

Quantitative approaches to analyzing imatinib-treated chronic myeloid leukemia

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Progress in understanding the genetic changes that drive tumorigenesis has enabled the development of molecularly targeted anticancer therapy. The first small molecule targeted to a specific protein was imatinib mesylate (Gleevec, STI571), which is used to treat chronic myeloid leukemia (CML). A recent article presents a computational model with which to study the treatment response in CML patients and investigates the effect that imatinib exerts on leukemic stem cells. Here, I discuss insights derived from this study and their implications for imatinib therapy against CML.

Introduction

Targeted anticancer therapy promises efficient and safe treatment by interfering with a specific molecular target that has a crucial role in tumor growth or progression. The clinical success of the small-molecule kinase inhibitor imatinib mesylate (Gleevec, STI571) in treating chronic myeloid leukemia (CML) sets the standard for such therapy [1]. Imatinib causes a rapid depletion of the leukemic cell burden and leads to remissions in all stages of the disease [2]. In most patients, however, imatinib fails to eliminate residual disease [3,4]. Furthermore, acquired resistance to imatinib is an emerging problem in the treatment of CML [5]. The ability to determine the disease burden by quantitative PCR of the *BCR-ABL* oncogene (a fusion gene comprising breakpoint cluster region and Abelson murine leukemia viral oncogene homolog 1), which drives CML, facilitates the development of quantitative approaches to investigate these issues [6,7]. The goal of such studies is to explain some key features of the data: (i) imatinib therapy leads to a biphasic decline of the leukemic cell burden during the first two years of therapy, after which the cell count seems to reach a constant level (Figure 1a); (ii) discontinuation of imatinib therapy causes a rapid resurgence of the leukemic cell load to levels beyond the pre-treatment baseline (Figure 1b); and (iii) evolution of resistance leads to an increase in the number of leukemic cells, despite continuous therapy (Figure 1c).

A computational model

A recent article describes a computational model with which to analyze the dynamics of imatinib-treated CML [7]. The model is based on a stochastic computer

simulation (first published in Ref. [8]) and considers two growth environments for hematopoietic stem cells; in one environment, stem cells proliferate and differentiate, whereas they are quiescent in the other. The propensity of individual cells to reside in these growth environments depends on a cell-specific affinity. Cells gradually lose this affinity in the proliferating environment, whereas they regain it in the quiescent one. The transition of cells between the two environments is modeled as a stochastic process, with transition probabilities depending on the affinity of the cells and on the number of cells per environment. Cycling cells produce proliferating precursors and non-proliferating differentiated cells (Figure 2a).

In this model, CML is explained by differences between normal and leukemic stem cells in the transition probabilities between the growth environments. These differences lead to an advantage of leukemic stem cells and a resultant increase in their frequency. Imatinib is assumed to inhibit this proliferative activity and degrade cycling stem cells, whereas quiescent stem cells are insensitive to treatment. The degrading effect of imatinib leads to the initial fast depletion of leukemic cells, and the regulatory response of the system due to reduced numbers of stem cells causes the second, shallower slope seen in the data (Figure 2b). The model also incorporates imatinib resistance mutations, which are assumed to lead to reduced values for the inhibition intensity and/or degradation intensity of the drug. Depending on the presence and degree of resistance, imatinib leads to a long-term depletion (no resistance) or increase (resistance) of leukemic cells (Figure 2b). Upon the cessation of therapy, the leukemic cell count returns to the pre-treatment baseline because quiescent stem cells cannot be depleted by imatinib (Figure 2c).

Assumptions and implications of the model

Research into whether imatinib induces apoptosis of leukemic stem cells has led to contradictory findings: whereas some studies show that primitive CML cells do not readily undergo apoptosis – even after prolonged *in vitro* exposure to imatinib [3,9,10] – others indicate that at least some leukemic cells undergo apoptosis or attenuation in response to imatinib treatment [11–13]. To clarify this issue, more detailed experimental observations are needed of the *in vivo* cell cycle and apoptosis characteristics of stem cells. Such studies are challenging because of the lack of clear markers with which to distinguish quiescent and

