

Combination Therapies against Chronic Myeloid Leukemia: Short-term versus Long-term Strategies

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Abstract

During therapy for chronic myeloid leukemia (CML), decline of the number of BCR-ABL transcripts has been shown to follow a biphasic pattern, with a fast phase followed by a slower phase. Hence, sustained remission requires a long phase of therapy. Data indicate that a combination of different available targeted drugs might prevent treatment failure due to drug resistance, especially at advanced stages of the disease. However, for long-term multiple-drug treatments, complications can arise from side effects. We investigate whether and how the number of drugs could be reduced during long-term therapy. Using computational models, we show that one or more drugs can be removed once the number of tumor cells is reduced significantly, without compromising the chances of sustained tumor suppression. Which drug to remove first depends on the number of mutations in the *BCR-ABL* gene that confer resistance to the drugs, as well as on how effectively the drugs inhibit Bcr-Abl protein tyrosine kinase activity and inhibit tumor growth. We further show that the number of CML cells at which the number of drugs can be reduced does not correlate with the two phases of decline of the BCR-ABL transcript numbers. Neither does it depend much on kinetic parameters of CML growth, except for the mutation rates at which resistance is generated. This is a significant finding because even without any information on most parameters, and using only the data on the number of cancer cells and the rate at which resistant mutants are generated, it is possible to predict at which stage of treatment the number of drugs can be reduced. [Cancer Res 2009; 69(11):4904–10]

Introduction

Chronic myeloid leukemia (CML) is a cancer of the hematopoietic system (1–4). It proceeds in three stages. During the chronic phase, the tumor cell population grows relatively slowly and the cells show a relatively high degree of differentiation. During the accelerated phase, the tumor grows faster and the percentage of undifferentiated blast cells increases. Blast crisis is the end stage of the disease, characterized by explosive growth and a relatively high proportion of undifferentiated blast cells. It is thought that the product of the *BCR-ABL* fusion gene is responsible for initiating and maintaining the disease (1). Consequently, small-molecule inhibitors of the Bcr-Abl oncoprotein have shown great promise in

treating this disease (5). The best known such drug is imatinib mesylate, which has shown significant treatment responses that can lead to molecular remission (defined by undetectable BCR-ABL mRNA transcripts using real-time quantitative PCR), especially when applied during the early phases of the disease (4, 6–12). The cancer cells, however, can evolve resistance to the drug, mainly through point mutations, and this is an important obstacle to sustained tumor suppression (6, 7, 9, 11–19). Recently, alternative small-molecule inhibitors have been developed that also target the Bcr-Abl oncoprotein (e.g., dasatinib and nilotinib), which could be used in combination with imatinib and each other (5) to prevent drug resistance. *In vitro* experiments have shown that “compared with single agents, each of the three combinations (imatinib mesylate + nilotinib, imatinib mesylate + dasatinib, and nilotinib + dasatinib) was more effective at reducing the outgrowth of resistant cell clones” (20).

During therapy, the decline of the BCR-ABL transcript numbers has been shown to follow a biphasic pattern in many patients, with a first and fast phase of decline followed by a second and slower phase of decline (21–24). Although debated, a reason for the slow phase of decline could be the presence of quiescent primitive CML cells (cancer stem cells) that do not respond to treatment and only get attacked by the drug when they exit the quiescent state and enter the cell cycle again (21–23). This in turn means that eradication of the cancer would require a long phase of treatment if this outcome is at all possible to achieve. If drug therapy has to be applied in the long term, complications can arise from side effects, especially when multiple drugs are given in combination. In this article, we investigate whether and how the number of drugs could be reduced during long-term therapy regimens. As cells die during treatment, mutant cells that are resistant to a subset of drugs can go extinct. For example, if three drugs are necessary to achieve a significant response to treatment, mutants that are resistant to two drugs might have gone extinct once the number of cancer cells has been reduced by several orders of magnitude. Hence, two drugs might be sufficient to continue therapy in the long term. We build on our existing mathematical framework to provide a quantitative analysis of such scenarios.

Materials and Methods

We use three different methods of calculating the number of tumor cells where it becomes possible to decrease the number of drugs by one without compromising the ability to suppress the tumor. These are the full stochastic method (see Supplementary Materials Sections 1–3), the ordinary differential equations for the expected values (see Supplementary Materials Section 4), and the “number of drugs” diagram that is explained in the next section (see Fig. 1).

Our stochastic model describes the dynamics of the cancer cell population during growth and during treatment (see refs. 21, 22, 25). We distinguish between active, cycling, and quiescent cells. These correspond to the primitive CML cell populations that are thought to drive and maintain the disease. Active cells divide with a rate l and die with a rate D .

