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Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications

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Treatment of chronic myeloid leukemia (CML) with the tyrosine kinase inhibitor imatinib represents a successful application of molecularly targeted cancer therapy. A rapid hematologic and cytogenetic response can be induced in the majority of people, even in advanced disease. However, complete eradication of malignant cells, which are characterized by the expression of the BCR-ABL1 fusion protein, is rare. Reasons for the persistence of the malignant clone are currently not known and provide a substantial challenge for clinicians and biologists. Based on a mathematical modeling approach that quantitatively explains a broad range of phenomena, we show for two independent datasets that clinically observed *BCR-ABL1* transcript dynamics during imatinib treatment of CML can consistently be explained by a selective functional effect of imatinib on proliferative leukemia stem cells. Our results suggest the general potential of imatinib to induce a complete elimination of the malignant clone. Moreover, we predict that the therapeutic benefit of imatinib can, under certain circumstances, be accelerated by combination with proliferation-stimulating treatment strategies.

Chronic myeloid leukemia (CML) is a clonal hematopoietic disorder induced by a chromosomal translocation generating the Philadelphia chromosome (Ph) and the oncogenic *BCR-ABL1* fusion gene. The translated BCR-ABL1 protein is responsible for an expansion of the malignant clone, resulting in the displacement of normal hematopoiesis[1–3]. Standard therapy for CML is the tyrosine kinase inhibitor imatinib[4–6], which induces a selective inhibition of proliferation[7,8] and increase of apoptosis[9–11] in *BCR-ABL1*–positive cells. Irrespective of these molecular insights into imatinib activity, the resulting dynamic effects on stem cells are not yet sufficiently well understood.

Molecular monitoring of tumor load in CML under imatinib treatment typically shows a biphasic decline of *BCR-ABL1* transcript levels during the first year of treatment as well as a rapid relapse upon treatment cessation[12]. The authors of a previous study[12] used a mathematical model to explain these observations based on the dynamics of two noninteracting populations of malignant and normal cells, assuming that imatinib has no substantial effect on stem cells. However, we show that the data presented is also consistent with a primary imatinib effect on *BCR-ABL1*–positive stem cells, with clinically relevant implications.

We demonstrate that the biphasic decline as well as the rapid relapse of *BCR-ABL1* levels can be explained by a new model of stem cell organization[13]. This model has previously been implemented and validated for mouse hematopoiesis[14,15], and we have now adapted it for the human situation. It assumes a heterogeneous population of hematopoietic stem cells (HSCs). Although all HSCs have the potential to repopulate the tissue whenever they are exposed to appropriate signals, their chances of using this potential differ. HSCs continuously switch between different signaling contexts (for example, residing within or outside a stem-cell niche), which leads to different developmental fates and causes phenotypic reversibility. Such a system-dependent activation-inhibition balance of cellular potentials generates a dynamically stabilized stem cell population with a large proportion of noncycling cells among those with high repopulating potential. In addition to HSCs, the model considers differentiated cells. This population arises from HSCs that have lost their long-term repopulating potential and comprises proliferating and nonproliferating precursor cell stages as well as terminally differentiated blood cells. For details see **Supplementary Note** online.

We applied this self-organizing stem cell concept to *BCR-ABL1*–positive CML. The malignant clone, which is created by a chromosomal translocation in a single cell, expands and in the long run outcompetes the normal cell population. In particular, we assume that the chromosomal translocation causes an impaired proliferation control (inducing enhanced cell-cycle activity) together with altered cell-microenvironment interaction. This implies a competitive advantage for the malignant clone in occupying appropriate stem cell–supporting niches and therefore ultimately results in an increase of *BCR-ABL1*–positive cells. Incorporating this premise, the model reproduced typical CML latency times of 5–7 years (ref. 16; **Supplementary Fig. 1** online).

Based on this model we explain the effect of imatinib treatment as a modulation of the competitive properties of *BCR-ABL1*–positive cells, applying the following assumptions: selectively for *BCR-ABL1*–positive cells, imatinib induces (i) inhibition of proliferative activity and (ii) degradation (for example, by inducing apoptosis, by attenuating apoptosis inhibition or by impairing function) of proliferating stem cells.

