Supplementary material

The Role of Cell Density and Intratumoral Heterogeneity in Multidrug Resistance

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Mathematical model

The model proposed here follows the structure of the mathematical model in

(1). In this model, we consider the population of the cancer cells and the drug, which is assumed to be a chemotherapeutic agent. The resistance level is denoted by a scalar x, which can be normalized as x ∈ [0, 1]. In our notations, x=0 corresponds to no resistance, while x=1 corresponds to maximum resistance.

Let *n(x, t)* denote the population density of cells with trait *x* (resistance level) at time *t*. We describe the dynamics of the cell population via the following selection/alteration integro-differential:

$$\frac{\partial n(x,t)}{\partial t} = \left[f\left(\rho(t)\right)\left(r(x)\left(1-\theta(x)\right) - c(x,\alpha)\right) - g\left(\rho(t)\right)d(x)\right] n(x,t) + f(\rho(t))\int_0^1 \theta(y) r(y) M(y,x) n(y,t)\,dy \quad (1)$$

where the total population is denoted as *ρ(t)*:

$$\rho = \int_0^1 n(x,t)\,dx \quad (2)$$

See Table S1 for explanations of the notations used. The assumptions for (1) can be described

as follows: *r(x)* denotes the natural division rate of a cancer cell with trait *x,* and *d(x)* denotes the natural apoptotic rate of a similar cell. These rates are time independent, so we assume no external forces are influencing the inherent growth and death rates. *c(x,α)* denotes the increased mortality rate of a cell of trait x and drug dose α. At this stage, we do not intend to study the treatment protocol, therefore the drug is assumed to be applied uniformly over time. $f(\rho(t))$ and $g(\rho(t))$ are included to add density dependence to the division and death rates. It is well known that density plays a role in both of these terms and their specific forms will play an important role in the overall dynamics. The cell division $r(x)$ and drug-induced death $c(x,\alpha)$ rates have the same density dependence factor *f*, since we assume that the chemotherapeutic drug affects primarily cells that are dividing. Hence, the induced death term should be proportional to the division rate. All parameters are nonnegative: *r, d, c,* g ≥ 0, *r, d, c* ∈ C([0, 1]) and *f*> 0.

We further assume that when cells undergo division, they may be mutated or modified. That is, if the parental cell has the specific trait *x*, the daughter cell can have the same level of *x*, which we call a faithful division, or have a different level (*y)*, which we refer to as a modification or alteration. This alteration can be the result of genetic or epigenetic changes. θ*(x)* denotes the total fraction of cells with trait *x* having any modification, where *0 ≤* θ*(x) ≤ 1*, and hence *1 −* θ*(x)* denotes the fraction of cells undergoing faithful division. The integral in system (1) takes into account all changes during division from different traits of *y*. *M(y, x)* denotes the probability that given a change, a parental cell with trait *y* will yield a daughter cell with trait *x*. The probability *M(y, x)* satisfies

(3) $M(x,y) \geq 0, \forall x, y \in [1,0]$

(4) $\int_0^1 M(x,y)dx = 1, \forall y \in [1,0]$

Equation (4) indicates that when a modification occurs, it must change to some $x \in [0, 1]$. Equation (1) is a selection/alteration model, which moves through the (*x, n(x, t)*) phase space by both Darwinian evolution and via mutations/changes. As time progresses, different traits become advantageous or disadvantageous, and the overall dynamics are determined by various rates, modification parameters, and the initial distribution of the cells. It is also worth noting that the model (1) can be thought of as the expected value of an individual stochastic model of cells described as above, where the expected value is over the number of cells of type *x*. Hence we can write (1) as:

(5)
$$\frac{\partial n(x,t)}{\partial t} = \left[f(\rho(t))\left(r(x)(1-\theta(x)) - c(x,\alpha)\right) - g(\rho(t))d(x)\right] n(x,t) + \theta(x) f(\rho(t)) \int_0^1 r(y) M(y,x) n(y,t) dy$$

