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

Running title: Selection pressure exerted by imatinib therapy

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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 sixty percent of patients experienced a disease relapse within six months of treatment cessation, while the remaining forty percent 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 of 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 is insufficient follow-up in the “Stop Imatinib” trial data 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 sustained complete molecular response after discontinuation 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.

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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 remission and prolonging progression-free survival (1-2). The phase III multicenter IRIS trial studied 1,106 previously untreated chronic phase patients who were randomized to receive imatinib versus IFN-α plus Cytarabine (AraC). The superiority of imatinib over IFN-α plus AraC was proven after 19 months median follow-up. Five years after the initiation of imatinib therapy, 40% of chronic phase patients achieved a complete molecular response (CMR). CMR is defined as undetectable minimal residual disease (MRD) by QRT-PCR (3). Estimated overall survival is 89% at 5 years and 85% at 8 years (4).

The achievement of CMR, however, is not a guarantee of disease eradication since undetectable MRD may persist, and little evidence exists whether treatment must be administered indefinitely to prevent a 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 reported (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) (**Fig. 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 an eradication of disease. Alternatively, leukemic cells may persist below the detection limit of QRT-PCR assays, but may be prevented from expanding to cause molecular relapse by immunological or other mechanisms (5, 8).

In this paper, we performed statistical analyses to investigate whether the STIM trial data supports 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

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prevalence of leukemic clones that have different growth characteristics as compared to the predominant clone at the start of treatment. This work is part of a growing literature of 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 represents an interim report of this clinical trial, is 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, where 59 out of 100 patients lost CMR during the follow-up time of the trial (**Fig. 1a**), we hypothesized that a statistical cure model may fit to 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, the possibility remains that the follow-up of the clinical trial is insufficiently long as to inform about the possibility of a cure. We thus probed whether the data on relapse-free survival was consistent with a model in which a fraction of patients can be cured. By utilizing the nonparametric 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 data (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 sufficiently long as to be able to statistically support 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 (**Fig. 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

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cells, which in turn produce terminally differentiated cells. This hierarchy applies both to 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.

Denote by x_0, x_1, x_2, and x_3 the abundances of normal hematopoietic stem cells, progenitors, differentiated cells, and terminally differentiated cells. 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. Then the system containing stem cells (SC), progenitor cells (PC), differentiated (DC) and terminally differentiated cells (TC) is 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

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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 differentially acts on different leukemic phenotypes and may differ in effect between patients, generates variability among the patient population with regard to the time of loss of CMR after imatinib treatment discontinuation. 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).

DOI: 10.3324/haematol.2012.062844

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 fit 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 (**Fig. 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 variation may lead to small changes in the estimated parameter values but does 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.

DOI: 10.3324/haematol.2012.062844

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, **Fig. 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 **Fig. 2a-e**. The densities of the production rates of progenitors and differentiated cells after imatinib cessation are correlated (**Fig. 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; 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 is 0.8 (9, 19). See **Table 1** for 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 a patient population.

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 administration of imatinib alone. We first fit 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 (**Fig. 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 then represent the sets of samples from the joint

DOI: 10.3324/haematol.2012.062844

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 0.72 based on the log-rank test, **Fig. 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 **Fig. 3a-e**. Again, the densities of the production rates of progenitors and differentiated cells after imatinib cessation are correlated (**Fig. 3f**), 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 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. Progenitors, for instance, were found to have a post-treatment production rate of 0.177 (mean; median 0.070) while the rate before initiation of therapy is 0.8. See **Table 2** for the full set of estimated parameter values.

These results suggest that imatinib may select for 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 (**Fig. 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 the predominant clone at the start of treatment (**Fig. 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

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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 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 immunologic, microenvironmental, or density-sensing processes (**Fig. 4c and d**). These processes were not explicitly considered in our mathematical modeling approach since at this time, insufficient quantitative data is 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 a cure of CML patients by imatinib therapy alone (**Fig. 1b**) and the absence of a cure (**Fig. 1c**). Even though the cure model (**Fig. 1b**) visually gives the impression of a better fit than the non-cure model (**Fig. 1c**), the follow-up time in the STIM data was not sufficiently long to statistically distinguish between these two models. We thus 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 presented 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, **Fig. 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 time of follow-up.

Our approach suggests a mechanistic hypothesis explaining the disparate outcomes of imatinib discontinuation trials (5-7) (**Fig. 4a and b**): we propose that the selection pressure exerted by imatinib leads to an increase in the frequency of leukemic clones

DOI: 10.3324/haematol.2012.062844

that have slower growth and differentiation properties as compared to the predominant clone at the start of treatment (**Fig. 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 in the patient population with regard to the aggressiveness of their disease, thereby generating a distribution of times at which patients experience loss of CMR after imatinib cessation (**Fig. 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 genetically, cells are 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 experimentally proven, then we might speculate 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 statistically prove that a cure is possible for a subset of those patients; however, at this time such a conclusion cannot be made.

DOI: 10.3324/haematol.2012.062844

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, can 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 – either due to 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 may 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 immunologic, microenvironmental, or density-sensing processes (**Fig. 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 clinical goal.

For simplicity, we have assumed that the leukemic cell characteristics are equal in all patients before the initiation of therapy. This modeling choice was made to prevent the need for estimating the distributions of leukemic cell division and differentiation parameters since no data is yet available for this goal. 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 low-risk, intermediate-risk or high-risk based on diagnosis markers including spleen size, platelet count, patient age, and blast count) have an enhanced risk of relapsing early. In addition, administration of imatinib therapy can then 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 in the prediction of treatment response and relapse time after 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 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).

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AUTHORSHIP AND DISCLOSURES

MT, JF, MG and FM performed modeling, JG and F-XM provided data.
All authors contributed to manuscript writing. The authors have no conflicts to disclose.