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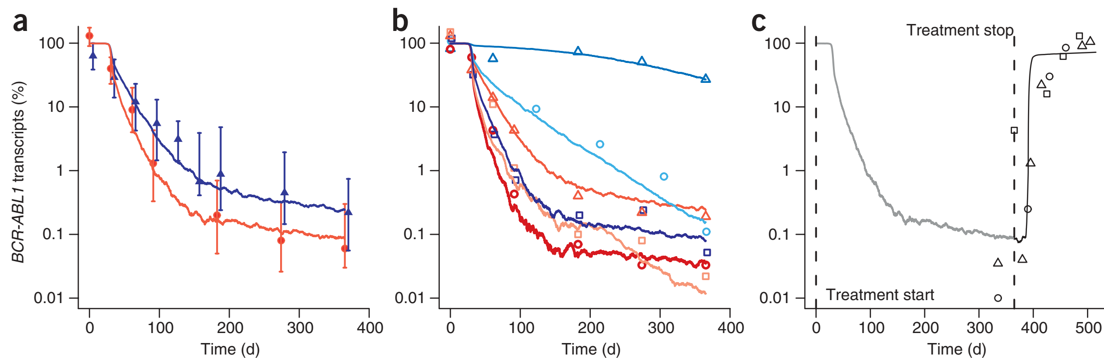

Figure 1 *BCR-ABL1* transcript dynamics. (**a**) CML under imatinib treatment. Data points represent median and interquartile range of *BCR-ABL1* transcript levels in peripheral blood, determined in two independent study populations: *BCR-ABL1/BCR* percentages in 68 individuals with imatinib-treated CML, previously published[12] (red) and *BCR-ABL1/ABL1* percentages in 69 individuals with imatinib-treated CML from the German cohort of the IRIS trial (blue). Solid lines represent corresponding simulation results of *BCR-ABL1* levels starting from a model system that had developed a CML with more than 99% *BCR-ABL1*–positive cells; applied degradation intensities $r_{deg} = 0.033$ (red line) and $r_{deg} = 0.028$ (blue line). 'Intensity' is a parameter that describes the probability of an individual cell being affected within a time step of 1 h. (**b**) Heterogeneity of responses. Selection of six qualitatively different *BCR-ABL1* dynamics in individuals (three from the previously published study[12] (red) and three from the German IRIS cohort (blue)). Corresponding simulations (color-matching solid lines) were obtained by quantitative variation of the imatinib-induced degradation (r_{deg}) and proliferation inhibition (r_{inh}) intensities: $r_{inh} = 0.05$, $r_{deg} = 0.033$ (dark blue); $r_{inh} = 0.005$, $r_{deg} = 0.012$ (medium blue); $r_{inh} = 0.005$, $r_{deg} = 0.028$ (light blue); $r_{inh} = 0.05$, $r_{deg} = 0.0375$ (dark red); $r_{inh} = 0.05$, $r_{deg} = 0.028$ (medium red); $r_{inh} = 0.005$, $r_{deg} = 0.0375$ (light red). (**c**) CML relapse upon treatment cessation. Simulated *BCR-ABL1/BCR* percentage after treatment cessation, starting from the indicated average simulation of imatinib treatment. Data points represent individual measurements in three different people, taken from the previously published study[12]. In contrast to the simulation (solid line), these individuals had different *BCR-ABL1/BCR* percentages (ranging from 0.01% to 5%) at or shortly before treatment cessation. Model details and parameters are given in the **Supplementary Note** and **Supplementary Tables 1** and **2**.

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Simulation results showed that these assumptions conformed to the clinically observed biphasic decline of *BCR-ABL1* transcript levels (**Fig. 1a**). The primary effect (steep decline) is induced by the initial reduction of proliferating *BCR-ABL1*–positive cells due to the assumed degradation effect. The later moderate decline is largely based on changes in the regulatory response of the system due to reduced stem-cell numbers (for a detailed explanation, see **Supplementary Note** and **Supplementary Fig. 2** online).

To compare the simulations with clinical data we used two independent datasets. The median *BCR-ABL1* dynamics published in an earlier study[12] relied on a selected population ($n = 68$), excluding cases with (transiently) increasing *BCR-ABL1* transcript levels. In addition, we present an unselected cohort of 69 people with newly diagnosed CML randomized for imatinib monotherapy recruited for the IRIS study in Germany[17,18] (**Supplementary Fig. 3** online). The quantitative difference in the dynamics of these two groups (most likely caused by the selection process applied to the cohort in the earlier study[12]) can be explained within our model by a small quantitative variation in the magnitude of the imatinib effect (for model parameters see **Supplementary Tables 1** and **2** online).

