to induce additional cell death ($V(t)$), which does not impact the expected measured tumour volume and instead impacts downstream immunogenic mechanisms. Dying cells are removed naturally within the tumour as described by Equation 2.

$$\frac{dZ(t)}{dt} = K_{2z} \cdot \text{Heaviside}(4.1 - \text{TIME}) - K_{2z} \cdot Z(t)$$

$$\frac{dV(t)}{dt} = \frac{Z(t)}{K_{2v} + Z(t)} - \frac{K_{V}\phi \cdot V(t)}{K_{V}\phi}$$

where $K_{2z}, K_{2z}, K_{2v}, K_{z}$ and $K_{v}\phi$ 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 1mg/kg, however at doses of 10mg/kg, the clearance of anti PD-L1 is non-linear. Assessment of cellular surface PD-L1 expression via flow cytometry indicated complete blockade of surface PD-L1 for between 7-14 days after a single 10mg/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 (Equation 3). This attempts to coincide with the first week of dosing.
Plasma Antibody concentration

\[
\frac{dI(t)}{dt} = K_{\phi I} \cdot \text{Heaviside}(8.1 - \text{TIME}) - K_{I\phi} \cdot I(t) \tag{3}
\]

Where \(K_{\phi I}\) and \(K_{I\phi}\) 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 to 4. A(t) then induces activation of T(t) and decays naturally. T(t) functions to target tumour 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 non-competitively inhibit the rate at which X(t) is synthesised by T(t). N(t) is assumed to be induced by DNAPKi explicitly (Equation 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 tumour diameter, combined with recent data suggesting that RT/DNAPKi may induce higher levels of cytolytic enzymes in NK cells compared with RT during MC38 tumour challenge (Nakamura et al., 2021).

**APCs**

\[
\frac{dA(t)}{dt} = K_{A\phi} \cdot \text{Heaviside}(4.1 - \text{TIME}) + \alpha \cdot P(t) \cdot R(t) - K_{A\phi} \cdot A(t)
\]

**CD8 T cells**

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

**Exhaustion receptors**

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

**Natural Killer cells**

\[
\frac{dN(t)}{dt} = \delta \cdot Z(t) \cdot \frac{1}{K_Z + Z(t)} - K_{Z\phi} \cdot N(t)
\]

\[
R(t) = 2 \cdot \text{Heaviside}(4.1 - \text{TIME}) \tag{4}
\]
where $K_{\phi_A}$, $\alpha$, $K_{\phi_A}$, $K_{\phi}$, $K_{\phi}$, $K_{\phi}$, $\beta$, $\gamma$, $\gamma_1$, $K_{\phi}$, $\delta$ and $K_{2\phi}$ 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 tumour 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 tumour growth in MC38 syngeneic models, where efficacy of each treatment was measured by the amount of time for the tumour to reach 1cm$^3$. Mice were sacrificed when the tumour reached over 1cm$^3$ or when the tumour was presenting as a wet ulcerated lesion. All studies followed similar protocols and were conducted in accordance with the Animals and Scientific Procedures act 1986, Home Office guidelines and a local ethical review body. A summary of the experimental procedures used regarding MC38 syngeneic models can be found in Nakamura et al. (2021). All mice which were given RT or RT-related combinations were subject to 10 Gray (Gy) of external beam radiation given as 2Gy fractions daily for 5 days. For mice given anti PD-L1 (ICI), 10mg/kg of ICI was injected intraperitoneally typically 1-2h prior to RT on a biweekly basis. All mice which were subject to DDRi treatments were given DDRi orally 1-2h prior to RT.