REFERENCES

1. Druker BJ TM, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001;344(14):1031-7.
2. Kantarjian H SC, Hochhaus A, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med. 2002;346(9):645-52.
3. Branford S SJ, Grigg A, Arthur C, Rudzki Z, Lynch K, Hughes T. BCR-ABL messenger RNA levels continue to decline in patients with chronic phase chronic myeloid leukemia treated with imatinib for more than 5 years and approximately half of all first-line treated patients have stable undetectable BCR-ABL using strict sensitivity criteria. Clin Cancer Res. 2007;13(23):7080-5.
4. Deininger M ea. International Randomized Study of Interferon Vs STI571 (IRIS) 8-Year Follow up: Sustained Survival and Low Risk for Progression or Events in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Imatinib. ASH Annual Meeting Abstracts. 2009;114(22):1126.
5. Mahon FX RD, Guilhot J, Guilhot F, Huguet F, Nicolini F, Legros L, Charbonnier A, Guerci A, Varet B, Etienne G, Reiffers J, Rousselot P; Intergroupe Français des Leucémies Myéloïdes Chroniques. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029-35.
6. Rousselot P HF, Rea D, Legros L, Cayuela JM, Maarek O, Blanchet O, Marit G, Gluckman E, Reiffers J, Gardembas M, Mahon FX. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. . Blood. 2007;109(1):58-60.
7. Ross DDM ea. The Majority of Chronic Myeloid Leukaemia Patients Who Cease Imatinib after Achieving a Sustained Complete Molecular Response (CMR) Remain in CMR, and Any Relapses Occur Early. . ASH Annual Meeting Abstracts. 2008;112(11):1102.
8. Ross DM BS, Seymour JF, Schwarer AP, Arthur C, Bartley PA, Slader C, Field C, Dang P, Filshie RJ, Mills AK, Grigg AP, Melo JV, Hughes TP. Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR. . Leukemia. 2010;24(10):1719-24.
9. Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, et al. Dynamics of chronic myeloid leukaemia. Nature. 2005 Jun 30;435(7046):1267-70.
10. Roeder I HM, Glauche I, Hochhaus A, Mueller MC, Loeffler M. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. Nat Med. 2006;12(10):1181-4.
11. Dingli D MF. Successful therapy must eradicate cancer stem cells. . Stem Cells. 2006;24(12):2603-10.
12. Komarova NL WD. Effect of cellular quiescence on the success of targeted CML therapy. PLoS ONE. 2007;2(10):e990.
13. Horn M LM, Roeder I. Mathematical modeling of genesis and treatment of chronic myeloid leukemia. Cells Tissues Organs. 2008;188(1-2):236-47.
14. Kim PS LP, Levy D. Dynamics and potential impact of the immune response to chronic myelogenous leukemia. . PLoS Comput Biol. 2008;4(6):e1000095.
15. Kim PS LP, Levy D. A PDE model for imatinib-treated chronic myelogenous leukemia. . Bull Math Biol. 2008;70(7):1994-2016.

16. Komarova NL KA, Wodarz D. Combination of two but not three current targeted drugs can improve therapy of chronic myeloid leukemia. . PLoS One. 2009;4(2):e4423.
17. Foo J DM, Clarkson B, Holyoake T, Michor F. Eradication of chronic myeloid leukemia stem cells: a novel mathematical model predicts no therapeutic benefit of adding G-CSF to imatinib. . PLoS Comput Biol. 2009;5(9):e1000503.
18. Lenaerts T PJ, Traulsen A, Dingli D. Tyrosine kinase inhibitor therapy can cure chronic myeloid leukemia without hitting leukemic stem cells. . Haematologica. 2010;95(6):900-7.
19. Michor F. The long-term response to imatinib treatment of CML. . British Journal of Cancer. 2007;96:679-80.
20. Elowitz MB LA, Siggia ED, Swain PS. Stochastic gene expression in a single cell. . Science. 2002;297(5584):1183-6.
21. Becskei A KB, van Oudenaarden A. Contributions of low molecule number and chromosomal positioning to stochastic gene expression. . Nat Genet. 2005;37(9):937-44.
22. Kaern M ET, Blake WJ, Collins JJ. Stochasticity in gene expression: from theories to phenotypes. . Nat Rev Genet. 2005;6(6):451-64.
23. Feinerman O VJ, Dorfman JR, Germain RN, Altan-Bonnet G. Variability and robustness in T cell activation from regulated heterogeneity in protein levels. Science. 2008;321(5892):1081-4.
24. Barnes DJ PD, Panousopoulou E, Schultheis B, Yong AS, Wong A, Pattacini L, Goldman JM, Melo JV. Bcr-Abl expression levels determine the rate of development of resistance to imatinib mesylate in chronic myeloid leukemia. Cancer Res. 2005;65(19):8912-9.
25. Skaggs BJ GM, Ryvkin A, Burgess MR, Xie Y, Han Y, Komisopoulou E, Brown LM, Loo JA, Landaw EM, Sawyers CL, Graeber TG. Phosphorylation of the ATP-binding loop directs oncogenicity of drug-resistant BCR-ABL mutants. Proc Natl Acad Sci USA. 2006;103(51):19466-71.
26. Notta F MC, Wang JC, Poeppl A, Doulatov S, Phillips LA, Ma J, Minden MD, Downing JR, Dick JE. Evolution of human BCR–ABL1 lymphoblastic leukaemia-initiating cells. Nature. 2011;469(7330):362-7.
27. Anderson K LC, van Delft FW, Bateman CM, Guo Y, Colman SM, Kempski H, Moorman AV, Titley I, Swansbury J, Kearney L, Enver T, Greaves M. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011;469(7330):356-61.
28. Ross DM HT, Melo JV. Do we have to kill the last CML cell? . Leukemia. 2010.
29. Kaeda J OSD, Szydlo RM, et al. . Serial measurement of BCR-ABL transcripts in the peripheral blood after allogeneic stem cell transplantation for chronic myeloid leukemia: an attempt to define patients who may not require further therapy. . Blood. 2006;107(10):4171-6.
30. Kantarjian H SN, Hochhaus A, et al. . Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2010;362: 2260-70.
31. Saglio G KD, Issaragrisil S, et al. ENESTnd Investigators. . Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med. 2010;362(2251-9).

DOI: 10.3324/haematol.2012.062844

TABLES

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 red entries are identified by the parameter search approach, all others from (9). When mean or median is not specified, then 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)

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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 red entries are identified by the parameter search approach, all others from (9). When mean or median is not specified, then 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)

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FIGURE LEGENDS

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 is 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 is 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.

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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 Fig. 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 Fig. 1b.

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 Fig. 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 Fig. 1c.

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Figure 4. Mechanisms of patient 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 like 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)**.

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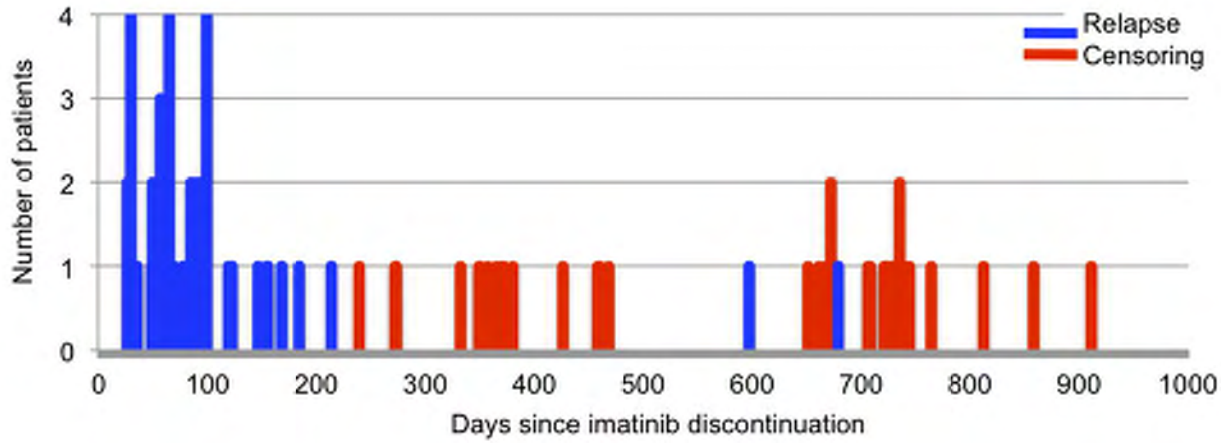
Fig. 1

a)



