Selection pressure exerted by imatinib therapy leads to disparate outcomes of imatinib discontinuation trials

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ABSTRACT

Background

Chronic myeloid leukemia is successfully managed by imatinib therapy, but the question remains whether treatment must be administered indefinitely. Imatinib discontinuation trials have led to two distinct outcomes: about 60% of patients experienced disease relapse within 6 months of treatment cessation, while the remaining 40% remained disease-free throughout the duration of follow-up. We aimed to investigate the mechanisms underlying these disparate clinical outcomes.

Design and Methods

We utilized molecular data from the "Stop Imatinib" trial together with a mathematical framework of chronic myeloid leukemia, based on a four-compartment model that can explain the kinetics of the molecular response to imatinib. This approach was complemented by statistical analyses to estimate system parameters and investigate whether chronic myeloid leukemia can be cured by imatinib therapy alone.

Results

We found that there are insufficient follow-up data from the "Stop Imatinib" trial in order to conclude whether the absence of a relapse signifies cure of the disease. We determined that selection of less aggressive leukemic phenotypes by imatinib therapy recapitulates the trial outcomes. This postulated mechanism agrees with the observation that most patients who have a complete molecular response after discontinuation of imatinib continue to harbor minimal residual disease, and might work in concert with other factors suppressing leukemic cell expansion when the tumor burden remains low.

Conclusions

Our analysis provides evidence for a mechanistic model of chronic myeloid leukemia selection by imatinib treatment and suggests that it may not be safe to discontinue therapy outside a clinical trial.

Key words: chronic myeloid leukemia, mathematical modeling, biostatistics, targeted therapy, minimal residual disease.

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The online version of this article has a Supplementary Appendix.

Introduction

Imatinib mesylate (Gleevec®, Novartis Pharmaceuticals, formerly STI571) is the current standard of care for patients with chronic myeloid leukemia (CML), inducing clinical, cytogenetic and molecular remissions and prolonging progression-free survival.[1,2] The phase III multicenter IRIS trial studied 1,106 previously untreated patients with chronic phase disease who were randomized to receive imatinib or interferon-α plus cytarabine (AraC). The superiority of imatinib over interferon-α plus cytarabine was proven after a median of 19 months' follow-up. Five years after the initiation of imatinib therapy, 40% of the patients in chronic phase had achieved a complete molecular response (CMR). CMR is defined as the state when residual disease cannot be detected by quantitative reverse transcriptase polymerase chain reaction analysis.[3] The estimated overall survival rate at 5 years is 89%, while it is 85% at 8 years.[4]

The achievement of CMR is not, however, a guarantee of disease eradication since undetectable minimal residual disease may persist, and there is little evidence on whether treatment must be administered indefinitely to prevent recurrence of disease. To determine whether imatinib can be discontinued safely without eliciting a loss of CMR, a pilot study and two clinical trials of imatinib cessation have been conducted.[5-7] About 60% of patients who discontinue imatinib after a period of stable CMR relapse within 6 months of treatment cessation, while approximately 40% of patients continue to present with undetectable disease with a follow-up beyond 18 months[5] (Figure 1A). This dichotomy between early relapse and stable CMR may support the hypothesis that in a minority of patients, prolonged imatinib therapy can lead to eradication of the disease. Alternatively, leukemic cells may persist below the detection limit of quantitative reverse transcriptase polymerase chain reaction assays, but may be prevented from expanding to cause molecular relapse by immunological or other mechanisms.[5,8]