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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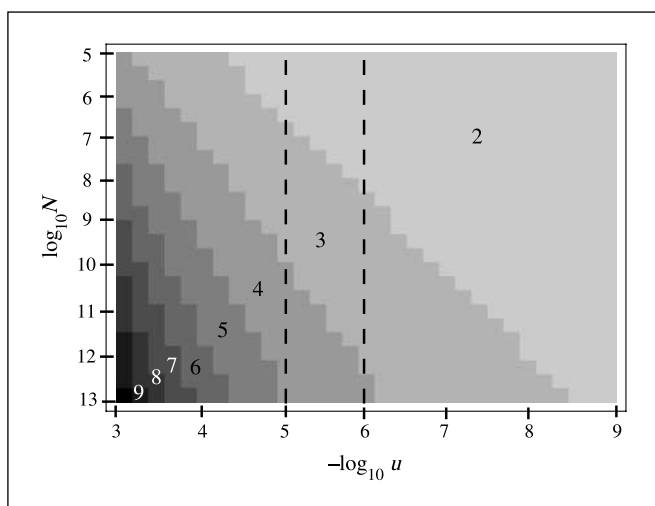


Figure 1. The number of drugs needed for the probability of treatment success $1 - \delta$ with $\delta = 0.01$, as a function of u and N . Other parameters are the following: $l = 1$, $d = 0$, $h = 2$, $\alpha = 0.01$, $\beta = 0.05$, $M_0 = 100$. The jagged appearance of the boundaries between the domains with different values of m is due to a discrete method used to calculate it and can be smoothed by using a simulation with a higher resolution.

They can enter quiescence with a rate α . Quiescent cells reactivate with a rate β . The model describes treatment with m drugs, which affects the model coefficients. That is, the overall cell death rate is given by $D = d + h$, where d is the natural and h is the drug-induced death rate. All the parameters are summarized in Table 1.

All cells are subdivided into resistance classes, ranging from fully susceptible to fully resistant. A cell can acquire resistance to a given drug through a mutation with a rate u . We assume that the cancer grows in the absence of treatment ($l > D$) until it has reached N cells. Then, treatment is started with m drugs (to prevent resistance), leading to an exponential decline ($D > l$). Further, we consider two-stage treatment strategies, where, during stage I, m drugs are applied in combination and then, during stage II treatment, the number of drugs is reduced.

The stochastic analysis described in detail in Supplementary Materials allows us to calculate the probability of treatment success (related to the extinction probability for cells), given the model parameters. We use these methods to calculate the number of tumor cells at which the number of drugs in a combination treatment can be reduced without compromising continued tumor suppression.

A simpler, approximate method of estimating the number of cells at which the number of drugs can be reduced without compromising tumor suppression is provided by deterministic modeling. There, we track the dynamics of the average population sizes for different resistance classes under stage I therapy and specifically calculate the expected number of mutants resistant to $m - 1$ drugs. We determine the time when the expected size of this population is reduced to below 1 cell and then declare that at this stage it is possible to remove one of the drugs without compromising the ability of treatment to suppress the tumor.

It is important to clarify the concept of treatment success used throughout the article. Using our models, we calculate the probability of treatment success based on the probability that the cancer cell population will be eventually driven extinct in the model. It therefore has a clear mathematical meaning. Although in the model the tumor cell population can indeed go extinct, it is unclear whether this is possible *in vivo* (factors not accounted for in the model could prevent tumor cell eradication) or whether this is possible to occur within a realistic period of time. In biological terms, the mathematical concept of treatment success translates into sustained remission without the resurgence of the disease as a result of drug resistance.

Finally, a note about the number of CML cells considered in the model. CML is made up of a heterogeneity of cells, ranging from

primitive cells to cells characterized by a relatively high degree of differentiation. Not all of these cells might contribute to maintenance and progression of the disease. Likely, the cancer stem cells and progenitor cells are most important in this regard, whereas the role of the more differentiated cells is less clear at the moment. Our model only considers the cells that drive the disease and that need to be attacked by drug therapy, and therefore, it is possible that this does not include the total number of CML cells found in a patient. Clearly identifying the role of the different cell populations for disease progression and correlating their numbers with BCR-ABL transcript numbers will be a very important step that will allow us to relate model predictions to clinical data in more precise way.

Results

The Number of Drugs Needed for Treatment

We start our discussion by determining the number of drugs, m , that we need for treatment under the assumption that treatment with m drugs continues indefinitely. We will fix parameters l , d , h , α , and β (see Table 1) and vary the mutation rate u (with which resistant mutants are generated) and the number of tumor cells at which treatment is started, N . For each pair (u, N) , we will find the minimum number of drugs that gives a probability of treatment success greater than $1 - \delta$. The result for a particular choice of parameters is presented in Fig. 1. We can see that for smaller mutation rates and smaller tumor cell numbers, two drugs are enough to treat successfully. For larger tumors or mutation rates, the number of drugs grows significantly.