The first dataset (Dataset 1) consists of data derived from four pooled experiments. Two experiments (KN2005 & DNAPK1908) were TTE experiments which assessed the efficacy of RT in combination with 75mg/kg of the DNAPKi – AZD7648. The other two experiments were based on pharmacodynamic assessment of different immunophenotypic biomarkers in response to RT/DNAPKi (KN2001) or 10mg/kg of the Ataxia Telangiectasia Mutated (ATM) inhibitor (ATMi) - AZD0156 (ATM1803). All datasets assessing RT/DNAPKi can be found in
Nakamura et al. (2021). Pharmacodynamic studies randomised 12 mice into control cohorts while TTE studies randomised 15 mice into treatment cohorts. For KN2001 and both pharmacodynamic studies, cohorts of 12 mice were randomly assigned into treated cohorts (Cohorts 2-4). Control mice were subject to oral vehicle controls, Cohorts 2 and 3 were subjected to RT or DDRi as monotherapy respectively, while cohort 4 was subjected to combination RT/DDRi treatments. For DNAPK1908, 12 mice per cohort were randomised into different treatment regimen, cohort 1 were assigned as control cohorts, while cohorts 2 and 3 were assigned RT and RT/DNAPKi dosage regimen respectively. Cohorts 4 and 5 assessed the effects of RT/DNAPKi during antibody mediated CD8 or NK1.1 blockade respectively and were excluded from this analysis.

The second dataset (Dataset 2), consists of data derived from four pooled experiments, the first two experiments (DDRIO1815 and DDRIO1821) were based on TTE experiments which assessed the efficacy of RT in combination with 100mg/kg olaparib (PARPi) in combination with ICI. The other two experiments were based on TTE experiments which assessed the efficacy of RT in combination with 2.5mg/kg ATMi with ICI (DDRIO1824 & DDRIO1835). Due to sparsity of data, heterogeneity of cohort sizes and inter-study 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 8 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 + DDRi (ICI/DDRi cohort) or RT + DDRi (RT/DDRi cohort) or RT + ICI (RT/ICI cohort), and one final cohort was given all three treatments (RT/DDRi/ICI cohort). However, one experiment (DDRIO1835) involving ATM 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 which were recorded as "no tumour" (1 mm) were included before model fitting.

**Model fitting and simulation.**

A list of the fixed model parameters, definitions, values and sources are shown in Table 2. Datasets 1 and 2 were fitted using NONMEM version 7.4.3 using FOCE followed by SAEM. Log likelihood values (-2LLIMP) and relative standard errors were calculated using importance sampling with an expectation only step, followed by a final covariance step. Inter-Individual Variability (IIV) was incorporated into the baseline size of the tumours quiescent core (Q₀), the baseline tumour growth rate (λ) and the rate of exhaustion receptor synthesis (β). IIV was assumed to be log-normally distributed and is represented by a 3x3 block matrix. Random unexplained variance (RV) was assumed to be additive. As there was no improvement in survival or tumour control after the administration of either DDRi or ICI as monotherapy (not shown), the data was excluded from the analysis prior to fitting.

100 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 (R Core Team, 2019), overlaying the 2.5%, 50% and 97.5% percentiles of the observed data with the corresponding areas from simulations.

DNAPKi was given at doses which lead to significantly higher free plasma concentrations than the cellular free in vitro IC₅₀ (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 IC₅₀ (Norris et al., 2014) while radiation is being administered. This relationship between Cₐᵥ and the IC₅₀ was incorporated into the model.

This analysis aims to address the impact of the amount of days which the DDRi is given concurrently with the RT on tumour growth. The function assuming a full 5 day course of DDRi is shown in Equation 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.

\[
\frac{dZ(t)}{dt} = K_{\phi Z} \cdot \text{Heaviside}(\frac{d}{5} - \text{Time}) - \frac{K_{\phi Z} \cdot Z}{Z_{\text{eq}}}
\]  

(5)

As this model fits an estimate for the pharmacodynamic IC\(_{50}\) of the drug dosage given a 10mg/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 \( K_{\phi} \) (Equation 3) by values between 0.1 and 1.0 in increments of 0.1.

