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# MATHEMATICAL MODELING OF CYCLIC TREATMENTS OF CHRONIC MYELOID LEUKEMIA

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Abstract. Cyclic treatment strategies in Chronic Myeloid Leukemia (CML) are characterized by alternating applications of two (or more) different drugs, given one at a time. One of the main causes for treatment failure in CML is the generation of drug resistance by mutations of cancerous cells. We use mathematical methods to develop general guidelines on optimal cyclic treatment scheduling, with the aim of minimizing the resistance generation. We define a condition on the drugs' potencies which allows for a relatively successful application of cyclic therapies. We find that the best strategy is to start with the stronger drug, but use longer cycle durations for the weaker drug. We further investigate the situation where a degree of cross-resistance is present, such that certain mutations cause cells to become resistant to both drugs simultaneously.

1. **Introduction.** Chronic Myeloid Leukemia (CML) is a cancer of the hematopoietic system that is initiated and driven by the product of the BCR-ABL fusion gene [17]. Small molecule inhibitors are a new class of agents that act by specifically inhibiting a certain enzyme that is characteristic of a particular cancer cell, rather than non-specifically inhibiting and killing all rapidly dividing cells. In the context of CML, small molecules specifically target the BCR-ABL gene product, and provide a successful treatment approach which can lead to a reduction of BCR-ABL+ cells below detectable levels, at least during the early stages of the disease. The drug Imatinib has been mostly used in this respect [37, 14]. It is the first member of the class of small molecule inhibitors; while it has some side-effects, in general it is reasonably well-tolerated [39, 2], compared to traditional chemotherapeutic agents, and it has not been found mutagenic [40].

As the disease advances, however, the chances of treatment failure rise due to the presence of drug resistance. In general, several causes of drug resistance in cancers have been identified. These include (i) genetic changes/variability, (ii) increased expression of target proteins, (iii) failure of the drugs to enter the target cell and/or drug ejection, (iv) failure of the drugs to reach the target cells. In this paper we will focus on the genetic cause of resistance which presents a significant problem for CML. It has been found that treatment failure often occurs because of the presence of mutants that are generated mostly through somatic point mutations [44] (in this paper we will not be concerned with germ-line mutations).

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Drug resistance can potentially be overcome by using multiple drugs, as long as a mutation that confers resistance against one drug does not confer resistance against any of the other drugs in use. In addition to Imatinib, the drugs Dasatinib and Nilotinib are alternative inhibitors of the BCR-ABL gene product [8, 48]. Even though these tyrosine kinase inhibitors address the same molecular target, they differ significantly in their activity spectra. In other words, a point mutation in the BCR-ABL gene may confer resistance, say, to Imatinib, but not to Dasatinib and Nilotinib [8, 38]. Approximately 50 mutations have been found that confer resistance against only one or two of the three drugs and not against the others (the degree of resistance to various combinations of these drugs depends on the drug dosage and has been studied in detail in [8, 38]). Unfortunately, the three inhibitors Imatinib, Dasatinib, and Nilotinib exhibit a degree of cross-resistance: one mutation, namely, T315I, has been identified which confers resistance against all three of those drugs [13, 41].

One of the current treatment strategies called a "cyclic treatment strategy" involves a sequential application of several (usually, two) different drugs. In this paper we will formulate a mathematical model that allows for a systematic study of cyclic drug treatments. We will use computational tools to optimize treatment regimens with the goal of minimizing the generation of resistant mutants, while keeping the cumulative drug doses below a certain level. Mathematical modeling of cancer therapy goes back to Goldie and Coldman [23, 27, 25, 10, 26] and Day [12]. This work had a widespread impact on the design of new chemotherapy regimens for testing in clinical trials in the late 70s and the 80s [35]. Stochastic models applied to generation of drug resistance and treatment optimization have been used in scheduling chemotherapy treatments. In particular, wet lab oncologists undertook the task of testing a specific, purely theoretical rule proposed by [12, 35]: the "worst drug rule". The gist of this rule is (a) "use more of the worst drug" and (b) "use the worst drug early". In this paper, we study a question similar to that raised by [12]: what is the optimal timing of treatment in the context of cyclic therapy? Our analysis employs a wider variety of methods and is more general and systematic. In particular, instead of testing a subset of 16 treatment strategies, as was done in [12], we test all cyclic treatment strategies to find the optimal cycle duration and the drug sequence. In addition, we extend the studies to drugs with cross-resistance.

The last four decades are characterized by significant developments in the field of anticancer therapy modeling. Different approaches to modeling and optimization of drug treatments in cancer have been proposed. [28] have introduced a model which treats the evolution of resistance as a dynamic process; they focus on gene amplification as an important mechanism leading to drug resistance, see also [3, 30]. This line of reasoning is continued in a number of papers [31, 47] in which optimal control problem resulting from protocol optimization is discussed; for a review of the optimal control theory in chemotherapy see [45]. Models for tumor growth incorporating age-structured cell cycle dynamics, in application to chemotherapy scheduling, have been developed by [18, 19]. Mechanistic mathematical models created to improve the design of chemotherapy regimes are reviewed in [21]. Another approach to scheduling of anticancer therapy in the presence of drug resistance is presented by [22] who suggest that the main goal of chemotherapy in the presence of drug resistant clones is to enforce a stable tumor burden by permitting a significant population of sensitive cells to survive in order to suppress proliferation of the less fit but resistant subpopulations. For a more complete review of recent developments in

the field of anticancer therapy modeling, see [43, 9, 6, 5, 46, 15]. As our techniques, we use stochastic and deterministic modeling of cancer growth and treatment, and concentrate on an intermediate, cellular scale of modeling. For the existing literature on this type of modeling, see e.g. [49].

## 2. The general methodology: Stochastic modeling of CML drug treatments.

2.1. **The master equations.** We start by introducing the mathematical formalism which describes a convenient way to reason about drug treatments in CML. Here we will concentrate on the case of two-drug treatments. Other applications of this method can be found in [34, 32]. The general methodology goes back to early works of [23, 25, 24, 10, 11, 26]. Let us suppose that a binary number $s$ takes values 00, 01, 10 and 11, and denote by $i_s$ the number of cells of resistance class $s$. There are four separate resistance classes: $s = 00$, fully susceptible; $s = 10$, resistant to drug 1 and susceptible to drug 2; $s = 01$, resistant to drug 2 and susceptible to drug 1; and $s = 11$, resistant to both drugs (or, fully-resistant).

