Supplemental materials for "Generalized principles of stochasticity can be used to control dynamic heterogeneity"

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1. Introduction

In Liao *et al.* B, we described how stochasticity in the timings of conversion for some phenotypes corresponds to stochasticity in the duration of quiescence. Supplemental section 2 explains that this situation guarantees that curves on metronomograms have an initial slope that is vertical. Given this condition, curves must explore the region $f_S > f_P$ above the central diagonal where reduction of target cell population is achieved.

Cell-cycle-specific phenotypes are only one example of a mechanism by which the time scales for phenotypic fluctuations and the time scales for the cell cycle can be related. In Liao *et al.* B, we provided broader examples of how these time scales could be similar. Supplemental section 3 explains that, in these situations, curves on the metronomogram may often be near the central $f_S = f_P$ diagonal, raising the possibility of beneficial schedules for therapy.

2. Cell-cycle specificity

In Liao *et al.* B, we explained that the variable duration of time that elapses before a quiescent cell enters another phase of the cell cycle can be equal to the variable duration of time that elapses before that cell undergoes a particular phenotypic transition. This relationship holds for a variety of phenotypes that are specific to particular cell-cycle phases. For the example of cell-cycle specific drug-sensitivity, we can demonstrate an interesting result for therapeutic scheduling. If the proliferative state is fully drug-sensitive, then a population of cells is guaranteed to be depleted by a cell-cycle specific drug delivered frequently. This is true even if the cells predominantly remain in a drug-resistant state and only occasionally attempt to proliferate.

In the following subsections we provide biological background information and then develop our argument by mathematically analyzing the shape of curves on the metronomogram.

2.1. Drugs that target specific phases of the cell cycle

Figure S 1(*a*) is a cartoon of the cell-cycle indicating passage through the phases G_1, S, G_2, and M, with rest in the quiescent phase G_0 (in some literature, the label G_1 is used to refer to both G_1 and G_0 as a single state). Classic antimetabolites, such as methotrexate and cytarabine, interfere with DNA synthesis (*S*

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phase) [1]. Anthracyclines, such as doxorubicin, are thought to exert cytotoxic effects by poisoning topoisomerase II, with particular activity in G_2/M [2]. Paclitaxel stabilizes microtubules, disrupting mitosis [3], [4]. In Figure S 1(*a*), *M*-phase is highlighted in orange to denote sensitivity to paclitaxel while other phases of the cell cycle, particularly "quiescence," i.e. G_0, are shaded blue to indicate relative insensitivity. In contrast to cells in culture *in vitro*, some cells in physiological and pathological conditions can rest for weeks in G_0 (counter-clockwise arrow) before occasionally entering the "cycling" phase (clockwise arrow) and dividing once, maybe a couple times. The cell-division time is roughly the time spent waiting in G_0 until entering the comparatively brief proliferative state. The time scale for an initially drug-resistant cell to achieve drug-sensitivity is likewise the time spent waiting in G_0 before entering *M*-phase. In this example, the time scale for switching to the drug-sensitive phenotype is comparable to the cell-division time *because* the drug-sensitive phenotype is associated with the brief replicative phase of the cell cycle. Other phenotypes associated with specific phases of the cell cycle in a similar way will also be described by fluctuations with time scales defined by the cell cycle time.

2.2. Qualitative form of curves on the metronomogram for cell-cycle specific drugs

Many of the mathematical techniques that will be helpful for studying and utilizing dynamic heterogeneity in cancer therapy have been used extensively in mathematical biology to study the special case of cell-cycle-specificity. In this supplemental section we provide a simplified calculation. We refer the interested reader to additional mathematical work modeling the cell cycle [5], [6], and variation in drug-sensitivity with cell-cycle phase [7], [4].

