Chronic Myeloid Leukemia: Origin, Development, Response to Therapy, and Relapse

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Abstract

Background: The introduction of imatinib, the first of a family of *abl* kinase inhibitors, opened a new era in the therapy of chronic myeloid leukemia (CML). The majority of treated patients achieve complete cytogenetic response, although the disease is often detectable by molecular techniques. **Materials and Methods:** Using a mathematical model for the architecture of hematopoiesis and progression of disease as well as clinical data, we develop a unified framework that models the origin and clonal expansion of CML, the response to *abl* kinase inhibitors, and relapse upon cessation of therapy. **Results:** The model predicts that a small pool of mutated stem cells is enough to drive CML. Inhibition of the *abl* kinase decreases the self-renewal capability of CML progenitors, altering their fitness compared with normal progenitors. Persistence of CML progenitors, however, is responsible for the rapid relapses observed upon cessation of therapy. We demonstrate how the architecture of hematopoiesis plays an instrumental role in growth of the CML clone and its response to treatment. **Conclusion:** A small pool of stem cells is enough to drive the chronic phase of CML. Imatinib reverses the fitness advantage of CML cells, allowing return of normal hematopoiesis in most patients. Persistence of CML progenitor cells seems to be responsible for the observed relapse kinetics.

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Key words: Abl kinase inhibitors, Biomathematical modeling, Hematopoiesis, Imatinib, Stem cells

Introduction

Chronic myeloid leukemia (CML) is one of the classic myeloproliferative disorders[1,2] that serves as a paradigm in hematology and oncology. The disease is characterized by the Philadelphia chromosome,[3] where the *c-abl* proto-oncogene, normally present on chromosome 9, is translocated to the major breakpoint cluster region (*bcr*) on chromosome 22. The result is the formation of the *bcr-abl* fusion gene and its aberrant expression as an oncoprotein.[4] This oncoprotein is the target of imatinib, the first of a series of recently designed reversible *abl* kinase–inhibiting drugs[5] that has high response rates in patients with this disease.[6,7] There is still some controversy regarding whether *bcr-abl* alone is responsible for the development of the disorder.[8] Recent work on the age-specific incidence of CML[9] and animal models suggest that aberrant expression of *bcr-abl* in hematopoietic stem cells (HSCs) might be enough to explain the chronic phase of the disease.[10,11] Chronic myeloid leukemia is a true HSC disorder because *bcr-abl* is found in both myeloid and lymphoid cells, including a small fraction of T and natural killer cells.[12] A characteristic feature of CML is expansion of granulocyte production with extramedullary hematopoiesis, leading to splenomegaly and an increase in the circulating number of granulocytes and their precursors. Normal marrow output in an adult is approximately 3.5×10^{11} cells per day, while output in patients with CML often exceeds 10^{12} cells per day,[8] a 3-fold increase.

The current consensus is that the HSC pool is not expanded in CML.[13,14] However, it has been shown that *bcr-abl* induces the production of interleukin (IL)–3 and granulocyte colony-stimulating factor (G-CSF) by progenitor cells that might act in an autocrine fashion to enhance self-renewal of more mature progenitors, leading to progenitor cell expansion.[15] Moreover, it is known that the combination of IL-3 and G-CSF can enhance the limited self-renewal