100 Simulations of each candidate dosage regimen were performed in NONMEM v 7.4.3. 100 simulations of dataset 1 was 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 tumour diameters. For assessment of different RT/PARPi/ICI schedules, 100 simulations of a dataset for a virtual study which assesses 12 mice per cohort, with tumour sizes observed every day between days 0-34 was used. Simulated tumours which were below 2.4mm at day 34 were considered complete responders. Overall response was characterised by tumours which achieved a tumour diameter reduction of 1.39mm from day 0 at any point during the simulated time course. To assess T cell infiltration, the area under the curve (AUC) 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 tumour 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 tumour 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 tumour volume in the context of MC38 syngeneic tumour models. Final parameter estimates are listed and described in Table 3 with relative standard errors (RSE %) for each estimated parameter, IIV is represented as the coefficient of variation (CV%). The model fit estimated the NK cell influx rate ($\delta$) 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 ($\gamma_V$) 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 which dying cells are present within the tumour. 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 tumour diameters of 11.44mm or smaller were included in the VPCs, corresponding to tumours which 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 (Figure 2). The model simulations capture the lack of variability in tumour sizes which 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 tumour 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 tumour responses would likely be beneficial when assessing the impact of different biomarkers on tumour 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 tumour trajectories. Upper, median and lower percentile estimates from simulations of the full dosage regimen (Figure 3A) indicates that tumours 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 tumour 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 show that the 97.5% percentile is expected to show growing tumours from this time point. This would potentially be indicative of tumours which 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 tumour. 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 tumour 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 tumour 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 individual values. In addition, given that ICI is given at doses which 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 IC$_{50}$, 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 which could implicate significant differences in efficacy between RT/ICI and RT/PARPi/ICI, as well as identify doses of RT/PARPi/ICI which could lead to sufficiently high levels of T cell responses comparable to those simulated in the current study. Comparisons of CRR, ORR and T cell AUC were used to infer differences in efficacy between different candidate dosage regimens. When assessing CRR (Figure 5A), assuming the full PARPi dosage schedule is given, the difference in percentage CRR between RT/ICI and RT/PARPi/ICI treatments increases between 0-5 mg/kg of ICI. However after 5mg/kg ICI, there is no net increase in CRR between RT/ICI and RT/PARPi/ICI larger than 15%. Similarly, the difference between ORR (Figure 5B) in RT/ICI treated mice and RT/PARPi/ICI treated mice, assuming the full PARPi dosage is given, doses of between 0-3mg/kg of RT/ICI lead to the largest observable
differences between RT/ICI and RT/PARPi/ICI, while doses above 4mg/kg exhibit no changes in the percentage difference in ORR between RT/ICI and RT/PARPi/ICI. When assessing T cell influx (Figure 5C), doses of 4mg/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 10mg/kg RT/ICI CRR rate. Collectively, this indicates the doses of approximately 4mg/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 (Figure 6), these included all fitted parameters. The majority of parameters modestly impacted the expected tumour size (Supplementary Table 1). The baseline tumour growth rate was found to have the largest impact on observed tumour size, with $K_{PT}$ having the second largest impact on tumour size. Interestingly, the model was not very sensitive to the T cell rate of exhaustion receptor expression. However, in the NLME model, this was the parameter which IIV 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 IIV 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 optimisation for both preclinical and clinical experiments require extensive experimental data. Mathematical modelling and simulation has the potential to identify
dosage regimens which 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 3 Rs of animal research (Reduction, Refinement, and Replacement), which minimise 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 optimising dosing schedules for pharmacodynamic study design, as well as minimising dosage while retaining efficacy.

