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Mathematical Modeling of Genesis and Treatment of Chronic Myeloid Leukemia

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Key Words

Chronic myeloid leukemia · Imatinib · Hydroxyurea · Interferon-α · Stem cells · Mathematical modeling · Simulation study

Abstract

Chronic myeloid leukemia (CML) is a clonal hematopoietic disorder induced by translocation of chromosomes 9 and 22, resulting in an overproduction of myeloid blood cells. CML-specific characteristics include a latency time of several years, a period of coexistence of malignant and normal cells and an eventual dominance of the malignant clone. Different drug therapies are available, most notably imatinib, which inhibits the oncogenic *BCR-ABL1* tyrosine kinase. Although the chromosomal aberration causing CML is well known, the resulting dynamic effects on the system behavior are not sufficiently understood yet. Here, we apply an already established mathematical model of hematopoietic stem cell organization. Based on parameter estimates for normal hematopoiesis, we systematically explore the changes in these parameters necessary to reproduce CML-specific characteristics regarding emergence and course of disease as well as a variety of qualitative and quantitative clinical data on CML treatment. Our results indicate that 1 or more of the following mechanisms are compatible with the induction of a dominant clone in the proposed model: a retarded differentiation process, a reduced turnover time or a defective cell-microenvironment interaction of the neoplastic cells. However, in order to explain the massive overproduction of malignant cells, an unregulated and abnormal activation of leukemia stem cells into cycle has to be assumed additionally. Based on our simulation results we conclude that CML dynamics can most appropriately be explained by a modulation of the cell-microenvironment interactions of leukemia stem cells, including both the process of stem cell silencing and activation into cycle.

Abbreviations used in this paper

CML	chronic myeloid leukemia
HSC	hematopoietic stem cell
HU	hydroxyurea
IFN-α	interferon-α
IM	imatinib
PCR	polymerase chain reaction
Ph	Philadelphia chromosome

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Introduction

Chronic myeloid leukemia (CML) is a clonal disorder of the hematopoietic system. It is induced by mutation on the hematopoietic stem cell (HSC) level and results in a malignant expansion and overproduction of (immature) myeloid blood cells [Mauro and Druker, 2001]. Cytogenetically, malignant cells are characterized by a reciprocal translocation of chromosomes 9 and 22 [Goldman and Melo, 2003]. It has been demonstrated that radiation is capable of inducing such a genetic aberration [Ichimaru et al., 1981]. However, the precise mechanisms as well as the frequency of the occurrence are only incompletely known. The shortened chromosome 22 is referred to as Philadelphia chromosome (Ph) which carries the *BCR-ABL1* fusion gene. Its gene product, the oncogenic BCR-ABL1 protein, is a constitutively activated tyrosine kinase, the expression of which has been shown to be responsible for the pathogenesis of CML [Deininger et al., 2000]. But even though these mechanisms within individual malignant cells are well understood, the resulting response of the system to the neoplasm on the cell population level is yet to be elucidated.

If left untreated, CML is a fatal disease. The only known curative treatment option of CML is stem cell or bone marrow transplantation [Goldman and Gordon, 2006]. This procedure, however, is associated with a high early mortality rate. Furthermore, several drug therapies are available. Hydroxyurea (HU) is a cytotoxic drug which specifically acts on proliferating cells, regardless of genotype, by inhibition of ribonucleotide reductase and, therefore, by inhibition of DNA synthesis. This effect prevents cell division and eventually leads to apoptosis [de Lima et al., 2003]. Currently, HU is primarily used as initial therapy to reduce the leukocyte count to normal levels before other treatment is applied. Interferon-α (IFN-α) has been used for many years in the management of patients in the chronic phase of CML, but the mechanisms by which it induces growth inhibitory effects in leukemia cells are not exactly known. Several different mechanisms have been suggested. It is widely accepted that IFN-α has an immunostimulating effect, that is, it renders leukemia cells visible to the immune system [Parmar and Platanias, 2003]. One indirect consequence of this immune effect may involve normalization of the adhesion to bone marrow stroma [Bhatia et al., 1994]. Downregulation of the expression of the *BCR-ABL1* oncogene might be another potential mechanism [Verma and Platanias, 2002].

Within the last decade, imatinib (IM) has rapidly become the front-line therapy for de novo CML. This drug inhibits the oncogenic *BCR-ABL1* tyrosine kinase by occupying the ATP-binding site of the BCR-ABL1 protein, thereby preventing phosphorylation of its substrates [Buchdunger et al., 1996]. This process ultimately results in the switching off of downstream signaling pathways that promote leukemogenesis [Savage and Antman, 2002]. Although these molecular mechanisms are well known, it is not sufficiently understood how they translate into the dynamic regulation of normal and leukemic cell growth. It is known that IM selectively acts on leukemia cells where it induces a proliferation inhibitory effect [Druker et al., 1996] as well as an increase in the apoptotic rate of actively proliferating cells [Oetzel et al., 2000; Vigneri and Wang, 2001; Holtz et al., 2007]. Molecular monitoring of tumor load revealed that IM induces a biphasic decline of *BCR-ABL1* transcript levels during the first year of treatment. It is characterized by an initially rapid followed by a moderate decline. Furthermore, a rapid relapse upon treatment cessation can be observed [Michor et al., 2005].

An important obstacle in the design of curative drug therapies is the fact that relevant details of the system behavior, such as resistance to IM treatment [Tauchi and Ohyashiki, 2004], are only insufficiently understood, irrespective of the above-mentioned molecular insights into pathogenesis of CML and IM activity.

It is the objective of this paper to apply an already established mathematical model of HSC organization [Roeder and Loeffler, 2002] to the situation of CML. Based on parameter estimates for normal hematopoiesis, we systematically investigate the changes in the cell-intrinsic parameters necessary to reproduce CML-specific characteristics. The model has to be consistent with a variety of qualitative and quantitative clinical data on CML treatment. Thereby, a comprehensive, predictive and interpretable picture of CML emergence, course of disease and treatment can be obtained. To be able to generate model predictions for new treatment options, an in silico disease model consistent with established treatment options is required. Therefore, also data on former first-line CML therapies, such as HU and IFN-α, are considered in order to guarantee the validity of the model.

