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We employ an in-silico modelling framework to elucidate the functioning of two broad classes of drug resistance mechanisms, which act in tumours to resist drugs. This reveals how different resistance mechanisms can exhibit clear-cut tissue level differences; whether the resistance mechanism is induced or intrinsic has important consequences; the significant role heterogeneity of resistance can play in tissue-level response; provides insights into how different mechanisms function together. These insights were obtained using dedicated multiscale models, which could be analysed transparently. Through the development of a simplified multiscale systems engineering approach, we can address basic biological questions providing the basis for understanding different aspects of cellular resistance mechanisms at the tissue level (bridging the gap from cells to tissues).

Intrinsic and induced drug resistance mechanisms: in-silico investigations at the cellular and tissue scales

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Abstract

Multiple cellular drug resistance mechanisms are present in a broad range of tumour types and act to counteract the effects of drugs. There are independent mechanisms by which drug resistance occurs; these include (i) the multi-drug resistance mechanism involving upregulation of ABC transporter proteins and (ii) intracellular mechanisms which sequester/degrade/detoxify drugs. In addition, drug resistance mechanisms could be either intrinsic, or directly induced by the drug. In this paper we focus on the behaviour of intrinsic and induced variants of these resistance mechanisms in solid tumours, by systematically elucidating their cellular and tissue level effects with an aim to bridge the gap between cell and tissue levels. This is achieved in a controlled in-silico setting, which allows for an investigation of the interplay between transport, resistance pathways, and tissue level effects.

Overall the paper (i) provides insights into the tissue level functioning of widespread classes of intracellular resistance mechanisms, showing important differences, (ii) systematically elucidates the difference between intrinsic and induced drug resistance mechanisms at the cell and tissue levels, (iii) demonstrates how spatial heterogeneity in intrinsic resistance in cells can significantly affect the response of solid tumours to drugs, and (iv) examines how different independent resistance mechanisms work in concert, to counteract drug dosages in tumours.

Keywords: induced drug resistance, drug sequestration, detoxification, intracellular signalling, drug transport, drug effect, cellular and tissue scale, solid tumour, tissue heterogeneity, combination of resistance mechanisms.

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We employ an in-silico modelling framework to elucidate the functioning of two broad classes of drug resistance mechanisms, which act in tumours to resist drugs. This reveals how different resistance mechanisms can exhibit clear-cut tissue level differences; whether the resistance mechanism is induced or intrinsic has important consequences; the significant role heterogeneity of resistance can play in tissue-level response; provides insights into how different mechanisms function together. These insights were obtained using dedicated multiscale models, which could be analysed transparently. Through the development of a simplified multiscale systems engineering approach, we can address basic biological

questions providing the basis for understanding different aspects of cellular resistance mechanisms at the tissue level (bridging the gap from cells to tissues).

1 Introduction

One of the most common ways of treating cancer is by chemotherapy. A variety of drugs such as doxorubicin, paclitaxel, cisplatin, have been used in this regard. These drugs are typically delivered through the blood stream. They are carried around by blood flow, permeate through the vessel wall, reach the tumour site, and then get transported through the interstitium before reaching cells whereupon, they act via an intracellular mechanism to determine the fate of cells. It is clear from the "pathway" of the drug that at every stage counteracting factors can arise, to compromise their efficacy. There could be an insufficient amount of drug reaching a tumour, poor transport in the interstitium may affect drug delivery to cells, or the drug could be degraded or sequestered in the interstitium [1-3]. Many of these aspects have been examined in studies which focus at length on drug transport.

A number of factors which are purely cellular in nature also play a role in reducing drug efficacy [4]. These include different intracellular factors such as drug sequestration, metabolism and efflux [5]. Other factors act to counteract the effect of the drug, by negatively regulating/affecting cell killing pathways or by activating pro-survival pathways. Multiple mechanisms for resistance work together in tumour cells, some of which are specific to particular drugs. Additionally, different variants of these mechanisms are present in different tumour types. A recent summary of drug resistance mechanisms in tumours is presented in [6-8]. It is worth noting that some of the factors responsible for resistance (e.g. upregulated ABC transporter proteins) are particularly accentuated in cancer cells when compared to wild-type cells.

The presence of multiple cellular resistance mechanisms poses significant challenges for elucidating and counteracting their effects. On the other hand, through a combination of a better understanding of these cellular factors using systems biology approaches, high-throughput screening methods and a broader range of anti-cancer agents and therapeutic methods, it is conceivable that approaches which are able to bypass or counteract these various resistance mechanisms can be developed. This will need a thorough understanding of these mechanisms, and how they work together in typical tumour environments.

In this paper we focus on two cellular mechanisms giving rise to drug resistance, one associated with drug efflux, and the other with drug metabolism/sequestration/detoxification. Increased drug efflux arises through increased expression of ABC transporter proteins, and this mechanism is a classic Multi-drug Resistance (MDR) mechanism. Likewise other

mechanisms exist which are responsible for metabolizing/sequestering/detoxifying drugs. Our particular focus will be to examine the functioning of these different resistance mechanisms at the cell and tissue levels and examine how they function to counteract different classes of drug stimuli.

At the outset, cellular resistance mechanisms can be divided into two broad categories from the perspective of a drug stimulus: intrinsic and induced. Intrinsic mechanisms are those which are already present when the cells are subject to a drug stimulus. Induced mechanisms are those which could be regarded as arising directly from the delivery of a drug stimulus. This subdivision is not always clear cut: for instance it is possible that a prior stimulus of drug can induce changes in cells in a tissue, either directly, or by altering the environment in which the cells reside. The latter may have an indirect effect on the subsequent evolution/growth of these cells. For the purposes of this study, a resistance mechanism is regarded as induced or directly induced, if the presence of a drug triggers signalling/gene regulatory pathways which act to resist drug action, over the timescale of action of the drug. On the other hand, the presence of drug in the environment can affect the division and evolution of cells, and this can alter and/or induce resistance characteristics (over a time scale longer than that associated with drug delivery), which may be relevant to the response of a tumour to a subsequent dosage of drug (with sufficient temporal separation). In such a case, the resistance characteristics acquired, may be regarded as intrinsic for subsequent rounds of drugs. Both kinds of resistances are relevant for tumours and have been discussed in the literature [6, 9].

The importance of drug resistance mechanisms has been recognised and different approaches are being actively investigated to address this, including combination therapies [10, 11]. While some of these resistance mechanisms have been documented and discussed in the biological and biomedical literature, and also notably from an evolutionary standpoint, there has been very little systematic dissection of how they act to counteract drug stimuli, especially at the tissue level. This is the natural level at which the effects of resistance need to be examined. In fact a closer look at the functioning of these mechanisms presents a number of unanswered questions. What are the different kinds of induced drug resistance mechanisms and how do they function at the cellular and tissue levels? How do these mechanisms function, both individually and in combination? How do they differ from intrinsic drug resistance through these mechanisms? How does variability of cellular resistance affect tissue level responses? Many of these issues need careful and systematic elucidation.

Although experiments in well controlled settings can provide insights into some of these important questions, mathematical modelling can be especially useful here, as it provides a controlled in-silico experimental setting in which these questions and relevant hypotheses can be investigated and the effects of auxiliary factors examined. In this regard, mathematical modelling serves as an essential complement to experiments. It also allows us to investigate the interplay between resistance and other factors, and in addition examine the effect of relaxing various auxiliary assumptions. Moreover, mathematical modelling allows us to probe different aspects of the interplay between cellular mechanism and system level behaviour which are either difficult or very expensive to probe experimentally.

In this paper, a modelling framework developed in our previous studies is expanded to address some of the questions mentioned above. The modelling framework contains both cell and tissue level descriptions, focussing on the issues we examine. In general, when studying drug resistance, making the transition between cellular and tissue level behaviour involves incorporating drug transport, cellular response, tumour heterogeneity, with drug resistant subpopulations alongside the complexity associated with the resistance mechanisms. As a starting point a homogeneous population of cells is assumed, in order to focus on examining the basic aspects of the interplay of drug dosage, drug transport, and cellular response (and the effects of resistance therein). This is, in some respects, the closest analogue to studying a drug dosage response and the effects of resistance at the single cell level. We also do consider the effects of spatial heterogeneity of resistance. However, we do not consider the effects of acquired resistance through evolution directly. Thus the present study is a drug and resistance pathway-centric rather than evolution centric study. Using this as a basis, it is possible to incorporate both aspects and their interplay subsequently.

The paper is organized as follows. In the next section we describe the approach we adopt in developing the models, and the mathematical models used. In the subsequent section we present the results from our computational analysis. We then provide a synthesis of our results.

2 Models and methods

The strategy for developing the modelling framework used in this study is as follows. We incorporate the essential elements relevant to the questions of interest. Thus the model incorporates elements both at the tissue level (drug transport) and the cellular level (drug triggering cell death via apoptosis, along with resistance mechanisms). The dynamic

information processing inside cells is described explicitly, but in a simplified manner. This is necessary to reasonably capture the nature of information flow from extracellular environment to cellular behaviour. The individual pathways are described in a simplified way, consistent with the nature of the investigation. The associated parameters, such as the kinetic rates of the pathways are varied as part of the investigation. Detailed mechanistic aspects of the pathways are not incorporated at this stage for multiple reasons: (i) We wish to ground our descriptions in the most basic aspects of these pathways, avoiding details which may not be experimentally confirmed or specific to only some cell types; (ii) The level of description incorporates the kinds of hypotheses which are explicitly or implicitly assumed broadly in the literature, but not much more; (iii) It can serve as a platform for systematic fine graining subsequently; and (iv) If it emerges in some contexts that the interplay between resistance and cell killing pathways is essentially more complicated which cannot be captured in simpler frameworks, then the role of that extra complexity will be clearly delineated and seen for what it is. At this stage, however, there is no evidence that this is the case, for the mechanisms examined in this study. Details of the mathematical models are described below.

