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Short title: Dynamics of long-term targeted therapy response

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Figure/table/references count: figures: 4, tables: 3, references: 45.
ABSTRACT

Treatment of chronic myeloid leukemia (CML) with the tyrosine kinase inhibitors (TKIs) imatinib and nilotinib represents a successful application of molecularly targeted anti-cancer therapy. However, the effect of TKIs on leukemic stem cells remains incompletely understood. Based on a statistical modeling approach using the 10-year imatinib treatment response of CML patients and a patient cohort receiving first-line nilotinib therapy, we found that successful long-term therapy results in a tri-phasic exponential decline of BCR-ABL1 transcripts in many patients. Within our framework, the first slope of \(-0.052 \pm 0.018\) (imatinib) and \(-0.042 \pm 0.015\) (nilotinib) per day represents the turnover rate of leukemic differentiated cells, while the second slope of \(-0.0057 \pm 0.0038\) (imatinib) and \(-0.0019 \pm 0.0013\) (nilotinib) per day represents the turnover rate of leukemic progenitor cells. The third slope allows an inference of the behavior of immature leukemic cells, potentially stem cells. This third slope is negative in most patients, positive in others, and not observable in some patients. This variability in response may be due to insufficient follow-up, missing data, disease heterogeneity, inconsistent adherence to drug, or acquired resistance. Our approach suggests that long-term TKI therapy may reduce the abundance of leukemic stem cells in some patients.
INTRODUCTION

Chronic myeloid leukemia (CML) is the first hematological malignancy treated with a molecularly targeted small molecule inhibitor, the tyrosine kinase inhibitor (TKI) imatinib mesylate\(^1\) (Gleevec). This agent induces clinical, cytogenetic and molecular remission and prolongs progression-free survival.\(^2-3\) The phase III multicenter International Randomized Study of Interferon versus STI-571 (IRIS) trial reported the superiority of imatinib over IFN-\(\alpha\) plus cytosine arabinoside (AraC) in 1,106 previously untreated chronic phase patients. Five years after the initiation of imatinib therapy, 40% of chronic phase patients achieved a complete molecular response,\(^4\) and estimated overall survival was 89% at 5 years and 85% at 8 years.\(^5\) Recently, trials utilizing the second-generation TKIs nilotinib and dasatinib as first-line therapy were initiated and showed promising results\(^6-7\). However, the question remains whether leukemic stem cells are sensitive to TKI therapy and whether this treatment represents a cure of the disease.\(^8-9\)

To study the dynamics of imatinib treatment response, we had previously analyzed data from the IRIS trial as well as a phase II trial (Therapeutic intensification in de-novo leukemia (TIDEL)\(^10\)) conducted by the Australasian Leukemia and Lymphoma Group. The TIDEL trial enrolled patients with newly diagnosed chronic phase CML using 600mg of imatinib per day initially, increasing to 800mg if specified response criteria were not met. Based upon 12 months follow-up data of a subset of these patients treated with 600mg of imatinib per day, our analysis demonstrated that imatinib therapy leads to a bi-phasic exponential decline of the leukemic cell burden.\(^11\) The bi-phasic shape of the treatment response curve was later reconfirmed using data of patients treated with 400mg per day enrolled in the German cohort of the IRIS trial;\(^12\) therefore, the bi-phasic nature of the treatment response is apparently not dependent upon the dosage of imatinib used as long as a biologically active concentration is administered.

We then designed a mathematical framework based on a four-compartment model which could explain the kinetics of the molecular response to TKIs in this patient cohort.\(^11,13\) Based upon our framework,\(^11\) the two slopes were interpreted as representing the decline of differentiated leukemic cells (the slope between the baseline measurement and the 3\(^{rd}\) month of treatment) and leukemic progenitor cells (the slope between the 6\(^{th}\) and 12\(^{th}\) months of treatment). We also analyzed the dynamics of the leukemic cell burden in three patients who discontinued imatinib therapy after one to three years of treatment, finding that treatment cessation led to a rapid rebound to levels at or beyond pre-treatment baseline. These rebound kinetics led us to hypothesize that the cell population driving the disease, leukemic “stem cells”, were not depleted by a large amount in these three patients since otherwise, imatinib cessation would have led to a rebound to levels significantly below pre-treatment baseline. However, further data is necessary to make general conclusions about the imatinib treatment effect on leukemic stem cells. Two types of data contain information about the behavior of leukemic stem cells during imatinib therapy. First, if a large number of CML patients discontinue imatinib and their relapse kinetics are closely followed,\(^14-16\) then the dynamics of leukemic stem cells during and after imatinib treatment can be inferred.\(^17\) Second, information on the response kinetics of patients receiving long-term imatinib
may reveal clues about the treatment effect on leukemic stem cells. Here we utilized 10-year follow-up data of patients enrolled in the IRIS trial, together with a statistical modeling approach and our mathematical framework,\textsuperscript{11,13} to investigate the effects of long-term imatinib therapy on CML stem cells. Furthermore, we employed a dataset of CML patients treated with first-line nilotinib\textsuperscript{6} (400mg twice a day) to elucidate the treatment response to a second-generation TKI.