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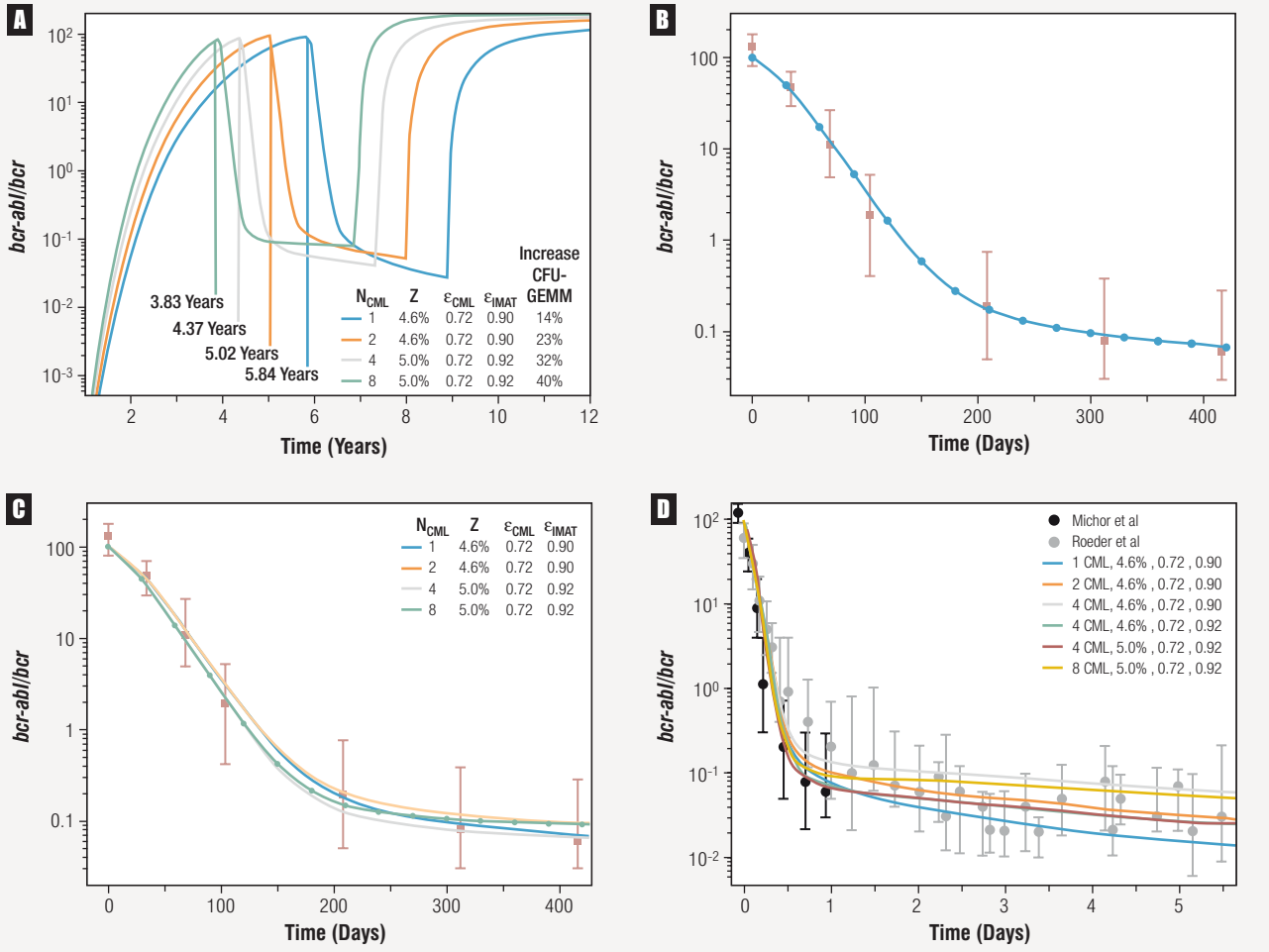
Figure 1 Chronic Myeloid Leukemia Dynamics Before and During Imatinib Therapy



(A) The time interval from initiation to full-blown disease depends on the number of CML stem cells (N_{CML}), which drive the disease; time to diagnosis decreases as N_{CML} increases. A larger N_{CML} also leads to a less than optimal depth of response to imatinib. **(B)** Our model was fitted to experimental data for *bcr-abl/bcr* obtained from cohorts of patients with CML treated with imatinib. We consider the data from Michor et al.[30] Solid squares and error bars represent the median and quartiles, respectively, for a given time point after the start of therapy. Solid circles joined with a dashed line represent the best fit obtained for $\varepsilon_{CML} = 0.72$ and $\varepsilon_{IMAT} = 0.90$, with 4.6% of the cells responding to imatinib, CML being driven by a single CML stem cell. **(C)** The model is robust with very little change in parameter estimates as N_{CML} is varied from 1 to 8. **(D)** Fitting of an independent dataset from Roeder et al[32] using the parameters obtained in **(C)** confirms the robustness and validity of the model.

capability of hematopoietic progenitor cells.[16] Therefore, current evidence suggests that, while CML arises in the HSCs, it is driven by progenitor cell expansion because of a higher probability of limited self-renewal of these cells.[17,18] In addition, when colony-forming unit generating granulocyte-macrophage isolated from patients with CML are exposed to pharmacologically achievable concentrations of imatinib in vitro, they display a decrease in self-renewal capability down to normal or subnormal levels.[19] A major difference between HSCs and progenitor self-renewal is that HSCs can self-renew for much longer than progenitor cells; hence, HSCs contribute to hematopoiesis for a long time (possibly the lifetime of the mammal), while progenitor cells contribute to hematopoiesis for a few weeks to months at most. There is still considerable controversy over whether imatinib actually induces cell death in the most primitive CML cells.

Under normal conditions, hematopoiesis can be metaphorically represented by a multi-compartmental model in dynamic equilibrium in which cells "move" from one compartment to the next as they become increasingly differentiated.[20] In a healthy adult, approximately 400 HSCs, which replicate on average once per year,[21,22] are responsible for the daily marrow output of approximately 3.5×10^{11} cells. In this article, we investigate how CML perturbs this system in order to understand clonal expansion as well as the response to therapy and relapse upon cessation of treatment.