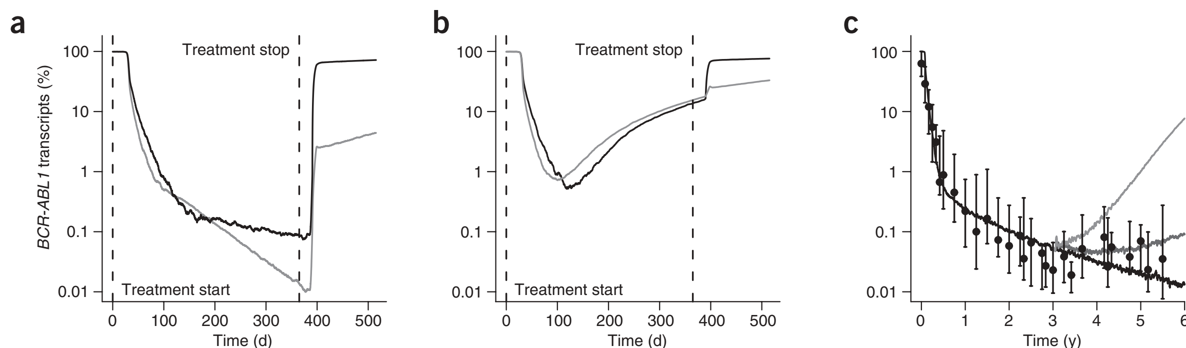

Figure 2 Model predictions for combination treatments and long-term dynamics. (**a**) Predicted effects for the treatment with imatinib alone (black line; $r_{deg} = 0.033$, $r_{inh} = 0.050$) in comparison with a combination of imatinib with an additional hourly entry of 0.1% of all (that is, both *BCR-ABL1*–positive and normal) dormant cells into the cell cycle (gray line), assuming no resistance. (**b**) The same treatment scenario as in **a**, but under the assumption of the emergence of a completely resistant clone ($r_{deg} = r_{inh} = 0$) at the beginning of treatment (black, imatinib alone; gray, imatinib plus nonspecific entry into cell cycle). (**c**) Long-term *BCR-ABL1* transcript dynamics. Data points represent medians and interquartile ranges of *BCR-ABL1/ABL1* percentages in subjects in the German cohort of the IRIS study. The simulations (solid lines) illustrate characteristic resistance scenarios for *BCR-ABL1* levels under imatinib: no resistance, assuming $r_{deg} = 0.028$, $r_{inh} = 0.050$ (black); induction of a partially resistant clone, assuming $r_{deg} = 0.0021$, $r_{inh} = 0.001$ (dark gray); and induction of a completely resistant clone, assuming $r_{deg} = r_{inh} = 0$ (light gray). Resistance is modeled by the incorporation of small clones (ten imatinib-resistant stem cells) 3 years after the beginning of treatment. Model details and parameters are given in the **Supplementary Note** and **Supplementary Tables 1** and **2**.