**MATERIALS AND METHODS**

We utilized data of 103 newly diagnosed patients who were treated with first-line imatinib for 12 months within the TIDEL trial\textsuperscript{10}. For each patient, RQ-PCR was used to determine the BCR-ABL1/BCR values at the baseline as well as months 1, 2, 3, 6, 9, and 12. We also utilized data of 29 newly diagnosed patients who were treated with first-line imatinib (400mg per day) within the IRIS trial for up to 10 years. The BCR-ABL1% values of these patients were recorded every three months. For the long-term imatinib cohort, values below detection of the RQ-PCR assay (values of zero in the data) were converted to positive BCR-ABL1 values that were likely present in the sample. This conversion was needed for the logarithmic transformation described below and justified since several studies\textsuperscript{14-16} have led to the conclusion that undetectable BCR-ABL1 transcripts are not synonymous with a cure of the disease, but that residual leukemic cells frequently remain in those patients. Furthermore, the limit of detection of each sample varies with the quality of the RNA and the efficiency of the reverse transcription reaction, which can lead to fluctuations in PCR positivity\textsuperscript{18}. The conversion is based on the BCR control gene transcript value and the standardized baseline value for our BCR-ABL1 detection assay\textsuperscript{4,8} (see Supplementary Information (SI)). This converted database was then used to examine the long-term imatinib treatment response. Our analyses are based on the assumption that the converted values and the true BCR-ABL1 abundance are directly proportional. Finally, we utilized data of a subcohort of newly diagnosed patients who were treated with first-line nilotinib (400 mg BID) at the M. D. Anderson Cancer Center with a follow-up of up to 66 months\textsuperscript{6} (29 patients). The BCR-ABL1% values of these patients were recorded every six months, with an additional time point at 3 months after treatment initiation. Details of the patient selection, statistical and mathematical modeling approaches are provided in the SI, and the individual BCR-ABL1/BCR% plots of the selected patients are shown in Supplementary Figures S1-S3.

**RESULTS**

To investigate the effects of imatinib and nilotinib on different subpopulations of leukemic cells, we utilized a statistical modeling approach to identify the shape of the treatment response curves in the short-term (12 months follow-up) and long-term (10 years follow-up) imatinib patient cohorts as well as the first-line nilotinib patient cohort (up to 66 months follow-up). Since no data was recorded between the baseline and the 3\textsuperscript{rd} month of treatment for patients in the long-term imatinib cohort, we were not able to
estimate the first slope of decline (between 0 and about 3 months of treatment) observed in the leukemic cell burden\textsuperscript{11} using this cohort. We therefore analyzed the short-term imatinib patient cohort to obtain the kinetics of the initial decline, and then utilized the 10-year follow-up data to infer the dynamics of the long-term response to treatment. Although the imatinib dose administered differs between the short- and long-term cohorts and therefore the slope of depletion may vary among those patients, the shape of the treatment response curve is apparently independent of the imatinib dose administered\textsuperscript{11-12} as long as a biologically active dose is used. The nilotinib patient cohort was analyzed to determine the shape of the nilotinib treatment response curve.

**Imatinib treatment response.** We investigated two statistical models to identify the one with the best fit using our short-term imatinib cohort (0 to 12 months of treatment), and then repeated this approach using the long-term imatinib cohort (6 months to 10 years of treatment). The two statistical models analyzed were (i) an exponential model, which predicts that the leukemic cell burden declines at a single exponential rate; and (ii) a bi-phasic exponential model predicting that the BCR-ABL1/BCR counts decline at two exponential slopes with a turning point (SI). We first fit these models to the short-term imatinib response data (Figure 1). Table 1 provides summary information for the statistical model fitting to individual patient data as well as the overall $R^2$ for each model. We found that the bi-phasic exponential model provides a larger final $R^2$ as well as better patient-specific $R^2_i$ than the exponential model. We then demonstrated, with a bootstrapping approach, that the final $R^2$ of the bi-phasic exponential model is significantly larger than the final $R^2$ of the single exponential curve (p-value = 0, see SI). A BIC criterion, which penalizes model size, also favors the bi-phasic model for the entire patient cohort (SI). We thus chose the bi-phasic exponential model as the best-fitting statistical model for the short-term imatinib treatment response data; this result is in agreement with previous findings.\textsuperscript{11} Figure S1 displays the individual patient data with the subject-specific fit of the bi-phasic model. Besides the analysis of the entire cohort, we also performed individual model fitting to compare, for each individual patient, the fit of the linear and bi-phasic models. Based on the BIC criterion, we obtained a better fit of the bi-phasic model for all 44 patients. The first slope of depletion for these 44 patients was identified as $-0.052 \pm 0.018$ per day (Table 3); all slopes are given in units per day. The second slope was negative in 35 patients, zero in one patient, and positive in 8 patients; the positive slopes were potentially due to the emergence of acquired resistance in these patients. However, we did not utilize this patient cohort to determine the second slope since the long-term imatinib cohort contained more information (i.e., a longer follow-up) and therefore, the second slope could be determined with greater precision using data of the long-term cohort.