Materials and Methods

Allometric scaling and experimental evidence suggest that hematopoiesis is maintained by an active pool of approximately 400 HSCs in healthy adults. These cells replicate approximately once per year, yet they are responsible for a normal marrow output of approximately 3.5×10^{11} cells per day. A recently developed multi-compartment model of hematopoiesis[20] has linked the slow replication of the HSCs with the high cellular output of the bone

marrow. In this model, cell division in any compartment i leads to 2 daughter cells that are transferred to the next downstream compartment ($i + 1$, compatible with differentiation) or that retain the properties of their parent and stay in the same compartment (self-renewal). The probability of differentiation is ε, whereas the probability of self-renewal is given by $1 - \varepsilon$. Both probabilities are considered to be constant across normal hematopoiesis. The normal value of ε was determined as $\varepsilon_0 = 0.85$. In the case of normal hematopoiesis, we could estimate the total number of divisions (K) that link the HSC with the circulating compartment as well as the exponential increase in replication rate that occurs between compartments ($r \approx 1.26$). Our estimate for K (approximately 31) is compatible with previous predictions,[23-25] while the estimated values of ε and r proved robust with respect to changes in the number of HSCs contributing to hematopoiesis, suggesting they are characteristics of hematopoiesis. The parameters of this model for healthy individuals were determined using data from polymorphonuclear leukocyte production, the number of active HSCs, the average output of the bone marrow, and the rates of cell division of stem cells and granulocyte precursors.[20]

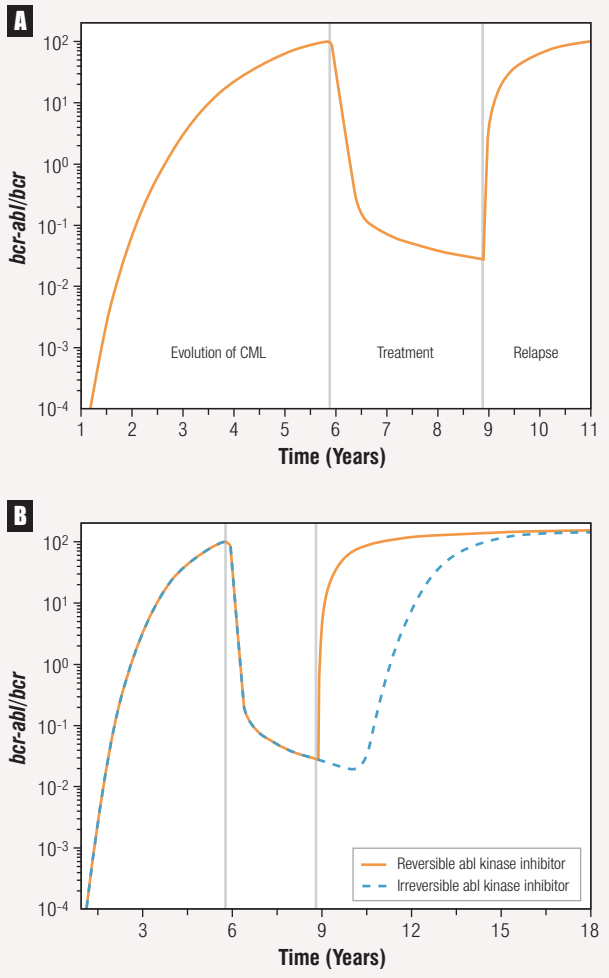
Model Constraints

The total number of active HSCs in patients with CML is not expanded,[13,14] but there is an increase in the number of myeloid progenitors by > 14%[26] because of higher self-renewal ($\varepsilon_{CML} < \varepsilon_0$).[17,27] We assume that whenever the marrow output exceeds 10^{12} cells per day, CML is diagnosed,[8] and treatment starts long before hematopoiesis reaches a stationary state under the reduced ε. The only known external hazard for the development of CML is radiation exposure, and from observations of the population exposed to the Hiroshima nuclear detonation, it appears that the time for the disease to become clinically evident ranges from 3.5 years to 6 years.[28] We use these experimental observations to constrain our parameter fits.

Disease Progression

Although the number of cells in compartment i, N_i, is an integer, we can approximate the average dynamics by a differential equation. During hematopoiesis, the number of cells in each compartment $i \geq 1$ changes as: (1) $\dot{N}_i = -d_i \times N_i + b_{i-1} \times N_{i-1}$ where $d_i = (2\varepsilon - 1)r_i$ represents the rate at which cells leave compartment "i" and $b_{i-1} = 2 \times \varepsilon \times r_{i-1}$ represents the rate at which cells originating from compartment "$i - 1$" are injected in compartment "i." The stationary state $\dot{N}_i = 0$ leads to the model of normal hematopoiesis.[20] In this case, N_0 is constant and given by $N_0 \approx 400$,[22] leading to an influx of $r_0 \times 2 \times \varepsilon \times N_0$ into the first compartment. Experimental data suggests that, even in CML, N_0 is not expanded; hence, we assume it remains approximately 400.[13,14] A mutation in the HSC compartment leads to one cancer stem cell, in our case a CML stem cell, and $N_0 - 1$, normal HSC. In such a scenario, we have to consider 2 different systems of equations: equation (1) for normal cells with $\varepsilon_0 = 0.85$ and a second equation, formally identical to the first, but for CML cells (N_i^{CML}) with $\varepsilon_{CML} < \varepsilon_0$. The dynamics of disease expansion are shown in Figure 1A and corresponds to the initial growth of the *bcr-abl/bcr* ratio.