Note that the existence of the four separate resistance classes is not meant to describe the full extent of the tumor's heterogeneity. It is well-known that tumor cells contain a large number of mutations (and other genetic alterations) which leads to a significant phenotypical heterogeneity. However here we classify all the tumor cells according to their resistance properties (and not according to any other characteristics). Therefore, in the presence of two drugs, there are four different classes of cancer cells. Within each class, the populations are strongly heterogeneous, and therefore the growth and mutation characteristics of those classes should be understood as "average".

Let $\varphi_{i_{00},\dots,i_{11}}(t)$ denote the probability that at time $t$ there are $i_s$ cells of resistance class $s$, for all classes $s$. Suppose that cancerous cells divide with the average rate $l_s$, and die with average rate $D_s$. Coefficients can depend on the treatment protocol. In particular, the death rate of cells, $D_s$, is comprised of the "natural" rate of cell death in an untreated tumor and the action of the drug(s), if any, upon the cells. Therefore, in general, this quantity is a function of time.

We further assume that divisions can lead to the generation of resistant mutants. By $u_1$ we denote the rate at which mutations resistant to drug 1 (and not to drug 2) are generated. Similarly, the rate $u_2$ describes the generation of mutations conferring resistance to drug 2 only. The possibility of mutations conferring resistance to both drugs simultaneously (the so-called cross-resistance, see [33]) is captured by the rate $u_{12}$; in the absence of cross-resistance, $u_{12} = 0$.

We assume that the timing of separate kinetic events is exponentially distributed, and construct a linear birth-death process (for a review of biological applications of the theory of birth-death processes see [36]). Its Kolmogorov forward equation can be written:

$$\dot{\varphi}_{i_{00},i_{10},i_{01},i_{11}} = \Big[\varphi_{i_{00}-1,i_{10},i_{01},i_{11}} l_{00}(i_{00}-1)(1-u_1-u_2-u_{12}) +$$
$$\varphi_{i_{00},i_{10}-1,i_{01},i_{11}} l_{10}(i_{10}-1)(1-u_2-u_{12}) +$$
$$\varphi_{i_{00},i_{10},i_{01}-1,i_{11}} l_{01}(i_{01}-1)(1-u_1-u_{12}) + \varphi_{i_{00},i_{10},i_{01},i_{11}-1} l_{10}(i_{11}-1)\Big] +$$

$$\Big[i_{00}(u_1\varphi_{i_{00},i_{10}-1,i_{01},i_{11}}+u_2\varphi_{i_{00},i_{10},i_{01}-1,i_{11}}+u_{12}\varphi_{i_{00},i_{10},i_{01},i_{11}-1})$$
$$+i_{10}(u_2\varphi_{i_{00},i_{10},i_{01},i_{11}-1}+u_{12}\varphi_{i_{00},i_{10},i_{01},i_{11}-1})$$
$$+i_{01}(u_1\varphi_{i_{00},i_{10},i_{01},i_{11}-1}+u_{12}\varphi_{i_{00},i_{10},i_{01},i_{11}-1})\Big]+$$
$$\Big[D_{00}\varphi_{i_{00}+1,i_{10},i_{01},i_{11}}+D_{10}\varphi_{i_{00},i_{10}+1,i_{01},i_{11}}+$$
$$D_{01}\varphi_{i_{00},i_{10},i_{01}+1,i_{11}}+D_{11}\varphi_{i_{00},i_{10},i_{01},i_{11}+1}\Big]- \tag{1}$$
$$\varphi_{i_{00},i_{10},i_{01},i_{11}}[i_{00}(l_{00}+D_{00})+i_{10}(l_{10}+D_{10})+i_{01}(l_{01}+D_{01})+i_{11}(l_{11}+D_{11})].$$

In this master equation, the first term in square brackets on the right hand side comprises all the processes of faithful cell division, the second term in square brackets includes all the mutation events, the third one represents all the cell death events, and the fourth term corresponds to no change in the system's state.

There are two ways in which we will use the above master equation: deterministic and stochastic.

2.2. **Deterministic approach.** From the master equation, information about all the moments can be extracted. In particular, the equations for the mean numbers of cells in each class can be written. They read:

$$\dot{x}_{00} = [l_{00}(1-u_1-u_2-u_{12})-D_{00}]x_{00}, \tag{2}$$
$$\dot{x}_{10} = [l_{10}(1-u_2-u_{12})-D_{10}]x_{10}+l_{00}u_1x_{00}, \tag{3}$$
$$\dot{x}_{01} = [l_{01}(1-u_1-u_{12})-D_{01}]x_{01}+l_{00}u_2x_{00}, \tag{4}$$
$$\dot{x}_{11} = [l_{11}-D_{11}]x_{11}+l_{00}u_1 2x_{00}+l_{10}u_2x_{10}+l_{01}u_1x_{01}. \tag{5}$$

These equations can be obtained directly from the master equation; for example, the first equation is nothing but equation (1) multiplied by $i_{00}$ and summed over all the indices. The initial conditions can be written as

$$x_{00}(0)=M_0, \quad x_{10}(0)=x_{01}(0)=x_{11}(0)=0; \tag{6}$$

in other words, we assume that at time zero, there are $M_0$ fully-susceptible cells, and no mutants initially present. The deterministic equations obtained in this way can help one reason about the expected dynamic of the colony growth and resistance generation. However, they cannot address questions of the probability of treatment success. If we are interested in the probability of treatment success (that is, if we want to quantify the likelihood of a successful treatment outcome), we need to use the stochastic approach, which is described next.

