Bridging *in vitro* and *in vivo* research via an agent-based modelling approach: predicting tumor responses to an ATR-inhibiting drug

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Abstract

Translating quantitative information between *in vitro* and *in vivo* research remains a scientifically and financially challenging step in preclinical drug development processes. However, well-developed *in silico* tools can be used to facilitate this *in vitro* to *in vivo* translation, and we here propose using an agent-based model to bridge the gap between *in vitro* and *in vivo* research. The agent-based model used in this study is governed by a set of empirically observable rules, and by adjusting only the rules when moving between *in vitro* and *in vivo* simulations, whilst keeping the fundamental mathematical model and parameters intact, the agent-based model can first be parameterised by *in vitro* data and thereafter be used to predict *in vivo* treatment responses.

As a proof-of-concept, this modelling approach is here validated against data pertaining to LoVo cells subjected to the ATR (ataxia telangiectasia mutated and rad3-related kinase) inhibiting drug AZD6738, but the modelling approach has the potential to be expanded to numerous applications. In this article we also highlight how agent-based models, that are currently underutilised in pharmaceutical contexts, can be used in preclinical drug development.

Keywords

Cancer, Mathematical Oncology, DNA Damage Response Inhibition, ATR Inhibitor, AZD6738.

1 Introduction

1.1 Bridging *in vitro* and *in vivo* research

Mathematical models, and corresponding *in silico* tools, can be used to simulate both *in vitro* and *in vivo* scenarios that involve cancer cell populations, or tumours, and their responses to anti-cancer treatments. However, cancer cells in an *in vitro* cell culture experience a microenvironment that is significantly different from the microenvironment experienced by cancer cells in a solid tumour *in vivo*. As these microenvironments influence cell proliferation and the delivery of oxygen, drug and nutrient molecules to cells, it follows that the dynamics of a cancer cell population *in vitro* differs from the dynamics of a solid tumour *in vivo*. Consequently, translating data obtained by *in vitro* experiments into quantitative information that can guide or predict *in vivo* experiments remains a challenging, but important, step in drug development processes.

Agent-based models (ABMs) are used in many applications in mathematical biology but are underutilised in the context of pharmaceutical drug development [1]. An ABM consists of multiple, distinct agents that may interact with each other and with their microenvironment. In the context of mathematical oncology, an agent typically corresponds to one distinct (cancer) cell or a group of distinct (tumour) cells [2]. ABMs are thus, by design, able to capture the heterogeneous nature of solid tumours. In this study, we introduce a novel modelling approach that uses an agent-based mathematical model to bridge the gap between *in vitro* and *in vivo* research. This modelling approach in conceptually illustrated in Figure 1. In the ABM at the core of this modelling approach, an agent consists of one cancer cell or a group of cancer cells, and the behaviour and fate of each agent is governed by a set of empirically observable and well-established *modelling rules* that incorporate both intracellular and microenvironmental dynamic variables, as is thoroughly described throughout Section 2. To account for differences between *in vitro* and *in vivo* scenarios, the modelling rules are appropriately adjusted when moving between *in vitro* and *in vivo* simulations. By only adjusting the rules, whilst keeping the fundamental mathematical model and parameters intact, when moving between *in vitro* and *in vivo* simulations, the mathematical framework can first be parameterised by *in vitro* data and thereafter be used to predict *in vivo* treatment responses.

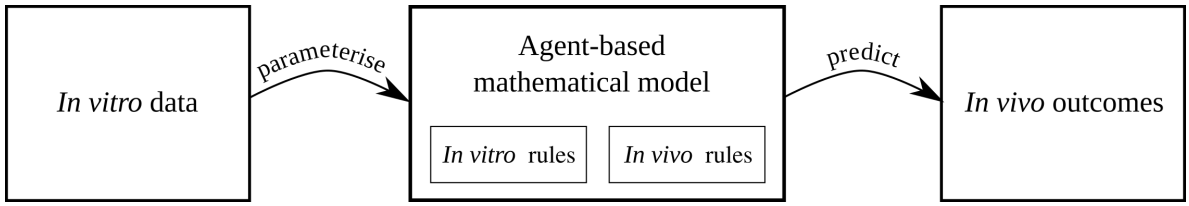


Figure 1: A schematic of the mathematical modelling approach used in this study. An agent-based mathematical model, that distinguishes between *in vitro* and *in vivo* modelling rules, is used to bridge the gap between *in vitro* and *in vivo* research. In this study, the mathematical model is first parameterised by *in vitro* data and is thereafter used to predict *in vivo* outcomes.

As a proof-of-concept of this modelling approach, we, in this study, simulate LoVo (human colon carcinoma) cells subjected to the anti-cancer drug AZD6738, which is described in Section 1.2. The *in vitro* and *in vivo* data used in this work are gathered from a previous study by Checkley *et al.* [3]. The ABM used in this study extends in two spatial dimensions and is an extension of a cellular automaton (CA) introduced by Powathil *et al.* [4].

1.2 DNA damage response inhibiting drugs

The deoxyribonucleic acid (DNA) in human cells is perpetually exposed to, potentially harmful, influences that can be derived from both exogenous and endogenous sources and events [5, 6]. Exogenous sources include ultraviolet radiation, ionising radiation and chemotherapeutic drugs [5], whilst erroneous DNA replication is an example an endogenous event yielding DNA damage [5]. Regardless of the source, a multitude of intracellular events are triggered when the DNA in a cell becomes damaged. Cells may, for example, respond to DNA damage by activating DNA repair mechanisms, cell cycle arrest or, in cases of severe DNA damage, apoptosis [7]. Such cellular responses to DNA damage are mainly governed by the DNA damage response (DDR), which comprises a complex network of signaling pathways [7]. The DDR has many functionalities and, amongst other things, it monitors DNA integrity and repairs DNA damage in order to maintain genomic stability in cells. Furthermore, the DDR pathway also governs DNA replication, cell cycle progression, and apoptosis [5, 8]. From this, it is clear that the DDR pathway is crucial for maintaining cell viability. When DNA repair in a cell is needed, the DDR activates relevant effector proteins [5]. Included in the group of DDR-associated effector proteins are approximately 450 proteins [8], out of which the two main regulators for cell cycle checkpoints are ataxia telangiectasia mutated kinase (ATM) and ataxia telangiectasia mutated and rad3-related kinase (ATR) [6]. ATM and ATR belong to the enzyme family phosphatidyilinositol-3-OH-kinases (PI3K), and they both play central roles when cells respond to DNA damage [7]. In this work, we study the effects of an anti-cancer drug, namely AZD6738, that works by inhibiting ATR activity.

DNA lesions in form of single-strand breaks are a common result of replication stress, and the repair of single-strand DNA breaks is mainly attributed to ATR activity. A drug that inhibits ATR activity consequently inhibits the repair of single-strand DNA breaks post replication stress. Cancer cells are associated with high replication stress and consequently ATR inhibitors have, during the last decade, been explored as anti-cancer agents [5, 7, 9]. With the premise that inhibiting DNA damage responses should increase the effect of some other main therapy, DDR inhibitors have been explored as both radiotherapy and chemotherapy treatment intensifiers [7, 9]. Two well-studied ATR inhibitors are AZD6738 and VX-970. AZD6738 is an oral ATR inhibitor, and its anti-tumour potential has been demonstrated in preclinical *vitro* and *in vivo* xenograft studies for various ATM deficient cell lines, including ATM deficient lung cancer, chronic lymphocytic leukemia and metastatic adenocarcinoma of the colon [3, 6, 10]. Combination treatments that combine AZD6738 with either radiotherapy or chemotherapy have produced synergistic results in preclinical settings [6], and AZD6738 is currently being evaluated in clinical phase I/II trials [5, 9]. VX-970 is an intravenous ATR inhibitor that has been investigated as a radiosensetiser and treatment intensifier in patient-derived triple negative breast cancer (TNBC) xenografts, where it should be noted that TNBC commonly features disruptions in the DDR

[11]. VX-970 has, furthermore, demonstrated tumour controlling effects in a phase I clinical trial, both as a monotherapy and in combination with the chemotherapy drug carboplatin [5]. A summarising table of clinical trials including ATR-inhibitors can be found in an article by Mei *et al.* [9].

2 Model and Method

An ABM, specifically a CA, is used to model a population of cancer cells (*in vitro*), or a solid tumour (*in vivo*), that evolves in time and two spatial dimensions. The model describes the behaviour of cancer cells using a set of modelling rules. In order to account for differences between *in vitro* and *in vivo* scenarios, these rules are appropriately adjusted when moving between *in vitro* and *in vivo* simulations, as is described throughout Section 2. Taking a minimal parameter approach, we aim to use as few rules and parameters as possible to capture the nature of the regarded system. We here chose to include model rules and parameters that pertain to the doubling time and cell cycle state of a cell (see Section 2.2), cell proliferation on the lattice (see Section 2.3), the distribution of oxygen and drugs across the lattice (see Sections 2.4 and 2.5 respectively) and cellular responses to local oxygen and drug concentrations (see Sections 2.4 and 2.6 respectively). In this work, details concerning nutrient distribution and its effect on tumour growth are not included. Instead, under a simplifying modelling assumption, the diffusion of oxygen forms a surrogate for distribution of nutrients. Differences between the *in vitro* and the *in vivo* modelling rules are pictorially summarised in Section 2.8, and the *in vitro*-parameterised model parameters are listed in Table 1.

The *in vitro* and *in vivo* data used in this study ar gathered from a previous study by Checkley *et al.* [3]. In the regarded *in vitro* experiments, populations of LoVo cells were plated and subjected to AZD6738, where population sizes of up to roughly 4000 cells were reported [3]. In the *in vivo* experiments, LoVo cells were subcutaneously injected in flanks of female Swiss nude mice in order to produce human tumour xenografts, and AZD6738 treatments started when the tumours had reached a volume of 0.2-0.3 cm^3 [3]. In this study we regard treatment responses in terms two dynamic variables: population or tumour size and percentage of DNA-damaged (i.e. γH2AX-positive) cells. The *in vitro* and *in vivo* data used in our current study is available in the supplementary material.

2.1 Cellular automaton lattice

In the model, one *agent* corresponds to one cancer cell (*in vitro*) or, due to computational costs, one group of cancer cells (*in vivo*) that is distinct from other agents in the system. The behaviour and fate of each agent is governed by a set of rules that takes into account both intracellular and environmental dynamic variables, that are integrated using multiscale and hybrid modelling techniques [2]. At the start of an *in silico* experiment, one initial agent is placed in the centre of the lattice. This initial agent produces daughter agents and ultimately gives rise to a heterogeneous population of agents. When the population has reached an appropriate size (chosen to match the *in vitro* and *in vivo* data), AZD6738 anticancer treatments commence. The CA lattice is a square lattice, and every lattice point is either empty or occupied by one agent. If a lattice point is empty, it consists of extracellular solution (*in vitro*) or extracellular matrix (ECM) (*in*

vivo). The ECM comprises multiple components such as collagen, elastin and fibronectin but we do not distinguish between these components in the mathematical model [12]. In the *in vitro* simulations, the dispersion of any molecules across the lattice is approximated as instantaneous, and thus the extracellular solution is considered to render the entire lattice homogeneous in terms drug and nutrient (here oxygen) concentrations at all times. In the *in vivo* simulations, however, drug and oxygen molecules are modelled as gradually diffusing over the ECM and tumour cells on the lattice, and consequently the *in vivo* lattice will be heterogeneous in terms of drug and nutrient (here oxygen) concentrations. Oxygen and drug distribution across the lattice are further discussed in Sections 2.4 and 2.5 respectively. Differences between the simulated *in vitro* an *in vivo* lattices are described below.

In vitro **lattice:** In our corresponding mathematical *in vitro* model, we regard cells on a two-dimensional square lattice with 100×100 lattice points, where the spacing in both spatial directions, x_1 and x_2, corresponds to one cell diameter.

In vivo **lattice:** Approximating the tumour as spherical, we, in this study, simulate (only) a central cross section of the tumour as an, approximately circular, disk of cells living on a two-dimensional square lattice. This lattice is specifically an $\tilde{L} \times \tilde{L} = 500 \times 500$ square lattice, with a spacing in both spatial directions $\tilde{x}_1$ and $\tilde{x}_2$ equal to 40μm, where the tilde-notations over the variables are used denote the *in vivo* setting. The dimensions are chosen in order to allow our agent-based model to simulate the required physical dimensions, whilst keeping computational costs low. Post simulation time, the two-dimensional cross section of cells is extrapolated to represent a three-dimensional tumour-spheroid. This disk-to-spheroid extrapolation process is outlined in the supplementary material.