2.1 Modelling overview

Our goal is to develop and analyse an in-silico model to obtain clear cut insights into the roles both intrinsic and induced drug resistance (realized through different mechanisms) may play in affecting cell and tumour tissue responses to drugs. In developing the in-silico model, we incorporate a basic description of the "pathway" of the drug and its action: release at the edge of the interstitium, transport in the interstitium, influx into and efflux from cells. In addition the effect of drug on the cell by triggering a killing pathway is incorporated via a simplified intracellular pathway description, and overlaid upon this is a description of the resistance mechanism (discussed in detail below).

Since the drug response mechanisms we are concerned with are cellular, this means that modelling of basic relevant parts of intracellular pathways is necessary. Minimal cellular descriptions leading to apoptosis and the resistance mechanisms are employed, in combination with interstitial drug transport and cell population-level descriptions. Thus the model allows us to focus on the resistance mechanisms and the effects of manipulating resistance pathways against a fixed background, and discern important relevant trends. We use the in-silico model to study both intrinsic and induced resistances, which can be realized through multiple distinct mechanisms, each of which is described in a simplified manner.

Using this approach, we can focus on the interplay of both intrinsic and induced resistance and cell killing mechanisms. We examine two distinct resistance mechanisms (see below) studying intrinsic and induced analogues of each. We also study the effect of a combination of resistance mechanisms. This provides direct understanding of how different resistance mechanisms function, and the differences between induced and intrinsic mechanisms. It also provides a platform for subsequently examining how additional factors might affect drug resistance and drug efficacy in a tumour specific manner. Figure 1 shows schematic interactions between different elements of the model. The elements of the model are the minimal essential ones needed for the purpose of this study.

Our model incorporates a basic description of the relevant cellular pathways. Thus cellular level descriptions are kept generic and coarse-grained to allow additional layers of detail to be incorporated subsequently. Coarse-grained descriptions of cell killing used two models in parallel, respectively incorporating simple monostable and bistable switches as in [12]. The reason why we employ two different coarse-grained models of cell killing is because there are two qualitatively different dynamical characteristics which have been postulated to underlie apoptosis: monostable switches, and bistable switches. Different detailed models have been built around these different core characteristics. At this point it is not clear which core characteristic is more appropriate, and also whether different cell types employ different core characteristics. Since the two core characteristics are essentially different from a dynamical systems point of view, we employ two separate models which describe both these characteristics. The fact that we get similar essential conclusions for both cases points to the robustness of our conclusions.

Two types of drug resistance are analysed. The first involves enhanced drug efflux via increased expression of ABC superfamily of transporters, in particular, P-glycoproteins. Overexpressed ABC transporters can reduce intracellular accumulation of most anticancer drugs, such as doxorubicin and paclitaxel to a level below their therapeutic threshold; additionally, several *in vitro* and *in vivo* studies have demonstrated that exposure to anticancer agents may enhance expression of ABC transporters, which reduces the intracellular concentration of anticancer agents [13, 14], as shown in Figure 1(a). Further, ABC transporter levels are enhanced in different tumour types intrinsically. Thus we study both intrinsic and induced ABC transporter-based resistance mechanisms. Such resistance mechanisms are classic Multi-drug Resistance (MDR) mechanisms. These mechanisms (at

least induced analogues) may be viewed as analogues of basic stress responses seen in bacteria and other cells.

The second is an intracellular mechanism involving detoxification/degradation/sequestration of drugs to reduce the intracellular drug concentration. When a drug enters the cell, it comes into contact with other molecules which may act to sequester, degrade or detoxify it. Again these mechanisms could be intrinsic or induced. One example of such a mechanism is the detoxification of irinotecan (CPT-11) in the treatment of colon cancer cells [15] (see Figure 1(b)). SN-38 (the active form of CPT-11) activates the transcription factor SXR (steroid xenobiotic receptor) which is recruited to the CYP3A4 gene (cytochrome p450 3A4) and up-regulates the expression of the gene. The expressed protein CYP3A4, an enzyme that metabolizes almost 50% of administrated drugs [15], triggers a negative feedback loop to reduce the amount of drug available inside tumour cells. Naturally intrinsic analogues of such mechanisms may also be present.

For convenience, the two patterns of drug resistance are termed as ABC transporter mediated and internal detoxifying drug resistance. We study both intrinsic and induced analogues of these mechanisms. We now present mathematical descriptions of the in-silico model: readers who are not interested in the details can skip the remainder of this section.

2.2 Model description

The model setting is similar to our previous modelling of tumour cells, which incorporated apoptosis as the primary cellular response, integrated with a population description of cell density and drug transport in the interstitium [12]. The model consists of stationary cells in an interstitium in a tumour-cord geometry (described in a 1-D cylindrical geometry for simplicity): drugs enter through the capillary, which is at one end of the interstitium. For simplicity, drug transport in the capillary is not explicitly described. In this paper, intracellular descriptions are augmented by descriptions of the two resistance mechanisms. The descriptions of the drug resistance mechanisms are presented in detail below followed by brief descriptions of the tumour cell density and intracellular signalling (see details in [12]).

2.2.1 ABC transporter mediated drug resistance

ABC transporters act to increase the efflux of drugs from the cell. The effects of induced increase of ABC transporter concentration have been examined in our earlier study [16]. The interaction between anticancer drugs and ABC transporters has also been studied earlier in an

ODE framework [17, 18]. In these studies, the pumping out of drugs was described by a Michaelis-Menten type equation, where the maximum efflux rate was dependent on the concentration of ABC transporters on the cell membrane. In our previous investigation, however (in contrast to previous studies), the model was formulated in a PDE based system with drug transport in the tumour being described by a diffusion-reaction equation. The same framework is employed here and the extracellular description of drug transport is the same as in this earlier study [16]. Thus, as before, the drug concentration in the interstitium is modelled by incorporating the diffusion, the uptake and efflux (dependent on transporter levels) from the cell. In addition, binding/unbinding of extracellular drug to/from albumin is included though this is not important for any of the results presented. The "baseline" intracellular drug concentration model describes the influx and efflux from the cell (other additional factors are incorporated subsequently). The equations for extracellular and intracellular drug concentration are:

$$\frac{\partial c_E}{\partial t} = D_E \nabla^2 c_E + c_t \left(\frac{[ABC]\lambda V_2 c_I}{k_2 + c_I} - \frac{V_1 c_E}{k_1 + c_E} \right) - k_a c_E + k_d c_B \qquad \text{Eqn. 1}$$

$$\frac{\partial c_I}{\partial t} = \frac{V_1 c_E}{k_1 + c_E} - \frac{[ABC]\lambda V_2 c_I}{k_2 + c_I} \qquad \text{Eqn. 2}$$

where c_E, c_B, and c_I represent the extracellular free, extracellular bound and intracellular drug concentration, respectively; c_t denotes the tumour cell density. The uptake of the extracellular free drug and pumping out of the intracellular drug are described by Michaelis-Menten type equations, with transmembrane transport parameters V_1 and V_2, and Michaelis-Menten constants k_1 and k_2. The maximum efflux rate is dependent on the concentration of ABC transporters, the scaling factor λ which relates the level of ABC transporters to efflux rates and the constant V_2. The scaling factor is chosen so that at basal conditions λ [ABC] equals 1 so that V_2 represents the maximal efflux rate at basal ABC transporter levels. k_a and k_d are the binding and disassociation rates for drug-albumin complex.

At the outer boundary of the tumour-cord, the no-flux condition is applied for both free and bound drug concentrations due to symmetry, whereas the well-known Kedem-Katchalsky equation [19] is specified at the inner boundary,

$$J_s = P_E\left(S - c_E(r_c)\right) + J_{F,s} \qquad \text{Eqn. 3}$$

which prescribes the flux J_s at the capillary wall as the sum of two terms. The first term is proportional to the difference between the drug concentration in the capillary S, and the extracellular concentration at the edge of the interstitium ($c_E(r_c)$) and depends on the

permeability of the capillary wall (P_E). The second term describes a convective flux through the capillary wall, where the transmural velocity is described by Starling's law. It is worth pointing out that more complex tissue configurations can be studied where drug may leak out of the outer boundary; additional factors such as lymphatic drainage can also be incorporated. It is recognized that these factors could play a modulatory and possibly non-trivial role, however, these details are omitted here with the understanding that they can easily be incorporated subsequently.

Dynamics of ABC transporter-mediated drug resistance:

We now discuss models for intrinsic and induced resistance mechanisms involving upregulation of ABC transporters. We first discuss induced resistances, The concentration of ABC transporter is initially at its steady state, determined by its intrinsic production rate ($R_{0,ABC}$) and natural degradation rate ($k_{d,ABC}$). The exposure to anticancer drugs can increase the ABC transporter level on the cell membrane [13, 14], which is described by an induction term related to the intracellular drug concentration (the second term in the RHS of Eqn.4). The induction term is assumed to be a linear function of intracellular drug concentration, even though an S-shaped function has been used to model the dynamics of ABC transporters [18]. Regardless of the exact form, this term describes a basic fact that the resistance is modulated by the time-dependent intracellular drug concentration in a reversible manner. The dynamics of the concentration of the ABC transporter is given by

$$\frac{d[ABC]}{dt} = R_{0,ABC} + R_{ABC}\varepsilon c_I - k_{d,ABC}[ABC] \qquad \text{Eqn. 4}$$

where $R_{0,ABC}$ is the intrinsic ABC production rate, R_{ABC} is the induction constant and ε is the conversion factor that relates ABC induction rate to the intracellular drug concentration and $k_{d,ABC}$ represents the ABC natural decay rate.

In the intrinsic analogue of this mechanism, ABC transporter levels are elevated relative to basal conditions. This is described in Eqn (4) by increasing the intrinsic production rate (relative to the baseline value), while setting the constant R_{ABC} to 0. In such cases the simulation of the model is performed with initial conditions corresponding to the steady state for the ABC transporter concentration, which remains fixed during the course of drug delivery. This can also be modelled by increasing the ABC transporter concentration in Eqn. 1. Overall, Eq. 4 incorporates both intrinsic and induced modes of ABC transporter based resistances. Note that since there is always a baseline level of ABC transporters, ABC transporter-based resistance implies an incremental effect relative to this baseline.