2.3. **Stochastic approach.** An alternative approach to studying the system of resistance classes is to calculate directly the probability of treatment success. This is done by using the probability generating function, defined in a usual way [20]:

$$\Psi(\vec{\xi};t)=\sum_{s=0}^{n}\varphi_{i_{00},i_{10},i_{10},i_{11}}(t)\xi_{00}^{i_{00}}\xi_{10}^{i_{10}}\xi_{01}^{i_{01}}\xi_{11}^{i_{11}},$$

with $\vec{\xi}=(\xi_{00},\xi_{10},\xi_{01},\xi_{11})$. The above transformation maps an infinite number of unknown functions onto one function of a continuous variable, $\Psi(\vec{\xi};t)$. This function satisfies a linear hyperbolic partial differential equation (see e.g. [4]), which can be

solved by the method of characteristics [32]. The equations for the characteristics are as follows:

$$\begin{aligned}
\dot{\xi}_{00} &= l_{00}(1-u_1-u_2-u_{12})\xi_{00}^2 + (l_{00}(u_1\xi_{10}+u_2\xi_{01}+u_{12}\xi_{11}) \\
&- (l_{00}+D_{00}))\xi_{00} + D_{00}, \qquad (7)\\
\dot{\xi}_{10} &= l_{10}(1-u_2-u_{12})\xi_{10}^2 + (l_{10}(u_2+u_{12})\xi_{11} - (l_{10}+D_{10}))\xi_{10} + D_{10}, \qquad (8)\\
\dot{\xi}_{01} &= l_{01}(1-u_1-u_{12})\xi_{01}^2 + (l_{01}(u_1+u_{12})\xi_{11} - (l_{01}+D_{01}))\xi_{01} + D_{01}, \qquad (9)\\
\dot{\xi}_{11} &= l_{11}\xi_{11}^2 - (l_{11}+D_{11})\xi_{11} + D_{11}, \qquad (10)
\end{aligned}$$

where the time-dependence of the coefficients is implicit. Solutions of this system with the initial conditions

$$\xi_{00} = \xi_{10} = \xi_{01} = \xi_{11} = 0 \qquad (11)$$

are used to calculate the probability of treatment success. We note that the quantity $\varphi_{0,0,0,0}(t) = \Psi(\vec{0};t)$ is the probability of having zero cells of all types at time $t$. This probability includes the scenario where the colony goes extinct spontaneously, as well as the scenario where the tumor grows and is subsequently treated successfully. The latter process has the meaning of the probability of treatment success. In other words, we have,

$$\varphi_{0,0,0,0}(t) = (d/l)^{M_0} + (1-(d/l)^{M_0})P_{success}(t),$$

where $M_0$ denotes the initial number of wild-type cells. Thus we have

$$P_{success}(t) = \frac{\xi_{00}^{M_0}(t) - (d/l)^{M_0}}{1-(d/l)^{M_0}}. \qquad (12)$$

In particular, one can study the limiting value of the probability of treatment success,

$$\lim_{t\to\infty} P_{success}(t), \qquad (13)$$

which corresponds to long-term treatment strategies where the drugs are used long enough for all the susceptible types to be eliminated.

## 3. Mutually strong drugs and treatment optimization.

### 3.1. Modeling cyclic treatment strategies.

Cyclic drug treatments are assumed to proceed as follows, see figure 2(a). Treatment starts at time $t_*$. Drug 1 is applied for a time-duration of $\Delta t_1$. Then the drug is discontinued and replaced by drug 2. After time-duration $\Delta t_2$, drug 2 is in turn replaced by drug 1. The total treatment duration is denoted by $T_{treat}$ and it consists of $2\mathcal{N}$ cycles of treatment (here the word "cycle" refers to a one-drug treatment with drug 1 or 2).

Mathematically, each treatment protocol corresponds to specific values of the death rates, $D_s$, at different moments of time:

$$D_s = d_s + h_s(t),$$

where the coefficients $d_s$ are natural death rates of the cancer cells, and $h_s(t)$ are the drug-induced cell death rates. The functions $h_s(t)$ depend on the particular treatment strategy used. As different drugs are applied, the "strength" of each drug, which depends on the concentration of the drug in the patient's blood, changes as some smooth function of time. The exact shape of these functions, and therefore, the shape of $h_s(t)$, depends not only on the treatment strategy (that is, whether drugs are applied in combination, or cyclically), but also on the way the drugs are administered, and on how quickly they are absorbed. For example, it can be

assumed that $h_s(t)$ for a susceptible class reaches a maximum sometime after the drug is taken, and decays until the next administration of the drug. However, in this paper we simplify this picture by assuming that the functions $h_s(t)$ are piecewise constant. They are assumed to have a constant nontrivial value for all the susceptible classes as long as the patient is treated with a given drug, and they become zero after the drug is discontinued. For the effects of pharmacokinetics on the dynamics of treatment see [19].

We proceed to describe a cyclic two-drug treatment protocol. As described above, for $0 < t < t_*$, we have $D_s = d_s$; normally, $d_s < l_s$, that is, the cancer is assumed to grow stochastically before treatment. At time $t_*$, the first drug is applied for a length of time, $\Delta t_1$. During this time, we have

$$D_{00} = d_{00} + h_1, \quad D_{10} = d_{10}, \quad D_{01} = d_{01} + h_1, \quad D_{11} = d_{11}, \tag{14}$$

where $h_1$ is the drug-induced death rate for drug one; see figure 2(b), on the left. After time $t_* + \Delta t_1$, drug 2 is applied for the duration $\Delta t_2$, resulting in

$$D_{00} = d_{00} + h_2, \quad D_{10} = d_{10} + h_2, \quad D_{01} = d_{01}, \quad D_{11} = d_{11}, \tag{15}$$

for $t_* + \Delta t_1 < t < t_* + \Delta t_1 + \Delta t_2$ (figure 2(b) on the right). Here, $h_2$ denotes the drug-induced death rate of drug two. After that, treatment is again switched to drug 1 for duration $\Delta t_1$, and so on, for a total time duration $T_{treat}$, with a total of $2\mathcal{N}$ cycles.

In this paper we assume that the division rates, $l_s$, and the death rates, $d_s$, of cells are time-independent. We will further assume that some of the coefficients in equations (7-10) are the same, namely, that $l_s = l$ and $d_s = d$, and denote

$$\gamma = l - d.$$

The time of the start of treatment, $t_*$, can be related to the initial colony size, $N$, by the deterministic relationship, $N = M_0 e^{(l-d)t_*}$.

In this study we are not making any assumptions on the timing of resistant mutation generation. New resistant mutants can stochastically arise before or during treatment (in fact, we have previously argued that a large percentage of resistance mutants are generated before treatment starts, [34]). Therefore, in our calculations of the probability of treatment success we will include both the pre-treatment regime and the during-treatment regime.