Rescaling the time by the term $\tau = \int_0^t f(\rho(s))ds$, and $\frac{\partial n}{\partial t} = \frac{\partial n}{\partial \tau} f(\rho(t))$, gives

(6) $$\frac{\partial n(x,\tau)}{\partial \tau} = \left[r(x)(1-\theta(x)) - c(x,\alpha) - G(\rho(t))d(x)\right] n(x,\tau) + \theta(x) \int_0^1 r(y) M(y,x) n(y,\tau) dy$$

where

(7) $G(\rho) = \frac{g(\rho)}{f(\rho)}$, where $\lim_{\rho \to \infty} G(\rho) = \infty$

Note that $\frac{d\tau}{dt} = f(\rho(t)) > 0$, so that we have not changed the direction of time. Henceforth, we study (6), with the notational convention that we replace τ by *t*, even though the change of

units is understood as above.

As mentioned, there are two types of modifications that could occur, heredity or temporal changes. θ*(x)* should be thought of as the summation of two separate parameters: θ*1(x)* and θ*2(x)*. When a similar effect is applied to the modification (M) function, the system (6) can be rewritten as:

$$\frac{\partial n(x,t)}{\partial t} = \left[r(x)\left(1 - \sum_{i=1,2} \theta_i(x)\right) - c(x,\alpha) - G\left(\rho(t)\right)d(x) \right] n(x,t) + \sum_{i=1,2} \int_0^1 \theta_i(y) r(y) M_i(y,x) n(y,t) dy \quad (8)$$

Drug-induced death rate: $c(x,\alpha)$

We chose to work with a basic mathematical description of a sigmoid function:

$c = \frac{\beta_1}{1 + \left[\exp(\beta_2)\right]^{\beta_3}}$. In the first case, c_1, the information concerning the death rate based on the trait is measurable for a given dose. Since the dependence on the dose is known to be a sigmoid function, we could propose the following structure: $c_1(\alpha,x) = \frac{\beta_1(x)}{1 + \left[\exp(\beta_2(\alpha))\right]^{\beta_3}}$, where $\beta_1(x)$ *and* $\beta_2(\alpha)$ decrease functions and β_3 is a parameter. Here, we chose the following functions: $\beta_1(x) = \frac{2.4}{\left(1 + x^2\right)}$, $\beta_2(x,\alpha) = -20(\alpha) + 10$ and $\beta_3 = 0.5$=>

$c_1(x,\alpha) = \frac{\left(\frac{2.4}{\left(1 + x^2\right)}\right)}{1 + exp(-20(\alpha) + 10)^{0.5}}$. Plotted in Fig. 2C.

The second case, c_2, is less trivial to define, since it includes less information on the trait. For this type of case, it is known that the survival curves as a function of the dose have sigmoid

shapes, for a given resistance level. Also, it is clear that the IC_{50} values for resistant cells are higher than for sensitive cells. Therefore, this rate of death has a 3D sigmoid function with an 'angle'. This 'angle' is basically described by $\beta_2(x,\alpha)$ in $c_2(\alpha,x) = \frac{\beta_1}{1+\left[\exp(\beta_2(x,\alpha))\right]^{\beta_3}}$, where $\beta_2(x,\alpha)$ is a decreasing function, and β_1 *and* β_3 are parameters. Here, we chose the following functions: $\beta_1(x) = 3$, $\beta_2(x,\alpha) = -20(\alpha) + \frac{50}{3}x$ and $\beta_3 = 0.4$ =>

$c_2(x,\alpha) = \frac{3}{1+exp\left(-20(\alpha)+\frac{50}{3}x\right)^{0.4}}$. Plotted in Fig. 2D.

In practice, the type of data sets, and information included, determine which function to use (c_1 or c_2) and help to estimate β_1, β_2 *and* β_3 based on the available biological data.