In this study, we performed statistical analyses to inves-

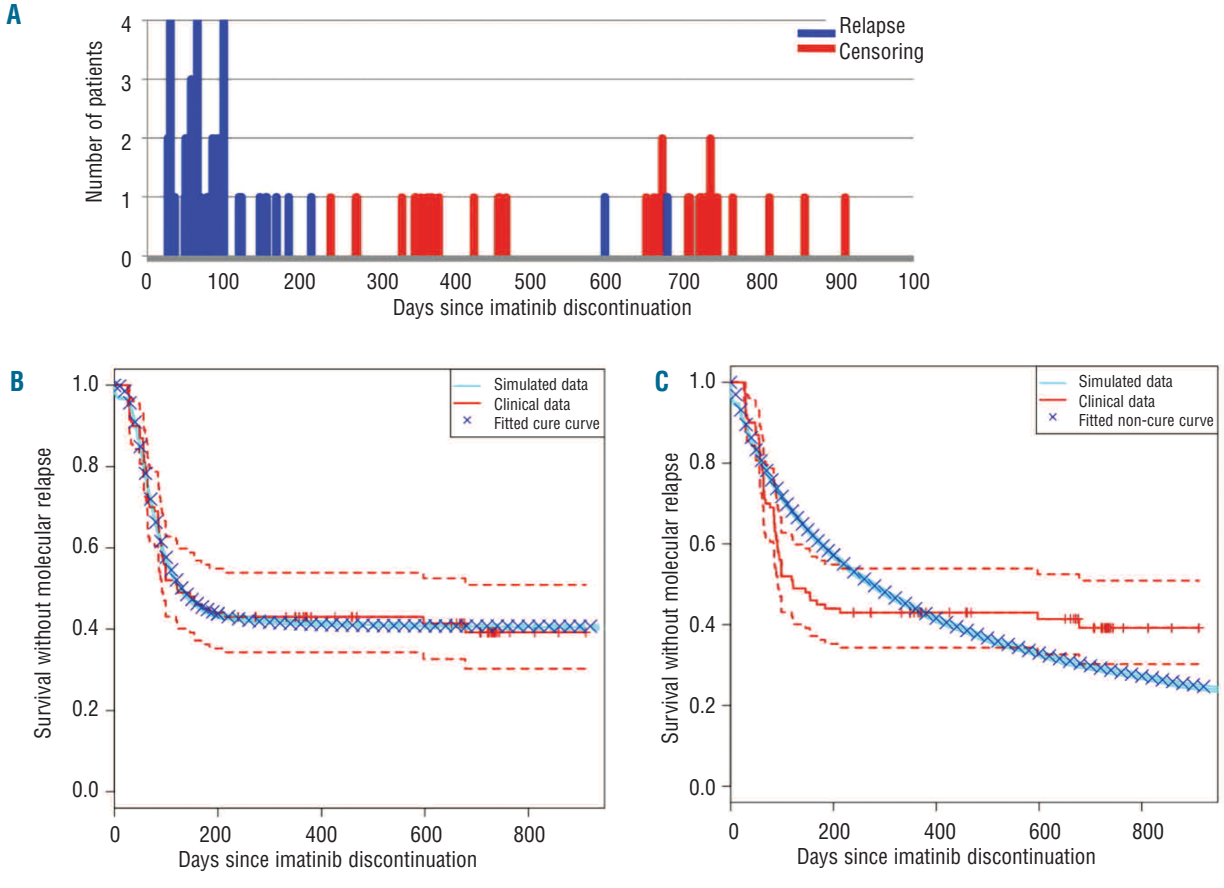

Figure 1. Outcomes of imatinib discontinuation in the clinical trial and generated by mathematical modeling. (A) Relapse kinetics of patients enrolled in the "Stop Imatinib" (STIM) trial. Blue bars represent patients who relapsed after imatinib discontinuation, while red bars indicate cessation of follow-up before a potential relapse. (B) Kaplan-Meier plots of survival without molecular relapse in the clinical trial. The fraction of patients without molecular relapse in the STIM trial after imatinib discontinuation is shown in red, with the 95% confidence interval represented as broken lines, and data of patients whose follow-up time ended before a potential relapse are shown as vertical ticks. The fitted log logistic cure model distribution is displayed as dark blue x's and the output of the mathematical framework under the assumption of a potential cure is shown in light blue. (C) Kaplan-Meier plots of survival without molecular relapse in the clinical trial. The fraction of patients without molecular relapse in the STIM trial after imatinib discontinuation is shown in red, with the 95% confidence interval represented as broken lines, and data of patients whose follow-up time ended before a potential relapse are shown as vertical ticks. The fitted lognormal distribution is displayed as dark blue x's and the output of the mathematical framework under the assumption of no cure is shown in light blue.

tigate whether the data from the Stop Imatinib (STIM) trial[8] support the conclusion that a subset of CML patients can be cured by administration of imatinib alone. We then performed a comprehensive investigation of a mathematical framework of CML treatment response. These analyses led us to propose the hypothesis that imatinib therapy exerts a selection pressure, which leads to a prevalence of leukemic clones that have different growth characteristics from those of the predominant clone at the start of treatment. This work is part of a growing body of literature on theoretical investigations of CML treatment response.[9-18]

Design and Methods

Testing for a cure

We first sought to determine whether the STIM trial data, which represent an interim report of this clinical trial, are consistent with the hypothesis that a subset of patients can be cured by imatinib therapy alone. Based on the Kaplan-Meier curve of the relapse data, in which 59 out of 100 patients lost CMR during the follow-up period of the trial (Figure 1A), we hypothesized that a statistical cure model may fit the data; such a model has the underlying assumption that a fraction of patients succeeded in eradicating their disease. However, since the distributions of relapse and censoring times partially overlap, it remains possible that the follow-up of the clinical trial is not long enouchg to provide information about the possibility of a cure. We thus probed whether the data on relapse-free survival were consistent with a model in which a fraction of patients can be cured. Using the non-parametric method based on the Kaplan-Meier curve, we found that there is no significant evidence that the follow-up is sufficiently long enough in the STIM trial (see *Online Supplementary Information for details*). This analysis led us to conclude that it may be possible that imatinib can eradicate CML in a subset of patients; however, the follow-up in the STIM trial is not long enough to provide statistical support to the existence of a cure. We, therefore, investigated two scenarios: (i) the situation in which a subset of patients (the "cure fraction") can be cured by imatinib (Figure 1B), and (ii) the situation in which all patients will eventually relapse, i.e. in which the cure fraction is zero. We conducted separate analyses for these scenarios.

The mathematical framework

We utilized a mathematical model of the treatment response of CML cells to imatinib therapy,[9,19] which describes four layers of the differentiation hierarchy of the hematopoietic system. Stem cells give rise to progenitors, which produce differentiated cells, which in turn produce terminally differentiated cells. This hierarchy applies to both normal and leukemic cells. Only stem cells have the potential for indefinite self-renewal, but progenitor and differentiated cells possess the capability to undergo limited reproduction, which, together with differentiation, leads to an expansion of the cell number at each level of the differentiation hierarchy.