It was observed from preliminary analyses that there was not a large degree of inter study variability between response rates, growth rates or baseline tumour 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 (Figure 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 inter study 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 tumours, models required re-fitting the external dataset in accordance with the baseline growth rate in order to improve model validation (Qiao et al., 2022). Thus, either 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 inter-study 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 response observed, which would facilitate identification of candidate biomarkers implicated in the MC38 tumour 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 in time as the exhaustion marker expression levels increase. However, this model ignores the direct effects which RT has on tumour cells. RT functions to directly kill tumour cells, which leads to changes in the distribution of oxygen within the tissue (Crokart 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 2Gy RT and 2Gy 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 dependant (Fok et al., 2019). Furthermore, fractionated radiotherapy also leads to repopulation of the tumour with changes in the distribution of cells which are radiosensitive and radioresistant, which also impacts the effects of each subsequent fractionated dose of RT (Baskar et al., 2014). A model specification which 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 optimisation strategies such as genetic algorithms (McCall, 2005) could be employed, which attempt to compromise between the beneficial effects of each fractionation step on tumour 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 optimisation algorithms (Jarrett et al., 2020).
performed in Cardilin et al. (2022) attempt to use exposure-response modelling of RT in combination with unspecified radiosensitisers to tune dosing schedules, however this analysis was performed using data from immunocompromised mice and thus removes the impact which the immune system has on local tumour control. Furthermore, the description of how RT induces cell death within the tumour is purely dependant on dose and does not take into consideration other aspects of the tumour microenvironment. A sophisticated description of how RT/DDRi impacts the tumour 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 which describes how RT/DDRi induces direct cell death, reoxygenation and redistribution could be coupled with this model in order 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 (Deng et al., 2016; Fok et al., 2019; Norris et al., 2014), 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 tumour models, followed by full PK-PD modelling. Datasets incorporating alternative schedules of RT/DDRi could also have been useful for model paramterisation, and the results of both the RT/DNAPKi and RT/PARPi/ICI simulations need to be validated experimentally in order to confirm or refine the model accordingly.

The results of the RT/PARPi/ICI cohorts (Figure 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 tumours. Simulations of RT/PARPi/ICI indicated that based on T cell influx, a 4mg/kg
dose of ICI in combination with a full PARPi dosage regimen would be capable of inducing a similar immune responses as a 10mg/kg dose of ICI (Figure 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 tumour 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 inter-dependency of model parameters and its impact on the total variability of the overall tumour response to RT and RT/DDRi combination.

In summary, this analysis describes a successful development of a mathematical model which captures tumour immune interactions in response to RT/DDRi/ICI, and suggests modification of RT/DNAPKi dosage regimen 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/PARPi/ICI cohorts to observe additional benefits of tritherapy, which could be of translational relevance.

Acknowledgements.

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Data availability statement:

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

Authorship Contributions:

Participated in research design: Ogungbenro, Yates, Aarons, Mistry

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

Performed data analysis: Hodson.

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

Conducted experiments: Staniszewska, Farrington.

References:


**Footnotes:**

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2 CONFLICT OF INTEREST STATEMENT: Staniszewska, Guzzetti and Davies are AstraZeneca employees and currently hold AstraZeneca shares and stock options. Farrington and Yates are former AstraZeneca employees. All other authors declare no conflict of interest.
Figure Legends:

Figure 1: Schematic representation of mathematical model of tumour and immune response fitted to tumour growth data in MC38 syngeneic mouse models. Fitted parameters are highlighted in bold with their respective roles within the model. Created with BioRender.com.

Figure 2: VPCs of model fit output from Dataset 1. A) Control cohorts, B) RT treated cohorts, C) RT/DNAPKi treated cohorts. 2.5, median and 97.5 percentiles from the observed datasets (dashed lines) are overlaid with the corresponding range of 2.5, median and 97.5 percentiles from simulated datasets (shaded regions).

Figure 3: Comparison of upper, median and lower bound estimates of full RT/DNAPKi treated cohorts (A) against a full schedule of RT with the addition of a single dose of DNAPKi on day 0 (B).

Figure 4: VPCs of model fit output from Dataset 2. A) Control cohorts, B) RT treated cohorts, C) RT/PARPi treated cohorts, D) RT/ICI treated cohorts, E) RT/PARPi/ICI treated cohorts. 2.5, median and 97.5 percentiles from the observed datasets (dashed lines) are overlaid with the corresponding range of 2.5, median and 97.5 percentiles from simulated datasets (shaded regions).