We then analyzed the long-term IRIS trial data (Figure 2). We again fit our two statistical models, using data starting from month 6 after the initiation of treatment. This choice was made since there was not sufficient data between months 0 and 6 in this cohort to determine the first slope observed in the short-term cohort, and since based on the analysis of the short-term data, the second slope initiates between months 3 and 6. The summary information of the fitting is displayed in Table 1. We again identified the model with the significantly larger final $R^2$ through a bootstrapping simulation (p-value 0.04,
SI), and also employed the BIC criterion to distinguish the models. We utilized the weighted least squares method to give less weight to the converted data points in the long-term patient cohort (see SI). Using these different approaches, we obtained consistent results: the bi-phasic exponential model was chosen as the model with a better fit to the long-term imatinib patient cohort. Figure S2 displays the individual patient data with the subject-specific fit of the bi-phasic model. Besides the analysis over the whole cohort, we also performed individual model fitting to compare, for each individual patient, the two statistical models. Based on the BIC criterion, the bi-phasic model represented the better fit for 14 out of 22 patients. The slope starting from the 6th month of treatment, which is equivalent to the second slope starting from the time of treatment initiation based on the short-term cohort, for these 14 subjects was -0.0057 ± 0.0038 (Table 3). This slope was negative in all 14 patients, suggesting that these patients did not harbor acquired resistance during that time. The last slope, which commences at about 34 months after the initiation of imatinib therapy and represents the third slope after treatment initiation, was negative in 13 patients and positive in one (patient 17). This last slope was -0.0006 ± 0.0006 averaged over all 14 patients and -0.0008 ± 0.0003 averaged over those 13 patients who had negative last slopes. The estimated turning point averaged over all 14 subjects was 34.5 ± 22.6 months, while the turning point for the patient with a positive last slope was 8.25 years (Table 3).

Based upon these statistical analyses of both short- and long-term imatinib treatment responses, we conclude that the imatinib treatment response of BCR-ABL1 transcripts in the peripheral blood of most patients displays three phases: during the first phase – between 0 and approximately 4 months after the initiation of therapy, the abundance of leukemic cells decreases with a slope of -0.052 ± 0.018 per day; this slope was identified using the short-term patient cohort since only this group contained sufficiently many early time points such that the slope could be robustly identified. During the second phase (between 4 and about 34 months after the initiation of therapy), the leukemic burden declines with a slope of -0.0057 ± 0.0038 per day; this slope was identified using the long-term cohort since the short-term cohort had a follow-up of only 12 months and might therefore lead to biased estimates of the second slope. During the third phase – starting at about 34 months after treatment initiation, the leukemic cell burden decreased in 13/14 patients with an average slope of -0.0008 ± 0.0003 while it increased in one patient with a slope of 0.0014. Overall (all 14 patients), the third slope is -0.0006 ± 0.0006 (Table 3).

Nilotinib treatment response. We then analyzed the nilotinib treatment response utilizing our nilotinib patient cohort and fit our two statistical models to this database. The summary information of the fitting is displayed in Table 2. We identified the model with a significantly larger final $R^2$, and also employed the BIC criterion to distinguish the models (SI). The bi-phasic exponential model was again chosen as the model with the better fit to the nilotinib response data. Figure S3 displays the individual patient data with the subject-specific fit of the bi-phasic model. In addition to the analysis over the whole cohort, we also performed individual model fitting to compare, for each individual patient, the performance of the two statistical models. Based on the BIC criterion, the bi-phasic model had a better fit for 28 out of 29 patients. There were 5 patients (IDs 1-5)
with a follow-up of at least 60 months. We hypothesized that these patients might have a sufficiently long follow-up such that a tri-phasic decline may be identified in these patients. We therefore compared the fit of linear, bi-phasic and tri-phasic models for these 5 subjects using the Joinpoint Regression Program\textsuperscript{19}. Based on the BIC criterion, three patients (IDs 1, 2 and 4) were identified as having a tri-phasic decline of their leukemic cell burden while two (patients 3 and 5) displayed a bi-phasic decline (Table S3). Thus, based on our analysis of the nilotinib cohort containing 29 patients, one subject had a linear decline, 25 subjects had a bi-phasic, and 3 a tri-phasic decline. The first slope for the 28 subjects who had more than one slope was -0.0424 ± 0.0154. (Table 3). This slope was negative in all patients, suggesting that these patients did not harbor acquired resistance during that time. The second slope was negative in 24 patients, zero in 2 patients (ID 6, 25) and positive in two (patient 26, 27). The second slope averaged over all 28 subjects was -0.0014 ± 0.0017, while it was -0.0019 ± 0.0013 averaged over the 24 patients with negative second slopes. The estimated turning points for all 28 subjects were 4.81 ± 2.20 months, while they were 4.33 ± 1.62 months for the 24 patients with negative second slopes (Table 3). The third slopes for patients 1, 2 and 4 were -0.00028, -0.00002 and -0.00697, respectively.