2.2.2 Internal drug resistance

We now present models for intrinsic and induced drug resistance mechanisms involving the degradation or sequestration of drugs by intracellular proteins. This is observed in multiple settings, for instance in the detoxification of irinotecan in colon cancers [15]. This involves an intracellular mechanism for detoxifying/sequestering/degrading drugs. This resistance could be enhanced directly through the drug presence. In this case, the effective intracellular drug concentration is reduced by a detoxifying effect which is assumed to be proportional to the intracellular drug concentration (c_I) and the concentration of relevant detoxifying protein (R_X), whose identity may vary from context to context. The dynamics of extracellular drug and intracellular drug incorporating the effect of detoxifying protein are given by

$$\frac{\partial c_E}{\partial t} = \boldsymbol{D_E \nabla^2 c_E + c_t \left(\frac{V_2 c_I}{k_2 + c_I} - \frac{V_1 c_E}{k_1 + c_E} \right) - k_a c_E + k_d c_B}$$

Eqn. 5

$$\frac{\partial c_I}{\partial t} = \frac{V_1 c_E}{k_1 + c_E} - \frac{V_2 c_I}{k_2 + c_I} - \boldsymbol{k_{in} R_X c_I} \qquad \text{Eqn. 6}$$

where k_{in} represents the effect of drug resistance on intracellular drug concentration. The extracellular drug concentration model is the same as that used previously.

In the above model, if either k_{in} or R_X is zero, this intracellular pathway would be deactivated. Increasing the value of k_{in} (for a fixed non-zero level of R_X) increases the strength of this intrinsic resistance mechanism. In addition to this, induced resistance mechanisms are also examined, which involve the regulation of R_X by intracellular drug and this is discussed next.

Dynamics of internal resistance:

In the induced case, the resistance pathway is induced by exposure to drug. For purely induced mechanisms, it is assumed that R_X does not exist in the cell at the initial (basal) state but is produced in response to intracellular drug and degrades naturally.

$$\frac{dR_X}{dt} = \boldsymbol{R_{R_X} \varepsilon c_I - k_{d,R_X} R_X} \qquad \text{Eqn. 7}$$

where R_{RX} and $k_{d,RX}$ are the induction constant and natural degradation rate, respectively. ε is the conversion factor. Naturally it is possible to include both intrinsic and induced analogues. Eq. 7 can be augmented to incorporate a basal induction rate (independent of drug), analogous to the situation with ABC transporters above (Eq. 7a).

$$\frac{dR_X}{dt} = R_{0,R_X} + R_{R_X}\varepsilon c_I - k_{d,R_X}R_X \quad \text{Eqn. 7a}$$

Such an equation would naturally encompass both induced and intrinsic modes: setting the basal induction constant R_{0,R_X} to zero would reduce to the purely induced case above, and setting the R_{RX} term to zero would remove the drug induced contribution. Removing both terms eliminates the detoxifying mode altogether. The only difference when compared with the ABC transporter case is that the baseline level of detoxification (i.e. no resistance) is taken to be 0, whereas a baseline level of ABC transporters are always present.

2.2.3 Combination of resistance modes

In reality, multiple resistance mechanisms co-exist in the cell. Therefore it is important to understand the simultaneous functioning of both ABC-transporter and detoxification mechanisms at the cell and tissue levels. This is achieved by simply incorporating both modes of resistance in the model, each weighted by their relative contribution. Both combined intrinsic resistance and combined induced resistance mechanisms are considered.

2.2.3.1 Intrinsic drug resistance

In the case of intrinsic drug resistance, a model which incorporates both modes of resistance is described by the following equations:

$$\frac{\partial c_E}{\partial t} = D_E\nabla^2 c_E + c_t\left(\frac{(1+\alpha\lambda_1)[ABC]_0\lambda V_2 c_I}{k_2+c_I} - \frac{V_1 c_E}{k_1+c_E}\right) - k_a c_E + k_d c_B \quad \text{Eqn.8}$$

$$\frac{\partial c_I}{\partial t} = -\frac{(1+\alpha\lambda_1)[ABC]_0\lambda V_2 c_I}{k_2+c_I} + \frac{V_1 c_E}{k_1+c_E} - k_{in}R_X c_I(1-\alpha) \quad \text{Eqn.9}$$

λ_1 is a factor which determines the strength of the intrinsic ABC transporter resistance (percentage increase in ABC transporter concentration over basal non-resistant cell levels), and $[ABC]_0$ in this equation represents the baseline, non-resistant level of ABC transporter concentrations and R_X , the sequestration protein concentration. The above model describes a mixture of both resistance modes: we clearly see a term corresponding to the ABC transporter resistance, which is beyond the basal term (associated with the constant λ_1). The parameter α denotes the relative "proportion" of the two resistance terms. Thus α=1 corresponds to purely intrinsic ABC transporter mechanism while α=0 corresponds to purely intrinsic internal mechanism. Note that the term could have been incorporated in the baseline induction rate of the ABC transporter generation also, but that would result exactly in the above equation.

2.2.3.2 Induced drug resistance

Incorporating both modes of induced drug resistance results in a combined model:

$$\frac{\partial c_E}{\partial t} = D_E \nabla^2 c_E + c_t \left(\frac{[ABC]\lambda V_2 c_I}{k_2 + c_I} - \frac{V_1 c_E}{k_1 + c_E} \right) - k_a c_E + k_d c_B \quad \text{Eqn.10}$$

$$\frac{\partial c_I}{\partial t} = \frac{V_1 c_E}{k_1 + c_E} - \frac{[ABC]\lambda V_2 c_I}{k_2 + c_I} - k_{in} R_X c_I \quad \text{Eqn.11}$$

$$\frac{d[ABC]}{dt} = R_{0,ABC} + R_{ABC} \varepsilon c_I * \alpha - k_{d,ABC}[ABC] \quad \text{Eqn.12}$$

$$\frac{dR_X}{dt} = R_{R_X} \varepsilon c_I * (1 - \alpha) - k_{d,R_X} R_X \quad \text{Eqn.13}$$

The factor α again determines the relative balance of the two mechanisms. Here [ABC] denotes the ABC transporter concentration which in general can vary with time. Thus α=1 corresponds to purely induced ABC transporter mechanism while α=0 corresponds to purely induced internal detoxifying mechanism.

2.2.4 Intracellular apoptosis signalling module

In the present model, there is no direct interaction between drug-resistance mechanisms and apoptosis signalling, but drug-induced resistance affects apoptosis signalling by reducing the intracellular drug concentration that serves as a stimulus to the apoptotic signal transduction. Other more complex interactions between drug resistance and signalling do exist in tumours (for instance other resistance mechanisms act at different points on the apoptosis signalling pathway inhibiting it) but this will be examined in future studies. This will need more detailed models of apoptosis pathways and the specific nature of the interaction.

Two coarse-grained models containing minimal elements of the underlying information processing are employed to describe the signalling leading to cell killing. The models describe two types of switches: a bistable switch and an irreversible monostable switch. Both these models incorporate characteristics common to all apoptosis models (threshold and irreversibility), but differ in basic dynamical characteristics. We study both these cases since each of these characteristics has been postulated to underlie apoptosis signalling.

Both models [20-23] are included so that the effects of drug resistance with both models can be examined. We can then establish if the differences between these two models has any important consequences for the questions of interest. A comparison of the information processing characteristics of these two types of switches, especially with reference to irreversible decision-making is presented in [24]. The model equations employed are

Bistable switch:

$$\frac{dR}{dt} = \frac{V_f(1-R)}{K_{m1}+(1-R)} + (p + qc_I)k_{fb}R(1-R) - \frac{V_r R}{K_{m2}+R} \quad \text{Eqn. 14}$$

Monostable switch:

$$\frac{dR}{dt} = k\left(\frac{c_I^n}{k_h+c_I^n} - R\right) \quad \text{Eqn. 15}$$

The response of *R* component triggers the downstream reaction, the output of which indicates the fate of tumour cells.

$$\frac{dR_1}{dt} = k_f R(1-R_1) - k_r R_1 \quad \text{Eqn. 16}$$

Under basal conditions, R_1 is very small and can only reach an appreciable level if the *R* level is sufficiently high for a considerable period of time. In the current model cell death is triggered if R_1 reaches a particular threshold $R_{1,th}$, and this is reflected at the population level in an irreversible manner. It is worth pointing out that other variations of bistable modules (including mechanistic ones) have exactly the same input-output characteristics as the module used here. Hence the qualitative characteristics are expected to be the same for other bistable modules, and employing comparable corresponding parameters would result in similar behaviour.

In summary, the signalling modules contain minimal elements of signal processing based on threshold and irreversibility. Two different mechanisms are incorporated to check whether the essential trends and results of this study are independent of the basic underlying apoptosis signalling model.

2.2.5 Tumour cell density module

Cell death at the population level is modelled in the same way as in the previous study [12], hence only a brief description is given here. A logistic growth equation is used to describe the population cell density

$$\frac{dc_t}{dt} = a_1 c_t - a_2 c_t - b c_t^2 \quad \text{Eqn. 17}$$

where a_1 and a_2 are intrinsic growth rate and degradation rate of tumour cells, respectively, and *b* is the saturation constant. The triggering of death is reflected as a sharp reduction in the growth rate (which results in the zero steady state being reached in the population module). It is assumed that resistant tumour cells possess the same growth dynamic properties (growth

and natural death rate) and the same apoptotic response, as the wild-type tumour cells. The population description is again a minimal description, which includes growth, death and saturating effects, with growth and/or death rates being modulated by the internal state of the cell.

2.2.6 Initial conditions

Concentrations of all states of drug and intracellular molecules are set to be zero initially, except the ABC transporter, whose initial level is determined by its intrinsic dynamics. When we examine intrinsic resistance, an elevated level of ABC transporter is incorporated for the ABC transporter resistance case and a basal concentration of detoxifying molecule is incorporated for the internal detoxifying resistance case. A uniform initial tumour cell density is assumed throughout the tumour cord.