The abundances of normal hematopoietic stem cells, progenitors, differentiated cells, and terminally differentiated cells can be denoted by x_0, x_1, x_2, and x_3. Their respective leukemic abundances are given by y_0, y_1, y_2, and y_3. We assume that homeostatic mechanisms maintain the hematopoietic stem cell population at a constant level and, therefore, introduce a density dependence term, ϕ, in the stem cell production rate. The *BCR-ABL* oncogene is present in all leukemic cells, leading to slow clonal growth of leukemic stem cells and accelerating the rate at which leukemic progenitors and differentiated cells are generated. The system containing stem cells (SC), progenitor cells (PC), differentiated cells (DC) and terminally differentiated cells (TC) is, therefore, described by:

	healthy cells	leukemic cells
SC	$\dot{x}_0 = [r_x\phi - d_0]x_0$	$\dot{y}_0 = [r_y\varphi - d_0]y_0$
PC	$\dot{x}_1 = a_x x_0 - d_1 x_1$	$\dot{y}_1 = a_y y_0 - d_1 y_1$
DC	$\dot{x}_2 = b_x x_1 - d_2 x_2$	$\dot{y}_2 = b_y y_1 - d_2 y_2$
TC	$\dot{x}_3 = c_x x_2 - d_3 x_3$	$\dot{y}_3 = c_y y_2 - d_3 y_3$

Here density dependence in the stem cell population is given by $\phi = 1/[1+p_x(x_0 + y_0)]$ and $\varphi = 1/[1+p_y(x_0 + y_0)]$. The potentially different carrying capacities of normal and leukemic stem cells are represented by the parameters p_x and p_y. Imatinib therapy reduces the production rates of leukemic progenitors and differentiated cells, and potentially also inhibits the expansion of leukemic stem cells. This change in rates leads to a bi-phasic decline of the leukemic cell burden. The parameters during imatinib therapy are denoted by r_y', a_y', b_y' etc, and the parameters after imatinib cessation are denoted by r_y'', a_y'', b_y'' etc. This mathematical framework can be used to study the dynamics of CML from its inception to diagnosis and its response to treatment as well as post-treatment behavior.[9,19]

The *BCR-ABL/ABL* ratio, for comparison with the clinical data, is calculated by taking into account the abundances of normal and leukemic terminally differentiated cells in the mathematical framework; since these cells are several orders of magnitude more frequent in peripheral blood than other, less differentiated cell types, the latter can be ignored when calculating the *BCR-ABL/ABL* ratio.

In the basic version of the framework, we model the leukemic stem cell pool as a homogeneous population whose growth and differentiation kinetics follow particular distributions; these distributions account for the heterogeneity within the population. However, the framework can be extended to explicitly describe separate stem cell types to account for heterogeneity. This more detailed framework provides more specifics than the general framework but does not significantly change the results. See the *Online Supplementary Information* for details of the mathematical framework and its extensions.

Results

We hypothesized that selective pressure exerted by imatinib therapy, which acts differently on different leukemic phenotypes and may differ in effect between patients, generates variability among the population of patients with regards to the time of loss of CMR after discontinuation of imatinib treatment. We characterized the extent of selection on the leukemic cell population by performing a comprehensive investigation of the parameter space in our mathematical framework to determine the joint density and range of parameters that can explain the observed variation in relapse times, both for the cure and the non-cure cases. We focused on the five parameters that influence the time of loss of CMR most significantly: the production rates of leukemic progenitors, differentiated and terminally differentiated cells after imatinib cessation as well as the growth rate of leukemic stem cells both during and after imatinib therapy (see *Online Supplementary Information* for details).

Our mathematical model represents between-patient heterogeneity via variability in patient-specific cell growth and differentiation kinetics. Two patients with identical parameter values will have identical model-predicted cell

growth profiles over time. Given a particular set of parameter values, the mathematical model can be computationally solved to evaluate the resulting relapse time; however, from a given relapse time it is not possible to determine a unique set of corresponding parameter values. In addition, the characteristics of the underlying parameter distributions for the growth and differentiation kinetics are unknown. For these reasons, we utilized a retrospective approach to determine the parameter distributions given the observed relapse time data. The first step in this process is to identify the distribution that best fits the data; this distribution depends on whether it is possible to cure CML under the assumptions of the model, as outlined in the following two sections.

The cure model

To investigate the scenario in which a cure may be possible, we first fitted the relapse data of the STIM trial using parametric cure models for censored data. Of the parametric distributions tested, the log-logistic distribution exhibited the best fit (Figure 1B). We then numerically solved the differential equation system to obtain the relapse time, defined as the time when the percentage of leukemic cells in peripheral blood exceeds 10^{-5}, for different parameter sets (see *Online Supplementary Information* for details). A fine grid (over 260 million samples) was used to sample the five-dimensional parameter space, and the corresponding times of loss of CMR or follow-up were determined using the mathematical model. Here CMR is defined as the time when the *BCR-ABL/ABL* transcript level decreases to 10^{-5}; this cutoff can be modulated to investigate alternative definitions. Such variations may lead to small changes in the estimated parameter values but do not alter our main conclusions (*data not shown*). We then selected the subset of outcomes from these times that recapitulated the fitted log-logistic cure curve up until the maximum follow-up and then retrospectively found S1, the set of parameters in the mathematical model that resulted in this subset of times. The set S1 then represents the sets of samples from the joint density of the five parameters in the mathematical framework for the scenario in which a cure may be possible.