Numerical results

Here, we assume two cases of initial conditions for (6):

(i) $IC_1 = 1, \forall x$

(ii) $IC_2 = \begin{cases} 0 & x \le 0.25 \\ 10.3465\left(x-0.25\right)^2 e^{\frac{-(x-1)^2}{0.1}} & x > 0.25 \end{cases}$

In our numerical simulations, we used the following form for the density function:

(9) $G(\rho) = \rho(\rho-2)^2$

and for the alteration kernel:

(10) $M\left(y, \frac{|y-x|}{\varepsilon}\right) = h(y)\exp\left(-\frac{|y-x|}{\varepsilon}\right)^2$

Note that (10), for a fixed value of y, is essentially a Gaussian distribution confined to [0, 1] with mean *y* and variance $\varepsilon^2/2$. As before, *h(y)* is chosen to ensure that (4) for all $y \in [0, 1]$.

All simulations were done using Matlab 2012(b). The functions and parameters that were used in all numerical analyses were: $r(x) = 2/\left(1.1 + 2x^5\right)$, $c(x) = 2/\left(1 + x^2\right)$, $d(x) = 0.05$ unless mentioned otherwise. In case 1 and Fig. 1: $c_{Drug_1}(x) = c(x)$, $c_{Drug_2}(x) = 0.5c_{Drug_1}(x)$, $\varepsilon = 0$, $\theta = 0$, $t_3 = 12.5$ in arbitrary units. Fig. 2 includes two drug-induced death rates as a function of the drug dose. The apoptosis assay was illustrated by the function $c_1(x,\alpha) = \dfrac{1.2c(x)}{1 + exp(-20(\alpha) + 10)^{0.5}}$, and the survival assay was illustrated by the function $c_2(x,\alpha) = \dfrac{3}{1 + exp\left(-20(\alpha) + \dfrac{50}{3}x\right)^{0.4}}$.

Fig. S1 illustrates the population response with these two drug-induced death rates, where $\varepsilon = 0, \theta = 0$ and the treatment time periods in all simulations and figures (prior to, during and post treatment, $t_1 = 2.5, t_2 = 5, t_3 = 12.5$) were chosen with the intention of demonstrating all of these cases and not in order to optimize a treatment protocol. In case 2 and Fig. 3: $\theta = 0.1,\ \varepsilon_0 = 0,\ \varepsilon_1 = 0.01,\ \varepsilon_2 = 1$ and $t_1 = 2.5, t_2 = 5, t_3 = 12.5$. For case 3 and Fig. S2, since the exact biological details about the relation between the genetic and epigenetic networks are not well known, we suggested two mathematical variants that mainly depend on external stress. The first includes two M functions with different time scale:

$$\frac{\partial n(x,t)}{\partial t} = \left[f\left(\rho(t)\right)\left(r(x)\left(1 - \sum_{i=1,2}\theta_i(x)\right) - c(x,\alpha)\right) - g\left(\rho(t)\right)d(x)\right] n(x,t) + \sum_{i=1,2} f\left(\rho(t)\right)\int_0^1 \theta_i(y) r(y) M_i(y,x) n(y,t)\,dy \tag{11}$$

We used $\varepsilon_{low} = 0.01,\ \varepsilon_{high} = 1,\ \ \theta_1 = 0.05,\ \theta_2 = 0.15$ in Figure S2 and Table S3.

The second includes only a single M function with varying ε and θ (Table 3), where these parameters depend on the external stress and time:

$$\frac{\partial n(x,t)}{\partial t} = \left[f\left(\rho(t)\right)\left(r(x)\left(1-\theta(stress,x,t)\right)-c(x,\alpha)\right)-g\left(\rho(t)\right)d(x)\right]n(x,t) + f(\rho(t))\int_0^1 \theta(stress,y,t)r(y)M(y,x)n(y,t)dy \quad (12)$$