3.2. **Modeling different types of drugs.** We will discuss two characteristics of drugs, their potency and their activity spectra. The conventional measure of *drug potency* used *in vitro* is the drug concentration needed to achieve a certain log kill. From the biochemical point of view this is related to the affinity, or on-rates, of the inhibitors. The mathematical definition uses the following way to quantify the potency. For the purposes of this study we assume that one drug is more potent than another if, under the given dosages, it results in a higher drug-induced death rate. The latter is measured as the (exponential) rate of the decay of a colony of target cells exposed to the given dose of the drug. Therefore, in what follows we will assume that higher potencies are correlated with higher values of $h_s$ for susceptible mutant classes.

The *activity spectra* of the drugs are described as follows. Drugs with broader activity spectra are characterized by a smaller number of mutations which confer resistance to such drugs. In other words, these drugs are active against a larger number of mutants (and fail against a smaller number of mutants). On the other

hand, the more specific, or narrow, drugs fail against a larger number of mutants. In this context we consider the values of cells' mutation rates with which various types of mutants are produced. If mutants resistant to drug 1 are produced with rate $u_1$, and mutants resistant to drug 2 are produced with rate $u_2$, then the inequality $u_1 < u_2$ means that drug 1 has a broader activity spectrum. That is, in our model drugs with a narrower activity spectrum correspond to larger mutation rates associated with the generation of corresponding resistant mutants.

3.3. **Insights from the deterministic theory: Mutually strong drugs.** Let us apply the deterministic methodology described in Section 2.2 to study the course of a cyclic treatment protocol. We are interested in maximizing the chances of treatment success, that is, in finding a protocol which is characterized by the highest probability of cancer cell eradication in a patient, while remaining within tolerance bounds in terms of its side-effects. In particular, we are interested in the production of fully-resistant mutants, because such mutants can become the reason for treatment failure. These mutants are produced by mutations of partially-resistant mutants, and also, in the presence of cross-resistance, by mutations of fully-susceptible mutants. This happens both before the treatment starts, and after it starts. The former process is treatment-independent. Therefore, in order to evaluate the effectiveness of different treatment protocols, it is sufficient to only consider the latter process. Solving system (2-6) for $t \in [0, t_*]$, we can calculate the total number of cells of each type, which is expected to be present in the colony by the time treatment starts. In particular, the numbers of partially-resistant mutants are given by

$$x_{10}(t_*) = N \log N u_1, \quad x_{01}(t_*) = N \log N u_2 \tag{16}$$

(here we used the smallness of the mutation rates with respect to 1). If by that time there are already fully-resistant mutants, then treatment will most probably fail (unless those mutants go extinct spontaneously). The goal of a cyclic treatment strategy is to minimize the production of fully-resistant mutants in the course of treatment, which helps to minimize the chances of treatment failure.

In figure 2 we plot the population sizes of the two partially-resistant colonies, $x_{10}(t)$ (dashed lines) and $x_{01}(t)$ (solid lines), in the course of treatment, for some fixed values of the parameters. Note that the terms "susceptible colonies", "partially-resistant colonies" etc have to be interpreted in terms of so-called "quasispecies" [16]. There is a "master sequence" which determines the typical properties of the cells in the given class (e.g. that they are resistant to a drug), and there is a "cloud" of other sub-types which evolved from the "master sequence" my mutations (or other genetic/epigenetic modifications). All these sub-types may have different characteristics, but still satisfy the main definition of the type (e.g. that they are resistant to a drug). Having a large degree of heterogeneity in a cancer does not contradict the kind of "coarse-grained" description used here. As mentioned before, the specific rates characterizing each type must be understood as the average rates.

In figure 2(a), treatment starts with drug 1, and we can see that during the first cycle the colony $x_{01}$ which is susceptible to this drug, decays exponentially, while the colony $x_{10}$, which is resistant to this drug, grows. In the second cycle, when drug 2 is applied, colony $x_{01}$ grows and colony $x_{10}$ decays. In figure 2(b) we present the scenario where the order of the drugs is switched (while all the parameters are kept the same).

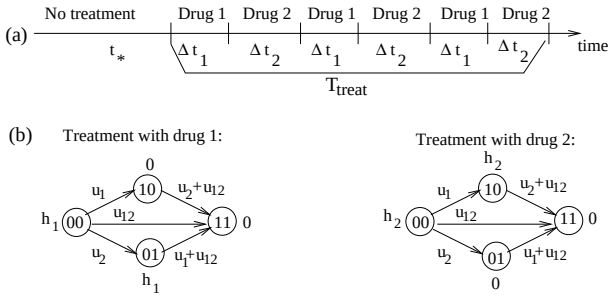


FIGURE 1. Cyclic two-drug treatments. (a) A cyclic treatment protocol. (b) The mutation diagrams and drug-induced death rates for treatments with drug 1 and drug 2. The resistance types are represented by circles with binary indices; the drug-induced death rates are marked next to the circles; the mutation rates are indicated next to the arrows.

Our task is to identify a treatment strategy which maximally suppresses the production of doubly-resistant mutants from the colonies $x_{01}$ and $x_{10}$. This is achieved by varying the cycle durations, $\Delta t_1$ and $\Delta t_2$, and the order in which the two drugs are applied. Some useful insights can be obtained by solving system (2-5) under treatment conditions, with initial values (16). After $2\mathcal{N}$ cycles, that is, for $t_{\mathcal{N}} = \mathcal{N}(\Delta t_1 + \Delta t_2)$, we have

$$x_{01}(t_{\mathcal{N}}) = A(u_2)e^{\mathcal{N}[(\gamma-h_1)\Delta t_1+\gamma\Delta t_2]}, \quad x_{10}(t_{\mathcal{N}}) = A(u_1)e^{\mathcal{N}[\gamma\Delta t_1+(\gamma-h_2)\Delta t_2]}. \quad (17)$$

It is clear that in order for the treatment in the absence of doubly-resistant mutants to work at all, we need to require that

$$h_1, h_2 > \gamma \quad \text{and} \quad \frac{\gamma}{h_2-\gamma} < \alpha < \frac{h_1-\gamma}{\gamma}, \quad (18)$$

where we used the notation

$$\alpha = \frac{\Delta t_2}{\Delta t_1}.$$

If conditions (18) are satisfied, functions $x_{10}(t)$ and $x_{01}(t)$ will on average decay. Conditions (18) are equivalent to condition

$$\frac{1}{h_1} + \frac{1}{h_2} < \frac{1}{\gamma}, \quad (19)$$

which can be viewed as a requirement for the two drugs to be sufficiently strong (compared to the colony growth-rate $\gamma$) such that they can eliminate a population of partially resistant mutants. We will refer to drugs that satisfy condition (19) as *mutually strong* drugs.