Materials and Methods

In this work, we apply a single-cell-based, stochastic mathematical model [Roeder and Loeffler, 2002]. The model has been developed for the HSC system and has already been validated for

animal data, for example to describe clonal competition processes in mouse chimeras [Roeder et al., 2005]. Furthermore, it has also been applied to 1 very specific treatment scenario in chronic myeloid leukemia [Roeder et al., 2006]. A schematic representation of the model can be found in figure 1. It is assumed that cells can reside in 2 signaling contexts (**A**, Ω), which can be interpreted as different growth environments, with **A** representing a stem cell-supporting niche within the bone marrow. A short explanation of the model can be found in the next paragraph. For a more detailed mathematical description, we refer to the Appendix and Roeder et al. [2006].

Each cell has a property a, which represents the affinity to reside in **A**. If a is greater than a given threshold a_{min}, the respective cell is denoted as a stem cell. Affinity a can be interpreted as the state of differentiation of a stem cell: the smaller a, the less stem cell potential is attributed to a cell. Differentiation (that is, decrease in a) is considered to be a reversible process until a has reached a_{min}. Whereas cells gradually lose affinity a under the influence of environment Ω [$a(t + 1) = a(t)/d$], they regain it in **A** [$a(t + 1) = a(t)\cdot r$]. The latter can be interpreted as a regeneration process on the individual cell level. Parameters d and r represent differentiation and regeneration coefficients, respectively. If a falls below a_{min}, it is set to zero. Such a cell loses its potential to change to **A** and, therefore, to regain a. It is no longer denoted as a stem, but as a differentiated cell, which initiates a clone that amplifies and finally dies after a fixed lifetime. Whereas cells in **A** are assumed to be quiescent, that is, in G_0-phase of the cell cycle, cells in Ω are actively proliferating with an average generation time τ_c. The transition of cells between the 2 signaling contexts is modeled as a stochastic process, that is, at every time step each cell has a certain probability to change from one compartment to the other. The transition probabilities depend on the individual cellular affinity a and the transition characteristics f_α and f_ω. These characteristics depend on the current numbers of cells in **A** and Ω, respectively (for a schematic, see fig. 2).

The described model is implemented as a C++ computer program. The source code can be obtained from the authors. Each individual cell in the system is simulated according to the above-outlined set of rules. These rules are applied at discrete time steps (Δt = 1 h) to simultaneously update the status of all model cells. The algorithm includes stochastic decisions, for example, with respect to transitions from one signaling context to the other. Due to this system-intrinsic stochasticity, even different simulation runs using identical model parameters generate quantitatively different outcomes. A population of patients can be represented by averaging the results of many single simulation runs.

The model assumes CML to be a clonal competition process between the malignant clone (Ph+), comprising all cells originating from 1 mutated cell, and normal (Ph–) hematopoietic cells, with potential quantitative differences in model parameters.

As mentioned above, the model has already been applied to the murine system [Roeder and Loeffler, 2002; Roeder et al., 2005]. In these publications, parameter values were fitted to quantitative data on specific mouse strains. In order to obtain a parameter set, which we assume to adequately represent a normal human hema-

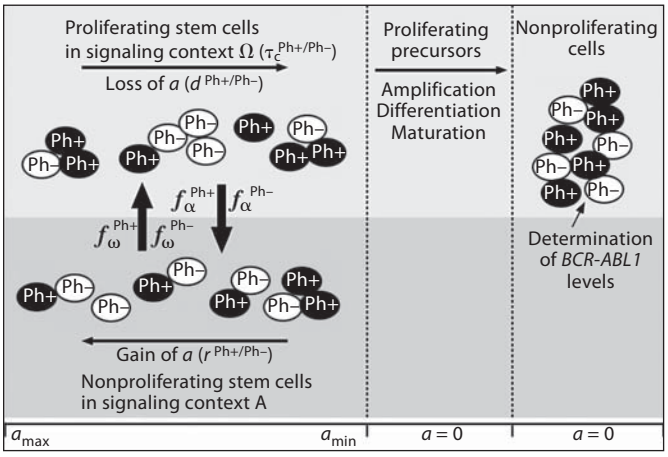

Fig. 1. Model scheme. Normal (Ph–) and malignant (Ph+) stem cells are assumed to coexist within 2 common signaling contexts (**A**, Ω). For a detailed model description, see text.

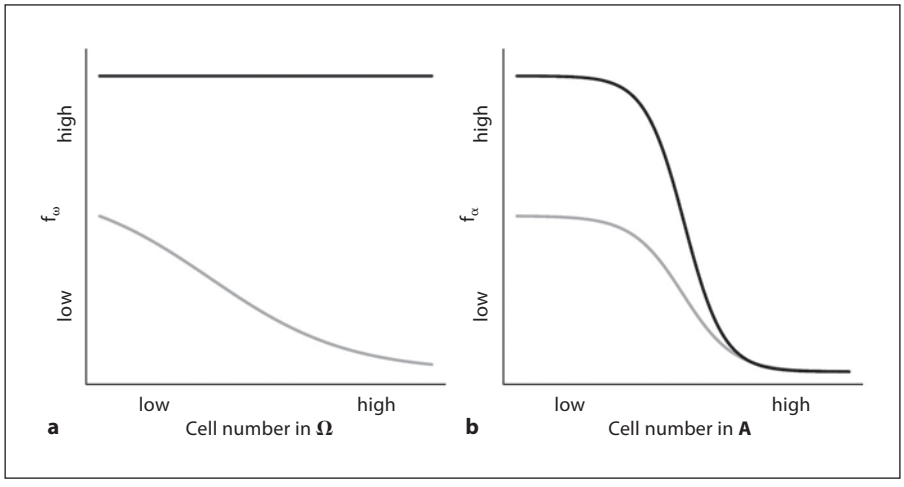

Fig. 2. Schematic of transition characteristics, showing examples of transition characteristics f_ω (**a**) and f_α (**b**) of normal (gray) and malignant (black) stem cells for the situation of untreated CML. For exact parameter values used in the computer simulations, see table 2.

topoiesis, the mouse parameter values served as a starting point. Although specific model parameters can be expected to differ considerably between mice and men, we assume the existence of fundamental interspecies similarities with regard to the general regulatory principles of hematopoiesis. Hence, it was an important goal to change only as few system parameters as necessary when applying the model to a new (here: the human) situation.