2.3 Numerical methods

The parameter values are presented in Tables 1 and 2, which are the same basal parameter values as previously used [16]. Doxorubicin is chosen as the anticancer drug for parameterization purposes, and the effects of parameters specifically related to resistance mechanisms are investigated. The role of the basal parameters in the model has already been studied previously [16]. All results are normalised by the following reference values: tumour-cord radius for the radial co-ordinate, 0.001 μg/mm^3 and 1 ng per 10^5cells for scaling extracellular and intracellular concentrations respectively, and 10^6 cells/mm^3 for tumour cell density. The input signal (denoted by S) is non-dimensionalized by the same representative factor as extracellular drug concentration. Since parameters in the drug resistance module are not directly available and only limited information can be found in the literature, values within estimated ranges are chosen to reflect a reasonable time scale and level of drug resistance. Furthermore, a sensitivity analysis has been carried out to determine whether the essential conclusions and trends are influenced by the specific values of these parameters. .

The models are solved numerically in MATLAB by employing the finite difference method to discretize the diffusion term in the radial direction [25], and adopting the solver ODE 15s to solve the resulting set of ODEs. Mesh independence is ensured by running the simulations with different mesh densities until results obtained from two successively refined meshes are approximately the same.

3 Results

In this section we present the results of our analysis on intrinsic and induced drug resistances at the cell and tissue levels, for the two resistance mechanisms. Our earlier study [16] had focussed exclusively on induced drug resistance associated with upregulation of ABC transporters. The baseline description and parameters are fixed at the same value as in the earlier study. The main parameters which we focus on are those associated with the resistance mechanisms/pathways. The effects of each of the intrinsic resistance pathways are examined first (in the absence of the other). This is followed by the effects of spatial heterogeneity associated with these parameters. The effects of the induced internal detoxifying resistance mechanism are then examined at the cell and tissue levels, thus complementing our earlier study of induced ABC transporter resistance. Finally the combined effect of the two resistance mechanisms is investigated. This is done separately for the intrinsic and induced cases.

Results presented are obtained for the monostable apoptosis model, as similar trends of tumour cell density distribution are found for the bistable apoptosis model (see Supplementary Material). The fact that two different models of apoptosis signalling result in very similar trends, suggests that details of the apoptosis model do not matter much for the issues of interest in this paper. It also suggests that having a more detailed model will also result in essentially similar results. Overall the trends associated with drug resistance which we study here can be understood in a clear-cut and straightforward fashion.

3.1 Intrinsic drug resistance mechanisms

3.1.1 ODE-based model formulation

We begin our investigation by studying the effects of intrinsic drug resistance mechanisms. The level of intrinsic drug resistance is associated with clear-cut parameters. We start from the simplest case: the effect of intrinsic resistance at cellular scale (studied through ODE models, focussing on the cellular level as in [16]) under a step signal. The drug stimulus is introduced via a production term and first order degradation term. As shown in Fig.2, intrinsic drug resistances, manifested as overexpression of ABC transporter and the presence of degradation/sequestration protein prior to drug injection at a constant level, lead to reduction in the intracellular drug concentration, thereby delaying or blocking cell apoptosis. However, they exert different effects on the extracellular drug concentration depending on

the nature of the resistance mechanism, resulting in an increase in extracellular drug concentration for the ABC transporter based mechanism and a reduction for internal mechanism. It is worth noting that increasing the signal intensity cannot compensate for the resistance effect of ABC transporter when the ABC transporter level is intrinsically beyond a critical level; however, increasing signal intensity can be an effective means to overwhelm resistant effect of the internal detoxifying mechanism, thereby resulting in cell death. This can be established analytically (not discussed here) and suggests that if cells have a high degree of intrinsic resistance (associated with ABC transporters), then they can become fundamentally immune to a specific drug attack. Directly inhibiting this resistance may be a necessary step to killing such cells.

Insight: While intrinsic resistance through both mechanisms act to counter drug stimuli, differences in the effects of intrinsic resistance through these mechanisms can be seen even through local stimulation.

3.1.2 PDE-based model formulation

3.1.2.1 Persistent infusion

The natural follow-up question is how intrinsic drug resistances behave at the tissue scale. This is addressed through a PDE model accounting for drug diffusion and its effect on cells in the tissue. Given homogenously elevated ABC transporter levels in the tissue, similar findings to wild-type (non-resistant) tumour cells are shown in Fig.3 (a) revealing that cells in the tissue are either alive or killed when subject to a step signal. Although the intrinsic drug resistance via ABC transporters alters the kinetics of drug transmembrane transport, a homogeneous distribution of drug concentration is still achieved under step signal, which is responsible for homogenous cell response. In contrast to the intrinsic ABC transporter drug resistance, Fig.3 (b) shows a heterogeneous response of tumour cells subject to step signals in the case of intrinsic internal resistance. Cells are killed in the region proximal to the capillary. The intracellular sequestration/degradation represents a sink of intracellular drugs, and results in concentration gradients along the radial direction. Consequently, the intracellular drug concentration reaches the requisite threshold in the proximal region, but remains below the threshold elsewhere. Therefore the “dose-response” characteristic of the tissue involves a transition from no killing (low dosage) to partial killing (intermediate dosage) to complete killing (high dosage).

Insight: Intrinsic resistance through the two different resistance mechanisms exhibit clear differences in the dose-response characteristics, for persistent infusion.

3.1.2.2 Pulse infusion

The preceding analysis serves as a stepping-stone to investigate the effects of more realistic drug regimens. Fig.4 shows the situation when cells with intrinsic drug resistances (both ABC transporter and internal detoxifying) are exposed to pulse (bolus) signals of increasing intensity (and fixed duration). The resulting trend reveals that cells in the tumour could survive the drug attack altogether, be partially killed in the proximal region, or be killed throughout the tumour cord, as the intensity is increased. Similar trends can be observed by varying the pulse duration while keeping the amplitude fixed (results not shown here). These results may be seen as an intuitive extension of the situation with step inputs examined above. As cell apoptosis is characterised by a threshold mechanism, it is only when the signal intensity exceeds a critical level can cell apoptosis be triggered. Noting that the resistance strength is uniform, cell apoptosis is induced primarily in the proximal region given the decaying drug concentration in the radially outward direction. A narrow range of signal intensity is required to achieve various tumour cell responses at the tissue scale for ABC transporter drug resistance, while a broader range of signal amplitudes is associated with inhomogeneous cell death in the intrinsic internal detoxification mechanism.

Insight: The trends of tissue response to a pulse stimulus are qualitatively similar for the two mechanisms, but with different quantitative sensitivity. Studies based on pulse stimuli alone can somewhat obscure the fact a clearer difference is seen in a simpler stimulus (persistent stimulus).

3.1.3 Heterogeneity of drug resistance

The results presented above were obtained by assuming spatially homogeneous levels of drug resistance. It is well known that there may be significant sources of heterogeneity in tumours owing to gradients of various species in the interstitium including oxygen [26]. This can also cause a marked heterogeneity of resistance levels across a tissue. The effect of heterogeneity of drug resistance characteristics in a tissue can be investigated by introducing the heterogeneity in a controlled manner, while keeping other factors fixed. The results can then be compared to the case of uniform resistance. The model still ignores multiple cell populations (locally), as well as other factors which may be relevant, such as direct transfer of P-glycoproteins between cells. Nevertheless, it allows us to focus on the effects which

arise primarily through the spatial variation of resistance. Two scenarios are assumed: one (S1) where tumour cells are more resistant in the region furthest from blood vessels, for instance due to a severe tumour microenvironment [27], while in the other case (S2) a higher level of expression of resistant proteins occurs in tumour cells closest to blood vessels, which might for instance arise from evolution of cells that were exposed to a sub-lethal prior dosage. Mathematically, the spatial variation of intrinsic resistance is represented by a step function, where a 20% increment in the baseline intrinsic level is imposed on the rear one-third region of the spatial domain for scenario S1 and the front one-third region for scenario S2. Both intrinsic ABC transporter and internal resistances are examined.

3.1.3.1 Persistent infusion

Given the same signal intensity (as adopted in the homogeneous case), it is found in Fig.5 (a) that tumour cells with higher level of ABC transporters are alive wherever they are located, with tumour cells in the rest of the domain being killed, thus resulting in heterogeneous responses to a step signal. As discussed above, a uniform distribution of drug concentration and uniform killing is achieved under persistent infusion (at this stimulus level) in the homogeneous case. In scenario S1, the more resistant cells in the further region can escape from cell apoptosis, as the intracellular drug concentration cannot exceed the relevant threshold. In the second case, it is easily seen that tumour cells stay alive in the proximal region to the capillary given the position of drug input and directionality of drug transport. In fact, the drugs pumped out of cells in this region are transported further in the interstitium, augmenting the intracellular accumulation of drugs therein, thus enhancing the possibility of cell killing there. Overall, heterogeneous distribution of intrinsic ABC transporter resistance can contribute to re-distribution of drug, posing an elevated threat of drug attack to cells with relatively low resistance level. At the same time, these extra resistant cells, irrespective of their location can successfully counteract drug stimuli.

Turning to the analogous situation for the internal detoxifying mechanism, Fig.5 (b) shows that tumour cells are killed in the proximal region irrespective of where the region of higher level of internal detoxifying resistance lies, for the stimulus studied. Excessive sequestration/degradation represents a sink term for intracellular drugs at cellular (local) scale, which impairs interstitial drug transport at tissue (global) scale, thus rendering tumour cells in the proximal region more prone to killing. In this case increasing the strength of drug stimulus further results in a scenario where only the resistant regions are alive (this is similar to the ABC transporter case), and when the signal strength is increased even beyond this, all

cells can be killed. This trend as the stimulus strength is increased shows the complex interplay between tissue-level effects of internal mechanisms and the heterogeneity of resistance.

Insight: The heterogeneity of drug resistance coupled with the specific nature of the drug resistance mechanism can strongly influence the response at the tissue level, something seen most clearly in the case of persistent infusion.