When comparing the Kaplan-Meier curve obtained from the set S1 to the Kaplan-Meier survival curve of the clinical data,[5] we found that there was no significant difference between the simulated curve and the clinical data (*P* value = 0.98 based on the log-rank test, Figure 1B). Thus, under the assumption of a cure, the joint density implied by the sample set S1 results in a Kaplan-Meier survival curve matching the clinical survival curve for relapse times. The characteristics of the five parameters are summarized in Table 1, and their marginal densities are shown in Figure 2A-E. The densities of the production rates of progenitors and differentiated cells after imatinib cessation are correlated (Figure 2F), but no other parameters show any significant associations.

Using this approach, we determined that the selection pressure of imatinib on leukemic stem cells leads to moderate changes in the growth rate of this population after discontinuation of the treatment; the growth rate of leukemic stem cells after cessation of therapy (mean of 0.0062, median of 0.0040 per day) is smaller than that before initiation of treatment (considered to be 0.008 per day for all patients[9,19]). The growth kinetics of progenitor, differentiated cell and terminally differentiated cell populations are sculpted to a larger extent by imatinib treatment. Progenitors, for instance, were found to have a mean post-treatment production rate of 0.2102 (median 0.1100) while the rate before initiation of therapy was 0.8.[9,19] Table 1 shows the full set of estimated parameter values. Note that differential growth rates and differentiation kinetics of leukemic cell types between patients also lead to disparate abundances of these types, which contributes to the variability in the relapse kinetics among the patients.

The non-cure model

We then sought to investigate the scenario in which eventual relapse is assumed for all patients, since their disease cannot be cured by the administration of imatinib alone. We first fitted the relapse data of the STIM trial using parametric survival models for censored data. Of the parametric distributions tested, the lognormal distribution exhibited the best fit (Figure 1C). We then again numerically solved the differential equation system to obtain the relapse time for different parameter sets, and selected the subset of outcomes from these times that recapitulated the fitted lognormal survival curve up until the maximum follow-up. The set S2 therefore represents the sets of samples from the joint density of the five parameters for the case in which a cure cannot be achieved.

When comparing the Kaplan-Meier curve obtained from the set S2 to the Kaplan-Meier survival curve of the clinical data,[5] we found that there was no significant difference between the simulated curve and the clinical data (*P* value

Table 1. Parameter values of the mathematical framework before initiation of imatinib therapy, during treatment, and after cessation of treatment under the assumption of the cure model. The five bold entries are identified by the parameter search approach, all others from Michor *et al.*[9] When the mean or median is not specified, the same value is assumed for all patients.

Parameter	Pre-imatinib treatment value	Value during imatinib treatment	Post-imatinib treatment value
Progenitor production rate	0.8000	0.0080	**0.2102 (mean) 0.1100 (median)**
Differentiated cell production rate	5.0000	0.0067	**1.5590 (mean) 1.0000 (median)**
Terminally differentiated cell production rate	100.0000	100.0000	**37.3900 (mean) 15.0000 (median)**
Stem cell growth rate	0.0080	**0.0006 (mean) 0.0004 (median)**	**0.0062 (mean) 0.0040 (median)**

Table 2. Parameter values of the mathematical framework before initiation of imatinib therapy, during treatment, and after cessation of treatment under the assumption of the non-cure model. The five bold entries are identified by the parameter search approach, all others from Michor *et al.*[9] When the mean or median is not specified, the same value is assumed for all patients.

Parameter	Pre-imatinib treatment value	Value during imatinib treatment	Post-imatinib treatment value
Progenitor production rate	0.8000	0.0080	**0.1771 (mean) 0.0700 (median)**
Differentiated cell production rate	5.0000	0.0067	**1.5186 (mean) 0.8000 (median)**
Terminally differentiated cell production rate	100.0000	100.0000	**39.4563 (mean) 20.0000 (median)**
Stem cell growth rate	0.0080	**0.0006 (mean) 0.0005 (median)**	**0.0079 (mean) 0.0070 (median)**