To discuss clinical consequences of cell-cycle specificity using the metronomogram, we express the cartoon in Figure S 1(*a*) quantitatively in terms of a mathematical model for the dynamics of the drug-sensitive population, *S*, and the drug-resistant population, *R*

$$\frac{dS}{dt} = c_R R + (r_S - m_S - c_S)S \tag{1}$$

$$\frac{dR}{dt} = (r_R - m_R - c_R)R + c_S S. \tag{2}$$

As previously described in Liao *et al.* A, a drug-sensitive cell replicates with rate coefficient r_S, dies (mortality) with rate coefficient m_S, and converts to the drug-resistant phenotype with rate coefficient c_S. The drug-resistant population is described by analogous rate coefficients r_R, m_R, and c_R. This model is illustrated in Figure S 2. Figure S 1(*b*) illustrates one parameter set we can use to convert the cartoon in Figure S 1(*a*) into rate coefficients for (1) and (2). The counter-clockwise loop describing G_0 in Figure S 1(*a*) is represented by the counter-clockwise loop associated with phenotypic interconversion passing into and out from the "resting" or nonproliferative drug-resistant state, in Figure S 1(*b*). The brief duration of drug-sensitivity in *M*-phase in Figure S 1(*a*) corresponds to the drug-sensitive state in Figure S 1(*b*), whose rate coefficients for replication and exiting out of the cycling state are comparable to each other and about an order of magnitude faster than the rate coefficient parametrizing the awakening of quiescent cells. The cell cycle can proceed as quickly as ~day *in vitro*, so we model the passage through *S*, G_2, and *M*, as well as the approach toward the less drug-sensitive state G_1, with rate coefficients of r_S and c_S ~1/day. To account for the weeks that a cell can rest in quiescence *in vivo*, we set the rate coefficient c_R describing conversion back to the cycling state at ~1/(2 weeks).

Using these parameter values, we obtain the curve in Figure S 1. At the earliest moments after drug-kill, the surviving population is drug-resistant. This means it is also "resting" in a nonproliferative state. The initial dynamics of the system involve moving cells from the drug-resistant population to the drug-sensitive compartment, rather than the net generation of additional cells. Consequently, the curve in Figure S 1(*c*) rises *vertically* from the origin, $f_S = f_P = 0$, ensuring that at least part of the curve explores

the potentially beneficial region where $f_S > f_P$ (above the diagonal). For the parameter values in this example, the curve then approaches a steady-state drug-sensitive fraction, crossing the diagonal $f_S = f_P$ line at $f_P \sim 0.1$ (arrow), corresponding to $\Delta t \sim 2$ days. In a cell population that predominantly rests in quiescence and occasionally, briefly passes through the drug-sensitive replicative phase for durations measured in days, the intervals of rest between drug kill should also be measured in days.

The initial vertical slope of the curve in Figure S 1(*c*) is the geometric expression of drug-resistance in quiescence. It means that there exists a dosing frequency such that reduction of the target population is possible. In more sophisticated models, where kill rates vary with drug-concentration, the corresponding idea is that reduction of the tumor burden is possible if a sufficiently high average dose over time or "area under the curve" can be administered to quickly kill cells in the drug-sensitive compartment. The cost for a cell to gain drug-resistance is to temporarily surrender its replicative capacity. This places a speed limit on the expansion of the drug-resistant population and ensures that depletion of the drug-sensitive population can, in principle, indirectly deplete the drug-resistant population as well. One can easily embark on mathematical investigations of cell-cycle-specific therapy, knowing ahead of time that a burden-reducing dosing schedule is at least mathematically guaranteed to exist. This feature may make this class of models particularly attractive for theoretical research. The phenomenon of bacterial persisters is usually modeled in a mathematically similar way [8], again, with the eradication of the drug-resistant population at least in principle possible for the same reasons.

3. Time scale calculations

In Liao *et al.* B, we demonstrated, through the use of theoretical and experimental examples, that time scales for phenotypic conversion generated by proteomic fluctuations can often be similar to the time scales for cell division. In this supplemental section, we explain the consequences of this relationship for therapeutic scheduling. In this parameter regime, curves on the metronomogram often straddle the $f_S = f_P$ diagonal. This means that some portion of a given curve may pass through the region $f_S > f_P$ (above the diagonal, reducing target population), and so it is useful to check through quantitative measurement whether (and where) the curve actually crosses the diagonal line to identify optimal scheduling.