2.2 Cell cycle model

In order to capture the influence of ATR and the ATR inhibitor AZD6738 on the cell cycle, we use a probabilistic, rule-based cell cycle model adapted from previous mathematical (non-agent-based) work by Checkley *et al.* [3]. In this cell cycle model, a cancer cell progresses through various states in the cell cycle, where the states correspond to different cell cycle phases. As is illustrated in Figure 2, a cell can be in an undamaged state (G1, S or G2/M), a replication stress-induced DNA damaged state (D-S) or a dead state. The cause of cell death is here unrepaired replication stress. As ATR is active in the checkpoint in the intra-S phase of the cell cycle, both under undamaged circumstances and in response to DNA damage [7], ATR inhibition will inhibit the cell from progressing to the G2/M state in the mathematical cell cycle model. A cell can take different possible paths through the cell cycle, and every time the cell cycle path forks, random number generation from a uniform distribution determines which path will be taken. Every cell commences its life in the G1 state of the cell cycle, but thereafter a cell can enter either the S state or the damaged S (D-S) state. The probability that a cell enters the D-S state is denoted Π_{D-S} and is fitted from *in vitro* data reported by Checkley *et al.* [3]. If a cell enters the D-S state, it has a chance to repair itself and enter the S state. If there is no drug in the system, this repair is always achieved, however the repair path is inhibited by the presence of the drug AZD6738. The higher the drug-concentration is, the more unlikely it is that a cell in the

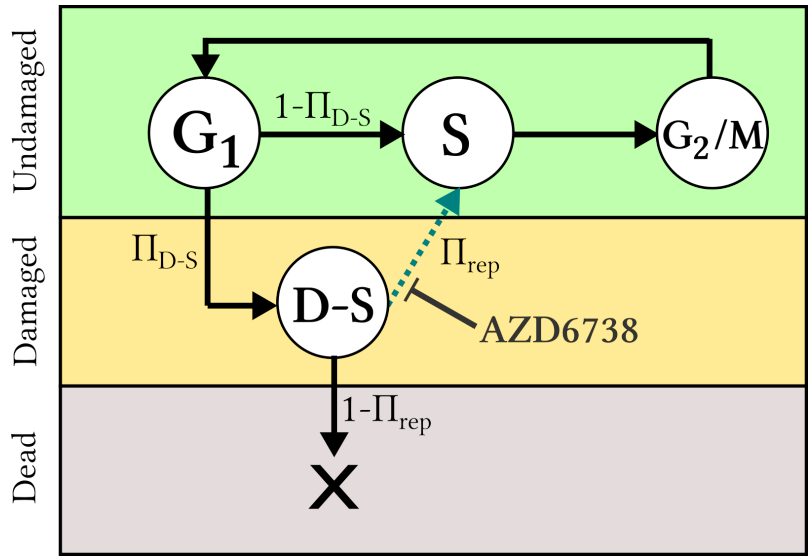


Figure 2: Cell cycle model: An agent, i.e a cell (*in vitro*) or a group of cells (*in vivo*), progresses through various states of the cell cycle, where the states correspond to cell cycle phases and are shown as nodes in the graph. Viable (undamaged or damaged) states are shown in circles, whilst the dead state is shown as a cross. Paths in the graph illustrate transitions between states, and symbols next to the paths denote the probabilities that the paths will be taken. The teal, dashed path indicates a replication path that can be inhibited by ATR inhibiting drugs, such as AZD6738, as is further described in Section 2.6.

D-S state will successfully repair itself to the S state. If a cell in the D-S state can not repair, it is sentenced to die. Whether a cell in state D-S repairs or dies is decided by comparing a random number, generated from a uniform distribution, to the cell's survival probability, which is influenced by the local drug concentration $C(\bar{x}, t)$, as is further described in Section 2.6. A cell that has successfully reached the S state continues to the G2/M state after which it duplicates and starts over in the G1 state again, ready to perform another cell cycle.

Each agent i on the lattice is assigned an individual doubling time τ_i, where τ_i is a random number generated from a normal distribution with mean value μ and standard deviation σ. A normal distribution is chosen in order to model the *in vitro* cells as being (not perfectly) synchronised, this is motivated by observations of the *in vitro* data, listed in the supplementary material. Each agent is also attributed an individual cell cycle clock, that determines when the agent should progress to a subsequent state in the cell cycle model. Every agent that is placed on the lattice commences its life in the G1 state, and progression to a subsequent state (i.e. S or D-S) is scheduled to occur once the agent has spent a certain fraction of its doubling time in the G1 state. The fraction of the doubling time spent in the G1, S (including D-S) and G2/M states are respectively denoted Θ_{G1}, Θ_S and $\Theta_{G2/M}$, where these values are approximate and chosen from literature to match values for typical human cells with a rapid doubling time of 24 hours so that $\Theta_{G1} = 11/24$, $\Theta_S = 8/24$ and $\Theta_{G2/M} = 5/24$ [13]. The fraction of an agent's doubling-time

spent in the D-S state, Θ_{D-S}, is on the other hand fitted by *in vitro* data produced by Checkley *et al.* [3]. In order to account for differences between *in vitro* and *in vivo* systems, cell cycle model rules are adjusted as described below.

In vitro **cell cycle model rules:** One agent corresponds to one cancer cell that is assigned an individual doubling time τ_i. The cell cycle path taken by cell i is governed by random number generations specific to that cell.

In vivo **cell cycle model rules:** One agent comprises a group of identical cancer cells. Each agent is assigned an individual doubling time, τ_i, and thus all cells belonging to agent i progress simultaneously uniformly through the cell cycle model. Random number generations specific to agent i determine which path the agent takes through the cell cycle.

2.3 Cell proliferation

When an agent has completed the mitosis state in the cell cycle model, a secondary agent, namely a daughter agent, is produced. Each daughter agent is placed on a random lattice point in the (approximately circular) neighbourhood of its parental agent. To accomplish circular-like growth, the model stochastically alternates between placing daughter agents on Moore and von Neumann neighbourhoods of parental agents, as is pictorially described in the supplementary material. A daughter agent is allowed to be placed on, up to, a νth order neighbourhood of its parental agent, but lower order neighbourhoods (i.e. neighbourhoods closer to the parent) are prioritised and filled up first. In other words, after all lattice points in neighbourhood j of the parental agent are occupied, daughter agents may be placed in neighbourhood $j+1$, as is illustrated in the supplementary material. Modelling rules concerning cell proliferation *in vitro* and *in vivo* are outlined below.

In vitro **proliferation rules:** In the experimental *in vitro* setup, there is no spatial constraint or nutrient deficiency that is inhibiting cell division within the time-course of the experiment. Consequently cells are allowed to divide freely in the *in vitro* model and we set ν to be equal to infinity in the *in vitro* case (with the restriction that agents can not be placed outside the lattice in the *in silico* implementation).

In vivo **proliferation rules:** *In vivo* tumours typically consist of a core with quiescent cells and a shell of proliferating cells. To accommodate for this, a daughter agent (representing a group of daughter cells) is allowed to be placed on up to a third order (von Neumann or Moore, alternately) neighbourhood of its parental agent, so that $\tilde{\nu} = 3$, in accordance with previous mathematical models [4]. For the *in vivo* experiment regarded in our current study, $\tilde{\nu} = 3$ matches the experimental data. However, for other experiments the value of $\tilde{\nu}$ may be adjusted to fit the specific cell-line and modelling scenario at hand. When an agent is in the G1 phase of the cell cycle, it scans its environment to see if it has enough resources, in terms of space and nutrients, to commence the process of producing a daughter cell. If not, the cell enters the quiescent phase [14]. Thus in the model, when an agent is in the G1 phase, it continues to progress through the cell cycle model, provided that some free space is available on the lattice

within in its $\tilde{\nu}$th neighbourhood. If this is not the case, the agent exits the cell cycle to enter a quiescent state G0. Should neighbourhood space be made available again, here as a result of anticancer targeting, quiescent agents may re-enter the cell cycle.

2.4 Oxygen distribution and influence on cells

Tumour growth and treatment responses are highly influenced by intratumoural oxygen levels and [15, 16, 17] severely hypoxic (cancer) cells proliferate slower than do well-oxygenated cells [14].[18].

In vitro **oxygen distribution and responses:** In the mathematical *in vitro* model, all cells are assumed to be well-oxygenated in accordance with the experimental *in vitro* setup performed by Checkley *et al.* [3]. Consequently, neither oxygen dynamics nor cellular responses to low oxygen levels are incorporated in the mathematical *in vitro* model.

In vivo **oxygen distribution and responses:** Within solid tumours, oxygen concentrations typically vary and hypoxic regions are common tumour features [19, 17, 20]. Avoiding complicated details of vasculature in the model, we here approximate oxygen as diffusing in from 'outside the boundary of the tumour'. Oxygen dynamics across the CA lattice is here described using a mechanistic diffusion equation, where the oxygen concentration in location $\bar{x}$ at time t is denoted by $K(\bar{x}, t)$ where

$$\frac{\partial K(\bar{x}, t)}{\partial t} = \nabla \cdot (D_K(\bar{x}, t)\nabla K(\bar{x}, t)) + r_K m(\bar{x}, t) - \phi_K K(\bar{x}, t) cell(\bar{x}, t). \tag{1}$$

The first term in Equation 1 describes oxygen diffusion across the CA lattice, the second term is an oxygen supply term and the third term describes oxygen uptake by cells. Accordingly, $D_K(\bar{x}, t)$ denotes the oxygen diffusion coefficient, and r_K and ϕ_K are supply and consumption coefficients respectively. The diffusion coefficient for oxygen is known from literature to be 2.5×10^{-5} cm^{2}s^{-1} [4]. Assuming that oxygen diffuses slower over cells than in the ECM, the oxygen diffusion coefficient is divided by a factor 1.5 if there is a cell in location $\bar{x}$ at time t. The binary factor $m(\bar{x}, t)$ is 1 if the regarded location $\bar{x}$ is outside the tumour boundary at time t and 0 otherwise, i.e. $m(\bar{x}, t)$ is 1 if the regarded lattice point is not occupied by an agent nor completely surrounded by agents. Similarly, the binary factor $cell(\bar{x}, t)$ is 1 if there is a viable cell in location $\bar{x}$ at time t, and 0 otherwise [4]. Equation 1 is coupled with no-flux boundary conditions, thus the total amount of oxygen in the system fluctuates over time [21]. A scaled oxygen variable $\hat{K}(\bar{x}, t)$ is introduced in order to express oxygenation levels in percentages (%). This scaled oxygen value is computed at every unique time step t_u by

$$\hat{K}(\bar{x}, t_u) = \frac{K(\bar{x}, t_u)}{max_{\bar{x}, t_u} K(\bar{x}, t_u)} \cdot h, \tag{2}$$

where $max_{\bar{x}, t_u} K(\bar{x}, t_u)$ denotes the maximum occurring $K(\bar{x}, t_u)$-value at the time point t_u [21] and h is a scaling factor [4]. Low cellular oxygen levels have been shown to delay cell cycle progression by inducing arrest in, particularly, the G1 phase of the cell cycle [14] and in our model, hypoxic cells with an oxygen concentration of 10 % or less display arrest (i.e. delay) in the G1 phase of the cell cycle. In mechanistic Tyson-Novak type cell cycle models [22, 23, 24],

the cell cycle is governed by a system of ordinary differential equations (ODEs) in which the G1 phase is inherently elongated under hypoxic conditions by incorporating hypoxia-induced factors into the ODEs [4]. In this model, we use a clock to model cell cycle progression and thus we introduce a G1 delay factor (G1DF) in order to achieve a longer G1-phase under hypoxic conditions where

$$G1DF(\hat{K}(x,t)) = \begin{cases} 2 & \text{if } 0\% \leq \hat{K}(x,t) < 1\ \%, \\ a_1 + \frac{a_2}{a_3 + \hat{K}(\bar{x},t)} & \text{if } 1\% \leq \hat{K}(x,t) \leq 10.5\ \%, \\ 1 & \text{otherwise.} \end{cases} \tag{3}$$

The G1DF is an approximation for how much the G1 phase is expanded in time as a function of oxygen concentration. It is matched to fit data points extracted from a previous mathematical study by Alarcon *et al.* [14], in which a Tyson-Novak cell cycle model is extended to incorporate the action of p27, a protein that is up-regulated under hypoxia and delays cell cycle progression. Data-fitting yields the parameter values $a_1 = 0.9209, a_2 = 0.8200, a_3 = -0.2389$ [19]. Thus the fraction of an agent's doubling time spent in the G1 state is $G1DF(\hat{K}(\bar{x},t)) \cdot \Theta_{G1}$, where $G1DF(\hat{K}(\bar{x},t)) = 1$ for normoxic cells.

2.5 Drug distribution across the lattice

Drug distribution significantly varies between *in vitro* and *in vivo* settings, as described below. In the regarded *in vitro* setup, the drug concentration can be regarded as homogeneous, whilst heterogeneous drug concentrations must be accounted for *in vivo*.

In vitro **drug distribution:** In the *in vitro* experiments performed by Checkley *et al.* [3], plated cell populations of roughly 1000 cells were treated with AZD6738 in the solvent dimethylsulfoxide (DMSO). In the mathematical model, we thus approximate the drug distribution across the lattice to be instantaneous (occurring at treatment time T_0) and homogeneous. We furthermore assume that the drug has a half-life time that exceeds the time course of the experiment, and note that there is no other drug elimination from the *in vitro* system (unless drug wash-out is applied). In our mathematical model, this is equivalent to there being no drug decay or elimination. Hence in the mathematical *in vitro* model, the drug concentration $C(\bar{x},t)$, in location $\bar{x}$ at time t is simply given by

$$C(\bar{x},t) = \begin{cases} 0 \text{ everywhere} & \text{if } t < T_0, \\ \mathbb{C} \text{ everywhere} & \text{if } t \geq T_0, \end{cases} \tag{4}$$

where $\mathbb{C}$ denotes the applied drug concentration (in units of molarity).

