Supplemental materials for "Conceptualizing a tool to optimize therapy based on dynamic heterogeneity"

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1. Introduction

In Liao *et al.* A, we developed a conceptual tool for understanding and utilizing dynamic heterogeneity in cancer therapy. The purpose of this supplemental information is to emphasize that an understanding of time scales for these mechanisms has consequences for clinical application of the concepts. In section 2, we explain that the time scale for generating drug-sensitive cells from initially drug-resistant cells imposes a natural bottleneck on the overall total course duration required to deplete the target population. This means that, at very high dosing frequencies, increasing the drug dose frequency does not significantly shorten total course duration. In section 3, we provide an estimate of the order-of-magnitude of the total course duration for the case of cell-cycle-specific drugs. The concepts in Liao *et al.* A provide an opportunity to investigate previous clinical and biological rationales for therapy with high-frequency dosing.

2. Relationship between duration of survival of a population and frequency of events of selection

2.1. An effect of "diminishing returns"

If frequent administration of low dose therapy is effective in reducing the number of resistant cells, will constant administration of low dose therapy be even more effective? Or, stated differently, how does one determine the optimal intervals between administration of a given low dose of drug? We will qualitatively describe an effect of "diminishing returns" in the relationship between the frequency of events of drug kill and the total duration that a population can survive.

At first glance, equation (9) in Liao *et al.* A, *appears* to indicate that the total course duration required to fully deplete a targeted cell population is proportional to the duration Δt of an individual rest period between two instances of cell kill

$$T_{CD} \sim \Delta t \frac{\ln\left(N_{IM}/N_F\right)}{\ln\left(N(0^+)/N(\Delta t^+)\right)}. \qquad (1)$$

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One natural strategy for reducing total course duration is, thus, to apply a given dose of drug at arbitrarily high frequencies, or equivalently, $\Delta t \to 0$. One might imagine that toxic side effects prevent implementation of this strategy. A "low" dose of drug may be tolerated when given every two weeks. However, when administered instead once a week, once each day, each hour, etc., the "individual" doses merge to become one very high dose. Long before the requisite total course duration, therapy must soon be stopped to allow patient recovery. This returns us to the more traditional strategy of maximum-tolerated-dose and "more is better" for which we tried to offer an alternative in Liao *et al.* A. While this toxicity argument is valid, surprisingly, the kinetics of phenotypic stochasticity provides another powerful argument for the lack of additional benefit of continuous administration of drug.

Notably, not even the simple model in Liao *et al.* A, argues for arbitrarily maximizing dosing frequency at all cost. As we show below, the strategy of increasing dosing frequency becomes marginally unproductive at very high frequencies. This effect of "diminishing returns" is surprising given that this modeling does not explicitly describe toxicity for the patient. Instead, this result is a consequence of the finite timescale that characterizes the acquisition of drug-sensitivity in initially drug-resistant cells. If two doses of drug are spaced too closely, the drug-resistant population surviving the first dose does not have enough time to generate drug-sensitive cells for the second dose to kill. This view is in contrast to the picture underlying the pharmacologic law of cell kill.

In this example, the drug-sensitive and drug-resistant cells have the same proliferation rate $r_S = r_R = r$, and we set their death rates to zero. Using (2), one can show that the total course duration for this situation is

$$T_{CD} = \frac{\tau}{r} \ln\left(\frac{N_{IM}}{N_F}\right) \tag{2}$$

a multiplicative factor of a dimensionless time τ defined at positions above the $f_S = f_P$ diagonal in Figure S 1(*a*). Positions that lie on the same dotted or dashed curve share the same value of τ. All points on a curve with a common value of τ have the same total course duration T_{CD}. The solid blue curve is an example of a solution to (1) and (2) from Liao et al. Moving from the right toward the left, the curve crosses the diagonal ($\tau \to \infty$), a dotted curve ($\tau = 4.6$), and then a dashed curve ($\tau = 2.2$). Now, the curve bends downward to make an eventual connection with the origin at (f_S, f_P) = (0,0). The curve hugs the dashes corresponding to $\tau = 2.2$. There are values of τ less than about 2.2 that the curve will never cross. Thus, arbitrarily large increases in dosing frequency do not afford corresponding, arbitrarily large improvements in total course duration.

Because the solid curve must bend downward to connect to the origin (0,0), there is a minimum value below which the total course duration T_{CD} cannot fall, regardless of the dosing frequency. This "bend" in the curve is a geometric manifestation of the finite time scale with which drug-resistant cells acquire drug-sensitivity.

Figure S 1(*b*) illustrates the same effect in another way. The population described in Figure S 1(*a*) is plotted as a function of time for 4 dosing frequencies, $1/\Delta t$ = 2, 4, 8, and 16. Here, time is measured in units of $1/r$. Each time the dosing frequency is doubled, the total duration required to achieve a log of depletion (here from 10^6 to 10^5 cells) decreases. At high frequencies, however, the sawtooth curves begin to pile up on each other, and, the total course duration is never shorter than about 5 units of time. As the teeth of the sawtooth plots become spaced at tighter pitches in time, their heights also become shorter. The overall population cannot be depleted faster than the rate at which drug-sensitive cells emerge from the drug-resistant bulk. This effect is similar to an effect of diminishing returns in bailing water from a ship. Water can only be bailed out of a ship as fast as it leaks in. When operated at faster rates, pumps expel air.