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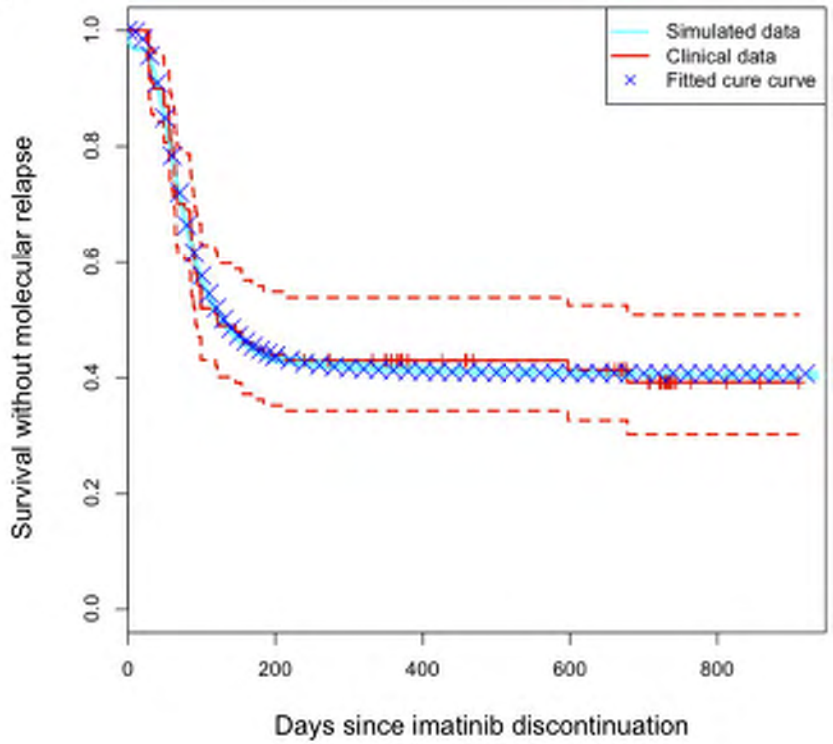

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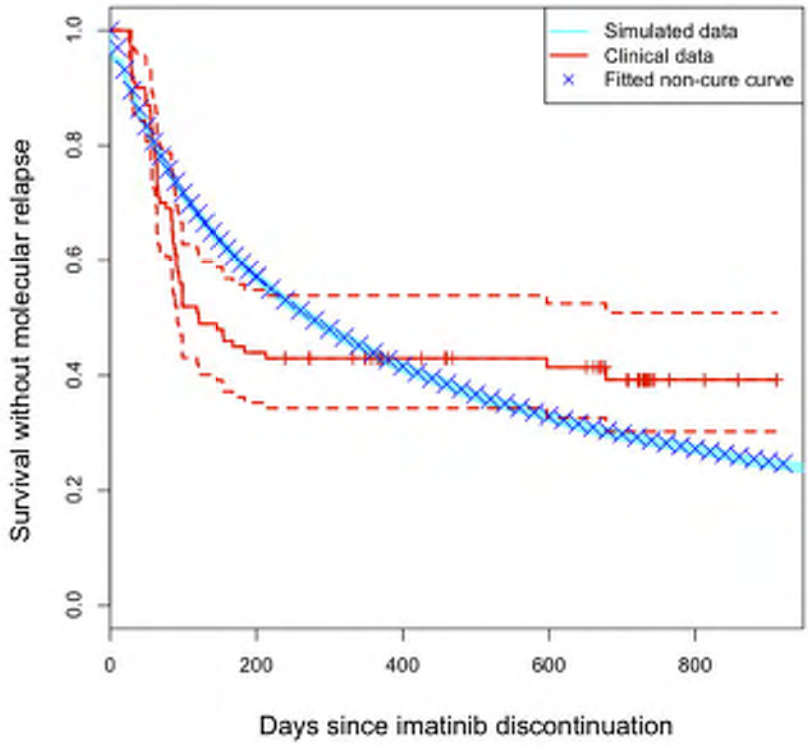