Figure 5: Simulations of alternative RT/PARPi/ICI dosing regimens and their respective impacts on A: Overall response rate (ORR), B: Complete response rate (CRR), C: Total T cell infiltration over the projected time course (AUC).

Figure 6: Local sensitivity analysis assessing the impact of 20% perturbations in selected parameter estimations which span all compartments in the model. Sensitivity was measured by the relative change in expected tumour diameter at day 21 compared to baseline parameter values. 20% increases in parameter estimates are shown by blue bars, 20% decreases in parameter estimates are shown by red bars.
Tables:

Table 1: Summary of data used for the analysis; cohorts in experiments and datasets.

<table>
<thead>
<tr>
<th>Cohort Allocation</th>
<th>Dataset 1</th>
<th>Dataset 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RT&lt;sup&gt;S&lt;/sup&gt;</td>
</tr>
<tr>
<td>KN2001 (DNAPKi)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>KN2005 (DNAPKi)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>ATM1803 (ATMi)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>DNAPK1908</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>DDRIO1815 (PARPi)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>DDRIO1821 (PARPi)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>DDRIO1824 (ATMi)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>DDRIO1835 (ATMi)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

1 15/12*/12**
2 12/6**
3 12/6**
4 12/6**
5 12/6**
6 12/6**
7 12/6**
1 75mg/kg AZD7648 given orally once per day for 5 days, 1-2h prior to RT, 2 10mg/kg AZD0156 given orally once per day for 5 days, 3 100mg/kg olaparib given once per day for 21 days 1-2h prior to RT, 4 2.5mg/kg AZD0156 given once per day for 21 days – all mice subject to ATMi monotherapy or combination therapies were censored from the analysis, 5 2Gy of external beam RT given once per day for 5 days, 6 10mg/kg anti PD-L1, intraperitoneal injection biweekly for three weeks 1-2h prior to RT, * Number of assigned mice for control cohorts in KN2005 and ATM1803, ** Number of assigned mice for each cohort in DDRIO1835.
Table 2: List of parameters fixed in the model and their sources