In vivo **drug distribution:** In the *in vivo* experiments performed by Checkley *et al.* [3], AZD6738, or vehicle in the control case, were administered via oral gavage once per day to female Swiss nude mice. In the mathematical *in vivo* model, we consider the drug to diffuse through the tumour from its surrounding, creating a drug gradient within the tumour. In the mathematical model, this drug dynamics is modelled using a partial differential equation (PDE), where the

concentration of the drug AZD6738 in lattice point n at location $\bar{x}$ at time t is denoted by $C(\bar{x}, t)$ and

$$\frac{\partial C(\bar{x}, t)}{\partial t} = \nabla \cdot \big(D_{AZD}(\bar{x}, t)\nabla C(\bar{x}, t)\big) + p(\bar{x}, t) - \eta_{AZD} C(\bar{x}, t), \tag{5}$$

where D_{AZD} is the diffusion coefficient of the drug AZD6738, and the supply coefficient $p(\bar{x}, t)$ is greater than zero at drug administration times only for lattice points outside the tumour. Assuming first order kinetics for drug elimination, the drug decay constant η_{AZD} is matched to the reported half-life time of 6 hours for AZD6738 *in vivo* [25, 26]. Note that the drug decay term here represents all drug elimination from the system, both metabolic and that caused by excretion.

The diffusion rate of a drug is predominantly affected by the molecular size of the drug. More specifically, the diffusion coefficient of a drug is inversely proportional to the square root of the molecular weight of the drug, so that large molecules diffuse more slowly than do small molecules [26]. Using this assumption, the drug diffusion coefficient is set in relation to the oxygen diffusion coefficient, as is done in previous mathematical studies [4]. Thus the relationship between the diffusion coefficients for the drug (AZD) and oxygen (O_2) corresponds to the square of the inverse relationship between the corresponding molecular weights, such that

$$\frac{D_{AZD}}{D_{0_2}} = \frac{\sqrt{\text{molecular weight}(\mathrm{O}_2)}}{\sqrt{\text{molecular weight}(\text{AZD})}} = \sqrt{\frac{31.998\text{g/mol}}{412.512\text{g/mol}}} \approx 0.27851, \tag{6}$$

where the molecular weights are collected from the PubChem database [27]. Details regarding pharmacokinetics are outside the scope of this study, bioavailability is instead calibrated using the extreme scenario in which the maximum drug dose is administered *in vivo*.

2.6 Drug responses

AZD6738 inhibits the repair from the D-S state to the S state in the cell cycle, as is illustrated in Figure 2, and maximal drug effect corresponds to complete repair inhibition. The drug effect is modelled using an agent-based adaptation of the sigmoid Emax model [28], in which the drug effect on a cell in position $\bar{x}$ at time t is given by

$$E(\bar{x}, t) = E_{max} \frac{C(\bar{x}, t)^{\gamma}}{EC_{50}^{\gamma} + C(\bar{x}, t)^{\gamma}}. \tag{7}$$

E_{max} denotes the maximal drug effect, here corresponding to complete repair inhibition ($E_{max} = 1$), EC_{50} denotes the concentration required to achieve half of the maximal drug effect, $0.5 \cdot E_{max}$ and γ is the Hill-exponent [28]. EC_{50} and γ are fitted from the *in vitro* data. When an agent is scheduled to progress from the D-S state in the cell cycle, it has a probability $\Pi_{rep} \in$ [0,1] to repair which is determined by the local drug concentration such that

$$\Pi_{rep}(\bar{x}, t) = 1 - E(\bar{x}, t). \tag{8}$$

Section	Parameter	Calibrated Value
2.2	μ, σ	24 h, 0.5 h
	Π_{D-S}, θ_{D-S}	0.75, 0.03
2.6	EC_{50}, γ	1 μM, 2
	$T_{L\to D}$	τ_i

Table 1: *In vitro* calibrated parameters.

Note that in the absence of drugs, the repair probability is 1. When a cell dies, it is transformed into a membrane-enclosed 'cell-corpse' [26]. In the *in vivo* setting, the cellular debris is digested by macrophages but in the *in vitro* setting such 'cell-corpses' linger on the lattice during the course of the experiment. Post the lethal event (i.e. the D-S to S repair failure) a cell is declared 'dead' in the model after a time $T_{L\to D}$ has passed (where L stands for 'lethal event' and D stands for 'death'). The parameter $T_{L\to D}$ is calibrated by *in vitro* experiments. The differences between modelling rules for *in vitro* and *in vivo* drug responses are described below.

In vitro **drug responses:** After failure to repair from the D-S state, a cell (i.e. and agent) is considered to be dead after a time $T_{L\to D}$ has passed. However, a dead cell is never physically removed from the lattice.

In vivo **drug responses:** An agent (i.e. a group of cells) is declared to be dead and removed from the lattice after an amount of time $T_{L\to D}$ post the lethal event (failure to repair).

2.7 Parameters

The parameters used in the mathematical framework are parameterised by *in vitro* data and are listed in Table 1. The *in vitro* and *in vivo* data produced by Checkley *et al.* [3] is listed in the supplementary material. The supplementary material also includes information regarding the model calibration process. In the context of quantitative pharmacology, knowledge about a model's robustness is crucial [29]. Accordingly, results from the uncertainty and sensitivity analysis are also included in the supplementary material. Three different uncertainty and sensitivity analyses techniques, suitable for agent-based models with stochastic elements, are used as described in the supplementary material. These three techniques are (*i*) consistency analysis, (*ii*) robustness analysis and (*iii*) Latin hypercube analysis [30, 31]. Thorough descriptions of how to perform and interpret these techniques are available in an introductory review [**?**]. In accordance with the performed consistency analysis, we run 100 simulations per *in silico* experiment in order to formulate results (here in terms of mean values and standard deviations) that mitigate uncertainty originating from intrinsic model stochasticity. Global sensitivity analysis (Latin hypercube analysis) demonstrates that *in vitro* simulation outputs, or in other words simulated treatment success, mainly depends on the cells' sensitivity to drugs, in terms of EC_{50}, and the treatment schedule in relationship to the cell population's overall cell cycle phase composition, influenced by μ and σ. Thorough investigations of all parameters listed in Table 1, using robustness analysis and Latin hypercube analysis, are available in the supplementary material.

2.8 Differences between *in vitro* and *in vivo* modelling rules

The differences between the *in vitro* and *in vivo* modelling rules in the ABM are pictorially summarised in Figure 3.

A note on simplifying modelling assumptions Note that an agent in the *in vivo* setting can, in general, be chosen to comprise either one cancer cell or a group of cancer cells. Note also that modelling the *in vivo* tumour as a (spatially) two-dimensional disk means that the distribution of nutrients and drugs is modelled across a two-dimensional (rather than a three-dimensional) space. Likewise, the parameter $\tilde{\nu}$, that governs the allowed distance between a parental agent and its daughter agents, only concerns proliferation on a two-dimensional plane (not in three-dimensional space). By increasing $\tilde{\nu}$, the tumour will grow quicker and comprise a higher fraction of cycling (to quiescent) agents. Thus both the parameters $\tilde{\nu}$ and μ (the average cell doubling time) influence the rate of tumour growth in the *in vivo* simulation, and should ideally be fitted to match detailed *in vivo* data that includes information regarding the intratumoural cell cycle phase composition. In the current study, we choose to keep μ at the *in vitro*-calibrated value and thereafter fit $\tilde{\nu}$ to match the *in vivo* data. A modeller can chose to use a three dimensional CA lattice, and thus explicitly model a tumour spheroid instead of a tumour cross-section. Computational costs, fineness of available data, and the desired level of simulation details should be used to guide the choice of CA agents and CA lattice.

2.9 Implementation

The mathematical model is implemented in an in-house C/C++ framework in order to produce *in silico* results. Partial differential equations are solved using explicit finite difference methods. Simulation cell-maps are visualised using ParaView [32]. Results reported in Section 3 are based on 100 simulation runs as, according to results from the uncertainty analysis, 100 runs are sufficient for mitigating uncertainty originating from intrinsic model stochasticity. Uncertainty and sensitivity analyses are performed using MATLAB [33, 30].

3 Results

The mathematical framework is first parameterised by *in vitro* data, and is thereafter used to predict treatment responses in human tumour xenografts *in vivo*. By only adjusting the modelling *rules* in the mathematical framework, whilst keeping the *in vitro* calibrated model parameters intact, the mathematical framework is used to predict treatment responses in human tumour xenografts *in vivo*.

3.1 Simulating *in vitro* experiments

The mathematical framework used in this study is parameterised by results from an *in vitro* experiment performed by Checkley *et al.* [3] in which populations of LoVo (human colon carcinoma) cells are exposed to the ATR inhibiting drug AZD6738. The *in silico* results in Figure 4 simulate the evolution of the *in vitro* cell population over time in terms of percentage of DNA damaged, i.e. γH2AX-positive, cells (Figure 4 Left) and in terms of cell count (Figure 4 Right). AZD6738

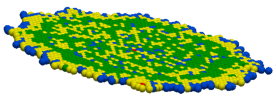
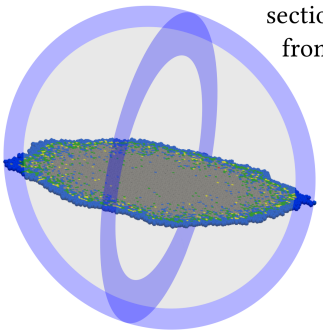
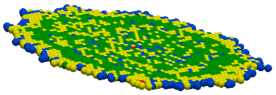
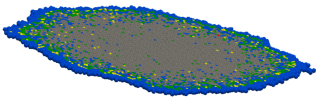
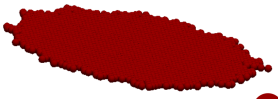
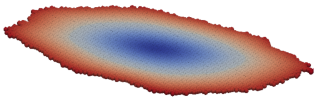
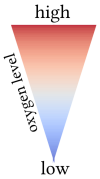
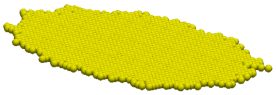
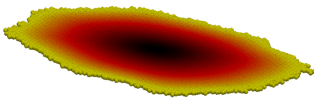
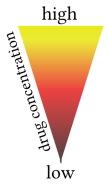
	in vitro rules	*in vivo rules*
Lattice setup	A monolayer of cells is simulated.  One agent represents one cancer cell.	A center tumour cross-section is simulated, from which a tumour spheroid is extrapolated post simulation time.  One agent represents a group of cancer cells.
Cell cycle heterogeneity	All cells are cycling.  G1 S D-S G2/M	Agents that are surrounded by other agents in ν or more orders of their circular neighbourhoods are quiescent.  G1 S D-S G2/M G0
Oxygen dynamics	All cells are well oxygenated, oxygenation levels are constant in time and space on the lattice.  Oxygenation level	Oxygen diffuses in from outside the tumour. Hypoxia induces G1 arrest.  
Drug dynamics	Drug concentration  Post drug administration time, drug concentration is constant in time and space on the lattice.	  Drugs diffuse in from outside the tumour, and drugs are eliminated from the system.
Cell removal	Dead agents are *not* removed from the lattice.	Dead agents are removed from the lattice after a time $T_{L\text{->}D}$.

Figure 3: A summary of the differences between the *in vitro* and *in vivo* rules used in the mathematical framework.

drugs are given at 0 hours, when the cell population has reached a size of approximately 1000 cells. Response curves for six different drug concentrations, including the zero-drug concentration control case, are shown. Each response curve is based on a mean value from 100 simulation runs, according to the uncertainty analysis described in the supplementary material. Also shown in Figure 4 are simulation standard deviations and *in vitro* results produced by Checkley et al. [3].

Using a minimal-parameter modelling approach, the mathematical framework is calibrated to best the maximum number of *in vitro* data points without introducing any variable model parameters. The parameter calibration process is described in the supplementary material. Our results demonstrate that, after *in vitro* parameterisation, our mathematical framework is able to capture the qualitative nature of *in vitro* LoVo cell population growth and drug (AZD6738) responses. Figure 4 (Left) demonstrates that the model is able to qualitatively reproduce the asymptotic fraction of DNA damaged cells in the system but fails to match early *in vitro* data points. The sensitivity analysis demonstrates that the treatment timing (in relation to the over all cell cycle phase composition of the cancer cell population) notably influences treatment responses in terms of percentage of γH2AX-positive cells. This indicates that in the *in vitro* experiments, a large fraction of the cells would have been in the DNA damaged D-S state when, or shortly after, the drug was applied. The experimental error bars in Figure 4 (Right) and the numerical cell count data, available in Table 1 in the supplementary material, demonstrate that the doubling time of the cell population drastically decreased towards the end of the experiment and, consequently, our agent-based model was not able to replicate cell count data at 72 hours as the modelling rules and parameters pertaining to cell proliferation were not updated over time.