Table S1. Variable References

Variable	Range	Biological Interpretation
x	[0, 1]	Resistance level
t	R_+	Time
α	[0, 1]	Drug dose
ε	[0, 1]	Reflects the variance in the 'alteration kernel function', M
n(x, t)	R_+	Concentration of cells with trait *x* at time *t*
r(x)	R_+	Natural division rate of cell with trait *x*
d(x)	R_+	Natural death rate of cell with trait *x*
c(x,α)	R_+	Drug-induced death rate of cell with trait *x* and drug dose α
f(ρ)	R_+	Density dependence within the cell division rate
g(ρ)	R_+	Density dependence within the death rate
θ*(x)*	[0,1]	Proportion of divisions of cells with trait *x* undergoing mutations/epimutations
M(y, x)	[0,1]	The alteration kernel function. Probability that a cell division results in a alteration from state *y* to state *x*, given that an alteration occurs
$\rho(t)$	R_+	Density of cells as a function of time

Table S2. Simulation details of all figures

Figure	Time (a.u)	c(x)	θ	ε	r(x)	d(x)
All figs.		$c(x)=\frac{2}{\left(1+x^2\right)}$	[0,1]	[0,1]	$\frac{2}{\left(1.1+2x^5\right)}$	0.05
Fig. 1	12.5	$c_{Drug_1}(x)=c(x),$ $c_{Drug_2}(x)=0.5c_{Drug_1}(x)$ $c_0(x)=0$ $IC_1=1,\ \forall x$ $IC_2=\begin{cases}0 & x\le 0.25\\ 10.3465\left(x-0.25\right)^2 e^{\frac{-(x-1)^2}{0.1}} & x>0.25\end{cases}$	0	0		
Fig. 2C,E	---	$c_1(x,\alpha)=\frac{1.2c(x)}{1+exp(-20(\alpha)+10)^{0.5}}$	0	0		
Fig. 2D,F	---	$c_2(x,\alpha)=\frac{3}{1+exp\left(-20(\alpha)+\frac{50}{3}x\right)^{0.4}}$	0	0		
Fig. 3A	$t_1=2.5,$ $t_2=5,$ $t_3=12.5$	$c_0(x)$	0.1	$\varepsilon_0=0$ $\varepsilon_1=0.01$ $\varepsilon_2=1$		
Fig. 3B		$c_{Drug_1}(x)$		$\varepsilon_2=1$		
Fig. 3C		$c_{Drug_1}(x)$		$\varepsilon_1=0.01$		
Fig. S1 A-B	$t_1=2.5,$ $t_2=5,$ $t_3=12.5$	$c_{1,Drug_1}(x,\alpha)=\frac{2c(x)}{1+exp(-20(\alpha)+10)^{1}}$ $c_{1,Drug_2}(x,\alpha)=\frac{1.2c(x)}{1+exp(-20(\alpha)+10)^{0.5}}$	0	0		
Fig. S1 C-D		$c_{2,Drug_1}(x,\alpha)=\frac{3}{1+exp\left(-20(\alpha)+\frac{20}{3}x\right)^{0.4}}$ $c_{2,Drug_2}(x,\alpha)=\frac{3}{1+exp\left(-20(\alpha)+\frac{50}{3}x\right)^{0.4}}$				
Fig. S2	$t_1=2.5,$ $t_2=5,$ $t_3=12.5$	$c_1(x)$	$\theta_1=0.05$ $\theta_2=0.15$	$\{0.01,1\}$ Table S3		