Figure 2 Myeloproliferation in the Presence of CML and Response to Therapy



(A) Starting with 1 CML stem cell, the disease takes almost 6 years to be clinically diagnosed (same parameters as in Figure 1A). When therapy with imatinib starts, the characteristic 2-slope response emerges as a consequence of the underlying architecture of the bone marrow. However, CML progenitors persist and, if imatinib is stopped after 3 years of therapy, relapse occurs rapidly, being driven by the progenitors; hence, the steeper slope compared to the initial growth of the disease. **(B)** Therapy with an irreversible *abl* kinase inhibitor will lead to a longer time to relapse when therapy is stopped; relapse occurring also at a slower rate.

Increased Cell Replication

The model allows us to estimate the average number of divisions C that a cell experiences during its trajectory from the stem cell compartment to the peripheral blood. If the number of compartments for normal hematopoiesis is given by K, then it follows that $C \geq K$. Let $D = C - K$. A cell in compartment i moves to compartment $i + 1$ with probability ε, or it makes an amplification step with probability $1 - \varepsilon$. The probability that a given cell undergoes D divisions that do not lead to the next compartment is given by a Poisson distribution with a characteristic parameter $\lambda = K(1 - \varepsilon)$: $P(D) = \frac{\lambda^D}{D!} e^{-\lambda}$.

Therefore, $C = K + [P(D)] = K + K(1 - \varepsilon)$. Hence, CML cells with a decreased ε will on average undergo more cell divisions (> 4), as indicated by experimental observations.[29]

Treatment with Abl Kinase Inhibitors

We simulate the effect of, eg, imatinib by modifying ε_{CML} to normal (or supra-normal) levels in the CML cells to which the drug binds. In this way, the advantage of the clone is lost.[19] Mature cells are not sensitive to the effects of the drug and simply undergo apoptosis as dictated by their natural life history. The tumor burden in CML is monitored using the *bcr-abl* to *bcr* ratio, determined via quantitative real-time polymerase chain reaction (RT-PCR), such that reductions in the absolute amount of *bcr-abl/bcr* detected serially in patients are considered to infer a decrease in tumor burden.

During imatinib therapy, a fraction, z, of CML cells respond to treatment. Thus, the number of CML cells in compartments $i \geq 1$, change as:

$$\dot{N}_i^{CML} = -(1-z) \times d_i^{CML} \times N_i^{CML} - z \times d_i^{IMAT} \times N_i^{CML} + (1-z) \times b_{i-1}^{CML} \times N_{i-1}^{CML} + z \times b_{i-1}^{IMAT} \times N_{i-1}^{CML}$$

where the definitions of d and b remain the same, except that ε must be replaced by the appropriate one for the *IMAT* (imatinib) and *CML* coefficients, respectively.

In our model, we obtain ε_{CML}, ε_{IMAT}, and z from a least squares fit to clinical data for *bcr-abl/bcr*.[30] In principle, our model has a total of only 7 parameters: The differentiation probabilities for the 3 types of cells, the increase in the replication rate $r = r_i/r_{i-1}$, the fraction of cells responding to treatment z, the number of compartments K, and the number of mutated stem cells. However, 3 of these parameters, ε_0, K, and r, are fixed from the outset based on data obtained in healthy individuals.[20] Fitting the model to the available clinical data for *bcr-abl/bcr* from the study of Michor et al[30] fixes ε_{CML}, ε_{IMAT}, and z; hence, we are left with a single undetermined parameter: the number of mutated stem cells.

We analyzed our model for a different number of mutated stem cells in compartment 0, taking into account that *bcr-abl* expression increases self-renewal in progenitor cells[19] but not in the HSCs.[31] The results of the best fits of z, ε_{CML}, and ε_{IMAT} are presented in Figure 2A for the 3 phases of the disease: before, during, and after imatinib treatment. Finally, we tested the model and parameter values obtained previously on an independent dataset published by Roeder et al[32] that has the advantage of up to 6 years of follow-up. A very good agreement is obtained, as shown in Figure 1D.

Results

Disease Progression

Details about the model are provided in the Materials and Methods section. We start from the view of normal hematopoiesis as a hierarchical multi-compartmental structure connecting HSCs to terminally differentiated cells. In each compartment, cells stochastically differentiate with the same probability ε_0 or self-renew otherwise. Chronic myeloid leukemia originates in the aberrant expression of the *abl* kinase, which takes place in some stem cells (CML stem cells). This acts to modify the probability of differentiation of the CML cells (ε_{CML}) across compartments. Hence, our free parameters are the number of CML stem cells that drive the disease, the probability of differentiation of the CML cells (ε_{CML}), and the impact of therapy (eg, via imatinib) on this differentiation probability (ε_{IMAT}). We fix the model parameters via a fit to the available experimental data for *bcr-abl* by quantitative RT-PCR from Michor et al,[30] shown in Figure 1B. The results indicate that CML cells exhibit a differentiation probability $\varepsilon_{CML} = 0.72$, while normal hematopoietic cells have $\varepsilon_0 = 0.85$.[20] Starting from a single mutated stem cell, it takes approximately 5.8 years for full-blown disease to appear, associated with a marrow output > 10^{12} cells per day. As the number of CML stem cells increases, the time required for the disease to become clinically diagnosed decreases, with 8 neoplastic stem cells giving rise to the disease in 3.8 years (Figure 1A). This is compatible with the data from Hiroshima after the nuclear weapon detonation in 1945.[28] The best fit to the data was obtained when the number of active CML stem cells was 1-2 cells.