3.2 Simulating *in vivo* experiments

Post the *in vitro* calibration, our mathematical framework is used to simulate *in vivo* experiments performed by Checkley *et al.* [3] in which LoVo xenografts, that are injected in mice flanks, are treated with AZD6738 once daily for 14 days. The results in Figure 5 simulate AZD6738 drug responses in terms of the percentage of DNA damaged, i.e. γH2AX-positive, cells (Figure 5 Left) and tumour volume (Figure 5 Right). Response curves to three different drug doses (0, 25 and 50 mg/kg) are shown, where the curves represent mean values for 100 simulation runs. Simulation standard deviations and *in vivo* data are also provided in Figure 5. Figure 4 (Right) demonstrates that our mathematical framework qualitatively agrees with the *in vivo* results reported by Checkley et al. [3] for approximately 12 days post tumour injection for control case tumours, and for approximately 8 days post tumour injection for tumours subjected to drugs. This can be explained by the fact that the behaviour of the agents in our current model does not change over time, when in fact tumours are highly adaptable and responsive to external pressures. It follows that details pertaining to tumour growth and drug sensitivity may vary over time, and in future work the agent-based model used in this study can be updated to incorporate variable modelling rules or parameters that change over time.

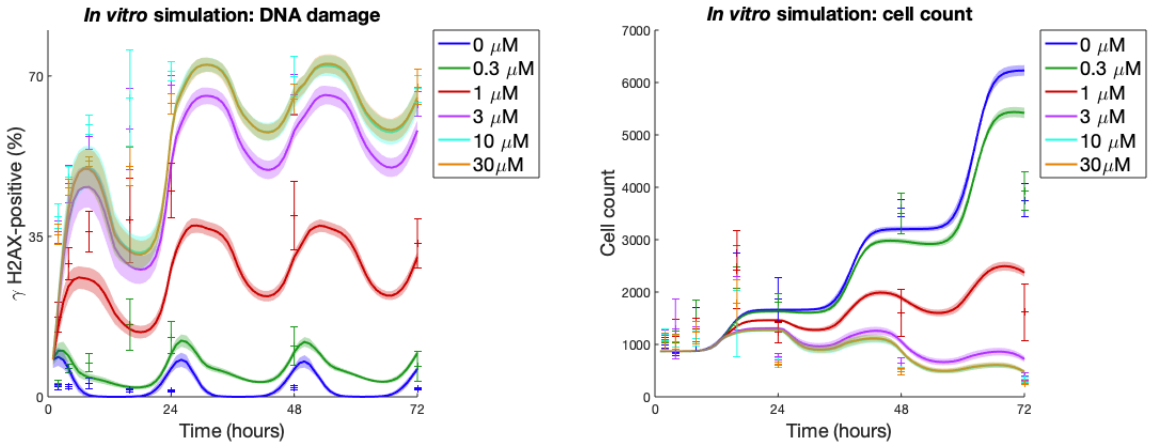


Figure 4: Simulated *in vitro* drug response curves. LoVo cells are exposed to drug (AZD6738) at 0 hours. Left: The percentage of γH2AX-positive (i.e. DNA-damaged) cells in the system over time. Right: Cell count over time. Simulated mean values and standard deviations for 100 *in silico* runs are shown with solid lines and shaded ribbons respectively. *In vitro* mean values and standard deviations are demonstrated with center points and error bars respectively [3].

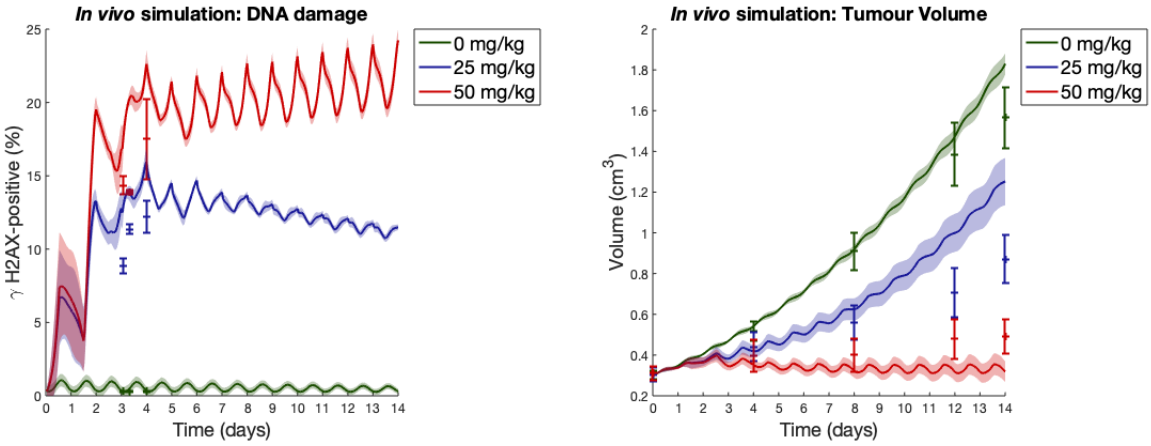


Figure 5: Simulated *in vivo* drug response curves. LoVo xenografts are exposed to drug (AZD6738) once daily for 14 days. Left: The percentage of γH2AX-positive (i.e. DNA-damaged) cells in the xenograft over time. Right: Tumour volume over time. Simulated mean values and standard deviations for 100 *in silico* runs are shown with solid lines and shaded ribbons respectively. *In vivo* mean values and standard errors are demonstrated with center points and error bars respectively [3].

4 Discussion

The *in silico* results obtained in this study were compared to *in vitro* and *in vivo* data in Section 3 and can, furthermore, be compared to previous mathematical modelling results produced by Checkley *et al.* [3]. In their study, Checkley *et al.* [3] modelled tumour responses to AZD6738 using coupled ordinary differential equations, where a pharmacokinetic/pharmacodynamic (PK/PD) model of tumour growth was integrated with a mechanistic cell cycle model. Their model, which is also calibrated by their *in vitro* experiments, is predictive of *in vivo* xenograft studies and is being used to quantitatively predict dose and scheduling responses in a clinical Phase I trial design [3]. Our *in vitro* modelling results qualitatively agree with those produced by Checkley *et al.* [3], as does our *in vivo* modelling results for up to approximately 8 days, although two different modelling approaches have been taken: Checkley *et al.* [3] regard the tumour as one entity with different compartments whilst we here use a bottom-up modelling approach and regard the tumour as consisting of multiple, distinct agents.

Moving drug-response investigations from *in vitro* to *in vivo* settings is a key step involved in the process of moving a drug from *bench-to-bedside*. However, data from *in vivo* experiments is often sparse, as gathering *in vivo* data is associated with practical, financial and ethical constraints. Plentiful and adaptable *in silico* data are, on the other hand, easy to produce, and thus sparse *in vivo* data can be complemented by *in silico* data. Consequently, mathematical frameworks, and their corresponding *in silico* experiments, can be used as an epistemic contribution to sparse data produced in wet labs. Well-formulated *in silico* experiments can be extended to investigate various dose-schedule scenarios in order to guide *in vitro* and *in vivo* experiments. Such *in silico* experiments may provide a testbed for simulating, and thus investigating, various monotherapies and combination therapies. We here propose creating *in silico* tools that are based on ABMs in which modelling rules are based on "fundamental" principles that describe how cancer cells in a system behave (where it is up to the modeller to decide which principles should be considered "fundamental" in the specific modelling scenario at hand). The ABM considered in this study is an extension of a mathematical model that has previously been used to study tumour growth and treatment responses to chemotherapy, radiotherapy, hyperthermia and hypoxia-activated prodrugs [4, 34, 35, 36, 37]. In recent years, several ABMs have been developed for the purpose of describing various aspects of cancer dynamics [38], and it should be noted that modelling approach proposed in Figure 1 is not conceptually limited to usage with the ABM described in this study. The choice of ABM should be influenced by the research question at hand, the desired level of model details and the available data.

Data-driven modeling, exploitation of existing data and proof-of-concept studies are important steps involved in current and future procedure for enabling mathematical modeling in systems medicine, as is argued in a report by Wolkenhauer *et al.* [39]. Despite the fact that mathematical modelling is, in general, becoming increasingly popular in the pharmaceutical industry, there are not that many ABMs present in the pharmaceutical scene [1]. We argue that this is a missed opportunity in the context of oncology, as ABMs naturally capture the heterogeneous nature of tumours, that is known to complicate treatments. As multiscale ABMs organically enable the integration of data across various scales in time and space, it follows that they are

useful to the interdisciplinary team that wishes to combine data and collective knowledge from its team members. Following interdisciplinary collaborations between clinicians, biologists and mathematicians, mathematical modelling may be used to enable *in silico* informed drug development.

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Competing Interests

No competing interests to declare.

Authors' Contributions

All authors conceived of the study and coordinated the study. All authors contributed to model design. SH performed the *in silico* experiments, sensitivity analysis and drafted the manuscript. All authors critically revised the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

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Targeting Cellular DNA Damage Responses: Predicting *in vivo* treatment responses using an *in vitro*-calibrated agent-based mathematical model

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– Supplementary Material

1. Experimental Data
2. Model Parameterisation
3. Proliferation of Agents on the Lattice
4. Cross-Section to Tumour Spheroid Extrapolation
5. Uncertainty and Sensitivity Analyses

1 Experimental Data

The experimental *in vitro* and *in vivo* data used in our current study are gratefully gathered from a previous study performed by Checkley *et al.* [1]. *In vitro* data are listed in Table 1 and *in vivo* data are listed in Tables 2 and 3.

Time (hours)	Cell count	Std.Dev (count)	γH2AX-positive (%)	Std. Dev (%)
0μM (control)				
2	996	59.72	2.14	0.56
4	850	62.30	2.20	0.45
8	1287.5	417.59	2.90	1.22
16	2742.75	439.69	1.44	0.33
24	1857.5	409.39	1.29	0.32
48	3605.25	167.38	1.93	0.44
72	3753	311.17	1.71	0.21
0.3 μM				
2	1081.88	53.63	2.93	0.59
4	1040.75	217.96	6.15	1.00
8	1447.25	392.45	7.41	1.99
16	2479.5	414.02	15.68	5.56
24	1805.63	161.41	12.91	3.42
48	3497.63	385.19	11.08	4.18
72	3928.25	376.08	6.57	3.30
1 μM				
2	1129.63	58.26	17.35	3.31
4	1153.63	331.31	29.12	3.47
8	1303.88	199.72	36.05	4.35
16	2420.25	744.38	38.51	9.25
24	1226.38	185.58	45.01	6.01
48	1600.38	456.80	39.47	7.47
72	1612.88	540.55	33.47	5.46
3 μM				
2	1171.14	97.71	36.01	2.42
4	1291.38	567.63	46.47	4.09
8	1224.63	113.30	56.72	2.62
16	1784.38	513.06	58.41	8.81
24	765.75	70.76	68.07	2.05
48	638.75	112.54	65.90	4.40
72	392.63	67.64	63.82	2.67
10 μM				
2	1191.13	110.15	39.38	2.62
4	1056.63	106.72	47.98	2.32
8	1113.63	144.42	59.35	1.99
16	1396	633.86	65.21	10.48
24	654.5	100.26	71.02	2.10
48	525.29	43.93	69.75	4.42
72	326.63	47.73	67.25	2.87
30 μM				
2	1055.13	155.16	35.37	2.21
4	1049.13	147.96	45.66	1.75
8	1228.75	211.96	51.37	1.11
16	1794.88	435.42	50.35	4.19
24	629	27.12	63.92	2.15
48	469.63	61.26	64.92	3.25
72	265.13	22.26	67.63	3.96

Table 1: *In vitro* data gathered from a previous study by Checkley *et al.* [1].

Time (hours)	γH2AX-positive (%)	Std. Error (%)
0 mg/kg (control)		
74	0.3200	0.0320
80	0.2770	0.0300
96	0.2970	0.0340
25 mg/kg QD		
74	8.8579	0.5364
80	11.3692	0.3272
96	12.1945	1.0949
50 mg/kg QD		
74	14.3417	0.6278
80	13.8967	0.1401
96	17.4986	2.7558

Table 2: *In vivo* data for DNA damage gathered and adapted from a previous study published by Checkley *et al.* [1].

Time (hours)	Volume (cm^3)	Std. Error (cm^3)
0 mg/kg (control)		
168	0.3028	0.0219
264	0.5189	0.0465
360	0.9095	0.0934
456	1.3857	0.1554
504	1.5646	0.1483
25 mg/kg QD		
168	0.3037	0.0342
264	0.4411	0.0704
360	0.5617	0.0840
456	0.7064	0.1221
504	0.8701	0.1187
50 mg/kg QD		
168	0.3106	0.0332
264	0.3971	0.0768
360	0.4002	0.0817
456	0.4783	0.0966
504	0.4923	0.0846

Table 3: *In vivo* data for tumour volume gathered and adapted from a previous study published by Checkley *et al.* [1].

2 Model Parameterisation

Using a minimal-parameter approach, seven model parameters are calibrated using the *in vitro* data previously produced by Checkley *et al.* [1], as listed in Table 1 in Section 2.7 in the main paper. Parameter sensitivity is explored in the sensitivity analysis (Section 5 of the supplementary material). The calibration process is outlined in Sections 2.1 through to 2.4. The *in vivo* calibration is described in Section 2.5.

2.1 Cell doubling

In the model, the doubling time of a cell i is denoted τ_i, where τ_i is stochastically picked from a normal distribution with mean value μ and standard deviation σ. Thus μ corresponds to the

average cell doubling time and σ corresponds to how synchronised the cells are. If σ is zero, then all cells have perfectly synchronised cell cycles and duplicate at the same time. Higher σ values achieve less synchronised cell cycles amongst cells and smoother cell count growth curves over time. The control case (i.e. no drug) cell count data is used to estimate μ and σ.