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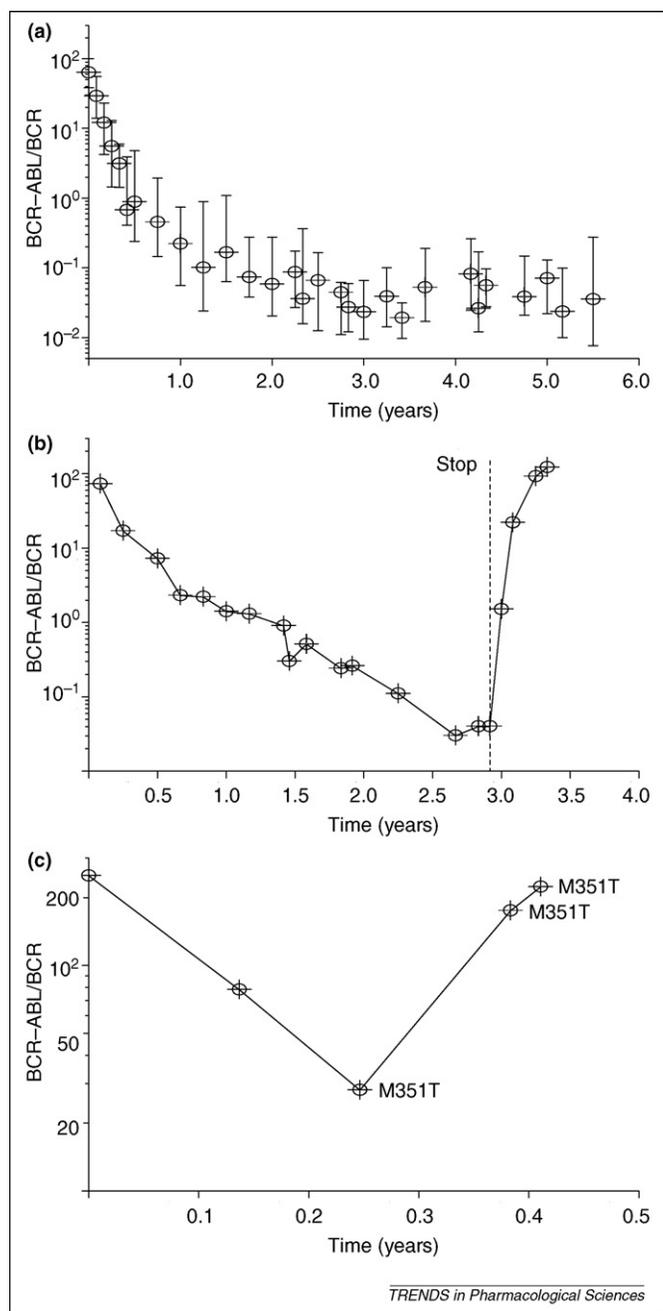


Figure 1. Response to imatinib therapy. (a) Shown are the median, 25 and 75 percentiles of 69 CML patients from the German cohort of the International Randomized Study of Interferon Versus STI571 (IRIS) trial [7]. Imatinib therapy leads to a biphasic decline in the leukemic cell burden during the first two years of therapy. Thereafter, the cell load seems to reach a steady state; this null hypothesis cannot be rejected with a conventional statistical criterion. (b) Upon discontinuation of imatinib therapy after three years, the leukemic cell count increases to levels at or beyond the pre-treatment baseline. (c) Evolution of resistance leads to a relapse of the disease. In the patient shown, a single point-mutation leading to a methionine–threonine substitution at position 351 in the ABL kinase domain (M351T) was found. The data shown in (b) and (c) were obtained from two patients in the IRIS trial [6].

proliferating stem cells, and the inability to define stem cells that occupy niches.

The dynamics of disease recurrence in patients who discontinue imatinib therapy can be used to infer the behavior of leukemic stem cells. Unfortunately, only three such patients were available for the quantitative analyses [6,7]. Although one patient experienced a rebound of the

leukemic cell count to a level close to the pre-treatment baseline, the other two patients suffered an increase to levels well beyond the baseline within weeks after discontinuation of therapy. The model by Roeder *et al.* [7] cannot explain an increase beyond the pre-treatment baseline because cycling leukemic stem cells are assumed to be depleted during therapy; because cycling stem cells make up only a small fraction of the leukemic burden [14], this decline can be invisible. The model by Michor *et al.* [6] assumes that leukemic stem cells continue to increase in number during therapy, which explains a relapse to levels beyond the pre-treatment baseline. So far, the conclusions of both models are based on these three patients only, and considerably more data must be collected to decide between the hypotheses. Such clinical trials are underway.

The long-term response to imatinib therapy has been suggested as a way to reject one of the two models. The model by Roeder *et al.* [7] predicts a continuous decrease in leukemic burden until the disease is eradicated; increases in cell number are possible only in the presence of resistance. An extension of the model by Michor *et al.* predicts that the leukemic cell burden slowly increases because of the intrinsic insensitivity of leukemic stem cells to imatinib [15,16]; this explanation of an increase does not require the presence of resistance. However, one cannot reject either hypothesis with the currently available data [7]; a simple power calculation using conventional criteria (5% confidence and 80% power) shows that, to test whether the cell levels reach 0.01% or 0 after prolonged treatment, a dataset many orders of magnitude larger is required. Therefore, this debate is currently futile and further experimental investigations are needed to study the long-term persistence of the disease.

