Dynamics of chronic myeloid leukemia response to dasatinib, nilotinib, and high-dose imatinib

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SUPPLEMENTARY INFORMATION

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STUDY GROUP

Patient selection
We utilized four cohorts in this analysis: 233 patients treated with dasatinib, 215 patients treated with nilotinib and 281 patients treated with high-dose imatinib within the phase II trial at the MD Anderson Cancer Center\textsuperscript{1–2}, as well as 22 patients treated with low-dose imatinib (400mg daily) within the IRIS trial\textsuperscript{3–4}. We employed the following criterion to select patients from the study group for inclusion in our various analyses. Only patients with measurements at the first five time points were included, which were at 0, 3, 6, 9, and 12 months.

Patient cohorts
Based on the above patient selection criteria, we obtained data of 92 patients treated with dasatinib, 75 patients treated with nilotinib and 123 patients treated with high-dose imatinib within the phase II trial at the MD Anderson Cancer Center\textsuperscript{1–2}. We also obtained data of 13 patients treated with low-dose imatinib (400mg daily) within the IRIS trial\textsuperscript{3–4}. For each patient, the disease burden was quantitated using the International Scale standardized methodology over the entire course of the trial.

DETAILS OF MATHEMATICAL MODELING APPROACH
To investigate the parameters of the mathematical model, we first conducted statistical model fitting to data of each patient cohort.

Individual model fitting
We fit two models to each individual patient to identify the better fit: a single exponential curve and a bi-phasic exponential curve with a turning point. These models were chosen since the leukemic cell burden is expected to decrease (or increase) at an exponential rate. When fitting these two models, we first performed a logarithmic transformation of the original data and then fit a bi-phasic linear or linear model to the transformed data.

The log-transformed data is of the form \((t_{ij}, y_{ij}), i = 1, ..., N; j = 1, ..., n_{i}\), where \(i\) is the patient-specific index and \(n_{i}\) is the total number of BCR-ABL1\% value measurements for patient \(i\); here \(y_{ij}\) is logarithmically transformed. Then the linear model for each patient \(i\) is given by

\[
M_0 : E(y_{ij}) = \alpha_i + \beta_i t_{ij}, \quad i = 1, ..., N; j = 1, ..., n_i,
\]

where \(\alpha_i\) and \(\beta_i\) are parameters to be estimated for each individual based on data of each individual patient \(i\); while the bi-phasic linear model is given by

\[
M_1 : E(y_{ij}) = (\alpha_i + \beta_i t_{ij}) I_{(t_{ij} \leq \tau_i)} + (\alpha_i' + \beta_i t_{ij}) I_{(t_{ij} > \tau_i)}, \quad \alpha_i + \beta_i \tau_i = \alpha_i' + \beta_i' \tau_i.
\]

where \(\alpha_i\), \(\beta_i\), \(\alpha_i'\), \(\beta_i'\) and \(\tau_i\) are parameters to be estimated for each individual based on data of each individual patient \(i\).

To determine the model with the best fit for each individual patient’s data, choosing among the above linear versus bi-phasic linear models, we utilized the joinpoint software, which is publicly available on the NCI website, http://surveillance.cancer.gov/joinpoint/. We chose to use this
software because it was designed to identify segmented models that best fit to longitudinal data. Within this framework, we utilized the permutation test approach since it controls the error probability of selecting the wrong model at a given significance level, for instance 0.05. This option was chosen over other approaches (such as the BIC) since the latter does not provide an estimate of the error probability.

For each model with \( k \) turning points, we estimated a total of \( 2k + 2 \) parameters (\( k = 0 \) or \( 1 \) in our study). For the parameter estimation, we utilized Hudson’s Method\(^5\), as it provides more accurate estimates compared to the Grid Search method\(^5\), even though it is computationally more expensive. For details of the parameter estimation and the hypothesis testing see Kim et al.\(^7\). In brief, for any linear model with \( k \) turning points, Hudson’s method first partitions the observed data into \( k+1 \) consecutive subsets. For each subset, an ordinary least squares method is applied to obtain the intercept and slope estimates over the data range of that subset. The turning points are then directly calculated as the intersections of two adjacent linear segments. If these intersection points divide the observed data into the same partitions as chosen originally, then the fit is admissible and its sum of squared error (SSE) is noted. Otherwise, the fit is not admissible and further adjustments are made. The least squares estimates for the linear model with \( k \) turning points are then obtained from the fit, which provides the smallest SSE over all feasible partitions.