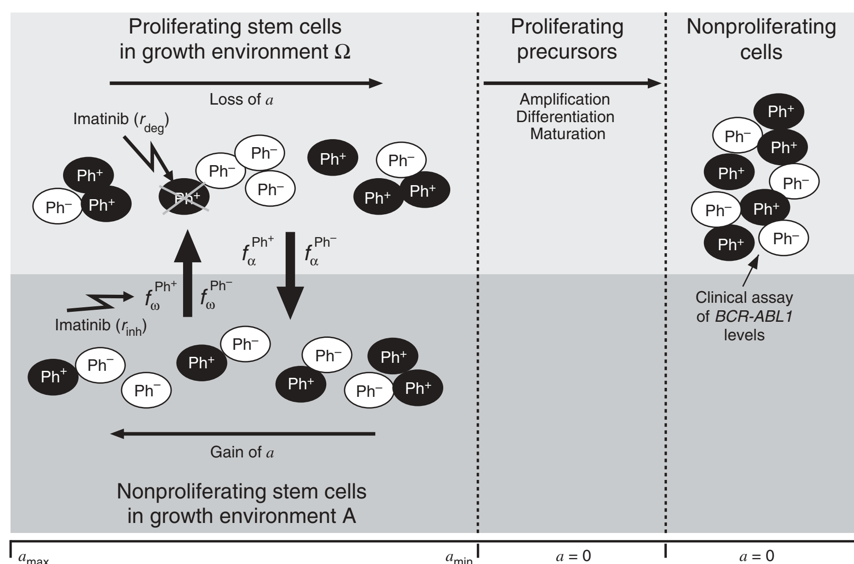

Figure 3 Model scheme. Normal (Ph⁻) and malignant (Ph⁺) stem cells are assumed to coexist within two common growth environments (A and Ω). The propensity of cells for residing in growth environment A is given by the cell-intrinsic affinity a. Whereas a is gradually lost in growth environment Ω, it is regained in growth environment A. Cells that have lost their potential to reside in growth environment A (that is, those with $a < a_{min}$) are denoted as differentiated. They initiate the formation of a clone, which transiently expands (proliferating precursors). Thereafter, the clone exists for a fixed lifetime without further expansion, comprising nonproliferating precursors and terminally differentiated cells (nonproliferating cells). Active proliferation of stem cells is only possible in growth environment Ω. Ph⁻ and Ph⁺ cells differ with respect to their growth environment transition characteristics f_α^{Ph-}, f_α^{Ph+}, f_ω^{Ph-} and f_ω^{Ph+}. Imatinib is assumed to exhibit two effects specific to Ph⁺ cells: a proliferation inhibition (affecting f_ω^{Ph+}) and a degradation of proliferating stem cells, modeled respectively by the inhibition intensity r_{inh} and the degradation intensity r_{deg}.

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In addition to the median *BCR-ABL1* transcript dynamics (**Fig. 1a**), our model consistently captures the heterogeneity of individual molecular responses to imatinib. Without a qualitative change of model assumptions, this heterogeneity can be explained by interindividual differences in the magnitude of the imatinib effects (**Fig. 1b**). Moreover, the model predicts that a considerable proportion of *BCR-ABL1*–positive stem cells escape the proliferation-specific degradation effect because they are in a dormant state. These noncycling cells, which have the potential for reactivation, are responsible for the rapid relapse after treatment withdrawal (**Fig. 1c**).

The proposed explanation of the imatinib effect as a selective one that depends on the proliferative status of the *BCR-ABL1*–positive stem cells has important implications for the design of treatment strategies. The model predicts that a continuous induction of cycling activity, in conjunction with imatinib treatment, will produce a more efficient reduction of tumor load than will treatment by imatinib alone, and that it will also result in a substantial deceleration of tumor regrowth after therapy termination (**Fig. 2a**). There is experimental evidence supporting this hypothesis[19]. However, this benefit might be reduced by imatinib-resistant clones. Such clones, arising from *BCR-ABL1* mutations, are observed in a considerable proportion of individuals with CML[20–22]. **Figure 2b** shows model predictions for the same scenarios as depicted in **Figure 2a**, but including the emergence of one completely imatinib-resistant clone at the beginning of treatment. Assuming partial resistance or resistance arising at later time points during treatment, the model predicts varying sizes of effects intermediate between the two illustrated scenarios. We also simulated the effect of combining imatinib with a nonspecific (that is, not *BCR-ABL1*–selective) deletion of proliferating cells. In contrast to cell cycle activation, this does not substantially change the molecular response to imatinib (**Supplementary Fig. 4** online).

Our results point to an explanation of the imatinib-induced *BCR-ABL1* dynamics that does not rely on a general insensitivity of stem cells to imatinib, but on a selective effect on proliferative cells. Such a selective sensitivity has been reported for *BCR-ABL1*–positive cells *in vitro*[23]. Although our approach is conceptually quite different from that proposed in the previous study[12], it similarly predicts the existence of imatinib-sensitive and imatinib-insensitive cell populations. However, we provide a possible functional explanation for a subpopulation of *BCR-ABL1*–positive (stem) cells that is not targeted, or is less effectively targeted, by imatinib.

As a major consequence of the assumed imatinib effect on long-term repopulating cells, we predict a sustained decline in *BCR-ABL1* transcript levels. However, the median imatinib effect within a treated population might be increasingly attenuated owing to the occurrence of resistance, as suggested by the heterogeneity in the response of individuals (see **Supplementary Fig. 3**). Modeling this heterogeneity by considering different degrees of resistance, our model explains a wide spectrum of clinically observed long-term dynamics, ranging from sustained *BCR-ABL1* declines to rapid relapse characteristics (see **Fig. 2c**).