3.1.3.2 Pulse infusion

Using the same setting described above, a more realistic situation of drug bolus/pulse signals is investigated. In the ABC transporter case, when compared to the baseline homogeneous resistance scenario under the same stimulus, it is surprising to find that cell death region (Fig.6 (a)) is extended until it reaches the boundary of the high resistance domain, leaving the high resistance tumour cells unaffected in scenario S1. In scenario S2, the cell death region is indeed extended, covering two-thirds of the tumour cord, in the region furthest from the capillary ($1/3R_t$ to R_t). The results under a pulse signal agree with those presented for a step signal in that the heterogeneous distribution of intrinsic ABC transporter resistance can play a positive role in drug redistribution (accumulation) in certain regions of tumour interstitium, thereby accentuating the drug effect in those locations.

In contrast to ABC transporter based drug resistance, heterogeneous distributions of internal detoxifying resistance (in either case) lead to a contraction of the region where cell death occurs, relative to the homogeneous resistance case. The presence of an extra sink of drugs (sequestration/degradation) in space in the tumour interstitium can act to reduce the drug action.

Insight: The interplay between heterogeneity and the nature of the resistance mechanism strongly affects the tissue level response to pulse stimuli too; this is seen by comparing the tissue level response in this case to that where resistance was homogeneous.

Taken together, the results suggest that spatial heterogeneity of resistance can significantly impact the response of a tissue to a drug stimulus, and that the specific influence depends both on the spatial profile of the heterogeneity and the nature of the resistance mechanism.

3.2 Induced internal drug resistance

Thus far, the effects of intrinsic resistances have been examined, both via enhanced expression of ABC transporters and by internal detoxifying mechanisms. With regard to

induced resistances involving these mechanisms, the drug signal is involved in inducing resistances directly through additional pathways of gene regulation/signal transduction. In these cases the induced resistance constitutes a negative feedback regulatory effect, the behavior of induced resistances involving ABC transporters has already been studied in our previous work [16]. Here, we complement that study by examining the behavior of induced internal resistance mechanisms at the tissue level.

For persistent infusion of drug, as briefly discussed in our previous work [16], low levels of persistent infusion would not result in any cell killing. However, as seen in Fig.7 (a), increasing the level of persistent infusion can either result in partial killing, for intermediate levels of infusion, or killing throughout the tissue, for high enough levels of infusion. In the former, cell death occurs in the region adjacent to the capillary wall. As the level of drug infusion is increased further, the region of cell death expands until it covers the entire tissue. This trend is in contrast with the corresponding trend for induced ABC-transporter based resistances.

The above result serves as a basis for investigating bolus (pulse) injections. Fig.7 (b) shows the effect of varying the time duration of a pulse signal of fixed amplitude. As can be seen, increasing the time duration results in a transition from no killing to partial killing (in the region closest to the capillary wall), and eventually to complete killing. A similar trend can be observed in Fig. 7(c) which involves varying the amplitude of a pulse of fixed duration (Fig.7 (c)).

Insight: Induced resistance through internal detoxifying mechanisms reveals particular dose response characteristics at the tissue level, which are different from the ABC transporter analogue; this is seen both for both persistent infusion and pulse stimuli.

3.3 Combined drug resistances

Thus far each of the resistance mechanisms has been investigated in isolation, allowing us to understand the effect of each resistance mechanism in a clear-cut manner. Naturally, both resistance mechanisms may co-exist in cells, and therefore the combined effect of both resistance mechanisms needs to be examined.

Generally, an enhanced drug resistance, resulting from a simple addition of the two resistance mechanisms, reduces the drug effect when similar levels of individual drug resistance are added, simply due to greater resistance. Here the effects of combined drug resistances are examined by varying the “proportion” of individual drug resistance strengths. In each case, a

representative level of individual resistance strength is chosen (the effect of which is understood from preceding analyses). A constant (α) lying between 0 and 1 defines the proportion of the individual drug resistance, with $\alpha = 1$ corresponding to purely ABC transporter drug resistance while $\alpha = 0$ corresponding to purely internal detoxifying drug resistance. Results for various α (0, 0.2, 0.5, 0.8 and 1) are presented for step and pulse stimuli in Fig.8 and in the supplementary material respectively.

3.3.1 Persistent infusion

The effect of combining intrinsic resistances of both types is presented in the left column of Fig.8. By recalling results of pure intrinsic ABC transporter and internal detoxifying resistances (which are limiting cases), it is shown in the left column of Fig.8 that cell death is still confined to the proximal region under a step signal when the resistance effect resulting from internal mechanism dominates, despite a slight extension of cell death region. Uniform cell killing occurs when α approaches 1. Therefore, with the presence of both types of drug resistance, the tissue level response tends to be in-between those of the individual ones, though quite a non-linear function of the proportion of individual drug resistance.

For a combination of induced drug resistances, it is non-trivial to predict the tissue response even under step signals given the heterogeneous response which can result in the individual resistance cases. The right column of Fig.8 shows spatial profiles of tumour cell density when the tumour is subject to step signals as α is varied. For a given signal intensity, cell death is partially located in the far region in the case of induced-ABC transporter resistance (as has been demonstrated and discussed in our previous paper [16]), and this remains the case for a dominant proportion of the ABC transporter resistance, the region of cell killing shrinking as the proportion of this mechanism is reduced. When the relative strengths of resistances are even (α=0.5), only a small fraction of cells are killed close to the blood vessel. With a further decrease in α, the induced internal resistance overwhelmingly determines the tissue response, resulting in cell death close to the capillary wall.

In contrast to the intrinsic case, for the situation considered it is found that the case with equal strengths of resistance gives rise to the least drug efficacy, which may be attributed to the role of induced ABC transporter resistance in interstitial drug transport (‘positive’ to certain extent) being complemented by its counterpart- internal feedback resistance (sequestering and detoxifying drug). In general we can say that the behavior of a combination of resistances can

be not only quite a nonlinear function of their proportion, but introduce some unexpected features as well.

Insight: When multiple routes to resistance are present, the dose response characteristics can be result in additional complexity at the tissue level (whereas the effects at the cellular level are simpler); the two resistance mechanisms can in certain cases counteract one another from the point of drug transport.

3.3.2 Pulse infusion

Finally, the response of combined drug resistance mechanisms is examined under a pulse stimulus. This builds on the results of persistent infusion and is briefly discussed in the Supplementary Material.

3.4 Sensitivity analysis

3.4.1 Parameters involved in the drug resistance mechanisms

The PDE model used in this study incorporates a compact description of key processes, and involves various parameters. The focus is entirely on the effects of resistance, which is overlaid on a baseline model which has been studied previously, and on key qualitative trends. Since the effects of key parameters in the baseline model have been studied in an earlier work [16], this will not be repeated here.

Changes in auxiliary parameters will alter numerical values of various quantities, however, the key trends in dose response characteristics are observed even when baseline parameters are altered within reasonable ranges. It is recognised that these baseline parameters can vary between tumour types and even within the same tumour type, therefore, the overall focus here is on qualitative trends (in dose response characteristics) rather than quantitative values.

The effect of perturbing the resistance strength, for a fixed stimulus was considered. By increasing the strengths of the intrinsic resistance, it is possible to understand how the resistance affects the tumour response. In the preceding analyses, representative values of resistance parameters and the associated responses have been used. Perturbing the resistance strength in general can perturb the tissue level response for a fixed stimulus (for eg. see Fig. S6). However the qualitative trends in the dose response characteristics are maintained. Furthermore the change in tissue level response for varying resistance parameter, for fixed stimulus results in a completely consistent pattern, with the variation of stimulus for fixed resistance. In general, the trends associated with the effect of increasing these resistance

parameters can be understood in a straightforward manner. Likewise, when examining a heterogeneous resistance profile, we are in effect perturbing the resistance in some part of the domain, increasing it beyond the rest of the domain, and compare this relative to the homogeneous case. In the induced resistance case (associated with the internal detoxifying mechanism), the same parameters for the internal pathway are used as in our previous study.

Overall the effect of varying these resistance parameters for fixed stimulus results in clearly discernible trends. When we study a range of signal strengths for a fixed set of resistance parameters, an input-output relationship (the output being where the cells are killed) can be obtained as we have done in this paper. A consistent pattern emerges when one varies signal strengths (for fixed resistance) or when resistance parameters are varied (for fixed stimulus).

4 Discussion

The effects of drug resistance are recognized in systems ranging from bacteria to eukaryotes. In the case of tumours, multiple cellular factors contributing to drug resistance have been documented, and resist the action of drugs in different ways. This includes efflux of drugs, deactivation/detoxification of drugs, drug target alteration, reducing the effect of cell killing pathways andactivating survival pathways. Understanding the functioning of these mechanisms and developing ways of counteracting them presents many challenges and requires a systems approach, with modelling and experiments. In this regard, it is important to understand the effects of these mechanisms at both the cell and tissue levels. Thus there is the need to bridge the gap between cellular pathway based analysis and equivalent studies in tissues. Previously, using a basic in-silico platform, we showed how similar induced resistance mechanisms at the cellular level can have very different tissue level consequences, even without any alteration to cellular pathways or interaction.

In this paper we focussed on two broad classes of “upstream” cellular resistance mechanisms, the upregulation of ABC transporters and enhanced detoxification/sequestration/metabolism mechanisms. Relative to a drug dosage these mechanisms can be regarded as intrinsic or directly induced by the drug. There is a real need to systematically tease out the effects of these different routes to resistance, in the context of chemotherapy, at cell and tissue levels.