By observing the control case cell count data in Table **??**, we note that the cell population roughly doubles between 2 and 24 hours (indicating an average cell doubling time, or μ, of approximately 22 hours, which is less than 24 hours). Furthermore, the cell population also roughly doubles between 24 and 48 hours (indicating that μ is approximately 24 hours). However, in the last 24-hour interval, between 48 and 72 hours, the control population increases by less than 5% (indicating that μ is more than 24 hours). From these three observations, we choose to make the modelling assumption that the average doubling time for cells should be around 24 hours, and σ-values in the parameter range [22,26] hours are investigated *in silico*. Due to the synchronised nature of the cell count data, σ-values between 0 and 2.5 hours were investigated *in silico*, where $\sigma = 0$ h corresponds to completely synchronised cells and $\sigma = 2.5$ h achieves a smooth cell count growth curve. After an iterative process of tuning parameters and running *in silico* experiments, the calibrated values are set to be $\mu = 24$ hours and $\sigma = 0.5$ hours. As discussed in the main article, the ABM can be improved to better fit wet lab data by including variable parameter values or rules, that are updated over time. However, in the current stage of our work, we decided to fix μ and σ.

2.2 Cell cycle progression

The *in vitro* data provides information on how many cells are in the damaged S state via the biomarker γH2AX. For the control case, the number of γH2AX positive cells in our mathematical model depends on two variables: (1) the probability (Π_{D-S}) that a cell enters the D-S state and (2) the amount of time ($\Theta_{D-S} \cdot \tau_i$) spent in the D-S state prior to repairing. Recall that Θ_{D-S} is the fraction of a cell's doubling time (τ_i) spent in the D-S state. As a first step, *in silico* experiments are performed in which we find various parameter pairs (Π_{D-S}, Θ_{D-S}) that agree with the control data. We thereafter note that the *in vitro* drug effect saturates for concentrations 3, 10 and 30 μM and assume that the maximal dose (30 μM) yields 100% D-S to S repair inhibition. Thus a second step we test the variable pairs (Π_{D-S}, Θ_{D-S}) for this 'maximal drug and no repair' scenario *in silico*, and we match these *in silico* results to the 30 M *in vitro* data. Here, we only use data from early time points (time $<$ 12 hours) in order to avoid the influence that dying cells have on the data and model outputs. After iterative *in silico* testing, the variable pair (Π_{D-S}, Θ_{D-S}) that best fits these both extreme cases is $\Pi_{D-S} = 0.75$ and $\Theta_{D-S} = 0.03$. The first extreme case refers to the 'no drug' *in silico* experiment matched to the *in vitro* control data, where we assume that all D-S cells repair to state S. The second extreme case refers to the 'maximum drug' *in silico* experiment matched to the 30μM control data, where we assume that no D-S cells repair to state S.

2.3 Drug response

Drug effects are modelled using the sigmoid E-max model [2], where the drug effect E is a function of the drug concentration C, so that

$$E(C) = E_{max} \cdot \frac{C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}},$$

where E_{max} denotes the maximal drug effect. Here we set $E_{max} = 1$ to corresponds to total D-S to S repair inhibition. EC_{50} denotes the drug concentration that achieves half of the maximal drug effect and γ is the Hill-coefficient. If drug effect is plotted over time, the EC_{50}-value determines the asymptotic behaviour of the effect whilst the γ-value determines how quickly the asymptotic value is reached.

From the *in vitro* data, we note that the drug concentration 1 μM achieves roughly half of the total drug effect in terms of γH2AX-positive cells. (Note from Table **??** that when the drug concentration is 10 μM or 30 μM, the percentage of γH2AX-positive cells is roughly 67% at 72 hours, and when the drug concentration is 1 μM, the percentage of γH2AX-positive cells is roughly 33% at 72 hours. Furthermore the lower drug concentration of 0.3 μM yields a percentage of roughly 7% γH2AX-positive at 72 hours, and the higher drug concentration of 3 μM yields a percentage of roughly 64% γH2AX-positive at 72 hours. Consequently, we use 0.3 μM as a lower bound and 3 μM as an upper bound for the parameter range in which we seek EC_{50}, and EC_{50} values in the range [0.3 μM, 3 μM] are investigated with various Hill coefficients to fit *in vitro* data for all (non-control) drug concentrations. In order to avoid the impact that dying cells have on the data used parameterise EC_{50} and γ, only early *in vitro* data (time $<$ 12 hours) is used to guide the calibration. After iterative *in silico* testing, the best variable pair (EC_{50}, γ) is determined to be $EC_{50} = 1$ μM and $\gamma = 2$.

2.4 Cell death

In the *in vitro* experiments, cells that are damaged (but not yet dead) are γH2AX-positive. In the model, the time it takes between the 'lethal event' (i.e. a cell's failure to repair) and a cell being 'dead' is denoted $T_{L\to D}$ and is matched from the *in vitro* experiment. After noting the asymptotic behaviour of the *in vitro* data, both in terms of cell damage and cell count, we estimate that the rate of cell elimination should roughly correspond to the rate of cell production, and thus $T_{L\to D}$ should be in the same order of magnitude as the doubling time. Consequently, values of $T_{L\to D}$ between 0 and 2 τ_i are explored *in silico* after which $T_{L\to D} = \tau_i$ is chosen as it best matches the *in vitro* data for all tested (non-control) drug concentrations.

2.5 *In vivo* calibration

For the control case, the *in vivo* model is directly calibrated by the *in vitro* data, and no further calibration is needed. For drug concentrations larger than 0 μM, we use the *in vivo* data for the highest administered drug dose to calibrate the model in order to disregard details concerning pharmacokinetics and bioavailability. In future work, our model can be integrated with pharmacokinetic modelling techniques.

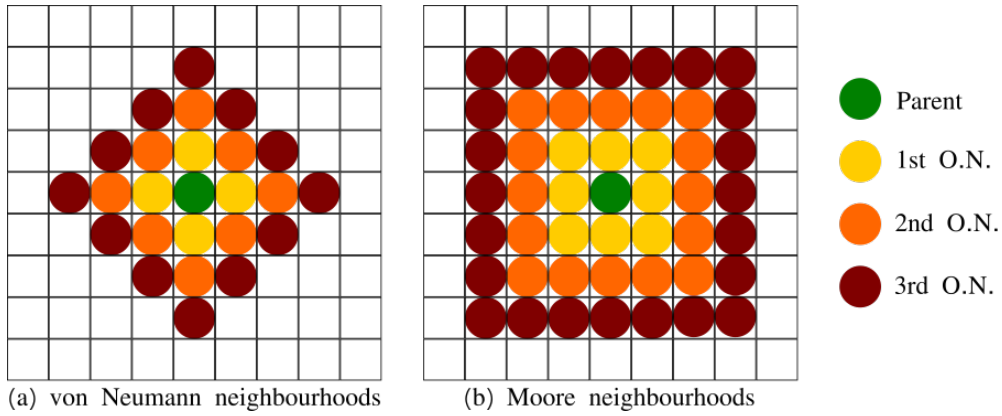

Figure 1: The 1st, 2nd and 3rd order neighbourhood (O.N.) of a parental agent using (a) the von Neumann convention and (b) the Moore convention.

3 Proliferation of Agents on the Lattice

When an agent divides, it places a daughter agent on the lattice. To achieve an approximately circular population growth on the square lattice, the placement of daughter agents alternates between following the von Neumann (Figure 1(a)) and Moore (Figure 1(a)) convention. The lattice points that are directly adjacent to the lattice point occupied by the parental agent (i.e. the agent that is dividing and thus producing a daughter cell) constitute the 1st order (von Neumann or Moore) neighbourhood of the parental cell, as is illustrated in Figure 1. If a parent is scheduled to place a daughter agent on the lattice according to the von Neumann/Moore convention, then a daughter agent will be placed on a random lattice point in the 1st order von Neumann/Moore neighbourhood. However, if all lattice point in the 1st order neighbourhood (O.N.) are occupied by other agents, the daughter agent is instead placed on a random lattice point in the 2nd O.N. Further, if the 2nd O.N. is also completely occupied by agents, then the daughter agent is placed on a random lattice point in the 3rd O.N., and so on up to the νth O.N. It is up do the modeller to decide the value of ν, and in this study ν equals infinity in the *in vitro* case (with the restriction that agents can not be placed outside the lattice in the *in silico* implementation) and $\tilde{\nu} = 3$ in the *in vivo* case.

4 Cross-Section to Tumour Spheroid Extrapolation

When implementing our mathematical *in vivo* model, only a central cross-section of the tumour is actually simulated *in silico* and post simulation time this cross-section area (that is approximately circular) is extrapolated to a tumour volume (that is approximately spherical). From the extrapolated tumour spheroid, the two outputs $\tilde{X}_1$ (percentage of γH2AX-positive cells) and $\tilde{X}_2$ (tumour volume) are gathered. This is done by using simulated areas to compute the total tumour volume,

$$\tilde{X}_2 = \text{Total Tumour Volume} = \frac{4\pi}{3}\left(\frac{\text{Total Simulated Area}}{\pi}\right)^{3/2}, \tag{1}$$

and the quiescent tumour volume,

$$\text{Quiescent Tumour Volume} = \frac{4\pi}{3}\left(\frac{\text{Quiescent Simulated Area}}{\pi}\right)^{3/2}. \tag{2}$$

From the above, the volume of cycling, or proliferating cells, is obtained by

$$\text{Cycling Tumour Volume} = \text{Total Tumour Volume} - \text{Quiescent Tumour Volume}. \tag{3}$$

Now the output $\tilde{X}_1$ can be computed where,

$$\tilde{X}_1 = \text{Percentage of } \gamma\text{H2AX-positive cells in sphere} = \frac{\text{Number of simulated } \gamma\text{H2AX-positive cells}}{\text{Number of simulated cycling cells}} \times \frac{\text{Cycling Tumour Volume}}{\text{Total Tumour Volume}}. \tag{4}$$

5 Uncertainty and Sensitivity Analyses

To evaluate the *in silico* findings obtained in our *in vitro* study, three uncertainty and sensitivity analyses techniques are performed. The three techniques are namely: (1) Consistency Analysis, which is used to determine how many *in silico* runs should be performed before defining results in terms of statistical metrics in order to mitigate uncertainty originating from intrinsic model stochasticity, (2) Robustness Analysis, which investigates model sensitivity to local parameter perturbations and (3) Latin Hypercube Analysis, which investigates model sensitivity to global parameter perturbations. These techniques are thoroughly described in a review/methodology paper [3] which provides background information concerning the origin of these techniques as well as detailed information on how to implement them. To perform uncertainty and sensitivity analyses we need to specify a set of inputs and outputs. Here, the output variables are X_1: the percentage of γH2AX-positive (i.e. damaged) cells at the end time of the experiment (72 hours), and X_2: the cell count (i.e. the number of non-dead cells) at the end of the experiment. The input variables are the seven model parameters listed in Table 1, in the main article, that we calibrate using *in vitro* data. These inputs are namely μ, σ, Π_{D-S}, Θ_{D-S}, EC_{50}, γ and $T_{L\to D}$.

5.1 Consistency Analysis

Results from the Consistency Analysis are provided in Figures 2, 3, 4, 5, 6 which show the $\hat{A}$-measures, in both computed and scaled forms, for the distribution sizes $n = 1, 5, 50, 100, 300$ respectively. By observing Figures 2 through to 6, it is clear that the statistical significance decreases with increasing distribution size n, as is shown in Figure 7 and Table 5.1 which show the maximal scaled $\hat{A}$-values for all tested distribution sizes. These results demonstrate that the distribution size $n = 100$ is the smallest tested distribution size that yields a small statistical significance (i.e. a maximum scaled $\hat{A}$-value smaller than 0.56) for both regarded output variables X_1 and X_2. From this we decide to base every *in silico* result (here in terms of mean values and standard deviations) on 100 simulation runs.

distribution size / output	n=1	n=5	n=50	n=100	n=300
X_1	1	0.92	0.61	0.55	0.54
X_2	1	0.84	0.59	0.55	0.54

Table 4: Maximal scaled $\hat{A}$-values produced in the Consistency Analysis for various distribution sixes n. The output variables are X_1, corresponding to the percentage of γH2AX positive (i.e. damaged) cells, and X_2, corresponding to the cell count.

5.2 Robustness Analysis

We use Robustness Analysis to investigate how sensitive the output is to *local* parameter perturbations, that is to say when input parameters are varied one at a time. Figures 8, 9, 10, 11, 12, 13, 14 provide boxplots and $\hat{A}$-measures that demonstrate the effect that local perturbations of the input variables μ, σ, Π_{D-s}, Θ_{D-S}, EC_{50}, γ and $T_{L\to D}$ respectively have on the output variables X_1 and X_2. Key findings are listed below, discussing the impact of one input parameter at a time.

- Remarks regarding input parameter μ: Figure 8 shows that, for small parameter perturbations, increasing the average doubling times of cells, μ, overall decreases the percentage of γH2AX positive cells and increases the cell count, however this decrease/increase is not linear. This indicates that the results of the *in vitro* simulation (and of the *in vitro* experiment nonetheless) are sensitive to the *timing* of the drug administration. In other words, Robustness Analyses demonstrates that treatment responses depend on how many cells are in the susceptible cell-cycle state at time of drug administration.

