

Implication of the Autologous Immune System in *BCR-ABL* Transcript Variations in Chronic Myelogenous Leukemia Patients Treated with Imatinib

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Abstract

Imatinib and other tyrosine kinase inhibitors (TKI) have improved treatment of chronic myelogenous leukemia (CML); however, most patients are not cured. Deeper mechanistic understanding may improve TKI combination therapies to better control the residual leukemic cell population. In analyzing our patients' data, we found that many patients who otherwise responded well to imatinib therapy still showed variations in their *BCR-ABL* transcripts. To investigate this phenomenon, we applied a mathematical model that integrates CML and an autologous immune response to the patients' data. We define an immune window or a range of leukemic loads for which the autologous immune system induces an improved response. Our

modeling results suggest that, at diagnosis, a patient's leukemic load is able to partially or fully suppress the autologous immune response developed in a majority of patients, toward the CML clone(s). Imatinib therapy drives the leukemic population into the "immune window," allowing the patient's autologous immune cells to expand and eventually mount an efficient recognition of the residual leukemic burden. This response drives the leukemic load below this immune window, allowing the leukemic population to partially recover until another weaker immune response is initiated. Thus, the autologous immune response may explain the oscillations in *BCR-ABL* transcripts regularly observed in patients on imatinib. *Cancer Res*; 75(19); 4053–62. ©2015 AACR.

Major Findings

On the basis of patient data and our model, we hypothesize that the autologous immune system plays a significant role in the dynamics of CML during imatinib therapy. Moreover, variations in *BCR-ABL* transcripts during imatinib therapy may represent a signature of the patient's individual autologous immune response. Immunotherapy may complement imatinib and other TKIs by helping to maintain a patient's autologous immune response when the leukemia stimulus alone is insufficient. Our mathematical model is a potentially valuable tool in studying and designing patient-specific schedules for these combination therapies.

Introduction

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder caused by the *BCR-ABL* fusion oncogene, which encodes for a constitutively active tyrosine kinase. Tyrosine kinase inhibitors (TKI), such as imatinib, are targeted therapies that have revolutionized the treatment of CML, producing durable remissions in many patients and resulting in substantially improved long-term survival rates (1, 2). Despite their success, their global therapeutic effect remains incompletely understood. Moreover, it is unclear whether TKIs alone are capable of eliminating the entire leukemic burden, as many patients in long-term remissions continue to harbor small residual leukemic loads even after many years of therapy (3). A better understanding of TKIs would allow us to improve the way that these drugs are administered and also to identify their limitations. If TKIs prove to be incapable of curing most patients, then understanding the drugs' mechanisms of action may inform our use of combination therapies.

There is compelling evidence that a patient's autologous immune response plays a significant role in the dynamics of CML. It is known that immune cells are capable of detecting and eliminating cancer cells (4). Immunotherapy is a major goal of immunology and cancer research because of the ability of the immune system to target and eliminate abnormal cells while leaving healthy cells intact. In the allogeneic setting, the immune system has demonstrated its power in the elimination of residual CML disease (5). Several combination therapies involving interferon- α (IFN α) are currently being investigated (6, 7, 8), in part, because of this drug's effects on the autologous immune system (8), which participates in the control of the

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

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Quick Guide to Equations and Assumptions

We developed an ordinary differential equation (ODE) model of chronic myelogenous leukemia (CML) and the immune system to study the dynamics of imatinib therapy. Specifically, we seek to understand patients whose BCR-ABL ratios vary nonmonotonically during therapy.

Let y_0 , y_1 , y_2 , and y_3 represent the concentrations of quiescent leukemic stem cells, cycling leukemic stem cells, progenitor leukemic cells, and mature leukemic cells. Let z denote the concentration of immune cells. We consider the following system of ODEs:

$$\dot{y}_0 = b_1 y_1 - a_0 y_0 - \frac{\mu y_0 z}{1 + \varepsilon y_3^2} \quad (\text{A})$$

$$\dot{y}_1 = a_0 y_0 - b_1 y_1 + r y_1 \left(1 - \frac{y_1}{K}\right) - d_1 y_1 - \frac{\mu y_1 z}{1 + \varepsilon y_3^2} \quad (\text{B})$$

$$\dot{y}_2 = a_1 y_1 - d_2 y_2 - \frac{\mu y_2 z}{1 + \varepsilon y_3^2} \quad (\text{C})$$

$$\dot{y}_3 = a_2 y_2 - d_3 y_3 - \frac{\mu y_3 z}{1 + \varepsilon y_3^2} \quad (\text{D})$$

$$\dot{z} = s_z - d_z z + \frac{\alpha y_3 z}{1 + \varepsilon y_3^2} \quad (\text{E})$$

In Eqs. A and B, a_0 and b_1 represent the transition rates of leukemic stem cells from quiescence to cycling and cycling to quiescence, respectively. We assume logistic growth of cycling stem cells, with growth rate r and carrying capacity K . Cycling stem cells die naturally at a rate d_1 . In Eq. C, the first term represents the differentiation of stem cells into progenitors. The coefficient a_1 is the product of the differentiation rate and the amplification factor upon differentiation due to cell proliferation. Progenitors die naturally at a rate d_2 . Equation D is similar to C, with differentiation rate a_2 and death rate d_3 . The last terms in Eqs. A to D represent the death of leukemic cells caused by an immune response. The mass action term $\mu y_i z$ represents the killing of leukemic cells by the immune system, where μ is the maximal rate (per immune cell) at which an immune cell will engage and kill a leukemic cell. Equation E represents the concentration of autologous immune cells. The first term s_z is a constant source term. Immune cells die at a rate d_z . The mass action term $\alpha y_3 z$ represents the expansion (proliferation) of the immune cell pool in response to its leukemia stimulus, which occurs with maximal rate per leukemic cell α . We include only the contributions of the mature leukemic cells y_3 to immune stimulation because they are a much larger population than the immature leukemic cells ($y_{\text{total}} \approx y_3$).