Before we proceed to optimize cyclic treatment protocols, we note two important implications of our model.

- Increasing the total treatment time, $T_{treat}$, will increase the chances of cancer elimination. This follows from the fact that under treatment with mutually-strong drugs, partially-susceptible colonies decay on average, and they grow in the absence of treatment. We note however that time $T_{treat}$ cannot be increased indefinitely because of drug toxicity consideration.
- Combining two drugs instead of using a cyclic treatment protocol will always correspond to a larger probability of colony elimination. Figure 2(c) shows the

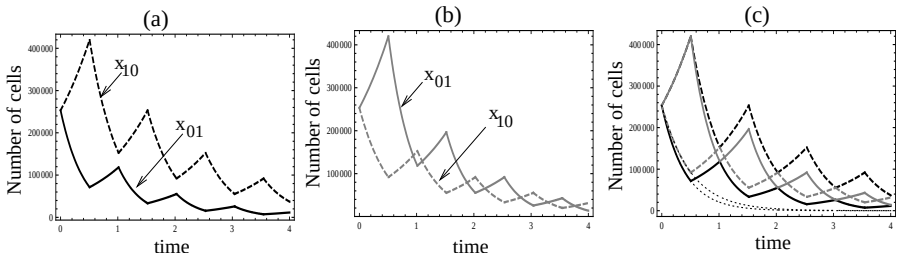


FIGURE 2. Cyclic two-drug treatments. The deterministic dynamics of the populations of partially-resistant mutants, $x_{01}$ (solid lines) and $x_{10}$ (dashed lines). (a) Worst drug first (black lines), (b) best drug first (gray lines), (c) lines from panels (a) and (b) are plotted together; also, the thin dotted lines present the same populations under a combination treatment. The parameters are $h_1 = 3$, $h_2 = 3.5$, $\gamma = 1$, the total number of cells at the start of treatment is $N = 10^{11}$, $u = 10^{-7}$, the numbers of partially-resistant mutants at start of treatment are $N_{01} = N_{10} = N \log Nu$, and $\Delta t_1 = \Delta t_2 = \log N/\gamma/50 \approx 0.5$. Neither of the two treatment strategies is optimal.

dynamics of the two partially-susceptible colonies under the cyclic treatment protocols of figures 2(a) and (b) together with the dynamics corresponding to the combination treatment where drugs 1 and 2 are applied simultaneously and continuously (dotted lines). The drug-induced death rates of all types are always smaller (or at least not larger) under combination treatment, thus eliminating the two colonies at a faster rate. Again, using two drugs simultaneously may not be feasible because of toxicity factors.

3.4. **The optimization problem.** In this paper we formulate and solve an optimization problem for cyclic treatment protocols with the goal of minimizing the generation of resistant mutants, while trying to minimize the drugs' side-effects. The requirement that the drugs' side-effects have to be kept under a certain tolerance level, imposes certain constraints on the timing and dosages of drug administration, especially in the context of traditional chemotherapeutic agents. The problem of optimizing treatment protocols with such drugs has been addressed in [29]. In this paper we focus our attention on the treatment of CML with small molecule inhibitors, which are generally considered only weakly-toxic, with relatively mild side-effects. To this end, we will assume that the side-effects of the drugs can be kept at the reasonable level by restricting the total treatment time (and thus restricting the cumulative drugs doses). In terms of our model, this means that the total treatment time, $T_{treat}$, cannot exceed a given value.

Then the optimal strategy is the pair $(\Delta t_1, \Delta t_2)$ which corresponds to the highest probability of treatment success for a given set of parameters, with the treatment time not exceeding $T_{treat}$. In order to find the optimal strategy for given parameters, we need to solve system (7-10) with initial conditions (11), and obtain the value $\xi_{00}(t)$, which is then used in formula (12). Once this is done for a number of choices $(\Delta t_1, \Delta t_2)$, we have to find the pair of cycle durations that give the maximum probability of treatment success.

3.5. **The mapping of treatment outcomes for different drug strengths.** We continue our analysis by a numerical exploration of treatment strategies for cyclic treatments with two drugs of different strengths. As described above, for

each pair of drug-induced death rates, $(h_1, h_2)$, we use the stochastic method of Section 2.3 to evaluate the probability of treatment success for different values of $\Delta t_1$ and $\Delta t_2$. We then find the optimal strategy (that is, the values $\Delta t_1$ and $\Delta t_2$ that maximize the probability of treatment success) for each pair $(h_1, h_2)$, and calculate the corresponding maximum probability of treatment success. The results are presented in figure 3, which shows schematically several regions on the $h_1 - h_2$ diagram, whose relatively sharp boundaries are defined by the difference in the treatment success probability for the optimal strategy. Figure 3 illustrates the limit of long treatment strategies; qualitatively similar maps can be obtained for different values of treatment durations, $T_{treat}$.

Regions A and B are characterized by the highest probabilities of treatment success, which for the particular choice of parameters in figure 3 are of the order one. There, we have $h_1^{-1} + h_2^{-1} < \gamma^{-1}$, that is, the two drugs are mutually strong, see inequality (19). This confirms our finding obtained by the deterministic methodology; parameters that correspond to the highest probability of treatment success in regions A and B satisfy

$$\frac{\gamma}{h_2 - \gamma} < \alpha < \frac{h_1 - \gamma}{\gamma},$$

see condition (18). Outside this region, the probability of treatment success drops by orders of magnitude. In the next section we will explore regimes A and B in more detail. Here we give some numerical illustration of the conditions listed here. Suppose that the net growth rate of cancer cells is $\gamma = 8.4$ yrs$^{-1}$; this means that the untreated tumor grows to the size of $10^{11}$ cells in 3 years. Further suppose that the drug-induced death rates of the two drugs are $h_1 = 59.1$ yrs$^{-1}$ and 33.7 yrs$^{-1}$, which means that the first drug is capable of eliminating the cancerous colony in 6 months (given that there is no resistance), and the second drug eliminates it in 12 months. In this case, the condition of mutual strength ($h_1^{-1} + h_2^{-1} < \gamma^{-1}$) is satisfied, and the two drugs belong to region A/B in the diagram of figure 3. For a relatively successful treatment, the ratio between the two cycle lengths must be somewhere in the window between 0.33 and 5.0. For example, if we apply each of the drugs for 1 month cycles (which corresponds to $\alpha = 1$), this protocol would satisfy the condition. On the other hand, if we chose the cycle length for drug 1 to be 4 months and for drug 2 to be 1 month, this would violate the inequality $\gamma/(h_2 - \gamma) < \alpha$, and the prediction is that the treatment would give a very poor outcome.