The value of the rate at which resistant mutants are generated plays an important role in this analysis, and it strongly influences the number of drugs needed for treatment. In one of our previous articles (25) where we first introduced our method, we argued that most of the time, resistance preexists treatment, that is, several resistant mutants are already present at the start of therapy. The same holds in the present investigation; the mutation rates are of utmost importance because it is by the way of mutations the resistant clones come about. In our mathematical approach, we consider several stages of tumor evolution, the first one being “before treatment.” According to the model, it is during that first stage that resistance is mostly generated by mutations and may cause treatment failure at later stages.

The Number of Tumor Cells at Which the Number of Drugs Can Be Reduced

Next, we assume that at some point during the treatment, the number of drugs is reduced. We will examine whether fewer drugs

Table 1. Variables and parameters of the model

Notation	Definition
$x(t), y(t)$	Populations of cycling and quiescent cancer cells
l	Cell division rate
d, h	Natural and drug-induced cell death rates
D	Total cell death rate
u	Mutation rate (per cell division)
α, β	Cell quiescence and awakening rates
m	The number of drugs used
N	The colony size at start of treatment
N_{off}	The colony size at time of drug removal
δ	The allowed loss in probability of treatment success

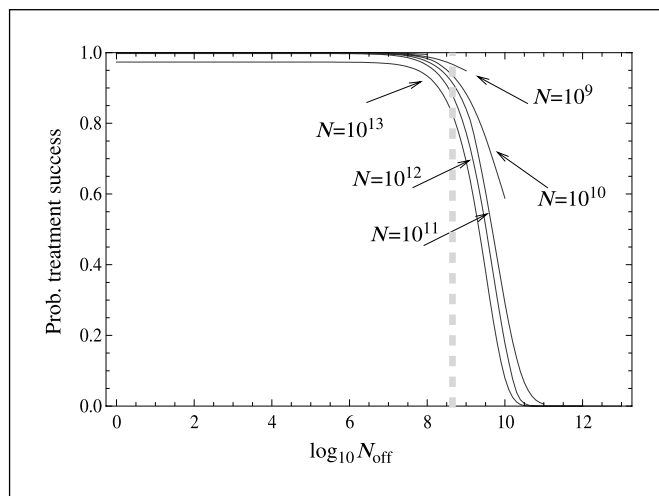


Figure 2. Probability of treatment success as a function of N_{off} , the number of tumor cells at which the number of drugs is reduced by one. Different curves correspond to different number of tumor cells on start of treatment, N . The initial number of drugs is $m = 3$, and $u = 10^{-6}$. The rest of the parameters are as in Fig. 1. The thick dashed vertical line corresponds to the quantity $N_{\text{off}}^{\text{det}}$, equation 8 of Supplementary Materials.

are sufficient to treat the cancer in the long term once the number of cells has shrunk. Let us calculate the number of tumor cells at which one drug can be taken away such that the probability of treatment success does not decrease by more than δ (see Supplementary Materials Sections 4 and 5 for details). Figure 2 presents the probability of treatment success as a function of the quantity N_{off} , the number of tumor cells at which treatment with three drugs is replaced by treatment with only two of those three drugs. This is plotted for different values of N , the number of tumor cells at which treatment is started. We observe the following interesting trends.

The probability of treatment success is flat for small values of N_{off} , and it is equal to the probability of treatment success for $m = 3$ drugs. In other words, if we remove one of the drugs when the number of cancerous cells is very low, it does not change the probability of treatment success. Note that in the particular example of Fig. 2, the probability of treatment success for low N_{off} is very close (closer than δ) to 1 for all curves except for the one with $N = 10^{13}$. If $N = 10^{13}$ and $u = 10^{-6}$, we can see from Fig. 1 that the number of drugs necessary for a success rate greater than $1 - \delta$ is $m = 4$, that is, if $m = 3$, the probability of success is smaller than $1 - \delta$. This can be seen in the limiting value of the probability of success rate as $N_{\text{off}} \rightarrow 0$ for $N = 10^{13}$ in Fig. 2.

On the other hand, if we take away one drug too early (N_{off} near N), then the probability of treatment success is lower than that with m drugs throughout the course of therapy. There is a relatively sharp transition between maximum possible probability of treatment success and a low probability of treatment success.