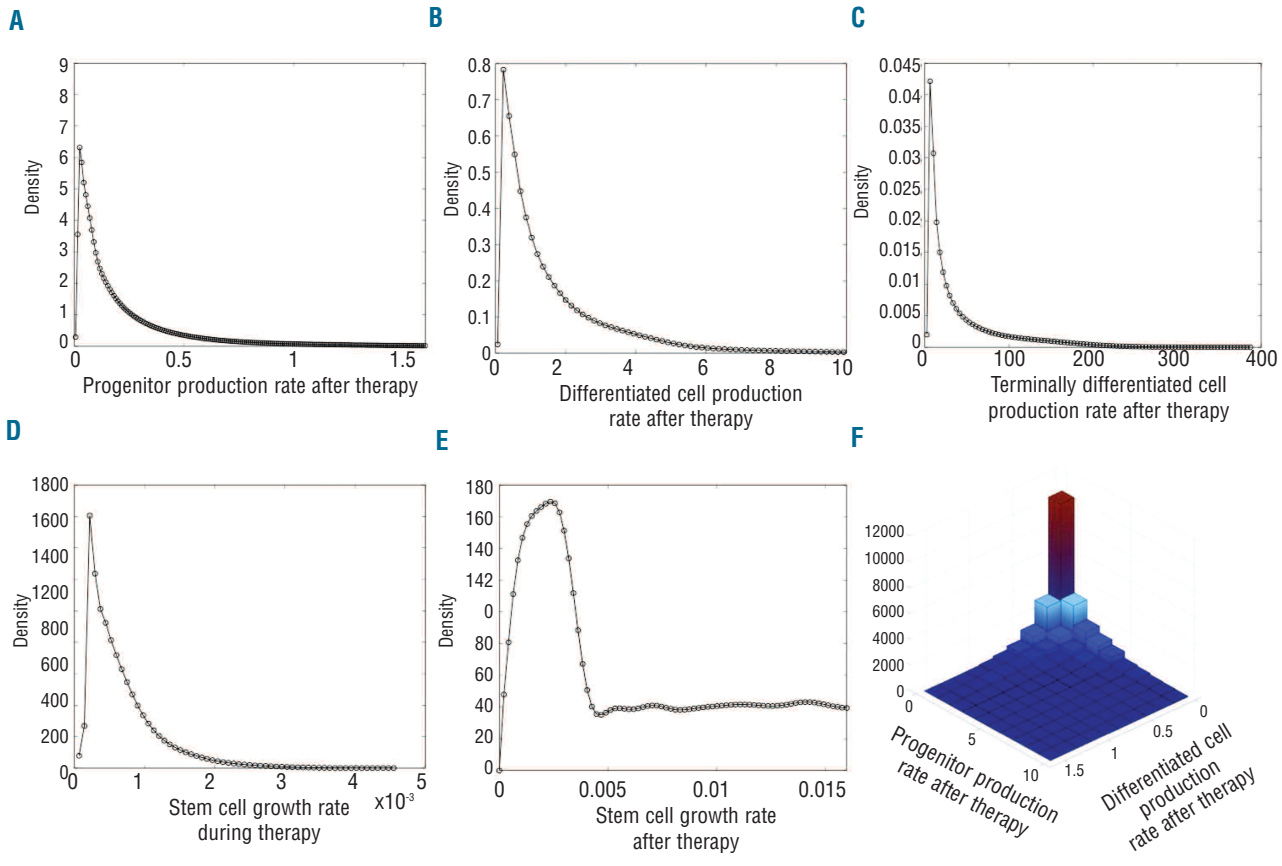

Figure 2. Predicted selection effect of imatinib therapy under the assumption of the cure model. The panels display the density of each parameter that reproduces the Kaplan-Meier survival curve shown in Figure 1B. The pre-imatinib treatment parameters are given in Table 1. (A) The density of the production rate of leukemic progenitor cells after imatinib cessation. (B) The density of the production rate of leukemic differentiated cells after imatinib cessation. (C) The density of the production rate of leukemic terminally differentiated cells after imatinib cessation. (D) The density of the growth rate of leukemic stem cells during imatinib therapy. (E) The density of the growth rate of leukemic stem cells after imatinib cessation. (F) The joint marginal histogram of the production rates of leukemic progenitors and differentiated cells after imatinib cessation, exhibiting a correlation between these two parameters in the sample set found to recapitulate the fitted log logistic cure survival distribution in Figure 1B.

= 0.72 based on the log-rank test, Figure 1C). Thus, under the assumption of no cure, the joint density implied by the sample set S2 results in a Kaplan-Meier survival curve matching the clinical survival curve for relapse times. The characteristics of the five parameters are summarized in Table 2, and their marginal densities are shown in Figure 3A-E. Again, the densities of the production rates of progenitors and differentiated cells after cessation of imatinib treatment are correlated (Figure 3F), but no other parameters showed any significant associations.

Using this approach, we determined that the selection pressure of imatinib on leukemic stem cells leads to very minor changes in the growth rate of this population after discontinuation; the growth rate of leukemic stem cells after cessation of therapy (mean of 0.008, median of 0.007 per day) is similar to that before initiation of treatment (0.008 per day for all patients). The growth kinetics of progenitor, differentiated cell and terminally differentiated cell populations, however, are sculpted to a larger extent by imatinib treatment. For instance, the post-treatment production rate of progenitors was 0.177 (mean; median 0.070) while the rate before initiation of therapy was 0.8. Table 2 shows the full set of estimated parameter values.

These results suggest that imatinib may select leukemic phenotypes associated with the production of fewer non-self-renewing cells, thereby leading to a slower expansion of cell numbers throughout the differentiation hierarchy (Figure 4A). Notably, the results of the scenario in which a cure is achievable and the scenario in which patients cannot be cured by imatinib alone lead to very similar results. In both cases, the selection pressure exerted by imatinib enhances the frequency of leukemic stem cell clones with growth properties that are less aggressive than those of the predominant clone at the start of treatment (Figure 4A). This selection effect on leukemic stem cells is more pronounced if we assume that patients who had not yet relapsed at the time of censoring were cured as compared to the assumption in which patients may relapse at a later time. In contrast, the estimated parameters governing the more differentiated cell types are very similar between the two scenarios (Tables 1 and 2). Thus, if a cure is achievable, the selection effect of imatinib on leukemic stem cells is expected to be stronger (mean of 0.0062 after treatment, 0.0080 before treatment) as compared to the case in which imatinib cannot eradicate the disease (mean of