<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>Fixed*</td>
</tr>
<tr>
<td>$T_0$</td>
<td>Initial active CD8 concentration</td>
<td>cells mm$^{-1}$</td>
<td>0</td>
<td>Fixed*</td>
</tr>
<tr>
<td>$X_0$</td>
<td>Initial exhaustion receptor level</td>
<td>receptors</td>
<td>0</td>
<td>Fixed*</td>
</tr>
<tr>
<td>$V_0$</td>
<td>Initial amount of dying cells</td>
<td>cells</td>
<td>0</td>
<td>Fixed*</td>
</tr>
<tr>
<td>$Z_0$</td>
<td>Initial DDRi concentration</td>
<td>μM</td>
<td>3.25</td>
<td>(Fok et al., 2019)$^9$</td>
</tr>
<tr>
<td>$I_0$</td>
<td>Initial ICI concentration</td>
<td>μg ml$^{-1}$</td>
<td>100</td>
<td>(Deng et al., 2016)$^5$</td>
</tr>
<tr>
<td>$K$</td>
<td>Rim carrying capacity</td>
<td>mm</td>
<td>2.4</td>
<td>(Salem et al., 2019)$^6$</td>
</tr>
<tr>
<td>$K_{PT}$</td>
<td>CD8 rim killing rate</td>
<td>cell$^{-1}$day$^{-1}$</td>
<td>0.001</td>
<td>(Kosinsky et al., 2018)$^{§§}$</td>
</tr>
<tr>
<td>$K_{PTQ}$</td>
<td>CD8 transfer of core to rim</td>
<td>cell$^{-1}$day$^{-1}$</td>
<td>0.001</td>
<td>Fixed**</td>
</tr>
<tr>
<td>$K_R$</td>
<td>Transfer affinity from the core.</td>
<td>mm</td>
<td>0.1</td>
<td>Fixed*</td>
</tr>
<tr>
<td>$K_{PA}$</td>
<td>Baseline influx of APCS</td>
<td>cells day$^{-1}$ mm$^{-1}$</td>
<td>0.001</td>
<td>Fixed*</td>
</tr>
<tr>
<td>$K_{Ap}$</td>
<td>APC natural death rate</td>
<td>day$^{-1}$</td>
<td>0.648</td>
<td>(Kronik et al., 2010)$^{§§}$</td>
</tr>
<tr>
<td>$K_{Np}$</td>
<td>NK cell natural death rate</td>
<td>day$^{-1}$</td>
<td>0.648</td>
<td>Fixed***</td>
</tr>
<tr>
<td>$K_{AT}$</td>
<td>APC activation of CD8 cells</td>
<td>day$^{-1}$</td>
<td>9.12</td>
<td>(Kronik et al., 2010)$^{§§}$</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
<td>Unit</td>
<td>Value</td>
<td>Notes</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------------</td>
<td>----------</td>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>$K_{T,e}$</td>
<td>T cell Exhaustion rate</td>
<td>day$^{-1}$ receptor$^{-1}$</td>
<td>0.0024</td>
<td>Fixed$^\dagger$</td>
</tr>
<tr>
<td>$K_{e,Z}$</td>
<td>DDRi infusion rate</td>
<td>μM day$^{-1}$</td>
<td>11.375</td>
<td>(Fok et al., 2019)$^\S$</td>
</tr>
<tr>
<td>$K_{Z,e}$</td>
<td>DDRi elimination rate</td>
<td>day$^{-1}$</td>
<td>3.5</td>
<td>(Fok et al., 2019)$^\S$</td>
</tr>
<tr>
<td>$K_{v,V}$</td>
<td>Dying cell induction rate</td>
<td>cells day$^{-1}$</td>
<td>1</td>
<td>Fixed$^{\dagger\dagger}$</td>
</tr>
<tr>
<td>$K_{V,e}$</td>
<td>Dying cell elimination rate</td>
<td>day$^{-1}$</td>
<td>0.17</td>
<td>(Kosinsky et al., 2018)$^{\S\S}$</td>
</tr>
<tr>
<td>$K_{e,I}$</td>
<td>ICI infusion rate</td>
<td>μg ml$^{-1}$ day$^{-1}$</td>
<td>69</td>
<td>(Deng et al., 2016)$^\S$</td>
</tr>
<tr>
<td>$K_{I,e}$</td>
<td>ICI elimination rate</td>
<td>day$^{-1}$</td>
<td>0.69</td>
<td>(Deng et al., 2016)$^\S$</td>
</tr>
<tr>
<td>$K_{X,e}$</td>
<td>Exhaustion marker elimination rate</td>
<td>day$^{-1}$</td>
<td>0.099</td>
<td>(Nakamura et al., 2021)$^\S$</td>
</tr>
<tr>
<td>$K_{Z}$</td>
<td>DDRi IC$_{50}$</td>
<td>μM</td>
<td>0.091</td>
<td>(Goldberg et al., 2020)$^{\S\S}$</td>
</tr>
</tbody>
</table>