DOI: 10.3324/haematol.2012.062844

Fig. 2

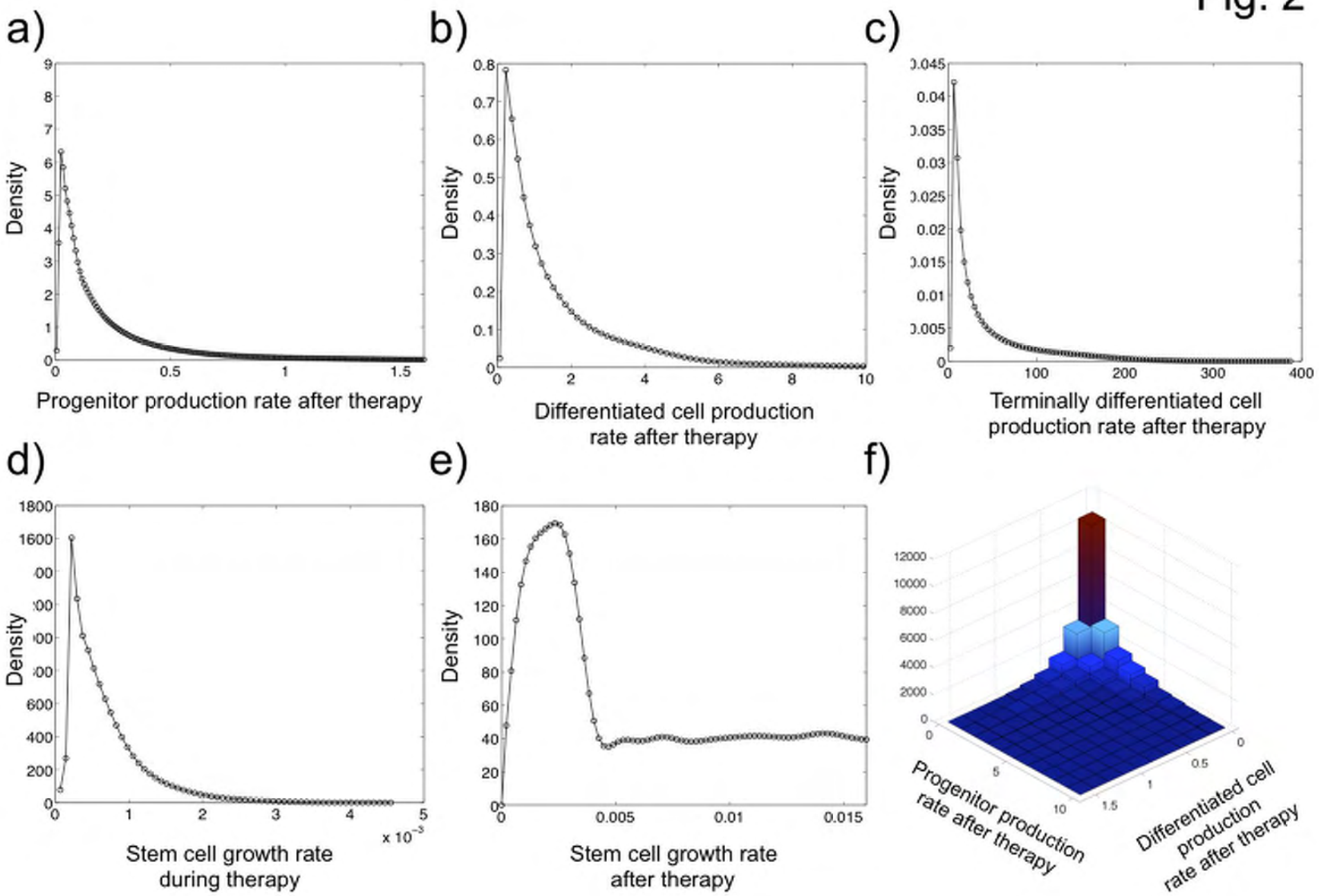

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Fig. 3

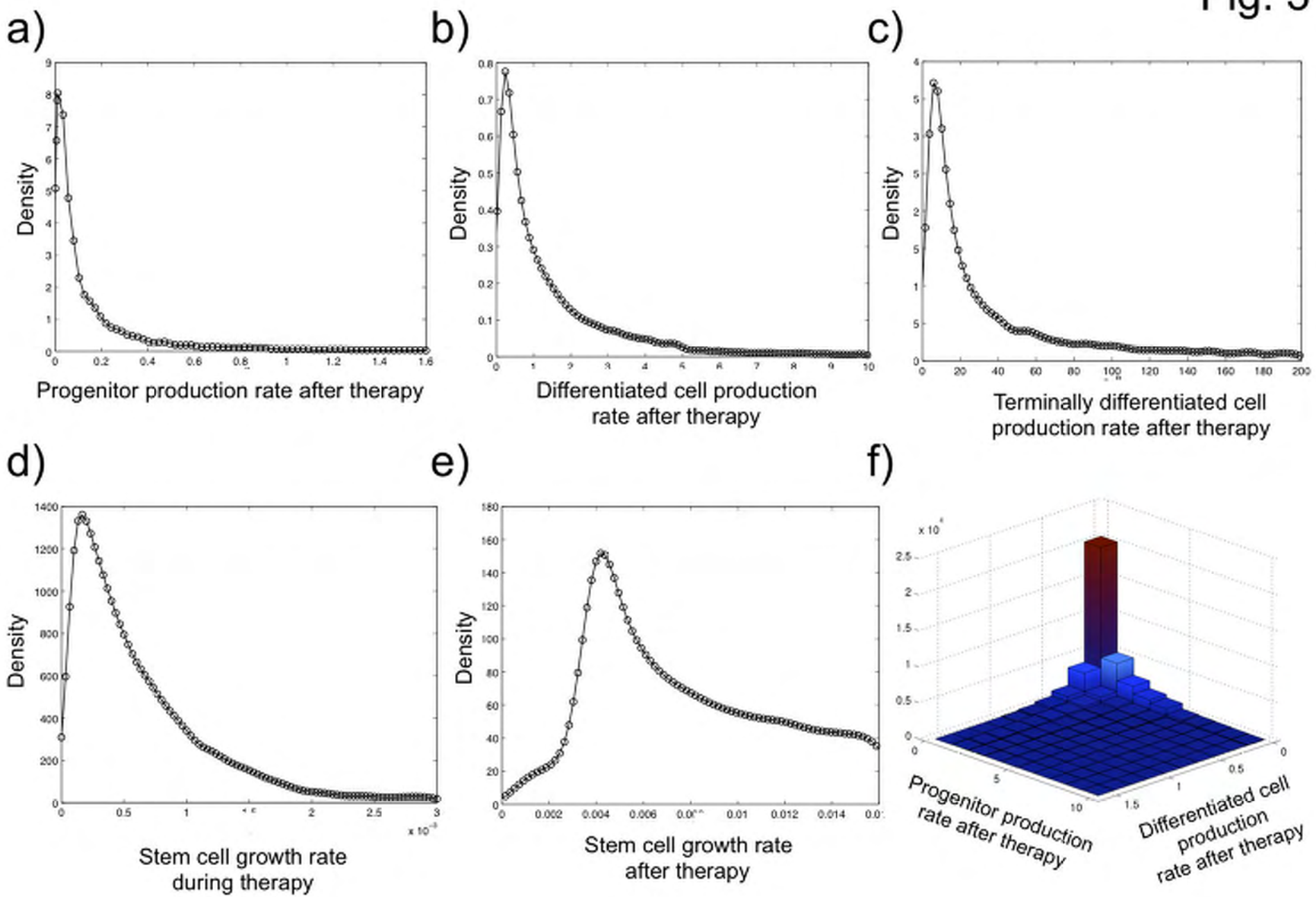

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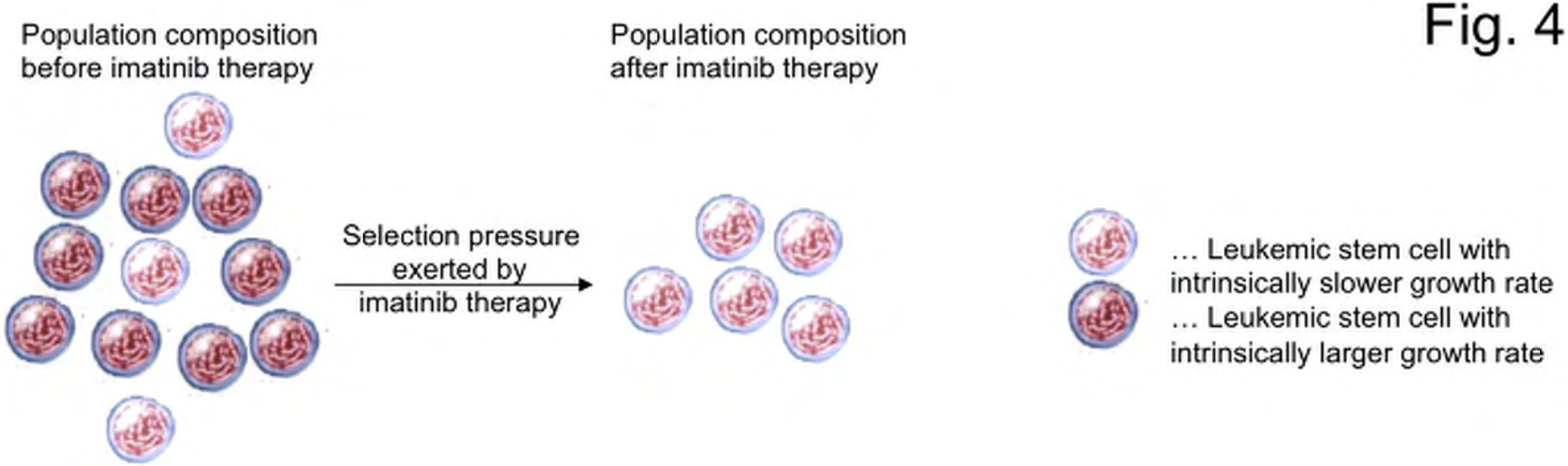
Fig. 4

a)