Our model is based on the assumption that immunosuppression acts in two ways. First, mature leukemic cells inhibit the expansion of immune cells. In Eq. E, the immune cell expansion term $\alpha y_3 z$ is divided by $1 + \varepsilon y_3^2$, where the constant ε determines the strength of the immunosuppression. Second, mature leukemic cells are assumed to decrease the killing capacity μ of activated immune cells, also by a factor of $1 + \varepsilon y_3^2$. This effect is represented in the last terms in Eqs. A to D. This approach is similar to the one used in ref. 9. By implementing immunosuppression in this way, we encode an autologous immune response that is effective only with intermediate levels of leukemic cells. When the leukemic load is small, only a small number of immune cells are stimulated to respond. On the other hand, although large leukemic loads provide a stronger stimulus, the leukemic cells are able to suppress the efficacy of the immune system. Thus, the immune response will be negligible when the leukemic load is either very small, at levels undetectable by the immune system, or very large, at levels that overwhelm and suppress the immune system. A strong immune response can occur only when the leukemic load y_3 is at an intermediate level, within a range $[y_{\text{min}}, y_{\text{max}}]$ that we call the immune window. In our model, we define the immune window as the range of y_3 , for which the rate of immune stimulation ($\alpha y_3 / (1 + \varepsilon y_3^2)$) exceeds the death rate (d_z). Imatinib therapy may be used to drive the leukemic load into this immune window, allowing the autologous immune system to assist the drug in the elimination of the leukemic cells.

Imatinib is known to block the kinase activity of the BCR-ABL protein, which results in a significant decrease in the proliferation rates of the BCR-ABL⁺ leukemic cells (1, 10) and apoptotic death (11). However, we focus here on the effects of imatinib on proliferation and leave incorporation of other mechanisms to a future work. We implement imatinib therapy, starting at time $t = 0$, by decreasing the differentiation/amplification rates a_1 and a_2 to lower values $a_1' = a_1 / \text{inh}_1$ and $a_2' = a_2 / \text{inh}_2$. It is unknown how imatinib affects leukemic stem cells and whether quiescent leukemic stem cells are affected at all, so we assume no direct effect of imatinib on these populations. However, our model provides a framework for testing various mechanisms of actions of imatinib, which we leave for a future work.

It is also unclear whether imatinib is capable of completely eliminating the leukemic cell burden or whether small residual populations will persist indefinitely. In our model, a leukemic load of zero can only be approached asymptotically, so we define cure as a cancer stem cell concentration less than $1.67(10)^{-4}$ cells/mL, which corresponds to less than one leukemic stem cell. We stop all simulations of the model whenever this is achieved.

disease. In addition, IFN α may drive quiescent leukemic stem cells into the cell cycle (12, 13), where they become exposed to the effects of TKIs.

In the Stop imatinib (STIM; ref. 14) and TWISTER (3) trials, patients who responded well to imatinib were taken off therapy in order to determine whether treatment-free remission (TFR) could be achieved. They found that approximately 40% of patients remained in TFR for at least 2 years after stopping treatment. Moreover, although not statistically significant, the TFR rate was higher in patients that had received IFN α prior to imatinib (14). In many of these patients, *BCR-ABL* DNA and mRNA were still detectable (3). Moreover, in ref. 15, patients still harbored *BCR-ABL*⁺ leukemic stem cells, despite having remained in TFR for up to 8 years. In these cases, because treatment did not completely eradicate the disease, some other mechanisms, such as the autologous immune system, must be preventing this residual cancer population from expanding. Motivated by these results, we constructed a mathematical model integrating CML and the autologous immune response.

Materials and Methods

A group of 104 patients with CML was monitored during imatinib therapy in the Centre Hospitalier Lyon Sud (Lyon, France). These patients were all treated with first-line imatinib 400 mg daily. Patients' *BCR-ABL* ratios were measured in the same laboratory according to the guidelines of European LeukemiaNet, with the same techniques at diagnosis, months 3, 6, 9, and 12 of therapy, and every 6 months thereafter; in order to limit variability, each measurement was run in duplicate, and the two resulting measurements were averaged. Overall, the patients had an average follow-up time of 62.76 months (range, 2.96–148.70), with an average of 12.69 measurements taken (range, 2–26). We excluded patients who changed TKIs for safety reasons ($n = 33$) and patients whose disease progressed ($n = 14$), as we focused exclusively in this study on patients obtaining a residual disease on imatinib. Thus, a population of 65 patients who responded well to imatinib remained for analysis.

BCR-ABL ratios were serially measured by quantitative RT-PCR in the peripheral blood of patients in the same laboratory according to the European standards of European Leukemia Net recommendations (16, 17) and expressed as a percentage on the International Scale (IS; ref. 18). Each sampling was run in duplicate in order to reduce variability and additionally run in parallel to the previous (frozen) sample from each patient in order to exclude technical problems, at each time point (except diagnosis) for all patients. A 2-fold variation was considered as significant (19).