After the parameter estimation outlined above, the following permutation test procedure is performed to determine the better fit between the linear model \( M_0 \) and the bi-phasic linear model \( M_1 \). This approach\(^5\) can be summarized in several steps:

1. Fit the original data set with the null hypothesis model with 0 turning points.
2. Permute the residuals from the null hypothesis model and add them back to the means from the null hypothesis model to obtain a new permutation data set.
3. For this permutation data set, fit both the null model with 0 turning points and the alternative model with 1 turning points and calculate a scalar goodness-of-fit measure. This measure is a ratio, \( \frac{SSEN}{SSEA} \), where \( SSEN \) is the sum of squared errors (SSE) from the null model \( M_0 \) and \( SSEA \) is the SSE from the alternative model \( M_1 \).
4. Repeat steps 2 and 3 \( N_p - 1 \) times. Denote the ratios, \( \frac{SSEN}{SSEA} \), from the permutation data sets \( p \) as \( \{T_p, p = 1, ..., N_p - 1\} \). Also calculate this ratio for the original data set and denote it as \( T_0 \). Values of the ratio close to 1 represent the case in which the alternative is not much better than the null hypothesis model, while larger ratios signify that the alternative is much better.
5. The p-value of testing the hypothesis \( M_0 \) versus \( M_1 \) for the original data set is determined from the permutation distribution of the goodness-of-fit statistics. Then p-value = (number of times that \([T_p \geq T_0]\) for \( p = 1, ..., N_p - 1\)) / \( N_p \).

The permutation tests are used to investigate whether there is enough evidence to require a model with a larger number of turning points than the one in the null hypothesis. This approach controls the error probability of selecting the wrong model at a significance level of 0.05.

For the dasatinib treatment response data, 35 out of 69 patients had the bi-phasic model as better fitting. For the nilotinib treatment response data, 26 out of 51 patients had the bi-phasic model as better fitting. For the high-dose imatinib treatment response data, 54 out of 123 patients had the bi-phasic model as better fitting. For the low-dose imatinib treatment response data, all 13 patients had the bi-phasic model as better fitting (Supplementary Table S3).

**Whole cohort model fitting**
We then identified the model with the best fit to the entire patient cohort. When fitting each model to data of each patient individually as stated in the above section, we obtained the corresponding SSE$_i$ as well as SST$_i$ (Total Sum of Squares) for each subject $i$. The total SSE and total SST of the model were then calculated as $\sum_i SSE_i$ and $\sum_i SST_i$ separately. We defined the final $R^2$ for each model over the whole cohort as $1 - \text{total } SSE/\text{total SST} = 1 - \sum_i SSE_i / \sum_i SST_i$. Let $R_0^2$ be the final $R^2$ of the model $M_0$ and $R_1^2$ be the final $R^2$ of the model $M_1$.

For the dasatinib treatment response data, 35 out of 69 patients had bi-phasic model as better fitting, $R_0^2$ was 0.59 , and $R_1^2$ was 0.92. For the nilotinib treatment response data, 26 out of 51 patients had bi-phasic model as better fitting, $R_0^2$ was 0.60, and $R_1^2$ was 0.93. For the high-dose imatinib treatment response data, 54 out of 123 patients had the bi-phasic model as better fitting, $R_0^2$ was 0.52, and $R_1^2$ was 0.89. For the low-dose imatinib treatment response data, all 13 patients had the bi-phasic model as better fitting. Also, for the dasatinib, nolotinib and high-dose imatinib cohorts, we obtained $N$ subject-specific $R^2 = 1 - \text{SSE}_i / \text{SST}_i$, $i = 1, ..., N$. The summary information (Minimum, 1st Quartile, Median, Mean, 3rd Quartile, Maximum) of the $R^2$ for each model of different analysis is reported in [Supplementary Table S2](#).

We found that the bi-phasic exponential model provided a larger final $R^2$ and smaller sum of BICs than the exponential model for all cohorts. The difference between the final $R^2$ was large enough to convince us of the better fit of the bi-phasic model in all four cohorts.

**Mathematical model**

We utilized a mathematical model of the treatment response of CML cells to TKI therapy, 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 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, $\phi$, 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:

$$
\begin{align*}
\frac{d x_0}{dt} &= \text{healthy cells} \\
\frac{d y_0}{dt} &= \text{leukemic cells}
\end{align*}
$$
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.