In this analysis, we investigated the relationship between the frequency of events that disrupt homeostatic heterogeneity and the total duration that a population can survive. The relationship involves a phenomenon of diminishing returns: arbitrarily shortening the time interval between successive selective events does *not* arbitrarily shorten the total duration the population survives. This apparent contradiction with (2) can be resolved by recognizing that factors in this equation include, not only Δt, but also the number of logs, $\ln(N(0^+)/N(\Delta t^+))$, by which the target population is reduced during each event of selection (cell kill or clearance). Since this factor is itself a function of Δt, the relationship between total course duration T_{CD} and interdose period Δt is not necessarily linear.

In geometric terms, arbitrarily shortening the time period Δt between events that disrupt homeostatic heterogeneity fails to provide arbitrarily short total course durations because the curve in the metronomogram (Figure S 1(*a*)) fails to cross all lines of equal τ as $f_P \to 0$. Another strategy that can alter the total duration a population survives is to adjust the kinetics of the restoration of homeostatic heterogeneity in the intervals of rest between successive events of selection. As we discuss in the accompanying manuscript, this strategy can push curves on metronomograms to cross lines of constant τ. Moving the curve to a smaller value of τ shortens the total course duration as dictated by (2).

3. Total course duration required to deplete the target cell population when administering a cell-cycle-specific drug

The metronomogram allows us to determine dose frequencies that will reduce tumor size. As the tumor recedes to sizes too small to easily detect, how can we determine the time it will take to completely "drain" the population of drug-resistant cells? To address this question, we will consider an example of cell-cycle-specificity. Classic chemotherapeutic drugs target processes specific to particular phases of the cell cycle. For example, DHFR targets DNA synthesis (*S* phase) while paclitaxel stabilizes microtubules, disrupting *M* phase. Cells are particularly sensitive to these drugs during their proliferative phase and relatively drug-resistant while "resting" in quiescence.

3.1. Fermi calculation of total course duration

This situation allows us to obtain one estimate for a relevant order of magnitude for total course duration. We proceed by comparing two time periods of a tumor burden's history. In the first time period, we consider the expansion of the tumor preceding detection and treatment. To simplify the description of the cell cycle, assume that upon entering the drug-sensitive, proliferative state, a cell is deterministically committed to replicating once, with both daughters then returned immediately to the drug-resistant, quiescent state. Suppose that cancer cell populations expand exponentially before initial treatment. Cells reside in the quiescent state for perhaps a couple weeks at a time, often longer or shorter, and then replicate during brief intervals of drug-sensitivity. We are describing a Poisson process in which exponentially distributed waiting periods are punctuated by occasional additions, one cell at a time, to the population. The use of Poissonian variation to model the time until re-entry into the proliferative phase of the cell cycle is discussed in Liao *et al.* B and [1].

Now, in the second time period of the tumor burden's history, treatment with a cell-cycle-specific agent begins. For simplicity, we consider the limiting case of frequent drug kill. Consider the application of a drug that targets, for example, *M*-phase, while leaving other phases, such as G_0 unaltered. A population of G_0 cells will then continue to display a distribution of "idle" waiting times with average duration, as before, of a couple weeks. During frequent administration of drug, however, a cell that emerges from G_0, will no longer be committed to replicating. Instead, it will be killed because it has entered a drug-sensitive state. Just as in our description of drug-free expansion, we are again describing a Poisson process, i.e. with exponentially distributed waiting periods, but in contrast, these waiting times are now punctuated by occasional *subtractions*, not additions, one cell at a time, from the population.

The initial expansion of the cancer cell population and the subsequent depletion of the population during therapy are both described here as Poisson processes with equal single-cell rate coefficients. The final population size of the expansion phase equals the initial population size of the treatment phase. The durations of the two phases should thus be identical. A one-size-fits-all value of T_{CD} cannot be prescribed because estimated durations of initial tumor expansion preceding diagnosis vary widely. However, at the level of an estimate of orders-of-magnitude, cancers can expand for years before treatment begins [2]. Thus, the total course duration necessary for eliminating a cancer cell population completely may also measure in the neighborhood of years.

We are not aware of a mathematical argument like the preceding discussion that we can use to estimate the total course durations in the more general case where therapeutic agents are not necessarily cell-cycle specific. However, we can develop intuition at an informal level as follows. Suppose, as discussed above, that rate coefficients for proliferation and phenotypic interconversion are similar to each other, measuring in days or weeks. Then, finite rate coefficients (i) describing expansion before treatment and (ii) describing the dynamics of the population during therapeutic treatment are measured in characteristic time scales of days or weeks. The tumor burden at the end of untreated expansion is the same as the tumor burden at the beginning of treatment. We can choose the initial population size from which the tumor initially expanded as the population size to which we aim to therapeutically reduce the tumor burden. In this case, the initial expansion of the tumor preceding treatment and its subsequent reduction through therapy are described by similar rate coefficients and ratios of cell population sizes. This leads to a concern that the durations for these two phases (expansion and therapeutic depletion) may be similar to each other, again measuring in years.