Based on these statistical analyses of the nilotinib treatment response, we concluded that a second-generation BCR-ABL inhibitor, nilotinib, elicits very similar treatment responses as the first-generation inhibitor, imatinib: the BCR-ABL1 transcripts in the peripheral blood of most patients decrease in two phases during short-term treatment. During the first phase – between 0 and approximately 4 months after the initiation of nilotinib therapy, the abundance of leukemic cells decreases with a slope of -0.0424 ± 0.0154 per day. During the second phase, the leukemic burden declines in 24/28 patients with an average slope of -0.0019 ± 0.0013 per day while it is zero in two patients and increases in two patients with slopes of 0.0011 and 0.0030. Overall (all 28 patients), the second slope is -0.0014 ± 0.0017 (Table 3). Out of 5 patients with a sufficiently follow-up, three displayed a tri-phasic trend; the third slopes for these three patients were -0.00028, -0.00002 and -0.00697, respectively.

**Mathematical modeling.** We then utilized our mathematical framework describing four subtypes of leukemic cells\textsuperscript{11,13} to predict the dynamics of BCR-ABL1/BCR values in the peripheral blood of newly diagnosed CML patients treated with a TKI. This model was designed to relate the available data on BCR-ABL1 transcript levels in peripheral blood to the kinetics of other, unobservable differentiation levels of leukemic cells (see SI). In the context of this mathematical framework, the three slopes observed in the data are interpreted as a decline in the abundance of differentiated (but not terminally differentiated) cells (first slope), progenitor cells (second slope), and finally a depletion (or expansion) of leukemic stem cells (third slope). In addition to these three cell types, we postulated the existence of a fourth differentiation level, made up of terminally differentiated cells that have an average lifespan of a day, whose depletion cannot be observed in the data due to limited resolution\textsuperscript{11,13}. This framework, together with the estimates of the three slopes obtained with our statistical model fitting approach, was then used to predict the kinetics of the treatment response (Figure 4). After treatment initiation, the leukemic cell population in peripheral blood declines at the death rate of
differentiated cells during therapy (equal to the first slope identified in the data, mean -0.052 ± 0.018 (imatinib) and -0.042 ± 0.015 (nilotinib)) until the latter reach a steady state with progenitor cells; from this time onwards, the kinetics display a shallower decrease signifying the depletion of progenitor cells during imatinib treatment (equal to the second slope identified in the data, mean -0.0057 ± 0.0038 (imatinib) and -0.0019 ± 0.0013 (nilotinib)). Finally, there is a second turning point in the dynamics of leukemic cells, which is caused by the progenitor cells reaching a steady state with their upstream population, potentially stem cells; after that time, the kinetics of treatment response are driven by the behavior of this population. Due to insufficient follow-up and/or missing data, this third slope could not be identified in all patients, but was observed in 14 imatinib-treated patients (mean -0.0006 ± 0.0003) and 3 nilotinib-treated patients (-0.00028, -0.00002 and -0.00697). It is possible that the population declining with the third slope does not represent leukemic stem cells but an immature population more differentiated than stem cells. In the latter case, we predict that the third slope signifies the decline (or increase) of this immature population, and that we would observe a fourth slope, representing the behavior of leukemic stem cells, if the detection limits of PCR assays improve and even longer follow-up was available.

In most patients of the long-term imatinib cohort, the third slope is negative, suggesting that imatinib may be capable of decreasing the abundance of leukemic stem cells in some patients. In a small subset of patients, however, there is a slow increase in the total leukemic burden during the third phase. In some cases, the third slope cannot be observed, probably due to insufficient follow-up or due to missing data. Our mathematical framework, together with data of the longest currently available follow-up of TKI-treated patients, thus predicts that in this subset of highly selected patients – those who tolerated continuous imatinib or nilotinib therapy for several years without the evolution of resistance, progression of disease, or adverse effects, TKIs may be able to decrease the abundance of leukemic stem cells in some patients.

DISCUSSION

It has been suggested that it is impossible to cure CML using targeted therapy because leukemic stem cells cannot be eradicated.20 For months or years after achieving a complete cytogenetic response, the majority of CML patients treated with imatinib has measurable disease by RQ-PCR and would relapse if imatinib therapy was discontinued.21-22 However, in the majority of patients who respond to imatinib, there is a progressive decline in the BCR-ABL1 counts over time, such that after several years of treatment an increasing number of patients achieve a complete molecular response within the limitations of the assay. Whether all CML cells have been eradicated in any of these patients is a question of significant clinical and scientific importance. Furthermore, the dynamics of responses to second-generation inhibitors have not yet been investigated.