* Assumes baseline immune response is negligible, ** Fixed to ensure that the core is released into the rim at the same rate 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. § Calculated from data in citation, §§ direct reference.
Table 3: List of parameters estimated when Dataset 1 was fitted.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value (RSE %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q_0$</td>
<td>Initial Core Depth (mm)</td>
<td>3.12 (2)</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Baseline tumour growth rate ($d^{-1}$)</td>
<td>0.221 (3)</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>RT mediated APC recruitment rate ($\text{cells}^{-1} \text{Gy} d^{-1}$)</td>
<td>1.52 (2)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>T cell mediated Exhaustion receptor recruitment rate ($d^{-1}$)</td>
<td>1.08 (13)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>DNAPKi mediated NK cell recruitment rate ($\text{cells} d^{-1}$)</td>
<td>73.8 (9)</td>
</tr>
<tr>
<td>$\gamma_v$</td>
<td>Dying Cell Exhaustion receptor IC$_{50}$ ($\text{cells}$)</td>
<td>0.0181 (32)</td>
</tr>
<tr>
<td>$\eta_{Q0}$</td>
<td>IIV in $Q_0$ ($\text{CV} %$)</td>
<td>18.1 (16)</td>
</tr>
<tr>
<td>$\eta_{\lambda}$</td>
<td>IIV in $\lambda$ ($\text{CV} %$)</td>
<td>25.3 (17)</td>
</tr>
<tr>
<td>$\eta_{\delta}$</td>
<td>IIV in $\delta$ ($\text{CV} %$)</td>
<td>141 (38)</td>
</tr>
<tr>
<td>Cov($\eta_{Q0}$, $\eta_{\lambda}$)</td>
<td>Covariance between $Q_0$ and $\lambda$ (unitless)</td>
<td>-0.0171 (34)</td>
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<tr>
<td>Cov($\eta_{Q0}$, $\eta_{\delta}$)</td>
<td>Covariance between $Q_0$ and $\delta$ (unitless)</td>
<td>0.00716 (207)</td>
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<tr>
<td>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 ($\text{mm}^2$)</td>
<td>0.197 (5)</td>
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Table 4: List of parameters estimated when Dataset 2 was fitted.

<table>
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<tr>
<th>Parameter</th>
<th>Definition (units)</th>
<th>Value (RSE %)</th>
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<tbody>
<tr>
<td>$Q_0$</td>
<td>Initial Core Depth (mm)</td>
<td>3.72 (2)</td>
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<tr>
<td>$\lambda$</td>
<td>Baseline tumour growth rate (d$^{-1}$)</td>
<td>0.212 (3)</td>
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<tr>
<td>$\alpha$</td>
<td>RT mediated APC recruitment rate (cells$^{-1}$ Gy d$^{-1}$)</td>
<td>1.39 (3)</td>
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<tr>
<td>$\delta$</td>
<td>T cell mediated Exhaustion receptor recruitment rate (d$^{-1}$)</td>
<td>1.67 (17)</td>
</tr>
<tr>
<td>$\gamma_v$</td>
<td>Dying Cell Exhaustion receptor IC$_{50}$ (cells)</td>
<td>0.727 (31)</td>
</tr>
<tr>
<td>$\gamma_i$</td>
<td>ICI Exhaustion receptor IC$_{50}$ ($\mu$g ml$^{-1}$)</td>
<td>3.65 (30)</td>
</tr>
<tr>
<td>$\eta_{Q0}$</td>
<td>IIV in $Q_0$ (CV%)</td>
<td>21.9 (16)</td>
</tr>
<tr>
<td>$\eta_{\lambda}$</td>
<td>IIV in $\lambda$ (CV%)</td>
<td>28 (20)</td>
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<tr>
<td>$\eta_{\delta}$</td>
<td>IIV in $\delta$ (CV%)</td>
<td>285 (23)</td>
</tr>
<tr>
<td>Cov($\eta_{Q0}$, $\eta_{\lambda}$)</td>
<td>Covariance between Q0 and $\lambda$ (unitless)</td>
<td>-0.0181 (44)</td>
</tr>
<tr>
<td>Covariance</td>
<td>Value</td>
<td></td>
</tr>
<tr>
<td>----------------------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>Cov($\eta_{Q0}$, $\eta_{\kappa}$)</td>
<td>-0.0594 (76)</td>
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<tr>
<td>Cov($\eta_{\kappa}$, $\eta_{\phi}$)</td>
<td>0.0473 (150)</td>
<td></td>
</tr>
<tr>
<td>$\sigma$ RV (mm$^2$)</td>
<td>0.5 (4)</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.