Using a parameter set capable of maintaining a steady state of normal hematopoiesis, the introduction of 1 Ph+ stem cell is assumed to represent the disease-initiating event. This stem cell is assumed to have a growth advantage compared to normal cells and is equipped with an inheritable marker, which allows tracking of its progeny, that is, the malignant clone, over time.

It is the aim of this study to investigate which model parameters are capable of inducing a growth advantage of leukemia cells that leads to the formation of a manifest CML clone. A set of model parameters which consistently describes the situation of untreated chronic myeloid leukemia is determined by applying the following qualitative criteria, all of which have been motivated by clinical and experimental observations.

(1) The emergence of the disease has to be accompanied by a long latency time of about 5–7 years [Ichimaru et al., 1981], characterized by a coexistence of normal and leukemia cells, until the proportion of neoplastic cells has reached more than 90%.

(2) If the disease remains untreated, the malignant clone eventually takes over the hematopoietic system, while the Ph– cells gradually disappear [Goldman and Melo, 2003].

(3) Compared to normal hematopoiesis, there is an increased absolute blood cell production primarily caused by an expansion of the malignant clone [Mauro and Druker, 2001].

(4) Quiescent leukemia stem cells show a delayed Ph positivity compared to actively proliferating cells, that is, the frequency of Ph+ stem cells in the quiescent pool lags behind that among proliferating cells [Dube et al., 1984].

All model parameters are systematically tested for the ability to reproduce these criteria. The agreement of simulation results and clinical/biological observations is judged by a consistency of average simulations with all above-stated criteria.

To further test the consistency of the derived parameter configurations beyond the emergence of CML, they are applied to different treatment strategies in silico. The simulation results are compared with qualitative and quantitative clinical data on CML patients. In particular, the following assumptions are applied for the simulation of treatment options.

HU treatment is assumed to induce an unselective kill of cells in S-phase, that is, a fixed percentage of S-phase cells per time step undergo apoptosis, regardless of their genotype. In contrast, IFN-α is assumed to affect leukemia cells only. At the moment of treatment initiation, the model parameters of malignant cells, which are capable of explaining the clonal dominance, are reset to the values assumed for normal cells. IM treatment is assumed to induce apoptosis and inhibition of the proliferative activity of proliferating Ph+ stem cells. Technically, the apoptotic effect is modeled by a selective kill of a fixed percentage of leukemia cells per time step (degradation rate r_{deg}), while the proliferation inhibition is modeled by a reduction of the activation of leukemia cells into cycle, that is, altering transition characteristic f_ω (transition from $\mathbf{A}$ to Ω), at the fixed inhibition rate r_{inh}.

Qualitatively, the model needs to reproduce that the majority of HU and IFN-α patients show a hematologic response, with HU inducing more rapid responses than IFN-α. In almost none of the HU patients, cytogenetic responses can be observed [Hehlmann et al., 1993]. In contrast, IFN-α is capable of inducing cytogenetic remissions in the majority of patients [Hehlmann et al., 1994]. Simulation of IM treatment needs to reproduce rapid hematologic and rapid cytogenetic remissions [Savage and Antman, 2002].

Furthermore, simulation results of IM treatment are compared with quantitative criteria, as highly sensitive measurements of tumor load, utilizing real-time quantitative polymerase chain reaction (PCR), are available [Michor et al., 2005; Roeder et al., 2006]. In these studies, *BCR-ABL1* transcript levels were measured at different time points during IM treatment. A typical biphasic decline of *BCR-ABL1* transcript levels during the first year of therapy as well as a rapid relapse upon treatment cessation can be observed [Michor et al., 2005]. An overview of the applied model assumptions and criteria can be found in table 1.

In order to compare clinically determined *BCR-ABL1* transcript levels to the mathematical model, *BCR-ABL1/ABL1* percentages are approximated using cell numbers in the population of nonproliferating differentiated cells according to the following relation: [number of Ph+ cells/(number of Ph+ cells + [2 × number of Ph– cells])] × 100%, motivated by the existence of 2 copies of each gene within individual cells and by a reported strong correlation between cytogenetics, assessing the proportion of Ph+ cells, and real-time quantitative PCR measurements of *BCR-ABL1* transcript levels in peripheral blood [Branford et al., 1999].

Results

CML Genesis

Based on a system of normal steady-state hematopoiesis (for model parameters, see table 2), 3 model parameters were found to be independently capable of inducing a competitive growth advantage, namely differentiation rate d, transition characteristic f_α and cell cycle duration τ_c. The possible parameter alterations can be found in table 3. Each of these 3 parameter changes, applied to exactly 1 proliferating stem cell (located in Ω), is capable of giving rise to a dominant clone. However, in each of these scenarios, this capability is realized in only about 20% of the cases, that is, in about 80% of the computer simulations there is only a transient low-level contribution of neoplastic cells. These cases, which are neglected in the following, are characterized by the extinction of the malignant clone owing to the stochasticity of the model.

Upon applying any of the parameter changes given in table 3, one obtains the results shown in figure 3. The percentage of the dominant clone relative to all cells within the computer simulation (average ± SD of 100 individual simulation runs) was determined using the population of nonproliferating differentiated cells. The disease-initiating single-cell mutation event occurred at time point

Table 1. Applied model assumptions and qualitative criteria for the simulation of CML treatment options HU, IFN-α and IM

Treatment	Assumptions	Criteria
HU	unselective kill of S-phase cells at a fixed rate per time step	rapid hematologic but no cytogenetic response
IFN-α	equalization of model parameters of normal and leukemia cells, which are capable of explaining the dominance of the malignant clone	hematologic and cytogenetic responses
IM	fixed degradation and proliferation inhibition rate selectively for leukemia cells	biphasic decline of *BCR-ABL1* transcript levels and rapid relapse after treatment stop

zero. Criteria 1 and 2 are met: after about 3 years, the proportion of malignant cells starts to rise. After about 5–7 years, clinically relevant levels of malignant cells can be detected. Finally, by outcompeting normal cells, the malignant clone takes over the hematopoietic system (fig. 3a). Criteria 3 and 4, however, cannot be met: compared to normal hematopoiesis, there is no increased production rate of malignant cells (fig. 3b). The overall count of normal (gray) and malignant (black) nonproliferating differentiated blood cells remains relatively constant over time. Furthermore, as indicated by figure 3c, quiescent HSCs (gray) do not show a delayed Ph positivity compared to actively proliferating cells (black). At each time point, the proportion of malignant quiescent stem cells is almost identical to the proportion of malignant proliferating precursors.