The condition $\varepsilon_{CML} < \varepsilon_0$ leads to an increased self-renewal capability of CML mutated cells. This is supported by experimental data showing a higher frequency of self-renewal in CML progenitors[19] and compatible with the concept of an enhanced fitness for mutations that increase the self-renewal capability of cells.[33] In addition, the parameters in Figure 1A, in which CML is initially driven by a single mutated stem cell, imply that patients with CML have a colony-forming unit generating granulocyte, erythrocyte, macrophage, and megakaryocyte (CFU-GEMM) pool that is expanded by approximately 14% compared with healthy adults, in excellent agreement with the data from.[14,26] Although slight changes in ε alter the kinetics of the disease (Figure 1C), the overall features remain unchanged, showing the robustness of the model.

Imatinib Treatment

In this section, we refer explicitly to imatinib, given that we are comparing our model results with experimental data involving patients treated with this drug. However, most of our results should also apply to other *abl* kinase inhibitors. Despite their increased potency and broader spectrum of inhibition, they should induce a qualitatively similar response of the hematopoietic system even in patients with imatinib-resistant disease.

At any time step, imatinib is taken up by normal and CML cells. It is active only in *bcr-abl*–expressing cells, where it modifies the value ε_{CML} in a fraction of the cells. Imatinib increases ε_{CML} to supra-normal levels, $\varepsilon_{IMAT} > \varepsilon_0 > \varepsilon_{CML}$ (Figure 1C), giving normal cells a fitness advantage.[19] In this respect, it is noteworthy that imatinib is the first drug in the history of cancer therapy that reduces the fitness of mutant cells compared with normal cells, enabling the latter to dominate most compartments and return hematopoiesis to normal or near-normal levels. Circulating cells have a finite life-time and are continuously being washed out. Therefore, the response to imatinib is also determined to a great extent by the underlying architecture and dynamics of hematopoiesis. As a result, the disease burden decreases by 3-4 log (Figures 1B and 1D), as observed clinically.[7,30] The steep slope in the response to imatinib is determined by the efficacy of the interaction between the drug and CML cells. Our model suggests that, at any time, approximately 5% of the cells respond to imatinib. A higher fraction of cells responding to the drug leads to a faster rate of decay. Such behavior is expected to take place with more recent drugs, such as nilotinib or dasatinib, which are known to bind more effectively to the *abl* kinase.[34]

Despite therapy, CML progenitors persist (albeit with a lower ε). As long as resistance to imatinib does not develop, the disease burden decreases (Figures 1 and 2) until it reaches a plateau. The level of this plateau depends on (1) the relative advantage of normal progenitor

cells compared with their CML counterparts in the presence of imatinib and (2) the number of CML stem cells that drive the disease.

The same parameter estimates obtained based on the data of Michor et al[30] are also compatible with the data reported by Roeder et al,[32] for an independent set of patients with CML treated with imatinib (Figure 1D) but with a (> 5 times) longer follow-up. Note that the values obtained for ε_{CML} and ε_{IMAT} (the disadvantage given to CML progenitors by imatinib) do not vary significantly as the pool of CML stem cells expands from 1 to 8 (Figure 1C), compatible with the fact that these 2 parameters are characteristics given to the cell because of *bcr-abl* expression in the absence and presence of imatinib, respectively.

Relapse After Cessation of Therapy

The model predicts that, when therapy is stopped, relapse occurs rapidly (Figure 2A), as observed clinically.[30] This suggests that the rapidity of relapse reported by Michor et al is based on a persistent pool of CML progenitors that rapidly takes over hematopoiesis when treatment is withdrawn. The reversible interaction between *abl* kinase inhibitors and *bcr-abl* allows CML cells to regain their enhanced fitness compared with normal progenitors when therapy is stopped. These observations also explain why relapse kinetics occurs promptly and is much faster compared with the initial growth of the tumor before therapy: the drug does not simply reduce the tumor burden and reset the clock. Results show that, if treatment is given for 3 years with an excellent response and then withheld, the disease burden will reach pre-therapy levels within approximately 2 years in the absence of acquired resistance to the drug (Figure 2A), although relapse will be detectable much earlier at the molecular level. Without CML progenitors, the process would take approximately 4 years. On the other hand, the reversible nature of imatinib (as well as other *abl* kinase inhibitors, albeit exhibiting longer binding times) is also directly responsible for the rapidity of the overall relapse upon cessation of therapy. Indeed, for an irreversible *abl* kinase inhibitor, a considerable delay in the time to relapse is predicted, as shown in Figure 2B.

