

## Chronic Myeloid Leukemia Blast Crisis Arises from Progenitors

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**Key Words.** Chronic myeloid leukemia • Hematopoietic stem cells • Hematopoietic progenitor cells • Differentiation

### ABSTRACT

Chronic myeloid leukemia (CML) progresses through three distinct clinical stages: chronic phase, accelerated phase, and blast crisis. The progression to accelerated phase and blast crisis is driven by activation of oncogenes, inactivation of tumor suppressor genes, and/or amplification of the *BCR-ABL* fusion gene, which causes the chronic phase of the disease. The cell of origin of blast crisis is a subject of speculation. Here, I develop a simple mathematical model of CML blast crisis to investigate whether blasts arise from leukemic stem cells or more differentiated leukemic cells. I use data of patients treated

with imatinib and previous agents to estimate the effects of therapy on the rate of progression. Imatinib reduces the progression rate 10-fold as compared with previous (ineffective) therapies. If blasts were produced by leukemic stem cells, there would be no difference in the rate of progression between patients treated with imatinib and previous therapies, because imatinib seems to be incapable of depleting leukemic stem cells. Imatinib does, however, deplete leukemic progenitors. Therefore, CML blasts are likely to arise from leukemic progenitors. *STEM CELLS* 2007;25:1114–1118

Disclosure of potential conflicts of interest is found at the end of this article.

### INTRODUCTION

Chronic myeloid leukemia (CML) is associated with the Philadelphia (Ph) chromosome, a t(9;22) translocation producing the *BCR-ABL* fusion gene [1–3]. The protein product of *BCR-ABL* is a constitutively active tyrosine kinase that drives the abnormal proliferation of leukemic cells [4]. There is experimental as well as theoretical evidence that the Ph chromosome is necessary and sufficient to initiate and sustain the leukemic phenotype of cells [5–8]. Imatinib (Gleevec, STI571; Novartis International, Basel, Switzerland, <http://www.novartis.com>), a potent inhibitor of BCR-ABL, induces remission in all stages of the disease [9]. However, residual disease is often detected in the bone marrow [10, 11]. Furthermore, a substantial fraction of patients develop acquired resistance to imatinib as a result of *BCR-ABL* amplification or mutations in its kinase domain [12, 13].

The cell of origin of CML has been the subject of much discussion [6, 10, 14, 15]. The Ph chromosome can be found in all cell types of peripheral blood, suggesting that CML is a stem cell disease [15]. Hematopoietic stem cells are the only normal cells in blood that renew themselves and are therefore considered to be the only cells that can accumulate (epi)genetic alterations [16, 17]. However, transgenic mouse models of CML indicate that myeloid progenitors transformed with *BCR-ABL* and *BCL-2* can drive leukemic expansion [6]. Progenitor cells might thus be able to acquire changes that restore stem cell capabilities and could be the cells from which CML originates [14, 18, 19].

Progression to blast crisis is driven by additional genetic and/or epigenetic events such as duplication of the Ph chromosome, trisomy 8, and mutations or deletions in tumor suppressor

genes like p16 and p53 [20–22]. Disease progression is supported by self-renewing blast crisis stem cells [3, 6, 15]. These blasts are immature hematopoietic cells and are assumed to arise from leukemic stem cells. However, the following new evidence suggests that blasts might be produced from leukemic progenitors instead [23]: progression to blast crisis is associated with expansion of leukemic progenitors rather than leukemic stem cells; BCR-ABL expression levels increase in leukemic progenitors but are constant in leukemic stem cells during disease progression; leukemic progenitors in blast crisis have elevated  $\beta$ -catenin expression, which is implicated in self-renewal; and CML progenitors indeed have self-renewal capacities in vitro. Other studies, however, have found that BCR-ABL expression levels increase in primitive leukemic cells rather than in progenitors during disease progression [12, 24–26]. Hence, the experimental evidence remains inconclusive and invites theoretical investigations.

It is important to identify the cell population that is responsible for driving the progression of chronic phase CML to blast crisis. Knowledge of the cell type and mutations responsible for disease progression might suggest new treatment strategies for accelerated phase and blast crisis patients and help us understand the natural history of CML. In this paper, I design a simple mathematical model of CML progression and investigate the cell of origin and the dynamics of blast crisis.