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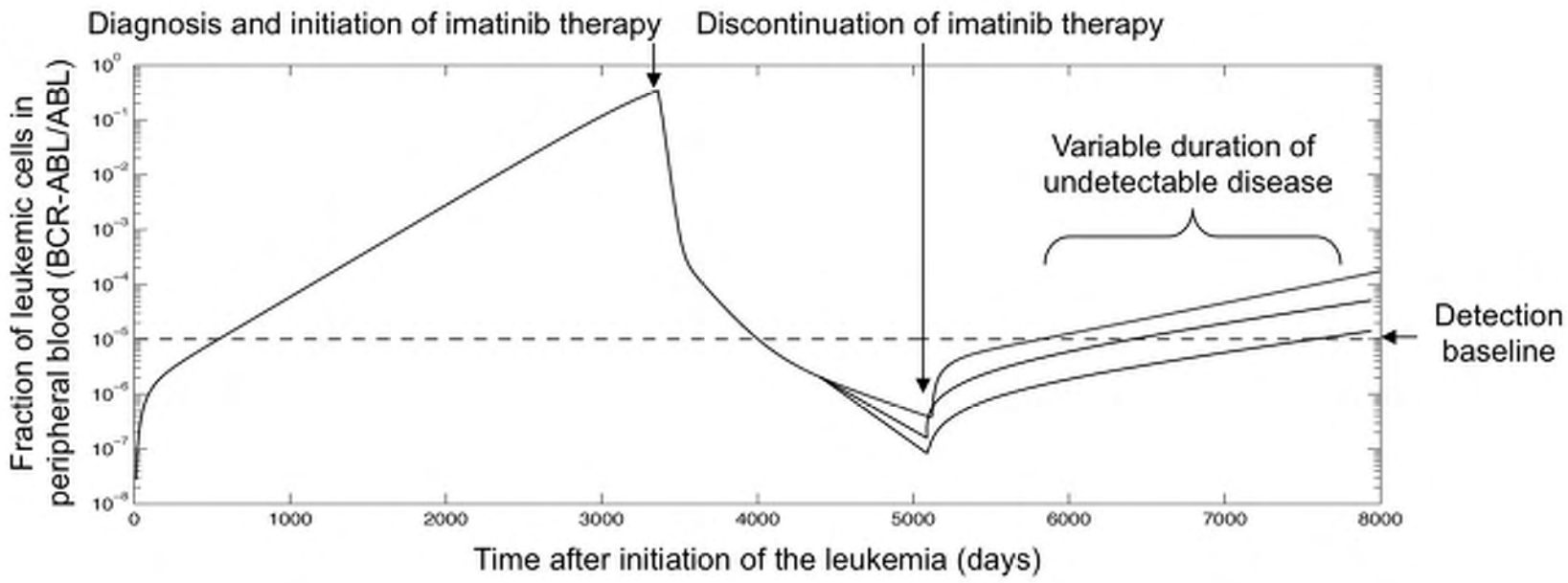
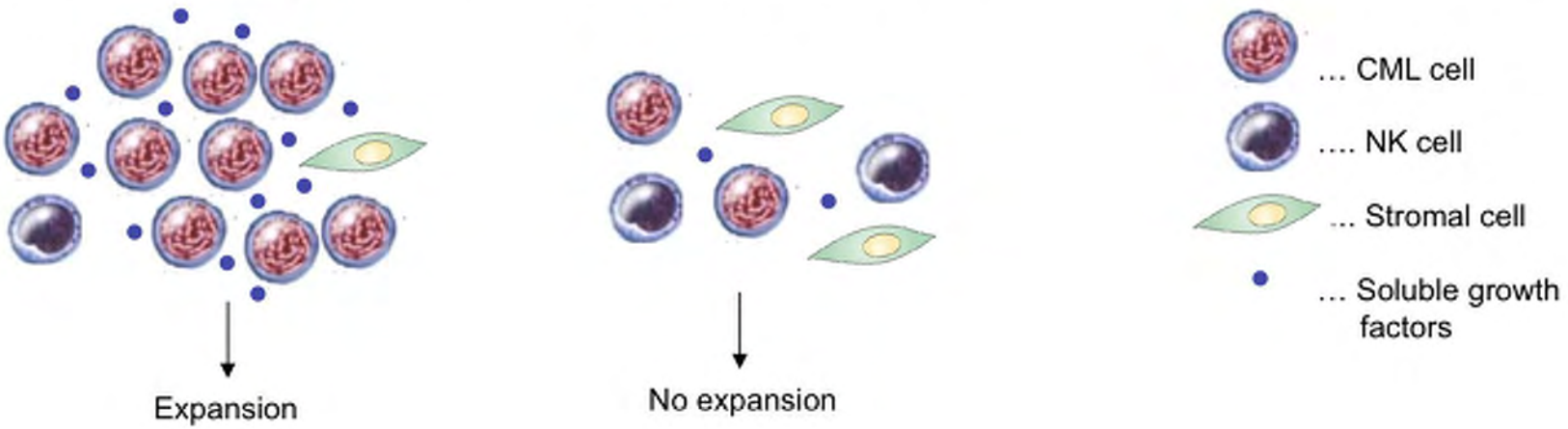


DOI: 10.3324/haematol.2012.062844

Fig. 4

c)