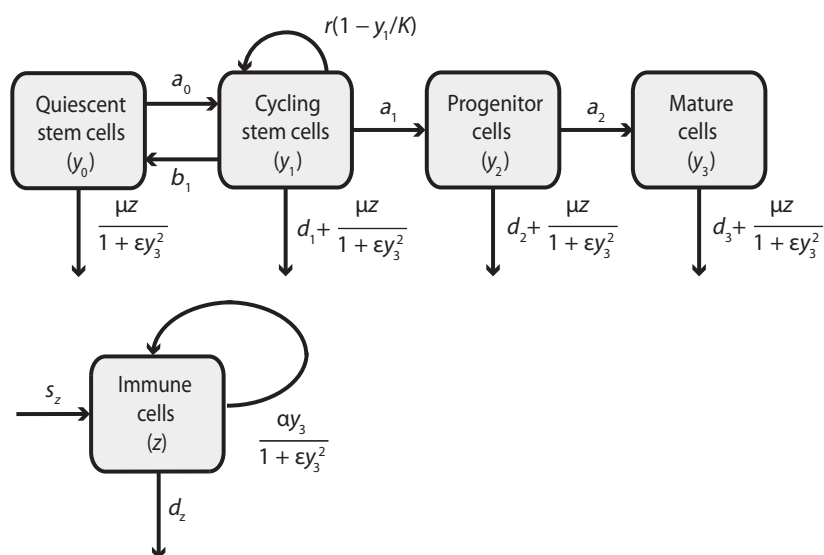
Our mathematical model divides leukemic cells into quiescent stem cells (y_0), cycling stem cells (y_1), progenitors (y_2), and mature cells (y_3). We also represent a single autologous immune cell population (z). For simplicity, we do not distinguish further between immune subpopulations. Leukemia cells stimulate immune cells to proliferate at a maximum rate α , while immune cells kill leukemia cells at a maximum rate μ . We incorporate immunosuppression by inhibiting the proliferation of the immune cells as well as their action on leukemic cells. Our model is summarized in Fig. 1. A more thorough description of the model is provided in the Quick Guide to Equations and Assumptions.

The *BCR-ABL* ratio is a blood measurement that quantifies the amount of *BCR-ABL* transcript relative to a control gene transcript, *BCR*, *GUS*, or *ABL* (here, *ABL*). Each leukemic cell possesses the *BCR-ABL* gene and the normal allele of the *ABL* gene, while healthy cells (x) possess two alleles of the *ABL* gene. Therefore, *BCR-ABL* transcripts are proportional to y_3 (the immature leukemia cell populations are much smaller than the mature population and can be neglected), while control transcripts are approximately proportional to $2x + y_3$. For simplicity, the number of healthy cells (x) is assumed to be constant and is estimated on the basis of the patient's initial *BCR-ABL* ratio at diagnosis. For all later measurements, the *BCR-ABL* ratio is approximated by

$$\text{ratio} \approx 100\beta \frac{y_3}{2x + y_3}$$

Figure 1.

Mathematical model including the intervention of the autologous immune system. In our model of CML and the immune response, we divide leukemic cells into quiescent stem cells (y_0), cycling stem cells (y_1), progenitors (y_2), and mature cells (y_3). Stem cells transition between quiescence and cycling, and some cycling stem cells differentiate into progenitors cells, which can further differentiate into mature cells. Leukemic cells can die naturally at rates d_i or as a result of an interaction with immune cells (z). Immune cells are supposed in this disease to be supplied at a constant rate s_z and to die at a rate d_z . They can also be stimulated by leukemic cells to divide to produce more immune cells. Large leukemic populations are able to suppress the autologous immune system by limiting immune cell expansion and limiting immune effector cells' ability to kill cancer cells.



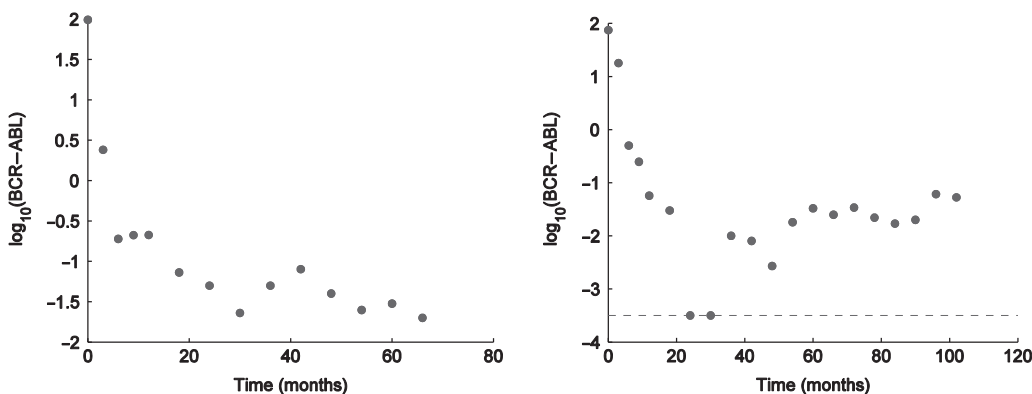


Figure 2. Oscillations of the BCR-ABL ratio in two representative patients. During TKI therapy, a patient’s progress is monitored by measuring their BCR-ABL ratio, which is a ratio of *BCR-ABL* mRNA expression to the expression of a control gene, in this case *ABL*. Both patients shown above were treated with standard imatinib 400 mg daily. During treatment, both patients showed multiple increases in BCR-ABL ratio without overt relapse. Here, dots represent clinical data, and the dashed line approximates the detection threshold or the lowest detectable leukemia level. Dots along this line indicate measurements of zero, meaning the leukemia was undetectable within the limits of the assay. These figures correspond to patients 4 and 12 in Supplementary Table S2.