In the results shown so far, only 2 of 4 qualitative criteria could be met. Hence, a second parameter alteration had to be considered. Because there is evidence that leukemia cells show an unregulated cellular proliferation [Eaves et al., 1986], the transition characteristic f_ω of malignant stem cells, which normally depends on the absolute number of proliferating stem cells, was set to constant at a high level. The described alteration results in a cell number-independent and, therefore, in an unregulated activation of malignant cells into cycle.

Using this additional parameter modification, the system dynamics change in such a way that all 4 criteria can be met. The alteration of f_ω has to complement the change in either differentiation rate d, transition characteristic f_α

Table 2. Model parameter set for normal hematopoiesis

Parameter	Value
a_{min}	0.002
a_{max}	1.0
d	1.05
r	1.1
τ_c	48 h
τ_S	8 h
$\tau_{G_2/M}$	8 h
$\tilde{\tau}_c$	24 h
λ_p	20 days
λ_m	8 days
$f_\alpha(0)$	0.5
$f_\alpha(\tilde{N}_A/2)$	0.45
$f_\alpha(\tilde{N}_A)$	0.01
$f_\alpha(\bullet\bullet)$	0.0
$\tilde{N}_A$	10^3
$f_\omega(0)$	0.5
$f_\omega(\tilde{N}_\Omega/2)$	0.3
$f_\omega(\tilde{N}_\Omega)$	0.1
$f_\omega(\bullet\bullet)$	0.0
$\tilde{N}_\Omega$	10^3

The given parameters are capable of maintaining a normal steady-state hematopoiesis in silico. The parameters are as follows: (a_{min}, a_{max}) = range of affinity a that characterizes the propensity of a cell to reside in **A**; d = differentiation coefficient; r = regeneration coefficient; τ_c = cell cycle duration of stem cells; τ_S, $\tau_{G_2/M}$ = durations of S- and G_2/M-phase; $\tilde{\tau}_c$ = generation time of proliferating differentiated cells; λ_p = transition time for proliferating precursor cell stages; λ_m = life time of nonproliferating precursor cell stages and mature, terminally differentiated cells; f_α, f_ω = transition characteristics for change from Ω to **A** and **A** to Ω; $f_\alpha(\cdot), f_\omega(\cdot)$ = function values of transition characteristic at given argument; $\tilde{N}_A$, $\tilde{N}_\Omega$ = scaling factors of transition characteristics.

Table 3. Parameter changes giving rise to a dominant clone

Parameter	Ph–	Ph+
$f_\alpha(\tilde{N}_A)$	0.01	0.015
d	1.05	1.045
τ_c	48 h	45 h

Each of these 3 parameter alterations, applied to a single normal (Ph–) stem cell, is capable of giving rise to a dominant clone (Ph+). Parameters are as follows: $f_\alpha(\tilde{N}_A)$ = function value of transition characteristic f_α (describing the transition from the proliferating to the quiescent compartment) at cell number $\tilde{N}_A = 10^3$; d = differentiation coefficient; τ_c = cell cycle duration.

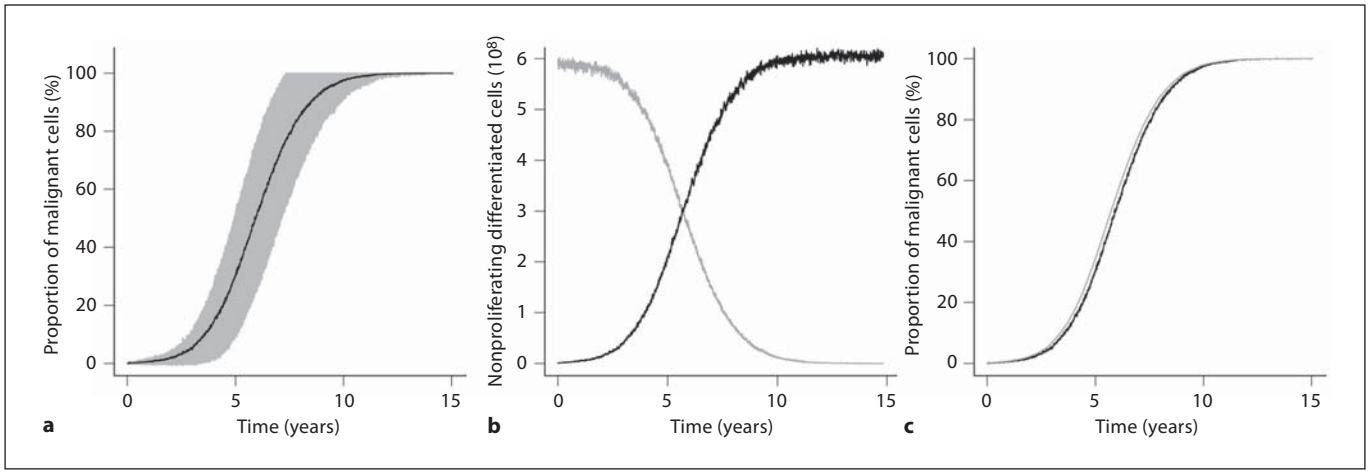

Fig. 3. CML genesis (1 altered model parameter), showing the scenario in which differences between normal and malignant cells are assumed for transition characteristic f_α. The other 2 scenarios are omitted because they are qualitatively identical. Each panel represents an average of 100 individual simulation runs. **a** Proportion of malignant nonproliferating differentiated cells (solid line) over 15 years postmutation. The gray shade indicates ± 1 SD. **b** Absolute cell counts in the nonproliferating differentiated cell compartment of normal (gray) and malignant (black) cells. **c** Proportion of cycling (black) and noncycling (gray) leukemia stem cells.