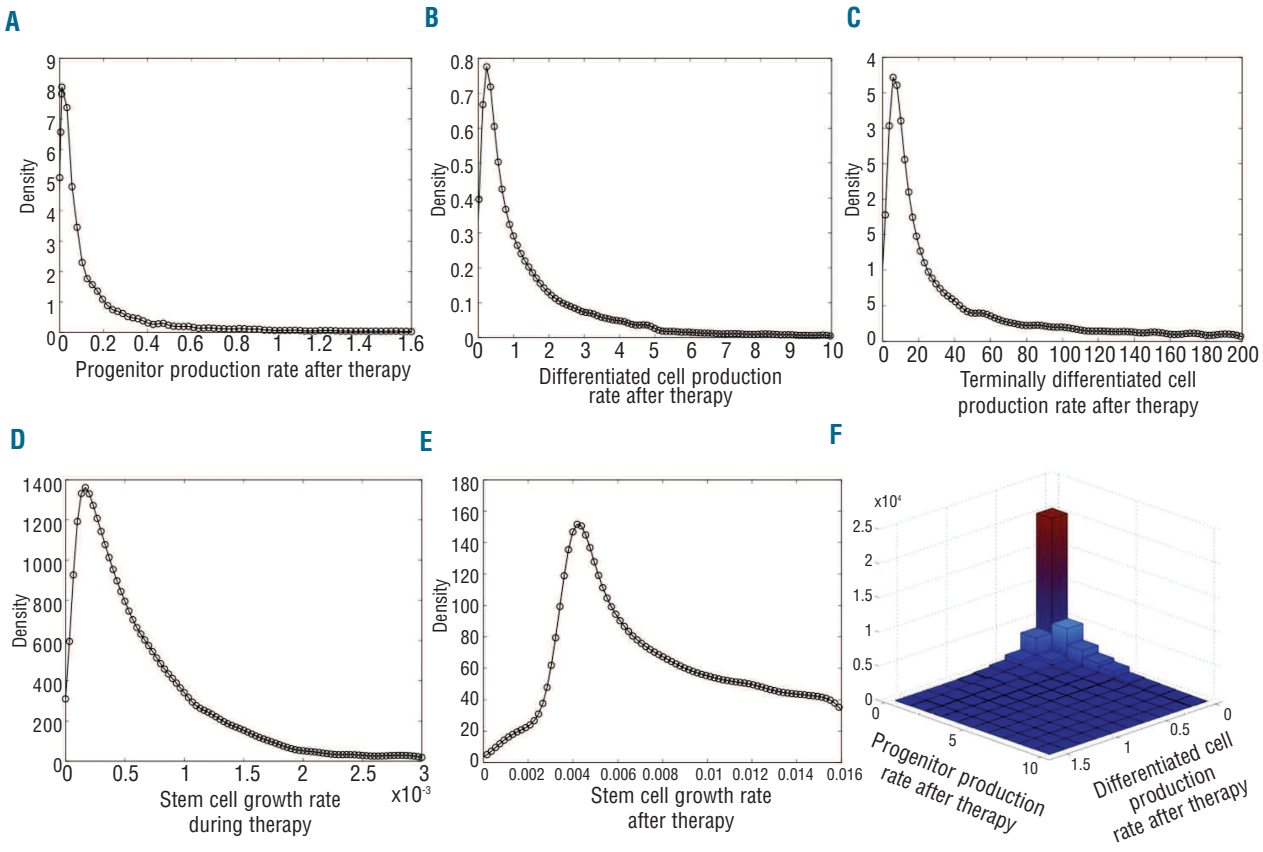

Figure 3. Predicted selection effect of imatinib therapy under the assumption of the non-cure model. The panels display the density of each parameter that reproduces the Kaplan-Meier survival curve shown in Figure 1C. The pre-imatinib treatment parameters are given in Table 2. (A) The density of the production rate of leukemic progenitor cells after imatinib cessation. (B) The density of the production rate of leukemic differentiated cells after imatinib cessation. (C) The density of the production rate of leukemic terminally differentiated cells after imatinib cessation. (D) The density of the growth rate of leukemic stem cells during imatinib therapy. (E) The density of the growth rate of leukemic stem cells after imatinib cessation. (F) The joint marginal histogram of the production rates of leukemic progenitors and differentiated cells after imatinib cessation, exhibiting a correlation between these two parameters in the sample set found to recapitulate the fitted lognormal distribution in Figure 1C.

0.0079 after treatment, 0.0080 before treatment). An alternative mechanism that may act in concert with selection of less aggressive phenotypes is the suppression of leukemic cells by immunological, microenvironmental, or density-sensing processes (Figure 4C and D). These processes were not explicitly considered in our mathematical modeling approach since at this time, insufficient quantitative data are available to estimate parameters associated with these mechanisms. These processes are the subject of ongoing investigation.