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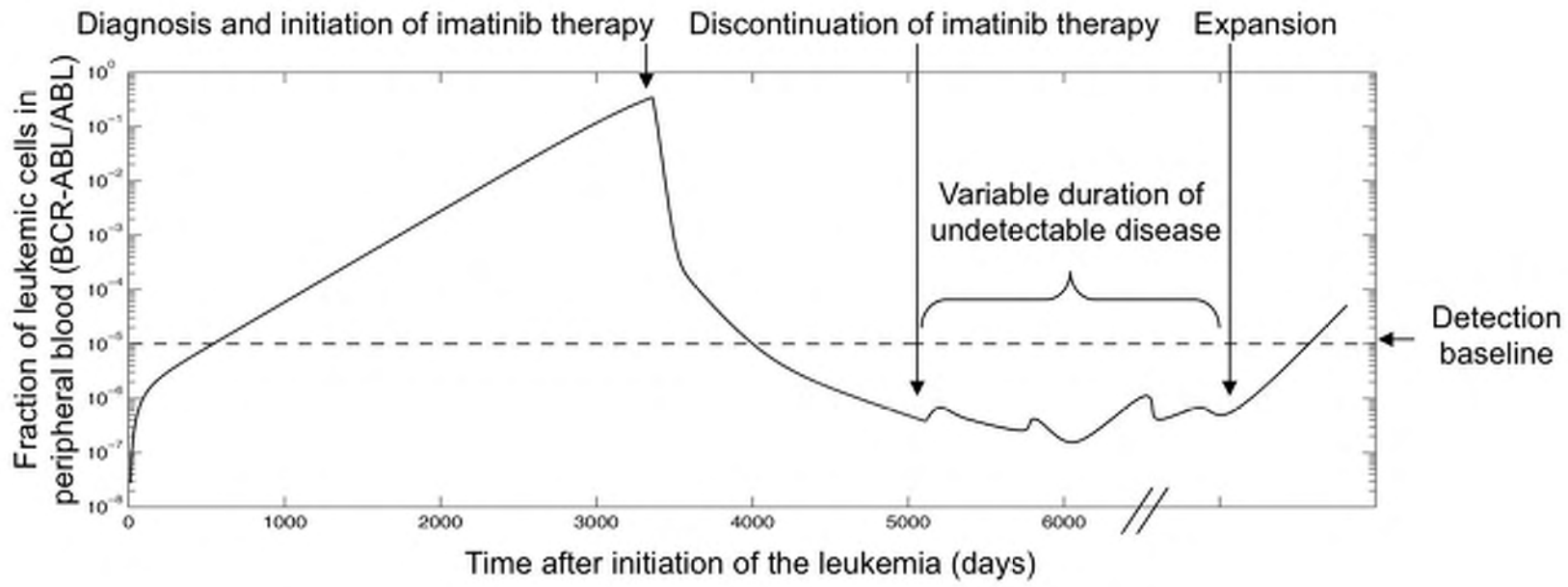

DOI: 10.3324/haematol.2012.062844

SUPPORTING INFORMATION

TABLE OF CONTENTS

The basic mathematical model

We utilized a mathematical model of the treatment response of CML cells to imatinib therapy (1-2), 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 both to 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 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. Imatinib therapy reduces the production rates of leukemic progenitors and differentiated cells, and potentially also inhibits the expansion of leukemic stem cells.

Denote by x_0, x_1, x_2, and x_3 the abundances of normal hematopoietic stem cells, progenitors, differentiated cells, and terminally differentiated cells. Their respective

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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. Leukemic stem cells grow at a slow pace until reaching their maximum number, which may be larger than that of normal stem cells; afterwards, their number is also held constant by a density dependence mechanism. Then the system containing stem cells (SC), progenitor cells (PC), differentiated (DC) and terminally differentiated cells (TC) is 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 dramatically reduces the differentiation rates of cells, a_y to a_y' and b_y to b_y'. This change in rates leads to a bi-phasic decline of the leukemic cell burden (1). 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. For comparison with experimental PCR data, we calculate the BCR-ABL to ABL ratio as $y_3/(2x_3 + y_3)$ times 100%; a healthy cell has two copies of ABL, while a leukemic cell normally has one copy of ABL and one copy of BCR-ABL. Since most cells that are sampled by the PCR assay are terminally differentiated cells, the calculation of the BCR-ABL/ABL ratio using the mathematical framework includes the abundance of terminally differentiated cells only. The time of loss of CMR is defined as the time at which the BCR-ABL/ABL transcript level exceeds 10^{-5}: when $\frac{y_3}{2x_3 + y_3} > 10^{-5}$. This definition of the BCR-ABL/ABL transcript level when reaching CMR can be varied to obtain a different cutoff; if a different cutoff is chosen,

DOI: 10.3324/haematol.2012.062844

then the estimation of the parameter values that replicate the trial dynamics (see Statistical analysis section) would lead to slightly different numerical results, but the basic conclusions would hold. This system of equations was numerically solved using a fourth-order Runge-Kutta integration scheme.

Extensions of the mathematical model

We investigated the possibility of an explicitly heterogeneous leukemic stem cell population within each patient as a possible mechanism for the imatinib selection effect. To this end, we considered a system with two leukemic stem cell populations: type-1 leukemic stem cells have intrinsic growth rate $r_{y,1}$ and type-2 leukemic stem cells have growth rate $r_{y,2}$ both before and after treatment with imatinib ($r_{y,1}'' = r_{y,1}$, $r_{y,2}'' = r_{y,2}$). The two cell populations have distinct growth kinetics, $r_{y,2} < r_{y,1}$, so that at diagnosis and the start of treatment, the majority of leukemic stem cells are of type-1. To bear out the imatinib selection hypothesis, we assumed that the effect of imatinib is stronger on the more aggressive type-1 cells than the more indolent type-2 cells such that $r_{y,1}' < r_{y,2}'$. Figures S1 and S2 demonstrate the effect of this hypothesis on the composition of the stem cell population. Figure S1A shows the terminally differentiated leukemic cell population over time for a system with a two-type heterogeneous leukemic stem cell compartment as described above, as well as for a homogeneous stem cell population. Note that heterogeneity in cell phenotypes is also included in the basic mathematical model incorporating only a single type of stem cells, since the kinetics of the stem cell population can be described by a distribution of growth and differentiation rates rather than a single value. Figure S1B displays the leukemic stem cell population sizes in both cases. Note that effects of a heterogeneous stem cell compartment on the observable quantities (terminally differentiated cells) may be minimal (Figure S1A), even though dramatic differences may be taking place at the level of leukemic stem cells (Figure S1B). Figure S2 shows the composition of the leukemic stem cell compartment in the heterogeneous system, both before imatinib treatment at detection and after treatment at the time of relapse. In this system, the effect of imatinib is to select a less aggressive population of stem cells, which was undetectable prior to treatment. This subpopulation

comprises the majority of the leukemic stem cell compartment after treatment, therefore lowering the effective stem cell growth rate.

The parametric cure model

Let us now consider the scenario in which imatinib has the potential to eradicate leukemic stem cells in some patients and CML can be cured by imatinib therapy alone. The possibility of cure is equivalent to the assumption that the population relapse time distribution is improper – i.e. as time goes to infinity, the probability of relapse is less than 1.

To investigate this scenario, we first fit parametric cure models to the data, assuming that the cure rate p is greater than zero. Based on the cure model, the patient population is made up of a mixture of two sub-populations, one that can be cured of their disease and one that will relapse sooner or later after imatinib discontinuation. We call subjects in the sub-population that will always relapse the 'susceptibles'. Then the survival function of the relapse time under the assumption of a cure model is a mixture of a parametric survival function for the susceptibles and a cure mass, i.e. $S(t) = p + (1 - p)\, S_0(t)$, where $S_0(t)$ denotes the survival function of the susceptibles and p represents the cure rate. We then used the maximum likelihood estimation method to fit the cure model to the clinical data and chose for $S_0(t)$ the most commonly used distributions including Weibull, lognormal, exponential, logistic and log logistic distributions. Of these distributions, the cure model with $S_0(t)$ being the survival function of the log logistic distribution exhibited the best fit with both the largest likelihood and lowest AIC (Akaike Information Criterion (3)) (Fig. 1b). The estimated cure rate based on this log logistic cure model was 0.41.

Testing for sufficient follow-up

To make any conclusions regarding the cure of some of the patients enrolled in the STIM trial, we tested whether there was sufficiently long follow-up in the clinical data.