- Remarks regarding input parameter σ: Figure 9 demonstrates that the level of cell cycle synchronisation amongst cells, quantified by the input σ, affects *in silico* outputs for small parameter perturbations. The results indicate that for highly asynchronised cells (i.e. high σ-values) the smoother growth curves yield higher cell counts at certain time-points (such as the end time 72 hours) and a lower percentage of γH2AX-positive cells. As discussed in the remark above, the timing between cell cycles and drug administration affect treatment responses.

- Remarks regarding input parameter Π_{D-S}: Figure 10 illustrates that increasing the probability that a cell enters the damaged S state, i.e. the variable Π_{D-S}, increases the percentage of γH2AX cells and decreases the cell count, as expected.

- Remarks regarding input parameter Θ_{D-S}: Figure 11 shows how the amount of time that damaged cells spend in the D-S state before attempting to repair, and thus the Θ_{D-S}-value, affects the output. Results show that the percentage of γH2AX positive cells increases with increasing values of Θ_{D-S}, as more damaged cells will accumulate in the D-S state. However, this does not affect the probability of cells repairing, so the cell count is not as sensitive to small perturbations of Θ_{D-S}. The value of $\Theta D - S$ implicitly affects the measured cell count at the end time of the experiment as a decreased/increased $\Theta D - S$-value yields a slightly decreased/increased time lag between a cell entering the D-S state and dying.

- Remarks regarding input parameter EC_{50}: Figure 12 demonstrates that output variables are highly sensitive to perturbations of EC_{50}. Increasing EC_{50} results in a higher percentage of γH2AX positive cells and a lower cell count. Thus the input parameter EC_{50} should be regarded as a highly influential on quantitative results.

- Remarks regarding input parameter γ: Figure 13 illustrates that output variables measured at the end time of the experiment are not very sensitive to small perturbations of γ. This

can be understood as the γ parameter inherently corresponds to 'how quickly' a drug achieves asymptotic behaviour in the E_{max} model, the model used in our mathematical framework to express cellular drug response.

- Remarks regarding input parameter $T_{L\rightarrow}$: Figure 14 shows how output variables change as a result of perturbations to the input variable $T_{L\rightarrow}$, that describes how long it takes for a cell that has failed to repair to die (i.e. how long a cell with a 'death-sentence' is picked up as γH2AX positive in the *in vitro* experiment). Results show that both the percentage of γH2AX positive cells and the cell count increases with increasing values of $T_{L\rightarrow}$, as dying cells will categorised as γH2AX positive longer before being categorised as dead. When calibrating the model, we avoid the effect of this input parameter by only regarding *in vitro* data at time points that are early enough to correspond to systems with no (or a negligible amount of) dead cells.

5.3 Latin Hypercube Analysis

Latin Hypercube Analysis is here used to investigate how sensitive output responses are to *global* parameter perturbations. We here investigate parameter values within ranges that we consider to be 'plausible' from the calibration process and the Robustness Analysis. Figures 15, 16, 17, 18, 19, 20, 21 provide scatter-plots that demonstrate correlations between the output variables X_1 and X_2 and the input variables μ, σ, Π_{D-s}, Θ_{D-S}, EC_{50}, γ and $T_{L\rightarrow D}$ respectively. The Pearson Product Moment Correlation Coefficients between the various input-output pairs are listed in Table 5.3. To determine threshold values for correlation coefficient descriptors, we compromise between suggested values by other authors [3], and take into account the fact that we are only regarding parameter values within 'plausible' ranges. With this as a guide, we here decide that our obtained correlation coefficients with a magnitude in [0,0.12] corresponds to the linear input-output relationship being 'negligible', [0.19, 0.35] 'weak', [0.48,0.59] 'moderate' and 0.84 'strong'. Key findings from the Latin Hypercube Analysis are listed below, where the impact of one input parameter is discussed one at a time.

- Remarks regarding input parameter μ: Figure 15 and the first column in Table 5.3 show that, for the allowed parameter range, μ and X_1 are moderately, negatively correlated as the correlation coefficient is -0.48 and the scatterplot displays an overall trend of the output (X_1) decreasing with increasing values of the input μ. The relationship between μ and the other output variable X_2 is, on the other hand, negligible. We explain this by the fact that treatment responses are sensitive to the timing of the drug administration, but there is a time-lag $T_{L\rightarrow D}$ between a cell's lethal event (failure to repair) and its death. As damaged (but not dead) cells are included in the cell count, the (μ,X_2)-relationship is more weakly linearly correlated than the (μ,X_1)-relationship.

- Remarks regarding input parameter σ: Figure 16 and the second column in Table 5.3 demonstrate that the linear relationships between input variable σ and the output variables X_1 and X_2 are both negligible, within the regarded input parameter value range.

- Remarks regarding input parameter Π_{D-S}: Figure 17 and the third column in Table 5.3 indicate that the relationships between the input variable Π_{D-S} and the output variables

X_1 and X_2 are, respectively, positively and negatively weakly linearly correlated. This agrees with the intuitive notion that if the probability that a cell enters the D-S state increases, cell damage (X_1) increases whilst the cell count (X_2) decreases.

- Remarks regarding input parameter Θ_{D-S}: Figure 18 and the fourth column in Table 5.3 show that the input variable Θ_{D-S} is has a negligible linear correlation with the output variables X_1 and X_2.

- Remarks regarding input parameter EC_{50}: Figure 19 and the fifth column in Table 5.3 demonstrate that the input variable EC_{50} impacts the output responses more than do other input variables, within the regarded ranges for input variables. EC_{50} is negatively, moderately linearly correlated with X_1 and EC_{50} is strongly, positively linearly correlated with X_2. These relationships are visually apparent in the regarded scatterplots.

- Remarks regarding input parameter γ: Figure 20 and the sixth column in Table 5.3 indicate negligible linear correlations between the input parameter γ and both output variables X_1 and X_2.

- Remarks regarding input parameter $T_{L\to D}$: Figure 21 and the last column in Table 5.3 demonstrate that the input variable $T_{L\to D}$ is positively, weakly, linearly correlated with the output X_1, whilst the linear correlation between $T_{L\to D}$ and X_2 is negligible.

input / output	μ	σ	Π_{D-S}	Θ_{D-S}	EC_{50}	γ	$T_{L\to D}$
X_1	-0.48	0.06	0.19	0.06	-0.59	0.05	0.35
X_2	0.12	0.01	-0.24	-0.02	0.84	0.12	0.00

Table 5: Pearson Product Moment Correlation Coefficients between input and output variables obtained in the Latin Hypercube Analysis.

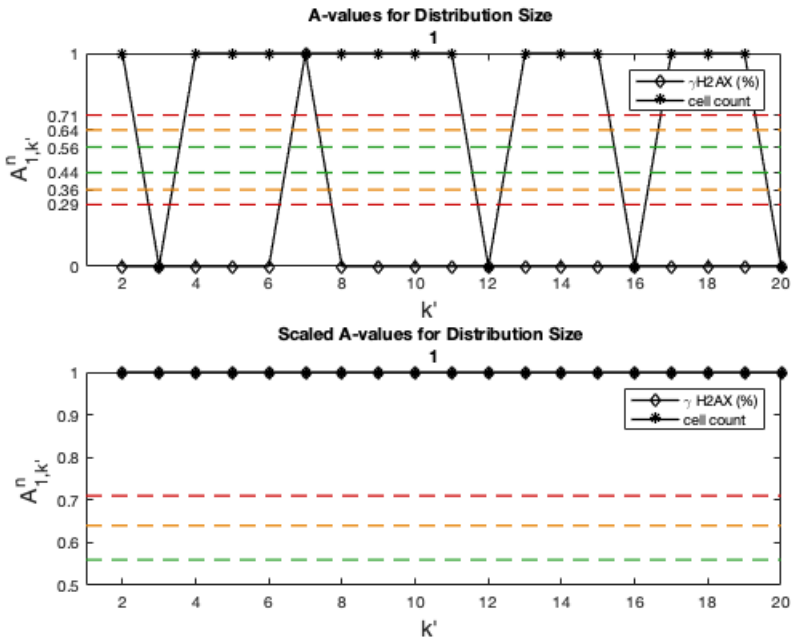

Figure 2: Consistency Analysis. $\hat{A}$-values in initial (top) and scaled (bottom) form for distribution size $n = 1$.

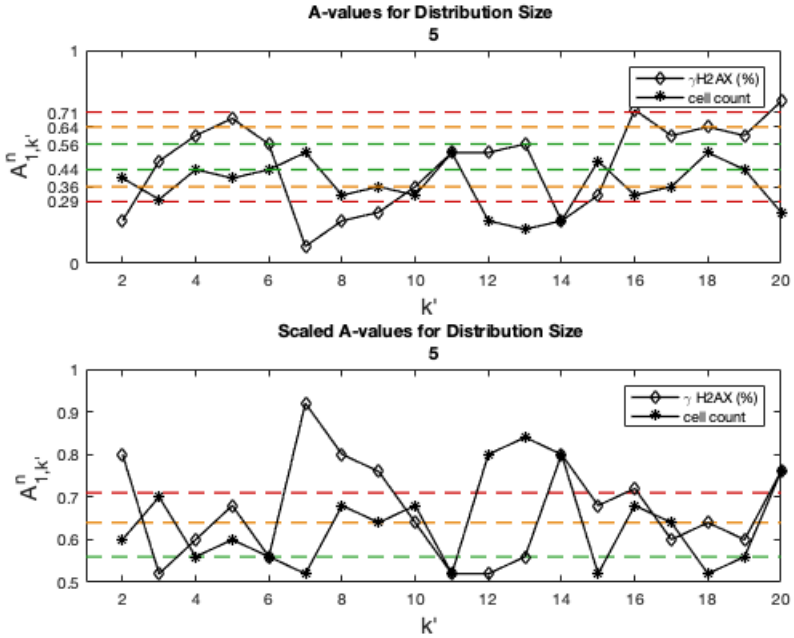

Figure 3: Consistency Analysis. $\hat{A}$-values in initial (top) and scaled (bottom) form for distribution size $n = 5$.

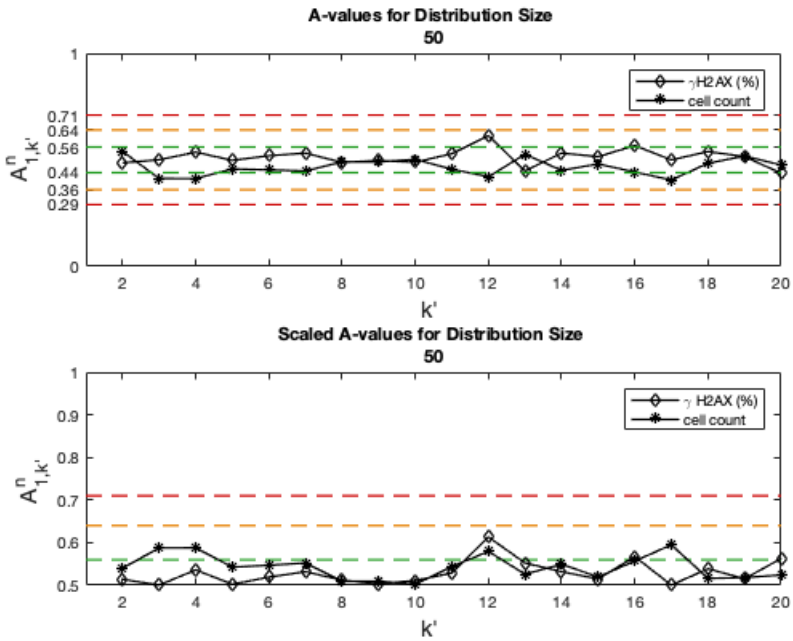

Figure 4: Consistency Analysis. $\hat{A}$-values in initial (top) and scaled (bottom) form for distribution size $n = 50$.

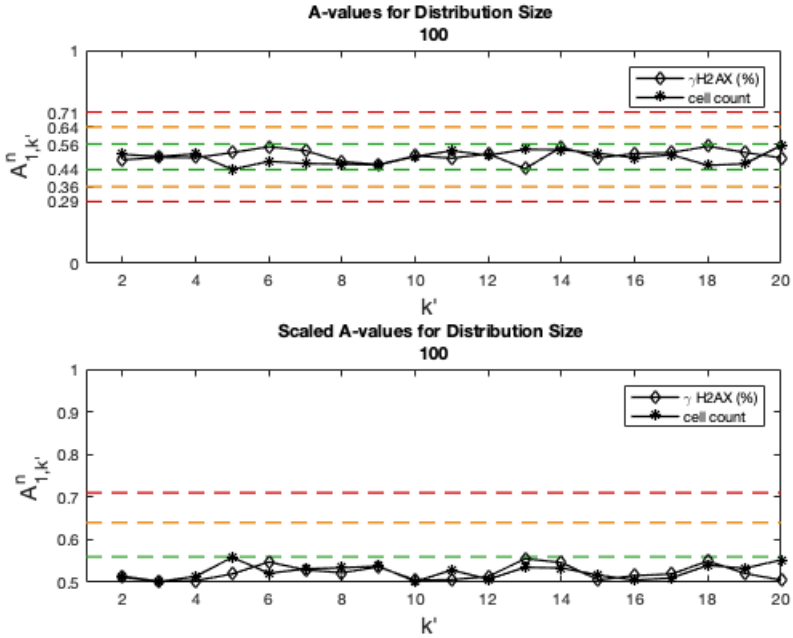

Figure 5: Consistency Analysis. $\hat{A}$-values in initial (top) and scaled (bottom) form for distribution size $n = 100$.