The multiplication factor β accounts for differences in mRNA expression between *BCR-ABL* and the control gene. We multiply by 100, in order to convert the ratio into a percentage when $\beta = 1$ and a value between 0 and 100β otherwise.

Results

Many patients who otherwise respond well to therapy exhibit oscillations in their BCR-ABL ratios. Of the 104 patients in our dataset, only 15 showed monotonically decreasing BCR-ABL ratios throughout therapy. Each of the remaining 89 patients showed increases in BCR-ABL ratios in, on average, 28.82% of their measurements. Two representative patients are shown in Fig. 2. These fluctuations occurred in many patients who responded well to imatinib therapy and did not have any adverse events. This lack of monotonicity in patients who responded well to therapy motivated this study.

We applied our mathematical model, which is summarized in Fig. 1, to the patient data in order to study these oscillations. As previously mentioned, our model represents leukemic cells of varying maturity and a single immune cell population. We applied Latin hypercube sampling in order to determine the effect of the drug (a_1' and a_2') and the immune parameters (μ , d_z , α , and ϵ). The parameters d_z , α , and ϵ determine the patient’s immune window $[y_{min}, y_{max}]$, or the range of leukemia loads that will stimulate a strong immune response. We define $[y_{min}, y_{max}]$ by the range of γ_3 , for which the level of immune stimulation exceeds the death rate. For each patient, we selected the parameter set that minimizes the squared log distance between the patient data and

the results of the model simulation (sampled at the same time as the data). All other parameters were held constant across all patients; their values can be found in Supplementary Table S1. The patient-specific parameter values are summarized in Table 1. Figures 3 and 4 show representative fits of our model to patient data. (The patient-specific parameters producing these fits are provided in Supplementary Table S2.) Keeping in mind that these fits are plotted on a logarithmic scale, we see that our model is able to reproduce many patients’ dynamics during therapy.

The patient data and modeling results suggest that patients who respond well to imatinib therapy go through three to four phases of tumor reduction. During the first few months, there is a rapid exponential decline in BCR-ABL ratio. In our model, this effect is due primarily to the action of the drug on the mature leukemic population. The immune response is negligible at this stage because the large leukemic load suppresses the immune system. Beginning around month 6, there is a second, slower exponential decline in BCR-ABL ratio. In some patients, the second phase is a plateau in BCR-ABL ratio rather than a decline (see Fig. 3D). The location of this plateau is determined primarily by the direct effects of imatinib on the leukemic cell population (parameters inh_1 and inh_2). This biphasic exponential decline has been previously observed in refs. 20, and 21. A few patients show a triphasic exponential decline (Fig. 4B-D), which was discussed in ref. 22. The duration of the biphasic or triphasic decline can vary significantly between patients, from the first 2 years (Fig. 3A, C, D, and F) to several years of therapy (Fig. 4B-D).

After this period of monotonic decline, many patients’ leukemia loads begin to vary nonmonotonically. These fluctuations are

Table 1. Summary of parameter values used in our model simulations

Parameter	$\log(inh_1)$	$\log(inh_2)$	d_z	$\log(\mu)$	$\log(y_{min})$	$\log(y_{max})$	$\log(y_{max}/y_{min})$
Mean	1.132	2.487	0.099	-7.047	3.970	5.024	1.053
STD	0.830	0.754	0.097	0.636	0.888	0.804	0.499
Max	2.772	3.880	0.371	-5.896	5.483	6.024	2.006
Min	0.024	1.073	0.005	-7.954	2.548	3.226	0.197

NOTE: Of the 65 imatinib patients who did not relapse, develop drug resistance, or progress, 22 changed their imatinib dose during therapy. An additional 6 patients had non-international standard (non-IS) measurements, and 11 patients had five or fewer measurements. We focused on the remaining 25 patients and present the mean, standard deviation, maximum, and minimum parameter values.