In regions C, D, E, and F the deterministic method predicts treatment failure, no matter what cycle lengths are used for the two drugs. Consistently with that, the stochastic method demonstrates a sharp drop in probabilities of treatment success as we cross the regions' borders. In particular, in Region C, each drug is strong enough to eliminate the mutants susceptible to it, if we apply the drug continuously, but in a cyclic treatment, we cannot reach extinction of both partially-resistant types, because condition (19) is violated. Regions D and E are characterized by very weak drugs; only one of the drugs is capable of eliminating mutants susceptible to it. Finally, in region F, neither of the drugs is capable of eliminating susceptible mutants, and the probability of treatment success is practically zero (and equals to the probability of the tumor's spontaneous extinction).

We can see that for all practical purposes, the drugs can be considered effective in the context of a cyclic treatment only if they satisfy condition (19). Moreover,

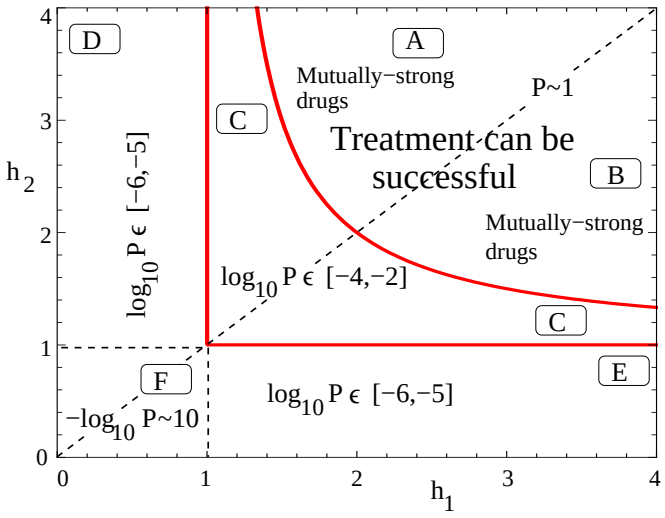


FIGURE 3. Optimal cyclic treatments for two drugs with different drug potencies and equal activity spectra. The two axes are the strengths of the drugs, $h_1$ and $h_2$. The lines correspond to $h_1 = \gamma$, $h_2 = \gamma$, $h_1 = h_2$ and $h_1^{-1} + h_2^{-1} = \gamma^{-1}$. The regions in the diagram marked by A,B,C,D,E, and F, are characterized by different optimal treatment strategies, and different maximal probabilities of treatment success. The parameters are $l = 1$, $d = 0$, $h_{12} = 0$, $u = 10^{-6}$, $N = 10^{10}$, $T_{treat} \to \infty$.

condition (18) should be satisfied to achieve a reasonable treatment schedule. The rest of this paper's analysis is therefore devoted to regions A and B.

4. **Analysis of drug treatments with mutually strong drugs.** Let us fix a certain treatment time, $T_{treat}$, and vary the number of cycles, $2\mathcal{N}$, used in the protocol. First we consider drugs of different potencies. We would like to determine the following features of the optimal protocol:

(i) What drug should be used first: best drug first (BDF) or worst drug first (WDF)?
(ii) What number of cycles, $2\mathcal{N}$, should be implemented within the allocated treatment time?
(iii) What is the optimal cycle duration ratio for the two drugs?

In figure 4 we consider two drugs: drug A has potency $h = 5\gamma$ and drug B has potency $h = 3\gamma$; the two drugs have the same activity spectra. The contourplots show the levels of the probability of treatment success calculated by using the stochastic methodology. Solid contours correspond to BDF strategies, and dashed contours - to WDF strategies. In figure 4(a), the treatment time is taken essentially infinite; in other words, doubling the treatment times does not change the probabilities of treatment success. It turns out that treating with BDF in this case is a better strategy; it corresponds to a finite number of cycles. If treating with WDF, the optimum corresponds to an "infinite" number of cycles, a theoretical confirmation of this result was obtained in [29] by using the deterministic methodology which works very well for long treatment times.

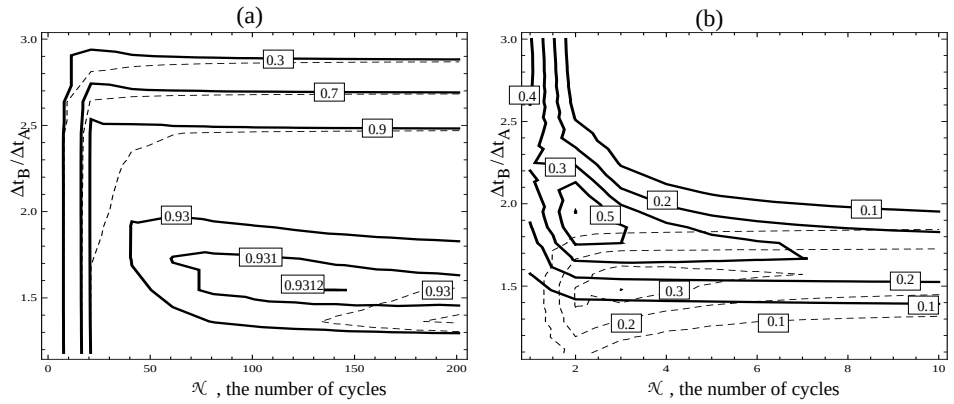


FIGURE 4. Drugs of different potencies in protocols of long and short durations. Drug A has $h = 5$, drug B has $h = 3$. (a) The treatment time is $T_{treat} = 74.83$, (b) $T_{treat} = 25$. The other parameters are $l = 1$, $d = 0$, $u = 10^{-8}$, $N = 10^{13}$. Solid lines correspond to BDF strategies, and dashed lines to WDF strategies.