In this paper, we performed statistical analyses of both short- and long-term imatinib as well as nilotinib treatment responses, encompassing patients who were treated with
600mg of imatinib and followed for 12 months, patients who were treated with 400mg of imatinib with a follow-up of up to 10 years, and patients who were treated with first-line nilotinib (400mg BID) with a follow-up of up to 66 months. Based upon these data and our mathematical framework,\textsuperscript{11,13} we concluded that the leukemic treatment response displays three phases: during the first phase, the abundance of leukemic cells decreases with a slope of -0.052 ± 0.018 (imatinib) or -0.042 ± 0.015 (nilotinib) per day, representing a depletion of differentiated leukemic cells by treatment. During the second phase, the leukemic burden declines with a slope of -0.0057 ± 0.0038 (imatinib) or -0.0019 ± 0.0013 (nilotinib) per day, signifying a decrease of the abundance of leukemic progenitors. Both the first and second slopes are significantly different between the imatinib and nilotinib patient cohorts, signifying variability due to differences in follow-up, missing data, pharmacokinetic effects or potentially due to different effects of imatinib versus nilotinib on the death rates of leukemic cells during therapy. During the third phase, the leukemic cell number decreases in a subset of imatinib-treated patients (mean -0.0008 ± 0.0003) and three nilotinib-treated patients (-0.00028, -0.00002 and -0.00697) while increasing in one imatinib-treated patient (0.0014). In a few patients, this third slope could not be observed, probably due to insufficient follow-up or missing data. The number of patients with a third slope was smaller in the nilotinib as compared to the imatinib cohort, since the follow-up of patients treated with first-line nilotinib was much shorter than the follow-up of imatinib-treated patients. Importantly, this third slope has never before been described in TKI response data and offers novel insights into the biology of the disease.

The existence of a third slope suggests that it may be possible to infer the kinetics of a population of immature leukemic cells, possibly stem cells, from long-term TKI response data. Our findings support the hypothesis that targeted therapy is capable of depleting leukemic stem cells at a very slow rate in a subset of patients. In a few patients, however, the third slope was positive or could not be identified with statistical significance. The variability in response to long-term targeted therapy may be due to inconsistent patient adherence to drug; indeed, poor adherence was hypothesized to be the predominant reason for the inability of some CML patients to obtain and/or maintain adequate molecular responses to imatinib therapy\textsuperscript{23}. Alternatively, this variability in patient response may be caused by the presence of low-level resistance in some patients and/or by heterogeneity in the disease characteristics among CML patients. In many situations, there is marked heterogeneity in phenotype even if genetically, cells are identical\textsuperscript{24-27}. Similarly, different patients may present with leukemic cell phenotypes with disparate growth and differentiation kinetics. This hypothesis is supported by experimental evidence suggesting that both the amount of BCR-ABL mRNA and second site mutations alter the fitness of leukemic cells\textsuperscript{28-29}. Furthermore, even leukemic stem cells within one patient may be highly heterogeneous, harboring clones with different growth kinetics\textsuperscript{30-31}. Inclusion of such intra-patient variability into our framework would not alter the results, since the mathematical framework describes the behavior of the dominant clone in each differentiation stage (see SI). Finally, this variability in the third slope observed among patients may be due to the limitations in the sensitivity of the RQ-PCR assay to detect BCR-ABL1 transcripts at such low levels (SI).
Note that the long-term TKI treatment follow-up data used for our analyses represents a strongly selected population of patients who were not only able to tolerate TKI therapy for a decade, but also achieved at least a complete cytogenetic response. Therefore, the conclusions based upon this patient group may not apply in general to all CML patients. Furthermore, the shape of the imatinib treatment response curve was identified based upon converted BCR-ABL1 transcript values (see SI); as long as the shape of the curve using converted values accurately represents the shape of the curve of true values, this data can be used to identify the behavior of immature CML cells during imatinib therapy. However, the majority of patients (over 93%) had at least one positive (non-converted) value after their specific turning points between the second and third slopes, and over 79% of those patients had multiple positive measurements thereafter. Therefore, the estimation of the third slope is not purely based on converted values.

Furthermore, a subset of nilotinib-treated patients, who did not have any converted data points, also displayed a tri-phasic decline of the leukemic cell burden.

Our results stand in contrast to extensive in vitro studies of CML stem cells suggesting that these cells are intrinsically resistant to TKIs. Such studies identified multiple pathways contributing to stem cell resistance, including decreased intracellular uptake and retention of cytotoxic drugs and tyrosine kinase inhibitors and resistance to apoptosis. A lower expression of human leukocyte antigen co-stimulatory molecules and targets of adaptive immunity may also protect stem cells from immune surveillance. Furthermore, treatment with TKIs in vitro results in an increase in quiescent immature CML cells that retain proliferative capacity after treatment is withdrawn. Finally, human CML stem cell survival was recently found to be independent of BCR-ABL activity, and although short-term in vitro imatinib treatment reduced the expansion of CML stem/progenitors, cytokine support permitted growth and survival in the absence of BCR-ABL activity that was comparable to that of normal stem/progenitor counterparts. These data suggest that stem cells may not be inhibited by imatinib therapy. These observations can be reconciled with our findings by the fact that in vitro studies may not predict the in vivo behavior of CML stem cells, that in vitro settings arguably utilize a more differentiated cell population than the most primitive leukemic stem cells present in patients, and that our study population represents a very selected group of patients which may not display the same disease characteristics and long-term response to TKIs as those patients from whom samples for in vitro studies were obtained. Furthermore, the possibility remains that the third slope represents the behavior not of leukemic stem cells, but of an immature subpopulation that is more differentiated than stem cells; in that case we would predict the existence of another slope signifying the behavior of leukemic stem cells.