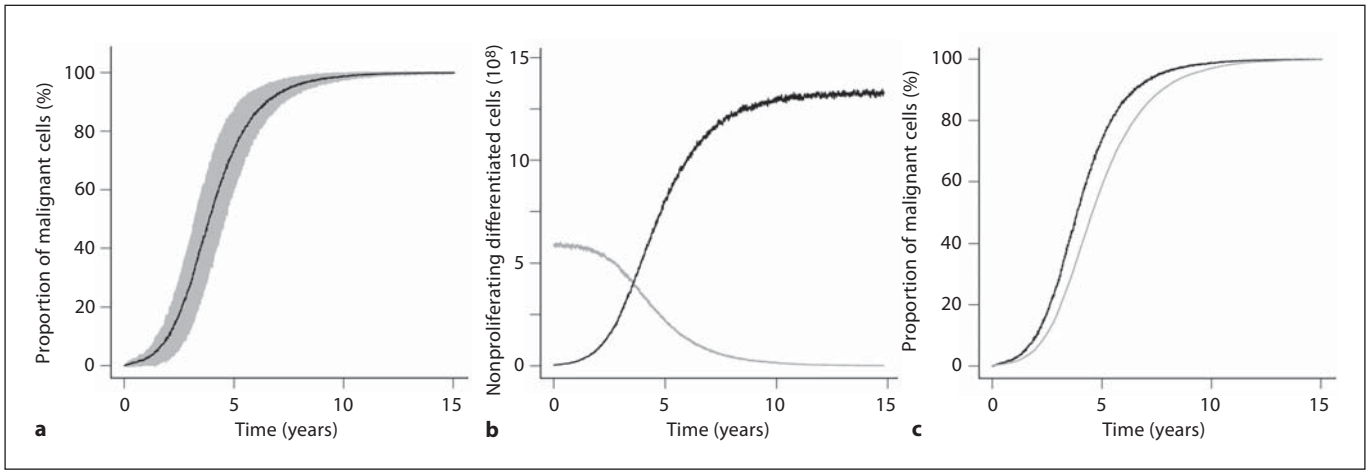

Fig. 4. CML genesis (2 altered model parameters), showing the scenario in which normal and malignant stem cells are assumed to differ in their transition characteristics f_α and f_ω (scenario 1). The other 2 scenarios are omitted because they are qualitatively identical. All graphs have been obtained averaging 100 individual simulation runs. **a** Proportion of malignant nonproliferating differentiated cells ± SD. **b** Absolute cell counts of normal (gray) and malignant (black) nonproliferating differentiated cells. **c** Proportion of actively proliferating (black) and quiescent (gray) malignant stem cells.

or cell cycle duration τ_c at the moment of the single-cell mutation. It should explicitly be noted that altering transition characteristic f_ω alone is not sufficient to induce a growth advantage necessary for the emergence of a dominant clone, because this parameter does neither influence criteria 1 nor 2. This means that changes in model parameters d, f_α or τ_c are required for clonal dominance, while the increased production of malignant cells, as well as the delayed Ph positivity of quiescent Ph+ stem cells are induced by the alteration of model parameter f_ω.

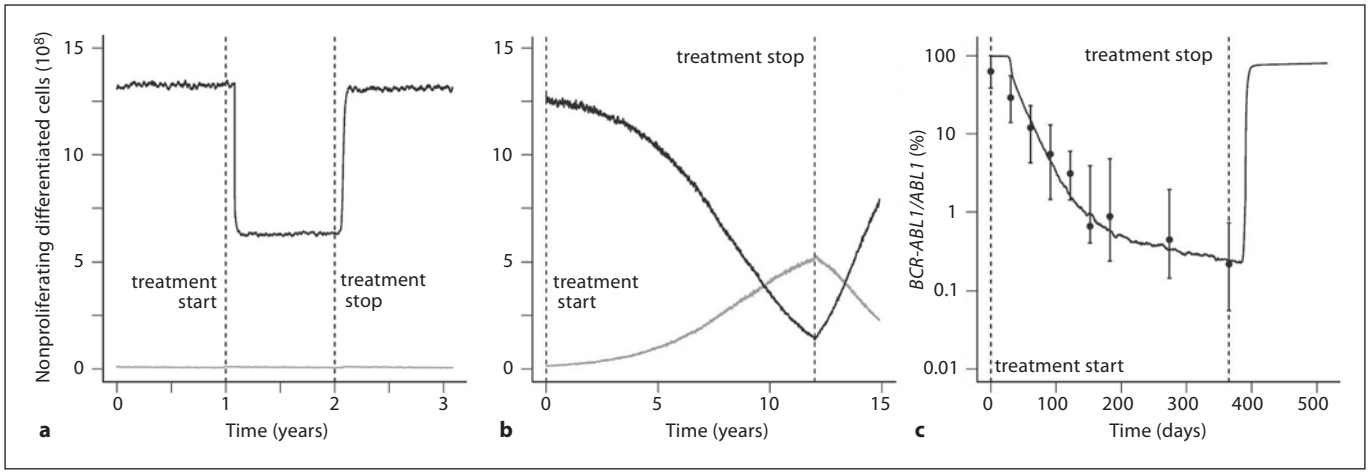

Fig. 5. CML treatment. All plots represent averages of 100 individual computer simulations. Shown is the scenario in which normal and malignant stem cells are assumed to differ in their transition characteristics f_α and f_ω (scenario 1). **a** HU treatment. Treatment is applied from year 1 to year 2 (as indicated by the dashed lines). Absolute numbers of normal (gray) and malignant (black) nonproliferating differentiated cells are shown. **b** IFN-α treatment. Treatment starts at time point zero and ends at the indicated time point. Normal (gray) and malignant (black) nonproliferating differentiated cells are shown. **c** IM treatment. Data points represent clinical measurements of *BCR-ABL1/ABL1* ratios, which are taken from Roeder et al. [2006]. The solid line shows the corresponding computer simulation.

Upon altering either of the parameter combinations f_α and f_ω (in the following referred to as scenario 1), d and f_ω (scenario 2) or τ_c and f_ω (scenario 3) for 1 proliferating stem cell, the results shown in figure 4 can be obtained (compare to fig. 3 for the situation of only 1 altered parameter value). Please note that in order to avoid redundancy, only 1 of the 3 scenarios is illustrated.

Scenarios 1, 2 and 3 are qualitatively identical, yet they represent different functional mechanisms potentially associated with the emergence of CML. Thus, an important goal was to discriminate between the scenarios by testing whether either of these is capable of reproducing clinically observed data on CML treatment strategies.