Discussion

In this paper, we investigated the disparate outcomes in the STIM trial and discussed two scenarios: the possibility of CML patients being cured by imatinib therapy alone (Figure 1B) and the absence of a cure (Figure 1C). Even though the cure model (Figure 1B) visually gives the impression of a better fit than the non-cure model (Figure 1C), the follow-up time in the STIM trial was not long enough to provide the data to distinguish between these two models statistically. We, therefore, discussed both cases. Notably, under the non-cure assumption, the survival function eventually decreases to zero. Thus no single parametric density will be able to approximate the tail plateau present in the Kaplan-Meier curve of the clinical data. We chose the lognormal distribution, which presented the best fit among a set of distributions and our analysis showed that there was no significant difference between the simulated curve and the clinical data (P value = 0.72 based on the log-rank test, Figure 1C). Note that all analyses were performed using data from all patients enrolled in the STIM trial and were not based solely on those who did or did not experience a relapse of their disease during the follow-up period.

Our approach suggests a mechanistic hypothesis explaining the disparate outcomes of imatinib discontinuation trials[5-7] (Figure 4A and B): we propose that the selection pressure exerted by imatinib leads to an increase in the frequency of leukemic clones that have slower growth and differentiation properties as compared to those of the predominant clone at the start of treatment (Figure 4A). This less aggressive phenotype may stem from a lessened

capability of leukemic stem cells to produce more differentiated populations and/or a decreased ability of progenitors and differentiated leukemic cells to undergo limited cell division. The selection of clones with altered growth kinetics then leads to variability among patients with regard to the aggressiveness of their disease, thereby generating a distribution of times at which patients experience loss of CMR after imatinib cessation (Figure 4B). This distribution, together with censoring due to limited follow-up in the STIM trial and a potential cure of a fraction of patients, causes a dichotomous outcome in that some patients relapse within the trial period while others remain in CMR during the period of observation. This postulation of selective pressures exerted by imatinib on different clones within patients represents a novel concept that differs from previously discussed effects of imatinib in that intra- as well as inter-patient valiability in the growth kinetics of leukemic cells is taken into account.

In many situations, there is marked heterogeneity in phenotype even if cells are genetically identical.[20-23] Similarly, the leukemic stem cell population may represent a continuum of phenotypes with disparate growth and differentiation kinetics; indeed, experimental evidence suggests that both the amount of *BCR-ABL* mRNA and second site mutations alter the fitness of leukemic cells.[24,25] Furthermore, it was recently demonstrated that leukemic stem cells in acute lymphoblastic leukemia are highly heterogeneous, harboring clones with varied growth kinetics.[26,27] If this is also the case for CML, which remains to be proven experimentally, we might then spec-