In figure 4(b) we decrease the treatment time by approximately a factor of 3. The effect is very noticeable. First of all, we can see that the maximum probability of treatment success achieved by shorter protocols is significantly lower than that for longer protocols. Further, we observe that treating with BDF has a significant advantage compared to treating with WDF. Also, the contourplot of the probabilities changes its shape significantly: for BDF treatments, the optimal treatment consists of only 2 cycles of each of the drugs, and for WDF treatments - of 3 cycles.

Figure 5 demonstrates the effects of decreasing the total treatment time in a systematic way. As $T_{treat}$ decreases, the probability of treatment success decreases (figure 5(a)), and the difference between the optimal BDF and WDF treatment protocols increases. The BDF strategy remains advantageous. The optimal number of cycles (figure 5(b)) decreases as the treatment time decreases. The optimal BDF protocol usually requires fewer cycles than the optimal WDF protocol. Very short treatment times require the optimal protocol to have only one cycle of drug application. For BDF treatments, the probability of treatment success experiences a slowing down in its growth as a function of $T_{treat}$. This corresponds to the regime where having only one cycle is no longer optimal, and the optimal strategy requires using more than one cycles.

Next we will assume that the two drugs have an equal potency and only differ by their activity spectra. As has been mentioned previously, a difference in the activity spectra of two drugs manifests itself in the different rates with which mutants resistant to each drug are produced. In figure 6, we consider two drugs, drug A with a lower activity spectrum (that is, it is active against fewer mutants), and B with a wider activity spectrum (that is, it is active against a larger number of mutants). Mutants resistant to drug A are produced with rate $u = 10^{-5}$, and mutants resistant to drug B are produced with rate $u = 10^{-9}$. In figure 6(a), we fix the treatment time to be approximately 16.49 times units, which for the parameter values chosen is comparable with the time it takes on average to eliminate

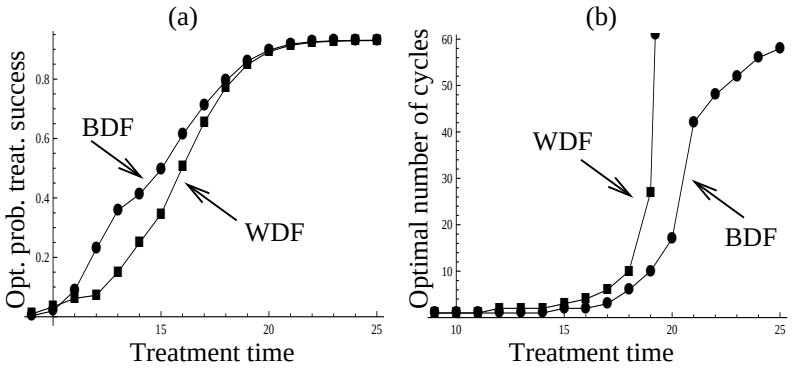


FIGURE 5. Drugs of different potencies in protocols of different durations. Drug A has $h = 5$, drug B has $h = 3$. (a) The optimal probability of treatment success as a function of treatment time. (b) The optimal number of cycles as a function of treatment time. The graphs are presented for the WDF and BDF strategies. The other parameters are $l = 1$, $d = 0$, $u = 10^{-8}$, $N = 10^{13}$.

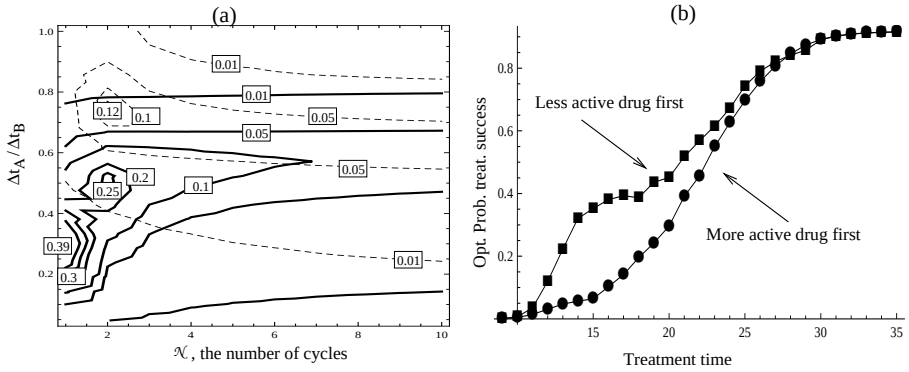


FIGURE 6. Drugs of different activity spectra in protocols of different duration. Drug A has $u = 10^{-5}$, drug B has $u = 10^{-9}$. (a) The contourplots of the probability of treatment success for treatment time is $T_{treat} = 16.46$; solid lines correspond to $u_1 > u_2$ (drug A first), and dashed lines to $u_1 < u_2$ (drug B first). (b) The optimal probability of treatment success as a function of treatment time. The other parameters are $l = 1$, $d = 0$, $h_1 = h_2 = 3$, $N = 10^{11}$.

the colony of susceptible cells with one of the drugs (approximately 12.66 units). The contourplots of the probability of treatment success obtained by the stochastic method are presented for the two cases: drug A first (solid lines) and drug B first (dashed lines). We observe that the optimal probabilities of treatment success in the two scenarios differ significantly. If we start with drug B, the optimal strategy is to use 2 cycles, and the corresponding success probability is about 0.12. If, on the other hand, we start treatment with drug A, then the best strategy is to use only one cycle, and the corresponding success probability is about 0.39, which is significantly higher. In both cases, the drug with the lower mutation rate must be used for longer.

In figure 6(b), we show that for most finite treatments protocols, it is advantageous to start with the drug characterized by a higher mutation rate, but use the other (more active) drug for longer cycle durations. The difference between the two types of protocols decreases as the treatment length increases, and for very long treatments, the two lines in figure 6(b) cross over. In the limit of long treatments, it becomes slightly advantageous to treat with the broader drug first [29].

If the treatment time is long, the extinction of the susceptible colony as well as both partially-resistant colonies is a certainty, and the probability of treatment success is defined by the dynamics of partially-resistant mutants, which are well described by the deterministic model. On the other hand, the dynamics of drug resistance for short treatment times has a purely stochastic component. If the treatment time is short, the probability of treatment success largely depends on the chance of the extinction of the susceptible and the partially susceptible colonies by the end of the treatment. For example, if the time $T_{treat}$ is so short that even the elimination of the fully-susceptible colony is unlikely, then the probability of treatment success is extremely low. This corresponds to the regime in figures 4(a) and 6(b) with treatment time below approximately 9; a rough estimate for this minimum treatment time which leads to clinically meaningful treatment probabilities is $\ln N/(h+d-l)$. For treatment times longer that this threshold, the main reason for treatment failure becomes the non-extinction of partially resistant mutants. While the fully-susceptible colony is killed by the drugs continuously, the two partially-resistant colonies are killed intermittently. The optimal treatment protocol maximizes the chances of eliminating these colonies by the end of the treatment.