CML Treatment

First, HU treatment was analyzed. An unspecific kill of both normal and leukemia cells in S-phase at a constant rate of 2% per time step was applied to all 3 aforementioned CML scenarios. Because no estimates of this rate were available in the literature data, it was chosen arbitrarily. Therefore, it allows only for a qualitative analysis of the system behavior.

Due to the constant kill rate, cell numbers reach a much lower steady-state level, that is, a significant hematologic response can be observed. After cessation of therapy, the cell count rapidly returns to its pretreatment level. As HU was assumed to affect hematopoietic cells regardless of genotype, the cell count of both leukemia and normal cells is reduced. Please note that this reduction is only detectable with respect to malignant cells owing to the small number of normal cells. However, there is no change in the proportion of malignant cells during HU treatment, which also points to an equal relative cell number reduction of both cell types. Hence, a cytogenetic remission cannot be induced. In all scenarios, the procedure yields the results shown in figure 5a, that is, based on HU treatment, discrimination between the 3 scenarios is not possible.

IFN-α treatment was simulated by altering the model parameters, which are capable of explaining the dominance of the malignant clone. That is, at the moment of treatment initiation, transition characteristic f_α (scenario 1), differentiation rate d (scenario 2) or cell cycle duration τ_c (scenario 3) of malignant cells are reset to the values assumed for normal cells. Transition characteristic f_ω, however, remains unchanged. For the sake of simplicity, all cells were assumed to be simultaneously affected. Due to the applied parameter alterations, the growth advantage of the malignant clone is undone, resulting in a slow reduction of leukemia cells. At the same time, the popu-

lation of normal cells recovers slowly. Hence, significant hematologic as well as cytogenetic remissions can be observed in silico. After cessation of therapy, even after continuous IFN-α administration for several years, a clinically relevant relapse can be expected. In any of the 3 CML scenarios the results shown in figure 5b can be obtained, that is, a discrimination of the 3 scenarios by IFN-α treatment is not possible.

Finally, the simulation results were compared to clinical data on *BCR-ABL1* transcript levels of IM-treated CML patients. It was found that only scenario 1 was capable of reproducing the typical biphasic decline of *BCR-ABL1* transcript levels during the first year of IM treatment as well as the rapid relapse upon treatment cessation (fig. 5c). In scenario 1, differences between normal and leukemic cells in the transition characteristics f_α and f_ω are assumed, which can be interpreted as differences in the stem cell-microenvironment interaction (for example, stroma attachment/detachment kinetics). Fitting the mathematical model to the quantitative clinical data [Roeder et al., 2006], degradation rate r_{deg} and inhibition rate r_{inh} were estimated to be 2.8 and 5.0% per time step, respectively. The first (steep) decline is caused by a massive initial reduction of proliferating leukemia cells due to the selective degradation effect. The second (moderate) decline is induced by the dynamic regulation of the system in response to the initial cell reduction. The model predicts leukemic stem cells to accumulate in compartment **A** (that is, quiescent stem cells) during IM therapy. These cells are responsible for the rapid relapse of *BCR-ABL1* transcript levels after treatment stop.

Discussion

It could be shown that CML development can be explained as a clonal competition process of normal and malignant cells, induced by quantitative differences in cellular properties. It can be stated that the situation of human hematopoiesis, in particular the situation of chronic myeloid leukemia, can be explained within the context of a general concept of tissue stem cell organization, which is accounting for cell-cell and cell-microenvironment interactions, and which allows for a flexible and reversible development of cellular phenotypes [Loeffler and Roeder, 2002; Roeder and Loeffler, 2002].

Quantitative differences in at least 1 of 3 model parameters (differentiation coefficient d, transition characteristic f_α, cell cycle time τ_c) were found to be capable of inducing a dominant clone, which at first coexists with normal cells for a couple of years, but ultimately takes over the hematopoietic system. It is sufficient to apply the parameter alteration to exactly 1 actively proliferating stem cell, representing a single-cell mutation, to induce a macroscopic CML.

Note that owing to clonal fluctuations, this potential is only realized in about 20% of the cases. Hence, our model predicts that the formation of Ph does not necessarily lead to the formation of CML. This result seems to be confirmed by reports that very low levels of Ph+ cells can be detected in healthy individuals, who, in the long run, do not develop CML [Biernaux et al., 1995]. It can be speculated that in about 80% of the cases the initially very small neoplastic clone might not be able to maintain its own population, but differentiates and finally undergoes apoptosis. Interestingly, another group, which used a fundamentally quite different mathematical model to analyze general issues of clonal domination in myeloproliferative disorders, found a similar percentage of about 80% [Catlin et al., 2005]. It must be mentioned, however, that the authors fitted their model to adequately reproduce mouse and cat data; they did not apply it to the human situation. Catlin et al. [2005] explain the dominance of the malignant clone without any modification of cellular properties but by extra stem cell-supporting resources (for example, alternative niches in the spleen or liver) that only neoplastic HSCs can make use of.

Within our proposed model, differentiation rate d can be interpreted as the velocity of stem cell differentiation. Parameter d of neoplastic cells needs to be decreased to induce the necessary growth advantage, which might correspond to a retarded differentiation process of malignant stem cells. In the clinical situation, highly immature myeloid cells can be found in peripheral blood [Goldman and Melo, 2003]. This observation might relate to an impaired differentiation process of leukemia stem cells. However, the impaired regulation might as well be induced at more mature cell stages.

Because 1 prominent mechanism involved in HSC quiescence is the adhesion to bone marrow stroma, transitions of cells from signaling context **Ω** (actively dividing cells) to **A** (quiescent cells) can be interpreted as stroma attachment processes. Based on this interpretation, an altered transition characteristic f_α in leukemia cells represents a defective adhesion to bone marrow stroma, which has also been reported experimentally [Gordon et al., 1987; Bhatia et al., 1995].

The third critical parameter alteration (cell cycle time τ_c) that was proposed by the model analysis has so far not been reported in the literature, as data on human in vivo

cell kinetics (for example, cycle time distributions) are lacking almost completely.