The main finding is that if we wait sufficiently long, the number of drugs used in combination treatment can be safely removed by one. Using similar methods, we can show that for some parameter combinations, there are conditions when more than one drug can be removed from treatment without compromising the ability of treatment to suppress the tumor. The number of tumor cells at which this can be done is calculated in similar ways (see Supplementary Materials Section 6 for details).

The Role of the Number of Tumor Cells and the Kinetic Rates

To implement a strategy when the number of drugs is reduced in the course of treatment, we need to be able to calculate when it is safe to remove a drug. Given parameter values, our methods allow us to perform corresponding calculations. However, reliable parameter estimation is usually problematic. Let us examine how strongly the number of tumor cells at which a drug can be removed from treatment depends on system parameters.

From Fig. 2, we observe that the probability of treatment success corresponding to different starting tumor cell numbers, N , are very similar for different starting cell numbers, N (see Supplementary Materials Section 5 for a quantification of this statement). One consequence of that is that for any tumor cell numbers at the beginning of treatment, one has to wait until the tumor reaches a defined number of cells, and then it becomes possible to remove one drug without compromising tumor suppression. To understand this result, we return to Fig. 1 and consider the case where $u = 10^{-6}$ (one of the dashed vertical lines in the figure). We can see that for $N = 10^{13}$, three drugs are not enough to treat with success rate of $1 - \delta$. For tumor cell numbers $\log_{10} N$ between about 9 and 12, three drugs are needed for the desired degree of success. For smaller tumor cell numbers, two drugs are enough. This simple reasoning explains intuitively why for any starting number of tumor cells within this range, a drug can be removed from treatment at about $N_{\text{off}}\delta$ for $u = 10^{-6}$. One needs to wait until the number of tumor cells is reduced enough such that $m - 1$ drugs can accomplish the job successfully and then take one drug off.

The above argument is not very precise because Fig. 1 has been created assuming that for each pair (N, u) , the tumor grows to N number of cells and is subsequently treated with m drugs. The composition of a tumor that grew to N_1 number of cells before start of treatment may be quite different from the composition of a tumor that was treated starting from $N_2 > N_1$ with m drugs and thus reduced to N_1 . However, we can use the chart in Fig. 1 for a rough estimate of reasonable population sizes.

We have seen that the number of tumor cells at which the number of drugs can be reduced without compromising tumor suppression strongly depends on the resistance mutation rate, u , and is not sensitive to changes in N . In Supplementary Materials

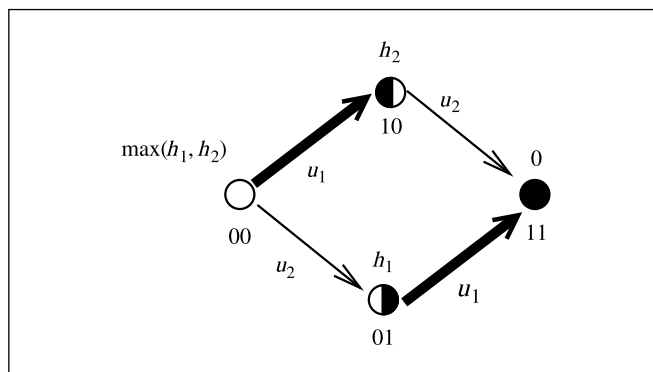


Figure 3. Mutation diagram for a two-drug case where drug 1 has a narrower specificity (a larger mutation rate u_1). Cell types are denoted by circles: fully-susceptible cells (combinatorial type 00) are denoted by an empty circle, partially resistant cells (10 and 01) are denoted by half-shaded circles, and fully resistant mutants (11) are black circles. The mutation rates and drug-induced death rates are marked. We have $u_1 > u_2$ (thicker arrow).