Discussion

In this work, we propose a unified framework for the dynamics of CML to include the expansion of the clonal population from a single mutant cell and the response to therapy using *abl* kinase inhibitors as well as relapse when treatment is withdrawn. To this end, we start from a hierarchical, multi-compartmental description of hematopoiesis and investigate the way in which CML cells differ from normal cells and how they influence the dynamics of hematopoiesis. Subsequently, we studied how *abl* kinase inhibitors influence the overall dynamics of CML. The model is compatible with the available experimental data on this disorder. While the total number of active HSCs in CML is not expanded,[14] there is an increase in the number of myeloid progenitors.[26] Expression of *bcr-abl* in progenitor cells enhances their self-renewal,[27] resulting in an increase in the size of each subsequent compartment: The effect is slowly transmitted and amplified throughout all downstream compartments with the result that the total daily marrow output increases. For the purposes of our model and in the absence of available quantitative data, we assume that, similar to normal hematopoiesis, CML-mutated cells express the same ε_{CML} in all compartments. This may be modified when experimental data become available. Moreover, our results do not critically depend on it, while the clinical data are compatible with an overall reduction of ε.

Our work shows that the disease can be driven by a very small pool of *bcr-abl*–positive HSCs that replicate slowly. Indeed, one CML stem cell might be enough to drive the disorder. *Bcr-abl* does not give a fitness advantage to the CML stem cell[31] within the most primitive pool, so expansion of this cell lineage within the HSC pool will proceed by neutral drift. However, whenever the CML stem cell pool expands, the time for diagnosis gets progressively shorter. The model illustrates how the architecture and dynamics of hematopoiesis enable the progeny of CML stem cells to dominate hematopoiesis and how therapy itself benefits from the same architecture to control the disease. Given the small number of CML stem cells driving the disease, one expects it will be very unlikely to eradicate these cells. Simple mass action considerations show how difficult it is for imatinib or other *abl* kinase inhibitors to interact effectively with such a small population of cells. The enhanced fitness bestowed by *bcr-abl* expression in CML progenitors is manifested as a higher self-renewal capability that leads to myeloproliferation and domination of hematopoiesis by the CML clone. The higher self-renewal capability of CML progenitors ($\varepsilon_{CML} < \varepsilon_0$) also results in a higher number of cell divisions for the clonal cells compared with controls, leading to shorter telomeres, as reported by Brummendorf et al.[29] The model predicts that, all else being equal, patients with a smaller pool of CML stem cells will have a larger reduction in tumor burden compared with those with a larger number of active CML stem cells. CML stem cell expansion by neutral drift within the active stem cell pool[35] provides a potential explanation for the variability in the depth of responses observed clinically despite the lack of mutant (drug-resistant) cells.

Tumor cells can acquire resistance to imatinib, an observation that fuelled the introduction of novel agents such as dasatinib and nilotinib to control most of the imatinib-resistant mutants of *bcr-abl*.[34,36] Nilotinib and dasatinib are more potent than imatinib in inhibiting the *abl* kinase.[34] Our model predicts that therapy with these agents should lead to a faster decline in the tumor burden with a higher fraction of cells responding to therapy ($z > 5\%$). However, the reduction of disease burden will depend on how much the drug is capable of reversing the value of ε_{DRUG} with respect to normal (ε_0). Simulations for a hypothetical drug that irreversibly inhibits abl (Figure 2B) suggest that the benefit of such an approach will be to substantially delay relapse of the disease together with a slower rate of disease expansion. Therapeutic approaches that lower the number of CML stem cells will be necessary to reduce the disease burden further.

The model presented here is not the first mathematical model for CML progression and response to imatinib. In a seminal paper, Michor et al[30] presented the first approach to tackle this problem. Shortly after, Roeder et al[32] pointed out some potential problems with this model and proposed a more elaborate approach. The work presented here is a step further, as the model is based on the architecture of hematopoiesis in healthy individuals. One remarkable feature of chronic phase CML is that, while marrow production is enhanced, the function of the produced cells is

fairly normal. The previous models[30,32] did not explicitly discuss marrow expansion while, in our model, myeloproliferation under normal cell replication rates occurs naturally. Furthermore, both models are based on the "2-slope" decay of the *bcr-abl/bcr* ratio in response to imatinib. This has been interpreted as a consequence of 4 operational compartments that comprise hematopoiesis: HSCs, progenitor cells, differentiated cells, and mature cells. The parameters of this 4-compartment model of hematopoiesis were adjusted to reproduce the "2-slope" decay for 1 year of therapy. Perhaps unsurprisingly, longer follow-up treatments cannot be explained without extending this model.[30] In fact, a close look at individual patient data indicates that the 2-slope proposition might not reflect the response to therapy well enough. This notwithstanding, yet another phenomenologic model of CML has been introduced by specifically imposing a 2-slope decay in response to imatinib.[32] As a result, the authors conclude that stimulating CML stem cells to replicate would increase imatinib sensitivity. However, stimulating stem cell proliferation could increase the risk of acquired resistance to imatinib with failure of therapy. A study combining G-CSF with imatinib therapy was initiated in patients with CML,[37] based on this model. The study was stopped because of a lack of benefit, and a potential for harm could not be excluded.[38]