The parameters during imatinib therapy are denoted by $r_y'$, $a_y'$, $b_y'$ etc.

**SUPPLEMENTARY REFERENCES**


**SUPPLEMENTARY TABLES**

Supplementary Table S1. The basic model of the differentiation hierarchy of normal and leukemic cells. The abundances of normal stem cells, progenitors, differentiated, and terminally differentiated cells are given by $x_0$, $x_1$, $x_2$, and $x_3$, while the respective abundances of leukemic cells are given by $z_0$, $z_1$, $z_2$, and $z_3$. Normal and leukemic stem cells divide at rates $r_x$ and $r_z$ per day, respectively. The rate constants for the production of progenitors, differentiated cells and terminally differentiated cells are given by $a_x$, $b_x$ and $c_x$ for normal and by $a_z$, $b_z$, and $c_z$ for leukemic cells. Stem cells die at rate $d_x$ progenitors at rate $d_1$, differentiated cells at rate $d_2$ and terminally differentiated cells at rate $d_3$ per day. Cells at all levels are assumed to potentially reproduce symmetrically and/or asymmetrically; the limited replication potential of more
differentiated cell types is then considered as part of the differentiation rates. Density dependence is achieved by the functions $q_z = 1/[1 + \rho_z(x_0 + z_0)]$ and $q_z = 1/[1 + \rho_z(x_0 + z_0)]$; these functions take into account crowding, limited resources, and interactions with the microenvironment. We assumed that the BCR-ABL1 oncogene increases the rate at which progenitors and differentiated cells are being produced; $a_z > a_x$ and $b_z > b_x$. Molecularly targeted therapy counteracts the effects of BCR-ABL1 by reducing the differentiation rates to $a'_z < a_z$ and $b'_z < b_z$ and possibly reducing the growth rate of leukemic stem cells to $r'_z < r_z$.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Leukemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem cells</td>
<td>$\dot{x}_0 = [r_z q_z - d_z] x_0$</td>
</tr>
<tr>
<td>Progenitors</td>
<td>$\dot{x}_1 = a_z x_0 - d_z x_1$</td>
</tr>
<tr>
<td>Differentiated cells</td>
<td>$\dot{x}_2 = b_z x_1 - d_z x_2$</td>
</tr>
<tr>
<td>Terminally differentiated cells</td>
<td>$\dot{x}_3 = c_z x_2 - d_z x_3$</td>
</tr>
</tbody>
</table>

**Supplementary Table S2. Summary statistics of the two statistical models for data in the dasatinib, nilotinib and high-dose imatinib patient cohorts.** The two statistical models investigated were a single-phasic exponential model (denoted as 1-phase in the following table) and a 2-phasic exponential model (denoted as 2-phase in the following table). Note that summary statistics for the low-dose imatinib cohort were not presented here because all 13 patients in the low-dose imatinib cohort had 2-phasic model as the best fitting model, thus 2-phasic model was obviously the best fitting model for this entire cohort.

<table>
<thead>
<tr>
<th></th>
<th>Dasatinib cohort (69 patients)</th>
<th>Nilotinib cohort (51 patients)</th>
<th>High-dose imatinib cohort (123 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-phase</td>
<td>1-phase</td>
<td>2-phase</td>
</tr>
<tr>
<td>Min* of $R^2_i$</td>
<td>0.230</td>
<td>0.011</td>
<td>0.575</td>
</tr>
<tr>
<td>1st Quartile* of $R^2_i$</td>
<td>0.901</td>
<td>0.449</td>
<td>0.913</td>
</tr>
<tr>
<td>Median* of $R^2_i$</td>
<td>0.960</td>
<td>0.573</td>
<td>0.964</td>
</tr>
<tr>
<td>Mean* of $R^2_i$</td>
<td>0.920</td>
<td>0.569</td>
<td>0.922</td>
</tr>
<tr>
<td>3rd Quartile* of $R^2_i$</td>
<td>0.984</td>
<td>0.736</td>
<td>0.982</td>
</tr>
<tr>
<td>Max* of $R^2_i$</td>
<td>1.000</td>
<td>0.968</td>
<td>0.999</td>
</tr>
<tr>
<td>**Final $R^2$</td>
<td>0.924</td>
<td>0.592</td>
<td>0.934</td>
</tr>
<tr>
<td>**Sum of BICs</td>
<td>-26.1***</td>
<td>96.7***</td>
<td>-9.86</td>
</tr>
</tbody>
</table>

* the Minimum/1st Quartile/Median/Mean/3rd Quartile/Maximum of the $R^2_i$, $i = 1, \ldots, N$, calculated from the corresponding fitted model for each patient, where $N$ is the total number of patients and $R^2_i = 1 - \text{SSE}_i / \text{SST}_i$.