Additionally to each of these parameter changes, transition characteristic f_ω of neoplastic cells was set to constant at a high level in order to induce an increased production of leukemia cells. This parameter alteration can be interpreted as an unregulated and increased activation of quiescent stem cells into cycle. Such observations can also be found in the literature [Eaves et al., 1986].

We would like to emphasize that within the proposed model, only the given parameter values are capable of inducing the desired model behavior, represented by the criteria stated above. Different parameter values necessarily result in a different competitive behavior of the clones (normal, malignant) and, for example, thus fail to reproduce criterion 1, that is, the delay from the initial malignant transformation to the formation of a manifest CML which is estimated to be about 5–7 years.

In the present work, we could demonstrate that the proposed model consistently explains a variety of clinical data on CML monotherapies such as HU, IFN-α and IM. Qualitative data on HU treatment can be reproduced based on the assumption of a constant kill rate of S-phase cells, independent of genotype. This conforms to biological insights regarding modes of action of HU [de Lima et al., 2003]. Qualitative data on IFN-α therapy can be explained under the assumption that the model parameters of malignant cells, which are capable of explaining the clonal dominance (for example, transition characteristic f_α), are reset to the values assumed for normal cells. Such a normalization of cellular properties could also be observed in biological experiments with respect to adhesion to bone marrow stroma [Bhatia et al., 1994]. This model assumption, however, which predicts a complete eradication of the malignant clone after about 15 years of treatment (fig. 5b), is likely to represent only the theoretically best possible scenario for IFN-α therapy. As there are no reports on the cure of CML with continuous IFN-α administration, it can be speculated that in the clinical situation the growth advantage of malignant cells is only reduced but not entirely eliminated. Please note that the assumption made for IFN-α treatment is sufficient to induce significant cytogenetic remissions, that is, no explicit cell kill has to be assumed.

Beyond the qualitative description of the HU/IFN-α effects, the model is capable of explaining quantitative clinical data on IM treatment [Roeder et al., 2006] by assuming 2 different functional mechanisms. First, a constant degradation rate was applied selectively for proliferative leukemia cells. This conforms to reports on an increased apoptotic rate of malignant cells [Oetzel et al., 2000; Vigneri and Wang, 2001; Holtz et al., 2007]. Second, selectively for leukemia cells, transition characteristic f_ω was decreased to a lower constant level. This can be interpreted as follows: IM reduces the rate at which leukemia cells are activated into cycle. Therefore, the excessive proliferation of malignant cells can be stopped. However, stroma detachment kinetics are still unregulated, that is, independent of cell numbers. The decrease in f_ω has no impact on the biphasic decline kinetics of *BCR-ABL1* transcript levels, but as a result a considerable number of malignant cells is kept in a quiescent state during therapy. Upon cessation of treatment, the therapy-induced reduction of cell cycle activity is replaced by a rapid activation of a large amount of malignant G_0 stem cells into cycle, resulting in the clinically observed rapid relapse of *BCR-ABL1* transcript levels. The potential accumulation of quiescent leukemia stem cells in the course of IM treatment leads to the model prediction of a benefit of the application of proliferation stimulating drugs additional to IM [Roeder et al., 2006]. This strategy has also been suggested experimentally. Jorgensen et al. [2006] and Holtz et al. [2007] performed in vitro studies and found that quiescent stem cells are resistant to IM therapy but can be activated into cycle by cytokines such as G-CSF, rendering them more accessible to IM therapy.

Only 1 of 3 model scenarios, which are capable of inducing CML, is additionally capable of explaining the quantitative clinical data on IM therapy. It is the scenario which assumes parameter differences between normal and malignant cells in transition characteristics f_α and f_ω. The other 2 scenarios, involving differentiation rate d and cell cycle duration τ_c, neither reproduce the typical biphasic decline kinetics of *BCR-ABL1* transcript levels nor the observed rapid relapse after termination of therapy.

Concluding from our theoretical results, we suggest that neither a retarded differentiation process of primitive stem cells nor different cell cycle time distributions in leukemia cells underlie the observed phenomena. Instead, we suggest differences in the dynamic regulation of cell-to-stroma attachment/detachment kinetics as possible key pathologic mechanisms. However, additional influences of, for instance, an impaired differentiation process, particularly of leukemia precursor cells, cannot be ruled out.

The quantitative model presented in this study represents human hematopoiesis in a simplified fashion. Therefore, transition characteristics f_α and f_ω subsume a

variety of cell-intrinsic and cell-extrinsic factors, for example, complex interactions of growth factors. In reality, this is most likely a high-dimensional system. Furthermore, the presented mathematical model does not discriminate between different functional end cells, that is, lineage commitment has been ignored completely at this stage. In hematopoietic disorders such as CML, however, the overproduction of particular lineages, for example myeloid blood cells, is an important endpoint, which was not investigated in this work owing to the limitations of the model. These processes, which can also be explained within the general concept of this model [Glauche et al., 2007a], have to be incorporated in a future version. Another simplification affects mature cell stages, which are called differentiated cells in the model. Feedback mechanisms from more mature cell stages into stem cell regulation processes are not yet included. The same is true for regulatory mechanisms of differentiated cell stages. As a technical consequence, kill rates have exclusively been applied to stem cells. There is no dynamic regulation in the model which compensates for an increased death rate of differentiated cells, for example by an increased amplification of previous cell stages. Consequently, treatment interventions may result in a short-term underestimation of absolute numbers of differentiated cell stages.

Despite these limitations, the stochastic model used in this paper is still the most comprehensive mathematical model which has so far been applied to the situation of CML. Previous model approaches could explain only a subset of the experimentally and clinically observed phenomena. Michor et al. [2005] used a 4-compartment model approach based on a hierarchical view of hematopoietic differentiation. They show that their model can reproduce clinical data on IM treatment on a short time scale of 1 year. The authors conclude from their model analysis that leukemia stem cells are not at all depleted by IM therapy. This statement has since been controversially discussed [Glauche et al., 2007b; Michor, 2007]. Furthermore, it is assumed that normal and malignant cells grow totally independent of each other. As this presumption seems to be unlikely, the authors recently presented a more sophisticated model, which assumes a competition process between normal and malignant cells [Dingli and Michor, 2006]. This model is in principle also able to consistently explain short- and long-term *BCR-ABL1* transcript dynamics.