Table S3. Simulation details of Figure S2

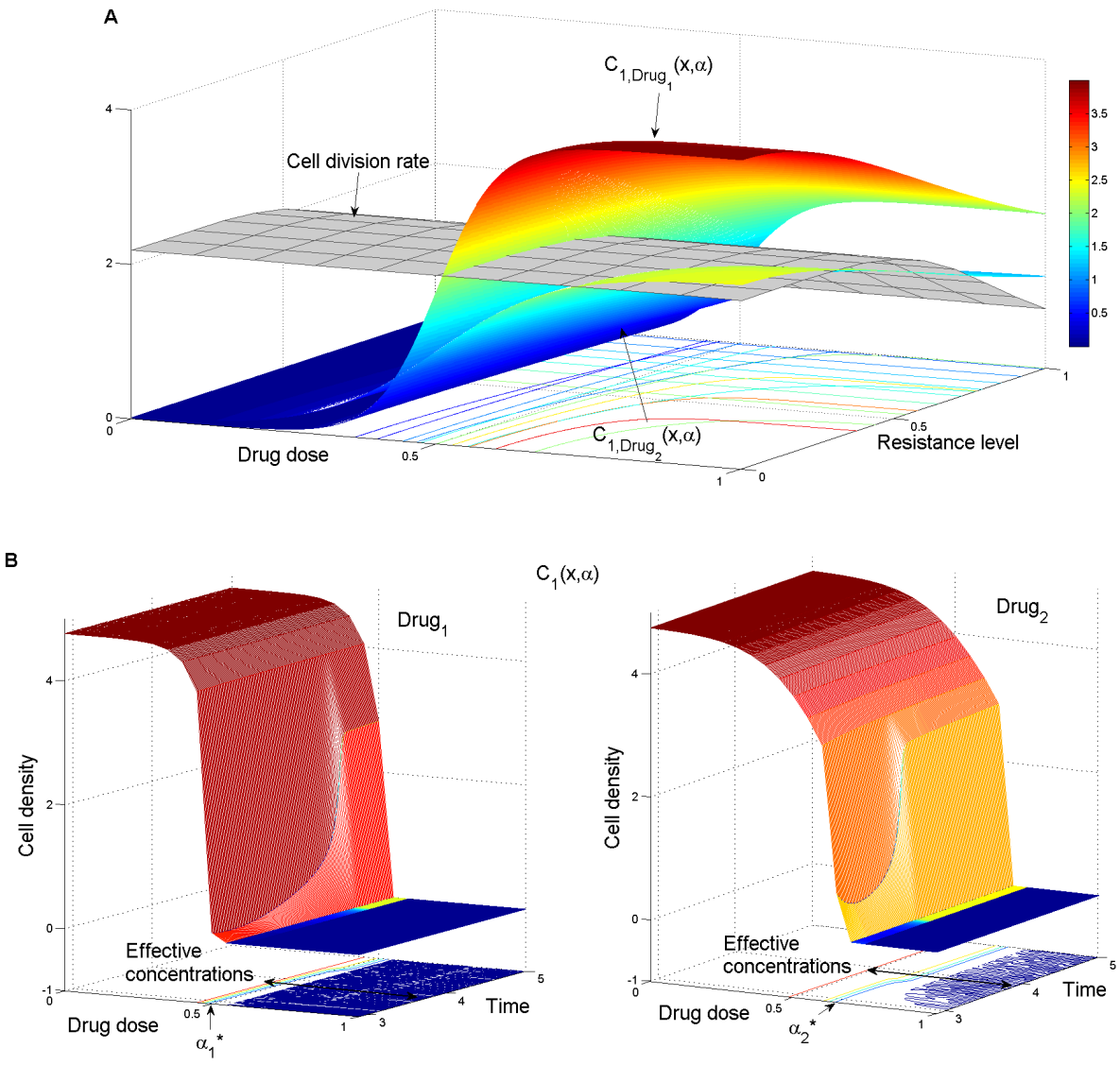
Panel	$t<t_1$	$t_1<t<t_2$	$t_2<t<t_3$
a	$1 >> \varepsilon_1,\ \varepsilon_2 = 0$	$\varepsilon_2 >> \varepsilon_1 > 0$	$1 >> \varepsilon_1,\ \varepsilon_2 = 0$
b	$1 >> \varepsilon_1,\ \varepsilon_2 = 0$	$1 >> \varepsilon_2 = \varepsilon_1 > 0$	$1 >> \varepsilon_1,\ \varepsilon_2 = 0$
c	$\varepsilon_1 >> 0,\ \varepsilon_2 = 0$	$\varepsilon_2 = \varepsilon_1 >> 0$	$\varepsilon_1 >> 0,\ \varepsilon_2 = 0$
d	$\varepsilon_1 >> 0,\ \varepsilon_2 = 0$	$\varepsilon_1 >> \varepsilon_2 > 0$	$\varepsilon_1 >> 0,\ \varepsilon_2 = 0$