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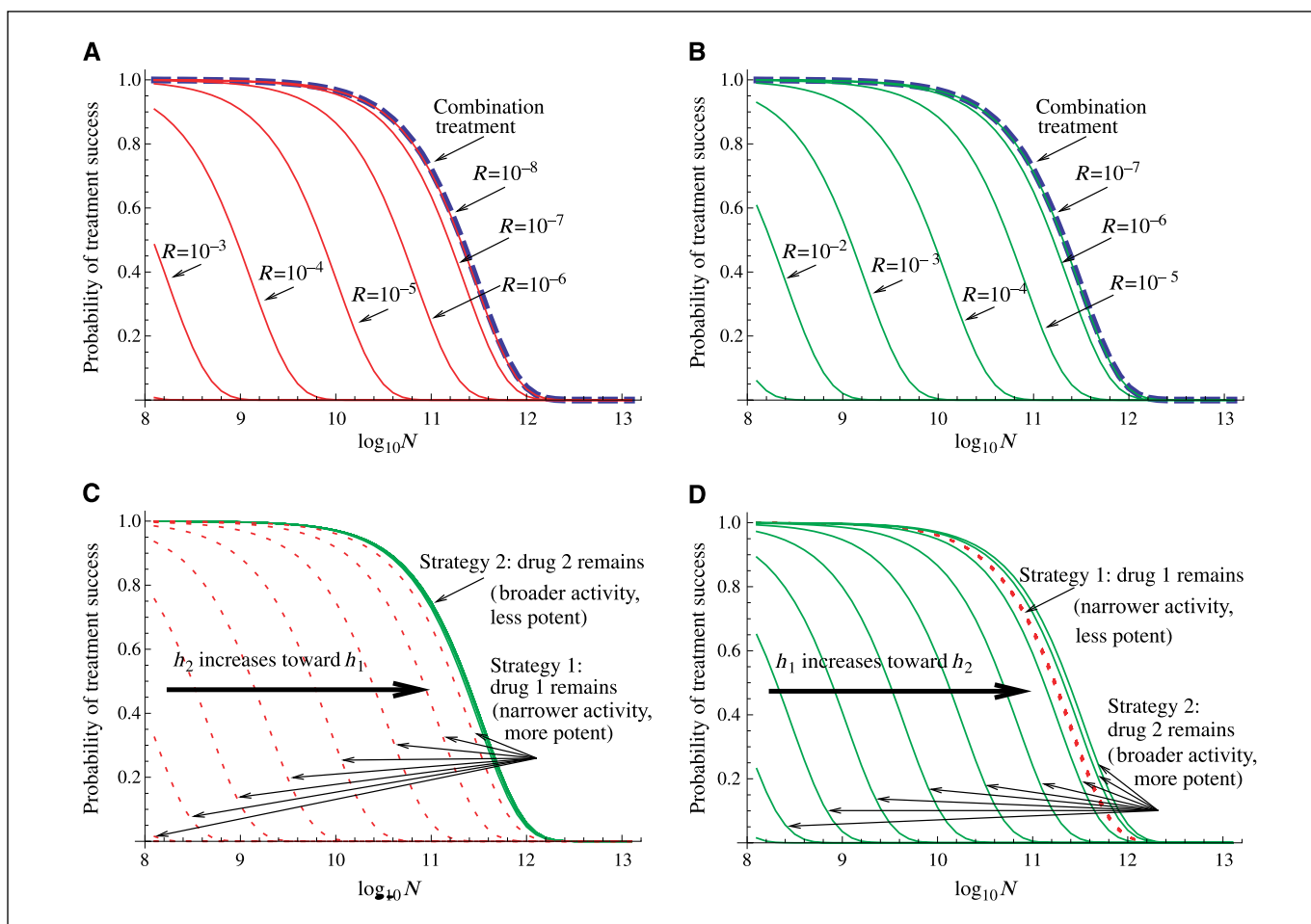


Figure 4. Drugs of different activity spectrum profiles: the probability of treatment success as a function of N , the number of tumor cells, for different treatment schedules. Drug 1 always has a narrower activity spectrum than drug 2. *Top*, the two drugs have equal potency ($h_1 = 5$). The dashed thick line corresponds to the combination treatment of two drugs. The other lines correspond to treatment schedules where one of the drugs is removed once the number of tumor cells is reduced by a factor R , indicated in the figure. *A*, drug 1 (the more active drug) is removed. *B*, drug 2 (the less active drug) is removed. *Bottom*, the two drugs have different potency. The dotted red lines correspond to strategy 1, where after a combination treatment only drug 1 remains. Solid green lines correspond to strategy 2, where only drug 2 remains. *C*, drug 1 has a larger potency than drug 2; different lines correspond to values of $h_2/l \in \{5, 10, \dots, 50\}$, such that $h_2 \leq h_1 = 50$. Note that for strategy 2, the green line, which appears thick, is made up of several lines that have very similar probabilities of treatment success and are hence close together. *D*, drug 1 has a smaller potency than drug 2; different lines correspond to values of $h_1/l \in \{5, 10, \dots, 50\}$, such that $h_1 \leq h_2 = 50$. The parameters are $d/l = 0.2$, $u_1 = 10^{-7}$, $u_2 = 10^{-6}$, $M_0 = 10^2$.

Section 5, we present a detailed study of how this quantity depends on other parameters. Here, we outline the main findings:

- For systems with higher rate quiescence, α , one has to wait longer before it is possible to reduce the number of drugs without compromising treatment success. However, the tumor cell number at which the drug reduction can take place remains virtually unchanged with α .
- The smaller values of β are (more quiescence in the system), the longer one needs to wait until a drug can be removed. But at the same time, the number of tumor cells corresponding to taking off a drug is largely independent from β .
- The time lapse before it is possible to remove a drug without compromising treatment success grows with d , but the tumor cell number is only weakly dependent on the death rate.