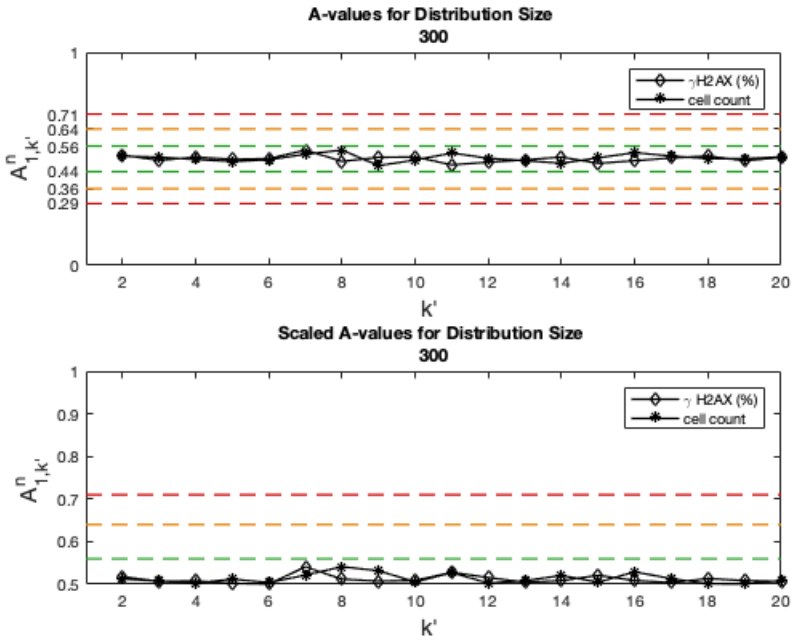

Figure 6: Consistency Analysis. $\hat{A}$-values in initial (top) and scaled (bottom) form for distribution size $n = 300$.

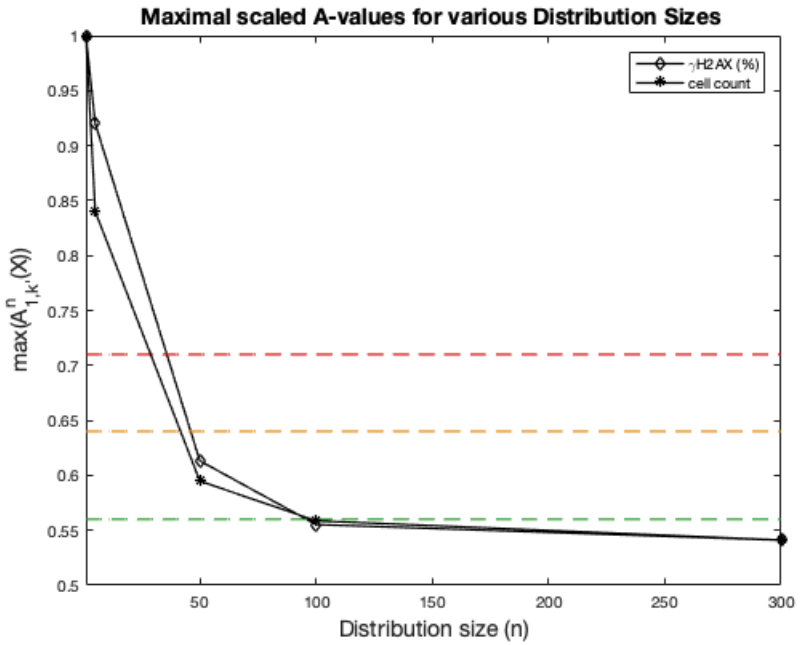

Figure 7: Consistency Analysis. Scaled $\hat{A}$-values for various distribution sizes tested in the Consistency Analysis.

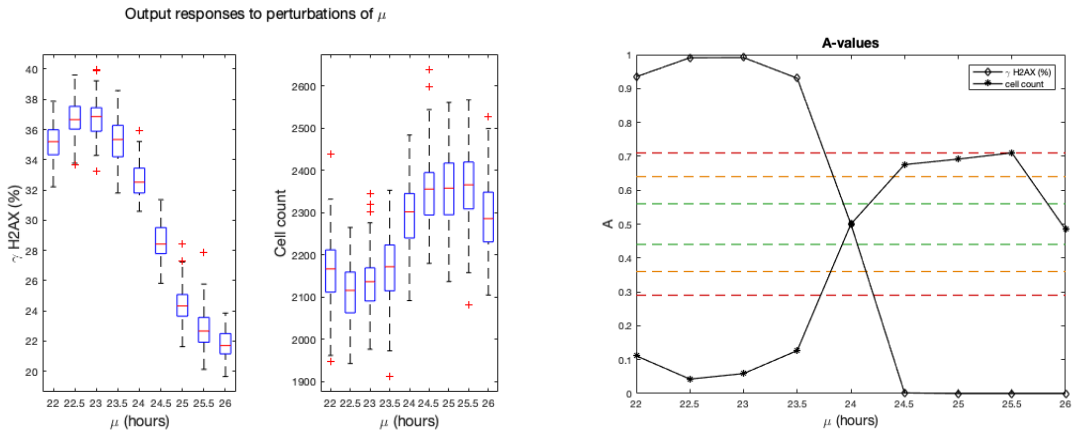

Figure 8: Robustness Analysis. Left: Output responses, in terms of percentage of γH2AX positive (i.e. damaged) cells, and cell count as a result of perturbations to the input variable μ. Right: Maximal $\hat{A}$-values resulting from comparisons between distributions with perturbed data and a distribution with calibrated (unperturbed) data.

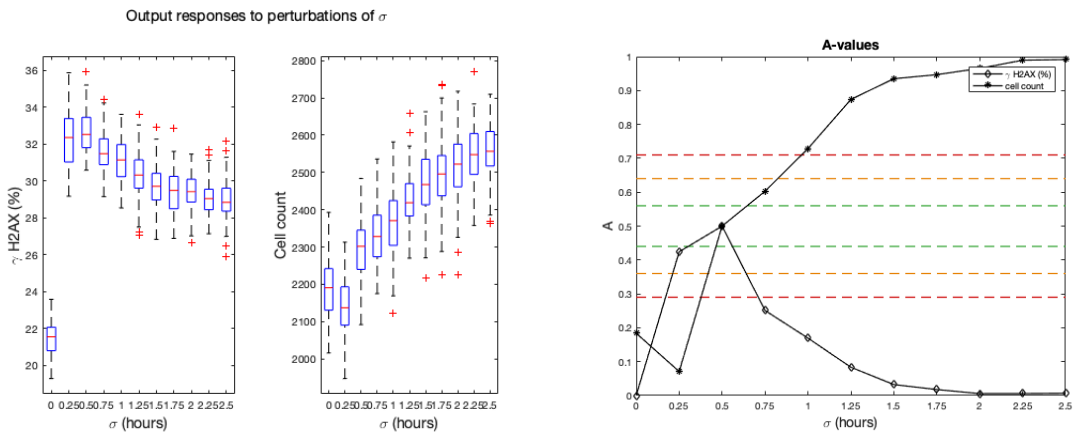

Figure 9: Robustness Analysis. Left: Output responses, in terms of percentage of γH2AX positive (i.e. damaged) cells, and cell count as a result of perturbations to the input variable σ. Right: Maximal $\hat{A}$-values resulting from comparisons between distributions with perturbed data and a distribution with calibrated (unperturbed) data.

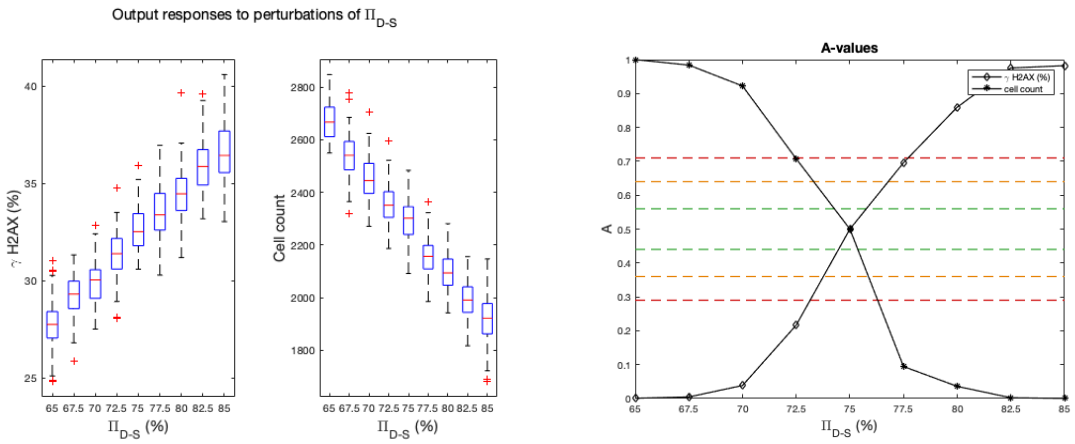

Figure 10: Robustness Analysis. Left: Output responses, in terms of percentage of γH2AX positive (i.e. damaged) cells, and cell count as a result of perturbations to the input variable Π_{D-s}. Right: Maximal $\hat{A}$-values resulting from comparisons between distributions with perturbed data and a distribution with calibrated (unperturbed) data.

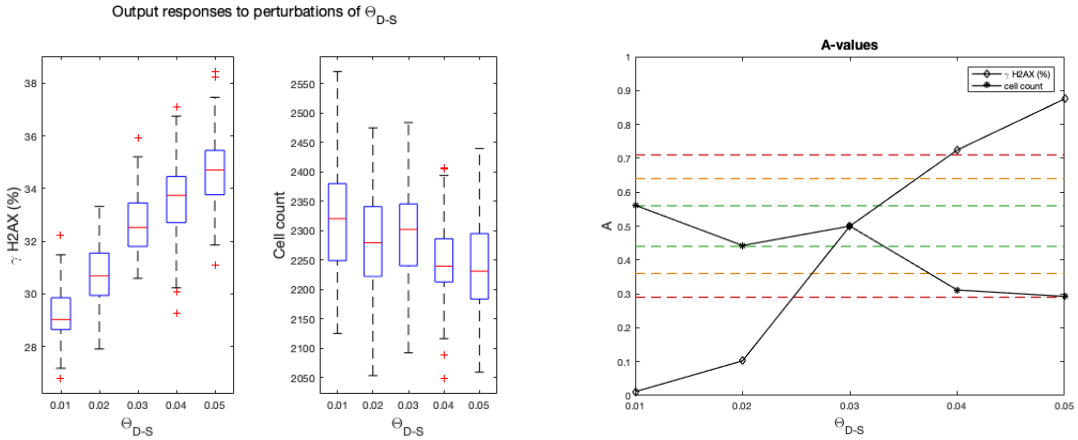

Figure 11: Robustness Analysis. Left: Output responses, in terms of percentage of γH2AX positive (i.e. damaged) cells, and cell count as a result of perturbations to the input variable Θ_{D-S}. Right: Maximal $\hat{A}$-values resulting from comparisons between distributions with perturbed data and a distribution with calibrated (unperturbed) data.

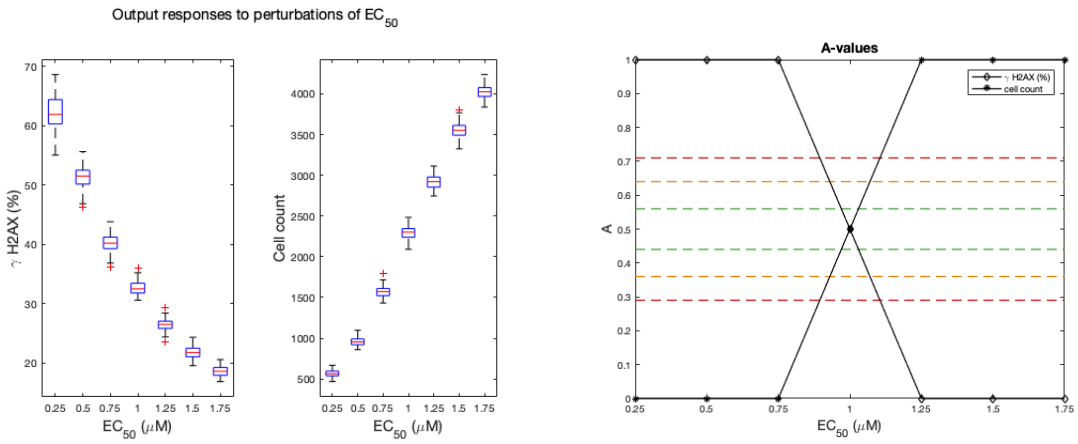

Figure 12: Robustness Analysis. Left: Output responses, in terms of percentage of γH2AX positive (i.e. damaged) cells, and cell count as a result of perturbations to the input variable EC_{50}. Right: Maximal $\hat{A}$-values resulting from comparisons between distributions with perturbed data and a distribution with calibrated (unperturbed) data.