The description of CML as a process of clonal competition for appropriate stem cell niches, which has also been discussed for myeloproliferative disorders in mice and cats[24], predicts the general possibility of cure by influencing the competition of *BCR-ABL1*–positive and normal cells. Relying on the imatinib-induced *BCR-ABL1*–specific proliferation inhibition and degradation, our model predicts that the malignant cell population can indeed be eradicated by continuous long-term imatinib administration if no resistance occurs. This process can even be accelerated by combining imatinib with stimulation of proliferation in HSCs (**Supplementary Fig. 5** online). However, as discussed, the development of resistance is likely to counteract these therapeutic effects. Still, an accelerated tumor reduction, as predicted for the combination treatment, is likely to reduce the probability of initiation of new resistance mutations. Our model predicts that clonal heterogeneity with respect to strength and time of appearance of resistance mutations has an important influence on achievable treatment responses. Depending on the degree of resistance, dynamics of relapses induced by pre-existing mutations (as suggested by others[25]) as well as by newly generated ones are consistent with the model.

Future model refinements need to address the question of whether one can predict the risk of onset of mutations and, if a resistant clone is present, whether one can predict its clonal development. However, further clinical and experimental data on the molecular response to imatinib, particularly on the onset and clonal development of resistant clones under treatment as well as on the pharmacokinetics of imatinib, is needed for this purpose.

The assumed competition for stem cell–supporting niches may not be the only explanation for the development of a CML. As suggested by

others[24], it may also be possible that malignant cells are able to invade microenvironmental regions that are not accessible to normal cells, thereby avoiding direct competition. However, based on currently available data—relying on a relative determination of tumor load (*BCR-ABL1/BCR* or *BCR-ABL1/ABL1*) only—it is not possible to discriminate between these two explanations. This would require, at the least, determination of the absolute size of the normal clone over time.

METHODS

Simulation model. The proposed model is based on the assumption that the development of hematopoietic stem cells depends on microenvironmental signals. In particular, two different growth environments (A and Ω) are assumed. The propensity of individual cells to reside in these two growth environments depends on a cell-specific affinity *a*. Whereas cells gradually lose this affinity in growth environment Ω, they regain it under the influence of growth environment A, which might be interpreted as a stem cell–supporting niche environment. If *a* falls below a given value a_{min}, it is set to zero and the cell loses the potential to change to growth environment A and, therefore, to regain *a*. These cells (denoted as differentiated) initiate clones that transiently amplify and finally die after a fixed lifetime. Whereas cells in growth environment A are assumed to be noncycling (that is, in G_0), cells in growth environment Ω are able to proliferate. We model the transition of cells between the two growth environments as a stochastic process, with transition probabilities depending on the individual cellular affinity *a* as well as on the transition characteristics f_α and f_ω. These characteristics depend on the actual numbers of cells in growth environments A and Ω.

In the context of this model, CML is explained by assuming quantitative differences between normal (Ph^-) and malignant (Ph^+) stem cells with respect to the transition characteristics f_α and f_ω (see **Supplementary Fig. 2**). These differences induce an advantage for malignant cells in the competition for available resources that are provided by the regeneration-supporting growth environment A.

Imatinib treatment is assumed to affect Ph^+ cells only. It induces a change in their entry into the cell cycle (altering f_ω with intensity r_{inh}) as well as elimination of proliferating cells in the stem-cell compartment (with intensity r_{deg}). For imatinib-resistant clones these effects are assumed to be reduced. **Figure 3** provides a conceptual schematic of the model, illustrating the differences between normal and malignant cells and the effects of imatinib treatment.

To compare clinically determined *BCR-ABL1* transcript levels to our model results, we approximated *BCR-ABL1/BCR* or *BCR-ABL1/ABL1* percentages using cell numbers in the population of nonproliferating differentiated cells according to the following relation: (number of *BCR-ABL1* positive cells / (number of *BCR-ABL1* positive cells + (2 × number of normal cells))) × 100%.

For a detailed description of model assumptions, mathematical equations, simulation algorithm and parameter choices, refer to the **Supplementary Note** and **Supplementary Tables 1** and **2**. The source code of the simulation program may be obtained from the authors.

Clinical measurements. We studied samples from subjects within the German cohort of the IRIS study by quantitative reverse transcriptase–polymerase chain reaction for residual *BCR-ABL1* transcripts at three-month intervals as previously described[18,26]. We analyzed a total of 894 peripheral blood samples. The median observation time was 60 months (range, 6–66 months). The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was reviewed by the ethics committees of all participating centers. All patients gave written informed consent according to institutional regulations.

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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