To perform our analysis using (1) and (2), we express the similarity between time scales for proteomic fluctuations and cell division by setting the rate coefficients for replication and phenotypic conversion equal, i.e. $r_R = c_R$ and $r_S = c_S$. For simplicity we also set $r_R = r_S$. This is a back-of-the-envelope approximation. As a further simplification, we consider the biological situation in which rate coefficients describing apoptosis and cell clearance are negligible ($m_R = m_S = 0$). The parameters we have chosen correspond to the solid curve in Figure S 3. In biological systems, the rate coefficients will vary. We adjust the rate coefficient, c_R, describing the conversion of drug-resistant to drug-sensitive cells. The solutions to (1) and (2) are plotted for values of $c_R = 0.5$, 0.75, 1.25, and 1.5. The curve with $c_R = 1$ straddles the $f_S = f_P$ diagonal. The dynamics for $c_R = 1.5$ includes a region above the diagonal, and the curve with $c_R = 0.5$ remains fully in the region where $f_S < f_P$, below the diagonal.

When rate coefficients for cell replication and phenotypic switching are of similar magnitude, curves hover near a crucial edge in the metronomogram where a slight change in parameter values can mean the difference between a situation in which tumor reduction can be achieved through judicious dose scheduling and a situation where such success is not possible. Since the curve is exploring a region where either outcome is possible, measurement is necessary to distinguish which outcome is actually realized in a particular biological system. In this perspective, similarities in time scales for the generation of heterogeneity and the generation of population number can manifest as the proximity of curves on metronomograms to the regions where $f_S = f_P$, i.e. regions where curves may cross the $f_S = f_P$ diagonal and potentially explore the beneficial region $f_S > f_P$ above the diagonal.

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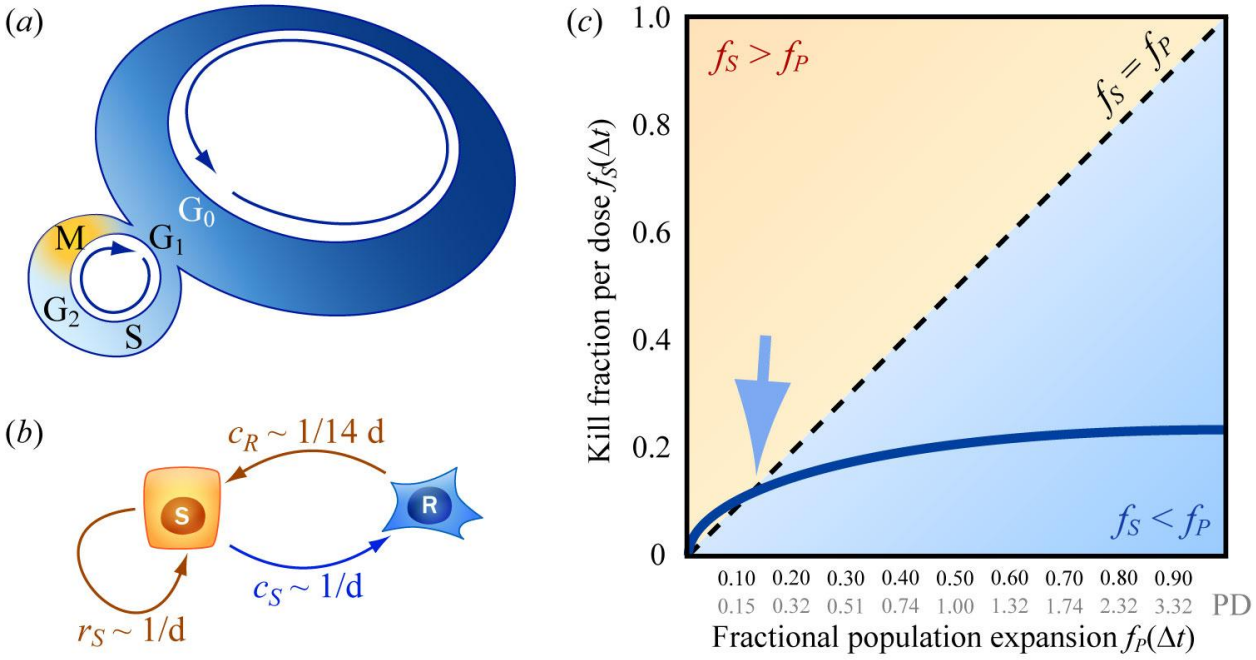