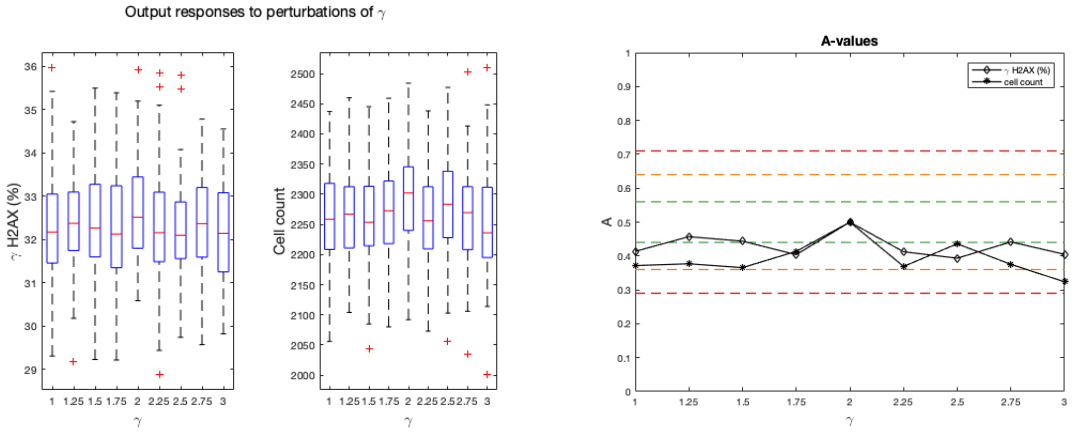

Figure 13: Robustness Analysis. Left: Output responses, in terms of percentage of γH2AX positive (i.e. damaged) cells, and cell count as a result of perturbations to the input variable γ. Right: Maximal $\hat{A}$-values resulting from comparisons between distributions with perturbed data and a distribution with calibrated (unperturbed) data.

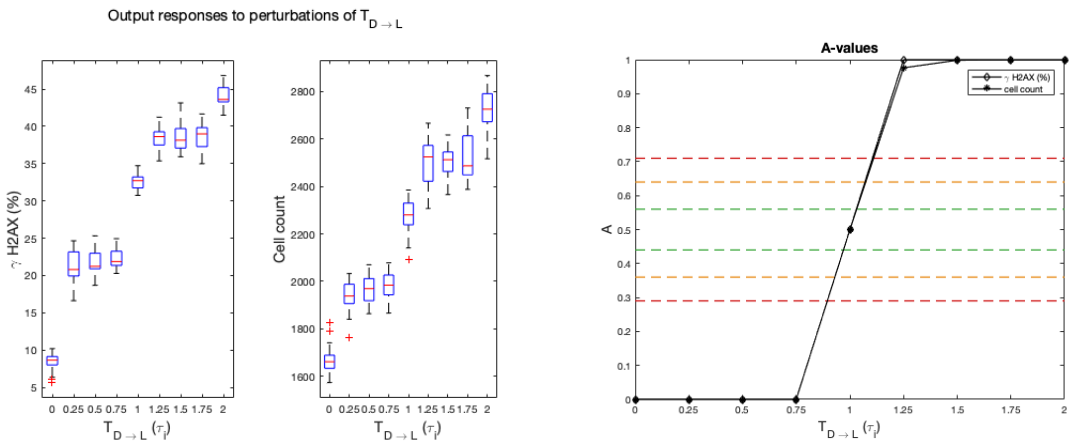

Figure 14: Robustness Analysis. Left: Output responses, in terms of percentage of γH2AX positive (i.e. damaged) cells, and cell count as a result of perturbations to the input variable $T_{D\to L}$. Right: Maximal $\hat{A}$-values resulting from comparisons between distributions with perturbed data and a distribution with calibrated (unperturbed) data.

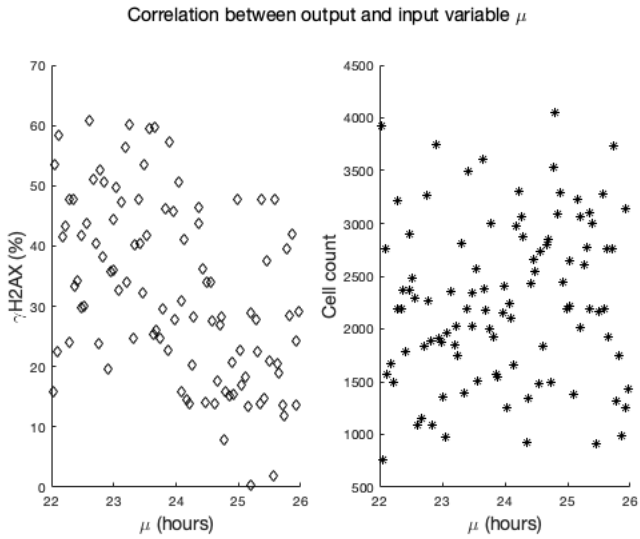

Figure 15: Latin Hypercube Analysis. Outputs in terms of 2AX positive cells (left) and number of viable cells (right) when global parameter perturbations are performed. The scatter-plots show the correlation between outputs and the input variable μ.

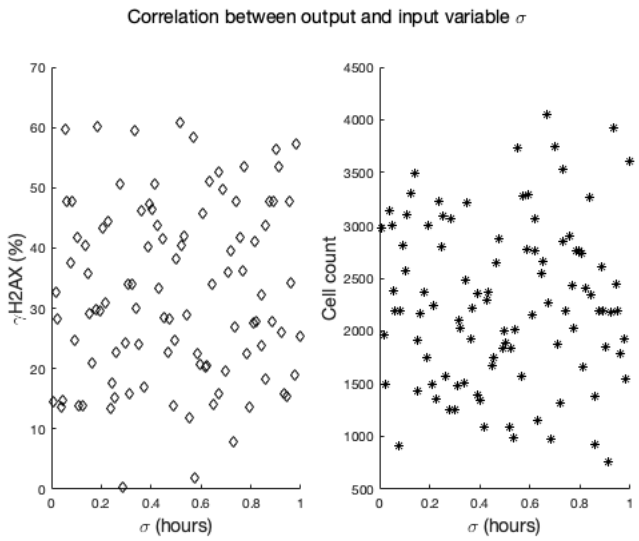

Figure 16: Latin Hypercube Analysis. Outputs in terms of γH2AX positive cells (left) and number of viable cells (right) when global parameter perturbations are performed. The scatterplots show the correlation between outputs and the input variable σ.

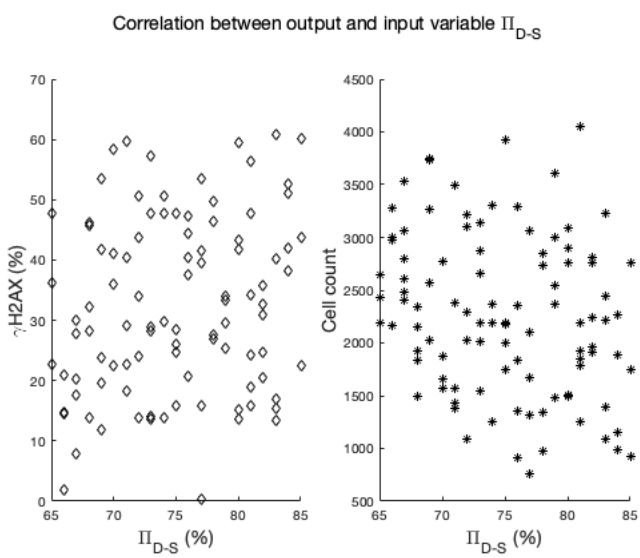

Figure 17: Latin Hypercube Analysis. Outputs in terms of γH2AX positive cells (left) and number of viable cells (right) when global parameter perturbations are performed. The scatterplots show the correlation between outputs and the input variable Π_{D-S}.

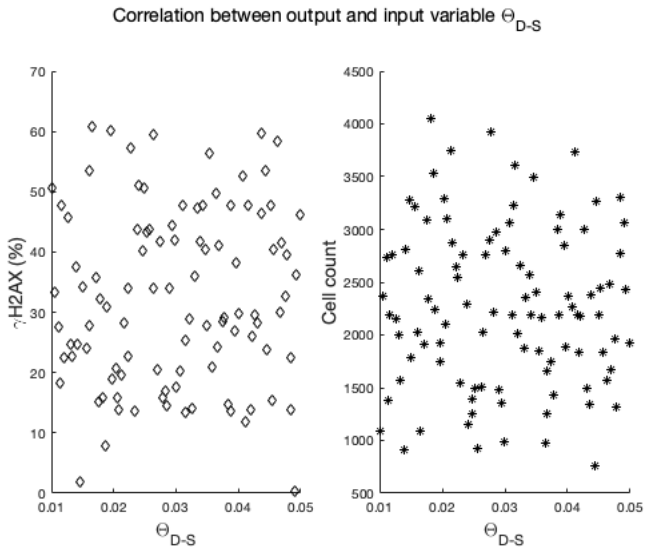

Figure 18: Latin Hypercube Analysis. Outputs in terms of γH2AX positive cells (left) and number of viable cells (right) when global parameter perturbations are performed. The scatterplots show the correlation between outputs and the input variable Θ_{D-S}.

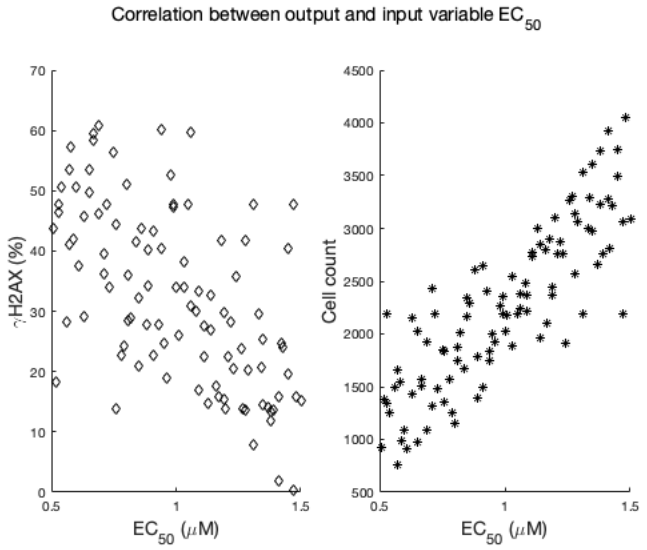

Figure 19: Latin Hypercube Analysis. Outputs in terms of γH2AX positive cells (left) and number of viable cells (right) when global parameter perturbations are performed. The scatterplots show the correlation between outputs and the input variable EC_{50}.

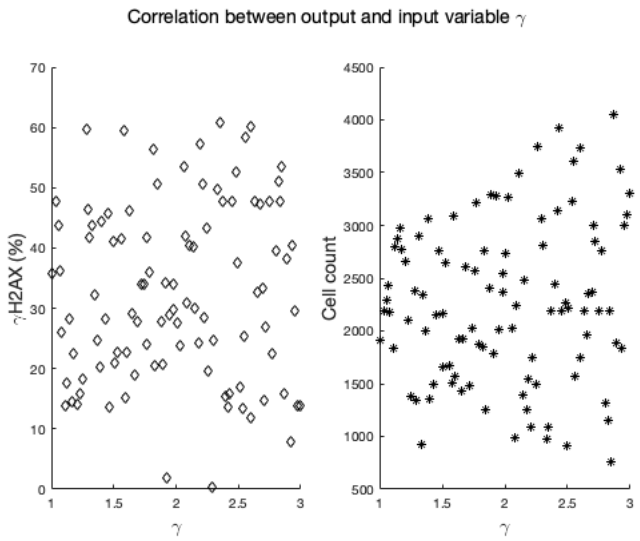

Figure 20: Latin Hypercube Analysis. Outputs in terms of γH2AX positive cells (left) and number of viable cells (right) when global parameter perturbations are performed. The scatterplots show the correlation between outputs and the input variable γ.

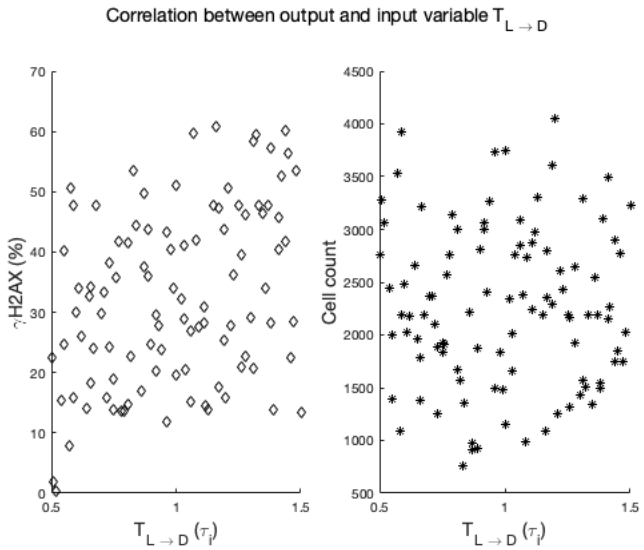

Figure 21: Latin Hypercube Analysis. Outputs in terms of γH2AX positive cells (left) and number of viable cells (right) when global parameter perturbations are performed. The scatterplots show the correlation between outputs and the input variable $T_{L \to D}$.

References

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