### MATERIALS AND METHODS

Denote the abundance of leukemic stem cells and leukemic progenitors by  $y_0$  and  $y_1$ , respectively. Leukemic stem cells grow at rate  $r$  and die at rate  $d_0$  per day. Leukemic progenitors are produced

by leukemic stem cells at rate  $a$  and die at rate  $d_1$  per day. The leukemic stem cell population is described by  $y_0 = (r - d_0)y_0$  and the leukemic progenitor population by  $y_1 = ay_0 - d_1y_1$ , where  $x = dx / dt$ . With the initial conditions  $y_0(0) = 1$  and  $y_1(0) = 0$ , the numbers of leukemic stem cells and progenitors at time  $t$  are given by

$$y_0(t) = \exp[(r - d_0)t]$$

and (1)

$$y_1(t) = \frac{a \exp[-d_1t]}{r - d_0 + d_1} [\exp[(r - d_0 + d_1)t] - 1].$$

This model assumes exponential expansion of leukemic stem cells and neglects density dependence effects within the bone marrow for mathematical simplicity. A model considering density effects is the topic of another paper [27].

Blasts can arise either from leukemic stem cells or from leukemic progenitors. Denote the abundance of blasts at time  $t$  by  $z(t)$  and their rate of production per cell division by  $\mu$ . If blasts arise from leukemic stem cells, then their abundance at time  $t$  is given by

$$z(t) = \mu \int_0^t y_0(\tau) d\tau = \frac{\mu}{r - d_0} [\exp[(r - d_0)t] - 1]. \quad (2)$$

If blasts arise from leukemic progenitors, then their abundance is given by

$$z(t) = \mu \int_0^t y_1(\tau) d\tau = -\frac{\mu a \exp[-d_1t]}{s(r - d_0)d_1} [s \exp[d_1t] - d_1 \exp[st] - r + d_0], \quad (3)$$

where  $s = r - d_0 + d_1$ . The probability of blast crisis at time  $t$  is given by

$$P(t) = 1 - \exp[-z(t)]. \quad (4)$$

## RESULTS

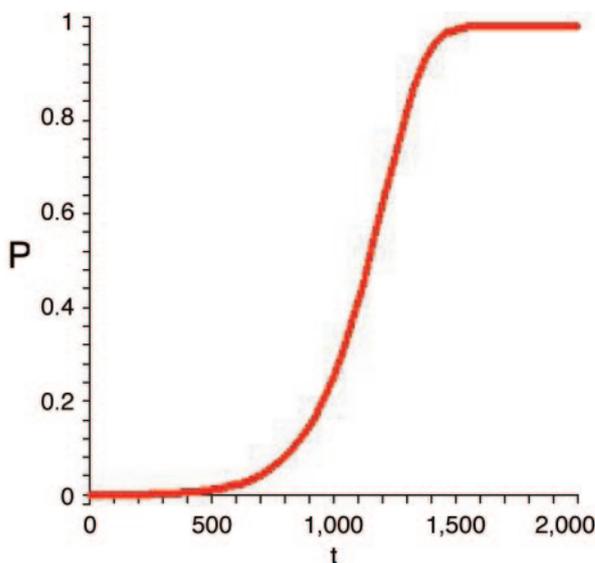
This model allows us to study the dynamics of blast crisis CML and to test the hypothesis that blasts arise from leukemic progenitors. A detailed quantitative analysis of the *in vivo* kinetics of chronic phase CML demonstrates that imatinib leads to an exponential decline of leukemic progenitors [28]. Their abundance decreases at a rate of 0.8% per day, which corresponds to an average lifespan of 125 days during therapy.