It could be shown in the present study that the applied stochastic model represents a powerful tool to study emergence and development of CML. Furthermore, this work provides the first systematic model analysis of the functional mechanisms potentially associated with CML emergence. It will be a future challenge to further contribute to a better understanding of the disease. For example, IM resistance, which currently represents a major obstacle to successful treatment, will be analyzed from a modeling point of view. So-called secondary mutations, which are thought to play an important role in IM resistance, are currently neglected in our modeling analyses. They will be considered in future studies. Furthermore, mathematical modeling might contribute to an optimization of treatment planning.

Appendix

The presented model is mathematically represented as a single-cell-based, stochastic process. This means that the development of each individual cell in the system is simulated according to a set of defined rules including stochastic decisions. These rules are applied at discrete time steps ($\Delta t = 1$ h) to simultaneously update the status of all model cells. Each cell is characterized by a triple $[a(t), m(t), c(t)]$, defined by its affinity $a(t) \in \{0\} \cup [a_{\min}, a_{\max}]$, its signaling context $m(t) \in \{\mathbf{A}, \Omega\}$ and its position in the cell cycle $c(t) \in \{0,1, \ldots, \tau_c\}$, with τ_c representing the cell cycle time. To realize an update step, the actual total number of stem cells in $\mathbf{A}$ and Ω $[N_A(t), N_\Omega(t)]$ is determined. Based on these numbers, the new status of each model cell $[a(t+1), m(t+1), c(t+1)]$ is calculated as follows.

(1) If the cell resides in signaling context $\mathbf{A}$, it changes to Ω or stays in $\mathbf{A}$ with probabilities $\omega = a_{\min}/a(t) \cdot f_\omega[N_\Omega(t)]$ and $1 - \omega$, respectively, where f_ω denotes the transition characteristic describing the change from $\mathbf{A}$ to Ω (see below). If the cell stays in $\mathbf{A}$, its affinity a is increased by multiplication with regeneration coefficient $r \geq 1$ $[a(t+1) = a(t) \cdot r]$, until a has reached its maximum value $a_{\max}$. If the cell changes to signaling context Ω $[m(t+1) = \Omega]$, its position in the cell cycle is set to the beginning of S-phase $[c(t+1) = c_1]$, which is calculated by $c_1 = \tau_c + (\tau_S + \tau_{G_2/M})$. Here, τ_S denotes the length of S-phase and $\tau_{G_2/M}$ defines the combined duration of G_2- and M-phase.

(2) If the cell resides in signaling context Ω, it changes to $\mathbf{A}$ or stays in Ω with probabilities $\alpha = a(t)/a_{\max} \cdot f_\alpha[N_A(t)]$ and $1 - \alpha$, respectively, where f_α denotes the transition characteristic describing the change from Ω to $\mathbf{A}$ (see below). Herein, a change to signaling context $\mathbf{A}$ is only possible in G_1-phase of the cell cycle, that is, $c(t) < c_1$. If the cell changes to $\mathbf{A}$, only its signaling context is modified $[m(t+1) = \mathbf{A}]$. If it stays in Ω, it is tested whether a has already reached its minimum value $a_{\min}$. If not, a is decreased by division by differentiation coefficient $d \geq 1$ $[a(t+1) = a(t)/d]$ and the cell cycle position is incremented $[c(t+1) = c(t) + 1]$. In case of cell cycle completion [that is, $c(t) > \tau_c$], $c(t+1)$ is set to zero and a new identical cell is generated (cell division). If, in contrast, a has reached the minimum value $a_{\min}$, it is set to zero and the cell is considered to start a terminal differentiation program. This means that the cell initiates a clone with a fixed life time $\lambda = \lambda_p + \lambda_m$. Herein, the first period λ_p represents the status of proliferating precursors where the clone still amplifies with a duplica-

tion time $\tilde{\tau}_c$. The second period λ_m represents the status of non-proliferating precursors and mature cells (for a schematic of the model, see fig. 1).

The transition probabilities α and ω depend on the actual affinity of the cell $a(t)$, on the fixed parameters a_{min} and a_{max}, and on the transition characteristics $f_\alpha[N_A(t)]$ and $f_\omega[N_\Omega(t)]$, respectively. The latter 2 functions depend on the total number of stem cells (N_A, N_Ω) in the respective target signaling contexts. They are modeled by a general class of sigmoid functions:

$$f_{\alpha/\omega}\left(N_{A/\Omega}\right) = \frac{1}{\nu_1 + \nu_2 \cdot \exp\left(\nu_3 \frac{N_{A/\Omega}}{\tilde{N}_{A/\Omega}}\right)} + \nu_4$$

The parameters ν_1, ν_2, ν_3 and ν_4 determine the shape of $f_{\alpha/\omega}$. $\tilde{N}_{A/\Omega}$ is a scaling factor for $N_{A/\Omega}$. It is possible to uniquely determine ν_1, ν_2, ν_3 and ν_4 by the more intuitive values $f_{\alpha/\omega}(0)$, $f_{\alpha/\omega}(\tilde{N}_{A/\Omega}/2)$, $f_{\alpha/\omega}(\tilde{N}_{A/\Omega})$ and $f_{\alpha/\omega}(\bullet\bullet)$: $= \lim_{N_{A/\Omega}\to\bullet\bullet} f_{\alpha/\omega}(N_{A/\Omega})$:

$$\nu_1 = (h_1 h_3 - h_2^2)/(h_1 + h_3 - 2h_2)$$
$$\nu_2 = h_1 - \nu_1$$
$$\nu_3 = \ln[(h_3 - \nu_1)/\nu_2]$$
$$\nu_4 = f_{\alpha/\omega}(\bullet\bullet)$$

with

$$h_1 = 1/[f_{\alpha/\omega}(0) - f_{\alpha/\omega}(\bullet\bullet)]$$
$$h_2 = 1/[f_{\alpha/\omega}(\tilde{N}_{A/\Omega}/2) - f_{\alpha/\omega}(\bullet\bullet)]$$
$$h_3 = 1/[f_{\alpha/\omega}(\tilde{N}_{A/\Omega}) - f_{\alpha/\omega}(\bullet\bullet)].$$

Examples of transition characteristics f_α and f_ω can be found in figure 2. For exact parameter values used in the computer simulations, see table 2.

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