Our findings provide a fresh perspective for the discussion of whether TKIs are capable of curing CML. The identification of a bi-phasic depletion of leukemic cells in response to imatinib therapy led to the design of several computational models; while our framework predicted that there would be three phases of BCR-ABL1 depletion in CML patients, representing the behavior of differentiated cells, progenitors and potentially leukemic stem cells, other models suggested that there are only two slopes, caused by a decline of cycling and quiescent leukemic stem cells, or by a decline of leukemic
progenitors and stem cells.\textsuperscript{40} The validity of any of these interpretations of the bi-phasic depletion of leukemic cells\textsuperscript{11-12,40} and thus the inferred effect of TKIs on leukemic stem cells remains unresolved. However, these computational models led to distinct predictions of the long-term imatinib response. Our analyses thus contribute to the discussion of the applicability of these alternative interpretations to long-term treatment response data.

Our findings are consistent with the results of imatinib discontinuation trials\textsuperscript{14-16}, which found that the majority of patients relapsed a few months after discontinuation of imatinib while about 40\% of patients remained BCR-ABL1-negative for the duration of their follow-up. However, several instances of late relapse and fluctuations of BCR-ABL1 levels after imatinib discontinuation also occurred, thus questioning the ability of imatinib to lead to a lasting cure of the disease in these patients. One treatment option that has been suggested to succeed in curing CML is allogeneic stem cell transplantation. Complete molecular response (CMR) is commonly achieved after allogeneic transplantation and is associated with long-term disease-free survival; furthermore, CMR induced by allografts was found to be more stable than CMR induced by imatinib therapy.\textsuperscript{41} However, late molecular relapses were reported even after this treatment option.\textsuperscript{9} In the allograft setting it is possible that ongoing immune surveillance is essential to suppress a pool of residual CML cells. Similarly, for the small number of patients who achieve CMR after administration of interferon-\(\alpha\) therapy, ongoing immune surveillance may be important. This agent may represent an attractive therapeutic option since several recent clinical studies\textsuperscript{15,42-44} as well as \textit{in vitro} data\textsuperscript{45} suggested that interferon-\(\alpha\) selectively impairs proliferation of primitive CML progenitors. While the ability of allografts and interferon-\(\alpha\) to cure CML remains incompletely understood, we believe that, based on our analyses, continuous TKI therapy has the potential to diminish the leukemic stem cell population at least in a subset of patients. The rate of depletion might vary between patients and may also depend on other factors such as the immune system and previous treatment with interferon-\(\alpha\), AraC or other agents. In addition, it remains a possibility that those patients who tolerate TKI therapy for up to ten years without adverse effects or progression of disease represent a distinct subset of patients whose leukemia is exquisitely sensitive to treatment. Future clinical studies will demonstrate the general applicability of these findings and will allow for the identification of predictors of the long-term response to TKI therapy.

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AUTHOR CONTRIBUTIONS

M.T., M.G., and F.M. designed the study, M.T. performed the analyses, M.G. and F.M. contributed analysis tools, S.B., T.P.H., A. Q.-C., J. C. and H. K. contributed data, and M.T., M.G., S.B. and F.M. interpreted the results and wrote the manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

No conflicts to report.

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### Table 1. Summary of the two statistical models for the imatinib response data.

#### The short-term (12 months) imatinib response patient cohort (44 patients)

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<tr>
<td>Mean* of $R_i^2$</td>
<td>0.98</td>
<td>0.79</td>
</tr>
<tr>
<td>3rd Quartile* of $R_i^2$</td>
<td>0.99</td>
<td>0.87</td>
</tr>
<tr>
<td>Max* of $R_i^2$</td>
<td>1</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>Final $R^2$</strong></td>
<td>0.98</td>
<td>0.81</td>
</tr>
<tr>
<td>*<strong>Sum of BICs</strong></td>
<td>-62.6</td>
<td>28.0</td>
</tr>
</tbody>
</table>