Leukemic stem cells, however, do not seem to be depleted during imatinib therapy. This conclusion is drawn from the relapse dynamics of patients who discontinue therapy. Despite being treated with imatinib for up to 3 years, the leukemic cell counts in those patients rise within weeks after stopping therapy to levels at or beyond pretreatment baseline [28]. As imatinib leads to an at least 5,000-fold reduction in the production of terminally differentiated leukemic cells from leukemic stem cells, discontinuation of therapy causes a rapid 5,000-fold increase in the number of leukemic cells. The cell count reached after discontinuation allows us to estimate the effect of treatment on the leukemic stem cell pool; a resurgence to levels well below pretreatment baseline means that leukemic stem cells were depleted during therapy, whereas a resurgence to levels beyond pretreatment baseline signifies that the leukemic stem cells continued to expand during therapy. The latter pattern is seen in all patients analyzed [28]. Therefore, we suggested that

**Table 1.** Annual rate of failure on imatinib

Year	Progression (%)	AP/BC (%)
1	3.4	1.5
2	7.5	2.8
3	4.8	1.6
4	1.5	0.9

The table shows the annual rate of progression and evolution to accelerated phase/blast crisis in patients treated with imatinib. Progression refers to loss of complete hematologic response or major cytogenetic response—defined as 0–35% Philadelphia-positive metaphases. Data from [32]. Abbreviation: AP/BC, accelerated phase/blast crisis.

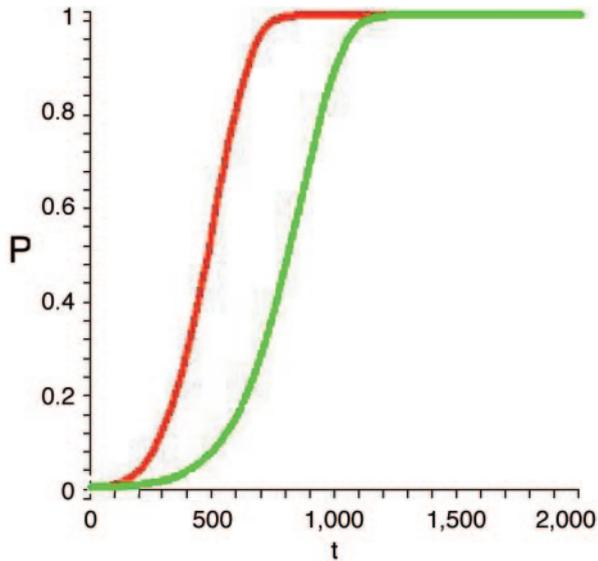


**Figure 1.** The figure shows the probability of progression to blast crisis over time when blasts arise by mutation from leukemic stem cells. Equations 2 and 4 are shown. The curve is the same for patients receiving imatinib therapy and for untreated patients, because imatinib cannot deplete leukemic stem cells. Parameter values are the growth rate of leukemic stem cells  $r = .008$ , the death rate of leukemic stem cells  $d_0 = 0.003$ , and the rate at which blasts arise per cell division  $\mu = 10^{-8}$ . See the text for a discussion of the parameter values. Abbreviations:  $p$ , probability of progression to blast crisis;  $t$ , time.

imatinib is incapable of depleting leukemic stem cells. This conclusion is supported by experimental *in vitro* studies finding that CML stem cells are insensitive to imatinib [11, 29–31].

The observed probability of progression to blast crisis is 1%–2% per year for patients on imatinib therapy and 10%–20% per year for patients on previous therapies such as  $\alpha$ -interferon plus cytarabine (Table 1) [32–34]. For the purpose of the analysis, I regard previous therapies as ineffective and compare the probabilities of progression to blast crisis with and without imatinib treatment, respectively, at 1%–2% and 10%–20% per year. Hence, imatinib reduces the probability of blast crisis 10-fold.

If blasts arise by mutation from the leukemic stem cell pool, then the probabilities of progression to blast crisis with and without imatinib should be the same, because imatinib does not seem to deplete leukemic stem cells. Treatment would not attenuate blast crisis if it did not change the abundance of the cells driving blast crisis. Figure 1 shows the probability of blast crisis at time  $t$  when blasts arise from stem cells (equations 2 and 4). The probability is the same with and without imatinib ther-