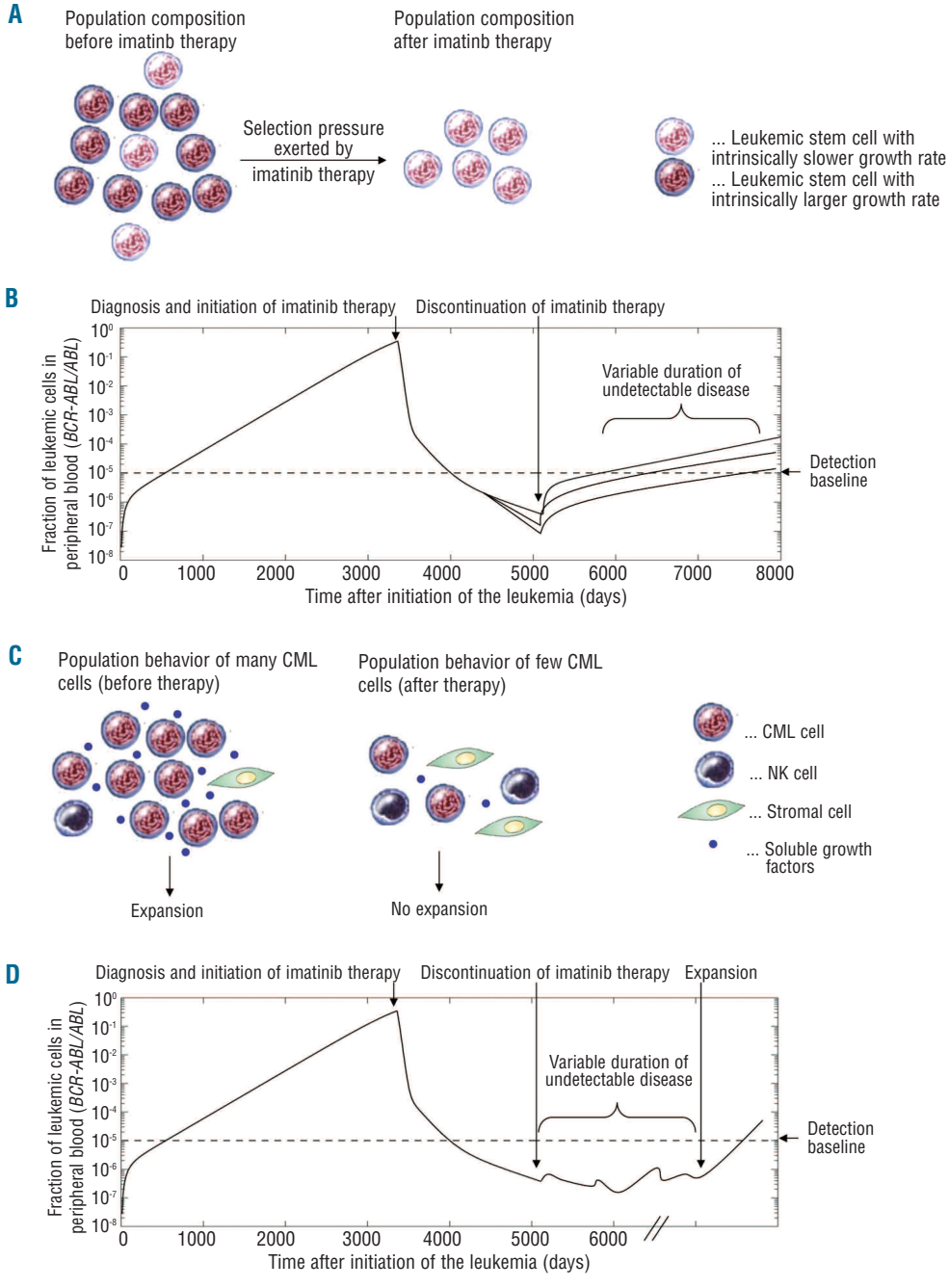

Figure 4. Mechanisms of patients' response to imatinib discontinuation. The figure outlines two proposed mechanisms explaining the disparate outcomes of clinical trials of imatinib cessation under the assumption of the non-cure model. (A and B) Selection of less aggressive leukemic phenotypes by imatinib therapy. We propose that imatinib treatment selects for leukemic cells with characteristics that are different from those of the predominant population at the start of therapy (A). This selection of less aggressive phenotypes leads to variable duration of undetectable disease as the leukemic cell population expands after imatinib cessation (B). This mechanism might act in concert with other factors influencing the kinetics of disease relapse, such as those discussed next. (C and D) Suppression of CML cell expansion by mechanisms such as immune surveillance, microenvironmental interactions, and quorum sensing. Despite the presence of minimal residual disease after imatinib cessation, the expansion of the leukemic clones may be inhibited by interactions of the leukemia with immune system cells such as natural killer (NK) cells or inhibition by stromal cells (C). These factors can suppress leukemic growth for a variable period of time before other events may lead to a relapse of the disease (D).

ulate that imatinib exerts a selection pressure leading to an adaptation of the leukemic stem cell population; this may explain the different kinetics of recurrence after discontinuation of treatment. Based on our results, we propose that imatinib therapy diminishes the clones with the most aggressive growth potential. However, unless treatment is administered in perpetuity, populations with less malignant properties may persist and lead to disease relapse after a variable duration of CMR. As a longer follow-up of the trial becomes available, we may be able to provide statistical proof that a cure is possible in a subset of these patients; however, at this time such a conclusion cannot be made.

Our results may also provide insights into other clinical characteristics such as non-response and early relapse. Such clinical scenarios, in the context of our framework, could be explained by heterogeneity in the leukemic stem cell population as well as differentiation kinetics both within and between patients, such that patients with an intrinsically more aggressive disease – due to either additional genomic alterations or epigenetic variability – are less likely to show an initial response. The patient- and clone-specific response to imatinib as well as immune system interactions could then explain the rates of relapse after treatment cessation.

An alternative mechanism that may act in concert with selection of less aggressive phenotypes is the suppression of leukemic cells by immunological, microenvironmental, or density-sensing processes (Figure 4C and D); in a small proportion of patients, these factors may even lead to disease eradication. There is a clinical distinction, however, between residual disease that can be maintained in perpetuity without treatment, thereby leading to a "cure", and the maintenance of an unstable equilibrium of the leukemic cell population which may break down to lead to relapses. Identification of the factors that cause either scenario is an important goal.

For simplicity, we assumed that the leukemic cell characteristics are the same in all patients before the initiation of therapy. This modeling choice was made to prevent the need to estimate the distributions of leukemic cell division and differentiation parameters since no data are yet available for this purpose. However, a parsimonious explanation of the trial outcomes is that patients with intrinsically more aggressive disease, as indicated by a high Sokal score (a prognostic test performed at diagnosis to characterize a patient as having a low risk, intermediate risk or high risk based on diagnostic markers including spleen size, platelet count, patient's age, and blast count), have a higher risk of relapsing early. In addition, administration of imatinib therapy can decrease the severity of the disease by selecting for less aggressive clones, which were present already at the beginning of therapy. We did not explicitly incorporate the Sokal score into our mathematical framework since it includes covariates such as age and spleen size, whose relationship to leukemic cell numbers and growth kinetics remain poorly understood. However, it would be of significant interest to incorporate information about the phenotypic characteristics of the leukemic cell burden of a patient into his/her Sokal score to aid the prediction of treatment response and relapse time after treatment discontinuation.

It has been suggested that it is impossible to cure CML using targeted therapy because leukemic stem cells cannot be eradicated.[28] The sole treatment likely to succeed is allogeneic stem cell transplantation; however, late molecular relapses have been reported even after this treatment option.[29] In the STIM trial, we observed that most patients relapsed after a few months, but several instances of late relapse and fluctuations of *BCR-ABL* levels after imatinib discontinuation also occurred. Additionally, 40% of patients did not relapse within our limited time of follow-up. Our mechanistic model invoking selection of less aggressive phenotypes by imatinib therapy may contribute to an explanation for these disparate outcomes, along with the effects of the immune system and microenvironment. Our work suggests the need to perform experimental investigations into the phenotypes of leukemic cells as well as statistical analyses of the patterns of relapse after nilotinib or dasatinib discontinuation, since these second-generation drugs are able to induce CMR in a greater fraction of patients than imatinib.[30,31]

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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