Both previous models assume that relapse is driven only by CML stem cells. Here, we propose that the fast relapse dynamics, often observed after stopping therapy, are because of the persistence of a significant number of CML progenitors. Recently, Rousselot et al[39] reported that some patients have not experienced relapse after a median of 18 months of stopping imatinib. Our model predicts that relapse can occur up to 4 years after stopping therapy. Consequently, we believe that it is premature to assume that patients are actually cured. Moreover, the fact that a significant number of patients in that report had received previous treatment with interferon-α (an agent that is known to induce delayed therapeutic effects and that can cure some patients with this disease) limits what can be inferred about the effect of imatinib in this group of patients.

Conclusion

Our model shows that the "2 slopes" emerge because of the combined effect of drug efficiency and the fact that only a fraction of the cells are responding to imatinib at any time. The model originates from a compartmental architecture of hematopoiesis that, under normal conditions, maintains a stochastic dynamic equilibrium between adjacent compartments, compatible with current thinking of hematopoiesis as a stochastic process.[40,41] It predicts that newer *abl* kinase inhibitors with a higher affinity for the kinase will lead to faster responses. Finally, for the first time, it was possible to assess how many active stem cells might actually drive a tumor. Our prediction of a small number of cells is in keeping with the clonal nature of a tumor as well as recent experimental data proving that a single cancer stem cell can lead to full tumor development in an animal model.[42] The apparent ease with which CML cells can be engrafted in immunodeficient mice to induce a disease similar to CML might suggest that the pool of CML stem cells is large. However, recent results show that such engrafted cells disappear after a few weeks, suggesting that the CML cells injected were not stem cells. The difficulty in successfully identifying CML stem cells correlates with our results, which predict that the number of CML stem cells is small.[43]

Abl kinase inhibitors reverse the fitness advantage of CML cells, inducing characteristic response profiles under therapy. When the fitness of CML progenitors is reduced, normal progenitors take over hematopoiesis with the normalization of blood counts and elimination of the Philadelphia chromosome by fluorescence in situ hybridization.[6,7] However, CML progenitors (not simply CML stem cells) persist and are responsible for rapid relapse when treatment is stopped, even in the absence of acquired resistance to the drug. If relapse was simply driven by the persistence of CML stem cells, the time taken for relapse would be significantly longer. Our work suggests that *abl* kinase inhibitors alone might not be able to eliminate CML; however, by reducing the disease burden, they might enhance the anti-CML immune response that could suppress the disease even further and perhaps operationally cure some patients.

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References

1. Fialkow PJ, Jacobson RJ, Papayannopoulou T. Chronic myelocytic leukemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. *Am J Med* 1977; 63:125-30.
2. Goldman JM. Chronic myeloid leukemia-still a few questions. *Exp Hematol* 2004; 32:2-10.
3. Rowley JD. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973; 243:290-3.
4. Groffen J, Stephenson JR, Heisterkamp N, et al. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. *Cell* 1984; 36:93-9.
5. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 1996; 2:561-6.
6. Druker BJ, Guilhot F, O'Brien SG, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med* 2006; 355:2408-17.
7. Hughes TP, Kaeda J, Branford S, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med* 2003; 349:1423-32.
8. Holyoake TL, Jiang X, Drummond MW, et al. Elucidating critical mechanisms of deregulated stem cell turnover in the chronic phase of chronic myeloid leukemia. *Leukemia* 2002; 16:549-58.
9. Michor F, Iwasa Y, Nowak MA. The age incidence of chronic myeloid leukemia can be explained by a one-mutation model. *Proc Natl Acad Sci U S A* 2006; 103:14931-4.
10. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* 1990; 247:824-30.
11. Zhao RC, Jiang Y, Verfaillie CM. A model of human p210(bcr/ABL)-mediated chronic myelogenous leukemia by transduction of primary normal human CD34(+) cells with a Bcr/Abl-containing retroviral vector. *Blood* 2001; 97:2406-12.
12. Martin PJ, Najfeld V, Hansen JA, et al. Involvement of the B-lymphoid system in chronic myelogenous leukaemia. *Nature* 1980; 287:49-50.
13. Udomsakdi C, Eaves CJ, Swolin B, et al. Rapid decline of chronic myeloid leukemic cells in long-term culture due to a defect at the leukemic stem cell level. *Proc Natl Acad Sci U S A* 1992; 89:6192-6.
14. Jamieson CH, Ailles LE, Dylla SJ, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med* 2004; 351:657-67.
15. Jiang X, Lopez A, Holyoake T, et al. Autocrine production and action of IL-3 and granulocyte colony-stimulating factor in chronic myeloid leukemia. *Proc Natl Acad Sci U S A* 1999; 96:12804-9.
16. Marley SB, Lewis JL, Gordon MY. Progenitor cells divide symmetrically to generate new colony-forming cells and clonal heterogeneity. *Br J Haematol* 2003; 121:643-8.