We conclude that although the time of drug removal is sensitive to exact parameter estimations, the number of cells at which the number of drugs can be reduced is only weakly dependent on parameter values.

Finally, we investigated how the number of tumor cells at which a drug can be removed depends on the location of the “transition” of the biphasic tumor decline (23, 24). It turned out that the point of transition is not indicative of when a drug can be removed. For some parameters, a drug can be taken away while still in the first, fast, phase of the decline, and for others one has to wait for a long time, well into the second phase, until a drug can be taken off.

Drugs with Different Specificity and Potency

In this section, we discuss some applications of our method to scenarios where the drugs have different characteristics. In particular, we will concentrate on the activity spectrum and potency of the drugs, which are defined as follows.

We will say that a drug is characterized by a broad activity spectrum if it is able to bind to a large variety of mutant Bcr-Abl proteins, with a subsequent successful destruction of the cell. On the other hand, a drug with a narrow activity spectrum is a more specific agent, which is active against a relatively small number of Bcr-Abl protein variants. In terms of our modeling approach, the

drug with a broader activity spectrum will be characterized by a smaller mutation rate, u , with which mutants resistant to the drug are generated. The more specific, or narrow, drug is characterized by a larger mutation rate associated with the generation of mutants resistant to the drug.

By potency, we mean how effectively a drug inhibits the Bcr-Abl protein tyrosine kinase activity and kills cells that are susceptible to the drug; this is reflected in the drug-induced cell death rate characteristic of each drug, where higher potency is correlated with higher values of h .

Consider a two-drug treatment with drugs 1 and 2. Suppose that drug 2 is characterized by a broader activity compared with drug 1. This setup is illustrated in Fig. 3, where a mutation diagram for all the resistance types is shown. Let us envisage a schedule whereby the tumor is first treated with a combination of drugs 1 and 2, and then the number of drugs is reduced by one. Which drug should remain and which one should be taken off?

First, we assume that the potency of both drugs is the same, that is, $h_1 = h_2$. To identify the more effective treatment strategy in the presence of two drugs with different activity spectra, consider Fig. 4. The graphs show the probability of treatment success as a function of N , the number of tumor cells, for different treatment schedules. In Fig. 4A, the drug with a broader activity spectrum (drug 2) is removed, and in Fig. 4B, the drug with a narrower activity spectrum (drug 1) is removed. The rightmost dashed thick line in both graphs corresponds to a combination therapy. The other lines show the probability of treatment success when a drug is discontinued; the drug is removed once the number of tumor cells is reduced by a factor R , which appears above each line. The goal is to keep the probability of treatment success as high as possible (the thick dashed line). We can see that in both cases, if the reduction happens sufficiently late in treatment (low values of R), the probability of treatment success will be unchanged. This is consistent with the results of the previous subsections.

We can see, however, that if the drug with a broader activity spectrum is removed (Fig. 4A), then we need to wait until the number of tumor cells is reduced by a factor of $R = 10^{-8}$; otherwise, the probability of treatment success is significantly lower compared with that of continuous combination therapy. On the other hand, if the drug with a narrower activity spectrum is removed (Fig. 4B), then we can withdraw the drug earlier in treatment (more precisely, when the number of tumor cells is reduced by a factor of $R = 10^{-7}$). This suggests that a more effective strategy is the one when the drug with a narrower activity spectrum is removed (Fig. 4B).

This result can be explained in the following way (see Table 2). Because of the differential activity spectra of the two drugs, the resistant mutants are accumulated at different rates before treatment. Mutants resistant to drug 1 are generated at a higher rate: $u_1 > u_2$. Consequently, by the time treatment starts, there

tends to be more mutants resistant to drug 1. During treatment stage I, both types of partially resistant mutants are killed at rate $h_1 = h_2$, such that by the time one of the drugs is removed (beginning of stage II treatment), there will be more mutants resistant to drug 1. To maximize treatment success, these mutants have to be removed; they are only susceptible to drug 2 (and resistant to drug 1). Therefore, it is more effective to continue treating with drug 2 to which these mutants are susceptible.

Next, let us add a layer of complexity and assume that the two drugs can differ not just in their activity spectra but also in their potency. In terms of our analysis, this means that the drug-induced cell death rates, h_1 and h_2 , are different. Below, we consider the two cases, $h_1 > h_2$ and $h_2 > h_1$ (see Fig. 4C and D).