Stem cell dormancy as a mechanism of insensitivity is still a hypothesis, and several alternatives have been suggested (note that the model by Michor *et al.* [6] does not study a particular method of stem cell insensitivity). For example, imatinib is a substrate for the multidrug resistance (MDR) protein P-glycoprotein [17] and is excluded from cells that express significant MDR levels, such as hematopoietic stem cells [18]. The second-generation BCR–ABL inhibitor dasatinib provides an opportunity to address this issue because it is not an MDR substrate. Therefore, it will be of interest to compare the kinetics of BCR–ABL mRNA decline in newly diagnosed dasatinib-treated patients with those of patients treated with imatinib. Such comparative upfront trials are getting underway.

Another possibility is that leukemic stem cells are less dependent on BCR–ABL for growth and survival than are committed progenitors, and therefore BCR–ABL inhibition does not eliminate leukemic stem cells. Indeed, there is evidence that primitive progenitors carrying the BCR–ABL gene rearrangement do not express the BCR–ABL hybrid mRNA or fusion protein [19]. There is also evidence, however, that BCR–ABL transcript levels are significantly increased within the most primitive CML cells and that this could contribute to the imatinib resistance of these cells [20].

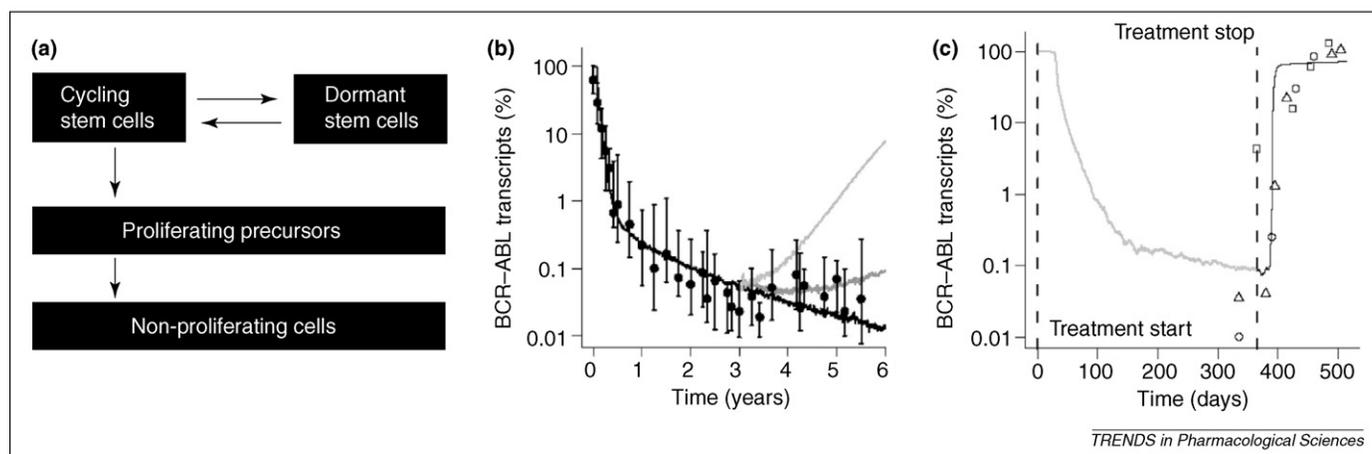


Figure 2. The model by Roeder *et al.* of imatinib-treated CML. (a) The model is based on a stochastic computer simulation that considers two different growth environments for stem cells: in one environment, stem cells are quiescent and regain the potential to proliferate, whereas, in the other, they divide and differentiate to produce cycling precursors and terminally differentiated cells. The *BCR-ABL* oncogene increases the transition rate of stem cells to the cycling environment. (b) In the context of this model, the first slope is explained by a degradation of leukemic stem cells by imatinib, and the second slope is caused by the inhibition of the proliferative activity of leukemic stem cells by imatinib. Data points represent medians and interquartile ranges of the percentage of cancer cells in the German cohort of the IRIS trial. The solid lines represent treatment response scenarios as predicted in Ref. [7]: no resistance (black), a partially resistant clone (dark gray) and a completely resistant clone (light gray). In this simulation, ten resistant stem cells were introduced three years after the start of imatinib therapy. (c) Upon discontinuation of therapy, the model predicts a rebound to levels at or below the pre-treatment baseline. Data points represent the relapse dynamics in three patients from the IRIS trial [6]. Panels (b) and (c) show the percentage of leukemic cells in peripheral blood over time.

Concluding remarks

Currently, the mechanism of the insensitivity of leukemic stem cells to imatinib therapy is the subject of speculation, and additional experimental and theoretical studies must be undertaken to elucidate this phenomenon. The article by Roeder *et al.* [7] represents an important step in that direction.

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