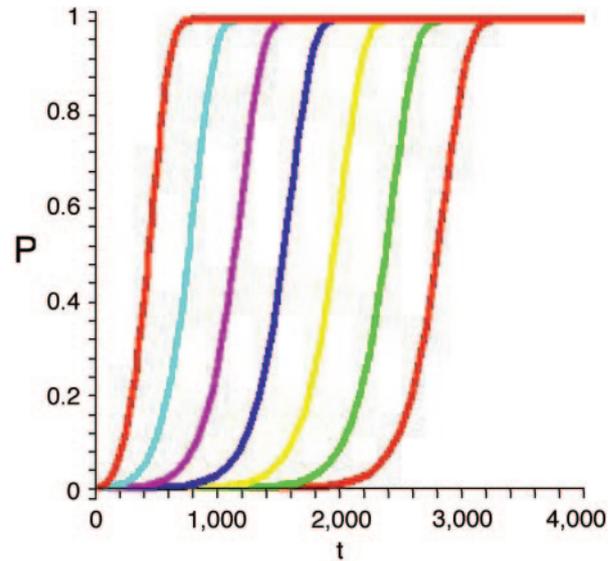
**Figure 2.** The figure shows the probability of progression to blast crisis over time when blasts arise by mutation from leukemic progenitors. Equations 3 and 4 are shown. The right curve shows the probability of blast crisis when the patient is treated with imatinib, and the left curve shows the probability of blast crisis when there is no treatment. The rate of progression with imatinib therapy is 10-fold lower than the rate of progression without imatinib therapy. Parameter values are the growth rate of leukemic stem cells  $r = .008$ , the death rate of leukemic stem cells  $d_0 = 0.003$ , the death rate of leukemic progenitors  $d_1 = 0.008$ , the rate at which blasts arise per cell division  $\mu = 10^{-8}$ , and the rate at which leukemic progenitors are produced by stem cells  $a = 0.1$  (right curve) and  $a = 1$  (left curve). Abbreviations:  $p$ , probability of progression to blast crisis;  $t$ , time.

apy, because imatinib is assumed not to change the behavior of leukemic stem cells.

If blast crisis is driven by leukemic progenitors, however, then the probability of progression with imatinib treatment is expected to differ from the probability of progression without imatinib treatment, because imatinib does deplete leukemic progenitors. Figure 2 shows the probability of blast crisis at time  $t$  when blasts arise from progenitor cells (equations 3 and 4). The probability is much (10-fold) lower when the patient is treated with imatinib than when there is no treatment.

Unfortunately, growth and death rates of leukemic stem cells have not been measured in vivo. However, the net proliferation rate can be inferred indirectly. The Philadelphia translocation might be the only genetic aberration needed to cause chronic phase CML [7, 8]. It takes  $t = 6-7$  years from the first incidence of the Philadelphia chromosome until diagnosis of the disease [35], and there are approximately  $y_0(\text{diagnosis}) = 250,000$  leukemic stem cells at the time of diagnosis [36, 37]. Assuming exponential growth of the leukemic cell clone, solving  $y_0(\text{diagnosis}) = \exp[(r - d_0) \cdot t \cdot 365.25]$  gives a net growth rate of  $r - d_0 \approx 0.005$  per day. The death rate of leukemic stem cells,  $d_0$ , is presumably lower than the death rate of leukemic progenitors,  $d_1 = 0.008$  (as measured in [28]). In Figures 1 and 2, I chose  $r = 0.008$  and  $d_0 = 0.003$  as a particular example. These rates are the same for imatinib-treated and untreated leukemia. The rate at which leukemic progenitors are produced from stem cells,  $a$ , depends on the presence of imatinib treatment. I assume  $a = 1$  for an untreated patient and  $a = 0.1$  for a treated patient.

The probability of blast crisis depends on the mutation rate of producing blasts. If one single base change can transform a cell into a blast and this cell has a high probability of survival, then the rate at which blasts arise is  $\mu = 10^{-8}$  (Figs. 1, 2) [38].



**Figure 3.** The figure shows the dependence of the probability of blast crisis on the rate at which blasts are produced from leukemic stem cells,  $\mu$ . Equations 2 and 4 are shown. Parameter values are the growth rate of leukemic stem cells  $r = .008$ , the death rate of leukemic stem cells  $d_0 = 0.003$ , and the rate at which blasts arise per cell division  $\mu = 10^{-12}$  (right curve),  $\mu = 10^{-11}$ ,  $\dots$ ,  $\mu = 10^{-6}$  (left curve). Abbreviations:  $p$ , probability of blast crisis;  $t$ , time.