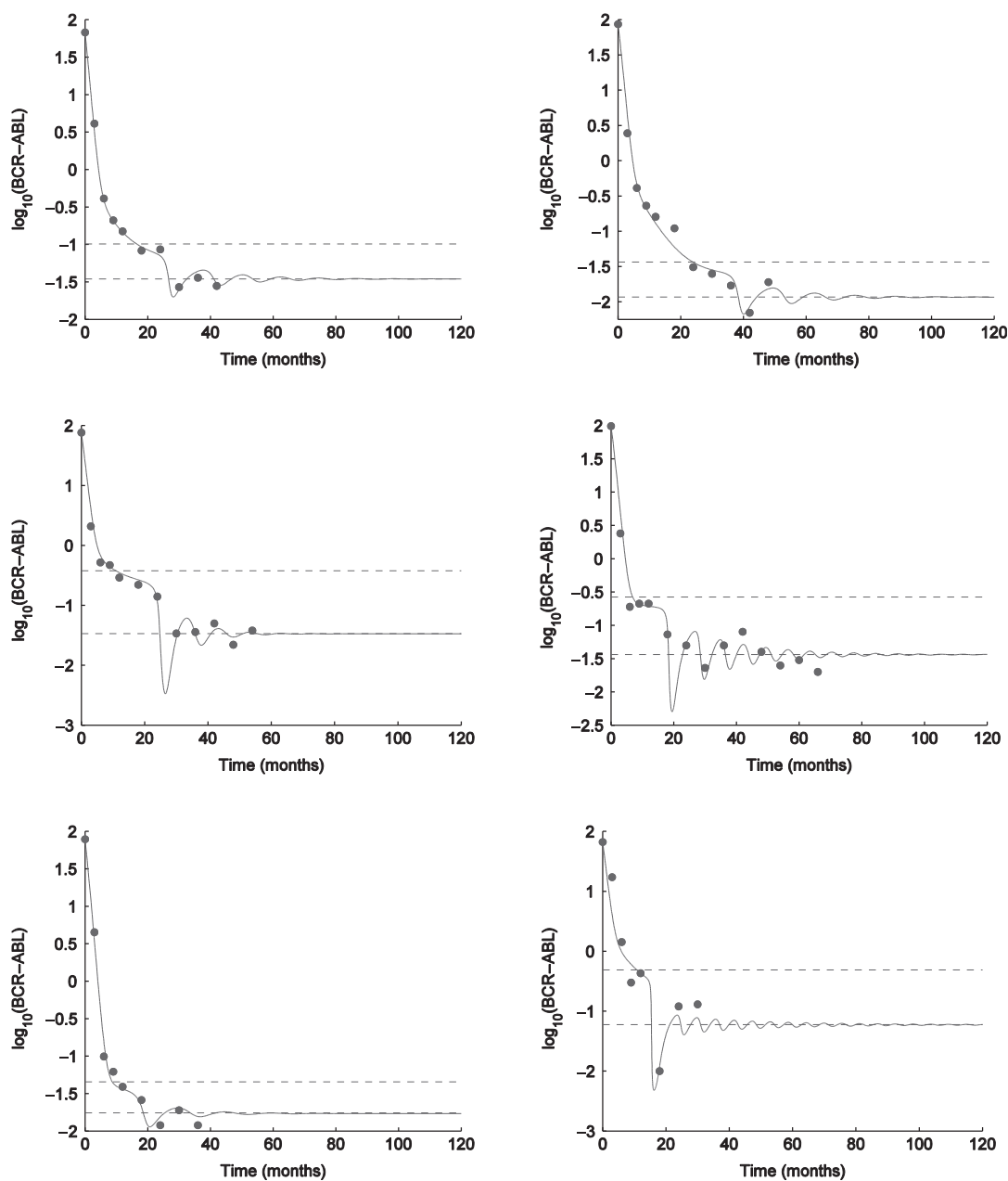


Figure 3.

Fits of our mathematical model to 6 representative patients. The base-10 log of the BCR-ABL ratio is plotted against time, in months. The dots represent patient data, and the solid lines represent our simulations. Dashed lines show the BCR-ABL ratios that correspond to the ends of immune window, y_{\min} and y_{\max} . These figures correspond to patients 1 to 6 in Supplementary Table S2.

often preceded by a sudden sharp decline in the leukemic population, as illustrated in Figs. 3F, 4B, D–F. If this effect is sufficiently strong, the leukemic stem cell population may be driven to less than one cell, which we interpret as cure in our model. Otherwise, the leukemic population is able to partially recover. Several oscillations in both the leukemic and immune cell populations follow, with their amplitudes decreasing over time as the populations approach an equilibrium, as seen in Fig. 5.

The patient-specific parameters are summarized in Table 1. Of the six parameters varied, the fits seem to be most sensitive to inh_1 and inh_2 , followed by y_{\min} and y_{\max} . The parameters d_z and μ seem to be less important. This is not surprising, as inh_1 and inh_2 determine the effect of the drug, and y_{\min} and y_{\max} determine at what point the autologous immune response becomes significant. Scatter plots depicting parameter sensitivities for a representative patient are shown in Fig. 6.