The drug with a narrower activity is more potent, that is, $h_1 > h_2$. As before, $u_1 > u_2$. In words, drug 1 has a narrower activity spectrum, but it is also more potent than drug 2. This case is similar to the case $h_1 = h_2$: the more effective treatment schedule involves continuing with the broader drug (drug 2) while removing the narrower drug (drug 1). The argument is as follows. By the time treatment starts, there are more mutants resistant to drug 1 than there are mutants resistant to drug 2 (see Table 2; as before, they are produced at a higher rate). During stage I treatment, the more abundant mutants are killed at a lower rate, h_2 , such that at the beginning of stage II treatment, mutants resistant to drug 1 are still more prevalent and they pose the biggest risk. Therefore, the drug applied in stage II treatment should be able to effectively kill these mutants. This is drug 2, as the more prevalent mutants are resistant to drug 1. The above ideas are illustrated in Fig. 4C, where we plot the probability of treatment success as a function of the number of tumor cells, for two strategies: “strategy 1” when drug 1 continues (*dotted red lines*) and “strategy 2” when drug 2 continues (*solid green lines*). We keep all the parameters fixed except varying the drug 1-induced cell death rate, h_1 (although it always remains below h_2). First, we can see that it is always advantageous to keep drug 2 and remove drug 1 (the corresponding probabilities of success are always higher). Another interesting observation is the following. As we vary the strength of drug 2, the probability of treatment success of strategy 1 changes dramatically, whereas this has almost no effect on strategy 2. This is because for strategy 1, the mutants resistant to drug 1 are only killed during stage I treatment by drug 2; thus, its strength is of a crucial importance. Under strategy 2, these mutants are continued to be exponentially killed throughout treatment.

The drug with a narrower activity is less potent, that is, $h_1 < h_2$. We can say that in this case, drug 1 is inferior to drug 2 because it has a narrower activity spectrum and it is also less potent. Under this scenario, interestingly, the choice of strategy will depend on the extent to which drug 2 is more potent than drug 1. During treatment stage I, the more abundant mutants (the ones resistant to drug 1) will be killed at a higher rate (h_2) than the less

Table 2. A summary of the dynamics of different resistant types during the pretreatment stage and two stages of treatment

Stage	Mutants resistant to drug 1	Mutants resistant to drug 2
Pretreatment	Generated at rate u_1 (faster, because $u_1 > u_2$)	Generated at rate u_2 (slower, because $u_1 > u_2$)
Treatment stage I	Killed with rate h_2	Killed with rate h_1
Treatment stage II (drug 1 only)	Not killed	Killed with rate h_1
Treatment stage II (drug 2 only)	Killed with rate h_2	Not killed

abundant mutants resistant to drug 2. Therefore, if by the time stage II treatment starts there are still more mutants resistant to drug 1, then drug 2 should be given. Otherwise, it is drug 1 that should be continued, and drug 2 removed. Figure 4D illustrates these scenarios. Again, strategy 1 corresponds to continuing drug 1, and strategy 2 leaves drug 2. Now, it is the potency of drug 1 that is varied (while remaining below the potency of drug 2). By analogy with the previous case, only strategy 2 is sensitive to changes in h_1 because in this case mutants resistant to drug 2 are only killed in stage I treatment by drug 1, and this has to be done as efficiently as possible. A more interesting observation that confirms the above verbal argument is that strategy 1 may or may not be the most efficient. In particular, it is the preferable strategy when drug 2 is significantly more potent than drug 1.³ In this case, during stage I treatment, the “better” (more specific and more potent) drug (which is drug 2 in our notations) effectively gets rid of the mutants resistant to drug 1. Then, the only problem is the mutants resistant to drug 2, which are subsequently killed by a long-term application of drug 1.

We conclude that if drugs with differential activity spectra are used, then the drugs with the broader activity should be continued, and the drug(s) with the narrowest activity can be removed. On the other hand, if the broader activity drug is also considerably more potent, the less potent and narrower drug should remain and the more potent and broader one can be removed.

Discussion

In this article, we built on an existing mathematical framework that describes the evolution of drug-resistant cancer cells in CML treated with small-molecule inhibitors (21, 22, 25, 26). This framework suggests that a combination of three or four different small-molecule inhibitors needs to be administered to avoid treatment failure as a result of drug resistance. It further suggests that this treatment might have to be applied in the long term. Here, we investigated the effect of withdrawing one or more drugs from the combination therapy regimen once the number of cancer cells has declined to lower levels. On the most basic level, the modeling tells us that the number of drugs can be reduced for long-term therapy without significantly reducing the chances of tumor suppression. This is important in the face of side effects that are likely to become problematic if treatment is continued in the long term. Using a variety of computational approaches, we calculated the threshold number of cancer cells at which the number of drugs can be reduced without significantly altering the chances of tumor suppression. We can generate charts (such as that presented in Fig. 1) that map the number of drugs needed for various numbers of tumor cells and mutation rates. It turns out that the number of tumor cells at which the number of drugs can be reduced does not correlate with the two phases of CML decline during treatment observed in clinical data. In addition, the number of tumor cells at which the number of drugs can be reduced does not depend strongly on model parameters, except the rate at which resistance mutations are generated. This is a significant finding because even without any information on most parameters, and using only the

data on the amount of cancer cells in the patient's blood and the rate at which resistance mutations are generated, it is possible to predict at which stage of treatment the number of drugs can be reduced.