** Final $R^2$, calculated as $1 - \sum \text{SSE}_i / \sum \text{SST}_i$, 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.
**** One patient in this cohort had SSE being exactly zero when fitting the 2-phasic exponential model which resulted in negative infinite BIC. The Sum of BICs here did not include the BIC from this patient.

**Supplementary Table S3.** Summary of statistical analysis of 1-phasic versus 2-phasic model comparison in all four patient cohorts. Note that all 2-phasic patients in each cohort had negative first slopes.

<table>
<thead>
<tr>
<th></th>
<th>Total # of patients</th>
<th># of 1-phasic</th>
<th># of 2-phasic</th>
<th>2-phasic, beta2 &gt;0*</th>
<th>2-phasic, beta2 &lt;0*</th>
<th>2-phasic, beta2 =0*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dasatinib cohort</td>
<td>69</td>
<td>34</td>
<td>35</td>
<td>15</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Nilotinib cohort</td>
<td>51</td>
<td>25</td>
<td>26</td>
<td>10</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>High-dose imatinib cohort</td>
<td>123</td>
<td>69</td>
<td>54</td>
<td>23</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>Standard dose imatinib cohort</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>1</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

* zero refers to any number with an absolute value ≤ 10⁻⁶.

**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1.** A bi-exponential and an exponential fit to the log-transformed nilotinib curves. The bi-exponential fit is the sum of two exponentials. The curves labeled “1” (black) and “2” (red) are the components of the bi-exponential and the curve labeled “3” (green) is the result. The curve labeled “4” (blue) is a single scaled exponential with an intercept (our model in Statistical Methods). Note its similarity to the bi-exponential. This is because curve “2” is virtually a straight line.

**Figure S2. Individual fitting for patients in the imatinib 800mg cohort.** The figure displays each individual's BCR-ABL1/ABL1% data (black circle), together with the bi-phasic fitting curves (red lines). For those patients whose data exhibit a better fitting with a single-phasic model, a dashed green line is shown. The green circles represents value of 0.00001, which is the original zero value.

**Figure S3. Individual fitting for patients in the dasatinib cohort.** The figure displays each individual's BCR-ABL1/ABL1% data (black circle), together with the bi-phasic fitting curves (red lines). For those patients whose data exhibit a better fitting with a single-phasic model, a dashed green line is shown. The green circles represents value of 0.00001, which is the original zero value.

**Figure S4. Individual fitting for patients in the nilotinib cohort.** The figure displays each individual's BCR-ABL1/ABL1% data (black circle), together with the bi-phasic fitting curves (red lines). For those patients whose data exhibit a better fitting with a single-phasic model, a dashed green line is shown. The green circles represents value of 0.00001, which is the original zero value.

**Figure S5. Individual fitting for patients in the imatinib 400mg cohort.** The figure displays each individual's BCR-ABL1/ABL1% data (black circle), together with the bi-phasic fitting curves (red lines). For those patients whose data exhibit a better fitting with a single-phasic model, a dashed green line is shown. The green circles represents value of 0.00001, which is the original zero value.
Imatinib800 1–phase 19

Imatinib800 1–phase 20

Imatinib800 1–phase 21

Imatinib800 1–phase 22

Imatinib800 1–phase 23

Imatinib800 1–phase 24

Imatinib800 1–phase 25

Imatinib800 1–phase 26

Imatinib800 1–phase 27
Nilotinib 2–phase 10

Months

Nilotinib 2–phase 11

Months

Nilotinib 2–phase 12

Months

Nilotinib 2–phase 13

Months

Nilotinib 2–phase 14

Months

Nilotinib 2–phase 15

Months

Nilotinib 2–phase 16

Months

Nilotinib 2–phase 17

Months

Nilotinib 2–phase 18

Months