This rate decreases if more than one change is necessary for transformation—for example, if both alleles of a tumor suppressor gene must be inactivated [20, 21] or if leukemic progenitors need to evolve self-renewal capacities and if blasts do not survive with probability one. The rate increases if the *BCR-ABL* oncogene elevates the genomic mutation rate [39]. Figure 3 shows how the probability of blast crisis depends on the parameter  $\mu$ .

## DISCUSSION

In this paper, I design a mathematical model of CML progression and investigate which population of leukemic cells is responsible for driving blast crisis. Blasts can, in principle, arise by mutations occurring in leukemic stem cells or in leukemic progenitors. The mutations arising in stem cells could be activation of oncogenes, inactivation of tumor suppressor genes, and/or other genetic changes deregulating cellular growth, whereas the mutations occurring in progenitors could additionally include changes facilitating self-renewal. In the model, the evolutionary process of mutation is encapsulated in the parameter  $\mu$ , which denotes the rate at which blasts arise. This parameter includes one or more mutations and also the probability that cells carrying such mutations survive to initiate blast crisis. The specific genetic changes needed to induce blast crisis are unknown, and their identification is an important goal in CML research.

Leukemic stem cells seem to be insensitive to imatinib therapy in vivo [28]. Several alternative mechanisms of stem cell insensitivity to imatinib have been brought forth. The stem cell dormancy hypothesis proposes that imatinib cannot inhibit quiescent stem cells [30, 40]. These dormant cells make up a significant fraction of the tumor stem cells—their abundance has been estimated to be approximately  $5 \cdot 10^7$  in an average patient [41], and depletion of proliferating stem cells might therefore not lead to visible declines in the cell number. Imatinib is a substrate for the multidrug resistance (MDR) protein p-

glycoprotein [31] and is excluded from cells that express significant MDR levels, such as hematopoietic stem cells [29]. Leukemic stem cells could be less dependent on BCR-ABL for growth and survival than are committed progenitors, and therefore BCR-ABL inhibition might 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 [42]. There also is evidence, however, that BCR-ABL transcript levels are significantly increased within the most primitive CML cells, and that this may contribute to their imatinib resistance [43]. Currently, the mechanism of insensitivity of leukemic stem cells to imatinib therapy is subject of speculation, and more experimental and theoretical studies must be undertaken to elucidate this phenomenon.

The probability of progression to blast crisis is 10-fold higher for patients who are not treated with imatinib than for patients receiving imatinib therapy. If imatinib does not significantly deplete leukemic stem cells, then blasts must arise from leukemic progenitors—otherwise, the probabilities of progression with and without imatinib would be the same. There are two caveats to this conclusion. The first is that imatinib could be capable of reducing the elevated mutation rates brought about by the *BCR-ABL* oncogene. Indeed, a CML mouse model suggests that *BCR-ABL* leads to a two- to threefold increase in the point mutation rate, which can be reversed by imatinib therapy

[44]. A significant reduction in the mutation rate by imatinib could, in principle, explain why the progression rate is reduced in patients receiving imatinib therapy. However, the reduction in the mutation rate reported from the mouse model cannot account for the 10-fold difference in the progression rate, and therefore this explanation is unsatisfactory. As long as the mutation rate is (near) constant among imatinib-treated and untreated patients, the conclusion that blasts likely arise from progenitors remains valid. The second caveat is that imatinib might reduce the rate of exponential expansion of leukemic stem cells without depleting them. The expansion of leukemic stem cells occurs very slowly (with a net growth rate of approximately 0.5% per day), and it is unlikely that robust expansion can be maintained when the growth rate is decreased. However, robust clonal expansion is necessary to explain the disease relapse in patients who discontinue imatinib treatment [28, 45]. Therefore, an explanation invoking decreased expansion of leukemic stem cells seems ungeneric. I conclude that CML blast crisis likely arises from leukemic progenitors.

### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The author indicates no potential conflict of interest.

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