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We utilized the nonparametric method, i.e. no assumptions were made on the type or shape of the underlying survival or censoring distributions, to estimate the cure rate *p* and tested whether the follow-up is sufficient in the STIM trial data. Throughout this paper, we assumed random (or non-informative) censoring, i.e. each subject's censoring time is statistically independent of its failure time.

We first obtained the nonparametric estimator of the cure rate for the STIM data and then tested whether there was sufficiently long follow-up to identify the existence of a cure, based on the measurement of the distance between the largest observed time and the largest uncensored relapse time. To be specific, let *n* be the total number of observations; $t_{(n)}$ be the largest observed (relapse or loss of follow-up) time; and $\hat{S}(t)$ be the Kaplan-Meier Estimator (KME) of the survival function for the relapse time. For the STIM trial data, n = 100 and $t_{(n)}$ = 911. Then the nonparametric estimator of the cure rate p is given by $\hat{p} = \hat{S}(t_{(n)})$ = 0.39 (4). We then applied the procedure outlined in Section 4.3 of (4) to test for the existence of sufficiently long follow-up, which is based on the measure of the distance between the largest observed time $t_{(n)}$ and the largest uncensored relapse time $t^*_{(n)}$. Let N_n be the number of uncensored observations in the interval $(2t^*_{(n)} - t_{(n)}, t^*_{(n)}]$ and $q_n = N_n / n$ be the proportion of uncensored observations in that interval. Following arguments in Section 4.3 of (4), large values of q_n generally lead to the conclusion that there was sufficiently long follow-up while small values of q_n represents insufficient follow-up. More specifically, if the observed value of q_n is greater than the 95% quantile of the simulated distribution of q_n, then there is strong evidence that there was sufficiently long follow-up in the sample. If, on the other hand, the observed q_n was less than the 5% quantile of the simulated distribution, then there was good evidence that follow-up was insufficient. However, if the observed value of q_n resided between the 10% and 90% quantiles, then we concluded that the data was inconclusive. In that case, it was doubtful that the data had leveled off sufficiently much to make any conclusions about the existence of a cure, but there was also no strong evidence that the follow-up was insufficient. For the clinical data utilized here, $t^*_{(n)}$ = 678; $(2t^*_{(n)} - t_{(n)}, t^*_{(n)}]$ = (445, 678]; N_n = 2 and q_n = 0.02.

DOI: 10.3324/haematol.2012.062844

We then conducted simulations to obtain the distribution of q_n, where survival times were generated from a log logistic distribution (the log logistic distribution was chosen because as outlined in the above section, it exhibited the best fit with both the largest likelihood and lowest AIC) for a number of observations of n = 100 and cure rate of p = 0.39. We simulated censoring times using a sampling with replacement procedure with samples representing the censoring times in the STIM trial data. We also simulated censoring times from a uniform distribution over the range of the censoring times in the STIM trial data and reached the same conclusions. With 100,000 replicates, the 5%, 10%, 90% and 95% percentiles of the simulated q_n values for this simulation setting were 0.01, 0.01, 0.59 and 0.59. Since q_n for the STIM trial data is 0.02, falling between the 10% and 90% percentiles, we thus obtained the conclusion that it was doubtful that the STIM data had leveled off sufficiently much to make any conclusions about the existence of a cure. Worded differently, there was no strong evidence for neither sufficient nor insufficient follow-up in the STIM trial data, which represents an interim report of this clinical trial. When longer follow-up becomes available, we will repeat the above procedure to test for the existence of sufficient follow-up in the updated data.

Statistical analyses

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 statistical approach to determine the parameter distributions given the observed relapse time data.

Since our goal was to identify the set of parameters that are consistent with the observed distribution of the time of CMR loss, we needed a complete estimate for the distribution of CMR loss times (i.e, defined for all times). Since traditional Kaplan-Meier

DOI: 10.3324/haematol.2012.062844

estimates are defined up to the maximum event time, we were unable to use them. Instead we utilized the maximum likelihood estimation method to fit a parametric distribution to the observed data and chose between the most commonly used distributions including Weibull, lognormal, exponential, logistic and log logistic distributions. Of these distributions, the lognormal distribution exhibited the best fit with both the largest likelihood and lowest AIC (Akaike Information Criterion (3)) for the non-cure model (Fig. 1c) while the log logistic distribution exhibited the best fit for the cure model (Fig. 1b), as outlined in the section above.

We then numerically solved the differential equation system to obtain the relapse time for different parameter sets. A fine grid was used to sample the five-dimensional parameter space, and the corresponding times of loss of CMR were determined using the mathematical model. We then selected randomized subsets (denoted as S.time) of outcomes from these times that recapitulated the fitted lognormal (for the non-cure model) or the fitted log logistic (for the cure model) survival curves up until the maximum follow-up. The set *S*, which contains parameter vectors resulting in the above selected survival times in the set S.time via the mathematical model, then represents a set of samples from the joint density of the five parameters. To compare the Kaplan-Meier curve obtained from our sampling procedure to the Kaplan-Meier survival curve of the clinical data, the one-sample log-rank test as implemented in the survdiff function in R was used.

More specifically, we defined possible ranges of the five parameters, based upon our mathematical model: a_y'' varies in the range (0, 1.6], b_y'' in (0, 10], c_y'' in (0, 200], r_y' in [0,0.003], and r_y'' in [0, 0.016], with the restrictions that $a_y'' \leq b_y'' \leq c_y''$ and $r_y' \leq r_y''$. A fine grid (over 260 million samples) was used to sample the five-dimensional parameter space from these ranges. Let *G* denote this grid. For each $g \in G$, we numerically solved the differential equation system to obtain the relapse time and the censoring indicator (T_g, C_g). The case of $C_g = 1$ denotes the situation in which CMR is lost; then the corresponding T_g represents the relapse time. The case $C_g = 0$ denotes the situation in which CMR is not reached; then the corresponding T_g represents the last follow-up time of the patient. Let *P* denote the set of (T_g, C_g) pairs obtained through this process. We

then tagged each relapse time in *P* according to its corresponding 18-quantile in the fitted density and considered it for acceptance into the sample set *S*.time. To ensure that S.time correctly recapitulates the fitted lognormal (for the non-cure model) versus log logistic (for the cure model) density function, samples were drawn from each bin uniformly at random, and a set of samples was accepted into the set *S*.time proportionally according to the contribution of each quantile to the density. The final set S, which contains parameter vectors corresponding to relapse time points in the set S.time, behaves like a random sample from the joint distribution of the parameter estimates. The marginal densities of each parameter were estimated using the non-parametric kernel density estimation technique (a.k.a. Parzen-Rosenblatt window method (5)). Multiple resamplings of the set *S* resulted in little or no change in the marginal densities, indicating robustness of the resulting distributions to this procedure. A randomly obtained sample of points chosen from a uniform distribution could have been used instead of the regular grid G for the purposes of obtaining (T_g, C_g). Using a regular grid is similar to latin hypercube sampling (6) and is more efficient than random sampling in most computational problems that require repeated evaluations from a sample space (7).