One approach involves designing controlled experiments on specific cell types, perturbing different resistance pathways and studying their effects. A complementary approach is to use modelling, creating an in-silico platform which automatically involves the maintaining of carefully controlled conditions. In a complex system such as a tumour, modelling can take

two forms: a comprehensive incorporation of many potential factors, or a simplified approach relevant to the question at hand. In the former case, a number of assumptions are incorporated regarding parameters, concentrations of various species etc., many of which are more or less arbitrary. We adopted the latter approach, which focuses on key aspects of pathways of interest: here many auxiliary factors are held fixed. Naturally, such an assumption may not literally hold good in some tissues. Nonetheless, if the dominant effects in-vivo are reasonably captured, such an approach can make useful conclusions. Such an approach can be seen as a platform for examining other effects. In the context of our investigations, we are able to investigate the effects of perturbing resistance pathways against fixed backgrounds. We use the in-silico platform as an experimental tool, which can complement in-vivo investigations. Throughout, our focus has been on examining trends in the tissue level dose response characteristics, as trends rather than actual numbers provide a more robust indicator.

ABC transporter and internal detoxifying mechanisms: For each of these mechanisms we examined both intrinsic and drug-induced variants. When we examine intrinsic analogues of both resistance mechanisms, we find a striking contrast: while both mechanisms function generally to resist the action of drugs, their tissue level behaviour and trends exhibited when drug dosage is increased is in striking contrast. It is possible to get partial cell killing in the domain even under persistent infusion (at the proximal end) for the internal detoxifying mechanism. The more realistic treatment case of bolus injections echoes these basic findings. Thus elevating these resistance levels can have non-trivial tissue level effects. Since these intrinsic resistance mechanisms may vary in strength in the tissue we examined situations where the cells exhibited heterogeneity of resistance. We find here that depending on the heterogeneity and the spatial variation of the resistance and the type of mechanism, the heterogeneity can reduce or enhance cell killing in certain regions in the tissue.

We examined induced internal drug detoxifying mechanisms to complement our study of induced ABC transporter resistance [16]. Again, tissue level models reveal clear non-trivial consequences and contrasts, seen both in persistent infusion and bolus injections. Examining the effects of combined routes to resistance, demonstrates a fairly nonlinear dependence on the relative strength of each mechanism. In some cases an equal combination of resistance mechanisms can result in the least degree of tissue level killing.

Our in-silico investigations allow us to make a number of conclusions. The first, most basic point is that in order to examine the effects of cellular drug resistance mechanisms, it is important to study them simultaneously at the cell and tissue levels and systematically

explore the connections between the two levels. Experimental dissection of resistance, likewise should incorporate both cellular systems biology approaches, with an analysis of the effect of transport and tissue characteristics. The second is that the nature of the resistance mechanism, and whether it is intrinsic or induced can have different tissue level dose response effects. The third point is that the contributions of multiple drug resistance mechanisms must be elucidated individually and in combination. Finally in tumours, either due to prior drug dosage or other conditions (for instance hypoxic conditions), cells can acquire a significant degree of intrinsic resistance. Since some of these factors are necessarily spatially varying, these may contribute to a significant spatial heterogeneity in resistance characteristics. Different sources to such resistance may result in completely different heterogeneous resistance profiles and affect tissue responses.

We have used an in-silico model as an experimental platform to investigate the functioning of pathways operating in real cells of different types. The complexity of real tumours brings with it other features: complex shape, irregular vasculature, and other factors having to do with cells: significant heterogeneity, multiple populations of cells, other interactions between cells etc. Our in-silico platform bypasses some of these effects, or does not incorporate them at this stage. While these features may be undoubtedly present, if they are more or less fixed, or do not significantly interact with the resistance mechanisms, then we may expect the essential conclusions to be extendable to these more complex situations. On the other hand it could be the case that in some contexts, some of these factors do indeed introduce significant interplay with the resistance mechanisms. If this is the case, then this interplay has to be teased out: this will involve the design of new experiments and models focussed towards this goal which can build on this study. In-silico platforms allow for the design of new experiments in this regard.

We have not directly examined the effects of drugs conferring resistance on cell populations, by affecting the microenvironment in which they evolve. Different studies in the literature focus on this aspect, often ignoring spatial aspects. A systems approach to understanding different aspects of drug resistance in tumours will involve the merging of the effects of drug dosage, drug transport, cellular pathways, and the evolution of cells. Likewise other aspects of multiple subpopulations of resistant cells will be examined subsequently.

Different approaches are being taken to circumventing drug resistance mechanisms. One approach includes altering the mode of drug delivery, for instance to bypass efflux [28, 29]. A second approach is to either suppress or inhibit the resistance pathway: this can also be

achieved by using some form of combination therapy. The use of P-gp inhibitor to suppress the efflux resistance, along with anti-cancer drug dosage has been reported to yield good results [30, 31]. While such results are encouraging, it is worth emphasizing that designing any such protocol in a systematic manner will benefit from accounting from tissue level effects of both drug dosage (including those in combination therapies) and drug resistance. Teasing out multiple sources to resistance and their effects in a tissue would greatly benefit the design of suitable protocols.

Overall through our study, we are able to obtain some basic understanding of how such cellular resistance mechanisms function at the tissue level. This can aid the design/creation of new strategies which circumvent these mechanisms, for instance by having an extra treatment to suppress these mechanisms or by devising new modes of drug delivery, or by using a combination of therapies. Finally it is also worth emphasizing that these studies can also be used as a basis of parallel investigations of resistance in other disease contexts, noting that the kinds of resistance mechanisms encountered here are by no means restricted to tumours. We anticipate that the systematic multi-level elucidation of cellular resistance mechanisms and their tissue level effects will provide a significant new window for both understanding and targeting these mechanisms, with both basic biological and biomedical significance.

Conflict of Interest

There are no competing interests.

Author Contributions

JK planned the work and the paper, CL performed all the computational work, JK wrote the paper with input from CL and XYX.

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Figure Legends

Fig.1 Schematic diagram of drug resistance integrated with drug transport and cellular apoptosis signaling (upper panel), and of representative mechanisms of drug resistance: ABC transporter-based and internal sequestration/detoxification at the cellular level each of which may be intrinsic/induced (see text): (a) Intrinsic ABC transporter drug resistance, (b) intrinsic drug sequestration/detoxification, (c) drug-induced ABC transporter resistance, and (d) drug-induced internal sequestration/detoxification resistance.

Fig.2 Effect of intrinsic ABC transporter drug resistance (left column) and intrinsic internal detoxification drug resistance (right column) on temporal profiles of (a) and (b) extracellular drug concentration, (c) and (d) intracellular drug concentration, and (e) and (f) tumour cell density under step infusion of drug intensity S = 1.5 (in dimensionless terms: see text for details) in the ODE-based model formulation. A decrease in tumour cell density is indicative of cell death (this is true for subsequent figures as well). A sufficient degree of resistance can counter this drug stimulus, in both cases.

Fig.3 Spatial profiles of tumour cell density at 24h under step infusion for varying signal intensity: (a) intrinsic ABC transporter drug resistance, (b) intrinsic internal resistance. The baseline concentrations of intrinsic ABC transporter and internal resistant proteins are assumed to be 0.45 and 0.1 respectively, unless specified otherwise. We see that for the intermediate signal strength, it is possible to have partial killing in the domain, in the right hand panel.

Fig.4 Spatial profiles of tumour cell density at 24h under pulse injections: (a) depicts the case of intrinsic ABC transporter drug resistance for varying signal intensity for fixed signal duration T = 6.5h; (b) depicts intrinsic internal resistance for varying signal intensity given the fixed signal duration T = 5h.

Fig.5 Spatial profiles of tumour cell density at 24h for (a) heterogeneous distributions of intrinsic ABC transporter resistance under step infusion with signal intensity S=1.4 (b) heterogeneous distributions of intrinsic internal detoxifying resistance under step infusion with signal intensity S=1.8. We find that in (a), the high resistant regions evade cell killing for the scenarios S1 and S2 (see text), and in contrast to the case of homogeneous resistance (Fig. 3), cell killing does not occur uniformly in the domain. In (b), cell killing appears near the region proximal to the capillary for both scenarios S1 and S2 (see text for discussion).

Fig.6 Spatial profiles of tumour cell density at 24h for (a) heterogeneous distributions of intrinsic ABC transporter resistance under pulse injection S=1.8, T=6.5h (b) heterogeneous distributions of intrinsic internal detoxifying resistance under pulse injection S=2.5, T=5h. When contrasted with the results of Fig. 4 at the same signal intensities and durations, the effect of the heterogeneity is seen clearly, manifesting itself in different ways for the two cases.

Fig.7 Spatial profiles of tumour cell density at 12h for induced internal detoxifying resistance (a) under persistent infusion for varying signal intensities, (b) under pulse injections for varying signal duration given the fixed signal intensity S = 4, (c) under pulse injections for varying signal intensity given the fixed signal duration T = 4h.

Fig.8 Spatial profiles of tumour cell density at 24h, left column: combination of intrinsic ABC transporter and internal resistance under step infusion with signal intensity S=1.6; right column: combination of drug-induced ABC transporter and internal feedback resistance under step infusion with signal intensity S=1.8. The proportion of the resistance contributions, α is varied here, with α=1 representing the ABC mechanism, and α=0 representing the internal detoxifying mode.

Table 1 Parameters and values used in extracellular drug transport and uptake. Doxorubicin (DOX) is chosen as the anticancer drug for parameterization purpose.