#### The long-term (10 years) imatinib response patient cohort (22 patients)

<table>
<thead>
<tr>
<th></th>
<th>Bi-phasic exponential model</th>
<th>Exponential model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min* of $R_i^2$</td>
<td>0.67</td>
<td>0.62</td>
</tr>
<tr>
<td>1st Quartile* of $R_i^2$</td>
<td>0.85</td>
<td>0.69</td>
</tr>
<tr>
<td>Median* of $R_i^2$</td>
<td>0.88</td>
<td>0.77</td>
</tr>
<tr>
<td>Mean* of $R_i^2$</td>
<td>0.87</td>
<td>0.77</td>
</tr>
<tr>
<td>3rd Quartile* of $R_i^2$</td>
<td>0.91</td>
<td>0.84</td>
</tr>
<tr>
<td>Max* of $R_i^2$</td>
<td>0.96</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Final $R^2$</strong></td>
<td>0.89</td>
<td>0.80</td>
</tr>
<tr>
<td>*<strong>Sum of BICs</strong></td>
<td>-23.3</td>
<td>-15.3</td>
</tr>
</tbody>
</table>

* the Minimum/1st Quartile/Median/Mean/3rd Quartile/Maximum of the $R_i^2$, $i = 1, ..., N$, calculated from the corresponding fitted model for each patient, where $N$ is the total number of patients and $R_i^2 = 1 - \frac{SSE_i}{SST_i}$;

** Final $R^2$, calculated as $1 - \frac{\sum_i SSE_i}{\sum_i SST_i}$, evaluates the overall fit of the corresponding model to the whole time series data with all patients;

***Sum of BICs is the sum of BICs over all subjects for each model;
Table 2. Summary of the two statistical models for the nilotinib response data.
The table displays information for the first-line nilotinib response patient cohort (29 patients).

<table>
<thead>
<tr>
<th></th>
<th>Bi-phasic exponential model</th>
<th>Exponential model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min* of $R_i^2$</td>
<td>0.74</td>
<td>0.31</td>
</tr>
<tr>
<td>1st Quartile* of $R_i^2$</td>
<td>0.95</td>
<td>0.47</td>
</tr>
<tr>
<td>Median* of $R_i^2$</td>
<td>0.97</td>
<td>0.64</td>
</tr>
<tr>
<td>Mean* of $R_i^2$</td>
<td>0.96</td>
<td>0.61</td>
</tr>
<tr>
<td>3rd Quartile* of $R_i^2$</td>
<td>0.99</td>
<td>0.73</td>
</tr>
<tr>
<td>Max* of $R_i^2$</td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>Final $R^2$</td>
<td>0.97</td>
<td>0.61</td>
</tr>
<tr>
<td>Sum of BICs</td>
<td>-83.22</td>
<td>34.48</td>
</tr>
</tbody>
</table>

* the Minimum/1st Quartile/Median/Mean/3rd Quartile/Maximum of the $R_i^2$, $i = 1, \ldots, N$, calculated from the corresponding fitted model for each patient, where $N$ is the total number of patients and $R_i^2 = 1 - \frac{SSE_i}{SST_i}$;

** Final $R^2$, calculated as $1 - \frac{\sum_i SSE_i}{\sum_i SST_i}$, evaluates the overall fit of the corresponding model to the whole time series data with all patients;

***Sum of BICs is the sum of BICs over all subjects for each model;
Table 3. Summary statistics of slopes and turning points for the imatinib and nilotinib response data. The table displays the first ($\beta_1$), second ($\beta_2$), and third ($\beta_3$) slopes as well as the first and second turning points ($\tau_1$ and $\tau_2$) for all patients whose treatment response data displayed a bi-phasic trend based on the BIC criterion (see SI), as well as for those whose second and third slopes were negative.

<table>
<thead>
<tr>
<th>Slopes and turning points</th>
<th>Short-term imatinib</th>
<th>Long-term imatinib</th>
<th>Nilotinib</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (sd)</td>
<td>median (number of patients)</td>
<td>mean (sd)</td>
</tr>
<tr>
<td>$\beta_1$ *</td>
<td>-0.0519 (0.0180)</td>
<td>-0.0525 (44)</td>
<td>Not observable due to data resolution</td>
</tr>
<tr>
<td>$\beta_2$ *</td>
<td>-0.0041 (0.0061)</td>
<td>-0.0044 (44)</td>
<td>Not observable due to data resolution</td>
</tr>
<tr>
<td>$\beta_3$ *</td>
<td>-0.0064 (0.0038)</td>
<td>-0.0056 (35)</td>
<td>Not observable due to data resolution</td>
</tr>
<tr>
<td>$\beta_1$ negative**</td>
<td>Not observable due to insufficient follow-up</td>
<td>Not observable due to insufficient follow-up</td>
<td>-0.0008 (0.0003)</td>
</tr>
<tr>
<td>$\beta_2$ negative**</td>
<td>Not observable due to insufficient follow-up</td>
<td>Not observable due to insufficient follow-up</td>
<td>-0.0008 (0.0003)</td>
</tr>
<tr>
<td>$\tau_1$ (months) *</td>
<td>4.23 (1.41)</td>
<td>4.14 (44)</td>
<td>Not observable due to data resolution</td>
</tr>
<tr>
<td>$\tau_1$ (months) negative**</td>
<td>4.00 (1.36)</td>
<td>3.91 (35)</td>
<td>Not observable due to data resolution</td>
</tr>
<tr>
<td>$\tau_2$ (months) *</td>
<td>Not observable due to insufficient follow-up</td>
<td>Not observable due to insufficient follow-up</td>
<td>34.5 (22.6)</td>
</tr>
<tr>
<td>$\tau_2$ (months) negative**</td>
<td>Not observable due to insufficient follow-up</td>
<td>Not observable due to insufficient follow-up</td>
<td>29.5 (13.4)</td>
</tr>
</tbody>
</table>