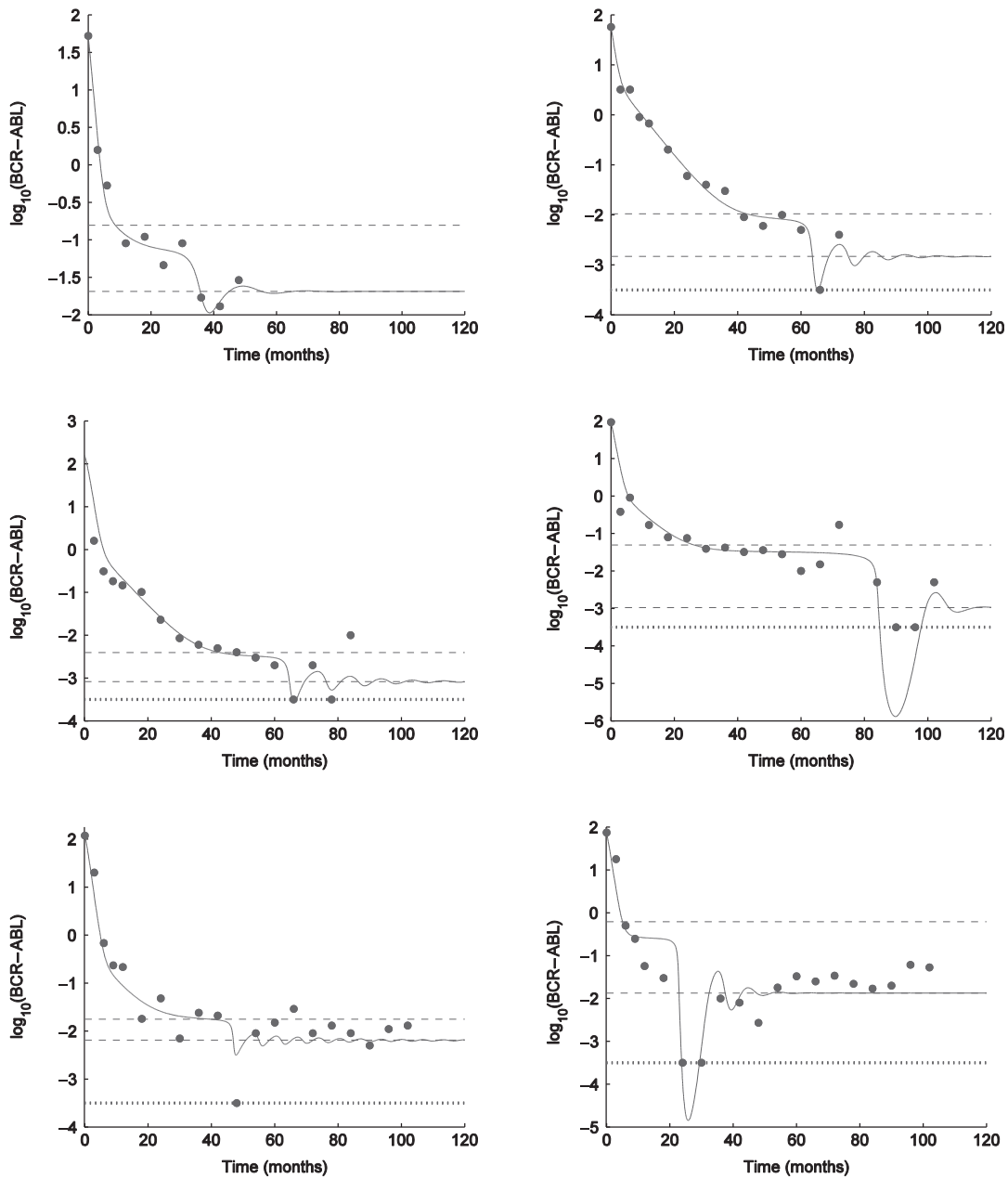


Figure 4.

Fits of our model to 6 additional patients. The base-10 log of the BCR-ABL ratio is plotted against time, in months. As in Fig. 3, the dots represent patient data, and the solid lines represent our simulations. Dashed lines show the BCR-ABL ratios that correspond to the ends of immune window, y_{min} and y_{max} . Dotted lines approximate the minimum leukemic level that is detectable by quantitative RT-PCR. Dots along this line represent zero measurements, meaning CML cells were not detected. These figures correspond to patients 7 to 12 in Supplementary Table S2.

The parameter values in Table 1 suggest that imatinib alone results in a 3.5-log decrease in the total leukemia load, on average (SD: 0.786, max: 5.158, min: 2.426). This effect is divided into a 2.5-log decrease in the proliferation of mature cells and a 1-log decrease in the proliferation of progenitors. Each patient's immune window covers approximately one order of magnitude of leukemic populations, generally falling between $10^{2.5}$ cells/mL

and 10^6 cells/mL. We assume an initial mature leukemic population of $1.5(10)^8$ cells/mL. Thus, imatinib must decrease the leukemia load by several orders of magnitude before the leukemia enters the immune window and an immune response is initiated. After the leukemic population enters this window, the leukemia and immune populations oscillate, with the amplitude of oscillations decreasing over time.

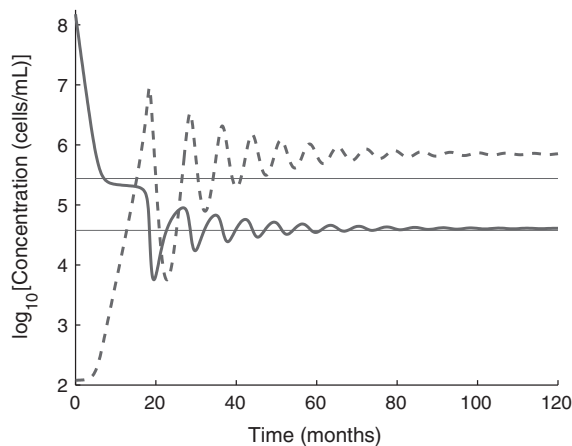


Figure 5.

Model simulation for a single representative patient. The base-10 log of the leukemia and immune cell populations, in cells/mL, are plotted as a function of time, in months. The thick solid line represents the total leukemic population ($y = y_0 + y_1 + y_2 + y_3$), and the thick dashed line represents the immune cell population (z). The thin solid lines show the immune window $[y_{\min}, y_{\max}] = [10^{4.58}, 10^{5.44}]$ cells/mL. For the first 20 months, the patient's leukemia load decreases monotonically, while the immune cells begin to expand. The leukemic population enters the immune window at around month 7. The immune cells mount an attack starting around month 18. This first attack results in the minimum leukemia load achieved during therapy, at around 10^4 cells/mL. The immune cells drive the leukemia load below the immune window, allowing the leukemic population to partially recover. The two populations oscillate with decreasing amplitudes as they approach their equilibrium concentrations of $(y, z) = (10^{4.61}, 10^{5.85})$ cells/mL. This simulation corresponds to the plot in Fig. 3D (patient 4 in Supplementary Table S2).