## 5. Conclusions.

In this paper we studied cyclic drug therapies with the aim to develop general guidelines on optimal treatment scheduling. Our work continues earlier studies of [12] and extends the results to cross-resistant drugs.

The main idea behind treatment with multiple drugs is as follows. One of major causes of cancer drug treatment failure is the development of drug resistance, which is often associated with genetic events that modify cellular phenotypes inside the tumor. Drug resistance can potentially be overcome by the combination of multiple drugs, where a mutation that confers resistance against one drug does not confer resistance against any of the other drugs in use. Cyclic drug therapies consist of several alternating treatment courses, such that each of the drugs gradually eradicates the population of susceptible cells, and the net effect of the alternating cycles of treatment is the decline of the tumor. We find that in order for a cyclic treatment to be feasible, the drugs' strengths must satisfy a certain condition, which we call the condition of "mutual strength". Our results suggests that for mutually strong drugs, the success of cyclic treatments depends on the exact scheduling, and in particular, on the following factors: (i) which drug is administered first, and (ii) the durations of the treatment courses.

The methodology used in this paper can be applied directly to cancer drugs with relatively mild side-effects, such as small molecule kinase inhibitors [50]. Kinases inhibitors are currently one of the most promising drug types [1], because of their high efficiency and low toxicity. One example is Imatinib [37, 14], the first selective tyrosine kinase inhibitor targeting Bcr-Abl protein, which has shown clinical efficacy in the treatment of Chronic Myeloid Leukemia (CML). Small molecule inhibitors such as Imatinib, have the ability to bind specifically to cancer cells, and spare healthy, non-cancerous cells. Currently, there are 11 kinase inhibitors that have received US

Food and Drug Administration approval as cancer treatments, and there are many more that are at different stages of development [50]. Because of relatively mild side-effects of these drugs, the main objective of protocol optimization is a maximal efficiency in killing cancer cells.

The main findings of this paper are as follows:

- Finding the optimal treatment protocol for CML cyclic treatments with small molecule inhibitors involves restricting the total drug dose (that is, restricting the total treatment length) to minimize side-effects, and then finding the cycle durations which maximize the probability of cancer cell elimination.
- In order for a cyclic treatment to be effective, the drugs' potencies must satisfy a certain condition (condition (19) in the paper), which we call the condition of "mutual strength". For realistic parameters, drugs which are not mutually strong will yield very poor probabilities of treatment success, if applied cyclically.
- The general rule for cyclic treatments with mutually strong drugs of similar activity spectra is: *use the best drug first, but use the worst drug for longer.*
- The shorter the total treatment time, the more important is the "best drug first" rule.
- The presence of cross-resistance does not change this rule.
- The general rule for cyclic treatments with mutually strong drugs of similar potency and different activity spectra is: *use the less active drug first, and use the more active drug for longer.*
- The optimal cycle durations for given parameters such as drug strengths and the mutations rates, can be calculated, and do not depend on the tumor size. They do depend on other parameters, and in particular, on the total treatment duration.
- Combination treatments, where both drugs are applied simultaneously, have a higher probability of treatment success compared to cyclic treatments (given that combination treatment is tolerated by the patient).

The mathematical model and the optimization techniques developed in this paper are described in the context of CML. There are several reasons why the model is specific to this cancer: (1) The stochastic model lacks spatial constraints, that is, geometric considerations of tumor growth and drug delivery are not included. For this reason, the model is best applicable to non-solid tumors. (2) It has been observed that in CML, the main source of treatment failure is resistance due to point mutations; in other cancers different mechanisms (such as gene duplication) might be important, which requires a different approach. An extension of the present model which included the mechanism of gene duplication was presented in [32]. (3) Small-molecule inhibitors are used to treat CML. One of the assumptions of the model is that the drugs must be non-mutagenic, and possess relatively mild side-effects. Most chemotherapeutic agents used to treat cancer are not as selective as small molecule inhibitors at killing cancerous cells. Their efficiency against cancerous cells has a side-effect of a high killing rate of healthy cells, and thus a prolonged administration of such drugs cannot be tolerated by patients. Our methodology can be applied, with some modifications, to drugs of high toxicity; a detailed account of the method is given in [29]. (4) There are more than one drugs available at the moment for treating CML. These drugs, while exhibiting a degree of cross-resistance, are still characterized by different activity spectra, with well-studied properties. It

is definitely possible to extend the model to other cancers, as long as these assumptions hold true. A change in these specific properties would require certain modifications in the model.

Another extension of our modeling approach is related to the the stem cell hypothesis [7, 42], which states that only a small subpopulation of cancerous cells "matters". In other words, it is assumed that only a fraction of the cells, if not eradicated by a treatment, can replenish the cancerous colony. According to this hypothesis, the other (non-stem) cancer cells have a limited proliferative capacity, and the main goal of treatment is to target the stem cells. The model described in the paper is capable of describing cancer treatments in the context of the stem cell hypothesis. We assume that the population or target cells (which is the cancer stem cells in this case) is capable of three types of division: (i) symmetric divisions by which a stem cells divides into two stem cells; (ii) asymmetric divisions where a stem cell divides into a stem cell and a daughter cell without the infinite proliferative capacity, and (iii) symmetric divisions whereby a stem cell gives rise to two (non-stem) daughter cells. From the point of view of the target population, events of type (i) contribute to the division rate, $l$; events of type (ii) do not change the number of stem cells, and events of type (iii) contribute to the "natural" death rate of cells, $d$. With these remarks, and with the understanding that the total population size is now the population size of the stem cells (which is usually assumed to be only a few percent of the total population), we can directly proceed with the application of the model to a cancer stem cell colony. The results of the model regarding the optimal treatment strategies remain unchanged.

In general, the methodology developed here can help create more detailed theories of cyclic drug treatments with non-symmetric rates, and different activities and potencies of the two drugs. It can also be extended to more than two drugs.

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