Supplementary references

1. Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, et al. Dynamics of chronic myeloid leukaemia. Nature. 2005 Jun 30;435(7046):1267-70.
2. Michor F. The long-term response to imatinib treatment of CML. . British Journal of Cancer. 2007;96:679-80.
3. Akaike H. A new look at the statistical model identification. Institute of Statistical Mathematics. 1974;19:716-23.
4. Maller R Z, X. Survival Analysis with Long-Term Survivors. 1 ed: Wiley, 1996.
5. Silverman B. Density Estimation for Statistics and Data Analysis. 1 ed: Chapman & Hall, 1986.
6. McKay MD B, RJ, Conover WJ. A Comparison of Three Methods for Selecting Values of Input Variables in the Analysis of Output from a Computer Code. Technometrics. 1979;21(2):239-45.
7. Niederreiter H. Random Number Generation and Quasi-Monte Carlo Methods. Society for Industrial and Applied Mathematics. 1992.

Supplementary figure legends

Figure S1. The effects of a heterogeneous leukemic stem cell population. **(A)** Terminally differentiated leukemic cell population over time, for a homogeneous

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leukemic stem cell compartment (r_y = 0.008, r_y' = 0.0015), and a heterogeneous two-type leukemic stem cell compartment ($r_{y,1}$ = 0.008, $r_{y,2}$ = 0.007, $r_{y,1}$' = 0.0005, $r_{y,2}$' = 0.0025). For both systems, a_y'' = 0.8, b_y'' = 5, c_y'' = 100, and all other parameters are the same as throughout the paper. **(B)** Leukemic stem cell population over time for a homogeneous stem cell compartment and for each type in a heterogeneous stem cell compartment. Parameters are as in (A).

Figure S2. The time evolution of a heterogeneous leukemic stem cell population. Composition of leukemic stem cell compartment (frequency of type-1 and type-2 cells) before imatinib treatment and at the time of relapse (post-imatinib). Parameters are as in Fig. S1.

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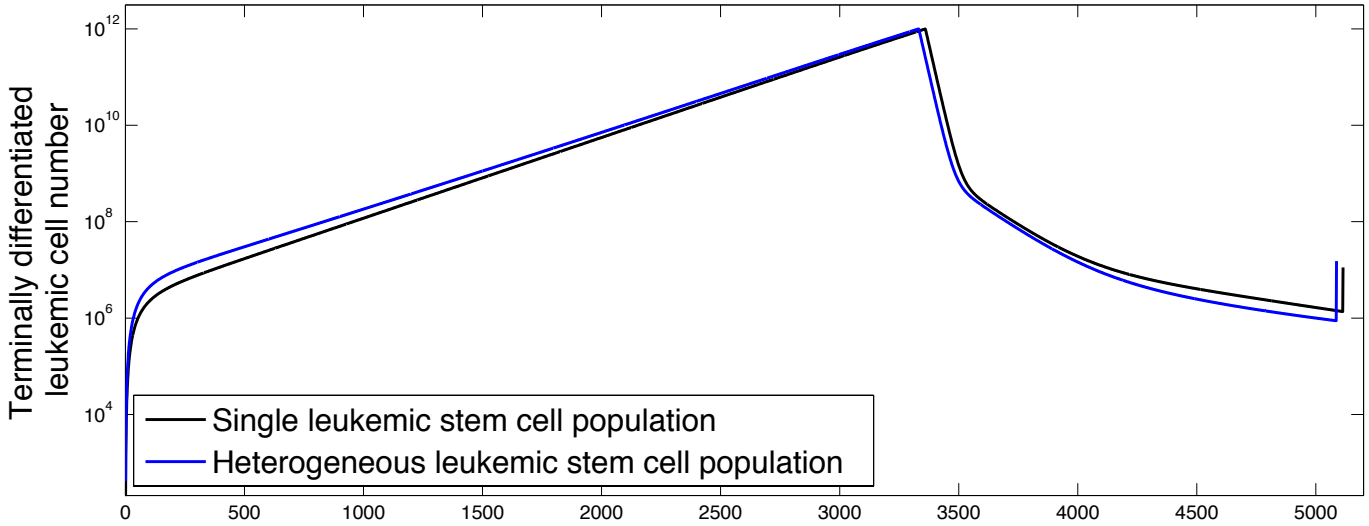
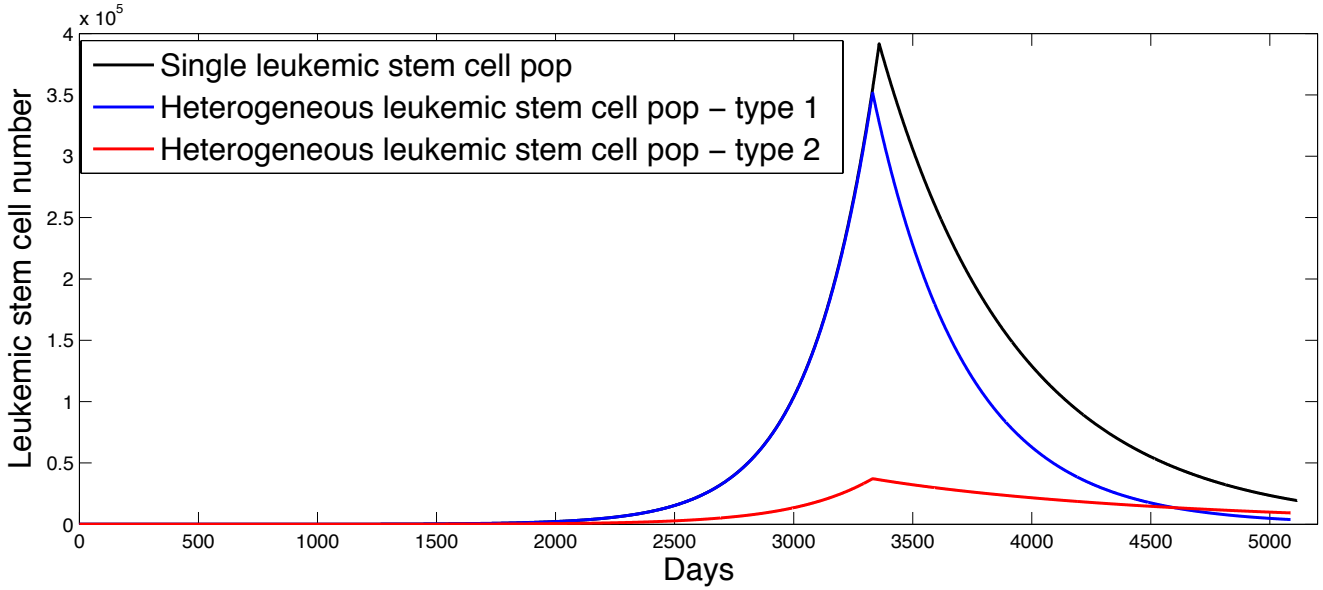
A



B



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