Parameter	Symbol	Value	Reference
Free DOX diff. coefficient	D_E	0.568 mm^2/hr	[32]
Bound DOX diff. coefficient	D_B	0.032 mm^2/hr	[32]
Rate of transmembrane transport	V_1, V_2 (V_1=V_2)	0.28 ng/(10^5cells)/min	[33]
Diffusive permeability for free DOX	P_E	10.0 mm/hr	[32]
Diffusive permeability for bound DOX	P_B	0.032 mm/hr	[32]
Michaelis constant for transmembrane transport	k_1	0.219 μg/ml	[33]
Michaelis constant for transmembrane transport	k_2	1.37 ng/(10^5cells)	[33]
Free DOX-albumin binding rate	k_a	3000 hr^{-1}	[32]
DOX-albumin dissociation rate	k_d	1000 hr^{-1}	[32]
Hydraulic conductivity	Lp	0.1 mm/hr/mmHg	[32]
Tumour vascular pressure	P_v	20 mmHg	[32]
Tumour IFP	P_i	15 mmHg	[32]
Osmotic reflection coefficient	σ_d	0.85	[32]
Osmotic reflection coefficient for free drug	σ_E	0.35	[32]
Osmotic reflection coefficient for	σ_B	0.82	[32]

bound drug			
Plasma colloid osmotic pressure	π_v	20 mmHg	[32]
Tumour colloid osmotic pressure	π_i	20 mmHg	[32]
Initial tumour cell density	$c_{t,0}$	10^6 cells/mm^3	[32]
Tumour cell growth rate	a_1	0.5 day^{-1}	Estimated
Saturation constant in logistic equation	b	0.02592 mm^3/(10^5cells)/day	Estimated
Tumour cell natural decay rate	a_2	0.24 day^{-1}	Estimated
Tumour capillary radius	R_C	10 μm	[32]
Tumour cord radius	R_T	120 μm	[32]

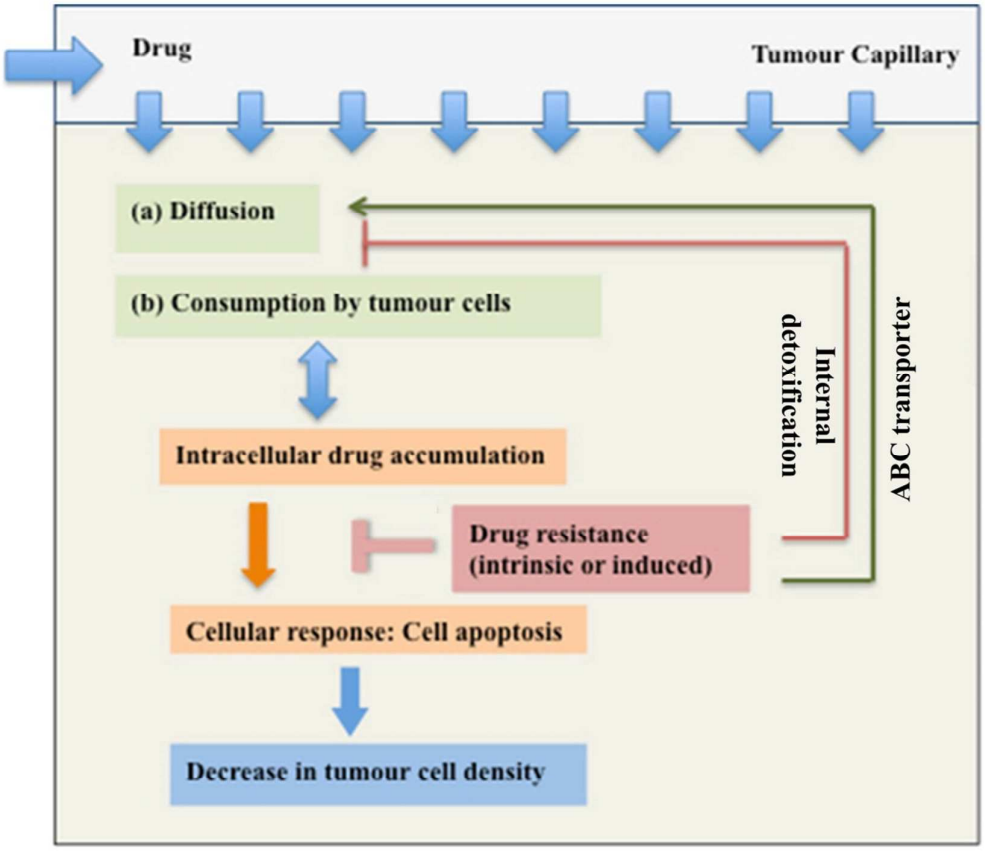
Table 1 Parameters and values used in intracellular signal transduction modules.

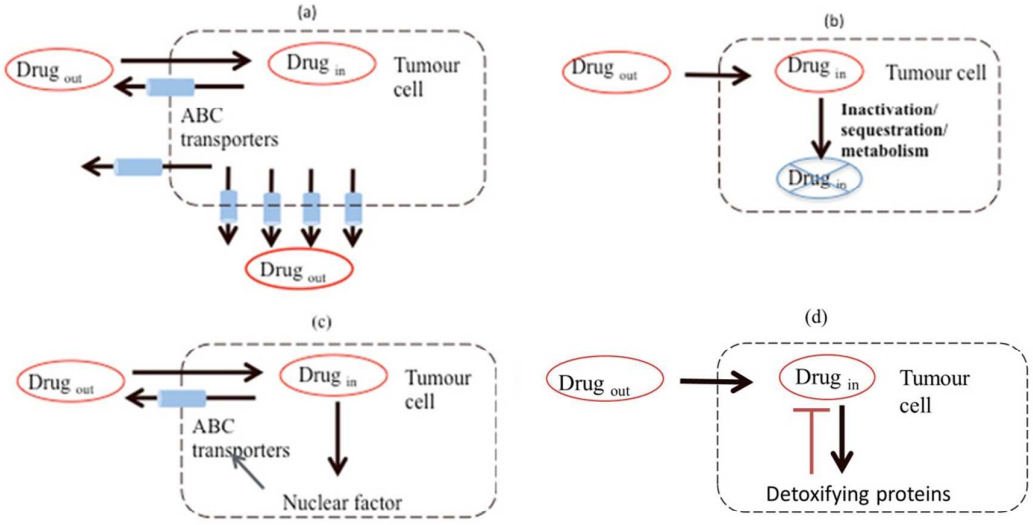
Parameter	Symbol	Value	Reference
Bistable switch			
Michaelis Menten constants	V_f	27 hr^{-1}	[34]
Michaelis Menten constants	V_r	0.459 hr^{-1}	[34]
Michaelis Menten constants	K_{m1}	100	[34]
Michaelis Menten constants	K_{m2}	0.01	[34]
Kinetic parameter mediating feedback strength	k_{fb}	2.927 hr^{-1}	[34]
Basal level in the bistable switch	p	0.7	Estimated
Parameter mediating input regulation in the bistable switch	q	0.3(ng/(10^5cells))$^{-1}$	Estimated

Monostable switch			
Kinetic parameter reflecting the time scale of the response	k	0.432 hr^{-1}	Estimated
Associated constant	k_h	1 ng/(10^5cells)	Estimated
Hill coefficient	n	10	Estimated
R_1 protein activation rate	k_f	3.6 hr^{-1}	Estimated
R_1 protein degradation rate	k_r	0.144 hr^{-1}	Estimated
R_1 Threshold for apoptosis switch	$R_{1,th}$	0.9	Estimated

Table 3 Parameters and values used in the drug resistance module

Parameters	Symbol	Value	reference
ABC-mediated module			
Intrinsic ABC production rate	$R_{0,ABC}$	0.1 day^{-1}	[17]
ABC degradation rate	$K_{d,ABC}$	0.3 day^{-1}	[17]
ABC induction rate	R_{ABC}	0.13 day^{-1}	[17]
Scaling factor	λ	3	Fixed constant
Scaling factor	ε	10 (ng/10^5cells)$^{-1}$	Arbitrary
Internal detoxifying module			
Resistant protein induction rate	K_{Rx}	0.13 day^{-1}	Arbitrary
Resistant protein degradation rate	$K_{d,Rx}$	0.3 day^{-1}	Arbitrary
Negative regulation factor	k_{in}	86.4 day^{-1}	Arbitrary
Scaling factor	ε	10 (ng/10^5cells)$^{-1}$	Arbitrary





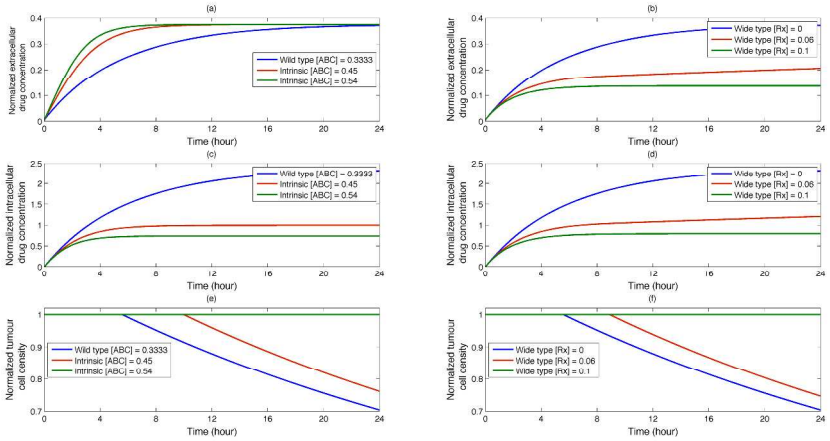


Figure 2
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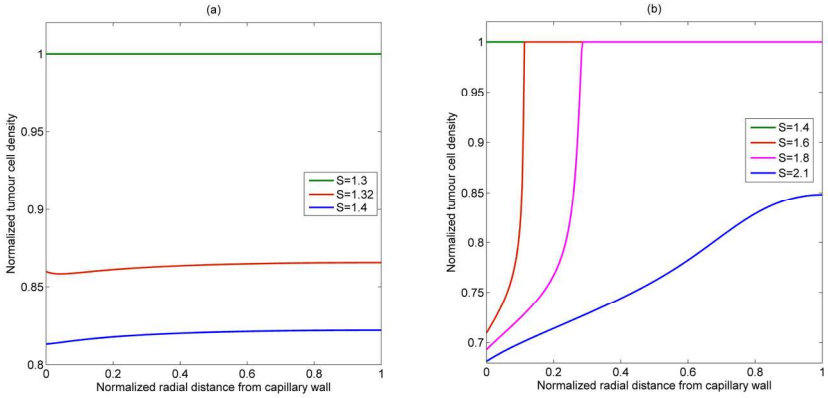