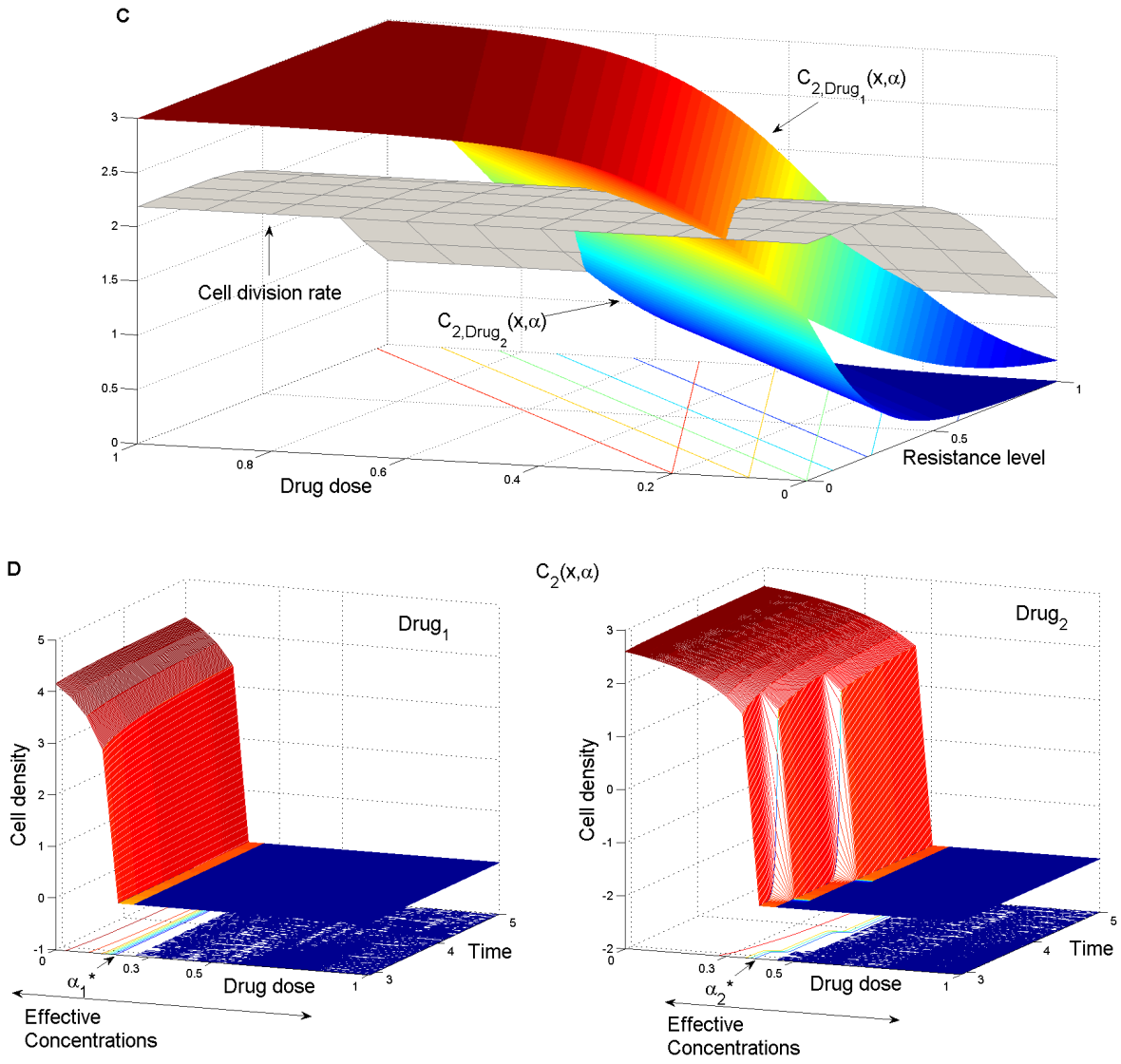
Table S4. Dynamics with variations in the percentage of cells altered

ε	θ	Cell density (ρ)
0.01	0.1	2.931
	1	2.903
0.1	0.1	2.823
	1	0.133

Simulation results at time t=10 in case a and t=100 in case b, where θ vary (θ =0.1, 1) with low/ high alteration rates ($\varepsilon_{low} = 0.01,\ \varepsilon_{high} = 0.1$, respectively). The densities (ρ) at the last point in time are listed. Note that for a longer time period (than listed here) the same qualitative results can be plotted.