Our method also helps determine which drugs should be removed and which ones continued. For example, in two-drug treatments, if one of the drugs has a narrower activity spectrum, and it is equally potent or more potent than the second drug, then it is the drug with the narrower activity that should be discontinued. On the other hand, if the narrower drug is significantly less potent than the broader activity drug, then it is the broader drug that should be removed from treatment. Experimental and clinical studies performing measurements of the rate at which resistance mutations are generated and the drug-induced death rates will be vital to make clinically relevant predictions. Cancer kinetic parameters (such as the division and death rate of the cancer cells, and the rates at which primitive CML cells enter and exit quiescence) are essential to predict the time course of treatment, such as treatment length and the duration of time until the number of drugs can be reduced without compromising continued tumor suppression.

For the purposes of this article, we considered combination therapies as a starting point and investigated whether the number of drugs in the combination can be reduced in the course of the treatment. The reasons that we chose combination therapies as our focus are the following. (a) There are *in vitro* data for CML suggesting that combining drugs results in a better treatment outcome (20). (b) Large research literature together with a long-term clinical experience in the field of viral disease treatment suggest that drug combination is the best strategy to suppress resistance (27, 28). One ready example is the case of HIV infection (27–30). In the early days of HIV treatment, drug combination treatments were considered controversial, whereas nowadays combination therapies have become the standard based on mathematical modeling and clinical trials (27, 28). (c) It is possible to show mathematically that combining cancer (small-molecule inhibitor) drugs will lead to a higher probability of treatment success compared with other therapeutic strategies where the drugs are given sequentially, which includes cycling of drugs, or treating to maximal benefit and then switching. In other words, drug combinations provide the strongest theoretically possible protection against resistant mutant generation; this is a subject of our manuscript in preparation. (d) When dealing with conventional chemotherapies, drug combinations can greatly amplify toxic side effects; however, with small-molecule inhibitors, side effects, although still present, are less of an issue, allowing for more freedom in combining two or more agents and treating continuously without breaks. All of the reasons listed above lead us to consider the combination therapy as a starting point. In future work, we plan to investigate optimization of other CML treatment strategies.

Our modeling assumed an optimal scenario where there is no cross-resistance between the different drugs used. That is, resistance against one drug does not affect the treatment with another drug. Although this is true for many mutations that confer resistance against small-molecule inhibitors, there is one mutation that confers resistance to all drugs used thus far (20). We are currently incorporating cross-resistance into our mathematical framework. This basically reduces the number of tumor cells at which resistance against an m -drug therapy becomes a problem. In other words, more drugs will have to be used in combination to

³ The latter result is reminiscent of the “worst drug rule” (33) which suggests that in cyclical treatments, the first drug to be applied is the worst drug. In contrast, our treatment schedules start with a drug combination and then followed by a single-drug treatment.

achieve successful treatment compared with a scenario where no cross-resistance is assumed. The extent of this complication depends on parameters. The basic insights described in this study and recapitulated above, however, remain robust. Development of alternative drugs that show no cross-resistance with the current array of small-molecule inhibitors of the Bcr-Abl oncoprotein (31, 32) would be very desirable because this would make combination therapy a more effective and feasible tool in the treatment of CML.

In a previous study, we have made the first step toward validating the mathematical model that is the basis for the computations in this article (22). There, we have used two sets of clinical data on the number of BCR-ABL transcripts in the blood of CML patients treated with the drug imatinib. The model does not only predict the correct mean behavior (the phenomenon of the biphasic decline of BCR-ABL transcript numbers) but also helps explain individual variation in patients. In the present article, the mathematical model is a modification of the same approach, which includes multiple drugs and a flexibility to change/switch drugs.

There are several types of potential data that can be used to improve the method. One is *in vitro* data where different treatment schedules are investigated (in the spirit of ref. 20). Another is actual clinical data similar to those in refs. 23, 24, where patients are treated with several drugs and then some drugs are discontinued. It is our hope that this article could encourage experimentalists and clinicians to consider pursuing some of these ideas.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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