Figure 3
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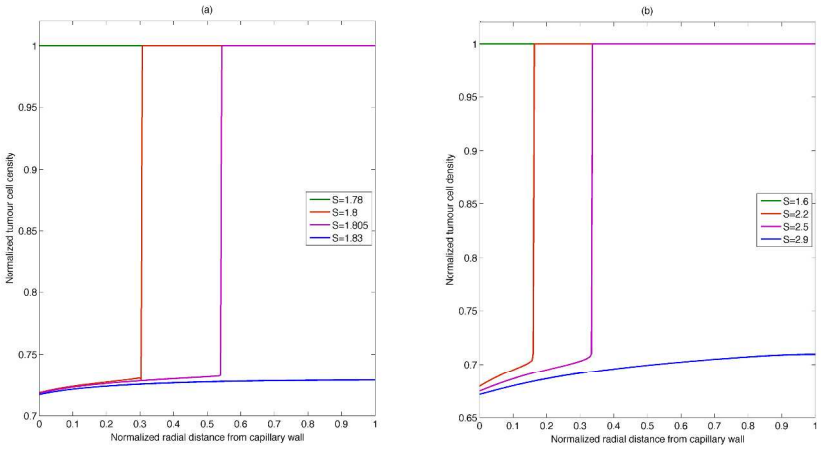

Figure 4
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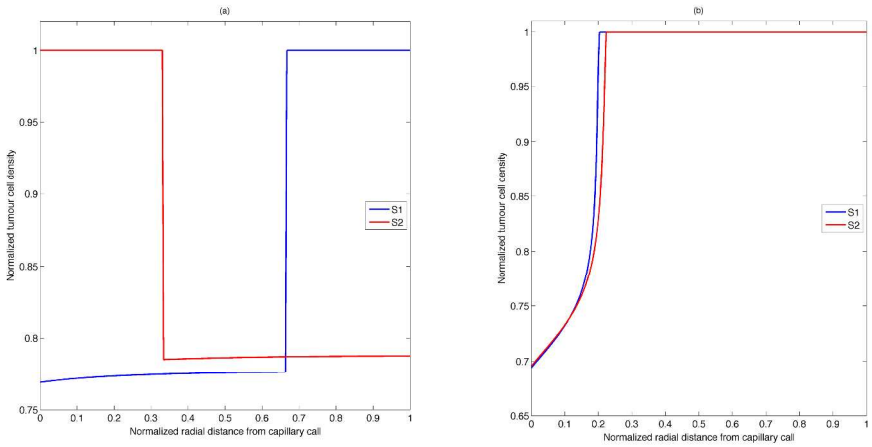

Figure 5
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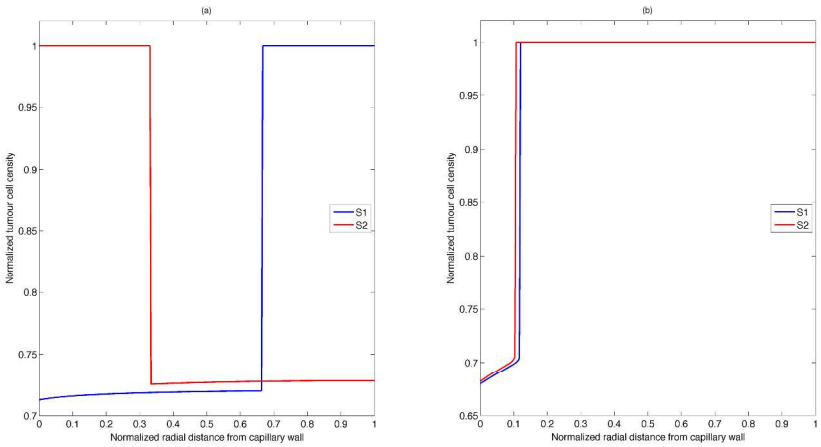

Figure 6
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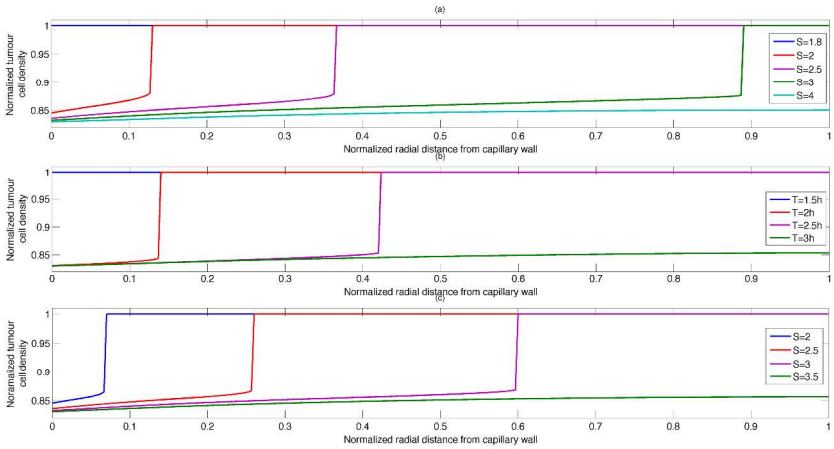

Figure 7
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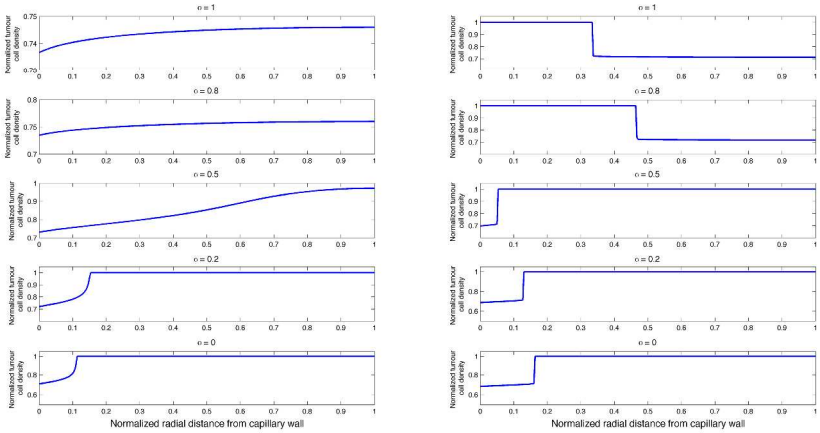

Figure 8
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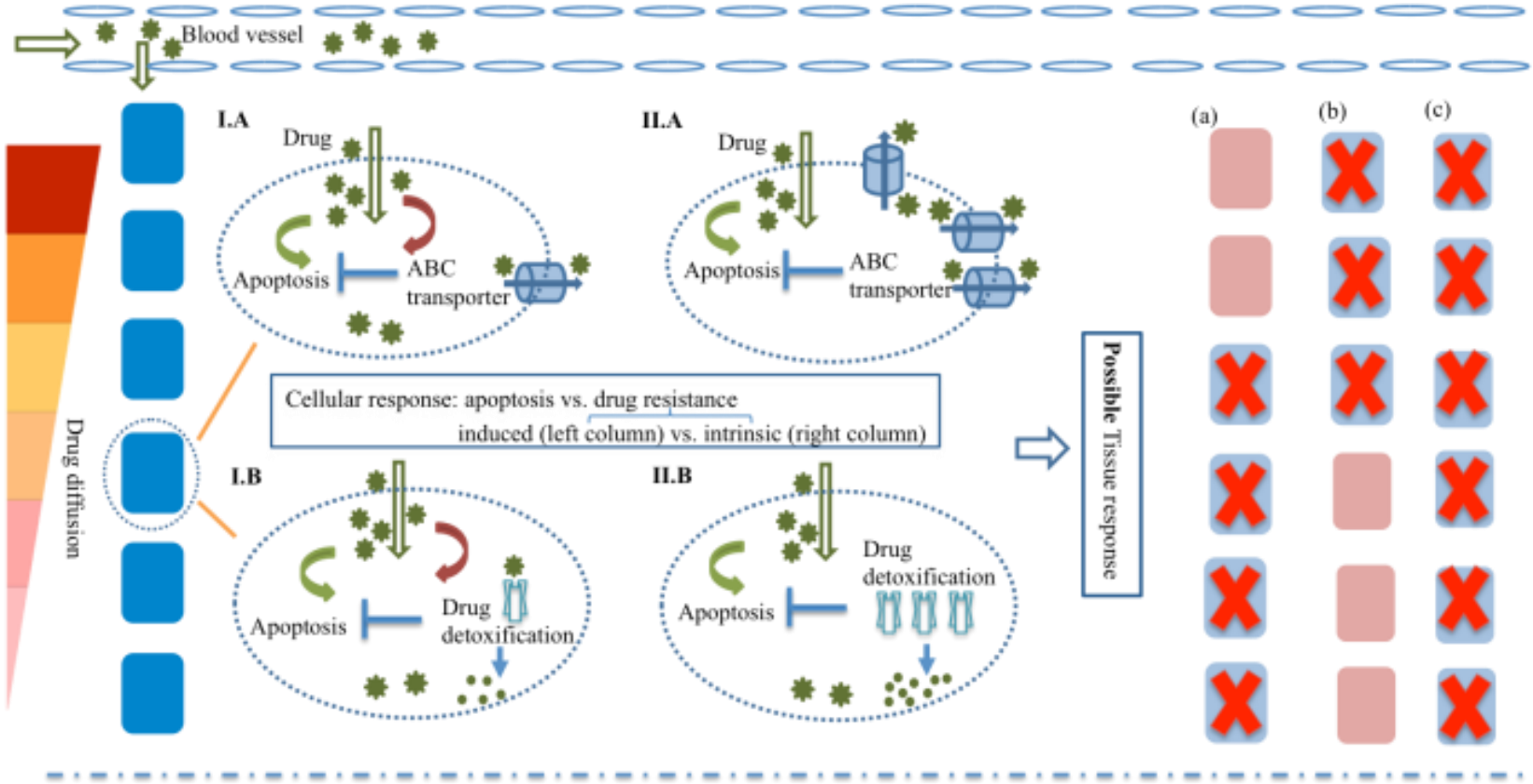

Basic in-silico models studying intrinsic and acquired variants of two types of cellular resistance mechanisms demonstrate important tissue-level differences and consequences.