Figure S1. Drug efficacy as a function of the dose and resistance level

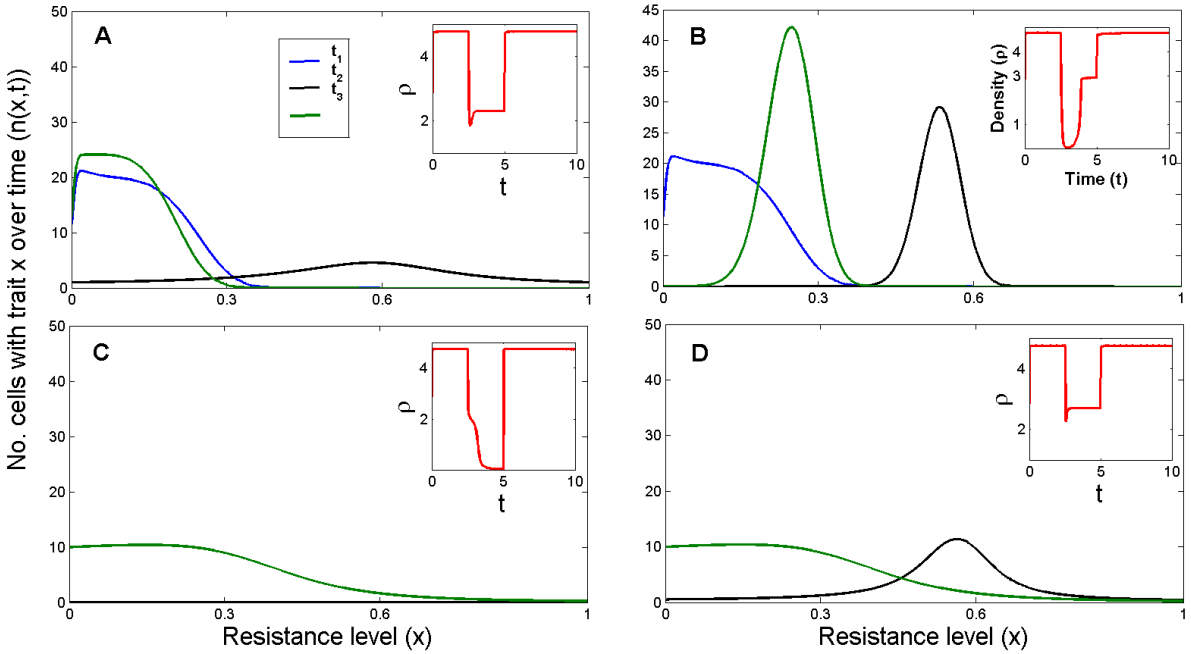




Panels A and C represent the two common types of drug-induced death rates, $c_1(x,\alpha)$ and $c_2(x,\alpha)$ that are plotted in Fig. 2C and D, respectively. Given the exact function of drug-induced death rate, in each panel (A and C) two different treatments were considered (Drug 1 and Drug 2), in a range of drug concentrations, for cells with different resistance levels. Panels B and D illustrate the population response during the treatments, and the density is plotted as a function of the drug dose and time. The shapes of the density curves vary according to the type of treatment and type of drug-induced death rate that were assumed. In all cases, a better

treatment would give lower cell density at a lower drug dose (α*), with a wide range of effective concentrations.

Figure S2. Dynamics with variations in the mutation rates over time with/without drugs



In all of these cases, there is an initial alteration (ε_1) fixed over time, and an additional alteration rate (ε_2) applied only when the drug (*C1*) was applied for a certain period of time (*t1<t<t2*, where *t2<t3*). See Table S2 for the variations in the alteration rates.