Figure S 1. Cell-cycle specificity. (*a*) Classic chemotherapeutic drugs target *S*, G_2, and *M* phases of the cell cycle. Here, drug-sensitivity in *M* phase is illustrated. (*b*) Two-compartment coarse-grained model. (*c*) Qualitative appearance of cell-cycle-specificity on a metronomogram.

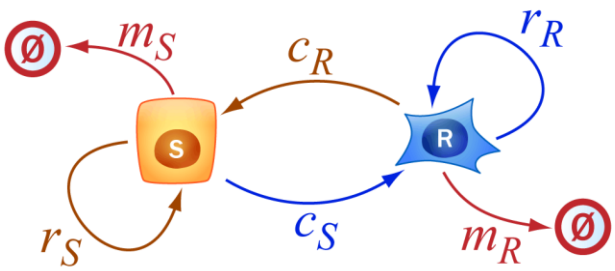

Figure S 2. Minimal Markov model described in Liao *et al.* A, figure 2(*c*). Drug-sensitive cells replicate with rate coefficient r_S, are cleared with rate coefficient m_S, and convert to the drug-resistant phenotypic state with rate coefficient c_S. Analogous rate coefficients describe the dynamics of the drug-resistant cells.

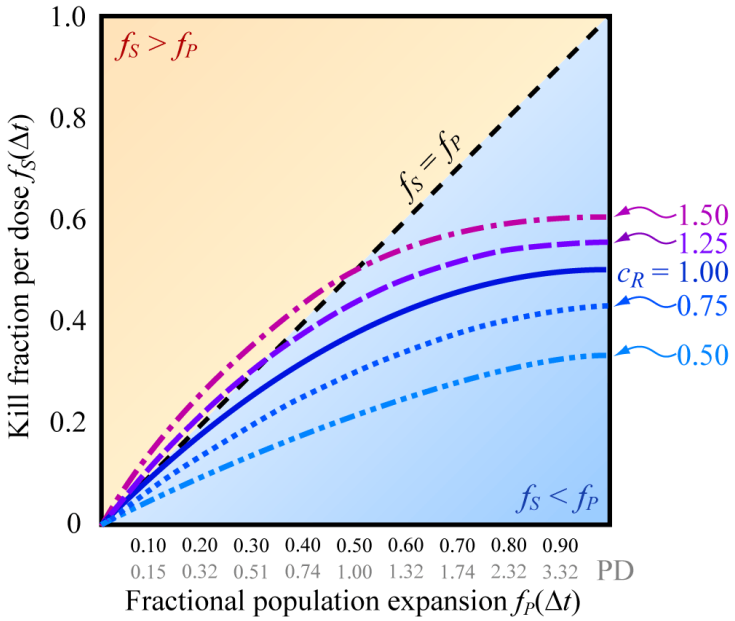

Figure S 3. Curves on a metronomogram straddle the central diagonal, $f_S = f_P$, when the rate coefficients describing phenotypic interconversion and the rate coefficients describing population expansion are of similar magnitude. The solid curve labeled $c_R = 1.00$ corresponds to the solution of the model in (1) and (2), with coefficients $r_R = r_S = c_R = c_S = 1$ and $m_R = m_S = 0$. The curves exploring greater and lesser values of c_R, with all other coefficients unchanged, cover a range of positions that sweeps across the $f_S = f_P$ diagonal line.