4. Previous rationales for high-frequency dosing schedules

High-frequency dosing schedules have been proposed and reported to provide clinical benefit for a variety of applications. In addition to the rationale based on dynamic heterogeneity and explained using the metronomogram presented in Liao *et al.*A, previous authors have offered the rationales listed in Table S1. These perspectives are not necessarily exclusive or alternative to each other. For example, the metronomogram described in the present work could be applied to optimally drain endothelial cells in the tumor stroma. The supplementary material provided in this appendix illustrates how the conceptual basis of phenotypic stochasticity can provide a counterintuitive solution to the questions of drug dose frequency. Likewise, using the concepts from these fundamental principles, the total course duration needed to obtain a complete eradication of the tumor can be estimated. The application of the quantitative concepts in Liao *et al.* A to previous biological rationales for high-frequency dosing schedules provides one direction for advancing metronomic therapy. In the accompanying manuscript, we describe how these fundamental principles can be generalized to a plethora of situations and phenotypes.

Table S 1. High dosing frequency: Rationales

Biological basis	Rationale	References
Present work:	Stochastic phenotypic interconversion: Kill cells soon after they gain drug-sensitivity, before repatriation to the drug-resistant population.	[3], [4], [5], [6]
Empirical clinical knowledge	Treatment of leukemia that has developed empirically includes a variety of stages including an initial period of induction and a long period (years) of low-dose, high-frequency maintenance therapy	[7]
Anti-angiogenesis	Prevent vascular repair. Endothelial cells respond to drug at low doses that do not kill epithelial carcinoma cells.	[8], [9], [10], [11], [12], [13], [14], [15]
Stromal-directed	Immune modulatory (anti-inflammatory and/or immunostimulatory). Target stromal fibroblasts.	[13], [14]
Cell-cycle specificity	Kill cells soon after they enter the proliferative state, before they return to the quiescent state.	[16], [17], [18]
Kinetic resistance	As tumor shrinks, the proliferative fraction near the periphery increases relative to the total tumor volume. Accelerate dosing frequency to keep pace with increasing effective proliferation rate.	[19]
Adaptive therapy	Computational result from a model in which the goal is to allow a drug-sensitive subpopulation to persist. The drug-sensitive subpopulation can then ecologically control a drug-resistant subpopulation.	[20], [13]

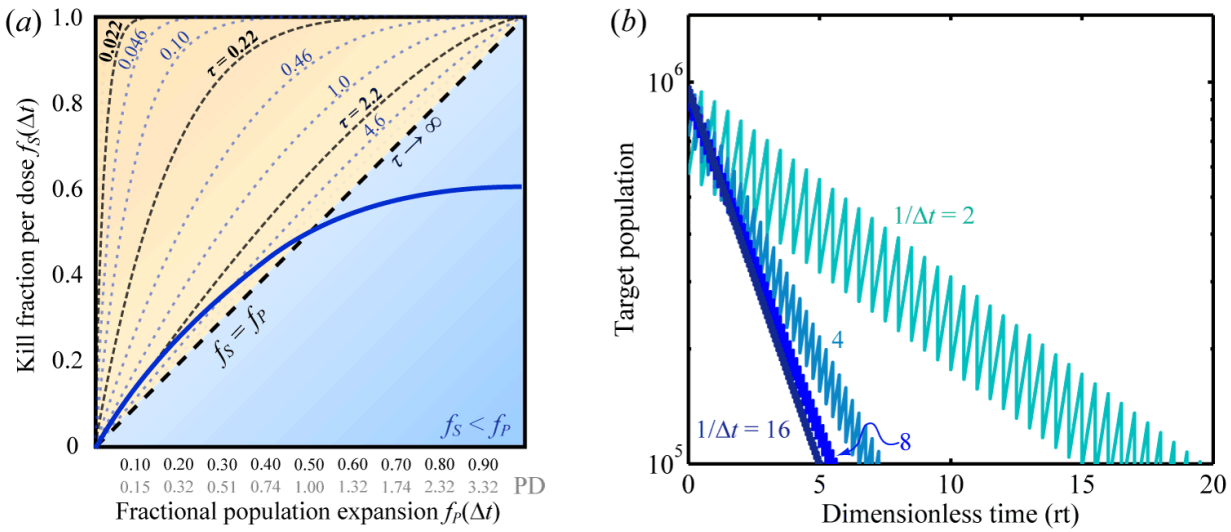

Figure S 1. Diminishing marginal returns in improvement in total course duration provided by increasing dosing frequency. (*a*) Positions sharing the same value of dimensionless time τ are plotted with dashed and dotted curves. (*b*) Total course duration required to reduce a cell population from 10^6 cells to 10^5 cells for dosing frequencies of $1/\Delta t$ = 2, 4, 8, and 16. Eventually, doubling the frequency of dosing does not significantly improve total course duration.

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