* the total numbers of patients used to calculate mean, median and standard errors are given by those who displayed a bi-phasic trend in their treatment response kinetics (see SI);

** mean and median are calculated using all individual patients whose response data displayed a bi-phasic trend based on the BIC criterion, and whose data also had negative slopes (see SI);
FIGURE LEGENDS

Figure 1. Dynamics of short-term imatinib treatment response. The figure displays the kinetics of the leukemic cell burden in patients treated with imatinib for one year after diagnosis in early chronic phase. (a-d) The panels show the percentage of cancer cells (BCR-ABL1/BCR as measured by RQ-PCR) of four individual CML patients (circles) and the fitted bi-phasic exponential model (red curves). This fitted model demonstrates that there is a bi-phasic exponential depletion of leukemic cells within the first year of treatment. (e) The panel displays the median (orange circles) and quartiles of the short-term imatinib response data together with the fitted bi-phasic exponential model (red curve). See Figure S1 for all patient plots and model fitting.

Figure 2. Dynamics of long-term imatinib treatment response. The figure displays the kinetics of the leukemic cell burden in patients treated with imatinib for ten years after diagnosis in early chronic phase. (a-d) The panels show the percentage of cancer cells (BCR-ABL1/BCR as measured by RQ-PCR) of four individual CML patients (circles) and the fitted bi-phasic exponential model (red curves, starting from month 6 of treatment). Converted data points are shown in green (see Methods and SI). This fitted model demonstrates that there is a bi-phasic exponential depletion of leukemic cells starting from six months after the initiation of therapy. (e) The panel displays the median (orange circles) and quartiles of the long-term imatinib response data together with the fitted bi-phasic exponential model (red curve). See Figure S2 for all patient plots and model fitting.

Figure 3. Dynamics of nilotinib treatment response. The figure displays the kinetics of the leukemic cell burden in patients treated with first-line nilotinib for up to 66 months after diagnosis in early chronic phase. (a-d) The panels show the percentage of cancer cells (BCR-ABL1/BCR as measured by RQ-PCR) of four individual CML patients (circles) and the fitted bi-phasic exponential model (red curves). This fitted model demonstrates that there is a bi-phasic exponential depletion of leukemic cells starting from the initiation of therapy. (e) The panel displays the median (orange circles) and quartiles of the nilotinib response data together with the fitted bi-phasic exponential model (red curves). See Figure S3 for all patient plots and model fitting.

Figure 4. A mathematical framework accurately predicts the dynamics of TKI treatment responses. (a-d) The panels display the abundance of healthy (black) and leukemic (blue) stem cells (SC), progenitor cells (PC), differentiated cells (DC), and terminally differentiated cells (TC) over time (years) since the initiation of imatinib therapy, as predicted by the mathematical framework\textsuperscript{10,12}. (e-f) The panels display the median (orange circles) and quartiles of the short-term (e) and long-term (f) imatinib response data together with the results of the mathematical framework. Based on the model presented in the SI, the mathematical model prediction is given by $\alpha y_3/(2x_3+y_3)$. Here $x_3$ and $y_3$ denote the abundance of normal and leukemic terminally differentiated cells while $\alpha$ specifies the average number of copies of BCR-ABL1 per cell times a factor representing batch effects between cohorts. Parameter values are $d_0 = 0.0008$, $d_1 = 0.0057$, $d_2 = 0.0519$, $d_3 = 1$, $r_x = 0.008$, $r_y = 0.01$, $p_x = 9\times10^{-6}$, $p_y = 1.15\times10^{-6}$, $a_x = 0.57$, $a_y = 0.57$.
\(b_x = 5.19, \ c_x = 100, \ a_y = 2a_x, \ b_y = 2b_x, \ c_y = c_x, \ a'_y = a_y/200, \ b'_y = b_y/300, \ c'_y = c_y, \ r'_y = r_y/15, \) and \(\alpha = 3\) in (e) and \(\alpha = 4\) in (f) (see SI). Apart from the dimension-less parameters, all values are given in units per day. Note that these parameter choices represent only one example that can recapitulate the dynamics of the treatment response seen in the clinic; other choices are possible.
Figure 1

BCR-ABL1/BCR (%) vs Months

(a) BCR-ABL1/BCR (%)

(b) BCR-ABL1/BCR (%)

(c) BCR-ABL1/BCR (%)

(d) BCR-ABL1/BCR (%)

(e) BCR-ABL1/BCR (%)

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Figure 2