17. Marley SB, Gordon MY. Chronic myeloid leukaemia: stem cell derived but progenitor cell driven. *Clin Sci (Lond)* 2005; 109:13-25.
18. Gordon MY, Marley SB, Lewis JL, et al. Treatment with interferon-alpha preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors (CFU-GM) from patients with chronic myeloid leukemia but spares normal CFU-GM. *J Clin Invest* 1998; 102:710-5.
19. Marley SB, Deininger MW, Davidson RJ, et al. The tyrosine kinase inhibitor STI571, like interferon-alpha, preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors from patients with chronic myeloid leukemia. *Exp Hematol* 2000; 28:551-7.
20. Dingli D, Traulsen A, Pacheco JM. Compartmental architecture and dynamics of hematopoiesis. *PLoS ONE* 2007; 2:e345.
21. Buescher ES, Alling DW, Gallin JI. Use of an X-linked human neutrophil marker to estimate timing of lyonization and size of the dividing stem cell pool. *J Clin Invest* 1985; 76:1581-4.
22. Dingli D, Pacheco JM. Allometric scaling of the active hematopoietic stem cell pool across Mammals. *PLoS ONE* 2006; 1:e2.
23. Vaziri H, Dragowska W, Allsopp RC, et al. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc Natl Acad Sci U S A* 1994; 91:9857-60.
24. Mackey MC. Cell kinetic status of haematopoietic stem cells. *Cell Prolif* 2001; 34:71-83.
25. Shochat E, Stemmer SM, Segel L. Human haematopoiesis in steady state and following intense perturbations. *Bull Math Biol* 2002; 64:861-86.
26. Primo D, Flores J, Quijano S, et al. Impact of bcr/abl gene expression on the proliferative rate of different subpopulations of haematopoietic cells in chronic myeloid leukaemia. *Br J Haematol* 2006; 135:43-51.
27. Marley SB, Davidson RJ, Goldman JM, et al. Effects of combinations of therapeutic agents on the proliferation of progenitor cells in chronic myeloid leukaemia. *Br J Haematol* 2002; 116:162-5.
28. Ichimaru M, Ishimaru T, Mikami M, et al. *Incidence of Leukemia in a Fixed Cohort of Atomic Bomb Survivors and Controls, Hiroshima and Nagasaki October 1950-December 1978: Technical Report RERF TR 13-81*. Radiation Effects Research Foundation, Hiroshima; 1981.
29. Brummendorf TH, Holyoake TL, Rufer N, et al. Prognostic implications of differences in telomere length between normal and malignant cells from patients with chronic myeloid leukemia measured by flow cytometry. *Blood* 2000; 95:1883-90.
30. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. *Nature* 2005; 435:1267-70.
31. Huntly BJ, Shigematsu H, Deguchi K, et al. MOZ-TIF2, but not Bcr-Abl, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. *Cancer Cell* 2004; 6:587-96.
32. Roeder I, Horn M, Glauche I, et al. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. *Nat Med* 2006; 12:1181-4.
33. Dingli D, Traulsen A, Michor F. (A)Symmetric stem cell replication and cancer. *PLoS Comput Biol* 2007; 3:e53.
34. O'Hare T, Walters DK, Stoffregen EP, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res* 2005; 65:4500-5.
35. Dingli D, Traulsen A, Pacheco JM. Stochastic dynamics of hematopoietic tumor stem cells. *Cell Cycle* 2007; 6:461-6.
36. Shah NP, Tran C, Lee FY, et al. Overriding imatinib resistance with a novel Abl kinase inhibitor. *Science* 2004; 305:399-401.
37. Heaney NB, Holyoake TL. Therapeutic targets in chronic myeloid leukaemia. *Hematol Oncol* 2007; 25:66-75.
38. Heaney N, Drummond M, Kaeda J, et al. A phase 3 study of continuous imatinib versus pulsed imatinib with or without G-CSF in patients with chronic phase CML who have achieved a complete cytogenetic response to imatinib. *Blood* 2007; 110:313a (Abstract 1033).
39. Rousselot P, Huguet F, Rea D, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. *Blood* 2007; 109:58-60.
40. Gordon MY, Blackett NM. Routes to repopulation--a unification of the stochastic model and separation of stem-cell subpopulations. *Leukemia* 1994; 8:1068-72; discussion 1072-3.
41. Abkowitz JL, Catlin SN, Guttorp P. Evidence that hematopoiesis may be a stochastic process in vivo. *Nat Med* 1996; 2:190-7.
42. Zucchi I, Sanzone S, Astigiano S, et al. The properties of a mammary gland cancer stem cell. *Proc Natl Acad Sci U S A* 2007; 104:10476-81.
43. Tauer J, Shultz L, Holyoake T, et al. Normal short-term but reduced long-term engraftment capacity of CML hematopoietic cells with skewed myeloid lineage differentiation is seen in an improved mouse model of human hematopoiesis. *Blood* 2007; 110:991a (Abstract 3383).