Discussion

Despite the success of imatinib and other TKI therapies, many questions about the underlying mechanisms of action remain. Mathematical modeling is a complementary tool to clinical and experimental data that can help us understand these mechanisms. Several mathematical modeling groups have already studied various aspects of CML (9, 20–26). We briefly review some of these contributions but note that a more thorough review can be found in ref. 27.

Michor and colleagues (20) constructed an ordinary differential equations (ODE) model of CML that divides leukemic cells into stem cells, progenitors, differentiated cells, and terminally differentiated cells. Upon analyzing patients' initial responses to imatinib therapy, they found that imatinib often leads to biphasic exponential declines in the leukemic cell populations. Their modeling results suggested that the first, steeper decline represents the action of imatinib on the differentiated leukemic cell population, while the second, slower decline represents an effect on the leukemic progenitors. They later hypothesized that long-term therapy leads to a triphasic exponential decline, where the third decline may represent an effect on immature leukemic cells and possibly leukemic stem cells (22).

On the other hand, Roeder and colleagues (21) developed an agent-based model of CML that divides leukemic stem cells into cycling and quiescent compartments. In their model, imatinib results in the degradation and inhibition of cycling leukemic stem cells while having no direct effect on quiescent leukemic

stem cells. They interpreted the biphasic exponential decline as an initial degradation effect, followed by a change in the regulatory response of leukemic stem cells that produces the second decline. A similar interpretation to the biphasic decline is proposed in ref. 24.

Although these modeling frameworks are capable of reproducing the dynamics of some patients during therapy, both are limited to those who show a monotonic decline in their leukemic burdens. Neither model includes a mechanism that would allow patients to show oscillations in leukemia load. However, in our data, we found that many patients who respond well to imatinib and achieve long-term remissions exhibit increases in leukemic burden. The fact that the Michor and Roeder models are unable to reproduce such oscillations suggests that there may be (an) additional mechanism(s) that contribute(s) to patients' dynamics during therapy.

Motivated by this, we developed a mathematical model that integrates CML and an autologous immune response. As previously mentioned, there is strong evidence that the immune system plays a role in the dynamics of CML (3–8, 15, 28, 29). In our modeling framework, we defined an immune window, or a range of leukemic loads that will provoke a strong autologous immune response. At diagnosis, the leukemic load is above this window, and the large leukemic population is able to partially or fully suppress the autologous immune system's response to CML. Imatinib therapy generally reduces a patient's leukemic load by several orders of magnitude, representing a significant reduction in immunosuppression. We hypothesize that imatinib may drive the leukemic population into the immune window, allowing a patient's autologous immune system to mount a response to CML.

In our model, oscillations in leukemic load occur after the leukemia enters the immune window. Without the autologous immune response, our model produces monotonically decreasing cancer loads, as seen in Supplementary Fig. S1. Once the autologous immune cells have expanded sufficiently, they attack the residual leukemic population. This first attack by the autologous immune system results in the minimum detectable leukemic load achieved during imatinib therapy. However, because the leukemia is driven below the immune window, the patient's immune cell population begins to contract. If the leukemia is not eradicated, it is able to rebound, until it reenters the immune window, thus stimulating another weaker immune response. The immune and leukemic cell populations continue to oscillate in this way, with the amplitude of these oscillations decreasing over time. Eventually, the oscillations dampen, and an equilibrium is achieved between the leukemic and autologous immune cells. Our modeling results suggest that oscillations in BCR-ABL ratio during therapy may be partially explained by the patient's autologous immune response to the residual CML population.

Moreover, the oscillations may be a signature of the autologous immune response that can be used to characterize a patient's individual immune system. This result is reminiscent of previous tumor-immune models, for example, Kuznetsov and colleagues (30).

On the basis of a patient's data over the course of TKI therapy, we determine their immune profile in the context of our model. Each patient's immune profile is different, as demonstrated by differences in the immune windows and in the timing and magnitude of the autologous immune response to CML. Our modeling framework provides a potential tool to help quantify

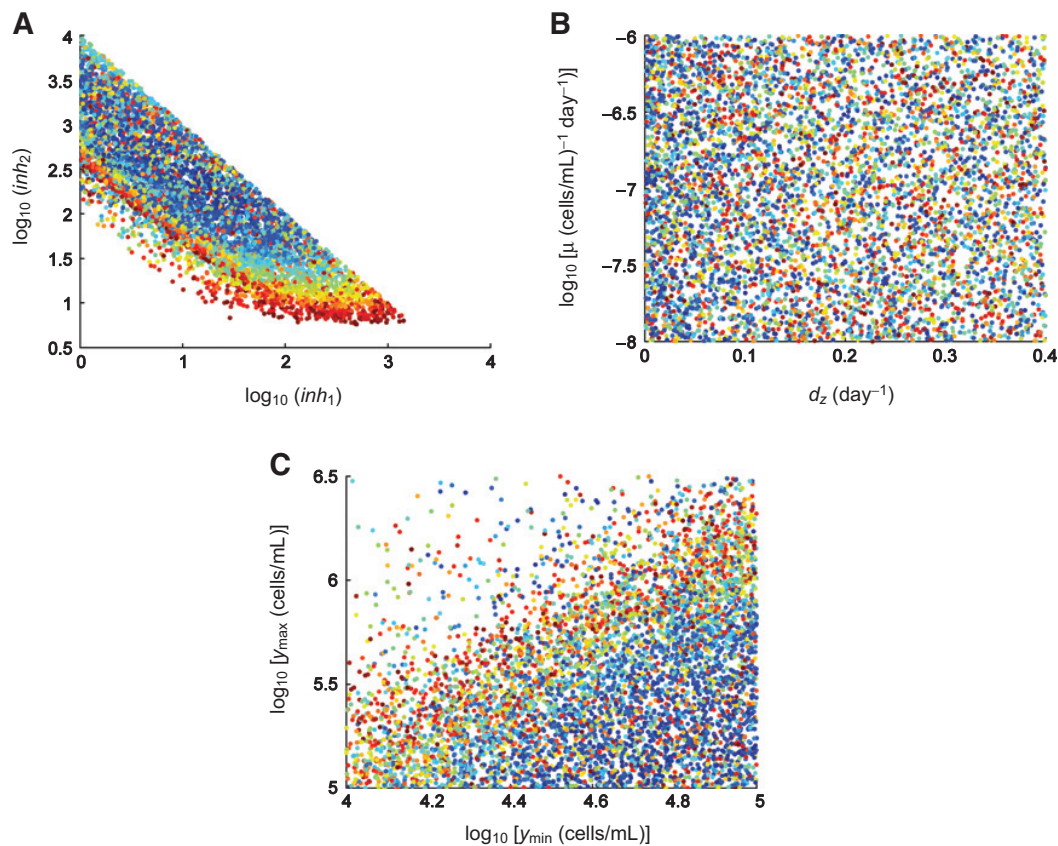


Figure 6.

Quality of the fit as a function of a pair of parameters, for a single representative patient. This is the same patient whose simulation is shown in Figs. 3D and 5 (patient 4 in Supplementary Table S2). Red dots indicate worse fits, and dark blue dots indicate better fits. Here, we only show simulations that resulted in a total cost of less than 10, where cost is the squared log distance between the patient data and model simulation. A, $\log(inh_2)$ versus $\log(inh_1)$. For this patient, inhibition values satisfying $\log(inh_1 \text{ } inh_2)$ in interval [2,4] were tested. These two parameters are strongly related to the quality of the fit. B, $\log(\mu)$ versus d_z . These two parameters seem to be the least important, as it is difficult to see any correlation between the fit and either of these parameters. C, Y_{\max} versus Y_{\min} . There is a definite relationship between the fit and these two variables. The worse fits tend to be in the upper left corner, while the better fits tend to be in the lower right corner. The parameters Y_{\max} and Y_{\min} determine the immune window and therefore affect the timing and magnitude of the autologous immune response.

these differences, which may play a significant role in designing personalized therapies or combination therapies aimed at further reducing or eradicating the residual CML burden. This framework will serve as a basis for future studies of treatment cessation and personalized combination therapies consisting of TKIs and immunotherapy.

Conclusion

The potentially significant role of the immune system in the dynamics of imatinib therapy suggests that immunotherapy may help to eliminate the residual leukemic burden. In our simulations, when imatinib therapy drives the leukemia into the immune window, an initially strong immune response occurs that weakens over time. Eventually, the immune cell population contracts, allowing the leukemia to partially recover. A combination of imatinib and immunotherapy may help to maintain a strong immune response, to prevent such a recovery in the leukemic population. As suggested in ref. 9, carefully timed vaccines may stimulate the patient's immune system when the

residual CML burden is no longer sufficient. A sustained immune response may result in a further decrease of the leukemic population and may even drive the leukemia to extinction. An optimal vaccine schedule would depend heavily on each patient's immune profile, and our model offers a tool for characterizing this.

Although we focus on the autologous immune response as a possible explanation of the oscillations that occur during imatinib therapy, many other factors may contribute to this behavior. The microenvironment of the leukemic cells is known to have a strong influence on both healthy and leukemic cells (31, 32), but is not included in our model. In addition, we do not account for patients who do not properly or regularly take their drugs, which is known to be an important factor (33). Moreover, for simplicity, we do not distinguish between various subtypes of immune cells, each of which may interact and play different roles in CML. Our model can be expanded in order to achieve a more accurate representation of the autologous immune response to CML. We leave this for a future work.

Still, the oscillations in patients' leukemic loads suggest an additional mechanism during therapy that has not previously

been included in mathematical models. Our modeling results support the hypothesis that the autologous immune system contributes to the dynamics of imatinib therapy. If this is the case, our model may serve as a valuable tool for characterizing a patient's immune response to CML. This immune profile may then help in designing personalized combination therapies in order to further control or eliminate the residual leukemic burden.

Disclosure of Potential Conflicts of Interest

F.E. Nicolini reports receiving a commercial research grant from Novartis Pharma; has received speakers bureau honoraria from Novartis Pharma, Bristol-Myers Squibb, and Ariad Pharmaceuticals; and is a consultant/advisory board member for Novartis Pharma and Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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Any opinions, findings, and conclusions or recommendations expressed in this article are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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